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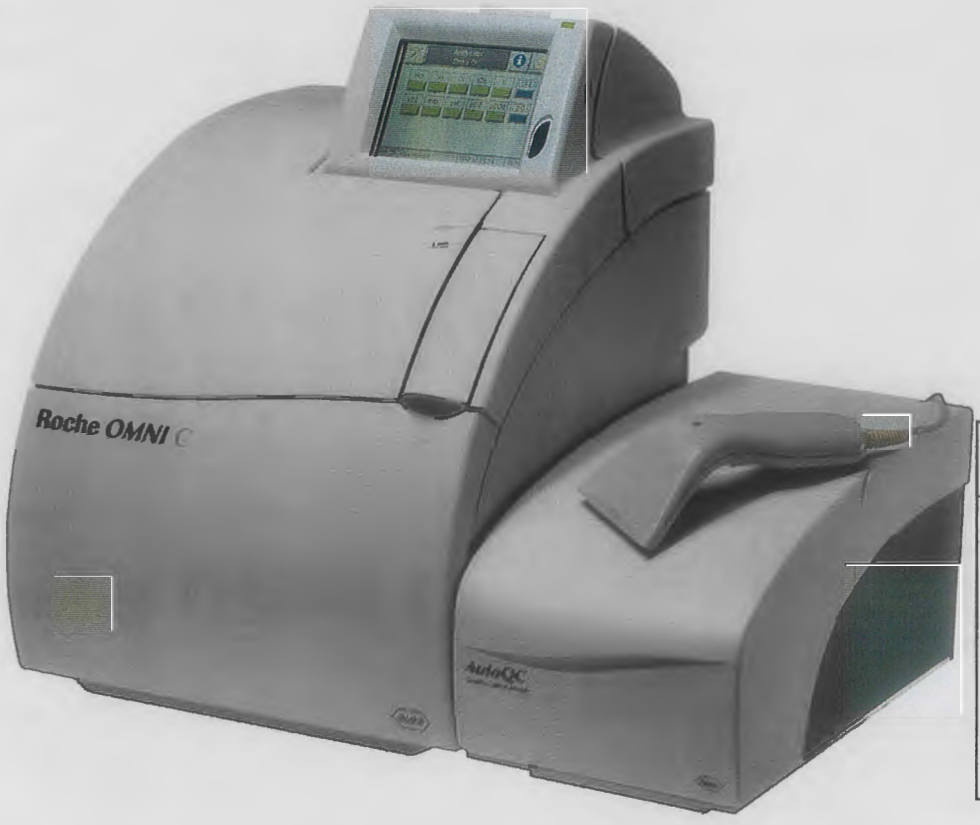

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T H Pullar Memorial Address
Symphony of Science - 1ST Movement - Regulation

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This address is given in honor of Dr Thomas Pullar who was a pathologist at Palmerston North Hospital from 1937 - 1962. Dr Pullar made an important contribution to the development of our profession by helping to establish educational training schemes for medical laboratory technologists, which included preparing new syllabuses and setting up examinations. He was a founder member of the New Zealand Society of Pathologists and was also involved in the formation of the Medical Laboratory Technologists Board, which leads me into the first movement of this Symphony of Science.

Medical Laboratory Science is a regulated profession. Before practicing as a medical laboratory scientist in New Zealand you must be registered by the Medical Laboratory Technologists Board (MLTB). To gain this registration you must satisfy the Board that you have a particular qualification, either a Bachelor of Medical Laboratory Science (BMLS) from Otago, Massey or the Auckland University of Technology, or you have undergone a course of training and passed examinations that in the opinion of the Board are substantially equivalent to those of the BMLS of Otago, Massey or Auckland University of Technology. You must also have undergone a course of training and have a minimum period of practical experience in a medical laboratory of 6 months full time so that you acquire the competencies set out in the MLTB document "Registration Requirements: Competencies, Learning Outcomes and Performance Criteria". You must produce 2 certificates of good character and swear that you have not been convicted of an offence punishable by imprisonment for a term of 2 years or more. If you imply that you are registered or qualified to be registered or engage in a registrable occupation when you are not registered, you are liable to a fine of up to $10,000. If your registration is fraudulently obtained you could be imprisoned for a maximum term of 3 years. So this is serious stuff.

Why is medical laboratory science a regulated profession? In 1972 an amendment to the Medical and Dental Auxiliaries Act resulted in Medical Laboratory and Medical Radiation Technologists, Podiatrists and Dental Technologists becoming registered professionals. I do not know the reason for this and am unaware of problems with the standard of healthcare these professions were providing. The Act is headed as "An Act to make for provision for the registration and discipline of persons engaged in occupations auxiliary to medicine or dentistry". The functions of the Boards were to register qualified persons and conduct examinations for entry into the occupation, to exercise disciplinary powers, to promote high standards of education and conduct and to advise the Minister on any matter affecting registration and education of these persons.

According to Barrie Edwards then Secretary of the New Zealand Institute of Medical Laboratory Technology, in a letter written to Mr. Stace of the Economic Development Commission in 1988, it was not our profession who wanted legislation. It was the Department of Health, who in a submission to the Board of Health Committee inquiring into Clinical and Public Health Laboratory services, stated that registration would protect members of the public by enabling them to distinguish between the qualified and the quacks and it would prohibit or restrict the performance by the unqualified of the kind of work with which the occupation is concerned. So even though it does not say so in the Act it appears that regulation was to protect the public.

The MLTB operates within the guidelines of 3 documents, the Medical and Dental

Auxiliaries Act, the Medical Laboratory Technologists Regulations and the Medical Laboratory Technologists Board Manual. This is very confusing with many items being mentioned in each document. Section 40 of the Act states that the Governor General may from time to time make regulations and it is these regulations that contain most of the detail. Section 4 of the Regulations states that certain matters are to be prescribed in the Manual. The qualifications, training and experience needed for registration appear here and interestingly the Board can change this document i.e. it does not require legislation to make changes to the manual unlike the Act or Regulations.

A large group of laboratory workers are exempted from regulation. Section 9 of the Regulations states that nothing in Section 32 of the Act (which is about offences by unregistered persons) shall prevent the performance of medical laboratory technology by a registered medical practitioner, a scientific officer (SO), a medical laboratory assistant or a trainee while responsible to and under the supervision of a medical laboratory technologist (MLT), SO or registered medical practitioner. Laboratory Assistants are exempt because they are defined as being engaged in work ancillary (subordinate) to medical laboratory technology under supervision and Scientific Officers are exempt because they are defined as university graduates in science or the equivalent, doing research or technical development work and not routine testing.

We may not have asked for our profession to be regulated, but having got it we have fought hard to keep it when under threat. In 1987 Geoffrey Palmer, then Deputy Prime Minister, made a speech in which he stated that New Zealand was over regulated. A working group on Occupational Regulation was set up to review legislation on occupational licensing. Questions that were asked included: what is the purpose of regulation, is that purpose being achieved, is the regulation actually in the interests of those who are regulated or the consumer? For 10 years regulation remained under a cloud and it was not until 1997 that those questions were finally answered.

So do we need regulation? Are we not just protecting ourselves - creating a closed shop and shutting out competition? True there is regulation in Canada, the United Kingdom and some states of the USA but Australia does not have it and they appear to have high standards in their laboratories with few problems. It has been said that if it was not for regulation employers might use unqualified staff in an effort to increase profits. I think it is more likely that employers will use qualified competent staff to reduce repeat testing thus minimizing costs. Anyway IANZ check that laboratories have appropriate staffing at the time of accreditation.

Some of you present probably think that the MLTB and its regulations are very restrictive and protective because the person you have employed from overseas has been denied registration but it is because they do not fulfil the criteria prescribed in the manual even though they have a science degree and experience.

One of the arguments put forward by the Ministry of Health (MOH) against regulation was that the public does not directly interface with MLTs. Rather it is the doctor who orders the tests and receives the results who does. In fact the MOH stated that in comparison to Medical Radiation Technologists and Podiatrists the risks posed by our work were much less. On the other hand because patients do not choose who performs their laboratory test you could argue this is all the more reason that those performing the tests should be regulated. We all know that doctors rely on the accuracy of our results - remember the PSA results in Gisborne.

Finally in 1997 the Ministry of Health included the following statement in a Cabinet paper prepared for Ministers. *"In the Ministry's view, the risks of wrong diagnosis (which could in some cases be life threatening) are managed through a variety of measures of which licensing is but one. The other measures include quality assurance processes and management structures which ensure that the more highly experienced staff act as "peer reviewers' of the analysis carried out by less experienced technicians. As noted above there will be occasions in which these other measures do not operate or can only operate retrospectively. In these situations reliance on a safe outcome is dependent entirely on the competence of the individual practitioner. In the Ministry's view these occasions are sufficiently frequent, and the possible outcomes of incompetent practice so severe, that a continuation of some form of occupational registration is justified."* So it does not matter what you or I think about regulation. Government has decided it is to stay and it only remains to see what changes are to be made to the present system.

What does the MLTB actually do you might ask? To give you an example, I will run through a recent meeting. It is 06.30 in the morning when Dennis and Ross, umbrellas up as they are from Auckland, leave home to catch the 08.00 am flight to Wellington where they meet the rest of the Board at the secretariat offices. So who are the MLTB? Well we are four practicing registered medical laboratory technologists, Dennis Reilly, Ross Anderson, Paul McLeod and Harold Neal, and four other persons, one of whom may be a registered MLT (myself), one of whom may be a pathologist, currently Brett Delahunt and two others, commonly referred to as "lay people". A layperson is defined as being neither registered nor qualified to be registered. I well remember being told that "lay people" were retired people with nothing better to do than get on these committees and make a nuisance of themselves. Nothing could be further from the truth in our case. Our laypersons are Tania Simpson a director of a Maori Policy and Conflict Management Company, and Carol Ramage, who was the General Manager of Green Lane Hospital. Both contribute much and bring different skills to the meetings. The Minister of Health has appointed us all. The most important person present however is Phil Saxby, Secretary of the Board.

On the agenda for registration there are 26 overseas applications, 27 from New Zealand, four applications to review and seven where additional information is to be requested. The applications from NZ graduates are straightforward in that they all have a BMLS and at least six months laboratory experience. The overseas applications are complicated in that they all differ in qualifications and experience. Some are easy to turn down as they do not have a qualification anywhere near equal to a New Zealand BMLS degree but for many there is considerable debate. Some applicants may have a BSc, B App Sci or even a Masters degree, most often in chemistry or microbiology, but they have not studied haematology, transfusion science etc. and therefore do not have a qualification equivalent to a New Zealand BMLS. They are not necessarily from 3rd world countries but may be from England or Australia.

Conscious of the shortage of Medical Laboratory Scientists in New Zealand and that the rules were rejecting applicants who probably could practice safely, the Board introduced a new category in 2001 to allow those who may not quite have the academic level of a BMLS, but have the equivalent of a National Diploma covering all laboratory disciplines, as well as having a lot of experience, to become registered. They must have completed a minimum of 10 years experience post qualification. This is a long time but the Board felt it should be conservative to start with in this new category. Where it looks as if a person may fit the bill but there is an element of doubt, such as coming from a country that we have no experience of, the Board will recommend that registration be subject to six months satisfactory work in a NZ laboratory to acquire the MLTB competencies.

There are still applicants who cannot be registered under present rules but who have a medical laboratory technology qualification and a lot of experience. The problem is that their qualification is not broad based which is a New Zealand requirement because we have general registration. It is proposed that a new category should allow holders of a post graduate qualification in medical laboratory technology and who have specialized laboratory experience at MLT level, to be registered. It is expected that these persons will work in specialized fields and this is perhaps pre-empting the changes about to take place in the regulations. Most of the first day is taken with assessing all of the applications.

Applicants can appeal if turned down. Eight people have done so in the last five years but only three proceeded to a hearing.

Next the financial report is considered. The Board is now a Body Corporate able to set its own fees and spend and invest as it wishes. The main income is from registration and Annual Practicing Certificate (APC) fees (about $208,000). The main expenditure is the cost of the Registration Board Secretariat (RBS), (the company that carries out the Board's administration), Board committee fees and travelling expenses (about $187,000)

It is then the Education committees turn. The Board moderates the NZ BMLS courses. This includes a visit, every five years to the universities offering the courses to check that their course meets the requirements of the MLTB Competency document. A visit is to be made to the Auckland University of Technology this year and the timetable and members of the review team are approved.

The syllabuses of the different disciplines are also examined every three years, the objective being to ensure that they are relevant and up to date. Reports have been received for two disciplines. The report on discipline A makes minor suggestions, which appear to have been acted upon. However the report on discipline B makes a number of recommendations about one of the university courses. It suggests there is a lot of out of date material. Apparently a new lecturer is to take over this discipline and the Board decides to ask the moderators to review the discipline again. The university will be informed that a follow up review will take place in six months time. The Board has the power to remove a course from those accepted as leading to registration. Reports are then received from the Board representatives on the university advisory committees. In this way dialogue is maintained between the MLTB and the universities providing the BMLS.

The Health and Disability Commissioner (HDC) has referred a complaint made against a MLT to the Board, who now have to appoint an investigator. The investigator must not be a member of the Board and will have to decide if a case should be brought against the MLT. If so a lawyer will be engaged to prosecute on behalf of the investigator. The Board will hear the case and adjudicate accordingly. There have been very few disciplinary cases against MLTs. There have been two in the last five years. The most recent one involved a person who was de-registered for six months for altering a laboratory result without authority.

The rest of the meeting is taken up with discussing the Health Practitioners Competence Assurance Bill (HPCA), which is going to require a huge amount of discussion and work to implement and some

other minor items on the agenda. The most pleasant part of the meeting is dinner at a reputable Wellington restaurant where we can relax and Brett will give us the latest gossip in the pathology world.

I would now like to outline the changes about to take place in our regulation. In June this year the Health Practitioners Competence Assurance Bill (HPCA) was introduced into parliament. This Bill is based on the Medical Practitioners Act 1995 and will repeal all existing health regulatory statutes. This involves 16 health groups. This time the Act has a statement of purpose. It is to protect the health and safety of members of the public by providing for mechanisms to ensure that health practitioners are competent and fit to practice their profession.

If the Act is passed Medical Laboratory Technology will become Medical Laboratory Science. Licensing will be replaced by certification in which professional title is protected but activities are not protected although the Minister of Health will be able to recommend that certain "invasive" activities that could cause serious or permanent harm should be restricted. For example cross matching for dispensing of blood and venipuncture could become restricted activities. If the Act is to protect the public then certification should include all qualified laboratory staff and three classes of registration are proposed:

- Medical Laboratory Scientists
- Medical Laboratory Technicians (currently Laboratory Assistants)
- Scientific Officers

You will be registered for a scope of practice for which you must have the prescribed qualification, training and experience to be competent to practice. This may include having to practice under supervision, for example Medical Laboratory Technicians. It is proposed that the current disciplines within the profession will be the scopes of practice although some individuals will have a more limited scope such as toxicology or mycology. Decisions have to be made on what you have to do to change your scope of practice; will there be an examination, a logbook or signing off by the charge scientist?

Some people will wish to practice within more than one scope, for example in small laboratories. What tasks will they be restricted to?

You will have an annual practicing certificate and this will only be issued if you can demonstrate that you maintain your competence. How is the Board going to monitor this? There is provision in the Act for the Board to set or recognize competence or re certification programs such as the NZMILS Continuing Professional Development Program. It could be argued that this program does not necessarily demonstrate that one is competent but that one is improving ones education. However completion of such a program plus signing off by the charge scientist may suffice. Will re certification programs also apply to Medical Laboratory Technicians? These are decisions the Board will now have to consider.

Mandatory reporting of a health practitioner, whose practice is below standard, has been deleted from the Act but if a health practitioner thinks that another health practitioner poses a risk of harm to the public they may notify the Registrar of the Board. If the Health and Disability Commissioner believes that a health practitioner poses a risk of harm they will have to notify the Registrar as will an employer if he/she dismisses someone for incompetence. When a Board has reason to believe that the practice of a registered health practitioner may pose a risk of harm to the public it will have to notify the ACC, Director General of Health, HDC and the persons employer.

If a complaint is made against a health practitioner it will go to the HDC who must inform the Board. If the HDC refers the complaint back to the Board, the Board appoints an investigating committee of two registered medical laboratory scientists and one layperson to investigate the complaint. This committee may recommend no further action, or conciliation, or that the Board reviews competence or fitness of the practitioner, or refer it to the police, or bring a charge against the practitioner before the Health Practitioners Tribunal. The Tribunal will have a chair and deputy chair who are barristers or solicitors of the High Court of not less than seven years practice and members from a panel of practitioners from each profession, maintained by the Minister of Health. Appeals against registration and the APC will be heard in the District Court whilst appeals against decisions of the Tribunal will be heard in the High Court. The Health Department has estimated that the cost of disciplinary hearings may be $20,000 - $25,000 per day, which would be considerably more than our present costs.

