

MINIREVIEW

Optimal Sampling Sites and Methods for Detection of Pathogens Possibly Causing Community-Acquired Lower Respiratory Tract Infections^{∇†}

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Acute respiratory tract infections (RTIs), both upper (URTIs) and lower respiratory tract infections (LRTIs), are the most common reason for consultation with a general practitioner. RTIs result in about 180 million antibiotic prescriptions per year in the EU-27 member states (ESAC website, 2008; www.esac.ua.ac.be), and 6.4 million antibiotic prescriptions were prescribed for acute bronchitis and cough in 2003 in adults between 16 and 64 years old in the United States (65).

The number of pathogens involved in LRTI, with various susceptibilities to antimicrobials, is large constituting an enormous challenge for diagnostic microbiology. In general, in only 50% of cases is an etiologic agent detected. Documented infection is uncommon in community-managed infection and is usually only defined in 25 to 50% of hospital-managed infections.

The upper end of the respiratory tract, the rhinopharynx, is widely open to the introduction of airborne microorganisms. It is, however, also a very efficient barrier for invading bacteria. The barrier function of the rhinopharynx results from the local lymphoid tissue producing phagocytic cells and secretory immunoglobulin (Ig) and from the rich commensal flora of aerobic and anaerobic microbes establishing an interfering colonization resistance. The number of these organisms varies from 2.6×10^4 to 4×10^8 CFU of cultivable bacteria per cm^2 (95). Aerobes tend to decrease and anaerobes tend to increase with age (98).

Colonization starts during the first year of life and is composed not only of commensal organisms but also of potential pathogens: group A β -hemolytic streptococci, *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Moraxella catarrhalis*. These are present permanently or intermittently, with rates varying with age (decreasing in adults), exposure to other children, geographic location, socioeconomic status, and vaccination status (39). In most cases, these organisms cause disease in only a small percentage of persons colonized. Viruses do not normally colonize the rhinopharynx. However, most bacterial

respiratory infections start with or are accompanied by their proliferation in the nasopharynx.

Thus, the URT with its commensal flora acts both as a defense mechanism and as a primary site for LRTI, which creates tremendous diagnostic challenges.

Any bacteriological examination of nonsterile respiratory specimens must indeed distinguish between organisms infecting the LRT and organisms colonizing the rhinopharynx. Normally sterile samples therefore are considered the “gold standard.”

Different specimens commonly collected to detect pathogens causing LRTI have been compared, but the results were not consistent (12, 43, 49, 58, 103) (Table 1; and see Table S1 in the supplemental material). This minireview presents an overview of the optimal detection of *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Moraxella catarrhalis*, *Mycoplasma pneumoniae*, *Chlamydia pneumoniae*, *Legionella pneumophila*, and respiratory viruses from specimens in patients with community-associated (CA)-LRTI (Table 2).

OPTIMAL SAMPLES FOR DETECTION OF RESPIRATORY PATHOGENS

Invasive techniques. (i) Sterile sampling sites. (a) Blood cultures. For the diagnosis of pneumonia, blood cultures have a very high specificity but are positive in only 4 to 18% of untreated cases (9, 106). In the study by Waterer et al. (106), a direct correlation was found between the severity (based on the Fine severity index) of pneumonia and blood culture positivity rate: the value of routine blood cultures was questioned for community-acquired pneumonia (CAP) for patients in lower risk classes. Two blood cultures should be obtained as early as possible in the disease and before any antibiotic treatment is started. Kalin and Lindberg (53) have shown that 13/38 (34%) blood cultures were positive when initiated within 4 days after the first symptoms of the illness and 3/26 (12%) were positive when initiated later. *S. pneumoniae* is identified in approximately 60% of positive blood cultures and *Haemophilus influenzae* in various percentages from 2 to 13% (66).

In a recent study, Butler et al. used latent class analysis to determine the sensitivity and specificity of blood cultures (14) and confirmed previous results.

(b) Thoracentesis. In 40% of pneumonia, there may be an accompanying pleural effusion. Although the specificity of pleural exudate culture is very high, the sensitivity is low be-

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TABLE 1. Comparison of specimens and sampling methods for the detection of different respiratory pathogens^a

