

CLINICAL UTILITY OF SYNDROMIC PANEL-BASED TESTING

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DISCLOSURE

- The presenter of this activity has no conflict of interest and nothing to disclose.

OBJECTIVES

- Discuss the advantages of commercially available Multiplex assays in diagnosing various infectious diseases
- Learn about real life challenges in interpretation of these tests
- Understand how the results of these tests impact our quality metrics and resource utilization

MULTIPLEX ASSAYS

- Commercial molecular assays that simultaneously detect and identify multiple pathogens associated with clinical syndromes.
- Syndrome based testing of Bloodstream, respiratory, gastrointestinal and central nervous system (CNS) infections

BLOOD STREAM INFECTIONS

- Sepsis is a common and potentially fatal illness
- Rapid institution of appropriate antimicrobial therapy is the cornerstone of treatment
- Increasing drug resistance
- Empirical broad spectrum antimicrobial use
 - Direct end organ toxicity
 - Costs
 - Resistance
 - C diff

TABLE 1 FDA-approved/cleared panel-based molecular assays for detection of select microorganisms and select resistance genes in positive blood culture bottles

Parameter	FilmArray BCID	Verigene	
		Gram-positive blood culture	Gram-negative blood culture
Total no. of targets	27	15	14
Ability to detect pathogen			
Gram-positive bacteria			
<i>Staphylococcus</i> species	✓	✓	
<i>Staphylococcus aureus</i>	✓	✓	
<i>Staphylococcus epidermidis</i>		✓	
<i>Staphylococcus lugdunensis</i>		✓	
<i>Streptococcus</i> species	✓	✓	
<i>Streptococcus agalactiae</i>	✓	✓	
<i>Streptococcus pyogenes</i>	✓	✓	
<i>Streptococcus pneumoniae</i>	✓	✓	
<i>Streptococcus anginosus</i> group		✓	
<i>Enterococcus</i> species	✓		
<i>Enterococcus faecalis</i>		✓	
<i>Enterococcus faecium</i>		✓	
<i>Listeria</i> species		✓	
<i>Listeria monocytogenes</i>	✓		
Gram-negative bacteria			
<i>Klebsiella oxytoca</i>	✓		✓
<i>Klebsiella pneumoniae</i>	✓		✓
<i>Serratia marcescens</i>	✓		
<i>Proteus</i> species	✓		✓
<i>Acinetobacter</i> species			✓
<i>Acinetobacter baumannii</i>	✓		
<i>Haemophilus influenzae</i>	✓		
<i>Neisseria meningitis</i>	✓		
<i>Pseudomonas aeruginosa</i>	✓		✓
Enterobacteriaceae	✓		
<i>Escherichia coli</i>	✓		✓
<i>Enterobacter</i> species			✓
<i>Enterobacter cloacae</i> complex	✓		
<i>Citrobacter</i> species			✓
Yeasts			
<i>Candida albicans</i>	✓		
<i>Candida glabrata</i>	✓		
<i>Candida krusei</i>	✓		
<i>Candida parapsilosis</i>	✓		
<i>Candida tropicalis</i>	✓		
Ability to detect presence of resistance gene			
<i>mecA</i>	✓	✓	
<i>vanA</i>	✓	✓	
<i>vanB</i>	✓	✓	
<i>bla</i> _{KPC}	✓		✓
<i>bla</i> _{NDM}			✓
<i>bla</i> _{OXA}			✓
<i>bla</i> _{VIM}			✓
<i>bla</i> _{IMP}			✓
<i>bla</i> _{CTX-M}			✓
Time to result (h)	~1	~2.5	~2

ADVANTAGES

- Very quick
- Highly sensitive and specific
- Detect common resistance genes
- Specific antibiotic therapy
- ? Decreased phone calls
- Minimal hands-on time
- ? Detection of targets despite prior antibiotics

LIMITATIONS

- \$\$\$ - These are add-on tests
- Optimal antibiotic therapy is a team effort
- Only certain resistance markers detected
- False positives
 - *Pseudomonas*, *Candida parapsilosis*, *Proteus* sp.
- Species level misidentification
 - *S. mitis* vs *S. Pneumoniae*
- Gene presence vs Gene expression
- Effect on mortality
- Effect on hospital length of stay
- Rapid clearance of blood stream

VERIGENE GP-BC CONCORDANCE

Organisms	No. of isolates				Concordance (%)
	Positive on culture	Correctly identified	Incorrectly identified	Targeted but not detected	
<i>Staphylococcus</i>					92.9
<i>S. aureus</i>	22	22	–	–	100
<i>S. epidermidis</i>	25	21	1	3	84.0
<i>S. lugdunensis</i>	2	2	–	–	100
Other CoNS	7	7	–	–	100
<i>Enterococcus</i>					84.6
<i>E. faecalis</i>	14	11	–	3	78.6
<i>E. faecium</i>	12	11	–	1	91.7
<i>Streptococcus</i>					82.2
<i>S. pneumoniae</i>	10	9	1	–	90.0
<i>S. pyogenes</i>	2	–	–	2	na
<i>S. agalactiae</i>	2	2	–	–	100
<i>S. anginosus</i> group	7	5	2	–	71.4
Other streptococci	24	21	3	–	87.5
<i>Listeria</i> spp.	1	1	–	–	100
<i>Micrococcus</i> spp.	1	1	–	–	100
Total isolates	129	113	7	9	87.6

Dodémont, M et al. *European journal of clinical microbiology & infectious diseases*. 34.3 (2015): 473–477.

