Laboratory diagnosis of pertussis: state of the art in 1997.

F M Müller, J E Hoppe and C H Wirsing von König

MINIREVIEW

Laboratory Diagnosis of Pertussis: State of the Art in 1997

FRANK-MICHAEL C. MÜLLER,1* JÖRG E. HOPPE, 2 AND C.-H. WIRSING VON KÖNIG3

University Children’s Hospital, Aachen,1 University Children’s Hospital, Tübingen,2 and Institute for Hygiene and Laboratory Medicine, Klinikum Krefeld, Krefeld,3 Germany

INTRODUCTION

In the late 1980s, three comprehensive articles reviewed the state of the art of the laboratory diagnosis of pertussis (21, 51, 85). Since then additional information on the clinical nature of the disease, and on serological and molecular methods of diagnosis have come to light.

Isolation of Bordetella pertussis from clinical specimens is the “gold standard” for the diagnosis of pertussis due to its high degree of specificity. The method is still widely used, although the sensitivity has been shown to be variable. Direct fluorescent-antibody (DFA) testing has a lack of sensitivity and specificity, but it can provide a rapid diagnosis. Considerable progress has been made in developing new methods with greater sensitivities, particularly the application of nucleic acid amplification methods. The evaluation of acellular pertussis vaccines in extensive studies represented an opportunity to evaluate different methods for the diagnosis of pertussis, especially the PCR method. For the detection of antibodies to B. pertussis, the enzyme-linked immunosorbent assay (ELISA) has been the method of choice for the last decade. In unvaccinated children increases in the levels of either immunoglobulin G (IgG) or IgA antibodies to a single or various antigens are required to meet the World Health Organization (WHO) definition of pertussis (121). In vaccinated children a single serum specimen may be diagnostic for pertussis.

Immunization has reduced the prevalence of pertussis in many countries, but epidemics still occur, even in countries where immunization is extensive. Pertussis in adults is far more common than was previously thought, even in adults who have had pertussis during childhood.

In view of the continuing importance and high prevalence of whooping cough in many countries, it seems appropriate to present an updated review of the laboratory methods used to diagnose this disease (52).

ISOLATION OF B. PERTUSSIS

At present, recovery of B. pertussis from respiratory secretions remains the gold standard for the diagnosis of pertussis. While the specificity of this method is very high, its sensitivity varies depending on a number of the following factors: collection methods, devices, transport and enrichment media, conditions and duration of transport, choice of media and selective agents, and incubation conditions and duration. Experience with the method is one of the most important variables. Attempts at isolating B. pertussis are most likely to be successful at the end of the incubation period, during the catarrhal stage, and at the beginning of the paroxysmal (convulsive) stage of the disease (9, 26, 42, 52, 59, 102). Isolation rates are negatively correlated with increasing age (high rate in infants [121]; low rate in adults [116]), with effective antimicrobial pretreatment (macrolides, co-trimoxazole, and tetracyclines but not penicillin or ampicillin [8, 42, 59, 102]), and with the number of pertussis vaccination doses received by the patient (lower isolation rate in completely vaccinated individuals [8, 35, 59, 102]).

Collection of specimens. Nasopharyngeal aspirates (NPAs) of nasopharyngeal secretions have been shown to be better specimens than secretions obtained on nasopharyngeal swabs (NPSs) (9, 34, 36). This is not surprising since more material is obtained in NPAs, which, in addition, allows PCR to be conducted in parallel. Many pediatricians are not familiar with the technique of nasopharyngeal aspiration, but simple techniques which can be used under office conditions should be developed and promoted.

In most countries, retrieval of secretions on NPSs is still the standard method for isolating B. pertussis. The use of throat swabs is less suitable (67), since B. pertussis exhibits a tropism for ciliated respiratory epithelium, which is not found in the pharynx (68, 116). Some investigators recommend taking duplicate NPSs or several swabs on successive days (1), but this is hardly feasible under routine conditions. Calcium alginate is the preferred swab material (49), but Dacron swabs are an acceptable alternative, particularly when both culture and PCR are to be done.

