**Table 2** Causative bacteria of childhood respiratory infections by disease location (Adapted from *The Guidelines for the Management of Respiratory Infectious Diseases in Children in Japan 2007*, Uehara and Sunakawa [eds.][2] with permission)

| | Group A Streptococcus | Group B Streptococcus | *Streptococcus viridans* | *Streptococcus pneumoniae* | *Staphylococcus aureus* | *Corynebacterium diphtheriae* | *Moraxella catarrhalis* | *Haemophilus influenzae* | *Bordetella pertussis* | *Pseudomonas aeruginosa* | *Klebsiella* | Anaerobic bacteria | *Mycobacterium tuberculosis* | *Nocardia* | *Actinomycetes* | *Legionella* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Acute nasopharyngitis (common cold) | ◎ | | | | | | | | △ | | | | | | | |
| Acute pharyngotonsillitis | ● | | | ○ | | ○ | | ○ | | | | ○ | | | | |
| Acute laryngitis (croup) | | | | | | ○ | | ○ | | | | | | | | |
| Acute epiglottitis | ○ | | | ○ | ○ | | | ◎ | | | | | | | | |
| Acute tracheitis | | | | ○ | ◎ | | | ○ | | | | | | | | |
| Acute bronchitis | △ | | | ○ | ○ | | ○ | ○ | | | | *Bacteroides* | | | | |
| Protracted bronchitis | | | | ◎ | | | ○ | ◎ | ○ | | | | | | | |
| Acute bronchiolitis | | | | ○ | | | | ○ | | | | | | | | |
| Pneumonia | ○ | ○ | ○ | ● | ◎ | | ○ | ● | △ | ○ | ○ | ○ | ○ | △ | △ | △ |
| Lung abscess | | | ○ | | ○ | | | | | | ○ | ○ | | ○ | ○ | |
| Pleurisy | | | | | | | | | | | | ○ | ○ | | | |
| Pyothorax | ○ | | | ○ | ◎ | | | ○ | | | | | | | | |

●◎○△: frequency of occurrence from high to low.

4 Causative bacteria of upper respiratory infections and their detection: The *Guidelines* describe detection methods for Group A Streptococcus (GAS), including rapid diagnostics, and for *Corynebacterium diphtheriae*.

5 Causative bacteria of bronchopulmonary infections and their detection: The major bacteria responsible for childhood bronchopulmonary infections are *S. pneumoniae*, *Haemophilus influenzae*, *Moraxella catarrhalis*, and *Staphylococcus aureus*. These organisms are isolated with blood agar medium and chocolate agar medium. The clinical laboratory should be contacted in advance about suspected cases of pertussis and *Legionella* infections, which require specialized media for isolation.

6 Selection and determination of causative bacteria of bronchopulmonary infections: As previously stated, contamination with bacteria from the upper respiratory tract is a problem when diagnosing the causative bacteria for bronchitis, pneumonia, and other bronchopulmonary infections. Clinical specimens for culturing the causative bacteria for pneumonia as proposed by Moffet[4] are presented in Table 3. Sputum and nasopharyngeal and throat secretions are categorized as being of dubious value for diagnosing the causative bacteria of pneumonia. Moffet states that bacteria cultured from blood, pleural fluid, and lung puncture are definitive. Blood culture is less sensitive than culture from lung puncture.[5] Surveys conducted by Uehara of the causative bacteria determined from blood, pleural fluid, and lung puncture at pediatric training hospitals throughout Japan showed that the number of cases caused by *S. aureus* became fewer and those caused by *S. pneumoniae* and *H. influenzae* increased, beginning in the 1990s (Fig. 2).[6] It must be noted that only a small number of the total cases were confirmed by these conclusive culture sources. Pneumonia is transmitted via the airways as well as the bloodstream. We were able to raise the significance of sputum from "3. Cultures of dubious significance", which included sputum and nasopharyngeal and throat secretions to "2. Occasionally significant culture sources".

7 Assessment of causative bacteria identified in sputum culture: As sputum consists of bronchopulmonary secretions covered by upper respiratory secretions, it is difficult to differentiate bacteria of bronchopulmonary origin and those of upper respiratory tract when it is cultured as is.[6–8] Washed sputum culture[8,9] and quantitative culture are used to detect the true causative bacteria of bronchopulmonary infections. In washed sputum culture, a sputum specimen is washed with sterile saline solution, airway secretions thought to originate from the lower airway based on cytological evidence are cultured, and the predominant bacterium as determined semi-quantitatively is considered as the causative bacterium.

**Table 3** Clinical specimens for identifying causative bacteria for pneumonia (created with modification from Moffet[4])

1. Conclusive Culture Sources
   - blood
   - pleural fluid
   - lung puncture
2. Occasionally Significant Culture Sources
   - transtracheal aspiration
   - tracheotomy aspiration
   - bronchoscopy aspiration
   - (washed sputum)
3. Cultures of Dubious Significance
   - tracheal aspiration
   - sputum
   - throat
   - nose/nasopharynx

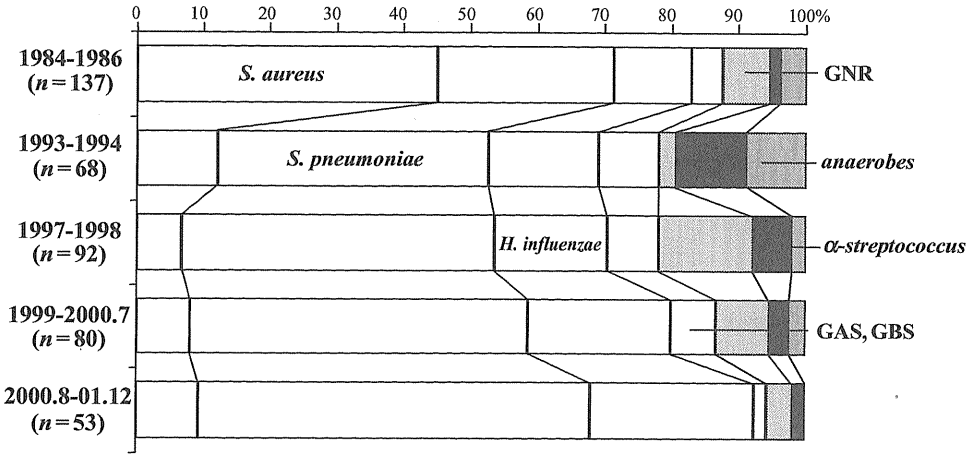


**Fig. 2** Causative bacteria detected from blood, pleural fluid and/or lung tissue samples from pediatric pneumonia patients. GAS, Group A Streptococcus; GBS, Group B Streptococcus; GNR, gram-negative rods. (Reproduced from *The Guidelines for the Management of Respiratory Infectious Diseases in Children in Japan 2007*, Uehara and Sunakawa [eds.][2] with permission.)

Pathogenic respiratory bacteria are predominantly isolated from purulent sputum and are often the likely causative bacterium. However, if the sputum is viscous, the isolated species may be from the oral flora. Broad classification of the causative bacterium can be made by Gram staining of sputum. The classifications defined by Geckler *et al.*[10] are used for the quality control of sputum. Sputum is Gram-stained and observed under weak magnification (×100). Evaluation is based on squamous epithelial cells and neutrophil counts. The predominant organism detected in a Gram-stained smear of a washed sputum culture is of greater significance as the likely causative bacterium of a bronchopulmonary infection when found in close contact with alveolar macrophages (Fig. 3).[9]

Table 4 lists criteria for determining causative bacteria.[8] For *M. catarrhalis* to be confirmed as the causative species, the bacterium must be the predominant species in sputum culture and detected in macrophages by sputum cytology.[7]

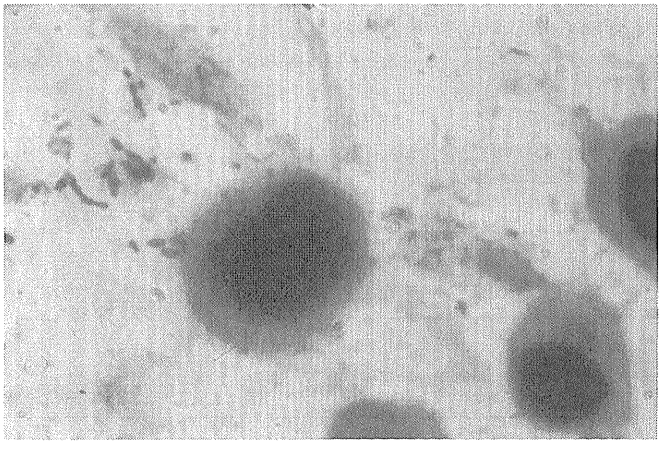

**Fig. 3** Alveolar macrophage and perialveolar existence of Gram-positive diplococci (*Streptococcus pneumoniae*) and Gram-negative bacilli (*Haemophilus influenzae*) on gram-stained washed sputum.

