Detection of multiple respiratory pathogens during primary respiratory infection: nasal swab versus nasopharyngeal aspirate using real-time polymerase chain reaction

T. J. Meerhoff · M. L. Houben · F. E. J. Coenjaerts · J. L. L. Kimpen · R. W. Hofland · F. Schellevis · L. J. Bont

Eur J Clin Microbiol Infect Dis 2010.

Testing for Respiratory Viruses in Children
To Swab or Not to Swab

JAMA Ped 2017.

Peter J. Gill, MD, DPhil, MSc; Susan E. Richardson, MD, FRCP(C); Olivia Ostrow, MD; Jeremy N. Friedman, MB ChB, FRCP(C)

Comparing Nose-Throat Swabs and Nasopharyngeal Aspirates Collected From Children With Symptoms for Respiratory Virus Identification Using Real-Time Polymerase Chain Reaction

Ped 2008.

Stephen B. Lambert, MBBS[a,b], David M. Whiley, PhD[a,b], Nicholas T. O'Neill, BNurs[a], Emily C. Andrews, BNurs[a], Fiona M. Canavan, BNurs[a], Cheryl Bletchly, PhD[c], David J. Siebert, MBBS[c], Theo P. Sloots, PhD[a,b,c,d], Michael D. Nissen, MBBS[a,b,c,d]

Nasal Swab versus Nasopharyngeal Aspirate for Isolation of Respiratory Viruses

J Clin Microbiol 2002.

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Dx lab for respiratory viral infection

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Test	Specimen Type[1,2]	Time to Results[a]	Cost[b]	Benefits	Limitations
Viral culture	NP swab, bronchial or NP washing, nasal or ET aspirate, sputum	2-10 d	$$	• High specificity (100%, reference standard)[13] • Sensitivity (44%-85% vs composite gold standard)[13,c] • Viral confirmation (detects live virus not only viral nucleic acids)[14] • Public health surveillance (gold standard) • Can detect new viruses	• Little utility in clinical management
Rapid antigen direct tests (eg, EIA and IC-LF)	NP swab, nasal aspirate, or washing	10-30 min (TAT for results for nonbatched testing)	$	• Point of care test with rapid result • Limited targets (influenza, RSV, and adenovirus) • High specificity (approximately 96%)[15] • Poor sensitivity (approximately 70% influenza A/B, approximately 85% RSV vs culture)[15]	• Low sensitivity • Negative test less helpful • Moderate cost • Wide variation in diagnostic test properties by test type • Limited to viruses with specific known antigens (ie, RSV and influenza A/B)
Immunofluorescence DFA staining Indirect fluorescent antibody staining	NP swab, nasal or ET aspirate, bronchial washing	4-5 h (TAT for hands-on test performance) 5-24 h (TAT for results for tests batched daily)	$$	• High specificity (approximately 99%) • Sensitivity (approximately 81% overall for all viral targets vs culture, 50%-80% vs PCR)[15,16]	• Moderate sensitivity • Limited to viruses with specific monoclonal antibodies available (8 viruses detected) • Labor intensive • Subjective
NAATs Individual target PCRs Multiplex PCR Rapid point of care PCR	NP swab, bronchial or NP washing, nasal or ET aspirate, sputum	4-5 h (TAT for hands-on test performance) 5-24 h (TAT for results for tests batched daily) 15-30 min (TAT for results for non-batched testing)	$$-$$$	• High sensitivity (85%-100% for multiplex PCR vs composite gold standard);[13] positivity rate increases by 50%-70%[13] • High specificity (98%-100%)[13] • ≥20 viruses detected (multiplex) • High sensitivity (approximately 95% vs individual target PCR)[17,18] and specificity (100%),[17,18]limited targets (influenza A/B, RSV)	• Costly • Diagnostic test characteristics varies for each virus tested • Unable to distinguish viral nucleic acids from live virus
Serologic tests	Serum	Days to weeks	NA[d]	• Differentiates between acute (IgM) and prior (IgG) infection	• Limited availability • Requires acute and convalescent serum, consequent delay in definitive diagnosis • Little utility in clinical management

Gold standard:
Flu, RSV, PIV1-3, AdV, HMP: +2 DFA or culture or 4 PCR
PIV4, Bocavirus, CoV, Enterovirus: +2 of 4 PCR

JAMA Ped 2017

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Type of molecular technology

- **Specific target gene**
  - **Bacteria:**
    16 S rDNA gene vs specific gene of that bacteria
  - **Fungus:**
    ITS of rDNA gene vs specific gene of that bacteria
- **Type of PCR:**
  Real time VS conventional, commercial VS in-house, qualitative VS quantitative, isothermal VS nonisothermal nested VS multiplex, allele-specific VS nonallele-specific Touchdown, assembly, colony, methylation-specific, loop -mediated isothermal amplification (LAMP)