Transport of specimens. Immediate plating of the specimen onto a suitable agar medium avoids the loss of bordetellae by minimizing exposure to cold temperatures, leads to earlier culture results, and is therefore preferable to transport of the specimen (21, 46, 58). When direct plating is not possible, a transport medium must be used. General nonnutritive bacteriological transport media, such as Amies medium, should be used only if they contain charcoal and the storage time does not exceed 24 h (116). Regan-Lowe transport medium (half-strength charcoal agar supplemented with horse blood and cephalexin) is a nutritive medium and inhibits the growth of the normal nasopharyngeal flora; it thereby improves the survival of the bordetellae and is the transport medium of choice (1, 50, 88). Preincubation of this medium at 36°C for 1 or 2 days before shipment enables early multiplication of the bordetellae and increases the bacteriological yield (50). On the other hand, the growth of the cephalexin-resistant components of the nasopharyngeal flora is favored by preincubation and may preclude the isolation of bordetellae due to overgrowth.

This is one reason for the improved yield by direct specimen plating; overgrowth is less of a problem since Bordetella colonies can be detected among colonies of normal flora (46). Cooled transport of the specimen suppresses overgrowth but...
decreases the number of bordetelae by >75% (80). Transport time should be as short as possible (86, 88, 102).

**Agar media.** Charcoal agar supplemented with 10% horse blood and cephalexin (40 mg/liter) is the medium of choice for the isolation of *B. pertussis* from clinical specimens (21, 116). It is superior to charcoal agar without blood (45), buffered charcoal-yeast extract agar (45), Bordet-Gengou agar (26, 47), and cyclohextrin solid medium (80), an entirely synthetic agar developed in Japan (4). Some investigators recommend the use of both charcoal horse blood agar and Bordet-Gengou agar in parallel (3, 58). Others use plates of charcoal horse blood agar with and without cephalexin in parallel (21, 36). Cephalexin slightly retards the growth of *B. pertussis* (88) but does not suppress it (116). Horse blood is more suitable than sheep blood or human blood as a supplement to charcoal agar (44). The shelf-lives of charcoal horse blood agar with cephalexin and of Regan-Lowe transport medium are 4 to 8 weeks (88).

**Enrichment media.** Ross demonstrated that even when swabs loaded with standard inocula of *Streptococcus pyogenes* were plated immediately, only a small percentage of the original inoculum could be recovered on solid media (92). The same is likely to apply to *B. pertussis* and NPSs. Therefore, some form of enrichment should be used to optimize the yield of culture in pertussis.

One possibility is the use of Regan-Lowe transport medium as an enrichment medium after inoculating an agar plate with the swab. The latter is then placed into Regan-Lowe medium and is streaked again onto an agar plate after 48 h of incubation (88). Charcoal horse blood broth is unsuitable for clinical specimens due to serious problems with the overgrowth of flora (48). Alternatively, Stainer-Scholte broth (98) may be used; it allowed for the isolation of a considerable number of additional strains in one study (119). Another alternative that has yet to be evaluated is the elution of NPSs in a suitable medium, e.g., serum inositol or Casamino Acids broth (15), followed by plating of the eluate. It is conceivable that elution increases the yield in comparison with that obtained by direct streaking of swabs.

**Inhibitors of nasopharyngeal flora in agar media.** Cephalexin was introduced by Sutcliffe and Abbott (103) as a selective agent in agar media for the isolation of *B. pertussis*. In this respect, cephalexin is superior to penicillin, methicillin, lincomycin, and cefsulodin (116). It is, however, by no means the ideal agent for this purpose since growth of cephalexin-resistant flora is not a rare event (38, 58, 88). Enterococci, coagulase-negative staphylococci, *Haemophilus influenzae*, and pseudomonads other than *Pseudomonas aeruginosa* are the most frequently encountered bacterial organisms (52a). In addition, yeasts and molds may grow on charcoal horse blood agar. To date, however, no better selective agent has been found.