ORIGINAL ARTICLE

Yasuko Hososaka · Hideaki Hanaki · Harumi Endo
Yumiko Suzuki · Zenzo Nagasawa · Yoshihito Otsuka
Taiji Nakae · Keisuke Sunakawa

Characterization of oxacillin-susceptible *mecA*-positive *Staphylococcus aureus*: a new type of MRSA

 OPEN ACCESS  PEER-REVIEWED

RESEARCH ARTICLE

Prevalence of vancomycin-variable *Enterococcus faecium* (VVE) among *vanA*-positive sterile site isolates and patient factors associated with VVE bacteremia

Philipp Kohler , Alireza Eshaghi, Hyunjin C. Kim, Agron Plevneshi, Karen Green, Barbara M. Willey, Allison McGeer, Samir N. Patel, for the Toronto Invasive Bacterial Diseases Network (TIBDN) 

Published: March 22, 2018 • <https://doi.org/10.1371/journal.pone.0193926>

CHALLENGES AND OPPORTUNITIES

- Polymicrobial bacteremias
- Fastidious organisms
 - *Coxiella*, *Rickettsia*, *Tropheryma*
- Direct MALDI-TOF from positive blood cultures
- Multiplex assay on partially incubated bottles
- Direct identification from blood samples

CNS INFECTIONS

- Meningitis / Encephalitis
- Very High Morbidity / Mortality
- Rapid and Specific Treatment Essential

CSF Parameter	Normal	Viral Infection	Bacterial Infection	Fungal / TB Infection
Opening Pressure (mmH ₂ O)	100 - 180	Normal - Elevated	200 - 500	150 - 340
WBC (cells/ μ L)	0-5	5 – 1000	100 – 1000	5 – 1000
Protein (mg/dL)	< 30	30 - 300	60 - 500	> 60
Glucose (%Blood glucose)	> 60	> 60	<45	<45

