

Public health impacts of culture independent diagnostic testing in Australia



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Culture independent diagnostic tests (CIDT) for detection of pathogens in clinical specimens have become widely adopted in Australian pathology laboratories. Pathology laboratories are the primary source of notification of pathogens to state and territory surveillance systems. Monitoring and analysis of surveillance data is integral to guiding public health actions to reduce the incidence of disease and respond to outbreaks. As with any change in testing protocol, the advantages and disadvantages of the change from culture based testing to culture independent testing need to be weighed up and the impact on surveillance and outbreak detection assessed. This article discusses the effect of this change in testing on surveillance and public health management of pathogens in Australia, with specific focus on gastrointestinal pathogens.

What is CIDT and how is it being used in Australia?

A CIDT is any diagnostic test that is performed directly on the clinical specimen without first requiring laboratory culture of a pathogen isolate^{1,2}. Common CIDT methods diagnose an infectious agent by detecting the nucleic acids of the pathogen (e.g. polymerase chain reaction [PCR]) or the pathogen antigen (e.g. enzyme immunoassays). Commercial and in-house CIDTs have been developed for a range of pathogens.

Many pathology laboratories across Australia have introduced PCR panels for identification of gastrointestinal pathogens (bacteria, parasites and viruses) in faecal specimens (John Bates for the Public Health Laboratory Network, personal communication). Most of these laboratories are continuing to perform culture of specimens, either concurrent with PCR or reflexively only on specimens that test positive by PCR. As with any change in testing protocol, the addition of PCR for pathogen diagnosis bears advantages and disadvantages, both for the diagnostic laboratory and for

end users of laboratory testing data, such as public health professionals and clinicians. As PCRs for gastrointestinal pathogens are the primary CIDT in use in Australian pathology laboratories, and gastrointestinal disease causes a significant burden of illness³, these will provide the focus for examples used in this article. However, PCR is becoming common for the diagnosis of a range of pathogens and most elements of this discussion are applicable across all pathogens.

CIDT has revolutionised pathogen detection

Many pathology laboratories have embraced PCR due to the low ongoing cost, speed, and ease of use^{1,4-6}. PCR requires less training and expertise, and is less subjective than culture, for which experience is required to identify appropriate colonies to select for further characterisation^{1,6}. PCR is generally more sensitive than culture, and can detect pathogens that do not grow easily in culture, have been treated with antibiotics or are in low numbers in the specimen^{5,6}. PCR is more likely to detect a pathogen in specimens with decreased viability due to a delay before testing⁷. PCR can also identify polymicrobial infections whereas culture would likely only detect the fastest growing pathogen⁵.

PCR also benefits public health. Faster identification of the causative agent in patients who would otherwise have gone undiagnosed due to a negative culture can result in earlier prevention of transmission. Outbreaks may be identified sooner¹. The use of tests with a higher sensitivity, such as PCR, provides a more accurate estimate of the burden of disease, which can inform targeted response and control measures⁴.

CIDT must be interpreted with caution

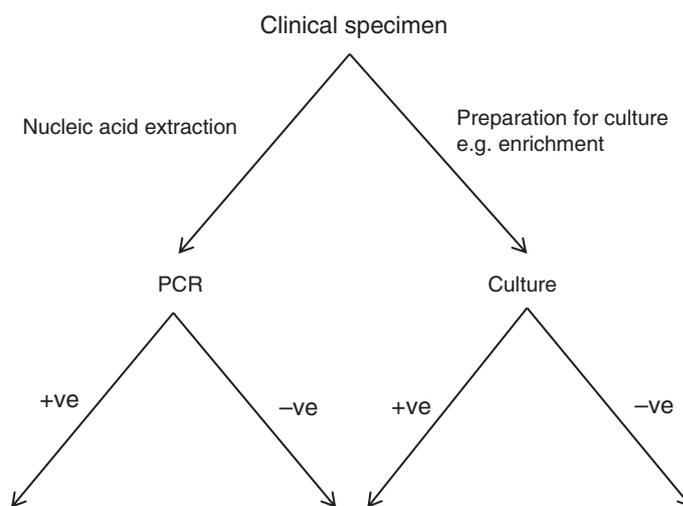
One of the most important uses of surveillance data is for the detection of an increase in disease through comparison with historical data^{5,6}. Therefore, any change in testing method will need to be reviewed in the context of previous results, to enable valid comparison of data from before and after the change in method. Since PCR is more sensitive than traditional culture methods, the detected incidence of pathogens will likely increase after introduction of PCR^{5,7}. However, other factors can also cause an increase in the detected incidence. Due to the rapid turnaround of results for PCR, clinicians may submit specimens from patients where previously they would not (e.g. a case where mild illness is likely to resolve before results would be available by culture). Pathology laboratories may advertise the introduction of a new

technology, such as PCR, resulting in an increase in specimen submission. Finally, there may be a real increase in the incidence of disease due to another cause.