**Incubation and identification.** Agar plates for the isolation of bordetelae should be incubated at 35 to 36°C (1). Desiccation of plates during the prolonged period of incubation (usually a maximum of 7 days, but longer incubation times were reported recently [57]) must be strictly avoided. *B. pertussis* is a fairly strict aerobe (18), and incubation in ambient air is preferable to incubation in an atmosphere with enriched CO$_2$ (44).

Bordetelae are presumptively identified by their typical colony morphology. *B. pertussis* develops mature colonies usually after 3 to 4 days; *B. parapertussis* develops mature colonies after 2 to 3 days. Gram stains of these colonies show gram-negative, small coccoid rods.

Since bordetelae are catalase positive, the catalase reaction may help to distinguish them from catalase-negative commensal organisms. A negative urease reaction distinguishes *B. pertussis* from *B. parapertussis* and *B. bronchiseptica*. For differentiation between *B. pertussis* and *B. bronchiseptica*, the oxidase reaction is helpful (*B. pertussis* is positive and *B. parapertussis* is negative). Both agglutinating and fluorescent antisera specific for both species are commercially available (51). Colonies of *B. parapertussis* sometimes weakly agglutinate *B. pertussis* antiserum (1). In order to avoid misidentification, it is not advisable to use both antisera in tests with the same isolate. Instead, the result of the oxidase reaction should guide the selection of the antiserum to be used, and normal saline is used as a negative control for the agglutination procedure. PCR can be helpful for the differentiation of *B. pertussis* and *B. parapertussis* colonies.

**DFA TESTING OF NASOPHARYNGEAL SECRETIONS**

Direct detection of *B. pertussis* and *B. parapertussis* by fluorescein-conjugated-antibody DFA staining of nasopharyngeal secretions can provide a rapid, presumptive diagnosis. However, several investigations have documented the lack of sensitivity and specificity of the existing commercially available polyclonal immunological reagents (20, 23, 36, 86).

Unlike culture and PCR, DFA testing does not incorporate multiplication of the organisms or their genetic material. This factor contributes to the low level of sensitivity of the method. Extensive cross-reaction with normal nasopharyngeal flora makes the results of DFA tests technically difficult to interpret, resulting in rates of false positivity of as high as 85% (20). A recently developed monoclonal antibody (BL-5) which specifically recognizes the lipoooligosaccharide of *B. pertussis* showed a sensitivity and a specificity of 65.1 and 99.6%, respectively, in comparison with the results of culture (70). This reagent is commercially available in some countries.

The technical skill and experience of the technicians who perform and read DFA test results are of crucial importance, and preparation of reagents, quality control, test performance, and subjectivity of interpretation of the DFA test results present some challenges even to good laboratories. Because of these limitations, DFA testing should always be used in conjunction with but not as a replacement for culture (68).

In the long run, DFA testing is likely to be replaced by PCR since the latter method combines rapidity with a much higher degree of sensitivity.

**DETECTION OF B. PERTUSSIS AND B. PARAPERTUSSIS BY PCR**

PCR is revolutionizing the diagnosis of many infectious diseases, particularly those caused by organisms that are difficult to cultivate. The best method for the detection of *B. pertussis* and *B. parapertussis* by PCR is still under investigation. Differences in the choice of target genes, amplification primers, detection systems, and the methodology itself preclude generally applicable recommendations. Various PCR procedures possess a diagnostic sensitivity which is at least comparable to and in many cases superior to that of culture.

**Sample preparation.** Two types of samples are used for pertussis PCR: NPA s and NPSs. NPA s should first be treated with a mucolytic agent (N-acetylcysteine or sodium hydroxide) and then centrifuged to pellet the bacteria. After careful removal of the supernatant, the pellet is resuspended in water and boiled. This procedure offers several advantages such as simplicity, concentration of the bacteria, and removal of potentially PCR-inhibiting substances. Additional steps, such as digestion with proteinase K (69) or phenol-chloroform extrac-
tion (38, 39, 41) of DNA before amplification and the use of Chelex (69, 81) or guanidium thiocyanate to remove potential inhibitors, were tested. Long-term storage by freezing of NPAs is possible to preserve the sample for PCR. Dry swabs can be suspended in physiologic saline supplemented with EDTA. Incubation at 100°C will lyse the bacterial cells. Processing of dry NPSSs is less complicated and requires fewer steps than processing of swabs stored in transport medium (38, 39, 109, 110).

