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International *Bordetella pertussis* assay standardization and harmonization meeting report. Centers for Disease Control and Prevention, Atlanta, Georgia, United States, 19–20 July 2007☆

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ABSTRACT

An international meeting on *Bordetella pertussis* assay standardization and harmonization was held at the Centers for Disease Control and Prevention (CDC), Atlanta, GA, 19–20 July 2007. The goal of the meeting was to harmonize the immunoassays used for pertussis diagnostics and vaccine evaluation, as agreed upon by academic and government researchers, regulatory authorities, vaccine manufacturers, and the World Health Organization (WHO).

The primary objectives were (1) to provide epidemiologic, laboratory, and statistical background for support of global harmonization; (2) to overview the current status of global epidemiology, pathogenesis and immunology of pertussis; (3) to develop a consensus opinion on existing gaps in understanding standardization of pertussis assays used for serodiagnosis and vaccine evaluation; and (4) to search for a multicenter process for addressing these priority gaps. Presentations and discussions by content experts addressed these objectives. A prioritized list of action items to improve standardization and harmonization of pertussis assays was identified during a group discussion at the end of the meeting. The major items included: (1) to identify a group that will organize, prepare, maintain, and distribute proficiency panels and key reagents such as reference and control sera; (2) to encourage the development and identification of one or more reference laboratories that can serve as an anchor and resource for other laboratories; (3) to define a performance-based assay method that can serve as a reference point for evaluating laboratory differences; (4) to develop guidance on quality of other reagents, e.g., pertussis toxin and other antigens, and methods to demonstrate their suitability; (5) to establish an international working group to harmonize the criteria to evaluate the results obtained on reference and proficiency panel sera; (6) to create an inventory to determine the amount of appropriate and well-characterized sera that are available globally to be used as bridging reagents for vaccine licensure; and (7) to seek specific guidance from regulatory authorities regarding the expectations and requirements for the licensure of new multicomponent pertussis vaccines.

1. Introduction

Pertussis (whooping cough) is a highly contagious, acute respiratory illness caused by the bacterial pathogen *Bordetella pertussis*. Despite high vaccination coverage, pertussis continues to be a major cause of morbidity and mortality in the United States, with 25,616 and 15,632 probable or confirmed cases reported in 2005 and 2006, respectively [1–5]. Among the reportable bacterial vaccine-preventable diseases in the United States with universal childhood vaccination, pertussis is the least well controlled [1]. Pertussis also remains a substantial public health problem in other developed regions, such as the European Union and Canada [6–10]. Studies suggest that there are approximately 48.5 million annual cases of pertussis worldwide, with 295,000 deaths [11–13]. However, the total burden of pertussis is likely underestimated, especially among adolescents and adults, in whom typical pertussis symptoms are often absent making diagnosis difficult [1,2,4,12–15].

The laboratory diagnosis of pertussis is challenging [16,17]. Culture is highly specific but requires a long incubation and sensitivity can be low [16,18]. Rapid, sensitive, and specific polymerase chain reaction (PCR) assays have been designed to detect *B. pertussis* [11,19–21]; however, these assays are not well standardized and problems have occurred with specificity, mainly during outbreaks [3,22–24]. Serologic testing with *B. pertussis* antigens, pertussis toxin (PT) in particular, can be sensitive and specific, but the tests are not widely available, have not been fully standardized and no universally accepted serologic correlates for protection are available [25–28].

The meeting sessions covered the following topics: global epidemiology of *B. pertussis* disease; pathogenesis and immunology of *B. pertussis*; assay standardization and harmonization; serodiagnosis of *B. pertussis*; current and future *B. pertussis* vaccine composition; and issues in vaccine evaluation with perspectives

☆ The findings and conclusions in this report are those of the author(s) of each section and do not necessarily represent the entire group of participants or the official position of the Centers for Disease Control and Prevention/Agency for Toxic Substances and Disease Registry. The findings and conclusions in this report have not been formally disseminated by the U.S. Food and Drug Administration and should not be construed to represent any Agency determination or policy.

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from regulatory authorities and vaccine manufacturers. Group discussions included serodiagnostics, vaccine evaluation, correlates of protection, and harmonization of assays. A list of action items was developed to identify existing knowledge gaps and to coordinate international efforts to standardize pertussis assays for immunologic and diagnostic use.

The meeting was opened by M. Lucia Tondella, PhD (CDC), followed by Nancy Messonnier, MD (CDC), who stressed the importance of having laboratory, epidemiologic and statistical groups working together to facilitate and support global harmonization. Dr. Messonnier also highlighted the necessity of improving pertussis diagnostics, pointing out that in 2007 alone, CDC experienced numerous occasions when problems with diagnostics impeded epidemiologic investigations, resulting in a substantial public health outcry [3]. CDC's Meningitis and Vaccine Preventable Diseases Branch has an extensive experience working on standardization and harmonization of assays on a variety of organisms, and this experience can be brought to bear at this meeting to determine the current status of pertussis serologic technologies in key laboratories, to facilitate discussion of the key issues in pertussis serology and to establish consensus on the action plan needed to address these issues. Presentations and discussions were summarized below.

2. Epidemiology—current status of global *B. pertussis* disease

Presenters: James D. Cherry, MD (David Geffen School of Medicine, University of California, Los Angeles) and Nicole Guiso, PhD (Institute Pasteur, Paris, France)

In the prevaccine era in the United States, pertussis was a disease with cyclic peaks averaging every 3.3 years. The average rate of reported cases was 157 per 100,000 population. More than 93% of reported cases occurred in children younger than 10 years of age. By the 1970s, pertussis was well controlled by immunization, and only 1000–2000 cases were reported per year; 50% of those cases occurred in infants. At present, more than 65% of pertussis reported cases are in persons older than 10 years of age. Since 1984 there has been a modest increase in reported pertussis from <1 to 9 cases per 100,000 population. In recent years (2004–2005) the reports have increased considerably; however, the number of cases is still approximately 15-fold less than in the prevaccine era. The cycles of reported pertussis today are essentially the same as they were in the prevaccine era [15,29].

Examination of reported pertussis in several countries between 1980 and the present found two different patterns. In some countries (Argentina, Bangladesh, Bolivia, Brazil, Cambodia, Ghana, Pakistan, Peru, Sudan, Vietnam), there was a reduction in total cases, similar to that seen in the United States between 1950 and 1975. A similar pattern was also noted in the United Kingdom, where pertussis was reasonably well controlled by vaccination in the 1960s and early 1970s, but then concerns for vaccine safety resulted in a marked decline in immunization and pertussis became epidemic. It was then brought under control in the early 1980s by increased vaccine usage. In Italy, where vaccination was not being consistently offered until the efficacy trials of 1990, a marked reduction in pertussis was related to the use of diphtheria and tetanus toxoids and acellular pertussis component (DTaP) vaccines. Success in the control of reported pertussis in all of the above countries with the exception of Italy was secondary to use of 3 doses of DTwP (whole-cell vaccines) in the first year of life.

