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75th Anniversary Issue

NZIMLS

THE NEW ZEALAND INSTITUTE OF MEDICAL LABORATORY SICIENCE (INC)

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New Zealand Journal of Medical Laboratory Science

Official Publication of the New Zealand Institute of Medical Laboratory Science Incorporated

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The *New Zealand Journal of Medical Laboratory Science* (the Journal) is the official publication of the New Zealand Institute of Medical Laboratory Science (NZIMLS). The Journal is peer reviewed and publishes original and review articles, case studies, technical communications, and letters to the Editor on all subjects pertaining to the practice of medical laboratory science. The Journal is open access (www.nzimls.org.nz/nzimls-journal) and is published three times per year in April, August, and November. Hard copies are circulated to all NZIMLS members and universities and research units in New Zealand and overseas. Current circulation is about 2,200 copies per issue. Printing is by Griffin Press, Christchurch on environmentally responsible paper using elemental chlorine free third party certified pulp sourced from well managed and legally harvested forests and manufactured under the strict ISO14001 Environmental Management System. The Journal is indexed by CINAHL, EMBASE, SCOPUS, Informit, Thomson Gale, EBSCO and Biosis Citation Index, and the Journal Editors are members of the World Association of Medical Editors (www.wame.org).

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Editorials

Original articles

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JOURNAL

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NEW ZEALAND ASSOCIATION OF BACTERIOLOGISTS

Volume I. APRIL, 1946 No. 1

EDITORIAL

The commencement of a Journal is never a step to be undertaken lightly, especially when subjects of a scientific nature are to be dealt with. However, it was the unanimous opinion of those present at the first Annual General Meeting of the Association, held in Wellington, that a Journal was a necessity as a means of keeping all the members of the Association acquainted with the progress of their fellow members and the dissemination of all knowledge thought to be of interest and use.

The progress of the Journal and its value will, however, depend on the active support of all members, senior and junior, for material to publish, for constructive criticisms and suggestions, and in the initial stages for a generous allowance for difficulties in publication.

At the present it is intended to make this a quarterly journal and to print it in Auckland with the assistance of members of that Laboratory. Should this venture prove a success, the problem of providing a suitable press will have to be faced and a discussion on the Journal and its future should be a subject for consideration at the next Annual General Meeting. In the meantime the Editor would be grateful for suggestions, notes, articles and references for the next issue, these to be to hand by June 1st for the July issue.

First Editorial, Volume 1, No. 1, April 1946

In this issue

Rob Siebers, Editor

Historically, Hb J variants have been identified by high performance liquid chromatography, though capillary zone electrophoresis is increasingly being used as a first line technique for Hb variant identification. Beverley Pullon searched a routine diagnostic in-house laboratory haemoglobinopathy database for Hb J variants and documented the zone, x-axis position, and Hb variant percentage of each variant. She identified four heterozygous cases for Hb J comprising seven different Hb J variants. This article describes characteristic capillary zone electrophoresis chromatogram patterns, zone, x-axis and Hb variant percentage, for seven Hb J variants, five of which have not been previously described on capillary zone electrophoresis.

Among the leading causes of nutritional anaemia in pregnant women are deficiencies of both vitamin B12 and folate. Benedict Ndem and colleagues assessed the possible influence of timely registration of antenatal care on the prevalence of folate and vitamin B12 deficiencies in a Nigerian population. They observed 1.7% folate deficiency, while the prevalence of vitamin B12 deficiency was 21.7% while anaemia was observed in 47.8% of the pregnant women. The prevalence for vitamin B12 deficiency occurred 13 times more compared to folate deficiency. Vitamin B12 and folate deficiencies were observed more among pregnant women who registered late for antenatal care.

In 2016 an investigation into one of the private laboratories in South Australia was undertaken by the Australian Commission on Safety and Quality in Healthcare to ascertain the mishandling of a significant complaint from patients and clinicians. An experienced review team made five recommendations to improve the laboratory's infrastructure. Alan Hicks highlights that the Australian Government does not mandate independent registration of Healthcare scientists which is an anomaly in the international community and creates the potential of risk in patients. In an accompanying Editorial, Michael Legge contrasts the regulation of medical laboratory science between New Zealand and Australia and argues that it is a clear indication of what happens when control systems and procedures are inappropriately applied or at worst ignored. He identifies the important issue of competency assessment, which is the case in New Zealand under a statutory requirement under the Health Practitioners Competence Assurance Act.

MCP-1(CCL-2) and GRO-α (CXCL-1) are chemokines that play an essential role in human body homeostatic and pathological processes. Both chemokines are critical in the pathogenesis of familial Mediterranean fever which Sham Kholoussi and colleagues from Egypt studied as diagnostic markers in a case-control study. They found that serum concentrations of GRO-α were significantly increased in patients with familial Mediterranean fever, compared to healthy controls while CP-1 and E-selectin values were non significantly elevated in familial Mediterranean fever patients. GRO-α may thus play a significant role in the development of familial Mediterranean fever and may be a marker of disease development, as well as a possible useful therapeutic target.

Dennis Mok and colleagues developed a good maintenance practice for Class II biological safety cabinets that can be implemented by the medical laboratory when accreditation for SARS‑CoV‑2 (COVID-19) testing is specified. They developed this by adapting appropriate criteria and requirements from International, national, and regional guidance. They have provided reasonably practical heightened control measures for the medical laboratory to implement to help ensure a safe laboratory environment.

Candida is one of the most commonly isolated yeasts in mucosal fungal microbiota. Vida Alizadeh and colleagues from Iran studied mucosal surfaces related to three different anatomical in 223 healthy people for the presence of *Candida* spp. %). *Candida* albicans was the predominant fungal species isolated, followed by C. glabrata, C. parapsilosis, Pichia kudriavzevi (formerly C. krusei) and C. tropicalis. Children had the highest incidence of *Candida* spp isolated from mucosal surfaces (46.2%) and the lowest was among adults (24.2%). There was a direct association between the age of subjects and the frequency of mucosal *Candida* isolates.

Laboratory requests can be justified and trimmed to what patients necessarily need. Reducing unnecessary laboratory requests can improve patient safety and both patient and provider satisfaction. Maryam Bahreini and colleagues from Iran assessed the status of laboratory requests in emergency departments of three multidisciplinary university hospitals based on ten most expensive and most requested laboratory tests and categorised by the ordering physicians and patients' chief complaints. The most requested tests were a complete blood count, sodium, potassium, urea, and creatinine. Moreover, the most expensive tests were coagulation profile, cardiac troponin, C-reactive protein, erythrocyte sedimentation rate, liver enzymes, alkaline phosphatase, and blood gases. They argue that it should be considered to rationally request the most frequent laboratory tests as many of them can be canceled by physicians and do not change the diagnosis, treatment, prognosis, and disposition in the emergency department.

To reach a level of mastery in reading anti-nuclear antibody (ANA) pattern patterns using indirect immunofluorescence (IFF) methodology is a complex and difficult process. Paul Austin and Helena Thompson-Faiva designed, implemented and assessed the value of a novel integrated training and assessment tool for ANA testing using IIF methodology. Five new recent BMLSc graduates employed at LabPLUS, Auckland were the test subjects of the novel training and assessment system. Individual performance data was collected, analysed and fed back to participants in real time both verbally and in graphical format. All five participants reached the set KPI values within one month of starting their assessment phases. All participants demonstrated an initial rapid reading agreement which then settled into a phase of gradual incremental improvement. Feedback from the participants was positive overall. The conceptualisation and implementation of a novel ANA IIF training and competency assessment system was an operational success. The features of the system gave it high flexibility and allows adaptation to other tests or areas either within or external to the diagnostic pathology laboratory.

Pregnant women are frequently exposed to yeast colonisation and infection compared to non-pregnant women. Mohammad Abu-Lubad and colleagues from Jordan investigated carriage rates of *Candida* species and their virulence factors in high vaginal samples from asymptomatic pregnant women as a probable predisposing factor for neonatal candidiasis. They found that in Jordanian pregnant women, non-*albicans Candida*, mainly *C. parapsilosis,* were the most frequently

isolated *Candida* species from high vaginal swabs. Routine screening and treatment are recommended for pregnant women, irrespective of symptoms.

Sunny Jamati and Maree Bell from Waikato DHB laboratory present a technical note detailing a freeze drying lyophilization process for their external INR and D-dimer quality assurance programme.

2021 is the 75th anniversary of the NZIMLS and the Journal. To celebrate, during 2021 a number of special articles will be published showcasing advances in the major medical laboratory science disciplines in New Zealand over the last 25 years. A previous special edition of the journal was devoted to the first 50 years. In this issue four such articles are presented, namely the Journal 1996-2021 by the Editor, Changes in transfusion science over the past 25 years by Holly Perry, celebrating 25 years of advances in microbiology by Julie Creighton, and cytology practice at Wellington Hospital – 1996-2020 by Sarla Naran and colleagues.

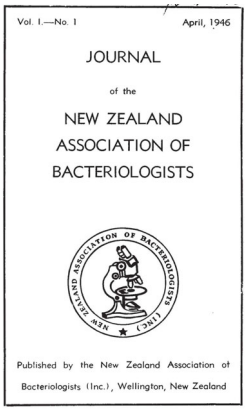
We welcome two new Editorial Board Members. First, Lisa Cambridge from Pacific Edge Limited in Dunedin as Deputy Editor. Lisa will learn all that editorial work is about and will assume the role of Chief Editor upon the ultimate retirement of the current Editor, Rob Siebers. Paul Austin joins as an Editorial Board Member. Paul has a wealth of knowledge and experience in serology, has published a number of articles and has reviewed on occasions for the journal. I welcome both to the team and am sure that they will prove their worth.

Advertisement from Volume 3, No. 3, July 1948

EDITORIAL

75th Anniversary of the Journal

Rob Siebers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JOURNAL

of the

NEW ZEALAND ASSOCIATION OF BACTERIOLOGISTS

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Since the mid 1920's medical laboratory workers in New Zealand had tried to set up a professional organisation. Due to lack of support from pathologists, the great depression, and World War II this did not eventuate until 11th May 1945 when a meeting of senior medical laboratory personal met in Wellington to discuss the desirability of forming a professional association. As a result, the New Zealand Association of Bacteriologists, forerunner of the New Zealand Institute of Medical Laboratory Science (NZIMLS), was formed at a conference in Wellington on 7th and 8th August 1945. At this conference it was unanimously decided that a journal was a necessity of the Association in order to disseminate all knowledge thought to be of interest and use to the profession of medical laboratory science. An editorial committee was established and in April 1946 the 1st issue of the *Journal of the New Zealand Association of Bacteriologists* was issued.

The Journal underwent name changes over the years and is now known as the *New Zealand Journal of Medical Laboratory Science* (the Journal). It has been published without a break since then and this year we celebrate 75 years of the Journal. Our Journal is one of the longest running journals devoted to medical laboratory science in the world. In this issue, and subsequent issues, a number of historical articles on the last 25 years of medical laboratory science in New Zealand, and the Journal and the NZIMLS are, and will be, presented. We hope you enjoy reading them and we will see you again in 25 years to celebrate 100 years of the Journal!

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EDITORIAL

Ensuring professional competency

Michael Legge

The case study by Alan Hicks in this issue clearly contrasts the regulation of Medical Laboratory Science between New Zealand and Australia (1). In addition, it is also a clear indication of what happens when control systems and procedures are inappropriately applied or at worst ignored. The author also identifies the important issue of competency assessment. New Zealand, like the UK, has both qualifications and requirements for competence assessment for all health professionals enshrined in law. The sharp contrast with Australia is where medical laboratory science degrees vary and there is no statutory requirement for demonstrating either competency or professional development. For medical laboratory scientists in New Zealand the Medical Sciences Council (MSC), a statutory requirement under the Health Practitioners Competence Assurance Act (HPCA Act, 2004), has the responsibility to oversee medical laboratory scientists and technicians. The MSC is responsible for issuing annual practicing certificates (APC) to all scientists and technicians who must be registered with the MSC to be employed in the health sector. Both professional groups are required to demonstrate competency (i.e. annual sign-off by their employer or delegated authority) as well as demonstrating continuous professional development by means of maintaining a portfolio of professional activities. The MSC randomly audits 10% of the portfolios each year and has the authority to take action for non-compliance and unprofessional activities.

There is a significant contrast with Australia, where there is no statutory requirements for assessing both competency and professional developments for scientists and technicians. Although the Australian Institute of Medical Laboratory Scienists and Clinical Scientists (AIMS) has on-line discipline-based assessments, there is no requirement to either belong to AIMS or to undertake the assessments, nor is there any protocol to assess competency. The recent introduction of the "National Certification for Medical Laboratory Scientists and Technicians" organised via AIMS is voluntary and has no requirements, including a participant audit process. Interestingly, the Australian Health Practitioners Regulation Agency (established under the Australian Health Practitioners Regulation legislation) works with 15 National Health Boards. However, medical laboratory scientists are not listed under the health professionals who are required to be registered with that Agency.

Returning to the case study by Allan Hicks, would the errors at SA Pathology have been mitigated by legislative requirements for registration, competency and professional development? A question that clearly cannot be answered based on what occurred. However, as the author concludes, an effective legislative framework not only enhances professional standards but will underpin public confidence in the profession as a whole as well as creating professional confidence.

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REFERENCE

1. Hicks A. Errors in pathology: a case study. *N Z J Med Lab Sci* 2021; 75: 16-20.

ORIGINAL ARTICLE

Chromatogram characteristics of seven Hb J variants on capillary zone electophoresis

Beverley M Pullon

ABSTRACT

Background: Historically, Hb J variants have been identified by high performance liquid chromatography, though capillary zone electrophoresis is increasingly being used as a first line technique for Hb variant identification. However, there is a lack of Hb J capillary zone electrophoresis electrophoretic chromatograms in the existing literature. The objective of this report is to describe chromatogram characteristics of seven Hb J variants on capillary zone electrophoresis.

Methods: A routine diagnostic in-house laboratory haemoglobinopathy database was searched for Hb J variants. Capillary zone electrophoresis characteristics of each case were documented, including the zone, x-axis position, and Hb variant percentage. Clinical characteristics, presenting red blood cell parameters, and serum ferritin levels were recorded.

Results: 14 heterozygous cases for Hb J comprising seven different Hb J variants were identified: four *HBA* and three *HBB* variants. *HBA:* Hb J-Cape Town (four cases) with 35.0-37.7% variant migrated in zone 11 at x-axis 121, 0.8-0.9% of Hb J-Cape TownA_2 migrated in the S zone at x-axis 217, and a further minor aberrant peak of 0.5% migrated in zone 12 at x-axis 84; Hb J-Tongariki (two cases) 34.3-36.2% variant migrated in zone 12 at x-axis 106 and 0.6-0.8% of Hb J-TongarikiA_2 migrated in the D zone at x-axis 203; Hb J-Toronto (one case) 23.1% variant migrated in zone 12 at x-axis 97 and 0.4% of Hb J-TorontoA_2 migrated in the D zone at x-axis 208; Hb J-Paris-I (one case) 27.8% variant migrated at x-axis 81 and 0.3% of Hb J-Paris-IA_2 migrated in the D zone at x-axis 198; *HBB*: Hb J-Baltimore (four cases) 50.3-54.1% variant migrated in zone 12 at x-axis 81; Hb J-Bangkok (one case) 51.9% variant migrated in zone 12 at x-axis 93; Hb J-Kaohsiung (one case) 47.3% variant migrated in zone 13 with an x-axis 66.

Conclusions: This article describes characteristic capillary zone electrophoresis chromatogram patterns, zone, x-axis and Hb variant percentage, for seven Hb J variants, five of which have not been previously described on capillary zone electrophoresis. This will be of value in expanding the capillary zone electrophoresis chromatogram library and aid in the provisional identification of Hb J variants.

Keywords: -α3.7 (rightward) deletion, capillary zone electrophoresis, chromatogram, Hb J variants, x-axis.

N Z J Med Lab Sci 2021; 75: 06-10

INTRODUCTION

Hb J are haemoglobin variants that migrate anodally to Hb A on alkaline gel electrophoresis. Over 50 different Hb J variants have been reported with mutations described on the *HBA1, HBA2* or *HBB* genes (1,2). The frequency of different Hb J variants varies considerably with geographical location and ethnicity (3-6). Hb J heterozygosity is usually associated with normal clinical and haematological findings (7). Though, some Hb J variants are commonly co-inherited with α-thalassemia which causes microcytic red blood cell indices (4), while some Hb J variants have abnormal properties which affect the haematological indices (8).

The majority of Hb J cases reported to date have been identified by alkaline Hb electrophoresis or cation exchange HPLC, with definitive identification by DNA molecular analysis. Consequently, there is considerable published literature detailing expected findings of HPLC retention times, percent of total haemoglobin, and characteristic elution peak shape by these methods for Hb J variants (9).

Capillary zone electrophoresis is an alternative first line technique that is emerging as a reliable method for Hb variant detection (10). However, there is a paucity of existing Hb J variant chromatograms using this methodology. Similar to HPLC retention times, capillary zone electrophoresis utilizes a specific x-axis migration position which along with the migration zone, Hb variant percentage, and characteristic capillary zone electrophoresis chromatogram pattern can aid in the provisional identification of Hb variants, including Hb J (11).

In our laboratory, where capillary zone electrophoresis is used for routine haemoglobinopathy screening, 14 heterozygous cases for Hb J were identified over a two-year period. These 14 cases comprised seven different Hb J variants: Hb J-Cape Town, Hb J-Tongariki, Hb J-Toronto, Hb J-Paris-I, Hb J-Baltimore, Hb J-Bangkok, and Hb J-Kaohsiung. This paper describes the chromatogram characteristics of these seven Hb J variants, five of which have not been previously described. The capillary zone electrophoresis migration patterns, zone, X-axis, and Hb variant percentage are reported to aid in provisional identification of Hb J variants and expand the library of variants. All Hb variants were confirmed by subsequent DNA molecular analysis.

MATERIALS AND METHODS

All subjects investigated had full blood counts analysed on an electronic Sysmex XN900 analyser *(Sysmex* Corporation, Kobe, Japan) and serum ferritin levels on a Cobas 6000 instrument (Roche Diagnostics, Indianapolis, Indiana, USA. Identification and quantification of abnormal haemoglobins, Hb A_2 and Hb F levels, were measured by capillary zone electrophoresis using the Hb E programme on a Sebia Capillarys 2 Flex Piercing analyser (Sebia, Lisses, France), and the x-axis migration position was recorded for each Hb variant peak. Alkaline (cellulose acetate, pH 8.5) Hb electrophoresis was performed using the Sebia Hydragel (E) Hb kit (Sebia, Lisses, France) and stained with amidoblack.

α-Thalassemia screening was performed using the immunochromatographic strip test (i+Med Laboratories, Bangkok, Thailand). Confirmatory testing was undertaken by assessing the *HBA* gene copy number using multiplex ligation-dependent probe amplification SALSA probe mix P140 *HBA* assay (MRC-Holland, Amsterdam, Netherlands), followed by quantification on an ABI PRISM® *3130xl* genetic analyser (Applied Biosystems).

Examination of haemolysate and tryptic peptides by electrospray ionization mass spectrometry was undertaken on an Agilent 6230 time-of-flight instrument operating under MassHunter software and connected to a 1260 Infinity binary pump (Agilent Technologies, Santa Clara, CA, USA) by the method of Brennan *et al.*(12).

DNA was extracted from peripheral blood and studies for mutation in *HBA1*, *HBA2* and *HBB* undertaken. Mutation analysis was determined by direct sequencing of overlapping PCR products spanning the entire α- and β-globin genes. Sanger sequencing methodology using the BigDye terminator v3.1 cycle sequencing chemistry (ThermoFisher, Waltham, MA, USA) was performed. The products were then separated by capillary electrophoresis on an ABI PRISM® *3130xl* genetic analyser (Applied Biosystems, Foster City, CA, USA). Reference sequences used were: NM_000558.4 for HBA1, NM_000517.4 for HBA2, and HBB NM_000518.4.

RESULTS

HBA Hb J variants

Four different Hb J *HBA* variants were detected with all cases heterozygous for Hb J. Hb J-Cape Town HBA1:c.278G>A (four cases); Hb J-Tongariki HBA1:c.347C>A (two cases); Hb J-Toronto HBA1:c.17C>A and Hb J-Paris-I HBA2:c.38C>A (one case each). Table 1 provides a summary of results.

All four Hb J-Cape Town cases had normal Hb, mean cell volume (MCV) and mean cell haemoglobin (MCH) with mild erythrocytosis and normal ferritin. Two cases were from European subjects and two were from a mother and daughter of African ethnicity. The Hb J-Cape Town variant levels were between 35.0-37.7% and migrated in zone 11 at x-axis 121. The corresponding minor Hb J-Cape TownA_2 variant levels of 0.8-0.9% were clearly observed in the S zone at x-axis 217. In addition, Hb J-Cape Town had a minor aberrant peak in zone 12 at x-axis 84 comprising approximately 0.5%. All cases were confirmed with –a3.7 (rightward) deletion (Figure 1A).

Hb J-Tongariki was discovered in a mother and daughter of New Zealand European descent. Both had microcytic red blood cell indices, normal Hb with a reduced MCV and MCH, and normal ferritin. Hb J-Tongariki variant levels of 34.3% and 36.2% migrated in zone 12 at x-axis 106. The corresponding minor Hb J-TongarikiA_2 variant levels of 0.6% and 0.8% were clearly observed in the D zone at x-axis 203. Both cases were confirmed with –a3.7 (rightward) deletion (Figure 1B).

Hb J-Toronto was identified in a European subject with normal RBC parameters and ferritin. The Hb J-Toronto variant level of 23.1% migrated in zone 12 at x-axis 97. The corresponding minor Hb J-TorontoA_2 variant level of 0.4% was clearly observed in the D zone at x-axis 208 (Figure 1C). There was a full complement of normal α-globin genes.

Hb J-Paris-I was identified in an Indian subject with normal RBC parameters and ferritin. The Hb J-Paris-I variant level of 27.8% migrated in zone 12 at x-axis 81. The corresponding minor Hb J-Paris-IA_2 variant level of 0.3% was clearly observed in the D zone at x-axis 198 (Figure 1D). There was a full complement of normal α-globin genes.

HBB Hb J variants

Three *HBB* Hb J variants were detected, with all cases heterozygous for Hb J. Hb J-Baltimore *HBB:* c.50G>A (four cases), Hb J-Bangkok *HBB:* c.170G>A and Hb J-Kaohsiung *HBB:* c.179A>C (one case each). All were associated with no abnormal clinical findings and normal haematology parameters. Table 1 provides a summary of results.

Hb J-Baltimore, identified in four New Zealand Europeans with variant levels of 50.3%, 52.1%, 52.2%, and 54.1%, migrated in zone 12 at x-axis 81 (Figure 2A). Hb J-Bangkok, identified in a Chinese subject, had a variant level of 51.9% and migrated in zone 12 with an x-axis of 93 (Figure 2B). Hb J-Kaohsiung, identified in an Asian subject, had a variant level of 47.3% and migrated in zone 13 with an x-axis of 66 (Figure 2C).

Table 1. Summary of data for Hb J cases identified in our laboratory.

Hb J-variant	HGVS[a]	Ethnicity[b]	CBC[c]	α-genotype	Alkaline Ep[d]	CE[e] zone Hb J (HbA_2 variant)	CE[e] x-axis Hb J (HbA_2 variant)	% Hb J (HbA_2 variant)
J-Cape Town	HBA1:c278G>A	European	Mild erythrocytosis	$-\alpha^{3.7}/\alpha\alpha$	HbA + Fast band	11 (S)	121 (217)	35.0 (0.8)
J-Cape Town	HBA1:c278G>A	European	Mild erythrocytosis	$-\alpha^{3.7}/\alpha\alpha$	HbA + Fast band	11 (S)	121 (217)	35.5 (0.9)
J-Cape Town	HBA1:c278G>A	African	Mild erythrocytosis	$-\alpha^{3.7}/\alpha\alpha$	HbA + Fast band	11 (S)	121 (217)	36.7 (0.9)
J-Cape Town	HBA1:c278G>A	African	Mild erythrocytosis	$-\alpha^{3.7}/\alpha\alpha$	HbA + Fast band	11 (S)	121 (217)	37.7 (0.9)
J-Tongariki	HBA1:c.347C>A	NZ[f] European	Microcytic indices	$-\alpha^{3.7}/\alpha\alpha$	HbA + Fast band	12 (D)	106 (203)	34.3 (0.6)
J-Tongariki	HBA1:c.347C>A	NZ European	Microcytic indices	$-\alpha^{3.7}/\alpha\alpha$	HbA + Fast band	12 (D)	106 (203)	36.2 (0.8)
J-Toronto	HBA1:c.17C>A	European	Normal	αα /αα	HbA + Fast band	12 (D)	97 (208)	23.1 (0.4)
J-Paris-I	HBA2:c.38C>A	Indian	Normal	αα /αα	HbA + Fast band	12 (D)	81 (198)	27.8 (0.3)
J-Baltimore	HBB:c.50G>A	NZ European	Normal	αα /αα	HbA + Fast band	12	81	50.3
J-Baltimore	HBB:c.50G>A	NZ European	Normal	αα /αα	HbA + Fast band	12	81	52.1
J-Baltimore	HBB:c.50G>A	NZ European	Normal	αα /αα	HbA + Fast band	12	81	52.2
J-Baltimore	HBB:c.50G>A	NZ European	Normal	αα /αα	HbA + Fast band	12	81	54.1
J-Bangkok	HBB:c.170G>A	Chinese	Normal	αα /αα	HbA + Fast band	12	93	51.9
J-Kaohsiung	HBB:c.179A>C	Asian	Normal	αα /αα	HbA + Fast band	13	66	47.3

[a]HGVS: Human Genome Variation Society. [b]Ethnicity classified according to Ethnicity New Zealand Standard Classification 2017 protocol (13). [c]CBC: complete blood count. [d]Ep: Electrophoresis. [e]CE: capillary zone electrophoresis. [f]NZ: New Zealand.

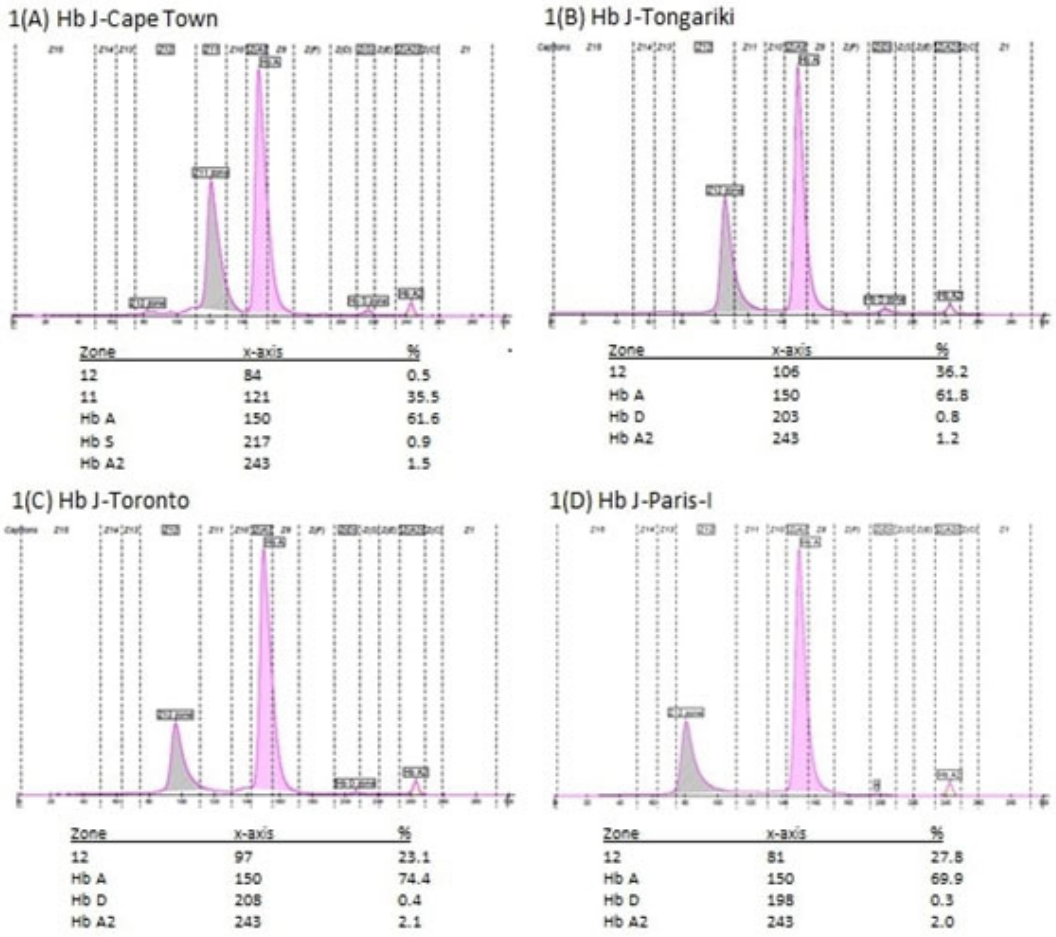

Figure 1. Capillary zone chromatogram migration patterns, zone, x-axis, and Hb variant percentage of *HBA* Hb J variants. (A): Hb J-Cape Town. (B): Hb J-Tongariki. (C): Hb J-Toronto. (D) Hb J-Paris-I.

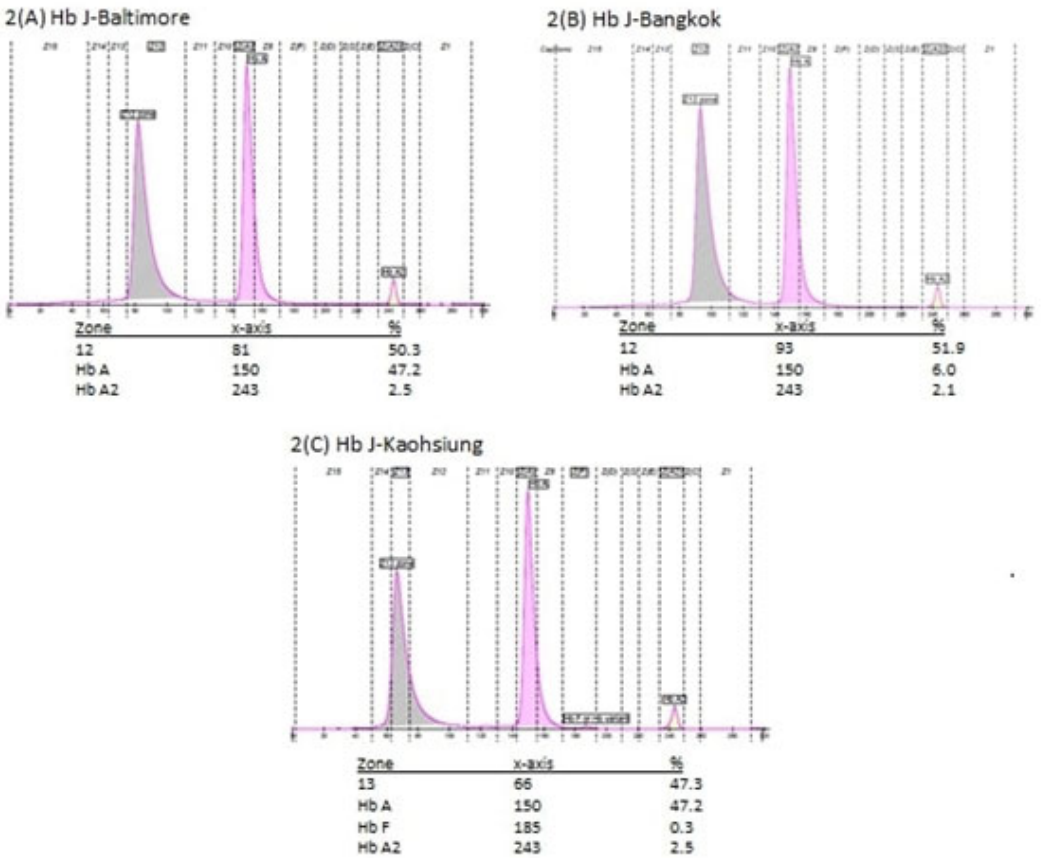

Figure 2: Capillary zone chromatogram migration patterns, zone, x-axis, and Hb variant level of *HBB* Hb J variants. (A): Hb J-Baltimore. (B) Hb J-Bangkok. C): Hb J-Kaohsiung.

DISCUSSION

There is sparse information on capillary zone electrophoresis characteristics of Hb J in the literature. This article details specific x-axis and zone migration position, and characteristic capillary zone electrophoresis chromatogram migration patterns on 14 heterozygous cases of Hb J covering seven different Hb J variants; Hb J-Cape Town, Hb J-Tongariki, Hb J-Toronto, Hb J-Paris-I, Hb J-Baltimore, Hb J-Bangkok, and Hb J-Kaohsiung. This appears to be the first report of the variants Hb J- Cape Town, Hb J- Tongariki, Hb J-Toronto, Hb J-Paris-I, and Hb J-Baltimore detected on capillary zone electrophoresis. This data will add to the body of knowledge and aid in the presumptive identification of Hb J variants.

A search of the literature and Sebia website customer extranet, Hemoglobin Atlas (14) uncovered nine reported capillary zone electrophoresis occurrences of Hb J from six separate publications: three *HBA* variants; Hb J-Abidjan HBA2:c.155G>A (or HBA1) (14), Hb J-Broussais (Tagawa-I) HBA2:c.273G>T (or HBA1) (11,14), Hb J- Sardegna HBA2:c.151C>G (15) and four *HBB* variants; J-Bangkok HBB:c.170 G >A (16,17), Hb J-Europa HBB:c.188C>A (14), Hb J-Kaohsiung HBB:c.179 A > C (6,17), Hb J-Lome HBB:c.180 G >C (15). All publications reported Hb J capillary zone electrophoresis migration zone and Hb variant percentage, but few gave x-axis information.

The ethnic distribution of the Hb J variants described here is consistent with that as reported by others (1,2). Similarly, most of the Hb J heterozygote cases had normal clinical and haematological findings, as described previously (1,2,7). Typically, described features of Hb J-Tongariki with microcytic RBC indices, normal Hb with a reduced MCV and MCH (4), were seen in both of our Hb J-Tongariki cases. Likewise, the characteristic findings of Hb J-Cape Town, a high oxygen affinity variant which is associated with a mild erythrocytosis and normal Hb levels, were observed in all four Hb J-Cape Town cases (8).

The Hb variant percentage is a differentiating feature between *HBA* and *HBB* variants. *HBA* variants have approximately 25%, and *HBB* variants approximately 40-50%. As anticipated, the expected Hb variant percentages were observed in our Hb J heterozygous cases, the *HBA* variants had between 23.1-27.8%, whilst the *HBB* variants had between 47.3-54.1%. However, when an *HBA* variant is co-inherited with an abnormal α-globin genotype, the Hb variant percentage increases (18). The relatively elevated percentage of Hb J-Tongariki (34.3% and 36.2%) and Hb J-Cape Town heterozygote cases (35.0%, 35.5%, 36.7%, and 37.7%), were attributed to co-inheritance of the –a3.7 (rightward) deletion. As described in previous reports, both Hb J-Tongariki and Hb J-Cape Town are commonly co-inherited with α-thalassemia (4,8).

Consistent with previous capillary zone electrophoresis reports of *HBA* Hb J variants, (11,14,15), there were two aberrant peaks on the capillary zone electrophoresis chromatogram, a major peak with a corresponding slow Hb A_2 peak (minor peak). Fucharoen ***et al.*** had observed this previously with *HBA* variants on capillary zone electrophoresis (16). They reported that the capillary zone electrophoresis system could clearly demonstrate this small second Hb A_2 peak for stable *HBA* variants. Conversely, these Hb A_2 variants were not observed on the HPLC formats (19,20). Hb J- Cape Town had an additional small peak in the Hb Bart's zone 12. As expected, only one aberrant peak was observed for the *HBB* Hb J variants on capillary zone electrophoresis, similar to previous reports (6,14,16,17).

Hb J variants have an electrophoretic mobility faster than Hb A, thus on capillary zone electrophoresis migrate ahead of Hb A. Like previous documented Hb J cases (11,14-17), the majority of our cases migrated in zone 12. The exceptions were Hb J-Cape Town and Hb J-Kaohsiung, which migrated in zone 11 and 13, respectively. Similarly, apart from Hb J-Cape TownA_2, which migrated in the S zone, all other slow *HBA* Hb A_2 peaks migrated in the D zone, which concurres with previous reports (11,14,15).

Although many of the Hb J variants in this study migrated in the same zone, when compared to published Hb variants, including Hb J variants, most had distinctive capillary zone electrophoresis chromatograms. Hb J-Tongariki could be distinguished from other zone 12 Hb variants by the variant level, 34.3% and 36.2%, x-axis migration position of the major peak at 106, and corresponding minor Hb J-TongarikiA_2 peak in the D zone at 203 on the capillary zone electrophoresis chromatogram. Likewise, Hb J-Bangkok with a variant level of 51.9% migrated in zone 12 with an x-axis migration position at 93 on the capillary zone electrophoresis chromatogram, whilst Hb J-Kaohsiung migrated in zone 13 with an x-axis migration at 66 on the capillary zone electrophoresis chromatogram and variant level of 47.3%.