For alginate NPSSs in a transport medium, DNA extraction appears to be necessary, because there are inhibitory factors in the calcium alginate fiber, in the aluminum shaft, and in the transport medium (112). Therefore, Dacron swabs are superior to calcium alginate swabs for PCR (71, 81). Schläpfer et al. (95) found that PCR was inhibited for 26% of samples prepared without DNA extraction. DNA extraction can be performed with phenol-chloroform, proteinase K, or Chelex (81, 91, 113). The effect of Chelex has been controversial: it may be inhibitory to the amplification reaction but, on the other hand, can increase the final diagnostic sensitivity (91).

**Primer selection and amplification conditions.** Primers derived from the following four chromosomal regions have been used: (i) pertussis toxin (PT) promoter region (10, 30, 53, 69, 90, 94, 95), (ii) a DNA region upstream of the porin gene (60, 61, 81, 97), (iii) repeated insertion sequences (7, 24, 38, 39, 84, 109, 110), and (iv) the adenylate cyclase toxin (ACT) gene (18). All of these primers except ACT primers, which do not distinguish between B. pertussis and B. parapertussis, are specific for B. pertussis. There is some evidence that assays with the repeat sequence as a target are more sensitive when a low number (n = 20 to 25) of amplification cycles is used, but assays with all targets are of comparable sensitivity when more than 35 cycles are used. The nested primer systems offer some advantages regarding sensitivity and specificity, but a disadvantage is that the uracil-N-glycosylase carryover prevention system, which destroys the products of previous amplifications, cannot be used before the second amplification step.

**Detection systems.** Most detection systems use ethidium bromide staining (10, 24, 38, 84, 109); several other detection systems that increase the sensitivity are in use. These include Southern blotting (18, 39, 95), dot blotting (30), digoxigenin immunoblot system (61, 81), liquid hybridization and hybridization of the PCR products with specific oligonucleotide probes (13, 24, 30, 39, 93, 109), and restriction enzyme analysis of PCR products (7, 84, 90, 91). Silver-stained polyacrylamide gel electrophoresis proved to be more sensitive than agarose gel electrophoresis (62).

**Controls.** Several steps are necessary to minimize the risk of false-positive and false-negative results. The following factors were identified as possible causes of false-positive results: the sample contains other bacteria with sequences homologous to those in B. pertussis, contamination of the sample with DNA or whole organisms of laboratory strains of B. pertussis or another Bordetella species, and product carryover contamination. In one pediatric outpatient clinic in Sweden, 32 of 35 (91%) NPSSs were PCR positive. B. pertussis had been isolated from only 12 of 35 (34%) of these NPSSs. Only the 12 patients infected with B. pertussis bacteria had symptoms typical of those of pertussis according to the WHO definition (121). Later, PCR-positive material was detected on several surfaces in the rooms where vaccinations were done and on the clothes and skin of the hands of personnel (104). Procedures to control contamination include physical separation of sample-processing areas from amplification and detection areas and incorporation of both known negative samples and coded (blinded) negative samples. Product carryover can be minimized by using the uracil-N-glycosylase carryover prevention system.

The following factors were identified as possible causes of false-negative results: insufficient number of organisms in the nasopharynx, very early or late stage of disease, an insufficient number of organisms in the sample because of an inadequate sampling procedure, the presence of an inhibitor in the sample, loss or damage of bacteria or DNA during processing or storage, technical problems with the assay and low sensitivity of the detection system, and infection of the patient with a B. pertussis strain with an altered or mutated sequence in the region defined by the primers (71). One of the major reasons is due to the presence in the crude clinical sample of substances inhibitory to Taq polymerase. The presence of hemoglobin in blood, incomplete removal of phenol, and the presence of insufficiently inactivated proteinase K are capable of inhibiting Taq polymerase and inhibiting amplification by PCR. Although it is not possible to control for all of these factors, the use of an internal control for each sample facilitates the interpretation of the PCR results (53, 63, 71, 97, 109).

