



Rational use of multiplex molecular assays for diagnosis of infectious disease syndromes

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The syndromic panel approach or “syndromic testing” is a clinical symptom-driven grouping of probable pathogens into one test. Multiplex molecular (mainly polymerase chain reaction or PCR) assays are available as rapid near-patient tests for respiratory, meningitis/encephalitis and infectious diarrhoea syndromes.

What we know about multiplex molecular assays

Multiplex molecular assays are:

- Sensitive and specific
- Reduce turn-around time
- Use less sample volume
- Improve the likelihood of identifying the infecting pathogen (*improve diagnostic yield*) compared to conventional testing protocols

Clinical impact

Receiving the correct answer within a clinically relevant time frame should enable the treating clinician to improve patient care and save healthcare cost by:

- Improved treatment decisions and patient management by starting adequate, targeted, and appropriate treatment earlier.
- Minimising the unnecessary or inappropriate use of antibiotics/antivirals, and thus also preventing microbial resistance.
- Prevention of unnecessary procedures or investigations, e.g. ancillary laboratory testing and imaging studies.
- Facilitating infection control via targeted patient isolation which can prevent the nosocomial spread of pathogens.
- Preventing or reducing inappropriate hospital stay.
- Acquiring knowledge of seasonality or disease prevalence that can influence healthcare of other patients.
- Earlier identification of outbreaks.

The correct and relevant utilisation of multiplex molecular testing and the interpretation of results are crucial. Some recent guidelines include guidance with regards to the indications and use of these tests in the context of a suspected infectious disease, but for many scenarios definitive and up to date guidance are lacking.

The syndromic testing panels currently readily available will be discussed in further detail.

Respiratory tract infection panels

- The first syndromic panels became commercially available approximately 10 years ago, and currently multiple different platforms and panel designs are available. These panels were a welcome replacement for insensitive and cumbersome viral cultures/stains and imperfect viral and bacterial serological assays.
- *Target:* Most common viral and bacterial pathogens that cause respiratory tract infections
- *Sample types:*
 - Nasopharyngeal swab (upper respiratory tract pathogens)
 - Nasopharyngeal aspirate or bronchial lavage (lower respiratory tract pathogens)

- *Sample volume required:* Typically around 300 µL
- *Who should get tested?*
 - Routine laboratory testing of people with uncomplicated influenza-like illness is not recommended as it provides no advantage in the management of individual patients.
 - Consider testing patients who meet the criteria for complicated or severe disease where a laboratory diagnosis will assist in patient management, e.g. complicated influenza is defined as:
 - people requiring hospital admission
 - people with symptoms and signs of lower respiratory tract infection (hypoxaemia, dyspnoea, tachypnoea, lower chest wall indrawing and inability to feed)
 - people with central nervous system involvement
 - people with a significant exacerbation of an underlying medical condition
 - Certain patients are at a higher risk of complications, e.g. young infants, the elderly, immunocompromised, and patients with co-morbidities.
 - Testing is indicated in clusters of cases where a diagnosis of the cause of the outbreak is needed (e.g. within institutions such as healthcare facilities, and nursing homes).
 - Repeat testing is not recommended within a 7 day period unless there is an acute change in the clinical condition of the patient suggesting a new infection.

Meningitis/encephalitis (ME) panels

- Laboratory diagnostic tests are compulsory to confirm central nervous system (CNS) infection. Molecular diagnostic tests have dramatically redefined both the diagnosis and management of CNS infections where rapid, accurate identification of a pathogen and prompt initiation of antimicrobial therapy can be potentially lifesaving.
- *Target:* Most common viral, bacterial and yeast pathogens that cause CNS infections in immunocompetent patients.
- *Sample type:* 200 µL cerebrospinal fluid (CSF) obtained via a lumbar puncture (LP)
- *Who should get tested?*
 - Individuals with signs and/or symptoms of acute meningitis and/or encephalitis.
 - Most panels are not intended for testing of CSF collected from indwelling CNS medical devices.
- *Interpretation of results:*
 - Molecular assays were shown to have incremental value compared to CSF culture and Gram stain, especially in patients with viral infections and those who received antibiotics prior to the LP procedure.
 - The risks of contamination necessitate the need for strict adherence to aseptic collection policies (e.g. the use of sterile gloves and a surgical mask) as individuals with active respiratory infections (rhinovirus may cross-react with enterovirus), active/latent herpes simplex virus infection (i.e. cold sores), and asymptomatic shedders of *S. pneumoniae*, *H. influenzae* or other organisms, may contaminate samples and thus cause false-positive results.
 - ME panels that include detection of cytomegalovirus (CMV) and human herpesvirus-6 (HHV-6) do not distinguish between latent/chromosomally integrated, secondary reactivation, or active infections – positive results should be carefully interpreted in conjunction with other clinical, laboratory, and epidemiological information.
 - Cryptococcal antigen testing continues to be the diagnostic standard for diagnosis of Cryptococcal meningitis.
 - CSF leucocyte count, glucose, and total protein levels are frequently within normal range or only slightly elevated despite CNS infection, particularly in neonatal meningitis and enteroviral meningitis.
 - A negative result does not exclude the possibility of CNS infection and should not be used as the sole basis for diagnosis, treatment, or other management decisions. There is a risk of false-negative results due to the presence of sequence variants or rearrangements in the gene targets of the assay, procedural errors, inhibitors in specimens, technical error, sample mix-up, low pathogen load, or infection caused by an organism not detected by the panel used. Low viral load in CSF for certain viruses (e.g. herpes simplex virus), especially in the first 3 days after symptom onset, may warrant additional testing with an alternative singleplex assay, testing alternate sources (e.g. blood) or repeating the LP procedure after a few days for follow-up testing.

