

# Newsletter

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## Anatomical Pathology achieve ISO 17025 accreditation

In July the Anatomical Pathology Department was successful in achieving ISO 17025 accreditation. This standard which incorporates much of ISO 9002 ensures that the Pathology Service has, and maintains, a quality system which is compliant with all the necessary requirements, while the individual scientific departments continue to provide a high level of technical and scientific competency. The other departments of IAHS Pathology are scheduled for accreditation in November.

## Laboratory Diagnosis of Meningococcal Disease

Recent media interest and concern from the general public has resulted in an increase in patient presentations for diagnosis or exclusion of invasive meningococcal disease. IAHS Pathology has subsequently noted a recent increase in requests for urine or blood meningococcal antigen testing or for "meningococcal screening". This has prompted a review of the appropriate diagnostic tests for invasive meningococcal disease.

Meningococcal polysaccharide antigen testing on urine, blood or CSF samples is not reliable, as the test has poor sensitivity and specificity. More useful tests for the diagnosis and exclusion of invasive meningococcal disease include:

**Blood cultures** – sensitivity falls dramatically if collected after antibiotic therapy, but still worth collecting as the specificity is 100%.

**Blood Meningococcal PCR** testing (EDTA anticoagulated blood). More sensitive than blood cultures, particularly in patients who have already received antibiotics. As the specimen has to be transferred for testing to the Dept. of Microbiology & Infectious Diseases at Liverpool Hospital, and the test generally requires overnight preparation, results are

usually available 24-48 hours after specimen collection. To minimise delays, it is best to contact the referring laboratory directly to expedite the process.

**Serum meningococcal IgM antibody** testing (blood in a serum gel tube) has a sensitivity of >97% on samples collected at least 5 days after illness onset, and a specificity of around 95%. Sensitivity is much lower on samples collected early in the illness and the test may need to be repeated on a later sample.

**CSF Gram stain, Culture and PCR** (if clinically indicated). A negative CSF Gram stain cannot exclude meningococcal meningitis as the sensitivity is only ~65%. The sensitivity of CSF culture is ~95% in untreated patients with meningococcal meningitis but falls dramatically if collected after antibiotic therapy. A negative CSF culture does not exclude meningococcal septicaemia, which often occurs without meningitis. CSF PCR results are less likely than culture to be adversely affected by prior antibiotic therapy.

**Gram stain, culture and PCR of other normally sterile sites**, such as aspirates from skin lesions (sensitivity ~60%), joint or pericardial fluid aspirates, if clinically indicated. Again, the sensitivity of Gram stain and culture falls dramatically after antibiotic administration.

**Throat swabs** with a specific request for meningococcal culture may also provide supportive evidence in clinically compatible cases. Meningococci may survive for a short period in the nasopharynx after antibiotic therapy and so throat swab cultures are less likely than cultures of other sites to be adversely affected by prior antibiotic therapy. A positive throat culture alone does not confirm a diagnosis of invasive meningococcal disease since meningococcal throat carriage can be found in up to 15%-20% of asymptomatic individuals. In this situation, follow-up meningococcal serology testing should help confirm a diagnosis of recent invasive disease with meningococcus.

For further information regarding the investigation and management of meningococcal disease please refer to: *Guidelines for the early clinical and public health management of meningococcal disease in Australia*. Commonwealth Dept. of Health and Aged Care. 2001. Available via CIAP.

## Viewing Pathology results via the IH Intranet.

At times when IAHS pathology results cannot be viewed via the WIS on the VMS computer system try using the "Courier" option via the IAHS Intranet as follows:

1. Contact Pathology Ext. 5283 who will provide you with a password
2. Click on Internet Explorer to go to the Illawarra Health Intranet home page.
3. Select Clinical Apps.
4. From the Clinical Apps menu select Pathology.
5. This will send you straight to the courier system.
6. Type PATHOLOGY in upper case in the User ID field and go to the "System" field.
7. In the "System" field, from the drop down menu select Network 2000+ Live, instead of Network 2000+ Live DCC
8. In the "Password" field type the password provided to you by pathology in upper case and click on "submit".
9. Type in the patient's MRN followed by .ILL (in upper case) or name in the relevant field and click on "Submit".
10. Click on the relevant episode number to view results from that episode.
11. Scroll down to view results at the bottom of the page.

