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Ted E. Schutzbank, PhD, D(ABMM)

Laboratory Director

Quantigen LLC

Utility of Syndromic Molecular Panels for Infectious Disease Testing

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Syndromic Infectious Disease Panels

- Detection of organisms specific to distinct syndromes
  - Respiratory infections
  - Gastrointestinal infections
  - CNS disease
  - Sepsis
  - Urinary tract infections (UTI)
  - Women's health
  - Sexually transmitted infections (STI)
- Test panels are not restricted to a specific organism type
  - Bacteria
  - Viruses
  - Fungi
  - Parasites

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Syndromic ID panels – Major Advantages

- Increased diagnostic yield to multiple targets from a single available sample
- Conserve and optimize analysis of rare and difficult samples (e.g. CSF)
- Simplify ordering algorithm
- Streamline workflow in the laboratory while reducing hands-on time
  - Consolidation of multiple flows into a single method
  - reduces turn-around time and labor/reagent/supply costs
- Savings compared to testing for organisms in individual assays
- Standardization of testing

Adapted from E. Palavecino, AACC Clin Lab News, April 1, 2015

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Syndromic ID Panels – Historical Challenges

- False positive results due to cross reactivity or non-specific amplification caused by multiple primers/targets in the reaction
- False negative results due to preferential amplification of one target over another
- Added cost of testing for inappropriate target organisms
- High cost of commercial kits and instruments

Adapted from E. Palavecino, AACC Clin Lab News, April 1, 2015

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Commercially Available Syndromic tests Respiratory Disease

TABLE 3 FDA-approved/cleared multiplex respiratory panels[a]

Parameter	FilmArray	Verigene	x-TAG RVP	x-TAG RVP Fast	NxTAG-RPP	eSensor RVP	ePlex
Analysis platform	FilmArray system or FilmArray Torch	Verigene system	Luminex 100/200	Luminex 100/200	Luminex Magpix	eSensor	ePlex system
No. of targets	20	16	12	8	20	14	17
Ability to detect pathogen							
Viruses							
Adenovirus	✓	✓	✓	✓	✓	✓ (differentiates subgroup B/E from C)	✓
Coronavirus							✓
Coronavirus HKU1	✓				✓		
Coronavirus NL63	✓				✓		
Coronavirus 229E	✓				✓		
Coronavirus OC43	✓				✓		
Human bocavirus					✓		
Human metapneumovirus	✓	✓	✓	✓	✓	✓	✓
Influenza A virus	✓	✓	✓	✓	✓	✓	✓
Subtype H1	✓	✓	✓	✓	✓	✓	✓
Subtype H3	✓	✓	✓	✓	✓	✓	✓
Subtype 2009 H1N1	✓					✓	✓
Influenza B virus	✓	✓	✓	✓	✓	✓	✓
Parainfluenza virus 1	✓	✓	✓		✓	✓	✓
Parainfluenza virus 2	✓	✓	✓		✓	✓	✓
Parainfluenza virus 3	✓	✓	✓		✓	✓	✓
Parainfluenza virus 4	✓	✓			✓		✓
Respiratory syncytial virus	✓			✓			
Respiratory syncytial virus A		✓	✓		✓	✓	✓
Respiratory syncytial virus B		✓	✓		✓	✓	✓
Rhinovirus/enterovirus	✓	✓	✓	✓	✓	✓	✓
Bacteria							
Chlamydophila pneumoniae	✓				✓		✓
Mycoplasma pneumoniae	✓				✓		✓
Bordetella pertussis	✓	✓					
Bordetella parapertussis-Bordetella bronchiseptica		✓					
Bordetella holmesii		✓					
Time to result (h)	~1	~2–3	~8	~6	~4	~6	~1.5

[a]The acceptable specimen type for all panels is a nasopharyngeal swab. RVP, respiratory virus panel; RPP, respiratory pathogen panel.

Ramanan et al, Clinical Microbiology Reviews, 31: 1 – 28, 2018)

Respiratory Syndromic Testing in the ED – Outcomes Study

Impact of a Rapid Respiratory Panel Test on Patient Outcomes

Beverly B. Rogers, MD; Prabhu Shankar, MD; Robert C. Jerris, PhD; David Kotzbauer, MD; Evan J. Anderson, MD; J. Renee Watson, BSM; Lauren A. O'Brien, PhD; Francine Uwindatwa, MS, MBA; Kelly McNamara, BSBA; James E. Bost, PhD

Arch Pathol Lab Med 139, 636-641, 2015

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Respiratory Syndromic Testing in the ED – Outcomes Study

- Mean time to the test result was shorter (6.4 hours versus 18.6 hours)
- The percentage of patients having a diagnostic test result in the emergency department was greater (51.6% versus 13.4%)
- There was no difference in whether antibiotics were prescribed, but the duration of antibiotic use was shorter
  - dependent on receiving test results within 4 hours
- If the test result was positive, the inpatient length of stay (P = .03) and the time in isolation (P = .03) were decreased
- **Overall conclusion: Use of panel based respiratory PCR testing in the ED decreases the duration of antibiotic use, the length of inpatient stay, and the time in isolation.**

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Commercially Available Syndromic Panels
GI Infections

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			
Campylobacter species	✓	✓	✓
Salmonella species	✓	✓	✓
Shigella species/enteroinvasive *E. coli*[b]	✓	✓	✓
Vibrio species	✓		✓
Vibrio cholerae		✓	✓
Yersinia enterocolitica	✓		✓
Escherichia coli O157		✓	✓
Enterotoxigenic *E. coli*		✓	✓
Enteropathogenic *E. coli*			✓
Enteroaggregative *E. coli*			✓
Plesiomonas shigelloides			✓
Shiga toxin-producing *E. coli* (stx_1-stx_2)	✓[c]	✓	✓
Clostridium difficile (toxin A/B)		✓	✓
Viruses			
Norovirus GI/GII	✓	✓	✓
Rotavirus A	✓	✓	✓
Astrovirus			✓
Adenovirus 40/41		✓	✓
Sapovirus			✓
Parasites			
Cryptosporidium species		✓	✓
Entamoeba histolytica		✓	✓
Giardia lamblia		✓	✓
Cyclospora cayetanensis			✓
No. of samples (throughput)	1–32 (scalable)	24	1–12 (scalable)
Time to result (h)	<2	~5	~1

[a]EP, enteric pathogens; GPP, gastrointestinal pathogen panel; GIP, gastrointestinal panel.
[b]The Verigene EP and Luminex GPP do not specifically target enteroinvasive *E. coli*.
[c]The Verigene EP has separate targets for stx_1 and stx_2.

Ramanan et al, Clinical Microbiology Reviews, 31: 1 – 28, 2018)

Current Commercially Available Syndromic Panels GI Infections cont.

BD MAX™ ENTERIC BACTERIAL PANEL

Salmonella spp.

Shigella spp./EIEC,

Campylobacter spp. (jejuni and coli)

Shiga toxin-producing organisms (STEC, Shigella dysenteriae)

BD MAX™ EXTENDED ENTERIC BACTERIAL PANEL

Yersinia enterocolitica

Enterotoxigenic E. coli (ETEC)

Plesiomonas shigelloides

Vibrio(V. vulnuficus/ V. parahaemolyticus/V. cholerae)

BD MAX™ Enteric Parasite Panel

Giardia lamblia

Cryptosporidium spp. (C. parvum and C. hominis)

Entamoeba histolytica

BD MAX™ ENTERIC VIRAL PANEL

Norovirus

Rotavirus

Adenovirus (40/41)

Sapovirus

Human Astrovirus

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Panel Based Gastrointestinal PCR Testing: Clinical Outcomes

Clinical Impact of a Multiplex Gastrointestinal Polymerase Chain Reaction Panel in Patients With Acute Gastroenteritis

Robert J. Cybulski Jr,[1,a] Allen C. Bateman,[1,a,b] Lori Bourassa,[1] Andrew Bryan,[1] Barb Beail,[1] Jason Matsumoto,[2] Brad T. Cookson,[1,3] and Ferric C. Fang[1,2,3,4]

[1]Department of Laboratory Medicine, University of Washington, [2]Harborview Medical Center Clinical Microbiology Laboratory, [3]Department of Microbiology, University of Washington, and [4]University of Washington School of Medicine, Seattle