- Panels optimised for acute meningitis together with additional targeted testing may be needed in the following circumstances:
 - Chronic meningitis (which can result from infection with *Mycobacterium tuberculosis* and fungal pathogens including, *Aspergillus*, *Histoplasma*, *Blastomyces*, and *Coccidioides*).
 - Suspected arboviruses after mosquito exposure, e.g. West Nile virus.
 - Shunt infections.

Gastrointestinal (GI) panels

- Recent guidelines for children and adults with suspected or confirmed infectious diarrhoea emphasise that the decision to test for pathogens should be based on a combination of epidemiologic risk factors for specific pathogens, risk of complications, immunosuppression, risk of transmission, severity and duration of symptoms, and need for treatment. They acknowledge that the optimal role of culture-based and molecular diagnostic testing has not been rigorously defined.
- *Target*: Common bacteria, viruses and parasites associated with gastroenteritis
- *Sample type*: 200 µL stool
- *Who should get tested?*
 - Diagnostic testing is not recommended in most uncomplicated cases of suspected infectious diarrhoea in previously healthy and immunocompetent patients. Most diarrhoeal illnesses are self-limiting and do not require evaluation or treatment beyond supportive care such as rehydration.
 - Clinical guidelines recommend that diagnostic testing in acute gastroenteritis be restricted to patients:
 - with moderate to severe symptoms
 - with prolonged symptoms lasting at least 1 week
 - younger than 5 years of age and the elderly
 - with immune deficiencies
 - returning from travels if treatment is indicated, or the traveller has had diarrhoea lasting 14 days or longer
 - with suspected epidemiologic exposures, e.g. from food/water borne and zoonotic sources
 - with diarrhoea and fever, bloody or mucoid stools, severe abdominal pain, signs of sepsis, or extra-intestinal manifestations, to exclude *Salmonella*, *Shigella*, *Campylobacter*, *Yersinia*, *Clostridioides difficile*, and Shiga toxin-producing *Escherichia coli*
 - when there is suspicion of an outbreak situation
 - when there is potential for the laboratory investigation to impact management
 - A stool culture may be needed in situations in which antibiotic susceptibility testing would affect clinical care of a patient or public health responses.
- *Interpretation of results*:
 - The greater sensitivity of these tests and ability to detect multiple pathogens may have clinical utility, but a more sensitive test may potentially lead to overtreatment. To balance benefits and harms of testing, clinicians should consider the patient's history, risk factors for severity of illness, and risk of complications.
 - The detection of organisms with less definitively established pathogenicity, e.g. enteroaggregative *E. coli*, enteropathogenic *E. coli*, and *Plesiomonas shigelloides* will need expert interpretation.
 - Some commercial assays are not able to specifically identify pathogenic vs non-pathogenic species of a pathogen.
 - Potential colonisers like *C. difficile* need to be interpreted in the clinical context to prevent overdiagnosis, e.g. children < 24 months of age are often asymptotically colonised, and testing healthy adult populations without previous use of antibiotics or hospitalisation or hospital exposure is not indicated.
 - Some organisms (e.g. *Salmonella* and norovirus) are capable of shedding for weeks or months after convalescence.
 - Repeat testing within a 4 week period is not recommended as it is unlikely to yield additional information.

Conclusion

Multiplex test results should be interpreted in the clinical context with consideration of the pre-test probability for each potential pathogen detected. Detection of nucleic acid from a pathogen consistent with the clinical presentation can support a diagnosis that informs management and limits unnecessary testing or treatment. However, non-pathogenic organism detection may lead to incorrect assumptions of cause-and-effect, increase utilisation of ineffectual and potentially harmful therapies, and impede evaluation and management of the true diagnosis.

References and guidelines

General

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