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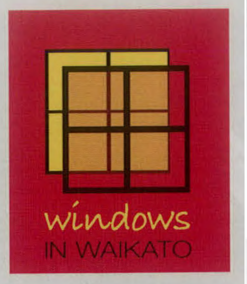

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Human parvovirus B19: a review of the disease and diagnostic tools.

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Abstract

Parvovirus B19, first discovered in 1975, is the only member of the Parvoviridae family known to cause human disease. It has a worldwide distribution with an epidemic occurring every 3-4 years. Sporadic cases occur in all age groups.

It has been identified as the causative agent of several distinct clinical syndromes, including erythema infectiosum (fifth disease), adult arthropathy, hydrops foetalis (with possible spontaneous abortion or stillbirth), and aplastic crisis in the immunocompromised or patients with underlying haematological disease.

The laboratory diagnosis confirms and differentiates parvovirus B19 infection with its broad spectrum of symptoms from other diseases with similar manifestations. The demonstration of specific parvovirus B19 IgM and PCR are suitable methods for the diagnosis of acute infection; however, the choice of diagnostic method is dependent on the clinical presentation. The disease and the relevant diagnostic tools will be reviewed.

Key words: parvovirus B19, diagnosis, PCR

Introduction

Parvovirus B19 is the only known human pathogenic parvovirus. In 1975 Yvonne Cossart and co-workers in England reported the discovery of Parvovirus B19 detected during routine screening for hepatitis B surface antigen, this was officially recognised by the International Committee on Taxonomy of Viruses, as a member of the *Parvoviridae* in 1985 (1). The addition of "B19 "to the name was to prevent any confusion with human papillomavirus. The B19 strain is only known to infect humans but other parvovirus strains can infect non-human species (2). Understanding parvovirus B19, its different clinical manifestations, epidemiology and pathogenesis is necessary for the application of diagnostic tools and for the interpretation of the results.

Biological properties

Parvoviruses are non-enveloped, 18 to 26 nm icosahedral viruses. The B19 genome is a single 5.5kb strand of DNA that encodes one major non-structural protein, two capsid proteins (VP1and VP2) and several other small peptides. The 3' end of the genome codes for the structural proteins VP1 (84Da) and VP2 (58kDa) that are in the same reading frame. The entire nucleotide sequence coding for VP2 is contained within that of VP1 that has an additional unique portion of 227 amino acids. The capsid is composed mainly of VP2 with only 5% or less VP1. The 5' end of the genome codes for the non-structural phosphoproteins NS1 and NS2. NS1 is required for parvoviral replication. Elimination of the 5' end of the genome encoding NS1 causes the remaining 3' end encoding VP1 and VP2 to produce empty capsids. This characteristic is being utilised in the development of a vaccine.

The virus binds to the P antigen or globoside in the human P blood group-system. P antigen is known to be abundantly expressed on erythroid cells (erythrocytes, erythroblasts, megakaryocytes, endothelial cells, placental cells, and foetal liver and heart cells) and is reported to be the cellular receptor for parvovirus B19. P antigen is necessary for parvovirus B19 entry into cells (3). Rare individuals who lack the P antigen are resistant to infection by parvovirus B19 (2).

Parvovirus B19 is a very resistant virus, tolerating pH as low as 3.5 and resisting denaturation at 56(C for one hour. The virus can however be inactivated by formalin, (-propiolactone and oxidising agents. Gamma irradiation will also inactivate B19 with 1.4 mR producing a 10-log10 reduction in infectivity (1).

Epidemiology

Parvovirus B19 has a worldwide distribution. Epidemics occur every three to four years in temperate climates, usually in late winter, spring and early summer. By age 15 approximately 50% of children are seropositive, with the rate steadily increasing to approximately 90% in the elderly (1,2,4-6). Of particular risk of infection are population groups involved in the care of children, such as teachers, day care workers and caregivers.

Routes of transmission

Parvovirus B19 virus replicates in the upper respiratory tract then spreads through the blood stream to the bone marrow where it targets early erythroid progenitor cells. The narrow cell specificity is determined by the receptor globoside (blood group P antigen) found predominantly on erythroblasts and megakaryocytes.

The virus causes a lytic infection resulting in an abrupt cessation of erythropoiesis. This resolves spontaneously in about one week as antibody production begins (1,6,7). Although transmission is via the respiratory route, the virus can be found in serum and can therefore also be transmitted by blood and blood products. Because of its heat resistant properties, parvovirus B19 has been transmitted by steam or dry heated factor VIII (1,5).

Vertical transmission from mother to foetus during the viraemic phase of the disease is also recognised as a potential hazard to the foetus (5). The effect on the foetus may be severe due to high red blood cell turnover and a deficient immune response. Virus particles have been detected in the foetal liver, thymus and myocardium (1).

Clinical manifestations

The clinical manifestations of parvovirus B19 are largely dependent on the immunologic and haemotologic status of the host. Asymptomatic infections occur in approximately 25% of adults with clinical presentations ranging from acute self-limiting disease to chronic illness (1). The most serious illnesses are during pregnancy, in patients with underlying haematological disease (e.g. sickle cell anaemia, hereditary spherocytosis, thalassemia or chronic haemolytic disease), and immunocompromised patients such as organ transplant recipients and patients with congenital or acquired immunodeficiency (1,2,4-8). Parvovirus B19 causes several well-defined clinical syndromes, namely erythema infectiosum, polyarthropathy syndrome, infection during pregnancy, and transient aplastic crisis. Other manifestations, such as thrombocytopenia, neurological disease, myocarditis, and paroxysmal cold haemoglobinuria, have also been reported (1).

In general treatment for parvovirus B19 infection is symptomatic, and supportive and dependent on the clinical presentation of the patient (6). Immunoglobulin from normal donors can be used in persistent parvovirus B19 infection in immunosuppressed patients (1).

Erythema infectiosum

Erythema infectiosum, or "Fifth disease", is a viral exanthem first described by Robert Willan in 1799, and subsequently named erythema infectiosum in 1899. Erythema infectiosum was finally linked to parvovirus B 19 in 1983 when anti B 19 specific IgM was detected by radioimmunoassay in the sera of 31 children with fifth disease (1).

Erythema infectiosum is the most common clinical manifestation of parvovirus B19; although up to 50% of infected children and adults have sub clinical or asymptomatic infection (2,5). During natural infection the incubation period is approximately 6 to 18 days. The prodromal illness lasts several days and is characterised by symptoms such as headache, myalgia, malaise, respiratory symptoms and sometimes nausea, vomiting and abdominal pain. The classic "slapped-cheek" rash appears two to five days later, after the disappearance of the virus from the circulation. This rash appears as a fiery red eruption on the cheeks, accompanied by relative circumoral pallor. The rash then spreads to the trunk and limbs fading from its erythematous maculopapular exanthemum, to its typically lacy appearance and subsiding over a 1 to 2 week period. Viral exanthems are common in childhood and prompt recognition of parvovirus infection in children is important if the primary caregiver is pregnant (9).

Polyarthropathy syndrome

Arthralgia is present in approximately 10% of children with B 19 infection. Adults, especially women, have a more severe second stage where the rash is more subtle or absent and the joint swelling and arthralgia more prominent. Joint involvement is usually symmetric and polyarticular, with the proximal interphalangeal and metacarpophalangeal joints most commonly affected. Duration of these symptoms is about 1 to 3 weeks, however 20 % of affected women may have persistent joint symptoms for 2 to 3 months, even up to 2 years (1,4,6). A higher proportion of children with juvenile rheumatoid arthritis have evidence of parvovirus B19 infection than healthy children (10).

In the acute stage parvovirus B19 can mimic rheumatoid arthritis and this infection should always be considered as part of the differential diagnosis of patients presenting with acute arthritis. Parvovirus B 19 is not associated with joint destruction and does not require the immunosuppressive therapy prescribed for rheumatoid arthritis.