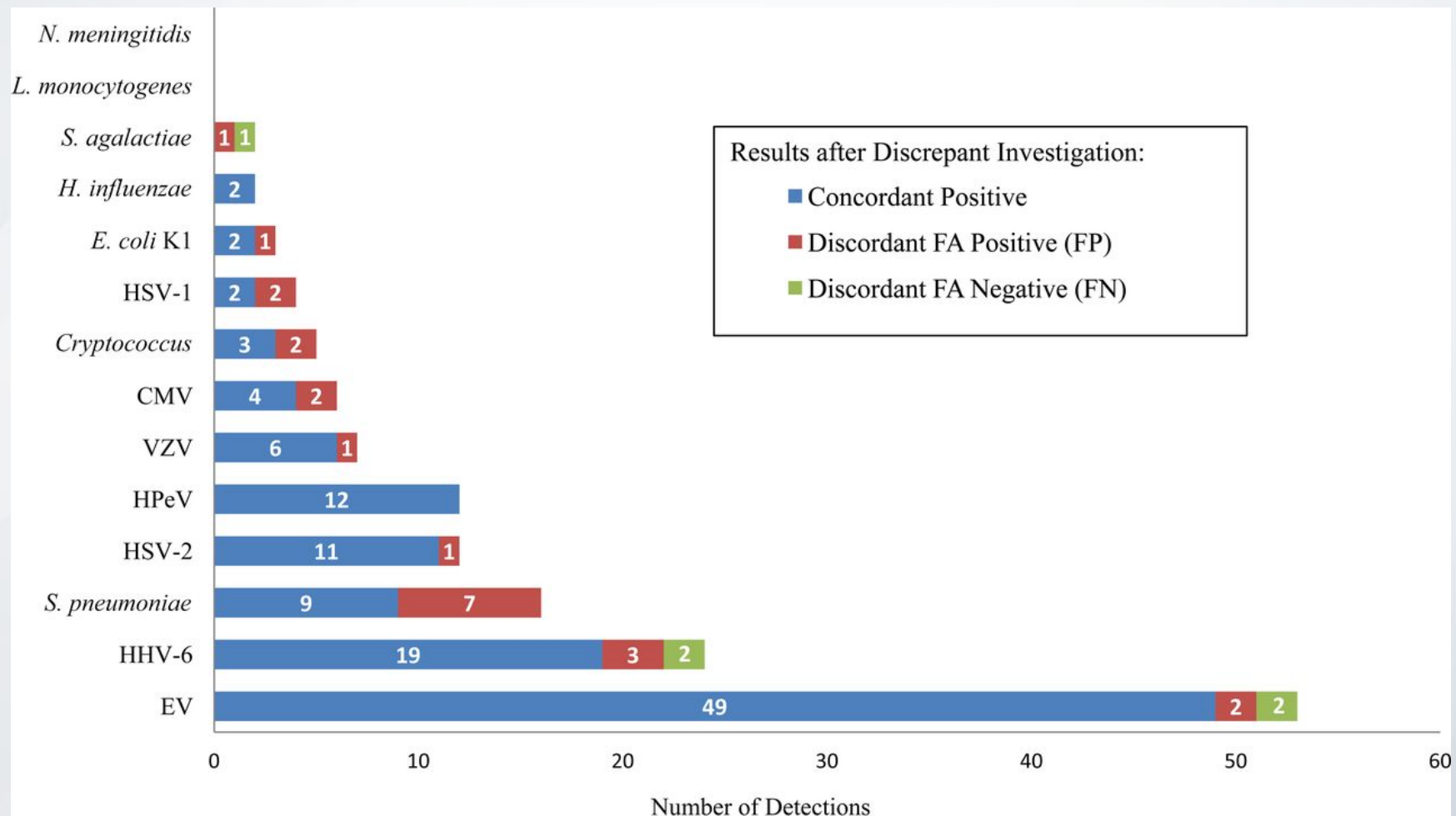
Parameter	FilmArray Meningitis/Encephalitis panel
Pathogen detected	
Viruses	Cytomegalovirus, enterovirus, herpes simplex virus 1, herpes simplex virus 2, human herpesvirus 6, human parechovirus, varicella-zoster virus
Bacteria	<i>Escherichia coli</i> K1, <i>Haemophilus influenzae</i> , <i>Listeria monocytogenes</i> , <i>Neisseria meningitidis</i> , <i>Streptococcus agalactiae</i> , <i>Streptococcus pneumoniae</i>
Fungi	<i>Cryptococcus neoformans</i> - <i>C. gattii</i>
Analysis platform	FilmArray system or FilmArray Torch
Acceptable specimen type	CSF
Time to results (h)	~1

ADVANTAGES

- Really Really Rapid TAT
- Only 200 μ L of CSF
- Multiple common targets

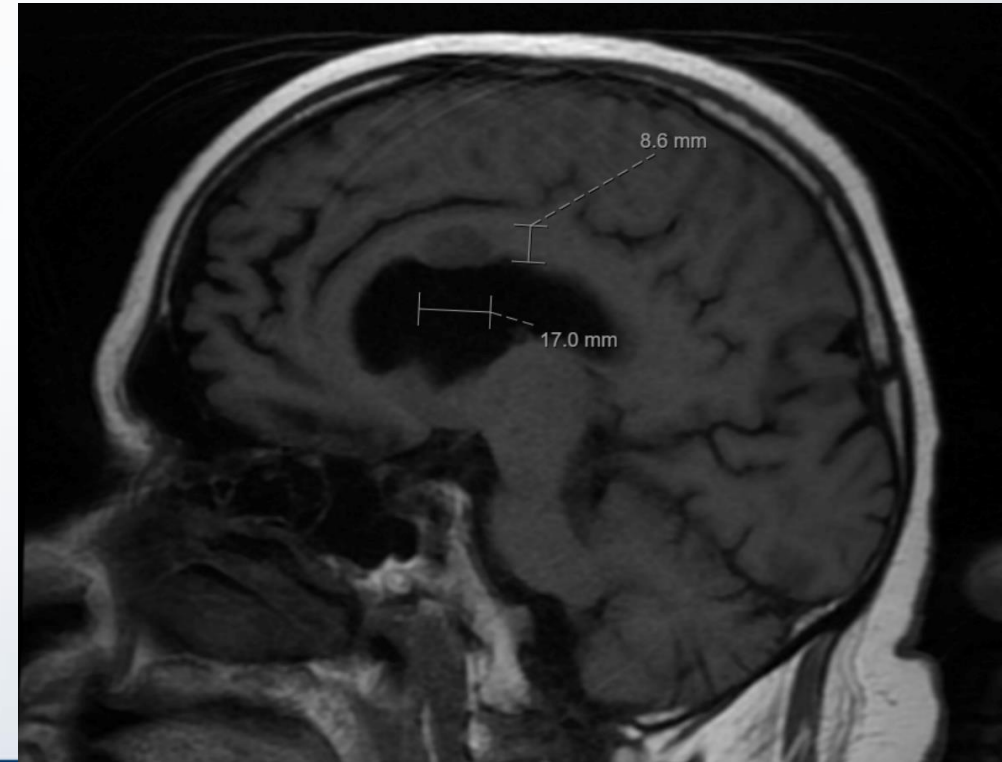
LIMITATIONS

- Bacterial meningitis is relatively rare in US
 - *S. pneumoniae*, *H. influenzae*, *N. meningitidis*
 - Dependent on immunization rates
- Post-surgical infections
 - *S aureus*, *Candida* sp, *Cutibacterium* sp, Gram negatives
- False positive results for *S. pneumoniae*
- \$\$\$ cf. CrAg
- False negative with *Cryptococcus* sp.
- Detection of multiple pathogens