Clinical Infectious Diseases, ciy357, https://doi.org/10.1093/cid/ciy357

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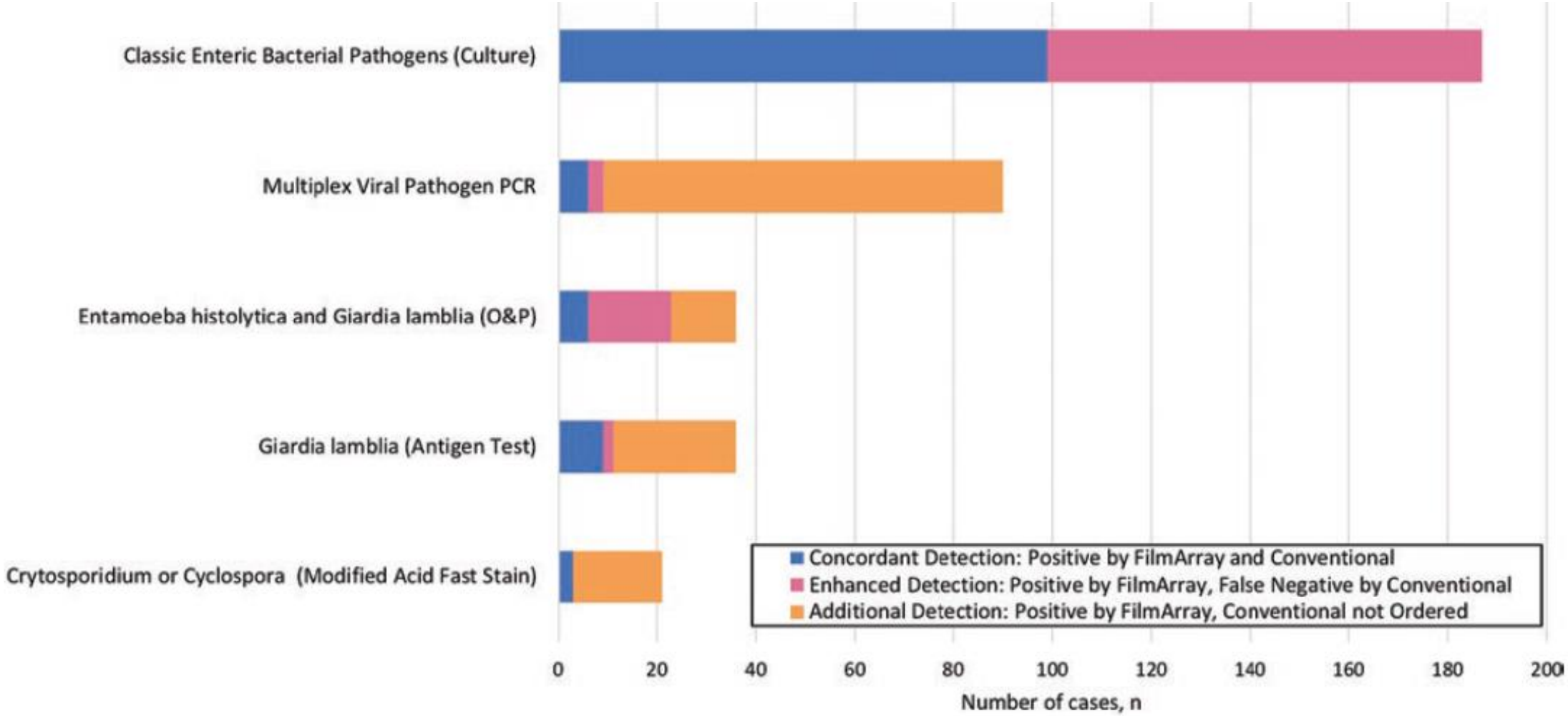
Improved Detection by Gastrointestinal Syndromic Panel PCR Compared to Conventional Testing



Cybulski et al

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Cybulski et al Study Results

- A total of 1887 consecutive fecal specimens were tested in parallel by Gastrointestinal Panel PCR (GIPCR) and stool culture.
- GIPCR detected pathogens in 35.3% of specimens, compared to 6.0% for culture
- GIPCR allowed increased recognition of coinfections
- Median time from collection to result was 18 hours for GIPCR and 47 hours for culture
- Median time from collection to initiation of antimicrobial therapy was 22 hours for GIPCR and 72 hours for culture.
- Patients diagnosed by GIPCR were more likely to receive targeted rather than empirical therapy, compared to those diagnosed by culture (P = .0148)
- Positive Shiga-like toxin-producing *E. coli* results were reported 47 hours faster with GIPCR and facilitated discontinuation of empirical antimicrobials

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PCR Panels - Blood Culture Applications

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			
Staphylococcus species	✓	✓	
Staphylococcus aureus	✓	✓	
Staphylococcus epidermidis		✓	
Staphylococcus lugdunensis		✓	
Streptococcus species	✓	✓	
Streptococcus agalactiae	✓	✓	
Streptococcus pyogenes	✓	✓	
Streptococcus pneumoniae	✓	✓	
Streptococcus anginosus group		✓	
Enterococcus species	✓		
Enterococcus faecalis		✓	
Enterococcus faecium		✓	
Listeria species		✓	
Listeria monocytogenes	✓		
Gram-negative bacteria			
Klebsiella oxytoca	✓		✓
Klebsiella pneumoniae	✓		✓
Serratia marcescens	✓		
Proteus species	✓		✓
Acinetobacter species			✓
Acinetobacter baumannii	✓		
Haemophilus influenzae	✓		
Neisseria meningitis	✓		
Pseudomonas aeruginosa	✓		✓
Enterobacteriaceae	✓		
Escherichia coli	✓		✓
Enterobacter species			✓
Enterobacter cloacae complex	✓		
Citrobacter species			✓
Yeasts			
Candida albicans	✓		
Candida glabrata	✓		
Candida krusei	✓		
Candida parapsilosis	✓		
Candida tropicalis	✓		
Ability to detect presence of resistance gene			
mecA	✓	✓	
vanA	✓	✓	
vanB	✓	✓	
bla_{KPC}	✓		✓
bla_{NDM}			✓
bla_{OXA}			✓
bla_{VIM}			✓
bla_{IMP}			✓
bla_{CTX-M}			✓
Time to result (h)	~1	~2.5	~2

Ramanan et al, Clinical Microbiology Reviews, 31: 1 – 28, 2018)

PCR Panels - Blood Culture Patient Outcomes

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

Clinical Infectious Diseases® 2017;64(1):15–23

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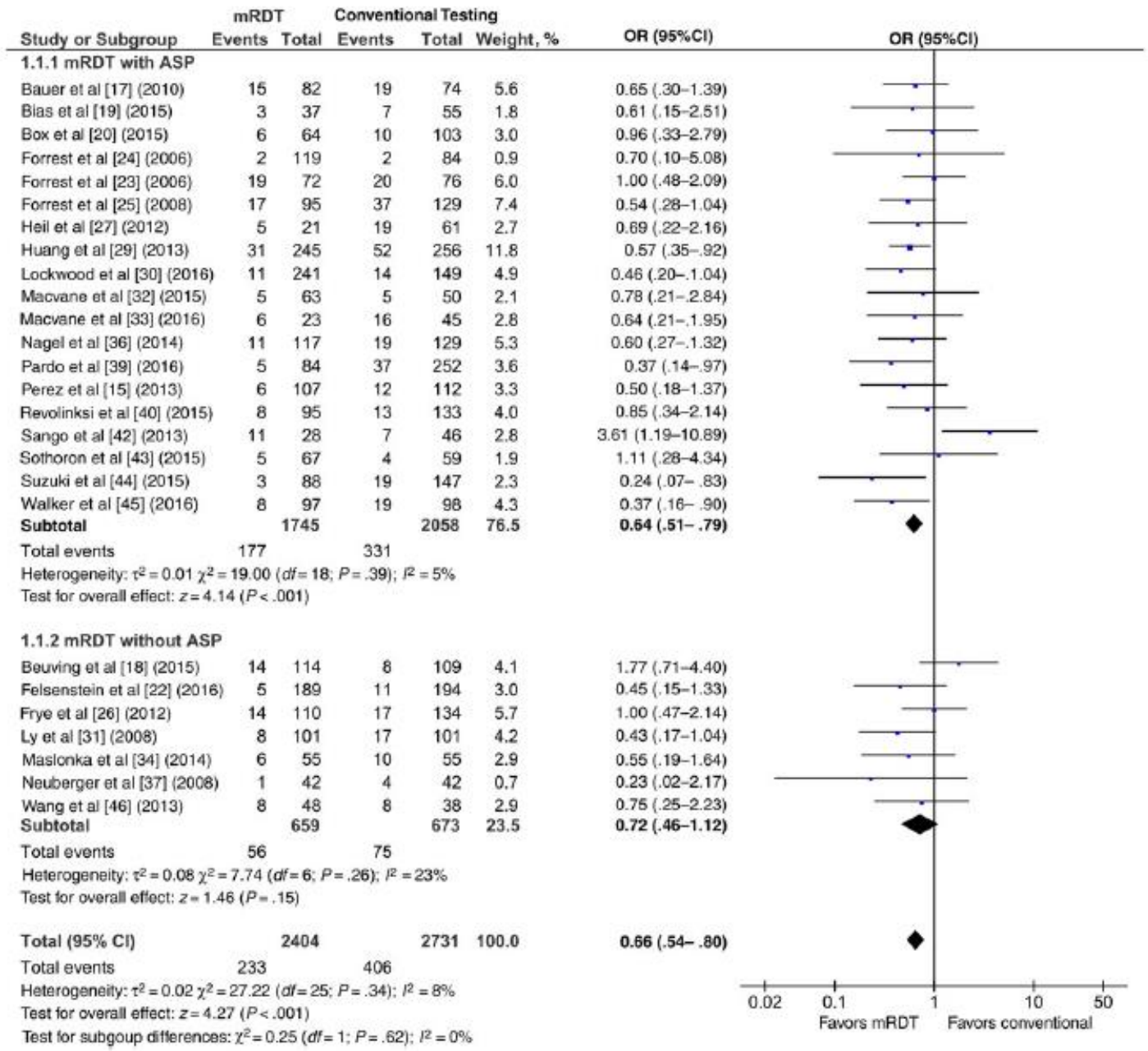
Mortality outcomes with molecular rapid diagnostic testing (mRDT) versus conventional testing in bloodstream infection

Odds ratios (ORs) were determined with the Mantel-Haenszel random-effects method.

