

Test & teach case

A 56 year old female presented with a five week history of watery diarrhoea, 10kg weight loss and fatigue.

A stool collected three weeks into her illness, "antibiotic-induced diarrhoea", revealed no leucocytes, erythrocytes, ova, cysts or parasites on wet film microscopy. Cultures were negative for *Salmonella* spp., *Shigella* spp., and *Campylobacter* spp., while *Clostridium difficile* culture and toxin assay were also negative.

Her diarrhoea persisted despite treatment with Metronidazole and Ciprofloxacin. A subsequent stool tested negative for *Giardia lamblia*, *Cryptosporidium parvum* and *Rotavirus antigens*.

A SEALS Clinical Microbiologist was consulted and informed that her illness began four days after her return from a holiday in Bali. A further stool specimen was ordered with a request for a permanent parasite stain and a modified acid fast stain. The photo shows the faecal smear stained with the modified acid fast stain.

What is the organism responsible for this patient's symptoms?

Is there any antimicrobial treatment available for this infection? If so, what?

Answers page 3.

Heparin Induced Thrombocytopenia - diagnosis and management

Heparin Induced Thrombocytopenia (HIT) is fortunately an uncommon complication of heparin therapy. The presentation is often thrombocytopenia that develops on heparin therapy and resolves after heparin cessation. It follows the development of heparin-dependent platelet-activating antibodies leading to platelet consumption and/or clearance, increased thrombin generation, and endothelial cell and monocyte activation. Rapid recognition and therapy are essential because HIT carries a high morbidity and mortality from thrombotic disease.

The pattern of thrombocytopenia is especially important in diagnosing HIT. The nadir platelet count is usually between $20-100 \times 10^9/l$, with at least a 50% reduction from baseline. Severe thrombocytopenia $<20 \times 10^9/l$ is unusual. The onset of thrombocytopenia is typically 5-10 days after commencement of heparin. Early onset (<5 days) or late onset (>10 days) thrombocytopenia are less common. The manifestations of thrombosis are protean and include vein thrombosis (deep vein thrombosis and/or pulmonary embolism), macrovascular arterial thrombosis especially in arteriopathic patients (stroke, MI, limb ischaemia), catheter-related thrombosis,

and microvascular thrombosis with skin necrosis and digital gangrene. A clinical probability scoring system provides guidance regarding diagnosis and management although these algorithms are still under formal evaluation¹.

The HIT antibodies bind to one or more neoantigens on the cationic protein platelet factor 4 (PF4) that are formed when PF4 binds to negatively charged glycosaminoglycans such as heparin. The prothrombotic state of HIT is associated with an increased risk of arterial and venous thrombosis (odds ratio 20-40-fold)².

Laboratory testing for HIT is a difficult area and relies on functional assays, which detect platelet activation, or antigenic immunoassays, which detect the presence of an antibody without regard for its functional activity. Functional assays have a greater specificity for HIT but require fresh normal donor platelets, special laboratory expertise, and are relatively difficult to standardise. Antigenic immunoassays have the advantage of better standardisation and do not require fresh platelets, but may give positive results in the absence of HIT.

Continued page 2.

In this issue...

- HIT – rapid diagnosis essential
- Merger Matters – initiatives at the coalface
- BNP tests help diagnose heart failure
- Viewpoint – harmonising our services
- Quality tests for pheochromocytoma
- Fred San Gil sees many changes
- NT-proBNP tests – view from the lab
- Comings and goings

Merger Matters

FOCUS ON THE LAB

Change can come from the top down or bottom up. This column shares some of the changes that have been initiated at the "coalface" with the intention of improving the quality and uniformity of testing.

Haematology

Haematology throughout the Area often uses similar technology, reagents, and in many instances the same batch of reagent, but does that mean the same test will have the same result from any one of the SEALS laboratories?

Haematology staff across the network have recently set up a regular exchange of quality control specimens for routine haematology and coagulation tests.

Interlaboratory variation in the interpretation of blood film morphology is also being examined. Progress is being made with the autovalidation of coagulation results. This project aims to produce results based on algorithms which have been agreed to by all laboratories across the SEALS network.

It is expected that these projects will further enhance the service by meeting clinical needs with standardised, validated and timely results.