**PCR results with clinical samples.** The results obtained by various investigators with different PCR systems performed with clinical samples are presented in Table 1. All PCR methods proved to be very specific, although some systems could not distinguish B. pertussis from other Bordetella species. When nasopharyngeal samples were analyzed by culture and PCR, PCR was found to be significantly more sensitive than culture overall (1,462 versus 931 samples; P < 0.001, chi-square test), although the samples in different studies were collected from different patient populations. PCR identified more pertussis patients than culture in each study. Only a minority of samples (2%) were PCR negative and culture positive, and all PCR systems were able to score 80 to 100% of culture-positive samples as PCR positive. However, there were large differences in the percentage of PCR-positive samples that were culture negative (2 to 51%). These differences may have been due to differences in study populations (vaccination status, antibiotic pretreatment, stage of disease, etc.), clinical samples (type, source), culture procedures, and the sensitivities of different detection systems. The interpretation of PCR-positive but culture-negative samples as either true positive or false positive is difficult. To confirm the result, PCR should be repeated or the result should be confirmed by another method (DFA testing or serology) (20, 24, 30, 91). A culture-negative but PCR-positive result should be considered a true-positive result only when the patient has symptoms typical of those of pertussis according to WHO criteria for clinical pertussis (91).

A PCR-positive but culture-negative result is common at later stages of the disease, in vaccinated patients, and in patients who are under antibiotic treatment or who have recently had close contact with patients with culture-proven pertussis (24).

The significance of results of PCR with clinical specimens from two large clinical populations with high levels of pertussis vaccination coverage has been analyzed (41). In Finland, of a total of 1,904 clinical samples tested for pertussis by PCR, 447 (23%) were positive. By culture and PCR a total of 829 samples were tested, and an additional 7 samples were only culture positive. The PCR positivity rate correlated with the number of B. pertussis colonies on plates. In Switzerland, 1,830 routine clinical samples were tested for B. pertussis by PCR only. A total of 683 were PCR positive, and 29 of 868 control samples were PCR positive. The comparison of culture, PCR, and enzyme immunoassay results demonstrated that none of the methods was able to detect all B. pertussis infections: 25% were confirmed by culture, 61% were confirmed by PCR, and 57% were confirmed by enzyme immunoassay.
Lichtinghagen et al. (62) compared the sensitivities of three different detection methods. After amplification by PCR, both a one-time PCR (35 cycles) with Enzymun testing and a nested PCR with either of the electrophoresis methods showed high sensitivities for the detection of *B. pertussis* in NPSs (62).

PCR is a rapid, sensitive, and specific technique that is likely to play an important role in the diagnosis of pertussis. PCR is faster than culture: 2.5 h (13) to 1 to 2 days versus 3 to 7 days. It remains positive longer than culture, and it provides positive results for vaccinated patients, for patients with antibiotic pretreatment, and at the late stage of the disease. Edelman et al. (19) compared culture and PCR results during and after erythromycin treatment. On the fourth day of treatment, 56% of the samples were positive by culture and 89% were positive by PCR, whereas after 7 days the rates were 0 and 56%, respectively (19). In one study the sensitivities of PCR for patients with symptoms for 10 days were 70, 50, and 10% for the age groups <1, 1 to 4, and >5 years, respectively (110). The explanation may be found in differences in immune response, which appeared to be faster and stronger with increasing age (110). However, PCR is much more expensive and sophisticated than culture, and PCR cannot distinguish between dead and viable organisms. It is evident that dead bacteria and their degradation products remain in the nasopharynx for some time after the active vegetation period has ended (19). PCR may also detect bacteria transiently colonizing the respiratory tract (40, 41). PCR has been useful in differentiating *Bordetella* isolates in patients for whom biochemical and serological tests gave inconclusive results (99). For molecular typing of clinical isolates pulsed-field gel electrophoresis was superior to PCR in one recently published study (79).