Hb J-Paris-I and Hb J-Baltimore both migrated in zone 12 at migration x-axis position 81, however, they were easily distinguishable by the Hb variant level and the presence/ absence of a slow Hb A_2 variant. Hb J-Paris-I had a variant level of 27.8% with a slow Hb A_2 variant peak in the D zone, whilst Hb J-Baltimore had variant levels of 50.3%, 52.1% 52.2%, and 54.1% and no slow Hb A_2 variant peak. Similarly, Hb J-Cape Town and a published case of Hb Fannin-Lubbock I (HBB:c.359G>A) (11,14) both migrated in zone 11 with migration x-axis position 121. Again, they were distinguishable by the Hb variant level and the presence/absence of a slow Hb A_2 variant. Hb J-Cape Town had a variant level of 35.0-37.7% with a slow HbA_2 variant peak in the S zone and a second minor Hb variant peak in zone 12, whilst Hb Fannin-Lubbock I had a variant level of 44.7% and no slow Hb A_2 variant peak (14).

Only one Hb J variant in this study did not appear to have a unique x-axis or differing Hb variant level when compared to published capillary zone electrophoresis data. Hb J-Toronto shared an x-axis migration position with Hb J-Broussais (11,14). Both are *HBA* variants, with the major Hb variant peak eluting in zone 12 at migration x-axis position 97. The Hb variant level was similar, Hb J-Toronto 23.1% and Hb J-Broussais 20.4%. The slow Hb A_2 variant peaks both migrated in the D zone with an identical variant level of 0.4%, but with marginally different x-axis. Hb J-TorontoA_2 migrated at x-axis 208 whilst Hb J-BroussaisA_2 migrated at an x-axis of 207 (14).

Generally, the capillary zone electrophoresis migration patterns, zone, and x-axis, along with the Hb variant percentage and clinical findings, can lead to an adequate presumptive identification of the Hb J variants. However, for those Hb J variants with an overlap of capillary zone electrophoresis migration patterns, definitive identification can only be achieved by DNA molecular analysis.

CONCLUSIONS

This article documents characteristic capillary zone electrophoresis chromatogram migration patterns, zone, x-axis and Hb variant percentage, for seven Hb J variants. For five of these, this is the first time that capillary zone electrophoresis features have been demonstrated. The characterisation of these Hb J cases will be of value to diagnostic laboratories in expanding the capillary zone electrophoresis reference library of Hb variants and facilitate provisional Hb J variant identification.

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ORIGINAL ARTICLE

Timely accessing of antenatal care and prevalence of vitamin B12 and folate deficiencies among pregnant women in a Nigerian population

Benedict N Ndem, Euphoria C Akwiwu, Patience A Akpan, Josephine O Akpotuzor, Iya Eze Bassey, Idongesit K Isong and Eme E Onukak

ABSTRACT

Objectives: Normal pregnancy is accompanied by increased physiologic requirements. Nutritional inadequacy during pregnancy carries the risk of poor pregnancy outcome, including anaemia. Among the leading causes of nutritional anaemia in pregnant women are deficiencies of both vitamin B12 and folate. This study assessed possible influence of timely registration of antenatal care on prevalence of folate and vitamin B12 deficiencies in a Nigerian population.

Methods: A structured questionnaire was utilized to obtain sociodemographic, obstetrics and supplement intake information among 180 study participants. Blood samples were collected from each participant for assays of Vitamin B12 and folate by enzyme linked immunosorbent assay methods and measurement of haemoglobin concentration by automation.

Results: This study observed 1.7% (3/180) folate deficiency, while the prevalence of vitamin B12 deficiency was 21.7% (39/180). Generally, anaemia was observed in 47.8% (86/180) of the pregnant women. Less than a third (26.7%) of the studied population registered for antenatal care within the first trimester. The highest registration (53.3%) occurred during the second trimester. Greater percentages of folate and vitamin B12 deficiencies were observed in association with late registration for antenatal care.

Conclusion: Gestational anaemia still remains a challenge in Nigeria. The prevalence for vitamin B12 deficiency occurred 13 times more compared to folate deficiency. Vitamin B12 and folate deficiencies were observed more among pregnant women who registered late for antenatal care.

Key words: Pregnancy, anaemia, vitamin B12, folate.

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INTRODUCTION

Anaemia remains one of the important health challenges in developing countries that disproportionately affects pregnant women and growing children. Nutritional deficiencies are among the underlying causes of gestational anaemia in sub-Saharan Africa (1,2). The presence of nutritional deficiencies during pregnancy contributes adversely to the outcome of pregnancy with regards to both maternal and infant health (3-6). The negative impact of micronutrient deficiencies on infant health are quite extensive and include increased risks of birth defects, sub-optimal foetal development and chronic health problems in childhood (1,7,8). Against this backdrop, preconception care with possible diet fortification or vitamin supplementation before pregnancy ought to be the ideal approach towards addressing gestational micronutrient deficiency. (5,9,10). However, this dimension of maternal healthcare is not common in developing countries like Nigeria. Inadequate healthcare infrastructure, low socio-economic status, and unplanned pregnancies contribute to the general healthcare-seeking attitudes of women of reproductive age in this part of the globe.

Although nutritional anaemia can be controlled by providing the deficient nutrient(s), either as therapeutic supplements or by fortification of commonly used foodstuff, reports from Nigeria indicate that significant proportions of the population are deficient (1,11). While deficiency states may be tolerable in the apparently healthy general population, the impact of such among pregnant women cannot be overemphasised. Where periconceptional micronutrient assessment and fortification may not be attainable at present in developing regions, supplementation during pregnancy ought to be encouraged at the earliest. Unfortunately, healthcare in resource-poor settings is neither adequate nor evenly distributed. It is rather available to a proportion of the general population and influenced by determinants of socioeconomic status. Even in developed regions, it is interesting to find that use of dietary supplements among pregnant women is also affected by socioeconomic factors, and significantly increases with the trimester of pregnancy (12,13). Unfortunately, with awareness campaigns shifting towards emerging health challenges, socioeconomic and demographic disparities perpetuate treatable health challenges such as gestational anaemia especially in developing regions of the world.

Notable among the causes of nutritional anaemia are iron, folate, and vitamin B12 (7,14). Already, reports of nutritional anaemia during pregnancy among Nigerian women implicate iron as a significant contributor while not much has been observed for folate and vitamin B12 (15,16). There are guidelines for diagnosis and treatment of vitamin B12 and folate deficiencies involving first line tests and confirmatory assays (17). For the purpose of screening out deficiency states, the first line tests were utilised in this study. The study also sought to observe possible impact of timely conventional antenatal care on the prevalence of folate and vitamin B12 deficiencies among pregnant women accessing antenatal care at a tertiary health institution in Southern Nigeria.

METHODS

The present study was conducted in the University of Calabar Teaching Hospital, Calabar, Nigeria; a tertiary hospital that caters for the health needs of the general public within the Calabar metropolis and its environs. This cross-sectional

descriptive study enrolled 180 pregnant women attending antenatal clinic at the hospital consecutively within the period of May 2017 to April 2018. The recruited subjects were apparently healthy pregnant women who had no known medial conditions, had no pregnancy complications, and were not on drugs, except for iron, folate and B12 supplements. They had commenced and sustained regular intake of folate and B12 supplements from the time of their enrolment for routine antenatal visit to term. Subjects with preconception intake and cases of supplement intake prior to commencement of antenatal were not part of the enrolled pregnant women. Ethical approval was obtained from the Health and Research Ethics Committee of University of Calabar Teaching Hospital. Informed consent was obtained from each participant enrolled in the research and confidentiality was maintained.

Biodata and information on time of antenatal registration and commencement of vitamin supplementation were obtained using a questionnaire with confirmation from patients' folders. Blood samples were collected from each enrolled subject at term. Firstline tests were conducted as a screening approach for the detection of deficiency states. The study had the limitation of not conducting confirmatory tests. Serum levels of vitamin B12 and folate were assayed by ELISA method using AccuDiag™ ELISA Kit (Diagnostic Automation/ Cortez Diagnostics, Inc. USA). Assay interference for vitamin B12 from lipemia, cobinamide, haemoglobin, bilirubin and rheumatoid factor range from <0.0001- 0.0008, while that of folate from bilirubin, biotin and lipemia are not detectable by these kit methods. Haemoglobin concentration was measured using an automated Sysmex K2–2IN haematology analyser (Sysmex Corporation, Japan). Statistical analysis using chi square testing was carried out by SPSS 22.0. A p-value of ≤ 0.05 was considered to infer a statistically significant difference.

RESULTS

The observed mean age of the pregnant women that participated in this study was 30 ±2.1 years. Table 1 shows that the participants were literate and comprised both government and non-government workers. There were more primigravida compared to multi-gravidae pregnant women. Among the pregnant women who enrolled in this study, only 26.7% (48/180) registered for antenatal within the first trimester. The second trimester recorded the highest registration of 53.3% (96/180), while third trimester registration was 20% (36/180). Tables 2 and 3 reflect prevalence rates for folate and vitamin B12 deficiencies as well as their contributions to anaemia among the studied population. It was observed that 1.7% (3/180) of the women had serum level of folate less than 6.8nmol/L. The prevalence of vitamin B12 deficiency in this study was 21.7% (39/180) and 86 (47.8%) of the pregnant women had haemoglobin concentration less than 110g/L. Greater percentages of folate and vitamin B12 deficiencies were observed in association with late registration for antenatal care (Table 4).

Table 1. Sociodemographic characteristics of the enrolled pregnant women.

Variables	Number n=180	Percentage (%)
Educational level		
Secondary Education	87	48.3
Tertiary Education	93	51.7
Occupation		
Civil Servants	76	42.2
Non-civil Servants	104	57.8
Gravidae		
Primigravida	121	67.2
Multi-gravidae	59	32.8
Time of ANC Registration/ commencement of supplementation		
First trimester	48	26.7
Second trimester	96	53.3
Third trimester	36	20.0

Table 2. Prevalence rates of anaemia, folate and vitamin B12 deficiencies among pregnant women in Calabar.

Parameter	Cut off value	Total No.	No. of pregnant women with deficiency	%
Folate	6.8nmol/L	180	3	1.7
Vitamin B12	148pmol/L	180	39	21.7
Anaemia	110g/L	180	86	47.8

Table 3. Contributions of folate and vitamin B12 deficiencies to anaemia among pregnant women in Calabar.

Parameter	Cut off value	No. of pregnant women with anaemia	No. of pregnant women with deficiency	%
Folate	6.8nmol/L	86	3	3.5
Vitamin B12	148pmol/L	86	39	45.3

Table 4. Association between commencement of supplementation with anaemia and deficiency states.

Gestational age	Number	Anaemia	Folate	Vit. B12
First month Second month Third month	9 (5.0%) 9 (5.0%) 30 (16.67)	0 0 2	0 0 0	0 0 0
1st trimester	**48 (26.7%)**	**2 (2.3%)**	**0 (0%)**	**0 (0%)**
Fourth month Fifth month Sixth month	36 (20.0%) 30 (16.67%) 30 (16.67%)	4 22 27	0 0 0	2 1 9
2nd trimester	**96 (53.3%)**	**53 (61.7%)**	**0 (0%)**	**12 (30.8%)**
Seventh month Eighth month Ninth month	30 (16.67%) 6 (3.33%) 0 (0%)	25 6 0	1 2 0	21 6 0
3rd Trimester	**36 (20.0%)**	**31 (36.0%)**	**3 (100%)**	**27 (69.2%)**
Grand total	180 (100%)	86 (100%)	3 (100%)	39 (100%)
Chi square test	x^2= 0.001	x^2= 0.001	x^2= 0.001	x^2= 0.016

DISCUSSION

Pregnancy, although a normal physiological process, is fraught with changes that often relate to increased nutritional demands. The extra demands on maternal body stores are necessary for proper development of the embryo. Consequences of inadequate nutritional supply during pregnancy extend to both maternal and infant health (5,18). Thus, maternal care during pregnancy considers possible supplementation of basic nutritional requirements that impact on healthy pregnancy, including Vitamin B12 and folate. In reality several factors influence dietary supplement use (12,13). In Nigeria, these supplements are prescribed to pregnant women as they commence antenatal care in health facilities. Time of antenatal care commencement, however, varies among pregnant women. This is largely due to the fact that in developing countries, such as Nigeria, national health insurance schemes do not cover everyone. While civil servants may be covered, a lot of non-government workers carter for the cost of their healthcare directly. Owing to this prevailing situation, financial constraints often hinder early hospital registration.

Anaemia in pregnancy is a global problem associated with increased morbidity and mortality, its persistence in developing regions, however, appears not to be abating. Anaemia among pregnant women in our locality has remained a significant health challenge in maternal health care. The prevalence for general anaemia in pregnancy for this study was 47.8%. The observed prevalence for vitamin B12 deficiency was 21.7%, while that of folate deficiency was 1.7%; indicating that vitamin B12 deficiency is about 13 times higher than folate deficiency among the studied population. More so, vitamin B12 deficiency contributed to 45.3% of anaemia in pregnancy, while folate deficiency stood at 3.5% out of the 86 gestational anaemic cases recorded in this study. In addition to malaria and iron deficiency, which have been reported previously (15,16,19), vitamin B12 deficiency has shown to contribute substantially to maternal anaemia compared to folate deficiency in the same studied population.

Nutritional deficiencies contribute to poor pregnancy outcome, and therefore require that determinants of status be investigated (8,20-23). The present study enrolled pregnant women on vitamin B12 and folate supplementation whose commencement of supplementation corresponded with the time of antenatal care registration. The approach revealed the importance of early antenatal registration in addressing nutritional anaemia among pregnant women in this region as greater percentages of folate and vitamin B12 deficiencies were observed in association with late registration for antenatal care. Considering that routine antenatal care in this part of the world does not extend to direct laboratory investigation of nutritional factors, deficiency states could go unnoticed until signs associated with severe conditions begin to manifest. A recent study in South-East Nigeria reported spina bifida cystica as the most common anomaly of the central nervous system, and that the majority of mothers of affected children had not taken periconceptional folic acid supplementation (24).

The observation that less than one third (26.7%) of pregnant women commenced antenatal visits and supplement intake in the first trimester of pregnancy poses a challenge to the eradication of nutritional deficiencies in pregnancy. The general implication of this finding is that early pathological defects associated with pregnancy may go unnoticed, contributing to avoidable adverse outcome of pregnancies in this region. More specifically, with regards to the present study, is that there may not be enough time to build up vitamin B12 and folate levels for healthy pregnancy outcome (25). Although supplements are quite readily available and affordable at the community pharmacy outlets, antenatal care affords the opportunity for enlightenment and encourages compliance (24,26). Thus, timely registration for conventional antenatal care would likely impact positively on early commencement of nutritional supplementation and compliance, particularly among women in first-time pregnancy.

In conclusion, gestational anaemia still remains a challenge in Nigeria as a 47.8% prevalence was recorded in this study. There was a 21.7% prevalence for vitamin B12 deficiency, and this frequency of occurrence is 13 times more compared to folate deficiency. Vitamin B12 and folate deficiencies were observed more among pregnant women who registered late for antenatal care.

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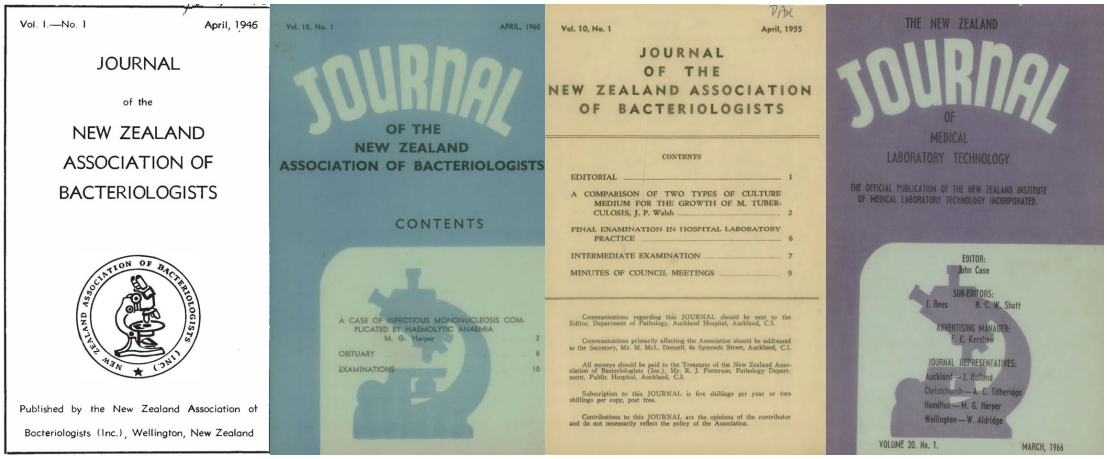

ORIGINAL ARTICLE

Analytical errors in pathology: a case study

Allan J Hicks

ABSTRACT

The Australian Government continues to be unsupportive of independent registration of the scientists through the Australian Health Practitioner Regulation Agency (AHPRA). They are satisfied with the controls of a Royal College of Pathologist, Australasia (RCPA), Registered Pathologist and National Association of Testing Authorities (NATA) accreditation.

In 2016 an investigation into one of the oldest private laboratories in South Australia was undertaken by the Australian Commission on Safety and Quality in Healthcare to ascertain the mishandling of a significant complainant from patients and clinicians. An experienced review team made five recommendations to improve the laboratory's infrastructure.

A major component of any professional registration is continuing education of staff and it is a requirement in both international and domestic standards. The review team made many references to the perceived lack of knowledge of the scientists in their report, but it does not form part of their recommendations. The Australian Government does not mandate independent registration of Healthcare scientists through AHPRA, which is an anomaly in the international community, and, as this report highlights, creates potential of risk to patients.

Keywords: Prostate Specific Antigen, Westgard Rules, Continuing Professional Education

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INTRODUCTION

In April 2016, a South Australian newspaper reported that there had been a medical misadventure at the state's largest private pathology laboratory, South Australia (SA) Pathology (1). This laboratory was established in the 1930's near the Royal Adelaide Hospital, and partnered with the existing hospital laboratories. It has evolved over the last 75 years and now includes training and research arms that support the healthcare system in South Australia.

SA Pathology performs a large range of clinical diagnostic tests, among them testing for levels of prostate specific antigen (PSA). PSA testing was initially described in America in the late 1980's and led to the development of a national Australian guideline for the monitoring of patients who had undergone radical prostate surgery (2). In 2015, SA Pathology used the Siemens ADVIA Centaur platform for this testing and, critically, this platform carries two alarms that inform staff that an assay is malfunctioning.

A report issued by the Australian Commission on Safety and Quality in Healthcare in October 2016 stated that these alarms were not being used by SA Pathology staff. *"The 10_x rule was no longer functioning*" and "*while the 4_{1s} rule was functioning, its reports were accepted despite the repeated warnings*" (4). As a consequence, the SA Pathology PSA test reports should have been considered unsafe as critical control measures may have violated two quality metrics and the review team identified that*; "…a lack of clinical expertise available when interpreting test results and examining the impact of quality assurance issues"* (4) may have contributed to this.

This is not unusual in a large laboratory which could be running hundreds of different chemistry tests on each of its analysers. The Barnes report for the British (UK) National Health Service (NHS) in 2014 concluded that the current quality assurance systems used in UK laboratories have gaps (5). This is not limited to the UK, a review of twenty-one large US academic medical centers showed that there is large variation in understanding and usage of quality control rules (6).

However, it wasn't until SA Pathology had received multiple (customer) complaints from clinicians and patients that the laboratory recognised the issue and began to act. At the beginning of February 2016 SA Pathology conducted an internal review. The problem was identified and confirmatory testing of the PSA assay with an external reference lab began at the end of the same month and released the following statement on its website as a Quality Improvement Program *"Whilst our PSA results have been highly accurate and reliable in the core range, we have moved to improve values below 0.15g/L, where some patients have required repeat testing"(7).*

Enquiries by reporters of the Adelaide Advertiser newspaper identified the truth of the confirmatory testing (1). An urgent review was commissioned by the Health Authority, which was convened in late April and its findings published on the 16th of July 2016 (4). The twenty-two page report, providing a comprehensive timeline of the issue, was conducted by a group comprising a senior clinical pathologist, a senior consultant urologist, a former Commissioner of the New South Wales Health Care Complaints Authority, and two members of the Australian Commission on Safety and Quality in Healthcare.

The report identified major deficiencies in analytical processes, governance and quality assurance of SA Pathology, which led to the following five recommendations:

Recommendation 1:	*Formal apology and implementation of lessons learnt*
Recommendation 2:	*New management structure for SA Pathology*
Recommendation 3:	*Immediately ensure appropriate pre-analytical, analytical and post-analytical quality control procedures are operational within SA Pathology which meet national standards and are reinforced and regularly audited*
Recommendation 4:	*National Accreditation to confirm that SA Pathology meets national laboratory standards.*
Recommendation 5:	*SA Pathology ensures that the Safety Learning System is fully implemented and that all incidents are logged in the Safety Learning System. Clinical staff are trained in open disclosure* (4).

The limited number of recommendations from this review of SA Pathology compares unfavorably to reviews of similar incidents in other laboratory services. A review of cellular pathology governance at Sherwood Forest Hospitals NHS trust by the Royal College of Pathologists in 2013 led to 57 recommendations (9). New Zealand Ministerial inquiries into the

under-reporting of cervical smears led to 46 recommendations (10), and the Health and Disability Commissioners report into PSA testing procedures at Gisborne Hospital, which encompassed administrative and clinical practices, provided 16 recommendations (11). These investigations resulted in fundamental changes to pathology services in those countries.

One of the common findings in these investigations was to highlight the lack of staff education regarding the issue at hand. In Australian laboratories, there is no legal requirement for technical staff to hold a practicing license or seek any continuing professional development (CPD) to maintain employment, as the Australian Government doesn't believe that medical scientists sufficiently influence patient outcomes to warrant it.*"The success of the National Australian Testing Agency (NATA)/ Royal College of Pathologist, Australasia (RCPA) laboratory accreditation scheme has given Australia one of the best pathology sectors in the world and the government's view is there is no evidence that scientist registration is required"* (12). In the recent National Pathology Accreditation Advisory Council Requirements (NPAAC) for Supervision in the Clinical Governance of Medical Pathology Laboratories: **S1.1"** *Every laboratory must be under the direction and control of a designated person who is a medical practitioner and who is responsible for and accountable for the clinical governance of the medical pathology services provided by the laboratory"* (13). The Pathologist has sole responsibility for supervision of the laboratory in Australia, NATA have the responsibility for the assessing the laboratory compliance with international standards and providing nationally recognised accreditation. These mechanisms seem to have been inadequate in this case, as observed by this comment by the review team: "*It appears that there was little understanding within SA Pathology of the clinical use to which the low level tests could be put and little appreciation of potential harm to patients*" (4). Given the above, were the number and type of recommendations resulting from the review of SA Pathology due to the vague nature of the incident, or the review parameters?

DISCUSSION

The central document that was analysed in this article was the Australian Commission on Safety and Quality in Health Care "*Review of serious failure in reported test results for PSA testing of patients by SA Pathology"*. It is a twenty-two page document released in July 2016 following three months of investigation into SA Pathology (4). An experienced team of clinicians and safety experts was assembled and charged with gaining information through meetings and interviews with key stakeholders, general observation of laboratory practices, and a review of all materials relating to the PSA testing incident. They had access to all stakeholders, including patients, clinicians, laboratory staff, SA Pathology Executive team members, and key members of the South Australian Healthcare departments. "*The terms of reference for this review ask it to "advise on improvements required relating to clinical governance systems and processes, incident management, professional standards and accountability within SA Pathology"* (4). The published recommendations were as follows:

Recommendation 1: *Formal apology and implementation of lessons learnt. That SA Pathology issue a public apology for distress and anxiety experienced by the patients because of the inaccurate PSA testing, and provide regular updates to the community on the implementation of lessons learnt from the incident and the new measures introduced to assure the quality control of clinical testing in SA Pathology laboratories* (4). This first recommendation was made in response to the lack of general disclosure given by SA Pathology following its discovery of inaccurate results. That discovery only resulted in communicating the unsafe test reports to referring clinicians. Sikaris *et al* recognised that this level of communication complied with the principle of open disclosure but critically added the need for a public apology stating: *"Although somewhat belated, the review recommends that an apology should now be offered"* (4).

In both the UK and New Zealand there is a robust system for open disclosure of incidents through those countries regulatory authorities: The Health and Care Professions Council (HCPC) in the UK and the Medical Sciences Council of New Zealand (MSCNZ). In Australia, laboratory accreditation is provided by the National Association of Testing Authorities (NATA) and its findings are not available to the public. Following an exhaustive search of both the SA Pathology (14) or the NATA website (15) the author could find no reference to the incident or any subsequent indications to the public that the review findings had been implemented.

Recommendation 2: New management structure for SA Pathology. The Program Director of South Australia Statewide Clinical Support Services engage an appropriately qualified and experienced person to implement an organization structure for SA Pathology that: aligns appropriately skilled staff placement with the operational needs of the service; provides adequate clinical expertise to monitor and inform the production of results; clearly defines the responsibilities and accountabilities of staff; and ensures the requirements of referring clinicians are reflected in the work rules of the service (4). Dr Sikaris made this observation of the management of SA Pathology: "During the review it became apparent that the structure of the organization did not provide sufficient clinical input and management accountability at appropriate levels" (4). In a concurrent review of the Governance and Management of SA Pathology by Dr. Peter Flett, a former Director General of Health in Western Australia, areas of concern were identified within the management structure of SA Pathology (16):

- A top down management process, which is identified on paper and allocated to pathologists and scientists but carries no accountability or responsibility.
- A horizontal management structure termed "Directorates" that identify senior pathologists and senior scientists as line managers, who cover all twelve laboratory sites, but they are confined and work one site. Hence management is off site and distant.
- A central large automated department is identified in each of the three metropolitan laboratories which is managed by a scientist, but the pathologists do not have active management influence within this area.

Interestingly, this deficiency identified in SA Pathology mirrors the deficiency identified in the PSA testing issue at Gisborne Hospital in 2003 which reported that: "*Communication between all levels of management and technical staff must be improved. Problems will recur if there is a continuation of the dysfunctional relationship evident in the past*" (11). It is worth noting that SA Pathology had a Quality Manager in position since 2009 and NATA would have conducted multiple periodic inspections to ensure compliance with International Organisation for Standardisation (ISO) 15189 standards. As part of the Flett review of SA Pathology a new, more conventional structure, was adopted in 2018 that included a Training Manger as an important addition. A laboratory without a dedicated Training Manager means that this responsibility is added to those of the Quality Manager.

Recommendation 3: *Immediately ensure appropriate pre-analytical, analytical and post- analytical quality control procedures are operational within SA Pathology which meet national standards and are reinforced and regularly audited. It is the role and responsibility of the senior management of a pathology service to see that policies, procedures and practices are in place that ensure staff understand the quality control*

system in use, and that staff understand their role in relation to quality control including reporting requirements. This review recommends that an immediate review is undertaken to ensure appropriate quality control procedures are operational within SA Pathology and staff are regularly assessed to ensure their understanding and compliance with quality control procedures (4).

It would be reasonable to assume that all training and competency documents would have been available to the review team as stated in ISO 15189 standard: ***5.1.6 Competence assessment***. *Following appropriate training, the laboratory shall assess the competence of each person to perform assigned managerial or technical tasks according to established criteria. Reassessment shall take place at regular intervals. Retraining shall occur when necessary* (17). Competency assessment documents are required for every test system (any process within the laboratory that produces a result) and are required to be reviewed following any change to the standard operating procedure. Therefore, all chemistry staff using the Siemens ADVIA Centaur must have had an annual competency document that recorded compliance with the six parts of full competency described in the standard:

a) direct observation of routine work processes and procedures
b) performance of equipment maintenance and function checks
c) recording and reporting of examination results
d) review of quality control records
e) assessment of problem solving skills
f) examination of specially provided samples e.g. proficiency testing samples

These records are explicitly stated in the NATA guidance for its assessors. Critically, the inadequacy of competency documentation appears to be a universal issue, as noted by Chittiprol *et al: "The most common areas of deficiencies among all the agencies include: testing personnel qualifications and competency evaluation"* (18). Throughout the report references are made about the apparent lack of knowledge of SA Pathology surrounding the PSA test among the staff at SA Pathology with a urologist interviewed by the review team stating that *"when he called SA Pathology he spoke to a scientist who appeared to have no understanding of the clinical implications of the inaccurate low level tests"*(4).

These observations, and the fact that NATA accreditation had been awarded to SA Pathology, appears conflicted. The third recommendation highlights a serious flaw concerning training and competency and, we contend, an underlying issue of CPD in Australian laboratory staff is being ignored. This is not uncommon and was identified in New Zealand following the incidents in Gisborne: *"Staff therefore had to ask for training opportunities and these were frequently declined"* (11).

The Therapeutic Goods Administration (TGA) of Australia released a Safety Advisory note in August of 2016 regarding a number of PSA testing kits that were showing errors.(19) This would constitute an excellent opportunity for education within a laboratory and in many other countries there is a requirement for CPD for medical scientists. A nationwide CPD scheme does exist for members of the Australian Institute of Medical scientists (AIMS) but is voluntary. Therefore, there is no mechanism to assess whether this important information reached the bench level staff. Due to the lack of any requirement for CPD, employers do not commit resources nor provide time for staff to complete these elements, despite this being an ISO 15189 requirement which states: **5.1.8 Continuing education and professional development**. *A continuing education programme shall be available to personnel who participate in managerial and technical processes. Personnel shall take part in continuing education. The effectiveness of the continuing education programme shall be periodically reviewed. Personnel shall take part in regular professional development or other professional liaison* (17).

International accreditation standards require staff education and records of training whenever a laboratory introduces a new test or changes the procedure around an existing one. This can be as simple as calling a huddle or as elaborate as giving an off-site presentation. In any case competency documents must be modified appropriately to reflect changes and the events recorded as CPD by the staff. Due to the fact that it is not a requirement for staff in Australia there was no mention made of this in Sikaris' report nor is it required of a NATA inspection, despite it being explicit in the ISO 15189 standards which NATA uses as its basis for accrediting Australian laboratories.

Recommendation 4: *National Accreditation to confirm that SA Pathology meets national laboratory standards, the service, as soon as practical, seeks independent assurance of technical competence through accreditation by the National Association of Testing Authorities (NATA) (4).*

NATA was established in 1947 and is a member of many international accreditation organizations. In order for any pathology laboratory to be approved by the Australian Government Department of Human Services (DHS) and to claim Medicare benefits, the laboratory must hold accreditation with NATA.*"NATA is the authority that provides independent assurance of technical competence through a proven network of best practice industry experts for customers who require confidence in the delivery of their products and services"* (15). In New Zealand prior to 2004, International Accreditation New Zealand (IANZ) held a similar position within the healthcare system as NATA does in the Australian Healthcare system today. *"Gisborne Hospital viewed IANZ as the 'primary watchdog for community safety' through its accreditation and assessment processes"* (11).

SA Pathology was established prior to NATA and has a close relationship with the South Australian Health system, so it can be assumed that SA Pathology must have maintained NATA accreditation for many years. Recommendation 4 appears to cast NATA assessments of SA Pathology in a critical light. NATA advises its assessors to audit, amongst other things, training and competence records. (20) The Sikaris review team would certainly have had access to the previous NATA reports, which would have provided them with a considerable amount of information. As it was in the report into the PSA testing errors in Gisborne, the authors make mention of reviewing the previous accreditation document and came to the following conclusion about laboratory accreditation: "*It is clear from subsequent events and investigations by International Accreditation New Zealand (IANZ), and from my investigation, that many of the concerns raised by previous assessments had a sequencing*" (21). The NATA Annual reports for 2016 or 2017 does not refer to the PSA test reporting discrepancies and customer complaint procedures. The assessors must have ratified the SA Pathology Chemical Pathologist directive to report PSA levels as low as 0.3ng/mL with the report mentioning that the manufacturers lowest checked value was much higher than this (4). It does not appear there was any documented additional education provided to laboratory staff about the clinical implications of the new testing criteria. This is require by ISO 15189 standards: **5.1.5** Training**, 5.1.6** Competence assessment, and **5.1.7** Reviews of staff performance (21) which state that: "*The effectiveness of the training programme shall be periodically reviewed"* and *"Retraining shall occur when necessary".* In the National Pathology Accreditation Advisory Council (NPAAC) is an Australian governmental ministerial advisory body responsible for publishing guidance for the pathology service. It explicitly mentions CPD standards in "*Requirements for Medical Pathology Services*": **C6.1(ii)** "*All qualified staff involved in the provision of Medical Pathology Services must provide documented evidence of participation in continuing professional*

development commensurate with their role and responsibilities" (23).

In the NATA document provided to inspectors, they are required to address the following questions pertaining to each ISO standard; (23). Remedial training is required when staff work in unfamiliar areas of the laboratory, work out of hours or at weekends. It is also required for all staff, especially if competency is lacking or when a new test is introduced, and this training must be documented. However, in the latest "*Guidance to NATA assessors*" document this is conflicting as there appears to be no requirement for an inspection team to review training or competency documentation as the only instructions provided are as follows:

Staff training and competence (20)

As a routine aspect of every assessment visit, an appropriate range of tests or inspections should be witnessed to ensure that:

- staff are familiar with test/inspection methods and are capable of carrying them out;
- appropriate training and education has been provided;
- staff are appropriately supervised and technical direction is provided; and
- staff understand test/inspection principles and limitations according to their responsibility.

Standard laboratory practice is to run periodic quality control (QC) materials for every test that is conducted which is detailed in ISO 15189 standard **5.6.2** Quality control (17). This is done to confirm that the analyser is providing a result that reflects the known value of the QC sample. Typically, there are statistical biases built into the system as no test is completely accurate but varies regarding its sensitivity and specificity. However, the review team observed that: "*In SA Pathology it does not appear that bench level staff were able to assess the significance of potential warnings being generated by analytical systems*" (4). The analyser software provides the user with information aligned to these rules and if the test violates these conditions then it will alarm to bring it to the scientists' attention. The "Westgard rules" are used on most laboratory analysers that run multiple QCs and usually require manual input to disable. The report implies that the technical staff ignored a warning from an analyser for some time, before it came to the attention of a senior member of staff or clinician who was aware of these implications.

The laboratory is required to record QC results, which may be done electronically, and are usually reviewed on a monthly basis by senior staff. The understanding of QC particular to any test system is one of the requirements of a competency assessment and these documents must be provided to accreditation inspectors if required. Once again this was highlighted by the review team "The clinical significance of the inaccurate low level PSA readings was not appreciated and action to investigate the cause was not pursued with any sense of urgency" (4). There are a number of ISO15189 standards that mention this practice such as:

4.9 Identification and control of nonconformities

The laboratory shall have a documented procedure to identify and manage nonconformities in any aspect of the quality management system, including pre-examination, examination or post-examination processes.

5.6.2.3 Quality control data

The laboratory shall have a procedure to prevent the release of patient results in the event of quality control failure.

5.7.1 Review of results

The laboratory shall have procedures to ensure that authorized personnel review the results of examinations before release and evaluate them against internal quality control and, as appropriate, available clinical information and previous examination results, and follow up with actions to address issues in a systematic and managed way, with closer monitoring in the implementation of any change in processes (17).

The review team's recommendation of seeking national accreditation with NATA appears redundant as SA Pathology was accredited by NATA at the time of the incident. The fundamental laboratory errors associated with PSA testing may have been missed by the previous NATA inspections but these reports are not publicly accessible. The NATA Annual reports for 2016 or 2017 do not refer to the SA Pathology PSA test reporting discrepancies and customer complaint procedures.

Recommendation 5: *SA Pathology ensures that the Safety Learning System is fully implemented and that all incidents are logged in the Safety Learning System. Clinical staff are trained in open disclosure. SA Pathology should cease using Q-Pulse as its exclusive incident reporting system and fully implement the state wide Safety Learning System (SLS) together with a program that ensures that staff understand how the system operates and the mandatory reporting requirements when clinical incidents are identified. SA Pathology should also review its open disclosure policy and how it will operate in the event of incidents involving patient results. SA Pathology should ensure that its systems allow for all relevant information to be provided to treating clinicians who will conduct the appropriate discussion with the patient (4).*

A standardised approach to safety is always desirable in a large organization as it reduces errors that might be easily be missed by divergent practices. The SLS was introduced into the South Australian Health system in 2010 and despite its use being a requirement of all organisations providing services on behalf of SA Health, it had not been adopted by SA Pathology at the time of the incident. They were still using Qpulse (24), which is a software solution for quality management, document control, and training and competency in use in many laboratories. The review team's recommendation for SA Pathology to surrender its use of Qpulse for incident reporting and adopting the universal SLS is sound. The approach would allow for more robust management of incidents by a team that are appropriately trained and unbiased, and it would also require little to no resource commitment from SA Pathology. This highlights another failure of SA Pathology management team to provide its staff with the required training that may have recognised this incident much earlier.

CONCLUSIONS

The 2015 PSA testing incident by SA Pathology was poorly managed by the executive of the organisation. There are many lessons that should be learnt from how it was handled that could have been implemented into the wider Australian pathology service. As it was in New Zealand fifteen years before however, it seems that the warning signs were not heeded. The Sikaris report found that neither of the regulatory controls in place were deficient, which contrasts with the findings of the review of the Gisborne Health Board which suggested that reliance on a single form of regulation would come with an element of risk. "*It is clear that accreditation by IANZ is no guarantee that all is well in the registered laboratory"* and " *It has become clear in the course of my investigation that, in light of IANZ's limited statutory role, this confidence may be misplaced*" (11).