Infection during pregnancy

Parvovirus B19 may be transmitted to the foetus from the mother during the viraemic phase of the disease, causing anaemia, hydrops foetalis, spontaneous abortion or stillbirth. About 4% of non-immune women acquire the disease during pregnancy, of which 30% will transmit the virus to the foetus. Of the pregnant women who transmit the virus to the foetus only 5 to 10% of infections will result in foetal damage (11-14). The majority of infections during pregnancy have favourable outcomes.

The time of greatest danger to the foetus is in the first 20 weeks of pregnancy where the overall risk of foetal loss, after maternal exposure is 3.4 to 9% (2,4,5,13). The risk of an adverse outcome of pregnancy to women infected after 20 weeks is reduced. Around 1 in 10 women infected before 20 weeks gestation will suffer a foetal loss due to B19 infection (11). The interval between onset of maternal infection and diagnosis of hydrops foetalis ranges from 2 to 17 weeks. Spontaneous reversal of foetal hydrops is quite common. If the foetus survives foetal hydrops, the prognosis is good with usually no long-term sequlae. Infection often occurs without any adverse effect on the foetus and no evidence of increased risk of congenital abnormality over that of the general population (2,5,11,12).

Parvovirus should be part of the differential diagnosis of non-immune hydrops foetalis (NIHF), as 8 to 10 % of cases of NIHF are caused by parvovirus (2). Foetal transfusion may be life saving but the hydrops may spontaneously resolve without treatment. No definitive guidelines have been established for determining which foetuses require transfusion, as controversy still exists regarding the management of the foetus. Because of the risk associated with intrauterine transfusion it should be considered only in cases with severe foetal anaemia or signs of foetal compromise (2). Infection by parvovirus B19 has recently been proposed as a causative factor for congenital red blood cell aplasia (2,5).

Transient aplastic crisis (TAC)

Transient aplastic crisis (TAC) is the abrupt onset of severe anaemia with absent reticulocytes. Aplastic crisis is a serious occasionally fatal consequence of parvovirus B19 infection and may occur in individuals with underlying haemolytic disease as a predisposing factor, and may be the first presentation of this disease. TAC may occur in either the viraemic phase or the secondary symptomatic stage of the infection. Haematologically competent patients show a varying degree of neutropenia and thrombocytopenia. Infection, however, in immunocompromised individuals may result in chronic disease (15-19).

Patients with aplastic crisis or persistent infection should be considered infectious (5). Although viraemia is rare (an incidence of 1 in 1,000 cases), infection may also be transmitted via blood and blood products (20).

Diagnostic tools

Diagnosis of parvovirus B19 infections is dependent on the time of infection and the type of samples available. Serological assays to detect antibodies are routinely used to diagnose recent B19 infections and to determine immune status. However, other diagnostic tools are also available, these can be divided into detection of the virus and serology.

Detection of the virus

Electron microscopy

Parvovirus B19 can be identified using standard negative staining electron microscopy methods. Identification is by direct visualisation of viral particles in infected tissues or serum (2). Electron dense material accumulates around the viral particles giving a negative contrast image.

Specific viral identification may be confirmed using immunoaggregation with poly-or monoclonal parvovirus B19 antibodies (immune electron microscopy). The absence of excess endogenous parvovirus B19 antibody is a prerequisite of successful immune electron microscopy. This method is most suitable for non-serum body fluids or tissues as the parvovirus B19 antibody is not present in these samples in high titers (6).

Combined pseudoreplica immunochemical staining is a useful alternative method as virus can be detected in various body fluids even in the presence of endogenous parvovirus B 19 antibody (6).

These methods, although highly specific, are not suitable for routine diagnosis as they require special equipment and experienced examiners (6).

Viral culture

Parvovirus is difficult to culture in vitro and does not grow in standard cell cultures used by diagnostic laboratories. Primary cell cultures of erythroid precursors derived from human foetal liver and

bone marrow or human erythroid leukaemic cell lines have been successfully used although the viral yield tends to be low (6). Parvovirus B19 has only been propagated in four cell lines; megakaryocytoblastoid cell lines UT7 and MB02, the erythroleukemic cell line JK1, and more recently in the erythroid cell line KU812Ep6 (21). Because of the difficulty in growing parvovirus, this is not an option for the routine diagnosis of parvovirus B19 infection (5).

Detection of parvovirus B19 DNA

As parvovirus B19 can only be cultured by specialist tissue culture, presence of the virus can be demonstrated by the detection of viral DNA using nucleic acid detection methods. Viraemia can be detected using nucleic acid hybridisation assays, but in immunocompetent individuals B19 DNA is only detectable for 2 to 4 days (1).

Synthetic oligonucleotide probes have been used in In Situ Hybridisation Assay (ISH). ISH has been used for the detection of parvovirus B19 DNA in bone marrow cells, foetal cells and tissues (5,6,22).

Viral DNA is detectable by PCR for several weeks or months following infection and is a particularly useful test for diagnosing foetal infection, and infection in the immunocompromised patient (8,13-16,19,22-26). By the time hydrops is diagnosed in foetal B19 infection, IgM antibodies may no longer be detectable in the mother's serum. Consequently PCR testing of amniotic fluid for viral DNA is more relevant (14).

The most sensitive diagnostic tool for detecting parvovirus B19 DNA is polymerase chain reaction (PCR) with or without subsequent southern- blot hybridisation (14). These tests also have the ability to detect B19 virus DNA in different clinical specimens. The human parvovirus genome consists of a single strand of DNA of approximately 5500 nucleotides with overlapping reading frames to encode its non-structural proteins (NSP) and two capsid proteins VP1 and VP2. Various primers directed against oligonucleotide sequences in both the non-structural and the viral capsid proteins have been used (6,13-14, 23-25,27). Samples that can be used for PCR are amniotic fluid, foetal blood, ascitic fluid, foetal biopsies, and placental tissues, with amniotic fluid being the most reliable specimen. Post mortem foetal heart, lung, and liver can also be used. For cellular or tissue samples, sample lysis and/or DNA purification is required. Serum has also been used with success in cases of chronic arthritis of recent onset and hydrops foetalis. Viral DNA is detected using either single step or nested PCR (5,16). The presence of inhibitors to Taq polymerase in clinical samples may affect the sensitivity requiring a second round of amplification or nested PCR (6).

Serology

Serology is the mainstay of diagnosis of parvovirus B19 infections. Parvovirus B19 IgM appears approximately 7 to 14 days post infection and is detectable in 90% cases by the 3rd day of the aplastic crisis, or at the time of rash in erythema infectiosum. IgM remains detectable for 2 to 3 months following infection, but may remain detectable for up to 9 months. IgG antibodies appear about 2 weeks post infection and persist for life (1,4-5,7).

During an acute parvovirus B19 infection antibodies are produced against the viral capsid proteins VP1 and VP2 (1,4,28). Both linear and conformational epitopes are produced. Although the early antibody response is to the major capsid protein VP2 and then to the minor capsid VP1, it is the immune response to the VP1 capsid protein that provides protective immunity. IgG antibodies to linear epitopes of VP2 disappear approximately 6 months after infection leaving only those antibodies that recognise undenatured VP2 (28-30).

Individuals with chronic parvovirus infection typically only produce antibody to VP2 (1,6). This lack of antibodies to VP1 is demonstrated in vitro by the serum's inability to neutralise parvovirus B19. There is no difference between antibody response in sub clinical and clinical infections.

Demonstration of an immune response to non-structural protein 1 (NS1) has been documented. IgG antibodies against NS1 may have utility as an indicator of chronic persistent forms of parvovirus B19 infection (28). NS1 antibodies may take (6 weeks to develop and the significance of the NZ1 antibody response, although obscure, may be of limited value in the diagnosis of chronic parvovirus B19 infection (31).

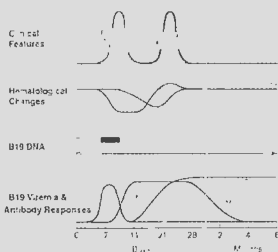

Figure 1. Diagram of clinical and virological events following B19 virus infection (from ref. 5).

Current serological detection of both parvovirus B19 IgM and IgG is by enzyme immunoassay (EIA), although radioimmunoassay has also been used (32). Serum is the sample of choice. Saliva has also been successfully used for IgM determination and is of particular value in children because of the ease of which samples can be obtained (11,33).