Abbreviations:
ASP, antimicrobial stewardship program
CI, confidence interval.



Study or Subgroup	mRDT Events	mRDT Total	Conventional Testing Events	Conventional Testing Total	Weight, %	OR (95%CI)
1.1.1 mRDT with ASP						
Bauer et al [17] (2010)	15	82	19	74	5.6	0.65 (.30–1.39)
Bias et al [19] (2015)	3	37	7	55	1.8	0.61 (.15–2.51)
Box et al [20] (2015)	6	64	10	103	3.0	0.96 (.33–2.79)
Forrest et al [24] (2006)	2	119	2	84	0.9	0.70 (.10–5.08)
Forrest et al [23] (2006)	19	72	20	76	6.0	1.00 (.48–2.09)
Forrest et al [25] (2008)	17	95	37	129	7.4	0.54 (.28–1.04)
Heil et al [27] (2012)	5	21	19	61	2.7	0.69 (.22–2.16)
Huang et al [29] (2013)	31	245	52	256	11.8	0.57 (.35–.92)
Lockwood et al [30] (2016)	11	241	14	149	4.9	0.46 (.20–1.04)
Macvane et al [32] (2015)	5	63	5	50	2.1	0.78 (.21–2.84)
Macvane et al [33] (2016)	6	23	16	45	2.8	0.64 (.21–1.95)
Nagel et al [36] (2014)	11	117	19	129	5.3	0.60 (.27–1.32)
Pardo et al [39] (2016)	5	84	37	252	3.6	0.37 (.14–.97)
Perez et al [15] (2013)	6	107	12	112	3.3	0.50 (.18–1.37)
Revolinksi et al [40] (2015)	8	95	13	133	4.0	0.85 (.34–2.14)
Sango et al [42] (2013)	11	28	7	46	2.8	3.61 (1.19–10.89)
Sothoron et al [43] (2015)	5	67	4	59	1.9	1.11 (.28–4.34)
Suzuki et al [44] (2015)	3	88	19	147	2.3	0.24 (.07– .83)
Walker et al [45] (2016)	8	97	19	98	4.3	0.37 (.16– .90)
Subtotal		**1745**		**2058**	**76.5**	**0.64 (.51– .79)**
Total events	177		331			
Heterogeneity: $\tau^2 = 0.01$ $\chi^2 = 19.00$ ($df = 18$; $P = .39$); $I^2 = 5\%$						
Test for overall effect: $z = 4.14$ ($P < .001$)						
1.1.2 mRDT without ASP						
Beuving et al [18] (2015)	14	114	8	109	4.1	1.77 (.71–4.40)
Felsenstein et al [22] (2016)	5	189	11	194	3.0	0.45 (.15–1.33)
Frye et al [26] (2012)	14	110	17	134	5.7	1.00 (.47–2.14)
Ly et al [31] (2008)	8	101	17	101	4.2	0.43 (.17–1.04)
Maslonka et al [34] (2014)	6	55	10	55	2.9	0.55 (.19–1.64)
Neuberger et al [37] (2008)	1	42	4	42	0.7	0.23 (.02–2.17)
Wang et al [46] (2013)	8	48	8	38	2.9	0.75 (.25–2.23)
Subtotal		**659**		**673**	**23.5**	**0.72 (.46–1.12)**
Total events	56		75			
Heterogeneity: $\tau^2 = 0.08$ $\chi^2 = 7.74$ ($df = 6$; $P = .26$); $I^2 = 23\%$						
Test for overall effect: $z = 1.46$ ($P = .15$)						
Total (95% CI)		**2404**		**2731**	**100.0**	**0.66 (.54– .80)**
Total events	233		406			
Heterogeneity: $\tau^2 = 0.02$ $\chi^2 = 27.22$ ($df = 25$; $P = .34$); $I^2 = 8\%$						
Test for overall effect: $z = 4.27$ ($P < .001$)						
Test for subgoup differences: $\chi^2 = 0.25$ ($df = 1$; $P = .62$); $I^2 = 0\%$						

Timbrook et al, CID 2017:64, 15-23

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Advantages and Disadvantages Current Commercially Marketed Testing Platforms/Tests

- Advantages
  - Standardized systems needing minimal effort to set up
  - Established test performance characteristics
  - Rapid turn-around time
  - Simple to use “sample to answer” format
  - Moderate complexity
- Disadvantages
  - High cost per patient test
  - Locked into target organisms selected by the manufacturer
  - Appropriateness of target organisms chosen by the manufacturer
  - Scalability

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Why Custom Syndromic Arrays?

- Flexibility
  - You test for only those molecular targets that are appropriate for your laboratory
- Higher throughput
  - Multiple patient samples can be tested per array
    - Sample number depends on number of molecular targets on the array
- Lower per patient cost per test

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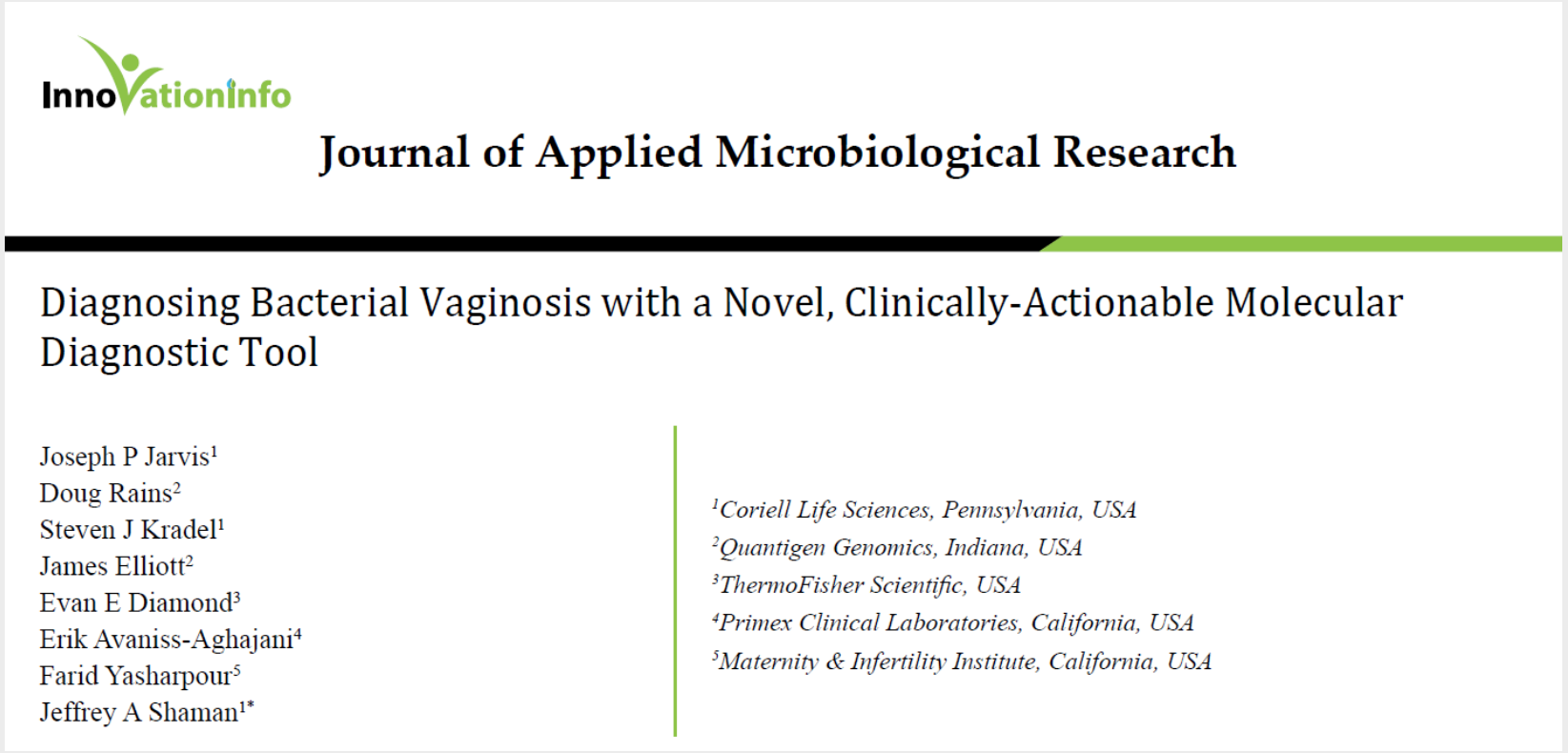
Why Custom Syndromic Arrays?