Current commercially available diagnostic PCR kits are generally unable to distinguish between dead and living cells⁵ or identify a pathogen beyond the genus level. Current methods for typing of pathogens, such as serotyping and genotyping, and assessment of antimicrobial susceptibility require a cultured isolate (Figure 1)^{2,5,6}. For common pathogens, such as *Salmonella*, this makes it difficult to detect outbreaks of a particular genotype against background surveillance data⁶. For outbreaks of foodborne gastroenteritis, this limits detection of outbreaks of common pathogens to those identified *via* complaints from the public or notifications from an event, food business or facility. Community outbreaks where cases appear to be unrelated (such as those involving commercial foods not eaten on premises) will not be detected if relying on PCR diagnostics alone. Without information on the specific genotype of the pathogen, it is difficult to prove the linkage of cases to pathogen isolates obtained after traceback to the potential food source. This can affect the success of interventions or litigation^{1,6}.

The impact of the introduction of PCR is different for each pathogen. For example, *Salmonella* is easy to grow in culture, so concurrent or reflex culture will result in an isolate for further characterisation most of the time. In contrast, as *Campylobacter* is fastidious⁸, culture has a much lower sensitivity than PCR, resulting in a higher proportion of specimens positive only by PCR even when culture is attempted.

If the specificity of the primers used in the PCR is not limited to the pathogen in question, the PCR may overestimate incidence of disease. For example, *Shigella* is genetically closely related to enteroinvasive *Escherichia coli* (EIEC)⁹ and the PCR primers used in commercial multiplex PCRs amplify the *ipaH* gene, which is common between *Shigella* and EIEC^{10,11}. Thus, culture-based phenotypic tests are required to differentiate between the two genera. However, *Shigella* can be difficult to culture¹², making it difficult to determine how many of the culture negative/PCR positive specimens are from true cases of shigellosis. While shigellosis is a notifiable disease in Australia, gastroenteritis due to EIEC is not, and the true incidence of EIEC and *Shigella* in Australia is unknown. However, studies from other developed countries show



Culture	PCR	Identification (notification) examples
+ve	+ve	<i>Salmonella</i> Enteritidis <i>Salmonella</i> Typhimurium MLVA 03-12-12-11-524 <i>Campylobacter jejuni</i> <i>Shigella flexneri</i>
+ve	-ve or not done	<i>Salmonella</i> Enteritidis <i>Salmonella</i> Typhimurium MLVA 03-12-12-11-524 <i>Campylobacter jejuni</i> <i>Shigella flexneri</i>
-ve or not done	+ve	<i>Salmonella</i> sp <i>Campylobacter</i> sp <i>Shigella</i> / EIEC not notified

Figure 1. Example of the flow of specimens and results through a pathology laboratory system. Steps conducted at different types of laboratory (primary vs reference) have not been separated as this will differ by and within jurisdiction. PCR, polymerase chain reaction; MLVA, multi-locus variable number tandem repeat analysis; EIEC, enteroinvasive *Escherichia coli*.

a higher incidence of *Shigella* than EIEC^{13,14} and a study from Victoria suggested that *Shigella* may be more common than EIEC in Australia¹⁵. In Queensland, the number of stools positive for *Shigella*/EIEC increased dramatically following the introduction of PCR¹⁶ and a similar increase is likely where PCR has been introduced into public health laboratories in other jurisdictions. Most jurisdictional public health guidelines require notification of only those *Shigella* positive stools with a confirmatory culture¹⁵. This minimises unnecessary follow up of cases of gastroenteritis caused by EIEC, but may result in loss of information about the true incidence and risk of *Shigella* transmission. Addition of primers that can distinguish between *Shigella* and EIEC to the gastroenteritis multiplex PCR is essential for accurate delivery of public health response.

Whole genome sequencing (WGS) of pathogens is currently under development for use in public health (see article by A. Jennison in this issue). WGS allows further characterisation of isolates and prediction of antimicrobial sensitivity which can be useful for public health and clinical treatment. However, the WGS techniques currently in use for high throughput testing of many clinical samples still require a pure culture so the introduction of PCR and subsequent reduced availability of cultures from clinical specimens in laboratories that have introduced CIDT will hinder the development of this technology.

Conclusion and future

Although it varies for each pathogen, the impact of the introduction of CIDT has both benefits and drawbacks for the laboratory, clinical diagnosis of disease and public health surveillance. It remains important that laboratories continue to perform concurrent or reflex culture in order to inform public health action, especially as WGS becomes more common. This period of extra testing is likely to be transitional, as techniques for sequencing directly from clinical specimens (metagenomics) are developed and neither culture nor PCR will be required for diagnosis of pathogens. In addition to bypassing the requirement for culture, metagenomics will allow detection of previously unknown pathogens *via* sequencing of all nucleic acid present in a specimen, expanding our knowledge of the agents causing infectious disease.

Conflicts of interest

The author declares no conflicts of interest.

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Biography

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