An experienced and qualified team of specialists spent three months succinctly tying up all the issues in only five recommendations. They decided that the IT system was inadequate, the organisational chart needed review and, despite a recent accreditation inspection, that it needed to be accredited again. That does not appear to be the case here, as analytical errors definitely affected fifty patients. There were certain questions that went unanswered by the review team, namely:

- How Siemens was made accountable for this error.
- Was there any investigation of the other laboratories using the same reagent.

- Did they review the previous accreditation report.
- Were the required training and competency records made available.

The Australian Health Practitioners Regulatory Agency supports many healthcare professions, all of which require some evidence of CPD. If the medical scientists involved had been provided with education and training, then many of the errors that contributed to this incident may have been prevented. The contention that supervision by an RCPA-accredited pathologist and NATA accreditation are the necessary and efficient controls required by laboratories was clearly shown to be inadequate by the fundamental failings of both precautions. The certification project currently implemented by the Australian Institute of Medical Sciences is a positive step but needs more support from industry or legislation to be truly effective. Any framework must ensure that clinical decision-making be made more frequently with the full support of those performing the testing, and licensing of medical scientists and require participation in a CPD scheme should become mandatory.

"Accreditation ensures a public service will be delivered at a standard which is appropriate. This in turn underpins the confidence of the public which then gives the government credibility allowing them to make policies which are robust and achievable". ***Greg Palmer, Laboratory Manager of SA Pathology in the 2016 NATA Annual report.***

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ORIGINAL ARTICLE

Serum levels of chemokines MCP-1, GRO-alpha and E-selectin correlates with familial Mediterranean fever

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ABSTRACT

Background: MCP-1(CCL-2) and GRO-α (CXCL-1) are chemokines that play an essential role in human body homeostatic and pathological processes. Both chemokines are critical in the pathogenesis of familial Mediterranean fever, an inflammatory disorder characterized by chronic inflammation attacks, neutrophil migration, and disruption of the process of apoptosis in damaged areas. E-selectin, one of the molecules that mediate leukocyte-endothelial adhesion, is also expressed through activated endothelial cells and is shed from these cells, its serum levels may indicate endothelial dysfunction in conditions such as familial Mediterranean fever disease.

Aim: In this study, we estimated the serum levels of MCP-1, CXCL-1, and E-selectin as diagnostic markers in familial Mediterranean fever patients, as compared to control subjects.

Patient and methods: 50 subjects were studied, 30 familial Mediterranean fever patients and 20 healthy controls, aged 1 to 18 years. Serum chemokines, namely, MCP-1, GRO-α, and E-selectin, were measured with Luminex® LabScan100.

Results: Serum concentrations of GRO-α was significantly increased in patients with familial Mediterranean fever, compared to healthy controls. MCP-1 and E-selectin values were also elevated in familial Mediterranean fever patients, but this was not statistically significant.

Conclusion: Our findings indicate that GRO-α may play a significant role in the development of familial Mediterranean fever. GRO-α may be a marker of familial Mediterranean fever disease development, as well as a possible useful therapeutic target. MCP-1 and E-selectin were non-significantly elevated in familial Mediterranean fever.

Keywords: Familial Mediterranean fever, MCP-1, CCL-2, GRO-α, CXCL-1, E-selectin, Luminex.

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INTRODUCTION

Familial Mediterranean fever is an inherited autoinflammatory disease frequently sharing similar clinical signs and symptoms with inflammatory bowel disease. The two diseases are characterized by neutrophil migration and chronic inflammation attacks, and in both conditions, disrupted apoptosis mechanisms are present in the damaged areas (1,2). Many inflammatory diseases, such as inflammatory bowel disease, are associated with familial Mediterranean fever in an increasing frequency. Moreover, in conditions where inflammatory bowel disease accompanies familial Mediterranean fever, the frequency of other inflammatory diseases, including juvenile rheumatoid arthritis, also increases (3,4).

Chemokines are a superfamily of small, secreted proteins that have been essential to many homeostatic and pathological human body processes. Chemokines have many roles in intercellular signalling and are especially important in the recruitment of leukocytes to inflammatory sites (5). The monocyte chemoattractant protein-1 (MCP-1/CCL-2) is part of the family of chemokines and a critical monocyte chemotactic factor (6). MCP-1 is activated either by oxidant stress, cytokines, or growth factors constitutively and after motivation. It is formed by various types of cells: epithelial, mesangial, smooth, astrocyte, monocyte, and microcellular (7,8). MCP-1 controls monocyte migration and invasion, natural killer cells (NK), and memory-T lymphocytes. MCP-1 recruits monocytes into active inflammatory concentration and has been shown to be the primary chemokine to recruit monocytes (9). MCP-1 uses the CCR2 receptor to mediate its effects, and, unlike MCP-1, CCR2 expression is relatively limited to certain types of cells. MCP-1 and its receptor CCR2 are induced during different diseases, including inflammatory bowel disease (10). In inflammatory bowel disease, the mucosa shows ulcerative lesions that are in the company of a prominent infiltrate of inflammatory cells. In many clinical and experimental studies on inflammatory bowel disease, various chemokines, including MCP-1, have been shown to be upregulated in mucosal tissues (11).

GRO-α (or CXCL1), a participant of the CXC family, is a 73-amino acid with growth-related properties and is produced by multiple cell lines. Its growth stimulating activity on malignant melanoma cells is well known. Still, many new functions and features of GRO-α were later discovered and associated with inflammation and cardiovascular disease (12). Among many other functions, the GRO family was shown to induce neutrophil chemotaxis, and respiratory burst (13), T-lymphocyte chemotaxis (14), and also, monocytes were shown to be a probable leukocyte target for GRO-α (15).

Endothelial adhesion of leukocytes is primarily regulated by the contact of adhesion molecules and their ligands on specific cells. Several molecules have been identified which mediate leukocyte-endothelial adhesion, including E-selectin intracellular adhesion. Activated endothelial cells express the E-selectin (16). Due to the shedding of selectin adhesion molecules from activated cells, these proteins can be found in soluble forms then released into circulation and may be used as endothelium activation markers and may also indicate endothelial dysfunction by their increased serum levels (17).

The objective of our study was to estimate the role of MCP-1, CXCL-1, and E-selectin in familial Mediterranean fever patients.

METHODS

Ethics

This research was accepted by the National Research Centre's ethics committee in Egypt and written informed consent was obtained from the control cases and the patient's guardian of all children involved in our study before their enrollment. Ethics number (13/146).

Subjects

Thirty familial Mediterranean fever patients and 20 healthy controls aged 1 to 18 years were recruited. Patients enrolled in the study were attending the hospital of the National Research Centre, Cairo, Egypt, from March 2015 to June 2016. All familial Mediterranean fever patients were free of any other diseases or medicines that could impair complement activity. Clinical manifestations and therapies of the familial Mediterranean fever patients are shown in Table 1. All tests were performed mainly for research purposes with no instant clinical benefit, and the patients were informed previously. All Familial Mediterranean. The control subjects had at least one month before blood sampling been free of any medication. In the case of patients, sampling was conducted on the second day of hospitalisation. Serum was separated after one hour of coagulation by centrifugation and serum aliquots were preserved at −30 ° C before cytokine analysis. Total leucocytic count was done using a Medonic m20 3-part differential CBC analyser according to the manufacturer's instructions (Boul Medical AB Domnarvsgaten 4, Spanga, Sweden).

Table 1. Clinical characteristics of patients and controls.

Characteristic	Familial Mediterranean Fever patients	Healthy controls
Number of subjects	30	20
Gender (number of male/female)	14/16	8/12
Age range (years)	1 - 34	3 - 35
TLC (× 10^3/mm3; mean ±SD)	7.8 ±2.6	8.6 ±2.2
Medications (colchicine*)	30/30	0/20

*The dose of colchicine ranged from 0.5-1.5 mg/day. TLC: total leucocytic count.

Luminex cytokine assays

The serum chemokines, MCP-1, GRO-alpha, and E-selectin, were assayed using the Premixed Human Multi-Analyte Assay Kit from R&D Systems (USA & Canada) and measured on a Luminex® LabScan100 analyser. According to the manufacturer instructions analyte-specific antibodies were pre-coated onto color-coded micro-particles. Micro-particles, samples, and standards were pipetted into wells, then washed and Streptavidin-PE added, which binds the biotinylated detection antibodies, then the micro-particles were resuspended in buffer and read using the Luminex® analyser.

Statistical analysis

Statistically analyzed data performed by using SPSS version 16.0 software. A nonparametric Mann-Whitney U test was used to compare concentrations of cytokines between FMF patients and healthy controls. Association between cytokine expressions with age, gender, and total leucocytic count of patients was tested using Spearman rank correlation. Data were presented as median except for total leukocyte count which are presented as mean±SD.A *P*-value <0.05 was deemed statistically significant. For each cytokine, the receiver operating characteristic (ROC) curve was designed to evaluate the effectiveness of these cytokines as biomarkers for familial Mediterranean fever patients against healthy controls. The values of the area under the curve (AUC) and 95% confidence intervals were determined for each cytokine.

RESULTS

Serum level of MCP-1 showed no statistically significant elevation in familial Mediterranean fever patients compared with healthy controls (Table 2). Concentrations of CXCL-1 showed a statistically significant increase in patients with familial Mediterranean fever compared to healthy controls. Moreover, there were insignificant higher levels (P<0.01) of E-selectin in the serum of familial Mediterranean fever patients.

Our results showed that the E-selectin level was inversely correlated with age and directly correlated with a total leukocytic count in familial Mediterranean fever patients (P < 0.05, Spearman correlation) (Table 3). Furthermore, our study indicated that there was a significant negative correlation between MCP-1 level and age of Familial Mediterranean fever patients (P < 0.01, Spearman correlation).

Our results indicated that a ROC curve of CXCL-1 showed a good significant AUC value of 0.830, an excellent sensitivity of 80%, and a moderate specificity of 71% (P < 0.001). Also, the ROC curve of MCP-1 had a non-significant AUC value of 0.649, a low sensitivity of 60%, and a moderate specificity of 68.8%. Moreover, E-selectin showed a ROC curve having a non-significant AUC value of 0.632, an average sensitivity of 66.7%, and a low specificity of 50% (Table 4 & Fig.1).

Table 2. Serum CXCL-1, MCP-1 and E-selectin in familial Mediterranean fever patients and controls.

Parameter	Groups	Median	Minimum	Maximum	*P*-value*
CXCL-1	Familial Mediterranean Fever patients	149.67	57.04	867.07	0.001*
	Healthy controls	45.23	9.07	334.88	
CCL-2	Familial Mediterranean Fever patients	286.04	93.69	1679.08	0.112
	Healthy controls	244.15	35.25	787.07	
E-selectin	Familial Mediterranean Fever patients	82937	28367	113000	0.176
	Healthy controls	58517	142	113000	

*P<0.05 by non-parametric Mann-Whitney U test

Table 3. Association of the three cytokines with clinical parameters of familial Mediterranean Fever patients.

Parameters	R (Spearman correlation)	*P*-value
CXCL-1 level - age	-0.286	0.150
CXCL-1 level - gender	0.016	0.478
CXCL-1 level - TLC	-0.007	0.490
Parameters	**R (Spearman correlation)**	***P*-value**
E-selectin - age	-0.388*	0.023
E-selectin - gender	0.268	0.089
E-selectin -TLC	0.370*	0.041
Parameters	**R (Spearman correlation)**	***P*-value**
CCL-2 level - Age	-0.465**	0.010
CCL-2 level - Gender	0.133	0.263
CCL-2 level - TLC	0.247	0.122

*Correlation is significant at the 0.05 level (1-tailed) **Correlation is significant at the 0.01 level (1-tailed)..

Table 4. AUC values of CXCL-1, CCL-2 and E-selectin.

Cytokines	AUC	S.E.	Sig.	Sensitivity	Specificity	95% Confidence interval
CXCL-1	0.830	0.067	0.001*	80%	71%	0.699-961
CCL-2	0.649	0.088	0.112	60%	68.8%	0.477-0.821
E-selectin	0.632	0.103	0.169	66.7%	50%	0.430-0.834

*Correlation is significant at the 0.01 level (1-tailed).

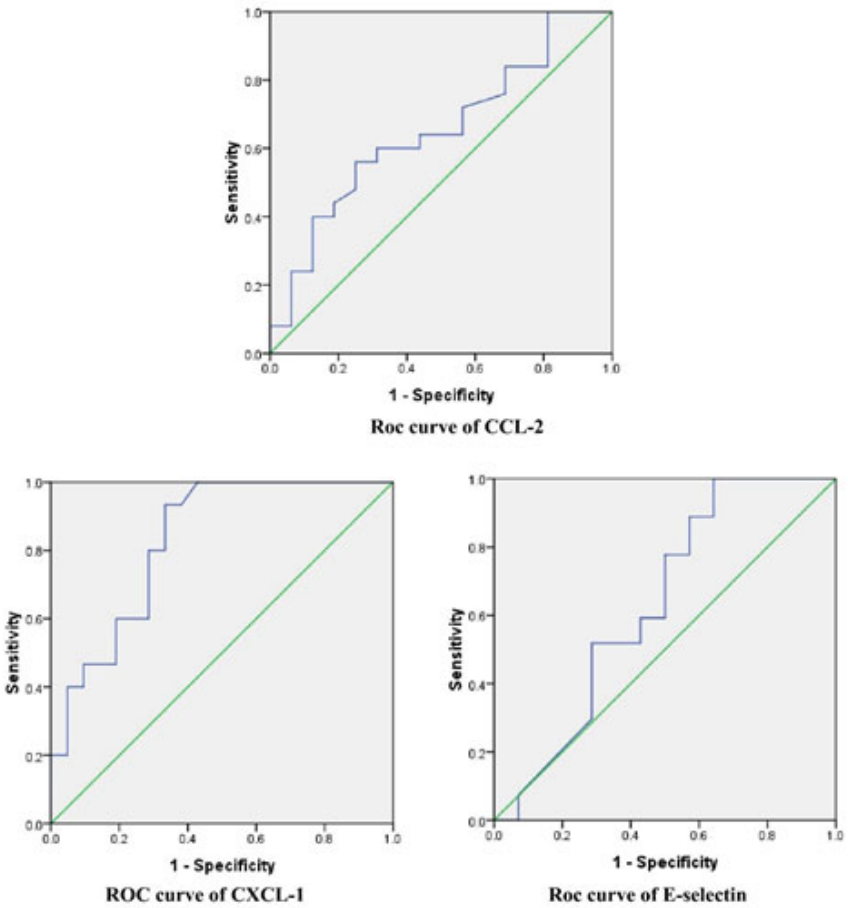

Figure 1. ROC curve of CXCL-1, MCP-1, and E-selectin for familial Mediterranean fever patients versus healthy controls. Diagonal segments are produced by ties.

DISCUSSION

Familial Mediterranean fever is an inherited autoinflammatory disease caused by pyrin mutation, which is involved in inflammasome complex formation (18). Pyrin is encoded by MEFV, the mutated gene found in familial Mediterranean fever patients. Pathogenic variants of MEFV prefer the active pyrin and induce proinflammatory cytokine release and proptosis (19). Accordingly, activated cytokine networks are implicated in the pathogenesis of familial Mediterranean fever (20,21). In our study, we estimated the role of MCP-1 (CCL2), GRO-a (CXCL1), and E-selectin as essential players in the process of chemotaxis and adhesion of recruited neutrophils and monocytes to the site of inflammation. We used the Luminex array system to estimate the serum level of these three cytokines, and we were able to identify some significant differences in levels between familial Mediterranean fever patients and healthy controls.

We found a negligible difference between patients with familial Mediterranean fever and controls in MCP-1 levels. A recent study showed that MCP-1 not only enhances the inflammatory response but can also have anti-inflammatory effects by inhibiting the migration of T cells in experimental colitis models when given under sub-physiological doses (22). Furthermore, MacDermott demonstrated that low-dose of MCP-1 also prevented the inflammation-enhanced carcinoma from the beginning. There is undoubtedly a need for more work to examine these effects in full (23).

Koga *et al*. showed a non-significant increase in MCP-1 serum levels in healthy control subjects compared with familial Mediterranean fever patients (24). Other studies on inflammatory bowel disease also showed contradictory results. Familial Mediterranean fever and inflammatory bowel disease have similar clinical and biological properties. Under both conditions, both are characterised by neutrophil migration and chronic inflammation attacks in the damage areas (1,2). Spoettl *et al.* found that MCP-1 can play a role in the disrupted intestinal differentiation occurring in the mucosa of patients with inflammatory bowel disease (10). Several studies have also identified elevated MCP-1 mRNA and protein expression in patients with inflammatory bowel disease mucosa (25). The small number of subjects involved in the various studies and perhaps the massive difference in diffusion rates of MCP-1 may explain this discrepancy (26).

In our study, the serum levels of GRO-α were significantly increased in patients with familial Mediterranean fever as compared to healthy subjects. Our results agree with Koga et *al.* who showed a significant increase in serum levels of CXCL1 (GRO-α) in familial Mediterranean fever patients (24). In conditions like Crohn's disease and ulcerative colitis, which carry very similar clinical and biological properties with familial Mediterranean fever, Mitsuyama *et al.* found that serum levels of GRO-α are significantly elevated in patients with inflammatory bowel disease and levels thereof perfectly correlate with disease grade. The serum GRO-α in other colitis was only marginally increased compared with familial Mediterranean fever and inflammatory bowel disease. In comparison to more acute colitis, specific inflammatory events that occur in acute and chronic diseases with familial Mediterranean fever and inflammatory bowel disease, distinguished mainly by chronic inflammatory cellular infiltrates that have the potential to produce GRO-α (27).

GRO-α is produced by multiple cell lines, and it has many functions and properties associated with inflammation. In familial Mediterranean fever patients, loss or gain of function mutation of pyrin protein renders it incapable of regulating inflammasome formation or leads to its over-activation (28). This increased inflammasome activity leads to increased activated caspase-1, which split pro-IL-18 and pro-IL-1β into their active matured variants. Proinflammatory cytokines IL-1, IL-18, and TNF-α, induce the expression of a large number of chemokines (29). Such chemokines can be identified in the blood with slightly lower rates than in tissues, thereby providing a gradient that signals cells moving into the tissues. Other studies indicate that monocytes also serve as target cells for members of the CX-C subfamily, suggesting that the classification of biological chemokine activities for different cell types along the lines of the conserved cysteine structural motif is oversimplified (30). This finding is consistent with the demonstration by Quigley *et al.* that in inflammatory bowel disease patients the circulatory GRO-α levels were higher than in healthy subjects (31). Previous studies focused on chemokine activities as soluble proteins, which were thought to serve as chemical factors attracting leukocytes exposed to the gradient of this soluble molecule. The presence of GRO homologs on the endothelial surface can be explained by several possible mechanisms. The protein can directly associate with the cell membrane via a transmembrane region (32).

Selectins are an adherence molecule familiar which controls the initial interaction of adhesion molecules, which regulate the initial interaction between leukocytes and vascular endothelium in acute and chronic inflammation tissues and sites. P- and E-selectin are two members of this family, expressed selectively in inflammation sites by endothelial cells. In particular, E-selectin is involved in migrating neutrophils to sites of acute inflammation and unique lymphocyte subsets into sites of chronic inflammation. The lymphocyte subsets are capable of binding E-selectin are cells that have undergone conversion to a "memory" phenotype, including both alpha/beta and gamma/delta T cells, and show preferential homing to inflammatory extra-lymphoid sites (33). In our study, we found that E-selectin expression with insignificant increases in patients than controls. E-selectin can be secreted into the bloodstream during only acute inflammation stages and can thus be involved in the accumulation of PMNs in the inflammatory areas.

We disagree with Erden *et al*. who demonstrated that genome-wide studies showed associations between single nucleotide polymorphisms within the ABO gene locus and the levels of E-selectin and P-selectin in the plasma, highlighting the possibility for a link between inflammation and ABO blood group (34). There has been previously shown that the soluble E-selectin level was increased in familial Mediterranean fever patients at the time of diagnosis. Many contribute to the possible link between the ABO blood group and colchicine resistance in familial Mediterranean fever patients (35). Also, E-selectin levels in active inflammatory bowel disease is increased significantly, and measurements of E-selectin may be clinically useful in inflammatory bowel disease for long-term monitoring (36).

In our study we found correlations between CXCL-1, MCP-1, and E-selectin with age, gender, and total leukocyte count. We found a negative relationship between MCP-1 & E-selectin with age. Only E-selectin has a positive correlation with the total leukocyte count. CXCL-1 showed no correlation with the three parameters. Our results indicated that a ROC curve of CXCL-1 showed a good significant AUC value of 0.830, a good sensitivity of 80%, and a moderate specificity of 71% ($P < 0.001$). In addition, the ROC curve of CCL-2 had a non-significant AUC value of 0.649, a low sensitivity of 60, and a moderate specificity of 68.8%. Moreover, E-selectin showed a ROC curve having a non-significant AUC value of 0.632, moderate sensitivity of 66.7%, and a low specificity of 50%.

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ORIGINAL ARTICLE

Implementation of Class II biological safety cabinet good maintenance practice: protective countermeasures against SARS-CoV-2 for ISO 15189:2012 accredited medical laboratories

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ABSTRACT

Objectives: The aim of this study was to develop a good maintenance practice for the Class II biological safety cabinet that can be implemented by the medical laboratory when accreditation for SARS-CoV-2 testing is specified.

Methods: The good maintenance practice was developed by adapting appropriate criteria and requirements from International, national, and regional guidance documents: International Standard ISO 15189:2012, Australian Standard AS 2252.2—2009, China National Standard YY 0569—2011, Japanese Industrial Standard JIS K 3800:2009, American National Standard NSF/ANSI 49-2018 and European Standard EN 12469:2000 as well as accreditation related documents (*n* = 76) of the 83/101 (82 %) accreditation bodies in 80/249 (32 %) countries.

Results: A total of 64/1515 (4 %) conformance requirements relating to equipment maintenance were identified in ISO 15189:2012. Clauses 4 and 5 of ISO 15189:2012 contained 8/64 (12.5 %) conformance requirements and 56/64 (87.5 %) conformance requirements, respectively. Comparative analysis of national and regional guidance documents determined that AS 2252.2—2009 contained 8 requirements, YY 0569—2011 contained 8 requirements, JIS K 3800:2009 contained 3 requirements, NSF/ANSI 49-2018 contained 10 requirements, and EN 12469:2000 contained 5 requirements. Accreditation related documents from 7/83 (8.4 %) national accreditation bodies in 7/80 (8.8 %) countries were found to contain supplementary criteria with the following results: Australia (AUS) had 2 requirements, Hong Kong (HKG) had 5 requirements, New Zealand (NZL) had 2 requirements, Singapore (SGP) had 3 requirements, Sri Lanka (LKA) had 2 requirements, the United Arab Emirates (ARE) had 2 requirements and Viet Nam (VNM) had 7 requirements. Overall, conformance requirements (*n* = 64) from ISO 15189:2012 and further testing requirements (*n* = 15) were found to be suited to good maintenance practice for Class II biological safety cabinets.

Conclusions: The current study strengthens the medical laboratory's good practice associated with protective countermeasures against SARS-CoV-2 during the entire examination process. The findings have provided reasonably practical heightened control measures for the medical laboratory to implement to help ensure a safe laboratory environment.

Key words: compliance, conformity, ISO 15189:2012, laboratory safety, quality management, SARS-CoV-2.
Supplementary information is available at https://www.nzimls.org.nz/journals-recent

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INTRODUCTION

The implementation of International Standard ISO 15189:2012 (1) prepared by the International Organization for Standardization (ISO) (2,p.857) for the medical laboratory quality management system remains the recognised benchmark for confirming the competence of medical laboratory practices in the pathology services industry (3,4). The maintenance of such a quality management system has posed formidable challenges to medical laboratories (5), mainly due to the continuous release of relevant guidance documents and recommendations that effect the implementation (6,7). More recently, the emergence of the 'Wuhan virus' (8), officially known as the 'SARS-CoV-2' by the International Union on Microbiological Societies (2,p.903) has resulted in further emphasis on the implementation of a safe environment for laboratory personnel during the entire examination process, especially during aerosol-generating procedures (9,10). Competent implementation of ISO 15189:2012 to ensure relevant infection control measures are effective remains a formidable task for medical laboratories. The medical laboratory needs to ensure the protection level is adequate for laboratory personnel during the examination processes, as specified in Subclause 4.1.1.4 e) of ISO 15189:2012 (1,p.7). This is especially important when the medical laboratory has specified accreditation for SARS-CoV-2 testing.

In addition, the medical laboratory must fulfil the relevant supplementary criteria of accreditation bodies that pertain to protective measures for laboratory personnel. More specifically, the medical laboratory must fulfil relevant conformance requirements (CRs) to ensure laboratory equipment is maintained and fully serviceable; and is at least maintained, repaired and used in accordance with the manufacturer's instructions, as specified in Subclause 5.3.1.5 (Equipment maintenance and repair) of ISO 15189:2012 (1,pp.24-25); however, the relevant CRs relating to equipment maintenance remain unspecified. Although it has been determined that the medical laboratory has 1515 CRs to consider during ISO 15189:2012 implementation (11), and the extent of requirement coverage by guidance checklists prepared by accreditation bodies to support the implementation has been quantified (12), there has been no attempt to quantify the CRs relating to equipment maintenance and supplementary criteria of accreditation bodies that are signatories to the International Laboratory Accreditation Cooperation mutual recognition arrangement.

To date there has been limited research into the management requirements for equipment maintenance associated with ISO 15189:2012 accreditation of the medical laboratory.

No previous study has focused on the routine maintenance aspects of the Class II biological safety cabinet, which is essential equipment that offers protective measures against SARS-CoV-2 during the examination processes. The primary aim of this paper was to develop good maintenance practice (GMxP) for the Class II biological safety cabinet. The development process allowed clarification of the requirements of Class II biological safety cabinet re-certification by quantifying the CRs and relevant supplementary criteria of accreditation bodies that are associated with ISO 15189:2012 accreditation purposes. The development comprised four steps. First, relevant CRs that relate to routine equipment maintenance were identified in Clause 4 (Management requirements) of ISO 15189:2012 (1,pp.6-19) and Clause 5 (Technical requirements) of ISO 15189:2012 (1,pp.19-39). Second, guidance documents prepared by accreditation bodies that are signatories to the International Laboratory Accreditation Cooperation mutual recognition arrangement from countries listed in International Standard ISO 3166-1:2020 (13) prepared by the ISO were analysed. Third, relevant national and regional guidance documents relating to maintenance of Class II biological safety cabinets were also analysed. Finally, the GMxP was developed by adapting appropriate requirements from Clauses 4 and 5 of ISO 15189:2012, relevant national and regional guidance documents, and accreditation related documents. The GMxP has been designed for medical laboratories to implement; if followed, medical laboratories could provide a reasonably practical level of safety to laboratory personnel during the SARS-CoV-2 testing as well as competently meeting the relevant CRs specified in ISO 15189:2012 with an appropriate level of scientific certainty.

MATERIALS AND METHODS

Elicitation of International Standard ISO 15189:2012 conformance requirements relating to equipment maintenance by content analysis

The technique of content analysis offers a reasonably practicable approach for analysing requirements in accreditation guidance documents (12) and International Standards (11,14). In this study, content analysis was used to identify relevant CRs that relate to routine equipment maintenance in Clauses 4 and 5 of ISO 15189:2012. Briefly, content analysis was used to identify the occurrences of the specific term 'shall', which indicates a requirement (15,p.13), and the specific term 'should', which indicates a recommendation (15,pp.13-14). The implied requirements indicated by the verbal form 'shall' were elicited as CRs, as previously described (11).

Guidance document selection criteria for routine maintenance of Class II biological safety cabinets

The criteria for selecting the guidance documents consisted of two fields. First, the guidance document is prepared by an accreditation body that is a signatory to the International Laboratory Accreditation Cooperation mutual recognition arrangement. Second, the country of the accreditation body is listed in ISO 3166-1:2020. In addition, guidance documents containing national or regional testing requirements that apply to maintenance of Class II biological safety cabinets were also included, as specified in Clause 1 (Scope) of ISO 15189:2012 (1, p.1).

Elicitation of supplementary criteria of guidance documents relating to routine maintenance of Class II biological safety cabinets

The technique of content analysis was also used to identify supplementary criteria and in guidance documents as well as their referred documents. Briefly, the implied requirements indicated by the verbal form 'shall' were elicited as supplementary criteria, as previously described (11). In addition, specific tabulated information prepared by accreditation bodies was also analysed for elicitation of supplementary criteria.

Comparative analysis of testing requirements relating to routine maintenance

The routine maintenance tasks were segmented into commonly defined headings (n = 20) for comparative analysis. The tabulated information represents the recommendations and testing requirements identified in accreditation related documents.

Development of good maintenance practice for Class II biological safety cabinets

To develop the GMxP, all supplementary criteria and CRs that fulfil relevant maintenance functions were gathered and practices that are reasonably practicable to ensure the relevant safety aspects are included in the proposed GMxP list for the medical laboratory to implement.

RESULTS

Identification of International Standard ISO 15189:2012 conformance requirement frequency relating to equipment conformity evaluation

Content analysis was used to detect CRs relating to equipment maintenance (Table S1). A total of 64/1515 CRs was identified in Subclauses 4.3 (Document control) (1,pp.10-11), 4.13 (Control of records) (1,pp.15-16), 5.2.1 (General) (1,p.21), 5.2.3 (Storage facilities) (1,p.22), 5.3.1.2 (Equipment acceptance testing) (1,p.23), 5.3.1.4 (Equipment calibration and metrological traceability) (1,p.24), 5.3.1.5 (Equipment maintenance and repair) (1,pp.24-25) and 5.3.1.7 (Equipment records) (1,p.25) of ISO 15189:2012. Clauses 4 and 5 of ISO 15189:2012 contained 8/64 (12.5 %) CRs and 56/64 (87.5 %) CRs respectively.

Selection of guidance documents for comparative analysis

A total of 83/101 (82 %) accreditation bodies in 80/249 (32 %) countries was identified as International Laboratory Accreditation Cooperation mutual recognition arrangement signatories to ISO 15189:2012 (16) (Table S2). Relevant guidance documents [national documents (n = 4) and regional document (n = 1)] were found to be associated with routine maintenance of Class II biological safety cabinets (Table 1). It was found that 29/83 (35 %) accreditation bodies of member countries of the European Committee for Standardization (2,p.857) used European Standard EN 12469:2000 (17) prepared by the European Committee for Standardization, 8/83 (10 %) accreditation bodies used EN 12469:2000 and 21/83 (25 %) accreditation bodies used a harmonised version of EN 12469:2000; 1/83 (1.2 %) accreditation bodies, China National Accreditation Service for Conformity Assessment used China National Standard YY 0569—2011 (18) prepared by the Standardization Administration of the People's Republic of China, 1/83 (1.2 %) accreditation bodies, the Japan Accreditation Board used Japanese Industrial Standard JIS K 3800:2009 (19) prepared by the Japanese Industrial Standards Committee, 2/83 (2.4 %) accreditation bodies, the National Association of Testing Authorities, Australia and International Accreditation New Zealand used Australian Standard AS 2252.2—2009 (20) prepared by Standards Australia, and 3/83 (3.6 %) accreditation bodies, the American Association for Laboratory Accreditation, Perry Johnson Laboratory Accreditation, and the ANSI National Accreditation Board, used American National Standard NSF/ANSI 49-2018 (21) prepared by NSF International.

Identification of recommendations and requirements of guidance documents relating to routine maintenance of Class II biological safety cabinets

Testing requirements (n = 20) were used for comparative analysis (Figure 1). It was determined that AS 2252.2—2009 contained 8 requirements (Table S3), EN 12469:2000

contained 5 recommendations and 5 requirements (Table S4), YY 0569—2011 contained 8 requirements (Table S5), JIS K 3800:2009 contained 6 recommendations and 3 requirements (Table S6), and NSF/ANSI 49-2018 contained 10 requirements (Table S7).

Identification of supplementary criteria of accreditation guidance documents relating to routine maintenance of Class II biological safety cabinets

Of the 83/101 (82 %) accreditation bodies, 7/83 (8.4 %) accreditation bodies (Table S2) were identified that provide additional supplementary criteria for accreditation when Class II biological safety cabinets are used in the medical laboratory. These include the National Association of Testing Authorities, Australia, the Hong Kong Accreditation Service, International Accreditation New Zealand, the Singapore Accreditation Council, the Sri Lanka Accreditation Board for Conformity Assessment, the Emirates International Accreditation Centre and the Bureau of Accreditation.

Proposed good maintenance practice for Class II biological safety cabinets

The GMxP was developed for the medical laboratory to implement as a baseline. Testing requirements (*n* = 15) containing specific calibration intervals (*n* = 4) and checking intervals (*n* = 14) (Figure 2) as well as CRs (*n* = 64) relating to equipment maintenance in ISO 15189:2012 (Table S1) were proposed for implementation.

Table 1. The availability of national and regional guidance documents for comparative analysis.

Countries	Guidance documents	References
Australia (AUS)	**Australian Standard AS 2252.2—2009** Controlled environments: part 2: biological safety cabinets Class II—Design	(20)
Belgium (BEL) *	**European Standard EN 12469:2000** Biotechnology - Performance criteria for microbiological safety cabinets	(17)
China (CHN)	**China National Standard YY 0569—2011** Class II biological safety cabinets	(18)
Japan (JPN)	**Japanese Industrial Standard JIS K 3800:2009** Class II biological safety cabinets	(19)
United States (USA)	**American National Standard NSF/ANSI 49-2018** Biosafety cabinetry: design, construction, performance, and field certification	(21)

*The European Committee for Standardization prepared European Standard EN 12469:2000 for its members; therefore, European Standard EN 12469:2000 has national standard status for the European Committee for Standardization members.

DISCUSSION

This study set out with the aim of determining good practice for routine maintenance of Class II biological safety cabinets in ISO 15189:2012 accredited medical laboratories. The term good practice' has been defined by the ISO as a 'method that has been proven to work well and produce good results, and is 'therefore recommended as a model' in Item 3.1.3 of International Standard ISO 14055-1:2017 (23,p.2) prepared by the ISO. This was achieved by the identification of relevant requirements that could provide support to routine equipment maintenance from Clauses 4 and 5 of ISO 15189:2012, national and regional guidance documents (*n* = 5), and accreditation related documents (*n* = 76). The current study found that CRs (*n* = 64) in Clauses 4 and 5 of ISO 15189:2012 and testing requirements (*n* = 15) from relevant guidance documents could offer enhanced protective countermeasures against SARS-CoV-2.

The proposed GMxP for Class II biological safety cabinets offers a practical approach that can be implemented by technical support services personnel. The implementation has two potential areas that are likely to add value to the medical laboratory. The first area is the enhancement of situational awareness in relation to routine maintenance of Class II biological safety cabinets for the laboratory personnel who contribute to the implementation of the medical laboratory quality management system and laboratory personnel who are the users of Class II biological safety cabinets. A good sense of situational awareness (24), especially in the safety aspects of laboratory personnel, is paramount to the maintenance of a safe working condition, as specified in Subclause 5.2.1 (General) of ISO 15189:2012 (1,p.21). The awareness of maintenance requirements should be part of the authorisation process for the laboratory personnel who undergo training before becoming authorised users of Class II biological safety cabinet, as specified in Subclause 5.3.1.3 (Equipment instructions for use) of ISO 15189:2012 (1,pp.23-24). However, the examination of electrical safety is already a routine maintenance practice for laboratory equipment (25), as specified in Subclause 5.3.1.5 of ISO 15189:2012, therefore it is not repeatedly stated in the proposed GMxP. It is important to note that the manometer that measures differential pressure across the exhaust and negative side of supply blower is subject to routine maintenance (26) and must be calibrated according to the manufacturer's recommendations, as specified in Subclause 5.3.1.4 (Equipment calibration and metrological traceability) of ISO 15189:2012 (1,p.24). Another conformity necessity is the serviceability of the annunciators and alarms management system (27), the associated items must be fully serviceable and inspected for functionality at least once per working day (Figure 2). The proposed GMxP could also support the medical laboratory in the implementation of International Standard ISO 15190:2020 (28) prepared by the ISO relating to relevant safety practices. The proper use of relevant engineering controls (29), such as Class II biological safety cabinets, as specified in Subclause 7.1.2 c) of ISO 15190:2020 (28,p.18), to suppress infectious aerosols, as specified in Subclause 7.4 c) of ISO 15190:2020 (28,p.21), could contribute directly to the implementation of good practice for a safe laboratory environment, as specified in Subclause 4.1.1.4 e) of ISO 15189:2012 (1,p.7).

The second area which is closely connected with the implementation is internal auditing of activities that are related to Class II biological safety cabinet work practices. The medical laboratory is required to conduct internal audits at planned intervals to determine whether equipment maintenance activities are within the specifications of the medical laboratory quality management system, as specified in Subclause 4.14 of ISO 15189:2012 (1,p.17). For laboratory personnel who have roles in conducting internal audits, the maintenance requirements should also be part of the mandatory training to ensure relevant work practices are audited thoroughly, as specified in Subclause 5.1.5 b) of ISO 15189:2012 (1,p.20). The internal auditors may also wish to ensure the Class II biological safety cabinets are subject to relevant certification annually, as specified in Subclause 7.7 c) of ISO 15190:2020 (28,p.22). The identification of 64/1515 (4 %) CRs in ISO 15189:2012 associated with equipment maintenance should provide a more standardised approach for the internal auditors to use, this is highly likely to produce consistent auditing of information (30).