Boards will have 5 - 14 members. The membership of the present Board will roll over but if it continues to have 8 members another layperson will have to be appointed. A registrar will also have to be appointed to the Board.

The HPCA bill has been introduced into Parliament. In the mean time there has been an election but submissions on the Bill are soon expected to be called for. The Act will come into force one year after the date it receives the Royal assent. This legislation will affect all those who work in a medical laboratory. The MLTB has a huge task ahead in implementing the likely changes and it has agreed to fund RBS $12000 along with seven other Boards, to employ someone for two years to manage the implementation of the Bill.

All technical staff will be registered including laboratory assistants. We will all practice within a scope of practice. We will not be registered for life as happens now but will be required to show that we continue to be competent to get our annual practicing certificate. For many people this is not a problem but some laboratory workers who have done no more than turn up at work to do some testing and mark time will have to become more active.

Employers will be affected if they continue to pay for annual practicing certificates, as there will be an increased cost if all staff is to be registered. It is also possible that fees will increase to pay for the increase in administration required by the Board. Competency reviews will mean a cost, be it paid to the MLTB or to an organization providing a program. Practitioners may undertake more training programs at a cost to themselves or employers.

The present Board has no major disagreements with the proposed Bill but other groups larger than us may well have and some items may change. Will the new requirements make a difference to the safe practice of Medical Laboratory Science in New Zealand? I doubt it mainly because I think our profession is already practiced to a high standard. Of course this may be because we have been a regulated occupation for the last 30 years. Because the MLTB has legal authority, it has refused to register people who do not have adequate qualifications and training, insisted that medical laboratory science diploma and degree courses are relevant and of a high standard before accrediting them and disciplined registrants who practice below the required standard. Therefore the aim of regulation has been achieved and that is to protect the public.

Salivary testosterone: is it an indicator of free testosterone concentration in plasma?

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Abstract

Total testosterone determinations in plasma are often used to assess the androgen status of patients. However it is now thought that the bioavailable or free fraction may be more clinically useful but methods for free testosterone measurement are difficult and time consuming although calculated values using total testosterone and sex hormone binding levels can overcome these problems.

An alternative is the use of salivary testosterone which may also be an indicator of bioavailable testosterone. This project compares salivary testosterone measurements with calculated free testosterone levels in plasma as well as the free androgen index in both male and female patients. It is concluded that the mathematically derived indicators of bioavailable androgen status using plasma total testosterone and sex hormone binding globulin are superior as they overcome the caveats imposed by saliva samples.

Keywords: testosterone, free testosterone, free androgen index, sex hormone binding globulin, saliva

Introduction

The sex steroids testosterone and estradiol are responsible for the secondary sex characteristics associated with each sex. These steroids are derived from cholesterol, as are all other steroids including cortisol responsible for fuel metabolism and aldosterone which controls salt balance. They are all produced by additions, alterations and deletions of the basic cholesterol molecules by the actions of enzymes, and are produced in a cascade system where one product becomes the substrate for the next member of the chain. Testosterone is derived from dehydroepiandrosterone sulphate (DHEAS) ↔ androstenedione ↔ testosterone, and the estrogens are further derived from testosterone. Testosterone is an anabolic steroid synthesized by the testes in males, the ovaries in females and the adrenal glands in both sexes. At puberty, and throughout most of the reproductive years, approximately 10-20 times more testosterone is synthesized in males than in females.

In males, testosterone and its 5 α reduced form dihydrotestosterone (DHT) are responsible for the male phenotype. At puberty, high levels of testosterone and DHT are responsible for the further development of male external genitalia, secondary hair patterns, stimulation of spermatogenesis, stimulation of anabolic activity leading to increased muscle mass, and behavioural changes. In pubescent females, testosterone effects are more subtle but equally important for proper musculoskeletal activity, general anabolic activity, and libido. In both sexes, testosterone enhances aerobic metabolism and increases protein synthesis.

Clinical syndromes in which plasma testosterone is increased include gonadal and adrenal tumours, adrenal hyperplasia, androgen insensitivity syndrome, severe acne, polycystic ovaries, and hirsuitism in females. Decreased levels of testosterone are associated with hypogonadism, hypopituitism, orchidectomy, oestrogen therapy, most cases of Klinefelter's syndrome, and decreased libido. Clinical applications in paediatrics include detection of precocious puberty, hypogonadism in adolescent boys, pituitary or hypothalamic disease, and virilization in girls.

Total testosterone determinations in plasma are often used to assess the androgen status of patients. The majority of testosterone in plasma (70%) is bound with high affinity to sex hormone-binding globulin (SHBG), 25-30% is bound with low affinity to albumin, and the remainder (1-2%) is free testosterone. There has been much discussion in the literature whether it is only the non-protein bound (free) portion that is available to tissues or the non-SHBG bound fraction, which is the free plus the albumin-bound portions (1-3). Most researchers maintain the bioavailable fraction in plasma comprises the free and albumin associated fractions, and it is thought that the bioavailable fraction of testosterone is more clinically relevant than total testosterone levels. However, the determination of bioavailable testosterone can be time-consuming and involved.

The traditional methods for the measurement of free testosterone are themselves indirect. The first available method used equilibrium dialysis where plasma was placed inside a semi-permeable membrane bag and then put into a protein-free buffer, whereby dialysis occurrs (4). Enhancements to this method involved the use of centrifugal force to speed the process, or the molecular sieving of plasma through steady state gel filtration. Other methods used ammonium sulphate precipitation of the protein bound fractions, but were also tedious. These methods are also theoretically problematical as they have the potential to disrupt the normal equilibrium between testosterone and its binding protein.

Methods for the direct measurement of free testosterone in plasma became available in the late 1980s. These involved the use of hormone analogues that have low affinity to the binding globulin but can compete with testosterone for antibody binding. This technology has also led to a great deal of discussion and controversy (5). Alternatively, bio-available testosterone can be calculated from total testosterone and SHBG measurements assuming constant plasma albumin levels. These calculations can be in the form of free androgen index (FAI) or as the actual free testosterone (FT) level. This appears to be a rapid, simple and reliable index of bioavailable T, in most cases more suitable for clinical routine use.

Over the past 20-30 years, numerous research studies have validated the use of saliva as a diagnostic medium in which to measure the biologically active fractions of steroid hormones in the bloodstream (6). As saliva is, in part, an ultrafiltrate of blood and steroids not bound by carrier proteins in the blood freely diffuse into saliva, the salivary testosterone level could also be an indicator of bioavailable testosterone. In this project we investigate this hypothesis with a view to providing clinicians with a useful, non-invasive indication of bioavailable testosterone.

Materials and methods

Subjects

EDTA plasma samples were obtained by venepuncture from 52 male and 21 female subjects attending Endocrine Outpatient Clinics between November 1998 - March 1999. At the same time, matched saliva samples were collected by direct salivation into sterile 5mL CSF

Labserv containers. All patients had given informed consent for these tests. Samples were frozen at -20(C until required for measurement. Freezing saliva also reduces its viscosity by partial mucin degradation.

Procedures

Plasma and salivary testosterone

Samples were measured by an "in-house" ELISA after organic extraction (7). Plasma and serum samples are treated as synonymous in this treatise. As saliva steroid concentrations are low, it was necessary to use a larger aliquot of the saliva to obtain significant sensitivity with our assay. Male plasma (50µL), female plasma (250µL) or saliva (500µL) were extracted (x2) with ether (3mL) and evaporated to dryness. Reconstitution was in 1mL phosphate buffered saline/bovine serum albumin (PBS/BSA) buffer for plasma and 250µL PBS/BSA for saliva samples.

Micotitre plates (96 well, Falcon 3912) were coated with testosterone-thyroglobulin conjugate in 6M aqueous guanidine hydrochloride overnight at 4°C (100µL/well). The plates were then washed and "blocked" in assay buffer (phosphate buffered saline containing 0.1% gelatin and 0.1% Tween 20). Aliquots (50µL) of reconstituted sample extracts were added to each well in duplicate and a 6-point calibration curve was performed on each plate. Following addition of standards (Sigma) and samples, 50µL of "in house" rabbit polyclonal antibody to testosterone-3CMO BSA (carboxymethyloxime BSA) was added to each well for a 90min incubation at room temperature. The plates were then washed 4 times in saline. A further 90min incubation with 100µL Silenus anti-rabbit HRP (horse rabbit peroxidase) was performed. The plate was finally washed and OPD (ortho-phenylenediamine) substrate added (100uL). Colour was developed and then stopped with 1.25M sulphuric acid (100uL). Absorbance was read at 492nm in a standard microtitre reader.

All specimens were run as routine samples using Bio-Rad Lyphocheck (ImmunoAssay Plus Control) Level 3 and Byk-Sangtec Diagnostica Steroid (Assayed Control Serum) Level I and II control material, and were subject to standard laboratory quality control criteria. An external quality assurance program was also assayed. Between-assay variation is <15%. Sensitivity calculated at two standard deviations from zero is less than 0.3nmol/L. Recovery of testosterone from saliva and plasma was found to be between 90% - 110%. More details on these statistics can be found in the original paper that describes the method used (7).

Plasma SHBG

Plasma SHBG was measured using a direct ELISA where microtitre plates were coated with DAKO SHBG polyclonal antibody diluted in PBS overnight at 4°C. Plates were 'blocked' and, following blocking, samples were diluted 1/1000 in assay buffer, added to the plate and incubated overnight at 4°C. Plates were washed and bound SHBG was detected using an "in-house" mouse monoclonal antibody to SHBG as previously described (8) and anti-mouse Ig peroxidase. After the final washing OPD substrate was added and colour developed. The plates were stopped and read at 492nm. The method is calibrated with pooled human pregnancy plasma, controls are in-house with an external quality assurance program, and between-assay variation is <15%. The lowest SHBG level detectable by this method is 3nmol/L (9).

Free testosterone

This was calculated according to the formula of Vermeulen and colleagues and results expressed as pmol/L (10).

$$\text{Free testosterone} = \frac{[-(23 + \text{SHBG-T}) + ((23 + \text{SHBG} - \text{T})^2 + 92 \times \text{T})^{1/2}]}{0.046}$$

Two binding constants (equilibrium association constants) have been assumed in using this formula, that of the interaction of testosterone with SHBG and with albumin. The value for testosterone/SHBG used is that chosen by Vermeulen of 1 x 10^9L/mol. It is probably the most commonly used and is in agreement with others (11,12).

The association constant for albumin used is 3.6 x 10^4L/mol. Vermeulen showed that within the physiological range of 40 - 50 g/L, the albumin concentration does not significantly affect free testosterone values. However, if the albumin concentration is expected to deviate significantly from normal, the actual albumin concentration should be determined and used in the calculation accordingly.

Free androgen index

The free androgen index (13) is calculated as

$$\frac{1000\text{x(T) nmol/L}}{\text{(SHBG) nmol/L}}$$

Statistical analysis

All methods and results have been compared using linear regression with correlation coefficients. Analysis of data has been grouped according to sex as well as treated together.

Results

The measured salivary and plasma testosterone concentrations and the SHBG concentrations, together with the derived free testosterone and free androgen index levels for the 73 subjects are available from the author on request. These have been statistically analyzed and the results expressed below as a series of graphs showing correlation coefficient data.

Free androgen index (FAI) Versus free testosterone (combined data)

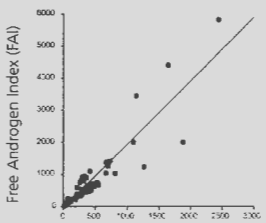

Figure 1. Correlation between free androgen index (FAI) and calculated free testosterone (FT). The equation is FAI = 1.98 FT- 64.7; r^2 = 0.84 (n = 73)

The precision of the derived concentration of free testosterone, as well as the free androgen index, is subject to the combined errors of the SHBG and total testosterone assays. Therefore, careful determination of both parameters is necessary. The data shown here represents a good correlation between calculated free testosterone and the free androgen index in plasma.

Free testosterone versus salivary testosterone (combined data)

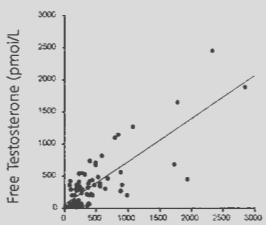

Figure 2. Correlation between free testosterone (FT) and salivary testosterone (ST). The equation is: FT = 0.67 ST + 49.9; r^2 = 0.64 (n = 73)

The results in figure 2 can be further broken down for females and males.

Free testosterone versus salivary testosterone in female patients.

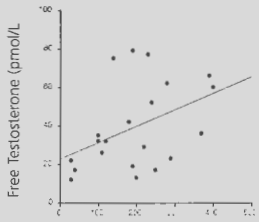

Figure 3. Correlation between calculated free testosterone (FT) and salivary testosterone (ST) for females. The equation is: FT = 0.08 ST + 22.7; r^2 = 0.18 (n = 21)

Free testosterone versus salivary testosterone in male patients

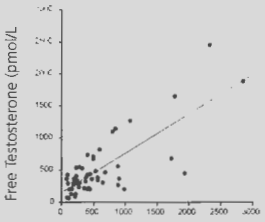

Figure 4. Correlation between calculated free testosterone (FT) and salivary testosterone (ST) for males. The equation is FT = 0.61 ST + 141; r^2 = 0.60 (n = 53)

Plasma total testosterone versus salivary testosterone (combined data)

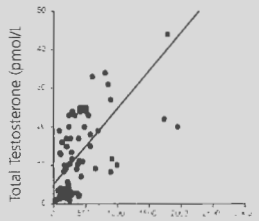

Figure 5.Correlation between total testosterone (T) and salivary testosterone (ST). The equation is: T = 0.02 ST + 5.1; r^2 = 0.57 (n = 73)

The results in Figure 5 are further broken down for females and males.

Plasma total testosterone versus salivary testosterone in female patients

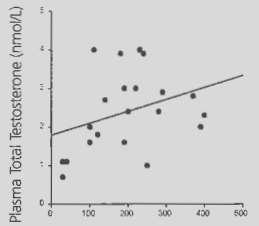

Figure 6. Correlation between total testosterone (T) and salivary testosterone (ST) for females. The equation is: T = 3.09 ST + 1.8; r^2 = 0.11 (n = 21)

Free androgen index (FAI) Versus free testosterone (combined data)

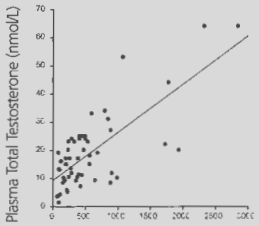

Figure 7. Correlation between total testosterone (T) and salivary testosterone (ST) for males. The equation is: T = 0.02 ST + 9.1; r^2 = 0.55 (n = 52)

Discussion

The calculated values of free testosterone and free androgen index showed good correlation (r^2 = 0.84). The calculation of plasma free testosterone makes several assumptions, and although calculated values generally correlate very well with those directly measured in plasma, discordant results are not infrequently encountered. This must be taken into account when initially evaluating results (5,14). Hence, it is not surprising that our experimental results when comparing calculated free testosterone (in plasma) with that measured in saliva also show some discordant results. It is interesting that the male value (r^2 = 0.60) shows significantly better correlation than the female value (r^2 = 0.18). Possible explanations are offered.

Firstly, the problems associated with measuring salivary testosterone lie mainly with the small detectable level of the steroid we are measuring as the concentration of testosterone one might expect to find in saliva should be only 1-2% of the total plasma concentrations. Increasing the saliva volume has overcome part of this problem with the resultant absorbances falling in the most sensitive part of our analytical curve. However the percentage error is still relatively large when we look at the levels between 30-3000 pmol/L that we are measuring (Table 1). A recent multi-centre evaluation has concluded that current methods for measuring men's and women's salivary testosterone concentrations are reliable enough to be quite useable for research purposes (15).

Secondly, the question of metabolic transformation of steroid hormones by the salivary glands, and the variable transfer of protein bound testosterone from the circulation into the saliva may also be contributing factors. Saliva is not a simple ultrafiltrate of plasma, it is a complex fluid formed by an active energy-consuming process, and is, in many ways quite dissimilar to an ultrafiltrate of plasma. To understand the potential and limitations of salivary hormone assays requires a knowledge of the salivary glands themselves, the mechanism of salivary formation and the composition of saliva (16).

Most of the saliva in man is produced by three pairs of salivary glands (parotid, submandibular and sublingual) and a small contribution is made by the buccal glands which line the mouth. Saliva also contains a small and variable amount of plasma exudate from abrasions in the oral cavity and of gingival crevicular fluid which leaks out from the tooth-gum margin. Saliva is formed in specialized secretory endpieces called acini. These acinar cells make up the largest part of the salivary gland. In healthy subjects the gingival crevicular fluid makes up about 0.5% of the total volume and plasma exudate from minor abrasions in the mouth may also contribute to saliva.

