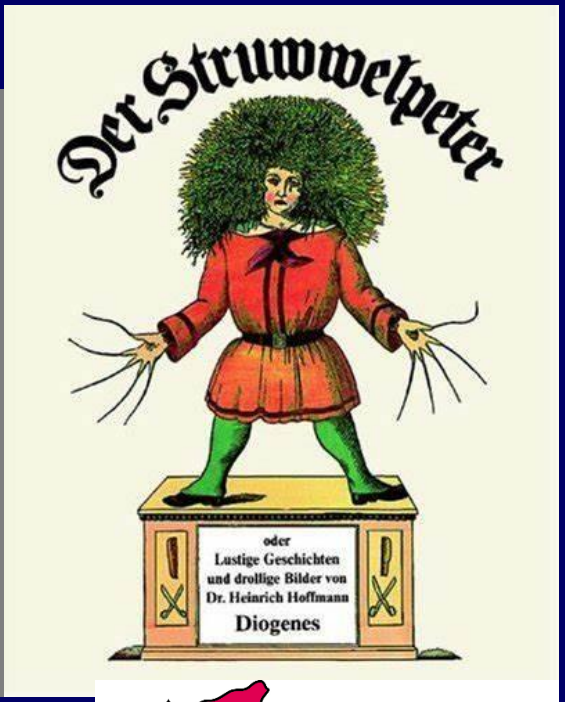
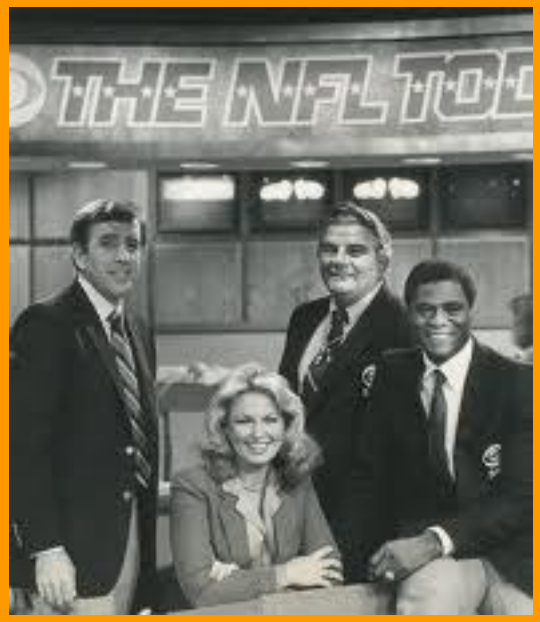
Critical Thinking with Multitest Syndromic Panels

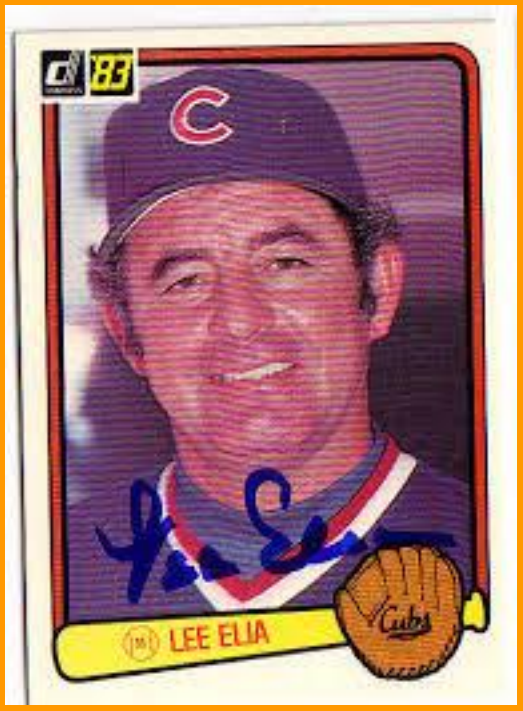


Erik Munson
WCLN Laboratory Technical Advisory Group
Department of Medical Laboratory Science
Marquette University
Milwaukee, Wisconsin



Pre-game Show

Sideline Reporter



Post-game Presser

OUTLINE

I. What are they?

II. Why beneficial?

III. How does critical thinking enter this conversation?

The presenter states no conflict of interest and has no financial relationship to disclose relevant to the content of this presentation.

What are they?

SYNDROMIC (MOLECULAR) PANELS

- Simultaneously tests for multiple pathogens on basis of site of illness (“shotgunning”)
- Can encompass multiple disciplines of microbiology

Bacteriology	Mycology
Virology	Parasitology

ANOTHER PERSPECTIVE

- Infectious agents of same organ system, though some agents may cause different manifestations
- “We’ve been doing this all along…”

Stool culture (x3)	Sputum culture
Blood culture	Urine culture
Fungus culture	Virus culture

- Without a diagnostic approach, providers would have to treat for everything
- Without a panel approach, providers would have to list infectious agent in requisition (kitchen sink)

AMERICAN SOCIETY FOR MICROBIOLOGY | Journal of Clinical Microbiology

Multicenter Evaluation of BioFire FilmArray Meningitis/Encephalitis Panel for Detection of Bacteria, Viruses, and Yeast in Cerebrospinal Fluid Specimens

Amy L. Leber,[a] Kathy Everhart,[a] Joan-Miquel Balada-Llasat,[b] Jillian Cullison,[b] Judy Daly,[c] Sarah Holt,[c] Paul Lephart,[d] Hossein Salimnia,[d] Paul C. Schreckenberger,[e] Sharon DesJarlais,[e] Sharon L. Reed,[f] Kimberle C. Chapin,[g] Lindsay LeBlanc,[g] J. Kristie Johnson,[h] Nicole L. Soliven,[h] Karen C. Carroll,[i] Jo-Anne Miller,[j] Jennifer Dien Bard,[k] Javier Mestas,[k] Matthew Bankowski,[l,m] Tori Enomoto,[l] Andrew C. Hemmert,[n] Kevin M. Bourzac[n]

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Escherichia coli K1
Haemophilus influenzae
Listeria monocytogenes
Neisseria meningitidis
Streptococcus pneumoniae
Streptococcus agalactiae
Cryptococcus neoformans

Cytomegalovirus
Enterovirus
Herpes simplex virus type 1
Herpes simplex virus type 2
Human herpes virus-6
Human parechovirus
Varicella-zoster virus

J Clin Microbiol. 54:2251-2261; 2016

Comparison of the Luminex xTAG Respiratory Viral Panel with In-House Nucleic Acid Amplification Tests for Diagnosis of Respiratory Virus Infections[▽]

Kanti Pabbaraju,[1]* Kara L. Tokaryk,[1] Sallene Wong,[1] and Julie D. Fox[1,2]

Provincial Laboratory for Public Health (Microbiology), Calgary, Alberta, Canada,[1] and Department of Microbiology and Infectious Diseases, University of Calgary, Calgary, Alberta, Canada[2]

Received 7 May 2008/Returned for modification 19 June 2008/Accepted 9 July 2008

Influenza A virus (H1)
Influenza A virus (H3)
Influenza A virus (H5)
Influenza B virus
Respiratory syncytial virus A
Respiratory syncytial virus B
Human bocavirus
Human metapneumovirus
Adenovirus
Enterovirus/rhinovirus

Parainfluenza virus 1
Parainfluenza virus 2
Parainfluenza virus 3
Parainfluenza virus 4
SARS-CoV-1
Coronavirus NL63
Coronavirus 229E
Coronavirus OC43
Coronavirus HKU1

J Clin Microbiol. 46:3056-3062; 2008

AMERICAN SOCIETY FOR MICROBIOLOGY | Journal of Clinical Microbiology®

COMMENTARY

Use of Rapid Diagnostics To Manage Pediatric Bloodstream Infections? You Bet Your ASP!