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Multiplexed Molecular Diagnostics for Respiratory, Gastrointestinal, and Central Nervous System Infections

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	Test System					
Characteristic	BD MAX	FilmArray	eSensor	Prodesse	Verigene	Luminex
Method	Real-time PCR	Nested PCR with melt curve analysis	PCR with electrochemical detection	Real-time PCR	PCR with low-density nucleotide array	PCR with liquid phase bead array
Degree of multiplexing	4 targets	14–22 targets	13 targets	3–4 targets	1–16 targets	9–20 targets
Panels	GI	Respiratory, GI CNS	Respiratory	Respiratory, GI	Respiratory, GI	Respiratory, GI CNS
Testing location	Clinical laboratory	Near patient facility or clinical Laboratory	Clinical laboratory	Clinical laboratory	Near patient facility or clinical laboratory	Clinical laboratory
Complexity	Moderate	Moderate	High	High	Moderate	High
automation	Full	Full	Partial	Partial	Full	Partial
throughput	Low-medium	Low-medium	Medium	Medium	Low	Medium-high
Time to results	~3 h	~1 h	~6 h	3–4 h	~2 h	~5–8 h

US FDA-approved syndromic panels for multiple pathogens.

Clin Infect Dis 2016

US FDA-approved respiratory panels

Pathogens	FilmArray	eSensor	Verigene	Luminex xTAG		
				RVP	RVP Fast	NxTAG
Viral						
Adenovirus	•	•	•	•	•	•
Coronavirus HKU1	•					•
Coronavirus NL63	•					•
Coronavirus 229E	•					•
Coronavirus OC43	•					•
Human bocavirus						•
Human metapneumovirus	•	•	•	•	•	•
Influenza A	•	•	•	•	•	•
Subtype H1	•	•	•	•	•	•
Subtype H3	•	•	•	•	•	•
Subtype 2009 H1N1	•	•				
Influenza B	•	•	•	•	•	•
Parainfluenza 1	•	•	•	•		•
Parainfluenza 2	•	•	•	•		•
Parainfluenza 3	•	•	•	•		•
Parainfluenza 4	•		•			•
Respiratory syncytial virus	•				•	•
Respiratory syncytial virus A		•	•	•		•
Respiratory syncytial virus B		•	•	•		•
Rhinovirus/enterovirus		•	•	•	•	•
Bacteria						
Chlamydia pneumoniae	•					•
Mycoplasma pneumoniae	•					•
Bordetella pertussis	•		•			
Bordetella parapertussis/Bordetella bronchiseptica			•			
Bordetella holmesii			•			

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US FDA-approved GI panels

Pathogens	FilmArray	Verigene	Luminex	BDMax	Prodesse
Bacterial					
Campylobacter	•	•	•	•	•
Salmonella	•	•	•	•	•
Shigella	•	•	•	•	•
Shiga-like toxin 1 and 2	•	•[a]	•	•	•
Enterotoxigenic *Escherichia coli*	•		•		
Enteropathogenic *E. coli*	•				
Enteroaggregative *E. coli*	•				
E. coli O157	•		•		
Vibrio	•	•			
Yersinia enterocolitica	•	•			
Plesiomonas shigelloides	•				
Clostridium difficile	•		•		
Viral					
Norovirus GI and GII	•	•	•		
Adenovirus 40/41	•		•		
Rotavirus	•	•	•		
Astrovirus	•				
Sapovirus	•				
Parasitic					
Giardia	•		•	•	
Cryptosporidium	•		•	•	
Cyclospora cayetanensis	•				
Entamoeba histolytica	•		•	•	

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Susceptibility testing

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Clinical and Laboratory Standards Institute (CLSI), the US

European Committee on Antimicrobial Susceptibility Testing (EUCAST), Europe

Both organizations recommend

Phenotypic resistance testing

Conventional tests: zone diameter & MIC breakpoints

Commercial tests:

