Syndromic Multiplex Panels – What's New, How Will We Use Them and What is the Impact on the Microbiology Laboratory

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Outline

- How changes in laboratory organization and POC will change microbiology test menus
- Respiratory Syndromic (or everything but the kitchen sink) Panels
- GI Panels and outcome studies
- Meningitis Panels
- Future Panels

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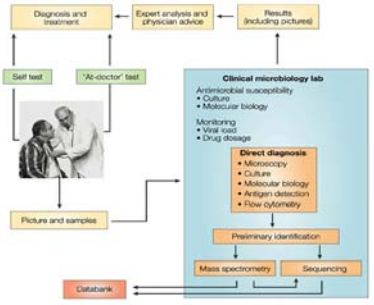
The future organization of clinical microbiology services – a Paradigm Shift



The Future of Molecular Biology

- Migration away from singleplex PCR to disease state testing
  - Eg. stool pathogen panels, sepsis panels, pneumonia panels
- Moving testing closer to patient
- Increased competition based on menu
  - Menu will be king, less capital for boxes
- Increased competition based on price
- Increased need for clinical data supporting use of molecular tests
- Movement to FDA approved kits

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Performance of POC Molecular Influenza Tests

- Binnicker et al., JCM 2015
  - Compared Liat (Roche, Indianapolis, IN) to laboratory based Simplexa (Focus, Chantilly, VA)
  - Enrolled 197 respiratory swabs
  - Found Liat was 99.2% and 100% sensitive for Flu A and B, respectively
  - Found Liat was 100% specific compared to Simplexa

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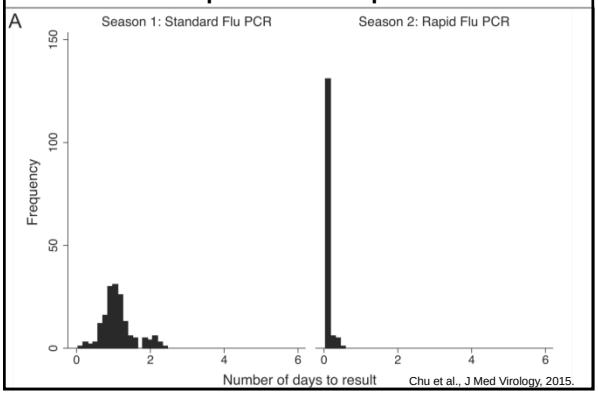
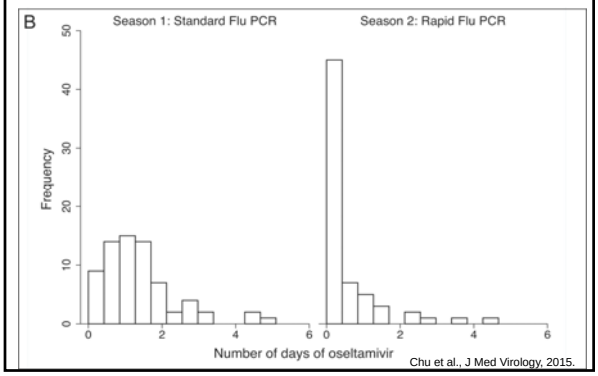
The Impact of Rapid PCR



Chu et al., J Med Virology, 2015.

The Impact of Rapid PCR



Chu et al., J Med Virology, 2015.

Respiratory Panels

Use of Large Multiplex Respiratory Panels

Point

- Influenza accounts for a minority of viral respiratory infections
- May contribute to increased patient satisfaction
- May contribute to better laboratory stewardship
- Identify outbreaks of resp viruses earlier

Counterpoint

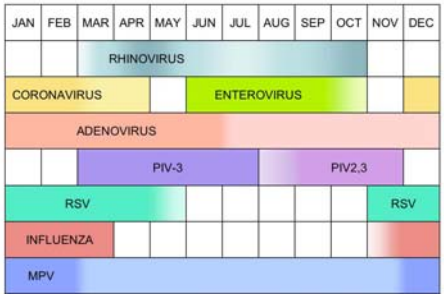
- Panels need to be linked to clinical syndromes
- Most panels are fixed design and do not allow reporting flexibility
- Testing for pathogens should be risk-guided
- When you hear hoof-beats, think horses, not zebras – ie common things are common
- Effects on cost are not clear
- Sensitivity of panels may be inferior to single-plex assays

Schreckenberger and McAdam, 2015. JCM: 53(10)