The protein concentration of gingival fluid is similar to that of plasma and hence it provides a route for entry of hormones into saliva. Any substance present in plasma is present in saliva to the extent of about 0.5%. However the contribution of gingival-crevicular fluid to the total saliva volume is highly variable, so too is the concentrations of

substances entering saliva via this route. The acinar cells or secretory tubules actively pump sodium ions from the blood into the lumen of the endpiece and the osmotic pressure causes water to flow from the blood resulting in the primary secretion being isotonic with plasma. As this precursor fluid moves down the ductal system of the salivary gland the cells lining the ducts pump sodium back into the blood.

The possible route which a steroid can enter saliva may be by passive intracellular diffusion. Here highly lipid-soluble materials (i.e. steroids) may diffuse into the acinar cells by virtue of their solubility in the lipid-rich cell membranes and then diffuse out the other side into the saliva. The rate of entry of steroids into saliva is also very rapid. An active transport mechanism clearly operates for some substances but there is little evidence for active transport of steroids into plasma. Salivary flow rate affects the levels of conjugated steroids with little effect on the neutral steroids. This suggests that the mechanisms of entry into the saliva are different. Charged steroids enter from the blood by ultrafiltration via diffusion through the tight junctions between the acinar cells whereas neutral steroids enter intracellularly by diffusion through the cells of the salivary glands. The pH of saliva varies between 6-8 whereas plasma has a pH of 7.6, hence salivary pH can alter salivary levels depending on the pKa of the analyte. Acidic or basic analytes with pKa in range 5-9 may be selectively concentrated in or excluded from saliva. Spurious entry of analytes can occur for example by oral ingestion or contamination by vigorous teeth brushing by blood contamination from the gums. However gingival crevicular fluid is also a normal "contaminant" or component of mixed saliva. As a matrix, viscous saliva can be difficult to handle and pipette although repeated freezing and thawing can precipitate the glycoproteins and mucins with negligible hormone losses.

It can be seen from our results that all the females in our study had salivary testosterone levels higher than their derived free testosterone concentration in plasma. Some recent studies have shown a good correlation between salivary testosterone and free testosterone, but there is not good agreement as to the relationships between percentages of total testosterone in plasma, free testosterone in plasma, and salivary testosterone (17). Others have also found total saliva testosterone concentrations exceed free testosterone plasma levels (18-20), sometimes by 2-3 fold those of plasma free testosterone. There is some postulation that this may be due to local conversion of androstenedione to testosterone in salivary glands. The high ratio between plasma androstenedione and testosterone (in women) may be responsible for this transformation. In normal men, such a discrepancy is not observed, free plasma and total saliva testosterone being similar. However we cannot exclude that the same process exists but is hidden by the probable reduction of testosterone into 5(-dihydrotestosterone or other non determined metabolites, or by the much higher levels of testosterone in saliva. Rey and colleagues found that the discrepancy between saliva testosterone and plasma free testosterone (derived) varied according to physiopathological conditions, suggesting that salivary testosterone levels do not result from a mere passive flux of testosterone between blood and saliva (20). In women, they noted a positive and significant correlation existed between androstenedione levels and the salivary testosterone - calculated free testosterone differences. They also noted (as have we) that some men had a "deficit" in salivary testosterone, this being more marked in impotent men, and appears to be reversed by the administration of testosterone. They concluded that saliva might be a medium of choice to evaluate "peripheral" events.

Another group reported excellent correlation between their (solid phase EIA) method for both plasma and salivary testosterone levels, but on close reading, their study only used normal male subjects (21). Johnson and colleagues performed a study similar to ours, but measured the plasma free testosterone levels, rather than calculated them (22). They concluded a significant correlation between salivary testosterone and plasma free testosterone over a wide range of concentrations, but were in agreement with others that the salivary testosterone concentration is 3-4% of the total plasma values in women and 1-2% in men. They also found higher concentrations of salivary testosterone than unbound testosterone (in plasma) in some patients and concluded that this may reflect non-linear transfer from plasma to saliva, metabolic transformation of steroid hormones by the salivary gland or the transfer of testosterone bound to protein from the circulation into saliva. They concluded that the salivary testosterone assay is as useful as the plasma free testosterone assay in determining androgenic status with elevated levels being seen in hirsutism, and low levels in prostatic cancer. Navarro and colleagues found that the percentage of salivary testosterone is higher than that for free testosterone in plasma, with respect to total plasma testosterone, and concluded that testosterone in saliva is influenced by the concentration of albumin in saliva and (or) metabolism of steroid hormones in salivary glands (23). They comment that further evaluations are required for a better understanding of the problem.

And finally, the measurement of total testosterone (and hence derived free testosterone) is generally not done well. Whilst we still use an extraction method in our laboratory and hence remove the matrix problems associated with calibration, comparisons carried out during the period of this project with some commercially available methods show that problems still exist with the measurement of testosterone.

Increasing numbers of laboratories are now using automated methods for measuring testosterone, most of which are direct and are subject to matrix effects which vary depending on the amount of sample taken for analysis and are therefore not linear. Extraction avoids these problems and allows a wider analytical range, in our case, from 0.3 to 50 nmol/L. Although automated methods provide controls to cover this range, little regard is placed on the main (male - female) decision points. Assays should be optimised to cover the required analytical range and controls placed strategically throughout that range and ideally at the main clinical decision points.

Assay systems behave differently (specificity, recovery, relationship to GCMS reference values etc.) with different matrices. Separation of systems with improved specificity and accuracy for the female matrix is urgently required to address problems of 'true' reference limits, interference with metabolites and spurious high values (with need for repeat extraction assays to confirm). Analysis of testosterone in the male matrix seems less problematical and is performed adequately by most automated systems.

As part of this project, we analysed 200 patient samples from a local reference plasma bank for both total testosterone and SHBG. We reaffirmed our own reference ranges for these analytes and used the results to establish our own reference ranges for the derived free androgen index, and calculated free testosterone. A copy of these reference ranges is appendixed. It should be noted that the assayed control serum (Byk-Sangtec Diagnostica) we use for testosterone is GCMS referenced.

Conclusions

Free androgen index is a simple ratio of testosterone and SHBG values that yields values similar to measured free testosterone concentrations. Although only an index of free testosterone levels, it has been found to be useful in the investigation of androgenic diseases. On the other hand, the mathematically derived free testosterone appears to give a good guide to free testosterone concentrations and has been used in several studies (24,25). It does however assume a constant plasma albumin concentration.

While we have shown that there is not a good correlation between calculated free testosterone and salivary testosterone, there is a trend for salivary testosterone to reflect free testosterone, more apparent in

men than in women. One limiting factor is probably the low level of testosterone in saliva and the sensitivity of testosterone methods. We have a relatively sensitive testosterone method in use in our laboratory (see methods section), yet we still have poor correlation with our female saliva samples. Many laboratories use some index of plasma free testosterone or androgen status within their clinical setting. However no external quality assessment scheme exists for free testosterone and there is no internationally recognised standard. The European Commission has started to address this issue. Until this happens it is up to individual laboratories to determine their own reference ranges using the best current available information. Many laboratories may be inappropriately combining results from different laboratories and there is also a need for standardization of the binding constant to use in the calculation.

While total testosterone is useful in assessing several clinical conditions it can be unhelpful in others including hirsuitism and the polycystic ovary syndrome (26). Many clinical investigators have shown a greater degree of abnormality in hirsute patients when free testosterone is determined rather than when total testosterone is measured alone. It matters little whether the free testosterone is directly measured or derived mathematically or if non-SHBG testosterone is determined (26).

In spite of some reports suggesting that salivary testosterone correlates well with free testosterone, few methods are sensitive enough for routine use. Although our salivary method is simple to perform, we consider that the determination of free testosterone using total testosterone and SHBG is superior because it avoids the caveats imposed by saliva.

Acknowledgements

I thank Drs John Lewis and Peter Elder, Mrs Tory Aebli and Mrs Wendy Whitau (Steroid and Immunobiochemistry Laboratory) for their assistance in this project; and Mrs Sophie Harrison Sands for editorial assistance. Helpful discussions were held with Dr John Middle of UKNEQAS (Birmingham UK) regarding quality control of testosterone assays.

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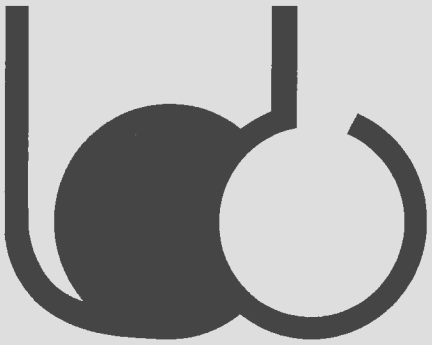
Appendix



Canterbury Health Laboratories

- New reporting format for plasma testosterone and sex hormone-binding globulin -

In consultation with our chemical pathologist and endocrinologists our Steroid laboratory will change the format for reporting testosterone and sex hormone-binding globulin (SHBG). We will report testosterone and SHBG as well as the derived free androgen index and free testosterone. A new reference range has been established on 100 male and 100 female samples from the Christchurch Endolab reference plasma bank and is shown below.

Adult females	
Testosterone	0.5-2.7 nmol/L
SHBG	20-90 nmol/L
Free androgen index	<80
Free testosterone	<50 pmol/L
Adult males	
Testosterone	9-38 nmol/L
SHBG	9-60 nmol/L
Free androgen index	>400
Free testosterone	250-800 pmol/L

For further information contact John Lewis on 0800 THE LAB or by email at johnl2@chhlth.govt.nz

9 January 2000

free T derived from Vermeulen et al JCEM (1999) 84, 3666

formula is free $T = [-(23+SHBG-T)+((23+SHBG-T)^2 +92xT)]^{1/2}/0.046$

T and SHBG are nmol/L, free T is pmol/L

Free androgen index = 1000xT/SHBG

Abstracts of presentations at the NZIMLS ASM, Wellington, September 2002

What's new in diabetes?

Dr Bob Smith. Wellington Hospital

Type 1 diabetes appears to have increased in NZ as in other parts of the world over the past 20 years. Type 2 diabetes has increased dramatically in the recent past and it is forecast that by 2020 there will be 97% more among Maori, 117% more among Pacific Islands people, and 42% more among Europeans in NZ. Best evidence is that the more frequent type 2 diabetes is linked to increased frequency of obesity which in turn seems best explained by a decreased level of energy expenditure and also by an increased intake of sugar and fat. In Japan paediatricians report that type 2 diabetes is more common than type 1 among their patients.

There have been changes in diagnostic criteria for type 2 diabetes and this has had some effect on the increased prevalence of the disorder. Recent Finnish studies have shown that diabetes has undoubtedly become more common with age but that the fasting level is less indicative than the 2-hour blood glucose level. This is pretty heavy criticism of the US preference for fasting levels. The hunt for a diabetes gene or genes is underway and there have been some small gains in knowledge of type 2 diabetes and a lot more for MODY which now has 5 proven monogenic varieties. A very interesting effect has been reported by Hattersley and his team of the effect of fetal genes affecting the outcome of birth weight in the offspring of diabetic mothers. LADA is a variety of type 1 diabetes which also seems to be increasing.

At a clinical level I would note the development of Local Diabetes Teams in most areas of NZ. This meeting of all stakeholders has encouraged the development and use of local services. A major initiative from the Ministry has been the setting up of a "get checked" scheme - a free-to-the-patient annual visit to GPs for a diabetes review which includes clinical and laboratory testing (HbA1c, lipids, urinary alb/cr) and a photographic retinal screening programme. Increased access to podiatry services for people with at risk feet is being worked through now. The goals are to get a measure on the size of the diabetes problem, to identify treatment strategies that work and to make them widely available and utilised. A major recent advance is the documentation in three countries that the progression (worsening) from impaired glucose tolerance to diabetes can be drastically reduced by life-style changes. Among new therapies are genetically modified quicker and slower acting insulins and a new class of oral agent the thiazolidinediones (glitazones) which work by reducing resistance to insulin.

Screening and diagnosis in Type 2 diabetes. Implications for laboratory services

Dr Michael Crooke. Wellington Hospital

Worldwide, the prevalence of diabetes is increasing. In 1985, WHO estimated that 30 million people had diabetes. Current estimates are 130 million and a rise to 300 million is predicted by 2025. Between 80-90% of these will have type 2 diabetes, although both the incidence and prevalence of type 1 diabetes are increasing.

The current prevalence of type 2 diabetes in New Zealand is estimated to be 160,000, with 50,000 of these undiagnosed. A further 300,000 are likely to have impaired glucose tolerance. Better estimates are hindered by the lack of a National Screening program. Projections to 2020 predict 250,000 with type 2 diabetes and 500,000 with IGT. In view of the obesity epidemic, these are likely to be underestimates and there is a trend towards type 2 diabetes appearing at earlier ages.

The pathology of diabetes

Dr Diane Kenwright. Wellington School of Medicine & Health Sciences

Pancreas. The islets of Langerhans are destroyed in type I diabetes mellitus. This occurs probably as a consequence of a genetic susceptibility, followed by the onset of autoimmune destruction triggered by some environmental factor such as a viral infection. Heavy lymphocytic infiltrates appear in and around islets. The number and size of islets are eventually reduced, leading to decreased insulin production and glucose intolerance. The islets of Langerhans are normal in number or somewhat reduced with type II diabetes mellitus. Fibrosis and deposition of amylin polypeptide within islets are most characteristic of the chronic states of type II diabetes.

Atherosclerosis. Persons with diabetes mellitus, either type I or type II, have early and accelerated atherosclerosis. The most serious complications of this are atherosclerotic heart disease, cerebrovascular disease, peripheral vascular disease and renal disease. The most common cause of death with diabetes mellitus is myocardial infarction. Peripheral vascular disease is a particular problem with diabetes mellitus and is made worse through the development of diabetic neuropathy, leading to propensity for injury.

Renal Complications. There are a variety of complications involving the kidney. Both nodular and diffuse glomerulosclerosis can lead to chronic renal failure. Diabetics are prone to infections, particularly pyelonephritis. Both bacterial and fungal infections can occur.

Ocular Complications. The eyes can be affected in several ways by diabetes mellitus. Diabetic retinopathy is one of the leading causes for irreversible blindness.. This retinopathy can occur with either type I or type II diabetes mellitus, usually a decade or so after the onset of diabetes. Most persons with type I diabetes and many of those with type II diabetes develop some background (non-proliferative) retinopathy. Proliferative retinopathy is more ominous and is more likely to occur when diabetes mellitus is poorly controlled.In severe retinopathy, neovascularization may lead to adhesions (synechiae) between iris and cornea or iris and lens. Neovascularization of the iris leads to secondary glaucoma with blindness. Cataracts are more common in diabetics. This predilection for development of cataracts is felt to result from hyperglycemia leading to accumulation of sorbitol that results in osmotic damage to the crystalline lens.

Studies of concordance, reproducibility and diagnostic cut-offs for Troponin I and Troponin T

Dr Chris Florkowski[1], CM Young[2], I Crozier2, PM George[1] & TM Walmsley[1]. Clinical Biochemistry Unit, Canterbury Health Laboratories[1] and Department of Cardiology[2], Christchurch Hospital, Christchurch

Consensus bodies have redefined myocardial infarction by a Troponin cut-off at three Standard Deviations above the population mean with an analytical CV<10%. Diagnostic cut-offs have been suggested for some Troponin assays, although it is not established that clinical laboratories can meet these performance criteria nor that these levels determine clinical outcomes.

Over a six week period, blood samples from inpatients and those attending the Emergency Department with requests for myocardial injury markers were analyzed for both Troponin I (Abbott AxSym) and Troponin T (Roche Diagnostics ELECSYS). Each patient admission / test request was assessed for biochemical concordance between the two assays based on currently used positive cut-offs for Troponin I (>1.0 ug/l) and Troponin T (>0.1 ug/l) and the newly recommended cut-off for Troponin T (>0.03 ug/l). A total of 1793 consecutive samples were analyzed from 550 visits in 507 patients. Sixty of the 550 patient visits (10.9%) had results where one Troponin type was positive at some stage whilst the other was consistently negative.

There is some discordance where TNI is negative though Troponin T positive (using the lower cut-off for TNT) associated with renal impairment. Each patient in the study has been assigned as having had a clinical myocardial event or not.

The Sirolimus Symphony: From Holt to HPLC in A+

MF Blake, GA Woollard. Department of Chemical Pathology,Auckland Hospital

Sirolimus (rapamycin) is a new immuno-suppressive drug currently under trial in New Zealand with renal, liver and heart transplant patients. It has been shown to be effective in the reducing episodes of acute rejection. It can be prescribed separately or in combination with other immunosuppressants. Sirolimus has a different mechanism of action to cyclosporin and tacrolimus. It inhibits the T-lymphocyte activation and proliferation that occurs in response to antigenic and interleukin IL-2, IL-4, and IL-15 stimulation. It also inhibits antibody production.

Patients taking sirolimus need to be monitored because it has a narrow therapeutic index and a variable pharmacokinetic profile (low bioavailability and unpredictable clearance). Optimal therapy is observed if blood levels are maintained between 5 to 15 mg/L. An immunoassay for this drug is not yet marketed and the accepted method for analysis at present is by HPLC with MS or UV detection (1). This method has been adapted and is being used routinely within the Department of Chemical Pathology at Auckland Hospital. The process involves extensive sample preparation. The data shows a batch to batch imprecision of 14%, analytical recovery 80% and linearity to above 75mg/L.