InnoVationInfo

Journal of Applied Microbiological Research

Diagnosing Bacterial Vaginosis with a Novel, Clinically-Actionable Molecular Diagnostic Tool

Joseph P Jarvis[1]
Doug Rains[2]
Steven J Kradel[1]
James Elliott[2]
Evan E Diamond[3]
Erik Avaniss-Aghajani[4]
Farid Yasharpour[5]
Jeffrey A Shaman[1*]

[1]*Coriell Life Sciences, Pennsylvania, USA*
[2]*Quantigen Genomics, Indiana, USA*
[3]*ThermoFisher Scientific, USA*
[4]*Primex Clinical Laboratories, California, USA*
[5]*Maternity & Infertility Institute, California, USA*

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BV Open Array Panel Target Organisms

Pathogenic	Commensal lactobacilli
Atopobium vaginae	Lactobacillus crispatus
BVAB2*	Lactobacillus gasseri
Gardnerella vaginalis Lactobacillus iners	Lactobacillus iners
Megasphaera 1 Lactobacillus jensenii	Lactobacillus jensenii
Megasphaera 2	Lactobacillus jensenii
Mobiluncus curtisii	
Mobiluncus mulieris	
Mycoplasma hominis	
Prevotella bivia	
Ureaplasma urealyticum	

*Bacterial Vaginosis–Associated Bacterium type 2

Jarvis et al.

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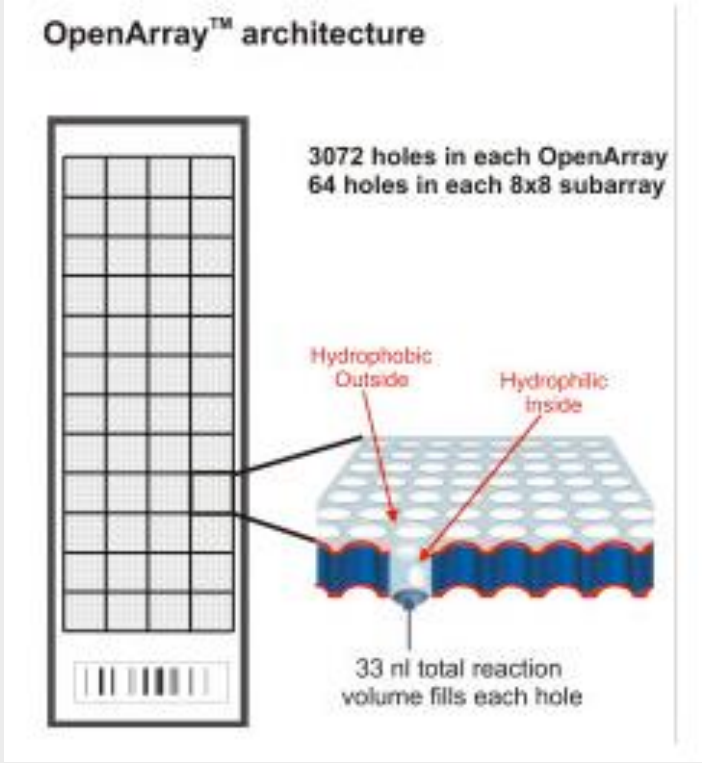
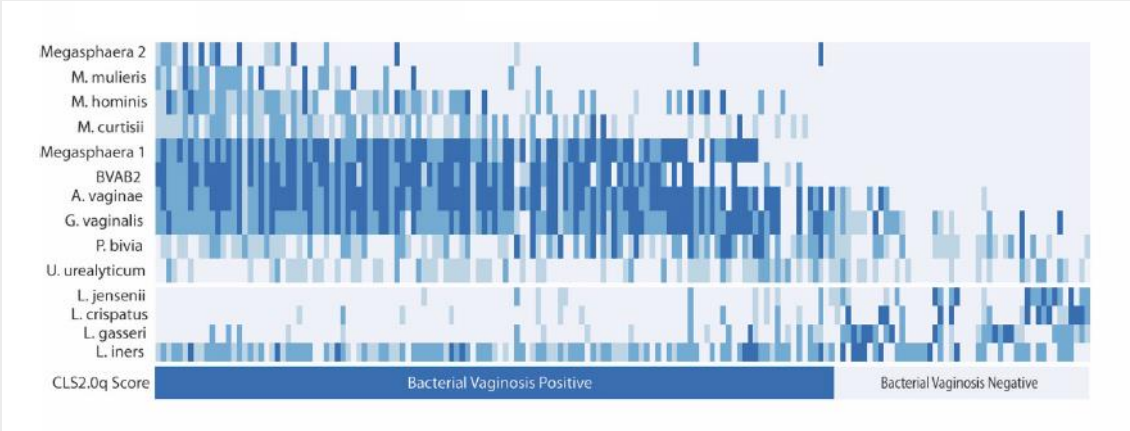
BV Open Array Panel Target Organisms





Jarvis et al.

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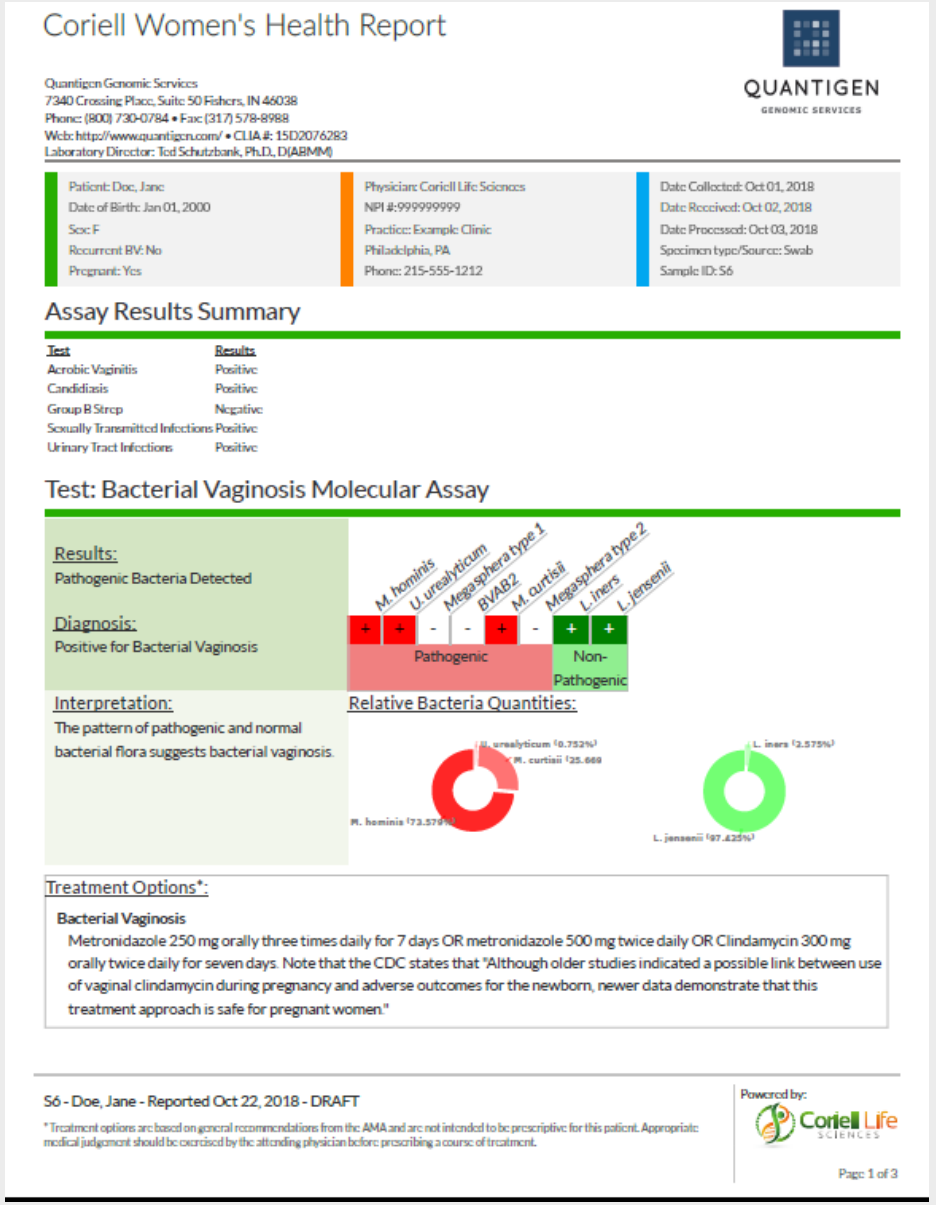
Example of a Custom Panel: Women's Health Testing



