AOTEA NEWS
– CLINICAL UPDATE

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SIMPLIFYING STI TESTING: CO-TESTING FOR CHLAMYDIA AND GONORRHOEA BY PCR

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Sexually Transmitted Infections hunt in packs, just like wolves. Now testing is simplified so that you can co-detect Chlamydia and gonorrhoea on swabs or urine from 1 May 2012.

Following a comprehensive validation study (see sidebar, pages 2/3), Aotea Pathology is pleased to offer PCR testing for both *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (NG).

This new test detects both gonorrhoea and Chlamydia from a single specimen, on all requests for Chlamydia and/or gonorrhoea. The assay is run on the Roche cobas 4800 instrument, which also tests for Human Papillomavirus (HPV). This technology brings improved sensitivity, the ability to use non-invasive sample types and room-temperature storage for collected samples simplifying testing for both clinicians and patients. PCR does not discriminate between live or dead bacteria allowing room temperature storage of specimens in cobas PCR media for up to 90 days. **Therefore PCR is now best practice for gonorrhoea testing, and replaces culture for routine diagnosis.**

SPECIMEN COLLECTION

The recommendations for gonorrhoea PCR testing match the Chlamydia Guideline, 2008 (1):

- **Females: A cervical swab if a pelvic examination is being carried out or a self collected low vaginal swab if not.**

  Note that urine is not recommended as a sole screening specimen for females due to low sensitivity.
- **Males: The first 10-20mL of voided urine decanted into a cobas PCR collection kit (delay passing urine for 1-2 hours before collection).**
- **Rectal, Pharyngeal and conjunctival swabs:** Swabs from these non-genital sites have been validated in house for the cobas 4800 CT/NG assay.

Collection sites should be determined by gender and infection risk based on a sexual history for the previous 3 months (2).

PCR is now best practice for gonorrhoea testing, and replaces culture for routine diagnosis. We concurrently test for Chlamydia trachomatis and Neisseria gonorrhoeae using the existing swab and urine collection kits.

A PLACE FOR CULTURE

PCR cannot provide antibiotic susceptibilities as no isolate is grown. Antibiotic resistance in Neisseria species is an issue due to its ability to acquire mutations that decrease antibiotic susceptibility and may cause treatment failure. For example, in 2005 the rate of Ciprofloxacin resistance in Aotea Pathology tested gonococcal isolates was 23%, but by 2011 this rate had risen to 52%. Because of this concern APL will continue to provide culture and antibiotic susceptibility testing in some circumstances.

When to ask for culture as well as PCR at first presentation:

- Patients with significant penicillin or a confirmed cephalosporin allergy.
- Neonatal conjunctivitis.
- For medico-legal requirements – e.g. sexual assault.

PRACTICE **POINTS**

- All Chlamydia specimens will automatically be tested for gonorrhoea regardless of request.
- **Specimen recommendations are:**

  Females: Cervical swab. A low vaginal swab may be self collected.

  Males: The first 10-20mL of voided urine decanted into cobas collection kit.
- Please contact the laboratory if you require culture and antibiotic susceptibility testing.

A patient may also be required to return for a culture:

- If treatment has failed – when symptoms persist. This should be done within 7 days of completion of treatment.
- Failed PCR tests – as indicated by the laboratory (3).

Please contact the laboratory to discuss culture testing, and note that the differential sensitivity between culture and PCR may cause discordant results.

TREATMENT

Co-infection is common with up to 40% of NG positive patients being infected with Chlamydia in our testing population. The current recommendations for empiric treatment cover both these considerations (3).

Ceftriaxone 500mg (IM) stat AND Azithromycin (oral) 1g stat

Including in pregnancy & breastfeeding

See the NZSHS guideline **www.nzshs.org/guidelines.html** for further treatment and test of cure information, or contact the Wellington Sexual Health Clinic for specialist advice on **(04) 385-9879**

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VIRAL HEPATITIS TESTING

In this update I aim to clarify the optimal use of laboratory tests in referral and care in patients with viral hepatitis. The emphasis, as usual, is on hepatitis B virus (HBV) and hepatitis C virus (HCV) which both commonly cause chronic infection. I also discuss the new standard of care around identifying pregnant women with high HBV viral loads, in whom antiviral therapy is now recommended to reduce transmission to the newborn.

The starting point is always serology and measurement of transaminases, which provide most of the information required to identify and diagnose viral hepatitis, but molecular tests for viral nucleic acid are central to the active management of hepatitis B and C.