Sputum collection in infants and children[7,8] is shown in Figure 4. Sputum collection should be attempted when the patient has a productive cough. If the patient is able to expectorate sputum, they should be instructed to discharge sputum into a sterile Petri dish with saline without contaminating the specimen by further productions of saliva, as far as possible. If the patient is an infant or preschool child who is unable to expectorate, the tongue should be depressed using a tongue depressor with a lamp to induce coughing. When the patient expectorates into the throat, a sterile swab should be promptly swiped around the sputum and placed in sterile saline. Recently, 1-mL disposable syringes have been used to aspirate specimens.[7]

8 The value of sputum washing and nasopharyngeal and pharyngeal culture:[11] Figure 5 shows the results of simultaneous culturing washed sputum, non-washed sputum, and nasopharyngeal and pharyngeal secretions for cases in which the causative bacteria was detected predominantly in washed sputum samples. Washed sputum samples showed better results than non-washed sputum samples. In the same patients, nasopharyngeal swabs showed better results than pharyngeal swabs, though detection was lower than in non-washed sputum samples.[11] Direct culturing of sputum

**Table 4** Criteria for determination of causative bacteria in bronchopulmonary infection (adapted from Uehara[8] with permission)

| |
|---|
| **① Pathogens occupying more than half of the colonies in culture or presenting >$10^7$ cfu/mL of washed sputum were regarded as "dominant".** |
| ② The same dominant pathogens were grown by repeated cultures. |
| ③ The pathogens were seen perialveorally in smeared specimens. |
| ④ Heavier growth of pathogens was observed with washed sputum than with nasopharyngeal or throat swabs. |
| **⑤ The pathogens in washed sputum correlated with the clinical course of the disease: signs and symptoms, acute phase reactants, and especially the purulence (neutrophilia) of the sputum.** |

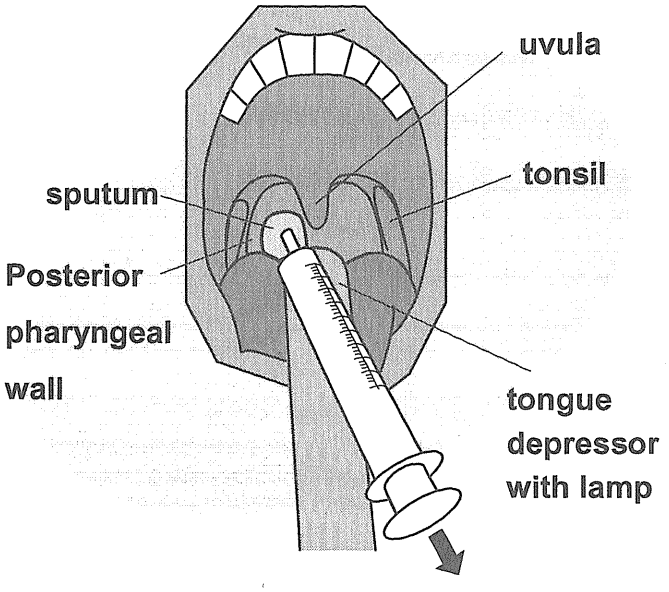


**Fig. 4** Placement of instruments for the collection of sputum from pediatric patients.

(non-washed) results in inferior identification of causative bacteria, as it is covered with bacteria from upper airway secretions. Sputum specimens should be pretreated to remove contamination from the upper airway as completely as possible before culturing. Use of nasopharyngeal and pharyngeal cultures is only of limited value in etiological diagnosis of bronchopulmonary infections. Nasopharyngeal culture should therefore be conducted when sputum cannot be collected. Nasopharyngeal culture, however, should be used to postulate rather than definitively identify the bacterium responsible for pneumonia.

9 Detection of bacterial antigens in urine: Pneumococcal antigen may show false-positive results in urine because of the high prevalence of *S. pneumoniae* in the upper respiratory tract of children.[12] Urinary antigens are of excellent value in diagnosing legionellosis. Urinary antigen testing for *Legionella* spp. should be performed as a precaution in the critical cases of pneumonia.

10 Blood culture: Although sensitivity is not as high as other methods, blood cultures are of extreme value in selecting drugs for treatment when identifying the causative pathogen. Blood culture should be conducted whenever possible. Blood culture is discussed in detail in *Cumitech 1C: Blood Cultures IV*, a publication of the American Society for Microbiology.[13]

### *Mycoplasma, Chlamydia*

*Mycoplasma pneumoniae* and *Chlamydia* infections are diagnosed by: (i) confirming significantly elevated or abnormally high serum antibody titers; and (ii) performing isolation culture, antigen detection, and nucleic acid detection on specimens from the infection site.

1 *Mycoplasma*: *M. pneumoniae* is the only significant pathogen involved in childhood respiratory infections. *Mycoplasma* infections are diagnosed by detection of *Mycoplasma* from the infection site and confirmation of increased antibody titers. *Mycoplasma* is detected in nasopharyngeal swab specimens, sputum, and pleural fluid. Detection is accomplished with direct fluorescent antibody assay, isolation culture, enzyme immunoassay, DNA probe assay, polymerase chain reaction (PCR), and other methods. Liquid pleuropneumonia-like organism (PPLO) media and other special media are used for isolation culture, which typically requires at least 7 days. PCR features excellent sensitivity and specificity. Serological diagnosis is accomplished with methods including particle agglutination (PA), cold agglutinin titer, complement fixation, indirect hemagglutination assay, and enzyme immunoassay.[14] Although serum antibody titer is at least fourfold higher in the acute and convalescent phases, increased immunoglobulin (Ig)M antibody levels must be identified to reach a definitive diagnosis. Infection may be strongly suspected if a PA titer of at least 320 or a complement fixation titer of at least 64 is detected in single serum. Infections in infants show poor antibody response.

2 *Chlamydia*: The three species *Chlamydophila pneumoniae*, *Chlamydophila psittaci*, and *Chlamydia trachomatis* are the causes of childhood *Chlamydia* respiratory infections. *Chlamydia* infections are diagnosed by detection of *Chlamydia* from

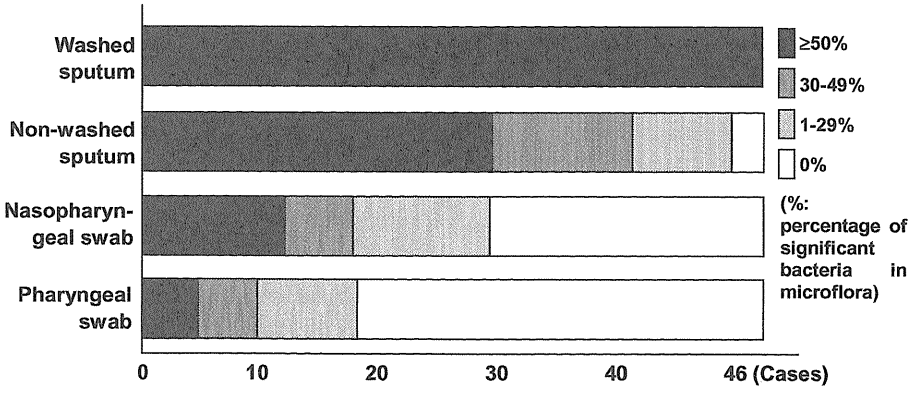


**Fig. 5** Simultaneous culturing of washed and non-washed sputum specimens and nasopharyngeal and pharyngeal swabs from cases in which causative bacteria could be identified from washed sputum cultures. (Reproduced from Takeda *et al.*,[11] with permission.)

the infection site and confirmation of significantly increased antibody titer. *Chlamydia* is detected in nasopharyngeal swab specimens, sputum, and pleural fluid. Direct fluorescent antibody, enzyme immunoassay, PCR, and other techniques are used for detection. Isolation culture in cell culture requires at least 7 days. PCR offers good sensitivity and specificity. The Committee on Serological Diagnosis of *Chlamydophila pneumoniae* infection (chaired by Toshio Kishimoto) sets related diagnostic standards in Japan.[15]

Although serum antibody titer is at least fourfold higher in the acute and convalescent phases, increased IgM antibody levels must be identified to achieve serological diagnosis. For initial infections, a diagnosis can be reached in a relatively early stage using IgM antibody assay. Infections in infants show poor antibody response.

It should be noted that legionellosis is attributable to aspiration of *Legionella pneumophila* and other *Legionella* spp. from water coolers and other climate-control equipment. Only a few infants have acquired legionellosis in a neonatal intensive care unit. Legionellosis is more often diagnosed through rapid antigen diagnostics of urine specimens (61%) than it is from serum antibody titers. Rapid antigen diagnostics should therefore be attempted in cases of critical pneumonia. Isolation culture requires special media (B-CYEα medium, World Health Organization [WHO] agar medium).

### *Viruses*

The characteristics of the viruses often isolated in childhood respiratory tract infections differ according to the infection site. Determining causative microorganisms according to symptoms alone is often difficult. The flow of testing is presented in the original *Guidelines*.

Medical staff collecting specimens for testing must be careful to perform collection at initial presentation in the early stage of the disease and to place specimens in a preserving solution for specimens for isolation (such as those designated by testing facilities). Specimens should be stored at low temperature (often 4°C). Specimens should be promptly shipped refrigerated to the testing facility. Serum specimens must be collected as paired sera once during the acute phase and again during the convalescent phase, 14–21 days after onset. A definitive diagnosis is reached when antibody titer is increased at least fourfold. The microplate method used by Numazaki *et al.* at the Virus Research Center of Sendai National Hospital[16] is well suited for the co-detection of viruses, is recommended by the WHO, and is increasingly used at Prefectural Institutes of Public Health in Japan, but the method is not feasible in all cases and must be selected according to the reason for culturing. The *2007 Guidelines* refined the list of rapid diagnostic testing, isolation culturing, nucleic acid detection testing, and serological detection methods for influenza virus, respiratory syncytial virus (RSV), and adenovirus pathogens.

