Molecular Diagnostics: The Benefits, Costs, and Reliability of Rapid Infection Detection

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Infectious Diseases
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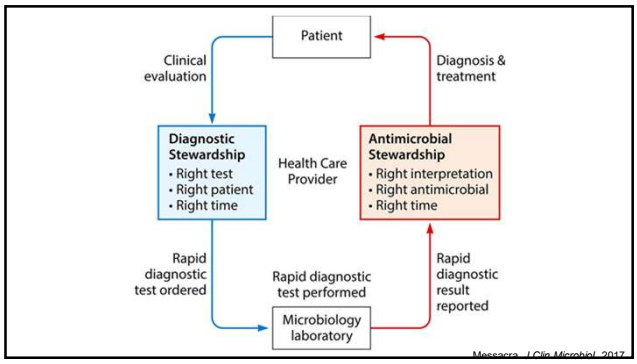
No disclosures

Just because you can does not necessarily mean you should...



Messacra, *J Clin Microbiol*, 2017

Outline

- Rapid Diagnostics
  - History
  - Molecular testing
    - Multiplex PCR
- Stool pathogen panel
  - *C diff* testing
- CSF pathogen panel
- Respiratory pathogen panel

1917 Pneumococcal polysaccharide by immunoassay in serum and plasma

1953 Structure of DNA

1960 Radioimmunoassay (RIA)

1971 Enzyme-linked immunoassay (ELISA)

→ Lateral flow Immunoassay

1985 Polymerase chain reaction (PCR)

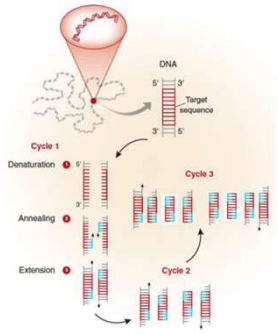
1988 Multiplex PCR

Diagnostics in Infectious Diseases

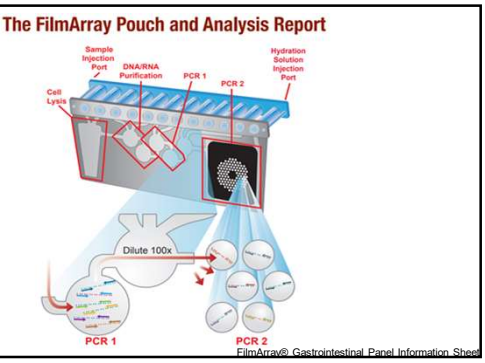
- Phenotype based
  - Culture & Biochem
  - Immuno-assays
- Genotype based
  - Molecular
    - NAAT
      - PCR

NAAT = nucleic acid amplification test
PCR = polymerase chain reaction

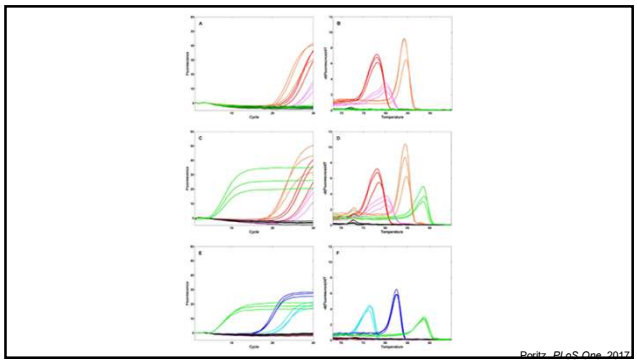
Weile, *Anal Bioanal Chem*, 2009



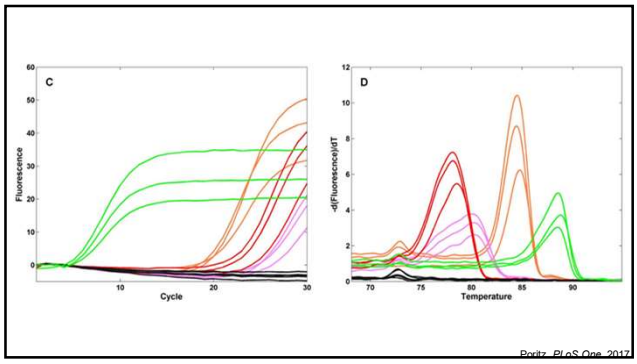
Garibyan, *J Invest Dermatol*, 2013



FilmArray® Gastrointestinal Panel Information Sheet



Poritz, PLoS One, 2017



Poritz, PLoS One, 2017

Hospital	Stool	CSF	Respiratory	Blood
Providence	---	✔	✔	TBD
Regional	---	---	✔	---
Central Peninsula	✔	---	✔	✔
South Peninsula	✔	✔	✔	✔