Culture and immunofluorescence are less sensitive than PCR (20). PCR is more rapid than the other methods currently in use, since neither multiplication of the organisms nor increased antibody titers are required. At present, different PCR methods for the detection of *B. pertussis* and *B. parapertussis* are still under investigation, but they have not been compared directly with each other. The recently finished field trials of acellular pertussis vaccines in different parts of the world provide more data on the role of PCR in the diagnosis of pertussis (91, 94, 110). The first collaborative study of different DNA extraction methods, primers, and detection methods is in progress in Germany (103a).

### SEROLOGIC METHODS

A few years after isolating the bacteria, Bordet and Gengou (11) published methods for detecting antibodies to *B. pertussis* in human sera. Detecting agglutinating antibodies to whole *B. pertussis* cells remained the hallmark of pertussis serology for more than 70 years. It was only in the 1980s that immunoassay methods were introduced and the interest in pertussis serology, both in vaccine trials and for diagnostic purposes, increased. During the last decade, ELISA has increasingly been regarded as the method of choice for measuring antibodies to *Bordetella* antigens (75).

#### Methods

Measurement of agglutinating antibodies to whole cells of *B. pertussis* has been modified (65) to a microagglutination method and is used for various purposes (43), although it seems to be less sensitive than enzyme immunoassays (77). The antibodies measured by microagglutination are mostly directed against fimbrial antigens, pertactin, and/or lipooligosaccharide (74).

Immunoblotting techniques have also been applied to per-
tussis serology (87, 105), and although this method does not lend itself to use for routine diagnosis, it has been used as a serological comparator in conjunction with culture and PCR (30, 31). Because highly purified antigens for quantitative ELISA methods are available, the theoretical advantages of qualitative immunoblotting techniques are limited.

Various methods for quantifying antibodies to PT have been developed, such as the CHO (Chinese hamster ovary) cell neutralization test (27) or direct neutralization tests of PT’s ADP-ribosylating activity (56). Although neutralization assays have given some disparate results, both methods seem to measure mainly IgG antibodies to PT, and these antibodies are mostly directed against the enzymatically active S1 subunit of PT (72).

Since 1980, various enzyme immunoassays (14, 27, 76, 82, 111, 114, 122) have been developed, with solid-phase ELISAs being the most widely applied assays. This method has been used in most recent vaccine trials (2, 28, 32, 55, 96), and thus, an impressive amount of data for evaluating ELISAs is now available. Most ELISA techniques use microtiter plates as a solid phase, although polystyrene balls and other solid supports have been described as well. As a conjugate, most assays use isotype-specific polyclonal antihuman antibodies, which are conjugated with various enzymes, e.g., alkaline phosphatase. Various methods for quantitating antibodies have been developed, and the reference line method for calculation has been proven to be superior to other calculation procedures, such as the use of single endpoints and other methods (89). WHO reference preparations for pertussis antibodies do not exist, although most laboratories performing pertussis serology have standardized their methods by using the reference sera from the Laboratory of Pertussis (Center for Biologies Evaluation and Research, U.S. Food and Drug Administration, Bethesda, Md.). Intra- and interassay coefficients of variance can be reduced to less than 10 and 15%, respectively, by use of a standardized ELISA technique. Another problem, which has only recently been addressed, is the lack of comparability between different assays, even when the same antigen is used and when other technical details are shared. An interlaboratory survey of various ELISA methods showed significant differences between participating laboratories. On the other hand, it was also possible to ensure comparability between different laboratories when some precautions were fulfilled (64).

Antigens used in antibody assays. Whole cells of B. pertussis have been used for agglutination methods as well as for other methods. This requires an exact definition of the strain used and its maintenance under controlled conditions to ensure equivalent levels of expression of various antigens on its surface (65).