In France, because of high vaccination coverage among children with DTwP vaccine during the last 40 years, pertussis predominantly affects children who are too young to be vaccinated and adults who are no longer protected by vaccine or disease-induced immunity [30–35]. A survey undertaken in 1993 and 1994 in a pediatric hospital network demonstrated an increase in the number of children hospitalized with pertussis suggesting the importance of introducing booster dose(s) to prolong vaccine immunity and reduce the exposure to *B. pertussis* of infants too young to be immunized [31]. Therefore, a fifth dose of acellular pertussis (aP) vaccine was introduced for 11–13-year olds in 1998, followed by the introduction of the so-called "cocoon strategy" in 2004. The current vaccine schedule consists of primary immunization for children aged 2, 3 and 4 months and boosters at 15–18 months, at 11–13 years old, and for young adults and healthcare workers in contact with infants [36]. No DTwP vaccine has been commercially available since 2005. The national hospital-based surveillance (RENACOQ network) has not shown any resurgence of pertussis. As observed in other countries, cycles of the disease have occurred every 3–5 years; however, the number of pertussis cases in 2005 did not reach the values observed in the earlier peak years which could be linked to the impact of the 11–13-year booster introduced 9 years previously [35,37].

Reported pertussis cases in Australia, Chile, Israel, Japan, Norway, and Poland have increased in recent years. In Australia, Israel, and Norway, the increase followed the introduction of DTaP vaccines. Possible reasons for the resurgence of reported pertussis are (1) greater awareness of pertussis; (2) waning of vaccine-induced immunity; (3) lessened potency of pertussis vaccines; (4) genetic changes in *B. pertussis*; and (5) the general availability of better laboratory tests for the diagnosis of pertussis. Of most importance is a general greater awareness of pertussis. The availability of better laboratory tests (PCR) and single-serum serology for diagnosing adolescent and adult pertussis has also contributed to the increase in reported cases. As evidence also suggests that in general DTaP vaccines may have less efficacy in children than the more potent DTwP vaccines [15,38,39], it is plausible that decreased efficacy or duration of protection could possibly contribute to the outbreaks that have been seen in pre-adolescence and adolescence.

Investigators in the United States and other areas of the world have used various laboratory methods to diagnose pertussis in subjects with prolonged cough illnesses. One of the most commonly used diagnostic markers is high single-serum titers to PT. Results of these studies suggest that about 13% of prolonged cough illnesses are due to *B. pertussis* infection. However, because not all adults have a PT antibody response following infection, the percentage is likely to be higher [15,29].

In summary, *B. pertussis* infections in adolescents and adults are very common. Rates of reported pertussis may be as much as 40- to 160-fold lower than actual illness rates, and unrecognized infections (e.g., asymptomatic or with nonspecific symptoms) are likely to be more common than symptomatic infections. Today, symptomatic adolescents and adults appear to be the major sources of infection for nonvaccinated children.

2.1. Discussion

Much of the discussion relating to epidemiology focused on the increase of pertussis in recent years despite adequate or increasing vaccine use in young children. In general, the most likely cause was thought to be greater awareness of pertussis. However, data from Australia (presented later in the meeting) suggested that false-positive laboratory tests contributed to the increase in reported pertussis in at least one setting. Discussion included the possibility that in some instances genetic change in the pertussis organism may have contributed to increases. In countries using DTwP, changes in vaccine manufacturers and even lots may have a significant relationship to efficacy. The increase in observed pertussis

associated with the introduction of acellular vaccines should be studied more carefully.

3. Pathogenesis and immunity in pertussis

Presenter: Erik L. Hewlett, MD (University of Virginia School of Medicine, Charlottesville, Virginia)

Multiple virulence factors produced by *B. pertussis* are able to disrupt the normal physiology of the host, causing the disease of pertussis [40]. This disease consists of a localized infection of the respiratory tract, with secondary systemic effects resulting from the actions of several bacterial products and from the host response to those factors. The molecular basis for the characteristic paroxysmal cough is not known, but is not mimicked by intravenous PT [41]. One likely role of PT is to impair the innate immune response of the host, causing leukolymphocytosis and blocking the migration of murine macrophages [42]. Reduced expression of L-selectin on the surface of leukocytes from infants infected with *B. pertussis* has also been demonstrated [43,44]. The leukolymphocytosis and several other effects of PT are mediated by the ADP-ribosyl transferase activity of the catalytic domain. The PT binding to its receptor elicits a separate set of responses, known as B-subunit effects. Recently it has been shown that PT B-subunit activates signaling through the T-cell receptor, without any contribution from ADP-ribosylation [45]. Similarly, PT and an enzymatically inactive (non-ADP-ribosylating) mutant stimulate cytokine production and dendritic cell maturation [46].

Evidence indicates that multiple virulence factors from *B. pertussis* have immunomodulatory effects. In several instances the effects appear to be antagonistic to one another. For example, it has been shown that filamentous hemagglutinin (FHA) is both pro-inflammatory and able to induce apoptosis in macrophages [47]. Similarly, adenylate cyclase toxin (ACT), which is known for its inhibitory effects and cytotoxicity on neutrophils and other phagocytic cells [48–50] also has pro-inflammatory effects, inducing IL-6 from respiratory epithelial cells [51] and cyclooxygenase-2 (Cox-2) in cells expressing the integrin, CD11b/CD18 [52]. The disease process of pertussis appears to be multifactorial, rather than the function of a single toxin/virulence factor. Therefore, the ideal immunologic response from the host consists of antibodies directed against multiple virulence factors, resulting in inhibition of bacterial adherence, neutralization of the actions of the toxins and clearance of the infecting organisms. In addition, a cell-mediated component in the host response to immunization and infection has been studied, but the relevant target antigen(s) to which this cellular response is directed and the contribution that it makes to bacterial clearance are unknown [53–55].

Several major issues in pertussis pathogenesis remain unresolved. Despite the numerous known biological effects of individual virulence factors, especially in vitro, the in vivo target tissue(s) of these molecules and the mechanism by which the characteristic cough is generated are mysteries. The molecular details of how Bvg, the two-component regulatory system, controls production of the virulence factors are not known. In summary, pertussis is a multicomponent disease, which requires more than a simple approach to its recognition, treatment and control. Recent analyses of the effects of known toxins and other virulence factors on the immune system have revealed that they are often multifunctional: cytotoxic, stimulatory, inhibitory, pro- and anti-inflammatory.

3.1. Discussion

Much of the discussion focused on the potential neurophysiologic effects of the multiple virulence factors; animal modeling, including new methods of infection through inhalation techniques; and novel imaging analysis that could help in understanding the pathogenesis of pertussis in humans. Recent research studies have shown presence of *B. pertussis* DNA from clinical specimens of children several months after infection. The possibility that the remaining DNA could be triggering the cough by an unrecognized mechanism was speculated.