Reference

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Through the eyes of the microscope

Gillian Rozenberg. Prince of Wales Hospital, Randwick, Australia

The Saal Foley Lecture is given by invitation at the Annual Scientific Meeting of the Australian Institute of Medical Scientists. The title I chose, "Through the eyes of the microscope" illustrates the contribution that experience in blood cell morphology continues to make in haematology where sophisticated blood cell anslyers are current. The presentation consists of two parts. The first illustrates how looking at a blood film can assist the clinician in areas where the automated blood cell analyser falters. The second introduces the field of white cell enumeration in bronchoalveolar lavage. Conditions considered in the first part include the nature of a lymphocytosis, white cell inclusions and malarial parasites as well as other blood cell analyser problems. The second part introduces the findings in normal bronchoalveolar lavages, in lung infections and in non-infectious lung diseases.

Paperless automated haematology - the Sysmex, MOLIS, MERU solution

Ross Anderson. Diagnostic Laboratory, Auckland

Over the last six months Diagnostic Medlab has introduced new Sysmex XE2100 and SP100 analysers coupled to two linked Haematology Sample Transporters (HST). All the samples are processed through the Routine HST with the results fed to the MOLIS computer which has received the test orders from the Delphic laboratory information system (LIS). MOLIS uses algorithm rules to determine which samples need reflex testing on the Expert HST and to automatically comment on normal or near normal samples before releasing them to the Delphic (LIS).

MERU is the paperless microscopy work area manager linked to MOLIS via Windows NT software. Each microscope work station has a PC running MERU as well as Delphic Latte. Cumulative results and scatter plots are available in MERU and it is through this software that all blood film examinations are reviewed, resulted and authorized.

An alternative qualification for laboratory assistants

Jim Clark. Auckland University of Technology

Laboratory assistants are offered a qualification by the New Zealand Institute of Medical Laboratory Science. The Qualified Technical Assistant (QTA) has served the profession well, but for those who wish to advance their career and become Registered Medical Laboratory Scientists the transition is not easy.

As well as the Bachelor of Medical Laboratory Science (BMLS), the Auckland University of Technology offers a generic Bachelor of Applied Science (BAppSci). This permits those who wish to do so, to exit the degree programme at a particular point and gain lesser qualifications such as a Certificate in Applied Science or a Diploma in Applied Science. If these options were offered to medical laboratory assistants, they could more easily continue their studies to progress through Certificate, Diploma to a full degree.

Pseudomonas aeruginosa, antibiotics and Cystic Fibrosis lung disease

Professor K Grimwood , Department of Paediatrics & Child Health, Wellington School of Medicine & Health Sciences, Wellington

Cystic fibrosis (CF) is an autosomal recessive disorder resulting from mutations in a gene on the long arm of chromosome 7. The gene product is the CF transmembrane conductance regulator, which regulates and facilitates electrolyte transport across epithelial cell membranes. While several organ systems are affected, lung disease is responsible for much of the morbidity and mortality associated with this illness. Pulmonary involvement is characterised by chronic lower airway infection from a limited number of respiratory bacterial pathogens, the most important of which is the gram negative bacillus Pseudomonas aeruginosa.

P. aeruginosa is detected in 60 to 80% of patients with CF who are sputum producers. Biofilm formation and the development of a mucoid phenotype by the original infecting strain mark the establishment of persistent infection. From this stage the organism is rarely eradicated by antibiotic therapy and the patient experiences a gradual deterioration in lung function and health.

It is poorly understood why *P. aeruginosa* is the predominant pathogen in cystic fibrosis. While the organism is most likely acquired from environmental sources, there is accumulating evidence to suggest however that some strains are transmissible when contact density is high. Once *P. aeruginosa* gains access to the lower airways, it has a wide array of virulence factors that allow adherence to mucus or epithelial cells and multiplication within the host, while evading innate and adaptive host defenses. The interaction between the microbe, its cell associated and soluble products, and an up-regulated but ineffective host immune response leads to persistent infection with progressive tissue injury, respiratory failure and ultimately death.

The failure of antibiotics to eliminate mucoid *P. aeruginosa* infection in CF has led some authorities to question their role in management of this disease. However, carefully conducted studies have shown a benefit from these agents. While *P. aeruginosa* possesses both classical and non-classical (biofilms, adaptive resistance) means of antibiotic resistance, there are also pharmacokinetic and pharmacodynamic reasons for the failure of antibiotics. However, studies indicate that subinhibitory anti-pseudomonal antibiotic concentrations reduce the expression of virulence factors, modify host immune responses and are associated with tissue protection in animal models and improvement in clinical studies. Similar observations have been made in-vitro and in animal models with other antibiotics lacking bactericidal activity against *P. aeruginosa*. The results of clinical trials using the azilide, azithromycin for example are eagerly awaited.

Important questions remain over the pathogenesis of *P. aeruginosa* CF lung infection, especially concerning acquisition and early establishment of infection. Means of preventing infection remain a research priority. For now reliable identification of early infection may allow aggressive therapy to delay the development of persistent infection. Once infection is established, antimicrobial treatment then aims to decrease the bacterial load, reduce virulence factor expression and moderate damaging airway inflammation.

Treating advanced colorectal cancer: the value of monitoring CEA

SK Wickremesekera, RS Stubbs. Wakefield Gastroenterology Centre, Wakefield Hospital, Wellington

Colorectal cancer is one of the commonest cancers seen in NZ and the Western World. Over 2000 new cases are diagnosed in NZ each year, of which around 50% will ultimately die of the disease. The liver is the commonest site of spread and almost half the patients who die with the disease, do so with liver-only metastases. Conventionally treatment options are limited to liver resection, which is applicable to only a small percentage of patients, and systemic chemotherapy which, even with the newer agents available, yields disappointing results. We have been involved in evaluating a number of new approaches to treating metastatic disease within the liver over the last 10 years and have obtained very encouraging results.

These options include hepatic cryotherapy, selective internal radiation therapy, hepatic artery chemotherapy and dendritic cell vaccines. Although treatment responses are generally evaluated by CT scanning, monitoring of serum CEA levels can be very valuable. Rising levels indicate progressive disease and falling levels are indicative of diminishing disease and hence a response to treatment.

Hepatic resection achieves 5-year survival rates of around 30% (0%) with median survival times of about 36 months (16 months). Hepatic cryotherapy can achieve 5-year survival rates of around 5% (0%) and median survival times of about 24 months (9 months). SIRT is unlikely to achieve 5-year survival but confers median survival times of around 18 months (6 months). Expected survival times without treatment are shown in brackets.

Dendritic cell vaccines are a new generation of vaccines aimed at inducing specific tumour immunity in patients with cancer. We have one of the largest clinical experiences of using these vaccines in colorectal cancer in the World. While we have not yet seen any dramatic responses to these vaccines, we do have some evidence of anti-tumour effect and slowing of tumour growth. It is likely DC vaccines will become an important modality for cancer treatment in the future.

The clinical significance of laboratory tests for Hepatitis A, B, C

Dr Nigel H Stace. Gastroenterologist and Senior Lecturer in Medicine, Wellington Hospital and Wellington School of Medicine & Health Sciences, Wellington

Hepatitis B virus (HBV) continues to be an important NZ infectious disease. The huge health care burden from the currently estimated 50,000 NZers with chronic HBV will in the long term only be reduced by vaccination of the whole population from a young age. The phases of HBV infection with evolving changes in lab detected markers are best understood if these phases are related to the corresponding changes in the immune response: immune tolerance, immune clearance, viral integration. The high prevalence in NZ of HBeAg negative chronic active HBV (about 30%) is a major clinical problem and can only be defined clinically with the help of multiple serological markers and quantitative HBV DNA assays. Co-infection of HBV with other viruses (HIV, HCV, HDV) is not infrequent. All Samoans with chronic HBV should be screened for anti-HDV. Whilst HBsAg patients with HDV are most likely to prematurely progress to cirrhosis and end stage liver disease, these patients usually do not have active HBV replication, having HBsAg without HBV DNA or HBeAg in serum. Liver biopsy is one of the most useful tools in clinical management especially when the report is

formulated in terms of the grade of information and the stage of fibrosis. HBV serotypes and genotypes are being increasingly investigated as predictors of disease and possibly treatment outcomes. Treatment with Interferon alpha or Lamivudine requires frequent assessment of serological markers and quantitative HBV DNA. Current dilemmas include the emergence of YMDD Lamivudine resistant mutants (at about 20% per year) and the ideal duration of therapy to minimise viral relapse. A current Phase 2 trial is exploring simultaneous Lamivudine and highly immunogenic HBsAg vaccine.

Hepatitis C is also evolving as a huge health resource burden with clinical management being similarly dependent on the availability of accurate laboratory serological markers, qualitative PCR for HCV RNA and liver biopsy. We now expect treatment to give sustained viral clearance rates (negative PCR for HCV RNA at 6 months after completion of therapy) of 30 - 60% for genotype 1 and 60 - 80% in genotypes 2 and 3. This has increased the demand for treatment and consequent dependence on laboratory assessment. Frequent serial laboratory monitoring is critical as Interferon causes a reduction in the WBC count (principally neutrophils) and platelets and Ribavirin, a potent foeto-toxin, causes a dose dependent haemolytic anaemia in all patients.

Though not often seen in hospital clinical practice in Wellington, we should never stop being vigilant against hepatitis A. Community outbreaks continue to occur with a need to quickly offer contacts hepatitis A immune serum globulin and/or vaccination. Most patients with chronic liver disease are encouraged to seek vaccination for hepatitis A and B if they are not immune.

When bugs go bad

John Aitken. Southern Community Laboratories, Christchurch

For the last 6 years a monitoring system has been gradually developed and extended throughout Southern Community Laboratories to track multiply resistant bacteria. The aim has been to collect and store isolates of medical interest, and to monitor and review any changes to resistance patterns over time.

A Christchurch based study was carried out in 1997 to monitor carriage of respiratory pathogens in the nasal secretions of children under the age of 6. In 1998 this pilot was extended to include Tauranga and Hamilton. In all 300 samples were examined, and carriage rate of beta lactamase positive respiratory pathogens was found to exceed 80% in this group. In 2001, Southern Community Laboratories took part in an international survey of penicillin resistant *Str. pneumoniae*. Submitted isolates showed a similar distribution of penicillin resistance to isolates collected from other countries, and confirmed an already observed increase in *Str. pneumoniae* resistance to penicillin in NZ.

Surveillance of multiply resistant gram negative bacilli commenced at The Princess Margaret Hospital in 1997, in conjunction with the Canterbury-wide *Acinetobacter baumaniii* outbreak, and was later extended to include the emergence locally of *Serratia* marcescens (profile A).

In August 1999 a small community outbreak of ESBL E..coli was identified in association with a refugee family from South East Asia, and this study lead to an extension of the monitoring system to include *E.coli* with antibiotic sensitivity patterns suggestive of multiple resistance. These markers include:

- gentamicin resistance
- resistance to augmentin and/or a cephalosporin
- resistance to norfloxacin/ciprofloxacin
- any unusual resistance pattern.

In 2001 this monitoring initiative was extended to Southern Community Laboratories microbiology departments in Auckland, Hastings and Dunedin. Isolates are sent to a referral site where initial findings are confirmed and further testing carried out. Where appropriate, infection control services are contacted and cultures for carriage undertaken. All isolates are stored at -70°c. If subsequent *E.coli* isolates from a region indicate the possibility of clusters, a representative sampling of isolates is sent to ESR for typing. Where plasmid transfer is suspected, samples are sent to the University of Washington for plasmid studies. Several clusters of multiply resistant *E.coli* infections have been identified by use of this method.

Resistance in E.coli isolates derived from human infections is not confined to medically important antimicrobials. Preliminary work on the detection of agriculturally-associated antibiotic resistance in *E.coli* isolated from human infections in New Zealand will also be presented.

Testing for macroprolactin

Marian Nouwens, Diagnostic Medlab, Auckland

The predominant form of prolactin in human serum is monomeric prolactin with smaller amounts of big prolactin and macroprolactin may also be present. It is thought that macroprolactin may have more than one aetiology. Macroprolactin is detected variably in different prolactin immunoassays. Because macroprolactin has little or no biological activity in-vivo its presence needs to be considered in the differential diagnosis of hyperprolactinaemia.

Our laboratory has been testing for macroprolactin on all elevated prolactin samples and specific requests from Endocrinologists and GPs for 8 months. Until recently the only method for detecting macroprolactin has been gel filtration chromatography which is time consuming and expensive. A screening test using PEG precipitation, which is simple and inexpensive has recently been described. This method has been validated for the Architect by other users and in our laboratory. Macroprolactin is precipitated using a 25% PEG solution and then the remaining prolactin concentration in the supernatants is determined. Fifteen percent of the samples we have tested are positive for macroprolactin.

The detection of macroprolactin is important to avoid unnecessary, costly, and invasive procedures in patients with elevated prolactin levels. The PEG precipitation method provides a simple and effect screening test for macroprolactin.

Electrocuting cats

Tara Moran, Diagnostic Medlab, Auckland

The purpose of this study was to determine the optimal oxidation potential for the electrochemical detection of urinary catecholamines as part of an HPLC method validation.

Catecholamines (adrenaline, noradrenaline and dopamine) in acidified urine were extracted using disposable micro-cation columns (a Bioradä ready-to-use reagent kit), then separated and detected on a Waters Breeze HPLC system with a Bioradä reverse phase column and an Antec Leyden electrochemical detector.

A complete cycle of RCPA specimens from the Biogenic Amines program was analyzed as part of the initial method validation, using an oxidation potential of 0.5V as prescribed in the Bioradä method. Subsequently, Bioradä quality control materials (Lyphocheck Quantitative Urine Controls 1 and 2) and further RCPA samples, were analyzed using varying oxidative potentials to ascertain the effect on catecholamine quantitations.

Analysis of the RCPA samples using the prescribed 0.5V oxidation potential, revealed inaccurate adrenaline results, but excellent accuracy for noradrenaline and dopamine. Altering the oxidation potential had minimal effect on noradrenaline and dopamine, but caused wide variations in the quantitation of adrenaline. The voltage that provided results closest to target values for all three analytes was 0.25V, considerably lower than the Bioradä prescribed oxidation potential.

The optimal oxidation potential for measuring catecholamines varies between electrochemical detectors. The prescribed voltage in the test methodology is not necessarily correct for every electrochemical detector, and recommended oxidation potentials should be interpreted only as a starting point.

Susceptibility testing of *Pseudomonas aeruginosa* isolates from patients with cystic fibrosis: is it possible?

Jan Bell, Adelaide, Australia

Pulmonary infections are the major cause of morbidity and mortality in cystic fibrosis (CF) patients, with *Pseudomonas aeruginosa* serving as the principal pathogen. Antimicrobial chemotherapy has made an important contribution to increasing the life expectancy of CF patients. Reporting of a multidrug-resistant *P. aeruginosa* strain may also affect infection control and isolation policies. The timely and accurate performance of susceptibility testing may therefore make a significant difference in the therapeutic management of patients with severe exacerbation. A standardized susceptibility methodology is highly desirable for tracking of resistance patterns in the CF population, especially as new therapeutic agents are developed and traditional antibiotics are delivered to more patients by aerosolization.

P. aeruginosa isolates from patients with CF are, however, often multi-resistant and have large amounts of mucoid exopolysaccharide and also have relatively slow growth rates in the laboratory. These characteristics may adversely affect the performance and interpretation of antimicrobial susceptibility testing. Disc diffusion susceptibility testing was recommended in 1994 as the being the most accurate susceptibility test method available for testing *P. aeruginosa* isolates from CF patients.

Editor' note: Only abstracts from presentations at the Wellington NZIMLS scientific meeting which contained data or useful information are published in this issue of the journal. This is in line with a recent Editorial decision (1). Many abstracts submitted still contain the two most commonly abused phrases (results will be presented, results will be discussed). These have been left out, as well as deleting these phrases from published abstracts.

Reference

1. Siebers R. Publication of abstracts of the Annual Scientific Meeting of the NZIMLS. *NZ J Med Lab Science* 2001; 55: 70.

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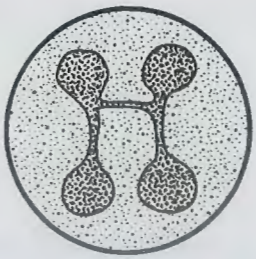

HSIG journal based - questionnaire

Reference:

Uggla B, Tidefelt, Vikerfors T, Fredlund H. Activation of granulocytes in patients treated with chemotherapy. *Clinical and Laboratory Haematology* 2002; 24: 29-31.

Q.1) What was the aim of the study?

Q.2) What is "Respiratory Burst"?

Q.3) What role do CD11 and CD18 play?