Pathogen	Sample ranking	Method	Age (yr)	Total no. of specimens/ no. of patients	Reference
<i>M. pneumoniae</i>	Sputum > TW > NPS > OPS	PCR	20–93	552/144	31
	OPS > NPS	PCR	NSp	132/66	43
	OPS > BAL > sputum	PCR	NSp	325/197	49
	Sputum > OPS	Gene-probe test	>18	160	57
	Sputum > NPA	Ag-EIA	>18	102/51	56
	Sputum > OPS	Culture, PCR, NASBA	NSp	302/180	61, 62
	NPS = OPS	PCR	NSp	63	69
	Sputum > NPA = OPS	PCR	22–29	96/32	79
	OPS > NPA	PCR	NSp	102	82
<i>C. pneumoniae</i>	NPS > TS	Culture, PCR	3–12	260	10
	Sputum > NPS = OPS	PCR, culture	3–79	319/129	12
	OPS > NPS	PCR	NSp	132/66	43
	Sputum > NPA > OPS	PCR	NSp	105/35	58
	NPS > OPS > sputum	PCR	20–93	468/156	103
RSV	PFS = NPA	DFA	0–5	910/455	1
	NPA > NPFS	PCR	0–18	338/169	19
	NPA > NPFS	DIF	0–18	338/169	19
	NPA = NPS	Culture, IFA	0–16	250/125	38
	NPA > NPS	DIF	0–2	366/183	64
	NPA > NPS	Culture, Ag-EIA, FAT	0–18	242/121	67
	Adenovirus	PFS = NPA	DFA	0–5	910/455
Parainfluenza virus 1, 2, or 3	PFS = NPA	DFA	0–5	910/455	1
Picornaviruses	Sputum > NS > OPS	Culture	5–15	66/22	50
Influenza virus	PFS = NPA	DFA	0–5	910/455	1
	NPS > NS > NPA	Quidel quickvue	0–18	366/122	2
	NPFS = NPA	PCR	0–18	338/169	19
	NPA > NPFS	DIF	0–18	338/169	19
	Sputum > NA > NPS > OPS	FLU OIA test	0–76	403/184	24
	NA > sputum > NPS > OPS	Culture	0–76	403/184	24
	NPS > NPA > OPS	PCR, Directigen Flu A+B	61–97	85/47	44
	NPS > OPS	Binax Now, Directigen Flu A+B, DIF	NSp	521/448	93
All viruses	NPA > NS = OPS	PCR	0–16	221/178	46
	NFS = NA > unpreserved saline	PCR	0–1.5	543/181	105
	NPA > NS	PCR, culture, DIF	≤5	950/475	97

^a BAL, bronchoalveolar lavage; DIF, direct immunofluorescence; DFA, direct fluorescent antibody assay; Ag-EIA, antigen enzyme immunoassay; IFA, indirect fluorescent antibody test; FAT, fluorescent antibody test; NA, nasal aspirate; NASBA, nucleic acid sequence-based amplification; NPA, nasopharyngeal aspirate; NPFS, nasopharyngeal flocked swab; NFS, nasal flocked swab; NPS, nasopharyngeal swab; NW, nasal wash; NSp, not specified; NS, nasal swab; OPS, oropharyngeal swab; PFS, pernasal flocked swab; TW, throat wash. (Adapted from reference 111 with permission of the publisher.)

cause of the low incidence of invasion of the pleura (92). Therefore Gram stains or cultures yielding bacterial pathogens from pleural fluid are likely to be an accurate reflection of the microbial cause of the pneumonia. Diagnostic thoracentesis should therefore be performed when a significant pleural effusion is present.

(c) *TNA*. Although not used extensively, in recent years there has been resurgent interest in and growing experience with transthoracic needle aspiration (TNA) for microbial diagnosis of pneumonia, especially in patients with severe pneumonia (89, 91).

TNA allows a specimen to be obtained from the infected focus without interference by commensal flora, except for possible skin contaminants. In studies reviewed by Skerrett et al.

(92), TNA yielded a positive culture in 33% to 80% of cases of patients with pneumonia.

From 13 studies (91) in which the results of blood cultures were known as well, the sensitivity of lung aspiration was estimated at 74% and that of blood cultures was estimated at 37%. Ruiz-Gonzalez obtained through TNA a microbiological diagnosis in 36/55 (65%) of patients with pneumonia of unknown etiology by conventional methods (89).

The superiority of direct access to a lung lesion through TNA is also illustrated in the study by Clark et al. (21), who identified an etiology of infection in 12/18 (23%) of infiltrates with a corresponding nondiagnostic bronchoalveolar lavage (BAL). Because of the inherent potential adverse effects, however, TNA can be considered only on an individual basis for

TABLE 2. Diagnostic approach for the most common specific agents in LRTIs^a

Pathogen(s)	Specimen(s)	Rapid test(s)	Conventional test(s)	Comment(s)
<i>S. pneumoniae</i>	Blood		Blood culture	Positive in 4–18% of cases when collected within 4 days
	Sputum	Gram stain	Culture	Only purulent samples acceptable, can be obtained in 35–40% of patients, informative if >90% gram positive, diplococci most relevant if Gram stain is informative.
	BAL, PSB Pleural exudates, TNA	Gram stain Gram stain	Culture Culture	Quantitative cultures Very specific, only considered if less invasive methods nondiagnostic
	Urine	Antigen test		Sensitivity in 50–80% of bacteremic cases, lacks specificity in children, more evaluation necessary
<i>H. influenzae</i>	Blood		Blood culture	Less frequently positive than for <i>S. pneumoniae</i>
	Respiratory specimens	Gram stain	Culture	
<i>Legionella</i> spp.	Urine	Antigen test		Sensitivity of 66–95%
	Respiratory specimens	NAAT	Culture	On appropriate media, late results
	Serum		IgM and IgG serology	Acute and convalescent specimens, retrospective diagnosis
<i>C. pneumoniae</i> and <i>M. pneumoniae</i>	Respiratory specimens	NAAT	Culture	On appropriate medium; low sensitivity
	Serum		IgM and IgG serology	Acute and convalescent specimens, lacks sensitivity and specificity, not appropriate for individual patient management, retrospective results
Respiratory viruses	Respiratory specimens	Direct antigen test NAAT	Virus isolation	Requirement for appropriate infrastructure, virus isolation less sensitive than NAAT

^a BAL, bronchoalveolar lavage; PSB, protected specimen brush; TNA, transthoracic needle aspiration; NAAT, nucleic acid amplification test (not generally available yet and not Food and Drug Administration cleared).

some severely ill patients with a focal infiltrate for whom less invasive measures have been nondiagnostic (89).