"In seeking 'laboratory improvement' there is a great danger of establishing exhaustive microbiology as an end in itself"

- Raymond C. Bartlett

Schreckenberger, *J Clin Microbiol*, 2015

42 year old male with no past medical history presents to your clinic with one day of diarrhea, nausea and vomiting. He was at a work event two nights ago where he enjoyed deli meats, different varieties of cheese and raw oysters...

Bacteria	Viruses & Parasites
Campylobacter spp	Adenovirus
C. difficile	Astrovirus
Plesiomonas shigelloides	Norovirus
Salmonella spp	Rotavirus
Vibrio spp	Sapovirus
V. cholerae	
Yersinia enterocolitica	*Cryptosporidium* spp
EAEC, EPEC, ETEC,	*C. cayetanensis*
STEC	*E. histolytica*
E. coli 0157	*G. lamblia*
Shigella spp/EIEC	

EAEC, Enteroaggregative *E. coli.* EPEC, Enteropathogenic *E. coli.* ETEC, Enterotoxigenic *E. coli.* STEC, Shiga-like toxin-producing *E.coli.* EIEC, Enteroinvasive *E.coli.*

- 1556 samples, from May - Sept
  - 62% peds, 38% age ≥18
  - 87% outpt, 11% inpt, 3% ED
- Positivity rate 54%
  - 832 single organism detections
  - 724 multiple organisms
- EPEC, *C. difficile*, EAEC

Buss. *J Clin Microbiol*. 2015

TABLE 5 Performance summary and characteristics of the FilmArray GI Panel versus those of comparator assays (stool culture or PCR and sequencing)

Analyte	No. of detections[a]		Sensitivity/PPA[b]			Specificity/NPA[b]		
	C	FA	TP/(TP+FN)	%	95% CI (%)	TN/(TN+FP)	%	95% CI (%)
Campylobacter spp.	35	58	34/35	97.1	85.1–99.9	1,497/1,521	98.4	97.7–99.0
C. difficile	165	204	163/165	98.8	95.7–99.9	1,350/1,391	97.1	96.0–97.9
P. shigelloides	3	18	3/3	100	29.2–100	1,538/1,553	99.0	98.4–99.5
Salmonella spp.	31	37	31/31	100	88.8–100	1,519/1,525	99.6	99.1–99.9
Vibrio spp.	0	2	0/0			1,554/1,556	99.9	99.5–100
V. cholerae	0	1	0/0			1,555/1,556	99.9	99.6–100
Y. enterocolitica	1	1	1/1	100	NA[c]	1,555/1,555	100	99.8–100
EAEC	83	109	82/83	98.8	93.5–100	1,446/1,473	98.2	97.3–98.8
EPEC	317	348	314/317	99.1	97.3–99.8	1,167/1,201	97.2	96.1–98.0
ETEC	22	31	22/22	100	84.6–100	1,525/1,534	99.4	98.9–99.7
STEC	33	38	33/33	100	89.4–100	1,518/1,523	99.7	99.2–99.9
E. coli O157	3	4	3/3	100	29.2–100	34/35	97.1	85.1–99.9
Shigella spp./EIEC (culture)[d]	49 (31)	49	47/49	95.9	86.0–99.5	1,505/1,507	99.9	99.5–100
Cryptosporidium spp.	18	24	18/18	100	81.5–100	1,532/1,538	99.6	99.2–99.9
C. cayetanensis	19	19	19/19	100	82.4–100	1,537/1,537	100	99.8–100
E. histolytica	0	0	0/0			1,556/1,556	100	99.8–100
G. lamblia	20	27	20/20	100	83.2–100	1,529/1,536	99.5	99.1–99.8
Adenovirus F 40/41	44	55	42/44	95.5	84.5–99.4	1,499/1,512	99.1	98.5–99.5
Astrovirus	7	8	7/7	100	59.0–100	1,548/1,549	99.9	99.6–100
Norovirus GI/GII	55	70	52/55	94.5	84.9–98.9	1,483/1,501	98.8	98.1–99.3
Rotavirus A	6	18	6/6	100	54.1–100	1,538/1,550	99.2	98.7–99.6
Sapovirus	46	59	46/46	100	92.3–100	1,497/1,510	99.1	98.5–99.5