Respiratory Tract Infections: Diagnostic Challenge

- The Problem
  - Respiratory tract most common site of infection
  - Symptoms alone are not sufficient for clinicians to determine optimal patient management
    - True infection? Bacterial? Viral?
  - Wide array of know respiratory pathogens
  - Flu/RSV remain most common pathogens ordered/detected
  - Other pathogens requested:
    - Human Metapnuemovirus, Parainfluenza, Adenovirus, Rhinovirus, *B. pertussis*

Respiratory Tract Infections: Diagnostic Challenge



Respiratory Tract Infections: Diagnostic Challenge

- Lab: Important considerations for diagnostic(s) choice
  - Sensitivity
    - DFA, RIDTs don't provide desired sensitivity
  - Cost to laboratory
    - Expensive to purchase multiple molecular platforms
    - Expensive to send out samples to reference lab for testing
  - Cost to patient
    - When physicians order flu testing, costs more to run a broad RVP
    - Patients may be under insured or have no insurance
  - Burden on Lab
    - QC testing, proficiency testing, tech training
    - Reporting of positive diagnostic results for testing not requested by clinician

Details for the four FDA-cleared respiratory panels

Assay[a]	Manufacturer	Methodology	Preextraction required	Viruses reported[b]
FilmArray RP[c]	BioFire Diagnostics	Endpoint melt curve analysis	No	AdV; CoV HKU1, NL63; influenza virus A (H1/2009, H1, H3); influenza virus B; MPV; PIV1, -2, -3, -4; RSV; RhV/EV
eSensor RVP	GenMark Dx	Voltammetry	Yes	AdV (C, B/E); influenza virus A (H1/2009, H1, H3); influenza virus B; MPV; PIV1, -2, -3; RSV (A/B); RhV
xTAG RVPv1	Luminex Molecular Diagnostics	Fluorescence-labeled bead array	Yes	AdV; influenza virus A (H1, H3); influenza virus B; MPV; PIV1, -2, -3; RSV (A/B); RhV/EV
xTAG RVP fast	Luminex Molecular Diagnostics	Fluorescence-labeled bead array	Yes	AdV; influenza virus A (H1, H3); influenza virus B; MPV; RSV; RhV/EV

Popowitch, et al. JCM 2013

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Comparison of the Biofire FilmArray RP, Genmark eSensor RVP, Luminex xTAG RVPv1, and Luminex xTAG RVP Fast Multiplex Assays for Detection of Respiratory Viruses

Virus	No. of true-positive specimens (n = 300 specimens tested)	% Sensitivity (95% CI) of:			
		FilmArray RP	eSensor RVP	xTAG RVPv1	xTAG RVP fast
AdV	35	57.1 (40.8, 72.0)	100 (88.2, 100)	74.3 (57.8, 86.0)	82.9 (66.9, 92.3)
Influenza virus					
A	30	86.2[c] (68.8, 95.1)	100 (86.5, 100)	100 (86.5, 100)	86.7 (69.7, 95.3)
A H1/09	16	73.3[c] (47.6, 89.5)	100 (77.3, 100)	100 (77.3, 100)	81.3 (56.2, 94.2)
A H3	14	100 (74.9, 100)	100 (74.9, 100)	92.9 (66.5, 99.9)	78.6 (51.7, 93.2)
B	22	77.3 (56.2, 90.3)	100 (82.5, 100)	95.5 (76.5, 99.9)	45.5 (26.9, 65.4)
MPV	26	96.2 (79.6, 99.9)	100 (84.8, 100)	100 (84.8, 100)	100 (84.8, 100)
PIV					
1	14	100 (74.9, 100)	100 (74.9, 100)	100 (74.9, 100)	NA[c]
2	13	92.3 (64.6, 99.9)	100 (73.4, 100)	100 (73.4, 100)	NA
3	13	100 (73.4, 100)	100 (73.4, 100)	100 (73.4, 100)	NA
RSV					
A	22	86.4 (65.8, 96.1)	100 (82.5, 100)	86.4 (65.8, 96.1)	86.4 (65.8, 96.1)
B	14	100 (74.9, 100)	100 (74.9, 100)	92.9 (66.5, 99.9)	85.7 (58.8, 97.2)
RhV/EV	43	83.7 (69.7, 92.2)	90.7 (77.8, 96.9)	93.0 (80.7, 98.3)	93.0 (80.7, 98.3)

Popowitch, et al. JCM 2013

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The Problem with multiplex RVPs