### *Testing for rapid diagnosis of childhood respiratory infections*

The *Guidelines* summarize: (i) trends in testing for the rapid diagnosis of childhood respiratory infections; (ii) the strengths and limits of immunochromatography; (iii) reagents for blood assay for *Mycobacterium tuberculosis* (BAMT), including whole-blood interferon-γ assay for diagnosing tuberculosis; and (iv) points to consider when performing rapid diagnostic testing.

## Upper respiratory infections

1 Common cold (nasopharyngitis): Colds, which are caused primarily by viruses, are not treatable with antimicrobials. Antimicrobials fail to improve the course or prognosis of colds and have been found not to protect against lower respiratory tract infections. Fever alone with no respiratory symptoms is differentiated based on the presence of occult bacteremia, urinary tract infections, and other conditions.

2 Pharyngitis/tonsillitis: These conditions are often of viral origin. Antimicrobial treatment is indicated for primarily GAS infections. The *Guidelines* now recommend penicillin (PC)-based antimicrobials[17] as first-line treatments for GAS based on the discussions of GAS treatment that have taken place since 2004, but also list cephem antimicrobials for short-term therapy. Cephem or macrolide antimicrobials are recommended for children with penicillin allergies, but some children are also allergic to cephem antimicrobials. Not a few GAS isolates in Japan show resistance to macrolide antimicrobials, making cross-resistance a concern.

3 Croup syndrome
   (1) Viral croup: Viral croup is to be treated symptomatically. Dexamethasone therapy is an option for severe cases.
   (2) Acute epiglottitis: The course of this serious disease can include asphyxiation occurring 10 h after onset. A tongue depressor must not be used. Securing the airway is an urgent priority. Lateral radiography of the neck can show any epiglottic enlargement. *H. influenzae* type b (Hib) is the causative microorganism in ≥90% of all cases. The disease is treated with the antimicrobials: ceftriaxone, cefotaxime, meropenem, or tazobactam/piperacillin. Now that the Hib vaccine (approved in January 2007 in Japan) has been found to be safe and effective, Hib epiglottitis can be almost completely prevented through vaccination.[18]
   (3) Laryngeal diphtheria: Although very rare (only one case has been officially reported over the past several years), the possibility of laryngeal diphtheria must be kept in mind in unvaccinated and older children. Antitoxin therapy should be administered first and foremost.
   (4) Bacterial tracheitis: Although very rare, bacterial tracheitis can cause asphyxiation. *S. aureus* and other organisms cause this disease.

## Bronchitis

1 Acute bronchitis:[19] Although acute bronchitis is usually viral, oral antimicrobials (consistent with those used for pneumonia) are used when bacterial bronchitis (*H. influenzae*, *S. pneumoniae*) is suspected based on fever, productive cough, or purulent sputum.

2 Protracted bronchitis (protracted, recurrent, and chronic bronchitis):[7] If infection is confirmed, the causative bacteria (*H.*

*influenzae* > *Streptococcus pneumoniae*) should be identified from the sputum and treated with the appropriate antimicrobial(s). Any underlying diseases (e.g. sinusitis, immunodeficiency) must be identified and superinfection by *Pseudomonas aeruginosa* or other organisms must be avoided.

## Bronchiolitis

Acute bronchiolitis[20] is common in infants and is primarily caused by RSV (45–75%). Fever infrequently exceeds 38.5°C, and chest radiography often shows hyperinflated lungs. Some serious cases in infants under 3 months old require respiratory management. Antigen testing is useful. Some infections are caused by the human metapneumovirus, which has become a recent focus of attention.[21] No consensus has been reached on the value of PCR detection of the human bocavirus. Palivizumab is an effective prophylactic for RSV infection in high-risk infants.

## Pneumonia

1 The definition of pneumonia: This acute respiratory infection is characterized by fever, rhinorrhea, and cough. Chest radiography, computed tomography (CT), and other imaging modalities show acute new infiltration in the lungs. Adventitious breath sounds and decreased respiratory sounds on chest auscultation can be observed in pneumonia.

2 Diagnosis of pneumonia: Patients suffering primarily from fever, cough, and dyspnea and who are suspected of having pneumonia based on chest findings should undergo chest radiography. Viral and *Mycoplasma* pneumonia are characterized primarily by interstitial lesions, and may show no abnormalities on chest auscultation. Once a definitive diagnosis of pneumonia is made based on imaging, the causative microorganisms should be identified in the blood and sputum (or in nasopharyngeal secretions). The need for antimicrobial(s) is determined in reference to pulmonary radiographs, acute phase reactants, and in consideration of the presumed causative microorganism. It must be remembered that infants and preschool children often cannot report dyspnea. When evaluating severity, features to check in addition to chest imaging include tachypnea (≥50 breaths/minute in children 1 year old and younger and ≥40 breaths/minute in children aged 2–5 years old) and retractions, nasal alar breathing, shoulder breathing, grunting, and cyanosis as signs of dyspnea (discussed later).

3 Causative microorganisms and examination

(1) Incidence of causative microorganisms: Based on the limited number of pneumonia cases for which the causative bacterium was confirmed through blood or pleural fluid culture in a nation-wide survey, the incidence of infections caused by *S. pneumoniae* and *H. influenzae* have exceeded those caused by *S. aureus* since the 1990s (Fig. 2).[6] Trends in *S. aureus* infection must be monitored.

Of the washed sputum cultures from bronchopulmonary infections, predominant bacteria were identified in about 30% of cases, and recent trends show that *H. influenzae* became more common than *S. pneumoniae*, and *M. catarrhalis*, in that order. *S. pneumoniae* pneumonia has been increasing since 1995 and accounted for about 30% of cases in which a causative organism was identified in 2005 (Fig. 6). For cases in which the causative pathogen of pneumonia was identified by washed sputum culture, about 30% of cases were attributed to bacterial pneumonia, 10–20% were attributed to *M. pneumoniae*, about 20% were viral, and the cause of the remaining 30% could not be determined. Trends in causative pathogens identified in washed sputum culture at three medical institutions associated with Chiba University showed *H. influenzae* and *S. pneumoniae* to be the major culprits since 1965, with some cases attributed to *M. catarrhalis*.

(2) Causative microorganisms and age distribution: The *Guidelines* summarize evidence about the age distributions associated with the causative microorganisms of pneumonia from the publication of McIntosh.[22] The Japanese evidence is similar. Although *C. pneumoniae* is well characterized, the data on other microorganisms do not differ substantially from those listed in medical texts, and no frequencies are stated. An investigation of the relationship of age in childhood pneumonia at Chiba Kaihin Municipal Hospital (1998–99) showed that of the 634 cases of childhood pneumonia treated, 170 (26.8%) were in 1-year-old children, 115 (18.1%) were in 2-year-old children, and 84 (13.2%) were in 4- to 11-month-old infants. A total of 512 (80.8%) were in children 4 years old and younger. Bacterial pneumonia was confirmed in washed sputum culture in 163 cases (25.7%). All cases were attributable to *H. influenzae*, *S. pneumoniae*, or combinations of these two, with the exception of three cases caused by *M. catarrhalis*, one caused by *Bordetella pertussis*, and two caused by GAS. Pneumonia was more commonly of bacterial origin in the younger age groups of hospitalized patients at Chiba Children's Hospital, while the incidence of *Mycoplasma* pneumonia increased with age (Fig. 7).

Although *C. pneumoniae* infections are relatively common beginning at young ages outside Japan, the prevalence of *C. pneumoniae* IgG antibody in Japanese children increases with age starting with an increased prevalence in 4–7-year-olds, a sharp increase to 44% in 8–11-year-olds, and about 50% above the age of 11 years.[23] The data provided by Kishimoto[24] on antibody incidence similarly indicate an increase in prevalence beginning at 6 years old. Grayston,[25] who stated that the incidences of bronchitis and pneumonia are about equal from 5 to 9 years old and that pneumonia is more common from 10 years old, reported that most cases of pneumonia are attributed to *C. pneumoniae* in older children.