Many commercial EIA assays are available with varying degrees of sensitivity and specificity (34-39). The source and nature of the viral antigens used are important variables to consider when evaluating an assay's performance (28). The use of recombinant antigen-based enzyme immunoassay has increased the sensitivity and specificity of the assays. Sensitivities to recombinant antigen based assays have been reported as 97% for IgM and 88% for IgG with specificities of 91 to 93% for the IgM assay and 94 to 98% for the IgG (34-35,37,39). This is a great improvement on earlier reported assays that have much lower sensitivities and specificities (34).

Capture EIA's, using native or recombinant antigens, are the best choice of assay, as they will detect IgM that reacts only with denatured VP1 or VP2 (28,30). There is also the requirement for at least one denatured antigen in the immunological detection of parvovirus B19 IgG (40).

The IgM capture assays reduce interference by rheumatoid factors by coating the solid phase with anti human IgM. On addition of the serum sample the anti- human IgM captures IgM. Antigen is added after excess serum is removed by washing. The parvovirus B19 antigen binds to the specific IgM. High specificity is obtained by the use of specific monoclonal antibody and baculovirus expressed B19 protein antigens that have been shown to be the most suitable antigens for detecting anti B19 IgM and B19 IgG (28,30,40). IgG is also detected by enzyme immunoassay, but in this case the solid phase is coated with the human parvovirus B19 recombinant antigen.

Diagnostic tests for specific IgM are not reliably detectable in the foetus as IgM antibodies only appear in the circulation after 22 weeks gestation. Serum should be collected from the mother and tested for both parvovirus B19 IgG and IgM, preferably in parallel with the original antenatal booking blood.

Demonstration of either a seroconversion from negative IgG and IgM to positive in both tests or an increase in the level of IgG present is indicative of recent infection. While the presence of parvovirus B19 IgM and IgG indicates a recent or current infection, the presence of parvovirus B19 IgG in the absence of IgM indicates immunity from past exposure. The presence of IgM without IgG should always be confirmed, either with a repeat serum sample to detect the IgG seroconversion or with DNA detection using PCR. Positive IgM, negative IgG serology may indicate either the sample was collected in the early stage of the infection (refer to Figure 1), or a non-specific IgM reaction caused by simultaneous IgM reactivity (38).

Simultaneous IgM reactivity of 4.65% against more than one virus has been reported (7,37). A positive parvovirus B19 IgG allows avidity testing to establish the causative virus. The viruses commonly implicated in simultaneous IgM reactivity are Epstein Barr virus, rubella, cytomegalovirus, human parvovirus B19, and measles infection (38).

Why should an individual produce IgM to more than one virus simultaneously? Thomas and colleagues demonstrated the need for T cell cross reactivity to cope with the large number of foreign epitopes encountered throughout ones life (41). This cross-reactivity in the early stages of a primary infection is not unexpected, as natural antibody will have a high degree of cross-reactivity due to low resolution of epitopes. The T-cell-independent IgM (TI) isotype response mounted early in infection (primary response) will exhibit some degree of cross-reactivity as a result of its still multivalent activity whereas the latter response is the high affinity T-cell-dependant IgG (TD) isotype. The TD response is a memory response (41).

Avidity tests have been introduced to discriminate between recently acquired and distant infection. Antibody production follows a characteristic pattern. Avidity assays make use of the gradually increasing binding force of IgG as the antibody response matures, permitting accurate timing of primary infection (42). Serological determination of the presence of antibody is dependent upon the formation of antigen antibody complexes and subsequent demonstration of their presence.

Avidity is the functional combining strength of an antibody with its antigen and is related to the affinity of the reaction and the valencies of the antibody and antigen (42-43). Addition of a protein denaturant such as urea, which disrupts the antigen-antibody link, will have little effect on high avidity antibody but great effect on weak avidity. The results of avidity tests are reported as either high or low. A high avidity index allows recent infection to be excluded. Low avidity indicates probable recent infection, however, low avidity in the immunocompromised should be interpreted with caution.

The differential assay of high avidity and low avidity IgG antibodies can be used to complement the IgM assay. If the presence of specific IgM is used as the criterion for parvovirus B 19 diagnosis, the positive predictive value is 98% (42). Avidity is a useful diagnostic tool for the assessment of the time of infection. This has particular importance for pregnant women in the first trimester for whom the time of infection may determine the management of their pregnancy. The presence of high avidity antibodies later in pregnancy does not exclude an infection early in pregnancy.

Differential diagnosis has implications especially for the pregnant woman, where the hydrops or stillbirth may have another aetiology and correct diagnosis is important for correct management. It is also important in cases of aplastic anaemia and immunocompromised patients where the correct diagnosis results in the appropriate treatment of the patient. Correct identification of the causative agent is also important in epidemiological studies. Positive results for parvovirus specific IgM therefore should be confirmed, particularly in pregnancy or in the absence of specific IgG antibody, by either a repeat blood sample or by PCR.

Selection of the most appropriate method for diagnosis relies on a good relationship between laboratory and clinician. Because of the diverse range of clinical conditions caused by parvovirus B19 each clinical presentation must be assessed separately for the correct selection of diagnostic tools.

Use of the diagnostic tools in the different clinical situations

Pregnancy

It is important to determine the immune status of a pregnant woman when exposure or infection is suspected. Serum testing at the time of exposure for parvovirus B19 IgG may allay many fears as between 50 and 70% of women in childbearing age are immune to parvovirus B19. Where foetal infection is suspected both IgG and IgM should be tested, preferably in parallel with an earlier antenatal sample allowing detection of any seroconversion.

The use of serum IgG avidity testing in conjunction with parvovirus B19 IgM detection may provide a less invasive alternative to obtaining amniotic fluid for PCR analysis.

Maternal serology in the third trimester may be a less sensitive determinant in suspected parvovirus B19 foetal death, as the IgM response may only last 2-4 months and the death could be due to longstanding foetal infection. A lower rate of foetal hydrops occurs in the third trimester due to the more mature immune response in the older foetuses. In this setting diagnostic procedure could be improved by the addition of PCR analysis for parvovirus B 19 infection in foetal and placental tissue, particularly in the case of third trimester intrauterine death where parvovirus B19 aetiology may be more common than previously reported (13,24).

Erythema infectiosum and polyarthropathy

Parvovirus B19 associated erythematous rash and arthritis occur in the second phase of illness at the same time as the development of parvovirus B19 specific IgG and are thought to be immune mediated. Positive IgM results with a negative IgG are uncommon and should be further investigated.

Human parvovirus B19 may persist in bone marrow and synovial tissues of patients with arthritis of unknown origin (27). In these cases nested PCR has been used to identify parvovirus B19 DNA from bone marrow and less successfully from synovial membrane.

Immunocompromised

In immunocompromised patients PCR is the method of choice as the onset of symptoms may not coincide with positive serology (7,8). The failure to produce neutralising antibodies to parvovirus B19 following infection in immunodeficient patients may result in persistent viraemia and chronic Pure Red Cell Aplasia (PRCA). Diagnosis can be established when the following criteria are met:

- bone marrow biopsy showing PRCA
- serum or bone marrow positivity for B19 DNA by PCR or dot -blot hybridisation
- no other explanation for the PRCA.

Serology is not useful as patients often lack both parvovirus B19 IgG and IgM (15). In HIV-infected individuals parvovirus B19 should be included in the differential diagnosis of chronic anaemia (16,17). Parvovirus has also been documented as the aetiological agent in organ transplant recipients (18,19).

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Drugs of abuse testing

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Abstract

For many years a wide range of drug tests have been performed by medical laboratories, including the investigation of drug overdoses, detection of illicit drugs and toxic chemicals, and the monitoring of therapeutic drugs. However with an increased awareness of social issues such as drug rape, workplace drug testing and schoolyard drug usage, medical laboratories are becoming more involved in testing for illegal drugs. The procedures for handling of these samples and funding of these tests are frequently confused. This paper describes the processes in place at LabPLUS, Auckland City Hospital.