Mark D. Gonzalez,[a] Melanie L. Yarbrough[b]

[a]Department of Pathology and Laboratory Services, Children's Healthcare of Atlanta, Atlanta, Georgia, USA

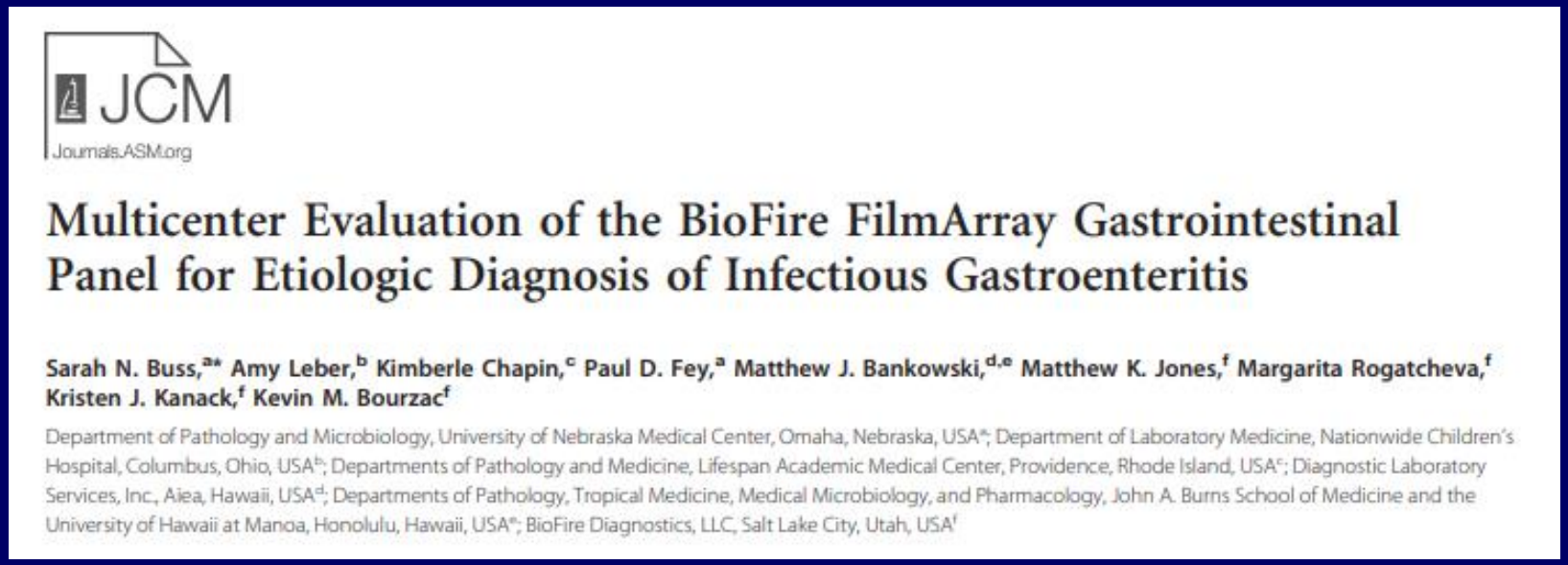
[b]Department of Pathology & Immunology, Washington University School of Medicine, St. Louis, Missouri, USA

TABLE 1 Summary of targets from the Verigene Gram-positive and Gram-negative blood culture tests

	Targets for Verigene:	
Analyte	**Gram-positive blood culture test**	**Gram-negative blood culture test**
Genus-level identification	*Listeria* spp., *Staphylococcus* spp., *Streptococcus* spp.	*Acinetobacter* spp., *Citrobacter* spp., *Enterobacter* spp., *Proteus* spp.
Species/group-level identification	*Enterococcus faecalis*, *Enterococcus faecium*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus lugdunensis*, *Streptococcus agalactiae*, *Streptococcus anginosus* group, *Streptococcus pneumoniae*, *Streptococcus pyogenes*	*Escherichia coli*,[a] *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Pseudomonas aeruginosa*
Antibiotic resistance markers	*mecA*, *vanA*, *vanB*	bla_{CTX-M}, bla_{IMP}, bla_{KPC}, bla_{NDM}, bla_{VIM}, bla_{OXA}

[a]The Verigene Gram-negative blood culture test does not distinguish between *E. coli* and *Shigella* spp.

J Clin Microbiol. 58:e02082-19; 2020



JCM
Journals.ASM.org

Multicenter Evaluation of the BioFire FilmArray Gastrointestinal Panel for Etiologic Diagnosis of Infectious Gastroenteritis

Sarah N. Buss,[a*] Amy Leber,[b] Kimberle Chapin,[c] Paul D. Fey,[a] Matthew J. Bankowski,[d,e] Matthew K. Jones,[f] Margarita Rogatcheva,[f] Kristen J. Kanack,[f] Kevin M. Bourzac[f]

Department of Pathology and Microbiology, University of Nebraska Medical Center, Omaha, Nebraska, USA[a]; Department of Laboratory Medicine, Nationwide Children's Hospital, Columbus, Ohio, USA[b]; Departments of Pathology and Medicine, Lifespan Academic Medical Center, Providence, Rhode Island, USA[c]; Diagnostic Laboratory Services, Inc., Aiea, Hawaii, USA[d]; Departments of Pathology, Tropical Medicine, Medical Microbiology, and Pharmacology, John A. Burns School of Medicine and the University of Hawaii at Manoa, Honolulu, Hawaii, USA[e]; BioFire Diagnostics, LLC, Salt Lake City, Utah, USA[f]

Aeromonas spp.
Campylobacter spp. (*jejuni*, *coli*, *upsaliensis*)
Clostridioides difficile toxin A, B
Plesiomonas shigelloides
Salmonella spp.
Vibrio spp. (*parahaemolyticus*, *vulnificus*)
Yersinia enterocolitica
Cryptosporidium spp.
Cyclospora cayetanensis
Entamoeba histolytica
Giardia lamblia

Yersinia enterocolitica
Enteroaggregative *E. coli*
Enteropathogenic *E. coli*
Enterotoxigenic *E. coli*
Enteroinvasive *E. coli* / *Shigella* spp.
Shiga-like toxin-producing *E. coli*
Adenovirus F 40/41
Astrovirus
Norovirus GI/GII
Rotavirus A
Sapovirus

J Clin Microbiol. 53:915-925; 2015


SOAP

I-Clicker Question 1

I-CLICKER QUESTION 1

Does your laboratory offer syndromic molecular panels on its test menu?

A. No, we don’t.

B. We don’t, but we want to.

C. Yes, we offer 1-2 of the tests that Erik just mentioned.

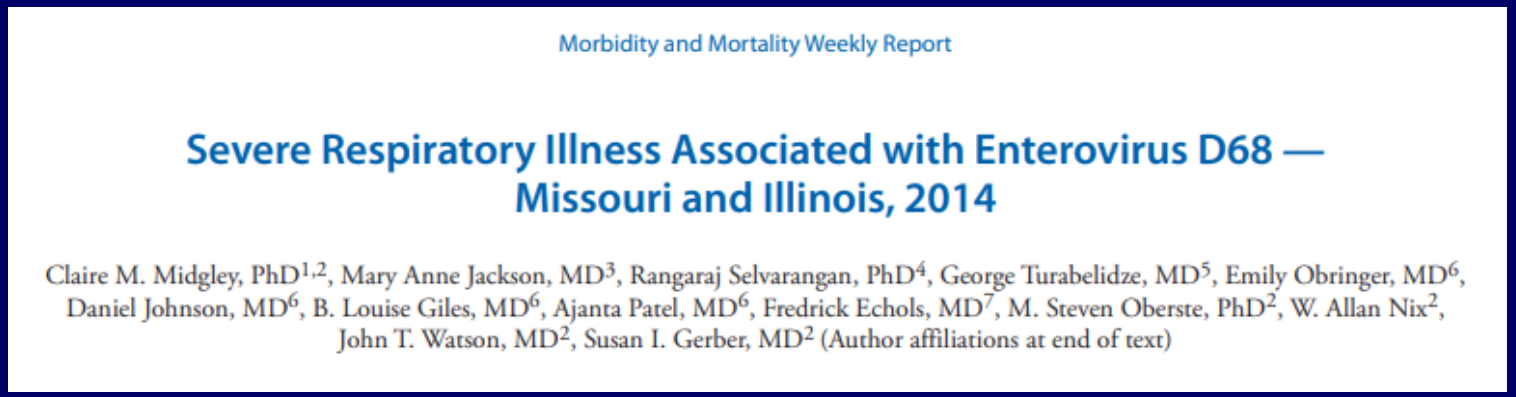
D. Yes, we offer 3-4 of the tests that Erik just mentioned.

E. I’m a bit scared about the surprise already.

Why beneficial?

BENEFIT #1: UNKNOWN OUTBREAK

- Respiratory illness in late summer 2014; variety of agents suspected at that time of year
- Identified with assistance of syndromic panel



Morbidity and Mortality Weekly Report

Severe Respiratory Illness Associated with Enterovirus D68 — Missouri and Illinois, 2014

Claire M. Midgley, PhD[1,2], Mary Anne Jackson, MD[3], Rangaraj Selvarangan, PhD[4], George Turabelidze, MD[5], Emily Obringer, MD[6], Daniel Johnson, MD[6], B. Louise Giles, MD[6], Ajanta Patel, MD[6], Fredrick Echols, MD[7], M. Steven Oberste, PhD[2], W. Allan Nix[2], John T. Watson, MD[2], Susan I. Gerber, MD[2] (Author affiliations at end of text)

- Ruling out known agents → emerging agent

MMWR Morb Mortal Wkly Rpt. 63:798-799; 2014

J Clin Microbiol. 53:3110-3115; 2015

BENEFIT #2: EXPAND MENU

- Central nervous system disease
- Past FDA clearance limited to enterovirus, HSV
- 145 neonatal specimens

  17 additional pathogens identified by panel
  Human parechovirus PCR not requisitioned in 7 of the 11 eventual detections

- Provider satisfaction (also in non-3º hospitals)