4 Clinical symptoms, laboratory test findings, and antimicrobial selection

(1) Clinical symptoms and physical findings encountered with different causative pathogens: Investigation of many

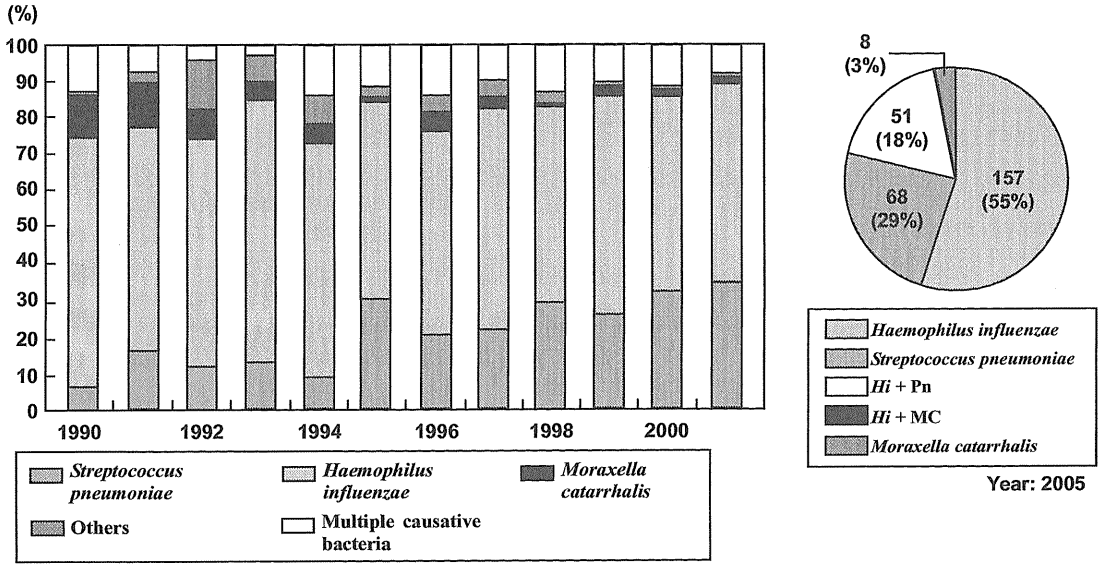


**Fig. 6** Trends in causative bacteria in childhood bronchopulmonary infections based on washed sputum culture (percentages among cases of known pathogens). MC, *Moraxella catarrhalis*; Pn, *pneumococcus*. (Prepared from data provided by Dr. Kurosaki of Chiba Municipal Kaihin Hospital; reproduced from *The Guidelines for the Management of Respiratory Infectious Diseases in Children in Japan 2007*, Uehara and Sunakawa [eds.][2] with permission.)

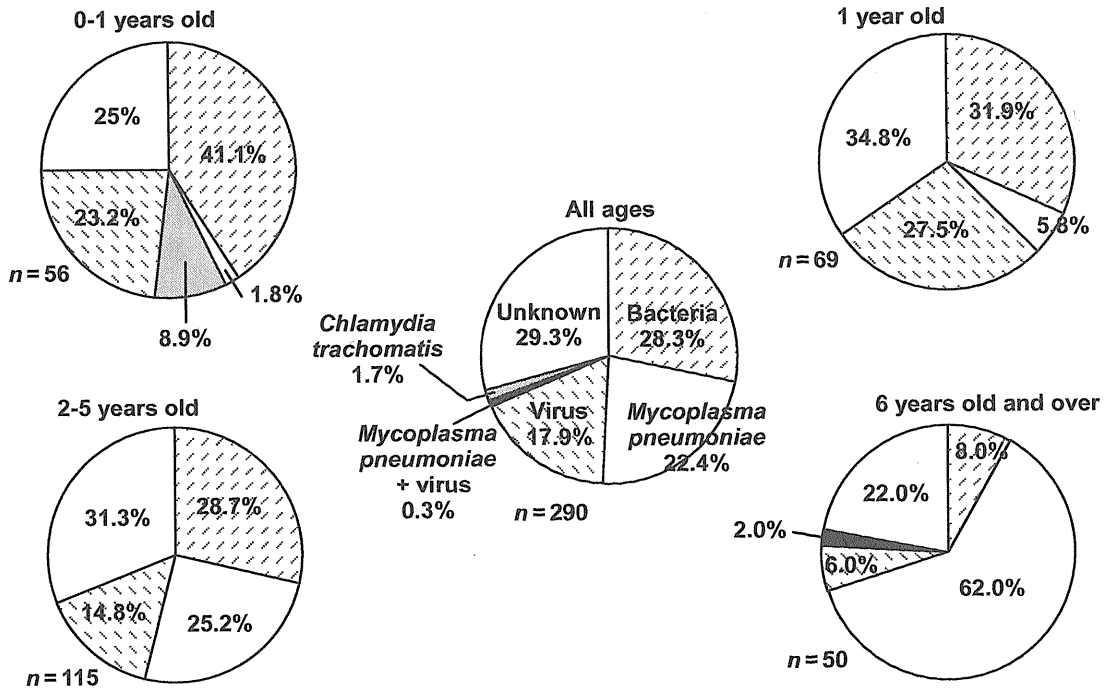


**Fig. 7** Causative bacteria of community-acquired pneumonia in children. (Data collected October 1988–March 2002 by A. Nakamura of Chiba Children's Hospital; reproduced from *The Guidelines for the Management of Respiratory Infectious Diseases in Children in Japan 2007*, Uehara and Sunakawa [eds.][2] with permission.)

**Table 5** Community-acquired pneumonia: determining severity through physical and laboratory observations

| | Mild | Moderate | Severe | Critical |
|---|---|---|---|---|
| General condition | Good | | Poor | |
| Cyanosis | Absent | | Present | |
| Respiratory rate† | Normal | | Rapid (over normal range) | |
| Forced respiration (grunting, nasal alar breathing, retraction) | Absent | | Present | |
| Extent of infiltration on chest X-ray examination | ≤1/3 of one lung | | ≥2/3 of one lung | |
| Pleural effusion | Absent | | Measurable quantity | |
| $SpO_2$ | >96% | | <90% | |
| C-reactive protein (mg/dL) | <3.0 | | >15 | |
| Neutrophils: Infant | 4000–8000 | | <500 or >10 000 | |
| Preschool-age child | 2500–5500 | | <500 or >10 000 | |
| School-age child | 3000–5000 | | <500 of >10 000 | |
| Criteria | All of the above criteria are met | Not mild or extreme | Any one of the above conditions are met | Accompanied by circulatory failure or when artificial respiratory care is required |

†Respiratory rate by age: (breaths/min): neonate, <60; infant, <50; preschool-age child, <40; school-age child, <30.

cases in which the causative pathogen has been identified has revealed that bacterial pneumonia often involves productive cough and that *M. pneumoniae* disease often lacks labored breathing and abnormalities on auscultation. *C. pneumoniae* infections result in low-grade fever and prolonged coughing. Diagnostics for causative organisms, however, are required because postulating the causative microorganism according to symptoms is difficult in individual patients.[26,27]

(2) Causative microorganisms and laboratory test findings on admission: Bacterial and viral pneumonia had been considered distinguishable by the intensity of the inflammatory response. In blood culture-negative bacterial pneumonia, although white blood cell counts, C-reactive protein levels, and erythrocyte sedimentation rates were significantly different from those of viral pneumonia ($P < 0.01$), overlap is seen in about one-third of patients, making differentiation of cause impossible in individual cases.[27] Bacterial culture is therefore necessary before antimicrobial treatment. The possibility of *Mycoplasma* pneumonia should be considered when C-reactive protein levels and erythrocyte sedimentation rates are high, but white blood cell counts are not elevated.

(3) Causative pathogens and findings from chest radiography: The cause of pneumonia cannot be clearly differentiated based on chest radiography performed on admission using the differentiation methods of Swischuk and Hayden[28] or the scoring method of Khamapirad and Glezen.[29]

(4) Classifications of pneumonia severity: Tachypnea: The WHO established management criteria for pneumonia in developing countries, with a focus on tachypnea and labored breathing. Kurosaki[27] compared respiratory rates (≥50 breaths/minute in children 1 year old and younger and ≥40 breaths/minute in children under 5 years old) to findings from washed sputum cultures and reported that tachypnea can be used as an index for determining the appropriateness of antimicrobial treatment before culture results become available for 1–4-year-old children.[30] Assessing the severity of pneumonia is a first step toward determining whether the patient should be treated as an outpatient or admitted, whether antimicrobials should be administered, and whether oral or intravenous (i.v.) administration is appropriate. Criteria for assessing pneumonia severity are shown in Table 5.

(5) Hospitalization eligibility criteria: Patients with a severity classification of mild should be treated on an outpatient basis, while patients with moderate or severe infections should be admitted for treatment.

(6) Important factors when considering initial antimicrobial therapy

(i) Intensity of bacterial pathogenicity: *S. pneumoniae* has the strongest pathogenicity of the three causative organisms of bronchopulmonary infections: *S. pneumoniae*, *H. influenzae* and *M. catarrhalis*. Antimicrobial therapy that considers *S. aureus* is occasionally recommended for infants and children with underlying diseases.

(ii) Relationship between age and causative organism: The organisms primarily responsible for pneumonia differ with age of children as follows.

Neonates: Group B Streptococcus, *Escherichia coli*, and other intestinal flora.

Infants to children aged 5 years old: viruses, *H. influenzae*, and *S. pneumoniae*.

Children 6 years of age and older: *M. pneumoniae*, *C. pneumoniae*, *H. influenzae*, and *S. pneumoniae*.

Macrolide antibiotics should be considered first in children at least 6 years old who do not exhibit productive cough.

(iii) Pharmacokinetics of oral antibiotics: The pharmacokinetics-pharmacodynamics (PK-PD)

theory indicates that new cephem antibiotics, when recommended, should be administered at a high dose.

(iv) Minimizing drug resistance: Care must be taken to use antibiotics appropriately (particularly oral cephem antibiotics).