SEROLOGICAL DIAGNOSIS

Hepatitis A and E

- These are the simplest to diagnose: the IgM is almost always positive at the time the transaminases are raised. The IgG is used to determine immunity, or to show seroconversion if the IgM results are inconclusive.
- Both are self-limited diseases that can be serious in adults, but children are usually asymptomatic. They occur as a result of contact overseas, and in local outbreaks as recently seen in Auckland.

Hepatitis B

- Surface antigen [HBsAg] is present in almost all actively infected persons with HBV and is the first test that should be requested if HBV infection is suspected. Positive individuals should have a care and monitoring plan developed, particularly women who are pregnant or about to become so.
- The assessment of surface antibody is a test that should be restricted to adults in occupational health settings, preferably within a few months of the final dose of vaccine; those who develop a clear response will be protected for life.
- Babies born to HBsAg positive mothers are a special group and should be tested at 5 months of age (along with HBsAg) to ensure that they are protected and have not become infected after delivery and neonatal immunisation.
- The e antigen predicts high titres of virus, but its absence does not exclude high viral loads, especially in adults who were infected in childhood.

Hepatitis C

- anti-HCV antibody is present in almost all those infected with HCV, but depending on the sex and age at acquisition, only 70-80% will have active viral replication. Like HIV, the antibodies are non neutralising, that is they do not control infection.

MANAGING THE PATIENT

Persons with chronic HBV and HCV are usually asymptomatic until the ongoing liver inflammation results in fibrosis, cirrhosis, hepatic failure or the development of hepatocellular carcinoma (see Figure 1). There are large numbers (up to 8% in some Pacific peoples) of either undiagnosed or diagnosed, but not followed-up, persons in the community. It is essential to monitor and/or treat to prevent these severe outcomes. The Hepatitis Foundation provides help with monitoring and motivating patients with chronic viral hepatitis.

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PRACTICE **POINTS**

- Doctors should be looking to identify patients with raised LFTs due to chronic viral hepatitis.
- All HBsAg-positive pregnant women should have their HBV viral load measured.
- There is somewhat of a hiatus in treating HCV infection, pending the availability of newer agents, but all motivated HCV PCR-positive patients should be referred for assessment and planning.
- Many HBsAg-positive patients are not being properly followed up or referred, and stand the risk of developing preventable liver disease.
- PCR tests should not be ordered without first determining the serology: they are never part of the initial diagnostic screen.

SIMPLIFYING STI TESTING: CO-TESTING FOR CHLAMYDIA AND GONORRHOEA BY PCR *Continued on from page 1 >*

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HOW GOOD IS PCR FOR GONORRHOEA?

In 2009 we installed the fully automated cobas 4800 platform (Roche Diagnostics, NZ) and began using the CT/NG PCR test initially for CT only and commenced evaluation of NG PCR in 2011. We compared the performance of the cobas 4800 NG PCR to culture in 17,400 visits.

Analysis of assay performance compared to culture is summarised in Table 1 . All specimen types surveyed reached the critical values of >95% for sensitivity and specificity and >90% for PPV and NPV indicating that the cobas NG assay may replace culture without the need for supplementary testing (Table 1)

Table 1: Performance of the cobas 4800 NG assay by specimen type

SPECIMEN TYPE	N	SENSITIVITY	SPECIFICITY	PPV*	NPV#
Urogenital	18,206	98.5%	99.9%	91.7%	100%
Non-genital (rectal, eye, throat)	610	100%	99.8%	91.7%	100%
All	**18,816**	**98.6%**	**99.9%**	**91.7%**	**100%**

*positive predictive value #negative predictIve value

Hepatitis B

HBV infection is a very complicated condition and thoughts regarding ideal management are constantly changing. Spontaneous or treatment-induced clearance of virus is uncommon, so the expectation is that long term follow-up and treatment will be required, even if interferon-based therapy is used. Unfortunately, many of those infected with viral hepatitis have financial and other barriers to accessing health care.

The highest priority patients for referral to specialists (gastroenterology or infectious diseases) are those with raised ALT, especially if over the age of 45 and preferably well motivated.