(ii) Nonsterile sampling sites: bronchoscopic PSB and BAL.

The specificity of bronchoscopy for the diagnosis of LRTI is not high because of contamination with the upper airway flora and because the patient may be put at unnecessary additional risk because of already compromised gas exchange.

Several techniques have been proposed to achieve accurate discrimination between colonization and infection. Diagnostic accuracy is improved by the use of a protected specimen brush (PSB) (110) and BAL, at first performed through a bronchoscope and later not bronchoscopically taken (NB-BAL) (6, 107). These procedures carry less risk and are usually more acceptable to patients than transtracheal aspiration and direct needle aspiration of the lung.

A major criticism directed at the PSB technique is the relatively small amount of distal bronchial secretions examined, particularly in comparison with the technique of BAL. Quantitative bacterial culture is important for the assessment of these techniques. The cutoff point for diagnosis of pneumonia has been set at 10³ CFU/ml or 10³ CFU/ml to 10⁴ CFU/ml (78, 92, 100).

Using 10³ CFU/ml as the threshold value for a positive culture, Cantral et al. (15) determined the sensitivity and the specificity to be 90% and 97%, respectively. With a threshold value for a positive culture of 10⁴ CFU/ml, the specificity of lavage cultures for potential pathogenic bacteria in relation to actual LRTI was 100% (78). Therefore, quantitative bacterial culture of potential pathogenic bacteria in BAL fluid is very specific but is positive in only about one-third of unselected immunocompetent adult patients with an LRTI (78).

Noninvasive techniques. (i) Sputum. The most widely accepted nonsterile sample for diagnosis of LRTI due to the so-called “typical bacterial respiratory pathogens” (*S. pneumoniae*, *H. influenzae*, and *M. catarrhalis*) is sputum.

To be of value for microbiological diagnosis, sputum specimens must be representative of lower respiratory secretions. The assessment of the acceptability in this regard is based on cytologic criteria (92).

Sputum should therefore be screened by microscopic examination for the relative number of polymorphonuclear cells and squamous epithelial cells in a low-power (×10) field. Invalid specimens (≥10 squamous epithelial cells and ≤25 polymorphonuclear cells/field) should not be examined further.

The main limitation is the difficulty to obtain good-quality, purulent sputum. Many pneumonia patients do not produce sputum, particularly older patients. Gleckman et al. (42) obtained satisfactory sputum specimens in 47/174 (32%) of patients, Roson et al. (85) in 210/533 (39%), Ewig et al. (37) in 23/42 (55%) patients, Kalin et al. (53) in 156/205 (76%) of adults, and Geckler et al. (41) in 90% of young military recruits.

When a purulent sputum sample can be obtained from patients with CAP and is processed in a timely manner, Gram stain may be very informative, if a predominant bacterial morphotype allows to infer the etiologic bacterial species and to interpret the results of sputum culture (71, 101).

In the past, there were great controversies about the value of the Gram stain. Reed et al. (80) reported the results of a meta-analysis of 12 studies and found wide variability in sensitivity (15% to 100%) and specificity (11% to 100%) for the sputum Gram stain. However Gram-stained sputum smears can only be validated by comparing the results with those of a reference, e.g., specimens devoid of commensal flora: blood- or pleural fluid cultures or TNA. Early studies (35, 41, 53) concerned a limited number of patients; later studies involved larger numbers of patients. In a prospective study of bacteremic pneumonia by Gleckman et al. (42), a predominant morphotype was observed in 79% of the acceptable specimens and a compatible organism was present in the blood of 85% of these patients. In a study by Roson et al. (85), the conclusion was that in good-quality sputum, through the detection of a single or predominant morphotype ($\pm 90\%$), the sensitivity and specificity for the detection of *S. pneumoniae* were 35.4% and 96.7%, respectively, and for *H. influenzae* they were 42.8% and 99.4%, respectively. When a purulent sample was available, the Gram stain gave a presumptive diagnosis in 175/210 cases (80%). The studies by Butler et al. and Miyashita et al. confirmed these results (14, 70).

There is a clear need for quality control. A low concordance of Gram-stained specimens examined by different technicians has been found (23), whereas others found the results to be reproducible (75).

In conclusion, provided a sputum specimen is of good quality, it may be a sample of choice on which a probable diagnosis of *S. pneumoniae* or *H. influenzae* LRTI can be made within 30 to 40 min by Gram stain and may be confirmed by culture.

(ii) NPA, NPS, NS, and OPS. In the literature, the distinction between an oropharyngeal swab (OPS) and a throat swab is not always clearly made. In this paper, OPS also includes throat swabs.

Although not optimal for the detection of typical bacterial pathogens, it is generally assumed that for respiratory infections due to viruses the optimal specimen is the nasopharyngeal aspirate (NPA); this might not be true for all respiratory viruses and for all detection techniques applied (24).