- Physician ordering preference is based on clinical presentation and local/seasonal epidemiological considerations
- Current multiplex products contain fixed panels at fixed costs – no flexibility
- No single test covers the continuum of respiratory multiplexing

BD Veritor System, QUIDEL, Nanosphere, GenMark Dx, QuickVue, Roche, Alere, GEN-PROBE, BIOFIRE, Cepheid, FOCUS, Luminex, HOLOGIC, Cepheid

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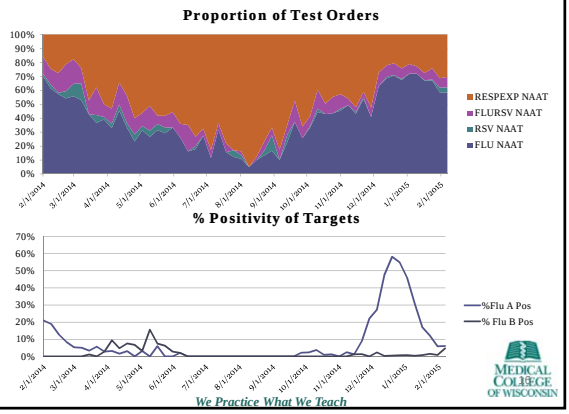

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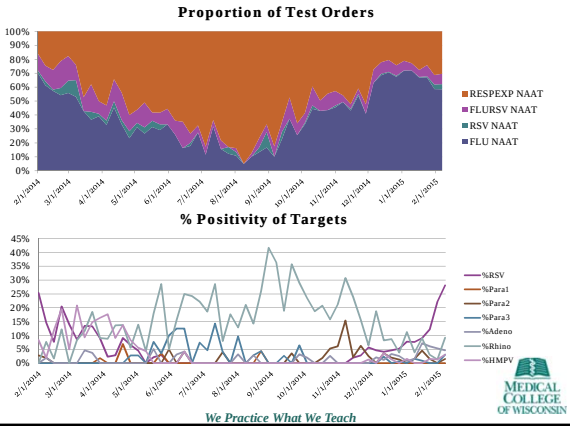

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Current respiratory Algorithm

- Benefits
  - Able to offer 4 orderable viral respiratory panels to clinicians
    - Limit unnecessary testing (test stewardship)
  - Minimize unnecessary cost to patient
- Limitations
  - Complicates ordering **->** Multiple LIS mnemonics
  - Costly to purchase "multiple boxes"
    - Multiple reagents **->** Inventory control, rapid scale-up
    - Maintain staff proficiency on multiple systems
    - QC and instrument comparability (COM.04250)
    - Space
  - $$$

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Gastrointestinal Assays

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Enteric pathogen "panels"

➢ Potential Benefits

✓ Higher sensitivity for detection/identification of enteric pathogens

✓ More rapid TAT

➢ Considerations

✓ Cost of molecular testing

✓ Technologist expertise
- Test complexity

✓ Level of automation
- Sample – Result? Off line extractions or PCR
- Volume!!!!

✓ Breadth of targets
- All inclusive (viral, parasitic, bacterial, toxin)
- Targeted (common causes of CA enteritis)

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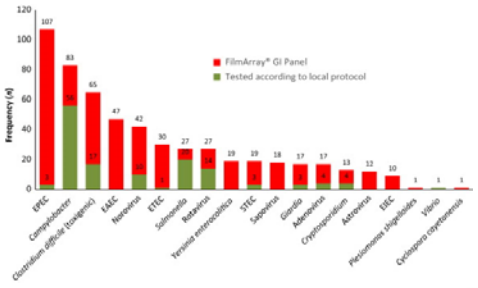
Frequency distribution of pathogens detected in 709 stool samples by diagnostic approach employed



Spina A. et al. CMI. 21(8): 719-728

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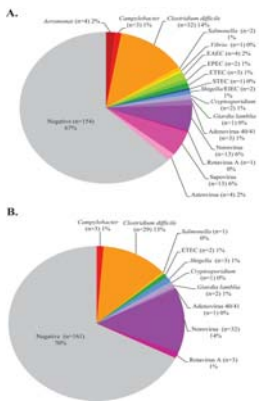

Distribution of pathogens detected by FilmArray (A) and Luminex (B) multiplex assays using prospective clinical specimens (n = 230)