4. Why are standardization and harmonization of *B. pertussis* assays necessary?

Presenter: Kathryn M. Edwards, MD (Vanderbilt Medical Center, Nashville, Tennessee)

Standardization and harmonization are necessary for several reasons: to assess the immunogenicity of new pertussis vaccines or new combination vaccines that include pertussis antigens, to compare the serologic responses to these new vaccines with those evaluated in earlier vaccine efficacy studies for the purposes of vaccine licensure, to establish serologic criteria for the clinical diagnosis of pertussis disease, and to conduct seroepidemiologic studies to assess the circulation patterns of pertussis in the community.

Multiple aP vaccines were licensed for use in infants, adolescents and adults [1,2,56]. Combination vaccines incorporating various antigens into a single injection have been designed. These combination vaccines may be associated with reduced serologic responses to one or more vaccine antigens. Therefore, antibody titers induced by these products must be compared with those seen after the separate administration of the individual vaccines before combination vaccines can be considered for licensure.

Safety and immunogenicity of 13 aP and two DTwP vaccines have been assessed in a single large clinical trial [57]. Although the trial evaluated different pertussis antigens administered in various concentrations, all the 15 vaccines were found to be safe and immunogenic. Several of these vaccines were then evaluated in efficacy trials in the European Union and Africa and were found to be efficacious in preventing culture-confirmed pertussis. Since placebo-controlled efficacy trials would now be considered unethical, new vaccines must be evaluated by comparisons with the immunogenicity data of the vaccines evaluated in earlier efficacy trials. Thus, the need for standardized assays is critical.

Standardized serologic assays are also needed to diagnose pertussis disease, particularly in adolescents and adults. A study was conducted to determine population-based antibody levels to three pertussis antigens, PT, FHA, and fimbrial proteins (FIM), for the purpose of establishing diagnostic cutoff points in adolescents and adults in the United States [58]. Enzyme-linked immunosorbent assays (ELISAs) were performed on sera from more than 6000 US residents aged 6–49 years. Quantifiable (>20 ELISA units [EU]/ml) anti-FHA and anti-FIM IgG antibodies were common but quantifiable anti-PT IgG antibodies were less frequent. An anti-PT IgG level of $\geq$94 EU was proposed as the diagnostic cutoff point in subjects 10 years of age and older. Application of this cutoff point to culture-confirmed illness yielded a high diagnostic sensitivity (80%) and specificity (93%). Anti-PT IgG assays with a single serum sample appeared to be useful for identification of recent *B. pertussis* infection in adolescents and adults. However, these assays must be standardized to remain useful in the diagnosis of pertussis.

Finally, standardized serologic methods for *B. pertussis* are needed to assess epidemiologic trends in pertussis disease in the population. This is particularly relevant with the universal recommendations for adolescent and adult pertussis vaccine in many developed countries. How universal vaccination will affect disease burden in the various age groups remains to be determined. In addition, comparisons of seroepidemiologic results from one country to another are dependent on standardized assays.

5. *B. pertussis* multilaboratory serologic studies: designs, status and outcomes

Presenters: Bruce D. Meade, PhD (Meade Biologics LLC, Hillsborough, NC), Dorothy Xing, PhD (National Institute for Biological Standards and Control (NIBSC), Potters Bar, UK) and Anna Giammanco, PhD (Department of Hygiene and Microbiology, University of Palermo, Italy)

Important insight on strategies for international standardization of pertussis immunoassays is provided by the results of an international collaborative study performed in 1995 [59]. In that study, 32 participating laboratories were asked to quantify specific antibody to PT, FHA, pertactin (PRN), and FIM in a panel of 21 blinded samples by using those ELISAs routinely performed in that laboratory. The laboratories employed a variety of procedures, antigens, conjugates and other reagents, and calculation methods. Results indicated an overall consistency among the laboratories. A reasonable quantitative agreement was achieved among the 19 laboratories with assays calibrated using the US reference pertussis antisera. This agreement demonstrated that use of a common reference serum had been successful at harmonizing results. To promote a higher level of international standardization of pertussis immunoassays, the highest priority recommendations would be to improve the availability of reference reagents and methods, including international reference sera, a proficiency panel of well-characterized sera, and well-characterized antigens.

In recent years, significant progress in the preparation and standardization of international reference sera has been made. The WHO collaborative study, "Evaluation of proposed international reference preparations for pertussis antiserum (human),"was a multicenter collaboration between NIBSC, UK; the Center for Biologics Evaluation and Research (CBER) at the Food and Drug Administration (FDA), US; the Institut für Infektiologie (IFI), Germany; and a number of international participants. Reference sera from the United States (US reference pertussis antiserum [human] lot 3 and lot 4 for IgG-antibodies and lot 5 for IgA-antibodies) were prepared by FDA, CBER more than 10 years ago, and since then have been commonly used as reference standards for pertussis serologic assays [58–63]. Currently, only limited quantities of these sera remain. With an increasing number of vaccine and diagnostic studies, more widely available international standard preparation(s) are needed. The WHO Ad Hoc Pertussis Working Group recommended the preparation and standardization of an international human reference antiserum to pertussis antigens to replace the current widely used CBER preparations before the supply is exhausted [64]. It was agreed that as far as possible, continuity with the existing CBER reference preparations should be maintained. These new international reference materials were intended for the following uses: (1) vaccine studies of products in current distribution, as well as those under development; (2) studies of serologic responses to infection; (3) epidemiologic surveillance; (4) future assessment of antibodies to other antigens apart from PT, FHA and PRN; and (5) evaluation of new generation vaccines.

Serum pools were kindly donated by Dr. Wirsing von König, IFI, Krefeld, Germany. They were prepared after recalcification of plasma samples selected from a blood bank during a pertussis outbreak in Germany. These sera were then lyophilized at NIBSC, resulting in four batches of freeze-dried preparations of 7800 ampoules of higher titer and 14,500 ampoules of lower titer IgG-anti-PT. Preliminary assessment of the freeze-dried materials in NIBSC showed that in comparison with the materials before freeze-drying, all freeze-dried material maintained their original ELISA binding activities. Furthermore, comparison of the candidate materials with the US reference sera showed that the dose–response lines did not deviate significantly from parallelism.

An international collaborative study to evaluate the candidate preparations was initiated in March 2007. The aims of the study were to (1) characterize candidate international reference preparations for pertussis antiserum (human); (2) compare candidate references with the US reference preparations, lots 3, 4, and 5; (3) compare candidate reference preparations with other available reference preparations, e.g., in-house preparations; and (4) define unitage for the candidate preparations for anti-PT, anti-FHA and anti-PRN, maintaining continuity with widely used reference preparations.

