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# New Zealand Journal of Medical Laboratory Science


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New Zealand Journal of

# Medical Laboratory Science

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# In this issue

## *Rob Siebers, Editor*

Every year Council of the NZIMLS invites a person who has made a significant contribution to laboratory medicine to deliver the prestigious TH Pullar Memorial Address at the Annual Scientific Meeting. This year's address was given by Jillian Broadbent who is well known to the profession in her role as CPD Co-Ordinator. In her talk Jillian presented some analogies between the America's Cup and how we work in medical laboratories nowadays.

Fellowship of the NZIMLS is the profession's highest professional qualification. Dennis Mok recently obtained his Fellowship and in this issue presents his Treatise on the extent of conformance requirement coverage provided by ISO 15189:2012 guidance checklists produced by accreditation bodies. The contributing objectives include the identification of conformance requirements in ISO 15189:2012 for the development of evaluation checklists and determination of the level of conformance requirement coverage by quantitative analysis.

Reference ranges which delineate as male or female are not currently useful for transgender individuals undergoing sex-hormone therapy, and may be misleading to clinicians. In this issue Melanie Adriaansen and colleagues bring this to the attention of medical laboratory scientists practicing in New Zealand, to raise awareness of prejudice and to shed light on current misunderstandings.

The use of maternal blood markers and early ultrasound measurements to screen for fetal neural tube defects and fetal aneuploidy (primarily trisomy 21, Down syndrome) is now a well-established practice in many countries. However, the origins and background relating to how the techniques became established and how the various action limits were derived is not universally known or understood. In this issue Michael Legge and Ruth Fitzgerald consider the historical origins of the maternal blood screening tests, the establishment of the analytical parameters currently in use, the development of the nuchal translucency test and the integration of multiple analytical parameters to facilitate the diagnosis of fetal abnormalities.

DFS70 antibody gives an ANA Immunofluorescent homogenous/speckled staining pattern frequently at high titre that is often seen in AARD patients. In this issue, Paul Austin and colleagues identified if DFS70 antibody was present in our test population (b) determined if DFS70 patterns could be identified with first-round Immunofluorescent ANA testing (c) established the relationships between Immunofluorescent ANA and chemiluminescence and line immunoassay methodologies for identification of DFS70 antibody and (d) propose a testing algorithm for implementation at LabPLUS that would allow the identification of DFS70 antibody.

The reticulocyte haemoglobin equivalent (RET_He) parameter provides the haemoglobin concentration of peripheral blood reticulocytes on Sysmex Haematology analysers. In this issue Charlotte Poffenroth and colleagues established a reference range for the RET_He and assessed its performance as a laboratory screening test for patient iron deficiency. They showed the potential for its use as a simple laboratory screening test for iron deficiency.

The complement dependent cytotoxicity (CDC) assay has been a mainstay of pre-transplant testing for anti -HLA antibodies for many years. In this issue Mary Chacko and colleagues quantified and compared positivity obtained on CDC using routine incubation, extended incubation and anti-human globulin. They found that anti-human globulin and extended incubation enhance the positivity of CDC by approximately 7%. However, neither anti-human globulin CDC nor extended incubation CDC showed any significant increase in positivity over each other.

Early reaction errors (ERE) are encountered on patient plasma samples using the photo-optical clot detection method used on the Sysmex CS2100i analyser. ERE are abnormal reactions that occur on some samples at the initial stages of the APTT coagulation reaction. This finding leads to additional sample preparation steps to resolve the issue or may lead to a sample recollect. To resolve this problem Mitchell Hill and colleagues assessed the STart Max semi-automated analyser (Stago) for use as it uses mechanical clot detection. The STart Max analyser was able to generate reportable APTT results on samples rejected for ERE using the Sysmex CS2100i analyser and was considered to be cost effective as an alternative method.

It is critically important for both patient management and infection control purposes that carbapenemase-producing organisms (CPO) are rapidly and reliably detected and identified in clinical laboratories. However, this can be problematic due to the diversity of carbapenemase enzymes, the different genera they can reside in, and the difficulties of discriminating CPO from carbapenem-resistant-non-carbapenemase producers. In this issue Julie Creighton and Clare Tibbs evaluated and compared the recently released MAST indirect carbapenemase test (ICT) and a modified carbapenem inactivation method (mCIM) test to determine their ability to detect carbapenemase production, and to reliably 'rule out' a non-carbapenemase producer. They found a high sensitivity and specificity for both assays across a range of Gram-negative bacteria. To reliably distinguish CPO from carbapenem-resistant-non-CPO, they recommend that the mCIM is used in tandem with the MAST ICT, or with another high performing assay, such as Carba NP, rather than as stand-alone tests.

*TH PULLAR ADDRESS*

# Bringing home the America's Cup


## *Jillian Broadbent, FNZIMLS*

***Canterbury Health Laboratories, NZIMLS CPD Coordinator***


## *THANKS*

Mr President, distinguished guests, fellow scientists, technicians, colleagues and friends, I feel enormously privileged to be standing in front of you today. I am immensely proud but also humbled to have been invited to deliver the TH Pullar address for the 2017 Annual Scientific Meeting.

## *HISTORY OF TH PULLAR ADDRESS*

As is customary, I will start with a brief history of the very significant contributions that Dr Pullar (or Thos as he was known) made in the field of pathology in New Zealand. He was a man of extremely high principles and a sound clinical pathologist. He was born in New Zealand in 1907 (110 years ago) but educated mainly in England and Scotland. In 1937 (80 years ago) he moved back to New Zealand and was appointed Pathologist to the Palmerston North Hospital, a position he held for 25 years. During that time he made a great contribution to the advancement of clinical pathology and medical education, his greatest contribution to our profession was through his involvement in the training of medical laboratory technologists. Any aspect of laboratory work was of importance to him but he was intensely involved in the training and welfare of medical laboratory technologists; he helped draft conditions of employment used in laboratories throughout New Zealand, was actively involved with preparing syllabi, and was an examiner for many years. Dr Pullar was a lifelong friend, teacher and champion of our profession. It has been tradition for the Annual Scientific Meeting to open with an address to commemorate this huge contribution made by Dr Pullar.

So, back to the address. When I received the invitation to speak from Fran on behalf of the Councillors for the NZIMLS, it ended "Was I able to accept?" What would your reaction have been?

Two thoughts immediately crossed my mind:
1. WOW, what an honour
2. EEK, why me?

And then I re-read the letter in which Fran had highlighted my career to date. I'm still not entirely sure why me, maybe it's just because I've 'been around' - been hanging around labs for 45 years in fact! And I've tested the waters of most facets of laboratory life!

- Trainee in a large teaching hospital
- Shift worker, weekends, night duties and on-call in a big laboratory
- Section head
- Lecturer
- Diagnostic and Biochemicals sales representative, which also included Point of Care instrumentation and molecular diagnostics
- Provided diagnostic applications and support for both large and small labs
- Practicing Scientist, Fellow of NZIMLS and currently on the Fellowship Committee
- CPD coordinator …. the face of CPD in New Zealand for the NZIMLS…and I've been into every laboratory in the country

So maybe I was "Lighting the Way", the theme for this year's conference.

## *WHAT TO TALK ABOUT??*

So it came to what to talk about that would portray that message – the nautical theme and the slogan 'lighting the way'. With the successful campaign of some New Zealand yachties in Bermuda recently winning the America's Cup, bringing it home and then touring the country with it, it seemed appropriate to think about that accomplishment and make some analogies in how we work in our laboratories these days.

Anyone going out on a boat knows there could be danger involved; you will either sink or swim, (as could I up here) so like any good boatie, I've brought my life jacket along with me just in case! Have you got yours? Even when you get on a plane these days they tell you your life jacket is under your seat. Go figure!

### History of America's Cup (or 'The Auld Mug')

- Oldest International Sporting Trophy founded in 1851.
- It is not only a test of sailing skill and boat and sail design, but also of funding and management skills.
- Over the years, not only the designs of the boats have changed, but the course and rules the yachts sail under have also changed. It is continually evolving and the holder of the cup has a lot of input into the changes made each time.
- The boats now have revolutionary winged keels, their hulls are currently made of fibreglass rather than aluminium or wood, and their sails are now made of Kevlar rather than canvas.

Technology and professionalism have become increasingly important in America's Cup racing. Does that sound familiar? I'll say it again … *technology and professionalism have become increasingly important!* It is this statement that really provided the opportunity for me to compare America's Cup racing with Medical Laboratory Science and our continual need to be updating our knowledge.

### Breakdown of Race

Becoming a Medical Laboratory Scientist or Technician is like being in an America's Cup Race – let's break down an America's Cup yacht race.

- there's the starting box – this is what subjects to take at school to make sure you are well prepared and won't be stalling or even barging at the start line – because there are time penalties involved with this – just like in America's Cup racing!
- a series of upwind and downwind legs – three years in our case - these could be your years at University, some of which will be easy, and some which may be a bit more difficult for you.
- a reach (the fastest point in sailing) for the finish – this is your placement year, you've done all the hard work and now it's time to put it all into practice and finish off the qualification.
- and then there's the aftermath, which isn't always a party and probably doesn't involve touring the country with an impressive piece of silverware! This is what happens when all that training is over. You now need to obtain registration with the Medical Sciences Council and then find yourself a job (of course, you may need to tour the country a bit to do this!)

So, have you won that cup? Actually no, you've really just won the Louis Vuitton Cup, you've now qualified for the right to compete for the America's Cup, it is still to come!

During your training years you will have needed to decide which side of the course to take, that is, what disciplines interested you the most, chase the wind shifts, ride the waves, survive the knockdowns, gybe and tack, and do all this without incurring any penalties or losing the advantage. No penalties because you need that job at the end of it all; you don't want your reputation or behaviour to impede or put off any future employers and you still haven't been through that rigorous check from the Medical Sciences Council to obtain your Annual Practicing Certificate - where one of the requirements is to be an upstanding citizen!

## The Annual Practicing Certificate: This is the real 'CUP'

This is what needs to be defended, so in other words, your Competency and Professional Development needs to be maintained for every year you want to keep that cup, or for every year you want to practice as a Medical Laboratory Scientist or Technician.

So, once you've won that cup, you now need to defend it. You will need to be continually working to keep that boat fine tuned and on course. Everything needs to be finely balanced, with plenty of diversification, and not all the eggs in one basket!

## Defence of Cup

You could become involved in chasing the dollars, or redesigning the boat, updating the technology, researching new materials and methodologies, training new recruits and making sure everything is shipshape – does this sound familiar? These are all aspects that the Team New Zealand people are going to be working on before the next defence and it certainly sounds like being a Medical Scientist to me especially with the diversification of employment opportunities that are out there nowadays. All the while you need to stay ahead of the times, stay up on your foils and be the absolute best you can possibly be, because there's an entire nation out there depending on you to keep that cup – in our case, the entire nation is our patients and their Doctors. How are we going to do this? With professional development of course!

I'm not going to talk about the changes in education programmes, the huge advances in technology, IT or management of laboratories because many before me have done that. Whilst we need to keep abreast of all this, the way to keep ahead is with professional development.

## So, let's talk about Professional Development – our duty to our patients

Racing along – we want to be like this, finely tuned, racing along, on top of our game! Professional development, continuing education, whatever you want to call it. It shouldn't be thought of simply as the number of points or hours we need to clock up to gain our APC and keep our 'licence to practice' current – it is our professional responsibility to ourselves, our work colleagues, our profession and most importantly our patients.

45 years down the track, am I still doing what I was doing when I first started in the laboratory? No, and neither are they racing America's Cup races in the same sort of boats they used 45 years ago, the hulls have changed, the materials have changed and the rules have changed!

So what has been going on? We've been professionally developing of course - even if we don't realise it, it is ongoing, we are doing it all the time.

We need to actively engage in learning to maintain and enhance our professional competence and performance. Professional development is the way we expand the depth and breadth of our expertise and keep abreast of the rapidly expanding knowledge related to technology and development in our field of Medical Laboratory Science (or any other profession for that matter).

I remember many years ago when I was head-hunted to be the Clinical Chemistry tutor for the Auckland School of Medical Technology, I declined saying that I didn't think I knew enough to be taking on that important role. The Head of the School told me I would be surprised at how much I did actually know. Nowadays, I believe the more we know, the more it hits home how much we don't know! Professional development is engaging in lifelong learning, and clearly, there are some of us around who have spent the majority of their life learning in this profession.

From Wikipedia: "professional development is learning to earn or maintain professional credentials; anything from academic degrees to formal coursework, conferences and informal learning opportunities situated in practice. It has been described as intensive and collaborative, ideally incorporating an evaluative stage." I believe this statement covers the CPD programme we use perfectly, you'd think Wikipedia had read our programme requirements.

## Adversity/Diversity

We have to make adversity work for us. One of our Pathologists at work made the statement to me that 'not everything goes to plan in life does it?' He was speaking from a medical point of view and he was referring to disease processes and outcomes, and he said that despite all the best intentions to be in control, there are two things that the medics can't control – childbirth and cancer!

Having managed to live through the former twice and then survive the latter 11 years ago, I feel I can speak from a bit of experience when I say we need to overcome obstacles and turn negatives into positives, to learn from everything both good and bad.

Had I not survived breast cancer, I would never have taken up paddling as a sport, and would never have made so many friends who, to use our team expression, are all in the same boat. I am now an International Athlete, I've found my sport, it keeps me fit and I can do it all sitting down. Breast cancer paddling has taken me all round the world. Dragon boat paddling is a very old sport, starting in China many years ago, and waka-ama (outrigger paddling) goes back beyond the first settlers in our own country. There is a continual learning process involved with these sports too - boats are becoming lighter and more streamlined, paddles are made from new materials, the stroke is being ergonomically improved - all the time the paddlers themselves are getting older ! There are new innovations involved with this sport, stand up paddle boarding being one of the latest.

There is even professional development in our dearly beloved game of rugby, look at how difficult it is for the referee these days to get it right, even with the aid of TMO technology! The rules are constantly undergoing revision and the players and referees need to keep up with this!

## Competency

Of course, as well as professional development, you also need to be competent. You can't have incompetent scientists and technicians in the lab, just like you couldn't have incompetent sailors crewing that finely tuned boat, or incompetent rugby players out on the field in a top level game of rugby! Proof of competence is a huge part of CPD and reassessment of this competence is paramount.

## Team Work

Which leads me on to team work. You are part of a team, even when you're on your own on a shift in the middle of the night! Not just the lab team - from the Phlebotomist right through to the Pathologist, but also from the ill or suffering patient, full circle through the entire medical team and back to the patient again. Our job is to help make that team job easy, it's a team thing and we need to be cohesive.

You need to learn to work as a team, a competent team. If you have one bad sailor, expect to be at the back of the field. And like all good teams, you need to train to be a team, every single person in any team relies on all those other blokes in the team to be competent too, and to be doing their job.

These guys can cycle, they can sail, they can trim a boat - but they still have to train, and they all need to be working together to make sure the end result is the best it could possibly be. (Did you know it is actually part of your professional duty to report incompetence?) Help others be good team members, go to a "Grand Round" and see how other members of our wider team all work together for the benefit of the patient!

Imagine, just imagine, if this guy, (Donald Trump) was in your team! And worse still, if he was the helmsman, you'd be lucky to even sail the same course as the other boats in the race! You'd even be wondering if you still had a job each day when you arrived at work.

## Things change!

We need to be able to change because things do change. Every lab, even in our own country, is very different and constantly evolving - one of the reasons why your competence needs to be signed off every year. Some things we can't control, and if we pitch pole, we need to be able to get back on board (the old sink or swim story). Just like if we decide to jump ship, there's not too much wrong with this, provided you know how to get back on board again. New diseases change our way of thinking, analysis methods have changed significantly, new disease detection methods have changed our way of diagnosis - we are often working at gene level now.

Look at how Health and Safety has changed over the years - no more mouth pipetting, eating tea in front of the analyser while on night shift, using a beaker on your bench as your ash tray! Never-the-less, sometimes we need to go backwards in order to go forwards - there's a reason the rearview mirror is so small and the windscreen is so big – where you are headed is much more important than what you've left behind. However, don't underestimate those years of experience, they count for a lot and you never know when you may need to look into that rear vision mirror!

Stay on top of problems, (your QC officer is speaking here), stay on top of your foils, make sure everyone in the team is paddling in the same direction, and always keep good, accurate records. If you're going to break any records, you need to know how you've done it, and so does everybody else.

It is absolutely essential for us to provide the highest quality services to our patients – it is our duty to our patients! Professional development is not insignificant, either nationally or internationally. It is there to meet government regulations, requirements and legislation. We owe it to our patients to learn as much about what we're doing as we can, and to be the best we can possibly be!

We can't all be a Peter Blake or a Peter Burling, but we all need to take responsibility for our own learning and development if we want to be part of a winning team, and not on a sinking ship!

And remember, without innovation there's only stagnation! Look at the success of those cyclors and how they managed to make Jimmy Spittle appear almost stagnant in the water!!

So, have you learnt anything from all of this?

To leave you with some nautical memories of "lighting the way" I'm going to give you a short boating lesson ……

In navigation on the water, we talk about cans and we talk about cones.

We talk about red and we talk about green.

We talk about port and we talk about starboard.

We talk about left and we talk about right.

We talk about red lights and we talk about green lights

So that gives us, CAN you see the little bit of RED PORT LEFT IN the bottle?

These are our channel markers, but of course, it depends on which direction you are heading ….so…when entering a port or harbour or going upstream, this is the way to use them. The red can shaped beacons go on your port (left) side. It's the opposite when you are leaving.

(You know, even navigation systems undergo change and require professional development. When I studied for my Boat Masters Certificate, starboard makers were painted black and not green, but they had green lights. One of the girls in our class asked the tutor "Sir, if the red buoys have red lights, why do the black buoys have green lights?" The tutor replied "well, they tried black lights, but people found them a little difficult to see!")

I've talked about Competence

I've talked about Professional Development

I've talked about Team Work and Training

I've talked about Record Keeping

All part of America's Cup Racing and all part of being a Medical Scientist

Thank you for the opportunity to share this boat race with you!

"Medical science has made such tremendous progress that there is hardly a healthy human left!" *Aldous Huxley*

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NZIMLS Executive Officer, Fran van Til, received the following emails from Joe Sullivan, who was unable to get back to New Zealand in time for the Annual Scientific Meeting:

*"It is a privilege to be asked to make an appearance and present Jillian Broadbent with a certificate for giving the TH Pullar address.*

*Congratulations Jillian on "Bringing home the America's Cup" I hope that you enjoyed doing this as much as we did.*

*.....please make sure my congratulations get to Jillian for her amazing work. She truly sounds like an inspiring woman that I hope to meet some day.*

*Joe Sullivan, Emirates Team New Zealand"*

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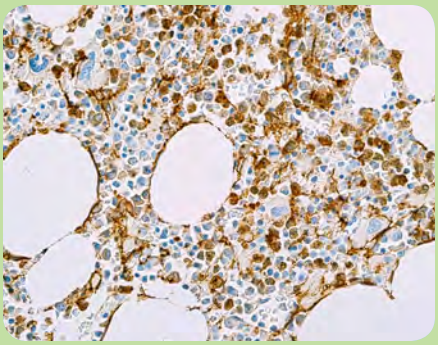
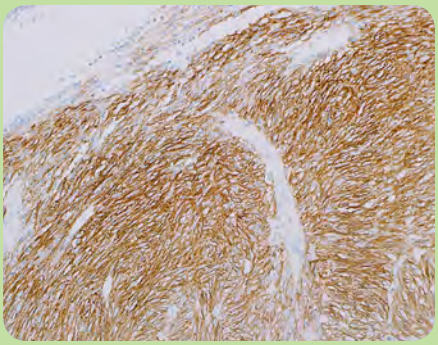


CD13 (EP117)



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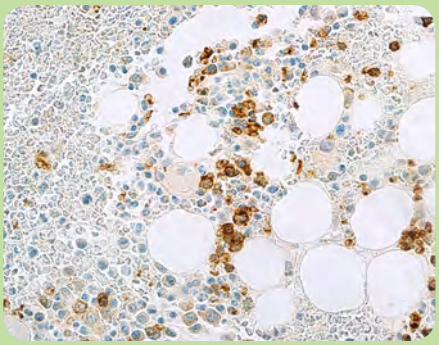

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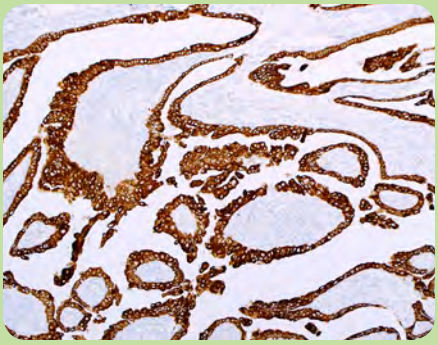
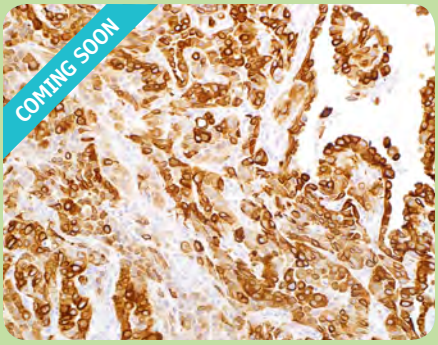
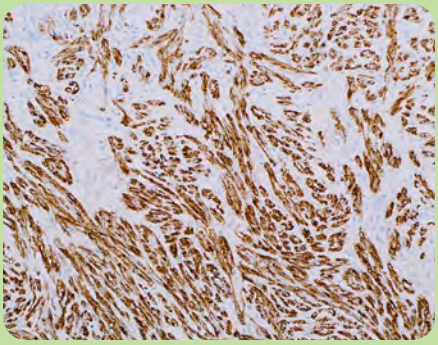


Cytokeratin 19 (EP72)

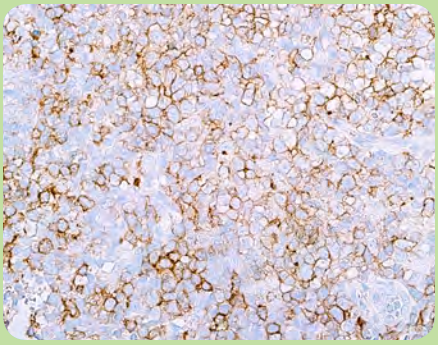



Cytokeratin 5 & 6 (EP24 & EP67)



NGFR (EP31)


Nestin (EP287)



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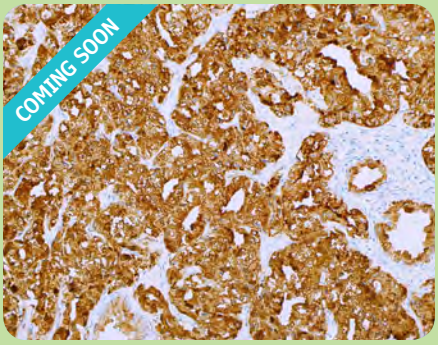


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FELLOWSHIP TREATISE

# ISO 15189:2012 implementation checklists for conformity assessment by accreditation bodies: a comparative analysis

*Dennis Mok*

*Medical Management Consulting, Birkdale, Australia*

## ABSTRACT

**Objectives:** The aim of this research was to determine the extent of conformance requirement coverage provided by ISO 15189:2012 guidance checklists produced by accreditation bodies. The contributing objectives include the identification of conformance requirements in ISO 15189:2012 for the development of evaluation checklists and determination of the level of conformance requirement coverage by quantitative analysis.

**Methods:** The conformance requirements were identified and located in Clauses 4 and 5 of ISO 15189:2012 by content analysis. The identified conformance requirements were used to develop evaluation checklists for further evaluability assessment. The distribution of conformance requirement coverage was allocated to the ISO 15189:2012 process-based quality management system framework for comparative analysis.

**Results:** A total of 51/109 (47 %) accreditation bodies offered ISO 15189:2012 accreditation to medical laboratories and 6/51 (12 %) of these accreditation bodies have published guidance checklists for use in preparation for accreditation assessment. An evaluability assessment of the checklists published by these 6/51 (12 %) accreditation bodies was conducted and the extent of coverage by the evaluand checklists was classified into four major stages based on the ISO 15189:2012 process-based quality management system framework. The overall conformance requirement coverage by the checklists was analysed with the following results: 'orange status' coverage (≤ 50 %) was provided by the Finnish Accreditation Service, the South African National Accreditation System and the National Association of Testing Authorities, Australia; 'yellow-green status' coverage (51 % to 84 %) was provided by the Danish Accreditation Fund; and, 'green status' coverage (85 % to 100 %) was provided by the Hong Kong Accreditation Service and the Singapore Accreditation Council. Three selected compliance management issues were also identified in areas with limited coverage (0 %); these include Subclauses 4.11, 5.6.1 and 5.9.1 of ISO 15189:2012. The implications of identified issues for the management of risk mitigation are highlighted and recommendations made.

**Conclusions:** Medical laboratories planning to conduct gap analysis in preparation for accreditation should take into account that the guidance checklists recommended by accreditation bodies are not intended to identify all relevant conformance requirements, and they need to conduct their own initial internal audits to support the implementation process.

**Key words:** continuous quality management, quality control, quality improvement, total quality management.

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## INTRODUCTION

Quality management plays a significant role in ensuring the diagnostic serviceability of the medical laboratory is maintained at a technically competent level at all times. The International Organization for Standardization (ISO) has been producing guidance documents to support continual improvements in quality performance in the medical laboratory since 1978 (1). The ISO collaborates extensively with many international organisations to produce relevant guidance documents (2,3). One such international non-governmental organisation is the International Electrotechnical Commission (IEC) (4). Together with the IEC, the ISO has produced guidance documents for the pathology services industry for implementation purposes by fulfilling conformance requirement (CR) coverage to an acceptable level. The foundation guidance documents were ISO Guide 25 (5) and ISO/IEC Guide 25 (6,7). However, these documents were not designed for purposes of medical laboratory accreditation. It was subsequently revised and replaced by ISO/IEC 17025 (8-10). ISO/IEC 17025 offered more specific requirements for accreditation purposes. As of 2003, the ISO released ISO 15189 guidance documents that are specific for the pathology services industry (11-14).

The latest edition, ISO 15189:2012 entitled 'Medical laboratories — Requirements for quality and competence' (14), aligns with the relevant requirements of current medical laboratory practices and remains the standard of choice for accreditation purposes. Implementation of ISO 15189:2012 requires the medical laboratory to fulfil specific CRs ranging from bench to strategic levels in relation to management system and technical competence. Specifically, Clause 4 (management requirements) of ISO 15189:2012 (14,pp.6-19) concentrates on the management system requirements containing 682/1 515 (45 %) CRs for the medical laboratory to consider if all areas are related to the areas of operations (15). By contrast, Clause 5 (technical requirements) of ISO 15189:2012 (14,pp.19-39), which relates to the implementation of technical competence requirements, contains 833/1 515 (55 %) CRs for consideration (15). Together with the specific requirements of accreditation bodies, ISO 15189:2012 enables accreditation bodies to customise the overall requirements for accreditation. The implementation of ISO 15189:2012 by the medical laboratory represents significant investment of effort and resources in order to competently accomplish all of the relevant CRs and while achieving desired economy, effectiveness and efficiency (16-18).

Since 2013, accreditation bodies have been granting ISO 15189 accreditations to medical laboratories that have achieved satisfactory on-site assessments globally. These specific accreditation bodies have the option of joining the International Accreditation Forum or the International Laboratory Accreditation Cooperation to demonstrate that they meet operational criteria as specified in ISO/IEC 17011:2004 entitled 'Conformity assessment — General requirements for accreditation bodies accrediting conformity assessment bodies' (19). Such accreditation bodies become signatories to an international mutual recognition arrangement that allows their accredited medical laboratories to produce mutually recognised test results. These accreditation bodies also provide guidance and recommendations to medical laboratories interested in becoming accredited, especially in the implementation of ISO 15189:2012.

Guidance documents for ISO 15189:2012 implementation are presented either in the format of checklists, such as the National Association of Testing Authorities, Australia (NATA) (20) or explanatory commentaries, such as the International Accreditation New Zealand (21). These specific guidance documents are supposed to provide self-assessments to produce the gap analysis results necessary to achieve accreditation. Despite the recent quantification that ISO 15189:2012 has 1,515 CRs for implementation purposes (15), there has been no detailed quantitative analysis of the extent of CR coverage provided by these guidance checklists nor has there been a suitable analytical tool available to conduct such an evaluation. The degree of coverage of the 1 515 CRs in ISO 15189:2012 offered by guidance checklists released by accreditation bodies remains unknown.

The focus of this dissertation is to quantitatively analyse the extent of CR coverage by ISO 15189:2012 guidance checklists produced by accreditation bodies. This is the first study to undertake an in-depth quantitative analysis of the comprehensiveness of guidance checklists. The evaluation of ISO 15189:2012 guidance checklists was conducted through content analysis (CA) and divided into four phases. First, an evaluation checklist was developed based on the quantification of 1 515 CRs (15) for Clauses 4 and 5 of ISO 15189:2012 (14,pp.6-39). An evaluation checklist can be defined as 'list of questions, each of which is designed to check for conformity of a product, process or service to one or more provisions within a particular International Standard' (22). A well-prepared checklist if deployed correctly can provide insights into workplaces by collecting objective evidence (23). Second, ISO 15189:2012 guidance checklists were identified that are intended to provide guidance from signatories to the International Accreditation Forum multilateral recognition arrangement (IAFMLA) or the International Laboratory Accreditation Cooperation mutual recognition arrangement (ILACMRA) using standardised selection criteria. Third, evaluability assessments of the selected ISO 15189:2012 guidance checklists were conducted using the 1 515 CRs framework-derived evaluation checklists.

The results were classified into four major stages based on the models of process-based quality management systems in ISO 9001:2015 entitled 'Quality management systems — Requirements' (24,pp.vii-ix) and ISO 15189:2012 (25) (Figure 1). Relevant subclauses were then allocated to each of the four stages for analytical purposes. Finally, the checklists' shortfalls were analysed in order to generate recommendations for organisations intending to use these guidance checklists for gap analysis. Overall, this research provides information on the usefulness of ISO 15189:2012 guidance checklists supplied by accreditation bodies for organisations who intend to use them.

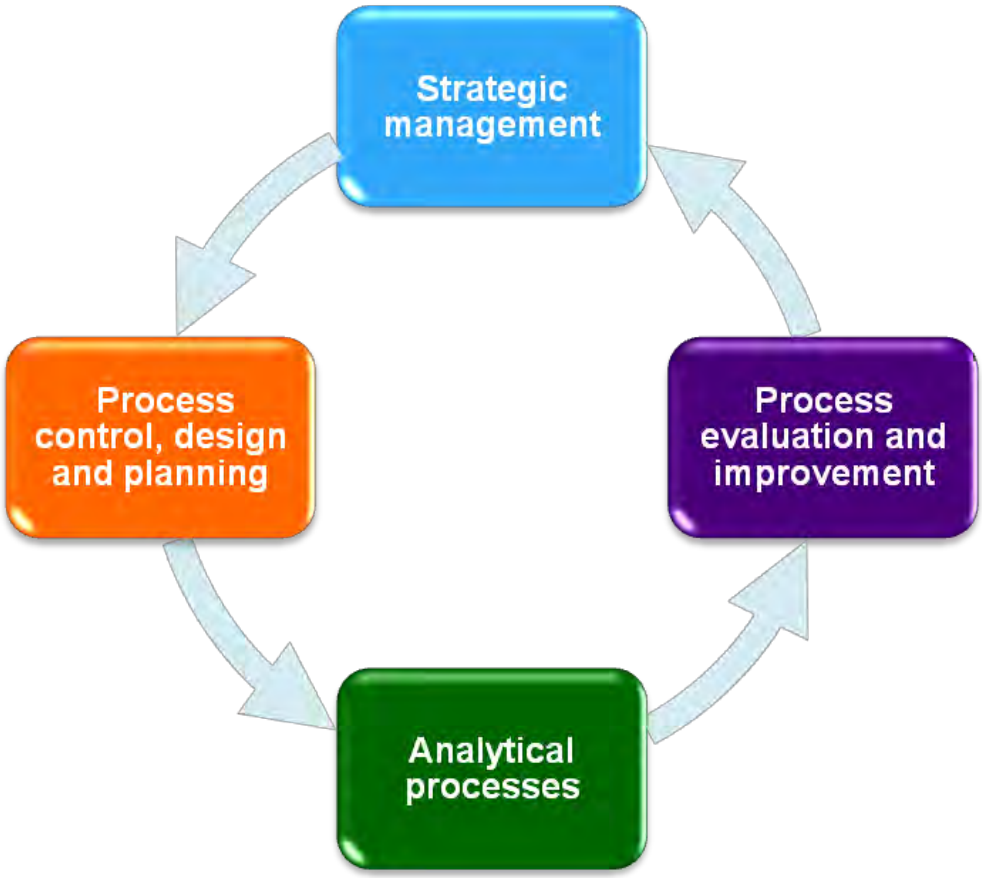



**Figure 1.** Representation of ISO 15189:2012 in a process-based quality management system framework. The four boxes represent the major stages of ISO 15189:2012 processes. This modified format is based on ISO 9001:2015 and ISO 15189:2012 models of process-based quality management systems (24,25).

## MATERIALS AND METHODS

### Content analysis of Clauses 4 (management requirements) and 5 (technical requirements) of ISO 15189:2012

CA is an established approach for analysing ISO 15189:2012 (15,26), and is highly suitable for the quantitation of CRs. In this investigation, CA was used to locate instances of the word 'shall' within Clauses 4 and 5 of ISO 15189:2012 (14,pp.6-39). According to the ISO, the use of the verb 'shall' indicates a mandatory requirement (27). The specific locations of the word 'shall' were identified using a computer-aided qualitative data analysis software, NVivo™ 10 (version 10.0.418.0 SP4) (QSR International, Doncaster, Victoria, Australia), as previously described (15). The implied CRs indicated by the word 'shall' were then elicited as previously described (15). The identification of CRs within Clauses 4 and 5 of ISO 15189:2012 (14,pp.6-39) was used for the development of evaluation checklists for the comparative analysis and evaluability assessment. An acceptable result was recorded when the evaluand subclause had an equivalent subclause on the evaluation checklist.

### Guidance checklist selection criteria for evaluability assessment

The criteria for selecting the evaluands consisted of five areas (Table 1). Briefly, ISO 15189:2012 guidance checklists were sought from accreditation bodies of signatories to the IAFMLA or the ILACMRA. Accreditation bodies were sought from countries and a dependent territory published in ISO 3166-1:2013 entitled 'Codes for the representation of names of countries and their subdivisions — Part 1: country codes' (28) and whose evaluand checklists were published in English, classified as 'eng' in ISO 639-2:1998 entitled 'Codes for the representation of names of languages — Part 2: alpha-3 code' (29).

### Point distribution for comparative analysis

The distribution of CRs in Clauses 4 and 5 of ISO 15189:2012 (14,pp.6-39) is represented by a radar chart. The advantage of a radar chart is that several dimensions can be viewed simultaneously (30,31). The radar chart represents the distribution of 1,515 CRs in Clauses 4 and 5 of ISO 15189:2012 (14,pp.6-39) by placing each subclause on a spoke. The subclauses are on a sequence of radii (n = 28) representing the number of CRs. The maximum magnitude of the point is joined by a continuous line between each data value for each spoke.

### Limitations of the evaluability assessment

The investigation had a major limitation: the evaluand checklists were highly unlikely to cover all aspects of Clauses 4 and 5 of ISO 15189:2012 (14,pp.6-39) because they were developed to guide the medical laboratory to address potential gaps. Different accreditation bodies have produced checklists with different levels of coverage; such as the NATA states that 'this worksheet provides only a brief summary of the clauses of the Standard' (20), and the Danish Accreditation Fund (DANAK) states that the checklist 'is much shortened compared to the text of DS/EN ISO 15189:2013 and it is therefore important that the checklist is used together with ISO 15189' (32).

## RESULTS

### Identification and location of conformance requirements in Clauses 4 (management requirements) and 5 (technical managements) of ISO 15189:2012

CA was used to detect the word 'shall' which implies the presence of a CR. A total of 1,515 CRs was identified in Clauses 4 and 5 of ISO 15189:2012 (14,pp.6-39) (Table 2); Clause 4 of ISO 15189:2012 (14,pp.6-19) contained 682/1 515 (45 %) CRs and Clause 5 of ISO 15189:2012 (14,pp.19-39) contained 833/1 515 (55 %) CRs. A positive correlation was found between these results and previously reported results (15).