Testing requirements (n = 20)	AS 2252.2 (n = 8)	EN 12469 (n = 5)	YY 0569 (n = 8)	JIS K 3800 (n = 3)	NSF/ANSI 49 (n = 10)
Airflow alarm serviceability	**Requirement**	**Recommendation** See †	See ‡		**Requirement**
Airflow balance				**Recommendation** See §	
Airflow direction orientation (downflow)		**Requirement**	**Requirement**	**Recommendation** See **	**Requirement**
Airflow direction orientation (sash seal)			**Requirement**	**Recommendation** See ††	**Requirement**
Airflow direction orientation (view screen)			**Requirement**	**Recommendation** See ‡‡	**Requirement**
Airflow direction orientation (working access opening)		**Requirement**	**Requirement**	**Recommendation** See §§	**Requirement**
Airflow rate (uniform downflow velocity)		**Recommendation** See ***			
	Requirement	**Requirement**	**Requirement**	**Requirement**	**Requirement**
Airflow rate (uniform inflow velocity)	**Requirement**	**Requirement**	**Requirement**	**Requirement**	**Requirement**
Carcass leaktightness				**Recommendation** See †††	
Electrical safety		See ‡‡‡	See ‡‡‡		See ‡‡‡
Front aperture containment efficiency	**Requirement**				
Installed filter system integrity	**Requirement**	**Requirement**	**Requirement**	**Requirement**	**Requirement**
Internal supply fan interlock alarm serviceability	**Requirement**	**Recommendation** See §§§	See ****		**Requirement**
Sash alarm serviceability	**Requirement**	**Recommendation** See ††††	See ‡‡‡‡		**Requirement**
Sound pressure level (L_p)	See §§§§	See §§§§	See §§§§	See §§§§	See §§§§
Surface integrity		**Recommendation** See *****	**Requirement**		
Vibration	See †††††	See †††††	See †††††	See †††††	See †††††
Work surface illuminance (E_v)	See ‡‡‡‡‡	See ‡‡‡‡‡		See ‡‡‡‡‡	See ‡‡‡‡‡
Work surface irradiance (E_e)	See §§§§§		See §§§§§		
Work zone intergrity	**Requirement**				

Figure 1. Selected routine maintenance testing requirements for Class II biological safety cabinets: Australian Standard AS 2252.2—2009, European Standard EN 12469:2000, China National Standard YY 0569—2011, Japanese Industrial Standard JIS K 3800:2009, and American National Standard NSF/ANSI 49‑2018.
†European Standard EN 12469:2000 recommends (routine maintenance testing) that the alarm indicators be checked to the manufacturer's specification [see Subclause K.3 (Class II MSCs) of European Standard EN 12469:2000].
‡China National Standard YY 0569—2011 specifies a requirement (factory testing) that the airflow alarm test be performed [see Subclause 5.3.7.5 (Airflow alarm) of China National Standard YY 0569—2011].
§Japanese Industrial Standard JIS K 3800:2009 recommends (routine maintenance testing) that the airflow balance test be performed [see Clause 9 (Inspection) of Japanese Industrial Standard JIS K 3800:2009].

[**]Japanese Industrial Standard JIS K 3800:2009 recommends (routine maintenance testing) that the airflow direction test be performed [see Subclause 8.9 a) of Japanese Industrial Standard JIS K 3800:2009].
[††]Japanese Industrial Standard JIS K 3800:2009 recommends (routine maintenance testing) that the airflow direction test be performed [see Subclause 8.9 d) of Japanese Industrial Standard JIS K 3800:2009].
[‡‡]Japanese Industrial Standard JIS K 3800:2009 recommends (routine maintenance testing) that the airflow direction test be performed [see Subclause 8.9 b) of Japanese Industrial Standard JIS K 3800:2009].
[§§]Japanese Industrial Standard JIS K 3800:2009 recommends (routine maintenance testing) that the airflow direction test be performed [see Subclause 8.9 c) of Japanese Industrial Standard JIS K 3800:2009].
[***]European Standard EN 12469:2000 recommends (routine maintenance testing) that the airflow rate be measured in accordance with the manufacturer's instructions [see Subclause K.3 (Class II MSCs) of European Standard EN 12469:2000].
[†††]Japanese Industrial Standard JIS K 3800:2009 recommends (routine maintenance testing) that carcass leak tightness test be performed [see Clause 9 (Inspection) of Japanese Industrial Standard JIS K 3800:2009].
[‡‡‡]European Standard EN 12469:2000 specifies a requirement (cabinet classification) [see Annex A.8 (Electrical safety) of European Standard EN 12469:2000], China National Standard YY 0569—2011 specifies a requirement (factory testing) [see Subclause 5.4.15 (Electrical safety) of China National Standard YY 0569—2011] and American National Standard NSF/ANSI 49:2018 specifies a requirement (worker comfort and safety) [see Annex F.8 (Electrical leakage and ground circuit resistance and polarity tests) of American National Standard NSF/ANSI 49‑2018] relating to electrical safety. The examination of electrical safety is a requirement of International Standard ISO 15189:2012 [see Subclause 5.3.1.5 (Equipment maintenance and repair) of International Standard ISO15189:2012].
[§§§]European Standard EN 12469:2000 recommends (routine maintenance testing) that the internal supply fan interlock alarm test be performed [see Subclause K.3 (Class II MSCs) of European Standard EN 12469:2000].
[****]China National Standard YY 0569—2011 specifies a requirement (factory testing) that the internal supply fan interlock alarm test be performed [see Subclause 5.3.7.2 (Internal supply/exhaust fan interlock alarm) of China National Standard YY 0569—2011].
[††††]European Standard EN 12469:2000 recommends (routine maintenance testing) that the sash alarm test be performed [see Subclause K.3 (Class II MSCs) of European Standard EN 12469:2000].
[‡‡‡‡]China National Standard YY 0569—2011 specifies a requirement (factory testing) that the sash alarm test be performed [see Subclause 5.3.7.1 (Front window operation port alarm) of China National Standard YY 0569—2011].
[§§§§]Australian Standard AS 2252.2—2009 specifies a requirement (worker comfort and safety) [see Subclause 5.3.3 (Sound level) of Australian Standard AS 2252.2—2009], European Standard EN 12469:2000 specifies a requirement (worker comfort and safety) [see Annex A.3 (Sound) of European Standard EN 12469:2000], China National Standard YY 0569—2011 specifies a requirement (factory testing) [see Subclause 5.4.3 (Noise) of China National Standard YY 0569—2011], Japanese Industrial Standard JIS K 3800:2009 specifies a requirement (factory testing) [see Subclause 5.7 (Noise) of Japanese Industrial Standard JIS K 3800:2009], and American National Standard NSF/ANSI 49‑2018 specifies a requirement (worker comfort and safety) [see Annex F.11.4 (Acceptance) of American National Standard NSF/ANSI 49‑2018] relating to sound pressure level generated by the cabinet.
[*****]European Standard EN 12469:2000 recommends (routine maintenance testing) that the external and internal surfaces are visually inspected for surface defects, cracks or other damage [see Subclause K.3 (Class II MSCs) of European Standard EN 12469:2000].
[†††††]Australian Standard AS 2252.2—2009 specifies a requirement (worker comfort and safety) [see Subclause 5.3.2 (Vibration) of Australian Standard AS 2252.2—2009], European Standard EN 12469:2000 specifies a requirement (worker comfort and safety) [see Annex A.3 (Sound) of European Standard EN 12469:2000], China National Standard YY 0569—2011 specifies a requirement (factory testing) (see Subclause 5.4.5 of China National Standard YY 0569—2011), Japanese Industrial Standard JIS K 3800:2009 specifies a requirement (factory testing) [see Subclause 5.9 (Vibration) of Japanese Industrial Standard JIS K 3800:2009] and American National Standard NSF/ANSI 49‑2018 specifies a requirement (worker comfort and safety) [see Annex F.10.4 (Acceptance) of American National Standard NSF/ANSI 49‑2018] relating to vibration level generated by the cabinet.
[‡‡‡‡‡]Australian Standard AS 2252.2—2009 specifies a requirement (worker comfort and safety) [see Subclause 5.3.4 (Lighting) of Australian Standard AS 2252.2—2009], European Standard EN 12469:2000 specifies a requirement (worker comfort and safety) [see Annex A.2 (Lighting) of European Standard EN 12469:2000], Japanese Industrial Standard JIS K 3800:2009 specifies a requirement (factory testing) [see Subclause 5.8 (Lighting intensity) of Japanese Industrial Standard JIS K 3800:2009] and American National Standard NSF/ANSI 49‑2018 specifies a requirement (worker comfort and safety) [see Annex F.9.4 (Acceptance) of American National Standard NSF/ANSI 49‑2018] relating to work surface illuminance level generated by the fluorescent lamp.
[§§§§§]Australian Standard AS 2252.2—2009 specifies a requirement (worker comfort and safety) [see Subclause 5.3.5 (Ultraviolet radiation) of Australian Standard AS 2252.2—2009] and China National Standard YY 0569—2011 specifies a requirement (factory testing) [see Subclause 5.4.14 (UV lamp) of China National Standard YY 0569—2011] relating to work surface irradiance generated by the ultraviolet lamp.

Testing requirements (n = 15)	Calibration interval (n = 4)	Checking interval (n = 14)	Procedures (n = 18)
Airflow alarm serviceability	≥ 1 per year		Calibration of the threshold limits according to the manufacturer's specifications.
		≥ 1 per each working day	Appropriate check to maintain serviceability quality.
Airflow direction orientation (downflow)		≥ 1 per year	Conformity with the manufacturer's specifications and a relevant certification standard.
Airflow direction orientation (sash seal)		≥ 1 per year	Conformity with the manufacturer's specifications and a relevant certification standard.
Airflow direction orientation (view screen)		≥ 1 per year	Conformity with the manufacturer's specifications and a relevant certification standard.
Airflow direction orientation (working access opening)		≥ 1 per year	Conformity with the manufacturer's specifications and a relevant certification standard.
Airflow rate (uniform downflow velocity)		≥ 1 per year	Conformity with the manufacturer's specifications and a relevant certification standard.
Airflow rate (uniform inflow velocity)		≥ 1 per year	Conformity with the manufacturer's specifications and a relevant certification standard.
Control knobs and switches serviceability		≥ 1 per each working day	Appropriate check to maintain serviceabililty quality.
Front aperture containment efficiency		≥ 1 per year	Conformity with the manufacturer's specifications and a relevant certification standard.
Installed filter system integrity		≥ 1 per year	Conformity with the manufacturer's specifications and a relevant certification standard.
Internal supply fan interlock alarm serviceability	≥ 1 per year		Calibration of the threshold limits according to the manufacturer's specifications.
		≥ 1 per each working day	Appropriate check to maintain serviceability quality.
Manometer serviceability	≥ 1 per year		Conformity with the manufacturer's specifications.
Sash alarm serviceability	≥ 1 per year		Calibration of the threshold limits according to the manufacturer's specifications.
		≥ 1 per each working day	Appropriate check to maintain serviceability quality.
Work surface irradiance (E_e)		≥ 1 per year	The work surface irradiance (E_e) conforms to ≥ 400 mW/m^2 when the E_e is unspecified by the manufacturer.
Work surface sterility ******		≥ 1 per week	Appropriate check to maintain sterility quality.

Figure 2. Suggested routine maintenance testing requirements for Class II biological safety cabinets.
******International Standard ISO 14698-1:2003 (22) provides relevant guidance information relating to determination of biocontamination on work surface [see Annex C (Guidance on determining biocontamination of surfaces) of International Standard ISO 14698-1:2003 (22, pp. 18-19)].

CONCLUSIONS

The present study was designed to develop GMxP for the Class II biological safety cabinet. This is particularly relevant for medical laboratories that provide SARS-CoV-2 testing service to ensure laboratory personnel to operate in a safe laboratory environment.

Overall, this study strengthens the idea that GMxP for the Class II biological safety cabinet should be implemented by medical laboratories as part of ensuring the practices relating to SARS-CoV-2 testing are performed with reasonably practicable protective countermeasures.

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ORIGINAL ARTICLE

Isolation of *Candida* species on human mucosal surfaces

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ABSTRACT

Objectives: Fungi are part of the healthy microbiota of the human mucosal surfaces. Fungal microbiota is often altered in disease states, especially when there is an imbalance in the host. (1). *Candida* is one of the most commonly isolated yeasts in mucosal fungal microbiota (2). When considering the role of mucosal surfaces as a reservoir from which infection of a susceptible host can occur, determining the fungal microbiome, especially *Candida*, is important. Therefore, in the current study, mucosal surfaces related to three different anatomical sites of the body were tested in 223 healthy people for the presence of *Candida spp.*

Results: Children had the highest incidence of *Candida* isolated in the mucosa surfaces (n=43, 46.2%) and the lowest was among the adults (n=22, 24.2%). *Candida albicans* (n=53, 58.2%) was the predominant fungal species isolated from mucosal surfaces, followed by *C. glabrata* species complex (n=18, 19.8%), *C. parapsilosis* species complex (n=10, 11.0%), *Pichia kudriavzevi* (formerly *C. krusei)* (n=6, 6.%) and *C. tropicalis* (n=4, 4.%). In this study the isolation of *Candida* species was similar between males (n= 37, 40.%) and females (n= 54, 59.3%).

Conclusions: There was a direct association between the age of subjects and the frequency of mucosal *Candida* isolates. *C. albicans* was the predominant species isolated from all age groups. This study showed no statistically significant relationship between subjects gender and the frequency of mucosal *Candida* isolates.

Keywords: Mucosal *Candida* isolates, normal flora, age, gender, DNA-sequencing, culture.

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INTRODUCTION

The genus *Candida* was created during the IX International Botanical Congress. *Candida* species are opportunistic fungal pathogens found as part of the normal microflora in human skin and mucosal surfaces and exist harmlessly in these anatomical sites (7-9). Mucous membrane surfaces constitute the largest interface between the host and the environment. There are several defense mechanisms that protect and combat commensal and opportunistic microorganisms from causing infection in a healthy individual (10).

Researchers have shown that *Candida* species are human and warm-blooded animal reservoirs (11,12). Since *Candida* species are commonly found in the human body, colonisation or infection occurs when this normal balance is disrupted (10). An example of this is long-term use of broad-spectrum antibiotics which may interrupt this balance. Other factors that can affect this imbalance include immunological functions, age, physiological changes, physical and mental disabilities, debilitating diseases, diabetes, the clinical use of immunosuppressive drugs, occupation, obesity, vascular disease, alcoholism, and avitaminosis. Damage to the mucus membranes and to the salivary glands also allow for *Candida* colonisation (10-15). They can be the agents of local or systemic opportunistic infections in hospitalized patients, those under intensive treatment, immunocompromised patients, and even in healthy individuals. Infections caused by opportunistic pathogens such as *Candida* spp. are frequent, especially in immunodeficiency disorders such as neutropenia, neoplasia, decompensated diabetes mellitus, malnutrition, organ transplantation, and AIDS (6,16 -19). Two of the most common factors influencing the microbiome of mucosal surfaces are gender (13) and age (15).

There is little information about *Candida* distribution inhabiting human mucosal surfaces. This is the first study on this topic in Iran. The purpose of this study was to determine the type and percentage of *Candida* population residing on mucosal surfaces (the mouth, nose, and vagina). In addition, to determine the potential influences of age and gender on the prevalence of *Candida* isolation on mucosal surfaces.

MATERIALS AND METHODS

Ethics

This study was approved by the ethical committee of Tehran University of Medical Science (Ethics Committee protocol: IR.TUMS.SPH.REC.1395.1759). Written informed consent was obtained from all subjects or their guardians prior to sample collection.

Sampling

A total of 223 healthy individuals (including 93 males and 130 females) categorised into three age groups were included. The distribution of subjects in each group was as follows:

- 103 children aged between 1 and 12 years old. Most were drawn from schools and kindergartens.
- 63 healthy adults aged between 18 and 45 years old. This group was composed of students of Tehran University, factory workers, health workers, and housewives
- 57 adults greater than 60 years old, which mainly consisted of retiries.

At the time of sampling, the age and gender of each subject were recorded. The mouth, nose, and vagina were sampled by means of a cotton-tipped swab moistened with sterile serum physiology.

Mycological techniques

All swabs were inoculated on Sabouraud Chloramphenicol Agar (SC, Merck, Germany) by swab-streak technique (3). All cultures were incubated at 25°C for 4 days. Isolated colonies were identified by growth on Corn Meal Agar Tween 80 (Micro Media, Hungary). Using a straight wire, we picked a colony off the surface from the SC medium and made a deep cut in the Corn Meal Agar (a horizontal furrow). Then we placed a flamed sterile coverslip over the line of inoculum. After incubation for 24 to 48 hours at 22°C the streaks were examined. Along with such streaks, *Candida albicans* produces characteristic mycelium-bearing ball-like clusters of budding cells and thick-walled round chlamydospores (4) which is distinct from other *Candida* species.

In this study, we picked several colonies from each SC medium and plated onto CHROMagar *Candida* medium (Paris, France). This medium contains chromogenic substrates which react with enzymes secreted by the target microorganisms to yield colonies of varying colors (5). CHROMagar *Candida* medium is species-specific, allowing the organisms to be identified to the species level by their color and colonial characteristics. It is able to detect and differentiate three species types: *C. albicans* by growth as light to medium green colonies, *C. tropicalis* by growth as steel blue colonies accompanied by purple pigmentation diffused into surrounding agar, and *C. krusei* by growth as large, fuzzy, rose-colored colonies with white edges, after incubation for 48 hours at 37°C. Other yeasts may develop either light to dark mauve or cream colors (e.g., *C. glabrata* comlex) on isolation media (5).
In this study, DNA sequencing was performed on isolates that were not identified by mycological techniques.

DNA extraction

An aliquot of 100 µL of yeast cell suspension was transferred to microtubes and incubated at 100°C in a boiling water-bath for 10 min, then centrifuged at 5,000*g* for 5 minutes. The supernatant (containing the DNA) was carefully transferred to a clean tube and was used for PCR.

RESULTS

Among the studied population, including children, adults and the elderly, divided into three age groups, 91 subjects were positive for *Candida* isolation. The highest prevalence of *Candida* isolation was related to the children age group (n=43, 46.2) and the lowest prevalence was related to the adults (n=22, 2.17%), and showed there was a direct association between the age of subjects and the frequency of mucosal *Candida* isolates ($p < 0.001$). *Candida albicans* (n=53, 58.2%) was the predominant isolated species from all age groups followed by *C. glabrata* species complex (n=18, 19.8%), *C. parapsilosis* species complex (n=10, 11.0%), *Pichia kudriavzevi* (formerly *C. krusei)* (n=6, 6.6%), and *C. tropicalis* (n=4, 4.4%). It should be noted that there was not a significant difference between the individual's age and the isolated organism (Table 2 and Figure 1).

In this study, 223 healthy subjects (93 males and 130 females) were examined. The isolation of *Candida* species was significantly similar between males (n= 37, 40.6%) and females (n= 54, 59.4%) and there was no significant difference in the mucosal *Candida* population between genders ($p<0.999$). *Candida albicans* (n= 53, 58.2%) was the most frequently isolated species and *Candida tropicalis* (n=2, 2.%) showed the lowest prevalence in both genders (Table 3 and Figure 2).

Considering *Candida* isolation based on anatomical sites, from 91 positive sites for *Candida* isolation, the highest prevalence of *Candida* isolation was related to the oral mucosa (n=62, 68.1%) and the lowest prevalence was related to the vagina (n= 10, 11.0 %) in females (Table 4 and Figure 3).

In the children among 43 *Candida* species isolated from three different mucosal surfaces (the mouth, nose, and vagina), the highest prevalence of *Candida* isolation was related to the oral mucosa, with 35 positive cases (81.4%), and the nasal mucosa with eight positive cases (18.6 %) was in second place. In this age group, none of the vaginal specimens had positive results for *Candida* isolation. Also, *Candida albicans* with 23 isolates (54.8%) was the predominant species isolated from three different mucosal surfaces of each person in this age group (Table 2).

In adults, among 22 isolated *Candida* species, the highest prevalence of *Candida* isolation was from the vagina (n=10, 5%) followed by the mouth (n= 9, 40.9%), and the nasal mucosa (n=3, 13.4 %). Furthermore, in this age group, *Candida albicans* with 16 isolates (72.7 %) was the predominant species from the mucosal surfaces of each person (Table 2).

In the elderly age group, among 26 isolated *Candida* species, the highest prevalence of *Candida* isolation was related to the oral mucosa with 18 positive cases (69.2 %), and the nasal mucosa with eight positive cases was in second place (30.8%). All vaginal specimens in this age group had negative results for *Candida* isolation. Also, in this age group, *Candida albicans* with 14 positive isolates (53.8 %) was the predominant species (Table 2).

The oral mucosa of male participants had the highest prevalence of *Candida* isolation with 28 of 37 isolated *Candida* species (75.7%). Also, in the male gender, the nasal mucosa with nine *Candida* isolates (24.32%) was in second place. This finding was similar in the female gender with 32 out of 54 *Candida* species isolated from the oral mucosa (59.3 %), and the nasal mucosa with 12 isolates (22.2 %) was in second place.

Table 1. GenBank accession numbers of DNA sequences included in this study

Fungal elements	GenBank accession numbers used in the sequence analysis
Candida albicans	MG913256, KY996543, MF614725, MF614723, MH729024, MG599201, MN4193373, MN318604, MH729028, KC905069, MG818819, MG818824
Candida tropicalis	MK793225, MK547223
Candida glabrata species complex	KU992391, KU992392, KU992393, LC311497, LR757911
Pichia kudriavzevi (Candida krusei)	MH545928, FJ515204
Candida parapsilosis complex	MK394127, KY102205, KP131738, EU564209

Table 2. The frequency of *Candida* species isolated from mucosal surfaces in different age groups

***Candida* species**	**Children**		**Adults**		**Elderly**		**Total**	
	Number	%	Number	%	Number	%	Number	%
Candida albicans	23	54.8	16	72.7	14	53.8	53	58.2
Candida parapsilosis species complex	2	4.8	2	9.1	6	23.1	10	11.0
Candida glabrata species complex	15	35.7	3	13.6	0	0	18	19.8
Pichia kudriavzevi (Candida krusei)	2	4.8	0	0	4	15.4	6	6.6
Candida tropicalis	0	0	1	4.5	2	7.7	4	4.4
Total	43	100	22	100	26	100	91	100

Table 3. Frequency of *Candida* species isolated from mucosal surfaces based on gender.

***Candida* species**	**Men**		**Women**	
	Number	**%**	**Number**	**%**
Candida albicans	21	56.8	32	59.3
Candida parapsilosis species complex	4	10.8	6	11.1
Candida glabrata species complex	8	21.6	10	18.5
Pichia kudriavzevi (Candida krusei)	3	8.1	5	9.3
Candida tropicalis	1	2.7	1	1.9
Total	37	100	54	100

Table 4. Frequency of *Candida* species isolated from mucosal surfaces based on anatomic site.

Anatomical site	***Candida albicans***		***Candida parapsilosis* species complex**		***Candida glabrata* species complex**		***Pichia kudriavzevi (Candida krusei)***		***Candida tropicalis***		**Total**	
	Number	%	Number	%	Number	%	Number	%	Number	%	Number	%
Mouth	36	67.9	6	60.0	14	77.8	6	100	0	0	62	68.1
Nose	10	18.9	2	20.0	4	22.2	0	0	3	75.0	19	20.9
Vagina	7	13.2	2	10.0	0	0	0	0	1	25.0	10	11.0
Total	53	100	10	100	18	100	6	100	4	100	91	100

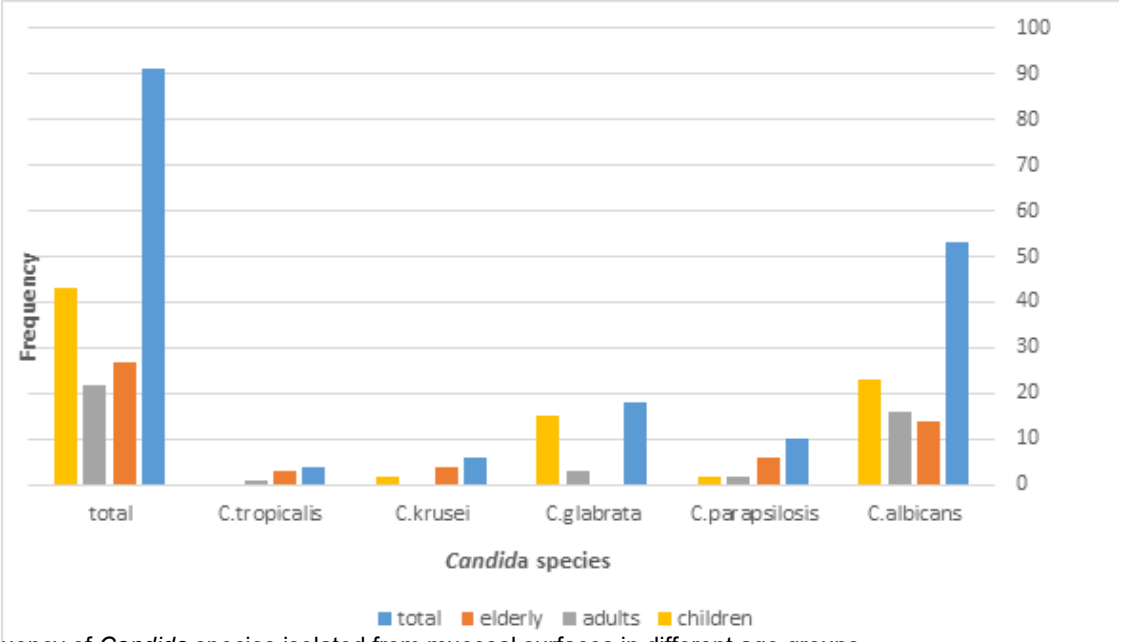

Figure 1. Frequency of *Candida* species isolated from mucosal surfaces in different age groups.

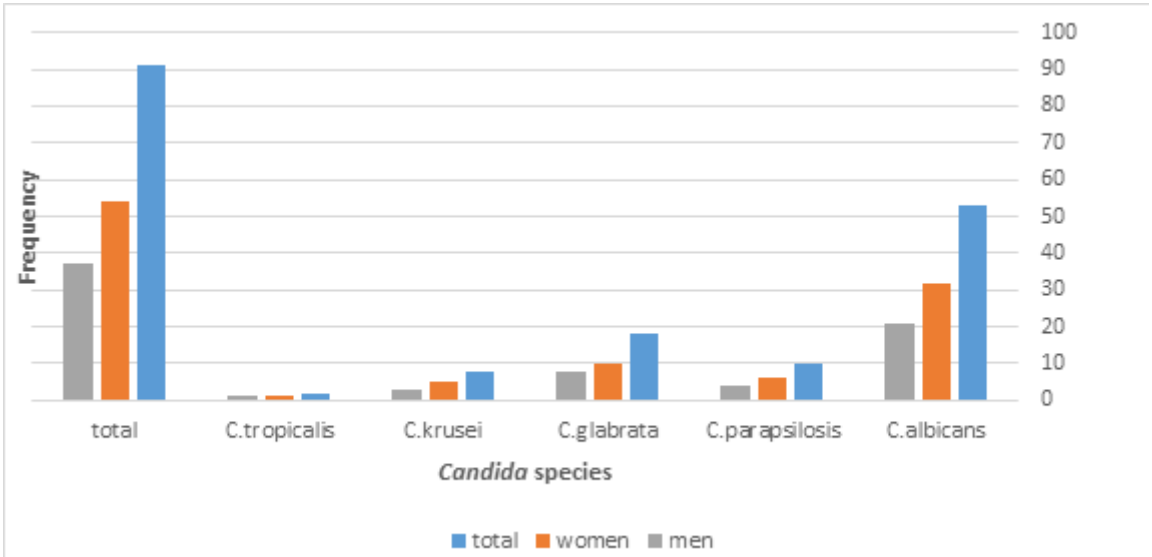

Figure 2. Frequency of *Candida* species isolated from mucosal surfaces based on gender.

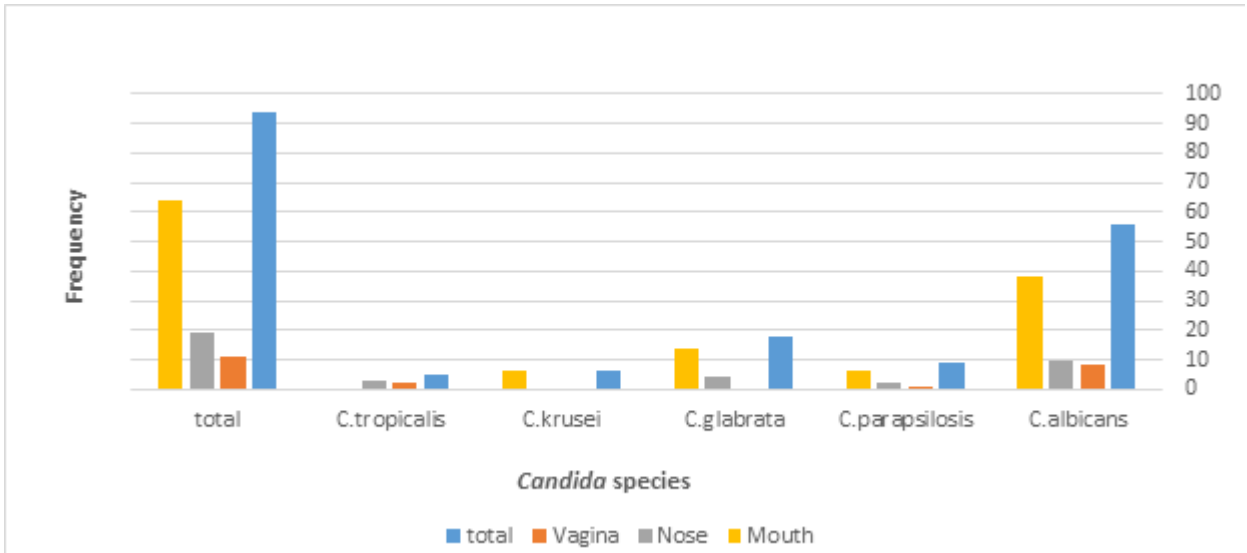

Figure 3. Frequency of *Candida* species isolated from mucosal surfaces based on anatomical site of the body.

DISCUSSION

Our study showed that *Candida* was most prevalent in children. This is mainly due to poor oral health among this age group (3-12 years). The elderly age group was the second most prevalent group. The main reason for this finding is due to the use of dentures in this age group (15, 21).

The mucosal *Candida* community was similar between males and females and there was no significant difference in *Candida* isolation between genders. Both groups share similar sociodemographic, lifestyle, and dietary conditions (20,23). The results of this investigation showed that the oral mucosa had the highest incidence of *Candida* isolation compared to the nasal mucosa and the vagina. This could be due to increased exposure of the mouth as the route of entry for a range of foods and other infecting microbes. Other factors that increase the microbial burden of mouth, such as poor hygiene, gingival disease, poor dentition and the effect of tooth decay and the use of dentures, the increased chance of yeast isolation from this anatomical site is explainable (15,25). *C. albicans* was the most frequently isolated organism (68.1%) from all mucosal surfaces in the present study (17,22). This finding is consistent with other published literatures (6,14).

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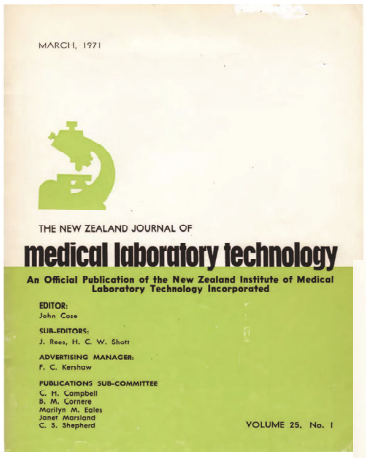

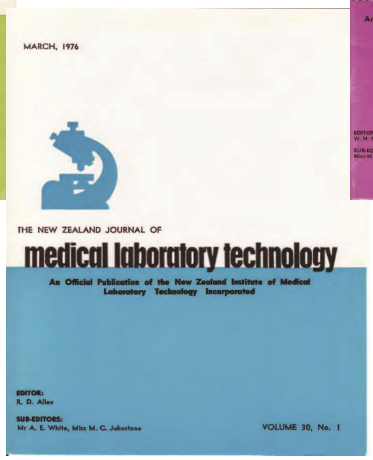

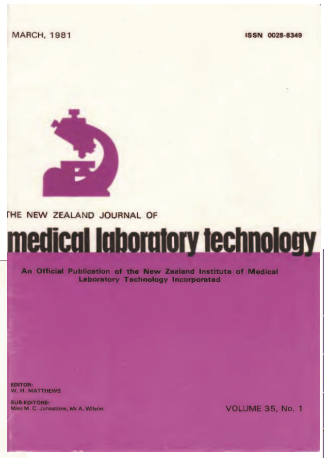

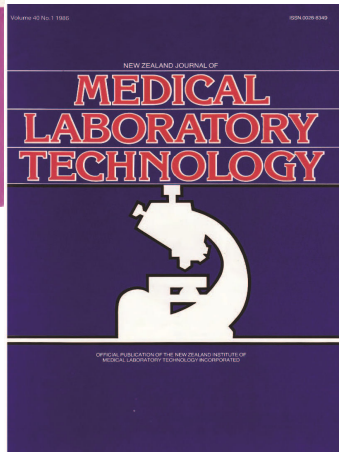

ORIGINAL ARTICLE

Assessment of laboratory test requests from three hospital emergency departments

Maryam Bahreini, Soheil Rafiee, Azar Hadadi, Pooya Payandemehr and Fatemeh Rasooli

ABSTRACT

Background: Laboratory requests can be justified and trimmed to what patients necessarily need. Reducing unnecessary laboratory requests can improve patient safety and both patient and provider satisfaction.

Methods: In this study, the current status of laboratory requests was assessed in emergency departments of three multidisciplinary university hospitals based on ten most expensive and most requested laboratory tests and categorised by the ordering physicians and patients' chief complaints in a 6-month period. These hospitals had annual visits to the emergency departments of between 20,000 – 72.000 patients.

Results: The most requested tests were as follows: complete blood count, biochemistry profile including sodium, potassium, urea, and creatinine. Moreover, the cumulating most expensive tests were coagulation profile, cardiac troponin, C-reactive protein, erythrocyte sedimentation rate, liver enzymes, alkaline phosphatase, and blood gases corresponding to the frequency of requests in the emergency department. Among the services, other than emergency medicine, requesting laboratory work in the emergency departments, internal medicine, neurology and surgery services had requested more laboratory tests comprising 44.0, 24.4, and 20.8 % of all specialties respectively. Moreover, the most frequent laboratory requests were from patients complaining of abdominal pain, chest pain, and penetrating or blunt traumatic injuries.

Conclusions: It should be considered to rationally request the most frequent laboratory tests as many of them can be canceled by physicians and do not change the diagnosis, treatment, prognosis, and disposition in the emergency department.

Key Words: diagnostic tests, routine, laboratories, emergency department.

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INTRODUCTION

Laboratory investigations are beneficial tools for screening, diagnosis or treatment monitoring, or assessing the complications of medications (1). Various approaches exist to request laboratory tests. According to Zhi et al., ordering tests with a clear indication referred to as restrictive criteria, while no contraindication is sufficient to order tests based on permissive criteria (1). Furthermore, objective criteria are investigator-independent while subjective criteria depend on expert review (1). Inappropriate testing can also be categorised into overutilisation including initial and repeated tests, and underutilisation, which is probably understudied (1). Overutilisation is thought to be more common, resulting from defensive medicine policies, the health system culture, unknown laboratory costs, and fear of receiving corrective feedback from the attending physicians during residency (2,3).

We aimed to assess the status of laboratory requests in three university hospital emergency departments categorised by patients complaints and the ordering physicians. The definite status of testing should be directed to optimise request protocols, improve patient safety, satisfaction of services providers, and decrease adverse events.

METHODS

The protocol of study has been reviewed and approved by the University of Medical Sciences Institutional Review Board and the ethical committee. It was in accordance with the ethical standards of the 1964 Helsinki declaration and its later ethical standards. Informed consent was obtained from the study participants.

In this cross-sectional study, we evaluated the topmost requested and most expensive laboratory requests during a fixed 6-month period in three university hospitals in the first 24 hours of patient entry to the emergency department in order to assess the present status of laboratory requests. These tests were ranked based on the cumulating most expensive tests corresponding to the frequency of requests in the emergency department.

The three hospitals were dedicated to a university with a similar educational system and mainly similar undergraduate and postgraduate circulating physicians among their rotations. They are multidisciplinary referral centres with annual visits to the emergency department of between 20,000 – 72,000 patients. Hospitals A and B are respectively known as referral healthcare centres for infectious diseases and internal medicine subspecialty services (haematology, rheumatology, gastroenterology, and endocrine diseases) while hospital C is mainly referred as a trauma and urology centre, although all of them are staffed by 24/7 board-certified emergency medicine specialists and various medical residents visiting a variety of patients' problems and diseases. Patient flow protocols are different among the hospitals, especially for patient output from the emergency department to admission wards. Additionally, laboratory tests were limited in hospital C to only emergency requests.

Subgroup analysis was performed to categorise chief complaints and the physicians who requested these laboratory tests. To export the right output from the Hospital Informatics System of the three hospitals, we had coordinated with the engineers of information technology in the hospitals and held several meetings to reach the desirable results.

Data was exported to SPSS, version 23.0 for analysis. Estimates were reported with the precision of 95% confidence intervals (95% CIs), significance <0.05 and type II error <0.2. We used descriptive statistics and independent sample t-test to analyse continuous variables and χ^2 test for categorical variables. In case of non-parametric distribution, Mann-Whitneytest and Kruskal–Wallis analysis of variance were considered. Quantitative data were reported as mean and standard deviation, and qualitative ones were presented by number and percentage.