Q.4) Discuss the choice of patients for the study.

Q.5) How was the activation of granulocytes achieved in this study?

Q.6) Were there any related studies conducted on granulocyte function in vivo?

Q.7) How was the granulocyte function measured?

Q.8) What was the result of the study?

Q.9) Why was the result of the study interpreted with caution?

Q.10) What did the study conclude?

Answers on page....107

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Abstracts of presentations at the Microbiology SIG meeting, May 10/11 2002, Christchurch

A fascia-nating story

Amber Killington. Medlab Central

This is a case study of a seventeen-year-old female involved in a car accident. This account opens up questions about issues related to the use of anti-inflammatory drugs and necrotising fasciitis.

The patient was treated in the emergency department and discharged on anti-inflammatory drugs. Three days later a piece of glass was removed from her swollen and bruised foot by her general practitioner, but her leg continued to deteriorate over the next few days. On day six the patient was readmitted to hospital where fluid from her leg was drained and submitted in blood culture bottles. No antibiotics were administered. Leg blisters were drained on day eight, but the fluid was not sent to the laboratory. Day nine involved a high leg amputation, which was submitted for microbiological culture and the administration of antibiotics commenced. Blood and tissue cultures grew *Streptococcus pyogenes* confirming a diagnosis of necrotising fasciitis. On day ten the patient passed away.

This case study highlights the unresolved issue of the use of anti-inflammatory drugs in patients who have skin lesions and infections where Streptococcus pyogenes has the potential of causing necrotising fasciitis. Perhaps anti-inflammatory drugs should be avoided or used with caution until further medical studies can clarify the association that they have with necrotising fasciitis.

Brucellosis

Dorothy Hartley Medical Laboratory, Wellington.

In March of this year a five year old boy, who had recently returned from a trip to India, presented with fevers, anorexia and malaise. His father was a GP and suspected malaria or typhoid fever, so he requested a parasite screen, blood cultures, and blood tests. The parasite screens were negative, the blood tests revealed a high white cell count and elevated liver enzymes, and after two days the two aerobic blood culture bottles were positive (BacT/ALERT system).

The Gram stain showed a tiny Gram negative cocco-bacillus, and after 24 hours the blood agar sub-cultures showed a very fine growth. Biochemical tests were performed and the most striking of these was the urea hydrolysis (Christensen's agar), which became positive in less than two hours. The combination of these results led us to believe that the organism could be a *Brucella spp* and the isolate was immediately referred to ESR for identification. It was confirmed as *Brucella spp* by PCR, and later speciated as *Brucella abortus* using biochemical and dye sensitivity tests.

The patient was treated with co-trimoxazole and rifampicin, and showed steady improvement.

Brucella abortus has been eradicated from New Zealand cattle but it is important to be alert for it in immigrants and overseas travellers. *Brucella spp* are highly contagious and, as they can be transmitted by aerosols, they pose a serious risk to laboratory workers. Unfortunately they are often misidentified initially and proper precautions are not taken. For this reason, it is advisable to refer any organism that may be *Brucella spp* without delay.

The detection of ESBL producing organisms

Dorothy Nisbet Microbiology Department, Wellington Hospital

The number of extended spectrum beta-lactamase (ESBL) producing gram negative bacilli isolated at Wellington Hospital has been increasing. As the result of noting that all the ESBLs isolated from urine specimens grew on both sides of our routine urine culture plates - a divided CLED/Aztreonam sheep blood agar plate - we decided to investigate whether we could use aztreonam plates as a screening medium for isolating ESBLs.

Seventy five multi-resistant oxidase negative, gram negative bacilli were supplied to us by ESR. These were subcultured onto blood agar and aztreonam agar plates for growth comparison and then tested for ESBL production using the NCCLS recommended method. This is a disc diffusion test which compares zone diameters between cefotaxime, cefotaxime plus clavulanic acid, ceftazidime and ceftazidime plus clavulanic acid. To confirm an ESBL producer the test specifies a 5mm or greater diameter for cefotaxime and or ceftazidime when tested in combination with calvulanic acid versus when tested alone.

Of the 75 organisms tested 59 (78%) were ESBL producers and 16 (22%) were non-ESBL producers.

Of the 59 ESBL producing organisms 55 (93%) grew well on aztreonam agar, 3 (5%) showed limited growth and 1 (2%) did not grow.

Of the 16 non-ESBL producers 6 (37%) grew well on aztreonam agar, 7 (43%) showed limited growth and 3 (20%) did not grow.

We concluded that aztreonam agar would be an effective medium for detecting possible ESBL producing organisms.

Rapid diagnosis of invasive Aspergillosis

Jennifer M Scotter.

Department of Pathology, Christchurch School of Medicine, University of Otago

Invasive Apergillus infections (IAIs) are a leading cause of morbidity and mortality in immune compromised patients, for example neutropenic patients with haematological malignancies. A major limiting factor in the successful treatment of IAIs is the lack of reliable, rapid early laboratory diagnostic tests. As a result, antifungal drug treatment tends to be poorly targeted or instigated too late for successful treatment of the disease. Consequently, the mortality rate from IAIs is high.

Rapid, non-culture based laboratory diagnostics for IAIs have been described since the early 1980s, and range from the detection of Aspergillus antigens such as galactomannan, through to in situ hybridisation, real-time PCR of Aspergillus DNA, and detection of Aspergillus RNA by nucleic acid sequence based amplification (NASBA).

While many of these new laboratory techniques show potential to improve patient outcomes, internationally standardised laboratory protocols need to be established, and well controlled clinical trials completed before the clinical usefulness of the tests can be determined. A study of febrile, neutropenic bone marrow transplant patients carried out by Canterbury Health Laboratories compared the sensitivity and specificity of the Platelia™ Galactomannan (GM) antigen detection kit and a PCR-ELISA assay for early diagnosis of IAI. The sensitivity of the two tests

were 60% and 100% respectively, and the specificity, 75 % and 95%, respectively. A total of 340 paired samples were tested. The PCR-ELISA also performed well on other sample types, for example tissue biopsies and CAPD fluids.

N. Cinerea

Marion Winn. Southern Community Laboratories, Dunedin

N. cinerea colonises the oropharynx in around 30% of people, but is an uncommon pathogen and is not often seen in the laboratory. It can be confused in laboratory tests with *N. gonorrhoeae*. Literature and studies available showed the two organisms both produce prolylaminopeptidase, and although *N. cinerea* supposedly gives a negative reaction in the acid from glucose test, it can actually give a weak positive reaction depending on the buffering capacity of the media as it forms its reaction by-product carbonic acid. As some commercial kits use these two properties of *N. gonorrhoeae* to distinguish them from other commonly pathogenic Neiserria species, care must be taken as *N. cinerea* may falsely identify through these systems as *N. gonorrhoeae*. Most *N. cinerea* strains are sensitive to colistin, and grow on simple media such as Tryptic soy agar, where as *N. gonorrhoeae* is resistant to colistin and is unable to grow on simple media. These are two simple ways of differentiating these two species. Using two identification systems will also support initial findings.

Case-study. On yer bike pedal trauma

Kara Ross. Microbiology Canterbury Health Laboratories, Christchurch

A 43 year old male medical doctor received a traumatic injury to his right shin, caused by a bike pedal. He presented at emergency nine days later with the laceration infected and a large area of erythema. He had been taking several antibiotics, including ciprofloxacin. A swab and some tissue were taken for culture and the next day the erythema was less marked and looked to be slowly resolving, so the patient was sent home.

The laboratory results showed no organisms in the Gram stain and the wound swab had no growth after 48hr of incubation. However, the tissue grew a heavy fungus after 72hr incubation. The fungus was difficult to identify using standard methods, and so was sent to the reference laboratory and was later reported as *Aspergillus sydowi*. The significance of this isolate and it's potential to become a pathogen are discussed.

TB survey 2001

Kathryn Coley. Microbiology Department, Health Waikato, Hamilton

This was a postal survey of 57 medical laboratories. The survey questioned aspects of mycobacteria culture including acid fast microscopy techniques (AFM), culture, laboratory safety, and Mantoux testing.

Two previous surveys were conducted in 1994 and 1998. The results of those surveys have been used by the New Zealand Mycobacteria Working Party, a group of clinicians and Ministry of Health officials involved with developing policies and setting guidelines. The objective of the current survey was to establish whether any changes in laboratory practice for mycobacteria culture had occurred in New Zealand in the past three years.

Of the 57 laboratories surveyed, 42 replied. Fifteen laboratories currently culture for mycobacteria, 12 laboratories perform urgent ZN microscopy only upon request. All 15 laboratories that cultured for mycobacteria performed AFM on specimens. Six laboratories perform ZN stain, 7 perform fluorochrome methods with 4/7 overstaining with ZN routinely to confirm a positive smear. Two laboratories used modified ZN.

Determination of positive cultures by all laboratories was with ZN stain, except for two laboratories using modified ZN. Only four laboratories excluded urine when performing AFM. One of these laboratories sometimes performed AFM on urine when requested or when there was a high suspicion of renal tuberculosis.

Thirteen of the 15 laboratories culturing for mycobacteria used both liquid and solid media. Two laboratories used either solid or liquid media. The majority of laboratories used the BD MGIT system (manual or automated) for broth culture. All laboratories participated in an appropriate QC program.

Laboratory safety has significantly improved. Ten laboratories now have a biological safety level 3 containment area. Most laboratories met with the minimum laboratory safety standard outlined in the 1996 Control of Tuberculosis Guidelines. However, one laboratory did not use sealed centrifuge buckets. Ten laboratories reported having an active staff screening program. Fewer laboratories reported Mantoux testing in 2001.

There has been a significant reduction in the number of laboratories performing mycobacteria culture; 55% in 1994 compared to 36% in 2001. The proportion of laboratories using both liquid and solid media has significantly increased; 16% in 1994 compared to 93% in 2001. The number of laboratories processing <15 specimens has almost halved; 72% in 1994 compared to 40% in 2001. This Has had the positive effect that most laboratories can now meet the turnaround times in regard to AFM and detection of positive culture stipulated in the guidelines. The majority of laboratories cultured for atypical mycobacteria at 30°C although less than half incorporate media for *M. haemophilum*. Most laboratories do not speciate atypival mycobacteria other than *M. avium* complex.

The results of the 2001 survey show that there has been general improvement in mycobacteria laboratory practice in regard to adherance to turnaround times outlined in the guidelines, culture techniques, and safety. It would appear that there is still room for improvement in some areas. Some procedures not included in this survey, but which need to be addressed in the future are, direct detection for *M. tuberculosis* complex, *M. avium* and *M. intracellulare*, and specimen processing methods, e.g. pooling of specimens, decontamination, and contamination rates.

Non-multiresistant EMRSA-15

Heather Davies. Nocosomal Infections Laboratory, ESR, Porirua

EMRSA-15 emerged in England in 1991 as a multi-hospital epidemic methicillin-resistant *Staphylococcus aureus*. It was first recognised in New Zealand in 1995, originally 'imported' on staff and patients from the United Kingdom. Isolated from 26 people in New Zealand in 1995, it is now widespread and in 2001 was isolated from 1,283 cases. Non-multiresistant EMRSA-15 (ciprofloxacin-resistant, erythromycin-susceptible) was first isolated in New Zealand in 1996. In the 2001 survey of all MRSA, 11.8% of EMSRA-15 were non-multiresistant.

The Draft Guidelines for the Control of Methicillin-resistant *Staphylococcus aureus* in New Zealand, April 2002 state:

"...given the transmissibility of this strain, it is recommended similar control measures are applied to all isolations of this strain irrespective of whether it is multiresistant or not...' and "... it is strongly recommended, at least in areas where EMRSA-15 is being isolated, that ciprofloxacin/fluoroquinolone susceptibility is routinely tested."

There was no distinction between non-multiresistant and multiresistant EMRSA-15 until surveillance changed in October 1998. However, since then there have been problems with referral of isolates for typing:

- Laboratories are not referring isolates to ESR because they are not multiresistant, i.e. resistant to ciprofloxacin only
- Laboratories which do not test for ciprofloxacin are not referring isolates which seem to be resistant to erythromycin only

- Laboratories which do not test for ciprofloxacin are not referring erythromycin-susceptible isolates which appear to be resistant to ß-lactams alone

ESR's ongoing surveillance is of multiresistant MRSA but we recommend sending all ciprofloxin-resistant MRSA to ESR in case they are non-multiresistant EMRSA-15.

Straight from the horse's mouth

Karen Cooper. Gribbles Veterinary Pathology, Auckland

A 12-year old male cat with a history of diabetes and Cushing's disease developed a pyothorax in December 2001. Cytological examination of the thoracic fluid revealed abundant neutrophils and macrophages, containing phagocytosed, pleomorphic, Gram-positive bacilli. A strictly aerobic, mucoid, pink, pigmented, catalase positive, weakly acid-fast, Gram-positive bacilli grew on Columbia blood agar and was identified as *Rhodococcus equi*. The cat was treated with intra-thoracic metronidazole and amoxyllin/clavulanic acid plus chest drainage, but deteriorated and was euthanatised.

R. equi is normally found in the soil and faeces of grazing herbivores. It is primarily an equine pathogen causing suppurative bronchopneumonia and abscesses in 2-4 month old foals. It is an uncommon isolate in other animal species but has been reported as causing various infections in cattle, llama, goats, and cats. *R. equi* is also occurring in humans with increasing frequency. It is found almost exclusively in immuno-compromised patients, especially those with HIV and most commonly produces a slowly progressive granulomatous pneumonia. Clinically it can resemble tuberculosis, actinomycete and fungal infections, which can result in delayed diagnosis. Recommended treatment is long-term combination antibiotic therapy, but is often non-responsive and infections frequently recur.

Mycobacteria identification using the RIDOM database

Leo McKnight. Mycobacteriology Reference Laboratory, Wellington Hospital

With the advent of molecular typing, the identification of mycobacteria has undergone a revolution. Since 1990, the number of mycobacteria species has increased by over a third to now number a total of 92. RIDOM believes an international gold standard for identification, using 16S rDNA gene sequencing, should be established.

However, there are two major problems with using public databases for sequence analysis. Firstly, public databases are not particularly user-friendly. The second and more critical problem is the quality of data they contain, sequences may be incomplete, faulty, or even have the wrong species label.

RIDOM's solution is to create a freely available user-friendly analysis site with it's own quality assured database. Database entry for established species is limited to type strains from major culture collections (these are strains, that the culture collection has undertaken a validation process to ensure the identification of the organism). Also, every sequence entry electorpherogram is available on-line so it's quality can be checked.

As well as being an on-line reference analysis site, it is also a wonderful general resource on the genus mycobacterium. You can find it on the web at www.ridom.de/.

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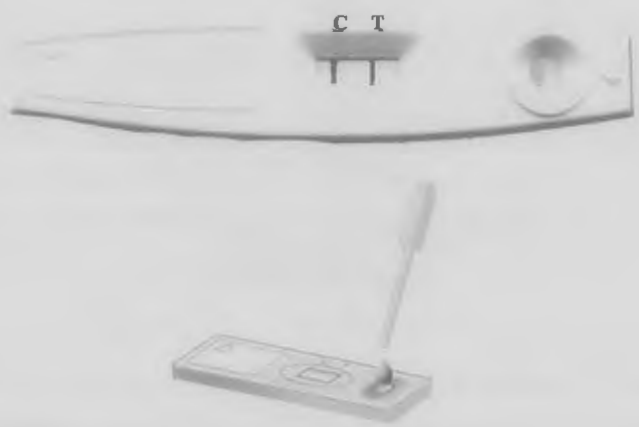

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Friday 2 May 2003

Registration fee

☐ $15 NZIMLS members ☐ $20 Non-members

Saturday 3 May 2003

Registration fee

☐ $65 NZIMLS members ☐ $85 Non-members

Talk Title .....

Subject (if not clear from title) .....

Length of Talk .....

Equipment required .....

First time speaker ☐ Yes ☐ No

Attending Dinner ☐ Yes ☐ No

Special Dietary Requirements .....

Please return to: **Tina Littlejohn**, Medlab Central, PO Box 293, PALMERSTON NORTH, Phone 06 952 3120
E-mail jandm@medlabcentral.co.nz By 18 April 2003

Fiji Medical Laboratory Technologists Association 17th annual conference

It is with sincere thanks to Brian McEwen and Paul Lloyd of Roche Diagnostics in conjunction with the Pacific Paramedical Training Centre management team that I was given the opportunity to attend the 17th annual Medical Technologists Conference in Fiji as well as visit both Lautoka and Suva Hospital laboratories in the capacity of QA Technical Advisor/Co-ordinator in Haematology responsible for the support of over 21 Pacific nations.

The first lap of the journey after staying the night in Nadi took us directly to Lautoka Hospital where I finally meet up with the lab staff who were as warm and welcoming as I had expected them to be. The temperature outside must have been close to 33 degrees so it was a pretty warm day all around. Blood film Microscopy is the basis of the Haematology QA programme and this visit provided the ideal opportunity to carry out a blood film morphology workshop for the staff, which was gratefully received. Such morphology workshops have become quite dynamic in nature and students as well as qualified staff enjoys the interaction that is subsequently generated through discussion. After touring the lab and becoming acquainted with the staff, we began our journey to the conference venue " The Naviti Resort".