The NPA, which is commonly used to isolate viruses in children, has not gained popularity as a sampling method to test for respiratory pathogens in adults. It is recommended to concentrate NPA specimens for rapid influenza virus testing by centrifugation and resuspension in a smaller volume whenever feasible. Alternatively, the collection of the NPA should yield a high-quality, undiluted specimen for rapid antigen testing (2).

Some studies collect both a NS and a OPS and combine

them in the same viral transport medium (11, 109). Dwyer et al. even recommend to take in adults a swab from each nostril and from the throat and combine them in the laboratory for influenza virus detection (36) during an epidemic, although, to our knowledge, no data are available to support this strategy.

It is difficult to compare recovery rates of respiratory pathogens from swabs because different types of swabs and transport media have been used. Indeed, proper transport of clinical specimens for culturing infectious agents may be the most important factor affecting the successful evaluation of these specimens. Because many samples are submitted from sites distant from clinical microbiological laboratories, it is essential that the viability of the organism be maintained.

For the isolation of *C. pneumoniae*, swab specimens should be collected only on swabs with a Dacron tip and an aluminum or plastic shaft. Swabs with calcium alginate or cotton tips and wooden shafts may inhibit the growth of these organisms (depending on the adhesive used) and are therefore unacceptable (34). Calcium alginate minitipped swabs, commonly used to collect nasopharyngeal specimens for *Bordetella pertussis*, may decrease recovery of lipid-enveloped viruses.

Since 2003, studies have been performed to evaluate the recovery of different organisms in new transport (83, 87, 102) and storage (20, 52) media.

For virus transport systems, no real standard has been defined, as is illustrated in a report published by the European Influenza Surveillance Scheme (EISS) in 2004. Different respiratory specimens and types of swabs and transport media were used among the EISS surveillance networks (68).

The Copan system combining flocced swabs and universal transport medium (UTM) (Copan, Brescia, Italy) for collection and transport is a universal system compatible with antigen detection kits, direct fluorescent antibody (DFA), culture and PCR (16, 17, 25). Nasal swabbing with the new flocced swabs (NFS) is equivalent to traditional rayon NPS with less patient discomfort. Significantly more epithelial cells are collected by these flocced swabs, providing better specimens for diagnosis. Furthermore, NPSs collected with flocced swabs (NPFS) detect a higher number of positives than NPSs collected with Dacron swabs. When comparing NFSs with NPAs collected from 455 children admitted to the hospital with an RTI, the sensitivities of the NFSs for respiratory syncytial virus (RSV), influenza virus A, parainfluenzavirus, and adenovirus were, respectively, 98.4%, 100%, 100%, and 88.9% (1). Additionally, when comparing NPFS with NPA for rapid diagnosis of respiratory viruses in children, Chan et al. (19) found that both NPA and NPFS were comparable for detecting influenza A virus by reverse transcription-PCR (RT-PCR) but an NPA was slightly more sensitive than NPFS for detecting RSV by both RT-PCR and direct immunofluorescence (DIF) and for detecting influenza A virus by DIF. They concluded that NPA remains the optimal specimen for diagnosis of respiratory infections by RT-PCR and DIF. However, the collection of NPFS was easier to perform in an outpatient setting and was more acceptable to the parents. On the other hand, Walsh et al. (105) demonstrated that NFSs in UTM-RT medium were better suited than NPAs from young children for the detection of respiratory viruses by PCR.

Nucleic acid amplification technique (NAAT) inhibitors occur also frequently and may be difficult to eliminate as pre-

sented previously (63). Therefore, both sampling devices and transport media should be checked for the presence of inhibitors.

OPTIMAL RECOVERY OF INDIVIDUAL RESPIRATORY PATHOGENS

***Streptococcus pneumoniae*.** It is generally accepted that the best nonsterile respiratory specimen for the recovery of *S. pneumoniae* is sputum. Sensitivity and specificity of sputum cultures are reduced by contamination with flora colonizing the URT. The value of sputum cultures in establishing a bacterial cause of LRTI depends on how the specimens are collected and processed and on whether a predominant bacterial morphotype has been observed in the Gram stain.

The yield of sputum cultures has varied widely ranging from <20% for outpatients (99) to >90% for hospitalized patients with pneumonia (35). Good concordance has been found between the results of cultures of sputum and tracheal aspirates (41), particularly when good-quality sputum specimens are washed and cultures are quantified (4).

Drew detected *S. pneumoniae* in up to 94% (29/31) of specimens from patients whose blood culture was positive (35). Others conclude that in cases of bacteremic pneumococcal pneumonia, *S. pneumoniae* may be isolated in sputum culture in only 40 to 50% of cases when standard microbiological techniques are used. Purulent sputa also can be obtained from patients without pulmonary pathology (59), and in some studies the predictive value of sputum culture is low (3), even as low as 5% in cases of nonsevere CAP (99). In one study (66) in which 19/48 (39.5%) of the bacteremic patients had sputum cultured, there was concordance of blood and sputum results for 9 (47%) of the pairs, and in another study (3) for 25/51 (49.0%) of the pairs.