RESULTS

Data was analysed and presented in detail for the three university hospitals. Tables 1 and 2 depict the top ten most requested and most expensive laboratory tests, respectively. The common chief complaints relating to the laboratory data are presented in Table 3.

The laboratory expense per each patient was US$ 9.6 for a total of 37,123 patients from hospital A. Hospital B acquired 163,064 laboratory data requests for 13,334 patients and the costs of laboratory tests per patient was US$ 7.35. Interestingly, expenses were much lower per patient at US$ 0.19 in hospital C.

Regarding the physicians requesting laboratory tests, the information from hospital A was not based on the ordering physician in the HIS system in a way that laboratory requests were assigned to the attending physician of anatomic clinical pathology service. However, the information of the treating physicians was obtained from the two other hospitals. In total, 57.0% were requested by the emergency medicine service in hospital B, followed by internal medicine 34.25 %, cardiology 3.29 %, surgery 1.27%, and neurology 1.07 % services. In hospital C, 98.1% of laboratory tests were ordered by emergency physicians. Among other treating services, internal medicine, neurology and surgery had requested more laboratory tests with 44.0, 24.4 and 20.8 % of the other services, respectively.

Table 1. Most requested laboratory tests in three university hospitals emergency departments.

Most requested laboratory tests									
	Hospital A			Hospital B			Hospital C		
	N=9,631,168	N	% †	N=163,064	N	%	N=28,761	N	%
1	CBC	17,359	15.3	CBC	17,358	10.6	CBC	2,395	8.3
2	Creatinine	14,781	13.1	Sodium	11,379	6.9	Creatinine	2,269	7.9
3	Potassium	14,767	13.0	Potassium	11,147	6.8	Urea	2,259	7.8
4	Sodium	14,732	13.0	Creatinine	10,051	6.1	Potassium	2,197	7.6
5	Urea	14,718	13.0	BUN	10,010	6.1	Sodium	2,181	7.6
6	PT/PTT/INR	12,626	11.1	VBG	9,263	5.6	Blood Glucose	1,980	6.9
7	Alkaline phosphatase	6,381	5.6	PTT	6,669	4.1	Troponin-I	1,122	3.9
8	SGOT, SGPT	6,382	5.6	PT	6,597	4.0	Calcium	1,017	3.5
9	Bilirubin*	5,990	5.3	UA	5,400	3.3	ABG/VBG	1,001	3.5
10	Calcium	5,394	4.8	Blood Glucose	3,442	2.1	UA	1,001	3.5
	Total	113,130	100		91,316	55.6		17,422	60.5

N: number. CBC: complete blood count. PT/PTT/INR: prothrombin time/partial thromboplastin time/ international normalisation ratio. SGOT/ SGPT: serum glutamic oxaloacetic transaminase/serum glutamic pyruvic transaminase. BUN: blood urea nitrogen. VBG: venous blood gases/ABG: arterial blood gases. UA: urine analysis. *: total and direct.
† The percent of laboratory data of the first hospital represents the percentage out of the total number of top ten laboratory tests to avoid reporting near zero percent because of the overcrowded nature and numerous total laboratory requests in six months of the centre.

Table 2. The most expensive laboratory tests in three university hospitals emergency departments.

Most expensive laboratory tests									
No	Hospital A			Hospital B			Hospital C		
		N	% †		N	% of Total		N	% of Total
1	PT/PTT/INR	12,624	13.1	Potassium	11,147	6.8	Troponin-I	1,122	3.9
2	Alkaline phosphatase	6,382	6.6	VBG	9,263	5.6	CRP	685	2.4
3	SGOT, SGPT	6,381	6.6	SGOT	3,483	2.1	ESR	579	2.0
4	CRP	6,334	6.6	SGPT	3,453	2.1	PT/PTT/INR	541	1.9
5	Bilirubin*	5,990	6.2	Alkaline phosphatase	3,418	2.1	Amylase	295	1.0
6	Calcium	5,394		Troponin-I	3,101	1.9	CPK	145	0.5
7	CBC	3,567	5.6	Calcium	2,853	1.7	D-Dimer	119	0.4
8	Lipase	1,031	1.1	Bilirubin*	2,536	1.5	CK-MB	73	0.2
9	Serum Iron	599	0.6	D-dimer	229	0.1	Urine Toxicology	42	0.1
10	CK-MB	349	0.4	CK-MB	175	0.1	CSF	41	0.1
	Total	48,651	100		39,658	24.3		3,642	12.6

N: number. CBC: complete blood count. PT/PTT/INR: prothrombin time/partial thromboplastin time/ international normalisation ratio. SGOT/ SGPT: serum glutamic oxaloacetic transaminase/serum glutamic pyruvic transaminase. BUN: blood urea nitrogen. VBG: venous blood gases/ABG: arterial blood gases. UA: urine analysis. *: total and direct.
†The percent of laboratory data of the first hospital represents the percentage out of the total number of top ten laboratory tests to avoid reporting near zero percent because of the overcrowded nature and numerous total laboratory requests in six months of the centre.

Table 3. Most requested laboratory tests categorized by chief complaints of patients.

Most requested laboratory tests categorized by chief complaints									
	Hospital A			Hospital B			Hospital C		
No	Complaints	N	%	Complaints	N	%	Complaints	N	% †
1	Abdominal pain	3,049	3.2	Abdominal pain	1638	13.2	Chest pain	37	23.7
2	Laceration	2,528	2.6	Multisystem trauma	1285	10.3	Urinary retention	25	16.0
3	Limb trauma	2,365	2.5	Dyspnea	805	6.5	↓ LOC	23	14.7
4	Generalized weakness	1,728	1.8	Chest pain	551	4.4	Stroke	14	9.0
5	Dyspnea	1,481	1.5	Limb pain	448	3.6	Abdominal pain	13	8.3
6	Chest pain	1,296	1.3	Stroke	434	3.5	Weakness	13	8.3
7	Multisystem trauma	1,262	1.3	Abnormal complete blood count	417	3.3	Headache	13	8.3
8	Suspected systemic infection	1,236	1.3	Gastrointestinal bleeding	406	3.3	Seizure	9	5.8
9	Flank pain	950	1.0	Flank pain	365	2.9	Renal colic	6	3.8
10	Headache	719	0.7	Vertigo	337	2.7	Multiple trauma	3	1.9
	Total	96,368	17.2		5638	53.7		156	100

N: Number of documented complaints. LOC: decreased level of consciousness.
† The percent of laboratory data of the third hospital represents the percentage out of the total number of top ten chief complaints to avoid reporting near zero percent because of the significant missing data of chief complaint.

DISCUSSION

In order to determine the status of laboratory requests in the emergency department the information of three university hospitals emergency departments was assessed. The top five most common requested tests in all three hospitals were complete blood count, and biochemistry profile including creatinine, potassium, sodium, and blood urea nitrogen, but the following most requested laboratory sequence was varied. For instance, prothrombin time and partial thromboplastin time were mostly requested in two of the hospitals. It can be caused by differences between the typical patient populations presented to these referral hospitals. On the other hand, it can be due to the limitations applied for the laboratory requests of hospital C in the emergency department. In this context it is clear that further stay in the emergency room necessitates more laboratory requests (1). Furthermore, the requested number of laboratory tests is affected by some emergency department protocols, thus limiting the request of non-urgent tests that occurred in hospital C, canceling elective laboratory tests in the emergency department. This strategy not only limits the burden of unnecessary laboratory requests by postponing them to outpatient visits, but also cause swifter flow of patients in hospital C, urging treating services other than the emergency department to mobilise their patients to the wards to perform further necessary but non-urgent tests during ward admission.
Some researchers have implemented short-term educational interventions aimed at reducing the number of inappropriate laboratory tests in a university hospital. They restricted available emergency laboratory tests and the frequency of repeated orders via an institutional protocol and showed a reduction of 19% in laboratory tests (95% CI: 18.8–19.2% in the year after the intervention (4. This effect on haematology test reduction was 7.6% (P = 0.009. Another study showed a decrease of 37% in requested laboratory tests after the institution of guidelines (p < 0.001 in a surgical intensive care unit (5.The most common requested tests were related to the patients with abdominal pain, chest pain, lacerations, multiple trauma ,and urinary retention in three hospitals which were ordered by emergency medicine, internal medicine, neurology, and surgery services, respectively. It is important to note that some complaints such as minor lacerations and urinary retention may not need any laboratory tests and institutional protocols are needed to determine the minimum necessary laboratory tests.

Our study showed that the most expensive requested tests were prothrombin time, partial thromboplastin time, international normalisation ratio, potassium, troponin, alkaline phosphatase, venous blood gases, and C-reactive protein in the three hospitals. Some of the most common requested tests are not expensive but this was due to the high volume of requests, which may not be necessary. In general, unnecessary tests are not only ineffective in patients' diagnosis and management, but also cause several shortcomings, such as increased patient's length of stay and results in increasing patient and personnel dissatisfaction due to financial issues. In this context, several studies have been designed to decrease unnecessary requested tests. For instance, Oliveira et al., showed that the most requested tests were complete blood count, sodium, and potassium, of which 41% of tests were considered unnecessary in this interventional assessment (4).

Nearly all interventions, such as education or feedback, lead to a reduction in laboratory requests according to a narrative review (6). Some authors assessed requested tests before and after educating internal medicine residents and showed that blood tests and the LOS decreased by 50% and 21%, respectively (7). Similar results were found in another studies by educating residents, limiting common laboratory or repeated test requests (8-10), and few beneficial protocols exist (11).

In addition, we confronted different patient outflow protocols from the emergency wards in a way that hospitals A and B, with more complicated infectious and internal medicine complaints, may have more length of stay in their emergency departments and therefore the rate and type of laboratory requests exceed expectations. However, hospital C, with more traumatic patient

visits, have a quicker turnaround rate in the emergency department with hospital bed management of emergency medicine attending physicians. Furthermore, there is an interesting alignment with the expensive laboratory tests requested by hospital C (Table 2) and the "most common complaints" in Table 3 that may be due to the less common complicated internal medicine patients referring to this hospital. Some authors considered that inappropriate laboratory requests were common in internal medicine due to the lack of awareness and knowledge and feelings of insecurity were causes of excessive requests among internal medicine residents (12). Finally, some authors discuss approaches to decrease inappropriate laboratory test utilisation and their financial effects in the University of Colorado including reimbursement rate reduction, decreasing the utilisation of unnecessary tests, test utilisation management by laboratory staff and installation of advanced information technology systems. The authors emphasise that combination of these approaches will be helpful (13).

A limitation of our study was that the hospital informatics system systems of the three hospitals were not unified; thus, the documentation pattern was different among them. Furthermore, some missing data existed, especially in the determination of patients' clinical complaints.

In conclusion, common laboratory requests were differently managed in the three hospitals. Applying limitations to the emergency department laboratory requests and relying on necessary tests can improve patient turnaround and overcrowding. Patient flow and hospital bed management by emergency physicians seem to be influential and are positive factors on optimal laboratory requests. Our study data is beneficial to recognise the type, physician, and services responsible for unnecessary laboratory test requests.

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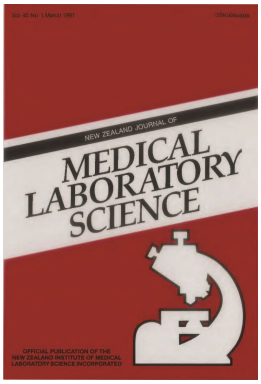

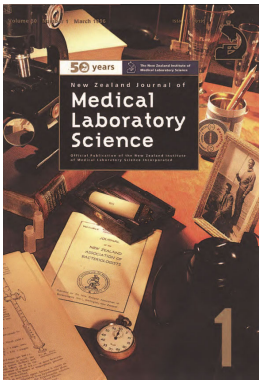

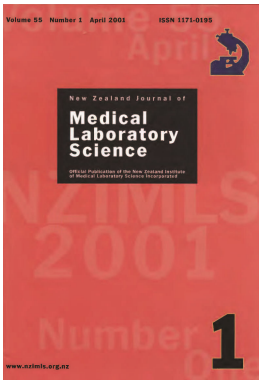

ORIGINAL ARTICLE

The design and implementation of a novel standardised training and assessment tool at LabPlus, Auckland Hospital, New Zealand for anti-nuclear antibody pattern reading using indirect immunofluorescence methodology in a non-automated digital microscopy setting

Paul M Austin and Helena T Thompson-Faiva

ABSTRACT

Background: To reach a level of mastery in reading anti-nuclear antibody (ANA) pattern patterns using indirect immunofluorescence (IFF) methodology is a complex and difficult process. In New Zealand, for new BMLSc graduates, this process becomes far more difficult as both the Universities offering the degree course do not invest strongly in the procedure during the first three years. Furthermore, the fourth year clinical placements, can, at best, only provide a brief introduction due to time restrictions. It therefore falls to the employing diagnostic laboratories to both train and validate individual practitioner competency. As a consequence of the low levels of theoretical and practical experiences of new graduates, we recognised that the historical methods of training and assessment were inappropriate for both the organisation and the new graduates.

Aims: To design, implement and assess the value of a novel integrated training and assessment tool for ANA testing using IIF methodology.

Methods: Five new recent BMLSc graduates employed at LabPLUS, Auckland were the test subjects of the novel training and assessment system. Individual performance data was collected, analysed and fed back to participants in real time both verbally and in graphical format. All participants, after completing the programme, were invited to respond to a questionnaire where each question (10 in total) had a choice of five selectable options. Responses were collated and results presented.

Results: All five participants reached the set KPI values within one month of starting their assessment phases. All participants demonstrated an initial rapid reading agreement which then settled into a phase of gradual incremental improvement. Feedback from the survey was positive overall, with highlights for participants being (1) the "real-time" graphical presentation of their progress (2) identifying with the process being supportive and improving their analytical skill sets and (3) the system was superior to other programmes they had been involved with.

Conclusions: The conceptualisation and implementation of a novel ANA IIF training and competency assessment system at LabPLUS, was an operational success. The system effectively allowed the objective assessment and development of participants in what is essentially a subjective test setting. The features of the system gave it high flexibility and allows adaptation to other tests or areas either within or external to the diagnostic pathology laboratory.

Key words: Anti-nuclear antibody, indirect immunofluorescence, training, competency, participant feedback.

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INTRODUCTION

Anti-nuclear antibody (ANA) testing visualising nuclear and cytoplasmic patterns from fixed human epithelial (HEp-2) cell lines using indirect immunofluorescence (IIF) methodology has been in use since the early 1970's and is widely regarded as the first choice diagnostic assay in the setting of a suspected systemic autoimmune-based rheumatic disease (SARD) (1).

Multiple technical factors impact on the output of the ANA test, principally (a) serum screening dilution (b) fixation and type of HEp-2 cell line (c) conjugate isotype and working strength and (d) IIF illumination source (2). It is due to these factors and, that the output of the assay relies upon the training, expertise and experience levels of the medical scientists (MLS's) / medical laboratory technicians (MLT's) tasked with the analyses. For every patient specimen under test the following assessments are made:

1. Determination of the presence of any artefacts or features that would compromise the subsequent visual assessment.
2. Discrimination of reactive versus non-reactive.
3. Assuming reactive, establishment of the location of reactivity (cytoplasmic versus nuclear or both).
4. Identification of the correct pattern(s). In our laboratory setting of 4 reportable cytoplasmic patterns and 8 nuclear, mathematically this equates to 12! or 479,001,600 possible combinations. Typically, for our ANA positive patients, on average we observe 1.5 any combination of cytoplasmic and / or nuclear patterns per patient, thus the formula becomes 12!/(12-1.5)! = 40 possible combinations per patient.
5. Determination of the strength of the observed reactivities.
6. Ensuring there are no abnormalities (e.g. suspected contamination) in the titration sequence that would preclude result reporting.

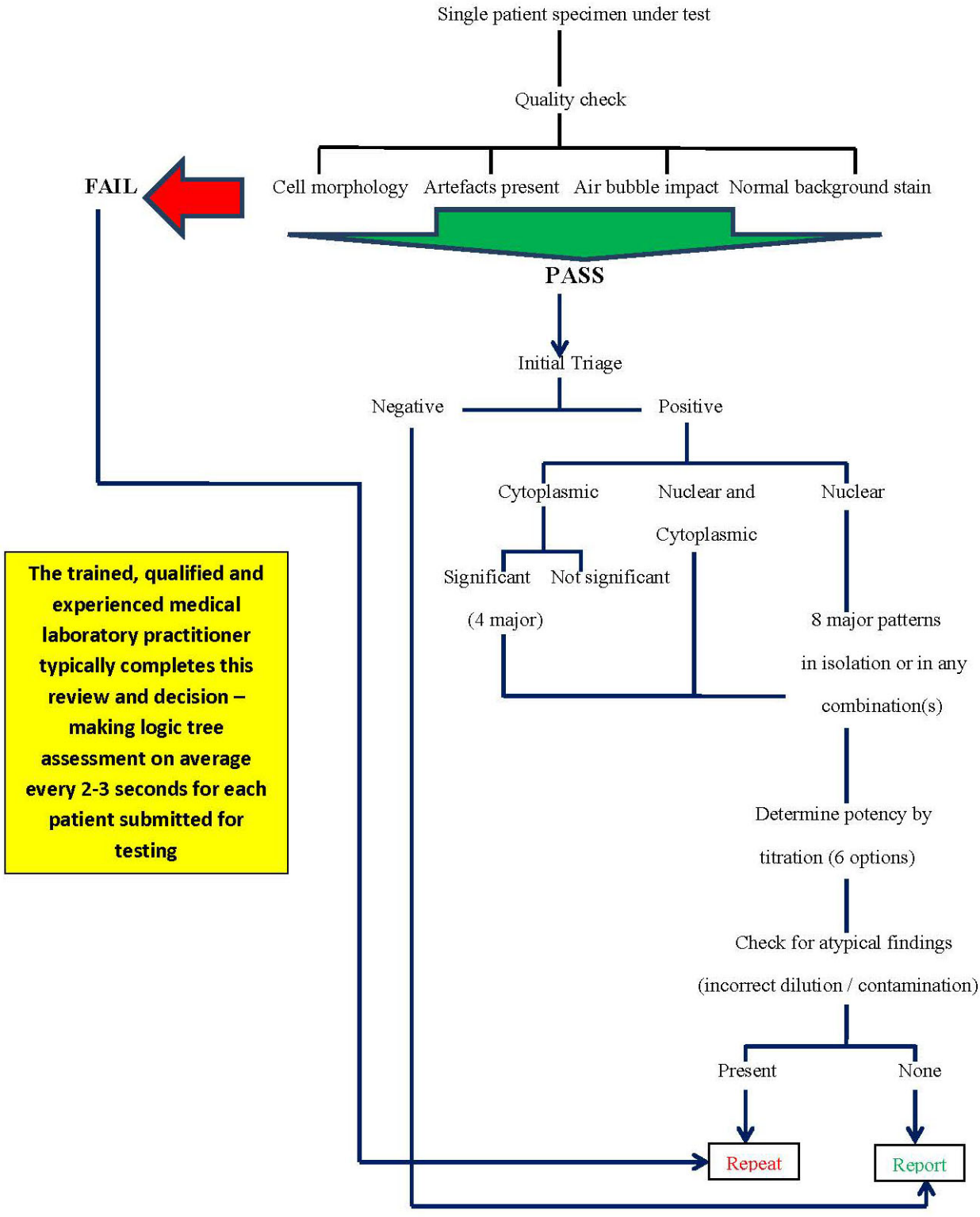

Figure 1. Logic assessment tree for reading the ANA IIF test.

To add to the challenge and complexity facing MLS's / MLT's some patterns may be masked and will only be revealed on a dilution sequence while for some cytoplasmic based antibodies (typically Ribo-P and Jo-1) there may be a complete absence of nuclear staining.

It follows, that harmonisation of ANA reporting across diagnostic laboratories continues to be a challenge. In 2015, in an attempt to address the lack of standardised reporting, the International Consensus on ANA patterns (ICAP) produced a set of standardised (AC1 – AC28) reporting codes based upon whether reactivity seen was nuclear or cytoplasmic based with or without mitotic reactivity. A further level of discrimination was applied by indicating some of the reportable patterns would only be recognised by expert-level readers (3-5). Despite the technical and interpretative challenges associated with the ANA IIF test as described, a recent review by the American College of Rheumatology indicated that the method should remain the gold standard despite other technologies being available (6).

There are numerous external quality assurance (EQA) proficiency programmes for ANA testing which, while useful and, participation being mandatory for continued laboratory accreditation (New Zealand-IANZ), are limited in terms of

assessing competence of individual practitioners across the wide potential spectrum of reportable ANA patterns. In New Zealand, a recent niche EQA ANA programme has been developed and implented to allow multiple reader inputs (7).

Over the past five years, a number of commercial ANA slide manufacturers have developed closed systems for automated reading of IIF ANA patterns. Typically, systems have algorithm–based triaging capacity (positive or negative) and then either software-driven pattern identification or user-selectable pattern identification. The latest versions of such systems usually have an on-board digital image library to assist with pattern identification. These systems are effective tools to not only assist in standardised ANA reporting but also to aid laboratories in staff training. In 2014 Bizzaro *et al* identified that, although systems available at that time could triage positive versus negative equivalent to manual reading, there was generally poor (52-79%) recognition across the six systems where mixed patterns were present (8). Such automated systems are expensive and are outside the purchasing ability of many laboratories performing ANA testing by IIF.

Over a 5 month period during 2017/2018 at LabPLUS, the serology unit (which performs ANA testing by IIF) lost 50% of its staffing level, the majority of those that left having posessed high experience and expertise in reading ANA patterns. It was realised that new staff recruitments would almost certainly have to be well supported in becoming proficient in performing the IIF test and reading ANA patterns. The reason for this, is that across the New Zealand Universities that offer the Bachelor of Medical Laboratory Science (BMLSc) degree, gaining practical expertise in ANA IIF methodology is either very limited or not included in their curricula. Rather, the only exposure students receive during their degree course is when they are on their fourth year clinical placements at diagnostic laboratories. Because the placement time is restricted to 16 weeks in total and, there are multiple assays that the student is required to demonstrate technical proficiency in, only a very limited exposure to ANA IIF testing is the usual outcome.

It was against this setting that the decision was made that the historical paper record system of training and recording ANA proficiency (sign-off being competent or not with a sub-classification into technical performance and pattern recognition) was deemed unsuitable for the needs of both the organisation and our new staff members. The challenge then became to design and implement a new sytem of training and assessment. This article details the training and assessment system that was developed and provides evidence of its effectiveness in producing staff that have high technical profiencies in the science and art of reading ANA patterns by IIF methodology.

METHODS

System design considerations

While acknowledging that newly qualified individual practitioners may bring with them (a) theoretic knowledge, (b) some IIF expertise (with or without ANA application) and (c) reading preconceptions through use of alternate cell lines and ANA frequencies in different test populations our design criteria required:

1. A standardised numeracy–based assessment tool that examined every component of ANA reading for both screening and screen reactive sera undergoing titration.
2. ANA pattern reading training delivery to be restricted to those practitioners with a minimum of 10 years experience and expertise.
3. A standardised KPI target based on compliance (% agreement) with senior staff reading. There were two senior staffs involved in the training assessment. They had a <1% inter-practitioner non-agreement in reading ANA patterns.
4. Availability and use of catalogued digital microscope images obtained by testing patient sera under our conditions. The digital microscopy image facility acted as an enabler during the active assessment process where features of individual patient images and pattern classification discussions could occur without image deterioration due to quenching.
5. Detailed collation of competency assessment data in real time with graphical output demonstrating practitioner development.
6. Specific one on one feedback and discussion during the training/assessment process using the data collected (point 4).
7. A system that was viewed as supportive and had a high empahsis on skill development.

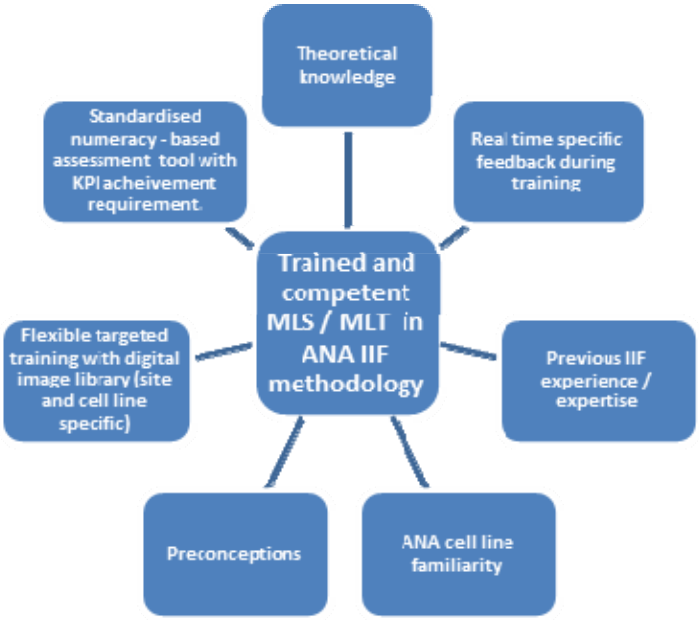

Figure 2. Inputs affecting the training of MLS and MLT practitioners in reading ANA patterns by IIF methodology.

Process - overview

For every practitioner, the following process was followed:

1. A two week one on one theoretical overview linking the technical aspects of the procedure with the visible outputs and the clinical significance of the patterns seen. The retained comprehensive digital image library was used for this purpose. Strong emphasis was made at this time that the final image quality and therefore the ANA pattern discrimination ability being directly linked to the 'hands-on' technical element of applying coverslips to slides with mounting media. Poor practitioner technique for this final pre-evaluation technical step may result in a combination of either (a) changes in refractive indicies diminishing fluorescence and distorting cell morphology and/or (b) physical damage to fixed cells giving rise to artefact's that may be mis-interpreted as true ANA patterns.
2. A one to three week period (dependent upon the individual practitioner) prior to the formal assessment process where practitioners could view slides to establish and fine tune their observation skills until such time they believe they could (a) confidently discriminate positives from negatives and (b) identify the commonest ANA patterns of Homogeneous, Speckled, Centromere, Nucleolar, Nuclear Membrane, Nuclear Dots, Cell-Cyclic and Hyper-expressed SSA (Hep-2000 cell line in use).

3. The formal period of assessment. The MLS/MLT would independently perform the first ANA slide reading, the senior MLS performing the second. Within 48 hours the assessment data was collated and there was a conversation between the assessee and the assessor based upon the data. The conversation/feedback loop was mandatory before the second reading / asssessment event.
4. The cycle of assessment/feedback continued until the required KPI's (≥ 85% reading concordance with senior MLS's and a minimum of 350 patients had been reviewed) had been met.
5. Issuing of a personalised certificate stating their competence achievement and graphical representation of their personal journey of development.

Process – system scoring

The assessment scoring is a standardised numeracy system whereby [1] point is allocated for agreement and [0] point for non-agreement with the senior MLS reader. There are different elements for review dependent upon whether the patient serum is undergoing a first-round screen or a full titration after an initial reactive screen.

Points for screening and titration reading are then added together and a batch compliance percentage is calculated. Subsequent batch readings follow the same process allowing the calculation of both individual batch and cumulative percentage complaince. The required KPI point is referenced against the cumulative percentage compliance.

Screening scoring

There are two elements for comparison and point accumulation, namely (a) agreement of positive versus negative for each patient and (b) for those scored positive by the senior MLS, pattern identification.

In the mock ANA read sheet example (Figure 3), 11/16 possible points were scored for positive/negative compliance and 4/8 points for pattern identification, totalling 15/24 possible points.

Titration scoring

There are three elements for comparison and point accumulation, namely (a) agreement of positive versus negative for each patient (b) for those scored positive by the senior MLS, pattern identification and (c) for those scored positive by the senior MLS, a point is awarded if the MLS/MLT end point dilution read is within a single dilution step of that determined by the senior MLS.

In the mock ANA read sheet example (Figure 3), 6/6 possible points were scored for positive/negative compliance, 3/5 points for pattern identification, and 4/5 points for end-point titre determination totalling 13/16 possible points.

Process – post-IIF read feedback/discussion

As aluded to earlier in this paper, the feedback/discussion phase occurs after the reading phase and before any subsequent readings. It allows a real time analysis of the strengths and weaknesses of the MLS/MLT trainee in a non-judgemental, unemotional supportive setting underpinned by an in-depth standardised objective data set.

In the mock ANA read sheet example (Figure 3), identified strengths would have been generally good pattern identification with excellent determination of end point in a dilution sequence. Areas to focus on would be (a) to have an awareness that masked patterns may be revealed on dilution (b) to understand that where there is SSA hyper-expression, the end point titre is established on the non-hyper-expressing cells and (c) as there was evidence of slight over reading and assignment of low titre speckled reactivity for a proportion of patient sera undergoing screening, a minor re-calibration in this area would be indicated.

Participant feedback

Five qualified MLS staffs (designated in this paper MLS 1 through MLS 5) were employed in serology over the period April 2017 to March 2018 to replace staff resignations that had occurred over the same time frame. All five went through the ANA IIF training and assessment programme over the period October 2017 to November 2020. There were differences across the five MLS candidates in terms of their knowledge base and laboratory experiences prior to their LabPLUS employment as detailed in Table 1.

All five MLS staffs achieved the desired KPI requirement. After course completion, each staff member was offered the opportunity to provide feedback on elements of the training and assessment scheme in terms of a questionnaire (Table 2).

Each question had a single selectable response, where responses (a) and (b) were designated below average, response (c) was average and responses (d) and (e) were above average. Using the assumption that there was equivalence between each response numbers were assigned a numeric value 1-5 in the order of response (a) – (e).

Furthermore, the ten questions made up four separate categorial groups for analysis:

Participant engagement:	Question 1
Specific elements:	Questions 2, 3, 7
Participant training confidence:	Questions 4, 5
System review:	Questions 6, 8, 9, 10

RESULTS

Participant training/competency assessments

All participants attained the KPI requirements of (a) a minimum of 85% agreement with those obtained by senior staffs and (b) a minimum of 350 patient specimens assessed (Table 3, Figure 4).

The average starting (threshold) agreement level was relatively high (80%). We believe this was principally due to (a) the structure of the ANA programme where there was a "lead-in" time before the formal assessment process commenced and (b) all participants had a minimum 2 month period of viewing non-ANA autoimmune IIF 's (neuronal antibodies, skin antibodies, adrenal antibodies and ANCA IIF) before commencing the ANA programme (Table 3).

Due to the high starting points for all candidates, identified improvements were relatively small being in the order of approximately 5-10% (Table 3). All candidates demonstrated a sharp initial reading agreement improvement which then settled into small gradual incremental improvements consistent with increases in volumes read and time invested (Figure 4). All candidates met the KPI requirements within a month of starting the training/assessment process (Table 3).

By way of comparison, the program was also applied to a medical registrar training for their theory and practical examinations. The registrar had no laboratory experience. In their case, the KPI was reduced to 70%. Their threshold point was 55% agreement and it took 3 months to attain the 70% KPI threshold, reading 750 patient tests during the process. The improvement in their case was three times higher than that seen in the MLS group.

Screen (S) / Titre (T)	MLS / MLT	Senior MLS
	1+ S	0
S2	0	0
S3	0	0
S4	1-2+ H/S	1+ H/S
S5	1-2+H/S	1+ S
S6	±	0
T1 – 80	3+ H	>3+H
T1-160	2-3+ H	3+ H
T1-320	2+ H	2-3+ H/S
T1-640	1+ H	1-2+ H/S
T1-1280	±	±
S7	>3+C	>3+C
S8	1+S	±
T2-80	±	0
T2-160	0	0
T2-320	0	0
T2-640	0	0
T2-1280	0	0
T3-80	1-2+ S	1+S
T3-160	1+S	±
T3-320	±	0
T3-640	0	0
T3-1280	0	0
T4 – 80	2-3+ H/N	3+ H/N
T4-160	2+ H/N	2-3+ H/N
T4-320	2+ H/N	2+ H/N/S
T4-640	1+ H/N	1-2+ H/N/S
T4-1280	±	±
T5 – 80	3+ S/SSA	1-2+S /SSA
T5-160	3+ S/SSA	1+S/SSA
T5-320	3+ S/SSA	0/SSA
T5-640	3+ S/SSA	0/SSA
T5-1280	2+ S/SSA	0/SSA
T6 – 80	2+ H/S	1-2+H/S
T6-160	1+ H/S	1+ H/S
T6-320	±	0
T6-640	0	0
T6-1280	0	0
S9	1+S	0
S10	0	0
S11	3+H	>3+H
S12	2-3+N	3+H/N
S13	1-2+ S /ND	2+S
S14	0	1+S
S15	1+S	±
S16	2+ NM	3+NM

Screen assessment (N=16)
11/16 Positive / Negative points
4 / 8 Pattern recognition points
15 / 24 possible points

Titre assessment (N=6)
6/6 Positive / Negative points
3 / 5 Pattern recognition points
4 / 5 End point titre agreement points
13 / 16 possible points

Screen and Titre assessment combined
N = 22 patients
15/24 + 13/16 = 28 /40 possible points per batch
Overall agreement: 70%

Figure 3. Mock ANA reading record sheet with assigned accumulated points based upon concordance of reading between the trainee practitioner and their trainer.

Participant feedback

Overall, feedback was positive. For the 10 questions, across the five participants a total of 50 points could be scored. The average was 40.4/50 (81%) with a participant range of 66% to 94% and a 95%CI range of 71.5% to 90.0% (Table 4[a]). All candidates had very high levels of engagement at the start of the process (Table 4 [a] and [b]).

The specific elements of (1) the availability of digital images as a training tool and (2) 'one on one' feedback sessions were well received [questions 2 and 3, Table 4 [a]). However, the specific feature of the programme that had almost complete approval (80% scored this feature as empowering) was the ability for participants to visually see and track their development over time [Question 7, Table 4 [a]).

Questions 4 and 5 were linked. They queried participant confidence levels regarding achieving the set KPI at the start of and mid-way through the training and assessment process. A single candidate (20%) felt confident at the start of the process. At the half-way point all remaining candidates had improved their confidence levels (Table 4 [a]). An associated question [9] asked participants to assess the difficulty level of the programme with hindsight. None gave a rating lower than 'Fair' and most felt it was challenging but achievable (Table 4 [a]).

Collectively, the four system review questions together (6, 8-10), across all participants scored 91 of a possible 100 points. (Table 4 [b]). Specifically, the majority of participants endorsed the use of a numeric-based system for objective assessment in what is essentially a subjective technical setting (Table 4 [a]). Equally high gradings were given for the core system principles (Question 8) and, in comparisons with other assessment schemes participants had been involved with (Question 10) (Table 4 [b]).

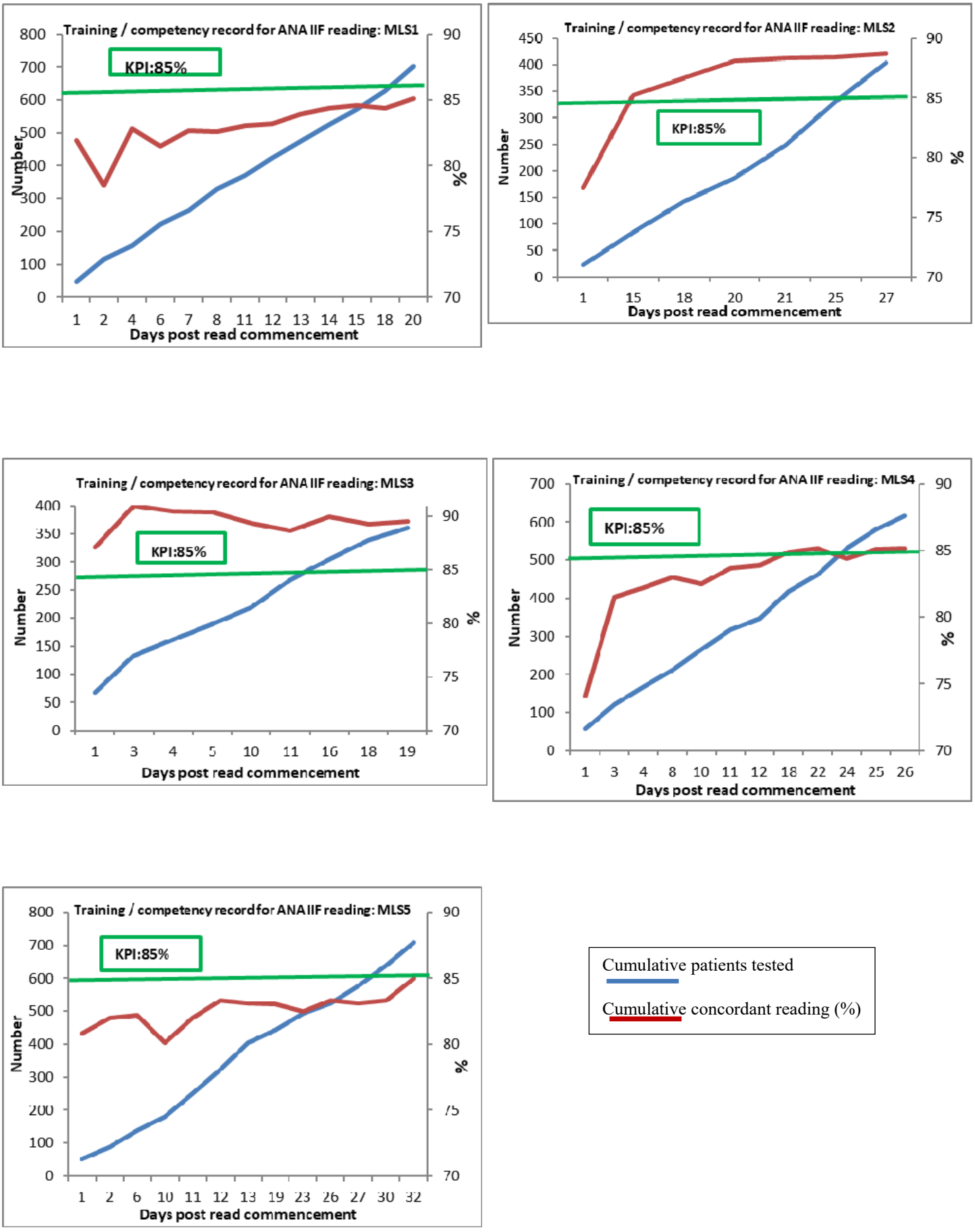

Figure 4. ANA IIF competency assessment journeys of five qualified MLS staffs over the period October 2017 – November 2020 at LabPLUS, Auckland Hospital presented in graphical form.