Samples provided to the participants were the four candidate reference preparations: the three US reference sera plus a negative control serum. All participants were quested to (1) carry out three independent assays for IgG anti-PT, FHA and PRN by ELISAs; (2) use their own methodology, reagents and calculation methods; and (3) include their in-house references and controls. Participants were also encouraged to perform other assays that could be of interest, e.g., IgG anti-FIM 2/3, IgA antibodies to the antigens, PT-neutralizing antibodies (CHO-cell assay). A total of 23 laboratories worldwide participated in this study, including 10 from Europe, 8 from North America, 2 from South America and 1 laboratory from each of Asia and Australia. All participants were requested to submit their raw results to NIBSC, where the statistical analysis will be performed. A study report will be submitted to the Expert Committee on Biological Standardization (ECBS) of WHO.

Another multilaboratory effort to standardize pertussis serologic assays was the European Sero-Epidemiology Network (ESEN) project, coordinated by the Health Protection Agency (HPA), Communicable Disease Surveillance Center (CDSC), London [62,65]. The aim of the ESEN project was to coordinate and harmonize serologic surveillance for a variety of infections in Europe. Age-stratified serum banks of a recommended sample size of 3300 individuals were collected by each participant country and one laboratory was selected to serve as a reference laboratory for each disease. All laboratories tested sera from their own country plus a shared panel of approximately 150 serum samples [62].

The standardization process allowed a comparison of national serosurveys for pertussis [62]. Because of the difficulty with standardization of cell extracts, the ESEN project emphasized purified antigens, focusing on IgG anti-PT assays because of the higher sensitivity and specificity for serodiagnosis [62]. Since most individuals had been exposed to pertussis vaccine or infection, the study focused on an estimation of the incidence of recent infections, as defined by the presence of IgG anti-PT antibodies above defined thresholds [61]. To accomplish this, quantitative assays were required and standardization efforts were based on analytical performance in the assay range near the diagnostic thresholds. In conclusion, the ESEN project demonstrated that standardization of pertussis IgG anti-PT assay was possible [62,66].

6. Serodiagnosis of *B. pertussis* in different countries

Presenters: C.H. Wirsing von König, MD (Institute fur Hygiene und Labormedizin, Krefeld, Germany), Nicole Guiso, PhD (Institute Pasteur, Paris, France), Linda Han, MD, MPH (Bureau of Laboratory Sciences, Department of Public Health, Boston, Massachusetts, US), and Linda Hueston, MS (Centre for Infectious Diseases and Microbiology (CIDM), Institute of Clinical Pathology and Medical Research (ICPMR), Westmead, Australia)

Appropriate application of a diagnostic laboratory test requires an understanding of the test as well as knowledge of the patient. Relevant criteria for the test include test format; specificity and sensitivity; and reference systems. Clinical interpretation of a test result concerning *B. pertussis* infection also requires an understanding of (1) the distinction between primary and secondary infection;

(2) the differential diagnoses, including other agents; (3) the kinetics of immune responses to infection; and (4) an understanding of the information provided by the test beyond that provided by the clinical history alone. The performance of laboratory tests can be displayed by cutoff values as shown in a reporter–operator characteristics (ROCs) curve, as well as by positive and negative predictive values. The differential diagnosis for *B. pertussis* as an agent of prolonged cough includes infectious agents such as adenovirus, respiratory syncytial virus, rhinovirus, human parainfluenzavirus, influenzavirus A and B, *Mycoplasma pneumoniae*, human metapneumovirus, and human coronavirus.

Several alternate approaches have been used for serodiagnosis. When paired sera were used, one study defined a case if there was 100% or more increase in antibody concentration or 50% or more decrease in antibody concentration. For single-sample serology, various cutoff-values have been proposed, such as >∼125 EU/ml IgG-anti-PT in the Netherlands; and >100 EU/ml IgG-anti-PT or IgG-anti-PT >50 EU/ml and IgA-anti-FHA > 50 in Germany. In most diagnostic laboratories, a so-called "grey-zone" is defined for sera that are positive and above a specified threshold but between the diagnostic cutoffs. This "grey-zone" is set in the Netherlands and Sweden to 20 EU/ml of IgG-anti-PT.

The sensitivity and specificity of single-sample serologic assays have been determined. One study was done in a population of children 0–11 years of age, mostly nonvaccinated (Germany), using a cut-off of ∼50 EU/ml (95th percentile of population). With a specificity of 95%, a sensitivity of 67% was found, which increased to 82% when IgA-anti-FHA was added [67]. Another study was done without age limit in a mostly vaccinated population (Netherlands) using a cutoff of ∼125 EU/ml (99th percentile of population). The study found a sensitivity of 76.4%, whereas a cutoff ∼62 EU/ml (∼95th percentile of population) had a sensitivity of 88.8% [61].

A recent German study evaluated 243 persons in hospital departments, pediatrician's offices, and child-care facilities, who received one injection of aP vaccines. After informed consent, blood was sampled before vaccination, and 1 month, 1 year, 2 years, 3 years and 4 years after vaccination. IgG- and IgA-antibodies to PT, FHA and IgG-anti-PRN were measured. In this population, when the distribution of IgG anti-PT levels prior to immunization was examined, the 95th percentile was 47 EU/ml, the 98th percentile was 103 EU/ml, and the 99th percentile was 163 EU/ml.

Applying a cutoff of 125 EU/ml to a seroepidemiologic study in a population of persons aged 20 to >65 years in various countries in the European Union resulted in the following percentage of the cohort showing values above the cutoff: Netherlands (1.4%), Finland (1.5%), Germany (former GDR) (1.7%), France (2.3%), Germany (former FRG) (3.0%), UK (5.0%), and Italy (6.5%) [66]. Seroepidemiologic studies can also demonstrate the cyclic nature of *B. pertussis* infections. In Germany, a population of 18–60-year-old blood donors was screened in different years. The percentage of this cohort with IgG-anti-PT ≥ 100 EU/ml was as follows: 4/600 (0.7%) in 2002; 6/1500 (0.4%) in 2003; and 70/2000 (3.5%) in 2005.

For a diagnostic laboratory, it is important to define how the customer will use the serologic information, specifically if it is used for clinical decisions and patient management or if it is used for epidemiologic studies. Similarly, clarity is needed regarding whether the definition should include all infections, symptomatic infections, infections with medical resource use, or severe infections. Diagnostic serology is also strongly influenced by the time when patients seek medical attention relative to onset of symptoms. In German studies performed among persons with PCR-positive cases, the median number of days of coughing before seeking medical attention was found to be 7.8 days for schoolchildren, aged 7–12 years, 12.5 days for adolescents, aged 12–18 years, and 17.3 days for adults, aged 18–81 years.