The conference was held at the Naviti Resort from the 22nd to the 23rd Sept on the Coral coast, an absolute island paradise for townies wishing to escape the humdrum of city life. The theme of the conference was titled "Emerging trends in Medical Laboratory Science" which introduced interesting presentation topics ranging from modular analytics, the development of quality systems, emerging trends in Haematology analysers, advances in autologous transfusion, total laboratory systems, and sperm banking to mention but a few. My presentation " Core laboratories and their impact on Technology and Technologists" is very much a topical issue these days and certainly a concept requiring serious consideration in laboratories that are continuously hounded by limiting resources and severe financial restraints. Advancement in automation, re-engineering of traditional laboratory structures and the development of core laboratory concepts within hospitals of the pacific are issues now being considered as a positive alternative in response to the challenge of budgetary restraints and the need for effective utilisation of existing resources. The presentation appropriate for the day was well received and generated positive interaction with the delegates present.

I was also able to present a second blood film morphology workshop in the afternoon. I believe that students learning blood film morphology and general blood film examination are not only required to identify abnormalities but to know within reason why such abnormalities have occurred and the physiology behind them. This gives a far greater understanding of the pathological process and its effect on normal haematological pathways.

The Naviti Resort is a spectacular tourist venue for those visiting Fiji, but such resorts can often give a false impression in terms of the economical standing and political stability of the country. Poverty is a reality in Fiji and the tragic situation is that it could remain so for some years to come.

The conference concluded on Sunday midday, releasing delegates to leisure activities.

With the white to golden sands, the blueness of the ocean and the incredibly tall but watchful coconut trees moving only slightly in the pacific breeze, one could probably settle here for a lifetime, if you enjoyed the heat that is, or not be phased at all by the prospect of being hit on the head by a falling coconut. This was a continual worry, but the locals assured me that falling coconuts were not an issue, except in strong winds.

Late Sunday afternoon we travelled from the Naviti Resort by car to Suva ...busy, hot and incredibly humid. The entire Monday was spent at Suva hospital visiting the laboratories as well as holding three blood film microscopy workshops for medical laboratory students and qualified staff working within the hospital itself. This was a very rewarding exercise and those who attended gained a comprehensive understanding of the case studies presented.

On Tuesday, I farewelled Suva to travel back to Nadi. Don't make the mistake and ask for a window seat, because every seat is a window seat as well as an isle seat. so it's a win win situation. The aircraft was pretty old and noisy but on a wing (I could only see one) and a discrete prayer it finally arrived in Nadi. Wednesday was back home to Wellington.

It was an excellent opportunity to be able to visit two laboratories that actively participate in the PPTC QA programme, meet the dedicated workers that provide the services and help in some way to enhance the quality of their performance.

Philip Wakem Dip MLT, MMLSc (Otago)
QA Technical Advisor/Co-ordinator for Haematology
Pacific Paramedical Training Centre

Abstracts of articles in the *British Journal of Biomedical Science* Official publication of the British Institute of Biomedical Science

Mahdi OS. Impact of host genetics on susceptibility to human *Chlamydia trachomatis* disease. *Br J Biomed Sci* 2002;59(2):128-32.

Abstract

Evidence that host genetic factors play a major role in susceptibility or resistance to many infectious diseases is increasing, due to major advances in genetic epidemiological methodology. Recent human genome mapping information and the identification of a large number of candidate genes provide the tools for such studies. The information obtained is important for understanding the pathogenesis of disease and for the design of preventive and therapeutic strategies. In the study of *Chlamydia trachomatis* disease pathogenesis, much research focuses on how bacterial factors modulate the immune response and thus contribute to the disease process. It is likely, however, that host factors also play a role, and therefore susceptibility to disease is the result of an environmental effect set against a background of genetic factors. This review outlines the evidence for the contribution of host genetic factors to susceptibility to *C. trachomatis* disease in humans.

Pitt SJ, Sands RL. Effect of staff attitudes on quality in clinical microbiology services. *Br J Biomed Sci* 2002;59(2):69-75.

Abstract

Technical quality of the work of clinical pathology laboratories is monitored regularly by both internal and external sources. Among the factors that might affect quality, laboratory staff attitudes are rarely considered. In this study, the psychological concepts of 'job satisfaction' and 'climate' are measured among microbiology biomedical scientists in the United Kingdom. A self-report questionnaire was developed and distributed (between November 1998 and February 1999) to biomedical scientists in 161 microbiology laboratories throughout the UK From 2415 questionnaires distributed, 931 replies were received — a response rate of 39%. A separate set of questions covering customer service and participation in internal and external quality assurance schemes was sent to laboratory managers. Biomedical scientists reported lower job satisfaction than did medical technologists in a previous study in the USA. Perception of climate was influenced by several demographic factors, the most important of which being the size of the laboratory. Optimal number of staff in a department was found to be less than 30. Aggregation of climate scores from members of the same department showed that a positive laboratory climate was important for good performance in internal and external measures of technical quality. For the best service, laboratory climate must be supported by a staff perception that the department is committed to enhancing quality — a climate for laboratory quality.

Nzeako B, Okafor N. Bacterial enteropathogens and factors associated with seasonal episodes of gastroenteritis in Nsukka, Nigeria. *Br J Biomed Sci* 2002;59(2):76-9.

Abstract

Each year, between April and October, many children of school age and some young adults in Nsukka, Nigeria suffer from gastroenteritis. The period covers the rainy season in this part of Africa, when manured farmland occasionally is flooded. In view of the number of people suffering diarrhoea and occasionally low-grade fever, it became necessary to investigate the nature of the bacterial agents responsible. Between April and October (1996-1998), 500 loose or watery stools were collected from patients, the ages of which ranged from one month to 31 years. Stools that contained parasites were excluded from the study. Samples were cultured on 5% blood agar and 1% egg-yolk agar (both containing 10 microg/mL ampicillin), MacConkey agar, Shigella Salmonella agar and in alkaline peptone water. Bacterial growths were identified using standard bacteriological procedures. Drinking water and some fruit and vegetables prevalent during this period of the year also were cultured. Of the 500 stool samples tested, 138 (27.6%) grew a range of organisms including *Aeromonas hydrophila* (65 [13%]), *Salmonella spp.* (55 [11%]), *Shigella spp.* (9 [1.8%]) and *enteropathogenic Escherichia coli* (9 [1.8%]). Drinking water and some vegetables grew *Pseudomonas aeruginosa* and *Enterococcus faecalis*, respectively. The highest isolation rate occurred during June and July, corresponding to the period of greatest flooding of arable land. Although no enteropathogens were isolated from the fruit and vegetables examined, they contained *E. faecalis* — an organism found in faeces. Our findings failed to explain why 72% of the samples grew no bacterial enteropathogens.

Ozolua RI, Omogbai EK, Famodu AB, Ebeigbe AB, Ajayi OI. Haematological influences of potassium adaptation in normotensive and renally-hypertensive Wistar rats. *Br J Biomed Sci* 2002;59(2):80-4.

Abstract

Dietary potassium is known to cause reduction in blood pressure in several models of hypertension in human and animal studies but its haematological effects are not known. Here, experiments are designed to study the haematological effects of potassium adaptation (achieved by administering 0.75% KCl solution in drinking water for five weeks) in Wistar rats. The animals are divided into four groups comprising controls, potassium-adapted, renal hypertensive, and renal hypertensive with later adaptation to potassium. Packed cell volume (PCV) and platelet count (PC), whole blood and plasma viscosities, and platelet aggregation in the presence of sodium nitroprusside, levcromakalim, and glibenclamide, are studied. Results showed comparable PCV and PC in all groups. While relative whole blood viscosity was significantly higher ($P<0.05$) in the hypertensive group, relative plasma viscosity was similar in all groups. Adaptation significantly reduced ($P<0.05$) the tendency of platelets to aggregate to collagen. Sodium nitroprusside significantly reduced ($P<0.05$) the pro-aggregatory effects of collagen only in the control group. Neither of the potassium-channel modulators (levcromakalim, glibenclamide) caused any significant alteration in platelet response to collagen at the concentrations studied. Although these results suggest that potassium adaptation may not affect haemorheology, the reduced ability of platelets to aggregate—by mechanisms not clearly understood — has implications for reduced thromboembolism and the attendant cardiovascular sequelae.

Bidarkundi GK, Wig JD, Bhatnagar A, Majumdar S. Clinical relevance of intracellular cytokines IL-6 and IL-12 in acute pancreatitis, and correlation with APACHE III score. *Br J Biomed Sci* 2002;59(2):85-9.

Abstract

Pro-inflammatory cytokines are involved in the pathogenesis of acute pancreatitis (AP). Here, we measure and correlate clinically the percentages of peripheral blood mononuclear cells (PBMC) that contain interleukin (IL)-6 and IL-12 and compare these with acute physiology and chronic health evaluation (APACHE III) scores in 30 patients with AP. Severity of AP is determined according to the Atlanta criteria. Patients with severe AP (n = 15) had significantly higher IL-6 values compared to those with mild AP (n = 15). IL-12 levels correlated well with aetiological factors (alcohol and biliary pathology) in patients with AP. Correlation was seen between IL-6 value and APACHE score in severe AP. A score of 30 points was used as the cut off between mild (<30) and severe (>30) cases, with a sensitivity of 80% and specificity of 100%. Cut off percentages for IL-6- and IL-12-positive PBMCs were >25% (positive predictive value [PPV]: 100%) and >9% (PPV: 70%), respectively. Based on these results, it would seem logical to use both APACHE III score and IL-6 percentage to assess severity in patients with AP.

Linhart A, Jindra A, Golan L, Jachymova M, Sedlacek K, Peleska J,et al. Association between tyrosine hydroxylase polymorphisms and left ventricular structure in young normotensive men. *Br J Biomed Sci* 2002;59(2):90-4.

Abstract

Tyrosine hydroxylase (TH) is a rate-limiting enzyme for catecholamine biosynthesis. Increased sympathetic activity is associated with an increased left ventricular (LV) mass. However, the influence of TH gene polymorphisms on LV structure and function has yet to be investigated. Here, we analyse the association of Val-81-Met and tetranucleotide TCAT repeat TH polymorphisms with LV structure and function (assessed by echocardiography) in 108 normotensive men aged < or = 35 years (mean age: 25+/-4 years) with body mass index (BMI) < or = 30 kg/m2 (mean BMI: 23+/-3 kg/m2). The distribution of genotypes was VV homozygotes (n=42), VM heterozygotes (n=49) and MM homozygotes (n=17). The Val-81-Met polymorphism showed significant linkage disequilibrium with the TCAT polymorphism (P<0.0001). No differences were seen between the subgroups with respect to age, BMI and blood pressure. Compared with the VV and VM genotypes, subjects with the MM genotype showed significantly (all P<0.05) increased LV cavity diameter (VV: 52.8+/-3.9 mm, VM: 52.9+/-3.6 mm, MM: 56.1+/-3.2 mm), global LV mass (VV: 159+/-31 g, VM: 165+/-36 g, MM: 187+/-30 g) and LV mass index (VV: 81+/-14 g/m2, VM: 84+/-17 g/m2, MM: 93+/-12 g/m2). No differences were seen between the subgroups in parameters of LV function. In addition, plasma epinephrine and norepinephrine levels were comparable in the three subgroups. The results suggest an important association between the MM genotype of Val-81-Met TH gene polymorphism and increased LV cavity dimension and mass in a young normotensive male population, indicating an important role for genetic determination of the sympathetic system in LV growth.

Martinez E, Puras A, Escribano J, Sanchis C, Carrion L, Artigao M, et al. Threonines at position 174 and 235 of the angiotensinogen polypeptide chain are related to familial history of hypertension in a Spanish-Mediterranean population. *Br J Biomed Sci* 2002;59(2):95-100.

Abstract

This study investigates the association between the allelic distribution of two polymorphisms of the angiotensinogen (AGT) gene (T174M and M235T in the polypeptide chain) and blood pressure (BP) in a Mediterranean population in the south-west of Europe. The sample consists of 1322 participants from urban and rural areas, from the province of Albacete (218,462 inhabitants), located in the south-east of Spain. The subsample of this study, adjusted by age (over 18 years old) and sex, consists of 401 individuals. A case-control study is conducted which analyses 205 individuals from the group with the highest BP (fifth quintile) and 196 from the group with the lowest BP (first quintile). In addition, a comparative and associated analysis of these polymorphisms with BP level and family history of hypertension is carried out. The T174 allele proved to be more common in the fifth quintile group, although not statistically so. When the presence of threonine was analysed in both polymorphism positions (174 and 235), the TTTT genotype was found to be more common in the fifth quintile than in the first quintile. Moreover, the TTTT genotype was significantly more common in individuals with a family history of hypertension, indicating that it could be considered a predisposing factor to high BP in individuals from such families. In addition, the T174M-T235T genotype was more common in the first quintile group, and showed significant association (P=0.05) with the group that had no family history of hypertension.

Clarke SC, Haigh RD, Freestone PP, Williams PH. Enteropathogenic *Escherichia coli* infection: history and clinical aspects. *Br J Biomed Sci* 2002;59(2):123-7.

Abstract

Diarrhoeagenic *Escherichia coli* remains an important cause of diarrhoeal disease worldwide. In terms of global public health, enteropathogenic *E. coli* (EPEC) and enterotoxigenic *E. coli* are the most important. However, enterohaemorrhagic *E. coli* has emerged as a cause of disease in developed countries in recent years, and a number of large outbreaks have been reported. Therefore, the importance of research into diarrhoeagenic *E. coli* remains an important issue. EPEC is the most widespread of the diarrhoeagenic *E. coli* and provides a good virulence model for other *E. coli* infections, as well as other pathogenic bacteria. Although the virulence mechanisms of *E. coli* are now better understood, there remains much to be learned before effective treatments can be developed. Type III secretion mechanisms, the locus of enterocyte effacement and various toxins are all involved in the pathogenesis of the various diarrhoeagenic *E. coli* and may provide targets for future therapies. This review aims to provide an update on the worldwide problem of diarrhoeagenic *E. coli* by focusing on EPEC, and describes the history of the organism, its incidence and the clinical aspects of infection.

Bignold LP. Hypothesis for the influence of fixatives on the chromatin patterns of interphase nuclei, based on shrinkage and retraction of nuclear and perinuclear structures. *Br J Biomed* Sci 2002;59(2):105-13.

Abstract

Nuclear chromatin patterns are used to distinguish normal and abnormal cells in histopathology and cytopathology. However, many chromatin pattern features are affected by aspects of tissue processing, especially fixation. Major effects of aldehyde and/or ethanol fixation on nuclei in the living state include shrinkage, chromatin aggregation and production of a 'chromatinic rim'. The mechanisms of these effects are poorly understood. In the past, possible mechanisms of fixation-induced morphological change have been considered only in terms of the theoretical model of the nucleus, which involves only a random tangle of partly unfolded chromosomes contained within the nuclear membrane. Such a model provides no basis for chromatin to be associated with the nuclear envelope, and hence no obvious clue to a mechanism for the formation of the 'chromatinic rim' in fixed nuclei. In recent years, two new models of nuclear structure have been described. The nuclear membrane-bound, chromosomal-domain model is based on

the discoveries of chromatin-nuclear membrane attachments and of the localisation of the chromatin of each chromosome within discrete, exclusive parts of the nucleus (the 'domain' of each partly unfolded chromosome). The nuclear matrix/scaffold model is based on the discovery of relatively insoluble proteins in nuclei, which it suggests forms a 'matrix' and modulates gene expression by affecting transcription of DNA. Here, a hypothesis for fixation-associated chromatin pattern formation based mainly on the first model but partially relying on the second, is presented. The hypothesis offers explanations of the variations of appearance of nuclei according to fixation (especially air-drying versus wet-fixation with formaldehyde, glutaraldehyde or ethanol); the appearances of the nuclei of more metabolically active versus less metabolically active cells of the same type; the appearances of nuclei after fixation with osmium tetroxide; and of the marked central clearing ('egg-shell' or 'orphan Annie' appearance) of tumour nuclei of papillary carcinoma of the thyroid gland. A similar process may underlie the phenomenon of 'chromatin margination' seen in apoptosis. Various tests of the hypothesis, such as time-lapse confocal microscopy of living nuclei during fixation, are suggested. The significance of the theory is that it suggests that chromatin patterns could be investigated in terms of qualitative and quantitative aspects of nuclear components, and hence be related to the results of studies of the structure and function of nuclei in health and disease.

Poch E, Gonzalez-Nunez D, Compte M, De la Sierra A. G-protein beta3-subunit gene variant, blood pressure and erythrocyte sodium/lithium countertransport in essential hypertension. *Br J Biomed Sci* 2002;59(2):101-4.