Open Forum Infect Dis. 4:S8; 2017

J Clin Microbiol. 56:e00018; 2018

BENEFIT #3: PATIENT SATISFIER

- Respiratory panel
- ER patients not there “for the sniffles”; illness perceived as severe
- Notification of diagnosis within 1-2 hours
- Publication of patient satisfaction surveys may influence choice of care provider in a competitive environment

J Clin Microbiol. 53:3110-3115; 2015

BENEFIT #4: MANAGEMENT (I)

- Central nervous system disease
- 8-12% fatality rate associated with bacterial
  <1% fatality rate associated with viral meningitis
- *Listeria* would not respond to empiric Rx
- Wisconsin study reported a 15-hour decrease in time to targeted therapy

Open Forum Infect Dis. 4:S8; 2017

J Clin Microbiol. 56:e00018; 2018

BENEFIT #4: MANAGEMENT (II)

- Lower severity (and absence of antimicrobial therapy) for viral meningitis
- Length of stay shortened to mean 3.7 days (viral)
  Length of stay mean 16.6 days for bacterial
- Neonatal length of stay

  44h when using syndromic panel
  72h when no viral agent detected by conventional

Open Forum Infect Dis. 4:S8; 2017

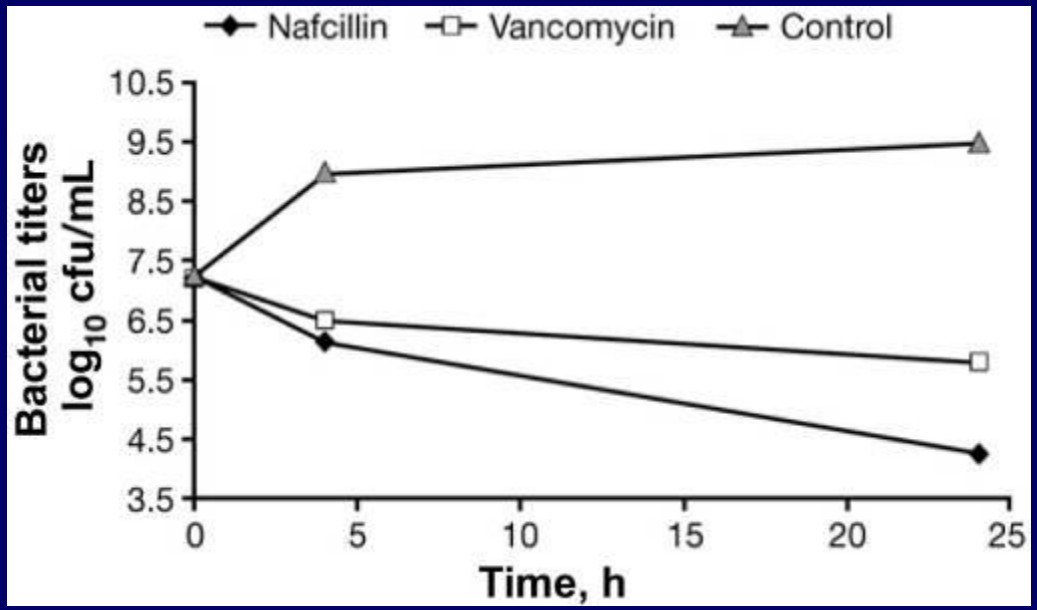
J Clin Microbiol. 56:e00018; 2018

BENEFIT #5: INTERVENTION (I)

Higher treatment failure rate in MSSA bacteremic patients treated with vancomycin than in those treated with nafcillin

REMEMBER...

	Nafcillin	Vancomycin
MSSA	Susceptible	Susceptible
MRSA	**Resistant**	Susceptible



Clin Infect Dis. 42 (suppl 1):S51-S57; 2006

BENEFIT #5: INTERVENTION (II)

Think Health Act Now!
CITY OF MILWAUKEE
HEALTH DEPARTMENT

How does critical thinking enter this conversation?

SYNDROMIC (MOLECULAR) PANELS

- Simultaneously tests for multiple pathogens on basis of site of illness (“shotgunning”)
- Can encompass multiple disciplines of microbiology

  Bacteriology Mycology
  Virology Parasitology

- Can become rather expen$ive
- Laboratories worry about reimbur$ement
- Difficult for laboratories to validate these panels

COMPREHENSIVE GI PANEL

Campylobacter spp.
Clostridioides difficile toxin
Plesiomonas spp.
Salmonella spp.
Yersinia spp.
Vibrio spp.
Vibrio cholerae
E. coli (aggregative)
E. coli (pathogenic)
E. coli (toxigenic)
E. coli (invasive)
E. coli O157
E. coli (shiga toxin)

Cryptosporidium spp.
Cyclospora spp.
Entamoeba histolytica
Giardia spp.

Adenovirus
Astrovirus
Norovirus
Rotavirus
Sapovirus

COMPREHENSIVE GI PANEL

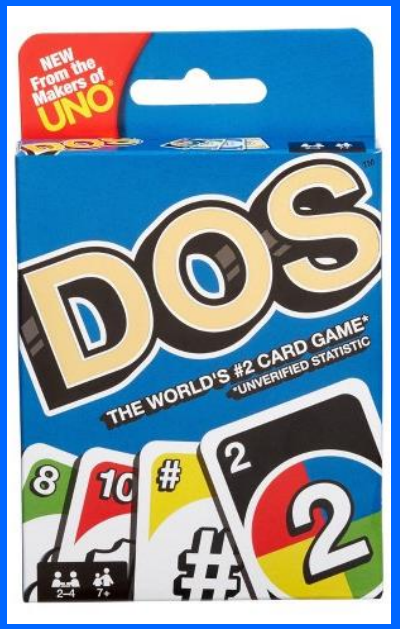
Campylobacter spp.	negative
Clostridioides difficile toxin	negative
Plesiomonas spp.	negative
Salmonella spp.	negative
Yersinia spp.	negative
Vibrio spp.	negative
Vibrio cholerae	negative
E. coli (aggregative)	negative
E. coli (pathogenic)	negative
E. coli (toxigenic)	negative
E. coli (invasive)	negative
E. coli O157	negative
E. coli (shiga toxin)	negative

Cryptosporidium spp.	negative
Cyclospora spp.	negative
Entamoeba histolytica	negative
Giardia spp.	negative
Adenovirus	negative
Astrovirus	negative
Norovirus	POSITIVE
Rotavirus	negative
Sapovirus	negative

WATCH OUT

Campylobacter spp.	negative
Clostridioides difficile toxin	POSITIVE
Plesiomonas spp.	negative
Salmonella spp.	negative
Yersinia spp.	negative
Vibrio spp.	negative
Vibrio cholerae	negative
E. coli (aggregative)	negative
E. coli (pathogenic)	negative
E. coli (toxigenic)	negative
E. coli (invasive)	negative
E. coli O157	negative
E. coli (shiga toxin)	negative

Cryptosporidium spp.	negative
Cyclospora spp.	negative
Entamoeba histolytica	negative
Giardia spp.	negative
Adenovirus	negative
Astrovirus	negative
Norovirus	POSITIVE
Rotavirus	negative
Sapovirus	negative



I-Clicker Question 2

I-CLICKER QUESTION 2

What do YOU do when your multiplex gastrointestinal panel detects *C. difficile* along with an agent of GI distress?

A. Report them both.

B. Report the most likely culprit (tell me which one).

C. Call the provider.

D. This situation never happens to us.

E. Send an Email to the inaugural winner of the Shult Award.

WHAT WOULD THE EiC DO?

"Would you recommend routine, simultaneous testing for the following combinations of pathogens?

Stool samples for *C. difficile* in combination with norovirus, *Salmonella*, *Campylobacter* species, and Shiga-toxin producing *E. coli* "

J Clin Microbiol. 53:3110-3115; 2015

FIXED PANELS CAN BE A PROBLEM

- Consider your patient population

  Age
  Previous Abx
  Hospital admission
  Cancer chemotherapy
  Gastrointestinal surgery
  GI tract manipulation