**Table 1.** Selection criteria for evaluand ISO 15189:2012 checklists.

| **Selection criteria** (n = 5) |
|---|
| The checklist is published by an accreditation body that is a signatory of the International Laboratory Accreditation Cooperation mutual recognition arrangement; |
| The country or dependent territory of the accreditation body is listed in ISO 3166-1:2013; |
| The checklist is published in English, classified as 'eng' in ISO 639-2:1998; |
| The checklist is freely and readily available; and |
| The checklist is not an exact duplicate of Clauses 4 and 5 of ISO 15189:2012. |

**Table 2.** Stage-by-stage coverage summary of ISO 15189:2012 conformance requirements.

| | | ISO 15189:2012 |
|---|---|---|
| Strategic management | **Subclause 4.1** *Organization and management responsibility* | 159/399 (40 %) |
| | **Subclause 4.2** *Quality management system* | 66/399 (16 %) |
| | **Subclause 4.3** *Document control* | 31/399 (8 %) |
| | **Subclause 4.4** *Service agreements* | 35/399 (9 %) |
| | **Subclause 4.13** *Control of records* | 59/399 (15 %) |
| | **Subclause 4.15** *Management review* | 49/399 (12 %) |
| | **Subtotal** | **399/399 (100 %)** |
| Process control, design and planning | **Subclause 4.6** *External services and supplies* | 26/477 (6 %) |
| | **Subclause 5.1** *Personnel* | 67/477 (14 %) |
| | **Subclause 5.2** *Accommodation and environmental conditions* | 88/477 (18 %) |
| | **Subclause 5.3** *Laboratory equipment, reagents, and consumables* | 154/477 (32 %) |
| | **Subclause 5.10** *Laboratory information management* | 142/477 (30 %) |
| | **Subtotal** | **477/477 (100 %)** |
| Analytical processes | **Subclause 4.5** *Examination by referral laboratories* | 32/387 (8 %) |
| | **Subclause 4.7** *Advisory services* | 11/387 (4 %) |
| | **Subclause 5.4** *Pre-examination processes* | 144/387 (37 %) |
| | **Subclause 5.5** *Examination processes* | 71/387 (18 %) |
| | **Subclause 5.6.1** *General* | 4/387 (1 %) |
| | **Subclause 5.6.2** *Quality control* | 12/387 (3 %) |
| | **Subclause 5.7** *Post-examination processes* | 21/387 (5 %) |
| | **Subclause 5.8** *Reporting of results* | 47/387 (12 %) |
| | **Subclause 5.9** *Release of results* | 45/387 (12 %) |
| | **Subtotal** | **387/387 (100 %)** |
| Process evaluation and improvement | **Subclause 4.8** *Resolution of complaints* | 4/252 (2 %) |
| | **Subclause 4.9** *Identification and control of nonconformities* | 23/252 (9 %) |
| | **Subclause 4.10** *Corrective action* | 10/252 (4 %) |
| | **Subclause 4.11** *Preventive action* | 10/252 (4 %) |
| | **Subclause 4.12** *Continual improvement* | 34/252 (13 %) |
| | **Subclause 4.14** *Evaluation and audits* | 133/252 (53 %) |
| | **Subclause 5.6.3** *Interlaboratory comparisons* | 26/252 (10 %) |
| | **Subclause 5.6.4** *Comparability of examination results* | 12/252 (5 %) |
| | **Subtotal** | **252/252 (100 %)** |
| | **Total (of 1 515)** | **1 515 (100 %)** |

## The frequency of conformance requirements in the ISO 15189:2012 process-based quality management system framework

CA successfully identified and located the CRs in the four-stage process-based quality management system framework (Figure 2). The 'strategic management' stage contained a total of 399/1 515 (26 %) CRs. The 'process control, design and planning' stage contained a total of 477/1 515 (31 %) CRs. The 'analytical processes' stage contained a total of 387/1 515 (26 %) CRs. The 'process evaluation and improvement' stage contained a total of 252/1 515 (17 %) CRs. The number of CRs identified in each subclause ranged from 4 CRs in Subclause 4.8 (resolution of complaints) (14,p.13) and Subclause 5.6.1 (general) of ISO 15189:2012 (14,p.33), to 159 CRs in Subclause 4.1 (organization and management responsibility) of ISO 15189:2012 (14,pp.6-9).

## Selection of evaluand checklists for comparative analysis

To ensure comprehensive coverage, accreditation bodies that are signatories to the IAFMLA (33) or the ILACMRA (34) were identified for selection purposes (Table 3). A total of 51/109 (47 %) accreditation bodies were identified that provide ISO 15189:2012 accreditation in accordance with the requirements of ISO/IEC 17011:2014 (19). A final 6/51 (12 %) accreditation bodies were selected because they met the selection criteria for evaluand checklists (Table 1).

## Evaluability assessment of evaluand checklists from selected accreditation bodies

Evaluand checklists from the 6/51 (12 %) accreditation bodies were used for the evaluability assessment. The CRs stage-by-stage coverage is presented in this sequence: 'strategic management', 'process control, design and planning', 'analytical processes' and 'process evaluation and improvement' (Table 4). The evaluability assessment indicated that the extent of coverage ranged from 353/1 515 (23 %) CRs to 1 479/1 515 (98 %) CRs. The interpretation of results was based on a three-colour colour-coded classification (Figure 3). Those accreditation bodies with the highest classification, indicated by green, achieved coverage of 85 % to 100 %. The Singapore Accreditation Council (SAC) (93 %) and the Hong Kong Accreditation Service (HKAS) (98 %) have 'green status'. Those accreditation bodies classified as yellow-green achieved coverage of 51 % to 84 %. The DANAK (55 %) has 'yellow-green status'. Those accreditation bodies classified as orange achieved coverage of 0 % to 50 %. The Finnish Accreditation Service (FINAS) (23 %), the South African National Accreditation Service (SANAS) (24 %) and the NATA (45 %) have 'orange status'.

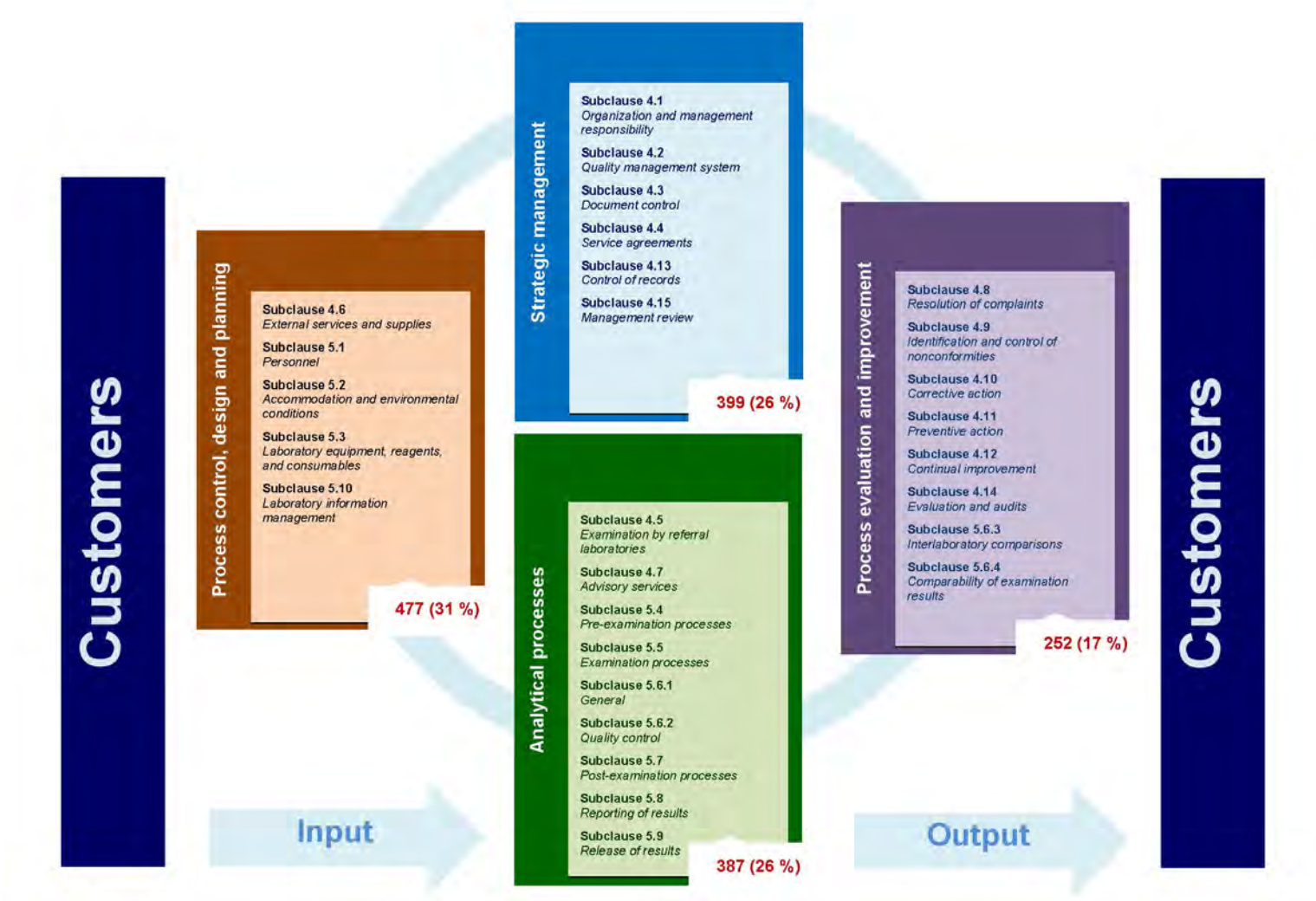



**Figure 2.** Distribution of conformance requirements among the four major stages of ISO 15189:2012 processes. The 'strategic management' stage contains 399/1 515 (26 %) conformance requirements. The 'process control, design and planning' stage contains 477/1 515 (31 %) conformance requirements. The 'analytical processes' stage contains 387/1 515 (26 %) conformance requirements. The 'process evaluation and improvement' stage contains 252/1 515 (17 %) conformance requirements.

**Table 3.** The availability and eligibility of ISO 15189:2012 guidance checklists for evaluability assessments by selected countries and dependent territory.

| **Countries/ Dependent territory** (n = 12) | **Accreditation bodies** (n = 20) | **Availability/ Eligibility** |
|---|---|---|
| Australia (AUS) | Joint Accreditation System of Australia and New Zealand | Unavailable/Ineligible |
| | National Association of Testing Authorities, Australia* | **Available/Eligible** |
| Belgium (BEL) | Belgian Accreditation Structure* | Available/Ineligible |
| Denmark (DNK) | Danish Accreditation Fund* | **Available/Eligible** |
| Finland (FIN) | Finnish Accreditation Service* | **Available/Eligible** |
| Hong Kong† (HKG) | Hong Kong Accreditation Service* | **Available/Eligible** |
| India (IND) | National Accreditation Board for Testing and Certification Laboratories* | Available/Ineligible |
| Malaysia (MYS) | Department of Standards Malaysia* | Available/Ineligible |
| Singapore (SGP) | Singapore Accreditation Council* | **Available/Eligible** |
| South Africa (ZAF) | South African National Accreditation System* | **Available/Eligible** |
| Turkey (TUR) | Turkish Accreditation Agency* | Available/Ineligible |
| United Arab Emirates (the) (ARE) | Dubai Accreditation Centre* | Available/Ineligible |
| United States (the) (USA) | American Association for Laboratory Accreditation* | Available/Ineligible |
| | AIHA® Laboratory Accreditation Programs | Unavailable/Ineligible |
| | American National Standards Institute | Unavailable/Ineligible |
| | American Society of Crime Laboratory Directors | Unavailable/Ineligible |
| | International Accreditation Service | Unavailable/Ineligible |
| | Laboratory Accreditation Bureau | Unavailable/Ineligible |
| | National Voluntary Laboratory Accreditation Program | Unavailable/Ineligible |
| | Perry Johnson Laboratory Accreditation | Unavailable/Ineligible |

* Signatory members of the International Laboratory Accreditation Cooperation mutual recognition arrangement.

† Hong Kong (HKG) is a special administrative region of China, therefore it is a dependent territory. The official name is Hong Kong Special Administrative Region of the People's Republic of China and is referred to as 'Hong Kong, China' by the International Accreditation Forum; and 'China, Hong Kong' by the International Laboratory Accreditation Cooperation.

**Table 4.** Coverage of ISO 15189:2012 conformance requirements by the evaluand checklists from selected accreditation bodies: the National Association of Testing Authorities, Australia (NATA); the Danish Accreditation Fund (DANAK); the Finnish Accreditation Service (FINAS); the Hong Kong Accreditation Service (HKAS); the Singapore Accreditation Council (SAC); and the South African National Accreditation Service (SANAS).

| | | NATA | DNK | FINAS | HKAS | SAC | SANAS |
|---|---|---|---|---|---|---|---|
| Strategic management | **Subclause 4.1** *Organization and management responsibility* | 47/159 (30 %) | 47/159 (30 %) | 12/159 (8 %) | 153/159 (96 %) | 156/159 (98 %) | 46/159 (29 %) |
| | **Subclause 4.2** *Quality management system* | 30/66 (45 %) | 40/66 (61 %) | 8/66 (12 %) | 64/66 (97 %) | 60/66 (91 %) | 12/66 (18 %) |
| | **Subclause 4.3** *Document control* | 30/31 (97 %) | 29/31 (94 %) | 5/31 (16 %) | 29/31 (94 %) | 31/31 (100 %) | 12/31 (39 %) |
| | **Subclause 4.4** *Service agreements* | 9/35 (26 %) | 7/35 (20 %) | 2/35 (6 %) | 34/35 (97 %) | 17/35 (49 %) | 4/35 (11 %) |
| | **Subclause 4.13** *Control of records* | 35/59 (59 %) | 46/59 (78 %) | 12/59 (20 %) | 59/59 (100 %) | 59/59 (100 %) | 9/59 (15 %) |
| | **Subclause 4.15** *Management review* | 41/49 (84 %) | 38/49 (78 %) | 7/49 (14 %) | 49/49 (100 %) | 49/49 (100 %) | 4/49 (8 %) |
| | **Subtotal** | **192/399 (48 %)** | **207/399 (52 %)** | **46/399 (12 %)** | **388/399 (97 %)** | **372/399 (93 %)** | **89/399 (22 %)** |
| Process control, design and planning | **Subclause 4.6** *External services and supplies* | 11/26 (42 %) | 8/26 (31 %) | 10/26 (38 %) | 18/26 (69 %) | 14/26 (54 %) | 9/26 (35 %) |
| | **Subclause 5.1** *Personnel* | 44/67 (66 %) | 53/67 (79 %) | 24/67 (36 %) | 66/67 (99 %) | 66/67 (99 %) | 23/67 (34 %) |
| | **Subclause 5.2** *Accommodation and environmental conditions* | 34/88 (39 %) | 49/88 (56 %) | 9/88 (10 %) | 88/88 (100 %) | 80/88 (91 %) | 11/88 (13 %) |
| | **Subclause 5.3** *Laboratory equipment, reagents, and consumables* | 57/154 (37 %) | 80/154 (52 %) | 4/154 (3 %) | 143/154 (93 %) | 148/154 (96 %) | 31/154 (20 %) |
| | **Subclause 5.10** *Laboratory information management* | 91/142 (64 %) | 96/142 (68 %) | 116/142 (82 %) | 140/142 (99 %) | 142/142 (100 %) | 8/142 (6 %) |
| | **Subtotal** | **237/477 (50 %)** | **286/477 (60 %)** | **163/477 (34 %)** | **455/477 (95 %)** | **450/477 (94 %)** | **82/477 (17 %)** |
| Analytical processes | **Subclause 4.5** *Examination by referral laboratories* | 20/32 (63 %) | 25/32 (78 %) | 6/32 (19 %) | 32/32 (100 %) | 24/32 (75 %) | 6/32 (19 %) |
| | **Subclause 4.7** *Advisory services* | 4/11 (36 %) | 7/11 (64 %) | 11/11 (100 %) | 11/11 (100 %) | 11/11 (100 %) | 3/11 (27 %) |
| | **Subclause 5.4** *Pre-examination processes* | 48/144 (33 %) | 90/144 (63 %) | 25/144 (17 %) | 143/144 (99 %) | 131/144 (91 %) | 68/144 (47 %) |
| | **Subclause 5.5** *Examination processes* | 7/71 (10 %) | 48/71 (68 %) | 7/71 (10 %) | 71/71 (100 %) | 71/71 (100 %) | 5/71 (7 %) |
| | **Subclause 5.6.1** *General* | 0/4 (0 %) | 1/4 (25 %) | 0/4 (0 %) | 4/4 (100 %) | 4/4 (100 %) | 0/4 (0 %) |
| | **Subclause 5.6.2** *Quality control* | 0/12 (0 %) | 6/12 (50 %) | 1/12 (8 %) | 12/12 (100 %) | 12/12 (100 %) | 1/12 (8 %) |
| | **Subclause 5.7** *Post-examination processes* | 1/21 (5 %) | 9/21 (43 %) | 2/21 (10 %) | 21/21 (100 %) | 19/21 (90 %) | 18/21 (86 %) |
| | **Subclause 5.8** *Reporting of results* | 26/47 (55 %) | 33/47 (70 %) | 26/47 (55 %) | 47/47 (100 %) | 47/47 (100 %) | 32/47 (68 %) |
| | **Subclause 5.9** *Release of results* | 25/45 (56 %) | 17/45 (38 %) | 0/45 (0 %) | 43/45 (96 %) | 45/45 (100 %) | 16/45 (36 %) |
| | **Subtotal** | **131/387 (34 %)** | **236/387 (70 %)** | **78/387 (20 %)** | **384/387 (99 %)** | **364/387 (94 %)** | **149/387 (39 %)** |
| Process evaluation and improvement | **Subclause 4.8** *Resolution of complaints* | 4/4 (100 %) | 4/4 (100 %) | 1/4 (25 %) | 4/4 (100 %) | 4/4 (100 %) | 1/4 (25 %) |
| | **Subclause 4.9** *Identification and control of nonconformities* | 22/23 (96 %) | 5/23 (22 %) | 8/23 (35 %) | 23/23 (100 %) | 20/23 (87 %) | 11/23 (48 %) |
| | **Subclause 4.10** *Corrective action* | 10/10 (100 %) | 9/10 (90 %) | 8/10 (80 %) | 10/10 (100 %) | 8/10 (80 %) | 2/10 (20 %) |
| | **Subclause 4.11** *Preventive action* | 8/10 (80 %) | 9/10 (90 %) | 3/10 (30 %) | 10/10 (100 %) | 9/10 (90 %) | 0/10 (0 %) |
| | **Subclause 4.12** *Continual improvement* | 4/34 (12 %) | 7/34 (21 %) | 12/34 (35 %) | 34/34 (100 %) | 16/34 (47 %) | 24/34 (71 %) |
| | **Subclause 4.14** *Evaluation and audits* | 72/133 (54 %) | 58/133 (44 %) | 24/133 (18 %) | 133/133 (100 %) | 128/133 (96 %) | 10/133 (8 %) |
| | **Subclause 5.6.3** *Interlaboratory comparisons* | 3/26 (12 %) | 13/26 (50 %) | 10/26 (38 %) | 26/26 (100 %) | 26/26 (100 %) | 0/26 (0 %) |
| | **Subclause 5.6.4** *Comparability of examination results* | 0/12 (0 %) | 5/12 (42 %) | 0/12 (0 %) | 12/12 (100 %) | 12/12 (100 %) | 0/12 (0 %) |
| | **Subtotal** | **123/252 (49 %)** | **110/252 (44 %)** | **66/252 (26 %)** | **252/252 (100 %)** | **223/252 (88 %)** | **48/252 (19 %)** |
| | **Total (of 1 515)** | **683 (45 %)** | **839 (55 %)** | **353 (23 %)** | **1 479 (98 %)** | **1,409 (93 %)** | **368 (24 %)** |

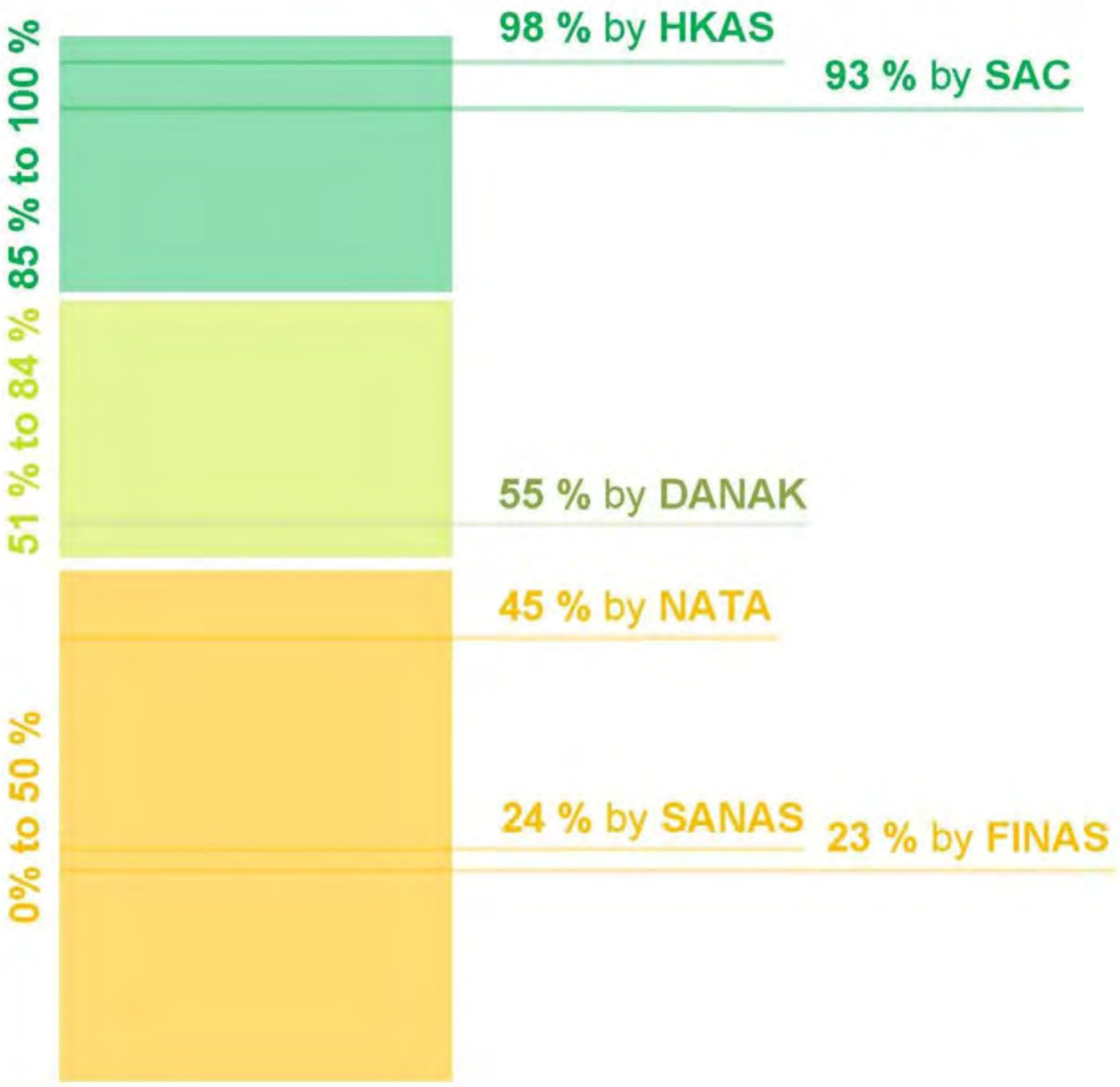
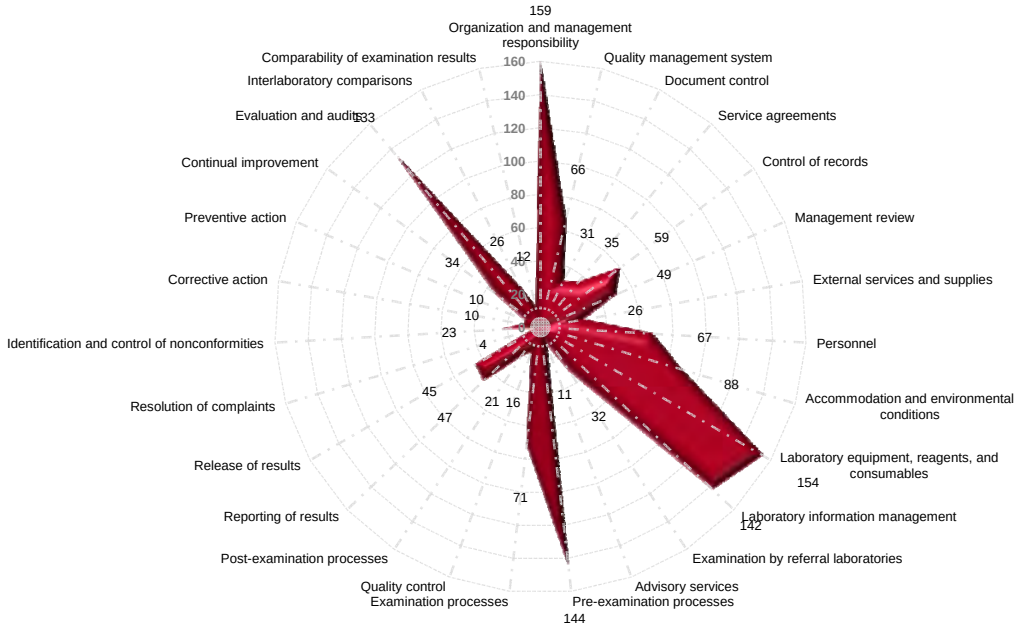



**Figure 3.** Interpretation of results by quantitation of conformance requirements in Clauses 4 (management requirements) and 5 (technical requirements) of ISO 15189:2012 using three-colour colour-coded classification. Green indicates the evaluand checklist achieved a total coverage of 85 % to 100 %: the medical laboratory is highly likely to make excellent progress and to achieve planned deliverables by fulfilling the checklist requirements. The Singapore Accreditation Council (SAC) (93 %) and the Hong Kong Accreditation Service (HKAS) (98 %) have 'green status'. Yellow-green indicates the evaluand checklist achieved a total coverage of 51 % to 84 %; the medical laboratory is highly likely to make very good progress and certain to achieve planned deliverables by fulfilling the checklist requirements. The Danish Accreditation Fund (DANAK) (55 %) has 'yellow-green status'. Orange indicates the evaluand checklist achieved a total coverage of 0 % to 50 %; the medical laboratory is likely to achieve good progress and almost certain to achieve planned deliverables. The Finnish Accreditation Service (FINAS) (23 %), the South African National Accreditation System (SANAS) (24 %) and the National Association of Testing Authorities, Australia (NATA) (45 %) have 'orange status'.

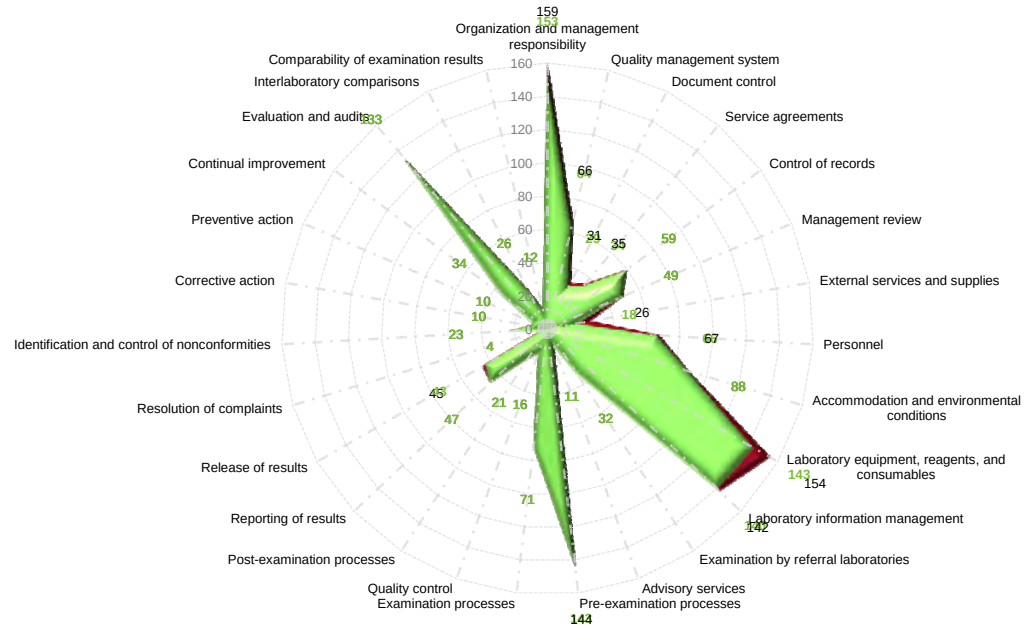
### Point distribution analysis of conformance requirements in Clauses 4 (management requirements) and 5 (technical requirements) of ISO 15189:2012

A radar chart was used to plot the results for point distribution analysis (PDA) of CRs in Clauses 4 and 5 of ISO 15189:2012 (14,pp.6-39) (Figure 4). The CRs coverage of each subclause of the six evaluand checklists were also plotted onto radar charts for PDA. The distribution area is superimposed on the radar chart representing coverage of the 1 515/1 515 (100 %) CRs for visualisation purposes. The radar charts illustrate the overall results of the CA of the selected evaluand checklists and incorporate all stages of the management system framework (Figures 5 to 10).




■ International Organization for Standardization

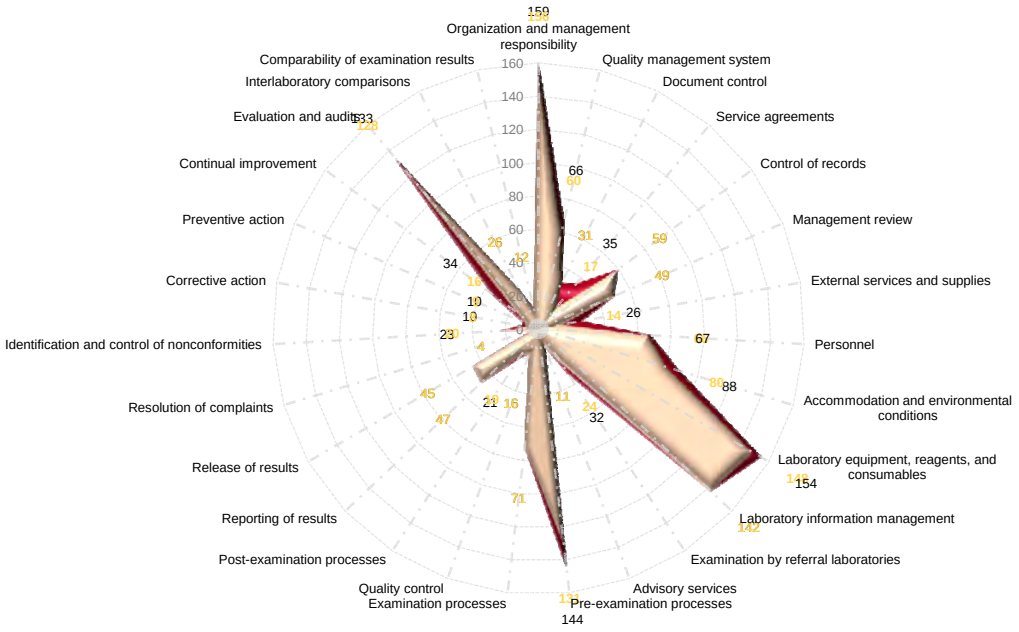
**Figure 4.** Relative point distribution analysis of conformance requirements of Clauses 4 (management requirements) and 5 (technical requirements) of ISO 15189:2012 of the International Organization for Standardization. The distribution of 1 515 conformance requirements is represented by a radar chart. The subclauses are presented on a sequence of radii representing the number of conformance requirements. The sequence of representation follows the four stages of ISO 15189:2012 processes, starting at twelve o'clock and progressing anticlockwise, from 'strategic management' to 'process control, design and planning', 'analytical processes' and 'process evaluation and improvement'.




■ Hong Kong Accreditation Service

■ International Organization for Standardization

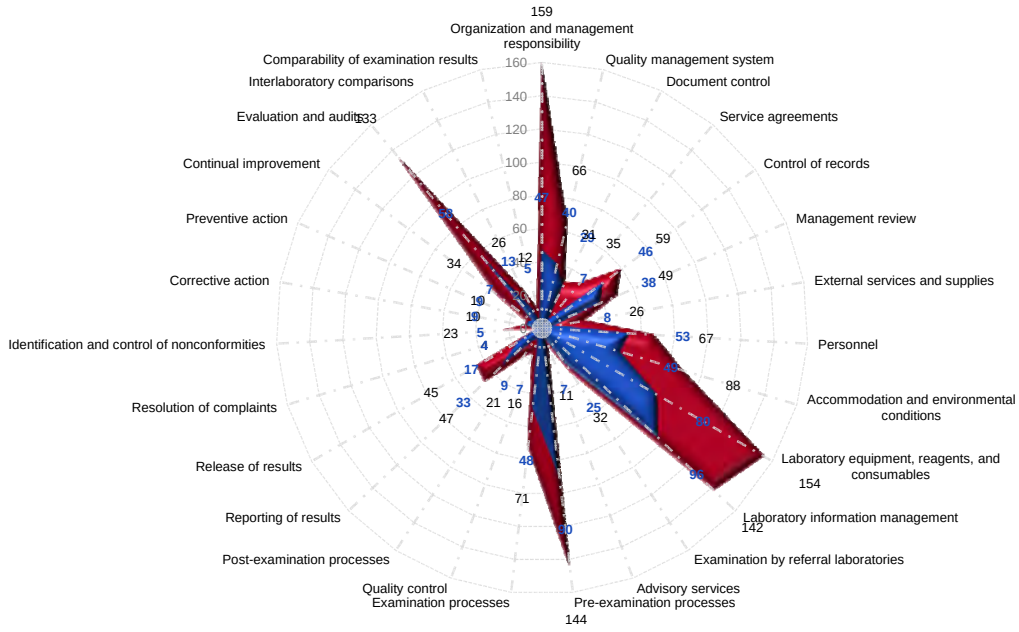
**Figure 5.** Relative point distribution analysis of conformance requirements of guidance checklist provided by the Hong Kong Accreditation Service. The distribution of conformance requirements by the Hong Kong Accreditation Service is represented in green and the International Organization for Standardization in red. Overall, the guidance checklist of Hong Kong Accreditation Service provided coverage of 98 % when compared with the International Organization for Standardization. The level of coverage by stage ranged from 69 % in Subclause 4.6 (external services and supplies) at 'process control, design and planning' stage to 100 % in Subclauses 4.13 (control of records) and 4.15 (management review) at 'strategic management' stage, Subclause 5.2 (accommodation and environmental conditions) at 'process control, design and planning' stage, Subclauses 4.5 (examination by referral laboratories), 4.7 (advisory services), 5.5 (examination processes), 5.6.1 (general), 5.6.2 (quality control), 5.7 (post-examination processes), 5.8 (reporting of results) at 'analytical processes' stage, and Subclauses 4.8 (resolution of complaints), 4.9 (identification and control of nonconformities), 4.10 (corrective action), 4.11 (preventive action), 4.12 (continual improvement), 4.14 (evaluation and audits), 5.6.3 (interlaboratory comparisons) and 5.6.4 (comparability of examination results) at 'process evaluation and improvement' stage.