Table 1. Backgrounds of 5 recently qualified MLS practitioners who participated in the novel ANA IIF Training and Assessment programme at LabPLUS.

	MLS 1	MLS 2	MLS 3	MLS 4	MLS 5
Qualification	BMLSc	BMLSc	BMLSc	BSc MLT (India) Grad dip. Sci. (NZ)	BMLSc
Time from graduation to LabPLUS employment	4 months	4 months	12 months	36 months	3 months
NZ University	Otago	Otago	AUT	AUT	AUT
Degree placements	Immunology / Haematology	Immunology / Microbiology	Haematology / Transfusion Science	Chemical Pathology	Immunology / Chemical Pathology
ANA IIF at University practicals	None	None	None	None	None
ANA IIF Theory at University	Brief	Brief	Brief	Brief	Brief
ANA IIF while on placement	80 Hours 15% placement time	128 Hours 25% placement time	N/A	N/A	32 Hours without formal training 6% placement time
Specific ANA IIF cell line experience	IC Hep-2000 (Placement)	IC Hep-2000 (Placement)	N/A	Biorad Hep-2 (2 years routine)	Biorad Hep-2 (Placement)
Microscopy expertise with cell morphology identification	Yes Haematology during placement	Yes Two years in University practicals	Yes As part of Haematology training	Yes In India (Haematology)	No

Table 2. MLS participant ANA IIF training and competency process feedback questionnaire.

1. Please select your engagement level during the training / assessment process (a) Very Low (b) Low (c) Average (d) High (e) Very High
2. What was your opinion of the retained digital image library as a training tool? (a) No value (b) Minimal value (c) Sometimes useful (d) Very helpful (e) Superb resource
3. What was your opinion of the value of the feedback / discussion sessions during the assessment process? (a) No value (b) Minimal value (c) Sometimes useful (d) Helpful (e) Very helpful
4. Define your confidence level in achieving the 85% KPI read agreement level before the formal assessment period started (a) Very concerned (b) Mildly concerned (c) Ambivalent (d) Confident (e) Very confident
5. Define your confidence level in achieving the 85% KPI read agreement level mid-way through the formal assessment period. (a) Very concerned (b) Mildly concerned (c) Ambivalent (d) Confident (e) Very confident
6. How do you rate the standardised numerical – based system as an assessment tool? (a) Unhelpful (b) Poor (c) Adequate (d) Good (e) Excellent
7. Please identify how you felt about being able to visually see and track your development over time? (a) Pointless (b) Little value (c) Ambivalent (d) Helpful (e) Empowering
8. What is your opinion of the statement ***"I viewed the process of training and assessment as supportive and skill-based driven"*** (a) Totally disagree (b) Disagree (c) Somewhat agree (d) Endorse (e) Fully endorse
9. Overall, with hindsight, what is your opinion reagrding the difficulty level for the training and assessment system (a) Near impossible (b) Easy (c) Fair (d) Acheivable with persistence (e) Challenging but acheivable
10. Compared to other training / competency assesments you have been involved in for subjective material, how did this system rate? (a) Much worse (b) Poorer (c) Same (d) Better (e) Significantly better

Table 3. Summary table of MLS participant's performance from their individual ANA IIF training and competency assessments.

	Threshold (start) read agreement (%)	Completion read agreement (%)	Improvement (%)	Days to meet KPI (≥85% read agreement) over 350 specimens assessed	Total patients tested	Time reading non-ANA autoimmune IIF before ANA training and assessment (months)
MLS 1	81.9	85.1	3.9	20	703	2
MLS 2	77.5	88.7	14.5	27	404	2
MLS 3	87.1	89.5	2.4	19	361	13
MLS 4	74.1	85.2	15	26	617	3
MLS 5	80.8	85.0	5.2	32	709	11
Mean	**80.3**	**86.7**	**8.2**	**25**	**559**	**N/A**
SD	4.9	2.2	6.1	5	166	N/A
95% CI	**76.0-84.6**	**84.8-88.6**	**2.8-13.5**	**21-29**	**413-705**	**N/A**

Table 4. MLS participant questionnaire responses with [a] individual gradings and [b] categorial groupings.

4 (a)

	Questions	MLS1		MLS2		MLS3		MLS4		MLS5			
Category: A = Answer S = Score		A	S	A	S	A	S	A	S	A	S	Total/ 25	Frequency (%)
Engagement	1	D	4	E	5	D	4	D	4	E	5	23	92%
Specific elements	2	C	3	E	5	D	4	E	5	A	1	18	72%
Specific elements	3	D	4	E	5	C	3	E	5	B	2	19	76%
Training confidence	4	B	2	A	1	B	2	D	4	C	3	12	48%
Training confidence	5	C	3	B	2	D	4	D	4	D	4	17	68%
System review	6	D	4	E	5	E	5	E	5	C	3	22	88%
Specific elements	7	E	5	E	5	E	5	E	5	C	3	23	92%
System review	8	E	5	E	5	D	4	E	5	D	4	23	92%
System review	9	D	4	E	5	E	5	E	5	C	3	22	88%
System review	10	E	5	E	5	D	4	E	5	E	5	24	96%
Total / 50			39		43		40		47		33		
Frequency (%)			78%		86%		80%		94%		66%		

4 (b)

Group	Questions	MLS1	MLS2	MLS3	MLS4	MLS5	Total scored	Total possible	Frequency (%)
Engagement	1	4	5	4	4	5	22	25	88
Specific Elements	2,3,7	12	15	12	15	6	60	75	80
Training Confidence	4,5	5	3	6	8	7	29	50	58
System Review	6,8,9,10	18	20	18	20	15	91	100	91

DISCUSSION

To achieve competency in consistently reading the ANA IIF assay is a challenge. Specifically, at our laboratory, acting in the capacity of a tertiary referral site for both New Zealand and neighbouring Pacific Islands we see a high frequency of ANA positive sera (approximately 30-40%). Associated high complexity with observed patterns is not an uncommon occurrence. In New Zealand, this challenge is exacerbated by the fact that the tertiary Universities offering an Immunology placement as part of the BMLSc degree resource this core autoimmune test (both theoretic background and practical exposure) at very low levels. Effectively, new BMLSc graduates come into the workforce without expertise in this technique. It thus falls to the diagnostic laboratory to both train and assess the competencies of newly recruited graduates.

In our laboratory we were faced with having to employ a number of newly qualified graduates, all of whom had limited experience with the ANA IIF method. We viewed this somewhat unique situation as an opportunity to comprehensively review our historical systems of training and assessment. With the transition from an experienced workforce to largely inexperienced one, historical embedded systems of annual assessment were deemed inappropriate.

We chose to maximise the resources we had to hand namely (a) highly experienced senior staffs and (b) a retained digital library of images. These assets were then augmented by designing a numeracy–based scoring system which targeted every aspect of reading the ANA IIF and was customised for either screen or titre reads. To bring the system together we focussed on supporting our new graduates by (a) delivering specific one on one feedback after each reading session and then (b) enabling them to visualise their own progression in real time via graphical output. The process as outlined in this paper has proved to be successful as evidenced by the performance graphs of all participants whereby their technical acheivements matched their questionnaire responses.

The challenges we face in New Zealand are seen elsewhere in the world. A survey in 2019 by the American Association of Medical Laboratory Immunologists (AAMLI) identified 65% of their respondents (almost exclusively USA laboratories) had “on the job” training with 10% stating they received “no training” (9). In the same survey, responders identified competency assessment as compliant performance in EQA programmes (9). The authors of the AAMLI paper concluded that improved training could be mediated through “hands-on” and “wet-bench” workshops. Improvements in EQA proficiency programmes would help in optimising the detection and measurement of HEp-2 based ANA IIF results and reports (9).

We believe that this is a unique publication in that it clearly outlines the processes to implement in both training and assessment phases. Additionally, it is our contention that for new inexperienced staffs the two phases should be viewed as integrated compatible elements of a whole as opposed to segregated un-associated elements. We further believe that compliant performance in existing EQA programmes for ANA speaks more to the systems employed within diagnostic units as opposed to being a measure of the competence of individual practitioners.

A possible weakness in this study was the low number of participants. However, when we consider the similarity of the training and assessment outcomes of the participants, matched with the uniformity of their questionnaire responses, from what can be considered a highly diverse group of newly qualified graduates, the small pool number is unlikely to have biased our conclusions.

The system we have developed and implemented has high flexibility. Dependent upon the desired setting, while maintaining the scoring component without modification, achievable levels can be set by simple manipulation of the desired KPI’s. The system can be easily adapted for both initial and re-assessments.

We chose a high KPI setting of 85% reading concordance because we wanted to develop future leaders (i.e. was part of a clear mid to long-term succession plan). It will be a relatively small step up from where practitioners currently sit to the required 95% agreement to be considered the second senior IIF reader as opposed to the first reader in our facility.

In conclusion, while “system fine-tuning” will invariably occur over subsequent years the underlying principles and core operational specifics will, in our opinion need little modification. Not only has this integrated system of training and assessment proven to be successful for the ANA IIF procedure, adaptation into other clinical areas (not restricted to the diagnostic pathology laboratory) where a subjective assesment is required should be easily attainable.

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ORIGINAL ARTICLE

Putative virulence factors of *Candida* species colonising asymptomatic pregnant Jordanian women

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ABSTRACT

Objectives: Pregnant women are frequently exposed to yeast colonisation and infection compared to non-pregnant women. This study was undertaken to investigate carriage rates of *Candida* species and their virulence factors in high vaginal samples from asymptomatic pregnant women as a probable predisposing factor for neonatal candidiasis.

Methods*:* High vaginal swabs were collected from 200 pregnant women from Al-Karak Governmental Hospital, Jordan from March to December 2018. *Candida* isolates were identified by their growth on CHROMagar *Candida* and Vitek2 automated system. Some virulence factors were determined.

Results: Among 200 swabs tested, 67 (33.5%) yielded *Candida* isolates as follows: *C. ablicans*, 28 (41.8%), while non-*albicans Candida* (NAC) were 39 (58.2%) isolates (p=0.04). *C. parapsilosis* was the most prevailed NAC species isolated, 29 (74.4%) followed by *C. tropicalis*: 6 (15.4%) while *C. glabrata* and *C. krusei*, each represented 2 (5.1%). Among *C. albicans* isolates, 27 (96.4%), 18 (64.3%), and 24 (85.7%) compared to 24 (82.7%), 4 (13.8%), and 25 (86.2%) among *C. parapsilosis* were positive for proteinase, phospholipase, and haemolysin respectively. The protease activity was the highest detected (88.1% of *Candida* isolates) compared to other enzymes but did not reach statistical significance between *Candida* species (p=0.215). Phospholipase activity was significantly detected among *C. albicans* isolates compared to other species (p <0.001). Insignificant differences in haemolysin production was observed among isolates (p= 0.134).

Conclusions: In Jordanian pregnant women, NAC, mainly *C. parapsilosis,* were the most frequently isolated *Candida* species from high vaginal swabs. Routine screening and treatment are recommended for pregnant women, irrespective of symptoms.

Keywords: *Candida albicans*; virulence factors; *C. parapsilosis*; asymptomatic pregnant women; non- *Candida albicans*.

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INTRODUCTION

The anatomical structure of the female genital tract provides a suitable environment for many pathogenic microbiota that colonise the vulvovaginal area. These microorganisms may either cause multiple infections or persist for years asymptomatically, especially opportunistic fungi (1, 2). *Candida* spp. are the most common opportunistic pathogens that colonise the vaginal tract in 20% of healthy asymptomatic women in childbearing age and up to 30% of pregnant women (3). Their colonisation and overgrowth causes vulvovaginal candidiasis which is associated with inflammation of the vagina and/or vulva, vaginal discharge, vaginal and vulvar pruritus, pain, burning, itching, and erythema (4). In fact, around 75% of all adult women experience at least one episode of vulvovaginal candidiasis, 50% of them are predisposed to recurrence (5) .

Candida infections impose a serious public health burden and socioeconomic challenge. Despite that more than 17 different *Candida* species are known, the vast majority of invasive infections are caused by *Candida albicans* and non-albicans *Candida* (NAC) species, including *C. glabrata*, *C. parapsilosis*, *C. tropicalis,* and *C. krusei* (6). They can asymptomatically colonise the gastrointestinal tract, oral cavity, and reproductive tract of healthy individuals. Asymptomatic colonisation of *Candida* spp., under certain conditions, may cause superficial infections such as oropharyngeal and vulvovaginal candidiasis up to systemic infections such as fungemia and invasive candidiasis(7,8). Several virulence factors are involved in establishment of *Candida* infections and permit *Candida* colonisation and invasion. These factors facilitate the adherence to target tissues, hyphal morphology, drug resistance, and cell lysis which include the exoenzymes aspartyl proteinase, phospholipase, and haemolysins (9). Aspartyl proteinase facilitate adhesion, invasion and tissue damage, while phospholipase mediates host cell membrane damage (10). Lysis of red blood cells by the haemolysin enzyme plays a role in fungal survival and facilitates hyphal invasion during disseminated candidiasis which is coupled with iron uptake (11).

The predisposing factors that might increase establishment of vulvovaginitis are consumption of contraceptives and broad spectrum antibiotics, uncontrolled diabetes, immuno-suppressive drugs, and pregnancy (1,12). The increase in development of vulvovaginal candidiasis in asymptomatic pregnant women, in contrast to non-pregnant women, is influenced by alteration of vaginal pH and decrease in antibody secretion in the vagina by gestational hormones (i.e. estrogen) which result in higher glycogen content in the vagina that serves as a carbon source for *Candida* species colonisation (12,13). Furthermore, stage of pregnancy and number of gravid influence the development of infection(14). During pregnancy, untreated vaginal colonisation of *Candida* is associated with certain complications, including delivery complications, preterm birth, low birth weight, chorioamnionitis, congenital cutaneous candidiasis, and systemic infections in the neonates leading to 25-35% mortality rates (13,15).

To date, two studies have focused on isolation of yeasts colonising the vagina of females residing in the central part of Jordan (16,17), without determining the associated virulence factors. Our study is the first one that addressed the determination of the carriage rates of *Candida* species in high vaginal samples from asymptomatic pregnant women, visiting prenatal clinic in AL-Karak Hospital in South Jordan. In addition, production of putative virulence factors by isolated *Candida* species was investigated. Our study aimed to provide

grounds to establish prenatal screening protocols for candidiasis in asymptomatic pregnant women and development of treatments regimes that reduce the rates of abortions and pre-term birth among vulvovaginal candidiasis infected women.

MATERIALS AND METHODS

Study design and sample collection

A cross-sectional study was conducted from March to December 2018.A total of 200 samples were collected from asymptomatic pregnant women during their regular antenatal visit to the gynaecologic clinic of the Al-Karak governmental hospital, south of Jordan. Vaginal samples were collected by the gynaecologist from pregnant women using a sterile cotton swab moistened with normal saline that were inserted and rotated gently to pick up the specimen. The age groups of included women and samples numbers were 20-26 years (65 samples, 32.5%), 27-33 years (75 samples, 37.5%), and 34-45 years (60 samples, 30%). Socio-demographic data, antenatal visits, and pregnancy complications were obtained from the study participants using structured questionnaires after informed consent was obtained from each participant.

Inclusion criteria

Inclusion criteria for the participants were all at the 35th week of gestation, healthy, not diagnosed with vulvovaginal candidiasis, gestational diabetes mellitus, any kind of immune deficiency, no history of previous preterm labour or spontaneous abortion, and not under medications or currently on treatment for antifungal therapy.

Ethical approval

This study approved by the Ethics Committee at the Faculty of Medicine, Mutah University, Jordan according to the institutional ethical considerations and guidelines.

Culture and identification of *Candida* species:

Vaginal swabs were inoculated on Sabouraud Dextrose Agar (SDA) (Oxoid, UK), and incubated at 37°C for 48 h. The grown creamy colonies were selected, re-cultured on SDA plates for purification, and identified by the automated VITEK2 compact system (bioMérieux, Marcy-l'Etoile, France). A suspension of each purified colony was plated on CHROMagar *Candida* (Oxoid, Ltd, Basingstoke, UK), a selective medium for *Candida* species. Isolation and identification was based on colony colour and morphology. Reference standard strains of *C. albicans* (ATCC 60193), *C. glabrata* (ATCC 22553), *C. parapsilosis* (ATCC 22019), *C. krusei* (ATCC 34135), and *C. tropicalis* (ATCC 1369) were included and cultured on CHROMagar medium as positive controls (Figure 1).

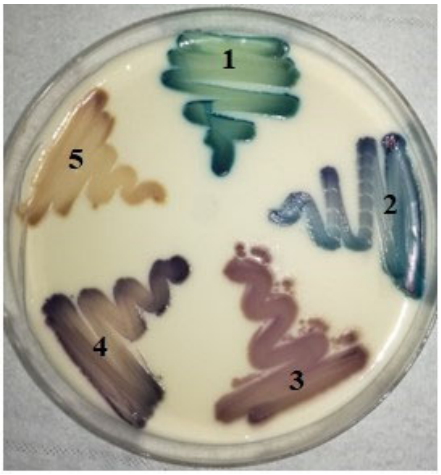

Figure 1. The appearance of different reference *Candida* species on the CHROMagar: green colonies of C. *albicans* (1), metallic blue colonies of *C. tropicalis* (2), fuzzy pink of C. *krusei* (3), mauve brown colonies of C. *glabrata* (4), and cream to white smooth colonies of *C. parapsilosis* (5).

Detection of virulence factors

Inoculum preparation: A suspension of 0.5 ml of McFarland yeast cells was made from SDA and incubated for 18 hours at 37ºC in brain heart fusion broth (BHI). The cells were standardised to approximately 10^6 CFU/ml at 530 nm wavelength according to the Clinical and Laboratory Standards Institute (CLSI) criteria (18) .

Production of aspartyl proteinases: The ability of collected *Candida* species to form a clear halo zone around their grown colonies on bovine serum albumin (BSA) agar was indicative on the extracellular production of aspartyl protease (19) . Briefly, 5µl yeast suspension from an 18hr culture containing 10^6 cells/ ml were spot inoculated onto 1% BSA agar plate and incubated for five days at 37ºC. After which the plates were flooded with 1.25% naphthalene black solution in 90% aqueous methanol for 15 min and decolorised for a further 36 hr with several changes using the latter solution. Each isolate was tested in triplicate. The halo zone (Hz) value was scored which represents the ratio of the colony alone to the diameter of the colony plus clear zone. Based on Hz values, aspartyl proteinase protein activities were classified into four categories: Hz=1(negative); 0.9-0.99 (+1), very low; 0.80-0.89 (+2), low; 0.70-0.79 (+3), high; and < 0.70 (+4), very high according to Price *et al.*(20) Reference strains of *C. albicans* (ATCC 10231) served as a positive control for aspartyl proteinase protein activity.

Production of haemolysin: The haemolysin activity was evaluated as previously described (10,11) . In brief, Sabouraud dextrose-enriched sheep blood agar was spot inoculated with 10µl of 10^8 cells/ml yeast suspension to yield a circular inoculation site of about 5 mm in diameter. Each isolate was tested in triplicate. The haemolytic activity was determined by the presence of a translucent halo zone around the inoculum. The haemolytic index (HI) was used to represent the intensity of the haemolysin production by dividing the total diameter of the colony plus the translucent halo zone over the diameter of the colony. The strains were classified according to the HI as a negative (HI=1.00), positive (1.00<HI<1.5), or strongly positive (HI>1.5). Reference strains of *C. albicans* (ATCC 32354) served as a positive control for the haemolysin activity.

Production of phospholipase: Production of phospholipase by *Candida* species was assessed by the egg yolk agar method (20). Egg yolk agar plates comprising 65g SDA, 1M NaCl, 0.005 M $CaCl_2$, and 8% sterile egg yolk emulsion (Oxoid) were spot inoculated with 1µl containing 10^5 CFU/ml and incubated at 37°C for five days. Each isolate was tested in triplicate. The diameter of the colony and a total diameter of colony and precipitation zone (Pz) were measured, and phospholipase activity was scored as previously in the aspartyl proteinase protein test. *C. albicans* (ATCC 90028) served as a positive control for phospholipas production.

Data analysis

All data were analysed using the Statistical Package for the Social Sciences (SPSS) software, version 18.0. Study variables (frequencies and percentages) were statistically analysed using chi-square test ($\chi 2$). *p*-values <0.05 were considered statistically significant.

RESULTS

Distribution of *Candida* species within different age groups of the studied asymptomatic pregnant women

Among a total number of the 200 vaginal swabs, 67 (33.5%) samples tested positive for *Candida species*. *C. albicans* was detected in 28 samples (41.8%), while NAC species were isolated from 39 samples (58.2%). *C. parapsilosis* accounted for 43.3% of the total isolated *Candida* species and 74.4% of the NAC isolates. Most of the vaginal *Candida* species were isolated from the pregnant women at the age group 34-45 years with a total of 30 (44.8%) isolates, followed by 24 (35.8%)

isolates at the age group 27- 34 years, while the least number of *Candida* isolates were at the age group 20 - 27. *C. albicans* was the most isolated vaginal *Candida* species in the age group 27-34 years (66.7.1%), while *C. parapsilosis* dominated among other species within the age group 34-45 years (60%). *C. Krusei* and *C. glabrata* were the least isolated *Candida* species within the different age groups (Table 1).

Aspartyl-proteinase and phospholipase activity among isolates of different *Candida* species

Proteinase and phospholipase activities were detected in 59 (88.1%) and 26 (38.8%) out of 67 *Candida* spp. isolates respectively (Table 2). Out of the 28 tested isolates of *C. albicans*, 27 (96.4%) showed proteinase activity, while among the 39 tested NAC species, 32 (82.1%) positive results were found as follows: 24/29 *C. parapsilosis* isolates (82.7%), 4/6 *C. tropicalis (*66.7%), and four isolates of *C. glabrata* and *C. krusei* were found to be proteolytic. The *C. albicans* isolates were the main producers of phospholipase18/28 (64.3%). Half of isolates of *C. krusei* (1/2 cases) and *C. tropicalis* (3/6 cases) were phospholipase producers. *C. parapsilosis* showed positive activity for phospholipase but with a lower percentage, 4/29 (13.8%). No phospholipase activities were detected in *C. glabrata* isolates. The activity scoring of the produced proteinase and phospholipase virulence factors is shown in Table 2.

Considering the scoring of proteinase activity (*Hz), a large number of *C. albicans* (25/27) and *C. parapsilosis* (21/24) isolates exhibited an enzymatic activity considered very high (+ + + +). Furthermore, all the *C. krusei, C. tropicalis,* and *C. glabrata* producers showed very high proteinase activity. Considering the scoring of phospholipase activity (*Pz), a considerable number of *C. albicans* (11/18) isolates exhibited an enzymatic activity considered very high (+ + + +). The protease activity was the highest detected (88.1% of *Candida* isolates) compared to the other enzymes but did not reach statistical significance between *Candida* species (χ^2=5.6, *p*=0.215). On the other hand, phospholipase activity was significantly detected among *C. albicans* isolates compared to other species (p <0.001).

Haemolysin activity among isolates of different *Candida* species

Among the 67 isolates of *Candida* spp., 54 (80.6%) were haemolysin producers (Table3). Regarding the 28 *C. albicans* isolates, 24 (85.7%) showed strong haemolysin activity compared to 4 (14.3%) isolates that were haemolysin negative. On the other hand, haemolysin production among the NAC species was detected in 30/39 (76.9%). Twenty-five (86.2%) out of 29 isolates of *C. parapsilosis* and 50% (3/6) of *C. tropicalis* showed strong haemolysin activity. One case each from C. *glabrata* and *C. krusei* revealed haemolysin activity to a lesser extent. This difference in haemolysin activity among *Candida* species did not reach the statistical significance (χ2=7.03; p= 0.134).

Table 1. Distribution of *Candida* species within different age groups of the studied asymptomatic pregnant women.

***Candida* species**	**Age groups (years)**			**Total N (%)**
	20 - 27	**27- 34**	**34-45**	
C. albicans	5 (38.5%)	16 (66.7%)	7 (23.3%)	28 (41.8)
C. krusei	1 (7.7%)	1 (4.2%)	0 (0%)	2 (3)
C. tropicalis	1 (7.7%)	2 (8.3%)	3 (10%)	6 (8.9)
C. parapsilosis	6 (46.1%)	5 (20.8%)	18 (60%)	29 (43.3)
C. glabrata	0 (0%)	0 (0%)	2 (6.7%)	2 (3)
Total	13	24	30	67(100)

Table 2. The distribution of proteinase and phospholipase activity and their scoring of the precipitation zones among isolates of different *Candida* species.

***Candida* species (n)**	**Scoring of proteinase activity (*Hz) No of positive isolates (%)**					**Scoring of phospholipase activity (*Pz) No of isolates (%)**				
	4+	**3+**	**2+**	**1+**	**Negative**	**4+**	**3+**	**2+**	**1+**	**Negative**
***C. albicans* (28)**	25 (89.3)	0 (0)	2 (7.1)	0 (0)	1 (3.6)	11 (39.3)	3 (10.7)	3 (10.7)	1 (3.6)	10 (35.7)
***C. krusei* (2)**	2 (100)	0 (0)	0 (0)	0 (0)	0 (0)	1 (50)	0 (0)	0 (0)	0 (0)	1 (50)
***C. tropicalis* (6)**	4 (66.7)	0 (0)	0 (0)	0 (0)	2 (33.3)	1 (16.7)	0 (0)	2 (33.3)	0 (0)	3 (50)
***C. parapsilosis* (29)**	21 (72.41)	0 (0)	2 (6.9)	1 (3.45)	5 (17.24)	2 (6.9)	1 (3.45)	0 (0)	1 (3.45)	25 (86.2)
***C. glabrata* (2)**	2 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (100)
Total	59 (88.1)				8(11.9)	26(39.0)				41(61.0)

*The value representing the ratio of the colony alone to the diameter of the colony plus formed zone. Score value=1 (negative); 0.9–1 (1+); 0.89–0.80 (2+); 0.79–0.70 (3+); ≤ 0.69 (4+).

Table 3. Distribution of haemolysin and its scoring among isolates of different *Candida* species.

Candida species (n)	Scoring of haemolysin activity (*HI) No of isolates (%)		
	Strongly positive	Positive	Negative
C. albicans **(2**8)	24 (85.7)	0 (0)	4 (14.3)
C. krusei **(**2)	0 (0)	1 (50)	1 (50)
C. tropicalis **(**6)	3 (50)	0 (0)	3 (50)
C. parapsilosis **(**29)	24 (82.8)	1 (3.4)	4 (13.89)
C. glabrata **(2**)	0 (0)	1 (50)	1 (50)
Total	51 (76.1%)	3 (4.47%)	13 (19.4%)

*HI: The strains were classified according to the HI as negative (if HI=1.00), positive (if 1.00<HI<1.5) or strongly positive (when IH>1.5). The experiments were performed in triplicate and the results given as the mean of the values obtained.

DISCUSSION

Candida colonisation of pregnant women genital tract without association of any signs of infection (asymptomatic) can predispose neonates to invasive candidiasis with mortality rates of 25-35%. Therefore, implementation of screening strategies for early detection of *Candida* species and treatment of vaginally colonised women by yeast will reduce vulvovaginal candidiasis predisposing factors and facilitates early resolution of infection. Intriguingly, detection of *Candida* colonisation of the vagina in asymptomatic women is failed when relying merely on vulvovaginal candidiasis symptoms and microscopic examination of vaginal secretions, credibility of colonisation detection is highly dependent on positive culture of yeasts (21). In Jordan, very limited studies focused on the association of vulvovaginal candidiasis with *Candida* species; however, none of these studied the production of virulence factors. Therefore, the present study was carried out to evaluate the carriage rate of *C. albicans* and other NAC species among asymptomatic Jordanian pregnant women. Herein, the carriage rate of *Candida* species among asymptomatic pregnant women was 33.5%. Although it was assumed that colonisation with *Candida* species is 20% in asymptomatic adult women and might reach up to 30% during pregnancy (2,3,22,23), our finding is comparable with *Candida* prevalence rates reported in two previous studies from Jordan in 1997 and 2017 (22-40%) (16,24), 31.4%, in Italy (13), and 20-56% in different states of Nigeria (4,5) . On the contrary, it was relatively higher than reported from Argentina (21), Ethiopia (14), Australia (25), Bulgarian (26)' and New York (27) with prevalence rates of 13.4%, 18.7%, 19.6%, 29%, and 29.4%, respectively.

Considering *Candida* species distribution in our study, *C. albi ns* represented 41.8% and NAC species accounted for 58.2% of the total identified *Candida* species. *C. parapsilosis* accounted for 43.3% of all detected *Candida spp.* (representing74.4% of NAC), 8.9% for *C. glabrata*, and *C. tropic is* and *C. krusi* represented by 3%. In a similar study of asymptomatic pregnant women in Australia(25), a higher colonisation rate was observed for *C. albicans* (73%) and *C. glabrata* (14.3%), similar rates were observed for the prevalence *C. krusei* and *C.* tropicalis (3.1%). On the other hand, a much lower prevalence than in our study was observed for *C. parapsilosis* 5.1%). The prevalence in our study was also lower than that by Nurat *et al.* with 54.3% positive cases for *C. albi ns*, 25.7% for *C. glabrata*, and 5.7% for *C. tropicalis* (5).

Compared to our study results, many discrepancies in *Candida* species detection and distribution were reported by previous epidemiological studies, including pregnant women, both symptomatic and asymptomatic. *Candida* were detected in 33.1% of Argentinean pregnant women, *C. albican* represents 86.4% and NAC 13.6% (21). In China 15% were *Candida*-positive, 79.9% were *C. albicans,* and 20.1 were NAC (3). 30-60.7% of Nigerian pregnant women were colonised with *Candida,* 73.8% were *C. albicans* and NAC accounts for 26.2% (4, 28). *Candida* species were isolated from 42% of pregnant women in Libya, *C. albicans* were isolated from 92% of patient samples and NAC were isolated from 8% of samples (29). 39-45% of Lebanese pregnant women were colonised with *Candida* species, 42-43% were *albicans* while 57-58% were NAC (2,22). in Egypt, the vaginal swab cultures revealed prevalence of *Candida* in 50.4% of patients with 60.3% being *C. albicans* (30). In a previous study from Jordan, *Candida* species were isolated from 68.2% of pregnant women, 60.7% were *C. albicans,* 14.3% were each of *C. glabrata* and *C. tropicalis*, 7.1% *C. krusei*, 3.6% *C. guilliermondii, and no C. parapsilosis* was detected (16) .

Compared to our study, *C. albicans* from vaginal samples in most of the above literate reports was the highly dominant isolated species followed by *C. glabrata*, *C. tropicalis*, *C. parapsilosis*, and *C. krusei*. Nevertheless, epidemiological data showed a mycological shift in regards to the vaginal colonisation toward the NAC species with incidence rate greater than 50% (31) . Variation in *Candida* species distribution between countries and increase in colonisation of NAC might be attributed to differences in geographic regions, sexual behaviours, culture, customs of different nations, differences in study design, target populations, risk factors, hygiene, disease history, contraceptives, prolonged antibiotic uses, and diagnostic methods (1,12,22).

Intriguingly, the higher incidence rates of the dominant *Candida* species in our study were for *C. albicans* and *C. parapsilosis* in the age groups of 27-33 and 34-45 years, representing 66.7 % and 60% of the totally isolated *Candida* species in each age group respectively. This is consistent with most studies that indicated that dominant *Candida* isolates were detected in the active reproductive and childbearing age periods of 26-45 years. (2,4,14)

The production of certain virulence factors determines the ability of *Candida* species to colonise and persist in the infection site. These virulence factors are hydrolytic enzymes that provide *Candida* species with nutrients through their extraction from the host cells (14,16,17,26,31,32) or facilitate their adhesion, tissue invasion, and blood dissemination; the most important hydrolytic enzymes being aspartyl proteinase, phospholipase, and haemolysin 32). Aspartyl proteinase protein makes colonisation easier through disrupting the integrity of the mucosal surfaces and interfering with the components of the immune response (33). Phospholipases aid in tissue damage

and invasion through their ability to hydrolyse host cell-membrane phospholipids to fatty acids, which help in exposing receptors to facilitate adherence of *Candida* (32,33). Aspartyl proteinase and phospholipase were produced by 88.1% and 38.8% of all *Candida* isolates, respectively. They were identified in 96.4% (27/28) and 64.3% (18/28) of *C. albicans* and in 82.7% (24/29) and 13.8% (4/29) of *C. parapsilosis*, respectively. The difference in protease production did not reach statistical significance among different *Candida* species. The phospholipase among the *C. albicans* isolates showed significant activity compared to the other species. These findings were higher than those in analogous reports from several countries(31,32) . However, haemolytic enzyme was found in 80.6% of all isolated species; 85.7% (24/28) and 86.2% (25/29) of *C. albicans* and C. *parapsilosis* were haemolysin positive respectively with no significant difference among *C. albicans* and NAC species. These results indicate the ability of all isolated *Candida* species in colonising their hosts through the ability to derange the host cell membrane and to extract iron from lysed red blood cells.

Altogether, our results revealed an increase in incidence rate of NAC species (58%) over *C. albicans* (42%) in Jordanian asymptomatic pregnant women. *C. parapsilosis* was the second most detected species with a comparable incidence rate as *C. albicans* (43%); and represented 72% of NAC species. This high detection rate of *C. parapsilosis* was inconsistent with most epidemiological studies, including Jordanian reports that indicated an increase in the incidence of NAC with *C. glabrata* as the second prevailed *Candida* species after *C. albicans* in asymptomatic pregnant women and women with vulvovaginal candidiasis and recurrent vulvovaginal candidiasis infections (2,3,14,16,17) . However, the high rate of *C. parapsilosis* incidence in asymptomatic pregnant women in our study unveil that its colonisation in the vagina is rarely associated with symptomatic vaginitis (5) .

A limitation of our study should be kept in mind, which is the lack of data about antifungal susceptibility profiles of the isolated strains as the different classes of antifungal drugs were not available during the research period. Consequently, the clinical relevance of our findings points out the need for further study for antifungal susceptibility testing for prompt treatment. Antifungal susceptibility testing could pave the way for further molecular studies to determine the origin of resistance, if any.

In conclusion, the significant isolation of *C. albicans* and NAC from Jordanian asymptomatic pregnant women raises the risk of the development of *Candida* associated infections with increase in the possibility of pre-term birth and neonatal abnormalities. Therefore, we recommend a screening program for all pregnant women to monitor and treat probable vaginal candidiasis. The implementation of early screening, detection, and adequate antifungal treatment could help in improving pregnancy outcomes, minimise the health-care budget, and the burden on the health sector.

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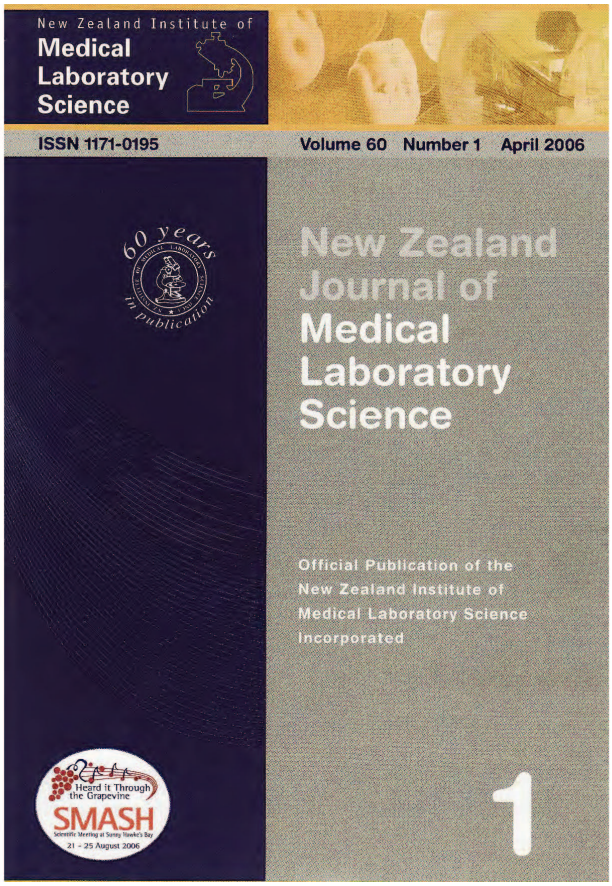

TECHNICAL NOTE

Lyophilisation (freeze drying) process for external INR and D-dimer quality assurance programme

Sunny Jamati and Maree Bell

For almost 30 years, Waikato DHB has offered a quality assurance (QA) programme across numerous disciplines. Originally lyophilisation of internally sourced coagulation and microbiology QA samples was performed twice a year for the current year, at an approved external laboratory. However, it was observed that the integrity of the lyophilised product deteriorated over extended periods. As a result, our laboratory made the decision to purchase their own freeze dryer in 2014 to enable lyophilisation to be performed internally throughout the year.