For ELISA and other purposes, whole-cell sonicates can be used (37), and these have been shown to have sensitivities comparable to those of purified virulence factors of the bacterium, but they tend to lack specificity. Another method with semipurified antigens used copurified extracellular products (120). For most ELISA techniques, however, purified antigens are used. These offer the advantage of allowing an antigen-specific immune response to be measured. For diagnostic purposes, various virulence factors of B. pertussis are available, such as PT, filamentous hemagglutinin (FHA), pertactin (69-kDa outer membrane protein) (12, 108), outer membrane proteins as well as type 2 and 3 fimbriae, which are used either as copurified antigens or as single antigens (75), ACT (6), lipooligosaccharide, porin, and chaperons (75). PT is the single antigen which is specific for B. pertussis, whereas FHA is shared with B. parapertussis and contains epitopes cross-reactive with noncapsulated H. influenzae and other bacteria.

The relevance of cross-reactive antibodies in FHA serology is, however, unclear and seems to have been overestimated.

Development of antibodies after natural infection and vaccination. After infection with the B. pertussis bacterium, antibodies can only be detected relatively late in the process of the disease, i.e., between 1 and 2 weeks after the beginning of the symptoms in nonvaccinated, primarily infected individuals (17). The production of measurable amounts of antibodies may be retarded in infants. The immune response is directed against various antigens of B. pertussis, with reactions to PT and FHA being the most invariable (>90%). Responses to pertactin, lipooligosaccharide, and fimbriae tend to be found somewhat less regularly (30 to 60%) (75). Although diagnostic methods are mainly based on the detection of antibodies of isotypes IgG and IgA, antibodies of all isotypes are produced after infection. Antibodies of isotype IgG are mostly of subclass IgG1, although antibodies to pertactin were 90% IgG1 and 10% IgG4 (83). The diagnostic significance of an IgM response to purified antigens as measured by ELISA has been debated (75). Antibodies of the IgE isotype are rarely produced after natural infection and do not seem to alter the course of the disease (115). IgG responses to PT and/or FHA can be detected in >90% of infected individuals, whereas IgA responses to PT are detected in 20 to 40% of infected individuals and IgA responses to FHA are detected in 30 to 50% of infected individuals. IgG responses against pertactin and fimbriae are detected in 30 to 60% of infected individuals, whereas IgA responses to these antigens occur less frequently (20 to 40% of infected individuals) (54, 107). Erythromycin therapy of the infection does not interfere significantly with antibody production (25).

After infection, the level of antibodies decreases for 1 to 3 years (106, 107), although in nonclosed communities, most sera from nonvaccinated adolescent and older individuals contain antibodies to PT, FHA, and pertactin. These are thought to reflect repeated subclinical infections with B. pertussis (29, 54, 55).

The immunologic response to vaccination is directed against the defined antigens of the vaccine when an acellular pertussis vaccine is used and is directed against various B. pertussis antigens, with a preponderance of activity directed against outer membrane and fimbrial antigens, when the whole-cell vaccine is used. The isotype of the immune response, when measured after administration of the second or third dose of the vaccine, is mostly of the IgG isotype, but IgA and IgE responses are also observed. IgA responses, which are sometimes used diagnostically as an indicator of infection, can be detected only in a small percentage of uninfected vaccinees. No single antigen or isotype, however, can absolutely distinguish between infection and vaccination (78).

Applications of measuring antibodies to B. pertussis antigens. Serology can primarily be used to diagnose pertussis in unvaccinated children. For this purpose, the WHO definition of pertussis requires increases in the levels of either IgG or IgA antibodies to a single or various antigens (121). PT and FHA were the first antigens used for ELISA, and these antigens still seem to be the most reliable ones for use in diagnosis (see above). According to the experiences of the first Swedish trial (2), the increase in the measurable amounts of antibodies necessary for diagnosis has mostly been between 50% (32) and 100% (28, 96). It is still not completely clear whether a high concentration in a single serum sample or in paired serum samples can be used for diagnosis and whether a decrease in antibody levels over a given period of time should also be used as a diagnostic criterion (107).