Coriell Women's Health Report

QUANTIGEN GENOMIC SERVICES

Quantigen Genomic Services
7340 Crossing Place, Suite 50 Fishers, IN 46038
Phone: (800) 730-0784 • Fax: (317) 578-8988
Web: http://www.quantigen.com/ • CLIA #: 15D2076283
Laboratory Director: Tod Schutzbank, Ph.D., D(ABMM)

Patient: Doe, Jane
Date of Birth: Jan 01, 2000
Sex: F
Recurrent BV: No
Pregnant: Yes

Physician: Coriell Life Sciences
NPI #:999999999
Practice: Example Clinic
Philadelphia, PA
Phone: 215-555-1212

Date Collected: Oct 01, 2018
Date Received: Oct 02, 2018
Date Processed: Oct 03, 2018
Specimen type/Source: Swab
Sample ID: S6

Assay Results Summary

Test	Results
Acrobic Vaginitis	Positive
Candidiasis	Positive
Group B Strep	Negative
Sexually Transmitted Infections	Positive
Urinary Tract Infections	Positive

Test: Bacterial Vaginosis Molecular Assay

Results:
Pathogenic Bacteria Detected

Diagnosis:
Positive for Bacterial Vaginosis

M. hominis, U. urealyticum, Megasphera type 1, BVAB2, M. curtisii, Megasphera type 2, L. iners, L. jensenii

+ + - - + - + +

Pathogenic | Non-Pathogenic

Interpretation:
The pattern of pathogenic and normal bacterial flora suggests bacterial vaginosis.

Relative Bacteria Quantities:

U. urealyticum (0.752%)
M. curtisii (25.069
M. hominis (73.579%)
L. iners (2.575%)
L. jensenii (97.425%)

Treatment Options*:

Bacterial Vaginosis

Metronidazole 250 mg orally three times daily for 7 days OR metronidazole 500 mg twice daily OR Clindamycin 300 mg orally twice daily for seven days. Note that the CDC states that "Although older studies indicated a possible link between use of vaginal clindamycin during pregnancy and adverse outcomes for the newborn, newer data demonstrate that this treatment approach is safe for pregnant women."

S6 - Doe, Jane - Reported Oct 22, 2018 - DRAFT

* Treatment options are based on general recommendations from the AMA and are not intended to be prescriptive for this patient. Appropriate medical judgement should be exercised by the attending physician before prescribing a course of treatment.

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PathGroup

Physician Centered. Patient Focused.

Application of OpenArray real-time PRC technology in Vaginal Microbiota Investigations

Sandeep Mukherjee, PhD

Disclosure

Scientific Director, Women's Health & Infectious Diseases,
PathGroup

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Overview

- Microbiomes in health and disease
  - Vaginal microbiota: finely balanced mutualistic association
  - Indigenous bacterial communities prevent colonization by pathogenic organisms
  - concept of normal versus abnormal microbiota
  - humans coexist with complex bacterial communities that are relatively unique to specific niches such as the gastrointestinal tract and the oral cavity
  - Impact in human health
    - Symptomatic/asymptomatic bacterial vaginosis, yeast infections, STI, UTI

Overview

- Culture has been inadequate (>99% cannot be cultivated)
- 16S rRNA gene sequencing
    - Unprecedented detail, ID low abundance taxa
    - Cpn60, rpoC, uvrB, RecA
- Several distinct vaginal communities with different species composition
    - Variation in different ethnic groups
- Differences in species composition correlate with response to "disturbances"

Vaginitis

- Inflammation of the vagina; discharge, itching and pain (11 million office visits per year)
  - Bacterial vaginosis: associated with an altered microbial flora (absence of gross appearance of inflammation)
  - Vulvovaginal candidiasis: caused by naturally occurring fungus of Candida spp.
    - 2nd most common cause of vaginitis symptoms
  - Trichomonal vaginitis: sexually transmitted parasite Trichomonas vaginalis
  - Aerobic vaginitis: Depletion of healthy Lactobacillus species with aerobic pathogens, mostly of intestinal origin
  - Vaginal atrophy (atrophic vaginitis): reduced estrogen levels after menopause
  - Overlapping symptoms

Bacterial Vaginosis

Shift in vaginal flora from homogeneous, lactobacillus dominated state to a heterogeneous, complex population of anaerobic & microaerophilic organisms - *Polybacterial dysbiosis*

- Upper genital tract infections
- Pelvic Inflammatory Disease (PID)
- Adverse pregnancy outcomes
- Increased risk of STI

CDC:

- most commonly reported microbiological syndrome among women of childbearing age
- 15% to 50% of vaginitis/vaginosis depending upon the patient population
- Prevalence: 29.2%, among women ages 14 to 49
- 84% of women with BV do not report symptoms

Lactobacillus spp.

- Hallmark of healthy vagina; dominant vaginal bacterial species in majority of women
- Vaginal pH ~3.5 to 4.5, lactic acid production through fermentation
- Protection against non-commensal & potentially pathogenic organisms
- Distribution of Lactobacillus dominated community types varies among different ethnicities
- 20% to 30% of asymptomatic healthy women lack significant numbers of Lactobacillus spp.

Related panels

- Aerobic Vaginitis
  - *Escherichia coli, Group B Streptococcus, Staphylococcus aureus, Enterococcus faecalis*
  - Localized vaginal inflammatory immune response
  - Confused with common vaginitis etiologies
  - 4.3% to 7.9% women attending vaginitis clinics were found to have moderate to severe symptoms of AV
- Genital Ulcer
  - Young, sexually active patients with genital, anal, or perianal ulcers
  - Genital herpes (HSV-1/HSV-2)
  - Syphillis (*Treponema pallidum*)
  - Chancroid (*Haemophilus ducreyi*)
  - *Chlamydia trachomatis*
  - Differentiate from non-sexually acquired genital ulceration

Rationale

- Conventional microbiological approaches – limited utility
- Quantitative/semi-quantitative PCR for BV diagnosis
  - Lack of standardization (different marker organisms, different thresholds)
  - Limited number of organisms
- Need for a multi-variate analysis of BV associated marker organisms
  - Microbiome?

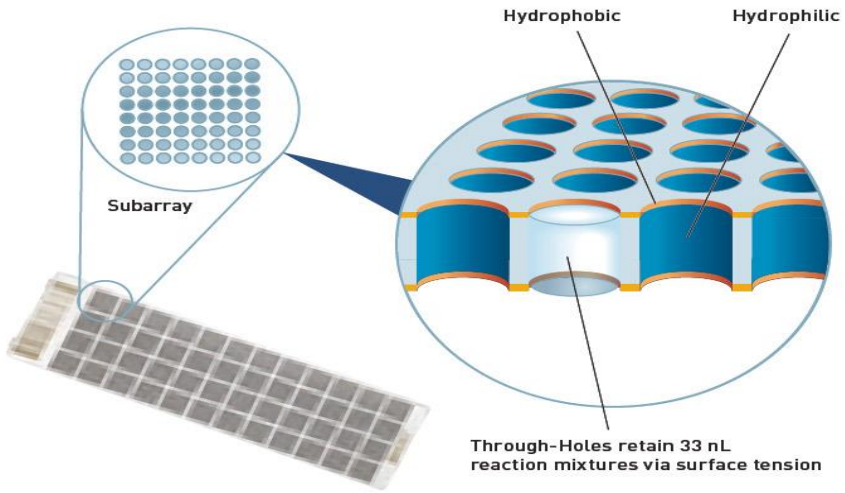
Potential Utility

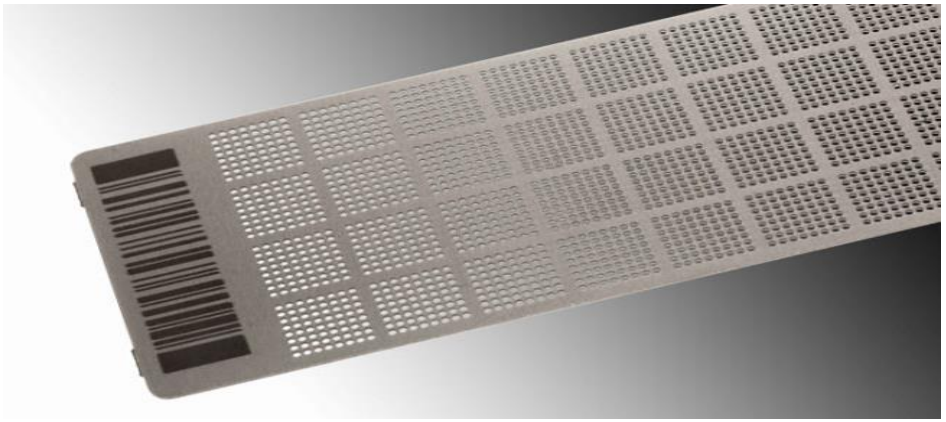
- Custom panel/s based on clinical association and potential utility
- Consolidation of targets
- Flexibility
- Sensitive, specific, rapid
- Detection of BV, non-BV targets
- Variability of specimen collection, multiplicity of targets