(v) Synthetic penicillin therapy for *S. pneumoniae* and *H. influenzae*: The Drug-Resistant *Streptococcus pneumoniae* (DRSP) Therapeutic Working Group of the Centers for Disease Control and Prevention of the USA, reasoning that pneumonia in children under 5 years of age is often bacterial in origin, advocates a β-lactam antibiotic (amoxicillin, amoxicillin/clavulanic acid, or cefuroxime for outpatient cases) for the initial treatment of pneumonia.[31,32] We set dosages for the treatment of cases for which *S. pneumoniae* was the predominant organism isolated from washed sputum based on the breakpoint for i.v. ampicillin defined by the Japanese Society of Chemotherapy of 2 μg/mL. Treatment with oral amoxicillin (30–40 mg/kg/day) and i.v. ampicillin (80–150 mg/kg/day) showed no significant differences for pneumonia with the following: penicillin-susceptible *S. pneumoniae* (PSSP), penicillin-intermediate resistant *S. pneumoniae* (PISP), and penicillin-resistant *S. pneumoniae* (PRSP).[32] (Note: The 2007 edition of the *Guidelines* lists penicillin G [PcG] resistance criteria that were revised in 2008. Following are the criteria in the 2007 edition of the *Guidelines*: PSSP, PcG-minimum inhibitory concentration [MIC] ≤ 0.06 μg/mL; PISP, PcG-MIC, 0.12–1 μg/mL; and PRSP, PcG-MIC ≥ 2 μg/mL). The *H. influenzae* ampicillin resistance criteria of the Clinical Laboratory Standards Institute (CLSI) of the USA[33] defines sensitivity as ≤1 μg/mL, moderate resistance as 2 μg/mL, and resistance as ≥4 μg/mL by the broth microdilution method. Most bronchopulmonary infections caused by β-lactamase-non-producing ampicillin-resistant (BLNAR) strains in Japan are treatable with i.v. ampicillin. Piperacillin, cefotaxime, and ceftriaxone offer reliable antibiotic activity against BLNAR strains. The response rate to piperacillin was 95%.[34] There are few patients with pathology caused by β-lactamase-producing *H. influenzae* strains that have shown clinical deterioration when treatment is initiated with oral amoxicillin or i.v. ampicillin. There is still time to switch antibiotics if resistance is identified after treatment is initiated.

(vi) Synthetic penicillin therapy for *M. catarrhalis*: Synthetic penicillin is clinically effective in treating *M. catarrhalis* infections even though the microorganism produces β-lactamase and is bacteriologically resistant to amoxicillin[35,36] because the produced β-lactamase has low activity.

(vii) Penicillin-binding protein (PBP) mutations: PBP of *S. pneumoniae* readily mutate in the presence of cephem antibiotics.[37] Mutation leads to increased resistance to β-lactam antibiotics and consequently DRSP strains. PBP mutations also underlie BLNAR and β-lactamase-producing amoxicillin-clavulanate resistant (BLPACR) *H. influenzae* strains. The increase in the prevalence of BLNAR strains is attributable to the widespread use of oral cephem antibiotics, which reaches a concentration that is only a fraction of that of amoxicillin.[37]

(7) Initial antimicrobial therapy when etiological pathogen is unknown: Antimicrobial agents recommended for initial treatment when the pathogen is unknown are shown for different age groups and for hospitalized patients and outpatients in Table 6. Agreement has been reached[38,39] on the appropriateness of the selections of initial antimicrobial agents given in the 2004 edition of the *Guidelines*. These selections must be continuously evaluated to take trends in causative microorganisms and drug resistance into account.

(8) Selection of antimicrobial agents when the etiological pathogen of pneumonia is known (monotherapy as a starting point): When the pathogen responsible for the pneumonia is known, the antimicrobial agent is selected in consideration of drug susceptibility and pharmacokinetics. Macrolide-resistant *Mycoplasma* strains have been increasing since 2000 (this is discussed later).

(9) Assessment of antimicrobial agent efficacy and duration of use: Antimicrobial agents for treating community-acquired pneumonia are normally sufficiently effective when administered for 3 to 7 days. Efficacy is assessed after 2 or 3 days (48–72 h after start of administration). Efficacy should be initially assessed after 2 days in younger children and severe cases. Assessment is performed to determine whether the initial antimicrobial agent is effective and whether the drug should be continued or switched. The duration of use will vary among individual patients. For common bacteria, use can be discontinued 3 days after the patient's fever breaks. A longer duration is required for *S. aureus* pneumonia. For *Mycoplasma* and *Chlamydia* infections, 10 days of new macrolide (clarithromycin) treatment or 3 days of azithromycin treatment (5 days in the USA) is recommended.

(10) Actions to take and selections to make when no response is achieved

(i) Actions to take when the patient does not respond to antimicrobial therapy: The correctness of the pneumonia diagnosis and the possibility of another disease producing pneumonia-like findings on imaging should be considered in order to distinguish pneumonia cases due to causative microorganisms other than common causative bacteria, such as viruses, tuberculosis, and fungi.

**Table 6** Initial antimicrobial therapy in children for unknown etiological pathogen

| | Severity | 2 months to 5 years old*1*2*5 | ≥6 years old |
|---|---|---|---|
| Outpatient | Mild | AMPC ± CVA or SBTPC p.o.<br>or<br>Broad-spectrum cephem p.o.*3 | Macrolide p.o.<br>or<br>Tetracyclin p.o.*4 |
| Inpatient | Moderate to Severe | ABPC ± SBT i.v. or PIPC i.v.<br>or<br>Broad-spectrum cephem i.v.*3 | ABPC ± SBT i.v. or PIPC i.v.*2<br>or<br>Broad-spectrum cephem i.v.*3<br>±<br>Macrolide p.o./d.i.v.<br>or<br>Tetracycline p.o./d.i.v.*4 |
| | Critical | Carbapenem d.i.v. ± Macrolide p.o./d.i.v.*6 | |

When the causative pathogen has been identified, change to the appropriate antimicrobial agent.
*1: With concomitant macrolide when *Chlamydia trachomatis* infection is identified.
*2: With concomitant macrolide when *Mycoplasma/Chlamydophila pneumoniae* infection is strongly suspected.
*3: The following offer superior antibacterial activity against *S. pneumoniae* and *H. influenzae*: Representative oral drugs: CDTR-PI; CFPN-PI; CFTM-PI. Representative intravenous drugs: CTRX; CTX.
*4: Use in children <8 years old only when other agents are ineffective or cannot be used.
*5: In principal, children <1 year old are hospitalized.
*6: With concomitant macrolide when Legionellosis cannot be ruled out.
AMPC, amoxicillin; CDTR-PI, cefditoren pivoxil; CFPN-PI, cefcapene pivoxil; CFTM-PI, cefteram pivoxil; CTRX, ceftriaxone; CTX, cefotaxime; CVA, clavulanic acid; d.i.v., drip intravenous; i.v., intravenous; PIPC, piperacillin; p.o., per os; SBTPC, sultamicillin.

(ii) Selection of antimicrobials when the patient does not respond to antimicrobial therapy:
- If a β-lactam antibiotic was initially used: Pneumonia is often caused by *H. influenzae* and *S. pneumoniae*, against which ampicillin and amoxicillin are recommended. These drugs are reportedly effective even against BLNAR and PRSP. For mild and moderate non-responsive cases, *Mycoplasma* or *Chlamydia* infection should be suspected, and the initial antimicrobial agent should be switched to or used in combination with a macrolide. A broad-spectrum i.v. cephem antibiotic or i.v. carbapenem antibiotic should be used when response is insufficient. For rapidly progressive, severe cases and critical cases, a carbapenem antibiotic and macrolide antibiotic should be used in combination. Addition of an anti-methicillin-resistant *Staphylococcus aureus* (MRSA) agent is to be considered.
- If a macrolide antibiotic was initially used: Treatment should be switched to a β-lactam antibiotic to treat macrolide-resistant *S. pneumoniae* and *H. influenzae*. Treatment should be switched to the optimal antimicrobial agent once the causative pathogen is identified. Table 6 provides recommendations for critical cases. When the condition of the patient is good and a *Mycoplasma* infection is suspected, switching to a tetracycline antibiotic should be considered to treat possible macrolide-resistant *Mycoplasma* infection.

(11) Outpatient parenteral antimicrobial therapy (OPAT): OPAT is sometimes used to treat patients with moderate pneumonia who are unable to be admitted. Such patients must visit the medical institution daily and be carefully monitored. Once-daily ceftriaxone has a long half-life and is commonly used.[40] A first-line treatment for bacterial meningitis, ceftriaxone should not be used readily and widely until the Hib vaccine has substantially reduced the prevalence of meningitis.

## Pleurisy and pyothorax

Although pyothorax prevalence in Japan has decreased with the waning incidence of *S. aureus* pneumonia, vigilance is required because the disease is still on the increase in countries outside Japan, despite widespread use of the pneumococcal conjugate vaccine.

## Pneumonia in patients with underlying diseases

The 2007 edition discusses pneumonia with accompanying underlying conditions (blood diseases, immunodeficiency, neonates, and cardiac diseases).