HBV DNA measurement is an essential part of assessment and management:

- To assess risk of progression and eligibility for antiviral treatment (risk increases from levels of 10^4 IU/ml)
- To assess treatment adherence and monitor development of resistance (usually 3-6 monthly on treatment)
- To identify mothers who should receive antiviral therapy in the second and third trimesters of pregnancy (if > 10^7 IU/ml)

Hepatitis B in pregnancy

As described in the PHARMAC schedule and the 2011 Immunisation Handbook, pregnant women who are surface antigen-positive should have their HBV viral load measured in the first trimester. Those with high viral loads may still transmit the infection to the baby (usually prenatally) despite neonatal immunoglobulin and vaccination. The rate of transmission with viral loads >10^8 IU/ml is over 5%, and is dramatically reduced with the use of antiviral therapy in pregnancy.

The PHARMAC schedule is somewhat ambiguous, so it would be wise to discuss all HBsAg-positive pregnant women with a gastroenterologist or infectious diseases physician.

Hepatitis C

In contrast to HBV, with HCV the aim of treatment is cure rather than control. There is a state of flux around treatment of HCV, with several exciting new drugs in the pipeline, but unfortunately it is impossible to guess when they will be available and funded in NZ. The new direct acting antivirals appear to have either fewer side effects or higher rates of cure than interferon-ribavirin.

The current emphasis is to identify and refer motivated patients with raised ALT who are keen to engage in treatment. It is likely that the new expensive drugs will be only available for patients with gentoypes 1 and 4, which do not respond so well to current regimens.

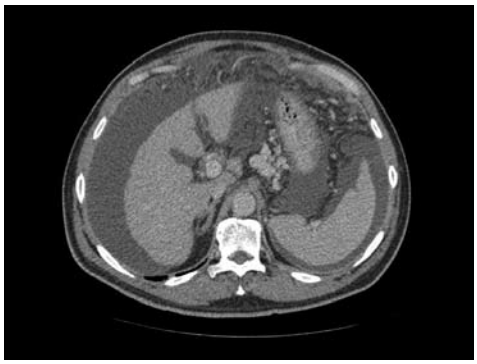

Figure 1 (right): CT scan of a patient with end stage liver disease due to HCV demonstrating a shrunken liver, splenomegaly and ascites. The patient was treated with liver transplantation.

Therefore a viral load test is needed to identify actively infected patients, and once referred, engaged patients will have a genotype test so that those with the favourable genotypes 2 and 3 can be treated sooner rather than later. Those with higher risk of liver fibrosis (older, with longer duration of infection) should also be actively considered for referral.

Regardless of genotype, once on treatment, the viral load is used to identify those doing well and those who are not responding. In practice this means serial viral load measurements at 4 and 12 weeks, and a test of cure after stopping therapy.

NEW DEVELOPMENTS WITH VIRAL HEPATITIS TESTING

The viral load tests are now being referred to Wellington Hospital Laboratory, which will mean shorter turnaround times and electronic delivery of results. As always, to get the best service, please provide full clinical details, including whether the patient is on or off treatment. Urgent tests can only be processed rapidly if there is sufficient information provided to enable us to prioritise.

At this stage the tests will be run every week at Wellington Hospital Laboratory: HCV on Tuesdays and HBV on Wednesdays, so by coordinating specimen collection and clinical decision making with the test runs it should be possible to keep the turnaround times short.

OTHER TESTS USED IN THE ADVANCED MANAGEMENT OF VIRAL HEPATITIS

- **Liver biopsy:** still recommended in many cases of HBV to determine need for therapy, and to determine if treatment can be delayed in HCV
- **Fibroscan:** used to avoid liver biopsy when there are clear results, but not available in the region yet
- **Ultrasound:** mainly used to detect portal hypertension and hepatocellular carcinoma, and to guide liver biopsy
- **IL28B gene polymorphisms:** to identify people more likely to respond to interferon in HCV therapy
- **Alpha fetoprotein:** used to monitor and detect hepatocellular carcinoma in those with liver fibrosis

References

PHARMAC schedule *http://www.pharmac.govt.nz/2011/12/01/SA1047.pdf*

Immunisation Handbook, 2011 *http://www.health.govt.nz/publication/immunisation-handbook-2011*

The Hepatitis Foundation *http://www.hepfoundation.org.nz/*

Hepatitis B management guidelines *http://www.nzsg.org.nz/uploads///Documents/HepBClinical.pdf*

However, the sensitivity of cobas 4800 PCR in female urine is only 78% compared to 100% in male urine (Table 2). Therefore we recommend against urine as a sole screening specimen in females.

Table 2: Performance of the cobas 4800 NG assay in male and female urine specimens

SPECIMEN TYPE	N	SENSITIVITY	SPECIFICITY	PPV*	NPV*
Female urine	564	77.8%	100%	100%	99.6%
Male urine	950	100%	100%	100%	100%

Cobas 4800 NG PCR detected 36% more confirmed NG infections than culture did in our validation cohort.