Abstract

Recently, a C825T polymorphism in the gene coding for the beta3 subunit of G proteins (GNB3) has been described in cells from patients with essential hypertension and enhanced Na+/H+ exchange activity. This study aims to evaluate the association between the 825T allele and activity of erythrocyte sodium/lithium countertransport (Na+/Li+ CT) and other sodium transport systems in red blood cells from patients with essential hypertension. A group of 77 patients (36 male, 41 female; aged 51.7 +/- 1.1 years) was studied. The maximal rates (Vmax) of Na+/Li+ CT, Na+/K+/Cl- cotransport and Na+K+ ATPase were evaluated in erythrocytes from all the patients. They were genotyped for the C825T polymorphism by a polymerase chain reaction (PCR) method, followed by digestion with BseDI. Body mass index (BMI) was higher in CT+TT patients than in CC patients (28.9 +/- 0.5 vs. 27.0 +/- 0.7 kg/m2; P=0.023). Hypertensives with the T allele (CT+TT genotypes) showed significantly higher systolic blood pressure (BP) values (156.9 +/- 2.1 vs. 148.9 +/- 2.8 mmHg; P=0.024), whereas differences in diastolic BP did not reach statistical significance (96.4 +/- 1.0 vs. 94.0 +/- 1.1 mmHg; P=0.120). No differences in the Vmax of Na+/Li+ CT between the genotypes was seen (CC: 236 +/- 19 and CT+TT 277 +/- 23 mmol/L cells per h; P=0.221). Similarly, no differences were detected in the Vmax of erythrocyte Na+/K+/Cl- cotransport and Na+K+ ATPase among the genotypes. There was no appreciable association between the G-protein beta3-subunit C825T polymorphism and erythrocyte Na+/Li+ CT and other sodium transport systems in the hypertensive patient sample studied; however, those with the T allele were more obese and had more severe systolic hypertension.

Humphreys H, Glynn G, Rossney A, McDonald P, Johnson H, McDonnell R, et al. Methicillin-resistant *Staphylococcus aureus*: laboratory detection methods in use in the Republic of Ireland and Northern Ireland. *Br J Biomed Sci* 2002;59(1):7-10.

Abstract

There is no universally agreed laboratory protocol for the detection of methicillin-resistant *Staphylococcus aureus* (MRSA) and hence a variety of approaches are used. As part of an all-island survey of MRSA in the Republic of Ireland (the South) and Northern Ireland (the North), a questionnaire was circulated to 14 participating laboratories in the North and 49 in the South, to determine the methods used to isolate MRSA from clinical specimens, identify *S. aureus* and test for susceptibility to methicillin. Almost two-thirds (64%) of laboratories in the North but only 16% of laboratories in the South use enrichment culture. There is heavy reliance on commercial kits to confirm the identification of *S. aureus* in the South but all laboratories in the North use the staphylocoagulase test. More than 90% of all laboratories use a disc method for susceptibility testing and 71% of laboratories in the North supplement this with the E-test; however, a range of methicillin disk concentrations are in use. There is a need to review current laboratory methods used to detect MRSA, with follow-up audit on their implementation. Additional resources may be needed in some laboratories to comply with revised guidelines, and reference facilities are required to assess new commercially available techniques and to confirm the identification of unusual or difficult strains.

Marshall T, Williams KM. Proteomics and its impact upon biomedical science. *Br J Biomed Sci* 2002;59(1):47-64.

Abstract

Proteomics is the protein equivalent of genomics and is the study of gene expression at a functional level. The proteome of an organism is the protein complement of its genome. However, unlike the genome, the proteome is dynamic: it varies according to the cell type and the functional state of the cell. In addition, the proteome shows characteristic perturbations in response to disease and external stimuli. Proteomics combines state of the art analytical methods with bioinformatics. Here, we review the concept and technology of proteomics with specific reference to applications in medical microbiology, cellular pathology, clinical chemistry, haematology/immunology, pharmacology and toxicology.

Other articles without abstracts

Rich M, Memish ZA. *Streptococcus pneumoniae*: presumptive identification and reporting. *Br J Biomed Sci.* 2002;59(1):65.

Matsuda M, Sekizuka T, Murayama O, Moore JE. Two Japanese urease-positive thermophilic *campylobacters*, CF89-12 and CF89-14, isolated in 1989 prove to be two distinct strains. *Br J Biomed Sci.* 2002;59(1):65.

Siebers R. Data in abstracts of research articles. Are they consistent with those reported in the article? *Br J Biomed Sci.* 2002;59(2):67-8.

Maratim AC, Kamar KK, Ngindu A, Akoru CN, Diero L, Sidle J.Safranin staining of *Cyclospora cayetanensis* oocysts not requiring microwave heating. *Br J Biomed Sci.* 2002;59(2):114-5.

Banchonglikitkul C, Smart JD, Gibbs RV, Cook DJ. Lectins as targeting agents — the in vitro binding of lectins to lesions in the eye and mouth. *Br J Biomed Sci.* 2002;59(2):115-8.

Bao Z, Wenli M, Rong S, Ling L, Qiuye G, Wenling Z. Re-use of a stripped cDNA microarray. *Br J Biomed Sci.* 2002;59(2):118-9.

Devanapalli B, Lee S, Mahajan D, Bermingham M. Lipoprotein (a) in an immigrant Indian population sample in Australia. *Br J Biomed Sci.* 2002;59(2):119-22.

Abstracts of articles in the *Australian Journal of Medical Science* Official publication of the Australian Institute of Medical Science

Shephard M, Allen G. Screening for renal disease in a remote Aboriginal community using the Bayer DCA 2000. *Austr J Med Sci* 2001; 22 (4): 164-70.

Abstract

End-stage renal disease among Aboriginal Australians has reached alarming proportions during the past decade. The early identification of this disease through community screening programs is a key strategy in reducing the long-term financial and cultural burden of the disease. The small point-of-care Bayer DCA 2000 analyser, which tests for urine albumin:creatinine ratio (ACR), was used as a marker for early renal disease in an adult screening program in a remote South Australian Aboriginal community. Nineteen percent of 149 adults screened had previous undiagnosed persistant microalbuminuria (ACR between 3.4 and 33.9 mg/mmol), while a further 9% had persistent overt albuminuria (ACR greater than or equal to 34 mg/mmol). Aboriginal health workers were trained in the operation of the DCA 2000 to enable screening to be an on-going, sustainable activity within the community setting. The DCA exhibited excellent analytical performance characteristics and was robust and reliable throughout the study period.

Shephard M, Tallis G. Assessment of the point-of-care Cholestech lipid analyser for lipid screening in Aboriginal communities. *Aust J Med Sci* 2002; 23 (1):

Abstract

Cardiovascular disease is the leading cause of mortality in Aboriginal Australians. Screening for cardiovascular disease risk factors, notably elevated blood lipids, is urgently needed. The small, portable Cholestech machine (Point_of_Care Diagnostics) can enzymatically measure total cholesterol, triglyceride and HDL cholesterol (without the prior need for precipitation of other lipoproteins) on 35µL of capillary or venous whole blood in under 5 minutes. It also calculates LDL cholesterol. Its suitability for use in Aboriginal communities was assessed. Fifty_one volunteers had their lipids measured on capillary and venous whole blood samples on the Cholestech. These results were compared with those obtained by Center for Disease Control (CDC)_certified methods on the Hitachi 917. The correlation (r2) between the Cholestech and Hitachi machines for both capillary and venous whole blood samples was 0.96 for total cholesterol, 0.99 for triglyceride, and 0.92 for HDL_ and LDL cholesterol. The mean percentage difference between results on the Cholestech and Hitachi for both sample types was less than 2% for total cholesterol and triglyceride analyses and less than 5% for HDL_ and LDL cholesterol. A positive bias of 6% was observed on the Cholestech at HDL cholesterol concentrations greater than 1.2mmol/L. Within_day precision (CV%) on whole blood samples ranged from 0.9 to 3.5% for total cholesterol, 1.6 to 2.5% for triglyceride and 6.3 to 7.9% for HDL cholesterol. There was no significant difference between capillary and venous whole blood lipid measurements performed on the Cholestech. With its simple operation, fully automated nature, sound analytical performance and ability to produce a full lipid profile in under 5 minutes, the Cholestech would be suitable for the Aboriginal health care setting.

Zarkos KB, Brown RD, McKenzie G. Hb A2 and Hb F determination: a comparison of the traditional methods with two HPLC methods. *Aust J Med Sci* 2002; 23 (1):

Abstract

The clinical relevance of quantitation of haemoglobin A2 (Hb A2) and foetal haemoglobin (Hb F) is dependent not only on the precision of the assay used but also the turn_around time. Furthermore, proper characterisation of abnormal haemoglobins allows for accurate diagnosis of individuals with severe haematological manifestations in families and carrier couples during prenatal screening tests. We compared results of two automated high performance liquid chromatography (HPLC) analysers with our manual cellulose acetate electrophoresis (CAE) method for Hb A2 quantitation and our manual alkaline denaturation test for Hb F quantitation. Measurement of over 200 blood samples showed that both the Bio_Rad Variant II and Primus Corporation CLC330 were reliable and accurate in obtaining quantitation of Hb A2 as compared to our CAE method (both Bio_Rad and Primus correlation $p < 0.001$) and Hb F as compared to our alkali denaturation method (both Bio_Rad and Primus correlation $p < 0.001$). Within_run percent coefficient of variation (CV) for the Bio_ Rad were 3.1 and 2.0 for a sample with a normal Hb A2 and one with a raised Hb A2 respectively, and for the Primus were 2.7 and 1.9. Between_run CVs for the Bio_Rad were 2.2 and 2.5, and the Primus were 5.6 and 5.5 respectively. The HPLC systems are highly automated and can include bar code reading, primary whole blood sampling with cap piercing and auto injection/gradient conditioning/peak integration/result calculation. Drawbacks on the analysers include lack of separation of Hb Lepore and Hb E from Hb A2, and elevated Hb A2 quantity in individuals with sickle haemoglobin (Hb S) and haemoglobin D (Hb D), due to the presence of abnormal haemoglobin adducts.

Bates J. Anthrax: An update for Australia. *Austr J Med Sci* 2002; 23 (2):

Abstract

The recent spate of anthrax mail attacks across the United States has lead to a heightened awareness of the bacterium Bacillus anthracis. Clinical microbiology laboratories are in the front line because samples from patients are likely to pass through their hands until a diagnosis is confirmed. The major problem facing clinical laboratories is that few of them are equipped to handle the organism (all manipulations should be conducted in a PC3 environment), and even fewer microbiology staff have ever seen the organism. This creates a potential problem for Australian microbiology staff. This paper sets out to partially redress this situation by providing some of the history of the organism, its characteristics and identification, and some of the unique characters of the organism that aid in its identification.

O'Brien JR, George NM. Speciation of the aerobic endospore forming rods of the genus Bacillus: Automated microbial system compared with conventional manual methods. *Austr J Med Sci* 2002; 23 (2):

Abstract

Speciation of 16 type strains of aerobic endospore forming Bacillus species were compared using the automated Vitek™ identification

system and manual conventional physico-chemical tests. The Vitek™ method utilised the Bacillus Biochemical panel in conjunction with the Vitek™ automated instrument. The manual identification system utilised a series of conventional physico-chemical tests and a modification of a dichotomous key. The latter system resulted in 100% correct identification whereas the Vitek™ Bacillus panel gave an unacceptably high percentage of incorrect speciation. The relative merits of other diagnostic schemes for species identification of members of the genus Bacillus were also reviewed.

Newman JD, Balazs NDH. Use of stabilized horse blood for hospital wide quality assurance of point-of-care glucose meters. *Austr J Med Sci* 2002; 23 (2):

Abstract

The purpose of this study was to provide a stabilized whole blood control to be used as quality assurance material for our hospital's point-of-care glucose meters. Equine whole blood was stabilized with 18 mmol/L sodium iodoacetate and distributed with instructions to each hospital ward for analysis by all staff using glucose meters. Results were forwarded to the laboratory for statistical analysis. This procedure has resulted in an inexpensive, safe method of providing quality assurance material to be used for point-of-care analysers, free of the potential problems due to matrix effects caused by using aqueous quality control material for whole blood analysers.

Answers to HSIG questionnaire

1. The aim of this study was to investigate whether or not granulocytes are functionally impaired immediately after or during recovery from chemotherapy.

2. Respiratory burst is the process in which granulocytes greatly increase their oxygen consumption in order to generate superoxide anions and hydrogen peroxide. These oxygen metabolites in turn give rise to other strongly antimicrobial reactive oxygen species; the process is necessary for the bacteria-killing function of granulocytes.

3. CD11b and CD18 are proteins located on the surface of leucocytes; they are important for adhesion to and migration over the endothelium, and for phagocytosis.

4. Granulocyte function was studied in blood samples from healthy donors, from patients with myeloproliferative disorders treated with low-dose hydroxyurea and from patients with acute leukaemia or multiple myeloma receiving high-dose chemotherapy. In the third group, blood samples were collected where possible from three phases of treatment: immediately before chemotherapy, day 1 to day 6 after chemotherapy and at the first sign of granulocyte recovery.

5. One Staphylococcus epidermidis strain and one Staphylococcus aureus strain were used as activators of granulocytes. The bacterial concentration was adjusted to a ratio of one bacterial cell to one granulocyte.

6. No, previous studies have investigated the effects of chemotherapy on normal garnulocytes in vitro only.

7. By measuring the amount of oxidative burst and the expression rate of CD11 and CD18 using flow cytometry. Each sample was divided into two tubes; one was analysed immediately after the addition of bacteria and the other after 30 minutes incubation. The difference in oxidative burst and CD11b/CD18 expression between the two tubes were calculated as percentages of the value from the first tube.

8. In samples collected during the recovery phase, there was a decreased ability to mobilize CD18 as compared with the phase after the start of chemotherapy.
No significant differences in respiratory burst activity or CD11b expression were seen between samples from the high-dose treatment, or between samples from the high dose group and those from the low-dose group or the healthy donors.

9. Due to the small number of patients studied.

10. The study concluded that granulocyte function is not greatly altered bye exposure to antineoplastis drugs.

For a copy of this journal article, contact Shadi Gadalla at Haematology Dept, DML, Auckland, email shadi0@hotmail.com

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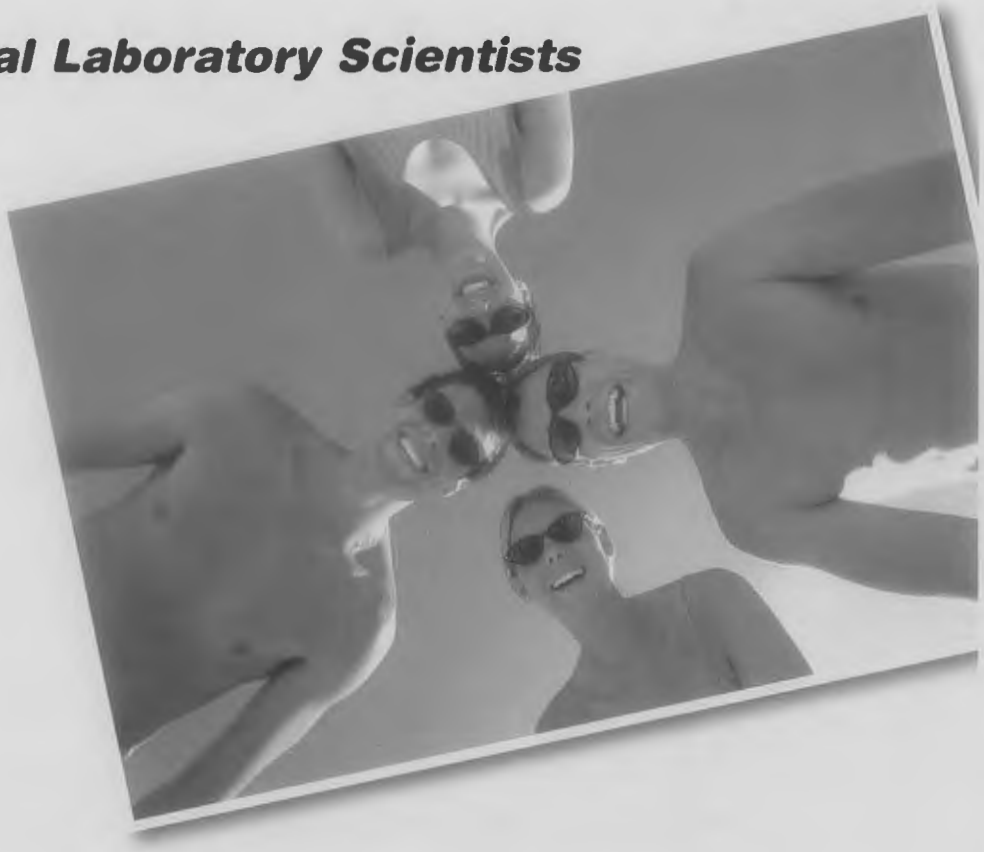

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Letters to the Editor

Continuing professional development

In the August 2002 edition, there was an article on Continuing Professional Development (CPD) by Graeme Broad (1). In this article he states that there is no formal CPD program in Australia.