CASE SCENARIO

- A 79-year-old male presents to the hospital with worsening confusion for 2 months. He has a history recent chemotherapy for breast cancer and is also on Prednisone for myasthenia gravis.
- MRI image is shown
- Possible mets vs other etiology
- CSF analysis is performed



CSF Parameter	Result
Opening Pressure	Not measured
WBC	34 cells/ μ L; 47% N, 35% L, 18% M
Protein	49.2
Glucose	55
Gram stain	Yeast

	Ref Range & Units	
Escherichia coli K1	Not Detected	Not Detected
Haemophilus influenzae	Not Detected	Not Detected
Listeria monocytogenes	Not Detected	Not Detected
Neisseria meningitidis	Not Detected	Not Detected
Streptococcus agalactiae (Group B)	Not Detected	Not Detected
Streptococcus pneumoniae	Not Detected	Not Detected
Cytomegalovirus	Not Detected	Not Detected
Enterovirus	Not Detected	Not Detected
Herpes Simplex Type 1	Not Detected	Not Detected
Herpes Simplex Type 2	Not Detected	Not Detected
Human Herpesvirus 6	Not Detected	Detected !!
Comment: Detection of HHV-6 may indicate primary, secondary reactivation, or the presence of latent virus.		
Human Parechovirus	Not Detected	Not Detected
Varicella Zoster Virus	Not Detected	Not Detected
Cryptococcus neoformans/gattii	Not Detected	Detected !!
Comment: Specimen submitted for Cyptococcal Antigen Titer testing. Additional report to follow.		

EDITOR'S CHOICE

Delayed Diagnosis of Tuberculous Meningitis Misdiagnosed as Herpes Simplex Virus-1 Encephalitis With the FilmArray Syndromic Polymerase Chain Reaction Panel

Carlos A. Gomez, Benjamin A. Pinsky, Anne Liu, Niaz Banaei [Author Notes](#)

Open Forum Infectious Diseases, Volume 4, Issue 1, Winter 2017, ofw245,
<https://doi.org/10.1093/ofid/ofw245>

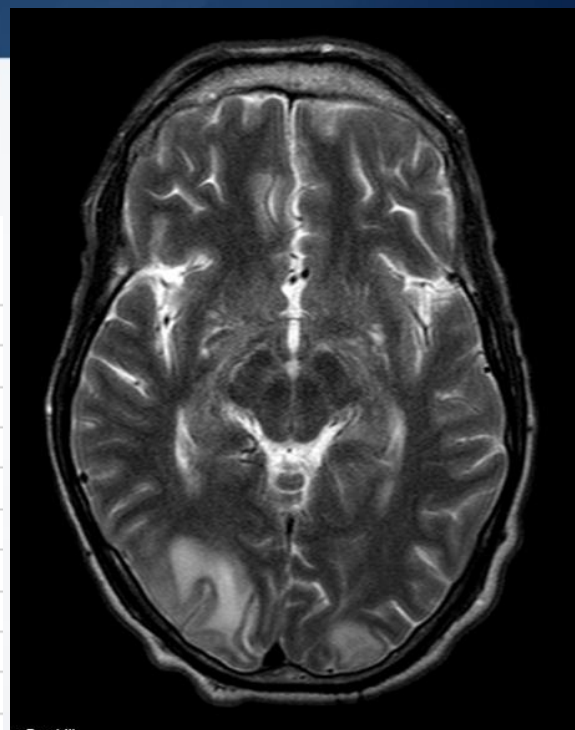
CASE SCENARIO

- A 39-year-old male with history of IVDU, HIV and syphilis presented to the hospital with 2 weeks of fever and confusion. This prompted MRI of the brain. The findings are shown on the next slide. He has no history of immunosuppression.