- Minority needs *C. difficile* + other GI agent testing

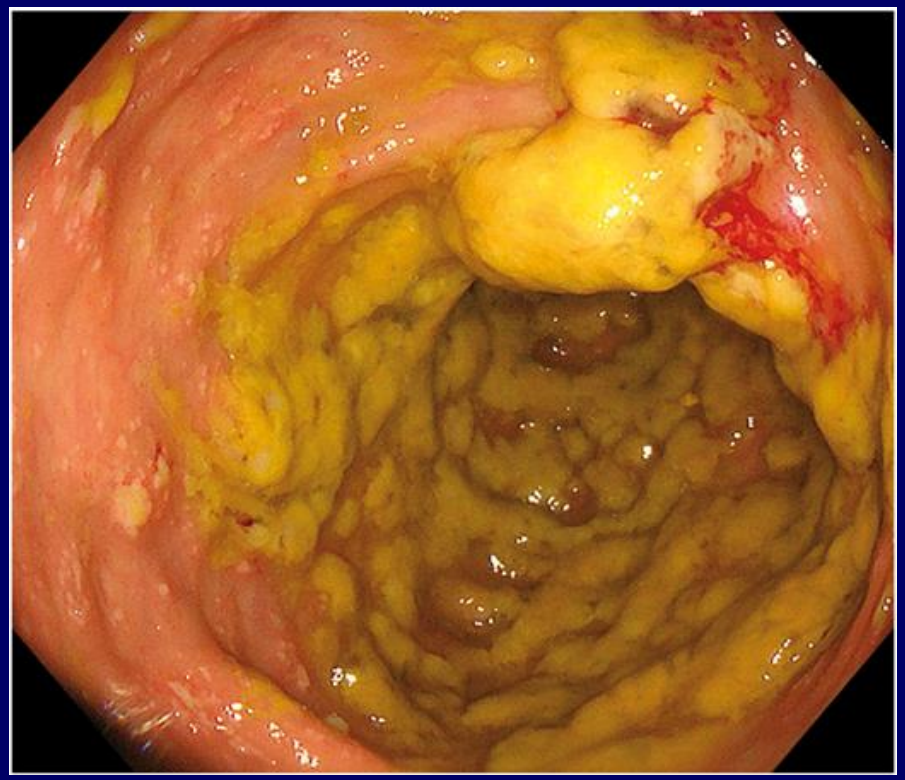
- Colonization of toxigenic *C. difficile*

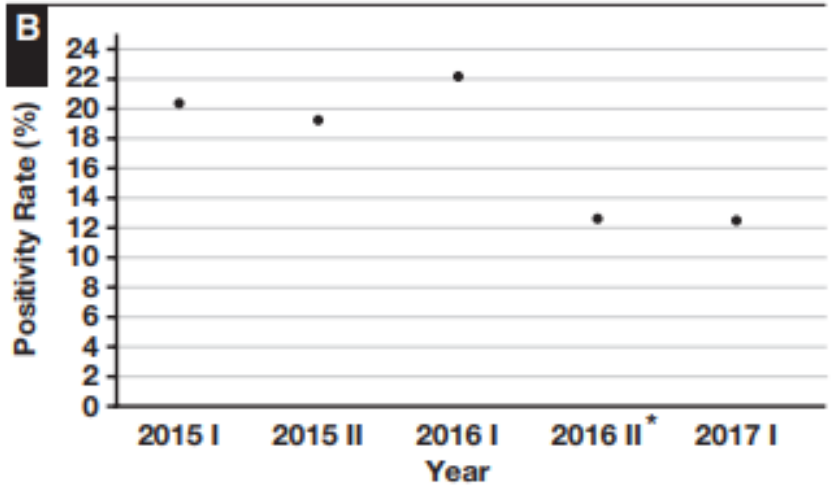
  Young children
  Children with inflammatory bowel disease

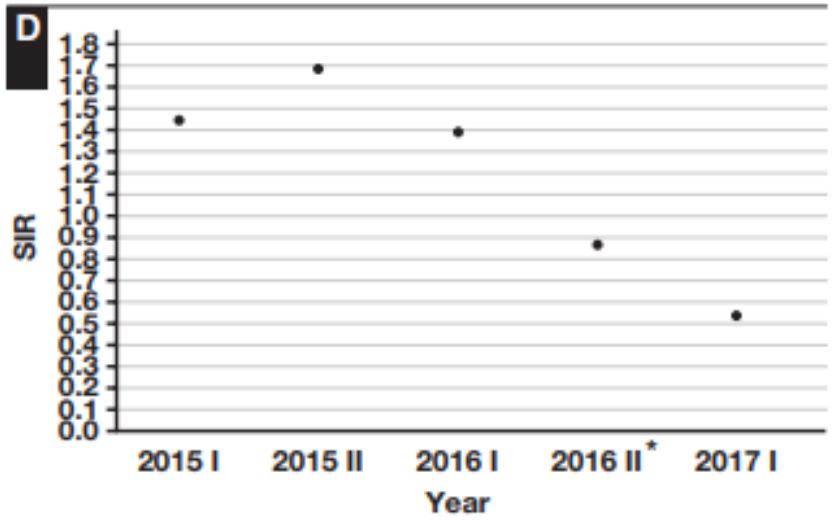
J Clin Microbiol. 53:3110-3115; 2015

Outcome of Electronic Order Alert Intervention Relative to Toxigenic *Clostridium difficile* PCR Analysis and Hospital-Onset *C difficile* Infection in a Multihospital Health Care System

Sonia Rodriguez, RN, BSN,[3] Nancy Riederer, RN, BSN,[4] Kimber L. Munson, PhD,[5] Denise Block, RN, BSN, CIC,[4] Gayle Land, RN, BSN, CIC,[4] Rosalyn Stone, RN, BSN, CIC,[4] Aurora Villalobos, RN, BSN, CIC,[4] Erin Dewey, MLS(ASCP),[1] and Timothy K. Block, MT(ASCP)[2]







Am J Clin Pathol. 151:622-627; 2019

SHARED SPECIMEN

Parameter	Bacterial Meningitis	Viral Meningitis
Estimated annual incidence in U.S.	4,000	30,000 to 50,000
CSF leukocytes	Neutrophils	Lymphocytes
CSF glucose	Low	Normal
CSF protein	Elevated	Elevated (slight)
CSF direct smear and culture	Usually positive	Negative

Additional assessment for erythrocytes, turbidity

AMERICAN SOCIETY FOR MICROBIOLOGY | Journal of Clinical Microbiology

Multicenter Evaluation of BioFire FilmArray Meningitis/Encephalitis Panel for Detection of Bacteria, Viruses, and Yeast in Cerebrospinal Fluid Specimens

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Streptococcus agalactiae
Cryptococcus neoformans

Cytomegalovirus
Enterovirus
Herpes simplex virus type 1
Herpes simplex virus type 2
Human herpes virus-6
Human parechovirus
Varicella-zoster virus

METHODS

- Eleven US medical centers over 8-month period
- 1560 prospective CSF subjected to Film Array

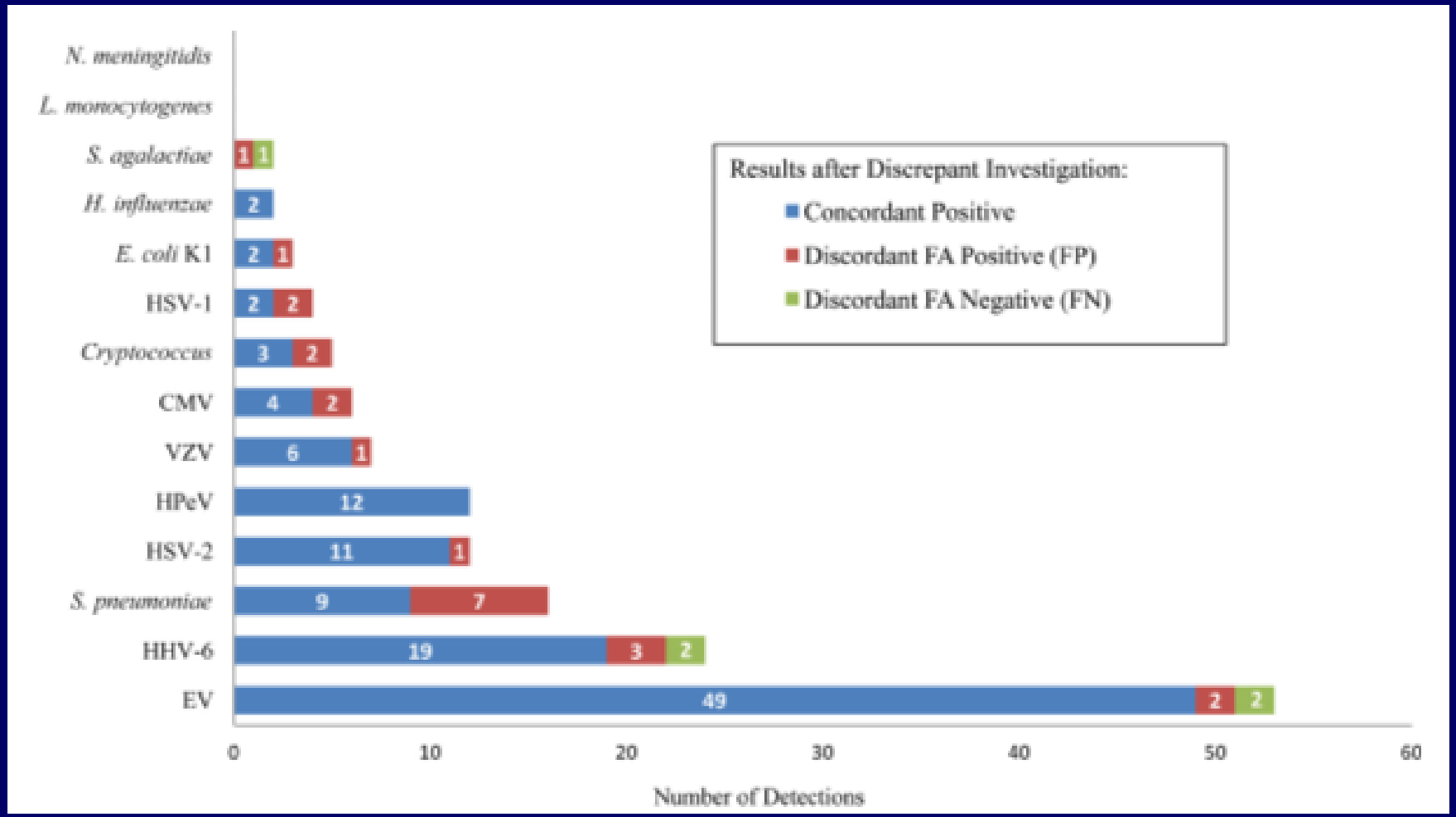
  Compared to culture (for bacteria)
  Compared to individual PCR (for viruses, yeast)