Continued from page 1

SEALS currently screens all samples submitted for HIT testing with the DiaMed particle gel immunoassay. Our own research indicates the Diamed assay is a good screening test for HIT (Sensitivity of 86%, Specificity of 84%, Negative Predictive Value of 99%). Positive and equivocal samples by DiaMed assay are subjected to confirmatory immunoassays after discussion with one of the Haematologists. Our functional assay is currently the gold-standard functional Serotonin Release Assay. Platelet aggregometry, so long a functional assay used in Australia, is no longer used routinely. Discussion with a Haematologist is recommended for interpretation of assay results.

Management of HIT patients requires some experience and again, specialist advice is recommended. Heparin therapy must cease immediately for all suspected (at least moderate probability score) and confirmed HIT patients. Low molecular weight heparins (such as enoxaparin, dalteparin) are to be avoided because of the high cross reactivity of the HIT antibody with these heparin derivatives. All patients with HIT probably require therapeutic anticoagulation unless absolute contraindications are present.

Patients with isolated thrombocytopenia without clinical thrombosis have a high risk of subsequent thromboembolism even on prophylaxis^{3,4}. Two agents are available for treatment in Australia including danaparoid (Orgaran®) and lepirudin (Refludan®). Argatroban is available overseas. Many patients will require warfarinisation after acute anticoagulation and stabilisation of their medical condition. Avoidance of heparin and LMWH is recommended. Detailed documentation along with clear explanation of the illness to the patient and their doctors is required.

REFERENCES

1. Warkentin T.E., An overview of the heparin-induced thrombocytopenia syndrome. *Seminars in Thrombosis and Hemostasis*, 2004; 30(3); 273-283.
2. Clinical picture of heparin-induced thrombocytopenia. Warkentin, T.E., Ch. 3: 43-86, in *Heparin-Induced Thrombocytopenia*, 2nd Edition. T.E. Warkentin & A. Greinacher Marcel Dekker, 2001. ISBN 0-8247-0658-7.
3. Wallis, D.E. et al, *Am J Med*, 1999; 106: 629-635.
4. Greinacher, A. et al, *Blood*, 2000; 96: 846-851.

Dr Tim Brighton, Haematologist, SEALS Kogarah and Randwick

Rosalie Gemmell, Senior Scientist, SEALS Kogarah

Sue Evans, Senior Scientist, SEALS Randwick

Clinical applications of BNP testing

Brain natriuretic peptide (BNP) is a protein released by the left ventricle in response to increased myocardial stretch. While physiological responses to heart failure have adverse consequences, the natriuretic peptides have positive effects. They are vasodilatory and cause a natriuresis and diuresis.

BNP is released as a pro-hormone which is cleaved into a 32-amino acid active molecule (BNP) and an inactive component, N-terminal proBNP (NT-BNP). Elevated levels of BNP can help in the diagnosis of heart failure. In normal people, BNP is barely detectable, but levels rise three to ten-fold in the presence of heart failure with pulmonary or peripheral congestion.

The main clinical utility for measuring NT-BNP is to exclude heart failure. In patients with "barn-door" heart failure, with clear pulmonary congestion on history, clinical examination and chest x-ray, measuring BNP is unlikely to add further diagnostic information. However,

BNP measurement can be useful in patients presenting with shortness-of-breath or oedema where heart failure is suspected but not proven e.g. in patients with chronic airway limitation where a secondary diagnosis of heart failure is suspected. In these patients, a NT-BNP result in the normal range is very effective at ruling out heart failure as a cause of dyspnoea. Studies consistently show a high negative predictive value for the test in this clinical situation.

Dr Susan Wright, Cardiologist, St George Hospital





Viewpoint

Since the last issue of the SEALS and IPATH Illawarra Pathology newsletters, these two entities have combined under the name of SEALS as a result of NSW Health's restructure of Area Health Services. As head of this new organisation much of my time is directed to bringing our services together. Much has already

been accomplished to this end, and this newsletter is but one example. There are still many challenges ahead but we are working hard to ensure that this process is transparent. I believe this merger provides us with an opportunity to make significant improvements which will directly benefit many of you. We have already extended the SEALS Call Centre facility to doctors in the Illawarra and Shoalhaven, for tests performed anywhere in SEALS from Macquarie Street to Nowra. Progressive integration of our clinical policies, processes, technologies, and IT systems, will eventually harmonise our services irrespective of location. Our aim, wherever practical, is to provide local services in partnership with local clinicians to best meet local need. Your continued support during this period greatly assists us in getting on with that task.