CSF		
Volume 1 CSF		5.5
Clarity, CSF		
Turbidity 1 CSF		Clear
Color 1 CSF		Colorless
Supernate 1 CSF		Colorless
Tube Number CSF		Tube 3
CSF RBC		4 ▲
WBC CSF Cell Count		190 ▲
% Neutrophils CSF		15 ▲
% Lymphocytes CSF		58
% Macro/Monos CSF		17
% Eosinophils CSF		10 ▲
Tube Number CSF Gl...		One
Glucose CSF		34 ▼
Tube Number CSF Pr...		One
Protein CSF		249.7 ▲

	Ref Range & Units		
Escherichia coli K1	Not Detected	Not	Detected
Haemophilus influenzae	Not Detected	Not	Detected
Listeria monocytogenes	Not Detected	Not	Detected
Neisseria meningitidis	Not Detected	Not	Detected
Streptococcus agalactiae (Group B)	Not Detected	Not	Detected
Streptococcus pneumoniae	Not Detected	Not	Detected
Cytomegalovirus	Not Detected	Not	Detected
Enterovirus	Not Detected	Not	Detected
Herpes Simplex Type 1	Not Detected	Not	Detected
Herpes Simplex Type 2	Not Detected	Not	Detected
Human Herpesvirus 6	Not Detected	Not	Detected
Human Parechovirus	Not Detected	Not	Detected
Varicella Zoster Virus	Not Detected	Not	Detected
Cryptococcus neoformans/gattii	Not Detected	Not	Detected

CRYPTOCOCCAL						
Cryptococcal Ag	Positive	!				
Cryptococcal Ag CSF		Positive	!		Positive	!
Cryptococcal Ag Titer	1:1024	▲			1:256	▲



Failure of multiplex meningitis/encephalitis (ME) NAT during cryptococcal meningitis in solid organ recipients

Benoit Pilmis^{1,2,3} | Marie-Elizabeth Bougnoux⁴ | Romain Guery¹ | Yaye Senghor⁵ | Alban Le Monnier⁵ | Fanny Lanternier^{1,6} | Stephane Bretagne^{6,7,8} | Alexandre Alanio^{6,7} | Olivier Lortholary^{1,6}

TABLE 1 Summary of the clinical/ features of four episodes of *Cryptococcus neoformans* meningitis

Patient Lead symptoms	No 1 Meningeal symptoms, fever	No 2 Meningeal symptoms, fever	No 3 Confusion	No 4 confusion, fever
Sex—Age	Male-53	Male-58	Male-62	Male-60
Medical history	KT	KT (PCLKD)	KT (diabetic nephropathy)	KT (diabetic nephropathy)
Duration of symptoms before diagnosis (days)	5	6	15	13
Immunosuppressive regimen	Corticosteroids Tacrolimus Mycophenolate mofetil	Corticosteroids Tacrolimus Mycophenolate mofetil	Tacrolimus Mycophenolate mofetil	Corticosteroids Tacrolimus Mycophenolate mofetil
CSF analysis				
WBC (/mm ³), (% Lymphocytes)	22 (91)	48 (75)	106 (90)	115 (95)
Ratio (Glycorrachia/glycemia)	0.51	0.33	0.3	0.34
Protein (g/L)	0.61	0.73	1.56	2.23
India ink staining	(-)	(-)	(-)	(-)
Culture (delay for culture positivity)	(+C <i>neoformans</i>) (7)	(+C <i>neoformans</i>) (4)	(-)	(+C <i>neoformans</i>) (7)
CSF CrAg (titer)	(+) (1:160)	(+)	(+) 1:20	(+) 1:256
FilmArray ME panel	(-)	(-)	(-)	(-)
Specific in house <i>C neoformans</i> qPCR	(-)	(-)	(NA)	NA
Serum CrAg (titer)	(-)	(+)	(+) 1:1280	(+) 1:128
Blood culture	(-)	(-)	(-)	(-)
Urinary culture	(-)	(-)	(-)	(-)

Abbreviations: KT, kidney transplantation; NA, Not available; PCLKD, Polycystic Liver and Kidney Disease; WBC, White Blood Cells.

GASTROINTESTINAL INFECTIONS

- 2 billion cases / yr worldwide
- Major cause of mortality among children < 5
- Primarily in developing countries
- Primary mode of transmission: Contaminated food and water
- Large number of pathogens
- Travel, Migration, Food supply chains

SYNDROMIC TESTING

- Targeted testing less feasible; costs can accumulate
- Traditionally, stool culture
- Replaced by multiplex
- Multiplex provides rapid TAT
- Minimal amount of stool
- Detection of outbreaks