Introduction

Traditionally, drug testing has been funded via bulk funding of clinical laboratories. However with many labs now operating on a fee for service basis, a re-examination of test costs and service billing has highlighted anomalies such as drug testing. The Community Referred Laboratory Contract clearly indicates the eligibility of patients who will be funded by the state as well as which tests are claimable by community laboratories. The non-claimable tests (non-schedule) are referred to several specialist laboratories on a regional basis. The referred tests are usually reported directly back to the requesting practitioners or via the referring community laboratories. The two broad categories used at LabPLUS are Clinical and Non-clinical drug testing.

Drug testing for clinical purposes

Clinical requests are those for medical or investigative reasons such as suspected overdose, abuse of illicit drugs, detoxification management, drug rape, suspected spiked drinks, poisoning, investigation of unusual behaviour or therapeutic drug monitoring. All these requests are non-evidential, non-legal and are within the payment criteria for primary referral testing (i.e. are government funded) or service billed if requested within a Hospital. They do not need proof of identity although medical ethics require that patient consent and full disclosure are part of the consultation process. The sample requirements are varied and exact information needs to be published by the laboratory. For example, investigation of spiked drinks/drug rape requires if possible 30 mL of urine and should be collected as soon as possible and no later than 8-12 hours after the event, because of the rapid clearance of GHB (gamma hydroxyl butyrate) by the body.

Non-clinical drug testing

Non-clinical drug testing requests are those required for legal or evidential reasons. These include local and overseas pre-employment, occupational health screening, workplace accident investigation, visa applications, family court requests, probationary service requests, drug rehabilitation programmes and school drug programmes. All these requests may have evidential or legal implications and therefore need proof of identity, supervision of sample collection to prevent adulteration or sample substitution, sample chain-of-custody documentation from client to laboratory and in some cases, confirmation analysis by Gas Chromatography Mass Spectrometry (GC-MS). In addition, court testimony by a laboratory expert such as a toxicologist may be required.

The funding of these tests is not part of the Ministry of Health's primary referral contract for laboratory testing. The cost is the responsibility of the client, company or agency making the request. Testing of these samples by a laboratory and charging to the Ministry of Health is illegal and has significant implications for the laboratory concerned. Repercussions will occur on both the practitioner and laboratory if erroneous results are released because of non chain-of-custody collection. For these reasons, LabPLUS, Auckland Hospital will refuse to analyse samples sent via the community laboratories for non-clinical testing if incorrectly collected or not paid for. It is important that all referring practitioners understand the difference and instruct their clients accordingly.

Custody and control documentation for evidential (non-clinical) drug analysis

"Chain of custody" is a legal term, which refers to the ability to trace a sample from time of collection, through transportation and testing to the final reporting of the results. The process involved at LabPLUS includes either an observed or supervised collection of the urine, clear documentation and security of the sample from the collection point to the toxicology laboratory. It is essential that the accompanying documentation is completed correctly as any departure from the procedure may invalidate the legality of the reported results.

LabPLUS specimen collection kits for evidential (non-clinical) drug analysis

LabPLUS supplies a kit for collection of urine samples and associated documentation for testing for drugs of abuse. The kit is sealed with an integrity seal and should the seal be broken, the kit should not be used.

The kit contains:

- A security bag.
- A set of three security seals A, B and C all bearing the same ID number.
- 2 specimen bottles for primary and reference specimens.
- Pressure bag and absorbent pad.
- Pressure bag clamp.
- Collection instructions.

Sealed beakers with a temperature strip are available for both male and female clients which are supplied separately to the collection kits.

1. 2 x specimen bottles for samples A and B
2. Pressure band and absorbent pad
3. Security Bag
4. Pressure bag clamp
5. A set of three security seals A, B, and C all bearing the same ID number

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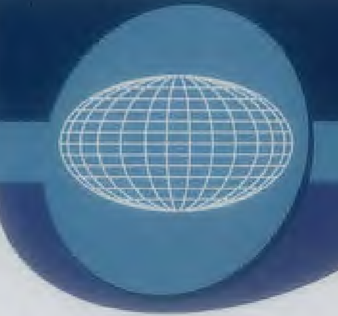

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Abstracts by New Zealand presenters at the South Pacific Congress, Brisbane, 2003

Dendritic cell immunotherapy for advanced low grade B cell lymphoproliferative diseases

D Ritchie, J Carter, M Findlay, P Dady, F Ronchese
Malaghan Research Institute, Wellington

Low grade B cell lymphoma remains incurable by conventional therapies. The median survival for patients with these conditions is approximately 6 years from diagnosis. We undertook a phase I/II study of dendritic cell (DC) immunotherapy in 15 patients with advanced B cell lymphoproliferative diseases.

Utilizing monocyte derived DC pulsed with keyhole limpet haemocyanin (KLH) and MHC class-I bound peptide eluted from autologous tumour biopsies we generated an autologous anti-tumour vaccine. Four doses of between 1 and 30 x106 DC were administered fortnightly. Immunological responses were monitored by a combination of delayed type hypersensitivity reaction and in vitro measurements of T cell proliferation to eluted peptide or KLH. Clinical responses were measured by serial clinical review and computed tomography. No severe adverse events were observed. DC yields were significantly lower in patients with advanced chronic lymphocytic leukaemia (CLL) consistent with heavy contamination of the cell isolates with tumor. Clinical responses were observed in two patients. These two patients also experienced positive DTH responses. No correlation was observed between clinical responses and immunologic responses.

We conclude that DC based immunotherapy is feasible and may be effective in selected patients. Patients with advanced CLL fail to provide sufficient DC from culture in order to benefit from the approach presented here and may require in vivo or ex vivo B cell purging to ensure production of sufficient DC numbers.

What can you do when troponins don't match?

M Reed
Southern Community Laboratories, Hawkes Bay

Cardiac troponin T (cTNT) and cardiac troponin I (cTNI) are currently the most sensitive and specific laboratory markers of myocardial injury. However, these immunoassays are susceptible to interferences from heterophile antibodies and/or human anti-animal antibodies (HAAA). We present a case of discordant troponins in a 70 year old male with a history of cardiac ischemia and multiple coronary artery stents.

An incidental finding of AST elevation in a haemolysis free specimen from an asymptomatic patient led to the discovery of concurrent elevation of CK, LDH and TNT. The patient was referred to the Emergency Department of the local hospital, where the CK and LDH elevations were confirmed, but the TNI was negative. The patient was discharged, and the TNT elevation dismissed as laboratory error.

Over the ensuing 4 months, the patient demonstrated sustained elevations of AST, CK, LDH, TNT and CK-MB mass in conjunction with elevation of NT pro-BNP and myoglobin, but at no time was an elevated TNI detected. Repeat samples, a variety of TNI assays, Polyethylene glycol (PEG) precipitation, Heterophile Blocking Tube (HBT) usage, immunofixation, and dilution have been utilised in an attempt to clarify this anomaly. Immunostaining for abnormal troponin forms is also being performed.

The importance of communication between clinicians, laboratories and clinical diagnostic suppliers in the endeavour to identify potential causes of discordant results is demonstrated by this case. Possible steps that may be undertaken when investigating possible heterophile or HAAA interferences are discussed.

Recombinant factor VIIa and the thrombelastography - a haemophilac case study

G Devenie, P Harper, L Young, D Lim, L Pitcher
LabPlus, Auckland Hospital, Auckland

Objectives: To investigate the effect of recombinant factor VIIa (rVIIa) therapy using the Haemoscope Thrombelastograph (TEG) analyser, in a child who has severe haemophilia, with inhibitors to factor VIII.

Findings: The baseline r time for the patient was 118 minutes - (normal = 5-12min). 30 minutes after rVIIa the r time was 19 minutes. This is not normal but a significant improvement on baseline. Subsequent samples showed that the r time progressively increases over time after the rVIIa dose. The recommended treatment guidelines is that rVIIa is given 2 hourly at 90u/kg. Our results showed that reducing this dose kept the TEG tracing close to the 30 minutes post recommended dose trace.