Lyophilisation (freeze drying) is the removal of ice or other frozen solvents from a material through the process of sublimation and the elimination of bound water molecules through the process of desorption (1) Controlled freeze drying keeps the product temperature low enough during the process to avoid alterations in the dried product appearance and characteristics. It is an ideal method for preserving an extensive selection of heat-sensitive materials such as plasma, proteins, microbes, pharmaceuticals, and tissues.

The lyophilisation process is an ongoing process that takes several days to complete. The journey begins with our patient samples. Samples are categorised based on their INR and D-dimer results. We aim to get a variety of results. Pathlab Hamilton assists us by sending samples with high INR results. Samples with similar results are pooled together and frozen at -20°C. The serology results for each pool must be negative for HIV, hepatitis B and hepatitis C. Pools with positive immunology serology are discarded.

The lyophilisation process takes four days. Each day has a crucial process required to yield an optimal final product for the participants to receive. On day 1, individual pools are thawed and combined to make a 40 ml pool. Glycine, Hepes, and sucrose are added to the pool to help support the coagulation proteins during the lyophilisation process. This allows consistent coagulation results to be obtained post-lyophilisation (a pre-frozen sample is tested as a baseline). Vials are labelled with the appropriate survey number and 1 ml is pipetted into each vial. A rubber stopper is placed halfway on to each vial. This is purposely done to allow the air to escape. The samples will explode if the air is not released. The vials are placed in a polystyrene box for 24 hours at -70 degrees (Figure 1.)

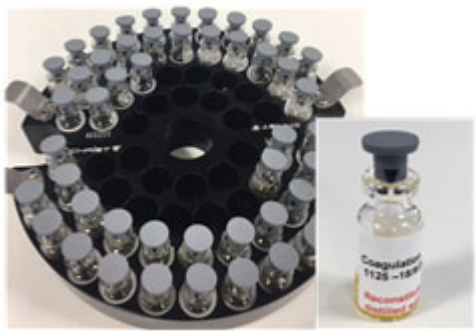

Figure 1.

On Day 2 the freeze dryer is turned on and warmed up. Samples are transferred quickly to the freeze dryer. It is important that the samples do not thaw, as this will ultimately result in a failed run due to 'caramelisation' of the plasma. The pressure dropping should cause the icicles on the vials to flake off, this indicates a successful run. This process takes 24 hours (Figure 2).

Figure 2.

On Day 3 the pressure is further lowered for the final drying step. This gives adequate final vacuum pressure to eliminate all capillary or molecular bound water (1). Finally, on Day 4 the lyophilisation process is complete. The rubber stopper is pushed down to seal the vial. A vial from the run is selected and reconstituted to make sure the pre- and post-lyophilisation results are comparable. The vials are stored at -20°C until the dispatch date (Figure 3).

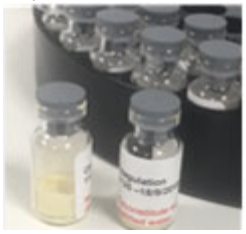

Figure 3.

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The journal 1996—2021

Rob Siebers, Editor

The New Zealand Journal of Medical laboratory Science (Journal) has been published continuously since 1946. Upon inception the 75th anniversary of the New Zealand Institute of Medical Laboratory Science (NZIMLS) and the Journal this article chronicles the preceding 25 years, 1996 to 2021. A previous article chronicled the Journal from 1946 to 1995 upon the 50th anniversary of the NZIMLS (1).

In 1996 the Editor was Rob Siebers from the University of Otago, Wellington. Rob took over the Editorship from Marie Gillies in 1994 and has been Editor continuously until present time. In case something unforeseen was to happen to the Editor, a Deputy Editor was appointed in November 2003. This was Ann Thornton from the University of Otago, Wellington and she served in that position until April 2011. From then on the following were appointed as Deputy Editor: Terry Taylor from SCL, Dunedin in August 2011; Collette Bromhead from Aotea Pathology, Wellington in August 2015; Michael Legge from University of Otago, Dunedin in April 2018; and Julie Creighton, Canterbury Health Laboratories in April and August 2019 as joint Deputy Editor with Michael Legge.

In order to offset production costs, the Journal has always carried commercial advertising. In 1983 Trish Reilly from Auckland was appointed as advertising manager to separate this aspect from editorial managing. Trish continued in this role until April 2012 when Fran van Til from the NZIMLS Executive Office took on this function. The Journal had been printed by Institute Press, Auckland since 1983 until November 2013 when printing was shifted to Christchurch with the current printers being Griffin Press. Since late 2013 formatting of the Journal, previously undertaken by the Editor, has been done by Sharon Tozer of the NZIMLS Executive Office.

From 1996 to 1998, four issues were published each year. Due to low submissions of articles, printing was limited to three issues per year from 1999 onwards. Published peer reviewed articles from 1996 to 2009 remained constant at an average of eight articles per year (range: 4-11). This increased in the 2010-2018 period when an average of 13 articles per year (range: 11-14) were published. An increase in published peer reviewed articles occurred in 2019 when 19 articles were published, rising steeply to 40 articles in 2020. The large number in 2020 may have been due to authors having to isolate during the Covid-19 pandemic and thus having increased time to write. This has also been experienced by many international journals.

In 2013 the Editor persuaded the NZIMLS Council to make the Journal platinum open access. In the platinum open access model, authors retain copyright to their articles under a creative commons attribution license and may freely distribute their articles anywhere provided the source (the Journal) is acknowledged. The open access model is widely used by many journals and publishers, however, in order to retain copyright the authors pay the publishers a fee upon acceptance of their manuscript, often to the tune of a thousand dollars or more. Under the platinum model authors retain copyright but do not pay author fees and the cost of producing the journal is born by the journal's owners, as is the case of the NZIMLS Journal. This makes our Journal attractive to overseas authors, especially those from countries where their institutions do not have the resources to pay for author fees and that is why we have seen an increase in submissions from overseas authors. The downside is that a large number of overseas submissions are of poor quality and currently we reject nearly half of overseas submissions after internal and external peer review. Our Journal is also indexed by SCOPUS, the 2nd most important database after PubMed and over the years we have seen an increase in citations to Journal articles by international journals. Since 2000 nearly 30% of Journal articles have been cited internationally at least once. Interestingly, the top four most cited Journal articles were from overseas, the top one from Nigeria (2).

The Journal also publishes other articles than peer reviewed ones. Thus, a number of Editorials, letters to the Editor, book reviews, and news items from the universities and Special Interest Groups have been published. Each issue also contains the Pacific Way column reporting on activities of the Pacific Pathology Training Centre, which provides training and support to medical laboratories in the South Pacific.. This column has been ongoing since 1983. Also published yearly is the TH Pullar Memorial Address, which is given by a prominent member or pathologist upon invitation by Council at the NZIMLS Annual Scientific Meeting. Since 2017, Michael Legge has written the Science Digest column, highlighting interesting snippets from international publications. Lately we have also published the abstracts from the Otago University BMLSc 4th year students' projects. A few of these have resulted in full publications.

In 2006 the Journal questionnaire was initiated in order for New Zealand medical laboratory personnel to obtain CPD points. Initially, it contained 10 questions derived from the Journal articles in a true/false format. Participants had to get at least 7 out of 10 questions right to obtain 10 CPD points. This was changed in 2007 when participants had to give answers to the questions and get at least 8 out of 10 correct. The questionnaire was popular with nearly 500 participants participating in the 1st questionnaire. Currently more than 1,000 attempt the questionnaire from each issue. In 2020, due to the Covid-19 pandemic, two journal questionnaires were introduced in each issue in order to give more opportunity to obtain CPD points as such opportunity was reduced as many scientific meetings were cancelled. Of note, if not for overseas articles, Journal questions would have not been able to be derived from a number of issues over the years.

The Journal has gone from strength to strength, especially over the last few years. It is truly an international journal, despite the New Zealand in its title. The Journal has adopted many editorial policies from the World Association of Medical Editors (WAME) of which the Journal's Editor is currently President of. The journal's success has been due to many people: the Editorial Board, Sharon Tozer at the NZIMLS Executive Office, the reviewers, the authors, and the support of the advertising companies. Hopefully, its success will continue over the next 25 years. I am sure it will!

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75TH ANNIVERSARY ARTICLE

Changes in transfusion science over the past 25 years

Holly Perry

Thanks to advances and modifications to transfusion policies and practices over the past 25 years, Aotearoa New Zealand now justifiably enjoys the reputation of being one of the best places in the world to receive a safe, effective, and timely blood transfusion.

This is due in no small part to the formation of a national blood transfusion service in 1998. Prior to 1998, each part of the country was served by its own regional blood service. Differences in testing policies existed between regions and there was no national computerised database in which to hold a patient's transfusion records prior to 1998. The "vein to vein" philosophy of New Zealand Blood Service (NZBS) sees the service take responsibility for a nationally standardised approach to the collection, testing and provision of all blood components and products transfused in New Zealand (1).

The creation of a national blood management system now ensures a patient's records can be accessed from anywhere in the country. This enhances transfusion safety for the patient; by establishing the history of a patient's blood group, pre-analytical errors such as wrong blood in tube can be detected. If a patient has a historical antibody that has currently fallen to undetectable levels, the presence of that antibody is recorded on the national database and the patient issued with appropriate safe blood, wherever they reside. In the past one would not have known this history unless the patient remained in a region served by one regional transfusion service throughout their life. When transfusion errors do occur, they are recorded and collated nationally and reported in the haemovigilance report published annually by NZBS. This national monitoring further enhances transfusion safety for patients through modification of practice.

Studies which track the risk of catching an infection from a blood transfusion reveal how much safer blood transfusions are from this standpoint. For example, in 1985 the risk of catching Human Immunodeficiency Virus (HIV) from a blood donation was approximately 1 in 100 (2), now it is less than one in a million (3). In 2001, the introduction of viral genome detection through the amplification technology known as Nucleic Acid Testing (NAT) resulted in a decrease in the time taken to detect blood donors infected with HIV and Hepatitis C virus (4). Later, Hepatitis B virus was added to the NAT regime, and transfusion-transmitted hepatitis is now a very unusual occurrence in New Zealand (3,5). Technological improvements in the testing of transfusion-transmitted infections are continuous and state of the art. As well as screening for well-established transfusion transmitted pathogens, the NZBS also monitors emerging agents, and when a new test is introduced, the whole of New Zealand benefits from the introduction of the screening test at the same time. It seems incredible now to think that different regions of New Zealand began their HIV and hepatitis screening at different times.

NZBS also introduced universal leucodepletion (removal of white blood cells from all blood components) early in the 21st century. Whilst this was driven largely by variant Creutzfeldt Jakob Disease fears in the last two decades of the 20th century, it brought a host of other safety advantages to blood components, including reduction of human leucocyte antigen (HLA) allo-immunisations, and consequent investigations of febrile reactions in transfused patients. The latter were time consuming for blood banks to conduct and as the workload of blood banks has steadily increased, and a febrile reaction is considered clinically insignificant, any drop in the number of transfusion reaction investigations of this type is helpful.

The international attitude to blood transfusion has changed and our country is no exception. The campaign conducted by NZBS in New Zealand hospitals carries the slogan "why give two when one will do?", exhorting clinicians to transfuse fewer red cells. This acknowledges that patients can have good quality of life with a lower haemoglobin level than previously believed, and that blood carries inherent risks, which increase with each unit transfused. This is a far cry from the belief held in blood banks during my training, when we were told if the patient needed only one unit, it was not really necessary to transfuse them at all! Before the formation of NZBS, many donations were discarded because they reached the end of their shelf life without being transfused. This wastage of our precious resource of blood donations has now been minimised, with red cells being re-directed before expiry to any area of the country.

Plasma is currently a major driver in the recruitment of blood donors. With the development of fractionated plasma products such as intravenous immunoglobulin being used to treat an ever-growing number of medical conditions, very large volumes of plasma are needed in New Zealand. Consequently, plasmapheresis is now a very common way to donate.

Technologies of column agglutination testing and solid phase red cell adherence to screen patients for unexpected blood group antibodies have made automation possible in a way that the old tube testing simply did not lend itself to. When automation first started making inroads into medical laboratory testing, I worried that this would make our job as transfusion scientists less interesting. On the contrary, the way the blood bank analysers are utilised means that the routine samples are taken care of on the analyser, leaving the scientist free to use their hands and brains on the more challenging samples from patients requiring identification of rare or multiple antibodies, investigation of haemolytic disease of the fetus and newborn, or investigation of autoimmune haemolytic anaemia.

Tissue typing is the arm of the blood service which matches donors with patients for solid organ and haemopoietic transplantation. In the past the tissue typing laboratory mainly used serological testing, with some manual molecular testing. As in many areas of the diagnostic laboratory, automated molecular testing is now the "go to" methodology. The polymorphism of Human Leucocyte Antigen (HLA) types seems to be almost unlimited, and to deal with this, companies manufacturing the testing kits are required to continually update their consumables and software.

The shelf life of platelets has been extended, thanks to bacterial detection testing and resuspension in platelet additive solution (PAS). Once again, the existence of a national service meant that the whole of New Zealand moved to the new component with its new shelf life at the same time. For this reason, New Zealand is frequently the envy of international blood banks who may or may not receive platelets in PAS, depending on who their supplier is. This must be challenging for clinicians in these countries, who undoubtedly want to treat all their patients with equal-quality blood components.

The introduction in 2008 of the Massive Transfusion Protocol caters for patients experiencing massive blood loss through a protocol which sees boxes of components delivered in a balanced ratio designed to maintain haemostasis (6). This has replaced the ad-hoc call for varying numbers of red cells, fresh frozen plasma, platelets, and cryoprecipitate. Whilst patients still need to be monitored for coagulopathy, the introduction of this regime has undoubtedly saved lives, and smoothed communication channels between the emergency room and the blood bank. Similarly, the introduction of prophylactic antigen matched (PAM) red cells (7) for patients who are transfusion dependent over long time periods has resulted in less alloimmunisation (8), and therefore less difficulty in sourcing compatible blood for people with multiple antibodies.

These many changes over the past 25 years mean that New Zealand can be proud to be a world leader in Transfusion in 2021. Most recently, New Zealand has engaged in research-led practice to provide convalescent plasma for patients seriously ill with COVID-19 (9). Whilst at the time of writing we have thankfully not had the need for this to become a routine treatment in New Zealand, it is an example of the proactive approach taken by our transfusion professionals. I have every confidence that our country will continue to evolve in the important field of transfusion medicine over the next 25 years and into the future beyond.

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News from the University of Otago Medical Laboratory Science Programme

Michael Legge

Following the retirement of Associate Professor Heather Brooks as the Director of the BMLSc programme, Dr Tania Slatter has been appointed as the full time Director of the BMLSc programme. On completing her Biochemistry BSc (Honours), Tania undertook research into lipid disorders in the Department of Biochemistry, University of Otago and completed her PhD in 2007. In 2016 she was awarded a Sir Charles Hercus Research Fellowship to investigate how brain tumours develop and how they could be effectively treated. In 2019 Tania was appointed as a Senior Lecturer teaching into the BMLSc programme for histotechnology, molecular diagnostics and chemical pathology. Associate Professor Heather Brooks has become the Assistant Director and Catherine Ronayne has been appointed as a lecturer specifically for the BMLSc programme.

The number of students undertaking the BMLSc programme continues to grow with 41 at second year, 36 at third year and 31 at fourth year for the 2021 academic year. Last year three students successfully completed rural placements, the first for the new programme, and this year there are five rural placements in Dunstan, Oamaru, Blenheim, Masterton and Hutt Valley.

Celebrating 25 years of advances in microbiology

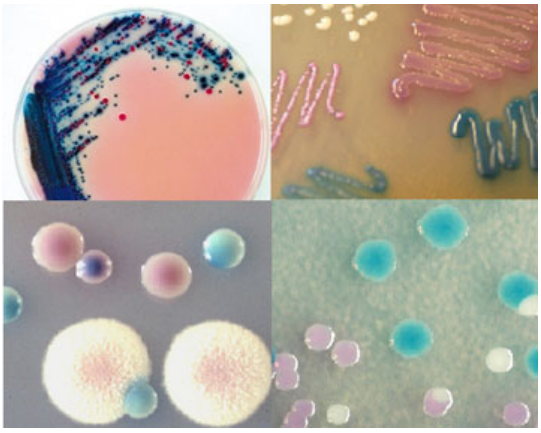
Julie A Creighton

There have been numerous amazing advances in microbiology over the last 25 years, many of which have been improvements on existing technology, but some have been innovative ideas stemming from scientists' naturally inquisitive minds. I have chosen four areas to highlight, which I think have had the greatest impact on the routine clinical microbiology laboratory: chromogenic agar, mass spectrometry, antimicrobial resistance, and whole genome sequencing.

Chromogenic agar

Routine sample processing in microbiology still relies on growing bacteria on solid media. The introduction of chromogenic agar has had a great impact on diagnostic clinical microbiology over the last 25 years. Various types of chromogenic agar are used across most areas of sample processing for the identification of diverse species.

In 1989 Dr Alain Rambach, a French scientist, patented a chromogenic media, known as Rambach agar, for the specific isolation of *Salmonella* species from faecal specimens. The media was a success, enabling the CHROMagar™ product line to expand and generate a plethora of industry competitors. The basic principle of chromogenic agar relies on targeting the presence of specific enzymes in bacteria e.g. beta-glucuronidase is specific to *E. coli* and active in approximately 95% of strains. The solid chromogenic media contains soluble molecules, called chromogens, which are colourless. The chromogens are composed of a substrate coupled with a chromophore (e.g. pH indicator). If a specific enzyme is present in the organism of interest, it reacts with the substrate, releasing the chromophore, enabling the unconjugated chromophore to exhibit its distinctive colour. The chromophore must be relatively insoluble so that it forms a coloured precipitate immediately around the targeted organism.



Chromogenic agar has three main areas of application (i) detection of enteric pathogens from faecal samples, e.g. *Salmonella* spp., *Shigella* spp., and Shiga Toxin-Producing *E. coli*, (ii) detection of specific pathogens from specific sample types, e.g. *Candida* spp. or Group B Streptococcus from vaginal swabs, or *S. aureus* from sputum samples, (iii) detection of antimicrobial resistant bacteria from screening samples, e.g. MRSA, VRE, ESBL or CPE. These applications allow the laboratory to quickly and efficiently "screen" for a specific organism or a group of bacteria (although confirmation of identification is still required), saving time and resources. The benefit of rapid pathogen identification enables timely follow up of healthcare interventions such as antibiotic treatment and implementation of infection control procedures or public health measures.

MALDI-TOF MS (Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry)

Bacterial and fungal identification has traditionally been based on phenotypic tests such as biochemical reactions, culture characteristics, and Gram stain pattern. Some identification tests can be rapid, for example the Spot Indole test to detect the enzyme tryptophanase in *E. coli*; however, many other tests take hours, overnight, or even days if the bacteria or fungi is fastidious or slow growing.

Mass spectrometry has been used for decades but its application to bacterial identification was limited due to low volatility and thermal instability of large molecules such as proteins and carbohydrates. The matrix-assisted laser desorption/ionization (MALDI) technique utilises a chemical matrix material which can absorb energy from the laser light, resulting in the creation of ions from large molecules without degradation. The laser is shot multiple times, which improves the signal-to-noise ratio, thus increasing the accuracy of the molar mass determination. The time-of-flight (TOF) analyser measures the traverse of particles from time of ionisation to the recording detector. The overall mass spectrum is analysed according to molecule mass charge and travel time. The resulting profile is compared to stored database reference profiles, generating a bacterial or fungal identification to either genera or species level. A numeric score helps to assess how closely the organism identification matches the reference spectra. A low score might indicate that the organism is not in the database, or that the biomass is insufficient (due to low density of organisms or immature growth).

MALDI-TOF MS technology has revolutionised organism identification in clinical microbiology over the last decade. Medical laboratories in New Zealand now have, or have access to, a MALDI-TOF instrument; either the Bruker-MS or the bioMérieux Vitek-MS. The instruments have high throughput, are accurate, low cost, and reduce waste, with identification of bacteria and yeast achieved in minutes. The identification of filamentous fungi can be more complex due to variable protein phenotypes. However, improved databases and the ability to have a user-developed database has improved accuracy and performance for filamentous fungi. The routine MALDI procedure is simple, starting with applying a small amount of organism directly onto an edged circle on a metal target plate. The smeared preparation is then overlaid with a 70% formic acid solution for initial extraction (step optional), air dried, then overlaid with matrix solution. The plate is dried again before placing into the MALDI-TOF instrument and running the chosen application. Different preparation protocols can be used for fluids such as blood cultures or urine, or additional extraction steps for fastidious organisms, or to obtain a better identification score. If the instrument is interfaced, then the organism identification result can be sent directly to the laboratory information system.

MALDI-TOF spectra can also be used for other applications, such as detection of a specific biomarker (e.g. to predict methicillin resistance in *S. aureus*), or for the detection of antibiotic degradation (e.g. detection of the carbapenem hydrolysis product following degradation by a carbapenemase enzyme in carbapenem resistant Enterobacterales), or for organism subtyping. There is no doubt that MALDI-TOF is an indispensable tool for microbiology and its future use will only be enhanced with continued library database extensions and new applications.

Antimicrobial resistance (AMR)

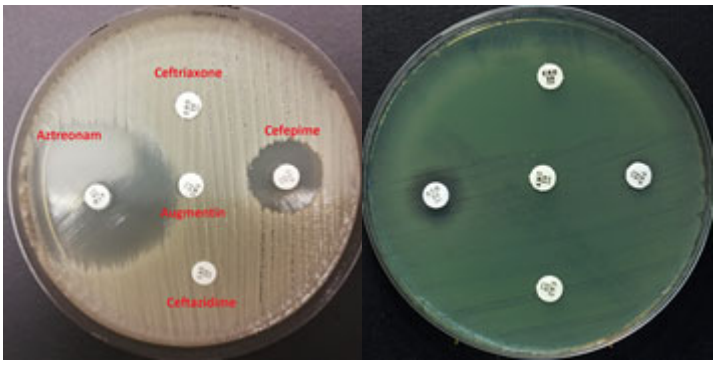
The escalating rates of antimicrobial resistance over the last couple of decades is a global health threat, affecting humans, animals, the environment, and economic productivity. Infections caused by multi-resistant organisms are associated with high morbidity and mortality rates. Drug resistance can be found in many microbes, including *Mycobacterium tuberculosis*, *Neisseria gonorrhoeae*, HIV, malarial parasites, and fungi. However, it is the rapid emergence of resistance and the expanding variety of resistance determinants in Gram-negative bacteria, particularly the Enterobacterales, that is classified as an urgent threat by the WHO. Enterobacterales such as *E. coli* and *Klebsiella pneumoniae* are common gut colonisers and frequently cause community-acquired infections such as urinary tract infections, blood stream infections, gastroenteritis, and hospital-acquired infections.

Extended-spectrum β-lactamases (ESBLs) are enzymes that can hydrolyse most β-lactam antibiotics except for carbapenems. Interspecies dissemination of ESBLs is facilitated by mobile genetic elements such as integrons, transposons, and plasmids. Plasmids can have a broad host range and frequently harbour resistance determinants to other antibiotic classes, leaving few treatment options. ESBLs were first described in 1986, but in New Zealand they were infrequently found, even up until 2000 (only 27 isolates reported to ESR). Since then, the prevalence of ESBL-producing Enterobacterales has rapidly accelerated. The most recent ESR survey was completed in 2016, collecting 521 ESBL isolates during a one-month period, from clinical samples only, suggesting a prevalence rate of 11.1/100,000 population. These numbers will be even more alarming today. Worldwide studies have shown the dissemination of genes encoding ESBLs in animals (farm, domestic, wild) and in the environment, creating community reservoirs for continued dispersion.

A consequence of high rates of ESBL-producing pathogens has been a corresponding increase in the use of carbapenem antibiotics, which in turn has led to the rapid selection of carbapenem resistant organisms. Carbapenem resistance can be due to a variety of resistance mechanisms, the most concerning of which are carbapenemase enzymes - found in various bacterial species, carried on a broad range of plasmid hosts, and are widely disseminated in nature and healthcare settings. Carbapenemases emerged decades ago but it wasn't until the 2000's that they started to proliferate (KPC along the Eastern seaboard of the United States, NDM in India). The prevalence and diversity of carbapenemases have increased worldwide and they are now endemic in many regions of the globe, presenting an enormous challenge to the health sector.

In New Zealand the first report of a carbapenemase-producing Enterobacterales (CPE) was in 2009. Between the years 2009 to 2014 only 35 CPE were identified, all most likely acquired overseas (hospitalisation or travel associated). Since 2014 the total number of CPE isolated each year has been steadily increasing, along with an expanding range of enzyme types (although still dominated by NDM and OXA-48-like), in a variety of Enterobacterales species. Overseas travel, especially from the Indian subcontinent, is still the highest risk factor for acquisition. Worryingly there have been several episodes of transmission events in NZ healthcare facilities as well as community transmissions. It remains to be seen if NZ's border restrictions have any impact on slowing our rising prevalence rates.

To curb AMR, future priorities should include faster laboratory detection, strengthening infection control bundles and international efforts to stimulate the research and development of innovative antimicrobials.



NDM-producing *Klebsiella oxytoca*

VIM-producing *Pseudomonas aeruginosa*

Whole genome sequencing (WGS)

The technology that has rapidly manifested around DNA sequencing is a game changer for microbiology. 25 years ago, pulsed-field gel electrophoresis (PFGE) was at the forefront of the molecular epidemiology era. It was considered the gold standard for assessing genetic relatedness among strains of bacteria following a possible hospital-acquired infection or disease outbreak (e.g MRSA and *Pseudomonas aeruginosa*). However, PFGE is time consuming, labour intensive, and is technically challenging. In addition, it has limited discriminatory power, especially with organisms that undergo significant amounts of horizontal gene transfer.

The human genome project, which identified and mapped the DNA sequence of the human genome, was started in 1990, but it took until 2003 to be completed. Since then rapid advances in the field of whole genome sequencing (WGS) have evolved, including the introduction of next-generation sequencing (NGS), meaning that an entire human genome could be sequenced within a day. WGS could distinguish bacterial relatedness at the nucleotide level, offering far greater resolution than PFGE. However, at the early stages of development instrumentation was very expensive, methods were not standardised and obtaining accurate data analysis could be difficult. Over the last few years the use of WGS has become more common place, especially in large institutions and universities, and we are now at the beginning stages of a transformation into mainstream clinical microbiology. WGS has proved to be a powerful tool for epidemiological investigations of disease outbreaks, with

improving technology allowing real-time analysis, detecting epidemiological risks as they arise. Since the SARS-CoV-2 epidemic, everyone has become familiar with the term “genomic sequencing”!

Aside from epidemiology, NGS can be utilised for resistance profiling. This technology is often superior to challenging phenotypic methods, but it relies on good sequence data and known database – which means that novel resistance mechanisms might be missed. WGS currently is too slow and expensive to replace routine antimicrobial susceptibility testing methods, and it is not suitable for treatment guidelines as gene absence cannot always predict susceptibility.

Metagenomic testing has had intense publicity in recent years, particularly around the gut microbiome. WGS performed on cultured bacteria is a current bottle neck in timely reporting for clinical microbiology. Metagenomic approaches, where the collective genetic material is analysed (e.g. detection of dysbiosis in vaginal discharge, or detection of organisms in sterile fluids) could be an exciting future application, providing rapid non-biased culture-independent diagnosis. Current limitations include method standardisation, issues with false positive results from contaminating DNA and samples with low biomass.

Nanopore, third generation sequencing, is the latest sensation. The MinION, Oxford Nanopore Technologies, uses a protein flow cell and electrical conductivity changes to identify DNA bases, producing long-read sequences. The MinION is small and can plug into a USB port on a laptop computer, making it a highly portable real-time sequencing device. IThe recently released “Flongle” is an adaptor for the MinION, designed for smaller DNA/RNA sequencing tests and lower cost. This technology could potentially be used for targeted sequencing for bacterial resistance genes, metagenomics or organism identification. With simultaneous sample processing, continued software and performance developments and cost decreases, this technology has the potential to transform our routine molecular workflow.

Oxford Nanopore MinION

If the last 25 years is anything to go by, the future for microbiology is looking incredibly exciting!

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75TH ANNIVERSARY ARTICLE

Cytology practice at Wellington Hospital 1996-2020

Sarla Naran, Sharda Lallu, Abed Kader and Peter Bethwaite

Time does not stand still. Technology and methodologies change, with a view to improving quality, adequacy, and diagnosis. In the last 25 years methods of cytology processing and reporting system have undergone major changes. In 1996, laboratory services at Wellington Hospital were operating under Capital and Coast District Health Board [CCDHB] management. At that time Dr Raj Gupta was charge cytopathologist until his retirement in 2003. During his service he had run a training programme for cytotechnologist, registrars, and students from Pacific Islands (PPTC). The laboratory was processing a variety of samples from various body sites such as cervical smears, sputum, urine, CSF, body cavity fluids, bronchoscopy samples, fine needle aspirations including ultrasound and CT guided fine needle aspirations (FNAs), and intra operative FNAs on rare occasions. These were a golden era for the practice of cytology as technology was advancing rapidly and scholars seemed to exude cytology knowledge and observation, and this was manifested in presentations at conferences and journal articles.

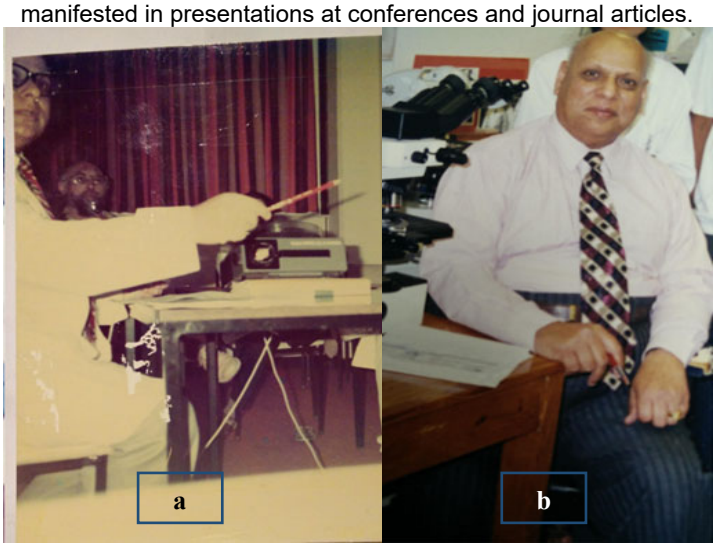

Figure 1 (a): Dr Raj K Gupta teaching gynae and non gynae cytology to the staff using codachrome teaching set on projector as a part of training in 1990's.
Figure 1 (b): Dr Raj K Gupta reviewing daily non gynae cases and abnormal gynae cases on double headed microscope with screener.

The Wellington Hospital cytology laboratory was processing gynaecologic cytology samples, largely from hospital outpatient and colposcopy clinics until 2002 when, as a result of recommendations stemming from the Gisborne Inquiry, the number of laboratories in the country processing gynaecological cytology samples (smears) was reduced from 26 to 8 as a result of the standard that then required laboratories to process a minimum of 15,000 gynaecologic cytology per annum. CCDHB's gynaecological cytology was then contracted to Medical Laboratory Wellington in Courtenay Place, which subsequently became Aotea Pathology and continued operating out of the Courtenay Place premises.

In 1996 gynaecologic [cervical] smears were predominantly "conventional smears" (introduced in 1946), obtained by Cervibroom or cytobrush, spread on glass slides, fixed in 95% ethanol in Coplin jars. In subsequent years, this advanced to fixation by commercially available spray fixative. Slides were stained manually with Papanicoloau [pap] stain, named for the Greek/American physician George Papanicoloau who was a pioneer of cytopathology and early cancer detection by cytologic methods. These glass slides were cover-slipped manually, screened by cytology scientists and rechecked by a second scientist for quality control. Abnormal smears were reviewed and reported by the cytopathologist, often at a double headed microscope with the cytology scientists in attendance for teaching purposes.

Cytology, like other laboratory disciplines, has been in a constant state of evolution over the past 100 years. There were rapid advances in microscopes, discoveries around dyes, staining, cell theories and publishing of colour atlases of cellular changes. An interesting story tells of the discovery of haematoxylin when two inebriated friends returning home from a public house stopped to urinate on a heartwood tree causing it to "bleed" and leading to the discovery of the natural plant pigment haematin used to manufacture haematoxylin, one of the most important dyes in tissue pathology.

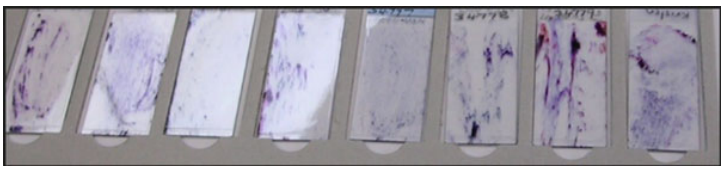

Figure 2: Conventional PAP stained cervical smears

From 1996 to 2012, the Wellington Hospital laboratory was processing non-gynaecological samples traditionally and manually using a very labour-intensive filter preparation technique. Sartorius filters were used through a filtration method to capture cells, using a cytosieve filter holder. The samples were either fresh or fixed with 30 % ethanol in saline. The prepared filters were stained using the pap stain and cover-slipped manually using Eukits and at a later date using EZ mount. The stained filters were screened by cytology scientists and preliminary handwritten reporting crafted on the request form, reviewed with the cytopathologist. The final reports were typed by secretarial staff, authorised by the cytopathologist on printed reports and then manual mailed. There was very little opportunity for database formation at the time and only crude manual card systems were maintained with very little opportunity for audit or complicated statistical analyses of trends. The samples were registered manually in a daybook which replaced by computer system in 2012.

RCPA QAP (Royal College of Pathologists of Australasia, Quality Assurance Programs were performed on provided glass slides until 2016, changed to USB stick and currently performed through virtual on-line images.

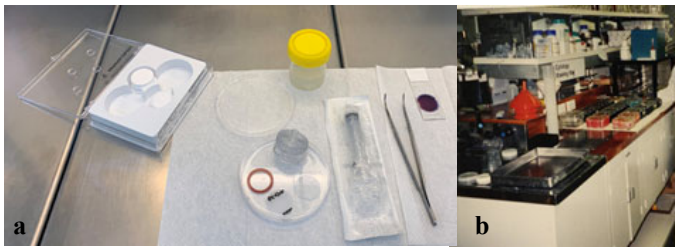

Figure 3 (a): Manual filter preparation of non gynae samples until 2012
Figure 3 (b): Manual PAP staining battery for gynae smears and filter preparations of non gynae samples

Cytospin preparations were done for low cellular samples, such as CSF specimens, using a Shandon cytocentrifuge (Shandon single disposable cyto funnel, sample chamber attached to metal chambers with white filter card).

The most profound development in cytology to occur at the end of the last century was the development of systems for liquid based cytology that allowed the mechanised preparation of evenly spread thin layered samples onto glass slides direct from sample vials. With advances in technology, FDA approved Liquid Based Cytology (LBC) techniques (ThinPrep and SurePath testing) were introduced to replace conventional cytology to improve adequacy, preservation, and to achieve a representative sample. ThinPrep (Hologic) was introduced in 1996 and SurePath (BD) in 1999.

These two LBC systems remain currently in use throughout the country for gynaecological and non-gynaecological sample processing. In 2012 Thinprep LBC was introduced at Wellington Hospital cytology lab. The samples were collected in cytolyt solution and slides were prepared using a TP 2000 machine. TP slides were stained on the automated Leica ST 5020 staining machine and covers lipped on the automated Leica CV 5030 cover slipper from 2013. This resulted in a significant reduction in specimen preparation time compared to the older manual filter methods previously employed.

Figure 4 (a): Hologic ThinPrep 2000 machine
Figure 4 (b): ThinPrep cytolyt vial for collection of non gynae samples, non gynae Transcyt blue filter and TP slide
Figure 4 (c): PAP stained ThinPrep slide

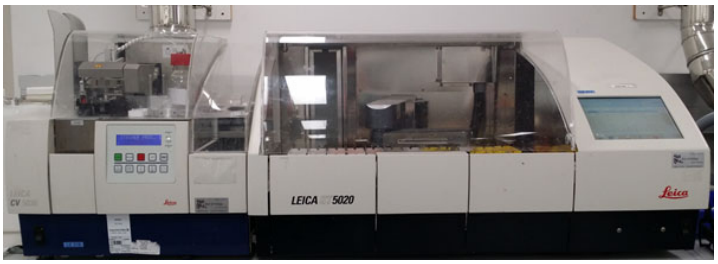

Figure 5: Automated stainer (Leica ST 5020) on right and automated coverslipper (Leica CV 5030) on left

During the first two decades of this century there has been enormous developments in laboratory information systems and CCDHB employed a number of these systems with increasing sophistication and functionality during this time. The database and search functions of these systems allowed for better analysis and quality control of results and greatly improved the scope and speed of histology-cytology correlation activities.