TABLE 4 FDA-approved/cleared multiplex gastrointestinal panels^a

Parameter	Verigene EP	Luminex GPP	BioFire GIP
Analysis platform	Verigene system	Magpix or Luminex 100/200 system	FilmArray system or FilmArray Torch
Acceptable specimen type	Stool in Cary-Blair medium	Fresh stool or stool in Cary-Blair medium	Stool in Cary-Blair medium
No. of targets	9	14	22
Ability to detect pathogen			
Bacteria			
<i>Campylobacter</i> species	✓	✓	✓
<i>Salmonella</i> species	✓	✓	✓
<i>Shigella</i> species/enteroinvasive <i>E. coli</i> ^b	✓	✓	✓
<i>Vibrio</i> species	✓		✓
<i>Vibrio cholerae</i>		✓	✓
<i>Yersinia enterocolitica</i>	✓		✓
<i>Escherichia coli</i> O157		✓	✓
Enterotoxigenic <i>E. coli</i>		✓	✓
Enteropathogenic <i>E. coli</i>			✓
Enteraggregative <i>E. coli</i>			✓
<i>Plesiomonas shigelloides</i>			✓
Shiga toxin-producing <i>E. coli</i> (stx ₁ -stx ₂)	✓ ^c	✓	✓
<i>Clostridium difficile</i> (toxin A/B)		✓	✓
Viruses			
Norovirus GI/GII	✓	✓	✓
Rotavirus A	✓	✓	✓
Astrovirus			✓
Adenovirus 40/41		✓	✓
Sapovirus			✓
Parasites			
<i>Cryptosporidium</i> species		✓	✓
<i>Entamoeba histolytica</i>		✓	✓
<i>Giardia lamblia</i>		✓	✓
<i>Cyclospora cayentanensis</i>			✓
No. of samples (throughput)	1–32 (scalable)	24	1–12 (scalable)
Time to result (h)	<2	~5	~1

^aEP, enteric pathogens; GPP, gastrointestinal pathogen panel; GIP, gastrointestinal panel.^bThe Verigene EP and Luminex GPP do not specifically target enteroinvasive *E. coli*.^cThe Verigene EP has separate targets for stx₁ and stx₂.

LIMITATIONS

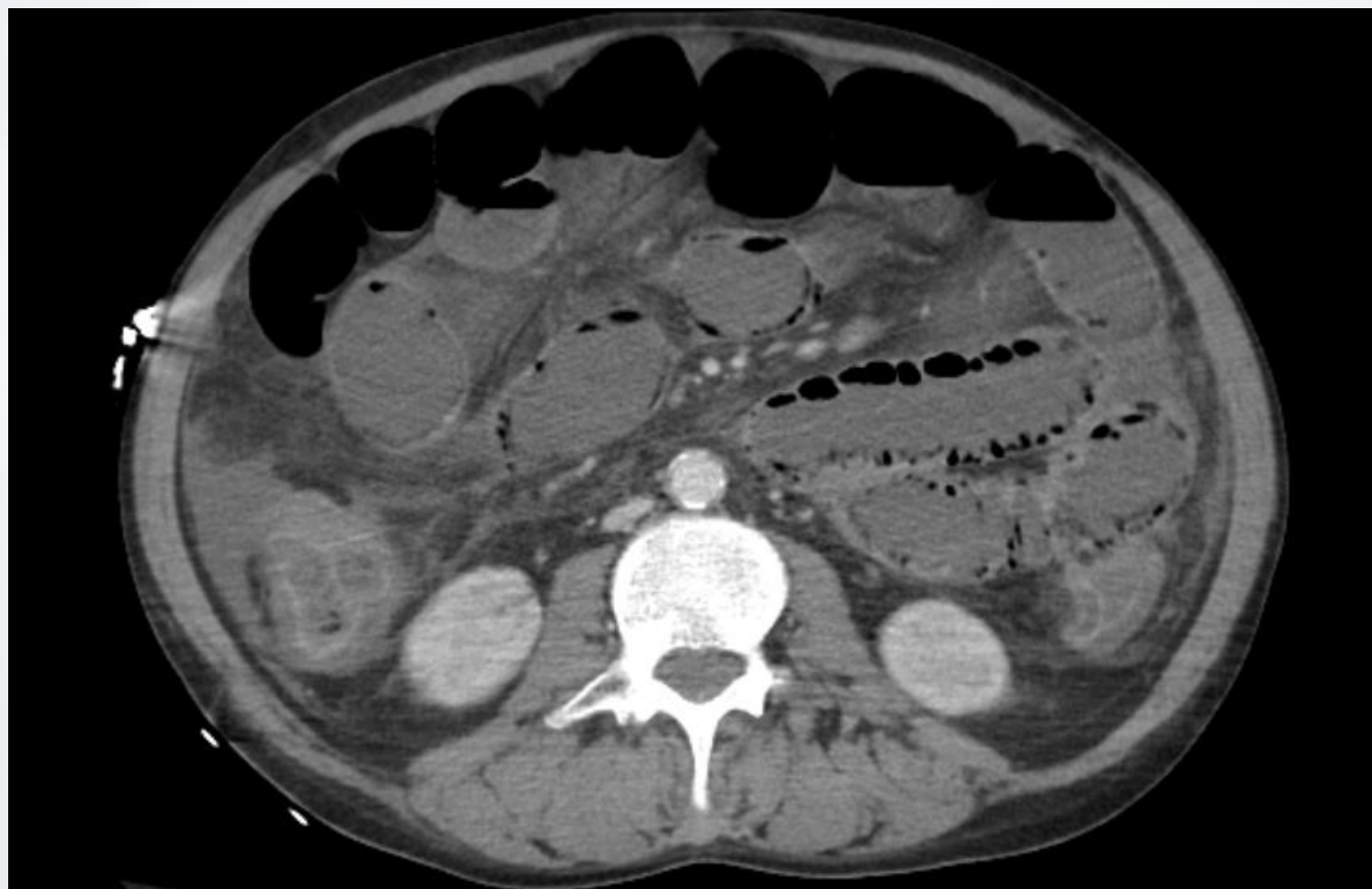
- \$\$\$
- ≥ 2 pathogens in up to 16% samples
 - EPEC and EAEC most frequent "co-pathogens"
 - Clinical significance of EPEC and EAEC unclear
 - 20% of children colonized
- Colonization vs Infection
 - Free DNA/RNA vs infectious organism
 - ?Isolate and / or ?treat
- Lack of Susceptibility Testing