In contrast with a widespread opinion, it is now also clear that sputum culture results are convincing when and only when the organism isolated is compatible with the morphology of the organism present in >90% of leukocytes in the Gram stain (14, 35, 42, 53, 85).

Very often, however, sputum is not available in CAP patients, especially in children, and upper respiratory specimens are collected, although these specimens have no place in the diagnosis of LRTI caused by bacterial pathogens such as *S. pneumoniae* since these specimens do not allow differentiation between carriage and infection.

***Haemophilus influenzae*.** As for *S. pneumoniae*, the best specimen with which to culture *H. influenzae* in LRTI is sputum, with sensitivities and specificities varying from 76 to 82% and from 99 to 100%, respectively (70, 85). Most studies with URT samples have been performed in studies looking for *H. influenzae* colonization in children. Again, URT specimens have no place in the diagnosis of LRTI caused by *H. influenzae*.

***Moraxella catarrhalis*.** The role of *M. catarrhalis* in LRTI is still questioned. However, the best specimen to culture this organism in patients with LRTI is sputum and URT specimens should not be used.

***Mycoplasma pneumoniae*.** Because of its fastidious nature, *M. pneumoniae* is not routinely cultured from respiratory specimens. Most studies are PCR-based on both LRT and URT specimens.

Gnarpe et al. (43) compared NPSs and OPSs for the detection of *M. pneumoniae* by PCR. A total of seven patients (10.6%) were positive for *M. pneumoniae* and of these, six were positive from OPSs and two were positive from NPSs. This difference was not statistically significant—probably due to the low numbers of positive specimens. Michelow et al. (69) investigated the optimal site and method of sampling the URT for the detection of *M. pneumoniae* by PCR. The diagnostic utilities of NPSs and OPSs were found to be equally effective. However, combining both sites yielded the greatest diagnostic sensitivity.

Reznikov et al. (82) compared NPAs to OPSs in children and found no significant difference in the detection of *M. pneumoniae* by PCR. They did note that NPAs were more likely to be rejected than OPSs because of PCR inhibitors or lack of respiratory epithelial material. Honda et al. (49) applied capillary PCR to sputum, BALs, and OPSs. Review of the differences in PCR positivity rates in function of the total number of specimens collected showed the highest rate of detection from OPSs (28.6%). However, there were some problems with proper collection of OPSs due to inadequate scraping of the mucosal surface, resulting in false-negative results due to the collection of an insufficient amount of DNA. OPSs therefore seem to be a valuable alternative.

On the other hand, when combining the results of two studies on LRTIs conducted by Loens et al. (61, 62), from 25 patients both an OPS and a sputum specimen were available for analysis by NAAT and culture. For both detection methods, sputa were the preferred specimens. Rätty et al. (79) collected sputum, an NPA and a OPS specimen from 32 young military conscripts with pneumonia during an *M. pneumoniae* outbreak and applied PCR and also concluded that sputum was the best sample to detect *M. pneumoniae*. Dorigo-Zetsma et al. also found that sputum was the specimen that was most likely to be PCR positive (62.5%, versus 41% for nasopharynx, 28% for OPSs, and 44% for throat washes (31). The superiority of sputum in these studies was in agreement with earlier studies when *M. pneumoniae* was detected by probe hybridization from sputum and OPSs (57) and by antigen detection from sputum and NPAs (56). The high diagnostic sensitivity of sputum PCR could be explained by the higher number of *M. pneumoniae* organisms in the pulmonary alveoli than on the epithelium of the URT, which has been demonstrated in experimentally infected hamsters by Brunner et al. (13) who quantified *M. pneumoniae* organisms in different parts of the respiratory tract by culture. In this model, the number of CFU from the lungs was 100 to 1,000 times higher than that from throat cultures. Collier and Clyde (22) recovered 10^2 to 10^7 CFU per ml from sputum specimens, while later studies estimated the number of CFU from OPSs to be 60 to 2,000 per ml (55) by culture and 20 to 3,830 CFU/ml by PCR (32).

Care should be taken when applying the NAAT to sputum samples since inhibitors in sputum occur frequently (63) and may be difficult to eliminate.

In conclusion, according to the state-of-the-art methodology, if a sputum sample is available, it might be the best specimen for *M. pneumoniae* detection by culture and NAATs. An NPS, NPA, or OPS might be the second-best option for analysis by NAATs.