Conclusion: The results in this patient have highlighted uncertainties over the relationship between rVIIa dose, TEG tracings and the patient's haemostatic response. Understanding the TEG tracings in relation to this treatment will be of great benefit.

Keywords: Thrombelastograph, haemophilia, Recombinant factor VIIa

The tattoo that turned nasty

Ioana Ioane, L Palmer, L Glogoski
Middlemore Hospital, Auckland

"The Tattoo That Turned Nasty" is a case study about a Samoan male who died after contracting necrotising fasciitis while having a series of traditional tattoos done. The presentation will give us a basic look at necrotising fasciitis, its clinical details, management and laboratory findings. We will also look at the causes and what can be done to prevent catching such a vicious disease. This case study will show how the different departments in the laboratory can be involved in such a short but dramatic case. Laboratory findings for the time period from admission to death will be reported for this patient. We will also review the custom of Samoan tattooing and the reason why it is still a strong tradition within the Samoan community.

Necrotising fasciitis is also known as soft tissue gangrene, streptococcal gangrene or the more popular name, the flesh eating disease. It is a bacterial infection that can cause a series of shutdowns throughout the body, for example, multiorgan failure and dangerously low blood pressure. It is caused by the spread of a number of different bacteria but the leading cause is known to be Group A Streptococcus. There is no vaccine but if caught early (before the body goes into toxic shock) there is a very good chance that the combination of antibiotics and surgery will have a positive effect. The Coroner's report will be presented with his recommendations of changes to tattoo laws and how these have actually changed the tattoo by-laws in New Zealand.

Bedding endotoxin levels are inversely related to disease severity in children with atopic eczema

R Siebers, R Kelly, D Sistek, P Fitzharris, T Stanley, J Crane
Wellington Asthma Research Group, Wellington School Of Medicine & Health Sciences, Wellington

Objective: Recent studies suggest that early life exposure to high levels of endotoxin from Gram negative bacteria might protect against the development of atopic diseases. Less is known about current endotoxin exposure and atopic eczema. The objective of the study was to determine whether current endotoxin or house dust mite allergen exposure influences eczema severity in atopic children.

Findings: We studied 36 children (mean age: 4.3yr, range: 1-10) with atopic eczema. Eczema severity was assessed by the SCORAD index. Mean SCORAD index was 32.4 (95% CI: 27.1-37.7). Bedding dust (1m2 for 2min) was analysed for endotoxin using a chromogenic kinetic limulus amoebocyte lysate test and for house dust mite allergen (Der p 1) by ELISA. Geometric mean endotoxin and Der p 1 levels from bedding were 4,677EU/g (95% CI: 3,206-6,839) and 2.45μg/g (95% CI: 0.83-7.26) respectively. The SCORAD index was inversely related to bedding endotoxin concentration (EU/g); r= -0.34, p= 0.044. This inverse relation was not affected by occlusive bedding cover use (n=8), or by positive skin prick to house dust mites (n=30). When expressed per unit area (1m2) the negative correlation was similar (r= -0.29) but not statistically significant (p= 0.083). Der p 1 levels, expressed as μg/g or μg/m2, were not related to the SCORAD index (r= 0.05 and 0.10 respectively).

Conclusions: This study suggests that higher bedding endotoxin levels are associated with decreased severity of eczema in atopic children. The explanation, immunological or otherwise, requires further examination.

Setting quality criteria for internal quality control

D Mikkelson
LabPlus, Auckland District Health Board, Auckland

The investment in quality in the modern medical laboratory consumes 20% plus of the total cost budget. Return on this investment requires robust processes to ensure that valuable quality data is not overlooked and the appropriate level of investigation is implemented in cases where quality criteria are not met. Setting quality control criteria in most laboratories is based on statistical methods that assume data is distributed in a Gaussian fashion. Ninety five % confidence limits are used as accept reject criteria and this is assumed to provide an appropriate level of quality for patient test results.

The above approach ignores the true driver for analytical quality in a medical laboratory, namely clinical usefulness. Criteria for clinical usefulness may vary depending on the clinical setting. Guidelines are available from many sources including published works, external QA data and local opinion. Procedures that are followed when QC data exceeds targets have varying statistical power and their effectiveness depends on policies and procedures implemented in your laboratory. Your procedures should be able to be followed by staff in stressful situations in reduced staffing times with appropriate confidence in detecting real errors and troubleshooting those observations effectively. Statistical laws are working against successful detection of quite major errors thus multiple reanalyses of material is required as first line follow up to increase the probability of successful true error detection.

Advances in electron microscopy referrals

S Cooke
LabPlus, Auckland Hospital, Auckland

Objective: To provide a fast and accurate method for data transfer of electron micrographs using internet technology. This involves the digital transfer of high resolution electron microscopy images for consultant referral and confirmation of pathological diagnosis.

Statement of findings: Electron microscopy images are placed on the LabPlus website by technical staff in co-operation with the website facilitators. By using the LabPlus website EM images can be displayed by a secure site to pathologists accessing the internet in any part of the world.

Key conclusions: LabPlus, Auckland Hospital, has developed an innovative tool using the best of internet technology to provide a service to our independent external pathologists. One advantage of this system is that the images can be on the website within an hour and can be discussed with pathologists in our lab either by phone or email the same day. Another advantage is that it gives easy access to a pool of consultative knowledge from around the world. Sampling errors are minimised by capturing images in conjunction with an experienced pathologist. Although the project is in its early stages it has been beneficial to our service and has given scope to future investigations with a view to enhancing what we can offer by way of adding written comments and reports, feedback forms for further imaging and investigations and also 3D enhancements.

An alternative freezing technique for muscle biopsies

Marney Lyons
LabPlus, Auckland Hospital, Auckland

Objective: To demonstrate an alternative freezing technique for muscle biopsies.

Statement of findings: Traditionally muscle biopsies are frozen in a bath of Isopentane cooled by liquid nitrogen. This alternative technique utilises a Hexane bath and dry ice and is performed in a biohazard cabinet. Hexane is chilled by the addition of 1.0cm cubes of dry ice. The hexane takes approximately 3 minutes to cool. It is only ready when ice lumps bubble slowly and a coating of frost is seen on the surface of the beaker that the mixture is ready for freezing. The muscle biopsy, no larger than 5x3x3mm is placed on to a clear surface e.g. parafilm. A cut should be made in the muscle biopsy at 90 degrees to the long axis of the muscle fibres. The muscle biopsy is frozen in the Hexane mixture and the forceps and nunc tube are also frozen in the same manner. The biopsy is then placed into the nunc tube which is then placed into the cryostat ready for sectioning. At all times equipment and materials are kept at the same temperature as the muscle biopsy i.e. frozen forceps and the use of a foam pad to insulate the Hexane bath from the stainless steel work surface. A 'chuck' is prepared in a cryostat by applying OCT to its surface. When the OCT at the base of the chuck has become opaque, the frozen muscle biopsy is then embedded on its end using frozen forceps. Frozen sections can then be cut and stained with a histochemistry panel. This method has consistently produced good results.

Key conclusions: This alternative freezing technique has the advantage of eliminating liquid nitrogen which is difficult to store as well as dangerous to use. The disadvantage of this technique is that Hexane is highly flammable and harmful by inhalation, ingestion or skin absorption and therefore must be handled in a fume cupboard and gloves must be worn.

Variations in silver-stained nucleolar organiser regions (AgNors) in neoplastic tissues as visualised by the scanning electron microscope (back-scattered mode)

J G McDermott

LabPlus, Auckland Hospital, Auckland

Objective: To observe variations in silver-stained nucleolar organiser regions (AgNORs) in neoplastic tissue using the scanning electron microscope (back-scattered mode).

Statement of findings: Nucleolar Organiser Regions (NORS) are loops of ribosomal DNA which contain the genes that transcribe to ribosomal RNA. They are situated on the short arm of the acrocentric human chromosomes 13, 14, 15, 21 and 22. By using the light microscope and silver-staining AgNOR technique, several of their associated nucleolar organiser region proteins, including nucleolin (C23) and numatrin (B23), can be visualised as tiny black dots contained within the nucleus. Colonic samples from archival material and controls taken from mid portion of jejunum of mouse were prepared for scanning electron microscope examination (back-scattered mode). Control samples were regular in structure, appeared uniform with smooth edges. In the malignant tumour (adenocarcinoma) samples, rapidly proliferating cells exhibited AgNORs with a bizarre three-dimensional structure resembling icebergs.