## Nosocomial pneumonia

Nosocomial pneumonia is defined as pneumonia acquired after a hospital stay of at least 48 h. Measures must be taken to prevent children from becoming infected due to the hospital environment and medical acts (including those leading to ventilator-associated pneumonia) as well as from other patients, attendants, visitors, and medical personnel. The *Guidelines* present measures for preventing respiratory infections acquired through different routes and discuss the person-to-person transmission of respiratory infections. The *Guidelines* also recommend the vaccination of medical personnel.

## Main diseases controlled by vaccination

The *Guidelines* discuss influenza, measles, pertussis, diphtheria, and tuberculosis. Also proposed are draft diagnostic criteria for pertussis based on epidemiological data that factor in the relative increase in the disease among older children and adolescents and DTP-vaccinated children and adults. Although affected older children and adults exhibit prolonged and severe coughing, no characteristic symptoms can be identified in children without a detailed interview. The disease lacks elevated white blood cell and lymphocyte counts. A novel trivalent vaccine for adolescents and adults (Tdap) has been developed in Europe and the USA.

## Pathogen resistance in community-acquired childhood respiratory infections

A classification system for *S. pneumoniae* and *H. influenzae* based on the analysis of antibiotic resistance genes is presented.[41] Antimicrobial agents currently used to treat resistant pathogens are listed (note: the antimicrobial susceptibility of *S. pneumoniae* and *H. influenzae* is discussed in Ubukata[37]). Most strains with an ampicillin-MIC $\leq 2$ μg/mL are treatable using oral amoxicillin or i.v. ampicillin, but when therapy must be changed, oral faropenem, cefditoren, or cefcapene or i.v. panipenem or vancomycin are recommended for resistant *S. pneumoniae*, and oral cefditoren or azithromycin or i.v. piperacillin, ceftriaxone, or meropenem are recommended for BLNAR strains. Clindamycin resistance among GAS and *S. aureus* and macrolide-resistant *Mycoplasma* is a problem. Macrolide-resistant *Mycoplasma* was first detected in culture and by PCR in 2000, and many strains are highly resistant to erythromycin.[42,43] Although the period of fever following macrolide administration is significantly longer than that for infections by susceptible strains (mean duration of fever: 4.3 days for resistant strains vs 1.4 days for susceptible strains), the clinical symptoms are not more severe.[43] Changing treatment to a tetracycline antibiotic should be considered if fever persists for more than 48 h after macrolide antibiotic initiation.

The *Guidelines for the Management of Respiratory Infectious Diseases in Children in Japan 2007* are summarized here with a focus on pneumonia. Only selected tables and figures to illustrate the *Guidelines* could be reproduced here due to limited space.

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# Evaluation of ELISA Kit for Detection of Pertussis-associated IgG Antibodies

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# Evaluation of ELISA Kit for Detection of Pertussis-associated IgG Antibodies

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**Abstract**

We had an opportunity to test a kit manufactured by Denka Seiken Co., Ltd. that measures anti-PT and anti-FHA antibodies (hereinafter referred to as "this product") and evaluate its fundamental performance. This product was an ELISA reagent with a 96-well microplate and we found that it was versatile and easy-to-handle, and had a high reproducibility of data. We also observed a high correlation of measured values between an in vitro diagnostic using a bead-based ELISA test and this product.

## Introduction

Although pertussis is a vaccine-preventable disease, it may become severe in some unvaccinated infants. Meanwhile, if adults are infected, their symptoms are not always typical coughs and it is difficult to diagnose. Furthermore, adult pertussis patients are sources of infection in unvaccinated infants.[1-4)]

In 1981, Japan lead the world in the introduction of diphtheria pertussis tetanus vaccine (DTaP) including the acellular pertussis vaccine, which mainly consisted of PT (pertussis toxin) and FHA (filamentous hemagglutinin), and the number of reported pertussis patients decreased significantly, but in recent years, the number of adult pertussis patients has relatively increased.[5)] It has been considered that they replace DT routine vaccination of period II with DTaP to discourage this tendency.[6)] IgG antibodies against PT and FHA must be measured to evaluate the vaccine effect, and these have been used to diagnose pertussis.[7,8)] In regards to the measurement of serum antibodies, although the measurement of agglutination titers by bacterial aggregation is more widespread in routine clinical practice in Japan, care should be taken in the interpretation of most agglutinin titers because some of the DTaP vaccines include agglutinin[7)]. It has been difficult to compare data of agglutination titers with that of Western countries because the ELISA test is usually used to measure anti-PT antibodies in the West.

In this study, we tested a kit manufactured by Denka Seiken Co., Ltd. that measures anti-PT and anti-FHA antibodies and evaluated its fundamental performance.

## I. Materials and Methods

### 1. Measurement Kit

The reagents used in this study were included in an ELISA kit newly developed by Denka Seiken

**Key words** : Pertussis kit, pertussis toxin (PT), filamentous hemagglutinin (FHA)

Co., Ltd. that measures serum anti-PT and anti-FHA IgG antibodies. This product consisted of the following reagents：① A PT immobilized plate, a freeze-dried plate, ② A FHA immobilized plate, a freeze-dried plate, ③ Buffer solution, liquid form, ④ Enzyme-labeled antibodies (for PT), liquid form, ⑤ Enzyme-labeled antibodies (for FHA), liquid form, ⑥ Substrate solution, liquid form, ⑦ Concentrated washing solution, liquid form, ⑧ Stop solution, liquid form

**2. Measurement Principle and Procedure for this product**

The measurement principle for this product is a general ELISA test. The following is the practical procedure provided by Denka Seiken Co., Ltd.

<Preparation of Reagents>

Dilute the concentrated washing solution ten times with purified water depending on the number of samples, and use as a washing solution. Other reagents should be used without dilution.

<Preparation of Pre-diluted Samples>

Add 2 mL of buffer solution into some small test tubes according to the number of samples. Then distribute 10 μL of each sample into each test tube and mix well. Use these solutions as pre-diluted samples.

<Procedure>

Use control for PT or FHA provided separately to prepare a calibration curve. Pull out PT or FHA immobilized plates from aluminum bags according to the number of samples and set in the plate holder. Prepare a well for each blank, pre-diluted sample and each antibody titer (0, 5, 10, 20, 40, 80 and 160 EU/mL) of the control for PT or FHA.

**<Step 1 (The Primary Reaction)>** Instillations of Pre-diluted Samples and Controls

1) Add 100 μL of corresponding control or pre-diluted sample to PT or FHA immobilized plates in a certain order and at regular intervals. No sample or control should be added to the wells of the blank.

2) Mix for a few seconds with a microplate mixer. Cover with plastic wrap, etc., and make them stand still for 1 hour at 20-30℃.

**<Step 2 (The Secondary Reaction)>** Instillations of Solutions of Enzyme-labeled Antibodies

1) Remove the reaction solution in each well by aspiration in the same order and at the same intervals as in <Step 1>.

2) Add approximately 200 μL of washing solution to each well and mix for a few seconds with a microplate mixer. Remove the solution again by aspiration. Repeat these washing procedures twice more. Flip the plates upside down and beat them on some clean paper towels to remove the residual washing solution completely.

3) Add 100 μL of solutions of enzyme-labeled antibodies (for PT or for FHA) to each well in the same order and at the same intervals as in <Step 1> and mix for a few seconds with a microplate mixer. Cover with plastic wrap, etc., and make them stand still for 1 hour at 20-30℃. No solutions of enzyme-labeled antibodies should be added to the wells of the blank.

**<Step 3 (Enzyme Reaction)>** Instillations of Substrate Solution

1) Remove the reaction solution in each well by aspiration in the same order and at the same intervals as in <Step 1>.

2) Add approximately 200 μL of washing solution to each well and mix for a few seconds with a microplate mixer. Remove the solution again by aspiration. Repeat these washing procedures four more times. Flip the plates upside down and beat them on some clean paper towels to remove the residual washing solution completely.

3) Add 100 μL of substrate solution to each well in the same order and at the same intervals as in <Step 1> and mix for a few seconds with a microplate mixer. Cover with plastic wrap, etc., and make them stand still for 1 hour at 20-30℃ under protection from light. In addition, add 100 μL of the substrate solution to the wells of the blank.

<Step 4> Instillations of Stop Solution and Measurement

1) Add 100 μL of stop solution to each well in the same order and at the same intervals as in <Step 1>. In addition, add 100 μL of the stop solution to the wells of the blank.

2) Measure with an automatic reader (dominant wavelength : 450 nm, reference wavelengths : 600～700 nm) within 30 minutes using the well of the blank as a control.

**<Evaluation of Measurement Results>**

1) Subtract the absorbance of the blank from that of each control.

2) Confirm that each value obtained by subtracting the absorbance of the blank from that of 10 EU/mL of antibody titer in each control is within 0.15-0.60.

3) Define the value obtained by subtracting the absorbance of the blank from that of sample as "a."

4) Plot antibody titers (EU) of each control on the horizontal axis and values obtained in 1) on the vertical axis to prepare calibration curves.

5) Based on these calibration curves, calculate the antibody titer corresponding to the absorbance of each sample (a) and display. The obtained values should be rounded down to whole numbers.

**3. Serum Sample**

We used serum samples from 158 patients who had visited National Hospital Organization Fukuoka National Hospital from 2005 to 2010 and had been suspected of pertussis infection.