***Chlamydomphila pneumoniae*.** The choice of the respiratory specimen may also have a major impact on the sensitivity of *C. pneumoniae* isolation and PCR. In a pneumonia treatment study with 260 previously healthy children aged 3 to 12 years, it was shown that the posterior nasopharynx may be superior to the throat as a source for isolation of the organism. Of 34 children from whom *C. pneumoniae* was isolated, NPSs were positive for all children, but OPSs were positive for only 50% of the same children (10). During a *C. pneumoniae* outbreak, Boman et al. (12), collected sputum, OPSs, and NPSs from 116 patients presenting with an RTI. When comparing the performances of PCR, culture, and antigen detection for samples from different locations in 61 patients for whom all three respiratory samples were available, 20 patients were considered to be infected by *C. pneumoniae*, for whom 7 NPSs, 10 OPSs, and 20 sputum samples were considered to be truly positive. The sensitivities of PCR, culture, and antigen detection by enzyme immunoassay (EIA) on NPSs were 35%, 30%, and 30%, respectively. The sensitivities of PCR, culture, and antigen detection by EIA on OPSs were 50%, 40%, and 25%, respectively. Finally, when looking at sputum, sensitivities of 95%, 100%, and 80% were found for PCR, culture, and antigen detection by EIA, respectively. The superiority of sputum for the detection of *C. pneumoniae* by PCR was confirmed by Kuoppa et al. (58), who examined a sputum sample, an NPA, and an OPS from 35 patients suspected of having a *C. pneumoniae* infection. *C. pneumoniae* DNA copies varied from 6.0×10^2 to 6.7×10^6 /ml. The majority of all samples had copy numbers below 1×10^4 /ml, but the greater part of the sputum samples contained large amounts of *C. pneumoniae* DNA, with an average of 8.6×10^5 copies/ml. However, these results are in contrast to those obtained by Verkooyen et al. (103), who examined sputum, NPS, OPS, and throat wash specimens by PCR and culture from 156 consecutive hospitalized CAP patients. The highest sensitivity in their study was obtained by applying PCR on NPSs. Surprisingly, none of the sputum samples tested became positive. These findings may indicate colonization of the organism in the URT rather than invasive infection of the LRT.

Gnarpe et al. (43) compared PCR results for *C. pneumoniae* from NPSs and OPSs in 66 patients presenting with pneumonia or URTIs. Of a total of 18 patients positive for *C. pneumoniae*, in 15 patients the OPS was the only positive specimen, whereas for 3 patients both the OPS and the NPS yielded a positive PCR result.

Gaydos et al. (40) reported 10 to >500 inclusion-forming units (IFU)/ml when isolating *C. pneumoniae* from NPSs using HEp-2 cells. This is in line with the 5 to 30 IFU/ml reported by Roblin et al. after isolation from fresh nasopharyngeal specimens from patients suspected of having a *C. pneumoniae* infection using HEp-2 cells, the optimal cell line (84).

In conclusion, sputum or an NPS may be the preferred specimen for detection of *C. pneumoniae* by NAATs.

***Legionella pneumophila*.** Sputum specimens are generally considered to be the best specimens for the isolation of *Legionella pneumophila* in patients with CA-LRTI caused by these organisms.

In a first very small study, OPSs were found to be suitable specimens for detection of *Legionella* by PCR (77). In a larger study of OPSs from 242 hospitalized CAP patients, Diederer

et al. questioned the suitability of OPSs as samples for *Legionella*-specific detection. Two different PCRs were applied, yielding a positive PCR result in only 3/11 confirmed cases of Legionnaires' disease (26).

Based on the available data, no anatomic site or method is clearly superior for optimal detection of *Legionella*. Combining test results from more than one site appears to improve the diagnostic accuracy.

Respiratory viruses. Antigens of the most common respiratory viruses such as influenza, respiratory syncytial virus (RSV), adenovirus, and parainfluenza viruses can be detected by DIF or by commercially available EIAs. The sensitivities of these tests vary from 50% to >90% (48, 92). Two of the main problems when evaluating different methods for the antigenic detection of respiratory viruses are the type of sample studied and the age of the patients. The majority of studies concerning EIA methods with the ability to detect simultaneously, although not differentially, the influenza A and B viruses have shown important variations in sensitivity according to the type of sample studied (24, 90). Thus, in the study by Schultze et al. (90) the optical immunoassay method (FLU OIA) (Biostar, Inc., Boulder, CO) displays overall sensitivities of 71.8% in pediatric samples and 51.4% in adult samples. Similarly, Covalciuc et al. (24) reported that, with the same method, the highest sensitivity is obtained with nasal aspirate (88.4%) and the lowest sensitivity is obtained with the OPS (62.1%). The sensitivity of the DIF test is lower in adults and older persons than in children (94).

Early studies have presented data favoring NPA specimens (38, 67) for respiratory virus isolation and detection in children. Frayha et al. (38) compared paired NPSs and NPAs from 125 children with URTI ($n = 32$) and/or LRTI ($n = 93$) for viral diagnosis by culture and indirect immunofluorescent assay (IFA) and found a higher isolation rate from NPAs.

Masters et al. (67) compared NPAs with NPSs from children with suspected bronchiolitis for the diagnosis of RSV. Irrespective of the detection method used, NPAs resulted in better recovery of RSV compared to NPSs. This is in line with results of Mackie et al. (64). NPAs tended to show a greater number of fluorescing cells per high-power field than pernasal swabs.