Following a DHB contractual tendering process for laboratory services, Wellington SCL was formed on the 1st of November 2015, which saw the provision of lab services across three DHB's (CCDHB. Hutt Valley DHB and Wairarapa DHB) and included processing of both community and hospital work. Aotea Pathology was incorporated into the new entity, based primarily on-site at Wellington Hospital. A decision was made that all gynaecological cytology would then be processed and reported at SCL Dunedin. Wellington SCL now operating across two floors of new purpose-build laboratory space in the CSB Building at Wellington Hospital. This new laboratory then processes and reports on all the regions non-gynaecological samples that are processed and stained using the BD PrepStain processor. The samples are collected in BD SurePath preservative fluid.

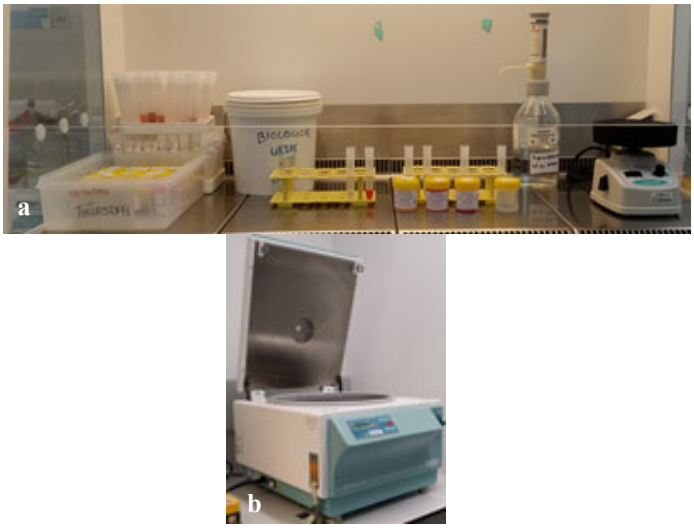

Figure 6 (a): SurePath preparation in Biosafety cabinet
Figure 6 (b): **C**entrifuge (Hettich)

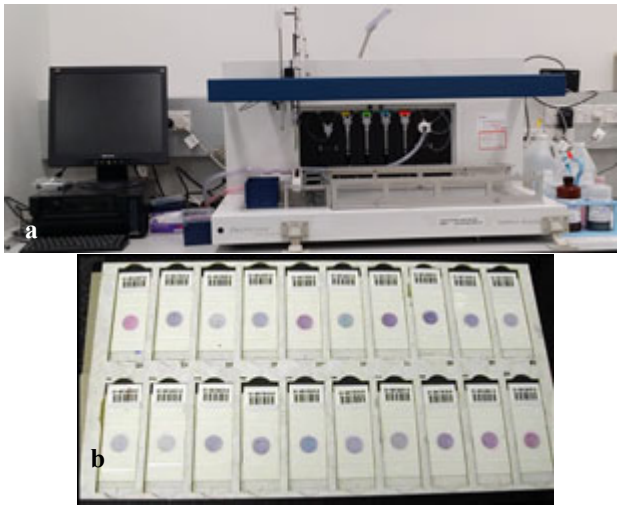

Figure 7 (a): BD SurePath processor
Figure 7 (b): PAP stained SurePath slides

In addition to the process and structural changes outlined above, the past 25 years have witnessed major changes in the demand, role, and scope of non-gynaecological cytology in this region. In 1996 one of the laboratories most common specimens were from fine needle aspiration of breast masses. However, the development of excellent thin core needle biopsy instruments combined with the development of a national breast screening programme have seen an 80% decline in the number of breast FNA samples submitted. Despite these changes in diagnostic fashion, demands for cytology services continue to grow annually. FNA has now become the triage tool of choice for assessment of thyroid nodules, and combined with the easy availability of portable ultrasound equipment, thyroid FNA's have numerically replaced breast FNA's over the past 25 years. In addition, increasing radiologist skills combined with better cross-sectional imaging and the development of endoscopic ultrasound have greatly increased the range and number of deep sites that are now routinely sampled by FNA.

At the end of the last century, the role of cytology was essential that of a screening or triage tool that would usually be followed up by tissue biopsy for histological confirmation in many instances. However, changes in medical practice over the past 25 years, combined with more sophisticated sampling techniques, and the exponential expansion of immunohistochemical antibodies have moved cytology to being more commonly the primary and only tissue diagnostic procedure performed, with treatment decisions made directly from cytology findings. These changes, in combination with the increasing use of molecular studies and flow cytometry, have meant that there are increasing testing demands being made on often limited cytology samples. Nowadays the cytology scientist and cytopathologist must not only provide diagnostic services but have become the curators of these limited samples and are involved in triage decisions about how these samples are best used.

The past 25 years have been exciting and challenging for the cytology service at Wellington Hospital. It has been sad to see the gynaecological cytology specimens be transferred out of region, but at the same time the scope and importance of the non-gynaecological cytology service has grown considerable and cytology is now longer the diagnostic Cinderella service of the past. It is our hope that the late Dr Gupta would be both surprised and pleased by the developments that have occurred in the nascent cytology department he helped to establish.

What of the next quarter-century? The future is much harder to predict than the past is to describe. We can make some reasoned guesses; there will be enormous changes in cervical cytology with the move to primary HPV testing that will likely see significant reductions in the laboratory screener workforce. Artificial intelligence technologies, which has been heavily applied to screening of cervical cytology specimens, will become more mainstream for all cytology specimens. We can be sure that the future will see much more molecular testing; the dire predictions from the early 2000's that molecular pathology would replace the need for routine morphologic assessment have not come to pass. However, the near future will see next generation sequencing on cytology samples rapidly translate from the research laboratory into routine diagnostic practice.

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BOOK REVIEW

The Great Secret
Author: Jennet Conaut, Grove Press UK.
ISBN 978 1 61185 644 6

By 1943 the Allied Forces had largely secured the Mediterranean war area and were advancing through Italy. The allied forces needed a suitable deep-water port to unload military supplies, tanks, vehicles, munitions etc, and Bari in southeastern Italy was an ideal location. The allies were so confident that the Germans posed no threat that they operated a 24 hour unloading of supply ships with no black-out for a fast turn-around. This was to be the strategic push for Rome. On December 2nd, 1943 the Luftwaffa bombed Bari sinking seventeen ships and killing over one thousand service men and civilians. Amongst the ships destroyed was the John Harvey containing a secret cargo. Soon after the bombings sailors began dying from conditions unrecognizable by the medical teams and a chemical weapons expert Lt Colonel Stewart Alexandra was sent to investigate the deaths. He quickly identified that the fatalities and disabilities were caused by mustard gas, which was denied by the military as well as the politicians, and all information immediately became classified. This also raised the issue of whether the Germans had used chemical weapons in the air raid. Alexandra subsequently determined the John Harvey was carrying mustard gas shells in case the retreating Germans resorted to chemical warfare during their retreat. His "Bari Report" was classified however, another clinician Colonel Cornelius Rhodes who was also a research scientist had access to classified material and identified that the toxic effects of mustard gas significantly depressed white blood cells. Although mustard gas had been identified in the1930s as a potential agent for 'curing cancer' its toxicity proved to be an ongoing issue and some small scale use with cancer patients at Yale University on a trial and error basis were hit and miss.

Rhodes connected the Bari reports and the Yale reports and proposed a centre for cancer research. Two industrialists, Alfred Sloan and Charles Kettering under-wrote the new cancer research institute – Sloan-Kettering Institute for Cancer research where the research continued using nitrogen mustards for cancer treatment. The increasing understanding of how nitrogen mustards inhibited cancer and in particular leukemia led to the new development of chemotherapeutic agents such as the folic acid antagonists.

The author writes clearly on the early history of cancer treatments initiated in an ad-hoc manner. The politics of war and secrecy are intermingled with the politics of medicine and research, two hotly contested battlefields. The remarkable well-referenced story of how a wartime disaster evolved into cancer chemotherapy makes this book an excellent read.

New Executive Officer for NZIMLS

Introducing Sharon Tozer

Many of you will already know me, and have spoken with me either on the phone, at an event or via email. I have been with NZIMLS since 2006, when I interviewed for the position of Database Administrator. At the time, another position was also advertised for a part-time accounts clerk. After interviewing with the (then) President, Robin Allen, Vice-president Kevin Taylor and Treasurer/Secretary Ross Hewett, it became apparent that I was also suitable for this position. I was offered, and accepted both positions, and the role of Membership/Finance Administrator was born. This role has developed into much more over the years, and I have evolved with the position, attending many courses and events to grow my skills to the advantage of the NZIMLS and it's members.

In 2009, I undertook part-time extramural studies at Massey University, studying towards a Diploma in Business Studies, endorsed in Accounting. A mother of 2 school-age children at the time, I found it at times challenging to study and be available for the children's sports, music and after school commitments, as well as working full time, however was very much supported by my husband, and completed my Diploma, graduating in November 2011.

In 2012, I took the entry examination to the New Zealand Institute of Chartered Accountants (now Chartered Accountants Australia New Zealand [CAANZ]) and gained entry as a registered Accounting Technician. In order to maintain this registration, I am required to complete CPD annually, and hold membership of CAANZ.

I also hold a Diploma in Bespoke Tailoring, from the Design and Arts College of New Zealand, and as an avid seamstress I am often found in my sewing room creating outfits for my other passion - rock and roll music and dance. I love the 1950s era, and along with my husband attend a Rock and Roll dance club and we regularly participate in car shows and events with either our classic 1965 Ford, or 1923 T-Bucket. I dabble a bit in 'Pin-up" and enter best dressed and Pin-up competitions. In 2020, during the "COVID Lockdown" I entered and won an international virtual pageant, "Miss Self-Isolation"! These pageants are fun to do, and really take me out of my comfort zone! They do give me a chance to show off my creations though! Riding is another passion, and whilst I don't get much of a chance to ride my horses now, I do enjoy them and they are very much family pets.

After the announcement of the retirement of Fran van Til, I interviewed for, and was offered the position of Executive Officer. I have very much hit the ground running this year, and whilst I have passed the membership part of my duties over to Jillian Broadbent, I still endeavour to maintain as much contact with our members as possible. I would like to take this opportunity to personally welcome all our new members, (over 600 new technicians so far this year). 2021 is going to be an exciting year for me, and I hope for our members too. So much is 'unknown' and unfolding daily, and we will all be met with challenges as we go through our day-to-day lives. In the meantime, if I can be of any assistance with NZIMLS related matters, you will find me at the end of the phone!

I look forward to being of service to you all in the coming years.

NZIMLS Journal Prize

The NZIMLS Council has approved an annual Journal prize to the value of NZ$300 for the best peer-reviewed article published by NZIMLS members in the Journal during the calendar year. The article can be a review article, original article, case study, research letter or technical communication. Excluded are Fellowship dissertations.

Many studies are presented at the Annual Scientific Meeting, SIG meetings, and the North and South Island Seminars, yet are rarely submitted to the Journal for wider dissemination to the profession. Consider submitting your presentation to the Journal. If accepted, you are in consideration for the NZIMLS Journal Prize and will also earn you valuable CPD points.

Please contact the Editor or any Editorial Board Member for advice and help. Contact details are on the NZIMLS web site (www.nzimls.org.nz) as are instructions to authors.

All articles published during the calendar year (April, August and November issues) will be considered. The Editor, Deputy Editor and the President of the NZIMLS, who themselves are ineligible, will judge all eligible articles in December. Their decision will be final and no correspondence will be entered into.

The winner of the 2020 Journal Prize is Andrew Wilson from the Wellington Regional Genetics Laboratory for his article "Validation of a pan-cancer targeted next generation sequencing panel in New Zealand" (N Z J Med Lab Sci 2020; 74(3): 210-214).

Publications by NZIMLS members

This column is to showcase recent international peer-reviewed publications by NZIMLS members. If you have had such a recent publication, please provide full details to the Editor at rob.siebers@otago.ac.nz

Legge M, Fitgerald R. Does the Human Assisted Reproductive Technology Act 2004 need a review? *Policy Quarterly* 2021; 17: 79-83.

Basu I, Nagappan R, Fox-Lewis S, Muttaiyah S, McAuliffe G. Evaluation of extraction and amplification assays for the detection of SARS-CoV-2 at Auckland Hospital laboratory during the COVID-19 outbreak in New Zealand. *Journal of Virological Methods* 2021; 289: 114042.

Moore JA, Pullon BM, Wang D, Brennan SO. Hb Waikato [α127 (H10)Lys→Gln; *HBA1*: c.382A>C]: A Novel High Oxygen Affinity Variant. *Hemoglobin* 2021;doi: 10.1080/03630269.2021.1873801. Online ahead of print.

Science Digest

Contributed by Michael Legge

Gestational diabetes and HbA1c

A common complication during pregnancy is the manifestation of gestational diabetes, which is often associated with both maternal and fetal complications. In addition, it may also be associated with an increased risk for the development of type 2 diabetes later in life. Previous research has indicated that fetal complications may develop prior to the onset and diagnosis of gestational diabetes.

A collaboration between research groups in the USA has published results from case-controlled women from 8-13 weeks' gestation to 34-37 weeks' gestation for HbA1c analysis, which included those who developed gestational diabetes as well as other clinical conditions (1). Those women with a pre-existing elevated HbA1c were excluded from the research. The researchers found that those women who subsequently developed gestational diabetes had significantly elevated HbA1c at 8-13 weeks' gestation compared to those who did not develop gestational diabetes. The initial elevation was consistent through-out pregnancy.

Their conclusion was that women who subsequently develop gestational diabetes later in pregnancy already have impaired glucose homeostasis either prior to, or during early pregnancy and that HbA1c screening in the first trimester may identify at risk pregnancies prior to gestational diabetes onset.

Animal origins of rubella?

Rubella was first described in 1814 and was associated with human fetal congenital defects, miscarriages and stillbirths from the 1940s to 1960s. At present the rubella virus is the only recognized member of the riboviral family *Matonavirdae* (genus *Rubivirus*). Despite effective immunization programs it is estimated that at least 100,000 cases of congenital rubella syndrome occur each year and may persist as a sub-clinical infection in the eye.

Recent research from the USA has identified two relatives of the rubella virus; ruhugu and rustella viruses in common animal species that share identical genomic architecture with the rubella virus (2). The ruhugu virus which is the closest relative of the rubella virus is found in healthy leaf-nosed bats in Uganda and rustella virus, which is an outgroup including rubella and ruhuga viruses, is found in placental mammals and marsupials at a German zoo plus in wild yellow-necked field mice near the zoo. Amino acid sequences of the fusion protein (E1) from all three viruses and two putative T-cell epitopes of the capsid protein of rubella and ruhuga viruses were all moderately to highly conserved. Modelling of E1 homotrimers in the post-fusion state has predicted that both the rubella and ruhuga viruses have a similar host membrane fusion protein capacity. The authors concluded that members of the *Matonaviridae* family have the potential for future zoonotic transmission to cross significant barriers and that rubella had a zoonotic origin.

Relationship of Von Willibrand Factor with bacterial pathogenesis

Von Willibrand Factor (VWF) is an essential factor for normal homeostasis and functions as a mechano-sensitive protein, which is dependent on shear-stress for normal function. VWF is most frequently associated with bleeding disorders, however it is also linked to higher risks of cardiovascular disease. In a recent review of VWF and bacterial pathogenesis the authors describe a mechanism for the pathogenesis of VWF and the inflammatory response (3). Pathogenic bacteria such as *Staphylococcus aureus* and *Streptococcus pneumoniae* can bind circulating VWF and subsequently bind the complex to endothelial cells, which subsequently interferes with normal endothelial cell function as well as platelet recruitment and normal blood coagulation, thereby promoting thrombus formation and occlusion of micro-capillaries. The authors conclude that haemodynamic flow conditions are critical to the bacterial-VWF complex formation but more precise data is still required.

Blood group A1 and acute respiratory distress syndrome

The ABO blood group system is defined by differing carbohydrate structures on red blood cells but the respective complex carbohydrates that define the ABO blood groups are also located on other body cells. In addition to defining ABO blood groups the ABO blood group system has been associated with the susceptibility to certain diseases.

Recent research has extended the association of blood group A1 with the severity of acute respiratory distress syndrome (ARDS) in adults (4). The authors using multiple (750,000) SNPs in the ABO gene, genotyped 3710 individuals divided into 3 cohorts of critically ill trauma and sepsis patients to determine the association of the A1 genotype with ARDS risk. In addition, the authors determined whether an association existed with *FUT2* – defined non-secretors. These lack the ABO antigens on the epithelium but not on the endothelium. In addition, the authors analysed plasma concentrations of endothelium derived glycoproteins.

The overall outcome from this research was that the A1 genotype was associated with a moderate to severe risk of ARDS (relative to blood group) in all three cohorts. The relationship of A1 was strongest with sepsis in non-pulmonary infections. This association persisted in non-secretors tending to indicate that a vascular based mechanism underlies the pathology. Additionally, the authors identified that the A1 genotype was associated with a higher risk of DIC.

Ethics in clinical autopsy

This is a relatively short publication where the authors provide a review of the rationale for an autopsy and a historical perspective relating to the use and progression of the autopsy as well as religious aspects relating to permission and philosophical concepts (5).

The role of relatives of the deceased in various countries is considered linked with consent or the necessity for consent. The use of autopsies in education is considered and interestingly the Australian "Autopsy Practice" document is provided as an example of important ethical aspects relating to the use of the clinical autopsy. Despite the relative brief nature of the review, it is well written, providing a good general overview and is well referenced.

Bilirubin in clinical practice

Bilirubin is a frequently requested analyte in the clinical biochemistry and is an important diagnostic aid from before birth (e.g., fetal blood group incompatibility) through to geriatric management (e.g., liver disease), as well as identifying certain inherited disorders (e.g., Gilbert's syndrome) and as a marker for a range of haemolytic disorders. But how well is this molecule understood?

A published review of bilirubin focused on its use in clinical practice and provides a succinct overview of bilirubin synthesis and links the synthesis to the clinical conditions where is has the most value (6). The review is relatively short, but well written and is well referenced.

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3. Steinert M, Ramming I, Bergmann S. Impact of Von Willibrand Factor on bacterial pathogenesis. *Front Med (Lausanne)* 2020; 7: 543.
4. Reilly JP, Meyer NJ, Shasaty MGs, et al. The ABO histo-blood group endothelial activation, and acute respiratory distress syndrome risk in critical illness. *J Clin Invest* 2021; 131: e139700.
5. Rugge M, Sacchi D, Cesaro S, et al. *J Clin Pathol* 2020; doi: 10.1136/jclinpath-2020-206793. (Online ahead of print).
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Advertisement from Volume 8, No 1, April 1953

THE Pacific WAY

A very warm welcome to you all and a very happy new year from us at the PPTC

COVID 19 continues to threaten the Pacific

The global pandemic has had a serious impact on PPTC operations, causing a sizable disruption to our overseas in-country training programmes, and to the Wellington Centre based courses, due to international border closures. We are however fortunate that both the PPTC's External Quality Assessment Programme and the Diploma programme have continued relatively uninterrupted. With the virus spreading globally all efforts are being made to ensure that countries are prepared and ready to respond, in situations where COVID infection has been detected. In the Pacific, countries are increasing their efforts to ensure that their health services have reached an acceptable level of preparedness to rapidly detect and respond to the threats of COVID-19.

The PPTC is continuously assisting in Pacific preparedness against COVID 19 and is currently working with WHO (World Health Organization), NZ Ministry of Foreign Affairs and Trade, Pacific Ministries of Health, the Pacific Community (SPC), the Australian Dept of Foreign Affairs and Trade (DFAT) and the Doherty Institute (Melbourne) to establish COVID diagnostic and treatment facilities in Pacific Countries, the majority of which would face the devastating effects of COVID in the event of community spread.

Selected PPTC activities (2020-2021) currently delivered to the Pacific can be listed as follows:

Tokelau: In 2020, the New Zealand Ministry of Foreign Affairs and Trade (MFAT) engaged the Pacific Pathology Training Centre (PPTC) to implement the procurement and refit of a shipping container that would accommodate a GeneXpert with associated training and support. As part of this activity, the PPTC has been required to work with the container supplier to ensure that the mini testing laboratory is fit for purpose, with remodelling based on detailed specifications provided by WHO and SPC. The PPTC has overseen the procurement of a biosafety cabinet and its installation into the container laboratory. Furthermore, the PPTC has procured additional laboratory equipment for basic diagnostic testing (as requested by Tokelau) and has overseen the supply and installation of this equipment into the container as well as its transport to Tokelau.

Kiribati: The Kiribati Medical Services is awaiting the refurbishment of their existing hospital facility to house a molecular testing platform (RT-PCR) to test and diagnose SARS-CoV-2 virus. This refurbishment has been delayed and will take approximately a year to complete. In order to ensure that testing can commence as soon as possible, it has been proposed that a container unit is refurbished as a mobile PCR laboratory while the hospital facilities are upgraded. The Pacific Community (SPC) and the Government of Kiribati has engaged the PPTC to oversee refurbishment of shipping container units, to be used as a mobile PCR laboratory. As part of this project, the PPTC is currently working with the container supplier to ensure that the testing laboratory is fit for purpose, with remodelling based on detailed specifications discussed with the Kiribati Health team, SPC and the Doherty Institute. The PPTC will oversee the shipping and transportation of the container units locally within New Zealand and internationally, to Tarawa, Kiribati. The container units are due to depart New Zealand on the 12th of March and is scheduled to arrive into Tarawa on the 27th of March. The laboratory units will then be setup by local trade staff with online support from the PPTC, making it ready for the mass testing of SARS-Cov-2 on RT-PCR platforms.

Niue Healthcare is provided by Foou Hospital, Alofi which has 20 beds, outpatient clinics, pharmacy, X-ray, ultrasound, and medical laboratory. Non-communicable diseases, such as diabetes, high blood pressure/hypertension, gout, asthma, and cancer are a major health care concern in Niue. Although there are currently no cases of COVID-19 in Niue the country needs to be prepared for its introduction as movement of people recommences with the likelihood of transmission. These preparations will include a testing strategy and capacity appropriate for the population of Niue and its visitors.

The COVID-19 pandemic has highlighted the need for improved biosafety within Niue's medical laboratory services. However, it has also provided an opportunity to build general capacity and sustainability. WHO has contracted the PPTC to design and arrange the construction of a mobile/container laboratory, with the inclusion and implementation of appropriate validated/verified equipment that will deliver the range of necessary tests. The PPTC will also provide the relevant training to staff as well as reagents and kits delivered on a regular basis to support this medical laboratory service for Niue's Foou hospital.

Progress is ongoing, with almost all laboratory equipment and consumables being delivered to the PPTC ready to be installed into the laboratory units. The units are being refurbished in Nelson and are expected to be in Wellington for the fit-out by early March. These are expected to depart Wellington by mid-March, arriving in Niue early April, after which the PPTC staff will provide on-line zoom training sessions to Niue Health staff to set-up the laboratory and the installed laboratory equipment.

Fiji Centre for Disease Control and Prevention (CDC): The PPTC has been commissioned through SPC supported by DFAT to conduct a virtual external Laboratory Quality Management Audit of the CDC based in Suva Fiji. An audit will be conducted by the PPTC Quality Manager/Consultant Mr Russell Cole in February using the SLIPTA audit tool modified by the PPTC to include all the elements of the ISO15189 standards.

EQAP COVID19: The SARS-CoV-2 pandemic has required the rapid introduction of large-scale molecular detection methods in diagnostic laboratories across New Zealand. diagnostic laboratories have had to meet unprecedented demand for testing to support the NZ COVID-19 Public Health response. However, limited reagent supply chains have led to a number of challenges, including the rapid introduction of kits and methods to ensure ongoing laboratory testing capacity. New Zealand is now well on the way towards "elimination" of COVID-19 with very low numbers of detected cases each day. Due to the 2019 Coronavirus outbreak, the PPTC has been asked to establish an EQA programme for the SARS-CoV2 disease for the Pacific region. The purpose of the SARS-CoV-2 EQA programme is to provide additional quality assurance for laboratories in the Pacific region, given that there are no positive results being detected.

With the assistance of ESR, who are supplying the programme materials and their technical expertise, a COVID-19 EQA panel has now been incorporated into the existing PPTC EQAP, which provides for other medical laboratory science disciplines, supported by the New Zealand Ministry of Foreign Affairs and Trade. Both WHO and MFAT contributed to the funding of the newly developed COVID-19 panel and its delivery. The PPTC dispatched two cycles of the COVID-19 panel to 23 Pacific Laboratories in 2020, and two additional cycles have been scheduled for 2021. It is hoped that further funding will be available to continue this programme beyond 2021.

Under the COVID-19 support provided to the Pacific Region by the New Zealand Government, the PPTC is supported by the Ministry of Foreign Affairs and Trade to provide ongoing laboratory technical advisory services to the Pacific Ministries of Health and to its regional stakeholders, including MFAT.

Can you help?

If any New Zealand medical laboratories have items of diagnostic instrumentation that have been recently upgraded or continue to be stored in the laboratory, but are actually surplus to requirements, the PPTC would be most grateful if such items could be donated through its Centre to Pacific Island laboratories where there is an exceptional need. Pacific laboratories have very restricted budgets and often cannot afford to replace troublesome instrumentation that continues to breakdown and which is often discontinued because it is so outdated.

The PPTC would also welcome teaching resources in terms of wall charts, Haematology case studies (stained blood films), projector slides, textbooks and journals (within 10 yrs of publication) etc , for teaching purposes in the Pacific, if you no longer have a use for them. Any contribution is so valuable to us.

Please contact:
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2021 NZIMLS CALENDAR
Dates may be subject to change

DATE	***COUNCIL***	***CONTACT***
May 2021	Council Meeting, Hamilton	sharon@nzimls.org.nz
DATE	***SEMINARS***	***CONTACT***
21—23 May	NICE Weekend, Wairakei Resort Taupo	raewyn.cameron@pathlab.co.nz
NZIMLS ANNUAL SCIENTIFIC MEETING AND ANNUAL GENERAL MEETING		
August 2021	At the time of printing, date and venue yet to be confirmed. Information will be emailed to members and on our Website once confirmed.	tbathgate@adhb.govt.nz sharon@nzimls.org.nz
DATE	***MEMBERSHIP INFORMATION***	***CONTACT***
January	Membership and CPD enrolment due for renewal	sharon@nzimls.org.nz
31 January	CPD points for 2020 to be entered before 31 January	cpd@nzimls.org.nz
15 February	Material for the April issue of the Journal must be with the Editor	rob.siebers@otago.ac.nz
15 June	Material for the August Journal must be with the Editor	rob.siebers@otago.ac.nz
18 June	Nomination forms for election of Officers and Remits to be with the Membership (60 days prior to AGM)	sharon@nzimls.org.nz
8 July	Nominations close for election of officers (40 days prior to AGM)	sharon@nzimls.org.nz
26 July	Ballot papers to be with the membership (21 days prior to AGM)	sharon@nzimls.org.nz
01 August	Annual Reports and Balance Sheet to be with the membership (14 days prior to AGM)	sharon@nzimls.org.nz
09 August	Ballot papers and proxies to be with the Executive Officer (7 days prior to AGM)	sharon@nzimls.org.nz
15 September	Material for the November Journal must be with the Editor	rob.siebers@otago.ac.nz
DATE	***NZIMLS QMLT EXAMINATIONS***	***CONTACT***
06 November	QMLT Examinations	membership@nzimls.org.nz

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BRIEF COMMUNICATION

CASE STUDIES

EDUCATIONAL ARTICLE

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SPECIAL ARTICLE

LETTER TO THE EDITOR

AUTHOR INDEX

Advertisement from Volume 29, No 2, July 1975

Journal Questionnaire

Due to reduced opportunities for obtaining CPD points because of the COVID-19 situation there are two journal questionnaires for the April 2021 issue. You can either do one (A) or the other (B) questionnaire, or both (A & B). There are 5 CPD points per questionnaire, thus if you do both and get at least 8 out of 10 questions right for each questionnaire then you get 10 CPD points. Otherwise, 5 CPD points for either questionnaire A or B.

Read the articles carefully as most questions require more than one answer. Answers are to be submitted through the NZIMLS website. Make sure you supply your correct email address and membership number. It is recommended that you write your answers in a word document and then cut and paste your answers on the web site.

You are reminded that to claim valid CPD points for successfully completing the journal questionnaire you must submit an individual entry. It must not be part of a consultative or group process. In addition, members who have successfully completed the journal questionnaire cannot then claim additional CPD points for reading the articles from which the questions were derived.

The site will remain open until Friday 16th July 2021. You must get a minimum of eight questions right per questionnaire to obtain five CPD points. The Editor sets the questions but the CPD Co-Ordinator, Jillian Broadbent, marks the answers. Direct any queries to her at cpd@nzimls.org.nz.

QUESTIONNAIRE A

1. The majority of Hb J cases have been identified by which techniques and with definitive differentiation by which analysis?

2. Which elements are among the notable causes of nutritional anaemia?

3. Infant micronutrient deficiencies include which increased risks?

4. Familial Mediterranean fever and inflammatory bowel disease are both characterized by what?

5. The chemokine MCP-1 is activated by what?

6. Endothelial adhesion of leukocytes is primarily regulated by what?

7. What are the most common factors influencing the microbiome of mucosal surfaces?

8. Damage to the mucus membranes and salivary glands allow for *Candida* colonization and can be agents of what?

9. In the *Candida* species on human mucosal surfaces, *Candida* was most prevalent in which two studied groups, and was mainly due to what?

10. What does the lyophilization process entail?

Answers for the November 2020 questionnaires are on page 76

QUESTIONNAIRE B

1. How can inappropriate laboratory testing be categorised?

2. Over utilisation of laboratory testing generally results from what?

3. What were the top five most common requested laboratory tests in the three hospital emergency departments study?

4. The most common requested laboratory tests were related to patients with which disorders?

5. What is widely regarded as the first choice diagnostic assay in the setting of suspected systemic autoimmune- based rheumatic disease and describe the main principles of this test?

6. What principally are the technical factors impacting on the output of the ANA test?

7. *Candida spp.* can asymptomatically colonise which anatomical sites in healthy individuals?

8. Asymptomatic colonization of *Candida spp.* may cause which superficial and systemic infections under certain conditions?

9. Which predisposing factors might increase establishment of vulvovaginitis?

10. Untreated vaginal colonization of *Candida spp.* during pregnancy is associated with which complications?

November 2020 Journal Questionnaires Answers

QUESTIONNAIRE (A)

1. Which mechanism has been suggested for lymphopenia in COVID-19 patients?
   Ability of the SARS-COV-2 virus to directly attack T-lymphocytes.

2. Which laboratory parameters have been suggested as markers for potential progression to critical illness in COVID-19 patients?
   Combination of platelet count, lymphocyte count, and IL-6.

3. A 100% increase was seen in which cardiac parameters in COVID-19 patients?
   LDH, myoglobin, CK-MB, and NT-BNP.

4. An increase in CK-MB in COVID-19 patients is evidence that suggests what heart pathology?
   Viral infiltration of cardiac tissue, or cardiac ischemia.

5. SARS-COV-2 is known to have an affinity for which receptor, where is this receptor located, and is a significant increase of what in in patients infected with COVID-19?
   ACE-2 receptor. Present in vascular epithelium. Increased risk of death in patients with cardiovascular disease.

6. What abnormalities in coagulation profiles are associated with COVID-19 severity?
   Increased D-dimer, increased prothrombin time, decrease in fibrinogen.

7. Studies have used which laboratory parameters to predict severity and/or mortality from COVID-19?
   Neutrophil to lymphocyte ratio, platelet to lymphocyte ratio, international normalized ratio, lymphocyte to monocyte ratio, and oxygenation index.

8. COVID-19 patients with which blood gases parameters have been classified as being severe/critical requiring intensive care management and mechanical ventilation?
   Oxygen saturation (resting stage) ≤ 93% or PaO_2/FiO_2 ≤ 300mm Hg.

9. In addition to the primary injury in spinal cord injury there is a strong association with which diseases, and what does this result in?
   Heart disease and Type 2 diabetes. Progressive loss of muscle mass and increased adiposity below the lesion.

10. Transition from lean tissue muscle mass to adipose tissue is associated with, and leads to what?
    Associated with progressive denervation leading to a decrease in the capillary circulation and progressive loss of mitochondria.

QUESTIONNAIRE (B)

1. Which laboratory parameters have been employed to further improve the clinical diagnosis of acute appendicitis?
   White blood cell count, C-reactive protein, IL-6, and procalcitonin.

2. Which imaging studies have been used for a more accurate diagnosis of acute appendicitis?**Ultrasonography, computerized tomography, and magnetic resonance imaging.**

3. Metabolic syndrome is a combination of which cardio-metabolic risk factors?
   Dyslipidaemia, hypertension, impaired glucose metabolism, insulin resistance, and obesity.

4. Neck circumference has been shown to correlate with which parameters?
   Age, weight, waist circumference, and waist-to-height ratio.

5. Which anthropometric indices are markers of central obesity?
   Waist circumference, waist-to-hip ratio, and waist-to-height ratio.

6. In New Zealand the majority of Legionella pneumonia infections are caused by which organism, what is the gold standard for diagnosis of Legionella infections, but which test is more sensitive?
   ***L. longbeachae*. Culture. PCR on respiratory specimens.**

7. Second generation sequencing techniques employ which techniques?
   Enzymatic or mechanical shearing of DNA in short fragments termed short reads, which are then sequenced in a massively parallel setting. These DNA fragments are then clonally amplified.

8. Which germline mutations are predominantly correlated with ductal carcinoma, and which mutation with both lobular and ductal carcinomas?
   BRCA1 and TP53. BRCA2.

9. In cold autoimmune haemolitic anaemia, cold agglutinins can cause clinical symptoms related to what?
   Agglutination of red blood cells in the cooler parts of the body and haemolysis in the warmer parts.

10. The term ‘angioimmunoblastic’ is derived from which words that refer to what?
    “Angio” referring to blood vessels and “immunoblast” to activated lymphocytes.

TH Pullar Memorial Address 1996-2020

Each year the NZIMLS Council invites a person who has made a significant contribution towards medical laboratory science in New Zealand to deliver the TH Pullar Memorial Address at the Annual Scientific Meeting (ASM). This prestigious address is in honour of Dr TH Pullar who passed away on the 29th August 1966. Thomas H Pullar was a friend, teacher and lifelong champion of New Zealand medical laboratory scientists and technicians. This memorial address is given at the opening ceremony of the NZIMLS ASM. Below is a list of the awardees since 1996 and the subsequent publication of their topic in the Journal.

Year	Awardee	Journal article
1996	Marilyn Eales	Eales MM. Going for gold. *N Z J Med Lab Sci* 1997; 51(1): 32-35.
1997	Mike Lynch	Lynch M. Training for paradise. *N Z J Med Lab Sci* 1998; 52(1): 3-5.
1998	Ross Hewett	Hewett R. Trends, technologies and medical laboratory scientist. *N Z J Med Lab Sci* 1999; 53(2): 39-45.
1999	John Aitken	Aitken J. Deconstructing the laboratory. *N Z J Med Lab Sci* 2000; 54(1): 3-6.
2000	Paul McLeod	McLeod P. Controlling change. *N Z J Med Lab Sci* 2000; 54(3): 94-97.
2001	Ross Anderson	Anderson R. What makes athletes super? Medical laboratory science has many answers. *N Z J Med Lab Sci* 2002; 56(1): 2-5.
2002	Shirley Gainsford	Gainsford SA. Symphony of science – 1st movement – regulation. *N Z J Med Lab Sci* 2002; 56(3): 82-84.
2003*		
2004	Rob Siebers	Siebers R. Outside, looking in. *N Z J Med Lab Sci* 2004; 58(1): 71-73.
2005	Christine Hickton	Hickton CM. From the back of beyond. *N Z J Med Lab Sci* 2005; 59(3): 50-53.
2006	Robin Allen	Allen R. Echoes from the past, implications for the future. *N Z J Med Lab Sci* 2006; 60(3): 87-91.
2007	Dennis Reilly	Reilly D. Stairway to science. *N Z J Med Lab Sci* 2007; 61(3): 84-85.
2008	Kevin Taylor	Taylor K. Finding Rumplestiltskin by crossing the Southern Alps. *N Z J Med Lab Sci* 2008; 62(3): 54-55.
2009	Chris Kendrick	Kendrick C. Back to the future – are we headed there? *N Z J Med Lab Sci* 2009; 63(3): 72-74.
2010	Christine Pry	Pry C. Believe in yourself, and anything is possible. *N Z J Med Lab Sci* 2010; 64(3): 67-68.
2011*		
2012	Dr Robin Fraser	Fraser R. Pathology: The study of structure and function in health and diseases. *N Z J Med Lab Sci* 2012; 66(3): 67-69.
2013	Don Mikkelsen	Mikkelsen D. Laboratory errors and accountability. *N Z J Med Lab Sci* 2013; 67(3): 114-115.
2014	Dr Alex Dempster	Dempster A. Looking forward with hindsight. *N Z J Med Lab Sci* 2014; 68(3): 94-97.
2015	Holly Perry	Perry H. The value of people: taking charge of our destiny. *N Z J Med Lab Sci* 2015; 69 (3): 78-80.
2016	Russell Cole	Cole R. The empowering impact of quality systems. *N Z J Med Lab Sci* 2016; 70(3): 89-90.
2017	Jillian Broadbent	Broadbent J. Bringing home the America's Cup. *N Z J Med Lab Sci* 2017; 71(3): 80-82.
2018	Ken Beechey	Beechey K. Show your true colours: values in the workplace. *N Z J Med Lab Sci* 2018; 72 (2): 85-88.
2019*		
2020**		

*No ASM in New Zealand. **No ASM due to Covid-19 pandemic.

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