■ Singapore Accreditation Council

■ International Organization for Standardization

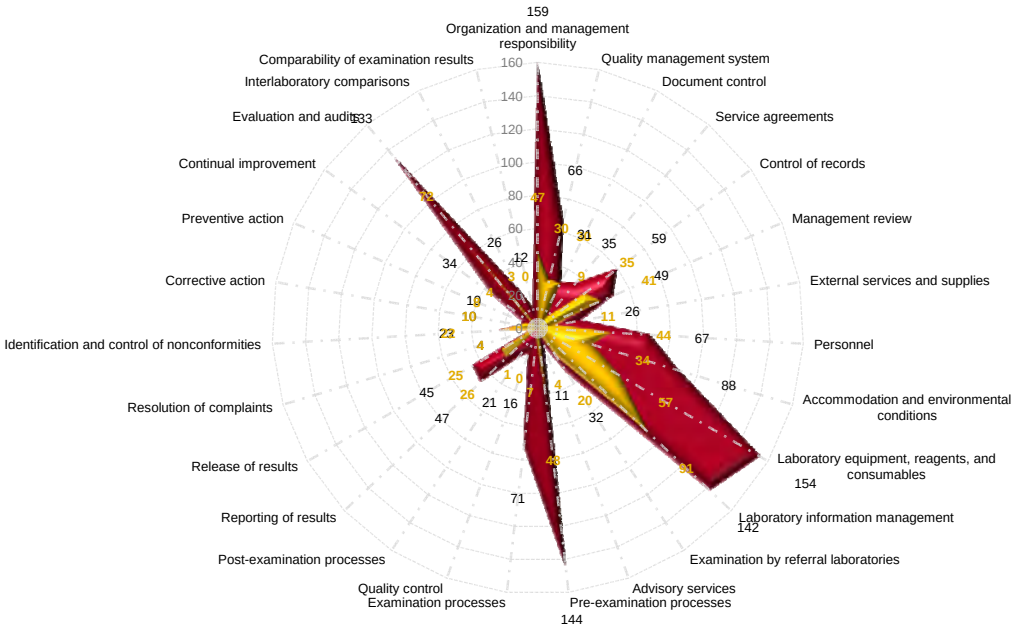
**Figure 6.** Relative point distribution analysis of conformance requirements of guidance checklist provided by the Singapore Accreditation Council. The distribution of conformance requirements by the Singapore Accreditation Council is represented in peach and the International Organization for Standardization in red. Overall, the guidance checklist of Singapore Accreditation Council provided coverage of 93 % when compared with the International Organization for Standardization. The level of coverage by stage ranged from 47 % in Subclause 4.12 (continual improvement) to 100 % in Subclauses 4.3 (document control), 4.13 (control of records) and 4.15 (management review) at 'strategic management' stage, Subclause 5.10 (laboratory information management) in 'process control, design and planning' stage, Subclauses 4.7 (advisory services), 5.5 (examination processes), 5.61 (general), 5.6.2 (quality control), 5.8 (reporting of results) and 5.9 (release of results) at 'analytical processes' stage, and Subclauses 4.8 (resolution of complaints), 5.6.3 (interlaboratory comparisons) and 5.6.4 (comparability of examination results) at 'process evaluation and improvement' stage.




■ Danish Accreditation Fund

■ International Organization for Standardization

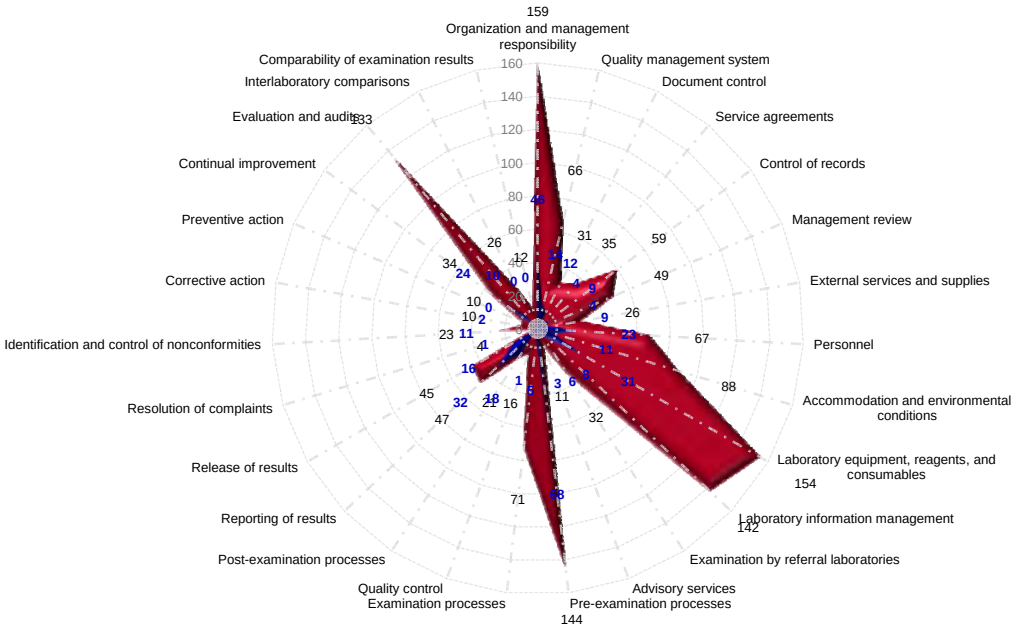
**Figure 7.** Relative point distribution analysis of conformance requirements of guidance checklist provided by the Danish Accreditation Fund. The distribution of conformance requirements by the Danish Accreditation Fund is represented in blue and the International Organization for Standardization in red. Overall, the guidance checklist of Danish Accreditation Fund provided coverage of 55 % when compared with the International Organization for Standardization. The level of coverage by stage ranged from 21 % in Subclause 4.12 (continual improvement) at 'process evaluation and improvement' stage to 100 % in Subclause 4.8 (resolution of complaints) at 'process evaluation and improvement' stage.




■ National Association of Testing Authorities, Australia

■ International Organization for Standardization

**Figure 8.** Relative point distribution analysis of conformance requirements of guidance checklist provided by the National Association of Testing Authorities, Australia. The distribution of conformance requirements by the National Association of Testing Authorities, Australia is represented in orange and the International Organization for Standardization in red. Overall, the guidance checklist of National Association of Testing Authorities, Australia provided coverage of 45 % when compared with the International Organization for Standardization. The level of coverage by stage ranged from 0 % in Subclauses 5.6.1 (general) and 5.6.2 (quality control) at 'analytical processes' stage and Subclauses 5.6.4 (comparability of examination results) at 'process evaluation and improvement' stage to 100 % in Subclause 4.8 (resolution of complaints) at 'process evaluation and improvement' stage.




■ South African National Accreditation System

■ International Organization for Standardization

**Figure 9.** Relative point distribution analysis of conformance requirements of guidance checklist provided by the South African National Accreditation System. The distribution of conformance requirements by the South African National Accreditation System is represented in dark blue and the International Organization for Standardization in red. Overall, the guidance checklist of South African National Accreditation System provided coverage of 24 % when compared with the International Organization for Standardization. The level of coverage by stage level ranged from 0 % in Subclause 5.6.1 (general) at 'analytical processes' stage and Subclauses 4.11 (preventive action), 5.6.3 (interlaboratory comparisons) and 5.6.4 (comparability of examination results) at 'process evaluation and improvement' stage to 86 % in Subclause 5.7 (advisory services) at 'analytical processes' stage.

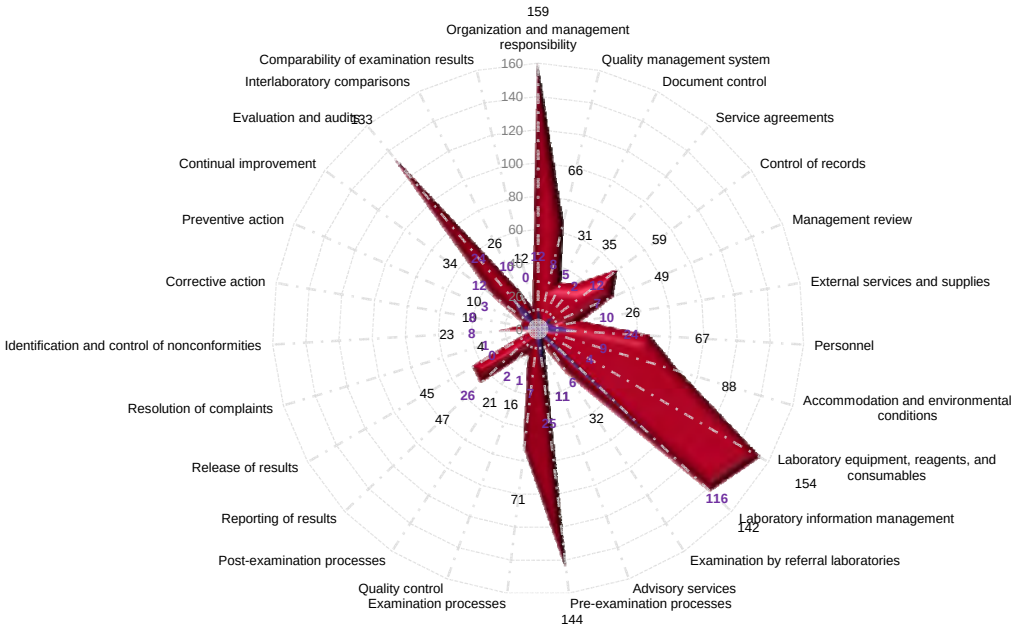



**Figure 10.** Relative point distribution analysis of conformance requirements of guidance checklist provided by the Finnish Accreditation Service. The distribution of conformance requirements by the Finnish Accreditation Service is represented in purple and the International Organization for Standardization in red. Overall, the guidance checklist of Finnish Accreditation Service provided coverage of 23 % when compared with the International Organization for Standardization. The level of coverage by stage ranged from 0 % in Subclauses 5.6.1 (general) and 5.9 (release of results) at 'analytical processes' stage and Subclause 5.6.4 (comparability of examination results) at 'process evaluation and improvement' stage to 100 % in Subclause 4.7 (advisory services) at 'analytical processes' stage.

## *DISCUSSION*

This CA study set out with the aim of quantitatively analysing the extent of CR coverage by ISO 15189:2012 guidance checklists provided by accreditation bodies. The recent emergence of a quantitative estimation of 1 515 CRs enables the formulation of an auditing tool to perform gap analysis on any ISO 15189:2012 guidance documents (15). This tool can assist organisations considering implementation of ISO 15189:2012 in the near future. The current study identified that 6/51 (12 %) accreditation bodies had readily available ISO 15189:2012 guidance checklists for medical laboratories to use for gap analysis (Table 3). The results provide four distinct areas for comparison purposes (Table 4). Each area is associated with a specific process of the ISO 15189:2012 process-based quality management system framework (Figure 1). Together with the information provided by PDA (Figures 5 to 10), the strengths and weaknesses of the evaluand checklists from the 6/51 (12 %) accreditation bodies are discussed below.

The use of the 1,515 CRs framework-derived evaluation checklists began with the in-depth quantitative analysis of CRs. The method to elicit CRs within Clauses 4 and 5 of ISO 15189:2012 (14,pp.6-39) involved the localisation of the word 'shall' in the text. Although the identification process located all related CRs (35,36), it is still possible to elicit CRs using other methods; but the relative percentages should remain very similar (Figure 2). It is important to note that quantitative analysis was conducted on Clauses 4 and 5 of ISO 15189:2012 (14,pp.6-39) only and the results of '1,515 CRs' represent the absolute minimum to consider. The potential variables address differences between countries and organisations and allow for customisation. This is set out in Clause 1 (scope) of ISO 15189:2012 (14,p.1), therefore the enumeration of '1,515 CRs' is the least quantity to consider if the medical laboratory wishes to conduct internal audit to all activities relating to the medical laboratory quality management system.

The evaluability assessment was conducted following the quantitation of CRs. It was decided that the document for evaluability assessment should be in checklist-based format and organised according to the identification and localisation of CRs in the four-stage process-based quality management system framework. It is understood that the format of the checklist varies depending on the intent of coverage comprehensiveness and it is possible to develop evaluation checklists using representative subclauses as audit criteria (37,38). This approach has been used by various organisations offering implementation support, such as the World Health Organization (39), where it has been demonstrated that it is impractical to provide complete coverage comprehensiveness in CRs to follow and difficult for implementers who need to establish the medical laboratory quality management system for planning purposes (40). With this in mind, the developed evaluation tool was able to offer assessment using a consistent, measurable and methodical approach as suggested (41). The evaluation findings were recorded as objective evidence to support conformity in the assessment results area against specific criteria, which are the subclauses of ISO 15189:2012. Overall, the use of checklists to conduct audit of fulfilment of CRs has met with marked effectiveness and efficiency.

Signatories to the ILACMRA must meet specific requirements imposed by the International Laboratory Accreditation Cooperation (42). One requirement is that all accreditation bodies are to operate in accordance with the requirements of ISO/IEC 17011:2004 (19). One such operating restriction is that accreditation bodies must ensure appropriate areas of expertise are available to the applicant while not providing direct consultancy. However, accreditation bodies can still provide general guidance in various formats to support medical laboratories for preparation for accreditation. Although the implementation process requires complex and detailed activities, the provision of support enables objectives and tasks

to be clearer and unequivocal. It was identified that 14/51 (27 %) accreditation bodies that are signatories to the ILACMRA have made available to provide general guidance documents to support medical laboratories for preparation for accreditation (Table 3). These documents represent extra effort on the part of accreditation bodies as they are not required by ISO/IEC 17011:2004 (19).

The presentation formats were largely divided into three types. The first type is provided by the International Accreditation New Zealand which operates within a specific accreditation process (43) while publishing explanatory commentaries for implementation purposes (21). The second type is provided by 7/51 (14 %) accreditation bodies that have listed duplicates of relevant subclauses of ISO 15189:2012 for medical laboratories to interpret; therefore, these checklists assemble in an exactly matching manner. The third type is provided by 6/51 (12 %) accreditation bodies that have checklists comprising modified ISO 15189:2012 content. The third type of accreditation bodies were eligible according to the selection criteria for evaluation (Table 1).

In relation to the overall distribution of CRs of Clauses 4 and 5 of ISO 15189:2012 (14,pp.6-39) in the four-stage process-based quality management system framework (Figure 2), the results are interpreted using a three-colour colour-coded classification (Figure 3). The evaluand checklists of the HKAS and the SAC achieved coverage of ≥ 85 % and 'green status'. The checklists comprised relevant contents of the subclauses rewritten as questions. This method may be useful for medical laboratories that have limited implementation experience because it enables the inclusion of almost all areas. The medical laboratories are highly likely to achieve all planned deliverables by using these checklists to track the CRs fulfilment progress in a highly structured manner. The evaluand checklists of the DANAK achieved coverage at 55 % and 'yellow-green status'. Although not as comprehensive as the HKAS and the SAC evaluand checklists, the DANAK's evaluand checklist can still give a good indication of whether CRs fulfilment is on the right track. Together with the medical laboratories own internal auditing process prior to the accreditation, it should enable the medical laboratories to make good implementation progress. The evaluand checklists of the NATA, the SANAS and the FINAS were classified as 'orange status'. Although their coverage of CRs ranged from 23 % to 45 %, it is important to understand that the checklists contain the majority of the main CRs for implementation. Although, the coverage figures appear less desirable for medical laboratories to use, medical laboratories are supposed to conduct their own initial internal audits with the support of guidance checklists. Using the results of both checking mechanisms, the medical laboratory is highly likely to achieve planned deliverables. When used together with the appropriate expertise from accreditation bodies and initial internal auditing information, medical laboratories are highly likely to undergo the accreditation in a more structured manner.

The distribution of 1,515 CRs in Clauses 4 and 5 of ISO 15189:2012 (14,pp.6-39) can be visualised by the use of radar charting (Figure 4). The main advantage of using a radar chart is to conduct relative PDA. The CRs distribution of the six selected accreditation bodies were charted and compared with the 1,515 CRs distribution as the standard. The evaluand checklist of the HKAS (44) (Figure 5) provided ≥ 93 % coverage of each subclause with the exception of Subclause 4.6 (external services and supplies) of ISO 15189:2012 (14,pp.12-13). The evaluand checklist of the SAC (45) (Figure 6) also provided ≥ 75 % coverage of each subclause with the exception of Subclause 4.4 (service agreements) (14,pp.11-12) at 49 %, Subclause 4.6 (14,pp.12-13) at 54 % and Subclause 4.12 (continual improvement) of ISO 15189:2012 (14,pp.14-15) at 47 %. The impressive coverage rate of both accreditation bodies allows medical laboratories to take a very structured approach to implementation. It is highly likely that the use of guidance checklists can support the implementation process effectively.

The guidance checklist of the DANAK (32) (Figure 7) achieved coverage of 55 % and 'yellow-green status'. However, it is important to note that the DANAK has declared that its guidance checklist 'is much shortened compared to the text of DS/EN ISO 15189:2013 and it is therefore important that the checklist is used together with ISO 15189' (32) and is a condensed summary of SFS-EN ISO 15189 entitled 'Medical laboratories. Requirements for quality and competence (ISO 15189:2012, corrected version 2014-08-15)' (46). Medical laboratories need to ensure their initial internal auditing is able to cover the CRs of Subclause 4.4 (14,pp.11-12), Subclause 4.9 (identification and control of nonconformities) (14,pp.13-14) and Subclause 4.12 of ISO 15189:2012 (14,pp.14-15) because the level of coverage by stage was ≤ 22 %. Overall, medical laboratories that use the checklist are still highly likely to make very good progress and certain to achieve planned deliverables.

The guidance checklists of the NATA (20) (Figure 8), the SANAS (47) (Figure 9) and the FINAS (48) (Figure 10) achieved ≥ 23 % of coverage and 'orange status'. This relatively low coverage rate is not an indication of the level of effectiveness. The NATA has declared that it is 'a brief summary of the clauses of the Standard' (49), while the SANAS indicates that it represents the general requirements only (47) and the FINAS has indicated that the checklist questions are representative of the relevant subclauses (48). Particular attention needs to be paid to the areas where ≤ 9 % of coverage was recorded. These include Subclause 5.6.1 (general) (14,p.33), Subclause 5.6.2 (quality control) (14,p.33), Subclause 5.6.3 (interlaboratory comparisons) (14,pp.34-35), Subclause 5.6.4 (comparability of examination results) (14,p.35) and Subclause 5.9 (release of results) of ISO 15189:2012 (14,pp.37-38). Medical laboratories need to ensure the initial internal audit process has the ability to cover these subclauses during the gap analysis.

Within the four major stages, there were specific areas of concern in the evaluand checklists involving subclauses (n = 3) that were unspecified (Table 5). It has been identified that these subclauses are associated with compliance requirements, including legislation and other non-legally binding documents (Table 5). Three potential implications are discussed below.

The first potential implication for compliance concerns Subclause 5.6.1 of ISO 15189:2012 (14,p.33), where the medical laboratory must not fabricate any results (Table 5). The intent relates to the potential for the emerging risk of theft, fraud and financial crime within the healthcare system (50). These risks can pose a serious threat to any organisation, especially in terms of reputational impact, organisational disruption and diminished patient safety (51). An effective method for medical laboratories to ensure comprehensiveness is to supplement any fraud and corruption detection elements with additional resources for internal auditors to implement mechanisms to identify corrupt or fraudulent activity (52,53). However, the additional resources need to implement an effective whistleblower protection policy to support internal auditors if findings require the involvement of law enforcement agencies (54,55). Further guidance can be sought from various external documents (56-62) (Table 5). The medical laboratory must generate reliable medical information, establish transparency in both financial and technical reporting and enforce accountability at all levels.

The second potential implication for compliance concerns Subclause 5.9.1 (general) of ISO 15189:2012 (14,p.37) where the medical laboratory must establish documented procedures for the release of examination results (Table 5). The medical laboratory must have manageable control of the release of information. The control process must be reasonably practicable to meet a privacy protection duty and the medical laboratory must be able to accept requests for the release of results, including from the laboratory information management system. One practical option is to achieve certification to demonstrate that the information security management system conforms to ISO/IEC 27001:2013 entitled 'Information technology — Security techniques — Information security management systems — Requirements' (63) and ISO/IEC 27001:2013/Cor.1:2014 entitled 'Information technology — Security techniques — Information security management systems — Requirements — Technical corrigendum 1' (64). Further guidance can be sought from various external documents (65-67) (Table 5). The medical laboratory must have effective documented procedures to grant access to relevant customers, meeting both contractual and legal requirements while operating within strict information security management system controls that address potential information security risks.

The third potential implication for compliance concerns Subclause 4.11 (preventive action) of ISO 15189:2012 (14,p.14), where the medical laboratory must determine actions to eliminate the causes of potential nonconformities in order to prevent their occurrence (Table 5). Subclause 4.11 of ISO 15189:2012 (14,p.14) contains 10/1 515 (1 %) CRs, and the one relating to the determination of preventive action poses a significant negligence risk. The medical laboratories must identify all potential causes and problems proactively (68,69). Compliance issues can be implicated if the medical laboratories violated the applicable standard of care or practice. An area that is an emerging concern is the prevention of human error. Lack of concentration and inadequate expertise are likely to cause diagnostic errors during processing (70,71). These two issues can be readily addressed. First, lack of concentration in the workplace is most likely due to human fatigue (72) resulting from sleep mismanagement (73). The control and monitoring of staff working hours is also an option to forecast and manage burnout of staff and, as an occupational risk control, highly likely to predict and prevent many unwanted issues (74). Second, an inadequate level of expertise at the allocated position should be addressed through the provision of ready access to training opportunities at the appropriate level to confirm competencies. Further guidance can be sought from various external documents (56,75-84) (Table 5).

## CONCLUSIONS

The aim of the current study was to determine the extent of ISO 15189:2012 CR coverage provided by the accreditation bodies' guidance checklists. Accreditation bodies from five countries and a dependent territory were identified as having suitable checklists for the evaluability assessment. The findings of this research provide insights into the CR detection limitations of the recommended guidance checklists issued by accreditation bodies. This research has two major practical implications. Medical laboratories need to be aware that guidance from relevant accreditation bodies may need to be supplemented by consideration of further compliance issues with international, national, regional or local regulations or requirements. A second important practical implication is the possibility that accredited medical laboratories need to develop comprehensive checklists as an internal auditing tool to confirm that all CRs are fulfilled competently. Overall, ensuring the ISO 15189:2012 conformity status is adequate at all times should be a priority for the accredited medical laboratory. It will be interesting to see whether the same accreditation bodies will provide direct guidance for the implementation of the next edition of ISO 15189, so that the same evaluability assessment can be conducted.

## AUTHOR INFORMATION

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OPINION ARTICLE

# Binary male-female laboratory reference ranges do not reflect reality for transgender individuals on sex-hormone therapy

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## ABSTRACT

Reference ranges which delineate as male or female are not currently useful for transgender individuals undergoing sex-hormone therapy, and may be misleading to clinicians. This article seeks to bring this issue to the attention of medical laboratory scientists practising in New Zealand, to raise awareness of prejudice and to shed light on current misunderstandings.

**Key words:** Transgender, reference range, non-binary

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## GLOSSARY

**Cisgender:** A term used to describe a person whose gender identity matches their sex assigned at birth (Duncan Matthews, personal communication, 2017, May 22nd).
**Fa'afafine:** A Samoan term used to recognise people born biologically as male but who embody the spirit of a woman, have female gender expressions and perform female as well as male gender roles (2).
**Gender:** Usually refers to the social and cultural construction of what it means to be a man or a woman, including roles, expectations and behaviours (3).
**Gender identity:** A person's internal, deeply felt sense of being male or female (or something other or in between). A person's gender identity may or may not correspond with their sex (2).
**Genderqueer:** A person who does not express a traditional gender identity. Some gender queer individuals may not change their physical sex or cross-dress, but can identify as genderqueer, gender neutral or androgynous (2).
**Intersex:** Describes a variety of conditions where a person is born with sexual anatomy that does not fit the typical biological definitions of male or female. Individuals may prefer to remain intersex rather than conform to male/female categories (2-4).
**Non-binary:** Relating to a gender or sexual identity that is not defined by traditional binary oppositions such as male and female (28).
**Sex:** Usually refers to a person's biological make-up and the distinction between male and female based on chromosomes and physical sexual characteristics (3,4). **Sexual orientation:** Describes the gender(s) that someone is attracted to. A person can be attracted to someone of the same sex or sexual identity as they are, (homosexual, for example gay, lesbian or queer), or the opposite sex or sexual identity (heterosexual), or attracted to both/all sexes (bi/pansexual) (2).
**Tangata ira tane:** A Māori term used to describe someone assigned a female gender at birth, who has a male gender identity (3).
**Transgender:** A person whose gender/sex identity is different from their physical sex assigned at birth (3).
**Transitioning**: Steps taken by transgender people to live in their chosen identity. These steps can be social or medical but they do not have to include hormone therapy or gender reassignment surgery (although they often do) (2,3).
**Trans man/trans boy**: Someone assigned a female gender at birth, who has a male gender identity (3).
**Trans woman/trans girl:** Someone assigned a male gender at birth, who has a female gender identity (2,3).
**Whakawahine (also Hinehi and Hinehua):** Māori terms for describing someone who was assigned a male gender at birth, who has a female gender identity (2,3).

## INTRODUCTION

Many human diagnostic markers have different reference ranges for males and females. However, this binary system of gender assignment makes no allowance for transgender people or other gender diverse groups and intersex people. As approximately 1.2% of New Zealand high school students identify as transgender (1), and because medical and emotional consequences may be high when an inappropriate reference range is applied, it is timely to review this topic in the diagnostic laboratory setting.

Gender is historically considered to be male or female, but the reality is that gender is more of a continuum. Although the majority of people identify as male or female, there is increasing awareness that sex and gender are flexible concepts, with a person's sex and gender determined not only by their X and Y chromosomes, but also by an inherent deep-rooted sense of one's self, expressed through behaviours. Most literature defines sex as a person's biological make up where XX is female and XY is male. Gender is usually defined as the social and cultural construction of what it means to be a man or woman (2-4). However, the transgender community, and increasingly biologists, do not separate out the terms sex and gender because of their significant overlap. For example, an individual with androgen insensitivity syndrome is genetically XY, but does not respond to testosterone at all and is considered to be female (provided the person also identifies as female). To address this inconsistency with sex and gender terminology throughout this paper, we will predominantly use the term 'gender'. When a person's assigned sex and gender identity do not align, an individual may identify as transgender, genderqueer, or gender fluid for example (5). There are now over 60 terms used to address different groups of gender non-conforming people including whakawahine (Māori) and fa'afafine (Samoan) (6). As well as this binary reference ranges are unable to provide for intersex individuals. The term intersex is applied to a person born with sexual anatomy that does not fit the typical biological definitions of male or female. Intersex individuals may choose to remain as intersex, rather than identify as male or female (2-4).

Laboratory request forms require that a patient's sex is identified so that male or female reference ranges can be applied. Using these reference ranges, abnormal test results are flagged allowing the clinician to recognise 'normal' and 'abnormal' results (7). This binary system of gender assignment does not allow for all individuals on the gender continuum, and most laboratory information systems (LIS) do not currently have the ability to extend beyond the binary male/female system (8,9). Pathology markers are important for monitoring health during gender changes, but because of the lack of reference ranges for transgender clients, clinicians are left to decide which results are normal for their patient (10). Published studies on transgender laboratory results are scarce. This article aims to explore the topic, with a focus on individuals undergoing hormone therapy because this group are advised to have regular laboratory testing and are most likely to frequently use laboratory services (2,3,11,12).

## GENDER AFFIRMING TREATMENTS

Transgender individuals can undergo a range of therapies to help have their physical bodies more closely align with their identified gender. Therapies for adults include hormone treatment, mastectomy, hysterectomy, orchiectomy, oophorectomy and gender reassignment surgery. Of these, hormone therapy is the least invasive and most accessible and can give transgender individuals relief from experiencing disconnection between their body and their identity (13). Table 1 shows the range of hormone options available, together with doses and delivery method.

**Table 1.** Hormone regime for transgender individuals receiving hormone therapy in New Zealand (2,11,27)

| Gender transition | Hormone class | Drug Options | Dose (pre-gonadectomy) |
|---|---|---|---|
| **Male to female** | Female sex hormone | Oestradiol valerate | 2-8 mg/day (oral) |
| | | Estradot | 100 µg 2x weekly (patch) |
| | Anti-androgen | Cyproterone acetate | 50 -100 mg/day (oral) |
| | | Spironolactone | 100-200 mg/day (oral) |
| **Female to male** | Male sex hormone (androgen) | Testosterone Sustanon | 250mg/ml. every 2 -3 weeks , intramuscular injection |
| | | Testosterone Cypionate | 200 -300 mg/ml every 2 -3 weeks , intramuscular injection |
| | | Testosterone | 2.5mg patch, 2-3 patches applied daily |

Both oestrogen and testosterone treatments work to induce secondary sex characteristics by directly stimulating receptors in target tissues (11). Treatment with oestrogen in adults causes breasts to form within 3-6 months, with maximum growth reached at two or more years. Over several years body hair becomes finer but facial hair will usually require other treatments such as laser hair removal. In adults receiving testosterone, skin starts to become oilier in the first 1-3 months. Increased muscle mass resulting in increased upper body strength is seen, together with fat redistribution from the hips and buttocks to the abdomen. Within the first 3-6 months the voice begins to crack and deepen. Menses stop within 1-6 months and facial hair development takes 1-4 years (11).

Children and adolescents can also identify as transgender and a different regimen of hormone therapy is applicable. Figure 1 shows the effect of gonadotropin releasing hormone (GnRH) in puberty. When an individual is committed to preventing the development of secondary sex characteristics, GnRH analogues ('blockers') can be administered to halt development of puberty. Administration of GnRH can stop menstruation and breast development in trans boys, and stop muscle development, voice dropping and beard growth in trans girls (2). Leuprorelin acetate is the most common GnRH analogue used in New Zealand (2) and acts to cause prolonged activation of the GnRH receptors. This leads to de-sensitisation of the receptors and indirectly causes decreased secretion of luteinising hormone (LH) and follicle stimulating hormone (FSH) (14).

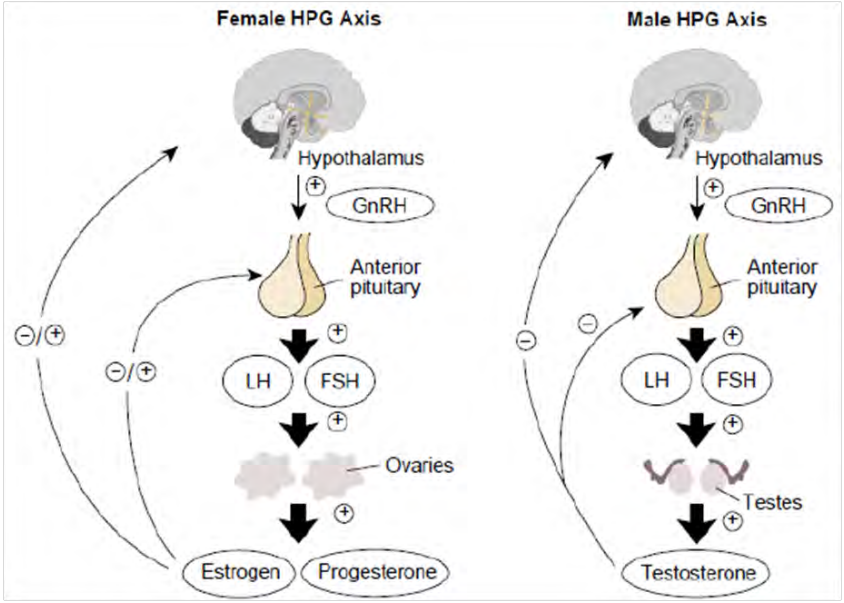


**Figure 1.** Hypothalamic-pituitary-gonadal axis in females and males. After puberty, the secretion of GnRH from the hypothalamus stimulates the release of LH and FSH from the anterior pituitary gland. This then stimulates the release of oestrogen and progesterone by the ovaries or testosterone by the testes. The negative feedback loop inhibits GnRH production by the hypothalamus. Reproduced with permission from Kong L et al. *Int J Mol Sci* 2014; 15: 21253-21269 (26).

## TRANSGENDER EXPERIENCES

Nationally and internationally, transgender people have felt unsafe sharing their gender identity due to prejudice received from healthcare providers (5). These social prejudices often stem from a lack of education around the topic of gender identity (9). The New Zealand Human Rights Commission reported in 2007 that all transgender interviewees experienced difficulties when accessing healthcare services (3). Their experiences were often "marked by discrimination [and] severe barriers to equitable services."(2). Discrimination can be as simple as a doctor assuming that a person's sex is fully aligned with their gender identity (5), using incorrect personal pronouns to address a patient, or assuming that someone who looks female cannot be named 'Trevor' and therefore has presented at the wrong clinic. In the laboratory discrimination could be rejecting specimens if their names do not match their gender identity or assuming that someone who is male cannot have a pregnancy test.

## DIAGNOSTIC MARKERS FOR TRANSGENDER PEOPLE

### Biochemistry and haematology

Table 2 shows the scope and frequency of laboratory testing recommended in New Zealand for individuals on hormone therapy for gender transition.

**Table 2.** Scope and frequency of laboratory testing for transgender individuals receiving hormone therapy in New Zealand (2).

| Gender transition | Schedule of testing | Parameter tested |
|---|---|---|
| Male to female | Baseline (before therapy commences) | free testosterone, prolactin, electrolytes, urea, creatinine, coagulation studies, lipid profile, fasting glucose, HbA1c, liver function enzymes, full blood count (FBC) |
| | 3 monthly for 1 year, then 6 monthly thereafter | free testosterone, prolactin, liver function enzymes (for patients taking cyproterone acetate), electrolytes for patients taking spironolactone, FBC, fasting glucose |
| | Annually | lipids, fasting glucose |
| Female to male | Baseline | free testosterone, plasma oestradiol, FBC, lipid profile, fasting glucose, HbA1c, liver function enzymes |
| | 3 monthly for 1 year, then 6 monthly thereafter | FBC, Liver function enzymes, Plasma oestradiol, Free testosterone |
| | Annually | Lipid profile, fasting glucose |

Hormone therapy for transgender individuals does not come without risks (15). The most significant risk identified is for individuals on oestrogen therapy (15), which is associated with increased incidence of venous thromboembolism (13,15). To assess baseline health status, and monitor health during treatment, it is recommended that lipids and liver function be obtained (2) as hormone treatments may cause liver damage (13). Hormone levels are monitored to assess the suitability of the treatment, together with markers that change as the transition takes place. Haemoglobin is one marker that changes during treatments that alter hormone levels. Because testosterone stimulates erythropoiesis (16), haemoglobin rises in individuals receiving testosterone (17). The association between polycythaemia and testosterone therapy in men has not been extensively studied but the link is suggested in several papers (2,15,18). Those on hormone therapy have increased incidence of insulin resistance and Type 2 diabetes (15,19). The cause of this is not well understood and it may result directly from hormone therapy or be a general response to hormone changes that are happening in the body (15). Weirckx *et al.* also suggested that the diabetic changes may only appear to have increased incidence because of the thorough monitoring of transgender bloods compared to the general population (19).

Roberts and Kraft analysed the bloods of 55 transgender women who had been on hormone therapy for longer than six months, and found that the haemoglobin and haematocrit had dropped to within female reference ranges (20). For transgender men, another study on 17 people found increases in the haemoglobin and haematocrit into the male reference range (18).

Lipid markers also change with hormone therapy. In the study of Roberts and Kraft, low-density lipoprotein values moved into the female reference range for individuals transitioning from male to female (20). However, in the individuals moving from female to male a small decrease in cholesterol levels and LDL-cholesterol was observed (18).

In the male to female individuals, alkaline phosphatase (ALP), potassium and creatinine remained within the male reference range (20). Interestingly, triglycerides were higher than either the male or female values in these individuals (20); however, oestrogen has been shown to increase triglyceride levels (21).

In puberty, FSH and LH levels are monitored by laboratory testing to assess if GnRH is suppressing the hypothalamic-pituitary-gonadal (HPG) axis from the normal state. FSH/LH levels should fall within pre-pubertal ranges to indicate treatment success. The management of hormone dose relies on laboratory testing to determine if levels for each individual are within the normal physiological ranges (19). The goal is to maintain the sex hormone levels within the range of the person's identified gender, and to suppress the endogenous hormones determined by the individual's genetic sex (12). GnRH analogues are considered a safe and mostly reversible treatment (2).

### Histology and cytology

Histology and cytology departments may also need to deal with binary gender identity classification problems. Transgender men will still require regular breast examinations if breast tissue remains (22) and cervical screenings every 3 years if they have not had a total hysterectomy (23). Confusion could arise if these samples are labelled as male. Long-term testosterone therapy can cause atrophy of the cervical epithelium and decreased vaginal secretions (2,15). This combined with the discomfort that trans men experience when having a cervical screening test leads to a higher rate of unsatisfactory sampling for cervical screenings- 10.8% compared with 1.3% for cis women. This can result in a recollect request and delayed test results (24). Women who have transitioned from male to female should have both breast and prostate screens, in accordance with NZ guidelines.