FA = Filmarray®, C = Comparator method

Buss. *J Clin Microbiol*. 2015

- Sensitivity/Positive Percent Agreement (PPA) 100% for 12 targets
  - *Y. enterocolitica* - only 1 detection
- ≥94.5% for 7 others
- Not calculated for *E. histolytica*, *Vibrio* spp and *V. cholera*

Buss. *J Clin Microbiol*. 2015

- Outpatient and ED
- Returning traveler
  - Immunocompromised hosts
  - Severely ill
- Bloody diarrhea - STEC, *E. coli* O157
- Patients with protracted diarrhea

What about *C diff* ???

Table 1. Summary of laboratory tests for *C difficile*

Laboratory Test	Sensitivity	Specificity	Time to results	Comment
ELISA for Antigen	High	Low	Rapid	Cannot distinguish between toxigenic and non-toxigenic strains
ELISA for Toxin	Low	High	Rapid	Easy to perform; poor sensitivity
PCR for toxigenic genes	High	High	Rapid	Cannot distinguish between active infection and asymptomatic carriage; more expensive than ELISA
Cytotoxin Assay	High	High	Slow (24-48hrs)	Gold standard; requires tissue culture facility

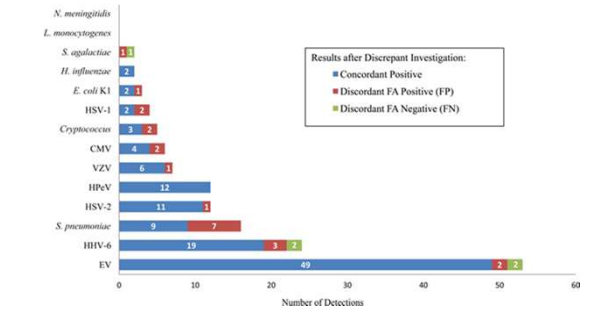
Solomon, *OFID*, 2014

20 year old previously healthy female presents to the ED with cough, fever and headache. Lumbar puncture is performed: WBC 5 cells/µl (98% lymphs), RBC 8 cells/µl, glucose 50 mg/dl and protein 45 mg/dl. Multiplex CSF PCR is positive for HHV-6...

Bacteria	Viruses
Escherichia coli	Enterovirus (EV)
Haemophilus influenzae	VZV
Listeria monocytogenes	HSV-1
Neisseria meningitidis	HSV-2
Streptococcus agalactiae (GBS)	CMV
Streptococcus pneumoniae	HHV-6
	Human parechovirus (HPeV)
Yeast	
Cryptococcus neoformans/gattii	

- 1560 prospectively collected samples
  - 136 (8.7%) positive
    - 5% in the adult population
- SEN/PPA 100% for 9/14, SPE/NPA >99.2%
- Enterovirus and HHV6 accounted for 54% of positive samples
- High number of false positives for *Cryptococcus* and *S. pneumoniae*

Leber, *J Clin Microbiol*, 2016

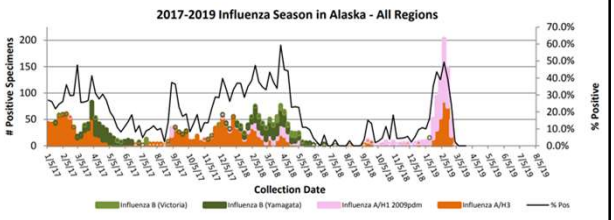


FA = Filmarray®, C = Comparator method

Leber, *J Clin Microbiol*, 2016

- If LP was done after antibiotics
- Suspect viral entity
- Immunocompromised hosts

72 year old man with history of COPD presents to ED in mid February with worsening shortness of breath. He did not get his influenza vaccine. Respiratory rate is 30 breaths/min and O_2 sat 86%, he becomes progressively more confused and requires intubation. CXR shows bilateral atypical pneumonia…