The diagnosis of pertussis in vaccinated children is more
TABLE 2. Laboratory diagnosis of pertussis

<table>
<thead>
<tr>
<th>Population</th>
<th>Test used for patients with the following duration of cough*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Untreated</td>
</tr>
<tr>
<td>Unvaccinated infants</td>
<td>PCR, culture, DFA</td>
</tr>
<tr>
<td>Unvaccinated children</td>
<td>PCR, culture, DFA</td>
</tr>
<tr>
<td>Vaccinated infants or children</td>
<td>PCR, culture, DFA</td>
</tr>
<tr>
<td>Adolescents and adults</td>
<td>PCR, culture, DFA</td>
</tr>
</tbody>
</table>

* When more than one method is listed, the first method listed is the preferable method. Methods listed in parentheses have a decreased sensitivity at a later stage of the disease.

a Treatment duration with adequate antimicrobial agent of <1 week.

b DFA is always used only in combination with culture or PCR.

c Young infants may be unable to produce specific IgA antibodies.

d Difficult, because these children will have an anamnestic response resulting in a rapid increase in antibody concentrations, precluding detection of a significant increase in antibody concentrations between acute- and convalescent-phase sera. Seroconversion and/or increases in the levels of IgA antibodies have been used in this population, as have single values of antibodies above a certain level (107) and single high values of antibodies 2 to 3 standard deviations above the mean value in vaccinated uninfected individuals (78). Serology from a single serum sample with an increased level of IgG anti-PT has also been used as an indicator of infection in vaccinated populations (66). The detection of antibodies of isotype IgA has been used as an indicator of infection (78), although IgA antibodies could also be detected in unvaccinated and asymptomatic individuals.

For future uses outside of vaccine trials, serology from a single serum sample may be available for the diagnosis of pertussis by using age-specific reference values for different populations, depending on the type of vaccine used. The ongoing serological surveys in U.S. and European populations will serve as a basis for providing such reference values.

In symptomatic adolescents and adults, serology may be the most important diagnostic tool, since pertussis in these individuals is usually diagnosed late in the course of disease and the individuals are thus frequently culture negative. In many cases, however, no increase in antibody levels can be detected; therefore, single serum samples containing high titers of antibodies must suffice to make a renewed recent contact with the bacterial antigen probable (5, 100, 117).

For epidemiological surveys, serology is the only means of gaining information about prior or renewed contact with B. pertussis antigens (16, 22). As a consequence, seroepidemiological studies with large numbers of samples are being planned in the United States and well as in the European Union.

Serological methods can also be used to measure the immunogenicities of pertussis vaccines, and these methods were widely used in the recently finished efficacy trials of acellular pertussis vaccines (73). Finally, to date serological methods represent the only surrogate marker of protection against pertussis, although no single serological marker has been identified, and this problem is very controversial (74, 90, 101, 118).

Various studies have been performed to compare the relative diagnostic sensitivities of culture and serology by ELISA (33, 36). PCR adds sensitivity to the detection of the organisms, and studies comparing the addition of PCR to the diagnostic armamentarium have been published (Table 1).

CONCLUSION

In a review article published in 1987 (85), the authors stated that new diagnostic methods with improved sensitivity, specificity, ease of use, rapidity, and cost-effectiveness were urgently needed for the laboratory diagnosis of pertussis. The recently developed PCR method fulfills only some of these criteria. The method has improved sensitivity and a high degree of specificity, and results can be obtained within 1 day, but the method is too sophisticated and not affordable for routine use in developing countries. Tests with the recently developed monoclonal immunofluorescent antibody (BL-5) were at least as sensitive and specific as culture and need to be further investigated in ongoing clinical studies. In the next decade, the diagnosis of pertussis may well be made by using a combination of methods (Table 2). In developing countries, isolation of B. pertussis will still be the method of choice because of its cost-effectiveness and practicality. A highly specific monoclonal antibody and serology for a single serum sample with age-specific reference values for unvaccinated populations may be an additional method. In developed countries, PCR will have its place in the differentiation of B. pertussis from B. parapertussis infection, in particular in patients with mild symptoms, patients receiving antibiotic treatment, and immunized individuals. Commercially available PCR kits that use the semiquantitative ELISA format for routine diagnosis of pertussis are under development. For epidemiological surveys and symptomatic adolescents and adults, serology will remain the most important method.

REFERENCES


