Additional Molecular PCR tests available at Southern.IML Pathology

Test	Medicare rebatable
Bordetella pertussis/parapertussis PCR	Yes
Chlamydia trachomatis PCR	Yes
Clostridium difficile toxin (CDT) PCR	Yes
Faecal pathogen PCR	Yes
Genital mycoplasma PCR	Yes
Group B streptococcus (GBS) PCR	Yes
Hepatitis B Viral Load	Must be Hepatitis B positive and on treatment
Hepatitis C PCR	Non-Medicare rebatable cost where Medicare criteria not met. Medicare criteria: ▸ patient is Hepatitis C antibody positive or ▸ patient is Hepatitis C antibody status indeterminate or ▸ to determine Hepatitis status in immunosuppressed or immunocompromised patient or ▸ detection of acute Hepatitis C prior to seroconversion when necessary for patient management or ▸ patient undertaking antiviral therapy for Hepatitis C
Hepatitis C Viral Load	Non-Medicare rebatable cost where Medicare criteria not met. Medicare criteria: ▸ pre-treatment evaluation for antiviral therapy for chronic Hepatitis C and test advised by the specialist who manages treatment of the patients hepatitis ▸ 12 week assessment on combination antiviral treatment If relevant information not supplied, the Molecular Biology department will fax a HCV Medicare criteria form to the referring doctor to complete before testing the sample (except for specialist doctors).
Herpes simplex PCR	Yes
Human papilloma virus PCR	Post-treatment for High-Grade Squamous Intraepithelial Lesions (HSIL) (CIN 2 or 3)
Influenza PCR	Yes
Neisseria gonorrhoeae PCR	Yes
Respiratory virus PCR	Yes
Trichomonas vaginalis PCR	Yes
Varicella zoster PCR	Yes

For further information, or to discuss a patient, please contact our clinical pathologists on 02 4224 7474

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Quality is in our DNA

Molecular diagnosis of infectious diseases – an update

Faecal pathogen PCR	Respiratory pathogen PCR	Genitourinary pathogen PCR	Vesicular rash PCR
Multiplex PCR test now available, adding 10 additional pathogens to menu.	Influenza menu expanded to include 14 more viral targets.	Additional genital pathogens added to menu.	Improved testing added for diagnoses.

Introduction

Nucleic acid testing (NAT), most commonly using polymerase chain reaction (PCR), has many advantages over traditional microbiological techniques. These include greater diagnostic sensitivity and specificity and more rapid availability of results. Molecular tests have therefore become the diagnostic tests-of-choice for many infectious diseases.

Southern.IML Pathology has been at the forefront in the provision of a high quality molecular diagnostic service to medical practitioners in private practice. Assays are currently available for a wide range of bacterial and viral pathogens and we are committed to the evaluation and implementation of additional molecular assays for the rapid and accurate diagnosis of infection.

Recently, Southern.IML Pathology has introduced a range of new molecular tests which detect multiple organisms at once ('multiplex' assays) and which are Medicare rebatable.

This newsletter outlines key testing developments in this area. If you have any questions, our clinical microbiologists would be delighted to discuss any of the above- mentioned assays in more detail.

Dr Ian Chambers
Dr Miriam Paul
Dr Michael Wehrhahn

Faecal pathogen PCR

- Multiplex PCR for 10 most prevalent enteric pathogens (new)
- *Clostridium difficile* PCR

Since January 2014, in addition to PCR for *Clostridium difficile*, a multiplex PCR for 10 additional faecal pathogens has been available through Southern.IML Pathology. This assay includes five bacterial pathogens: *Campylobacter, Salmonella, Shigella, Aeromonas* and *Yersinia* and five protozoan pathogens: *Giardia, Cryptosporidium, Entamoeba histolytica, Dientamoeba fragilis* and *Blastocystis hominis*. One important advantage is the more rapid time to results, particularly for the bacterial pathogens, with results expected within 24 hours rather than 48-72 hours. Also of special note is the far greater ability to detect *Dientamoeba fragilis*. This is an under-diagnosed pathogen which may cause nausea, diarrhoea, fatigue and which may be the cause of failure to thrive in some children. Until now, it has been poorly detected without the aid of a formalin preserved/SAF collection. Similarly, the faecal PCR allows the rapid differentiation of *Entamoeba histolytica* (the cause of amoebic dysentery) from the more common, morphologically identical but non-pathogenic *Entamoeba dispar*.