Key conclusions: The variations in AgNORs in malignant tumours may be because the NOR associated proteins are being produced at an abnormally rapid rate. Because of this the NOR associated proteins may be incorrectly formed structurally, present in the incorrect amount or possibly in an incorrect ratio to each other. This may lead to abnormal quantities of free carboxyl or sulphydryl groups and would explain the abnormal silver deposits (bizarre iceberg structures) observed in this study. The scanning electron microscope (back-scattered mode) has the potential of providing more accurate information on the tumour AgNOR. This may lead to additional prognostic data being made available to clinicians.

Salivary cortisol measurement using Roche E170

R Sargon

LabPlus, Auckland Hospital, Auckland

Salivary cortisol measurements offer distinct advantages over blood sampling: (a) sampling is non-invasive, acceptable in children and suitable for home or work environments, and (b) salivary cortisol reflects free cortisol, the active fraction. Bedtime salivary cortisol has been reported to be superior to 24h urine free cortisol, and equal to bedtime plasma cortisol measurement, as a screening test for Cushing's syndrome. Salivary cortisol is gaining in popularity for assessing stress responses. However, as the concentration of cortisol in saliva is only 2 to 5% of that in serum, many automated cortisol methods lack adequate sensitivity. Salivary cortisol was measured on the Roche E170 analyzer using Roche serum cortisol assay reagent. Samples were collected using salivettes. Cortisol stock solutions in ethanol were prepared from Sigma hydrocortisone powder and diluted into E170 diluent. The E170 measured cortisol in the range 2 to 40 nmol/L with essentially zero bias and with acceptable precision and linearity. Stock cortisol added to E170 diluent at a level of 10 nmol/L gave a recovery of 9.03 nmol/L (between batch CV 2.6%), at 20 nmol/L recovery was 19.22 nmol/L (between batch CV 3.0%) and at 40 nmol/L recovery was 40.16 nmol/L (between batch CV 1.7%). Mean bedtime salivary cortisol in 11 normal subjects was 5.15 nmol/L, with range 2.52 to 11.02 nmol/L, which is in agreement with published data. Serial measurements in a normal subject during the course of a day showed the expected diurnal rhythm in both saliva and serum cortisol. Salivary cortisol was elevated in a patient with Cushing's syndrome and showed a marked decrease with therapy. These results indicate that the Roche E170 cortisol assay can be used to measure salivary cortisol with clinically acceptable accuracy and precision.

Microbiology

Southland parasitology workshop

A Parasitology Workshop was held at HealthLab Kew,Southland Hospital on 15/16 August 2003. It was organised by a very dedicated small group of Microbiology staff at HealthLab Kew who decided that, because they didn't have much chance of all attending a workshop, they would organise their own! A total of 23 people attended with other participants coming from Otago Health Laboratory, Southern Community Laboratory, and MedLab Southland.

Graeme Paltridge from Canterbury Health Laboratories presented the workshop. Graeme is widely respected for his knowledge of parasitology. He is a contributor in the Parasitology section of the 8th edition of the 'Manual of Clinical Microbiology"

The workshop commenced on the Friday evening with pizza, registrations and a general catch-up gossip. Graeme then spent 2 hours going through the very extensive workbook he had produced, answering questions as he went. The workbook formed the base for the Saturday slide demonstrations.

The Saturday was a 'semi dry' workshop. Graeme had numerous slides prepared which he set up under the microscope linked to an overhead TV screen. This enabled everyone to see exactly the same thing and for Graeme to point out details (unfortunately our curtains didn't quite cut out all the sun!). The slides covered intestinal protozoa, helminths, malaria, and miscellaneous specimens. The overhead screen allowed for good group interaction and plenty of discussion.

Evaluation forms from participants gave excellent feedback and all seemed to find the workshop worthwhile. Requests for a follow-up workshop have been noted but I think we'll wait until we're in our new hospital and the venue is better! The day finished with an enjoyable meal out and then we adjourned to watch the Bledisloe Cup at the Otago Health staff motel.

Overall the workshop was a great success but it woudn't have been possible without Graeme's help, and the sponsorship we received from Global Science, Biolab, Ngaio Diagnostics Ltd, Fort Richard Laboratories Ltd, Kendra Laboratory Products, and Bristol-Myers Squibb.

Anne Buchanan, Charge medical laboratory scientists, Microbiology, Invercargill

Haematology

Special Interest Group

HSIG journal based learning - questionnaire

Moorthy VS, Good MF, Hill AVS. Malarial vaccine developments. *Lancet* 2004; 363: 150-6.

1. What are the three intermediate goals of vaccine research?
2. In what phase of the trials was Spf66 shown to lack efficacy?
3. How many merozoites per original sporozoite are released into hepatic venous circulation?
4. What is sub unit vaccination?
5. To increase antibody immunogenicity we need what?
6. "The newest generation of subunit vaccines are ____ _____"
7. ELISPOT assay is used to assess T-cell responses. **T/F**
8. Natural exposure to P falciparum in human hosts, gradually elicits what?
9. The aim with most vaccines is to induce antibody and T-cell responses to one or a few antigens. **T/F**
10. Name two pre-erythrocytic vaccines.
11. What is MSP-1?
12. Name the recombinant viral vaccine that encodes 7 antigens from various life-cycle stages.
13. A sexual stage vaccine would not protect vaccinated individuals from disease but would protect communities from infection. **T/F**
14. Approximately how many P falciparum antigens are there?
15. In the past five years the number of groups doing research has reached ______.

For a copy of this article please contact Virginia Nairn, Haematology, Diagnostic Medlab, Auckland. (09) 571 4072 or vnairn@dml.co.nz

Answers on page.....23

Internet sites for medical scientists

Another year has slipped by and a new one beginning. I hope 2004 will be a little less frantic than 2003, but already my senses tell me it 'aint going to be so. Here are some of the sites I have had the need to visit since my last web site page. I hope you will enjoy them.

This has some interesting e-books, e-journals and images.

www.dartmouth.edu/~biomed/

This is the Merck manual for diagnosis and therapy.

www.merck.com/pubs/mmanual/sections.htm

Nice anatomical pathology cases here.

www.path.upmc.edu/casemonth/cp-casemonth.html

This is a search engine for the site above. Allows cases to be accessed according to user preferences.

www.path.upmc.edu/cases/engine.html

An Internet pathology lab for medical education.

http://medlib.med.utah.edu/WebPath/webpath.html

For the uninitiated out there, the Ministry of Health website. Home for dozens of reports, tool kits etc.

www.moh.govt.nz

One issue that will have to be addressed by every laboratory seeking IANZ accreditation will be to determine and publish the uncertainty of measurement for tests and testing systems. Listed below are some sites for general terms and information and a NATA technical circulars on the subject.

http://www.nata.asn.au/fs_publications.htm

http://www.fasor.com/iso25/bibliography_of_uncertainty.htm

http://www.measurementuncertainty.org/mu/guide/index.html

http://physics.nist.gov/cuu/Uncertainty/

While I am enjoying bring this page to you, I have not had any feedback at all

So here is the challenge: each reader is to send me one site that they have visited that would be of interest to our scientific readers. My email address is graemeb@medlab.co.nz