- 9.0% overall detection rate

  22 bacteria detected
  114 viruses detected
  5 yeast detected

J Clin Microbiol. 54:2251-2261; 2016

>90% sensitivity for most analytes
>99% specificity for all analytes



J Clin Microbiol. 54:2251-2261; 2016

WATCH OUT

- 3/14 targets had ≥10 positive reference specimens
  [16/22 targets had ≥10 positive reference specimens for GI panel]
- Detections of unknown clinical significance

  HHV-6 — Commonly encountered in childhood
  Found in up to 40% normal brain tissue
  Second-most-frequent positive result

  CMV — High seroprevalence; latency in WBC

  C_T not available for Film Array
- General prevalence

J Clin Microbiol. 56:e00018; 2018

LAB MATH (population)

$$\text{Positive Predictive Value} = \frac{\text{true positives}}{\text{true positives + false positives}}$$

$$\text{Negative Predictive Value} = \frac{\text{true negatives}}{\text{true negatives + false negatives}}$$

INFLUENCE OF PREVALENCE

- Assay with 87.0% sensitivity; 90.0% specificity
- Test 1000 people in two scenarios

20% prevalence	2% prevalence
200 true-positives	20 true-positives
77 false-positives	98 false-positives
693 true-negatives	879 true-negatives
30 false-negatives	3 false-negatives
72.2% Positive Predictive Value	16.9% Positive Predictive Value
95.8% Negative Predictive Value	99.7% Negative Predictive Value

CANNOT REPLACE OTHER ASSAYS

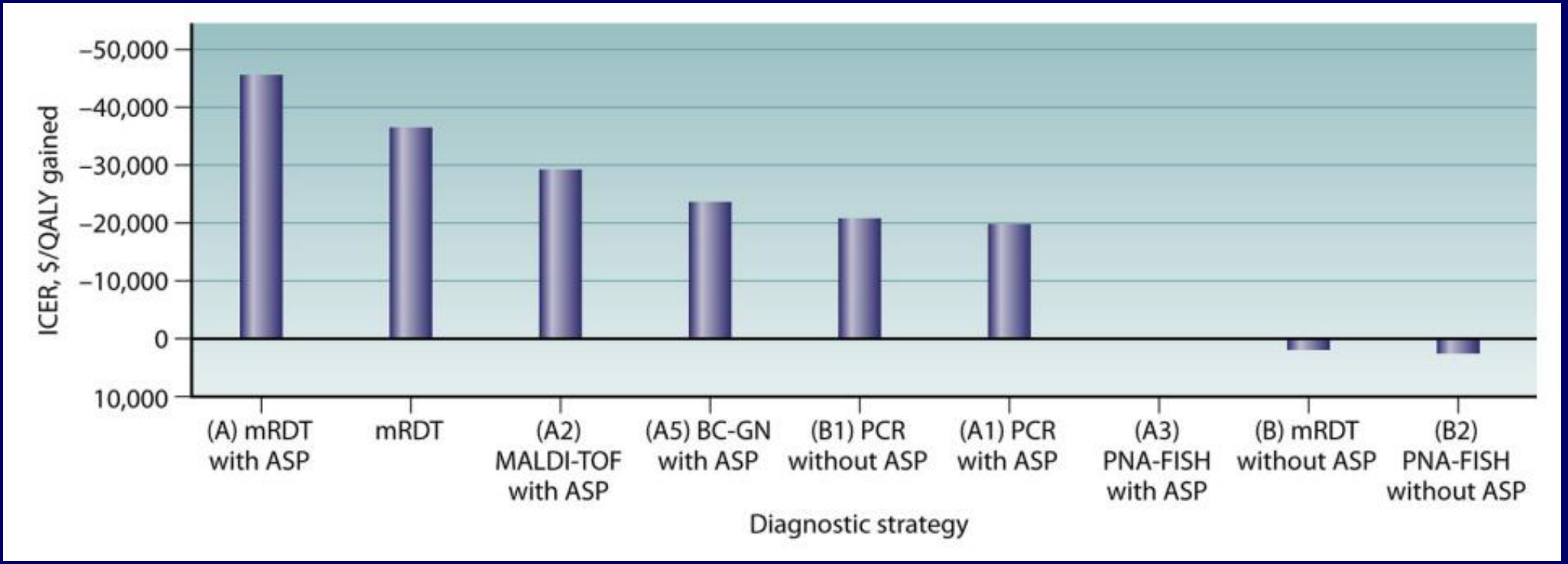
- Gram stain essential for interpreting PCR results
- Culture still needed for non-targeted organisms and antimicrobial susceptibility testing
- Cryptococcal antigen still very good
- Targeted PCR testing in i'competent adults may be more cost-effective

  CSF nucleated cell counts could be a way to minimize unnecessary testing in i'competent adults

J Clin Microbiol. 54:2222-2224; 2016

POSITIVE BLOOD CULTURES

Savings per death averted



Clin Microbiol Rev. 31:e00095-17; 2018

Clinical Infectious Diseases

MAJOR ARTICLE

The Effect of Molecular Rapid Diagnostic Testing on Clinical Outcomes in Bloodstream Infections: A Systematic Review and Meta-analysis

Tristan T. Timbrook,[1,4] Jacob B. Morton,[1,4] Kevin W. McConeghy,[2] Aisling R. Caffrey,[1,2,4] Eleftherios Mylonakis,[3] and Kerry L. LaPlante[1,2,4]

[1]Rhode Island Infectious Diseases Research Program, Providence Veterans Affairs Medical Center, [2]Center of Innovation in Long Term Services and Supports, Providence Veterans Affairs Medical Center, [3]Infectious Diseases Division, Warren Alpert Medical School of Brown University, Providence, and [4]College of Pharmacy, University of Rhode Island, Kingston

Mortality Risk [Odds Ratios (95% confidence interval)]

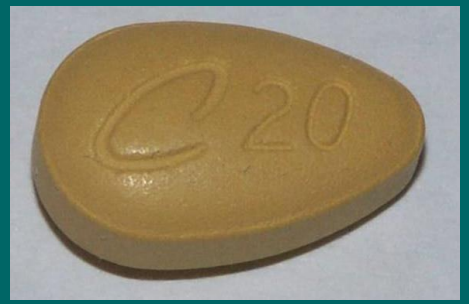
mRDT vs. conventional microbiology	0.66 (0.54-0.80)
mRDT with antimicrobial stewardship	0.64 (0.51-0.79)
mRDT w/out antimicrobial stewardship	0.72 (0.46-1.12)

Time to effective Rx ↓ 5.03 hours; LOS ↓ 2.48 days

Clin Infect Dis. 64:15-23; 2017

Surprise


#DontbTriched
by this tiny parasite named Trichomoniasis


C20


VIAGRA
(sildenafil citrate)

"You know, ever since we started doing your new Trich test, we still notice guys with obvious urethritis, but still have negative results for chlamydia, gonorrhea, and Trich. I really think that it's *Mycoplasma*; can you test for this?"

R. Gremminger, M.D.
circa 2010


ROAD
HAZARD
AHEAD


NIH
National Library of Medicine
National Center for Biotechnology Information
Log in
PubMed®
Search
Advanced
PubMed® comprises more than 35 million citations for biomedical literature from MEDLINE, life science journals, and online books.
Citations may include links to full text content from PubMed Central and publisher web sites.