Dr Roger Wilson, Executive Director

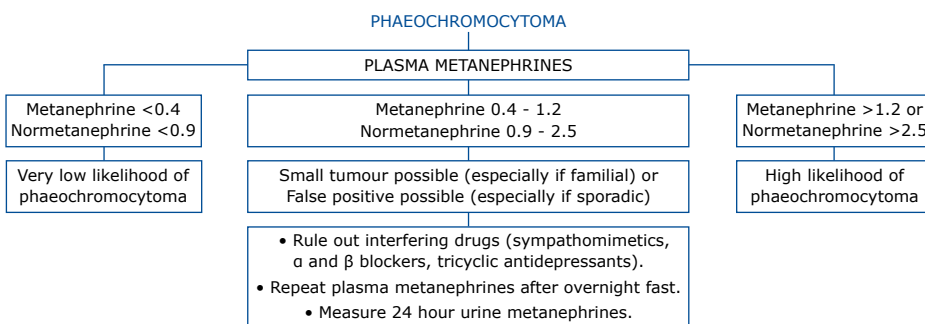
Quality Corner

TESTING FOR PHAEOCHROMOCYTOMA

The measurement of plasma metanephrines is the most sensitive test for the detection of phaeochromocytoma and is recommended as the best initial diagnostic test. Patients with a tumour often have levels more than three to four times the upper limit. The specificity of this test is not as high as its sensitivity and it thus has a false positive rate of around 15%. False positive levels are usually less than three to four times the upper limit and can occur in non-fasting specimens, from increased catecholamine metabolism, and from interference by some drugs.

Plasma metanephrines increase with age and the calculation of age corrected values for sporadic cases can help reduce the incidence of false positives. The best approach to a possible false positive is to repeat the test on a fasting specimen after avoiding interfering drugs, and to measure 24-hour urine metanephrines. Urine metanephrines have very high specificity for phaeochromocytoma and are usually normal in patients without a tumour.

The following testing algorithm is suggested:



NOTES:

1. Urine catecholamines (adrenaline, noradrenaline and dopamine) provide little additional information in the investigation of phaeochromocytoma.
2. A fasting specimen is preferred for plasma metanephrines. However, non-fasting specimens may also yield useful information if levels are below the upper limit, or are

elevated more than three to four times above it. A list of medication should be provided.

3. Appropriate comments will be included with each report to assist with interpreting results and any further testing.

*Dr Dilo Pillai, Senior Hospital Scientist,
Clinical Chemistry, SEALS North*

Editorial...

Focus is the quarterly newsletter of the South Eastern Area Laboratory Services (SEALS).

Editorial team: Peter Taylor, Stuart Purvis-Smith, Sue Acland, Anne-Maree McDougall, Desiree Berry, George Gray and Nicole Flynn.

Copy editor: Kath O'Sullivan, Active Voice www.activevoice.net.au

Design: Carly Hood, Active Voice

Please send your comments and contributions to Peter.Taylor@sesiahs.health.nsw.gov.au

Deadline for Issue 2: 19 January 2007

Test & Teach

ANSWERS

Cyclospora cayentanensis is an intestinal coccidian protozoan, increasingly recognised as a treatable cause of persistent diarrhoea in returned travellers. Diagnosis is made by modified acid fast staining of faecal smears and should be specifically requested, as the oocysts can be missed on routine faecal microscopy.

The recommended treatment for immunocompetent patients is a seven day course of oral Cotrimoxazole. In the case presented the symptoms resolved within four days of starting therapy.

This case illustrates the importance of including relevant clinical details such as a travel history on pathology request forms. If these details were known at the time of the original examinations, the diagnosis could have been made earlier.

REFERENCE

Pingé-Suttor V. et al, *Med J Aust*, 2004; 180:295-6.



Staff Profile

DR FRED SAN GIL

Fernando "Fred" San Gil was born in Spain but migrated to Wollongong in 1960 when he was one year old. Fred is married with two teenage daughters. Travelling (anywhere) is one of his great passions and he is a keen snooker player.

