Pertussis, or whooping cough, is a highly contagious disease caused by *Bordetella pertussis*. This update summarises developments in laboratory testing of pertussis that assist clinicians with the confirmation of the clinical disease and the investigation of outbreaks.

### Current laboratory methods

Tests currently used to confirm pertussis infection are shown in the Table 1.

#### Specimen collection

Proper technique and timeliness of specimen collection are important (Figure 1). Nasopharyngeal aspirates are the preferred specimens for polymerase chain reaction (PCR), but are often difficult to collect except from very young children. Aspirates produce a higher recovery of organisms than swabs, and specimens can be split for multiple tests. A swab of the nasopharynx is better than a swab of the anterior nostril for PCR. The polyester swab should be gently inserted into the base of a nostril, advanced as far as possible and rotated in the posterior pharynx for ten seconds before withdrawing. Throat swabs are also acceptable specimens for PCR. Nasopharyngeal aspirates or swabs are the only suitable specimens for culture.

Bordetella pertussis is fastidious and quite difficult to grow in the laboratory. It can be recovered from patients only in the first 3 to 4 weeks of illness, and is particularly difficult to isolate from previously immunised persons (Figure 1).\(^1\)

#### Polymerase chain reaction

Laboratory confirmation of the diagnosis by PCR or serology should be attempted, especially when atypical pertussis is suspected clinically. The use of PCR has made the rapid diagnosis of pertussis possible and is more sensitive than culture.\(^1,2\) The PCR assay is also less affected by antimicrobial therapy. However, as with culture, the sensitivity of PCR decreases with the duration of symptoms. There are occasional false-positive PCR results caused by contamination, which may occur at any stage between sample collection and the laboratory.\(^2\)

#### Serology

Natural infection with *B. pertussis* is followed by an increase in the serum concentration of IgA, IgG and IgM antibodies. In contrast to natural infection, primary immunisation induces mainly IgG and IgM antibodies.\(^1\) The greatest specificity for the serological diagnosis of *B. pertussis* infection is achieved by the measurement of IgG and IgA antibodies against pertussis toxin. Either a significant increase in serum antibody level (preferably) or single high level, in sera obtained at least 2–3 weeks into the illness may be used for diagnosis. It is rarely possible to demonstrate seroconversion because initial symptoms are non-specific and the first (acute) serum is often not collected until 2–3 weeks after the onset of cough. Anti-pertussis toxin IgG levels of >100–125 European or International Units (using standardised methodology) have been shown to be specific for recent exposure to *B. pertussis*, but this criterion was established in the context of vaccine trials and may be less sensitive and reliable for routine diagnosis.

Many different commercial and in-house serological tests – usually enzyme immunoassays (EIA) – are currently in use; they employ various antigens including pertussis toxin alone or in combination with other, less specific, *B. pertussis* antigens or a crude preparation of whole bacterial cells. The sensitivity and specificity of EIA-based assays vary considerably, but may be as low as 50–60%. The absence of established cut-off points or diagnostic criteria limit the usefulness of serological confirmation.\(^1,3\) Despite these limitations, serological testing (most
commonly conducted by a commercial assay, which detects IgA against a whole cell pertussis antigen) has been the basis for notification of the majority of pertussis cases in older children and adults, in Australia.

**New methods to confirm pertussis infection**

Efforts to control outbreaks of pertussis in a community are costly and require: intensive surveillance; detailed alerts to health-care professionals; enhanced vaccination coverage and public education; and aggressive measures involving treatment, prophylaxis and the isolation of suspected cases.4,5

During the past decade, the demonstration of polymorphism in *B. pertussis* genes encoding the expression of pertussis toxin and pertactin (another immunogenic *B. pertussis* virulence factor) led to the suggestion that vaccine-driven evolution has resulted in decreased vaccine efficacy.6,7 Several research groups have also accumulated data suggesting that isolates circulating in a community may be antigenically distinct from vaccine strains and from strains circulating before the introduction of the pertussis vaccination.7,8 Recent evidence from Europe and Australia indicates that we may face the emergence of successful clones of *Bordetella* harbouring new variants of pertussis toxin.7,9,10

Genetic and antigenic variations in virulence factors of strains circulating in the population can be monitored to detect potential escape from immune protection. However, identification of these variants currently requires time-consuming and expensive sequence analyses. Moreover, as PCR increasingly replaces culture for diagnosis of pertussis, fewer clinical isolates are available for testing. To address this problem, researchers at the Centre for Infectious Diseases and Microbiology – Public Health, in partnership with colleagues from the Universities of Sydney and New South Wales, have been developing new

### Table 1. Tests currently used to confirm pertussis infection

<table>
<thead>
<tr>
<th>Test</th>
<th>Sensitivity %</th>
<th>Specificity %</th>
<th>Positive predictive value %</th>
<th>Negative predictive value %</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture*</td>
<td>15*</td>
<td>100</td>
<td>100</td>
<td>88</td>
<td>Time consuming and rarely offered</td>
</tr>
<tr>
<td>Polymerase chain reaction (PCR)</td>
<td>94</td>
<td>97</td>
<td>84</td>
<td>99</td>
<td>Rapid confirmation of diagnosis; expensive; not affected by antibiotic therapy</td>
</tr>
<tr>
<td>Serology (IgA/IgM antibody)</td>
<td>Variable**</td>
<td>Variable</td>
<td></td>
<td></td>
<td>No single test is universally accepted or standardised nationally;*** May remain negative in infants</td>
</tr>
</tbody>
</table>

*Discussion with a pathology service provider is advisable before a specimen collection.

**Higher in children.

***Performance characteristics vary significantly between different serological assays, but attempts to standardise interpretation are underway.

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**Figure 1. Decision aid for the choice of laboratory investigations in the diagnosis of pertussis. PCR: polymerase chain reaction.**
culture-independent methods for molecular subtyping of B. pertussis directly from clinical specimens. This method will allow monitoring future epidemiological changes that predict significant antigenic variation and the potential escape from immune protection.

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References