This assay is able to provide sensitive and accurate results on non-invasive specimens, such as self-taken vaginal swabs for females and urine for males, increasing accessibility of screening to a wider population, especially for patients unable or unwilling to undergo a pelvic exam. Had NG PCR been performed on all the non-invasive specimen types received for CT only, without an accompanying culture, an additional 10,118 patients would have been screened for NG and around 85 additional positive cases identified.

HPV TESTING FOR POST TREATMENT AND "HISTORIC" TEST-OF-CURE

In addition to using high risk HPV (HrHPV) testing to triage all low grade and atypical smears in women over 30 years of age. HrHPV testing is also funded for use in two "test-of-cure" situations. Smear takers need to be aware of these situations and to request testing in appropriate situations.

Molecular testing for high risk (oncogenic) HPV types is easily undertaken on liquid-based cervical cytology specimens and Aotea Pathology examines for the presence of one or more of 14 genital HPV subtypes that have shown an association with high grade dysplasia and cancer. The presence of high risk HPV subtypes in cervical epithelial cells is a more sensitive triage test for the possible presence of HSIL than cytology examination alone.

Conversion to HrHPV negative status after ablative treatment of CIN 2/3 or after long term follow-up for high grade disease correlates with a low chance of developing further high grade cervical disease – a state of "cure" – for a period of up to 5 to 7 years; any future disease being the result of infection with other high risk HPV's. Again negative HrHPV testing is a more sensitive marker of "cure" than negative cervical cytology alone.

The first "test-of-cure" situation involves women eligible for "historical testing"; these are women who have had a previous high grade lesion more than three years ago with negative smears subsequent to this episode. Many women find follow-up yearly smears an imposition and compliance is variable. With HrHPV testing a woman could be returned to normal (3 yearly) screening if her test results are negative for both cytology and Hr HPV on two occasions, 12 months apart. **This historic test-of-cure HPV testing is not ordered as a reflex by the laboratory but rather relies on smear takers to identify if a woman, at any age, has previously been treated for CIN 2/3 and is on annual smears.** Smear takers should inform her of the option of an HPV test with her smear and request this on the laboratory form or eLab visit. There is no cost to the patient for HPV testing in this situation however the woman should make the informed choice about whether to opt for HrHPV testing or to continue to have annual smears. If the cytology and HrHPV test are negative, a repeat negative cytology and HPV test in a further 12 months time is required before a return to normal screening.

The second "test-of-cure" situation arises in women having had ablative treatment of CIN 2/3 within the last three years. The acquisition of the negative HrHPV status is a good proxy for effective treatment and viral clearance. As it can take up to 12 months to clear the virus after ablative treatment HPV testing should wait a year post therapy. As women have usually been referred back to their smear taker for follow up by this point again the **onus is on the smear taker to recognise this situation and request cytology and HrHPV testing 12 and 24 months after ablative treatment**.

What is the implication of a positive HrHPV test in these test-of-cure situations? Those women in the 'historical' category, who test HrHPV positive, continue having annual cytology with HrHPV testing until two negative consecutive events are achieved for both 12 months apart. These women are likely to be at very low risk of CIN 2/3 or worse and a positive HrHPV test where cytology remains negative is not a cause for immediate concern or referral to colposcopy. Women who test positive for HrHPV at 12 or 24 months post ablative treatment for CIN 2/3 or have positive cytology should be referred back to colposcopy as here there is significant risk of residual disease requiring assessment and further treatment even if the cytology is negative.

If confusion remains there is useful information available on the NCSP website (www.nsu.govt.nz) or please contact Aotea Pathology for assistance.

Below: Cobas 4800 instrument used for HPV, Chlamydia and gonorrhoea testing at APL

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PRACTICE **POINTS**

- Women who are currently on annual cervical smear recall because of high grade disease in the past can elect to have funded HrHPV testing; if a woman is HrHPV and cytology negative on two occasions 12 months apart she can return to three yearly screening. Smear takers need to offer this option to women and request this testing when sending in cervical cytology samples.
- Women who have had recent ablative treatment for proven high grade disease need to have HrHPV testing requested by smear takers when undertaking a cervical smear at 12 and 24 months after treatment. The acquisition of the negative HrHPV infection status is considered the best proxy for effective treatment and viral clearance – that is "cure".