At this stage it is advisable not to try to print results viewed in the Courier format as the patient identifier may not print on the page with the results and the results may not print out completely. Détente systems are working on these problems and IAHS Pathology will advise when reports can be printed out using the Courier system. Printing of results from ward terminals is best done using the WIS.

### MARKERS OF KIDNEY DAMAGE IN RENAL DISEASE

As the supply of Kidneys for transplants (13,000 /year in the U.S.A) is limited, most patients with end-stage kidney disease will end up having dialysis.

The rate of increase in Kidney disease is alarming and public awareness will create new demands on laboratories for early and more sensitive detection of kidney disease and for identification of new markers.

**Serum creatinine** has long served as the mainstay for detecting impaired kidney function. Unfortunately serum creatinine is an insensitive marker of kidney injury. There are two main reasons why this is insensitive.

- The first is that the reference interval is relatively wide because it must encompass a large range of creatinine production, which is related to muscle mass.

- The second is the inverse relationship between GFR and serum creatinine predicts that large reductions in GFR from normal produce only small absolute increases in serum creatinine. In small and elderly persons with small muscle mass, serum creatinine will remain within the usual reference limits despite substantial kidney damage.

The first sign of glomerular disease is increased urinary excretion of **albumin**, initially in the **microalbumin** range 30-300 mg/day, which progresses over 15- 20 years to proteinuria detectable by routine dipsticks. Annual testing for diabetes is useful to detect microalbumin levels in urine.

There is an ongoing search for better markers of impaired renal function or injury.

One promising candidate has been **CYSTATIN C**, a small plasma protein of 13 Kda that inhibits cysteine proteases. It is produced by all nucleated cells and is small enough to be freely filtered at the glomerulus.

The serum Cystatin C concentration correlate inversely with GFR, and assays for this are available commercially. Recent studies have shown decreased Cystatin C production in transplant patients, with low GFR, limiting its role in advanced renal disease. However it is most useful in detecting early renal dysfunction.

A **glycoconjugate of tryptophan** also has been tested as a marker of renal dysfunction, but further evaluation of the assays and clinical studies needs to be conducted.

Lipocalcin- type urinary prostaglandin synthase (LPGDS) is a 26 Kda secretory glycoprotein that was termed  $\beta$  - trace as it was first discovered in the CSF, are increased in individuals with early kidney damage. Its level was reported to be significantly higher in persons with renal failure, than in normal persons.

Further studies are being done to see if this could be used as a marker for renal glomerular damage.

Finally, many laboratories are now screening for mRNAs or proteins (proteomics) that are differentially expressed during kidney disease. Although it is easy to find mRNAs that are differentially expressed in the kidney using PCR technology, few of the proteins they encode will be detectable in serum or urine, and even fewer will be regulated by injury. **Dr. Farid S. Zaer**

### REFERENCES:

1. Jones CA et al: Serum creatinine in the U.S. population: Third national health and nutrition examination survey. Am J. Kidney Dis 1998; 32:992-999
2. Oda H. et al Development and evaluation of a practical ELISA for human urinary Lipocalcin – type prostaglandin synthase. Clin.Chem 2002; 48: 1445-53
3. Newman DJ. Cystatin C, Ann. Clin. Biochem 2002; 39: 89-104
4. Takahira R et al: Tryptophan glycoconjugate as a novel marker of renal function. Am J. Med 2001: 110: 192-197.
5. Taft JL et al: Proteinuria in diabetic patients. Diabetes 1994; 43: 1046 - 51

## **FAMILY PHYSICIAN'S ROLE IN THE DIAGNOSIS OF CANCER**

Even though cancer is now one of the leading causes of morbidity and mortality in the Western world, it is still seen as peripheral to primary care and predominantly an area of medicine practiced by specialists.

Is there a role for the family physician in the diagnosis and management of cancer?

The answer is "yes"!