Highlights

- "Pan-bacteria" control
- Normalization to standardize results
- Integrated interpretation – "normal", "borderline", "abnormal"
- ThinPrep or BD Universal Swab

OpenArray from Life Technologies





- OpenArray Digital PCR plates
  - 48 subarrays; 64 through-holes/subarray
  - 3,072 individual real-time PCR assays in parallel on a single OpenArraay
  - 4 arrays can be run at the same time (12,288 assays)
  - 33 nL reaction volume
  - Hydrophilic interior & external hydrophobic coatings
- 48 specimen per OpenArray
  - 192 specimens per run (6 hours)
  - 26 organisms per specimen
- Simple workflow

Assay Workflow

Automated liquid handling

DNA isolation

Load samples

Apply lid and fill

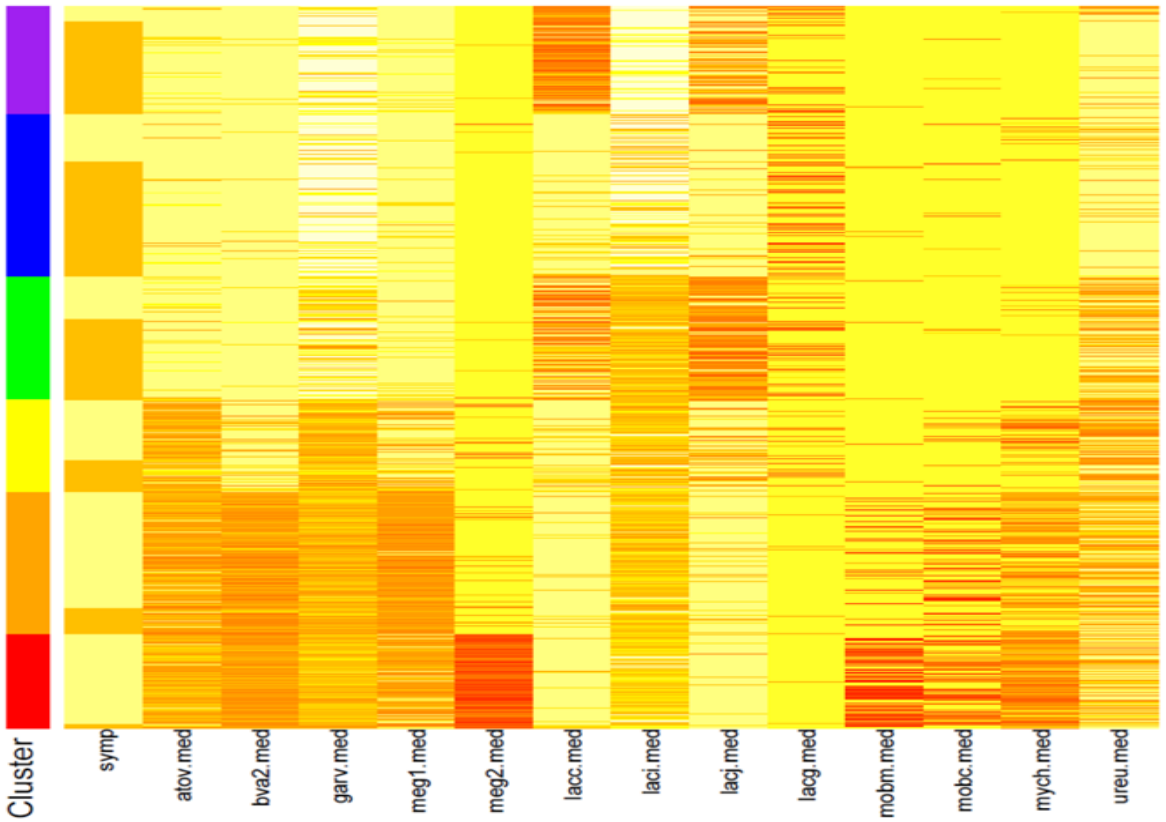
Cycle, image, analyze data

PathGroup
Physician Centered. Patient Focused.

Assay design/Verification

- relative quantitative data for each organisms by comparing to total bacterial load
  - Internal control
- Compared this data in symptomatic/asymptomatic subjects
- statistically determined cut-offs based on a bimodal distribution of the data to determine normal versus elevated levels
- Statistical analyses to identify patterns of clustering of organisms between symptomatic/asymptomatic status
- reference lab results for comparison
- samples from our routine screening population

Statistical Clustering



- Heat map representation/cluster analysis
- probability of being symptomatic, observed symptomatic status and observed covariates, as predicted by Probability model
- Six separate groups, based on composition of the bacterial communities

PathGroup
Physician Centered. Patient Focused.

Technical Specifications

- Prediction of symptomatic status
  - Specificity: 88.5%
  - Sensitivity: 90.1%
- Analytical Sensitivity:
  - ThinPrep: 1.5 X 10^4 - 7.5 X 10^3 targets/ml
  - Swab: 2.5 – 5.0 X 10^4 targets/ml
- Specificity: ~100%

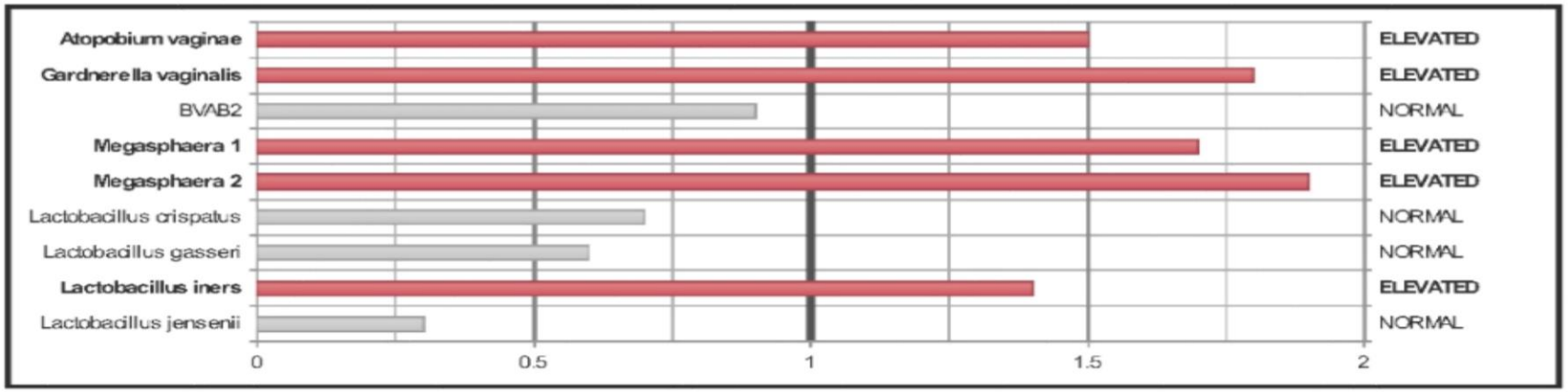
BV+ Abnormal Report

DNA ASSAYS REPORT

Specimen Source: Cervical
Clinical History:

TEST NAME	SPECIMEN	RESULTS
Bacterial Vaginosis+ with Lacto Profiling	Universal Swab	ABNORMAL

Normal Bacterial Flora[1]