However, it is important to note that while PCR improves the detection of those pathogens which are targeted, it cannot detect those which are not and does not provide information on antibiotic susceptibility. It is recommended that faecal PCR is not requested on its own as conventional methods, such as microscopy and culture, will continue to have the great virtue of being able to detect the unexpected and potentially allow susceptibility testing. This should be borne in mind, particularly when investigating gastrointestinal illness in returning travellers, immigrants and the immunocompromised.

To comply with Medicare, the request must be written as faeces MCS, OCP and PCR and only one sample per 7 days will be rebatable. When less common pathogens, e.g helminthes are suspected, 2 OCP collections within a 7 day period are covered by Medicare.

Molecular diagnosis of infectious diseases – an update

Respiratory pathogen PCR

- *Bordetella pertussis* and *Bordetella parapertussis*
- Respiratory viruses (RV PCR)

In Winter 2013, Southern.IML Pathology expanded its respiratory PCR repertoire from influenza A (and its subtypes), influenza B and *Bordetella pertussis* and *parapertussis* to include another 14 viral targets in addition. This 16 target assay includes parainfluenza 1-4, respiratory syncytial virus (RSV) A and B, human metapneumovirus (HMPV), adenovirus, the three most common coronaviruses, the enterovirus group (which includes coxsackie A and B viruses, echoviruses and the remaining enteroviruses), rhinovirus and the relatively newly identified bocavirus. Since its introduction, more than 5000 samples have been tested using these 16 target assay and approximately half of these have had one or more viruses detected.

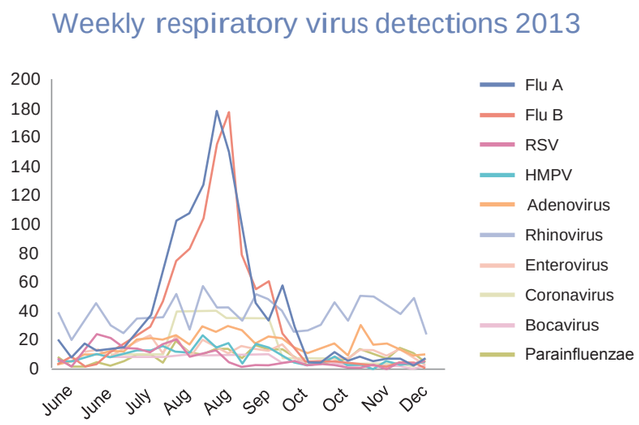


Figure 1: Diagram showing the different viruses being detected and their peaks

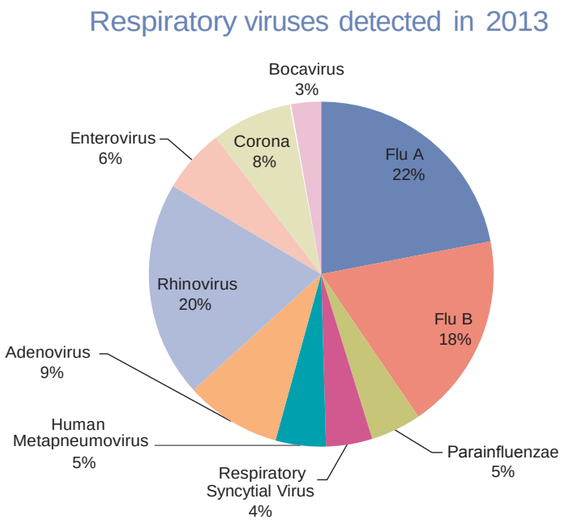


Figure 2: Pie chart demonstrating proportions of different viruses

Many patients have had the aetiological agent causing their clinical syndromes clarified: for example, flu-like illness has been confirmed as influenza or parainfluenza; pharyngitis has mainly been caused by adenovirus, RSV and enterovirus; croup has been mainly due to parainfluenza 1 and bronchiolitis has been due to RSV, human metapneumovirus, parainfluenza 3 and adenovirus. It is hoped that the confirmation of suspected viral illness, especially when severe, will assist in limiting the use of antibiotics and in turn reduce antibiotic resistance in the community. While nasopharyngeal aspirates and bronchoalveolar lavage samples have traditionally been the best samples for detecting viruses, with the advent of dry flocked swabs which have a far greater surface area to pick up virus, nasopharyngeal and even mid-nasal and throat swabs frequently detect virus.