Parker, ASVL, 2019

Viruses	
Adenovirus	Human Rhinovirus/Enterovirus
Coronavirus HKU1, NL63, 229E, OC43	Influenza A, A/H1, A/H1-2009, A/H3, B
Human Metapneumovirus	Parainfluenza 1-4
Respiratory Syncytial Virus	Mers-CoV*
Bacteria	
Bordetella pertussis	*Chlamydophila pneumoniae*
Mycoplasma pneumoniae	*Bordetella parapertussis**

*Biofire® Filmarray® Respiratory Panel 2 *plus* only

TABLE 1. Comparison of positive and negative results by FilmArray RP and Prodesse assays[a] for viruses detectable by both assays

Virus	No. of specimens with results:				Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Kappa (95% CI)
	PRO$^-$/FA$^-$	PRO$^+$/FA$^+$	PRO$^+$/FA$^-$	PRO$^-$/FA$^+$					
Adenovirus	181	6	5	0	54.5	100	100	97.3	0.69 (0.43–0.96)
Influenza A virus H1-2009	191	1	0	0	100	100	100	100	1
Influenza B virus	191	1	0	0	100	100	100	100	1
Metapneumovirus	180	12	0	0	100	100	100	100	1
Parainfluenza virus 1	187	3	0	2	100	98.9	60.0	100	0.75 (0.39–1)
Parainfluenza virus 3	179	10	0	3	100	98.4	76.9	100	0.86 (0.71–1)
RSV	181	10	0	1	100	99.5	90.9	100	0.95 (0.85–1)

[a] Prodesse assays were considered the gold standard for comparison. Viruses targeted by both FilmArray RP and Prodesse assays, but not detected during this study, were as follows: influenza A H1 seasonal virus, influenza A H3 seasonal virus, and parainfluenza virus 2. PRO, Prodesse; FA, FilmArray RP; PPV, positive predictive value; NPV, negative predictive value.

Loeffelholz, *J Clin Microbiol*, 2011

TABLE 1. Viruses detected by FilmArray RP, xTAG RVP, and standard culture/antigen

Virus	No. detected by:		
	Culture/antigen (n = 185)[a]	FilmArray RP (n = 200)[b]	xTAG RVP (n = 200)[c]
Influenza A virus	32	32	33
Influenza B virus	7	7	7
RSV	36	45	37
Rhinovirus/enterovirus	6	43	41
Parainfluenza virus	14	16	15
Adenovirus	11	10	10
Metapneumovirus		7	6
None (negative)	82	62	68
Total no. of viruses	106	160	149

[a] Culture, n = 180; influenza virus antigen, n = 3; RSV antigen, n = 2.
[b] $P < 0.00001$ (chi-square test) for culture versus FilmArray RP.
[c] $P < 0.00001$ (chi-square test) for culture versus xTAG RVP.

Rand, *J Clin Microbiol*, 2011

- Be aware of what rapid testing is available at your facility

Antimicrobial and diagnostic stewardship:

- How will the test change your treatment?
- Consider costs
- Understand the limitations of these tests
  - PCR detects both alive and dead organisms
  - Be aware of specific test characteristics

Test	PAMC	AKH	CPH	SPH
Influenza A & B	✔	✔	✔	✔
C. diff	✔	✔	✔	✔
MRSA (nasal)	✔	✔	✔	---
GBS (vag/rectal)	✔	✔	---	✔
CT/GC	✔	✔	✔	---
MTB-Rif	✔	---	✔	---
HSV 1,2 (mucous)	✔	---	---	✔

Gram Positive Bacterial	Gram Negative Bacteria
Enterococcus	*Acinetobacter baumannii*
Listeria monocytogenes	*Haemophilus influenzae*
Staphylococcus spp	*Neisseria meningitidis*
Staphylococcus aureus	*Pseudomonas aeruginosa*
Streptococcus spp	*Enterobacteriaceae*
Streptococcus agalactiae	*Enterobacter cloacae* complex
Streptococcus pneumoniae	*Escherichia coli*
Streptococcus pyogenes	*Klebsiella oxytoca*
	Klebsiella pneumoniae
	Proteus
	Serratia marcescens

Resistance Genes	Yeast
mecA - methicillin resistance	*Candida albicans*
van A/B - vancomycin resistance	*Candida glabrata*
KPC - carbapenem resistance	*Candida krusei*
	Candida parapsilosis
	Candida tropicalis

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Recommended Reading

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