**4. Standard Substance**

We dissolved the standard serum JNIH-10 (PT : 250 EU/vial, FHA : 400 EU/vial) obtained from the National Institute of Infectious Diseases in Japan into 1 mL of purified water and used this as a standard substance.

**5. International Standard Serum**[9)10)]

We dissolved the international standard serum 06/140 (PT : 335 IU/vial, FHA : 130 IU/vial) obtained from the National Institute for Biological Standards and Control (NIBSC, U.K.) into 1 mL of purified water and used this as an international standard serum.

**6. Measurement of Serum Samples, the Standard Substance and the International Standard Serum**

We measured a twofold dilution series of the standard substance JNIH-10 in accordance with the above procedure and evaluated the range of absorbance and the effects by dilution. We then measured a dilution series of the international standard serum 06/140 as well and compared it with the results in JNIH-10 to evaluate the correlation between the standard unit in Japan (EU) and the international standard unit (IU).

All the serum samples were evaluated by JNIH-10. Antibody titers (EU/mL) were calculated using calibration curves prepared by controls for PT or FHA provided separately.

**7. Evaluation of Changes in Measured Values by Repeated Measurement of the Same Sample**

We measured two samples of known anti-PT and FHA antibody titers, a total of four samples, four times and calculated the coefficient of variation (CV, %) to evaluate within-run reproducibility of this product. We used two types of samples of known antibody titers, 20 and 80 EU/mL.

**8. Correlativity with the Control Product**

We used Reagent Wako for Measurement of Pertussis Bacteria Antibody Titer (Wako Pure Chemical Industries, Ltd.) as a control product and evaluated correlativity with this product. Since the measurement range of this product was 1～160 EU/mL and that of the control product was 1～100 EU/mL, if the antibody titer of the sample showed as out of the range for both or either of them, the sample was excluded. We calculated the slopes of regression lines and correlation coefficients for 137 samples of anti-PT antibody titers and 145 samples of anti-FHA antibody titers.

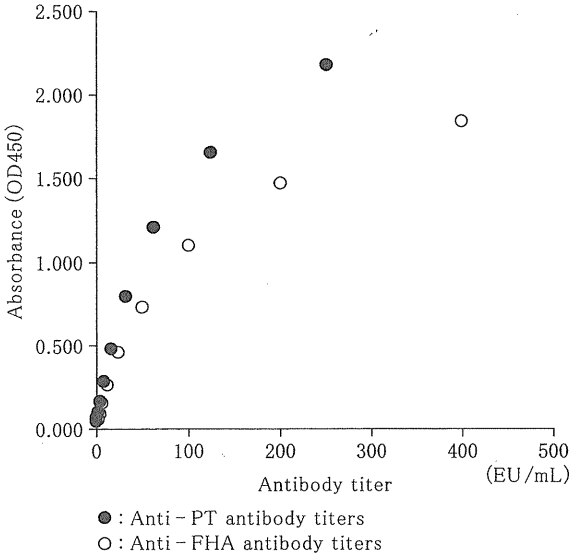


**Fig. 1**　Changes of absorbance in dilution series of JNIH-10

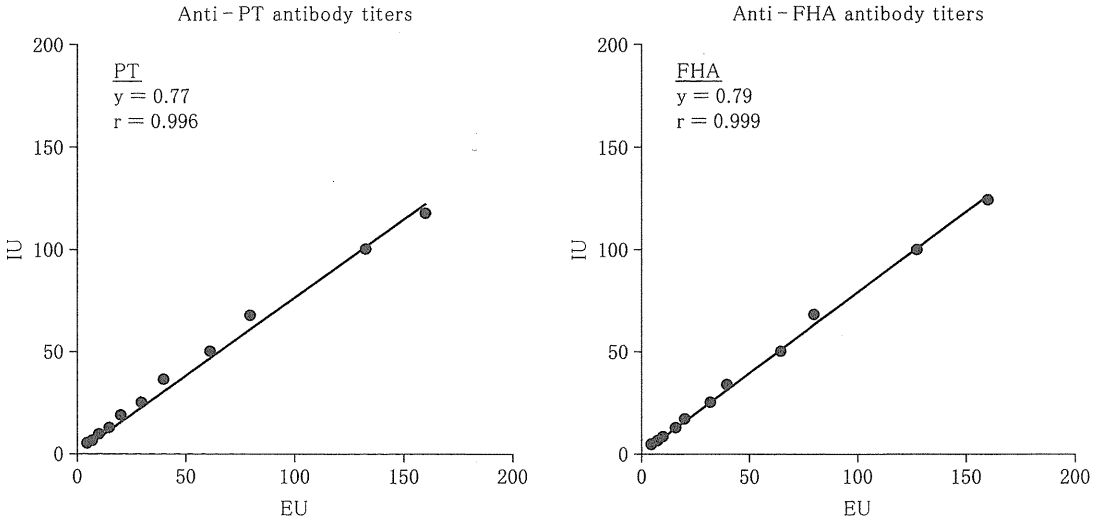


**Fig. 2**　Comparison between the standard unit in Japan (EU) and the international unit (IU)

## II. Results

### 1. Changes of Absorbance in a Dilution Series of JNIH-10

We prepared a twofold dilution series of JNIH-10 and measured absorbance in accordance with the above procedure (**Fig. 1**). As a result, the absorbance obtained from the pre-diluted sample of the undiluted solution of JNIH-10 (diluted to 201-fold with the buffer solution) showed around 2OD in both anti-PT and FHA antibodies. In the lots of reagents shown in Fig. 1, the absorbance of anti-PT antibodies was usually slightly higher than that of anti-FHA antibodies in the same EU values, but their absorbance was considered to be practically equivalent. It can be considered that increases of absorbance of anti-PT and anti-FHA antibodies slow down as increases of antibody titers and the

**Table 1**　Simultaneous reproducibility

| Samples | Lot# of reagents | CV values (%) | | |
|---|---|---|---|---|
| | | First | Second | Third |
| PT (20 EU/mL) | 1 | 1.3 | 2.9 | 2.1 |
| | 2 | 3.6 | 2.7 | 4.1 |
| | 3 | 2.8 | 2.0 | 5.5 |
| PT (80 EU/mL) | 1 | 4.5 | 3.9 | 1.0 |
| | 2 | 2.6 | 3.9 | 3.2 |
| | 3 | 2.4 | 0.5 | 1.9 |
| FHA (20 EU/mL) | 1 | 2.2 | 2.1 | 2.9 |
| | 2 | 2.4 | 1.1 | 1.4 |
| | 3 | 2.0 | 2.7 | 2.4 |
| FHA (80 EU/mL) | 1 | 2.0 | 2.4 | 2.9 |
| | 2 | 0.9 | 1.9 | 1.5 |
| | 3 | 2.5 | 1.2 | 1.3 |

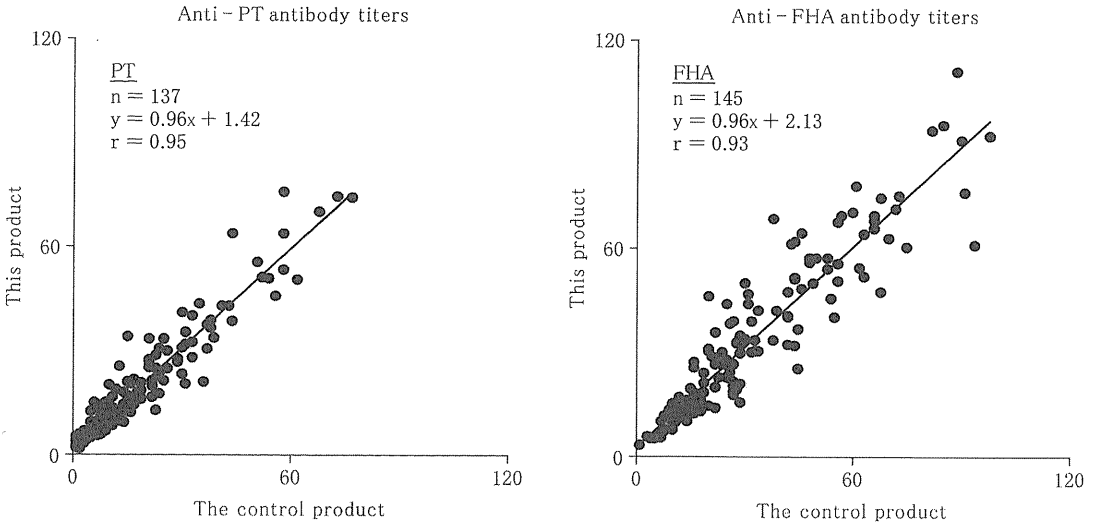


**Fig. 3**　Correlation with the control product

dilution curves reach plateau. This is a common phenomenon in the plate ELISA test.

**2．Comparison between the Standard Unit in Japan (EU) and the International Unit (IU)**

We simultaneously measured (seven times per lot) the dilution series of JNIH-10 (**Fig. 2**, x-axis) and the international standard serum 06/140 (Fig. 2, y-axis) using this product to compare them. The results showed a good correlation, $y=0.77x$ ($r=0.996$) in PT and $y=0.79x$ ($r=0.999$) in FHA.