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Book review

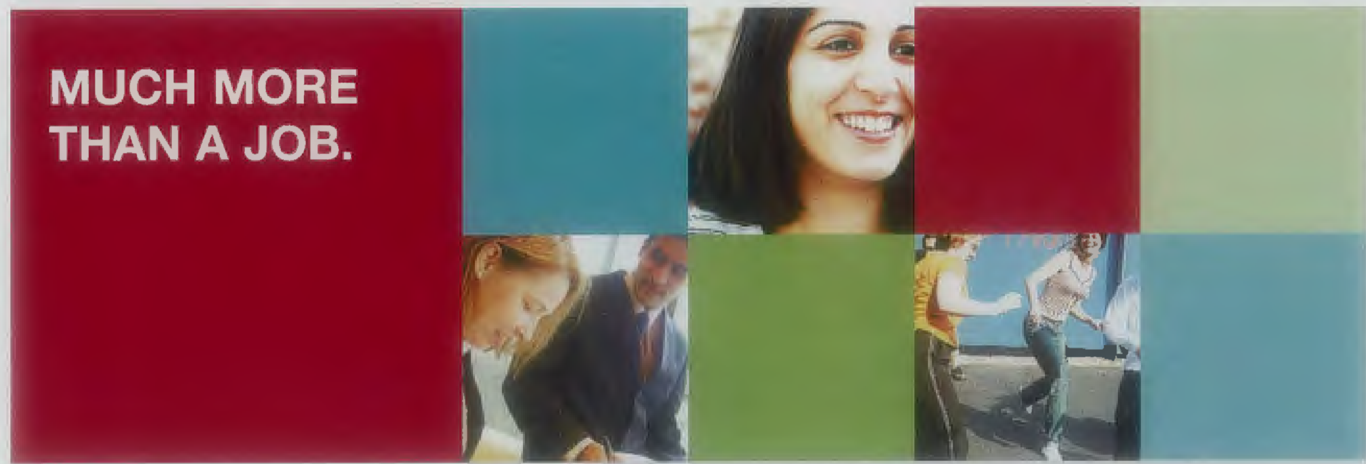
The Urinary Sediment - An Integrated View (2nd Edition 2000) by Giovanni Fogazzi. Published by Oxford University Press. ISBN 0192630741.

Because of my interest in urine microscopy, I have been asked by a number of Microbiology laboratory technologists if I could recommend a book on urine microscopy. The 2nd edition of The Urinary Sediment, which is a photographic atlas of urinary sediment, and priced around $200, is one I can thoroughly recommend. In nearly 200 pages, the book contains over 180 excellent colour illustrations, from casts to contaminants. All three authors are internationally recognised in their fields of nephrology.

The book is divided into 7 chapters, including sections on formed elements, diseases of the kidney and urinary tract, interpretation of findings and a look at automation. The introduction by JS Cameron (Renal Unit, Guy's & St Thomas' Hospitals, London) on the history of urine analysis, makes for interesting reading. I was particularly impressed with the section on dysmorphic red blood cells, which includes many phase contrast colour photos and detailed electron microscopy illustrations.

As the book was aimed primarily at nephrologists, the emphasis is on clinical significance. However, it is also very well suited as a 'beside the microscope' reference atlas for laboratory scientists and is an excellent teaching reference.

Reviewed by Julie Vincent, Microbiology, Canterbury Health Laboratories, Christchurch.



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What has been happening at the PPTC lately?

Te Pacific Paramedical Training Centre had a very busy and successful year in 2003. There were four highlights that I would like to specifically mention.

1. Blood bank technology course

Again we are very indebted to the NZ Blood Service for running this course for us and our special thanks go to Carolyn Graham and John Dagger of the Wellington Regional Centre for their support and all the hard work put in to the conducting of the training.

There were six participants on the course representing Tuvalu, Palau, Yap [in the Federated States of Micronesia] Vanuatu and Cambodia, you can see them all in the photo below along with the teaching staff.

2003 Blood bank Technology Course Participants and staff.

It was pleasing having the two participants from Cambodia, sponsored by WHO, on the course as they added a new flavour especially when we all went out to diner at a local Cambodian restaurant!

PPHSN LabNet 2003 meeting, Suva

The Pacific Public Health Surveillance Network was set-up in 2000 by the SPC and WHO as a voluntary network of Pacific Island Countries, dedicated to the promotion of public health surveillance. Currently its focus is on communicable diseases, especially outbreak-prone ones that represent major public health problems in the Pacific. The current target diseases are: Measles/Rubella, Dengue, Typhoid, Cholera, Influenza and Leptospirosis.

It was recognised right from the commencement that laboratory confirmation of these infections is essential and so one of the key tasks was to set-up LabNet an email list which aims to strengthen links between the laboratory personnel so that they can share information and improve testing capability of their laboratories.

In September 2003 representatives from most laboratories in the Pacific met in Suva for several days to discuss the current capacity for their laboratories to successfully diagnose the various target diseases. Laboratory specific training needs were on the agenda as were issues relating to quality assurance.

It was agreed that the surveys of the PPTC's Regional EQA Programme should be expanded to include the target diseases whereever possible. The PPTC also agreed to offer courses relevant to these epidemic prone diseases in its curriculum.

WHO laboratory management workshop

In late October WHO sponsored a workshop in Manila on the Management of Clinical Laboratories. Representatives from the laboratories of most Pacific Island countries attended this workshop which had as one of its objectives the acceptance of the new ISO 15189 standard for medical laboratories as the standard which laboratories in the region should be working towards implementing. The group, which included representatives from China, Mongolia, Vietnam, Cambodia and the Philippines as well as the Pacific Island countries, agreed to this concept but it was realised that the implementation of this standard would require not only time but also appropriate training in Quality Management. The PPTC's EQA surveys and training programme was again seen as a means of assisting the laboratories in the Pacific region in attaining this goal.

Laboratory quality systems

This project commenced in 1998 with the aim of introducing a Laboratory Quality System (LQS) into the operations of the National Health Laboratory, Apia, Samoa and the Vaiola Hospital Laboratory, Tonga. The project, funded by the New Zealand Government, was successfully completed in September 2003 and certificate presentation ceremonies were held in each country with representatives of WHO and the New Zealand Government plus local Ministry of Health representatives attending. An approach has been received from the Ministry of Health in Kiribati to carry out a similar programme.

Quality Systems Certificate Presentation in Samoa: Dr Ron Mackenzie, Chairman of the PPTC, presenting Dr V F Asaua, ACEO Laboratory Services with the Certificate.

Quality Systems Certificate Presentation in Tonga: Laboratory staff with Mr Jonathan Curr, Acting NZ High Commissioner, Dr Litili 'Ofanoa, Director of Health, Dr Ron Mackenzie and John Elliot of the PPTC.

2004 courses at the PPTC
The following courses are scheduled for 2004.

Course	Month
Laboratory Diagnosis of Sexually Transmitted Infections	March
Blood Cell Morphology	April
Blood Bank Technology	August
Laboratory Management and Quality assurance	November

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Interviews with King's College Hospital representatives will be held in New Zealand in July 2004.

To find out more please contact
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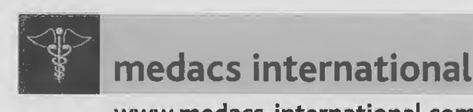
E-mail ruth.jameson@medacs.com
Freephone from NZ 0800 446 440 outside office hours select extension 211.



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Answers to HSIG journal based learning questionnaire

1. Induction of strong, strain-transcending, durable immune responses. Identification of protective antigens for stage-specific immunity. Successful combination of candidate immunogens.
2. Phase III.
3. 20,000 - 30,000.
4. Subunit vaccination - part or complete antigens are identified from a pathogen's proteomic complement, which can induce protective immunity to the whole pathogen on vaccination.
5. Increased understanding of antigen processing, adjuvants and their effects on innate immunity, genetic engineering techniques, and novel delivery systems.
6. "DNA based".
7. True.
8. Short-lived strain-specific malaria immunity.
9. True.
10. RTS,S/AS02 and ICC-1132.
11. Merozoite surface protein - 1.
12. NYVAC Pf-7.
13. Would protect communities from infection.
14. 5,300 P falciparum antigens.
15. Eleven.