ROAD
FLOODED


ROUGH
ROAD


DANGER


UFO
PRESENCE

J Infect Chemother (2012) 18:494–500
DOI 10.1007/s10156-011-0362-7

ORIGINAL ARTICLE

CAUTION I

Evaluation of Seeplex® STD6 ACE Detection kit for the diagnosis of six bacterial sexually transmitted infections

Abstract Traditionally, the diagnosis of bacterial sexually transmitted infection (STI) has been dependent on the isolation of the causative pathogens by culturing endocervical or urethral swab specimens on selective media. While such procedures typically provide excellent diagnostic accuracy, they are often time-consuming and expensive. Amultiplex polymerase chain reaction (PCR) assay, based on a semi-automated detection system, was evaluated for the detection of six STI causative organisms. The Seeplex® STD6 ACE (auto-capillary electrophoresis) Detection assay employed six pairs of dual priming oligonucleotide (DPO™) primers specifically targeted to unique genes of *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Mycoplasma genitalium*, *Ureaplasma urealyticum*, *Mycoplasma hominis*, and *Trichomonas vaginalis*. A total of 739 specimens (304 cervical swabs and 435 urine samples) collected for 4 months were tested, and results were compared to those obtained with a combined monoplex PCR. The concordance between the multiplex PCR and monoplex PCR assay was 100% for both sensitivity and specificity. We also tested for the presence of two pathogenic bacteria (*C. trachomatis* and *N. gonorrhoeae*) and compared the results obtained with the multiplex PCR and BD ProbeTec duplex strand displacement amplification (SDA). The results of the multiplex PCR and duplex SDA were 99.7% concordant for *C. trachomatis* and 100% concordant for *N. gonorrhoeae*. The multiplex PCR assay using the Seeplex® STD6 ACE Detection kit proved to be a novel cost-effective and fast diagnostic tool with high sensitivity and specificity for the simultaneous detection of six STI pathogens.

Keywords Diagnosis · Sexually transmitted infection · Bacterial and parasite infection · Multiplex PCR

SUBOPTIMAL REFERENCE METHOD

Monoplex PCR assay

In contrast to multiplex PCR, only one pair of primers was used to detect the target organism in the monoplex PCR using Seegene DPO™ technology [12]. PCR amplification was performed with the Seeplex® *C. trachomatis* Detection kit, Seeplex® *N. gonorrhoeae* Detection kit, Seeplex® *M. genitalium* Detection kit, Seeplex® *U. urealyticum* Detection kit, Seeplex® *M. hominis* Detection kit, and Seeplex® *T. vaginalis* Detection kit (Seegene) respectively, according to the manufacturer's instructions. The internal control was present in the PCR mixture. Therefore, the internal control was used as the sole check for possible PCR inhibition.

Table 1 Comparison of results between multiplex polymerase chain reaction (PCR) and monoplex PCR ($n = 739$)

Target pathogen	Monoplex PCR	Multiplex PCR	
		Positive	Negative
Chlamydia trachomatis	Positive	40	0
	Negative	0	699
Neisseria gonorrhoeae	Positive	32	0
	Negative	0	707
Mycoplasma genitalium	Positive	2	0
	Negative	0	737
Ureaplasma urealyticum	Positive	157	0
	Negative	0	582
Mycoplasma hominis	Positive	82	0
	Negative	0	657
Trichomonas vaginalis	Positive	7	0
	Negative	0	732

J Infect Chemother. 18:494-500; 2012

CAUTION II

Original research article

INTERNATIONAL JOURNAL OF STD & AIDS

International Journal of STD & AIDS
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Multiplex PCR testing for nine different sexually transmitted infections

Abstract

Current sexually transmitted infection (STI) testing is not optimal due to delays in reporting or missed diagnoses due to a lack of comprehensive testing. The FilmArray® (BioFire Diagnostics, LLC, Salt Lake City, Utah) is a user-friendly, fully automated, multiplex PCR system that is being developed for rapid point-of-care use. A research-use-only STI panel including multiple PCR primer sets for each organism was designed to detect *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Treponema pallidum*, *Trichomonas vaginalis*, *Mycoplasma genitalium*, *Ureaplasma urealyticum*, *Haemophilus ducreyi*, and herpes simplex virus (HSV) types 1 and 2. Standard clinical testing included Gram stain, nucleic acid amplification, wet mount examination, herpes simplex virus culture, and syphilis IgG. Standard clinical tests were not available for all the organisms tested by the FilmArray STI panel. Two hundred and ninety-five clinical specimens from 190 subjects were directly compared to standard testing. Urine ($n = 146$), urethral/cervical swabs (31), oral swabs (60), rectal swabs (43), and ulcer swabs (15) were tested. Among the tested samples, FilmArray detected *C. trachomatis* in 39 (13%), *N. gonorrhoeae* in 20 (7%), *T. vaginalis* in nine (3%), HSV 1 in five (2%), HSV 2 in five (2%), *U. urealyticum* in 36 (12%), *M. genitalium* in eight (3%), and *T. pallidum* in 11 (4%). Concordance between the FilmArray STI panel and standard nucleic acid amplification testing for *C. trachomatis* was 98% and for *N. gonorrhoeae* was 97%. Multiplex PCR STI testing has the potential to improve public health by providing rapid, sensitive, and reliable results within the clinic or nearby laboratory.

Keywords

FilmArray, sexually transmitted diseases, sexually transmitted infections, STI, diagnostic test performance, multiplex PCR

NO REFERENCE METHOD

Table 1. Standard clinical testing methods employed at the [redacted] clinic at the time of the study. Urethral Gram stains were used in patients with suspected urethritis, in addition to nucleic acid amplification testing (NAAT). NAAT for *T. vaginalis* was not employed as a standard clinical test due to the expense and low prevalence of this disease in the patient population. Amplification testing for *M. genitalium* and *U. urealyticum* were not commercially available at the time of the study. *H. ducreyi* has not been identified in this population.

Organism	Standard testing
Chlamydia trachomatis	NAAT (Roche Amplicor)
Neisseria gonorrhoeae	a) Urethral Gram stain, b) NAAT (Roche Amplicor)
Treponema pallidum	a) Syphilis IgG (Captia), b) RPR staging of all IgG positives c) TP-PA tie breaker, if necessary (reverse sequence syphilis screening)
Trichomonas vaginalis	Wet mount examination
HSV1	HSV culture
HSV2	HSV culture
Mycoplasma genitalium	None
Ureaplasma urealyticum	None
Haemophilus ducreyi	Clinical examination, Gram stain

RPR: rapid plasma reagin; HSV: herpes simplex virus.

Int J STD AIDS 27:1275-1282; 2016

LOW NUMBERS

Table 1. Standard clinical testing methods employed at the [redacted] clinic at the time of the study. Urethral Gram stains were used in patients with suspected urethritis, in addition to nucleic acid amplification testing (NAAT). NAAT for *T. vaginalis* was not employed as a standard clinical test due to the expense and low prevalence of this disease in the patient population. Amplification testing for *M. genitalium* and *U. urealyticum* were not commercially available at the time of the study. *H. ducreyi* has not been identified in this population.

Organism	Standard testing
Chla	
Neis	
Trep	
Trich	
HSV	
HSV	
Myc	
Urea	
Hae	
RPR:	

Table 4. Results for specimens (by type) tested by the FilmArray STI panel. Two hundred and ninety-five specimens from 190 subjects were selected for testing on the FilmArray device. The table header shows the total number of samples tested for each specimen type. The table body shows the number of tested specimens that were positive by the FilmArray.

Organism	Urine $n=146$	Urethral/cervical swab $n=31$	Rectal swab $n=43$	Oral swab $n=60$	Ulcer swab $n=15$	Total $n=295$
Chlamydia trachomatis	23	5	10	1	0	39
Neisseria gonorrhoeae	9	1	6	4	0	20
Treponema pallidum	2	0	2	2	5	11
Trichomonas vaginalis	5	3	0	1	0	9
HSV1 or HSV2	5	1	2	1	1	10
Mycoplasma genitalium	5	1	1	1	0	8
Ureaplasma urealyticum	9	9	10	8	0	36
Haemophilus ducreyi	0	0	0	0	0	0
Total	58	20	31	18	6	133

Int J STD AIDS 27:1275-1282; 2016

APMIS
ACTA PATHOLOGICA, MICROBIOLOGICA
ET IMMUNOLOGICA SCANDINAVICA

APMIS 123: 879–886

DOI 10.1111/apm.12430

CAUTION III

Evaluation of the new AmpliSens multiplex real-time PCR assay for simultaneous detection of *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, *Mycoplasma genitalium*, and *Trichomonas vaginalis*

In this study, we performed an evaluation of the new CE-marked multiplex real-time AmpliSens N.gonorrhoeae/C.trachomatis/M.genitalium/T.vaginalis-MULTIPRIME-FRT PCR assay compared to APTIMA tests, i.e., APTIMA COMBO 2 assay, APTIMA *Trichomonas vaginalis* assay (FDA-approved), and two different APTIMA *Mycoplasma genitalium* assays (research use only; one of them only used for discrepancy analysis). Vaginal swabs (n = 209) and first-void urine (FVU) specimens from females (n = 498) and males (n = 554), consecutive attendees (n = 1261) at a dermatovenerological clinic in Sweden, were examined. The sensitivity of the AmpliSens PCR assay for detection of *C. trachomatis* (6.3% prevalence), *M. genitalium* (5.7% prevalence), *N. gonorrhoeae* (0.3% prevalence), and *T. vaginalis* (0.08% prevalence) was 97.5% (95% confidence interval (CI): 91.2–99.6%), 81.9% (95% CI: 70.7–89.7%), 100% (95% CI: 40.2–100%) and 100% (95% CI: 16.5–100%), respectively. The specificity of the AmpliSens PCR assay was 100% (95% CI: 99.6–100%) for all agents. The analytical sensitivity and specificity for *N. gonorrhoeae* detection was excellent, i.e., 55 international gonococcal strains detected and 135 isolates of 13 non-gonococcal Neisseria species were negative. In conclusion, the multiplex real-time AmpliSens N.gonorrhoeae/C.trachomatis/M.genitalium/T.vaginalis-MULTIPRIME-FRT PCR assay demonstrated high sensitivity and excellent specificity for the detection of *C. trachomatis*, *N. gonorrhoeae*, and *T. vaginalis*, and excellent specificity but suboptimal sensitivity for *M. genitalium* detection.