Three specimen collection methods involving NPSs, NSs, and NPAs used with 122 children (2) were also evaluated for the detection of influenza A and B viruses by a rapid antigen test in a pediatric emergency medicine setting. Influenza A and B viruses were detected in 85% of NPSs, 78% of NSs, and 69% of NPAs (3 to 5 ml). The relatively low sensitivity of the NPA compared to the swabs was surprising to the authors. A study published by Cazacu et al. (18) which examined NPA specimens collected in the emergency department of a large pediatric hospital supports the finding of Agoritsas et al. (2) on the sensitivity obtained with NPA specimens for rapid influenza virus detection. It is possible that the volume of saline used in NP wash collection procedures diluted out target influenza virus antigens for rapid immunobased methods, resulting in decreased sensitivity. This effect might not be seen in DFA or culture assays for influenza virus because specimens for fluorescent antibody assays are commonly centrifuged to concentrate cellular material and relatively large inocula are used in culture assays. This limitation can be overcome if the NPA material is centrifuged and the pellet is resuspended in a

smaller volume of saline (2). This finding may also be relevant to rapid antigen testing of swabs diluted by being placed in a volume of transport medium larger than is needed to perform a rapid test.

In a clinical study comparing influenza virus detection on four different specimens (NPSs, OPSs, nasal aspirates, and sputum) by two detection methods, sputum and nasal aspirates, respectively, were shown to be superior to NPSs and OPSs (24). The suitability of sputum for virus isolation was already described in the 1970s (50) when Horn et al. examined sputum, NSs, and OPSs from 22 children (5 to 15 years old) during 72 attacks of wheezy bronchitis. Twenty-nine (41%) isolates were obtained from 70 sputa, 15 (23%) from 64 NSs, and 14 (22%) from 64 OPSs.

To determine the role of the specimen type, Weinberg et al. analyzed the sensitivity and specificity of three immunoassay kits for rapid detection of influenza A and B viruses on NPS and NPA specimens and found sensitivities varying between 27 and 100% in NPSs and between 50 and 81% in NPAs (108). The authors investigated the differences in test characteristics according to age group as well and reported a trend toward a lower sensitivity in the older age group for all three tests. A limitation of this study is the unequal number of specimens tested by the different methods. However, the lower sensitivity of the rapid tests was confirmed in a study conducted by Ruest et al. (88) using NPAs from children and adults admitted to the hospital.

Reina et al. (81) divided the patients into two different groups, both for reasons of age (children and adults) and for the type of clinical specimen to be analyzed (NPA and OPS) when evaluating the Directigen Flu A+B test against cell vial culture. It seems obvious, once more, that the type of specimen and, therefore, the viral load present, is what probably determines the sensitivity of the different antigen detection methods against the majority of respiratory viruses (90).

Influenza virus real-time PCR and Directigen Flu A+B EIA were performed on NPSs, NPAs, and OPSs collected from residents of nursing homes with clinical suspicion of influenza during seven probable outbreaks (44). PCR detected influenza virus RNA in 80% (68/85) of specimens from 38/47 residents. NPSs were equally sensitive to NPAs by PCR, but the latter appeared impractical due to the common underlying disability of residents. Furthermore, the NPS PCR cycle threshold values were continuously 4.7 cycles lower than in paired OPSs, indicating a 10- to 100-fold-higher sensitivity of NPS. PCR and immunoassay sensitivity was highest on NPS, and positive results were rarely found in OPSs and NP washes. The higher detection rate of influenza A and B viruses in NPSs was confirmed when Smit et al. applied different tests on both an NPS and an OPS (93).

The superiority of NPA for the detection of respiratory viruses by PCR was illustrated in the study by Gruteke (46): the percentage of diagnosed episodes was 84% on NPA compared with 58% when only transnasal swabs or OPSs were available.

When analyzing paired NPAs and NSs for the presence of respiratory viruses by culture, immunofluorescence and multiplex PCR, Sung et al. (97) found a higher overall sensitivity of viral detection with NPA specimens, but when PCR was used, the sensitivity obtained with NPAs was significantly higher than that obtained with NSs only for RSV.

In conclusion, OPSs and NSs might be unreliable when used in rapid tests to detect outbreaks. Alternative sampling techniques could be studied to increase immunoassay sensitivity, including the use of nasopharyngeal and nasal flocculated swabs. When NAATs are to be applied for respiratory virus detection, NPAs, NPSs, or sputa seem to be suitable specimens both in children and in adults.

OTHER SPECIMENS

Urine for antigen testing. Currently, one test is often used to detect the pneumococcal cell wall polysaccharide common to all serotypes in urine: the *S. pneumoniae* urinary immunochromatographic membrane test (ICT). It can be performed on single specimens within 15 min using unconcentrated urine and has a sensitivity of 70 to 80% in adult bacteremic pneumonia (28) with a high specificity (>95%). Gutierrez et al. (47) performed the ICT on concentrated urine samples obtained from 452 adults with CAP. Pneumococcal antigen was detected in 19/27 (70%) of patients with documented pneumococcal pneumonia, in 69/269 (26%) of patients with no pathogen identified, and in 16/156 (10%) of samples from patients with CAP due to other causes, indicating a problem of specificity. Stralin et al. (96) found the urinary antigen (uAg) test to have a sensitivity of 79% and a specificity of 83%. The specificity of the test could be increased when weak-positive results were considered negative. In a comprehensive study of the value of the *S. pneumoniae* uAg test, Roson et al. (86), Van der Eerden et al. (101), and Ortega et al. (73) concluded that the test should be applied to specimens of severely ill adult patients for whom demonstrative results of a sputum Gram stain are unavailable.