Etest, MicroScan, Phoenix, Sensititre, Etest, MicroScan,

Phoenix, Sensititre, Vitek Legacy, & Vitek 2 systems

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Test/Report Group	Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm				Interpretive Categories and MIC Breakpoints, µg/mL				Comments
			S	SDD	I	R	S	SDD	I	R	
PENICILLINS											
A	Ampicillin	10 µg	≥17	–	14–16	≤13	≤8	–	16	≥32	(4) Results of ampicillin testing can be used to predict results for amoxicillin. See general comment (2).
O	Piperacillin	100 µg	≥21	–	18–20	≤17	≤16	–	32–64	≥128	
O	Mecillinam	10 µg	≥15	–	12–14	≤11	≤8	–	16	≥32	(5) For testing and reporting of *E. coli* urinary tract isolates only.
β-LACTAM COMBINATION AGENTS											
B	Amoxicillin-clavulanate	20/10 µg	≥18	–	14–17	≤13	≤8/4	–	16/8	≥32/16	
B	Ampicillin-sulbactam	10/10 µg	≥15	–	12–14	≤11	≤8/4	–	16/8	≥32/16	
B	Ceftolozane-tazobactam	30/10 µg	≥21	–	18–20	≤17	≤2/4	–	4/4	≥8/4	(6) Breakpoints are based on a dosage regimen of 1.5 g every 8 h.
B	Ceftazidime-avibactam	30/20 µg	≥21	–	–	≤20	≤8/4	–	–	≥16/4	(7) Breakpoints are based on a dosage regimen of 2.5 g (2 g ceftazidime+0.5 g avibactam) every 8 h over 2 days.
B	Piperacillin-tazobactam	100/10 µg	≥21	–	18–20	≤17	≤16/4	–	32/4–64/4	≥128/4	
O	Ticarcillin-clavulanate	75/10 µg	≥20	–	15–19	≤14	≤16/2	–	32/2–64/2	≥128/2	

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Table 3A. Tests for Extended-Spectrum β-Lactamases in *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Escherichia coli*, and *Proteus mirabilis*

NOTE: Following evaluation of PK-PD properties, limited clinical data, and MIC distributions, revised breakpoints for cefazolin, cefotaxime, ceftazidime, ceftizoxime, ceftriaxone, and aztreonam were published in January 2010 (M100-S20) and are listed in Table 2A. Cefuroxime (parenteral) was also evaluated; however, no change in breakpoints was necessary with the dosage. When using the current breakpoints, routine ESBL testing is no longer necessary before reporting results (ie, it is no longer necessary to edit results for cephalosporins, aztreonam, or penicillins to resistant). However, ESBL testing may still be useful for epidemiological or infection control purposes. For laboratories that have not implemented the current breakpoints, ESBL testing should be performed as described in this table.

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Prediction of Susceptibility to First-Line Tuberculosis Drugs by DNA Sequencing

The CRyPTIC Consortium and the 100,000 Genomes Project

RESULTS

A total of 10,209 isolates were analyzed. The largest proportion of phenotypes was predicted for rifampin (9660 [95.4%] of 10,130) and the smallest was predicted for ethambutol (8794 [89.8%] of 9794). Resistance to isoniazid, rifampin, ethambutol, and pyrazinamide was correctly predicted with 97.1%, 97.5%, 94.6%, and 91.3% sensitivity, respectively, and susceptibility to these drugs was correctly predicted with 99.0%, 98.8%, 93.6%, and 96.8% specificity. Of the 7516 isolates with complete phenotypic drug-susceptibility profiles, 5865 (78.0%) had complete genotypic predictions, among which 5250 profiles (89.5%) were correctly predicted. Among the 4037 phenotypic profiles that were predicted to be pansusceptible, 3952 (97.9%) were correctly predicted.

CONCLUSIONS

Genotypic predictions of the susceptibility of *M. tuberculosis* to first-line drugs were found to be correlated with phenotypic susceptibility to these drugs. (Funded by the Bill and Melinda Gates Foundation and others.)

Fungus and virus

Genotypic resistance tests

- Limited availability
- Costly
- Labor-intensive
- Wide variation in diagnostic criteria

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Wrap-up

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Conclusion in diagnosis

- **Too high sensitivity:**
  Not true causative pathogen, asymptomatic carriage
  Pathogen of past infection, not current infection
- **Low sensitivity in some IDs:**
  CDI
  Encephalitis caused by AdeV, JEV, arbovirus
  Lyme disease
- **Variable sensitivity & specificity in specimen & virus, type/gene target of PCR:**
  Respiratory & stool speciemens
  Each kind of virus
- **Cultures as gold standard:**
  Bacteria & fungus

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Beyes' theorem

- Please don't send the test if no pretest Dx is made
- Pretest Dx is up to good clinical approach

Test performance

- Due to high sensitivity, the innocent bystander will be detected which is not the true causative agent
- Due to low sensitivity in some IDs, clinician must not send the test
- Due to variable sensitivity & specificity, clinician must know the contributing factors
- Most IDs: conventional culture's still gold standard

Which side are you regarding molecular microbiology utilization?

A. Pro

B. Con