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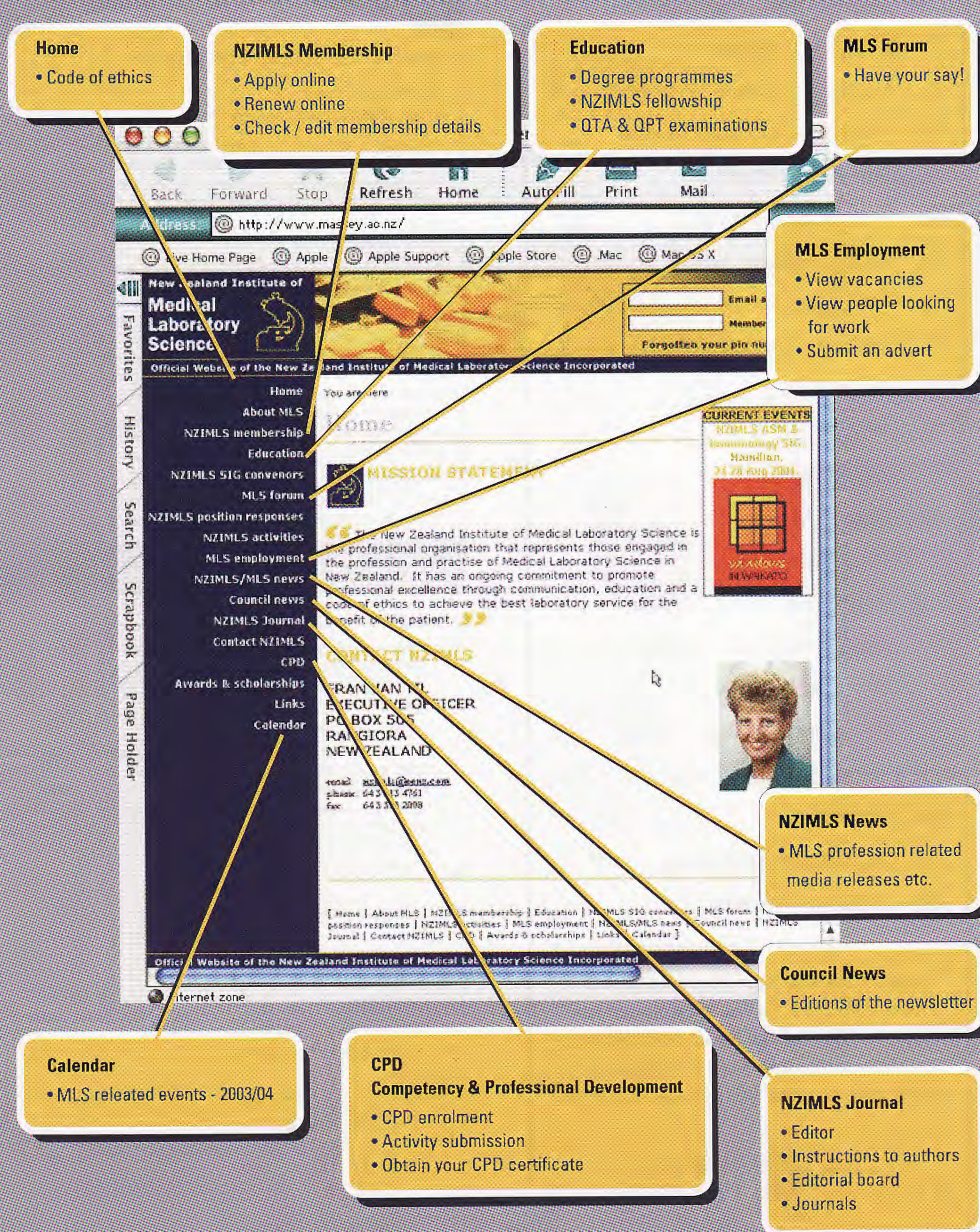

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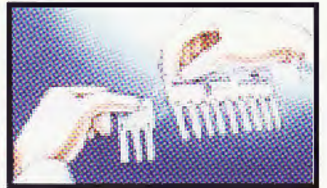
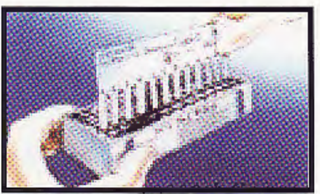


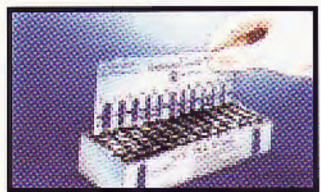
If needed, you may bend and break the comb for individual testing.

Draw and add specimens and controls to Row A.

Insert Comb into Row A and incubate.



Insert the Comb into Row B and incubate.



The colour reaction occurs in Row F.

Read the results. Keep the comb for long term documentation and patient follow-up.

For more information, or to arrange an evaluation, contact Med-Bio Limited:

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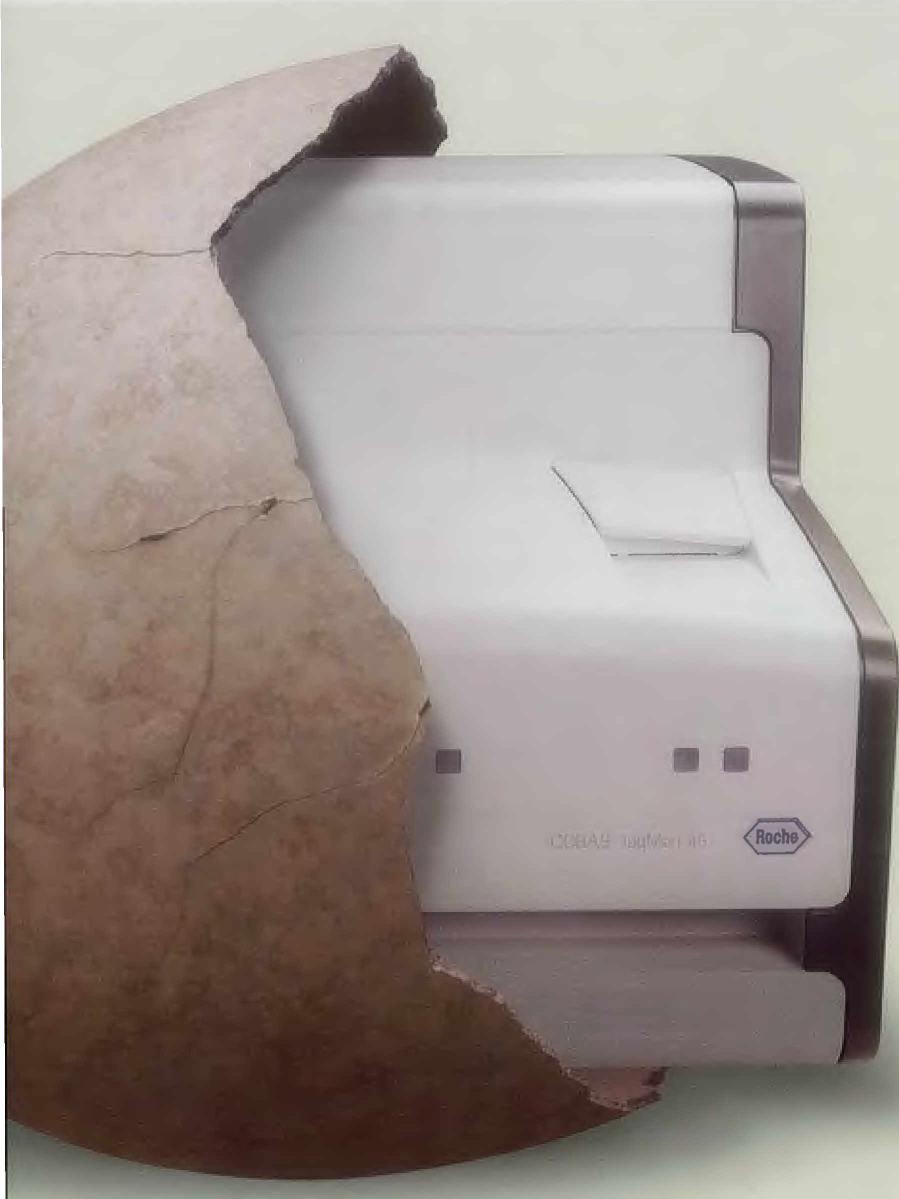
Diagnostics

The evolution of PCR



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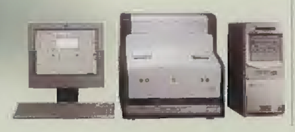

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