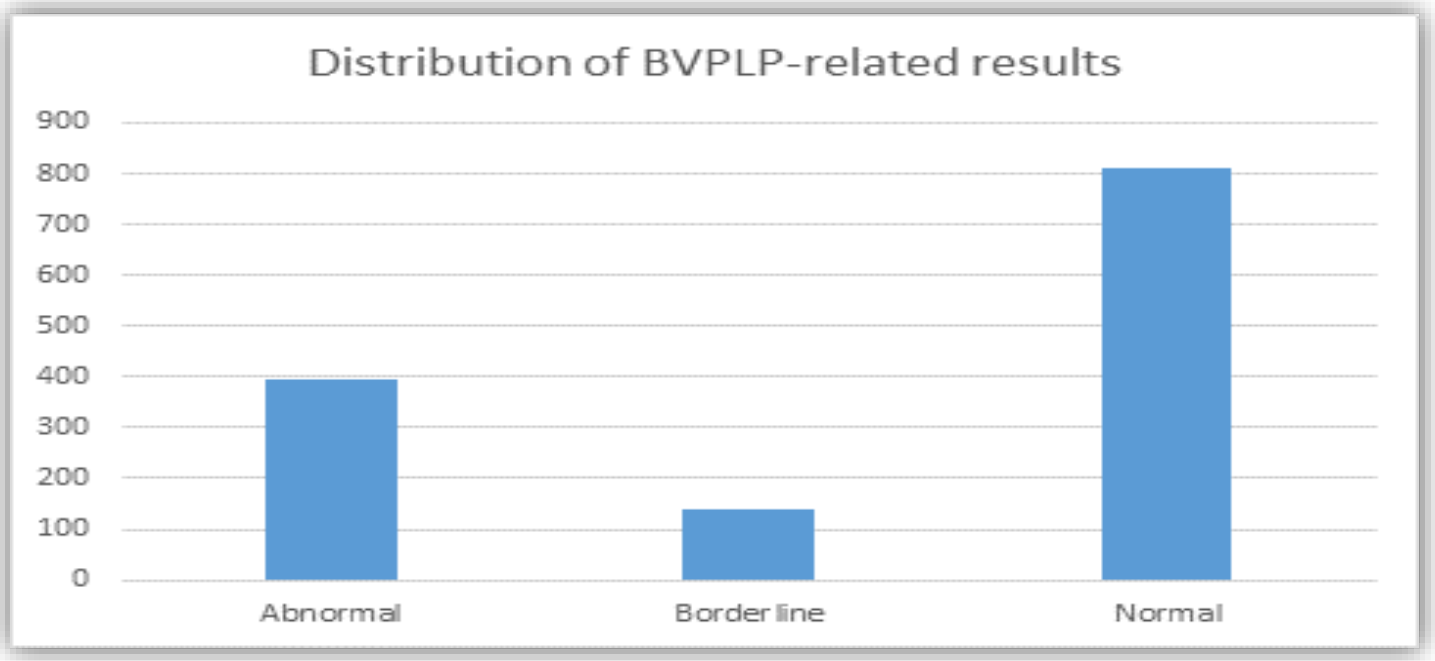


Pathogenic Flora[2]

Mobiluncus mulieris	NOT DETECTED
Mobiluncus curtisii	NOT DETECTED
Mycoplasma hominis	**DETECTED**
Ureaplasma urealyticum	**DETECTED**

PathGroup
Physician Centered. Patient Focused.

BV+ Performance



>150,000 subjects tested

Normal = 60.3%
Borderline = 10.2%
Abnormal = 29.5%

CDC Statistics

- Prevalence estimated at 21.2 million (29.2%) among women ages 14 to 49
- 84% reported no symptoms

PathGroup
Physician Centered. Patient Focused.

Related panels

Panel Name	Panel Components	
Bacterial Vaginosis+	*Lactobacillus* Profile *Atopobium vaginae* *Megasphera 1 & 2* *Gardnerella vaginalis* *BVAB2*	*Ureaplasma urealyticum* *Mycoplasma hominis* *Mobiluncus curtisii* *Mobiluncus mulieris*
Candida	Candida albicans Candida glabrata Candida krusei	Candida parapsilosis Candida tropicalis
Leukorrhea	Chlamydia trachomatis Neisseria gonorrhoeae	Trichomonas vaginalis
Vaginitis	Candida species Trichomonas vaginalis	Gardnerella vaginalis
Aerobic Vaginitis	Enterococcus faecalis Escherichia coli	Group B Streptococcus Staphylococcus aureus
STI/STD	Chlamydia trachomatis HSV 1&2	Neisseria gonorrhoeae Trichomonas vaginalis
Genital Ulcer	Haemophilus ducreyi HSV 1&2	Treponema pallidum Trichomonas vaginalis

Conclusion

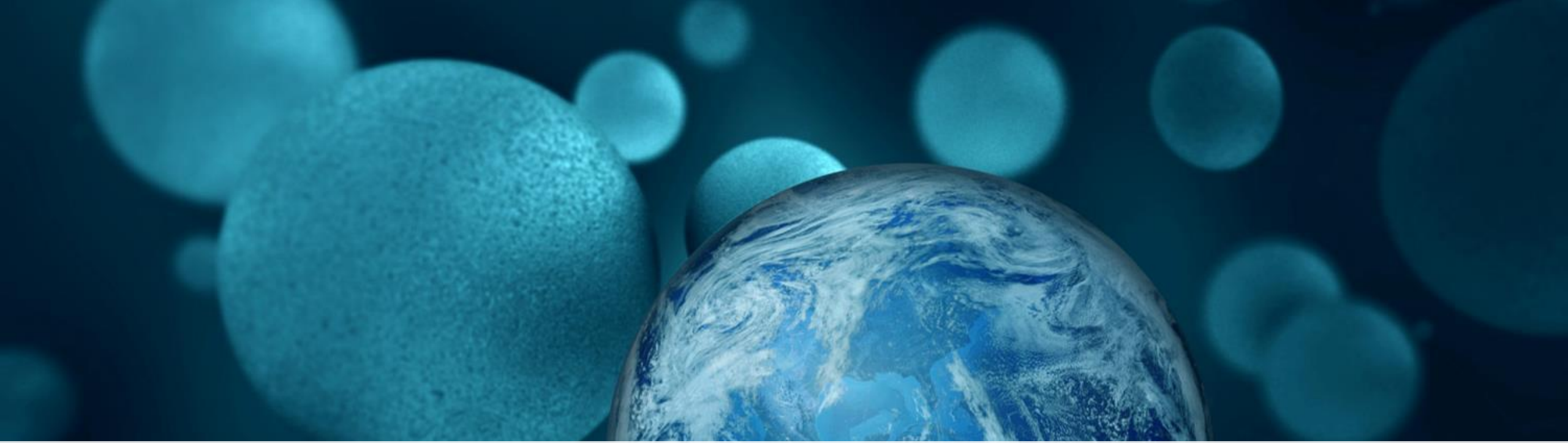
- High-throughput
- Sensitive/Specific
- Investigation of vaginal infections by evaluating a large panel of pathogenic and commensal organisms
  - Syndromic testing
- Faster TAT, without subjectivity associated with culture
- Operational efficiency
- Integrated, pre-microbiome

References

- Charles P. Cartwright et al. **Development and Validation of a Semiquantitative, Multitarget PCR Assay for Diagnosis of Bacterial Vaginosis.** J Clin Microbiol. 2012 Jul;50(7):2321-9.
- Srinivasan et al. **Bacterial communities in women with bacterial vaginosis: high resolution phylogenetic analyses reveal relationships of microbiota to clinical criteria.** PLoS One. 2012;7(6):e37818.
- van de Wijgert JH et al. **The vaginal microbiota: what have we learned after a decade of molecular characterization?** PLoS One. 2014 Aug 22;9(8):e105998.
- Ma B et al. **Vaginal microbiome: rethinking health and disease.** Annu Rev Microbiol. 2012;66:371-89.

Acknowledgements

- **Pranil Chandra**, *DO, FCAP, FASCP*
- **Scott Wheeler**, *PhD*
- **James Prescott**, *PhD*
- **Zeq Ma**, *PhD*
- Mathew Rodgers
- Criziel Quinn
- Vickie Clinard
- Joni Williams
- Melody Hedjnal
- Dustin Murdock



ThermoFisher SCIENTIFIC

Microfluidics qPCR Solution for Respiratory Pathogen Detection

Kelly Li, PhD
Associate Director, Clinical R&D, Genetic Sciences Division

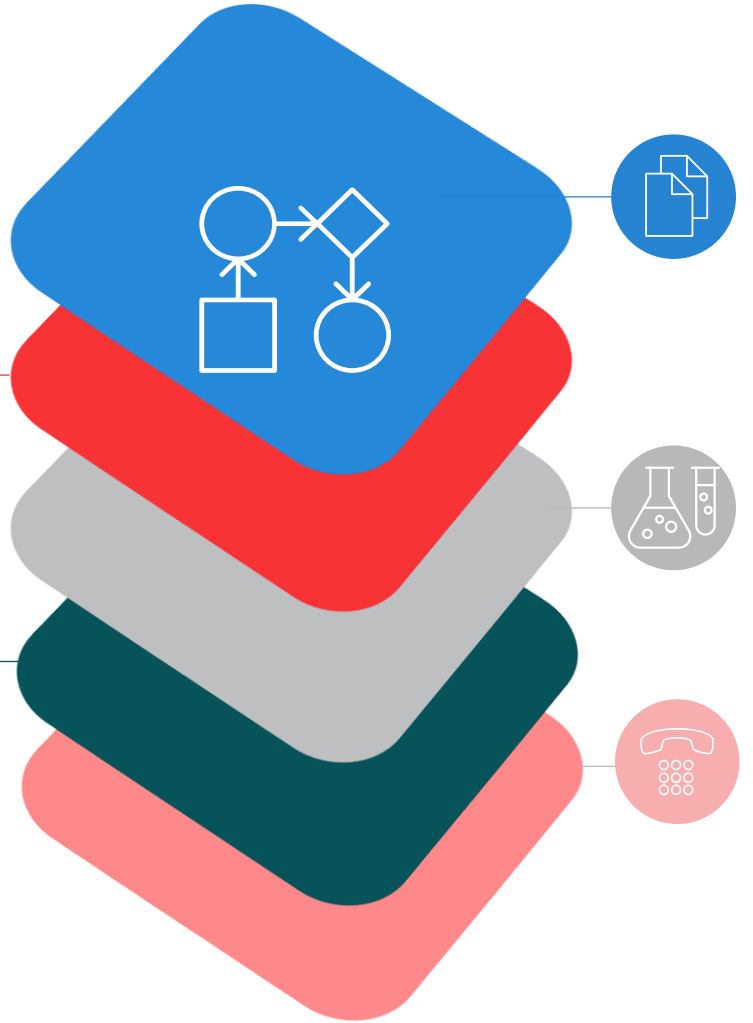
Nov 7th, 2018
Webinar: The Impact of Flexible Panel-Based Solutions for Pathogen Detection