Reeti Khare et al. J. Clin. Microbiol. 2014;52:3667-3673

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Performance

Target	FilmArray				Luminex				FilmArray		Luminex	
	TN	TP	FP	FN	TN	TP	FP	FN	Sensitivity (% [95% CI])	Specificity (% [95% CI])	Sensitivity (% [95% CI])	Specificity (% [95% CI])
Aeromonas spp.	248	5	0	16					23.8 (10.2, 47.1)	100 (98.2, 100)		
Campylobacter spp.	240	28	1	1	241	23	0	6	96.6 (81.4, 99.9)	99.6 (97.4, 100)	79.3 (61.3, 90.5)	100 (98.1, 100)
Clostridium difficile	229	33	5	3	229	33	4	3	91.7 (77.4, 97.9)	97.9 (95.0, 99.2)	91.7 (77.4, 97.9)	98.3 (95.5, 99.5)
Plesiomonas shigelloides	265	2	0	0					100 (29, 100)	100 (98.3, 100)		
Salmonella spp.	246	24	0	0	246	20	0	4	100 (83.7, 100)	100 (98.1, 100)	83.3 (63.5, 93.9)	100 (98.1, 100)
Yersinia enterocolitica	242	27	1	0	243	13	0	14	100 (85.2, 100)	99.6 (97.5, 100)	48.1 (30.7, 66)	100 (98.1, 100)
Vibrio spp.	270	0	0	0					ND	ND		
Vibrio cholerae	270	0	0	0	270	0	0	0	ND	ND	ND	ND
EAEC	256	0	0	0					ND	ND		
EPEC	243	0	0	0					ND	ND		
ETEC	267	0	0	0	270	0	0	0	ND	ND	ND	ND
STEC	240	28	2	0	241	27	1	1	99.2 (96.8, 100)	99.2 (96.8, 100)	96.4 (80.8, 99.9)	99.6 (97.5, 100)
E. coli O157	258	11	0	0	257	10	1	1	100 (70, 100)	100 (98.2, 100)	90.9 (80.1, 99.9)	99.6 (97.6, 100)
Shigella/EIEC	258	10	1	1	259	9	0	2	90.9 (60.1, 99.9)	99.6 (97.6, 100)	81.8 (51.1, 96)	ND
Cryptosporidium spp.	264	5	0	0	265	5	0	0	100 (51.1, 100)	100 (98.3, 100)	100 (51.1, 100)	100 (98.3, 100)
Cyclospora cayetanensis	269	0	0	0					ND	ND		
Entamoeba histolytica	269	0	0	1	268	1	0	0	0 (0, 83.3)	100 (98.3, 100)	100 (16.8, 100)	100 (98.3, 100)
Giardia lamblia	263	7	0	0	263	7	0	0	100 (60, 100)	100 (98.3, 100)	100 (60, 100)	100 (98.3, 100)
Adenovirus 40/41	258	9	2	1	259	8	0	2	90 (57.4, 99.9)	99.2 (97.1, 100)	80 (47.9, 95.4)	100 (98.2, 100)
Norovirus GI/GII	225	41	0	3	194	41	32	3	93.2 (81.3, 98.6)	100 (98.0, 100)	93.2 (81.3, 98.6)	85.8 (80.7, 89.8)
Rotavirus	255	14	1	0	254	13	1	1	100 (74.9, 100)	99.6 (97.8, 100)	92.9 (66.5, 99.9)	99.6 (97.6, 100)
Sapovirus	237	28	0	3					90.3 (74.3, 97.4)	100 (98.1, 100)		
Astrovirus	257	11	0	0					100 (70, 100)	100 (98.2, 100)		

Khare R. et al. J. Clin. Microbiol. 2014;52:3667-3673

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Benefits of GI Panels

Goldenberg et al. 2015. J Infection. 70(5).

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Cost Savings

Isolation days	3.0 days	2.5 days	2.0 days	1.5 days	1.0 days
Total isolation days GPP testing pathway	1715	1581	1447	1313	1179
Total isolation cost under GPP testing pathway	£151,602	£139,754	£127,944	£116,057	£104,209
Total laboratory testing costs for GPP	£56,243	£56,243	£56,243	£56,243	£56,243
Total costs for GPP testing pathway	£207,845	£195,997	£184,187	£172,300	£160,452
Total costs for conventional testing pathway	£228,661	£228,661	£228,661	£228,661	£228,661
Net savings using GPP testing pathway	£20,816	£32,664	£44,474	£56,361	£68,209

Goldenberg et al. 2015. J Infection. 70(5).