**Table 3.** Reference ranges from sources (a) Waitemata District Health Board Haematology Department; (b) Waitemata District Health Board Biochemistry Department; (c) LabPlus, Auckland Hospital Biochemistry Department.

| Analyte | Units | Age (yrs) | Adult male | Adult female | Uncertainty of measurement | Source |
|---|---|---|---|---|---|---|
| Haemoglobin | g /L | >16 | 130 - 175 | 115 - 155 | 2% | a |
| Red Cell Count | x $10^{12}$/L | >16 | 4.3 – 6.0 | 3.6 – 5.6 | 2% | a |
| Haematocrit | | >16 | 0.40 – 0.52 | 0.35 – 0.46 | 3% | a |
| Creatinine | µmol/L | >16 | 60 - 105 | 45 - 90 | 10.00% | b |
| Progesterone | nmol/L | | | 0 – 6 (no ovulation)<br>6 – 80 (ovulation)<br>32 – 728 (pregnancy) | 0.8nmol /L at 3nmol/L<br>18% above 24nmol/L | c |
| Testosterone | nmol/L | 15 – 19<br>20 – 50<br>> 50 | 7.6 – 28<br>8.7 – 29<br>6.7 - 26 | 0 – 1.8 | 12% | c |
| Oestradiol | pmol/L | | < 160 | 150 – 2,000 (cyclic pre-menopause)<br>< 110 post-menopause | 10% to 14% | b |

### Difficulties in interpretation of laboratory results

As shown by the results of the studies by Jacobeit *et al.* (18), and Roberts and Kraft (20), neither male nor female reference ranges appear to be most appropriate for use with transgender individuals, as some markers fall into the male reference ranges, and others within the female reference ranges. Individual's results for markers such as haemoglobin, haematocrit and lipids may also change over time, and not necessarily be indicative of disease, as it would be in a cisgender individual. Feldman and Goldberg (8) recommended that the best categorisation to use on current binary laboratory forms is:

- Use the gender assigned at birth if the individual is not taking hormones and has not undergone orchiectomy/ oophorectomy.
- If the individual is on hormones and has undergone orchiectomy/oophorectomy, use the gender aligned with hormone treatment (e.g. M for testosterone).
- Vary the gender if the individual is currently transitioning. It is best practise to explain to the individual why a certain option has been selected, and ask if that is okay with them.

However, these recommendations do not easily accommodate transitioning individuals, and also force transgender individuals who have not undergone medical steps to transition to remain identifying as their sex assigned at birth.

## *THE FUTURE*

From a clinician's perspective, it would be ideal to have an option to select both male and female reference ranges simultaneously. This option would be selected by clinicians when working with a patient who does not fit typically in the binary system and would offer safer and more effective treatment. This solution would allow the clinician to apply the reference range most appropriate for each analyte making result interpretation and patient management easier (Dr Jackie Hilton, personal communication, 2016 December 9th). It would also allow the clinician to see where in the natal sex reference range a transgender person has moved from, and where in the new range they are aiming for. Furthermore, intersex individuals could have reference ranges applied to them as an intersex person and maintain their intersex status.

The 2015 Auckland transgender community forums (25) voiced the need for *all* healthcare services to have multiple gender boxes available including laboratories. New laboratory testing forms should aim to have multiple gender/sex boxes available such as 'male', 'female' and 'non-binary' as a third category. Non-binary would bring up both male and female reference ranges, but these reference ranges could also be selected by clinicians for any patient. Ideally abnormal test results would be flagged based on what is abnormal for the individual rather than what is abnormal for their sex. A progressive laboratory information system would also be welcoming of, for example, allowing males to receive pregnancy tests and females to receive prostate-stimulating-antigen (PSA) tests.

There are plans for the New Zealand National Health Index (NHI) system to include values for both sex and gender identity. Initially, the individual's sex would also appear in the individual's gender value, but the individual would be able to access and update both their sex and gender to match their identity via an online portal requesting system. This system will allow for multiple gender identity options including transgender, whakawahine, fa'afafine and genderqueer. NHI information is not deleted, but time stamped and saved and then only available by specific requests allowing a person's gender history to remain confidential. Other related information can also be held within the NHI system and can be used, for example, to alert a trans man who has not had a full hysterectomy to have his cervical screening test (4) or to prevent reminders being sent to trans women who do not have a cervix (Robin Steel, personal communication, January 9th, 2017). This approach to sex and gender will also provide data on issues affecting sex and gender minority groups within healthcare, allowing these groups to become more visible. With the current NHI system, individuals who wish to have their name changed on their clinical records have usually been able to do so (2). Clinicians are able to contact the Ministry of Health and alter the gender marker on an NHI on behalf of their patient (3) (Duncan Matthews, personal communication, 2017, May 22nd).

## *CONCLUSIONS*

Transgender and other non-binary individuals are entitled to receive the same healthcare as any other individual, without discrimination. Providing laboratory information systems with a way to record more than the binary male or female, and supplying both male and female reference ranges simultaneously may assist in this. Transgender individuals will be long term users of laboratory services and therefore the laboratory community should be approaching ways to best serve this group with our services.

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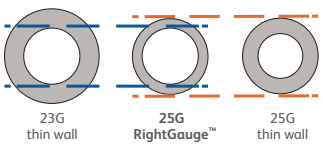


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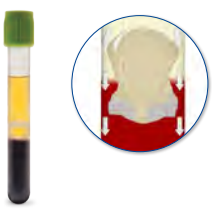

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REVIEW ARTICLE

# Deciding by numbers: the genesis of prenatal serum screening cut-off limits for Down syndrome and neural tube defects

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## ABSTRACT

The use of maternal blood markers and early ultrasound measurements to screen for fetal neural tube defects and fetal aneuploidy (primarily trisomy 21, Down syndrome) is now a well-established practice in many countries. However, the origins and background relating to how the techniques became established and how the various action limits were derived is not universally known or understood. In this paper, we consider the historical origins of the maternal blood screening tests, the establishment of the analytical parameters currently in use, the development of the nuchal translucency test and the integration of multiple analytical parameters to facilitate the diagnosis of fetal abnormalities.

**Key words:** prenatal diagnosis, Down syndrome, neural tube defects, chromosome aneuploidy, cut-off limits.

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## INTRODUCTION

Historically the outcome of a pregnancy was unknown, with the majority of parents anticipating a trouble-free pregnancy and birthing resulting in a healthy child. For some parents, however this was not the outcome and the child was born with a disability ranging from mild to severe. While certain disabilities could be corrected, or treated there remained a group of disorders for which there was little or no correction such as chromosome disorders, severe neural tube defects and certain inborn errors of metabolism.

It is interesting to note that the understanding of human chromosomes, neural tube defects and inherited metabolic diseases has evolved over the last 50 years. For example, in the mid-1950s there was considerable debate regarding the number of human chromosomes ranging from 46,47 and 48 (1,2,3)· with a final (and correct) resolution of 46. This resolution led to the discovery that Down syndrome was the result of inheriting an extra chromosome 21 giving rise to the terminology “trisomy 21” (4). Notwithstanding this discovery, there is historical evidence of Down syndrome, which pre-dates the publication of John Down in 1866. Both visual (as early as 1500BC) and archeological evidence (ca 5-6$^{th}$ century) respectively indicates that children with Down syndrome have a long historical lineage (5,6). Equally neural tube defects can be dated back to at least ancient Egypt with some archeological evidence from 10,000BC (7).

Despite this long history, Down syndrome and neural tube defects remained “an accident of birth” until the advent of second trimester amniocentesis. Limited amniocentesis had been used to detect and monitor haemolytic disease of the newborn (Rhesus disease) in the third trimester of pregnancy (8.9). Later, it was demonstrated that fetal chromosome analysis could be undertaken using amniotic (shed fetal) cells (10). These diagnostic landmarks brought about significant changes in perceptions, attitudes and diagnosis of Down syndrome and other fetal chromosome abnormalities, which was reinforced by the introduction of obstetric ultrasound in the 1970s. This offered for the first time, the opportunity to test for and identify fetal chromosome disorders at 14 to 16 weeks of pregnancy thereby fitting the time frame in the UK for termination of pregnancy. As there is a strong association of fetal Down syndrome and other chromosomal disorders occurring in women over the age of 35 years (discussed in more detail later) amniocentesis provided a powerful diagnostic tool in early pregnancy. This was subsequently extended to diagnose certain inherited metabolic disorders (11). However, as women are deferring pregnancies until later i.e. 35 years or older, a progressive increase in the incidence of Down syndrome is now being observed (12).

Although amniocentesis provided a reliable diagnostic procedure, it is invasive and has associated risks especially in the early days prior to the use of ultrasound and was time consuming. A major change in approach to prenatal diagnosis occurred in 1973 when a-fetoprotein was discovered in maternal blood and elevation of the protein had a strong association with fetal neural tube defects (anaencephaly and severe spina bifida) in early pregnancy (13). Subsequently low maternal serum a-fetoprotein levels in maternal blood were identified as having a strong association with fetal Down syndrome (14,15) thereby providing a screening test for Down syndrome for all women from an ante-natal blood sample in early pregnancy. Further research identified other maternal blood biochemical markers, which increased the accuracy of the screening test and the subsequent development of unified cut-off or action limits to identify pregnancies associated with Down syndrome. In this paper, we describe the development of the ante-natal screening programme and discuss the evolution of the action or decision making limits used in the maternal pre-natal screening programme.

## BACKGROUND

At conception and early embryonic development humans have a high rate of embryonic wastage primarily due to chromosome abnormalities with an estimated loss of approximately 70% of all embryos within six weeks of fertilization (16,17). As the pregnancy proceeds some chromosomal abnormalities persist and some will result in early fetal loss as a miscarriage (18). Amongst the most common of the chromosome abnormalities that continue during pregnancy are trisomy 21 (Down syndrome), trisomy 18 (Edward syndrome); trisomy 13, (Patau syndrome); Turner syndrome (typically inheritance of one X chromosome instead of two) and triploidy (more than one set of

chromosomes). Normally humans have 46 chromosomes (23 from each parent including either two X chromosomes (female) or XY chromosomes (male). These are all balanced with approximately equal contribution from each parent. In trisomy, an additional chromosome has been inherited such as three chromosomes 21 in the majority of cases of Down syndrome and creates a case of where more is not beneficial. In general, the term used to indicate that there are errors in chromosome numbers is aneuploidy but does not indicate a specific number or condition. One of the significant risk factors for fetal chromosome abnormalities is maternal age, with increasing maternal age strongly associated with increased risk of fetal chromosome abnormalities (19). Typically, at the age of 20 there is a risk of any fetal chromosomal abnormality of 1 in 526, by 35 this has changed to 1 in 192, and by 40 it is 1 in 66 (20). However, the most common fetal chromosome abnormality at birth is Down syndrome with an overall incidence of 1 in 800 live births (21) But the risk varies considerably with age, for example at age 20 the risk is 1 in 2300, however over the age of 45 the risk is 1 in 40 (21). This increasing risk of fetal chromosome abnormalities associated with maternal age has given rise to the development of techniques to identify potentially 'at risk' reproductively older women (over the age of 35 years) and provide diagnostic services to identify or reassure fetal normality. In addition to fetal aneuploidy, fetal neural tube defects (primarily anencephaly and spina bifida) at an incidence of approximately 1 in 1300 births are potential causes of fetal abnormality but there is some population variation (22) and prenatal detection could provide a useful diagnostic technique in identifying pregnancies affected by these abnormalities.

## *PRENATAL TESTING – BACKGROUND*

Prenatal genetic testing has made significant progress since its early introduction in the 1970s when fetal chromosome analysis became feasible with the development of amniocentesis and the ability to culture fetal cells present in the amniotic fluid sample. This service was available from 1975 in Christchurch and provided an analytical service for both the North and South Island (23). Prior to the advent of ultrasound, the amniocentesis procedure was not without risk and the risk of penetrating the placenta or the fetus with the needle was always a possibility. Miscarriage risk was always a complication; however, with the advent of obstetric ultrasound both placenta and fetus could be localized with the associate reduction in risks to the fetus. Ultrasound also reduced the risk of maternal blood contamination of the amniotic fluid sample thereby increasing the accuracy of the fetal chromosome analysis by eliminating the possibility that the chromosomes were from the mother. The development of prenatal chromosome analysis was viewed as providing a 'choice' especially for those women over the age of 35 years where the risk of a fetal chromosome abnormality starts to increase significantly (21). A culture within the medical and scientific community evolved relating to 'prevention' and many women were offered prenatal chromosome analysis from which the outcome was termination of the fetus that had a karyotype associated with known pathology. This was significantly reinforced with discovery of a-fetoprotein (AFP) in amniotic fluid in 1972 and subsequently in the maternal blood in 1973 (13,24) with the significant association of elevated AFP with neural tube defects (anencephaly and spina bifida). This discovery was a milestone in the advancement of prenatal diagnosis at a time when obstetric ultrasound was still in its infancy. For the first time a biochemical analysis could reliably predict fetal outcome without the need of fetal cell culture and maternal serum screening using AFP became part of the obstetric management, often used in conjunction with amniocentesis. A major advantage of AFP testing was that the optimum time for diagnostic use (15 to 19 weeks-gestation) coincided with the optimum time for fetal karyotype analysis and fitted comfortably with the legal time frame for termination of pregnancy in the United Kingdom, where the research was conducted. However, there was variability in interpreting individual results within the reference ranges for AFP both in amniotic fluid and maternal serum largely due to variation in methods used in different testing centres, which was overcome by a statistical manipulation of reference ranges, rather than use the conventional statistical technique of percentiles or standard deviation around the mean. Subsequently it was proposed that results could be expressed in relation to multiples of the median (MoM) linked with gestational age (25). Intensive research ensued to seek new biochemical markers of fetal disorders and to improve the reliability of the AFP testing. Whilst there was a clear association with elevated AFP and the neural tube defects data emerged that there was another cohort of AFP results that were in the lower region of the maternal serum AFP values and were identified to be associated with fetal chromosome abnormalities (14,15). Analysis and refinement of these data identified a correlation between low maternal serum AFP and Down syndrome (14). Subsequent research provided a risk estimate for Down syndrome using low AFP values and maternal age (26).

## *DIAGNOSIS BY NUMBERS*

The advent of numerical data to undertake prenatal diagnosis was a major shift in concepts of diagnostic techniques in obstetrics. Karyotype analysis was visual, early (first and second trimester) examination of the fetus essentially relied on the 'laying on of hands', reliable gestation dates became essential for the accurate interpretation of the biochemical results, and diagnosis shifted from clinician based to laboratory based information systems. Paralleling the development of the biochemical diagnostics was the development and implementation of the use of ultrasound to measure fetal biparietal diameters and crown rump length, which correlated with gestational age thereby providing accurate gestational dates to assess the biochemical results. Although an accurate independent technique could be used to establish gestational age, another issue arose that different laboratories had variable reference ranges for AFP results, raising the possibility of misinterpreting diagnostic results. This dilemma was largely overcome by using the multiple of the median. Using this technique, the individual result is assessed as to how far it deviates from the median, this normalized the result and provided a technique to compare results both from individual diagnostic centres but to also undertake retrospective analysis of data. Threshold levels could be established as multiples of the median for a given gestational age, where the most likely cut off would detect the maximum number of neural tube defects (25). Re-analysis of data from the prenatal diagnosis of NTD revealed a set of data with very low maternal serum AFP levels (contrary to the high levels associated with NTD) these data were found to correlate with fetal chromosome abnormalities (15,27) and the possibility of a maternal serum marker as a screen for Down syndrome quickly became a reality, with the finding initiating a new analysis of numerical data (26). As the AFP data was now in the form of MoM large multi-centre collaborative studies were undertaken with pooled data confirming the original reports. As the incidence of Down syndrome is linked to advancing maternal age a regression curve model was developed, which integrated the risk factor for increasing frequency of Down syndrome with the maternal serum AFP value (26). Additional discovery of maternal serum biochemical markers associated with Down syndrome and other fetal chromosome abnormalities continued with elevation human chorionic gonadotrophin (βhCG) values associated with Down syndrome (28), low maternal blood unconjugated oestriol (29) and the development of the 'triple screening test' for Down syndrome integrating maternal serum AFP, βhCG and unconjugated oestriol (30). Using the triple screen plus ultrasound dating and the risk factor associated with maternal age >39 years the expected detection rate for Down syndrome was predicted to be 93% with a false positive rate of 24%, which increased with advancing maternal age (31). In 1992, a fourth marker maternal blood marker was identified to correlate with the antenatal prediction of a Down syndrome fetus, Inhibin A, which is a placental protein and is elevated above the median in the majority of cases of Down syndrome (32).

This led to the development of a four-marker biochemical screening system utilizing ultrasound, AFP unconjugated oestriol, βhCG and Inhibin A. Taken overall the detection rate was 92% and the false positive rate improved to 19.1% (33). A further placental protein was identified as a potential maternal serum marker. Pregnancy-associated plasma protein A (PAPP-A), which was decreased in both Down syndrome and other chromosomal abnormalities in the first trimester. (34). Decisions had to be made on the most appropriate time to screen, which markers had the greatest utility and statistical modeling to define cut-off limits appropriate for the stage of gestation. Using a meta-analysis approach, it was determined that screening in weeks 9 to 11 using maternal serum PAPP-A, free βhCG, and unconjugated oestriol a detection rate of 65% was predicted with a false positive rate of 5% and if ultrasound nuchal translucency (see next section) was included the combined prediction rate rose to 88.3% (35). Various mathematical models were applied to the maternal serum markers relating to the best possible diagnostic combination and the combination across the first and second trimester of nuchal translucency, PAPP-A, AFP, unconjugated oestriol, βhCG and inhibin-A gave a 93% detection rate with a false positive rate of 2.6% (36). The overall screening results have been analysed in the UK in two programmes; SURUSS (Serum, Urine and Ultrasound Study) and FASTER (First and Second Trimester Evaluation of Risk Trial), which indicated either the first or second trimester integrated screening progrmames were effective but did not support the use of individual screening tests (37).

## *NUCHAL TRANSLUCENCY*

This is classed as a 'soft' marker for fetal aneuploidy as it is non-specific and often transient. The nuchal translucency can be visualized using ultrasound in both normal and aneuploidy fetuses, and must be used before 14 weeks gestation as it can no longer be seen by the end of the second trimester and nuchal fold measurement would be required (38,39). In Down syndrome and other aneuploidies the nuchal translucency behind the neck of the fetus 'thickens' due to an accumulation of fluid and about 20% of fetuses with increased nuchal translucency will have chromosomal abnormalities (40) with the incidence increasing as the nuchal translucency thickens. However, this is not a specific test and increased nuchal translucency has been reported in congenital heart defects, certain autosomal disorders and some structural abnormalities (41,42). There is some debate relating the most appropriate cut-off in relation to the 95$^{th}$ or 99$^{th}$ centile, however, a consensus is that above the 99$^{th}$ centile (3.5mm) represents an increased nuchal translucency (38,43). Used in conjunction with the biochemical markers across first and second trimester and factoring maternal age, the combination of nuchal translucency, PAPP-A, maternal serum AFP, unconjugated oestriol, βhCG and inhibin-A gave a 93% detection rate with a false positive rate of 2.6% (37).

## *WHAT CAN GO WRONG?*

As with all biochemical tests there are factors, which will influence the result and the use of the above maternal blood screening tests are no exception. The mathematical calculation of risk is complex involving maternal age, multiple analytical parameters, the conversion of data to MoMs and a comparative analysis to a population based model using multivariate Gaussian statistics. This relies on the use of software of which there were a number of versions some of which did not give consistent comparative results (44). In addition, although MoMs have been generally accepted as a normalizing method, questions arose about the universal acceptance of MoMs due to how differing centres calculated their threshold values and considered that many threshold MoM values related to differing percentiles of gestational age–dependent distributions (45). Additional factors were identified as introducing variance of the results. Variable results from AFP, βhCG and unconjugated oestriol from four population groups in the USA, (Caucasian, Afro- Caribbean, Hispanic and Asian) were identified and although this variability did not bias screening for Down syndrome it was considered that higher normal values for serum AFP in Asian populations may have to use adjusted median values (30,46). An additional report indicated that smokers may also have to use separate MoMs as they identified that smoking modified the risk estimate for Down syndrome from 1 in 250 to 1 in 200 when compared with non-smokers (47). Later research indicated that smoking reduced the serum PAPP-A by 15% in the first trimester and βhCG by 18% in the second trimester which may impact on the mathematical modeling of MoMs (46). Serum AFP, βhCG and unconjugated oestriol are lower in Type 1 diabetes which may require adjustment to the MoMs to provide a reliable risk prediction (48). Result adjustment for maternal weight as a compounding factor for serum AFP has been proposed but not a significant influence on the AFP (49). At the time, no reliable data was available for multiple pregnancies and adjustment of data was considered unreliable in predicting fetal risk and first trimester nuchal translucency was considered to provide the only test for these pregnancies. Notwithstanding the issues outlined above, other factors relating to fetal distress, death, miscarriage, etc. will all influence the biochemical marker results and possibly skew the interpretation of risk. Although nuchal translucency and nuchal fold measurements rely on biophysical data concern has been expressed that the training and quality of results may lead to unsatisfactory measurements and potential misinterpretation especially in situation where nuchal measurements are being used in isolation (50,51).

## *WHY SCREEN FOR NEURAL TUBE DEFECTS AND FETAL ANEUPLOIDY?*

What constitutes a NTD? These are defects, which occur during early embryo development when the area (the neural tube) in the embryo is destined to become the spine, spinal cord, brain and skull are formed. Failure to close at the specified time during embryogenesis can result in a wide range of abnormalities ranging from anencephaly (failure of the top of the skull and brain to develop) to varying degrees of the spine failing to close (spina bifida) of which meningomyelocele (failure of the posterior or caudal part of the spine to close) is the most common (52). In a Caucasian population, the incidence of neural tube defects is around 1 in 1300 live births, however this estimate varies with ethnic origin and geographical location, for example: in Northern China the incidence is 9 per 1000 and in Japan 0.6 per 1000 births. Analysis of birth records in Christchurch, New Zealand between 1970 to 1975 indicated that the overall incidence of neural tube defects was 5.86/1000 births (53). The majority of NTDs are non-syndromic and although genetic factors are considered to influence NTDs there is also an environmental consideration and a link with maternal age (22,54). This is indicated in Ireland where the incidence of NTDs in 1974 was 8 per 1000, however in 1994 the incidence was 3.3 per 1000, which has been associated with a significant increase in folate (a water-soluble vitamin strongly associated with decreasing incidence of NTD) the in the diet (52,55). Folate therapy however, must be commenced prior to becoming pregnant and cannot correct an existing fetal neural tube defect. The ability to screen in early pregnancy to detect the more severe forms of NTDs provides the intending parents options on deciding pregnancy outcomes and possible future care of a child with a disability.

Aneuploidy is an error in cell division whereby the resulting daughter cells contain the incorrect chromosome number, either extra or less. In the majority of cases the fetus will not survive in-utero and will miscarry (56). Those that do survive to birth will have defects associated with the incorrect chromosome number, which accounts for 0.16 to 0.27% of live births in humans (56). The most well-known of which is Down syndrome. Typically, infants with Down syndrome may have heart defects, thyroid gland dysfunction, digestive tract problems, facial and hearing defects as well as intellectual disability and repeated infections. Unfortunately, the prenatal diagnosis of Down syndrome cannot indicate the severity of the intellectual

disability associated with trisomy 21; however, heart and other structura l defects are often identified during prenatal ultrasound scans. Trisomy 18 (Edward syndrome) which affects 1 in 3000 live births, however, is associated with a high rate of fetal and neonatal death as well as multiple abnormalities and those that do survive have severe to profound developmental disabilities (57)). Trisomy 13 (Patau syndrome), which affects 1 in 5000 infants, may survive beyond birth to one year but will die due to the multiple abnormalities associated with this disorder. As pregnancies are deferred and maternal age increases so will the associated risk of fetal aneuploidy and the necessity to consider options at a late stage in a woman's reproductive life.

## THE FUTURE OF PRENATAL DIAGNOSTICS

Although severe NTD can now be identified using ultrasound and the techniques are becoming increasingly sophisticated and sensitive in identifying fetal abnormalities, it still does not provide a definitive fetal diagnosis for chromosome abnormalities. Currently the two most reliable techniques are chorionic villus sampling and amniocentesis with fetal karyotype analysis. These are close to 100% accurate but clearly carry risk, particularly of miscarriage. New technologies however, could well eliminate these risks and provide safer options in prenatal diagnosis. It was established over 20 years ago that fetal cells were detectable in the maternal circulation often as early as six weeks' gestation (58) and that fetal DNA can be detected in the maternal circulation giving rise to speculation that this discovery could lead to the development of non-invasive prenatal diagnosis (59). Subsequent work using ivf and embryo replacement, established that fetal DNA could be detected in the maternal circulation as early as day 18 post-conception and concluded that the fetal DNA detected resulted from the trophoblast as the fetal circulation proper is not established until around day 28 post conception (60). However, other workers have indicated that the fetal DNA exists in fragments 80% of which are less than 200bp and represents approximately 6 per cent of the circulating cell free DNA in the maternal circulation, the remainder being maternal (61). Notwithstanding the disproportionate relationship between fetal and maternal cell free DNA reports from large population cohorts indicate that use of fetal cell free DNA for identifying the most frequent fetal trisomies (13,18 and 21) had a higher predictive value than serum biochemical and nuchal transparency analysis (62,63). Further refinement of the fetal cell free DNA techniques has indicated that identification of fetal microdeletion syndromes was possible (64). These microdeletions are independent of maternal age and would not normally be detected using current cell free fetal DNA (cfDNA) analysis or the normal techniques used in chromosome karyotyping (64,65).

The potential of non-invasive prenatal testing (NIPT) for fetal aneuploidy using cfDNA is already raising questions however relating to patient information, technical competency, limitations on identifying relevant clinical conditions, and ethical concerns (66). In addition, it has been proposed that in maternal low-risk populations the positive predictive value was found to be less than 50% (62). The Society of Maternal-Fetal Medicine (2016) has recommended that the cell free DNA technology should not be used for low-risk pregnancies and that cfDNA screening results should be confirmed by an alternative technique such as chorionic villus sampling (67). Further research has identified that here is a possibility of the detection of occult maternal malignancies that may be discordant with the fetal karyotype and these results required follow-up (68). An alternative consideration is the presence of circulating fetal cells in the maternal blood that are detectable from approximately seven weeks of pregnancy and can persist for years (69,70). The ability to isolate such cells provides an alternative opportunity to isolate fetal DNA and analyze it using molecular biology technologies and bioinformatics techniques. A more exciting future is the ability to 'switch off' the expression of the extra chromosome in the trisomies. Recent research using cell culture has demonstrated that it is possible (*in-vitro* at least) to silence the additional chromosome 21 associated with Down syndrome using a mechanism that normally silences one of the X-chromosomes in women (71,72). Whether this may translate in to a future treatment and the implications associated with human genome modification still remain to be considered as has been recently highlighted with the molecular 'engineering' of non-viable human embryos (73,74).

## DISCUSSION

Scientific developments in prenatal diagnosis have made a major contribution to the identification of fetal abnormalities and genetic diseases at a time when decisions can be made on pregnancy outcomes. For many this has meant the termination of the pregnancy when an affected fetus has been identified. However, central to the use of early pregnancy prenatal screening is the issues relating to understanding the nature of the procedures, consent and the decision-making processes. Over time the concept of prenatal diagnosis has changed from 'prevention' to offering choices of pregnancy outcomes. What is the nature of 'consent' when ultrasound scans and 'routine' blood samples are taken and testing initiated? This concept is critical in the use and acceptance of the screening procedures. For example, if the results of the screening indicate an abnormality such as a fetal aneuploidy, will the woman proceed to chorionic villus sampling or amniocentesis to verify or otherwise the screening result? In a reproductively older woman with a first pregnancy will the risks of a miscarriage (albeit small) out-weigh the potential benefit? Entering in to a prenatal screening programme the outcome must be understood in order that autonomous choice and informed consent are within an ethical and legal framework. A survey of New Zealand maternity carers identified that there was strong support for the introduction of NIPT in the first trimester; however, of particular concern was that of the 108 maternity carers interviewed, only 20% would refer to a genetic counselor, and the majority would provide support themselves, raising questions relating to whether there is relevant expertise within that group (75). In addition, it is important to consider individual beliefs and cultural norms in relation to the information provided and the management of the results and subsequent out comes resulting from prenatal testing (76). The decision-making process based on a set of complex data analysis will for many be difficult to reconcile especially when the expectation is a routine clinic visit which may not have any other personal support and time is not on the side of extensive consultation.

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*ORIGINAL ARTICLE*

# Diffuse fine speckled (DFS70) antibody in ANA testing: identification and proposed management in a New Zealand tertiary hospital setting

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## ABSTRACT

**Objectives:** DFS70 antibody gives an ANA Immunofluorescent (IIF) staining pattern (homogeneous / speckled), frequently at high titre that is often seen in AARD patients although DFS70 antibody is rarely associated with systemic autoimmune – based rheumatic disease. The purposes of this study were to (a) identify if DFS70 antibody was present in our test population (b) determine if DFS70 patterns could be identified with first-round IIF ANA testing (c) establish the relationships between IIF, CMIA (Chemiluminescence) and LIA (Line Immunoassay) methodologies for identification of DFS70 antibody and (d) propose a testing algorithm for implementation at LabPLUS that would allow the identification of DFS70 antibody. This is the first published study on a NZ patient cohort.

**Methods:** Patients referred for routine ANA testing were selected as presumptive DFS70 antibody positive and were then subsequently tested by both CMIA (INOVA Diagnostics BIOFLASH™) and LIA (Euroimmun EUROLINE Profile 3 Plus DFS70™) methodologies.

**Results:** DFS70 antibody was proven to occur in NZ patients referred for ANA testing. IIF methodology in the hands of experienced operators correctly identified approximately 80% of DFS70 antibody positive patients. As second round confirmatory assays, both the CMIA and LIA methodologies were effective in the role.

**Conclusions:** On the basis of the results of the study a testing algorithm was designed to allow the identification and reporting of DFS70 antibody. It is believed that when testing is implemented there will be significant fiscal benefits from reduced inappropriate testing as well as improved rheumatology outpatient clinic patient triaging over time. We further believe that implementation of DFS70 antibody testing and reporting will, for a proportion of patients reduce their anxiety and greatly reduce if not completely eliminate the opportunity for inappropriate treatment based on apparently significant ANA results.

**Keywords:** AARD, IIF, CMIA, LIA, DFS70 antibody, NZ patient cohort, testing algorithm.

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## INTRODUCTION

The detection of antinuclear antibodies (ANA), directed against intracellular antigens is a key serological feature of ANA associated rheumatic diseases (AARD) (1-3). Although the acronym 'SARD' (systemic autoimmune rheumatic diseases) is often used synonymously with AARD, the latter also encompasses RA, AAV and APS diseases (1) which is why AARD was adopted for this study. The most commonly used method for the detection of ANA is indirect immunofluorescence (IIF) using Human Epithelial Polyoma type-2 (Hep-2) cells (4,5) and this has been the screening test of choice for ANA for many years (5). A recent review by the American College of Rheumatology indicated that this should remain the gold standard for ANA testing despite new techniques and technologies being available (6).

Although high in assay sensitivity, the main drawback of ANA testing is the limited specificity for SARD (2,4,7), as the presence of ANA directed against intracellular antigens is associated with a wide range of disorders (8). Studies have also shown that ANA can return positive in up to 20% of apparently healthy individuals (HI) (2,4,8) with the majority of these positives resulting from antibodies against the dense fine speckled 70 antigen (anti-DFS70) (2,8).

Anti-DFS70 was first identified in 1994 in a patient with interstitial cystitis (3,4) but has since been found in sera of patients with a variety of conditions (2) including healthy individuals and those with no evidence of AARD (2,9-10). Due to the high prevalence of anti-DFS70 antibodies in ANA positive healthy individuals (1), it has been postulated that the presence of anti-DFS70, particularly when this occurs in isolation from other clinically relevant autoantibodies (2,9), could be used to help exclude the diagnosis of AARD (2,10). This is important, as the distinction between ANA positive HI's and those with early/undiagnosed AARD is vital in appropriate triage and referral of patients to tertiary services (3).

Although multiple studies have been performed worldwide and evidence has been provided to suggest that anti-DFS70 antibodies are not associated with AARD, there is no literature or study that is specific to New Zealand. As a tertiary hospital – based referral laboratory, LabPLUS processes ANA screening tests from patients with high and low pre-test probabilities of having AARD as well as those patients with identified AARD's who are under treatment.

The aims of this study were to (a) identify if DFS70 antibody was present in our test population; (b) determine if DFS70 patterns could be identified with first-round IIF ANA testing; (c) establish the relationships between IIF, CMIA (Chemiluminescence) and LIA (Line Immunoassay) methodologies for identification of DFS70 antibody; and (d) propose a testing algorithm for implementation at LabPLUS that would allow the identification of DFS70 antibody.

## METHODS AND MATERIALS

### Patient cohort

Over the period November 2014 – November 2015, sera from 57 patients tested for ANA by IIF were retained at -80$^0$C. The cohort was selected on the basis that all sera had homogeneous and speckled patterns that implied (by comparing with literature – based images) a high pre-test probability for the presence of DFS 70 antibody.

Five patients had >1 bleed giving a total of 63 sera. The cohort comprised 13 males (median age 51 years; range 6 – 83) and 44 females (median age 52 years; range (17-95). Patient diagnosis (of AARD or otherwise) was determined by clinical chart review of medical records. Patient identity was not disclosed and data was used anonymously. As this was a retrospective study with no modification on clinical decision making or individual follow up, patient consent was not required.

All 63 sera were tested by both CMIA and LIA methodologies. Sera tested by CMIA and LIA underwent a single freeze-thaw cycle. Patients were identified as having DFS70 antibody if both CMIA and LIA methodologies were reactive. Equally, if both methodologies were non-reactive, patients were identified as not possessing DFS70 antibody. Patients demonstrating discordant CMIA / LIA results were identified as having an undetermined DFS70 antibody status.

### Diagnostic assays

#### Indirect Immunofluorescence (IIF)

Patient sera were initially screened at a 1:80 dilution and those giving an initially reactive screen result had testing repeated at dilutions of 1:80, 1:320 and 1:1280. The assay used was supplied by Immunoconcepts™ (USA) and the cell line was their SSA transfected Hep-2000™ product. Testing was performed in accordance with the manufacturer's instructions. Serum dilutions, incubations and wash steps were performed using a Theradiag CARIS® 4 – probe robotic pipetting system. Resultant fluorescence was viewed independently by two senior medical laboratory scientists with a Zeiss LED AXIO Lab.A1 microscope. Images were captured with a Zeiss Axio Cam MRC® digital camera and ZEN® image processing software.

#### Chemiluminescence (CMIA)

All specimens were processed in accordance with manufacturer's instructions on the QUANTA Flash® benchtop CMIA analyser (Werfen group / INOVA Diagnostics, USA). Briefly, sera is pre-diluted by the instrument and then mixed with paramagnetic beads coated with recombinant DFS 70 antigen. Following 37$^0$C incubation, beads are magnetised and washed. This is followed by second 37$^0$C incubation with an isoluminol derivative labelled anti-human IgG conjugate. Again, beads are re-magnetised and washed. Triggers (high pH, hydrogen peroxide and an unstated catalyst) are added to the reaction cuvette which initiates the light reaction, recorded and interpreted by the instrument as Relative Light Units (RLU's). The RLU produced is directly proportional to the concentration of DFS70 IgG in the original serum specimen. The assay cut-off for reactivity is 20 RLU.

#### Line immunoassay (LIA)

All specimens were processed in accordance with manufacturer's instructions using the EUROIMMUN ™ EUROLINE ANA Profile 3 plus DFS70 LIA® and the EUROLineScan® band interpretation software. Briefly, 1:100 diluted sera are incubated with re-hydrated strips containing recombinant DFS70 antigen. After a 30 minute ambient incubation and wash step strips are re-incubated (30 minutes) with an alkaline phosphatase labelled anti-human IgG reagent. Strips are re-washed and incubated for 10 minutes with a substrate (NBT-BCIP) reagent. Strips are then dried, mounted, scanned and digitally evaluated using the EUROLineScan® software. Line intensity readings of >11 units are considered specific.

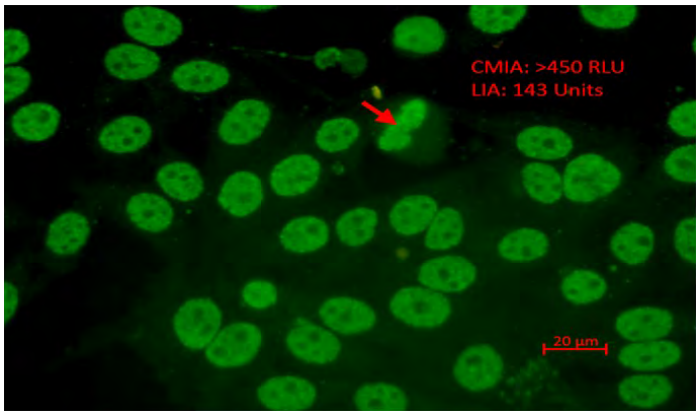
#### Statistical analyses

Statistical analyses were performed using GraphPad PRISM 7™ software (La Jolla, California, USA).

