Very often it is difficult to make a clinical diagnosis of a specific viral infection, as many viruses have clinically similar presentation (e.g. hepatitis viruses, Chapter 38) and the same virus can have many different clinical presentations (e.g. entero-viruses, Chapter 5). It is therefore essential to seek specific laboratory diagnosis to enable correct management of the patient. This may be important from an epidemiological perspective as well. It has been shown that even experienced clinicians are not able to clinically diagnose cases of rubella or measles correctly. A specific diagnosis of a viral rash is important, not only to the clinical management of the patient, but essential for control of infection, outbreak control and the public health perspective of continuing to ensure the efficacy of vaccination programmes.

Antibodies are produced as a host response to viral infection. Immunoglobin A is produced at the local site of infection and provides local immunity, for example in the gut or respiratory tract. The generalized humoral immune response is mounted by B lymphocytes and the first antibody to appear is of the IgM class, which can be detected as early as a couple of days after an acute infection. Some of the B lymphocyte clones then switch over to producing IgG antibody, which appears from 7–15 days after onset of infection. Both classes of antibodies continue to rise in response to the infection, peaking at about 6 weeks post infection. Viral specific IgM then declines and is normally undetectable by about 3 months after infection. IgG antibody persists for life and is responsible for providing lifelong immunity to the particular virus. Figure 47.1 shows the sequence of serological response after viral infection.

**Acute or recent infection can therefore be diagnosed by:**

- demonstrating the presence of virus specific IgM (IgG may or may not be present)
- showing a rise in antibody titre between an acute and convalescent specimen, or
- a high antibody titre in a convalescent specimen.

**Past infection or immunity is diagnosed by:**

- demonstration of virus specific IgG alone (and absence of IgM).

Serology is used widely to diagnose viral infections as many of the viruses cannot be easily cultured. ‘Serology’ means the study of serum and can be used to detect both antibody and antigen (e.g. hepatitis B surface antigen). Several techniques have been developed, but the fundamental principles are similar for all.
Principle of serological techniques

Assays may be qualitative (e.g. give only a yes or no answer) or be quantitative (e.g. measure the antibody level).

As a rule, assays that utilize the presence of IgM or IgG to make a diagnosis are usually qualitative, as presence or absence of these antibodies is sufficient to make a diagnosis.

On the other hand if diagnosis relies on detection of a rising or a high antibody titre, then the assay needs to measure the level of antibody response (quantitative). Antibody ‘titre’ is expressed as the inverse of the highest serum dilution at which the antibody is detected. For example, influenza A antibody titre of 128 means that antibody to influenza A was detected until a 1 in 128 serum dilution, but not in higher dilutions.

Many of the quantitative assays have been developed by exploiting the functional properties of antibody response (e.g. complement fixation, haemagglutination, neutralization tests). Table 47.1 shows diagnostic uses of the serological techniques.

Techniques

All serological techniques that detect antibody are based on the principle of adding specific viral antigen(s) to patient serum. If virus-specific antibody is present in the serum then it will bind to the antigen to form an antigen/antibody complex. An indicator system (depending on the technique) is then used to detect whether such a complex has been formed. These techniques can be reversed to detect the presence instead of viral antigen, such as hepatitis B surface antigen, in the patient’s serum.

Enzyme-linked immunosorbent assays (EIA or ELISA)

These are the most widely used serological assays in routine diagnostic laboratories. There are several variations on the technique but essential steps are shown in Fig. 47.2 a to d.
Table 47.1. Diagnostics uses of the serological techniques.

<table>
<thead>
<tr>
<th>Test</th>
<th>Example of use</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complement fixation test (CFT)</td>
<td>Respiratory viruses – measures total antibody, is quantitative; to diagnose recent infection acute and convalescent serum samples are required to show rise in titre.</td>
</tr>
<tr>
<td>Enzyme-linked immunosorbent assays (EIA or ELISA)</td>
<td>IgG/IgM antibody – rubella, measles, mumps, HIV, hepatitis A etc.</td>
</tr>
<tr>
<td></td>
<td>Antigen – hepatitis B surface antigen in serum samples, norovirus and rotavirus antigen in faeces.</td>
</tr>
<tr>
<td>Immunofluorescence (IF)</td>
<td>IgG/IgM antibody – EBV, VZV.</td>
</tr>
<tr>
<td></td>
<td>Antigen – RSV, influenza and other respiratory viruses in respiratory secretions.</td>
</tr>
<tr>
<td>Latex and gel particle agglutination</td>
<td>Antibody – rubella, toxoplasma.</td>
</tr>
<tr>
<td></td>
<td>Antigen – rotavirus, norovirus.</td>
</tr>
<tr>
<td>Western blot (WB) and line assays (LIA)</td>
<td>Used to confirm HIV and HCV-screen positive specimens.</td>
</tr>
<tr>
<td>IgG avidity assays</td>
<td>To confirm recent CMV, rubella and toxoplasma infections.</td>
</tr>
</tbody>
</table>

Fig. 47.2. a–d Figurative representation of enzyme-linked immunosorbent assay (EIA).

(a) Antigen is attached to the base of a plastic microtitre well (solid phase).
(b) Patient’s serum is added to this microtitre well. If specific antibody is present in the serum it will attach to the antigen on the solid phase. Excess serum is washed off.
(c) Anti-human antibody coupled to an enzyme is added to bind to this antibody/antigen complex. Excess enzyme is washed off.
A substrate for the enzyme is added, a colour change indicates a positive reaction due to the action (on the substrate) of the enzyme which has been bound to the antigen/antibody complex.

The colour change in the EIA can be detected by eye or measured in a spectrophotometer, and the intensity of the colour can indicate how much antibody is present in the serum. Figure 47.3 shows positive (coloured) and negative (colourless) reactions in the EIA test.