### **OFFICE – BASED CANCER SCREENING**

#### **The Pap Smear (Exfoliative cervical cytology):**

This is a common and widely used technique by GP's in screening for pre-malignant and malignant lesions of the cervix. It has proven to be the most successful screening program world wide in reducing the incidence of cervical cancer. Several publications outlining guidelines are available that will enable physician's in developing a personal comprehensive screening program in his or her practice.

In order that physicians receive good and valuable reports from their pathologists the following simple rules should be adhered to:

1. Adequate sampling requiring cells from the transformational zone to be collected.
2. Endocervical cells must be obtained in a specimen to make it adequate
3. Air drying must be avoided as cells appear distorted and hard to read
4. To avoid air drying, fix immediately in 95% ethanol for 15 minutes or by spraying it with an aerosol or pump fixative.
5. The cytobrush is superior to either the Ayres spatula or cotton swab in retrieving cells from the endocervical canal.
6. Proper labeling of the slides and all clinical information including patient's age and last menses must be provided.

In recent years attempts to automate the process of cervical cell evaluation for pre-malignancy has been attempted, but both Flow cytometry and Image analysis have not been as successful as traditional screening.

In addition to the traditional technique of slide based exfoliative cytology, a newer technique of "Liquid based cytology" is now available and superior to the traditional method. Your pathologist or pathology department can provide further information on this new technique.

#### **OTHER EXFOLIATIVE CYTOLOGY:**

The other two common office procedures are respiratory cytology and urinary cytology.

Sputum analysis is a common way to obtain information about respiratory tract involvement by microorganisms, tumour or toxins.

The samples that yield positive malignant cells are in middle age and elderly smokers with blood tinged sputum or secondary infections in tumours obstructing the airways. Urine cytology is not a screening test for the general

population for the detection of bladder cancer. It may be useful in other conditions such as infections or stones.

The best yield for the detection of bladder cancer is in a selected population of individuals with a high risk of bladder cancer, or as a means of follow up in TCC – insitu or even post resection of the tumour mass.

Bladder washes and biopsies are generally done by the urologist and have a higher yield.

#### **FNAB (Fine needle aspiration biopsy)**

This technique is now conducted mainly by Pathologists, radiologists or oncologists. The radiologist may perform this procedure for non-palpable lesions.

Physicians may aspirate palpable lesions of the breast or thyroid gland. In addition other lumps or bumps may also be aspirated and this includes subcutaneous lesions, skin nodules and lymph nodes. It is only indicated in the presence of a mass that is highly suspicious for a neoplasm.

The main contraindication to doing an FNAB is in highly vascular tumour such as carotid body neoplasm or aneurysm or vascular malformation. The correct technique requires practice and it is best to make at least 3 passes to obtain diagnostic material.

#### **GENERAL PROCEDURES:**

Skin biopsies are useful in the diagnosis of skin cancers and the excised ellipse must be marked with a suture or paint to indicate the 12 o'clock pole, making assessment of the margins of excision easier.

Removal of anal varicosities may require endoscopy or sigmoidoscopy, any suspicious area may be biopsied if the physician has some training in conducting such a procedure. This may be left to a gastroenterologist if the clinician is not comfortable taking biopsies. All such biopsies are submitted in formalin.

#### **BLOOD TESTS:**

Most family physicians will perform routine blood tests, but there are some tests that are now considered mandatory in the diagnosis of cancer.

1. Tumour markers include
  - a) **Prostate specific antigen** (Free and Total) in the screening, diagnosis and follow up in men suspected of having prostate carcinoma.
  - b) **Carcinoembryonic antigen**: This is generally preferred in the follow up of patients having bowel cancer that has been resected.
  - c) **CA19-9**: This is a marker that is elevated in >70% of pancreatic carcinoma. Very high levels indicate the lesion to be malignant and possibly

unresectable. Intermediate levels are of no value. Low levels may indicate benign tumours.

- d) **CA 125:** Now used in the screening of ovarian cancers and has the added value in follow up of persons who have had their tumour resected. The screening is restricted to women with pelvic masses.
- e) **AFP:** This has been suggested as a marker for screening populations at high risk for hepatocellular carcinoma e.g. in Hepatitis B or cirrhosis of the liver. This is also elevated in patients with testicular tumours along with HCG. These two markers are used in monitoring response to therapy in such patient's.