C DIFF

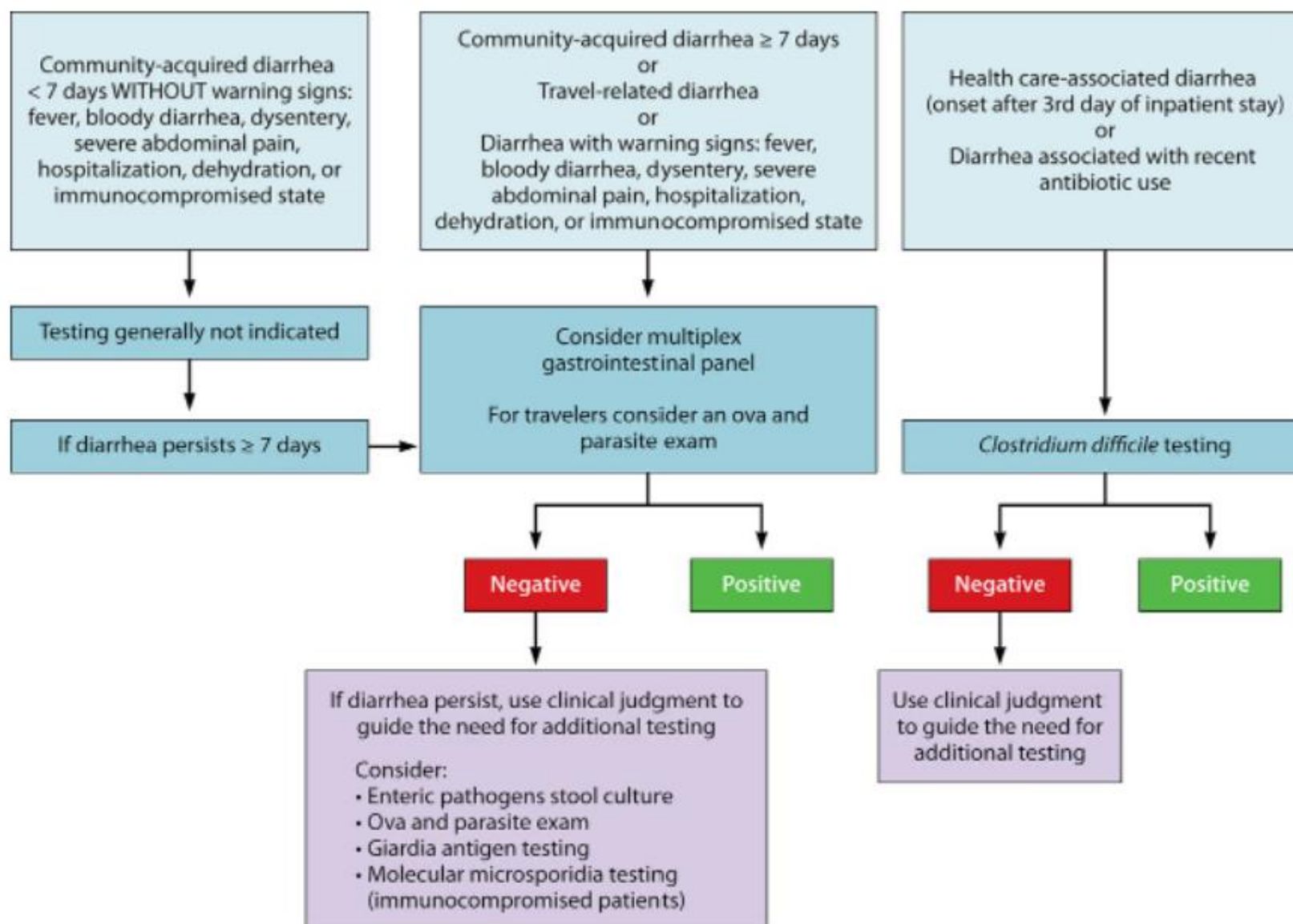
- Most common hospital acquired infection
- Associated with high rates of antibiotic utilization
- However, 20% patients colonized
- Simultaneous administration of:
 - Laxatives
 - Contrasts
 - Tubal Feeding
- Quality metrics – Are we measuring the right target?

