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MOLECULAR DIAGNOSTICS IN MICROBIOLOGY

# Molecular testing for viral and bacterial enteric pathogens: gold standard for viruses, but don't let culture go just yet?

MAXIM G. BLOOMFIELD[1], MICHELLE N. D. BALM[1,2] AND TIMOTHY K. BLACKMORE[1,2]

*[1]Department of Clinical Microbiology, and [2]Department of Infectious Diseases, Wellington Regional Hospital, Capital and Coast District Health Board, Wellington, New Zealand*

**Summary**

Contemporary diagnostic microbiology is increasingly adopting molecular methods as front line tests for a variety of samples. This trend holds true for detection of enteric pathogens (EP), where nucleic acid amplification tests (NAAT) for viruses are well established as the gold standard, and an increasing number of commercial multi-target assays are now available for bacteria and parasites. NAAT have significant sensitivity and turnaround time advantages over traditional methods, potentially returning same-day results. Multiplex panels offer an attractive 'one-stop shop' that may provide workflow and cost advantages to laboratories processing large sample volumes. However, there are a number of issues which need consideration. Reflex culture is required for antibiotic susceptibility testing and strain typing when needed for food safety and other epidemiological investigations. Surveillance systems will need to allow for differences in disease incidence due to the enhanced sensitivity of NAAT. Laboratories should be mindful of local epidemiology when selecting which pathogens to include in multiplex panels, and be thoughtful regarding which pathogens will not be detected. Multiplex panels may not be appropriate in certain situations, such as hospital-onset diarrhoea, where *Clostridium difficile* testing might be all that is required, and laboratories may wish to retain the flexibility to run single tests in such situations. The clinical impact of rapid results is also likely to be relatively minor, as infective diarrhoea is a self-limiting illness in the majority of cases. Laboratories will require strategies to assist users in the interpretation of the results produced by NAAT, particularly where pathogens are detected at low levels with uncertain clinical significance. These caveats aside, faecal NAAT are increasingly being used and introduce a new era of diagnosis of gastrointestinal infection.

*Key words:* Clinical laboratory techniques, diarrhoea, gastroenteritis, infection control, nucleic acid amplification techniques, public health.

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## INTRODUCTION

Infectious diarrhoea is one of the major causes of morbidity and mortality in the developing world and remains a leading cause of infectious morbidity in developed countries.[1] Older studies suggest that only 25–58% of patients with acute infectious diarrhoea have the causative pathogen identified by conventional tests.[2] Conventional bacterial testing algorithms are often complex, requiring selective media, enrichment broths, microscopy and antigen detection methods which are labour intensive and may require up to 96 hours to obtain a final result. Nucleic acid amplification tests (NAAT) for enteric pathogens (EP) now are generally more sensitive with a faster turnaround time than bacterial culture, and long ago eclipsed traditional virological methods. Rapid NAAT results may improve patient care through earlier management decisions including reducing unnecessary hospitalisation, diagnostic procedures or inappropriate antibiotic use.[3]

This review will focus on the NAAT available for the diagnosis of bacterial and viral EP, with particular attention to cost, clinical utility and the uncertainties/limitations that diagnostic laboratories must resolve prior to widespread implementation of NAAT for the detection of EP. It will not address the diagnosis of enteric parasites as this topic will be discussed elsewhere in this issue.

## MOLECULAR METHODOLOGIES AND APPROACHES

In recent years, an ever-increasing number of molecular testing systems for diagnosis of acute infectious diarrhoea have been developed (Table 1).[4] Single pathogen detection by NAAT has greatest utility for pathogens with distinctive clinical characteristics such as *Clostridium difficile* or norovirus. A huge expansion has recently occurred with the development of multiplexed NAAT, offering 'one-stop shop' testing of a range of viral, bacterial and parasitic EP (Table 2).[5] Robust nucleic acid extraction methods and the inclusion of extraction and amplification controls are required for NAAT of faeces due to the large amount of background nucleic acid and presence of many inhibitors of polymerase chain reaction (PCR) amplification. Many commercial and in-house tests can be run on automated or manual extraction systems which enhance the quality and yield of target nucleic acid. Commercial real-time PCR multiplex panels have the advantage that most kits are developed to work on a number of common real-time PCR instruments and thus may be readily introduced into laboratories using existing, often expensive, equipment. The number of targets which can be detected in each reaction is currently limited by the number of fluorescence detection channels available. Some commercial assays provide panels for 3–5 pathogens each with several parallel multiplex reactions with fluorescence-based detection of amplicons; others use tandem PCR. The latter technique uses an initial multiplex PCR reaction, followed by single target PCR with the product detected with an intercalating dye.

Another method combines multiplex PCR with hybridisation to microarray or macroarray for detection of multiple targets.

