

Pandemic (H1N1) 2009 influenza testing in the critical care setting



Summary

Polymerase chain reaction (PCR) testing is currently regarded as the 'gold standard' for the laboratory detection of pandemic H1N1 (2009) influenza virus.

The results of testing are dependent on the quality of the sample. This is affected by the site where the sample is taken, the skill of the person taking the sample, the storage conditions of the sample and the time between the sample being taken and it being tested.

In those patients with an illness consistent with pandemic flu but where there is a negative nose or throat swab, then a sample from the lower airways should be sent for testing. Treatment should continue until the result of this sample is known.

A negative result may also indicate the absence of infection and multiplex PCRs for respiratory viruses should be considered in all cases.

Serial testing should only be undertaken with the agreement of the local virologist/microbiologist and against agreed treatment/management objectives.

Oseltamivir resistance testing should be considered in patients with confirmed H1N1 who deteriorate or fail to respond clinically to treatment with oseltamivir.

Issues

Intensivists taking part in the fortnightly HPA pandemic flu critical care teleconference have expressed concerns about the sensitivity of the PCR tests for influenza. There have been instances where combined throat and nasal swabs have been negative but lower respiratory samples have been positive, constituting a false negative with implications for clinical and infection control decisions.

A number of intensivists are carrying out serial sampling so guidance is now needed on the value and place of sequential testing.

Background

PCR is considered by many to be the 'gold standard' against which all other methods of testing should be compared. It is more sensitive than immunofluorescence or any of the current, commercially available near-patient antigen testing kits^{1,2,3}.

The current PCR tests are very sensitive but the seasonal assay will not detect the pandemic flu virus, so a specific assay for this virus has been developed and used during the pandemic.

The pandemic H1N1 PCR, developed by the HPA, is comparatively more sensitive than the seasonal H1N1 influenza assay, but is specific for the different, pandemic H1N1 target.

PCR testing is not available in a number of district general hospitals and it is likely that influenza testing in this setting may be undertaken using less sensitive, non-specific PCR or non-PCR tests. These are not recommended in the ITU setting.

Animal studies carried out in mice, ferrets and non-human primates have shown that the pandemic flu virus replicates well in the lungs and is found in higher titres in the trachea and lungs than the nasal turbinates⁴.

Viral load can be assessed semi-quantitatively using the CT value of the PCR test and this could be useful in determining the efficacy of treatment and the duration of infection control measures, but the clinical value of this needs to be determined. At the moment, well evaluated quantitative tests for viral RNA are not available. These are being developed by the HPA and will be available in the reference laboratory for use where quantitation is indicated, but it is not expected that this will be used routinely in diagnosis or surveillance or rolled out to the testing network laboratories.

Note: a positive detection of viral RNA by PCR does not equate to viable virus, especially late on in the recovery phase.

If the patient is on oseltamivir, failure to improve on treatment could be indicative of antiviral resistance and antiviral resistance testing should be undertaken.

There is increasing evidence that oseltamivir resistance can readily emerge in immunocompromised patients. It is therefore recommended that people in this group are either treated with zanamivir or oseltamivir and zanamivir combination in line with locally agreed protocols.

Discussion

Detecting the presence of influenza virus will depend on two main factors:

- The sensitivity of the test.
- The quality of the sample.

The current PCR tests are considered to be very sensitive and will detect very small amounts of viral RNA. The quality of the sample is therefore an important aspect to be considered.

Sample quality will vary depending on the competence of the sample taker, the site, the point in the illness the sample is taken from, the storage conditions and time between the taking of the sample and testing.

For the current pandemic flu virus, animal studies have suggested that the virus may be present in greater amounts in the lower airways and may in fact be localised to the lower respiratory tract in critically ill patients with a viral pneumonitis. **In a critically ill case the upper respiratory samples may well be negative.**

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It will also be important to consider other viral infections as causes of the patient's illness if negative influenza results are obtained.

Testing in district general hospitals may not be done by PCR or using the HPA PCR test. Using less specific tests could have a significant impact on the sensitivity of the testing.

Severity of illness or prediction of severity is difficult to evaluate confidently based on CT value of pandemic flu PCR alone, especially when sampling methods are not standardised. The CT values are not comparable between laboratories or between patients. Reproducible quantitative PCR testing is a potential area for development.

Most patients should be considered infectious while they have symptoms. It is important to note that a positive detection of viral RNA by PCR does not equate to viable virus, especially later on in recovery phase.

Immunocompromised patients can shed virus for prolonged periods of time, even after the resolution of symptoms. It is important that this group of people are encouraged to adopt a high standard of respiratory hygiene and that infection control precautions are likely to be required for longer.

There is evidence that clinical diagnosis is not reliable in differentiating individual respiratory virus infections either in adult or paediatric settings. Indeed, more than one respiratory pathogen may be active at the same time in any patient, especially among children. Laboratory testing for an array of respiratory viruses (including influenza) is indicated in the ITU setting.

Recommendations

- Administration of oseltamivir should not be delayed while waiting for sampling results.
- In an intensive care patient with a history and clinical picture strongly suggestive of influenza a lower respiratory tract specimen is preferable to an upper respiratory tract specimen. A negative influenza test result based on samples from the upper airways may be unreliable and should be repeated, if possible, using a sample from the lower airways. In this situation antiviral treatment should not be stopped until a second negative result is obtained.
- Samples should be tested as soon as possible – if this is not possible then samples should be stored at 4°C until they can be tested.
- Viral yield is likely to be highest early in an illness and usually drops significantly once treatment is instituted. Samples should therefore be taken as soon as possible in the course of the illness.
- If clinicians decide to consider serial sampling, this must be discussed with the local microbiologist/virologist in advance. There are some important reasons to consider repeat testing such as concerns over sensitivity of the

sampling, to further the understanding of the disease progression and to carry out oseltamivir resistance testing. However, it is important to be clear about why serial sampling is being undertaken rather than simply to perform a routine of repeated testing.

- Multiplex PCRs for respiratory viruses should be considered in all cases, as an alternative diagnosis may be found or a dual infection may be present. This service is generally available throughout the UK in virology laboratories.
- Oseltamivir resistance should be considered in patients with confirmed H1N1 who deteriorate or fail to respond clinically to treatment with oseltamivir. Further sampling should be undertaken.
- Respiratory hygiene and infection control precautions are likely to be required for longer periods in patients who are considered to be immunocompromised.

References

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Version control

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