**3．Evaluation of Changes in Measurement Values by Repeated Measurement of the Same Sample**

As shown in **Table 1**, the coefficient of variation (CV, %) obtained with a four-time measurement of two types of samples with known antibody titers were 0.5～5.5% in anti-PT antibody titers and 0.9～2.9% in anti-FHA antibody titers. These results showed a good reproducibility in all evaluated lots of reagents.

### 4. Correlation with the Control Product

As shown in **Fig. 3**, the linear regression formula of PT (n=137) was y=0.96x+1.42 (r=0.95) and that of FHA (n=145) was y=0.96x+2.13 (r=0.93), and both of them showed high correlations.

## III. Discussion

Since this product adopts a general 96-well microplate, it is considered to have good versatility and can easily conform to automation in sites such as testing centers, which treat many samples. From the results of fundamental performance in this study, this product is a stable reagent with a good reproducibility and is expected to enable the comparison of data with that by Reagent Wako ELISA test due to a high correlation with that test, which is currently the only reagent used to measure anti-PT and anti-FHA antibodies in Japan. Furthermore, this product is also considered to allow the possibility of comparing data with anti-PT antibody titers obtained in IU in other countries[11)12)]. We believe that this product can be widely used in support of clinical diagnosis of pertussis, as well as in the evaluation of vaccine effects, epidemiological studies and for other purposes.

However, the results of this study were insufficient to calculate a conversion factor between EU and IU. The purities of antigens, purification methods, strains, compositions of buffer solutions, etc., used in the test differ for every manufacturer of reagents, which affects the reactivity of reference and standard substances. Since serum antibodies are polyclonal, the composition rates of recognition epitopes may differ in every serum. In view of the fact that a certain tendency is expected to be present in the products by the same manufacturer, we estimated that the IU value was approximately 70-80% of the EU value in this product.

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ウイルス感染症と疫学・臨床像

# 麻疹

庵原俊昭

## 原因

- RNA ウイルスであるパラミクソウイルス科モルビリウイルス属麻疹ウイルスによる全身性ウイルス感染症である.
- 麻疹患者と接触後，発熱，上気道炎症状（カタル症状）出現までの潜伏期間は 8～12 日間，家族内接触後の発疹出現までの潜伏期間は 7～18 日（中央値 14 日）である[1].
- 麻疹の発疹は，麻疹ウイルスに対する免疫反応により出現する.

## 疫学

- 日本では，この 20 数年間に数回麻疹流行を経験したが，流行株の遺伝子型はすべて異なっていた（❶）[2].
- 日本で使用されている麻疹ワクチンの遺伝子型はすべて A 型であるが，ワクチン接種により誘導された抗体は，遺伝子型の異なる野生株に対して有効である.
- 自然宿主はヒトだけであり，多くのヒトが適切にワクチン接種を受けると，地域から野生株が排除される．麻疹排除の基準，排除に向けての診断方法

### ❶ 日本で流行した麻疹ウイルスの遺伝子型と麻疹の診断

**遺伝子型**

- 米国で分離された Edmonston 株由来の麻疹ワクチン株である AIK-C 株，Schwartz-FF8 株はともに遺伝子型 A である.
- 日本で分離された田辺株由来の麻疹ワクチン株 CAM-70 株も遺伝子型 A である.
- 1985～1990 年に日本で流行した野生株の遺伝子型は D3 であった.
- 1990～1997 年に流行した野生株の遺伝子型は D5 パラオ型であった.
- 2001 年ころに流行した野生株の遺伝子型は中国由来の H1 であった.
- 2006 年からの 3 年間に流行した野生株の遺伝子型は D5 バンコク型であり，1996 年ころに流行した野生株（D5）と異なる lineage であった.

**麻疹の診断**

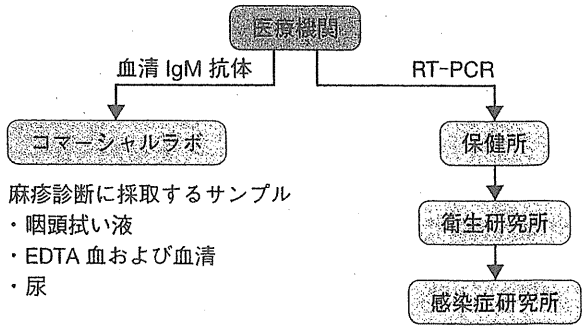
- 麻疹ワクチン接種で誘導された免疫は，遺伝子型の異なる野生株に対しても有効である.
- 麻疹初感染または PVF の急性期血清抗体パターンは，IgM 抗体陽性，IgG 抗体陰性または弱陽性である（一次免疫応答）.
- 麻疹 SVF の急性期血清抗体パターンは，IgM 抗体陽性，IgG 抗体高値であり，IgG 抗体は急速に上昇する（二次免疫応答）.
- 初感染，PVF，SVF の急性期には，末梢血単核球，咽頭拭い液，尿から麻疹ウイルスが分離され，ウイルス RNA は検出される.

PVF：一次性ワクチン不全，SVF：二次性ワクチン不全

### ❷ 麻疹排除に向けての対策と評価（日本）

**対策と評価**

- 95％以上の接種率で MR ワクチンの 2 回接種
- 実験室診断による麻疹の全数把握（WHO 認定の麻疹センター〈国立感染症研究所〉が承認した施設〈衛生研究所〉での診断）
- 麻疹の発症率 1/100 万人
- 外国から輸入された野生株による発症で，流行は第 2 波まで
- 麻疹患者サンプルは保健所を介して衛生研究所に送付




麻疹診断に採取するサンプル
- 咽頭拭い液
- EDTA 血および血清
- 尿

❸ 麻疹ワクチン接種率と麻疹発症年齢

| 接種率 | 麻疹流行間隔 | 野生株ウイルス量 | 小児の感受性者数 | 成人の感受性者数 |
|---|---|---|---|---|
| 0%～低率 | 1～2年ごと | ＋＋＋＋ | ＋＋＋＋ | ＋ |
| 部分接種*1 <90% | 数年～10年ごと | ＋＋ | ＋＋ | ＋＋＋ |
| 全般接種 ≧90% | なし 輸入例と関連*2 | ＋ | ＋ | ＋ |

*1 中途半端な接種率のとき流行間隔は延長するが，発症者に占める成人，ワクチン接種歴のある児（者），1歳未満児の割合が高くなる．

*2 輸入症例の発症があっても，それに続く集団発生は小さい規模で終わる（例数<100例，流行期間<3か月）

を❷に示す[3]．

- 麻疹ウイルス野生株が排除されている国は，フィンランド，韓国，全アメリカ地域に属する国である．
- 麻疹ウイルスは主として飛沫・接触で感染し，時に空気感染する．
- 麻疹患者の周囲への感染可能期間は，カタル症状出現1～2日前（発疹出現3～5日前）から発疹出現後4日ころまでである．
- 麻疹ワクチンが行われていなかった時代の発症者は1歳過ぎの幼児であったが，集団免疫率*1を下回る接種率で麻疹ワクチン接種が行われている現在の主たる発症者は，思春期から若者と1歳未満の乳児である（❸）．
- 麻疹は，感受性者が集まる都会から地方へと流行が拡大する．
- 麻疹の基本再生産数*2は16～21であり，集団免疫率は90～95%である．
- ワクチン不全の原因の多くは，免疫減衰による二次性ワクチン不全（SVF）である．
- 麻疹ワクチン接種世代の麻疹抗体価は，多くの人が自然感染していた世代よりも低値である[4]．
- 近年，麻疹の移行抗体は低下し，生後6か月を過ぎると約半数が，10か月を過ぎると多くの子どもが移行抗体を消失している．

## 臨床症状（❹）

- 麻疹患者と接触8～12日後に発熱，咳，鼻汁，結膜炎などのカタル症状が出現する（カタル期）．
- 2～3日後に一度解熱するが，翌日には再度39～40℃に上昇し（二峰性発熱），二峰目の発熱出現と同時に顔面から斑丘疹が出現し，下方へ拡大する（発疹期）．
- 典型的な麻疹の皮疹は色素沈着を残して消退する（回復期）．
- 麻疹に特徴的な頬部に出現する白色の粘膜疹（Koplik斑）は，発疹出現前の解熱時に出現し，3日間程度持続する．
- 麻疹の合併症として，中耳炎，肺炎，クループ症候群，角膜潰瘍，下痢，脳炎がある．
- 肺炎には免疫不全者に認める巨細胞性肺炎，ウイルス性肺炎，細菌の二次感染による細菌性肺炎がある[5]．

*1
**集団免疫率（herd immunity : $H_0$）**
流行を止めるために必要な集団の特異免疫陽性者の割合であり，野生株の排除をめざすには，集団免疫率よりも高い接種率でワクチンを接種する必要がある．
$H_0 = (1 - 1/R_0) \times 100$
の関係があり，基本再生産数が高い感染症ほど集団免疫率が高くなる．麻疹と百日咳の集団免疫率がいちばん高く，90～95%である．

*2
**基本再生産数（basic reproduction number : $R_0$）**
1人の患者が周囲の免疫のない人に感染させる数で，数が大きいほど感染力が強く，短時間の接触で感染する．
基本再生産数がいちばん高い感染症は麻疹と百日咳であり，麻疹は20分間同じ部屋に一緒にいると周囲に感染させる．

SVF：secondary vaccine failure