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**Table 1** Available nucleic acid amplification assays for enteric pathogens

| Manufacturer and test name | Analytical turnaround time | Method | Workflow and instrument considerations | | | | |
|---|---|---|---|---|---|---|---|
| | | | Integrated 'black-box' system | Separate nucleic acid extraction required | Generic thermal cycler required | Dedicated instrument purchase required | Post-PCR handling required |
| R-Biopharm RIDA GENE-kits | 1.5 h | Multiplex real-time PCR | No | Yes | Yes | No | No |
| Serosep EntericBio Gastro Panel 1 | ~1.5 h | Multiplex real-time PCR | No | No | Yes | No | No |
| Seegene Seeplex Diarrhoea ACE Detection | ~10 h | Multiplex PCR | No | Yes | No | Yes | Yes |
| AusDiagnostics Faecal Bacteria | ~3 h | Multiplex tandem PCR | No | Yes | No | Yes | No |
| AusDiagnostics Faecal Pathogens A/B/C | ~3 h | Multiplex tandem PCR | No | Yes | No | Yes | No |
| Luminex xTAG GPP | ~5 h | Multiplex PCR, suspension array detection | No | Yes | Yes | Yes | Yes |
| Genomica CLART EnteroBac | ~5 h | Multiplex PCR and array detection | No | Yes | Yes | Yes | Yes |
| Nanosphere Verigene Enteric Pathogens Test | ~2 h | PCR, hybridisation to gold nanoparticle | Yes | No | No | Yes | No |
| BioFire FilmArray GI Panel | ~1 h | Nested PCR, Multiplex PCR, melt curve analysis | Yes | No | No | Yes | No |
| BD Max Enteric Bacterial Panel | ~3 h | Multiplex real-time PCR | Yes | No | No | Yes | No |
| Genetic Signatures EasyScreen | 3–5 h | Multiplex real-time PCR | No | Yes | Yes | No | No |
| Fast-Track Diagnostics Gastroenteritis | ~3 h | Multiplex real-time PCR | No | Yes | Yes | No | No |

Table adapted from Reddington *et al*.[4] and individual assay product information sheets. h, hours; PCR, polymerase chain reaction.

The technique still includes nucleic acid extraction and amplification, but products are hybridised to an array with automated reading and interpretation of results. Advantages of these systems include a high throughput of samples and the detection of a much larger range of targets in a single reaction. However, they also require specialised instruments, separate nucleic acid extraction prior to PCR, and post-PCR handling of samples, which increases the potential for contamination.

Fully integrated ('closed') platforms have been developed which incorporate extraction, amplification and detection phases within one 'black box'. These can be operated by less skilled personnel, require less hands-on time and provide simple detected/not detected results. This automation allows the tests to be performed by less experienced staff in a general laboratory area with potentially significant cost and time savings. Several commercial systems are available with a large menu of bacterial, viral and parasitic EP. The major disadvantage of these platforms is that the initial investment may be significant and the cost of reagents is often high. Additionally, sample throughput is usually low, limiting the utility of 'black box' testing to urgent one-off tests.

Consistent with many other areas of microbiology, molecular methods offer significant gains in sensitivity compared to conventional methods for detection of EP. One of the original studies investigating NAAT for EP diagnosis demonstrated an increase in pathogen detection from 53% to 75% with NAAT compared to conventional methods.[6] Although a comprehensive discussion of the literature is beyond the scope of this review, numerous studies have demonstrated higher sensitivity of NAAT for detection of both bacterial and viral pathogens, often with a near doubling of positive results compared to conventional methods.[7–15] An exception may be for *Salmonella* species: selective enrichment culture appears to have similar sensitivity to NAAT.[11–14] Unfortunately, it is not possible to definitively determine the comparative sensitivity and specificity of the different NAAT assays because of a lack of a reference standard, but the authors have not noted any major difference between the commercial assays. Molecular methods also greatly reduce analytical turnaround times over traditional culture (Table 1), although how these translate into reductions in clinical turnaround time depends on the testing workflow adopted.

## NAAT FOR EP IN THE CLINICAL MICROBIOLOGY LABORATORY

### Can laboratories afford to not introduce NAAT?

The greatest advantage of NAAT is its high negative predictive value, allowing negative results to be reported quickly, and removing the need for further workup in the majority of specimens. Beal *et al*. found that 62% of bacterial stool cultures grew organisms requiring further workup, which ultimately proved to be insignificant.[16] The extra work required to identify non-significant colonies in stool culture increases turnaround time of negative results to 48–96 hours. Conversely, targeted culture of NAAT positive specimens allows efficiency gains in human and material resources. A 2 year prospective study using molecular screening of stools followed by culture confirmation of positive samples showed significant reductions in testing burden along with enhanced detection of all pathogens tested, with the exception of *Salmonella enterica*.[11]

The role of NAAT-based diagnosis of infectious diarrhoea is evolving. The choice of testing strategy is complex; it is influenced by existing critical equipment, equipment purchase versus rental policy, available human resources, and physical layout and space of the laboratory. It is also dependent on the expected volumes of faecal samples, and the local epidemiology of EP. The trend towards centralisation of laboratory services and reduction in staff costs favours automation. This results in a greater reliance on the specimen collection, preservation and transport systems. Moreover, automation requires a standardised broad brush diagnostic approach, as it becomes