The test lacks specificity in children as a result of the carrier state of pneumococci in the nasopharynx in this population (33).

uAg detection is currently the most helpful rapid test for the diagnosis of *Legionella* infection. It is recommended for patients with enigmatic pneumonia hospitalized in an intensive care unit, in the presence of an epidemic or failure to respond to a β -lactam antibiotic (111). Several test formats have been developed, the EIA format being more suited to test a larger number of specimens and taking a few hours to complete. The major limitation of uAg tests is that currently available tests are intended to detect *Legionella pneumophila* serogroup 1 antigen, which is the most common cause of *Legionella* infection. The other serogroups of *L. pneumophila*, however, or the other species of *Legionella* are not reliably detected by this test, although cross-reactions with these species also do occur (7). These tests are particularly useful since culture of *Legionella* spp. is slow and takes 3 to 4 days. *Legionella* uAg detection is frequently the first positive laboratory test in this infection.

The sensitivities of *Legionella* uAg tests vary between 55.5 and 66.6% in unconcentrated urine specimens and between 86.6 and 91.6% in concentrated urine specimens (29, 30). The assay may be negative in some patients during the first 5 days of the disease and remain positive for between 6 and 14 days (8). In a large outbreak of Legionnaires' disease in Holland, the antibiotic management of the patients could be guided by the results of the rapid uAg testing, reducing both mortality and need for intensive care (112). In this outbreak

for patients with mild Legionnaires' disease, test sensitivities range from only 40 to 53%, whereas for patients with severe Legionnaires' disease who needed immediate special medical care, the sensitivities were 88 to 100% (112). Additionally, Dirven et al. (27) showed that testing of a second urine sample collected 3 days later increased the sensitivity by 10%. Furthermore, it was shown that for patients with a negative uAg test result, deferring anti-*Legionella* therapy for 24 h may be justified since the outcome in these patients was not influenced (87) even if they were subsequently shown to have a *Legionella* infection. In a prospective study of sporadic CAP in adults, *Legionella* uAg detection also influenced the management of 7/9 patients (60).

In conclusion, the use of the rapid uAg tests reduces mortality and the need for intensive care and avoids unnecessary or inappropriate use of antibiotics in patients with CAP.

Serum samples for serology for respiratory infections. The serologic measurement of specific antibody responses has limited application for an etiologic diagnosis of LRTI, because diagnostic results are only available retrospectively.

Efforts were made to diagnose infections caused by slowly growing or difficult-to-grow organisms by serology. This holds particularly for *Mycoplasma pneumoniae*, *Chlamydia pneumoniae*, and *Legionella* infections and viruses. It should be remembered that the most reliable serologic evidence of an ongoing infection is based on a fourfold increase in titer of IgG (or IgG plus IgM) antibodies during the evolution of the disease episode based on two serum samples collected with an interval of 7 to 10 days or longer, and/or the appearance of IgM antibodies during the evolution of the disease. IgM tests are usually less sensitive and specific than fourfold changes in antibody titers between paired specimens separated by several weeks (92). Solitary high IgG titers have no diagnostic value (33). IgM antibodies against *M. pneumoniae* require up to 1 week to reach diagnostic titers and sometimes much longer (104).

Reported results for the sensitivity of *M. pneumoniae* serology are variable (5, 72, 74). The serologic responses to *Chlamydia* and *Legionella* species take even longer (45, 77).

The acute antibody test for *Legionella* in Legionnaires' disease is usually negative or demonstrates very low titers (76). As for other etiologies, the presence of high titers of IgG and/or IgM above a certain threshold present early during the disease has been interpreted as diagnostic (54), but at least one study showed that this titer had a positive predictive value of only 15% (76).

For *M. pneumoniae* and *C. pneumoniae*, a great number of antigen preparations have been proposed: whole organisms, protein fractions, glycoprotein fractions, and recombinant antigens. Some commercialized assays lack both sensitivity and specificity, emphasizing the need for more validation and quality control (5, 51). Furthermore, PCR has been proven to be more sensitive and specific than serology (72).

Serologic tests for the management of the individual patient with an LRTI are therefore not recommended. Serology for infections caused by *M. pneumoniae*, *C. pneumoniae*, and *Legionella* spp. is more useful in epidemiologic studies than in the routine management of the individual patient.

CONCLUSION

Studies on optimal specimens and transport for detection of pathogens causing CA-LRTIs have been hampered by the sample size, lack of matched controls, sample quality, and divergence of detection methods. Distinct differences were observed comparing different specimens for detection of respiratory agents based on conventional detection methods, such as culture and serology. However, with more sensitive detection methods, such as nucleic acid amplification methods, the differences in recovery rates between respiratory specimens might be more subtle. Clinicians, microbiologists and epidemiologists, collecting respiratory specimens for diagnosis of CA-LRTIs should be aware of the performance characteristics of these tests.

DG Research of the European Commission is funding GRACE (Genomics to Combat Resistance against Antibiotics in Community-Acquired LRTI in Europe), a large project on LRTI in the community. One of the goals is to enroll 3,000 LRTI patients and matched controls to study the etiology of LRTI in the community by applying conventional and NAATs to detect the causative organisms. GRACE is the largest study ever on CA-LRTI and should therefore help identifying the optimal microbiological method for diagnosis of CA-LRTI.

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