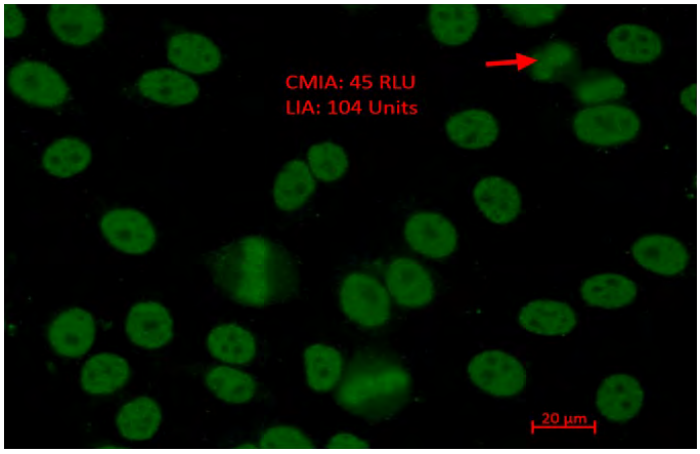
## RESULTS

### IIF

The success rate of identifying DFS70 antibody in the selected patient cohort was 75% (47/63 sera). Patients with multiple bleeds had the same pattern and end-point titres on all occasions. The staining pattern for DFS70 antibody (homogeneous / speckled with discrete moderate sized speckles visible in both interphase and mitotic cells) was consistent irrespective of assay strength as determined by CMIA and LIA methodology (Figure 1).




(a)




(b)

**Figure 1.** DFS70 antibody IIF staining pattern on Hep-2000 cell substrate. Micrographs of DFS 70 antibody staining on Hep-2000 cell line X400 magnification where antibody is present in both (a) high and (b) low levels. Discrete speckling is visible in both interphase and mitotic (arrow) cels.

### CMIA and LIA

A single patient did not have sufficient specimen to allow LIA testing. The qualitative CMIA result for the patient was 'not detected' with a RLU value of <3.0. All sera (N=10/ 10) with a CMIA RLU value of <3.0 were negative for DFS70 antibody by LIA, and on this basis, this patient serum was characterised as DFS70 antibody negative.

Method agreement was 94% (10 dual methodology DFS70 antibody negative; 48 dual methodology DFS 70 antibody positive). The two methods demonstrated a moderate to strong correlation (r = 0.811) when $\log_{10}$ transformed sample to cut-off (S/CO) ratios from DFS 70 antibody positive sera were subjected to LR analysis (Figure 2). The five patients with >1 specimen, all of whom were DFS70 antibody positive effectively had stable antibody levels over time (Table 1).

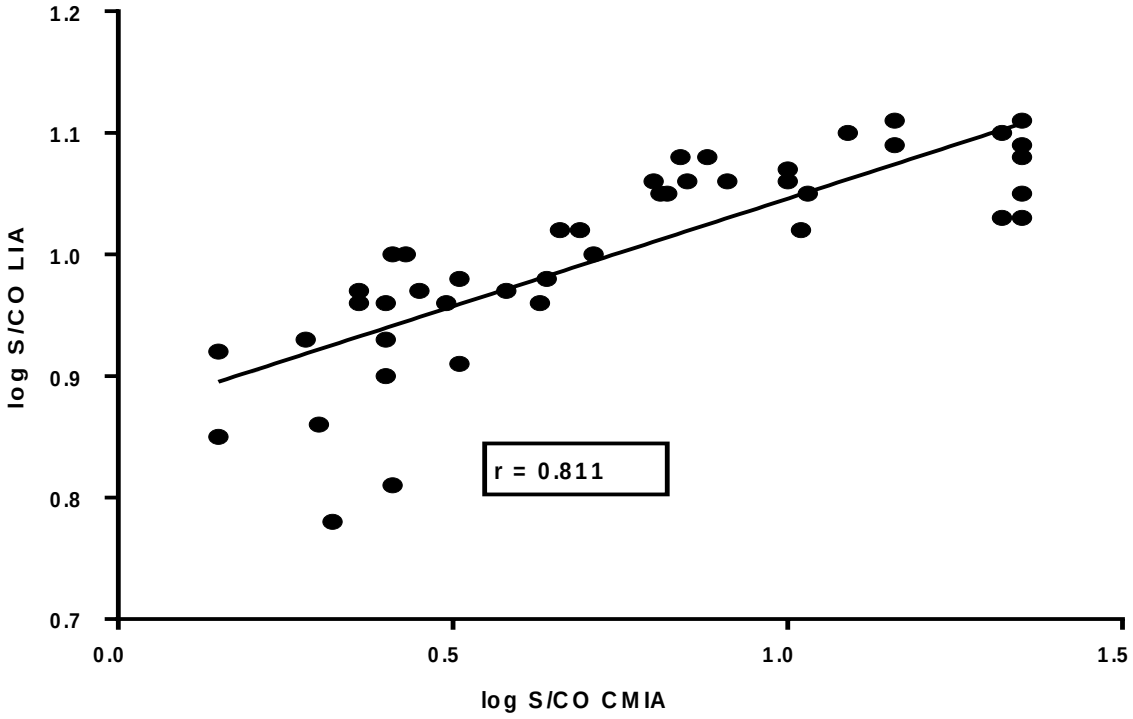



**Figure 2.** Methodology (CMIA / LIA) correlation in a cohort of patients demonstrating dual method reactivity for DFS70 antibody.

**Table 1**. Stability of DFS70 antibody levels in five patients.

| Patient | Bleed | CMIA (RLU) | LIA (Units) |
|---|---|---|---|
| 1 | Initial | 128 | 122 |
| | +40 days | 138 | 132 |
| | +55 days | 139 | 126 |
| 2 | Initial | 55 | 110 |
| | +50 days | 52 | 110 |
| 3 | Initial | 243 | 140 |
| | +37 days | 213 | 124 |
| 4 | Initial | >451 | 132 |
| | +35 days | >451 | 119 |
| 5 | Initial | 56 | 102 |
| | +16 days | 76 | 102 |

**Table 2.** Patients with discordant methodology results for DFS70 antibody.

| Patient | CMIA RLU Cut-off: >20 | LIA Units Cut-off: >11 |
|---|---|---|
| 1 | 12 | 68 |
| 2 | 12 | 48 |
| 3 | 18 | 74 |
| 4 | 11 | 46 |

There were 4 patients with discordant methodology outcomes, all of whom were reactive by LIA and negative by CMIA (Table 2). Interestingly, the mean ± SE CMIA RLU (13.25 ± 1.601) from this group was significantly different ($P<0.05$; Students – t- test) from both the dual methodology DFS70 antibody positive group (155.9 ± 20.1) and the dual methodology DFS70 antibody negative group (<3.0 ± 0.0), implying the presence of low level specific DFS70 antibody. None of the four patients were reactive for either dsDNA or ENA antibodies.

## Patient cohort

Due to difficulties in accessing clinical notes, 51 of the original 57 patients were included and analysed with respect to their pathology status. For both males and females there was an inverse relationship between numbers of patients tested and frequency of DFS70 antibody per age class as patient age increased. Over the age range of 30-54 years there was a 1:1 ratio of patients tested: patients DFS70 antibody positive for both sexes (Figure 3).

Two DFS70 antibody positive patients had low titre (7-12 IU/mL [<7]) dsDNA antibody (RIA) results without detectable ENA antibodies. Both patients did not have clinical features of a CTD. A further DFS70 antibody positive patient with a history of SLE was negative for dsDNA antibody (RIA) but had isolated Ro52 antibody detected on ENA antibody panel testing.

A single DFS70 antibody negative patient with stage 4 kidney diseased due to lupus nephritis had high titre (>45 IU/mL [<7]) dsDNA antibody (RIA) results with isolated Ro52 antibody detected on ENA antibody panel testing.

Within our selected patient cohort we identified a high rate (>75%) of non-specific ANA requesting (with respect to clinical features of a possible AARD) which applied in the settings of DFS70 antibody being present or absent (Figure 4).

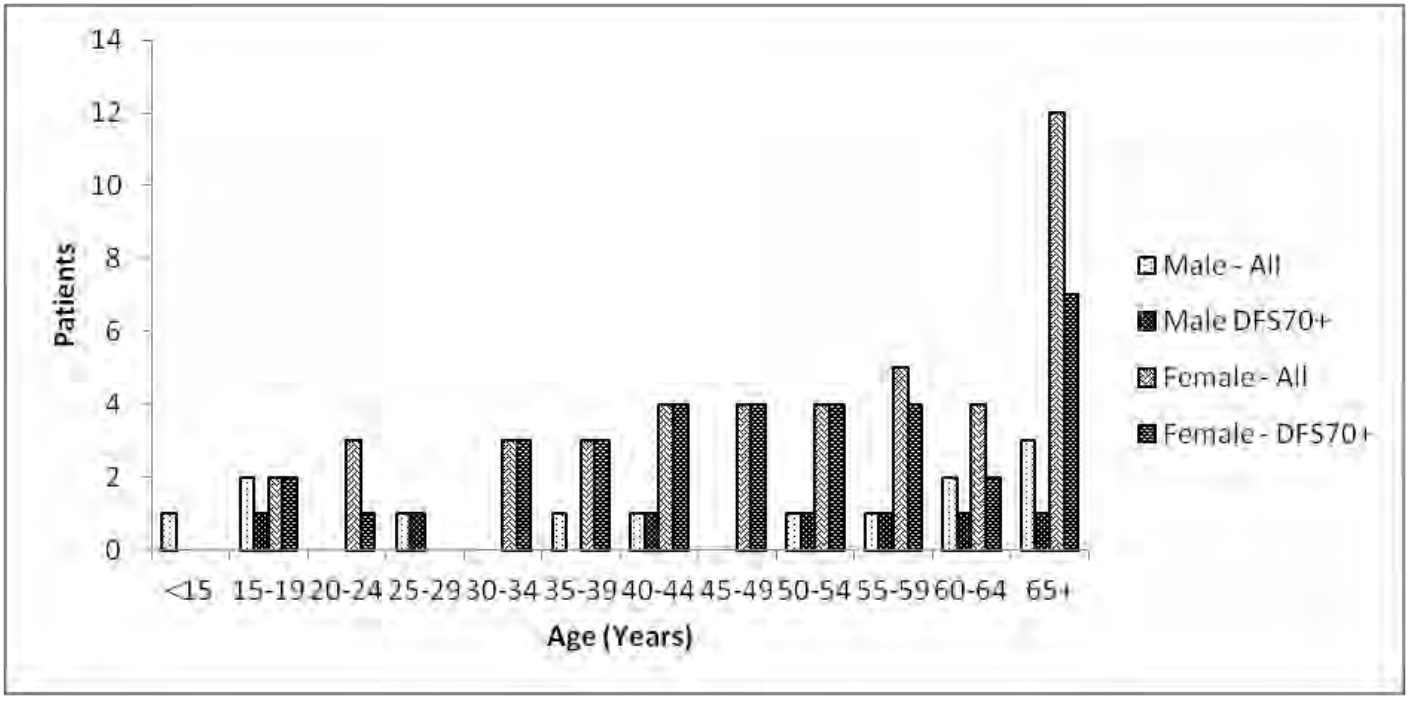



**Figure 3.** Frequency of DFS70 antibody in a selected patient cohort.

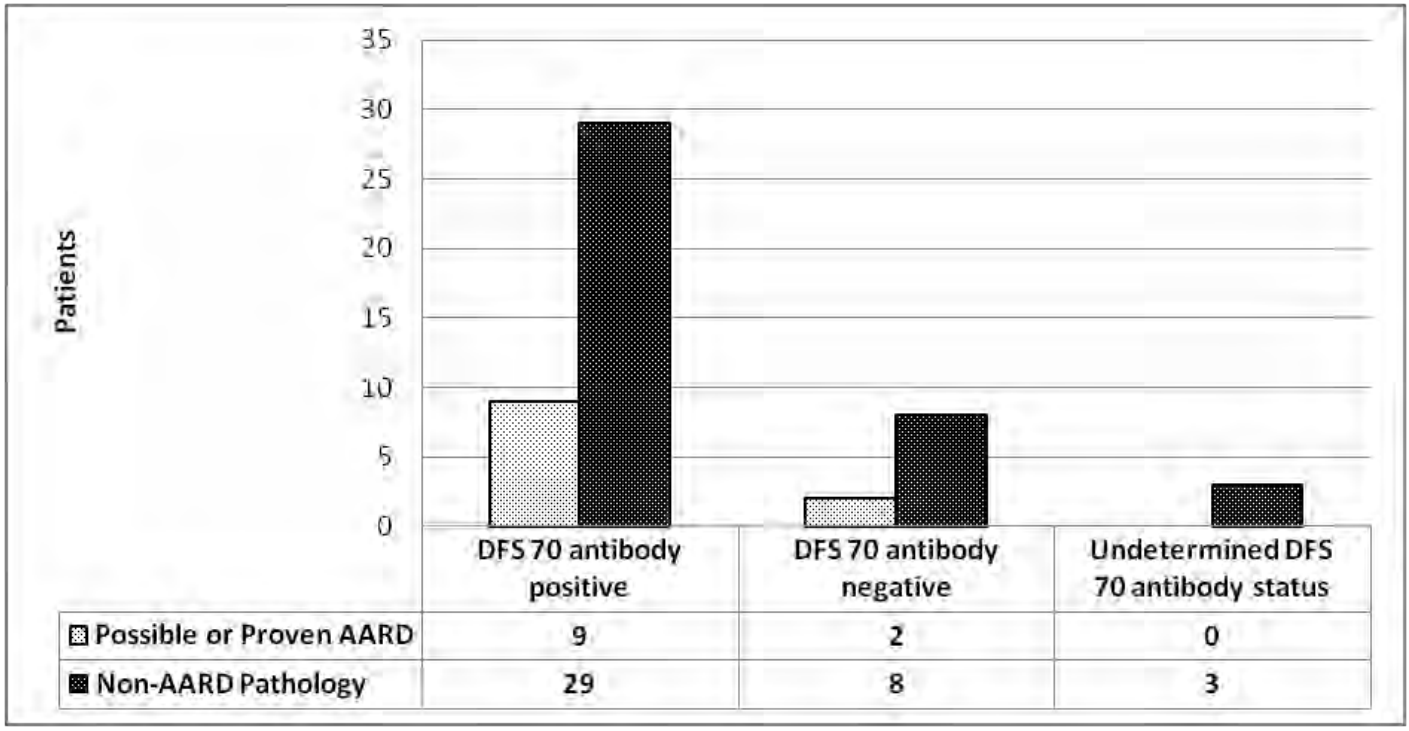



**Figure 4.** Relationship of the frequency of DFS70 antibody to AARD pathology in a selected patient cohort.

## *DISCUSSION*

This study proved that a proportion of patients who are referred for ANA testing at LabPLUS have DFS70 antibody and, the titres and patterns (range 1:320 - >1:1280; homogeneous - speckled) reported are also seen in patients with AARD's. We believe this is the first published study in a NZ patient cohort. This study further demonstrated that the DFS70 antibody, when present, remains at a consistent titre over time. Published studies to date have used characterised disease and non-disease single bleed patients as opposed to a longitudinal series of specimens from single patients (2,11-13). Although our patient numbers with multiple bleeds were small (N=5), they all demonstrated detection stability of DFS70 antibody over time using multiple methodologies, which we consider a valuable finding with respect to implementation of testing protocols.

A key question that had to be answered was which methodology should be used for DFS70 antibody identification. Using IIF methodology, backed up by experienced microscopists the standard DFS70 antibody pattern was recognised in approximately 8 out of 10 patients, a frequency which is consistent with that seen in other studies (10-11). This study is not a method comparison; however, in our hands, after reviewing results from the INOVA Diagnostics BIOFLASH™ CMIA system and the EUROIMMUN EUROLINE Profile 3 plus DFS70™ LIA we found very similar levels of performance which is in agreement with a review article by Karsten *et al*. in 2016 who concluded that the method of detection for DFS70 antibody was less relevant than that for ANA testing (1). As both methods use recombinant DFS 70 antigen and independent studies have demonstrated DFS70 antigen homology (14-15) good method agreement is not an unexpected finding.

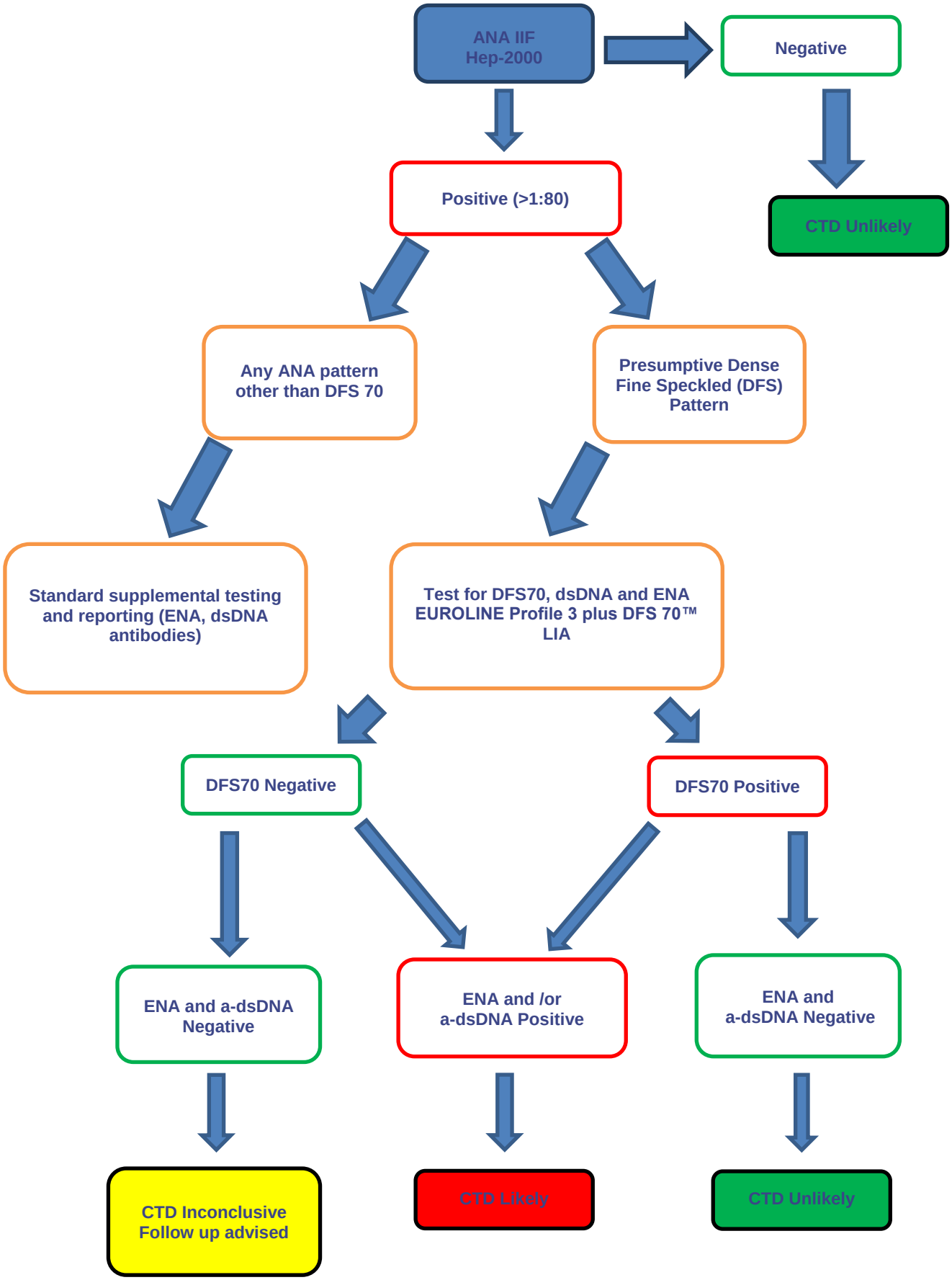



**Figure 5.** Proposed modified ANA testing algorithm for use at LabPLUS to identify and report DFS70 antibody.

Our study implied that the EUROLINE Profile 3 plus DFS70™ LIA may have slightly superior assay sensitivity compared to the CMIA assay. This was an unexpected finding, given that CMIA methodology has both high assay sensitivity and wide analytical measuring ranges (16). At the time of submission of this paper, we were unable to find any publications comparing the performance of CMIA and LIA methodologies, although Mutlu *et al's* study in 2016 suggested that LIA (different manufacturer: Immco Diagnostics) had lower sensitivity than ELISA (11). Mahler *et al's* finding of good correlation between ELISA and CMIA in 2012 (12) was reflected in our study which showed good correlation between CMIA RLU's and LIA densitometry values. A particular strength of our study design was subjecting all presumptive IIF DFS70 antibody positive sera to both specific techniques. This allowed confidence in choosing an assay that not only would detect DFS70 antibody when it was present but equally important for our purposes would give a negative result when the antibody was absent. For a second-round (post-IIF) confirmatory procedure, we consider either method to be suitable; but when we add the parameters of result TAT and expected low test numbers, LIA methodology was identified as the best fit for our testing environment and patient matrix.

As stated earlier, unexpected high titre ANA results frequently causes patients to be referred to Rheumatology services in the Auckland region. Complete reliance on clinical presentation for exclusion of AARD is not suitable in all cases as autoantibodies can develop and be detected years before clinical manifestations are seen (16). Furthermore, we have seen evidence (unpublished) that different preparation techniques of Hep-2 cell substrates by manufacturers may impact on DFS70 antibody detection. In our own setting, we have experienced two separate cases where DFS70 antibody was present giving a high ANA titre (1280) on a SSA transfected Hep-2 (Hep-2000™) cell line but gave low titre (80) on an un-transfected Hep-2 cell line implying the transfection process may inadvertently make the cell line more permissive to DFS70 antibody binding. To refute or validate this observation, testing of higher numbers of characterised DFS70 antibody positive sera on both transfected and un-transfected Hep-2 cell lines will be required. On limited evidence it appears as though DFS70 antibody may play a part in the reduced standardisation of ANA results across the Auckland region by those laboratories using IIF methodology highlighting the urgency for both identification and reporting. Implementation of DFS70 antibody testing and reporting has shown to have significant fiscal benefits (reduced testing costs for both ENA and ds DNA antibodies) as well as gains in Rheumatology out-patient clinic triaging efficiency (4).

In our setting we noted a high rate of non-specific ANA requesting. While requestor education will address this issue, it is expected this will be a mid to long-term project. In conjunction with the education initiative we propose the immediate implementation of a testing algorithm using IIF as the first-round screen and then LIA to confirm presumptive IIF DFS70 antibody positive sera (Figure 5). This proposed algorithm is similar to others in the literature (1,4) but it is uniquely adapted to our institution, our patient matrix, and the findings from this study. The proposed algorithm has the benefit of fitting seamlessly into our established testing procedures while retaining the safety of not excluding patients from ENA and dsDNA antibody testing based upon DFS70 antibody testing outcomes. We believe that benefits will accrue from (a) reductions in unnecessary follow up testing, (b) reduced rheumatology outpatient clinic appointments, and (c) enhanced clinical understanding for a proportion of patients where there are significant differences in ANA reported results from laboratories across the Auckland region using IIF methodology.

Beyond the likely fiscal benefits and system efficiency gains that will likely accrue from DFS70 antibody testing and reporting we must also consider the implications for patients. In New Zealand, medical laboratory practitioners (scientists and technicians) are governed by a code of ethics (17) of which points 4 and 5 combined require that our core values must be that of beneficence (to do good) and non-maleficence (to do no harm) whilst ensuring that the clinical information provided be both valued and precise. Having identified that DFS70 antibody is present in our test population and knowing that standard ANA reporting may lead to increased patient anxiety and potentially inappropriate treatment, we believe that for ethical code compliance it is mandatory that our testing strategy be modified to identify and report DFS70 antibody when present. We encourage all laboratories using IIF methodology to adopt this position.

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## 2017 NZIMLS CALENDAR

*(Dates may be subject to change)*

| DATE | COUNCIL | CONTACT |
|---|---|---|
| 23/24 November 2017 | Council Meeting | fran@nzimls.org.nz |
| **DATE** | **SEMINARS** | **CONTACT** |
| 11 November | Immunology SIG Seminar, Hutton Theatre, Otago Museum, Dunedin | helen.vanderloo@sclabs.co.nz |
| **DATE** | **CONFERENCE** | **CONTACT** |
| 21-24 August | Annual Scientific Meeting, Airforce Museum, Christchurch | jacquie.leaman@sclabs.co.nz<br>fran@nzimls.org.nz |
| **DATE** | **MEMBERSHIP INFORMATION** | **CONTACT** |
| 28 January | Membership and CPD enrolment due for renewal by 28 February 2017 | sharon@nzimls.org.nz |
| 31 January | CPD points for 2016 to be entered before 31 January 2017 | 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 |
| 23 June | Nomination forms for election of Officers and Remits to be with the Membership (60 days prior to AGM) | fran@nzimls.org.nz |
| 13 July | Nominations close for election of officers (40 days prior to AGM) | fran@nzimls.org.nz |
| 2 August | Ballot papers to be with the membership (21 days prior to AGM) | fran@nzimls.org.nz |
| 10 August | Annual Reports and Balance Sheet to be with the membership (14 days prior to AGM) | sharon@nzimls.org.nz |
| 17 August | Ballot papers and proxies to be with the Executive Officer (7 days prior to AGM) | fran@nzimls.org.nz |
| 15 September | Material for the November Journal must be with the Editor | rob.siebers@otago.ac.nz |
| **DATE** | **NZIMLS EXAMINATIONS** | **CONTACT** |
| 04 November 2017 | QMLT Examinations | fran@nzimls.org.nz |

ORIGINAL ARTICLE

# The reticulocyte haemoglobin equivalent (RET_He) and laboratory screening for iron deficiency

*Charlotte Poffenroth[1], Craig Mabbett[2] and Christopher Kendrick[1]*

*[1]Massey University, Palmerston North and [2]LabCare Pathology, New Plymouth*

## ABSTRACT

**Background:** The reticulocyte haemoglobin equivalent (RET_He) parameter provides the haemoglobin (Hb) concentration of peripheral blood reticulocytes on Sysmex Haematology analysers. In the iron deficient (ID) state, reticulocytes and mature red cells have a lower Hb concentration due to the reduced bioavailability of iron. Reticulocyte haemoglobin parameters have been available on cell counting analysers for years. Recently there has been renewed interest surrounding the suitability of the RET_He as a laboratory screening test for ID. This work presents the findings of a study in which the RET_He was evaluated for its use as a marker of ID.

**Aim:** To establish a reference range for the RET_He and to assess its performance as a laboratory screening test for patient iron deficiency.

**Methods:** One hundred and seventy-eight patient samples submitted for iron studies to Labcare Pathology and Taranaki MedLab were retested to provide a CBC with a RET_He measurement using the Sysmex XN-2000 (Sysmex, Japan) analyser. All samples had previously been tested for ferritin, serum iron, transferrin and saturation. The results for the RET_He were stratified against iron studies, the CBC results and patient clinical details. A reference range for the RET_He was established using 66 samples from selected patients at Taranaki Base Hospital.

**Results:** A working cutoff value for ID for the RET_He was established as <26 picograms (pg) of Hb per reticulocyte. Values at or below this cutoff were mainly from patients with either iron deficiency, anaemia of chronic disease or known haemoglobinopathy patients. The reference range for the RET_He with a 95% confidence interval was 30.3-35.0 pg.

**Discussion:** This study showed the potential for the use of the RET_He as a simple laboratory screening test for iron deficiency. With a cutoff of <26pg, the RET_He correlated well for red cell microcytosis but was not specific for iron deficiency. Used as a screening test for ID, RET_He results of <26pg could signal the need for iron studies with results above this cutoff acting as a negative predictor of ID in anaemic patients. Used in this way the RET_He could better guide the use of iron studies for patients in whom ID may be suspected.

**Keywords:** Reticulocyte, RET_He, iron deficiency, anaemia, iron studies.

***N Z J Med Lab Sci 2017; 71: 120-123***

## INTRODUCTION

Iron deficiency (ID) and iron deficiency anaemia (IDA) affects approximately 2 billion people globally making it the most common of the nutritional deficiencies (1,2). Iron is essential for normal biological function and untreated IDA has been associated with developmental delays in the young (2). Iron deficiency can be difficult to diagnose using traditional biochemical markers of iron metabolism and the presence of the morphologically characteristic hypochromic microcytic red blood cells (RBC) in the blood film are typically only apparent once iron deficient erythropoiesis is advanced (3).

The complete blood count (CBC) is the most frequently ordered of all laboratory tests (1) and so expansion of the clinical utility of results produced as part of the CBC could be beneficial for patient diagnosis and management. The reticulocyte haemoglobin equivalent (RET_He) is a red cell parameter available on the XN-2000 and other Sysmex haematology analysers and provides a measure of the bioavailability of iron during erythropoiesis (4,5). Reticulocytes have a short life-span of 1-2 days in the peripheral blood before full maturation, and during iron deficient erythropoiesis, reticulocytes have reduced levels of haemoglobin production. The RET_He parameter has the potential for greater clinical use as an adjunct to current biochemical-based assays for ID. It has previously been proposed as a laboratory tool to distinguish between IDA and anaemia of chronic disease (ACD) with both aetiologies producing morphologically similar hypochromic microcytic RBC populations (1). ACD results from the inability of erythropoiesis to utilise body iron while IDA results from the lack of body iron.

The aim of this study was to establish a reference range for the RET_He at Taranaki Base Hospital and to assess its clinical value as a tool for the identification of ID.

## MATERIALS AND METHODS

The study utilised samples submitted to LabCare Pathology and Taranaki MedLab for iron studies over a period of eight weeks. Results were collated twice daily from the laboratory information systems and EDTA anticoagulated blood samples were run on the XN-2000 (Sysmex, Japan) using the RET channel analysis feature. Testing was performed twice daily to ensure samples were less than six hours old to reduce any effects of sample ageing. A total of 178 patient samples were included in the study and all laboratory testing results and patient clinical details were collated in Microsoft Excel.

In the RET channel, blood cells are exposed to a surfactant reagent that lightly perforates the membrane of the RBC, WBC and platelet populations. In the machine the blood sample, surfactant and a fluorescent dye (Fluorocell RET) are incubated

together for a short period, allowing the dye to penetrate the cells (1). The stained cells are then cycled through a flow cell and past a beam of high intensity laser light. Reticulocytes containing RNA, fluoresce producing forward and side scattered light that is captured by light-detectors producing results that are presented graphically in the form of a 2D-scatterplot. Total cell numbers are counted with the forward light-scatter providing cell size and side-scattered light indicating the presence of cytoplasmic nucleic acid (DNA/RNA). In the RBC population the degree of fluorescence is proportional to the cytoplasmic RNA and provides the reticulocyte population. Results are presented as picograms (pg) of Hb per reticulocyte (1).

The biochemistry analyser used for iron studies at LabCare Pathology was the Cobas 6000 (Roche Diagnostics, Germany) with the iron panel providing results for serum ferritin, serum iron, serum transferrin and transferrin saturation. Ferritin was measured by electrochemiluminescent immunoassay, iron by colorimetric assay and transferrin using an immunoturbidimetric assay (6-8).

A RET_He reference range was constructed from the results of 66 EDTA anticoagulated peripheral blood samples. The samples were selected from patients presenting to Taranaki Base Hospital with a normal CBC and medical conditions unlikely to impact on their iron status or reticulocyte parameters.

The statistical software program MedCalc®, was used to provide the reference range with a 95% confidence interval and an online Clinical Calculator software package (9) used to calculate sensitivity, specificity, positive and negative predictive values for selected RET_He cutoff values.

## *RESULTS*

In the RET channel of the Sysmex XN-2000 analyser, normal and ID samples vary in the scatterplots they produce as a result of the differences in the RBC and reticulocyte populations. In Figures 1 and 2 the reticulocyte population (pink/red) is presented along with the mature RBC population in blue. Figure 2 shows the presence of microcytic RBC & reticulocyte populations lower on the Y axis of the scatterplot in contrast to the iron replete example in Figure 1. The microcytic RBCs in Figure 2 are the result of iron deficient erythropoiesis caused by reduced cellular Hb levels.

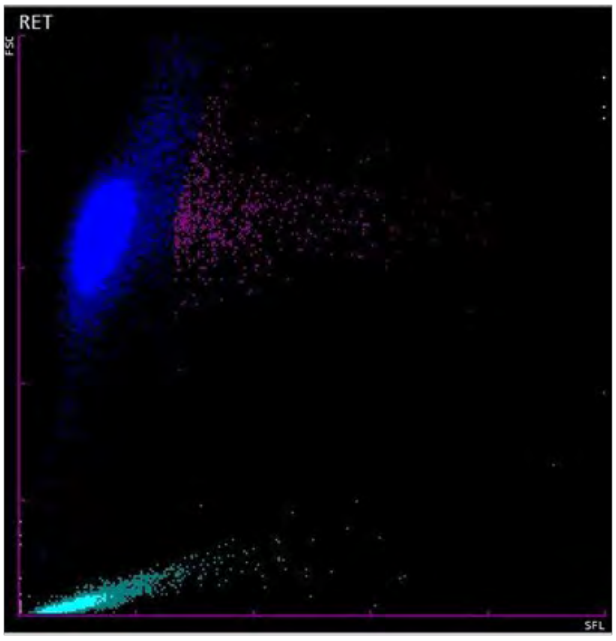


**Figure 1.** RET scatter gram - normal iron.

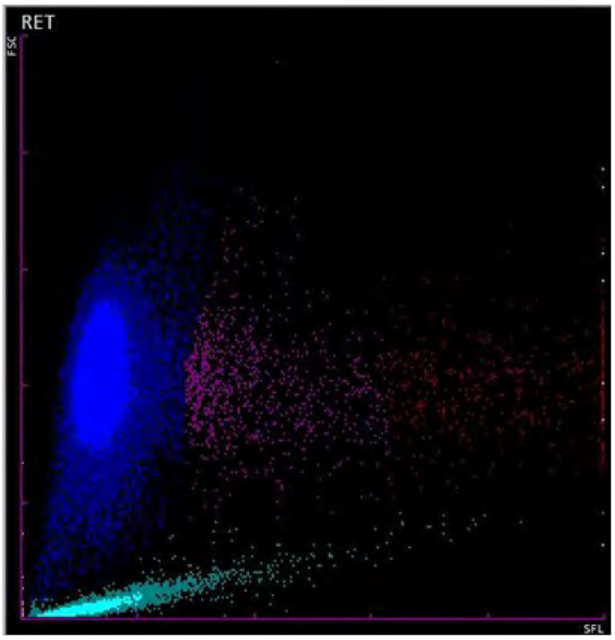


**Figure 2.** RET scatter gram - iron level deficiency.

Participants had an average age of 44 with a male to female ratio of 1:2. The RET_He results were stratified against the iron studies, CBC results and relevant clinical information. This divided patients into normal, ID/ACD, IDA and haemoglobinopathy clinical groupings. The diagnostic cutoff values for ID were those used at LabCare Pathology with ferritin <20 ug/L and saturation <16%. Using the World Health Organisation (WHO) criteria, patients were classified as anaemic if the Hb was <120g/L for non-pregnant females and <130g/L for males (9). A C-reactive protein assay (ref range <5 mg/L) was used to establish cause when clinical details were not available for some apparent ID patients.

A summary of the results of the laboratory testing divided participants into four groups and is presented in Figure 3.

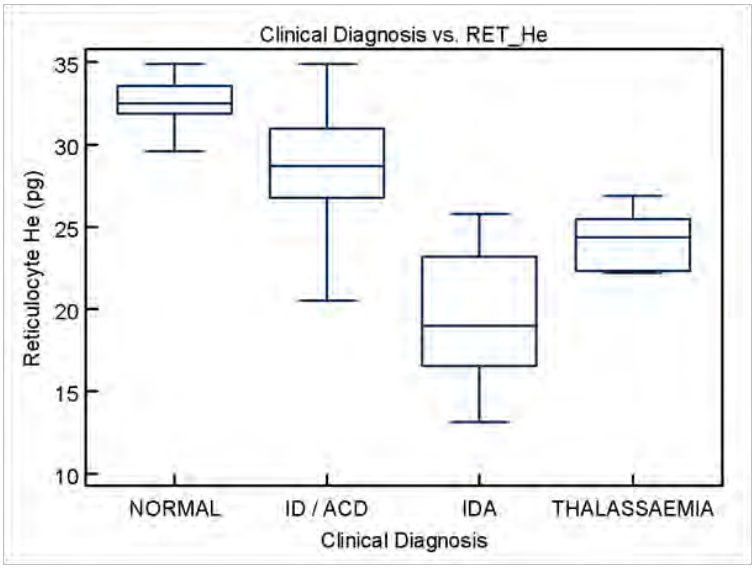


**Figure 3.** Clinical diagnoses vs RET_He results.

The data used to establish rates of True and False positives for the patient groups is presented in Tables 1 and 2. A RET_He cutoff of <26 pg favoured the detection of more true positives (21) but also more false positives (10). The cutoff of <25pg detected less true positives (19) but also had less false positives (7).