As chair of the Australian Professional Acknowledgment of Continuing Education (APACE) scheme for the Australian Institute of Medical Scientists (AIMS), I would like to correct this statement. The APACE scheme in its current format has been running for 4 years with over 80 members attaining certification for their continuing education activities. It is a voluntary program that recognizes continuing education, formal courses and a wide range of professional activities that contribute to professional growth. Participants have a maximum period of 2 years to accumulate the necessary points and can then apply for APACE certification. The APACE committee is currently receiving at least 10 applications a month for certification as our members are realizing the need for formal recognition of their continuing education and professional activities.

Reference

1. Broad G. Continuing Professional Development (CPD). *N Z J Med Lab Science* 2002; 56: 64-5.

Robyn Wells
Chair
AIMS APACE committee

An open letter to all phlebotomists in New Zealand

Dear Phlebotomist,

An exciting chapter is unfolding in the field of Phlebotomy within NZ. The Phlebotomy Special Interest Group, now called N.Z.A.P (New Zealand Association of Phlebotomy), has been working hard on your behalf over the past year to develop a nationally-recognised qualification in Phlebotomy.

To be eligible for this qualification, you will need to be currently working in the field of Phlebotomy (Pathology Specimen Collection) in New Zealand and for a period of not less than two years (full or part time). The qualification will be a "Qualified Technical Assistant" style of qualification, possibly called "Qualified Phlebotomy Technician" (QPT) and will be administered under the auspices of the New Zealand Institute of Medical Laboratory Science (NZIMLS).

A syllabus is being developed to outline the course requirements for training. It is envisaged that all participating laboratories will design their training programmes to include the modules outlined in the Syllabus. A Log Book is also being developed to assist you in self-directed learning and as a record of your technical skills and knowledge.

"Train the Trainer" days are being planned for early next year. This is to facilitate each participating laboratory to understand the Standards of Competency required and to assist where necessary to develop their Training Programmes.

Requirements for the qualification are as follows:

- Working currently as a Phlebotomist in NZ (2 years)
- Completing the Log Book
- Passing an examination - both written and practical assessments.
- Being a member of NZIMLS.

To become a member of the Institute, a reduced fee has been set at $30 for the 2002-2003 year. As from April 2003, the fee will be $56. As a member, you will receive the NZIMLS journal 3 times per year. This will feature articles of special interest to Phlebotomists. When you apply to sit the exam (possibly November, 2003), you will be required to submit $125 with your application.

To join NZIMLS, please make your application to:
The Executive Officer of the NZIMLS: Fran Van Til, P.O. Box 505, Rangiora. Phone: (03) 313 2097 E-Mail: fran@eenz.com

New products and services

The VIDAS EMERGENCY Panel

The VIDAS EMERGENCY Panel offers 6 parameters for the exclusion of life-threatening conditions in patients presenting to Emergency Departments: D-Dimer, Troponin I, Myoglobin, CK-MB, Digoxin, and hCG.

The miniVIDAS and the VIDAS are reliable systems that are always available to deliver cost effective and high quality results. This makes the VIDAS instrument well suited to any laboratory that services Emergency and Critical Care Departments.

The VIDAS D-Dimer parameter is internationally referenced for the exclusion of venous thromboembolism (VTE) from diagnosis. Its excellent negative predictive value (NPV) helps reduce the need for expensive diagnostic imaging procedures.

VIDAS Troponin I is a front-line test that is both quantitative and highly reproducible. Laboratories can provide rapid, accurate results and avoid a 2-stage testing strategy.

The miniVIDAS and the VIDAS are proving to be essential elements in urgent or emergency testing strategies. For more information please contact:

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Tel: 1 800 333 421
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Dade Behring's new Sysmex® CA-7000 instrument brings unprecedented speed to the coagulation analyzer market

At the July 2002 meeting of the American Association of Clinical Chemistry (AACC), Dade Behring introduced the Sysmex® CA-7000 analyzer, the newest addition to the family of coagulation analyzers offered by Dade Behring. For use in large-scale laboratories, the CA-7000 is capable of attaining the fastest throughput of any coagulation analyzer on the market, with results every seven seconds, or 550 results per hour, when testing PT and aPTT simultaneously.

"In today's demanding medical care environment, the lab's ability to rapidly and accurately process tests can affect treatment decisions for hundreds of patients, from a single lab site," said Jim Reid-Anderson, President and CEO, Dade Behring. "Every technological advance in diagnostics, such as the CA 7000 instrument, translates into the global effort to improve patient care. It is our corporate commitment to meet customer and patient needs with advanced technologies such as this."

The CA-7000 enables laboratorians to continuously load samples, reagents and reaction cuvettes without interrupting workflow. Up to 100 patient samples can be processed simultaneously in 10 racks; racks can be continuously fed as processing is completed, making the CA 7000 instrument ideal for a large reference lab or busy hospital lab.

The CA-7000 provides the most current technology in clotting, chromogenic and immunologic* methodologies, and can process both routine and specialty assays, using complete, 20-parameter random access capabilities. With up to 20 QC files per parameter, lab customization and new lot crossover studies are simple to conduct.

The CA-7000 instrument's second-generation optional cap-piercing feature improves pre-analytical processing time, while reducing biohazard risk. "The ability to provide cap piercing is a valuable feature not available on all analyzers," said Jackie Hauser, Marketing Manager, Dade Behring. "As importantly, in this instrument, the cap-piercing capability is a feature that does not detract from the CA-7000's exceptional speed."

Additionally, she indicated, the CA-7000 instrument has the ability to calibrate assays while running routine specimens. This saves valuable laboratorian time, and prevents workflow backups.

The CA-7000 analyzer's sophisticated automated reagent management system enables laboratorians to positively confirm reagent lots and type, while eliminating operational errors. Dual integrated patient barcode readers positively confirm patient identities, which further reduces errors and increases security. The instrument also features automatic repeat, redilution and reflex testing, which saves time that would otherwise be spent in identifying and reanalyzing samples.

"The CA-7000 represents the next step in coagulation analyzers," said Ms. Hauser. "Its exceptional speed offers labs the ability to significantly advance their productivity with a single instrument."

With 2001 revenues of $1.23 billion, Dade Behring is among the world's largest clinical diagnostics companies. It offers a wide range of products and systems for diagnostics testing, making it today's best resource in this field. The company is headquartered in Deerfield, Illinois, and has operations in 43 countries. Additional company information is available on the internet at www.dadebehring.com.

Dade Behring's new MICroSTREP plusTM dried panel is a new diagnostic aid in the fight against antibiotic resistance

The U.S. Food and Drug Administration (FDA) has cleared for use in laboratories a new dried MIC panel, MICroSTREP plus™, that provides antibiotic susceptibility testing across a range of 18 antibiotics. The product is manufactured and marketed by Dade Behring. Unlike other products on the market, the MICroSTREP plus panel can test all pneumococcal and streptococcal isolates; other dried products may only have clearance for testing *Streptococcus pneumoniae*. It also features an expanded number of drugs recommended for the treatment of these infections.

"This new panel is an important contribution to the fight against a growing health care problem-bacterial resistance," said Jim Reid-Anderson, President and CEO, Dade Behring. "As such, MICroSTREP plus is a demonstration of our commitment to offering diagnostic products that can improve health care and the quality of life."

Both pneumococcal and streptococcal infections can lead to complications ranging from pediatric ear infections to life-threatening conditions like meningitis or sepsis, making bacterial resistance to treatment a serious concern. Increasingly, highly resistant strains of these bacteria are developing, making early prescribing decisions critical to a good outcome. "To the health care professional today, identifying an antibiotic that is an effective treatment option is extremely important.

Otherwise, patients may have to go through multiple treatment regimes," said Bob Brightfelt, President, Global Research and Development. "We are extremely concerned about antibiotic resistance, particularly since it represents a growing proportion of the clinical laboratory's daily workload." The new MICroSTREP plus panel can aid in the process of making a prescribing determination, he said, by providing a larger choice of antibiotic susceptibility results to manage the patient's infection.

The ability of the MICroSTREP plus panel to utilize MIC testing (Minimum Inhibitory Concentration) is a particular advantage, since this method is especially valuable in managing serious infections.

Antibiotics in the panel include: ampicillin, augmentin, azithromycin, cefaclor, cefepime, cefotaxime, ceftriaxone, cefuroxime, chloramphenicol, clindamycin, erythromycin, meropenem, penicillin, tetracycline, trimethoprim/sulfamethoxazole and vancomycin as well as newer drugs such as gatifloxacin and levofloxacin. In addition to using the panel for data to assist in reaching more accurate prescription decisions, researchers may find it useful in studying emerging resistance.

While some methods of antibiotic susceptibility testing may involve labor-intensive manual steps with inherent interpretive variability, the new MICroSTREP plus panel's clear-cut end points facilitate laboratorians' ability to provide a precise and accurate result.

The dried panel has a full 12-month shelf life, and can be stored at room temperature.

With 2001 revenues of $1.23 billion, Dade Behring is among the world's largest clinical diagnostics companies. It offers a wide range of products and systems for diagnostics testing, making it today's best resource in this field. The company is headquartered in Deerfield, Illinois, and has operations in 43 countries. Additional company information is available on the internet at www.dadebehring.com.

Recent Fellows of the NZIMLS receiving their certificates at the Wellington 2002 ASM, presented by Les Milligan, NZIMLS President to (1) Ann Thornton, Wellington School of Medicine, Wellington; (2) Vasanthan Thuraisamy, Waikato Hospital, Hamilton; and (3) Jan Deroles-Main, Medlab Central, Palmerston North.

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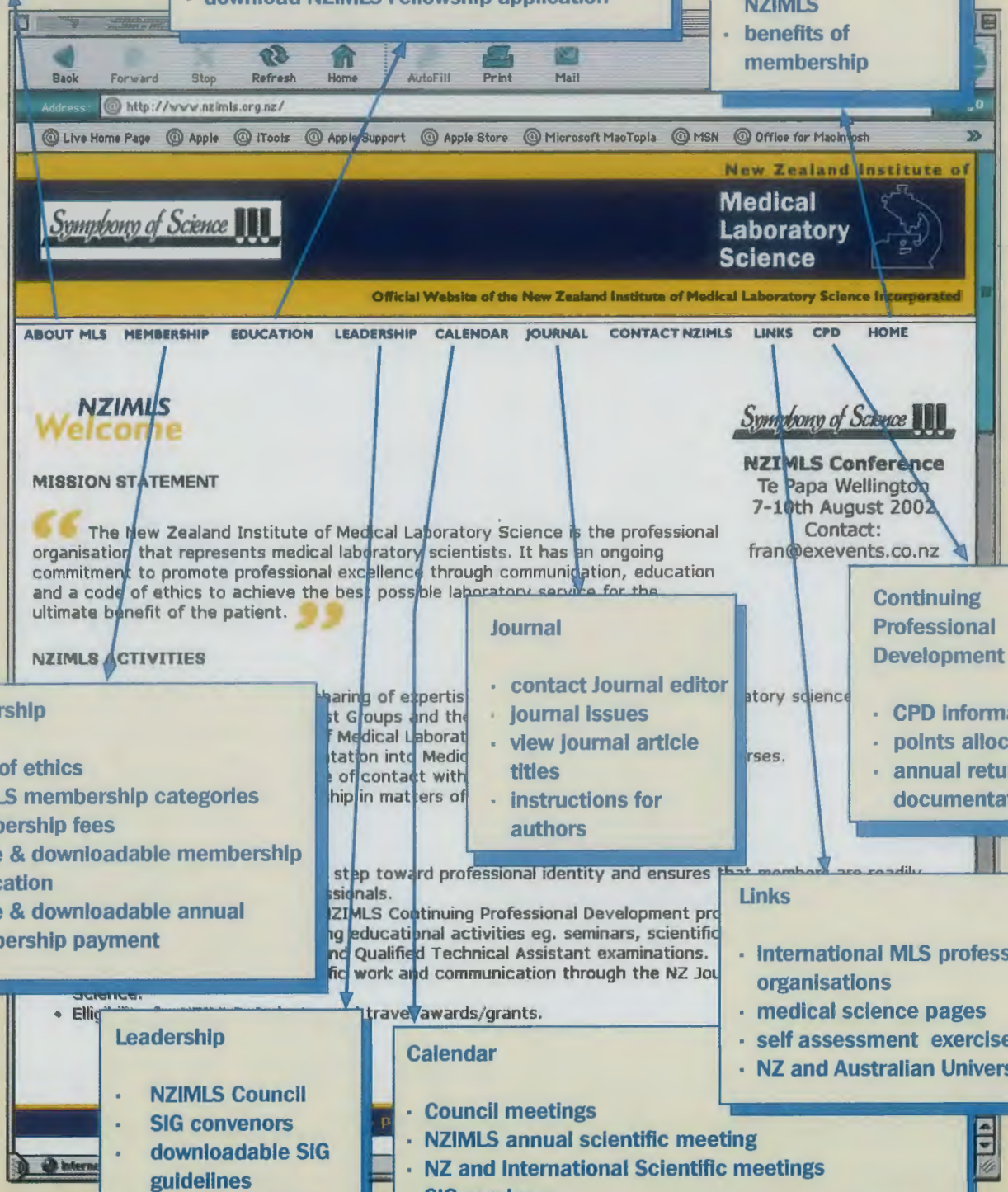

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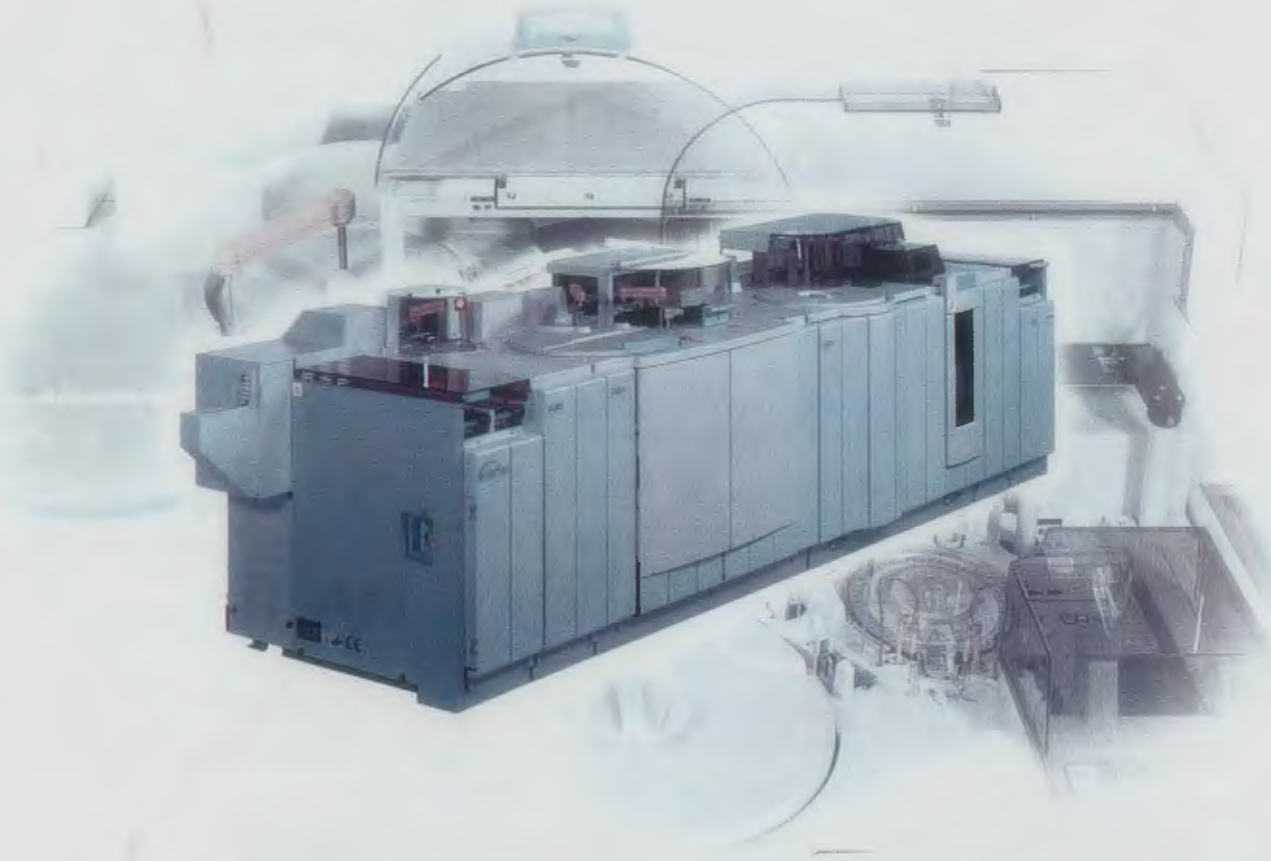

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