In France, serologic testing is performed for epidemiologic studies and surveillance. Serology consists of measurement of IgG anti-PT antibody titers using the reference ELISA and purified PT provided by the manufacturers [68]. A positive case is defined by either a twofold change in the titers between two serum samples obtained at a 1-month interval or a titer >100 EU/ml in a single serum sample, only if the serum is collected after more than 3 weeks of cough and 3 years after a vaccine booster. In some French laboratories, an "in house" Western blot assay using purified PT, as well as various commercial tests have been used for routine diagnoses. These assays are not validated and have also been responsible for false-negative or false-positive results. For this reason, microbiologists have preferred real-time PCR for routine diagnoses. Serology based on measurement of anti-PT titers may be less useful in the future, since children, adolescents and now adults are vaccinated with aP vaccines containing PT. The proportion of laboratory tests performed to diagnose pertussis in France has changed over the years, according to the RENACOQ database [37]. In 1997, 84% of pertussis cases were diagnosed by culture, 50% by PCR and 23% by serology, whereas in 2005; 67% of cases were diagnosed by culture, 90% by PCR and only 1% by serology.

In the United States, the Massachusetts State Laboratory Institute has been using a single-serum anti-PT IgG EIA on patient samples since 1987. Other versions of the current assay have been used in the past, including measurements of IgA and IgM to PT, IgG to FHA, and anti-PT IgG in paired sera. Ultimately, all these other assays were discontinued when data indicated that they did not contribute much additional information beyond that obtained from the single anti-PT IgG alone.

Plates are coated with commercially prepared PT. Standards are derived from pools of positive patient samples and are calibrated against the US reference lot 3. A concentration (μg/ml) is assigned, calculated from a five-point standard dilution curve. The cutoff point for positivity was established on 100 adults, including blood donors and healthy volunteers with anti-PT IgG levels ranging from 0.1 to 15 μg/ml [63]. A cutoff point was chosen based on the 99% upper tolerance limit, such that there is 95% confidence that 99% of the population falls below the cutoff. The cutoff point was set at 20 μg/ml, which is equivalent to 200 CBER units per ml. This is actually quite high relative to what other groups have chosen [58,61].

Clinicians are asked to order a serologic assay only for patients who are older than 11 years of age and only when coughing has been present for more than 14 days. Specimen volumes have been increasing steadily over the last 15 years, so that in 2006, more than 9000 specimens were tested. Seropositivity has been fairly constant at approximately 10% of the submitted samples.

Many of the challenges associated with the assay arise from manipulations needed to produce a quantitative test result. It may make sense, at least in Massachusetts, to consider some modification of the assay to make it more qualitative, simple, and robust, and able to be performed consistently and accurately with less effort than is currently expended.

At least two knowledge gaps prevent wider application of the assay. First, the impact on assay results of adolescents and adults having recently received tetanus toxoid, reduced diphtheria toxoid and acellular pertussis (Tdap) vaccination is not fully understood. The Massachusetts Immunizations Program contacts every seropositive patient and asks about recent vaccination history. In the last 2 years, since Tdap has been available, only two seropositive patients with recent Tdap vaccination have been identified. One person was vaccinated the day before blood was drawn for serology, so the antibodies detected were unlikely to have been due to vaccine. In Massachusetts, Tdap-associated IgG may be less of a problem than was originally anticipated. Possibly, post-Tdap

immunization IgG levels do not frequently exceed the relatively high assay cutoff value. Alternatively, clinicians may not be submitting samples from recent vaccine recipients, recognizing that those individuals are likely to be protected from disease.

A second knowledge gap is the uncertain applicability of IgG EIA results to children younger than 11 years of age. Results are considered uninterpretable for that age group, because of the possible presence of vaccine-associated antibody. Massachusetts State Laboratory Institute data on seropositivity by age demonstrate a peak in seropositivity among adolescents and young adults, and also a peak at 5–6 years of age. Admittedly, no clinical information is available for the 5–6-year-old patients because they are not considered to be pertussis case-patients and are not investigated. In addition, their vaccination status is unknown. Nevertheless, the peak in seropositivity that occurs at this age may correspond to the fifth dose of DTaP. Interestingly, the peak is not very wide, spanning only 2–3 years, so the application of the assay could likely be extended down to some ages less than 11 years.

Australia has a 16-year experience of using commercial serologic kits for the routine diagnosis of pertussis. IgA has been used rather than IgG kits because IgA is believed to be short-lived (12–14 weeks) in comparison to IgG (26–52 weeks), and, in children at least, IgA is not produced in response to the vaccine.

Unusually large numbers of pertussis IgA-positive tests were reported to public health authorities during 2006. At the time, only two IgA commercial serologic tests were used, ELISA kit 1 (82% of laboratories) and ELISA kit 2 (15% of laboratories). This prompted a widespread evaluation, comparing various commercial available ELISA kits with complement fixation (CF), IgA immunofluoresence (IF) and IgA Western blot (WB) kits. Ninety healthy adults with no clinical history of respiratory illness in the preceding 12 weeks and negative by CF, IF and WB formed the negative group for the evaluation. The positive group consisted of 27 children and adults with a clinical history of pertussis of less than 6 weeks' duration and positive by CF, IF and WB. The respective antigens, sensitivity and specificity of each commercial IgA ELISA kit were as follows: (1) kit 1, whole cell, 66.7%, 86.7%; (2) kit 2, FHA&PT, 59.3%, 94.4%; (3) kit 3, FHA&PT, 74.1%, 98.9%; (4) kit 4, FHA&PT, 77.8%, 97.8%; (5) kit 5, FHA&PT, 77.8%, 97.8%; (6) kit 6, FHA&PT, 37.0%, 92.2%; (7) kit 7, PT, 33.3%, 90.0%; (8) kit 8, FHA&PT, 59.3%, 87.8%; (9) kit 9, FHA&PT, 63.0%, 85.6%; (10) kit 10, FHA&PT, 85.2%, 83.3%; and (11) kit 11, FHA&PT, 66.7%, 76.7%. These results demonstrate a wide variability among the commercial tests evaluated.

In 2006, CIDMS laboratory tested 1547 serum samples for pertussis IgA using the ELISA kit 1. Following the evaluation of various commercial tests, over 90% of these samples were retested and 73% were found to be false-positives. The false-positive specimens reacted with the FHA antigen band only, suggesting that PT would be a better choice for an ELISA antigen. However, the only kit that used PT as an antigen performed poorly, which emphasizes the need for better standardization. In addition, a group of 70 negative samples was tested by the ESEN IgG ELISA [62] using a 100 EU/ml cutoff; 20 samples had IgG levels >100 units, which would have been interpreted as evidence of recent infection when clearly these individuals were not symptomatic. This highlights the need for caution in determining a cutoff for recent infection in adults, particularly in highly vaccinated populations.