**Table 1.** True and false positives using a <25pg cut-off for the RET_He.

| RET_He | True positives | False positives | Totals |
|---|---|---|---|
| **< 25pg** | 19 | 7 | 26 |
| **> 25pg** | 2 | 150 | 152 |
| **Totals** | 21 | 157 | 178 |

**Table 2.** True and false positives using a <26pg cut-off for the RET_He.

| RET_He | True positives | False positives | Totals |
|---|---|---|---|
| **< 26pg** | 21 | 10 | 31 |
| **> 26pg** | 0 | 147 | 147 |
| **Totals** | 21 | 157 | 178 |

Sensitivity, specificity and positive and negative predictive values for the RET_He were calculated for the two cutoff options <25pg and <26pg rf. Table 3 (10). Results showed that a cutoff of <26pg had a small advantage for the detection of ID.

**Table 3**. Summary of sensitivity, specificity, positive (PPV) & negative predictive values (NPV) for two cut-off values.

| RET_He | Sensitivity | Specificity | PPV | NPV |
|---|---|---|---|---|
| **< 25pg** | 0.905 | 0.955 | 0.730 | 0.987 |
| **< 26pg** | 1 | 0.936 | 0.677 | 1 |

The results for the RET_He from 66 patient samples were used to calculate a reference range with a 95% confidence interval of 30.3-35.0 pg with a mean of 32.7 pg. The coefficient of skewness (-0.034) showed the data set was normally distributed (Figure 4b) with support from a low coefficient of Kurtosis or Z score of (-0.335) (Figure 4a).

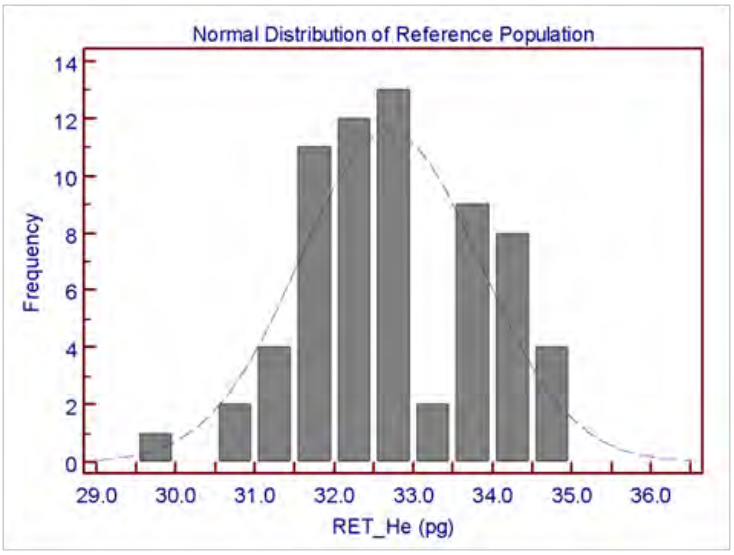


**Figure 4a.** Z score and population distribution for the RET_He reference range.

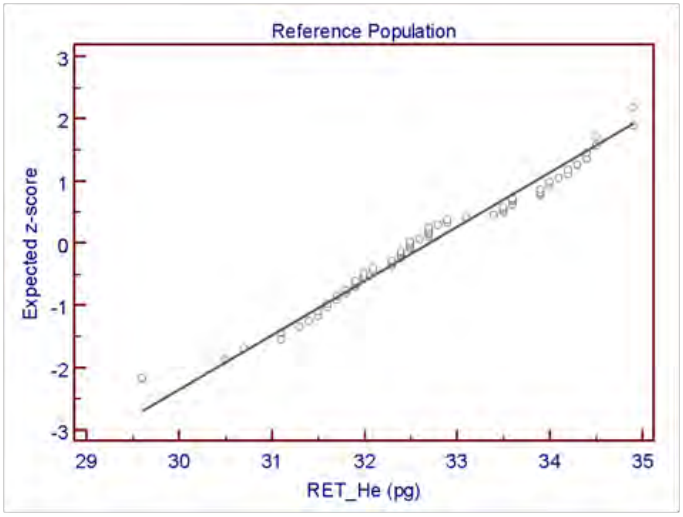


**Figure 4b.** Population distribution for the RET_He reference range.

## *DISCUSSION*

The gold standard for the assessment of body iron is a bone marrow biopsy but it is an invasive procedure rarely used in the diagnosis of ID related disorders (11). Instead the quantitation of iron is traditionally performed using biochemistry-based assays. Ferritin is the long term storage form of iron and in the plasma it reflects total body iron stores. Its use as a marker for ID is complicated, as together with transferrin, both are also acute phase reactants that are elevated in infection, chronic disorders and other inflammatory states. As a result, the diagnosis of ID using iron studies is not always straight forward.

This study correlated patient clinical information against the results of iron studies, CBC data and the RET_He parameter. Results indicated that a RET_He cutoff of <26pg was able to identify ID patient groups and best supported the use of the RET_He as a screening test for iron deficiency. The RET_He uses the mean cell volume (MCV) and so results can be affected when there is microcytosis unrelated to IDA, such as, in double RBC populations, in cases of RBC aggregation and when there is hyper or hyponatraemia (12). To aid interpretation, the RET_He results should be considered together with the results of the red cell distribution width (RDW).

In this study the RET_He failed to provide a clear division for the ID and ACD groups affecting the overall specificity of the parameter. Given this, the follow-up of patients with a RET_He less than or close to the 26pg cutoff should include iron studies (13).

The RET_He reference range for the population in this study was 30.3-35.0 pg with a 95% confidence interval and is comparable to the range of 28.9-36.3 pg developed by LabPlus in New Zealand for a demographically similar population group (14). That study recommended a RET_He cutoff value for ID of <25pg slightly lower than the cutoff of <26pg in this work. A possible limitation of this study could have been the participant group selected for the reference range. Made up of selected inpatients at Taranaki Base Hospital instead of healthy members of the public, the reference range data could have been skewed. This does not appear to have been the case with other researchers producing reference ranges comparable to this study (2,5,11,12).

Laboratory cost containment has always been an important consideration in laboratory testing. A cost advantage for the RET_He as a screen for ID may be significant as compared to traditional iron studies. In this study iron study costs were estimated to be approx 1.5 times that of the CBC + RET. A closer consideration of the cost/benefit of the RET_He over the use of traditional iron studies as a screen for ID may or may not support the findings of this study.

## CONCLUSIONS

This study highlighted the clinical potential of the RET_He. Its use as part of a screening algorithm together with Hb, MCV and RDW, could better guide laboratory recommendations for iron studies, reducing costs when iron studies are not warranted. The study showed that the RET_He with a cutoff of <26pg was highly sensitive but not specific for the detection of ID. Its future clinical utility could be as a screening test for ID but also as a negative predictor of ID when the RET_He results fall within the reference range in anaemic patients. Its utility as an early marker for ID has been previously reported and has been confirmed with the demographic investigated in this study. The future acceptance of the value of the RET_He by clinical staff may be hampered by a lack of awareness of its potential for patient diagnosis and treatment. The education of clinical staff could start by reporting the parameter in anaemic patients with results below the RET_He cutoff, triggering a comment about additional laboratory testing to rule out possible ID .


## ACKNOWLEDGEMENTS

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ORIGINAL ARTICLE

# A comparison of positivity using routine incubation, extended incubation and antihuman globulin in the complement dependent cytotoxicity (CDC) assay

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## ABSTRACT

**Objectives:** This study quantified and compared positivity obtained on complement dependent cytotoxicity (CDC) using routine incubation, extended incubation and anti-human globulin.

**Methods:** This was a retrospective study which included results from all samples processed for CDC as part of pre-transplant screening in our laboratory in 2013. Samples were processed in parallel for routine incubation CDC, extended incubation CDC and anti-human globulin CDC techniques. Positivity in terms of percentage dead cells was recorded for each technique. All samples that showed positive results (>/=10% dead cells) by at least one method were included in statistical analysis to compare degree of positivity. Negative samples and those that failed validation controls by any technique were excluded. Results of extended incubation CDC were analysed in 131 samples, routine incubation CDC in 103 and anti-human globulin CDC in 111. Results were recorded as percentage dead cells and these values were compared between techniques using the paired t test.

**Results:** Comparison of reactivity of extended incubation CDC and anti-human globulin CDC with routine incubation CDC, showed a highly significant difference ($p<0.0001$ and 0.003 respectively) with a mean increase in positivity of 7% over routine incubation with both extended incubation CDC and anti-human globulin CDC. Comparison of extended incubation CDC to the anti-human globulin CDC showed no significant difference $p=0.19$).
Routine incubation missed positivity in 30% of the positive samples tested.

**Conclusions:** Anti-human globulin and extended incubation enhance the positivity of CDC by approximately 7%. However, neither anti-human globulin CDC nor extended incubation CDC showed any significant increase in positivity over each other.

**Key words:** anti-human globulin, complement dependent cytotoxicity, crossmatch, extended incubation, human leukocyte antigens.

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## INTRODUCTION

The complement dependent cytotoxicity (CDC) assay since the late 1960s has been a mainstay of pre-transplant testing for anti - HLA antibodies (1,2) Many laboratories continue to report on the CDC with routine incubation timings, while others use various enhancement techniques to improve its sensitivity, two favoured ones being the use of extended incubation CDC and antihuman globulin CDC (2). Laboratories worldwide use either of these methods to enhance the CDC. There are few studies that have actually measured the degree to which these methods enhance the positivity obtained on routine incubation CDC.

Our study estimated the increase in positivity obtained using each of these enhancement methods over the standard CDC, and also compared them with each other to find if either enhances positivity of the other.

## MATERIALS AND METHODS

This was a retrospective study that collated data from all cross-matches performed in our laboratory in 2013, as part of pre-transplant screening for living donor renal transplants. All samples were processed for donor recipient crossmatch using routine incubation CDC, extended incubation CDC and anti-human globulin CDC. The cross-match procedure was as follows:

### Primary sample and processing

Blood for lymphocyte separation was collected from the donor (who was instructed to arrive in the morning in a fasting state) in Acid Citrate Dextrose 1 and processed immediately. Lymphocytes were separated into 5% McCoy's by density gradient centrifugation using Lymphoprep (Axis-Shield PoC AS, Norway). Contaminating erythrocytes were lysed using tris ammonium chloride solution. Platelets were aggregated using thrombin and removed by sedimentation. Viability was checked after the primary separation and should be at least 95%. In case the quality of separation was unsatisfactory, further processing using Percoll (Sigma Aldrich, USA) was undertaken. Lymphocytes were adjusted to a concentration of 1.5-2.5 million/ml for the crossmatch. Ten ml of blood collected from the patient in a clot enhancer tube and allowed to clot at room temperature following which serum was separated and used for the cross-match.

### Cross-match procedure

One microliter of cell suspension was dotted onto Terasaki trays. One microliter each of serum in neat (N), N/2 and N/4 dilutions, and rabbit complement (One Lambda, USA; lot #030 was used during the entire study period) were added. For routine incubation timings, incubation periods of half an hour following addition of serum and one hour following complement were followed. These incubation periods were doubled for extended incubation cross-matches.

For the anti-human globulin cross-match, cells and serum wee incubated for forty-five minutes followed by three manual washes on the tray using 5% McCoy solution. Subsequently the tray was re-oiled followed by addition of anti-human globulin (goat IgG anti human kappa, One Lambda, USA; lot #007 was used for the entire period of the study) in dilutions of N/32, N/64 and N/128. Undiluted complement was added followed by ninety minutes of incubation. Subsequently vital dye (aqueous eosin) was added and the reaction was fixed by adding 5% formalin after 5 minutes and read. The trays were read on an inverted phase contrast microscope and the percentage dead cells in each dilution were recorded.

All incubations occurred at controlled room temperature (22 to 26 degrees Celsius). Appropriate positive and negative controls were dotted in parallel in each tray. In the negative control wells, serum from a non-sensitised male was added in similar dilutions as the corresponding test wells. In addition, to control for background cell death, in one well, phosphate buffered saline was added to the cell suspension instead of serum. Each sample was processed by the three techniques in parallel and read by the same individual. Reactivity was measured in terms of percentage of dead cells in test wells over that in the negative controls. The CDC is a subjective test. Therefore, the test was read by a staff member with at least five years of experience in reading CDC crossmatches.

For this study, the maximal reactivity obtained by each method on a sample was recorded. Any sample that showed positivity of 10% or more on at least one technique was included for analysis. A cut off of 10% was used as this was considered the minimal degree of positivity that was consistently interpreted as positive by different observers. Tests that had failed controls or where background cell death exceeded 10% were excluded from comparison for that technique. Also, where patient samples were limited, routine incubation CDC was not performed. Towards the end of the year, in recognition of the lack of sensitivity, routine incubation CDC was discontinued. A paired t test was used to determine any significant difference in reactivity between the two methods.

## *RESULTS*

354 CDC crossmatches were performed during the study period. 131 samples (37%) were positive by at least one method and were included in our analysis. All of these had been processed by the extended incubation CDC protocol.

In addition, 103 of these samples had been processed using routine incubation CDC, and 111 using anti-human globulin CDC (Figure 1).

Only 72 out of 103 (70%) were positive on routine incubation CDC. 14 samples were positive on extended incubation CDC but negative on anti-human globulin CDC, with positivity on the former ranging from 10-15%. Seven of these were also positive on routine incubation. Nine samples were likewise positive on anti-human globulin CDC but negative on extended incubation CDC, with positivity with anti-human globulin ranging from 10-15% in all but one sample. This sample alone showed strong positivity of 60% with anti-human globulin which was not detected by extended incubation. None of the samples that were positive with anti-human globulin CDC but not with extended incubation. CDC were positive on routine incubation (Tables1 and 2, and Figure 2).

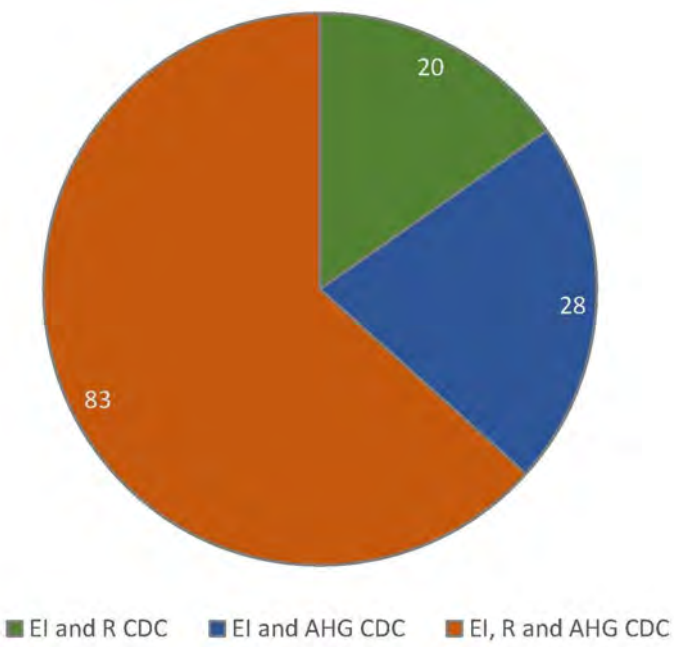


**Figure 1.** Pie chart showing the number of samples included under each method. (EI=extended incubation; R=routine incubation; AHG=anti-human globulin).

**Table 1**. Distribution of reactivity among samples processed by each technique.

| Reactivity (% dead cells) | Routine incubation Number of samples (%) | Extended incubation Number of samples (%) | Anti-human globulin Number of samples (%) |
|---|---|---|---|
| </=20% | 79 (77) | 90 (69) | 76 (69) |
| 21-50% | 3 (3) | 9 (7) | 8 (7) |
| 51-80% | 5 (5) | 9 (7) | 9 (8) |
| 81-100% | 16 (15) | 23 (17) | 18 (16) |
| **Total** | **103 (100)** | **131 (100)** | **111 (100)** |

**Table 2.** Number of positive samples missed by each technique.

| Technique | Number of samples processed | Number of samples where antibodies were missed (%) |
|---|---|---|
| Routine incubation CDC | 103 | 31 (30) |
| EI CDC | 131 | 9 (7) |
| AHG CDC | 111 | 14 (13) |

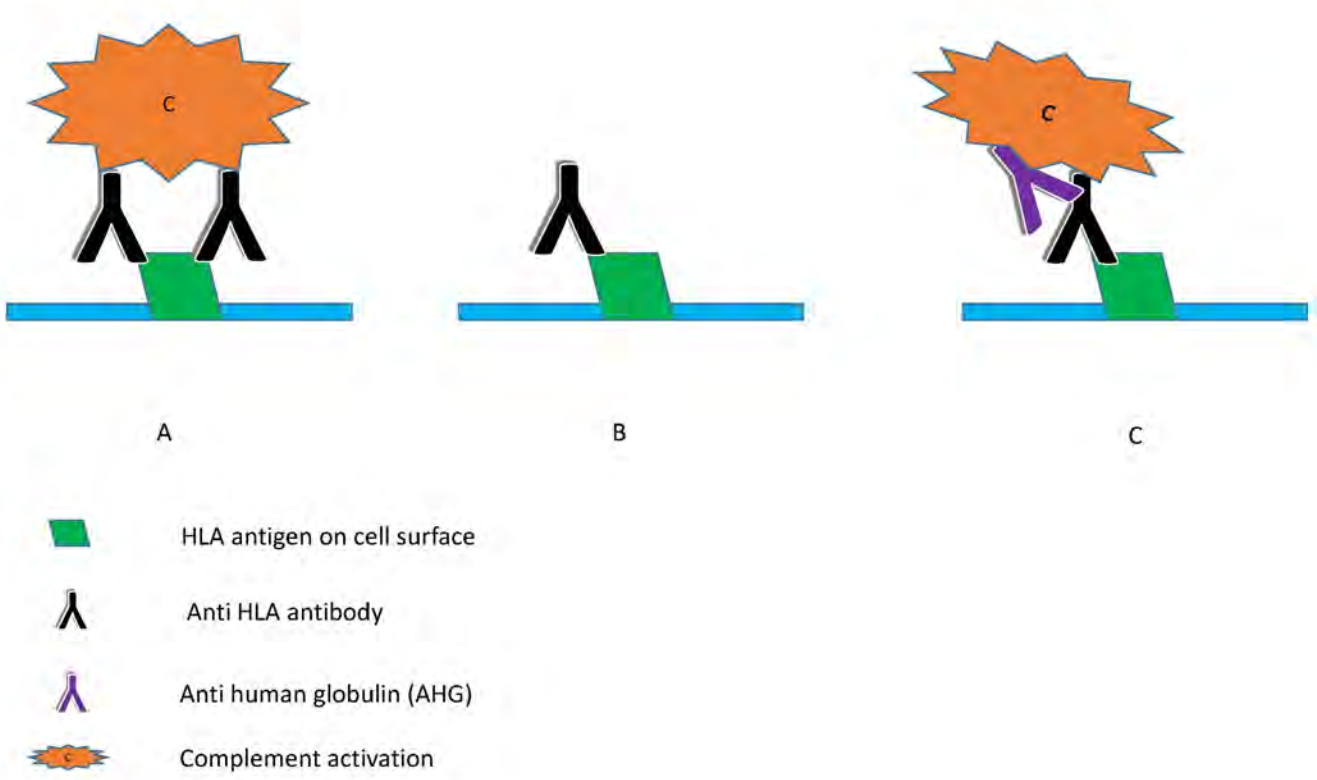


**Figure 2.** Diagram showing the mechanism of complement fixation by IgG and the CYNAP phenomenon. A) IgG antibodies bound to different epitopes on the same molecule together activate complement. B) IgG antibody binding to a single epitope on a molecule cannot activate complement on its own. C) Antihuman globulin binds to the primary antibody and associates with it to activate complement.

Comparison of reactivity of extended incubation CDC with routine incubation CDC, showed a highly significant difference ($p<0.0001$; $t = 4.81$) with a mean increase of 7% (standard error 1.49). Comparison of anti-human globulin CDC with routine incubation CDC also showed a significant difference ($p=0.003$; $t = 3.03$) with a mean increase in reactivity of 7% (standard error 2.3). Comparison of extended incubation CDC to the anti-human globulin CDC showed no significant difference (two tailed $p=0.19$; $t=-1.33$) with a mean of differences equal to 2.3% (standard error 1.76) (Figure 3).

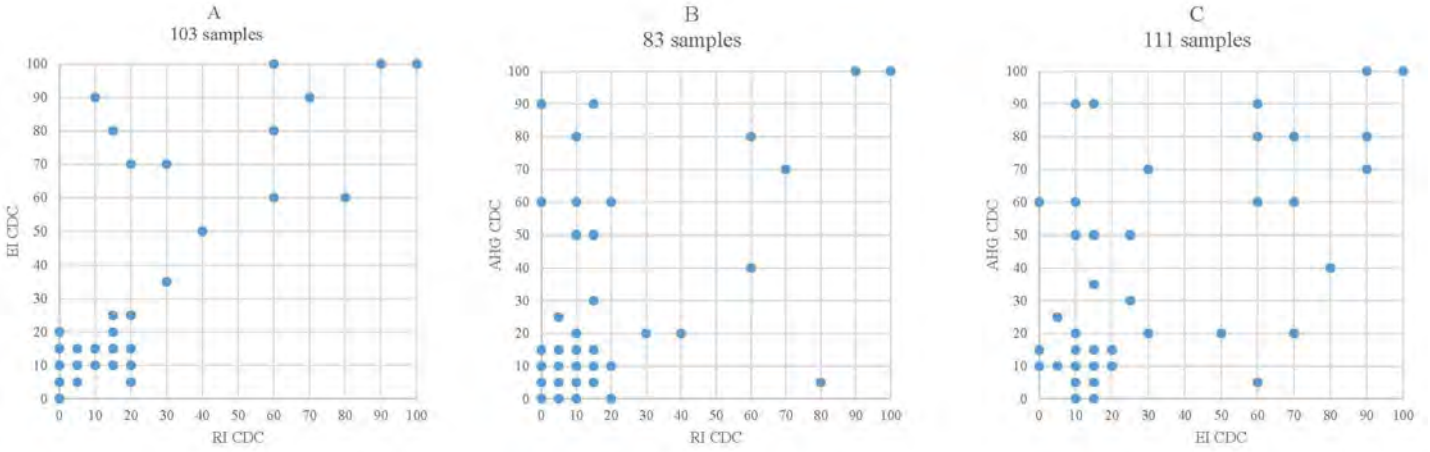


**Figure 3.** Scatter plots comparing positivity exhibited by samples with various techniques. A) routine incubation CDC (x axis) versus extended incubation CDC (y axis). B) Routine incubation CDC (x axis) versus anti-human globulin CDC. C) Extended incubation CDC (x axis) versus anti-human globulin CDC (y axis). (RI=routine incubation; EI=extended incubation).

## *DISCUSSION*

The CDC crossmatch has been a decisive test in pre-transplant compatibility testing ever since its high prediction for early acute rejection in renal transplants was brought to attention by Patel and Terasaki (1). Among the various platforms available in the market today, CDC alone has shown consistent and strong correlations with post- transplant outcomes.(1,3-5) However, CDC is often criticized for its lack of sensitivity as compared to other platforms. Modifications aimed at enhancing antibody detection of CDC almost immediately followed its inception. These include the anti-human globulin technique, and extended incubation (6,7).

In our study, as expected, both enhancement methods produced a significant and comparable increase in positivity over routine incubation CDC with a mean increase in reactivity of 7% for both extended incubation CDC and anti-human globulin CDC over routine incubation CDC. However, there was no statistically significant enhancement in positivity observed with either of the enhancement techniques as compared to the other, even though anti-human globulin CDC is generally declared to be more sensitive.

The use of anti-human globulin enhances the detection of weak or low titred antibodies as well as non-complement fixing antibodies, including cytotoxicity negative adsorption positive (CYNAP) antibodies (8-11). Complement activation requires binding of complement by at least two nearby Fc segments, requiring that they be within a distance of 14 angstroms on the cell surface (9,10). IgM antibody by virtue of its large pentameric structure has five Fc fragments per molecule and will fulfil this requirement. IgG, with its monomeric structure (with only one Fc fragment per molecule) will only fulfil this requirement where there are multiple antibodies clustered on a single HLA molecule. Consequently, where there is IgG with only a single specificity, complement activation may not occur. Anti-human globulin circumvents this by binding to the primary antibody, allowing participation of its own Fc fragment with that of the primary antibody in complement activation, thereby allowing detection on CDC (10,11) (Figure 3). Notably, sera that have multiple specificities binding to the same antigen, based on this principle *are not enhanced by using anti-human globulin as they already effectively activate complement* (9). This probably explains why our study did not show any overall enhancement of positivity when using anti-human globulin CDC as compared to extended incubation CDC. Extended incubation on the other hand increases the time period allowed for antigen-antibody interaction and complement fixation. This probably improves detection of weak antibodies but still does not allow for detection of non-complement fixing antibodies (8-10).

Cross *et al.* first introduced the anti-human globulin technique, and observed that it had higher sensitivity and enhanced the reaction as compared to extended incubation CDC. They also noted that all patients who were transplanted across a positive anti-human globulin CDC but negative extended incubation CDC developed accelerated or acute rejection within two months post-transplant, whereas those who were transplanted across a positive extended incubation crossmatch but negative anti-human globulin CDC had neither hyper-acute or accelerated rejection (7). Interestingly, all the sera in their study that were solely positive on extended incubation were also auto -reactive. This would explain absence of rejection in these cases, as auto-antibodies are generally considered harmless and not a contra-indication for transplant (2,12). The clinical significance of CYNAP antibodies is debated. It is suggested that these antibodies pose some risk but are not an absolute contra-indication for transplant (2).

Cross and others have demonstrated enhancement of the reaction, detection of antibody in higher dilutions, and enhanced binding of complement when using anti-human globulin even when direct CDC was positive (6,10). However, methodological differences are known to cause wide variation in the sensitivity of CDC crossmatches and others have demonstrated achievement of sensitivity comparable to anti-human globulin CDC merely by increasing serum and complement incubation timings (2,13).

As in Cross's study, certain sera in our study showed positivity on only one of the enhancement techniques. Missed positivity was more frequent with anti-human globulin. Cross *et al.* showed that crossmatches that were positive on extended incubation alone in their study became negative in two cases on introducing a wash step (7). This suggests that weakly bound antibodies may be lost during the wash steps in the anti-human globulin CDC technique. Our finding that at least some of these samples that were negative on anti-human globulin CDC were positive on routine incubation as well as extended incubation further implicates the wash step in the loss of antibody. On the other hand, positivity that was evident on anti-human globulin CDC but not on extended incubation CDC may have been due to CYNAP antibodies, though this could not be proven.

The antibodies picked up by either technique alone in our study were weak, with one exception. This case showed strong positivity with anti-human globulin which was not evident on extended incubation CDC. Moreover there was an apparent prozone effect with positivity increasing from 5-10% in neat serum to 60% with N/4 dilution. The positivity was confirmed on the Luminex crossmatch assay using donor lysate which showed class I positivity with a median fluorescent intensity median fluorescent intensity (MFI) of 3928.

## CONCLUSIONS

As has been previously reported, anti-human globulin CDC and extended incubation CDC enhance the positivity of the routine incubation CDC. The increase in positivity using both enhancement methods was approximately 7%. Positivity obtained on anti-human globulin CDC and extended incubation CDC was comparable. i.e. anti-human globulin CDC was not found to show any significant enhancement of positivity over that obtained on extended incubation CDC.

We also found that both anti-human globulin CDC as well as extended incubation CDC used in isolation could potentially miss some positive samples. As none of our positive patients were transplanted without undergoing desensitisation, the clinical implications of this remain uncertain. Towards the end of the year, our laboratory discontinued the routine incubation CDC in view of its lack of sensitivity. We continue to perform extended incubation CDC and anti-human globulin CDC on all samples in parallel.

## AUTHOR INFORMATION

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# Science Digest

## *Contributed by Michael Legge*

### Links to malaria protection

*Plasmodium falciparum* (*P. falciparum*) is a leading cause of childhood malaria e.g. a child growing up in the Congo has a 22.5% cumulative risk of dying from malaria. Although sickle cell heterozygotes are the textbook example of protection against malaria there are obvious consequences with this untreated disorder. To complete their life cycle the parasites must bind to red cell receptors to enter the red cell and these are species dependent. *P vivax* binds to the Duffy receptor, which has been largely lost in African populations providing protection against *P vivax*. *P falciparum* targets glycophorins, which form the MNS blood group system. Recent research has identified that the glycophorin copy number variants were associated with resistance to *P falciparum,* which were higher in African populations (11%) compared to non-Africans (1.1%). One particular allele, the Dantu allele, is strongly associated with *P falciparum* resistance and is believed to represent human adaption to a *P falciparum* sub-species. The Dantu allele is restricted to East Africa suggesting it has only emerged recently.

### The fetus has an active immune system

The fetal immune system has been regarded as passive but the quandary has been how does the fetus avoid maternal immune attack and how can it prepare for birth? Research from Singapore using fetal tissue has identified that the fetal immune system was active from as early as 13 weeks gestation producing a range of active immune cells as well as active dendritic cells. The research identified that the dendritic cells in culture produced more than the usual T-regulating cells and that different genes were switched on in the fetal dendritic cells than in adult dendritic cells. The fetal cells synthesized high levels of arginase-2, which breaks down argenine a key messenger for tumour necrosis factor alpha (TNF alpha), which triggers inflammation. The authors speculate that understanding the development of the fetal immune system could lead to a better understanding of adult immune system diseases as well as providing an explanation for some types of miscarriages.

### New link between metabolism and immunology

Pre-beta-cell acute lymphoblastic leukemia (ALL) typically has mutations in transcription factor genes involved in beta-cell development. When leukaemia cells from 279 patients with pre-beta-cell ALL were analyzed, 209 had inactivating lesions in genes encoding beta-cell transcription factors) PAX-5; IK2F1; EBF1 and TCF3). Following chromatin-immunoprecipitation and sequencing all of the transcription factors bound to promoter regions of glucose uptake and influenced glucose metabolism as well as genes encoding negative regulators of . The changes resulted in associated reduction in glucose uptake and ATP depletion and it was subsequently identified that PAX-5 could act as a ‘gatekeeper’ to restrain glucose uptake and ATP supply in pre beta-cells. In addition the researchers found that PAX-5 and IK2F1 determined the responsiveness of beta-lymphoid ALL to determined the responsiveness of beta-lymphoid ALL to predispose treatment by inducing cell death by positively regulating NR3C1 levels. Overall, the research demonstrated beta-lymphoid transcription factors exert tumour suppression by limiting the supply of glucose and ATP to prevent malignant transformation of pre-leukaemic cells.

### Does gut metabolism signal pathogenic bacteria protection?

It is well established that there is crosstalk between gut bacteria and the immune system. Part of the crosstalk involves bacterial derived metabolites. The short chain fatty acid, butyrate is a common metabolite that can bind to G-protein-coupled receptors on both colonocytes and immune cells leading to antimicrobial immune responses. Recent research from Belgium using an animal model has identified that butyrate directly influences colonocyte oxygen consumption through the beta-oxidation pathway leading to a symbiotic effect in normal gut by maintaining obligate anaerobes rather than facultative anaerobes such as pathogenic *Escherichia* and *Salmonella* by limiting the availability of oxygen in the gut, Additionally; gut nitrate is essential for facultative anaerobes, which is formed via nitric oxide synthase 2. The researchers identified that butyrate activates peroxisome proliferator-activated receptor-gamma (PPAR-gamma) in colonocytes which inactivated nitric oxide synthase 2 thereby reducing the nitrate production. In addition PPAR-gamma also activated beta-oxidation in macrophages. The researchers conclude that increased butyrate-producing bacteria in the gut, was associated with a lower risk of intestinal inflammation and gut-barrier dysfunction and that these interactions may have significance in obesity and diabetes mellitus.

### A possible new bio-terrorism threat

Researchers in the USA have demonstrated that it is possible to use DNA sequences to hack in to analytical sequencing instruments. They developed a technique to encode malicious software in to strands of DNA. This then becomes a programme that corrupts gene-sequencing software. And takes control of the underlying computer. While this type of ‘attack’ is unlikely at present, computational biologists indicate that external sourced DNA could potentially be difficult to vet and that the hacking process has the potential to corrupt forensic and diagnostic DNA testing as well as gaining access to intellectual property resulting from DNA analysis. Although the design of the hacking sequence of the DNA was difficult the researchers indicate that the potential for such an attack is feasible. Currently the experimental hacking DNA hacks in to the memory of the computer running the compression software to run its own arbitrary commands.

ORIGINAL ARTICLE

# Stago Start Max analyser validation and early reaction errors (ERE) in haemostasis testing at Wellington SCL

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*[1]Massey University, Palmerston North and [2]Southern Community Laboratories, Wellington*

## ABSTRACT

**Background:** At the Southern Community Laboratories (SCL) Wellington laboratory, the Sysmex CS2100i analyser (Siemens) is responsible for the testing of all samples submitted for haemostasis evaluation. One of the limitations of this equipment is a tendency to produce invalid international normalised ratio (INR) and activated partial thromboplastin (APTT) tests on some samples due to early reaction errors (ERE). This requires additional sample processing and sometimes a patient re-bleed. The STart Max (Stago) semi-automated benchtop analyser showed promise as a suitable alternative method since it used mechanical clot detection, rather than an optical method, and potentially the ability to eliminate problematic ERE's. This in turn might reduce delays in reporting results and sample recollection.

**Methods**: The STart Max analyser underwent validation using the methodology outlined in the IANZ specific criteria for accreditation (Medical Testing 7). Validation included the development of reference ranges for the prothrombin time/international normalised ratio (PT/INR) and the APTT. Accuracy & precision characteristics were assessed using patient samples, external quality control samples and samples that had previously produced ERE results on the CS2100i. All results were statistically evaluated using Analyse-it software.

**Results**: Results for the PT/INR and the APTT showed good correlation with the Sysmex CS2100i analyser (*r*-value >0.95) and external QC samples. However, for the APTT, there was a significant difference between the two methods (1-11 secs). The reference ranges for the STart Max were found to be similar to those in use for the INR on the Sysmex CS2100i. For APTT, the reference ranges did not show uniform similarity between the two methods. Tests for precision produced a coefficient of variation (CV) of < 4% in all tests except for the elevated range of the APTT where this was 4.96%. The STart Max analyser was able to generate reportable results for all samples that generated ERE results on the Sysmex CS2100i analyser.

**Conclusions** : The generation of patient sample results affected by unresolvable ERE results with the Sysmex CS2100i analyser highlighted the need for an alternative method in the laboratory. This study has shown that the STart Max analyser produced comparable results to those from the CS2100i. With the exception of the APTT, a regional biological reference range can be used for reporting results from the STart Max analyser. The STart Max analyser also showed that it was able to generate reportable APTT results on samples rejected for ERE using the Sysmex CS2100i analyser. The results of this study has allowed the validation of the STart Max analyser for use at Wellington SCL.

**Key words:** haemostasis, international normalised ratio, activated partial thromboplastin, early reaction errors.

***N Z J Med Lab Sci 2017; 71: 129-135***

## INTRODUCTION

Early reaction errors (ERE) are encountered on patient plasma samples using the photo-optical clot detection method used on the Sysmex CS2100i analyser (Siemens) currently installed at Wellington SCL. Early reaction errors are abnormal reactions that occur on some samples at the initial stages of the APTT coagulation reaction (1). This finding leads to additional sample preparation steps to resolve the issue or may lead to a sample recollect. To resolve this problem, the STart Max semi-automated analyser (Stago) was assessed for use as it uses mechanical clot detection and was considered to be cost effective as an alternative method.

The literature reports conflicting information about the advantages of photo-optical and mechanical clot detection systems for coagulation testing. Discrepancy between the two methodologies has been demonstrated for some samples linked to the turbidity, colour, haemolysis, and other sample-related factors; while others report that the two methods are equivalent (2-7). This study looked to determine if the STart Max analyser would provide a solution to ERE produced by some samples on the high throughput Sysmex CS2100i analyser used at Wellington SCL.

## MATERIALS AND METHODS

The equipment used in the study included the Sysmex CS2100i and the Stago STart Max coagulation analysers. The validation procedure used for the STart Max analyser was taken from the IANZ specific criteria for accreditation (Medical Testing 7) (8). Plasma samples used in the study were separated from citrate anticoagulated whole blood collected from community and hospital patients in the greater Wellington region served by the Wellington SCL laboratory. For all samples, testing was performed in duplicate and the mean for each pair of tests was derived. If there was more than a 10% difference in the clotting times of duplicate samples, tests were either repeated, or if insufficient, excluded from data sets.

Reagents used for testing included Siemens Thromborel® S for the PT/INR, Dade® Actin FS and $CaCl_2$ for the APTT. Some samples (for the reference range and ERE) were aliquoted and stored frozen at -20°C until testing was performed. Frozen samples were thawed in a 37°C water bath and all testing was completed within two hours, post-thaw. All statistical calculations were performed using Analyse-it™ software.