Distribution of pathogens detected by the FilmArray GI Panel in diarrheal stool specimens that were negative for *C. difficile* and/or rotavirus by conventional testing

Pathogen on FilmArray GI Panel	Number of samples testing positive for indicated analyte on the FilmArray GI panel that were originally negative or not tested for:		
	C. difficile, N = 142	Rotavirus[a], N = 16	All negative patients, N = 158
Norovirus	9 (2)[a]	1	10
Rotavirus	8 (2)		8
EPEC	8 (3)		8
EIEC/*Shigella*	2 (2)	1	3
EAEC	2		2
ETEC	2 (1)		2
Astrovirus		2 (1)	2
Salmonella		1	1
Cryptosporidium	1		1
Aeromonas	1		1
C. difficile	2	1 (1)	3
Adenovirus		1	1
Total pathogens	35/142 (24.6%)	7/16 (43.8%)	42/158 (26.6%)
Total patients	29/142 (20.4%)	6/16 (37.5%)	35/158 (22.2%)

Rand et al. 2015. DMID. 82(2).

Advantages and Disadvantages

Advantages	Disadvantages
Laboratory perspective	
Potential for lower cost with larger volumes	Currently significantly more expensive with low volumes
Potential for enhanced efficiency and higher throughput	Culture confirmation still required for antibiotic susceptibility testing ± strain typing
Rapid reporting of negative samples and reductions in turnaround times	Demand for testing may grow due to perception of rapidity of result
Bundled test that can be applied to all faeces samples	Need to retain other individual tests for certain situations, e.g., hospitalised patients
Individual patient management	
Rapid diagnosis	Does not alter management for vast majority of patients
Enhanced sensitivity for STEC, and *C. difficile* in particular	Over-diagnosis and low-level positives of uncertain significance, which may result in unnecessary treatment
Less dependent on clinical details or clinician request	Will miss non-wild type or unusual bacteria not included in panel
Results less affected by concurrent antibiotic therapy	Potential to increase antibiotic prescribing
	No antibiotic susceptibility result
Infection prevention and control and public health	
Rapid detection of outbreaks	No culture isolate available for strain typing
More reliable detection of pathogens potentially missed by culture, e.g., sorbitol fermenting STEC	Limited to organisms included in panel, which may not account for local epidemiology
Increased detection of EP	Not suitable for return-to-work clearance
Shorter period of isolation for patients with negative results	Loss of opportunity to passively screen for MDROs on stool
More sophisticated cohorting of patients according to pathogen	

EP, enteric pathogens; MDROs, multi-drug resistant organisms; NAAT, nucleic acid amplification tests; STEC, Shiga-like toxin producing *Escherichia coli*.

Molecular testing for viral and bacterial enteric pathogens: gold standard for viruses, but don't let culture go just yet?.
Bloomfield, Maxim; Balm, Michelle; Blackmore, Timothy

Pathology. 47(3):227-233, April 2015.

Meningitis Panels

Biofire ME

Table 8. Comparator Methods for FilmArray ME Panel Clinical Evaluation

FilmArray Analyte	Comparator Method	Comparator Test Location
E. coli K1	CSF bacterial culture	Source Laboratory
H. influenzae		
L. monocytogenes		
N. meningitidis		
S. agalactiae		
S. pneumoniae		
CMV	Two PCR assays with bi-directional sequencing[a]	BioFire Laboratory
EV		
HSV-1		
HSV-2		
HHV-6		
HPeV		
VZV		
C. neoformans/gattii		

BioFire ME Bacterial Performance

Table 9. FilmArray ME Prospective Clinical Performance Summary[a]