Genitourinary pathogen PCR

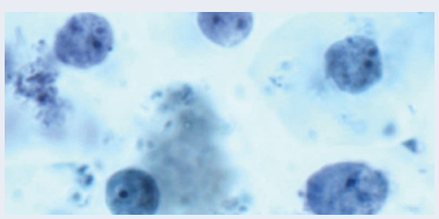
- *Chlamydia trachomatis* and *Neisseria gonorrhoeae* PCR
- HSV 1 and 2 PCRs
- Group B streptococcus PCR
- *Trichomonas vaginalis* PCR
- Genital mycoplasma PCR
  - *Mycoplasma genitalium* and *hominis*
  - *Ureaplasma urealyticum* and *parvum*

In addition to the longstanding use of PCR for the detection of the common sexually transmitted infections, *Chlamydia trachomatis* and *Neisseria gonorrhoeae*, Herpes simplex virus 1 and 2 and Group B streptococcus for prenatal screening, Southern.IML Pathology has recently been enhancing diagnosis of other important genital pathogens, such as *Trichomonas vaginalis* and the genital mycoplasmas. First pass urine, genital swabs (flocked swabs best) and ThinPrep® samples are all suitable for testing.

Two parasites now best detected by PCR

1) *Trichomonas vaginalis* (detected with Trichomanas PCR)

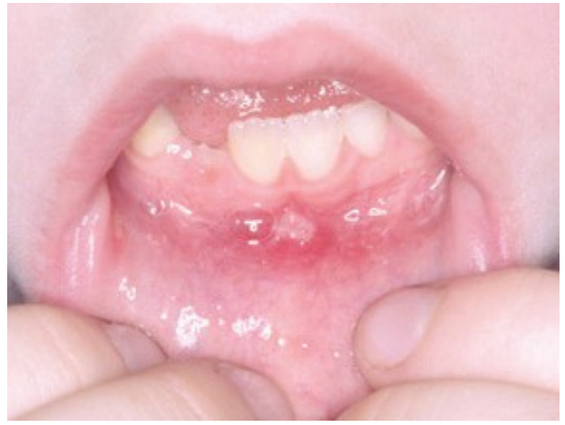
2) *Dientamoeba fragilis* (detected with faecal PCR)



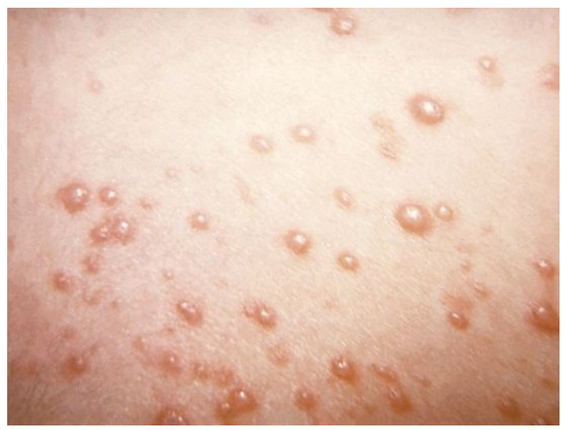
Vesicular rash PCR

- Herpes simplex (type 1 and 2) PCR
- Varicella zoster virus PCR
- Enterovirus PCR

In the last 12 months, Southern.IML Pathology has been using an assay that is performed on all requests for Herpes simplex virus 1 and 2 or Varicella zoster virus, as the vesicular rash caused by these three viruses may be identical. The respiratory virus PCR which includes enterovirus is similarly sensitive at detecting virus from flocked swabs of the vesicles of suspected Hand, Foot and Mouth disease.



Oral lesion of hand, foot and mouth disease



Vesicular rash seen with chicken pox