**DIAGNOSTICS 2
THE DIAGNOSIS OF INFECTION AND THE USE OF
THE VIROLOGY LABORATORY**

Dr Mark Atkins (m.atkins@imperial.ac.uk)

**Learning objectives**

* Appreciate the range of viruses that can cause human disease.
* Understand what tests are available for diagnosing viral infections.
* Know what clinical samples to take to enable you to make the correct diagnosis.

When diagnosing a viral illness, taking a good history and performing a clinical examination can often give you the diagnosis. Some infections are easy to diagnose such as shingles and chicken pox. However many have more subtle signs. When talking a history it is important to include, vaccination history, travel (especially in the previous 3 weeks), contact with animals/pets, contact with infected persons and occupation. This information may give you some important clues.

Diagnosis depends on the clinical findings, the detection of specific antibodies and/or the detection of a virus in the appropriate clinical sample.

# Virological tests- ideal tests should have the following qualities

## High specificity i.e. have a low level of cross reactivity.

## Sensitive- detect the virus or the antibody at very low levels

## Rapid- results should be available in a timely fashion.

## Non-invasive. This reduces the risks of the procedure and makes then easier to repeat if necessary.

## Cost effective. Most virology tests only cost a few pounds each but some of the molecular tests are significantly more expensive, so use them wisely.

# Diagnostic methods

## Cell culture

## Electron microscopy (EM)

## Antibody detection e.g. HIV antibody

## Antigen detection e.g. HBsAg in hepatitis B infection or RSV antigen in respiratory sample

## Genome detection – e.g. using PCR to detect viral DNA or RNA

## Quantification of antigens and genomes (now essential for diagnosis and monitoring of HIV, HBV and HCV)

# Samples to take will depend on the disease being investigated

## Throat swab - for virus isolation (in virus transport medium, VTM) - useful in the diagnosis of enteroviruses and respiratory viruses.

## Stools - for EM and Rotavirus EIA (in sterile pot) - for the diagnosis of enteroviruses and viruses that cause diarrhoea such as rotavirus, astrovirus, adenovirus, noroviruses, etc.

## CSF - PCR for herpes and enteroviruses (in sterile container, VTM (viur transport medium) not required) - for the diagnosis of viruses causing meningitis or encephalitis such as HSV, VZV, enteroviruses, mumps, etc.

## Nasopharyngeal aspirate (NPA) - for respiratory viruses using Immunofluoresence (IF) or PCR, such as RSV, influenza A&B, adenovirus, parainfluenza viruses, SARS etc.

## Urine - virus isolation or PCR depending on which viruses you are interested in (in sterile container), e.g. BK virus, CMV, etc.

## Blood (clotted) - for antibody detection

## Blood (EDTA) - for PCR. Used for detection and quantification of HIV, HBV and HCV.

Biopsy samples can be useful in certain circumstances e.g. brain biopsy in encephalitis.

# Electron microscopy

## Virus structures can be visualised using an electron microscope

## Used mostly for stool samples.

# Immunofluorescence (IF)

## Useful for the direct detection of viral antigens in clinical samples (eg respiratory viruses)

## Can be used for typing and culture confirmation

## Relatively quick and inexpensive but subjective and very dependent on the skill of the technician and the quality of the sample

# Enzyme Immuno assays (EIA’s)

# Detection of antibodies and antigens using immunoassays.

## EIA’s (enzyme immunoassays)

## Western blots

## RIBA’s (recmbinant immunoblot assays useful for eg typing anti-HIV 1 &/or 2)

## Specific, sensitive and relatively easy to automate.

## Can be adapted to detect specific antibody classes e.g. IgM IgG or IgA.

## Sensitive and can quantify amounts of antibody (e.g. anti –HBs antibody)

## Adaptable to antibody or antigen detection

## Examples include HIV antibody and antigen, Hepatitis A,B,C serology, rubella, mumps, parvo etc

# EIA’s for HIV antibody detection

## Detection of specific antibody is an indirect method of detecting infection.

## Non-specific reactions can be a problem, therefore it is important to use multiple formats (generally use 3 different assays).

## Interpretation of results must take the clinical circumstances into account.

# Viral gene detection and quantification

## Polymerase chain reaction PCR (a target amplification system)

## bDNA (signal amplification system)

## Both assays used to measure “viral load” in HIV, HCV HBV infection.

# Polymerase chain reaction

## Target amplification to allow detection and quantification over very large dynamic ranges (> 5-8 logs)

## Can be very sensitive (as low as 1 genome copy)

## Can subtype viruses from PCR products

## Problems with contamination. This can be overcome using “Real Time” PCR.

# “Real Time” PCR. Advantages

## Quantification during linear phase gives better reproducibility, precision and dynamic range.

## Readily adapted to multi-plexing i.e. detect multiple viruses in the samples simultaneously.

## Closed tube monitoring eliminates contamination

# Summary

Many virus infections can be diagnosed by the detection of specific antibodies. This is particularly so for HIV, HCV and HAV.

The detection of viruses in the appropriate clinical sample using cell culture, immunofluorescence or PCR can be diagnostic during acute or chronic infections.

Modern detection methods e.g. PCR can be applied to most viruses. These are very powerful diagnostic tools but they must be used with caution.

## Antibody and antigen detection will be supplemented by genome detection and quantification for a wider range of viruses

## Resistance testing, genotyping and other DNA/RNA based tests are become more widely used.

## However, as with all tests, the results need to be interpreted in the clinical context of the patient.