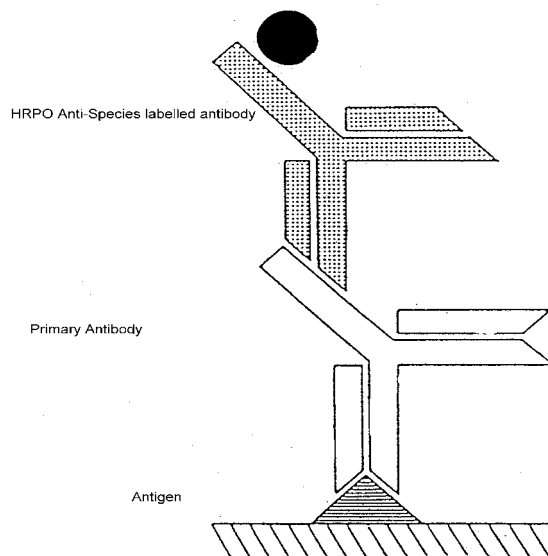


Enzyme Linked Immuno Sorbent Assay (ELISA)

The MVMS routinely uses an antibody capture ELISA system to detect antibody which is specific for the agents under investigation in rodent sera.

A schematic representation of such an ELISA is given below.



Theory

In this system Antigen (this may be purified or semi-purified virus, or a recombinantly produced protein specific for the virus) is bound to the well of a plastic ELISA plate. The animal serum at a predetermined dilution is added to the well, if any specific antibody (Primary antibody) is present it will bind to the antigen. The test serum is then removed and the ELISA plate is washed thoroughly and a species specific enzyme conjugated antibody is added (sometimes called conjugate or secondary antibody). After a suitable interval this is also removed and the plate washed. A substrate is then added which will give a colour reaction in the presence of HRPO and the resultant colour change is measured by a spectrophotometer. If there is no antibody present in the test sample, the labelled antibody will not be able to bind and will be washed out which means that there will be no HRPO present to react with the substrate and no colour change will take place.

Although the theory is extremely simple, there are a number of aspects of the assay which have to be optimised and a number of controls and checks which have to be in place before a meaningful interpretation of the test results can be made.

Antigens

Most viral antigens are prepared by growing the virus in cell culture and then purifying the virus to some degree prior to using it in the test system. Purifying viral antigen to the point where there are **absolutely no** cellular components left is extremely difficult, costly and

Enzyme Linked Immuno Sorbent Assay (ELISA)

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time consuming and in general this is not a practical option for most testing laboratories. As a result most antigens are purified to an extent, but will undoubtedly contain some cellular components as contamination. A **cell control** is used in all of our assays where antigen is produced by growing virus in cell culture. This allows us to detect sera which contain antibodies to cellular components and allow for this in our interpretation of the results.

Performance

Assay performance can be improved by increasing the "signal to noise ratio". This means that we take steps to maximise the intensity of reactions given by positive sera whilst decreasing the background reactions given by negative sera. This is done by using the purest antigen available at an optimum concentration to allow more specific antibody to bind and by the addition of blocking agents to test serum and conjugate diluents to minimise the amount of non-specific immunoglobulin binding which may occur.

Interpretation

Despite all of this, no serological test is fool proof and false positive, false negative and non-specific reactions still occur. Some of the reasons why are outlined below.

False Negatives

- Antibody levels were below those detectable by the assay system used
- Animals were sampled during or very shortly after the active phase of infection, before the development of serum antibodies
- Samples came from immunodeficient or immunosuppressed animals
- Antibodies were formed to a serotype that does not cross react with the assay used
- If there is a low prevalence of antibody positive animals, the sample number was insufficient
- Sentinels not adequately exposed
- Incorrect sample preparation or storage

False Positives

The causes of false positives in ELISA assays are not always clearly understood. They occur when antibody in a specimen binds non-specifically to the antigen but not to the cell control. They may also be caused by:

- Improper sample preparation and storage
- Improper preparation or performance of the assay
- Presence of maternal antibody

Non-Specific Results:

- Improper sample preparation and storage
- Low level bacterial contamination
- Severe haemolysis
- Antibody present in the sample which is directed against murine cellular components – especially true of aged animals and an increased incidence of NS results has been noted in some transgenic lines.



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Action

Interpretation of results should always be done with caution. False positive results should be suspected if the reaction intensities are low and/or the number of positive results is low, taking into account the expected prevalence of the disease agent during an outbreak. In the event of an unexpected positive result being found, rule number one is **DON'T PANIC**. Positive results must be confirmed by further serological testing (same or different test method) and/or other methods such as pathology, culture, electron microscopy, PCR.

Even if a positive is confirmed, take time to consider the situation and evaluate if the infection which has been found is really a threat to the work being carried out and plan your response accordingly.

Selected Reading

"Immunoassay, a Practical Guide", Chan DW, Perlstein NT (1987), Boston: Academic Press

"Infectious Diseases of Mice and Rats", National Academy Press

Serologic Screening for murine pathogens: Basic Concepts and Guidelines. Lab Animal (1988), 89: 40-47

The Mouse in Biomedical Research, Vol II Diseases.



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