In general, diagnostic laboratories prefer commercially available assays. Two studies so far have evaluated selected commercially available ELISAs for *B. pertussis* serology: Kösters et al. used paired sera from 20 patients (children), sera from 15 vaccinees, 7 interlaboratory comparison samples, and 4 reference preparations [69]. Schellekens et al. used paired sera from 41 PCR-positive patients (median age 3 years) and 65 control patients with respiratory symptoms (median age 30 years) [70]. Another study with various currently existing serologic tests is ongoing and results are expected to be available in early 2009.

In summary, *B. pertussis* serodiagnosis still suffers from many unsolved problems, such as that diagnostic testing is performed in immunologically non-naive populations with antigens that are components of aP vaccines. Generally an anamnestic immune response develops more rapidly than symptoms, and immune responses to vaccine antigens cannot be distinguished from response to infection. Problems also exist with serodiagnosis of *B. parapertussis* infections. Serologic interpretation requires knowledge of pertussis immunization, information not typically known by the testing laboratory. Reliable, standardized ELISA systems are not commercially available at this time. Additionally, the clinical course appears to differ between primary and non-primary infections. For most subjects, clinical case definitions for pertussis remain nonspecific. There are currently no suitable serologic tests for recently vaccinated patients, since tests based on nonvaccine antigens have not been satisfactory for diagnosis. Finally, when using single-sample serology, population-based cut-offs will need re-verification after any change made in a vaccination schedule.

6.1. Discussion

Discussion focused on the use of antigens not present in vaccines for serologic diagnosis of pertussis. Cherry et al. [71] showed that patients with vaccine failure responded poorly to the ACT antigen, suggesting an induced tolerance due to the phenomenon called "original antigenic sin." In this phenomenon, vaccinated patients respond to antigens that they have been primed with and do not respond to new antigens (e.g., ACT) associated with infection. Therefore, it was suggested that nonvaccine antigens may not be as good as PT for serologic diagnosis of pertussis in vaccinated populations. Other points of discussion included the potential use of reverse vaccinology approach to identify new antigen candidates to be used in serologic diagnostics and lack of specificity of other antigens other than PT, such as FHA and ACT. The lack of sensitivity and specificity of commercial tests currently available worldwide was broadly discussed. It was pointed out that one commercial IgG and IgA anti-PT test available in the United States has been standardized to provide adequate sensitivity and specificity for the diagnosis of pertussis in persons older than 10 years of age.

7. Correlates of protection

Presenter: James D. Cherry

In studies of whole-cell pertussis vaccines, agglutinin titers of 1:320 or greater were found to be protective against pertussis when children were exposed within the household [72]. Agglutinating antibodies were thought to be primarily directed against the agglutinogens FIM-2/3, PRN and lipopolysaccharide.

Only two of the nine aP vaccines efficacy trials conducted during the 1980s and 1990s were done in such a way that data relating to serologic correlates could be developed. These trials were centered in Erlangen, Germany, and Stockholm, Sweden [27,28,73,74]. To determine serologic correlates, antibody titers to specific antigens were determined at the time of exposure. Both studies indicated that PRN was most important in protection and that antibody to FIM was next most important. Also noted in both studies was an apparent antagonism between antibody to PT and FIM. In clinical trials in which children with both mild and severe disease were included in the analyses, efficacy jumped considerably for vaccines that contained PRN as well as PT and FHA when compared with PT and PT/FHA vaccines. In the Erlangen trial, the imputed titers at the time of exposure in 6 DTP and 11 DTaP vaccine failures were examined. In the majority of the failures, subjects had high levels

of antibody to PRN, which is difficult to explain in relation to the overall data on serologic correlates. This indicates that there is much yet to be learned about serologic correlates. Of interest is the fact that when a DTwP vaccine (Evans vaccine) was compared with a two-component DTaP vaccine, a three-component DTaP vaccine and a five-component DTaP vaccine, the greatest efficacy was noted with the DTwP vaccine. This vaccine elicited minimal antibody responses to PT and FHA (10 and 34 EU/ml, respectively) and high values to PRN and FIM (150 and 677 EU/ml, respectively).

7.1. Discussion

Discussion centered on diagnosing pertussis serologically using antigens contained in the vaccines and antigens not contained in the vaccines. Although still controversial, if acute-phase serum is collected early, titer increases against both vaccine antigens and nonvaccine antigens are useful for diagnosis. Further discussion related to cell-mediated responses associated with infection, use of animal models in studies of infection and consideration of additional antigens that might be included in vaccines.

8. The composition of *B. pertussis* vaccine with considerations for the future

Presenter: John B. Robbins, MD (National Institutes of Health, Bethesda, Maryland, US)

Dr. Robbins presented evidence supporting the hypothesis, based on the classic article by Pittman [75], that an inactivated and immunogenic pertussis toxoid (PTox) is both essential and sufficient for a pertussis vaccine [76]. Furthermore, he discussed data suggesting that multicomponent pertussis vaccines include nonprotective antigens [77,78] and may be more difficult to standardize [79].

US pertussis cases reported to CDC have increased despite a high immunization rate among infants and children [1–3,5,56]. Booster doses, starting in adolescence and offered every 10 years, will maintain a high level of immunity in the population, and Dr. Robbins recommended that a genetically inactivated toxin should replace the chemically inactivated toxoids for mass immunization of infants, adolescents and adults [80,81].

With respect to the goals of the meeting, the importance of assays to measure serum PT IgG was emphasized because PT IgG is the only reliable assay for serologic diagnosis of pertussis after the acute phase of pertussis has passed [67,68], and because the concentration of vaccine-induced IgG anti-PT correlated with the efficacy of monocomponent PTox [82]. The correlation indicates that the level of IgG anti-PT may predict the efficacy of aP vaccines [82].

8.1. Discussion

A number of participants raised questions related to the hypothesis that PTox alone is sufficient for a pertussis vaccine. It was mentioned that the study supporting the hypotheses did not include serology. Other studies, including serologic ones, showed evidence that addition of FHA in the vaccine provides some protection against infection. Data (not included in this report) based on extensive investigations in Sweden and presented at the meeting in a supplemental presentation by Rose-Marie Carlsson, MD, Swedish Institute for Infectious Disease and Control, contradicted many of the conclusions presented in this session. Some participants also questioned the appropriateness of the analogy between diphtheria and PToxs. The presentation on pathogenesis and immunity as well as the presentation on correlates of protection at this meeting are also at variance with the hypothesis that PTox alone is sufficient for a pertussis vaccine. The discussion was cut short to allow adequate time for consideration of issues related to assay standardization, the main focus of the meeting.

9. Vaccine evaluation based on *B. pertussis* immunogenicity data (Discussion)

Some conclusions previously reached by a WHO-convened working group on the clinical evaluation of new aP vaccines served as an introduction to discussion. One such conclusion was that additional placebo-controlled protective efficacy studies were not feasible since withholding pertussis vaccine was no longer a possibility. Relative efficacy studies of adequate size and statistical power also seemed unlikely. The approval of new aP vaccines would have to rely on comparative immunogenicity data.