### HAEMATOLOGIC NEOPLASMS:

Since haematopoietic tissue is in fluid state and the cells are dyscohesive, flow cytometry is the preferred method of diagnosing such cancers. Flow cytometry can also be used in diagnosis of neoplasms in body fluids (ascitic or pleural). All abnormal or markedly elevated counts in peripheral blood must be followed up by flow cytometric analysis of peripheral blood for leukaemia, myeloproliferative or lymphoproliferative states.

In non-haematopoietic tumours, flow cytometry can be used to assess the DNA content and ploidy status indicating if the cells are benign or malignant.

Physicians must know that these modalities are available and must order tests directly or in consultation with Pathologists.

Finally understanding the laboratory and the techniques and the facilities available is the most important undertaking a physician can do.

The Pathologist offers Intraoperative consultation (frozen sections), FNAB facilities, advanced testing including Immunohistochemistry and in-situ hybridization and advice on a range of pathological conditions that is so vital to the proper and safe practice of medicine.

Newer and more advanced methods are now being developed and these include molecular methods and Proteinomics.

Targeted drug therapy is now evolving and is used in the treatment of certain breast cancers, stromal tumours and myeloproliferative states. This form of therapy can only be instituted after correct pathological evaluation.

Dr. Farid S. Zaer

#### Customer feedback:

Is there anything which you would like to see us feature in this Newsletter?

Or is there anything which you think we could do to provide a better service, to you, our customers.

If so, please contact:

George Gray

Quality Manager Telephone: 42225283

### Recent Changes to Antibiotic Assays

Over the last couple of months readers will have noticed some changes in the way we report our **Gentamicin** assay results. We have removed the "Reference Range 0-2mg/L" from our laboratory reports. This is because the concept of a "reference range" does not apply to antibiotic assay results. The target serum Gentamicin concentration that should be aimed for depends on the dosing regime that is used for the patient. In addition accurate knowledge of the time interval between specimen collection and the end of the last dose infusion is critical to the interpretation of serum antibiotic levels. For patients receiving a "once daily" Gentamicin dosing regime, guidance on interpretation of serum levels and any dosage adjustment that may be required can be found in *Therapeutic Guidelines – Antibiotic*, Version 11, 2000.

This document can be viewed on-line via the CIAP site on the Illawarra Health Intranet. For 8 or 12 hourly Gentamicin dosing regimens, monitoring trough (or pre-dose) serum levels is recommended. The target trough Gentamicin level for most indications is < 2mg/L. When Gentamicin is used for synergy in the treatment of endocarditis in patients with normal renal function, 8 or 12 hourly dosing is recommended and the target trough level should be < 1mg/L.

**Amikacin** is a rarely used antibiotic for which monitoring of serum levels is also indicated. The main indication for the use of Amikacin is the treatment of gram-negative infections where the causative organism is resistant to Gentamicin and Tobramycin. **For the last 12 months or so serum Amikacin assays have been performed on-site at our Wollongong laboratory rather than having to be referred to a Sydney teaching hospital laboratory for testing.** This has had the obvious benefit that results are available in a shorter time than they would be if the test were performed in another laboratory. Again a "reference range" does not apply to Amikacin levels. Interpretation of results and dosage adjustment methods can also be found in *Therapeutic Guidelines – Antibiotic*, Version 11, 2000.

Another recent change in our antibiotic assays is the shift of the **Vancomycin** assays to a random access analyser in the Biochemistry Department. This has reduced the time to result availability and means that Vancomycin assays are now routinely available on weekends and outside of normal laboratory hours if required. For patients receiving Vancomycin, monitoring of trough serum levels is recommended. To ensure therapeutic efficacy, the recommended target range for trough Vancomycin levels is 5 - 15 mg/L.

Further advice regarding antibiotic levels and appropriate dosing can be obtained by discussion with the Clinical Microbiologists.

Telephone. 42225729/5395