The EIA can be constructed to detect either IgG or IgM depending upon whether the anti-human antibody is directed to the IgM or IgG class. Positive and negative controls are added to the assay runs to ensure the quality of the assay system. Most of the EIAs in use have a very high sensitivity and specificity (>95%), some even approaching 100%.

The assay can also be done in reverse to detect viral antigens simply by coating the solid phase by antibody (mono- or polyclonal) specific for the antigen to be tested.

Advantages of EIAs are:
• they are rapid – most can be done within 2–3 hours
• they can be easily automated
• they are objective – the reaction can be read by spectrophotometer.

Immunofluorescence tests (IF or IFT)
These assays use the same principle as EIA, and like EIA they can be constructed to detect either viral antibody or antigen in the patient specimen. However, instead of the enzyme/substrate detector system of EIA, fluorescein-labelled anti-human
antibody is used to detect a positive reaction, which appears as apple-green fluorescence under a light microscope. Figure 47.4 shows a positive VZV IF reaction.

To look for viral antigen, cells from the patient’s secretions (e.g. nasopharyngeal aspirate) are fixed to a spot on the glass slide and fluorescein-labelled monoclonal antibody against the virus (RSV, influenza A etc.) is added. A mixture of these monoclonal antibodies can be added at the same time to detect a panel of viruses (e.g. respiratory viruses, all at one go).

Immunofluorescence tests are also rapid serological tests, but the disadvantage is that they require subjective interpretation and are therefore labour intensive to carry out and are dependent upon operator expertise.

**Latex agglutination (LA) and gelatin particle agglutination test (GPAT)**

Here the antigen or antibody is adsorbed on an inanimate particle (latex or gelatin) and a positive reaction is indicated by agglutination of the particles.

**Complement fixation test (CFT)**

This test is based on the principle that when an antigen/antibody complex is formed it will ‘fix’ (bind) complement, so free complement is not available to lyse sensitized red cells that are added as indicator.

Complement fixation tests have been extensively used in the past to aid clinical diagnosis; however, because of their complexity and relative insensitiveness they are now being replaced by newer tests such as EIA.
Haemagglutination (HA) and haemagglutination inhibition tests (HAI)
These tests detect antibodies to viruses (rubella, influenza) that possess a haemagglutinin antigen. These are also relatively insensitive and can give non-specific reactions, and have mostly been replaced by more sensitive and specific techniques.

Neutralization tests (NT)
Virus-specific neutralizing antibodies, if present in the serum, will neutralize the virus so it is not able to grow in culture. This is a very specific but labour-intensive and technically demanding technique, and is being replaced by more modern techniques.

Western blot (WB) or line immunoassays (LIA)
Specific viral proteins are transferred on blotting paper either from a gel (western blot) or produced by recombination or peptide synthesis (line immunoassays). Further steps are similar to those of EIA (see above). The viral antigen band on the blotting paper develops colour if specific antibody to that particular antigen is present in the serum.

The advantage of these techniques is that the assays are able to distinguish antibody directed against specific virus proteins and are therefore very specific.

Antibody avidity assays
The host antibody response matures over several weeks post acute infection. Therefore, antibody detected >3 months after acute infection binds strongly (high avidity) to antigen(s) used in laboratory assays; as a corollary the antibody in the first 3 months has weak binding (low avidity) and can be easily dissociated from the antigen/antibody complexes.

This is used as a principle in tests devised to measure IgG avidity. These tests are helpful in distinguishing primary infections from reinfections or reactivations, as in the latter IgG is of high avidity.

Use of non-serum samples for antibody tests
Enzyme-linked immunosorbent assay antibody tests have been adapted for use on urine (HIV) or salivary samples (HIV, Hepatitis B and C, measles, mumps and rubella). This is of great advantage in those who are needle phobic or difficult to bleed, such as intravenous drug users (IVDUs) or neonates/small children. A special kit is required to collect the salivary sample for antibody testing.

Automation in serology
All the steps in the EIA lend themselves to automation, and several systems are now available on the market. This has enabled the laboratories to process thousands of specimens very quickly and improve turnaround times for results. The latest generation of automated machines are called ‘random access’, as the specimens do not have to be
batched and an urgent specimen can be put on the machine at any time without disrupting the other assays already on it. Results can be obtained in under an hour.

Many of this automated serology equipment in use in the virology laboratory is common to clinical chemistry and immunology, therefore the technology is further driving the way that specialist laboratories work. Many hospitals now have 'blood sciences laboratories' where automated machines are linked with specimen tracks that act as assembly lines to load the specimens on to the machines.

Most laboratories also have interfaces linking their machines to a laboratory computer system; linking of the laboratory computer system to a hospital-based IT system allows the clinician to access these results as soon as they are ready. This also has the advantage that if barcodes are used for specimen recognition, samples are not registered to the wrong patients accidentally.

**Future of serology**

- Despite the availability of rapid molecular diagnosis (Chapter 49) serology remains an important tool for the diagnosis of acute and chronic viral infections.
- It is of particular value in assessing the immune status of patients, either as a result of natural infection or post-immunization.
- Serological screening of blood for bloodborne viruses is mandatory in many countries around the world.
- It is a very important public health tool for epidemiological studies to provide prevalence of infection data.

**Conclusion**

The technically demanding serological assays of the past have largely been replaced with rapid diagnostic techniques in serology. Automation of EIA has further revolutionized the way that the laboratories operate, and a result can be given for patient management to the clinician within hours of the specimen arriving in the laboratory.

Despite the popularity of molecular diagnostic techniques for viral diagnosis, serological techniques will continue to form an important part of the laboratory’s armoury for some time to come.