### International Sensitivity Index and Local Mean Normal Prothrombin Time

The thromboplastin used by Wellington SCL was Thromborel S and was calibrated against a reference thromboplastin (Siemens PT Multicalibrator) to derive the International Sensitivity Index (ISI). To establish the local mean normal prothrombin time (MNPT), 20 “normal” citrated plasma samples were analysed and the geometric mean calculated. The ISI and local MNPT were then used to calculate the International Normalised Ratio (INR) for patient samples.

### Accuracy

Twenty randomly selected patient samples that were representative of the measuring range for each of the two tests (PT/INR, APTT) were run in parallel on the STart Max and CS2100i analysers. In addition, 16 lyophilised plasma samples were provided by the Royal College of Pathologists of Australasia, Quality Assurance Programme (RCPAQAP). These samples were provided with the PT, INR and APTT results from 31 laboratories that had tested the samples using the Stago STart 4 (previous model to STart Max). Scatter plots and difference plots were used to analyse the paired samples.

### Precision

The reproducibility of each test was assessed by 10 repeated measurements of the same patient plasma. Samples with normal and elevated results were chosen for the PT/INR & APTT assays. Precision was assessed using the coefficient of variation (CV) calculated for each of the tests.

### Reference interval

To establish the reference ranges for the PT/INR and APTT for the STart Max analyser, 120 patient samples were selected using the laboratory IT3000 middleware. Patients were included if they were >16 years of age and had a normal coagulation screen performed within 4 hours post collection. Patients were excluded if they had a history of bruising, bleeding or thrombosis; were post-operative; had clinical data that suggested either drug therapy; an active deteriorating or resolving disease process; or had other concurrent abnormal results or results associated with a recognised disease process (e.g. abnormal renal or liver function tests, abnormal cardiac markers).

### Internal QC and Measurement of Uncertainty (MU)

Internal QC limits for the STart Max analyser were established using Siemens Ci-Trol 1 and 2. The mean and standard deviations of the ten replicates of testing were used to establish a target and to set allowable limits for the internal QC of the analyser. Measurement of uncertainty (MU=2(CV)) was calculated using the CV of the normal QC replicates to show the dispersal of results from the estimated value.

### ERE’s

Fourteen samples that had shown an ERE on the Sysmex CS2100i analyser had been collected from the 1st February to the 30st April 2017 and stored frozen. All samples were thawed and rerun on the STart Max analyser.

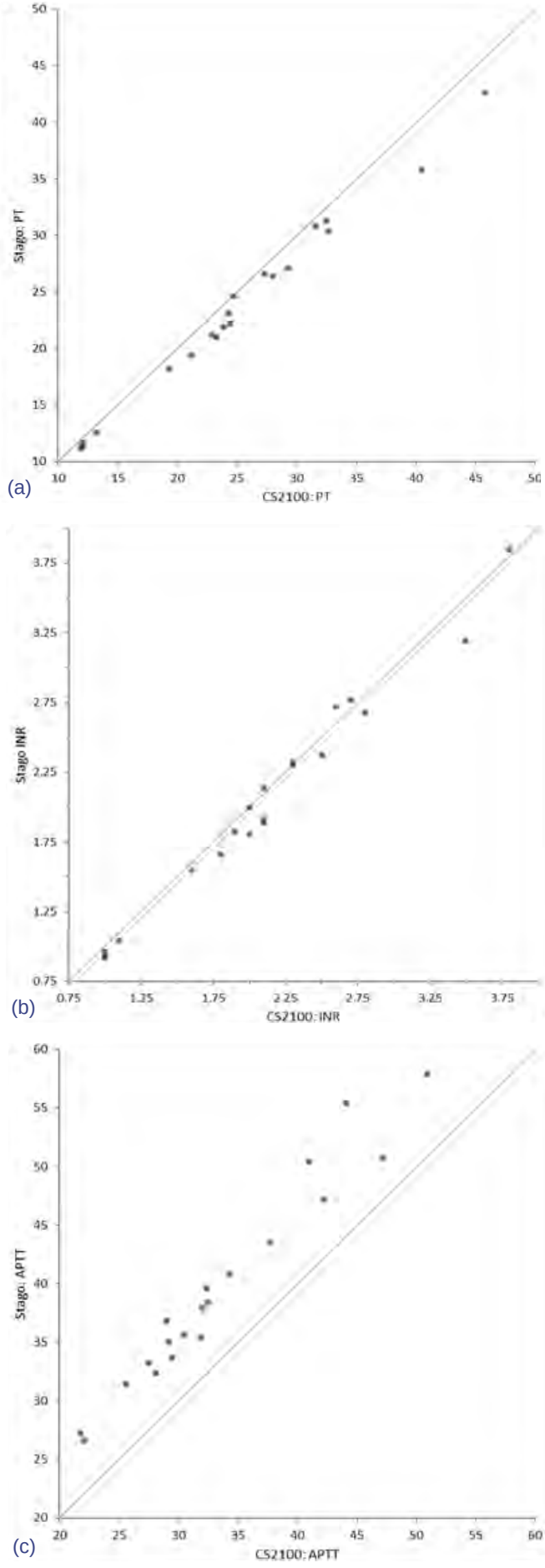
## *RESULTS*

### ISI and local MNPT determination

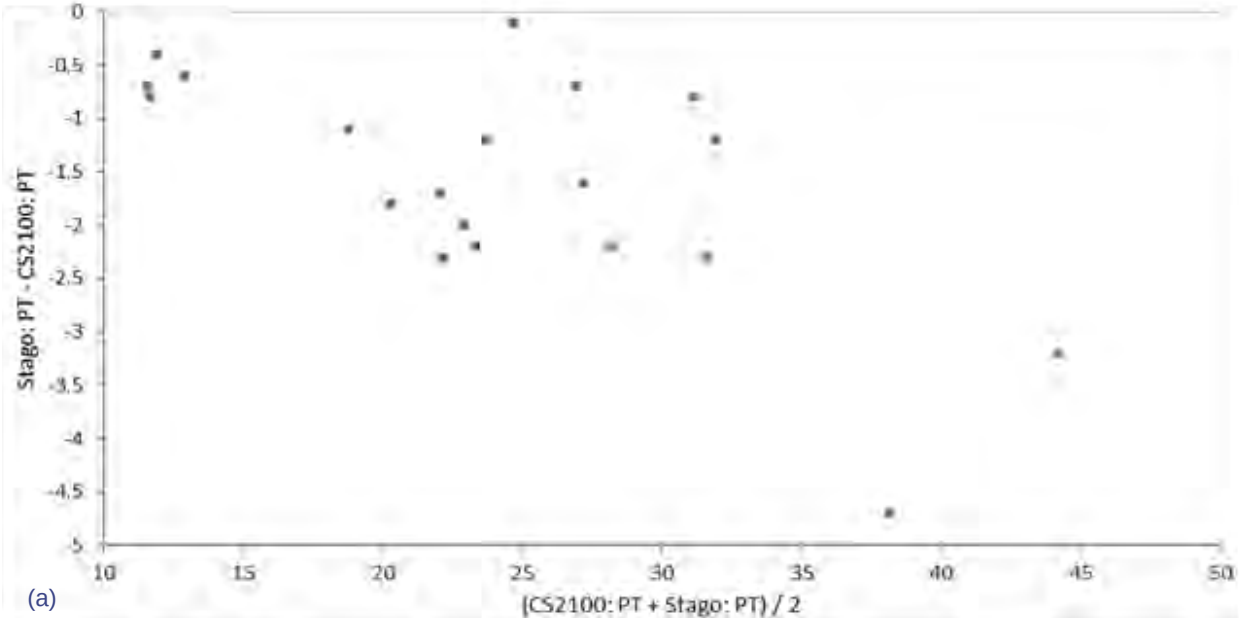
Results for the MNPT from the STart Max analyser provided a geometric mean of 12.1 seconds. An ISI value of 1.05 was determined from the PT multi-calibrator. The MNPT and ISI values were programmed into the STart Max software and used for subsequent PT/INR testing.

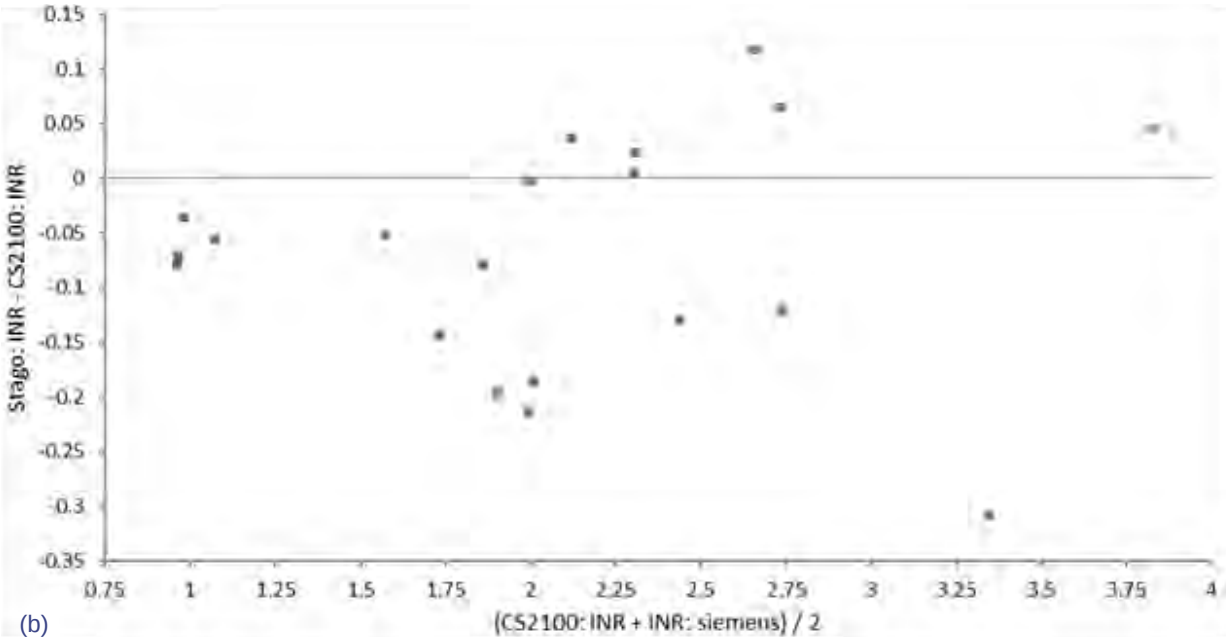
### Accuracy

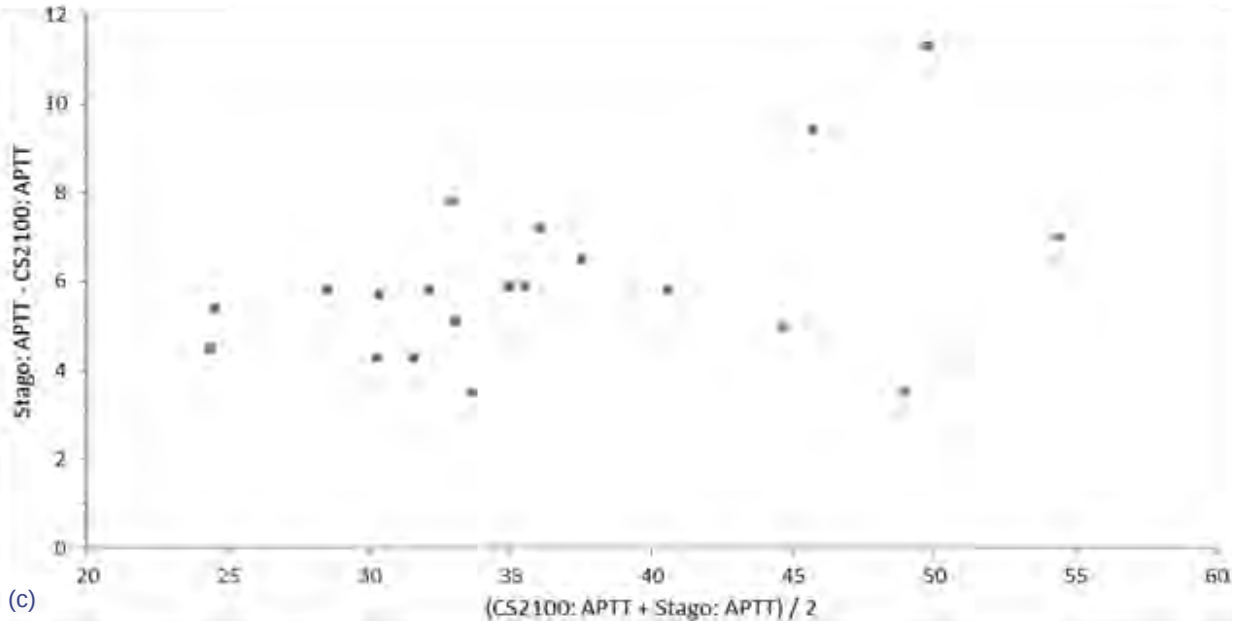
The results produced by the STart Max analyser for of the PT/INR and the APTT for 20 randomly selected patient samples were compared with the results for the same samples produced by the CS2100i (Figures 2 a-c). The r values (a) PT 0.996 (b) INR 0.990, (c) APTT 0.979 showed strong correlations. Difference plots were prepared for each of the tests and are presented in Figures 1 a-c.




**Figures 1 a-c.** Scatter plots for 20 patient samples tested for PT (a) and INR (b) and APTT (c) on both the STart Max and CS2100i analysers.

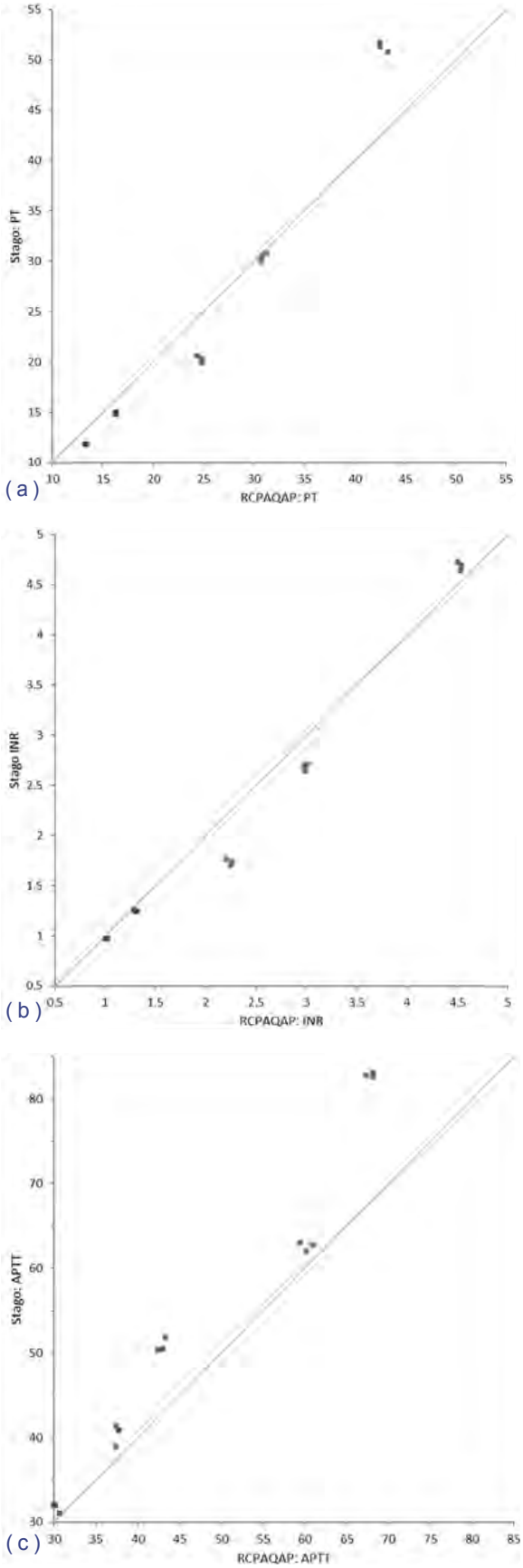



**Figures 2 a-c.** Difference plots of 20 patient samples tested for PT (a) and INR (b), and APTT (c) on the STart Max and CS2100i analysers.

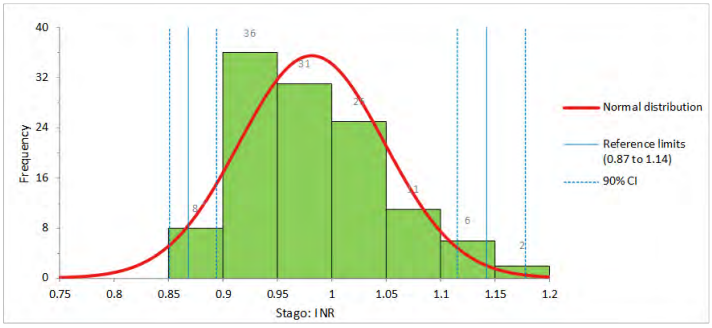
The results for 16 RCPAQAP external quality control samples were compared with the mean values provided from the 31 users of equivalent STart 4 coagulation analysers. Results are presented in Figures 3 a-c with *r*-values of 0.981 for the PT, 0.986 for the INR and 0.978 for the APTT.




**Figures 3 a-c.** Scatter plots for RCPAQAP samples tested for PT (a) and INR (b) and APTT (c) using the STart Max and CS2100i analyser.

## Reference intervals

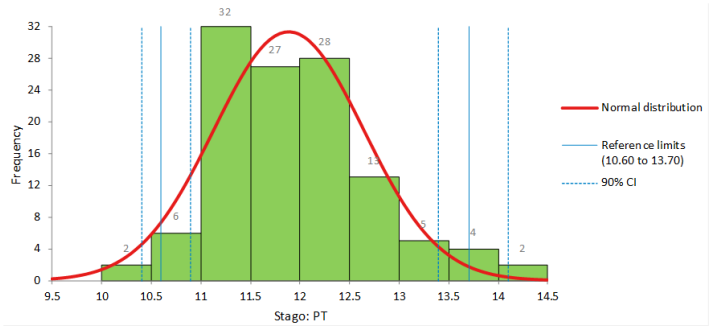
Of the patient samples selected for the reference interval, 119 were included in the validation series. A 95% confidence interval was determined based on the standard deviation of the population mean for the INR 0.9-1.1 (Figure 4), PT 11-14 secs (Figure 5) and the APTT 24 -36 secs (Figure 6). The difference between these and the biological regional reference ranges (used in reporting Sysmex CS2100 results) are shown in Table 1.




| | N | Mean | SD | Skewness | Kurtosis |
|---|---|---|---|---|---|
| Stago: INR | 119 | 0.98 | 0.07 | 0.7 | 0.34 |

| | Minimum | 1st quartile | Median | 3rd quartile | Maximum | IQR |
|---|---|---|---|---|---|---|
| Stago: INR | 0.9 | 0.93 | 0.97 | 1.02 | 1.2 | 0.09 |

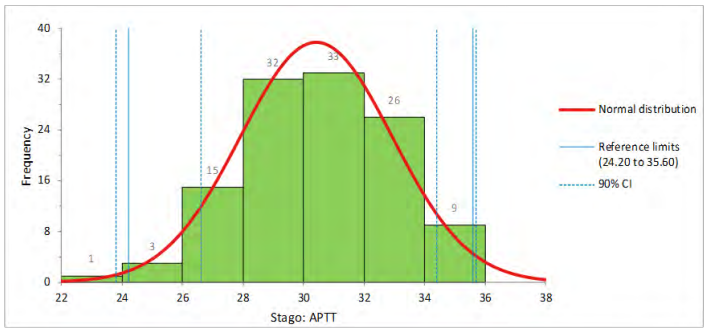
**Figure 4.** INR reference interval.




| | N | Mean | SD | Skewness | Kurtosis |
|---|---|---|---|---|---|
| Stago: PT | 119 | 11.89 | 0.76 | 0.7 | 0.32 |

| | Minimum | 1st quartile | Median | 3rd quartile | Maximum | IQR |
|---|---|---|---|---|---|---|
| Stago: PT | 10.4 | 11.30 | 11.80 | 12.30 | 14.1 | 1.00 |

**Figure 5**. PT reference interval.




| | N | Mean | SD | Skewness | Kurtosis |
|---|---|---|---|---|---|
| Stago: APTT | 119 | 30.42 | 2.51 | -0.1 | -0.07 |

| | Minimum | 1st quartile | Median | 3rd quartile | Maximum | IQR |
|---|---|---|---|---|---|---|
| Stago: APTT | 23.8 | 28.70 | 30.40 | 32.28 | 35.7 | 3.58 |

**Figure 6.** APTT reference interval.

**Table 1.** Summary of the reference range values for the STart Max vs CS2100i analysers.

| Test | STart Max | CS2100i |
|---|---|---|
| **PT (sec)** | 11 – 14 secs | 10 – 14 secs |
| **INR** | 0.9 – 1.1 | 0.9 – 1.2 |
| **APTT (sec)** | 24 – 36 secs | 22-30 secs |

## Precision

The precision evaluation results for the STart Max analyser are presented in Table 2. The CV's for PT normal and elevated results were 1.33% and 2.28% respectively. The CV's for the APTT for normal and elevated results were 1.15% and 4.96% respectively.

**Table 2.** Precision evaluation testing for the STart Max analyser.

| Test | PT (secs) | | APTT (secs) | |
|---|---|---|---|---|
| | **Normal** | **Elevated** | **Normal** | **Elevated** |
| **1** | 11.7 | 31.2 | 28.9 | 59.7 |
| **2** | 11.7 | 29.5 | 28.3 | 54.4 |
| **3** | 11.7 | 29.5 | 29.2 | 55.6 |
| **4** | 12.0 | 30.4 | 28.8 | 61.4 |
| **5** | 12.1 | 30.5 | 29.2 | 64.1 |
| **6** | 12.0 | 29.6 | 28.6 | 615 |
| **7** | 11.7 | 29.2 | 28.5 | 59.8 |
| **8** | 11.8 | 29.3 | 29.1 | 60.5 |
| **9** | 12.0 | 29.7 | 28.4 | 61.0 |
| **10** | 11.8 | 29.1 | 28.6 | 62.6 |
| **Mean** | 11.85 | 29.80 | 28.76 | 60.06 |
| **2 SD** | 0.32 | 1.36 | 0.66 | 5.96 |
| **CV** | 1.33% | 2.28% | 1.15% | 4.96% |

## Internal QC and Measurement of Uncertainty (MU)

Internal QC results from the STart Max analyser are presented in Table 3. For Ci-Trol 1, the CV's were; PT (1.46%) and APTT (1.27%). For Ci-Trol 2 the CV's were; PT (1.70%) and APTT (1.64%). Measurement of uncertainty (MU=2(CV)) was estimated based on Ci-Trol 1 results for the PT (2.92%) and the APTT (2.54%).

**Table 3.** CV's for Ci-Trol 1 & 2 (CT) using the STart Max analyser.

| | PT | | APTT | |
|---|---|---|---|---|
| **Replicate** | **CT1** | **CT2** | **CT1** | **CT2** |
| **1** | 12.2 | 40.3 | 30.8 | 55.8 |
| **2** | 11.9 | 40 | 30.1 | 54.8 |
| **3** | 12.2 | 40.5 | 30.3 | 55.9 |
| **4** | 12.1 | 39 | 29.8 | 54.6 |
| **5** | 12.1 | 41 | 30.3 | 57 |
| **6** | 12.1 | 40.1 | 29.7 | 54.7 |
| **7** | 12.1 | 39.9 | 30.4 | 55 |
| **8** | 12.2 | 39.6 | 30 | 55.1 |
| **9** | 12.6 | 40.8 | 29.9 | 57.1 |
| **10** | 12.1 | 41.3 | 29.5 | 55.8 |
| **Mean** | 12.16 | 40.25 | 30.08 | 55.58 |
| **2 SD** | 0.36 | 1.37 | 0.76 | 1.82 |
| **CV** | 1.46% | 1.70% | 1.27% | 1.64% |

### ERE's

The 14 stored patient samples that had previously flagged as an ERE on the CS2100i were retested using the STart Max (Table 4). The APTT had been affected in all cases and in one sample the PT/INR was also affected. When reanalysed on the STart Max analyser all 14 patients produced reportable results for the APTT, PT and the INR. In Table 4 the ERE codes are presented in the column on the left: Slow Reaction (0008.0128.0001), Start Angle 1 (0008.0128.0002), Start Angle 2 (0008.0128.0004), Early % (0008.0128.0016).

**Table 4.** Early Reaction Error (ERE) samples from the CS2100i rerun on the STart Max analyser.

| ERE samples | CS2100i | | | STart Max | | |
|---|---|---|---|---|---|---|
| | PT (secs) | INR | APTT (secs) | PT (secs) | INR | APTT (secs) |
| 0008.0128.0001<br>0008.0128.0016 | 14.4 | 1.2 | *** | 14.6 | 1.2 | 51.3 |
| 0008.0128.0016 | 16.2 | 1.4 | *** | 12.9 | 1.1 | 27.8 |
| 0008.0128.0016 | 20.5 | 1.8 | *** | 18.3 | 1.6 | 55.6 |
| 0008.0128.0016 | 13.1 | 1.1 | *** | 12 | 1 | 44.5 |
| 0008.0128.0016 | 14.1 | 1.2 | *** | 13.6 | 1.1 | 28.2 |
| 0008.0128.0016 | 14.4 | 1.2 | *** | 12.9 | 1.1 | 42.6 |
| 0008.0128.0016 | 12.6 | 1.1 | *** | 11.6 | 1 | 41.7 |
| 0008.0128.0001<br>0008.0128.0002<br>0008.0128.0016 | *** | *** | *** | 106 | 11.88 | 211 |
| 0008.0128.0004 | 13.4 | 1.2 | *21.9 | 13.1 | 1.1 | 25.5 |
| 0008.0128.0004 | 20.5 | 1.8 | *28.4 | 15.7 | 1.3 | 37.8 |
| 0008.0128.0004 | 16.8 | 1.4 | *45.7 | 14 | 1.2 | 55.6 |
| 0008.0128.0004 | 14.1 | 1.2 | *34.5 | 13.3 | 1.1 | 37.7 |
| 0008.0128.0004 | 20.1 | 1.7 | *32.2 | 17 | 1.4 | 41.8 |
| 0008.0128.0016 | 15 | 1.3 | *** | 13.8 | 1.2 | 21.8 |

* and *** = ERE

## *DISCUSSION*

Haemostasis testing is subject to inter-laboratory distortion due to pre-analytical and analytical variables, including differences in method and endpoint detection technologies such as photo-optical vs. mechanical clot detection. In addition, fully automated vs. semi-automated equipment and reagent variables can influence the results (9). This study was undertaken to validate a backup system to the Sysmex CS2100i analyser at Wellington SCL. The analyser selected was the Stago STart Max machine and one of the drivers for an alternative to the CS2100i was to enable the reporting of results affected by the ERE seen on this analyser.

This work evaluated the accuracy of the STart Max analyser compared to both the Sysmex CS2100i analyser and STart 4 analyser users in Australasia for the PT/INR and APTT tests. The *r*-values of >0.95 for each of the tests indicated a linear correlation between the STart Max and the other analysers. There was, however, poor agreement between the two data sets for the APTT, with the difference plot showing a positive bias and a clinically significant results difference (up to 11 secs). Difference plots for the PT and the INR showed only marginal differences and were not considered to be clinically significant.

Reference intervals for the STart Max analyser were established for the PT/INR and the APTT. Since the STart Max used a different reaction principle, it was expected that the results would differ significantly using regional reference ranges. This proved not to be the case for the PT/INR allowing the use of the existing reference range for these tests on both analysers at Wellington SCL. The finding that the APTT results from the STart Max showed a considerable shift from those from the CS2100i meant that an independent reference interval for STart Max APTT would need to be used.

Precision evaluation of the STart Max analyser for the PT/INR and the APTT against normal and prolonged ranges, showed a CV of approximately 2% for most tests. The exception was in the elevated range of the APTT where the STart Max showed a CV of 4.96% for the 10 replicates of the same prolonged sample.

Internal QC targets and allowable limits were established based on the mean and standard deviation of 10 replicates for two QC levels. The CV was used to calculate the MU, which was <5% for each test. Since the STart Max was a semi-automated method, there was likely to be some degree of intra-user variability attributable to the manual pipetting required. As such, the targets, allowable limits and the MU established during this commissioning exercise may not be reflective of true values. A bigger data set will be required to provide a more accurate evaluation once the analyser goes into regular use.

Finally, the STart Max analyser produced reportable results in all of the samples that had produced an ERE on the Sysmex CS2100i machine showing an advantage for mechanical clot detection ahead of the photo-optical technology for these samples in this study. A number of theories have been proposed to describe why ERE are encountered using the Sysmex CS2100i. A review of the clinical records of the patients included in the study showed some commonalities.

Some patients had been treated with unfractionated heparin and some were on dialysis. For others there was a history of calcium antagonist, ACE inhibitors and beta-blockers (Metoprolol, Amlodipine, and Cilazapril) medications. In others, records showed a history of propofol usage, something previously reported as a possible cause of coagulation testing error (10).

## CONCLUSIONS

In this study the Stago STart Max analyser produced precise and accurate results for each of the method validation stages. The PT, INR and APTT test results were statistically comparable to those obtained from the Sysmex CS2100i analyser. With the exception of the APTT, the existing biological reference ranges for the population served by Wellington SCL could be used to report the STart Max results. For the APTT, a new reference interval was established. Since the scatter plot for the APTT indicated a constant proportional bias, future work to perform regression analysis on a larger validation series would be required. The use of the regression equation ($y = mx + c$) might uncover a closer correlation between the two methods may yet enable the future reporting of the APTT using a single reference range for both machines.

In this study the Stago STart Max analyser demonstrated its suitability as a tool for use in routine coagulation testing and that it could be used interchangeably with the Sysmex CS2100i analyser. When samples affected by ERE using the Sysmex CS2100i machine were retested on the STart Max analyser, all samples generated a valid reportable result. Early reaction errors result in delays in reporting and/or unnecessary sample re-collections. This could elevate clinical risk with the inability to report a reliable result, particularly when the ERE cannot be resolved. This study has shown the Stago STart Max to be a robust analyser that offers a cost-effective alternative to the elimination of clinical risks associated with ERE affected sample results in the haemostasis laboratory. Its introduction at Wellington SCL is a quality improvement measure which will have a positive impact on future patient care.

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*ORIGINAL ARTICLE*

# Evaluation of the MAST indirect carbapenemase test and comparison with a modified carbapenem inactivation method for the detection of carbapenemase enzymes in Gram-negative bacteria

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## ABSTRACT

**Introduction:** Carbapenemase-producing Enterobacteriaceae (CPE) are no longer rarely encountered in New Zealand and worrying aspects of increasing prevalence include two hospital-associated outbreaks, infections in patients with no travel history, and a report of household transmission. It is critically important for both patient management and infection control purposes that carbapenemase-producing organisms (CPO) are rapidly and reliably detected and identified in clinical laboratories. However, this can be problematic due to the diversity of carbapenemase enzymes, the different genera they can reside in, and the difficulties of discriminating CPO from carbapenem-resistant-non-carbapenemase producers. Thus, the aim of this study was to evaluate and compare the recently released MAST indirect carbapenemase test (ICT) and a modified carbapenem inactivation method (mCIM) test, in order to determine their ability to detect carbapenemase production, and to reliably 'rule out' a non-carbapenemase producer.

**Methods:** A total of 100 non-duplicate isolates, consisting of 80 *Enterobacteriaceae*, 12 *Pseudomonas aeruginosa*, and 8 *Acinetobacter baumannii* were included in the study. The panel included 63 carbapenemase-producing strains and 37 non-carbapenemase producing multi-drug resistant strains. Each isolate was tested by the MAST ICT and a mCIM assay, with sensitivities and specificities determined.

**Results**: Both the MAST ICT and mCIM tests performed with 100% sensitivity, detecting all carbapenemase-producing strains. For the non-carbapenemase-producing strains, 3 false positive results were observed with the mCIM assay, giving a specificity of 91.9% and PPV of 95.5%. The MAST ICT was more subjective to interpret, with the assay initially producing 11 equivocal or false positive results (specificity 70.3%). Upon repeat testing, 4 strains were negative; giving a final specificity of 81.1% and PPV of 90.0%.

**Conclusion**: Our evaluation of the MAST ICT and a modified CIM assay found high sensitivity and specificity for both assays across a range of Gram negative bacteria. To reliably distinguish CPO from carbapenem-resistant-non-CPO, we would recommend that the mCIM is used in tandem with the MAST ICT, or with another high performing assay as such as Carba NP, rather than as stand-alone tests. Advantages of these tests include ease of use, simple to interpret, inexpensive and an ability to detect carbapenemase production, regardless of class type, in *Enterobacteriaceae* as well as Pseudomonas and Acinetobacter. An all-in-one test format rather than having to use multiple inhibitor-based tests, is favourable for laboratories with limited resources and experience.

**Key words:** indirect carbapenemase test, carbapenem inactivation method, carbapenemase, Gram- negative bacilli.

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## INTRODUCTION

Carbapenemase-producing Enterobacteriaceae (CPE) are no longer rarely encountered in New Zealand (NZ) (1). Although most findings of CPE have been from patients who have recent overseas travel history, worrying aspects include infections in patients with no travel history, two hospital-associated outbreaks, two probable cross-transmissions, and a report of a household transmission (1-3). A worldwide increasing prevalence of these often extremely multi-drug resistant organisms is an immediate threat to global health systems (4). Furthermore, there is a very real concern that the spread of CPE might mimic that seen with extended-spectrum beta-lactamase (ESBL) spread, especially the globally successful $bla_{CTX-M}$ epidemic (5). It is critically important for both patient management and infection control purposes that carbapenemase-producing organisms (CPO) are promptly and reliably detected and identified in clinical laboratories. However, this can be a challenge for many laboratories as the carbapenemase enzymes are a diverse group; being found in a variety of Gram-negative bacteria, they can sometimes express only low levels of carbapenem resistance and test susceptible to extended spectrum cephalosporins (e.g. OXA-48-like group), or conversely be transmitted on a plasmid which also harbours other resistant mechanisms such as ESBLs (6).

Complicating detection are organisms which are resistant to carbapenems due to other resistant mechanisms such as outer membrane porin mutations or up-regulated efflux pumps, often in combination with AmpC hyper production or ESBLs. Organisms that can sometimes possess these mechanisms, such as *Enterobacter cloacae* complex, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* complex, are more frequently encountered in most New Zealand clinical laboratories, making for problematic differentiation.

There are a range of phenotypic, enzymatic and molecular methods available for clinical laboratories to choose from, many of which are commercially available (7). Unfortunately, many of the phenotypic based tests are only recommended for *Enterobacteriaceae* and do not include Pseudomonas or Acinetobacter. For laboratories with limited expertise and resources it is important to be able to utilise methods which are accurate, simple, inexpensive, and can reliably discriminate non-carbapenemase resistance from the more concerning carbapenemase producers.

One method which is suitable for a variety of Gram negative bacteria is the recently released Indirect Carbapenemase Test (ICT) from MAST (Liverpool, UK). Using an indirect method to detect a β-lactamase is not new, being described by Moland *et al.* (8) as a method to detect Class A carbapenemases. The indirect method is a simple disc diffusion test whereby a reporter organism, such as *E.coli* ATCC 25922, is the lawn culture and the test organism is spread onto a bacterial permeabiliser-containing disc (e.g. EDTA), then the inverted disc is placed adjacent to an indicator disc (e.g. imipenem). The plate is incubated and any resulting distortion of the indicator disc zone is considered positive for carbapenemase production (8). Another simple phenotypic test, also suitable for Pseudomonas and Acinetobacter, is the carbapenem inactivation method (CIM), which was first described by van der Zwaluw *et al.* (9) in 2015. This method was subsequently modified by Tamma and colleagues (10), essentially replacing water with tryptic soy broth to suspend organisms, and incubating for four hours instead of two hours.

Thus, the aim of this study was to evaluate and compare the MAST ICT and a modified CIM (mCIM) test, in order to determine their ability to detect carbapenemase production, and to reliably 'rule out' a non-carbapenemase producer; against a panel of isolates, including 63 CPO and 37 non-CPO, constituting a diverse range of Gram-negative species and resistant markers.