Key words: Sexually transmitted infections; AmpliSens; APTIMA COMBO 2 assay; APTIMA *Trichomonas vaginalis* assay; APTIMA *Mycoplasma genitalium* assay.

DISCREPANCY PREPONDERANCE

Table 2. True positive and negative results and the results obtained using the AmpliSens multiplex real-time PCR assay, divided into specimen type

True results[1]	AmpliSens result	No. of samples			
		Chlamydia trachomatis	*Neisseria gonorrhoeae*	*Mycoplasma genitalium*	*Trichomonas vaginalis*
Vaginal samples					
+	+	12	0	13	0
–	–	197	209	192	209
+	–	0	0	4	0
–	+	0	0	0	0
Total		209	209	209	209
FVU, females					
+	+	28	2	22	1
–	–	469	496	472	497
+	–	1	0	4	0
–	+	0	0	0	0
Total		498	498	498	498
FVU, males					
+	+	38	2	24	0
–	–	515	552	525	554
+	–	1	0	5	0
–	+	0	0	0	0
Total		554	554	554	554
Overall results					
+	+	78	4	59	1
–	–	1181	1257	1189	1260
+	–	2	0	13	0
–	+	0	0	0	0
Total		1261	1261	1261	1261

APMIS 123:879-886; 2015

PERFORMANCE INDICES

Diagnostic method and specimen	Number of specimens				Sensitivity		Specificity		Predictive value (%)	
	True positive	False positive	False negative	True negative	%	95% CI	%	95% CI	Positive	Negative
	Infected patient status algorithm									
Culture	12	0	0	286	100	69.9-100	100	98.3-100	100	100
PCR - Urethral swab	11	13	1	273	91.7	59.8-99.6	95.5	92.2-97.5	45.8	99.6
PCR - Urine	11	9	1	277	91.7	59.8-99.6	96.9	93.9-98.5	55.0	99.6
ATV - Urethral swab	11	38	1	248	91.7	59.8-99.6	86.7	82.1-90.3	22.5	99.6
ATV - Urine	11	23	1	263	91.7	59.8-99.6	91.9	88.0-94.7	32.1	99.6
	Molecular resolved algorithm									
Culture	12	0	30	256	28.6	16.2-44.8	100	98.2-100	100	89.4
PCR - Urethral swab	23	0	19	256	54.8	38.8-69.8	100	98.2-100	100	93.1
PCR - Urine	20	0	22	256	47.6	32.3-63.4	100	98.2-100	100	92.0
ATV - Urethral swab	40	9	2	247	95.2	82.6-99.2	96.5	93.2-98.3	81.7	99.2
ATV - Urine	31	4	11	252	73.8	57.7-85.6	98.4	95.8-99.5	88.6	95.8

Am J Obstet Gynecol. 200:188.e1-188.e7; 2009 (adapted)

I-Clicker Question 3

I-CLICKER QUESTION 3

Does your laboratory test for organisms such as (genital) *Mycoplasma hominis* and *Ureaplasma urealyticum*?

A. Yes, we routinely do so.

B. Yes, but we do not get requests for these very often.

C. No, we do not offer this testing.

D. Sort of; this is a send-out test.

E. I need more pastries.

Mollicutes PATHOGENICITY

CAUTION IV

Disease	Causality by:		Comments
	U. urealyticum	*M. hominis*	
NGU	+++	-	*Ureaplasma* proportion unknown
Prostatitis	++	-	no evidence for chronic prostatitis
Epididymitis	+++	-	particularly in HIV-positive
Urinary calculi	++	-	largely animal studies
Pyelonephritis	-	+++	acute cases and exacerbations
Reiter's disease	+	-	more *Ureaplasma* data needed
Involuntary infertility	+	-	role in sperm motility

Mandell Principles and Practice of Infectious Diseases

Mollicutes PATHOGENICITY

Disease	Causality by:		Comments
	U. urealyticum	*M. hominis*	
Low birth weight	-	-	causal relation unproved
Chorioamnionitis	++	-	quoted as “few cases”
Repeated stillbirth/ spontaneous abortion	-	-	causal relation unproved
Involuntary infertility	+	-	also role in sperm motility
Postpartum fever	+	+++	*M. hominis* major cause
Postabortal fever	-	+++	*M. hominis* proportion unknown
PID	-	++	probably small proportion
Vaginitis/vaginosis	-	-	*M. hominis* association with vaginosis
Cervicitis	-	-	NONE
Bartholin abscess	-	-	*M. hominis* involvement doubtful

Mandell Principles and Practice of Infectious Diseases

Diseases Characterized by Urethritis and Cervicitis

Urethritis

Urethritis, as characterized by urethral inflammation, can result from either infectious or noninfectious conditions. Symptoms, if present, include dysuria, urethral pruritis, and mucoid, mucopurulent, or purulent discharge. Signs of urethral discharge on examination can also be present among persons without symptoms. Although *N. gonorrhoeae* and *C. trachomatis* are well established as clinically important infectious causes of urethritis, *M. genitalium* has been strongly associated with urethritis and, less commonly, prostatitis (*691–697*). Data are inconsistent regarding other *Mycoplasma* and *Ureaplasma* species as etiologic agents of urethritis (*707*). The majority of men with *Ureaplasma* infections do not have overt disease unless a high organism load is present.

Cervicitis

Diagnostic Considerations

Because cervicitis might be a sign of upper genital tract infection (e.g., endometritis), women should be assessed for signs of PID and tested for *C. trachomatis* and *N. gonorrhoeae* with NAAT on vaginal, cervical, or urine samples (*553*) (see Chlamydial Infections; Gonococcal Infections). Women with cervicitis also should be evaluated for concomitant BV and trichomoniasis. Because sensitivity of microscopy for detecting *T. vaginalis* is relatively low (approximately 50%), symptomatic women with cervicitis and negative wet-mount microscopy for trichomonads should receive further testing (i.e., NAAT, culture, or other FDA-cleared diagnostic test) (see Trichomoniasis). Testing for *M. genitalium* with the FDA-cleared NAAT can be considered. Although HSV-2 infection has been associated with cervicitis, the utility of specific testing (i.e., PCR or culture) for HSV-2 is unknown. Testing for *U. parvum, U. urealyticum, Mycoplasma hominis*, or genital culture for group B streptococcus is not recommended.

Sexually Transmitted Diseases Treatment Guidelines, 2021

PELVIC INFLAMMATORY DISEASE

- Upper female genital tract inflammatory disorders

  Endometritis
  Salpingitis
  Tubo-ovarian abscess
  Pelvic peritonitis

- *N. gonorrhoeae*, *C. trachomatis* many cases
- Vaginal organisms (*G. vaginalis*, anaerobes, enteric GNR, *H. influenzae*, *S. agalactiae*)
- Some associations with *M. hominis*, *U. urealyticum*, *M. genitalium*, cytomegalovirus

Sexually Transmitted Diseases Treatment Guidelines, 2021

PELVIC INFLAMMATORY DISEASE

- Most-specific diagnostic criteria:

  Histopathologic evidence of endometritis
  Thickened, fluid-filled tubes (MRI, sonography)
  Laparoscopic findings consistent with PID

- Supplemental findings include:

  C. trachomatis, *N. gonorrhoeae* cervical infection
  Abnormal cervical mucopurulent discharge
  Increased leukocytes in vaginal fluid
  Elevated C-reactive protein

Sexually Transmitted Diseases Treatment Guidelines, 2021

PARTICIPANTS: 304 ♀; 435 ♂

Table 1 Comparison of results between multiplex polymerase chain reaction (PCR) and monoplex PCR ($n = 739$)