All the aP-containing vaccines that have been licensed thus far in the United States and European Union incorporate the same pertussis antigens made by manufacturer-specific processes as were included in at least one of the successful vaccine efficacy trials in infants. Therefore, immunogenicity data have been used to link the demonstration of efficacy for the tested aP vaccine to the new aP vaccine even when the total antigen compositions of the two vaccines differ.

Comparisons of immune responses between the new and reference aP-containing vaccines raise several difficult questions. For example, with no identified immunologic correlates of protection against pertussis, it is not known which antigen(s) elicits immune responses that are important for prevention of clinically apparent infection. It is also not known whether the primary comparison should be in terms of percentages with postvaccination titers above the assay cutoff, on seroconversion rates (which are open to various definitions) or to geometric mean antibody titers. Whichever immunologic parameter is chosen for the primary comparison, it is difficult to decide what might constitute a clinically important difference. In addition, the predefined criteria for noninferiority might be met for one or more, but not all immunologic parameters, with unknown implications for protection. Because of these uncertainties, the overall judgment of the likely protective efficacy of an aP-containing vaccine should take into account all aspects of immune responses. Thus the immunogenicity data should be described in terms of percentages of subjects reaching assay cutoffs, percentages achieving fourfold increments in antibody concentrations (or a similar definition of seroconversion), geometric mean antibody titers and reverse cumulative distributions.

The uncertainty regarding the predictive value of these immunologic comparisons supports the importance of postlicensure surveillance programs to assess the effectiveness of aP vaccines against pertussis disease. However, it is very unlikely that vaccine-specific estimates of effectiveness could be generated, since this would require data from a region or country in which the vaccines used all contain the same aP antigens from the same manufacturer. Because of the complexities of such programs and the infrastructure needed to generate reliable data on disease, such data are most likely to come from surveillance conducted by public health agencies.

In the future, new aP-containing vaccines might include the same range of aP antigens previously shown to be efficacious, but some or all of these may be made by a different manufacturer and/or by a different process. New aP-containing vaccines could also have a different composition of pertussis antigens (in type and/or amount) compared with vaccines that were previously evaluated for protective efficacy. There is no agreed regulatory position on the minimal data that would be required to support approval of these products; however, practical limitations point to the need to consider how

immunogenicity data could be best used to provide reassurance regarding likely efficacy.

If a new aP-containing vaccine contains only antigens that were included in at least one vaccine evaluated in previous protective efficacy studies then immune responses could be compared between the new vaccine and an approved vaccine that has the most similar aP antigen content. If the new aP-containing vaccine contains fewer antigens, different amounts of antigens and/or additional antigens as those in vaccines previously shown to provide protective efficacy there is a need for a careful justification of all the differences between the new and past efficacious vaccines, which would likely have to be based on nonclinical studies. In particular, the response to the PT component should be fully assessed for efficacy in animal models and by estimating the functional antibody to PT (for example, by using the CHO cell neutralization assay). The purity, integrity and functional activity of all antigens should be assessed by physical–chemical evaluation, measurement of residual toxicity (if applicable) and assessment of immunogenicity based on binding and functional assays and protective effects in relevant immunogenicity test has been used for monitoring consistency of production in routine control procedures with comparison with a clinical trial lot (or equivalent). However, the criteria for evaluating equivalency must be defined and no defined common specification has been set.

Recent evidence suggests that both antibody and cell-mediated immunity contribute to the protective process to a variable extent. Following the decision made at the WHO Ad Hoc Working Group meeting (1998, NIBSC, UK), international collaborative studies on protection models for aP vaccines were initiated in 1999, and the outcome of these studies was discussed in subsequent WHO working group meetings. The harmonized procedure for the intranasal challenge assay (INCA), defined in 2003, has been shown to be effective for monitoring the activity of different vaccines and to be transferable between laboratories [64,83,84]. Future work on optimizing experimental conditions to allow calculation of a relative potency is needed and a reference vaccine needs to be agreed upon. This model should be useful for development and characterization of new products or formulations, performing preclinical evaluation or stability and lot consistency monitoring.

The current safety tests for aP vaccines include assays for residual active PT and reversibility of detoxification based on histamine sensitization activity, an endotoxin assay and a general toxicity test. The absence of other toxins at significant levels (heat-labile toxin, ACT, tracheal cytotoxin) has been controlled through process validation. Problems associated with the histamine sensitization test include variation in test performance among laboratories and the absence of an internationally defined acceptance criterion for the assay. The influence of significant interactions between PT and other vaccine components over histamine sensitization assay is unknown. The assay could be improved by (1) defining assay sensitivity by including reference groups; (2) narrowing the range of permissible sensitivity; (3) expressing the results in international unit (IU) of PT activity relative to a common reference; and (4) establishing limits based on a panel of products with a known safety history.

In summary, current quality control tests and specifications for aP vaccines are product-specific, and the clinical relevance of this approach remains uncertain. An effective potency assay for new products and formulations should be defined, and suitable reference preparations should be identified. For the current histamine sensitization test, an upper limit for active residual PT should be defined and use of a reference preparation should be encouraged. Finally, a specific assay for residual active PT that does not require use of animals should be identified. The challenge in testing of aP-based combination vaccines still remains.

10. Vaccine evaluation: perspectives from regulatory agencies, and vaccine manufacturers

Presenters: Drusilla Burns, PhD (Center for Biologics Evaluation and Research (CBER), Food and Drug Administration, Bethesda, Maryland, US), Dorothy Xing, PhD (National Institute for Biological Standards and Control (NIBSC), Potters Bar, UK), Jan T. Poolman, PhD (Bacterial Vaccine R&D, GlaxoSmithKline Biologicals, Rixensart, Belgium) and Stephen W. Hildreth, PhD (sanofi pasteur, Swiftwater, Pennsylvania)

10.1. A view from the regulatory authorities: FDA, US

To begin with a brief overview of pertussis vaccine evaluation in the United States, aP vaccines were first licensed for toddlers in 1991, for infants in 1996, and for adults and adolescents in 2005. Serologic responses to aP vaccination have been used as a measure of lot-to-lot clinical consistency, to bridge populations, and to evaluate booster immunizations, new combinations and concomitant vaccination. Relevant parameters measured have included geometric mean concentrations as well as percentage of responders. In addition, reverse cumulative distribution curves have proved to be an informative way to display serologic response data.

10.2. A view from the regulatory authorities: NIBSC, UK

The composition and formulation of aP vaccines vary widely between manufacturers and products, and no globally accepted reference preparations and release criteria have been established; thus, current reference materials and release criteria are product-specific. This causes difficulty in standardization of the laboratory tests for control of these vaccines. The basis of current regulatory approaches is to demonstrate that newly manufactured lots are comparable either to lots shown to have acceptable clinical trial performance or to lots considered equivalent to these clinical trial lots.