Fred completed a Science Degree at the Australian National University in 1978 and a Graduate Diploma in Medical Technology

at the University of South Australia a year later. In 1994 he undertook a Master of Science, followed by a PhD, at the University of Wollongong.

Since beginning his career as a Technical Officer in Biochemistry in 1980, he has progressed to his current position of Department Manager of Clinical Chemistry. In this role he oversees Biochemistry services for the Wollongong, Shellharbour and Shoalhaven laboratories.

Over the years Fred has seen many changes. The most significant has been the rapid progression from manual testing to highly automated analytical platforms with diverse test menus for all pathology disciplines. This is driven in part by mounting financial pressures on pathology and the ever-increasing demand for faster results. The growing and ageing population will also impact pathology providers. In the last five years alone the number of tests performed in the Clinical Chemistry department has increased from 80,000 to 130,000 tests per month.

Noticeboard

COMINGS AND GOINGS



SEALS welcomes **Associate Professor Szu-Hee Lee** as Senior Staff Specialist Haematologist at SEALS Central. Professor Lee

graduated from Cambridge in 1976, obtained his PhD in 1986, and his FRCPA in 1991. He joins SEALS from the IMVS in Adelaide.

Professor Beng Chong has been appointed Acting Director of Haematology, following the resignation of Dr Kwan in June.



Dr Paul Kitching joined Anatomical Pathology (AP) SEALS North, in November 2005. Educated and trained in Cardiff and London,

Dr Kitching joins SEALS after eight years in Bath. He reports general pathology and covers the clinical meetings for Medical Oncology, Gastrointestinal, Liver, Urology and Lymphoma.



Dr Dianne Reeves was appointed Staff Specialist at AP SEALS North in November 2005. Dr Reeves graduated from the University of

Newcastle and completed pathology training at Liverpool and SEALS Randwick. She reports general adult Surgical and Non-Surgical Pathology, Ocular Pathology and Cytology.

Dr Grant McBride has been appointed Acting Director of AP SEALS South bringing valuable experience from his previous appointments in Australia and Hong Kong.

We note with regret the resignations of Senior Staff Specialist **Dr Peter Kyle** and Director of Haematology **Dr Yiu Lam Kwan** from SEALS Central, of 16 and 28 years service respectively.

We also regretfully announce the resignation of **Dr Gary Schier**. Gary has worked with SEALS for 40 years, and was recently acknowledged at the long service award ceremony at Wollongong. He will be missed and long remembered.

Collections & Contacts

THE NT-proBNP ASSAY FOR HEART FAILURE – THE LABORATORY VIEW

The assay for serum levels of the biologically inactive 76 amino acid NT-proBNP has been available at the SEALS Sutherland Centre for Immunology since July 2004 and is measured on the Roche E170 automated immunoassay analyser.

The half life of NT-proBNP in the circulation is 120 minutes and is mainly cleared by renal excretion. The 32 amino acid, biologically active, C-terminal fragment BNP has a half life of 20 minutes. Thus the serum values of NT-proBNP are approximately six times higher than BNP values. The NT-proBNP is stable in whole blood for 72 hours at room temperature whereas BNP is stable for 24 hours with the addition of EDTA stabiliser. The increased levels of NT-proBNP are related to disease severity as assessed by the New York Heart Association functional class.

Because BNP values obtained with various assays are not comparable and there is no conversion factor for the comparison of BNP and NT-proBNP¹, the same assay and analyser must be used when comparing serial measurements of NT-proBNP. The NT-proBNP assay has excellent precision with a CV <2% at all levels.

Age (years)	<45	45-54	55-64	64-74
Female	0-13.7 pmol/L	0-20.0 pmol/L	0-29.1 pmol/L	0-33.6 pmol/L
Male	0-7.4 pmol/L	0-9.9 pmol/L	0-19.0 pmol/L	0-28.4 pmol/L

Published data indicate that heart failure is unlikely at NT-proBNP levels <35 pmol/L¹.

REFERENCE

1. *Heart*, 2006; 92:843-849.

*Dr. Desiree Berry, Principal Scientist
SEALS Sutherland Centre for Immunology*