Target pathogen	Monoplex PCR	Multiplex PCR	
		Positive	Negative
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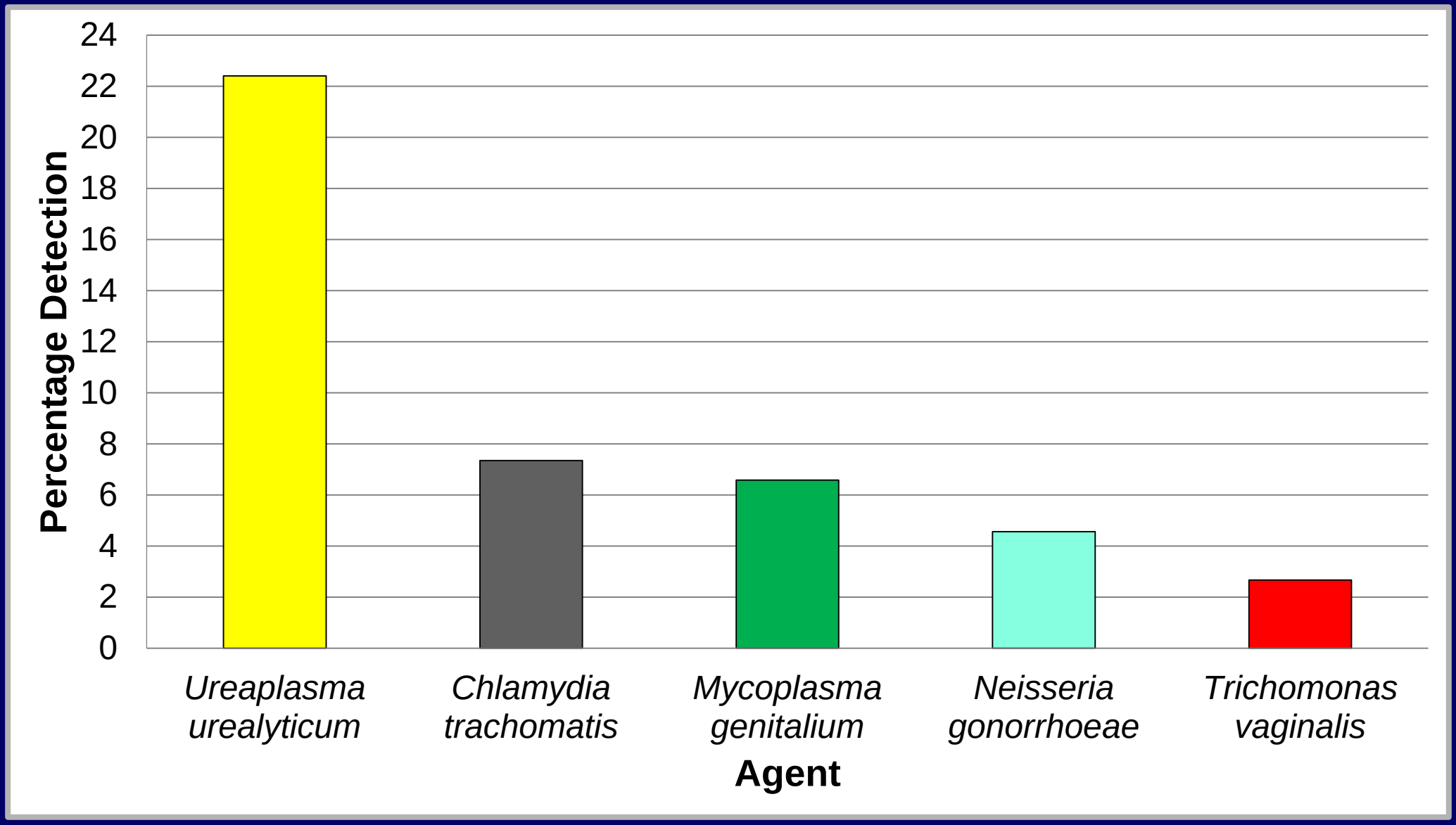
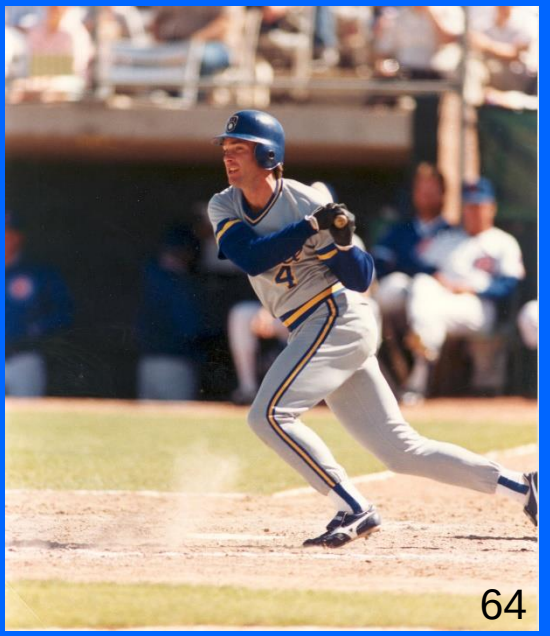
	Negative	0	732

J Infect Chemother. 18:494-500; 2012


Percentage Detection
24
22
20
18
16
14
12
10
8
6
4
2
0
Ureaplasma urealyticum
Chlamydia trachomatis
Mycoplasma genitalium
Neisseria gonorrhoeae
Trichomonas vaginalis
Agent

I-Clicker Question 4



I-CLICKER QUESTION 4

Other than at the height of your SARS-CoV-2 powers or peak respiratory season, do you have a molecular test that exhibits >22% positivity (over a non-seasonal continuum)?

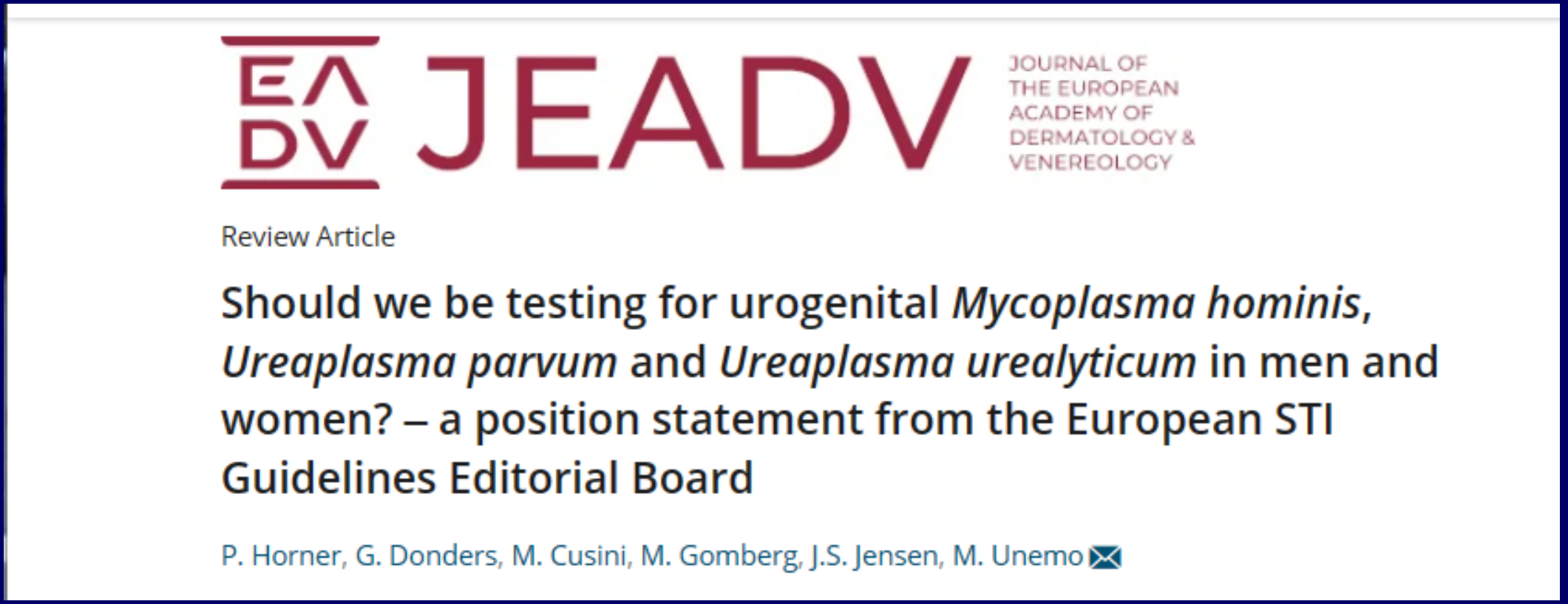
A. No.

B. No, you have a problem with your test.

C. Yes.

D. Yes, and I would love to tell you about it.

E. I want to go to lunch.



Routine testing, treatment of a/symptomatic women and men for *M. hominis*, *U. urealyticum*, *U. parvum* not recommended

Asymptomatic carriage common; majority does not develop disease

Extensive detection and treatment may introduce economic burden (♀)

J Eur Acad Dermatol Venereol. 32:1845-1851; 2018

TAKE HOME

- Benefits do exist within the paradigm of molecular syndromic panels
- Limitations do exist within the paradigm of molecular syndromic panels
- Review literature with a critical eye (how does this impact your offering?)
- Don’t offer a test “just because you can”…
  Determine first if you should