Current routine control tests for aP vaccines include characterization of antigens, assay of immunogenicity, and assay of residual PT bioactivity by the histamine sensitization test and identity testing of antigenic components. A modified intracerebral challenge assay (MICA, modified Kendrick test) has been used in Japan, Korea and China as the potency assay for release with a specification $\geq$4 unit/dose; vaccines regulated using this approach have been shown to be effective in controlling pertussis. In other countries, no nationally defined official potency test has been used at present. An

10.3. A view from the vaccine manufacturers: GlaxoSmithKline (GSK)

For the immunologic evaluation of DTaP combinations and co-administrations with DTaP-based vaccines, GSK referred back to the original DTaP efficacy trials. The cornerstone of comparisons among combined vaccines to their separately administered licensed counterparts is the evaluation of the antibody responses to PT, FHA and PRN [85]. Noninferiority of the percentage of vaccine responders as well as of geometric mean titers forms the basis for the licensure of new combinations and co-administrations. Reverse cumulative antibody distribution curves are also useful for such comparisons. Further characterizations of new DTaP combinations by GSK involve the evaluation of clinical T-cell responses as well as preclinical evaluations in a mouse lung clearance model [85].

With respect to vaccine composition and protection, the available data suggest potent DTwP and DTaP containing PT and PRN induce high levels of protection against whooping cough

[27,28,86,87]. The mechanisms linked to PRN-mediated protection relate to the observations that PRN expression affects ACT activity [88] and induces opsonophagocytic antibodies [89]. The DTaP efficacy trials have been contradictory with regard to the efficacy of DTaP2 (PT + FHA without PRN). Initially the efficacy of DTaP2 was reported to be 85% in Senegal, according to WHO criteria, but this was later corrected to 74% since the original diagnostic criteria were different from other trials [90]. This 74% efficacy of DTaP2 in Senegal probably is similar to the 59% efficacy observed with another DTaP2 in Sweden because of the finding that PT immune responses in Senegal are more than 1.5 times higher than in Western Europe [91]. In conclusion, DTwP and DTaP with ≥3 components are the preferred options for immunization against whooping cough. DTaP5 has demonstrated efficacy comparable with DTaP3, although it is uncertain if FIM3 is a protective antigen [92]. The duration of protection with DTwP and DTaP can be estimated at 6 years or more [93]. To further control pertussis in newborns, adolescents and adults, DTaP booster immunizations can be recommended [8].

10.4. A view from the vaccine manufacturers: sanofi pasteur

Sanofi pasteur has been using immunoassays for *B. pertussis* – IgG ELISAs for PT, FHA, PRN, and FIM antigens – to support the evaluation of vaccine-induced immune responses. The assays used are essentially those identified by Manclark et al. [94]. Key reagents are qualified and standardized, coating antigens are highly purified and assessed both by physical chemistry and immunologic methods to ensure purity, antigenicity, and specificity. Assay reference sera are bridged to international references, and the performance has demonstrated to remain stable. Control sera are shown to be specific and represent suitable ranges across the range of the assay. All components are qualified, with performance panels of sera to ensure consistency. All assays have been fully validated by precision (inter- and intralaboratory), accuracy (using spike recovery), specificity (assessing matrix effects and performing competition studies), linearity/dilutability, limit of detection (LOD), limit of quantitation (LOQ), and robustness.

From the perspective of a vaccine manufacturer such as sanofi pasteur, issues and concerns for the task of global standardization should focus on critical reagents and data reduction methods. For coating antigens, consideration needs to be given to the impact of antigen source as well as the criteria for quality, and consumption rate. For references, most laboratories have been forced to develop internal references that are bridged to international standards; guidance is needed on how best to select these internal references and the sustainability of international references to support frequent calibrations. Similarly, guidance is needed on the best characteristics for controls and implementation of quality control and assay acceptance criteria. Finally, for data reduction, guidance is needed to establish which data reduction systems are most suitable in today's laboratory.

11. Harmonization of serologic assays for *B. pertussis*. Group discussion and action items

Chairpersons: Freyja Lynn, BS (National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland, US) and Annette Morris, BS, BEd (Canadian Center for Vaccinology, Dalhousie University, Nova Scotia, Canada)

To achieve harmonization of assays for the clinical diagnosis of pertussis and evaluation of immunity postvaccination, several issues will have to be addressed. First, consensus on methodologies, assay validation, and analysis of results is required to facilitate comparisons among data generated in different laboratories. The requirements for a diagnostic assay will likely differ in some aspects from assays used to perform postvaccination immunity evaluation. Stability of assay results over time will be dependent on the consistent availability of appropriately characterized reagents and materials. Studies are required to determine the effect on assay results of different sources and lots of antigen, conjugate and other reagents. A proficiency panel of human sera is vital to harmonization of methods between laboratories, although creating a panel with sufficient volume for worldwide use will be a challenge. A reference laboratory or a series of connected reference laboratories will be essential as resources for methodology, change control and management and evaluation of the proficiency panel. Finally, a repository for the distribution of standardized materials, such as antigens, conjugates, and reference materials, will be crucial for standardization and harmonization of *B. pertussis* assays.

A steering committee should be established for the purpose of identifying and prioritizing the steps toward harmonization, including identification of resources required for implementation. If harmonization is to be accomplished, it must be a global effort with the collaboration of many research, government and corporate organizations. The WHO is currently evaluating a collaborative study of proposed international reference preparations for human pertussis antiserum. If these are shown to be acceptable, the availability of international reference material will be valuable for promoting harmonization. Additionally, the collaborative study data, available mid-2008, will provide information on comparability of results in participating laboratories and will help point to where priorities should be focused.

Actionable items to accomplish standardization and harmonization of *B. pertussis* immunoassays identified during group discussion included the following: (1) identify a group that will organize, prepare, maintain, and distribute a common pertussis performance serum proficiency panel that can be used to help laboratories to develop their assays and monitor stability; (2) assemble a working group to assist with the details of the panel; (3) encourage the development and identification of one or more reference laboratories that can serve as an anchor and resource for other laboratories; (4) focus on key reagents such as reference and control sera; (5) define a performance-based assay method that can serve as a reference point for evaluating laboratory differences; (6) develop guidance on quality of other reagents, e.g., PT and other antigens, and methods to demonstrate their suitability; (7) establish an international working group to harmonize the criteria to evaluate the results obtained on reference and proficiency panel sera; (8) create an inventory to determine the amount of appropriate and well-characterized sera that are available globally to be used as bridging reagents for vaccine licensure; and (9) seek specific guidance from regulatory authorities regarding the expectations and requirements for the licensure of new multicomponent pertussis vaccines.

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