## MATERIALS AND METHODS

### Bacterial isolates

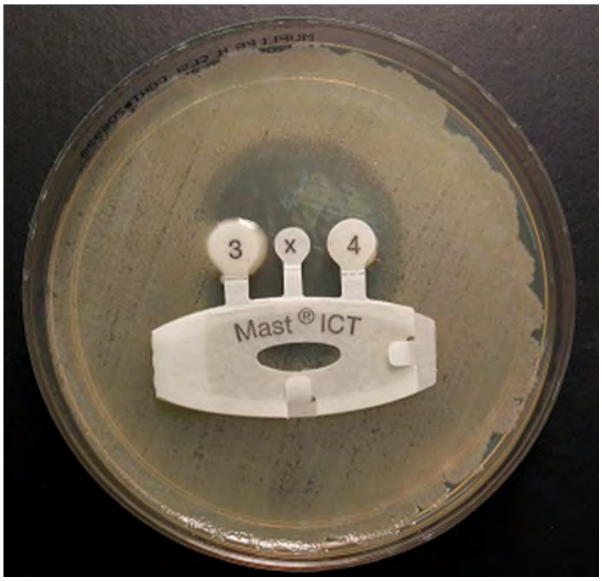
A total of 100 non-duplicate isolates, consisting of 80 *Enterobacteriaceae*, 12 *Pseudomonas aeruginosa*, and eight *Acinetobacter baumannii* were included in the study (Table 1). The study isolates were collected between 2008 and 2017, with the carbapenemase-producing isolates collected consecutively. The panel included 63 carbapenemase-producing strains: NDM (*n* = 18), OXA-48-like (*n* = 19), KPC (*n* = 6), IMP (*n* = 5), VIM (*n* = 4), OXA-23 (*n* = 3), and one each of OXA-24, OXA-25, OXA-27, OXA-58, GES-5, IMI-1, VIM/IMP, OXA-48/NDM; and 37 non-carbapenemase producing isolates that produced other resistant mechanisms such as ESBL, plasmid-mediated AmpC, and derepressed AmpC. Thirteen of the non-carbapenemase strains were non-susceptible to one or more carbapenem. All bacterial isolates used in the study were either isolates obtained from Canterbury Health Laboratories or reference strains provided by the Institute of Environmental Science and Research Limited (ESR). *Klebsiella pneumoniae* BAA1705 (KPC positive) and *K. pneumoniae* BAA1706 (KPC negative/ESBL positive) were used as positive and negative controls respectively. Isolates were stored at -80°C and subbed twice onto Columbia base blood agar before tests were performed.

## MAST ICT

The MAST ICT consists of a paper device which has three disc-like tips; one for a negative control, one for the test organism, and a middle disc containing a carbapenem antibiotic. The organism tips contain ESBL and AmpC inhibitor products as well as a permeabilising agent. The test was performed according to the manufacturer's instructions. Briefly, 3-5µl of test organism was spread onto tip 2 and *E.coli* ATCC 25922 was similarly spread onto tip 1. The device was then inverted and placed firmly onto a Mueller Hinton Agar plate which had previously been seeded with a lawn of the reporter organism, *E.coli* ATCC 25922. The plate was then incubated at 35°C aerobically for 16 to 20 hours. For *Pseudomonas* spp., the lawn organism was *K. pneumoniae* ATCC 700603, and the plate was incubated anaerobically. A positive result was indicated by a distortion of the carbapenem zone approximate to the test organism tip (Figure 1). The negative control should show no zone distortion.

***mCIM***: The mCIM test was performed with a slight modification to previously published methods. Briefly, approximately 5µl of test organism was evenly suspended in 400µl of tryptic soy broth in an Eppendorf tube. A 10µg meropenem disc was added and the tube was vortexed for a few seconds before incubating for 4 hours ± 15 mins. The meropenem disc was carefully removed and placed onto a Mueller Hinton Agar plate which had previously been seeded with a lawn of the reporter organism, *E.coli* ATCC 25922. The plate was then incubated at 35°C aerobically for 16 to 20 hours. A zone of <20mm indicated a positive result. Each test was read by three scientists, at least two of whom were blinded to the organism's resistance mechanisms. All initial equivocal interpretations and any discrepant results were repeated.

(a)




(b)


**Figure 1.**
(a) OXA-232 producing *K.pneumoniae* (weak positive)
(b) pAmpC producing *K. pneumoniae* (false positive)

**Table 1.** MAST ICT and mCIM results for carbapenemase and non carbapenemase-producing isolates

| Resistance mechanism(s) | Species | n | Number of Positive Test Results | |
|---|---|---|---|---|
| | | | MAST ICT | mCIM |
| **Carbapenemase** | | | | |
| NDM | *K.pneumoniae* | 6 | **6** | **6** |
| | *K. oxytoca* | 2 | **2** | **2** |
| | *E.coli* | 4 | **4** | **4** |
| | *C. freundii* | 2 | **2** | **2** |
| | *P. mirabilis* | 1 | **1** | **1** |
| | *P. stuartii* | 1 | **1** | **1** |
| | *M. morganii* | 1 | **1** | **1** |
| | *P. aeruginosa* | 1 | **1** | **1** |
| NDM/OXA-48-like | *K.pneumoniae* | 1 | **1** | **1** |
| | | 1 | | |
| OXA-48-like | *K.pneumoniae* | 1 | **11**[a] | **11** |
| | *E.coli* | 8 | **8**[b] | **8** |
| KPC | *K.pneumoniae* | 6 | **6** | **6** |
| IMP | *E.coli* | 2 | **2** | **2** |
| | *K.pneumoniae* | 1 | **1** | **1** |
| | *K. oxytoca* | 1 | **1** | **1** |
| | *P. aeruginosa* | 1 | **1** | **1** |
| VIM | *K. pneumoniae* | 1 | **1** | **1** |
| | *P. aeruginosa* | 3 | **3** | **3** |
| VIM/IMP | *P. aeruginosa* | 1 | **1** | **1** |
| GES-5 | *P. aeruginosa* | 1 | **1** | **1** |
| IMI-1 | *E. cloacae* | 1 | **1** | **1** |
| OXA-23 | *A. baumannii* | 3 | **3** | **3**[c] |
| OXA-24 | *A. baumannii* | 1 | **1** | **1** |
| OXA-25 | *A. baumannii* | 1 | **1** | **1** |
| OXA-27 | *A. baumannii* | 1 | **1** | **1** |
| OXA-58 | *A. baumannii* | 1 | **1** | **1**[c] |
| **Total (% sensitivity)** | | **63** | **63 (100%)** | **63 (100%)** |
| **Non-carbapenemase** | | | | |
| ESBL | *E.coli* | 4 | **0** | **0** |
| | *P. mirabilis* | 2 | **0** | **0** |
| | *P. vulgaris* | 1 | **0** | **0** |
| | *K. pneumoniae* | 1 | **0** | **0** |
| | *K. oxytoca* | 1 | **0** | **0** |
| | *C. koseri* | 1 | **0** | **0** |
| ESBL/pAmpC | *E.coli* | 2 | **0** | **0** |
| | *K.pneumoniae* | 1 | **0**[d] | **0** |
| ESBL/AmpC | *E.coli* | 2 | **0**[d] | **0** |
| | *C. freundii* | 1 | **0** | **0** |
| | *E. cloacae* | 1 | **1** | **0** |
| | *P. aeruginosa* | 1 | **0** | **0** |
| pAmpC | *E. coli* | 3 | **0** | **0** |
| | *P. mirabilis* | 3 | **1** | **0** |
| | *K. pneumoniae* | 1 | **0**[d] | **0** |
| | *K. oxytoca* | 1 | **1** | **0** |
| | *C. koseri* | 1 | **0** | **0** |
| AmpC derepressed | *E. cloacae* | 3 | **1** | **1** |
| | *E. aerogenes* | 1 | **0** | **0** |
| | *E.coli* | 1 | **0** | **0** |
| AmpC/porin/efflux | *P. aeruginosa* | 4 | **2**[d] | **2** |
| | *A. baumannii* | 1 | **1** | **0** |
| **Total (% specificity)** | | **37** | **7/37 (81.1%)** | **3/37 (91.9%)** |

N=Number of strains tested; [a] Two isolates weak positive; [b] One isolate weak positive; [c] Zone sizes 19mm; [d] One isolate initially tested equivocal, repeat tested negative

## RESULTS

Results of the trial are displayed in Table 1. Both the MAST ICT and mCIM tests performed with excellent sensitivity, detecting 63/63 (100%) of the carbapenemase-producing strains, giving a negative predictive value of 100%. Regarding the MAST ICT, three of the OXA-48-like producers (one *K. pneumoniae* carrying OXA-162, one *K. pneumoniae* carrying OXA-232 and one *E.coli* carrying OXA-181) were determined to have a weak positive result by at least one of the readers. Repeat testing produced a clear positive result for the *K. pneumoniae* carrying OXA-162, but the other two remained weak positive/equivocal. Carbapenem MIC values did not have any correlation to strength of zone distortion (data not shown). In comparison, reading and interpretation of the mCIM was less subjective, with the zone diameter reading being more clear-cut, yielding no equivocal results.

For the non-carbapenemase-producing strains, three false positive results were observed with the mCIM assay, including two strains of *P. aeruginosa*, which had high-level resistance to imipenem and meropenem, and a hyper-AmpC producing *E. cloacae*, which was non-susceptible to ertapenem. Repeat testing of these three strains reproduced identical results with no discernible zone size, giving final specificity and positive predictive value (PPV) of 91.9% and 95.5% respectively. For interest (results not shown), we also tested these three strains with Tamma's method (10) using 1 µl of organism suspended in 2 ml of TSB. The *E. cloacae* and one of the *P. aeruginosa* yielded zones of 19mm (negative by Tamma's criteria) and the other *P. aeruginosa* yielded a zone of 17mm (indeterminate by Tamma's criteria). However, it is unknown how this lower inoculum version may have affected the overall sensitivity of the assay for all of the test strains.

Compared with the mCIM, the initial specificity of the MAST ICT was lower at 70.3%, with the assay producing 11 equivocal or false positive results, including the same three isolates that were also mCIM false positive. Upon repeat testing, four strains which were initially interpreted as equivocal by some readers were negative on repeat testing, giving a final specificity of 81.1% and PPV of 90.0%. Of note is that the majority of strains that produced a false positive result in the MAST ICT assay were hyper-AmpC producing.

## DISCUSSION

Clinical isolates possessing a carbapenemase is becoming more common in NZ. Worrying aspects of this increasing prevalence include hospital, healthcare and household transmission events (1). Therefore it is imperative that all NZ hospital and community laboratories should be adept at detecting CPO and have the ability to rapidly discriminate carbapenemase producers from non-carbapenemase producers. Indeed, there has been a recent call for the implementation of a nationally coordinated response plan, akin to the preparation that would be required for an Ebola outbreak (11). Included in the recommendations by Blakiston and colleagues is the development of minimum patient screening criteria and laboratory testing standards for CPE.

Due to the diverse nature of carbapenemase genes and the many Gram-negative species that can harbour them, there is no single 'gold standard' phenotypic test available, making for complicated laboratory identification and confirmation. Many phenotypic tests have sensitivity issues, especially with the detection of OXA-48-like enzymes, or tests have been designed for a geographical area with a predominating gene-type e.g. KPC producers in the USA, rather than for a diverse range of CPO or Gram-negative organisms (12).

Van der Zwaluw's (9) initial presentation and evaluation of the CIM test included a large panel of carbapenemase-producing *Enterobacteriaceae* as well as *P. aeruginosa* and *A. baumannii*. Their original method consisted of a full 10 µl loop of organisms suspended in 400 µl of water, the addition of a 10 µg meropenem disc and incubation for a minimum of two hours at 35°C. A positive result was classified as allowing uninhibited growth, whereas a negative result was classified as a clear inhibition zone. The authors reported sensitivities of 100% for *Enterobacteriaceae* and *P. aeruginosa*, but two OXA-23-producing *A. baumannii* were not detected.

Similarly Tijet *et al.* (13) evaluated the CIM and compared to Carba NP, against 182 *Enterobacteriaceae*; 100 of which were carbapenemase producers. They used a zone size of ≥20 mm to indicate a negative result. The authors found excellent sensitivity and specificity (99% and 100% respectively) with the CIM assay, which was superior to the performance of the Carba NP, the latter failing to detect 11/100 CPE (mainly OXA-48-like).

A modified version of the CIM (mCIM) was compared to 10 other phenotypic assays by Tamma *et al.* (10), including various modifications of the CarbaNP, Blue Carba and CIM assays. The mCIM substituted 400 µl of water for 2 ml of tryptic soy broth, adding just 1 µl of organism instead of 10 µl and extending the incubation period from 2 h to 4 h ± 15 min. In addition they incorporated zone diameter interpretations of 6 to 15 mm = positive, 16 to 18 mm = indeterminate and negative if ≥19 mm. This study determined that excellent sensitivity and specificity was achieved by the mCIM as well as Rapidec Carba NP, modified Carba NP and the manual Blue Carba. Extending the work by Tamma's group was a more comprehensive study by Pierce *et al.* (14), which involved a multi-laboratory evaluation on a variety of *Enterobacteriaceae*, as part of a CLSI working group. Overall, a high level of sensitivity, specificity and reproducibility was found by this group for the mCIM. In addition, laboratory staff found the mCIM simple to perform, with less subjective results compared to their experiences with Carba NP or MHT (14). This mCIM assay has subsequently been added to the 27th edition of the CLSI M100 supplement (15). A disadvantage of both studies is the exclusion of non-fermentative Gram-negative bacilli.

There are scant published studies evaluating the use of the ICT for carbapenemase detection. Mathers *et al.* (16) evaluated an in-house ICT method against 127 *Enterobacteriaceae* with ertapenem MICs ≥1 mg/L, 56 of which were $bla_{KPC}$ positive. They found the overall sensitivity to be 90.0%, but ranged as low as 84.3% for *Klebsiella* spp. The authors concluded that this ICT method may not be adequate for KPC-producing *K. pneumoniae*. It is important to note that Mather's ICT version consisted of just EDTA as the bacterial cell lysing agent, whereas the cell permeabilising agent is not disclosed in the MAST ICT documentation.

Our evaluation of the MAST ICT and the mCIM would indicate that both methods have a high capacity to detect CPO, with a sensitivity and negative predictive value of (100%). An area of concern with the MAST ICT would be the number of equivocal reactions (false positives) and the weak positive results for some of the OXA-48-like group; which are one of the most common enzymes currently found in NZ (1,17). The weak positive results were indistinguishable from false positive/ equivocal results (direct communication from MAST), as shown in Figure 1. Problems with the detection of OXA-48-like genes are a well-recognised problem with many phenotypic assays. Any equivocal results should be regarded as initially positive until further confirmation is known. Most of the mCIM tests produced no discernible zone, but of note are the two *A. baumannii* strains, one with OXA-23 and one with OXA-58, which both gave zone diameters of 19 mm. While our study used a cut-off value of <20 mm for the mCIM, these isolates would have been considered negative by Tamma (10) and Pierce (14); although their studies both used a lower bug/broth ratio and described zone interpretations for *Enterobacteriaceae* only, so it is unclear how these method variations would relate

to Pseudomonas and Acinetobacter. In addition, Van der Zwaluw, using the original CIM assay, also reported two false negative results with OXA-23-producing *A. baumannii* (9). Further research is needed in order to set mCIM method variations and zone interpretations for all Gram-negative species.

In our study the specificity and PPV of the mCIM was high (91.9%, 95.5%), indicating an excellent ability to reliably 'rule out' a non-carbapenemase-producer. The positive false results can mostly likely be attributed to hyper-AmpC production in a high-inoculum test environment. On the other hand the MAST ICT produced appreciably more equivocal or false positive results, delivering a final specificity of 81.1%. While four false equivocal results were retested as negative, repeat testing involves another day waiting for results. To reliably distinguish CPO from carbapenem-resistant-non-CPO, we would recommend that the mCIM is used in tandem with the MAST ICT, or with another high performing assay as such as Carba NP, rather than stand-alone tests.

Limitations of this study include the inability of either method to differentiate carbapenemase class, requiring further phenotypic testing and molecular sequencing. The number of Class A carbapenemase genes in the study were small as were the number of unusual enzymes such as GES and IMI; however, the panel reflects the most common carbapenemase genes that are currently found in NZ (1,17).

To our knowledge, this is the first report on the performance of the recently released MAST ICT assay. Our findings would suggest that both the MAST ICT assay and modified CIM assay have a high level of sensitivity and specificity across a range of Gram-negative bacteria. Advantages of these tests include ease of use, simple to interpret, inexpensive, and an ability to detect carbapenemase production, regardless of class type, in *Enterobacteriaceae* as well as Pseudomonas and Acinetobacter. An all-in-one test format rather than having to use multiple inhibitor-based tests, is favourable for laboratories with limited resources and experience. All phenotypic testing should be tempered with the requirement for molecular confirmation.

## ACKNOWLEDGMENTS

We would like to thank the staff at Canterbury Health Laboratories who helped with assay reading.

## AUTHOR INFORMATION

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**MINUTES OF THE NZIMLS ANNUAL GENERAL MEETING HELD AT THE RUTHERFORD HOTEL, NELSON ON THURSDAY 24 AUGUST 2017 AT 7.30AM**

**PRESENT**
The President presided over approximately 33 members.

**APOLOGIES**
Apologies were received from: Jacquie Leaman and John Sheard
Accepted

**PROXIES**
Nil

**MINUTES OF THE AGM HELD 18 AUGUST 2016**
Motion:
Moved T Barnett, seconded MA Janssen
*That the minutes of the AGM held on 18 August 2016 be received.*
Carried
Motion:
Moved T Barnett, seconded MA Janssen
*That the minutes of the AGM held on 18 August 2016 be accepted as a true and correct record.*

**BUSINESS ARISING FROM THE MINUTES**
Nil

**REMITS AS CIRCULATED**
Motion:
Moved T Barnett, seconded R Hewett
"THAT Policy Decision Number 3 be reaffirmed
*Policy Decision No 3 (1972): Council will make and administer awards to members of the Institute; the details of each award will be recorded and may be amended from time to time by resolution of Council. The summary of these details shall be published annually in the Journal.*
Carried
Motion:
Moved T Barnett, seconded R Sloper
"THAT Policy Decision Number 5 be reaffirmed."
*Policy Decision No 5 (1978): That invitro diagnostic companies should not be approached to aid in the finance of Special Interest Group meetings; companies may be invited to SIG Seminars and although donations may be accepted money is not to be solicited.*
Carried
Motion:
Moved T Barnett, seconded R Siebers
***Section 8 Cessation of Membership***
*Subsection (c) of Section 8 Cessation of Membership to be reworded:*
*c) Any member whose subscriptions are unpaid after the expiration of three (3) calendar months from the date fixed for the payment of subscriptions shall cease to be a financial member of the Institute and shall cease to receive the publications and membership benefits of the Institute. If subscriptions remain unpaid after the expiration of a further six (6) calendar months, he/she shall be struck off the Roll by the Council provided that in absolute discretion of the Council such member's name may be returned to the Roll at any time upon payment of all arrears due by the member at the time of restoration.*

*Subsection (d) to be removed, thereby re-indexing subsequent sections.*
Carried
Motion:
Moved T Barnett, seconded R Sloper
*THAT Subsection (a) of Section 19 Subscriptions and Levies to be reworded:*
*The rates of subscription for the various classes of member shall be such amounts as may from time to time be fixed by the Institute in General Meeting provided that no alteration of such rates shall be made unless not less than sixty (60) days' notice in writing of such proposed alteration shall have been sent to each member prior to such meeting, and the proposed alteration is approved by two-thirds (2/3rds) of those present personally, or by proxy, at such meeting. Any alteration shall take effect from 1st January following approval of the proposed alteration.*

*Subsection (d) to be reworded:*
*d) Subscriptions for new and reinstated members elected up to nine (9) months after the date of renewal shall be at the annual rate. Subscriptions for new or reinstated members elected after nine (9) months of the financial year has lapsed, shall be invoiced at one-half of the annual rate. Members shall be deemed financial for the current year on payment of the subscription.*
Carried

**PRESIDENTS REPORT**
Motion:
Moved T Barnett, seconded R Hewett
*That the President's Report be received.*
Carried

**ANNUAL REPORT**
Motion:
Moved T Barnett, seconded S Melvin
*That the Annual Report be received.*
Carried
The attendees were questioned if the information within the Annual Report gives members what they are looking for and is in a format that is clear and readable. In Greg Warren's opinion, it covers all that is required and in a clear, readable format.

**FINANCIAL REPORT**
Motion:
Moved T Barnett, seconded R Hewett
*That the Financial Report be received.*
Carried

**ELECTIONS:**
Elected unopposed:

| | |
|---|---|
| President | Terry Taylor |
| Vice President | Mary-Ann Janssen |
| Treasurer / Secretary | Tony Barnett |
| Region 1 Representative: | Sujata Hemmady |
| Region 3 Representative: | Raylene Sloper |
| Region 5 Representative: | Sue Melvin |

Result of the election for Region 2:

| | | | | | |
|---|---|---|---|---|---|
| Kate McLaughlin | 09 | Jo Madden | 17 | Sean Munroe | 36 |

Therefore, Sean Munro was elected as the Region 2 Representative on the NZIMLS Council.

Result of election for Region 4:

| | | | | | |
|---|---|---|---|---|---|
| Clare Tibbs | 16 | Matthew Pynegar | 23 | John Sheard | 24 |

Therefore, John Sheard is elected as the Region 4 Representative on the NZIMLS Council.

**HONORARIA**
Motion:
Moved R Siebers, seconded R Hewett
*That no honoraria be paid.*
Carried

**AUDITOR**
Motion:
Moved T Barnett, seconded T Taylor
*That the Auditor for NZIMLS be Nexia New Zealand for the 2017-2018 audit.*
Carried

**GENERAL BUSINESS**
Greg Warren noted the good will of employers and suggested to Council that they find a good way to communication with employers in the future. He considered that it is important to have a voice both ways with employers and Council. It was noted that most communication with employers is via the Laboratory Managers meeting and the Pathology Roundtable group. Council do need to step back a little, be mindful and not been seen to be in cahoots with any particular employer group. Council will be considering this suggestion with a view to opening a communication stream.

John Aitken spoke of the conference budgets that are available to clinical pathologists and specialists that medical laboratories provide services for. He suggested that this type of provision should be available to medical laboratory staff. It was noted that employers do have funding for medical laboratory personnel to attend conference and this is also outlined in contracts. Mr Aitken considered that there needs to be a national policy for medical laboratory scientists and technicians to be allocated funding to attend conferences and seminars.

John Aitken went on to acknowledge that medical laboratory scientists/technicians are very much a silent group and he sees others introducing data that is produced by a scientist. An MLS does not get acknowledged for developing this and the others are only taking credit for this themselves. Mr Aitken feels it is time for us to redefine the relationship with the employers and public. Let's rethink engagement with other groups and do in such a way that we do not upset out employers. Ross Hewett advised the meeting that attendance at conferences/SIG seminars is included under the MECCA agreement. Amounts are ring-fenced for CPD and there is fixed amount for scientists and technicians. However, often by the end of the financial year there are funds still available. The reason for this is because staff are not applying for these funds. Mr Hewett noted that the amount available for these educational programmes is approximately $600.00.

Jillian Lanham, Waikato DHB to check with her employer as to what funding is available to her for conference attendance.

Navin Karan from the PPTC thanked the NZIMLS for their donation of $6,000.00 to the PPTC for the EQ programme in PNG.

**2018 Annual Scientific Meeting**
To be held in Christchurch

**2019 Annual Scientific Meeting**
South Pacific Congress, Gold Coast, Australia 17 – 20 September 2019.

The meeting closed 8.15am

# Fellowship of the NZIMLS

The NZIMLS encourages members to consider Fellowship as an option for advancing their knowledge and career prospects. Fellowship provides an attractive option to academic postgraduate qualifications at a fraction of the cost.

Fellowship of the NZIMLS may be gained by thesis, by peer reviewed publications; or by treatise in case of a member holding an appropriate postgraduate or professional qualification.

Fellows may use the nominals FNZIMLS if a current financial member of the Institute.

### Thesis

The thesis must be based on the style of Master of Science by thesis requirement of New Zealand universities.

### Publications

A minimum of ten peer reviewed publications in international or discipline acknowledged biomedical journals. The candidate must be the 1st or senior author of at least six of these publications. A comprehensive review of the submitted publications is also required.

### Treatise

By submission of a treatise in the form of a dissertation of 3000 - 5000 words on a medical laboratory science subject. The dissertation may take the form of a review, a scientific study, development of a hypothesis, or any other presentation that meets with the approval of the Fellowship Committee.

Candidates applying for Fellowship by this route must be holders of at least a Master's degree in medical laboratory science or a closely related subject, or have a professional qualification such as Fellowship of the following professional bodies: Australian Institute of Medical Science; Institute of Biomedical Science; Faculty of Science of the Royal College of Pathologists of Australasia, Australiasian Association of Clinical Biochemists; Royal Institute of Biology, London.

For full Fellowship regulations and the application process visit the NZIMLS web site at www.nzimls.org.nz

### Current Fellows of the NZIMLS

Jenny Bennett
Jillian Broadbent
Brett Delahunt (Honorary)
Jan Deroles-Main
Susan Evans
Sheryl Khull
Christine Leaver
Dennis Mok
Maxine Reed
Robert Siebers
Andrew Stewart
Vasanthan Thuraisamy
Jacqueline Wright
Sheryl Young
Mark Bevan
Ailsa Bunker
Jennifer Castle
Marilyn Eales
Christine Hickton
Michael Legge
Ron Mackenzie
Howard Potter
Mohammad Shahid
Mary Stevens
Vanessa Thomson
Emil Wasef
Rubee Yee

### New Fellow

Congratulations to Dennis Mok, Australia who has been awarded Fellowship for his treatise "ISO 15189:2012 implementation checklists for conformity assessment by accreditation bodies: a comparative analysis".

### Honorary Fellow

Upon recommendation of the Fellowship Committee, Council has awarded an Honorary Fellowship to Professor Brett Delahunt of the Wellington School of Medicine & Health Sciences for his significant contributions to our profession and to pathology. This was presented to him during the opening ceremony at the NZIMLS Annual Scientific Meeting In Nelson in August 2017.

Brett Delahunt obtained his BSc(Hons) at Victoria University before studying medicine at Otago University, gaining a BMedSci and MB ChB in 1978. He then studied anatomic pathology, gaining Fellowship of the Royal College of Pathologists in 1985. He worked for many years as an Anatomic Pathologist at Wellington Hospital and was its Clinical Director until 1998 when he became Professor of Pathology and Molecular Medicine at the Wellington School of Medicine. During this period he obtained his MD degree by thesis, was elected FRCPath and FFSc(RCPA), and was recently elected as Fellow of the Royal Society of NZ. He has received numerous honours over the years, in particular Knight of the Order of St John, and the NZ Order of Merit for services to pathology.

His main speciality over the years has been in urological pathology, particularly in prostrate cancer of which he is a renowned international expert and has played leading roles on it for the WHO, The RCPA, and the International Society of Urological Pathologists. His research interests involve all aspects of urologic pathology with emphasis on the diagnosis, classification, genetics and prognosis of prostatic and renal neoplasia. He has over 300 peer reviewed publications including five in our Journal. He is Chief Editor of the journal *Pathology*, and is Editorial Board Member of nine other international pathology journals.

His major contribution towards the profession of medical laboratory science has been his role on the Medical Laboratory Technologists Board, now the Medical Sciences Council. He was appointed in 2000 and served on the Board for a record 13 years, the last three years as Deputy Chair. During his time on the Board he has been Medical Laboratory Sciences Representative Board Member; Medical Sciences Secretariat Board of Management 2010-2013; Member, Health Practitioners Competence Assurance Act Implementation Committee 2004-2006; Member, Qualifications Assessment Committee (later Registration Committee) 2000-2013; Member, Professional Standards Committee 2000-2002; Convenor, Professional Standards Committee 2010-2013; and Member, Education Committee (later Accreditation Committee) 2003-2005.

Other contributions to our profession include six years on the Otago University Board of Studies for Medical Laboratory Science, and Consultant Pathologist to the Pacific Paramedical Training Centre from 1985 to 1995. Professor Brett Delahunt has always valued the contributions of members of our profession to pathology and has been a champion of our profession over many years.

Professor Brett Delahunt (left) receiving his Honorary Fellowship from Ross Hewett, President of the NZIMLS.

# Journal Questionnaire

Below are ten questions based on articles from the November 2017 issue. 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.

The site has been developed for use with Microsoft's Internet Explorer web browser. If you are having problems submitting your questionnaire and you are using the Firefox web browser, try re-submitting using Microsoft's Internet Explorer.

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 23nd February, 2018. You must get a minimum of eight questions right to obtain five CPD points.

The Editor sets the questions but the CPD Co-ordinator, Jillian Broadbent, marks the answers. Please direct any queries to her at cpd@nzimls.org.nz.

## *NOVEMBER 2017 JOURNAL QUESTIONNAIRE*

1. Which therapies can adult transgender individuals undergo to help have their physical bodies more closely align with their identified gender?
2. What are amongst the most common of chromosome abnormalities that continue during pregnancy?
3. Typically, infants with Down syndrome may have which symptoms?
4. What is the main drawback of ANA testing, and why?
5. The reticulocyte haemoglobin equivalent provides a measure of what?
6. What could the future clinical utility of the reticulocyte haemoglobin equivalent be?
7. The use of anti-human globulin in the complement dependant cytotoxicity assay enhances what?
8. The finding of early reaction errors on the Sysmex CS 2100i analyser leads to what?
9. What is an area of concern with the MAST indirect carbapenemase test?
10. To reliably distinguish carbapenemase-producing organisms from carbapenem-resistant-non- carbapenemase-producing organisms, what do the authors recommend?

## *AUGUST 2017 JOURNAL QUESTIONNAIRE ANSWERS*

1. Name the elements of the ADKAR model.
   **Awareness; Desire; Knowledge; Ability; Reinforcement.**

2. The ADKAR model in conjunction with a business model should assist management in what?
   **Successful transition of changes in planning change management activities; Diagnosing gaps; Developing corrective actions; Supporting managers and supervisors.**

3. What factors influence the knowledge element of the ADKAR model?
   **Current knowledge base of an individual; Capability of this person to gain additional knowledge; Resources available for education and training; Access to or existence of the required knowledge.**

4. In the change management of healthcare study what did participatory action research allow for?
   **Open communication and ongoing support for individuals who chose to move to another career**.

5. Name four reported criteria of participatory action research.
   **Educative; Deals with individuals as members of social groups; problem-focussed, context-specific and future-orientated; Involves a change intervention;**
   **Aims at improvement and involvement;**
   **Involves a cyclic process in which research, action and evaluation are inter linked;**
   **Founded on a research relationship in which those involved are participants in the change process.**

6. Increased oxidative stress of the red cell membrane due to sulphonamide antibodies results in what?
   **Extravascular and intravascular destruction of red cells.**

7. What are known markers of phagocytes removal?
   **Autologous IgG and complement C3 fragments.**

8. In the anti-sulphonamide antibodies article what may have explained the study's finding of lower lymphocyte counts?
   **Lymphocytes are responsible for antibody production and may be used up in the process of sulphonamide antibodies production.**

9. What may explain why the neutrophil count was significantly higher in patients with sulphonamide antibodies?
   **Neutrophils may be needed to phagocytize sulphonamide antibodies-coated red blood cells leading to their ultimate extravascular destruction.**

10. What were the significant haematological findings of the presence of sulphonamide antibodies?
    **Anaemia, thrombocytopenia, increased neutrophil count and decreased lymphocyte count.**

# THE Pacific WAY

## *GREETINGS TO YOU ALL, FROM THE PPTC*

### Laboratory Quality Management 2017

A Laboratory Quality Management course was provided by the PPTC in August 2017 at its centre in Wellington, and the following three students attended:
Rejieli Nagiri, Fiji ; Samson Kangapu, Papua New Guinea; and Edgard Tay, East Timor.

This course was carried out over four weeks and during this time, the PPTC provided a comprehensive theoretical component and a series of case scenarios to those students who attended. The purpose of this specialised training was to provide a holistic approach to quality management in a medical laboratory setting. The students, on completing the course, were equipped with sufficient knowledge to be able to confidently lead their team with the ISO 15189 requirements, enabling their laboratory to function efficiently in providing a continuous uninterrupted service so as to ensure the delivery of quality diagnostic test results to clinicians using the medical laboratory services for patient management.

Students and Staff – Laboratory Quality Management 2017

### Microbiology 2017

A Microbiology course was provided by the PPTC in October 2017 at its centre in Wellington, and the following seven students attended:
Thomas Kunjil, Nellie Tumu, Papua New Guinea; Tupou Chan, Samoa.; Ashwini Vinod, Fiji; Chanborann Chaing, Sineang Morn, Cambodia/Diagnostic Microbiology Development Programme (DMDP) Swartika Sivangni Prasad, Fiji/ Fiji Vet Pathology Lab.

This course was also carried out over four weeks allowing students to gain a comprehensive theoretical understanding and extensive practical experience in the diagnostic medical field of Medical Microbiology.

Students and Staff – Microbiology 2017

### Pacific Paramedical Training Centre Based Courses

**Training Courses 2017:**

- **Blood Transfusion Science**
  30 October - 24 November 2017

**Training Courses 2018:**

- **Laboratory Health & Safety; and Quality Management Systems**
  9 April - 4 May 2018 *(4 weeks)*
- **Haematology and Blood Cell Morphology**
  21 May – 29 June 2018 *(6 weeks)*
- **Effective Laboratory Management**
  2 – 27 July 2018 *(4 weeks)*
- **Biochemistry**
  13 August – 7 September 2018 *(4 weeks)*
- **Microbiology**
  24 September – 19 October 2018 *(4 weeks)*
- **Blood Transfusion Science**
  5 – 30 November 2018 *(4 weeks)*

For further information contact:
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**Overseas Travel**
**From July 2017 to the present:**

July 10th – 14th TONGA
Health and Safety Assessment, Tonga
John Elliott

July 17th – 21st
Accreditation and Haematology HOD training, Solomons
Russell Cole and Phil Wakem

July 24th – 28th
Microbiology HOD training, Samoa
Navin Karan

31st July – 4th August
Biochemistry Service Development , Tonga
Filipo Faiga
Accreditation Audit , Vanuatu
Russell Cole

4th – 8th Sept
Biochemistry Service Development, Samoa
Filipo Faiga
TB Surveillance, Vanuatu
Navin Karan

25th – 29th Sept
HOD Biochemistry Training , Solomons
Filipo Faiga
HOD Microbiology Training, Samoa
Navin Karan

9th – 13th Oct
Provincial Laboratory Assessments, Tonga
Phil Wakem and Navin Karan

## Diploma of Medical Laboratory Science Programme 2017 - 2018 Cycle

The PPTC is currently offering six POLHN Modules leading to the Diploma in Medical Laboratory Science [PPTC] and students are currently nearing the completion of Haematology and Biochemistry the second and third of the six modules to be launched in the 2017-2018 cycle.

The Diploma course is to be delivered over a two year period to students who have registered and have been accepted for the programme.

Four students from the Federated States of Micronesia graduated with the PPTC's Diploma in Medical Laboratory Science. (2015 – 2016)

## Diploma of Medical Laboratory Science Graduates 2015 - 2016 Cycle

- Ivapene Faumuina-Aiafi Samoa
- Makerita Ieremia Samoa
- Maeva Ah Yen Samoa
- Perenise Eti Samoa
- Tupou Chan Tung Samoa
- Roseleen Deo Fiji
- Andrea Kisina Tonga
- Pukelotu Kakau Tonga
- Mo'unga Tu'inukuafe Tonga
- Atevalu Lino Tonga
- Vaimoana Lomu Tonga
- Mo'unga Tu'inukuafe Tonga
- Sokopeti Fakaosifolau Tonga
- Elizabeth Tekanene Kiribati
- Obwaia Buren Kiribati
- Ianeta Tewaaki Kiribati
- Iasko Ada Pohnpei
- Elizabeth Rogers Pohnpei
- Adesina Maruame Pohnpei
- Johnny Amor Pohnpei
- Bradley Edgar Pohnpei

## United Nations Development Programme

Both Russell Cole and Navin Karan facilitated training in Fiji for the regional TB laboratory technicians on the 7th and 8th of August with the following objectives:

- To disseminate the findings, elaborate on ways to improve areas of deficiencies and determine the way forward in regards to External Quality Assessment (EQA).
- To provide information and train on Gene Xpert operations, testing algorithms, operating procedures and maintenance.

This workshop was very successful and 20 delegates from Pacific laboratories attended.

Regional TB Laboratory Technicians training for the Pacific Island Countries, Nadi, Fiji, 7-11 August 2017

## Blood Transfusion Specialist

**The appointment of a new PPTC Consultant (Blood Transfusion Specialist) for 2018**

In Nov 2017 the CEO will advertise for a Qualified Medical Laboratory Scientist preferably with 5 or more years post graduation experience to commence employment with the PPTC mid January 2018.

The successful applicant will have comprehensive experience particularly in Blood Transfusion Science and to a lesser extent in Haematology and is prepared to travel extensively throughout the Pacific Region. Knowledge of Quality Laboratory Management and a comprehensive computer skill set will also be a requirement.

This is a full time position, the advertisement for which will be placed on the NZIMLS website in mid November.

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