Analyte		Sensitivity (compared to culture) TP/(TP + FN)	%	95% CI	Specificity (compared to culture) TN/(TN + FP)	%	95% CI
Bacteria							
E. coli K1	Fresh	1/1	100	-	1014/1014	100	99.6-100
	Frozen	1/1	100	-	543/544	99.8	99.0-100
	Overall	**2/2**	**100**	**34.2-100**	**1557/1558[b,c]**	**99.9**	**99.6-100**
H. influenzae	Fresh	1/1	100	-	1013/1014	99.9	99.4-100
	Frozen	0/0	-	-	545/545	100	99.3-100
	Overall	**1/1**	**100**	**-**	**1558/1559[d]**	**99.9**	**99.6-100**
L. monocytogenes	Fresh	0/0	-	-	1015/1015	100	99.6-100
	Frozen	0/0	-	-	545/545	100	99.3-100
	Overall	**0/0**	**-**	**-**	**1560/1560**	**100**	**99.8-100**
N. meningitidis	Fresh	0/0	-	-	1015/1015	100	99.6-100
	Frozen	0/0	-	-	545/545	100	99.3-100
	Overall	**0/0**	**-**	**-**	**1560/1560**	**100**	**99.8-100**
S. agalactiae	Fresh	0/1	0.0	-	1013/1014	99.9	99.4-100
	Frozen	0/0	-	-	545/545	100	99.3-100
	Overall	**0/1[e]**	**0.0**	**-**	**1558/1559[d]**	**99.9**	**99.6-100**
S. pneumoniae	Fresh	2/2	100	34.2-100	1008/1013	99.5	98.8-99.8
	Frozen	2/2	100	34.2-100	536/543	98.7	97.4-99.4
	Overall	**4/4**	**100**	**51.0-100**	**1544/1556[f]**	**99.2**	**98.7-99.6**

Analyte		Positive Percent Agreement (compared to PCR with bi-directional sequencing)			Negative Percent Agreement (compared to PCR with bi-directional sequencing)		
		TP/(TP + FN)	%	95% CI	TN/(TN + FP)	%	95% CI
Viruses							
CMV	Fresh	2/2	100	34.2-100	1010/1013	99.7	99.1-99.9
	Frozen	1/1	100	20.7-100	544/544	100	99.3-100
	Overall	***3/3***	***100***	***43.9-100***	***1554/1557[a]***	***99.8***	***99.4-99.9***
EV	Fresh	43/44	97.7	88.2-99.6	965/971	99.4	98.7-99.7
	Frozen	1/2	50.0	-	542/543	99.8	99.0-100
	Overall	***44/46[b]***	***95.7***	***85.5-98.8***	***1507/1514[b]***	***99.5***	***99.0-99.8***
HSV-1	Fresh	1/1	100	-	1013/1014	99.9	99.4-100
	Frozen	1/1	100	-	543/544	99.8	99.0-100
	Overall	***2/2***	***100***	***34.2-100***	***1556/1558[c]***	***99.9***	***99.5-100***
HSV-2	Fresh	6/6	100	61.0-100	1008/1009	99.9	99.4-100
	Frozen	4/4	100	51.0-100	540/541	99.8	99.0-100
	Overall	***10/10***	***100***	***72.2-100***	***1548/1550[d]***	***99.9***	***99.5-100***
HHV-6	Fresh	13/15	86.7	62.1-96.3	997/1000	99.7	99.1-99.9
	Frozen	5/6	83.3	43.6-97.0	535/536	99.8	99.0-100
	Overall	***18/21[e]***	***85.7***	***65.4-95.0***	***1532/1536[e]***	***99.7***	***99.3-99.9***
HPeV	Fresh	9/9	100	70.1-100	1003/1006	99.7	99.1-99.9
	Frozen	0/0	-	-	545/545	100	99.3-100
	Overall	***9/9***	***100***	***70.1-100***	***1548/1551[f]***	***99.8***	***99.4-99.9***
VZV	Fresh	3/3	100	43.9-100	1010/1012	99.8	99.3-99.9
	Frozen	1/1	100	-	543/544	99.8	99.0-100
	Overall	***4/4***	***100***	***51.0-100***	***1553/1556[m]***	***99.8***	***99.4-99.9***

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Yeast Performance

		Yeast					
C. neoformans/gattii	Fresh	0/0	-	-	1015/1015	100	99.6-100
	Frozen	1/1	100	-	540/544	99.3	98.1-99.7
	Overall	***1/1***	***100***	***-***	***1555/1559[n]***	***99.7***	***99.3-99.9***

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How does ME compare in Published Studies?

- Too early to establish performance and clinical outcomes
  - But…

Table 1. Enterovirus Detection in Frozen, Archived, De-identified Patient CSF Samples

Test	EV Detection	Error	Additional Detection
ARUP LDT	50/50	NA	NA
FA ME	50/50	0/50	*S. pneumoniae* (2), HSV2 (2), EBV (1)
GX-EV	47/48	2/50	NA

ME: Meningitis/Encephalitis; LDT: Laboratory Developed Test

Lunt, T et al. ASM Poster #1668. 2015

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Future Panels

- Zoonotic Diseases Panel
- Arthropod-bourne Diseases Panel
- Pneumonia Panel
- STI Panel
- Atypical Pneumonia Panel
- BV Panel

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