CASE SCENARIO

- A 51-year-old male with common variable immunodeficiency and history of multiple opportunistic infections presents with acute on chronic abdominal pain with blood in stools. He has had multiple prior admissions for the same problem. However, this time, he is in septic shock. A CT abdomen is performed, and findings are as follows:



Component	Ref Range & Units	2 yr ago (11/26/19)	2 yr ago (5/31/19)	3 yr ago (3/28/19)	3 yr ago (3/9/19)	3 yr ago (1/19/19)	3 yr ago (1/9/19)	4 yr ago (12/19/17)
Campylobacter	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected
Salmonella	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	Detected !	Not Detected
Shigella	Not Detected	Not Detected		Not Detected	Not Detected	Not Detected	Not Detected	Not Detected
Vibrio	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected
Yersinia enterocolitica	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected
Shiga-Like Toxin 1	Not Detected	Not Detected		Not Detected	Not Detected	Not Detected	Not Detected	Not Detected
Shiga-Like Toxin 2	Not Detected	Not Detected		Not Detected	Not Detected	Not Detected	Not Detected	Not Detected
Norovirus	Not Detected	Detected !		Detected !	Detected !	Detected !	Detected !	Detected !
Rotavirus	Not Detected	Not Detected		Not Detected	Not Detected	Not Detected	Not Detected	Not Detected



1. This algorithm should not be used for chronic diarrhea (duration >30 days).
2. For ova and parasite exams, submit 3 stool samples collected on separate days for maximum sensitivity.
3. During the summer, consider molecular detection of Shiga toxin in fecal samples for children with diarrhea even if they do not have bloody diarrhea, are not toxic-appearing, and diarrhea has been present <7 days.

RESPIRATORY INFECTIONS

- Common cause of morbidity and mortality
- Wide array of pathogens
- Similar clinical syndrome
- Multiplex assays can identify many common pathogens
- Rapid TAT
- Almost POC testing
- Higher sensitivity compared to Ag or cultures
- Potential for cost savings
- Potential for decreased antimicrobial use

LIMITATIONS

- Co-infections
- Colonization vs Infection
- Susceptibility testing
- \$\$\$
 - Still less than shell vial culture or and DFA staining
- No specific treatment for most viruses
 - Potential for decreased antibiotic prescriptions
 - Lower odds for admission
 - Lower odds for unnecessary imaging
 - Lower length of hospital stay
 - Increase in duration of isolation
 - Provides sense of closure

CASE SCENARIO

- A 43-year-old lady with DM2 and Class III obesity presents with severe shortness of breath, fever and cough. She is found to be severely hypoxic and intubated in the ER. CXR & CT are performed.



RESPIRATORY SPECIMEN TESTING

Respiratory Viral Panel TEM-PCR About this test		
Performed at: Eurofins Viracor 		
Received: 04/08/2022 08:20 AM CT Final Approved: 04/08/2022 09:10 PM		
Result (Unit)	Flag	Ref Range
Adenovirus		
Not Detected		Not Detected
<i>Detects Serotypes B and E. Detection of Serotype C may be limited. If Adenovirus infection is suspected and a Not Detected result is returned the sample should be re-tested for adenovirus using an independent method (e.g. Eurofins Viracor Adenovirus Quantitative Real-time PCR test).</i>		
Enterovirus/Rhinovirus		
Not Detected		Not Detected
Human bocavirus		
Not Detected		Not Detected

Human coronavirus

Not Detected

Not Detected

This test does NOT assay for the novel 2019 Coronavirus out of China. This test detects the respiratory Coronaviruses: types 229E, OC43, NL63, and HKU1.

Human metapneumovirus

POSITIVE

 Not Detected

Influenza A - Human

Not Detected

Not Detected

Influenza A - H1N1-09

Not Detected

Not Detected

Influenza B

Not Detected

Not Detected

Parainfluenza

Not Detected

Not Detected

Respiratory Syncytial

Not Detected

Not Detected

Sputum Culture

Methicillin Susceptible
Staphylococcus aureus

FUTURE OPPORTUNITIES AND CHALLENGES

- Multiplex assays for Sterile body fluids
 - Pleural fluid
 - Peritoneal fluid
 - Synovial fluid
- Colonization vs Infection
- Effect of prior antimicrobial therapy
- Cost
- Multiplex assay after inoculation in blood culture bottles
- Polymicrobial infections

THANK YOU!

AVISH.NAGPAL@SANFORDHEALTH.ORG