Applied Biosystems - Microbial Detection Solutions

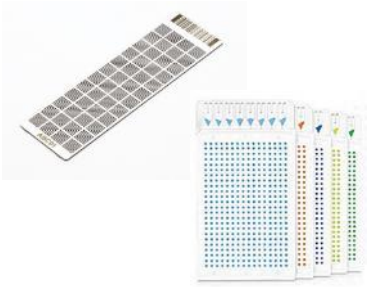
End to end solution approach for supporting pathogen testing

COVERAGE
Allows to select clinical research studies and population demographics relevant genomic targets

INSTRUMENTATION
Combining flexible throughput capabilities with a streamlined workflow



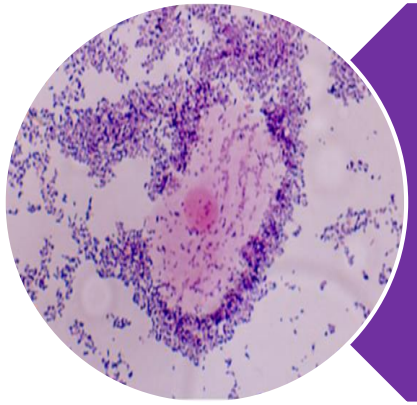
PLATFORM
Form factors to suit different sample number, target number and throughput needs



CONSUMABLES
Supporting workflow reagents including controls, sample prep, master mixes

SERVICE & SUPPORT
Instrument OQ/IQ/PV, training and support your analytical validation

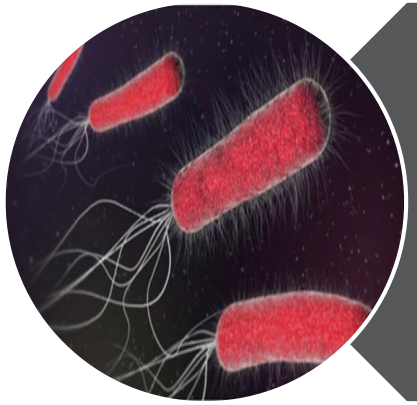
Uro-Genital Pathogen Detection Solutions on Nanofluidics



Vaginal Microbiota

Collection of 34 Pathogens (24 Bacteria, 7 Fungi, 1 Protozoa, 2 Virus)

www.thermofisher.com/vm



Urinary Tract Microbiota

Collection of 17 Pathogens (16 Bacteria, 1 Fungi)

www.thermofisher.com/utm

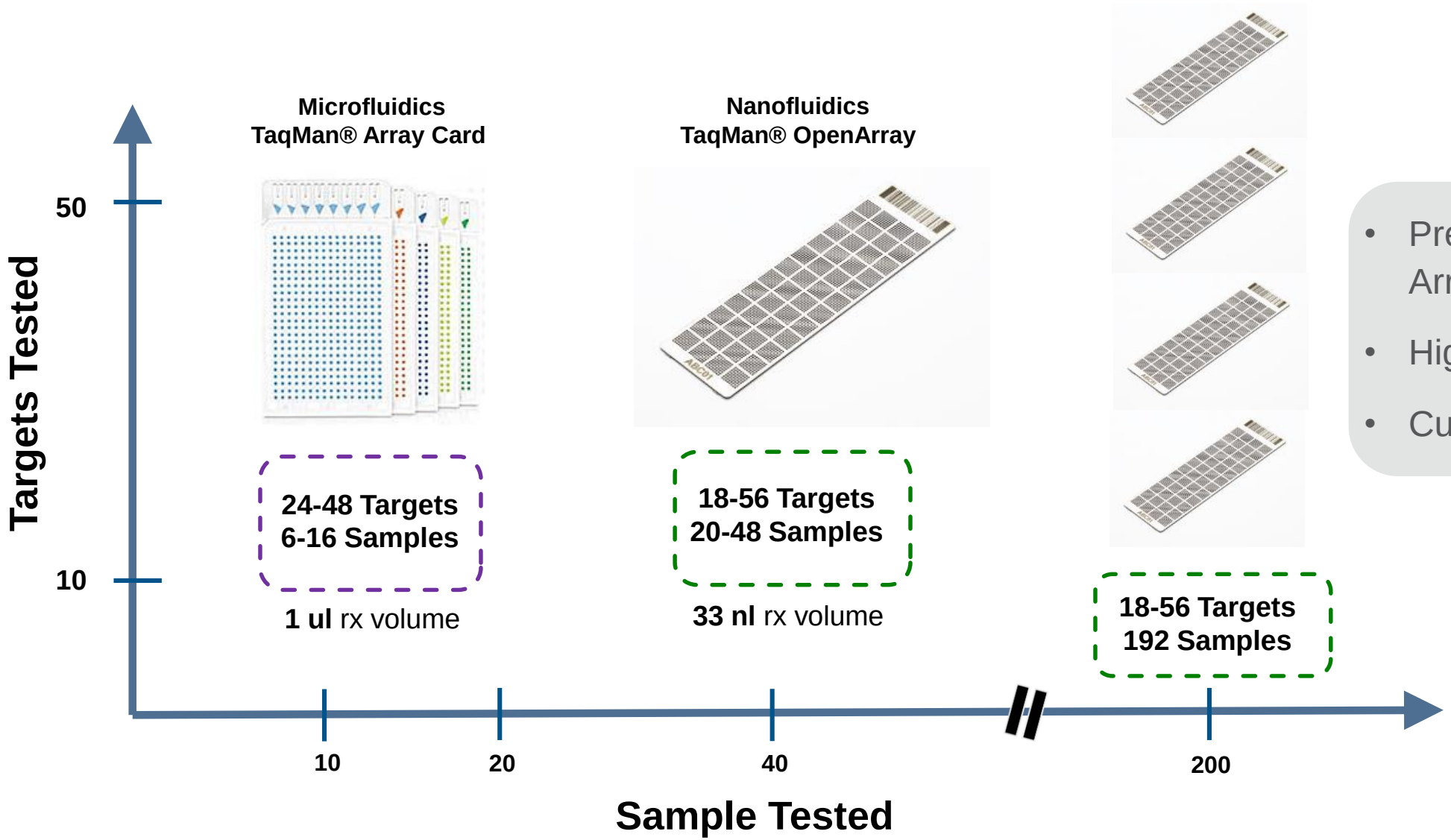
Organism type	Organism name
Bacteria	*Atopobium vaginae*
	Bacteroides fragilis
	BVAB2
	Chlamydia trachomatis
	Enterococcus faecalis
	Escherichia coli
	Gardnerella vaginalis
	Haemophilus ducreyi
	Lactobacillus crispatus
	Lactobacillus gasseri
	Lactobacillus iners
	Lactobacillus jensenii
	Megasphaera 1
	Megasphaera 2
	Mobiluncus curtisii
	Mobiluncus mulieris
	Mycoplasma genitalium
	Mycoplasma hominis
	Neisseria gonorrhoeae
	Prevotella bivia
	Staphylococcus aureus
	Streptococcus agalactiae (group B)
	Treponema pallidum (Syphilis)
	Ureaplasma urealyticum
Fungi	*Candida albicans*
	Candida dubliniensis
	Candida glabrata
	Candida krusei
	Candida lusitaniae
	Candida parapsilosis
	Candida tropicalis
Protozoa	*Trichomonas vaginalis*
Virus	HSV1
	HSV2

Organism type	Species
Bacteria	*Acinetobacter baumannii*
	Citrobacter freundii
	Enterobacter aerogenes
	Enterobacter cloacae
	Enterococcus faecalis
	Enterococcus faecium
	Escherichia coli
	Klebsiella oxytoca
	Klebsiella pneumoniae
	Morganella morganii
	Proteus mirabilis
	Proteus vulgaris
	Providencia stuartii
	Pseudomonas aeruginosa
	Staphylococcus saprophyticus
	Streptococcus agalactiae
Fungus	*Candida albicans*
Control	Xeno

Current solution includes optimized protocols for sample prep and qPCR workflows as well as synthetic controls and master mix.

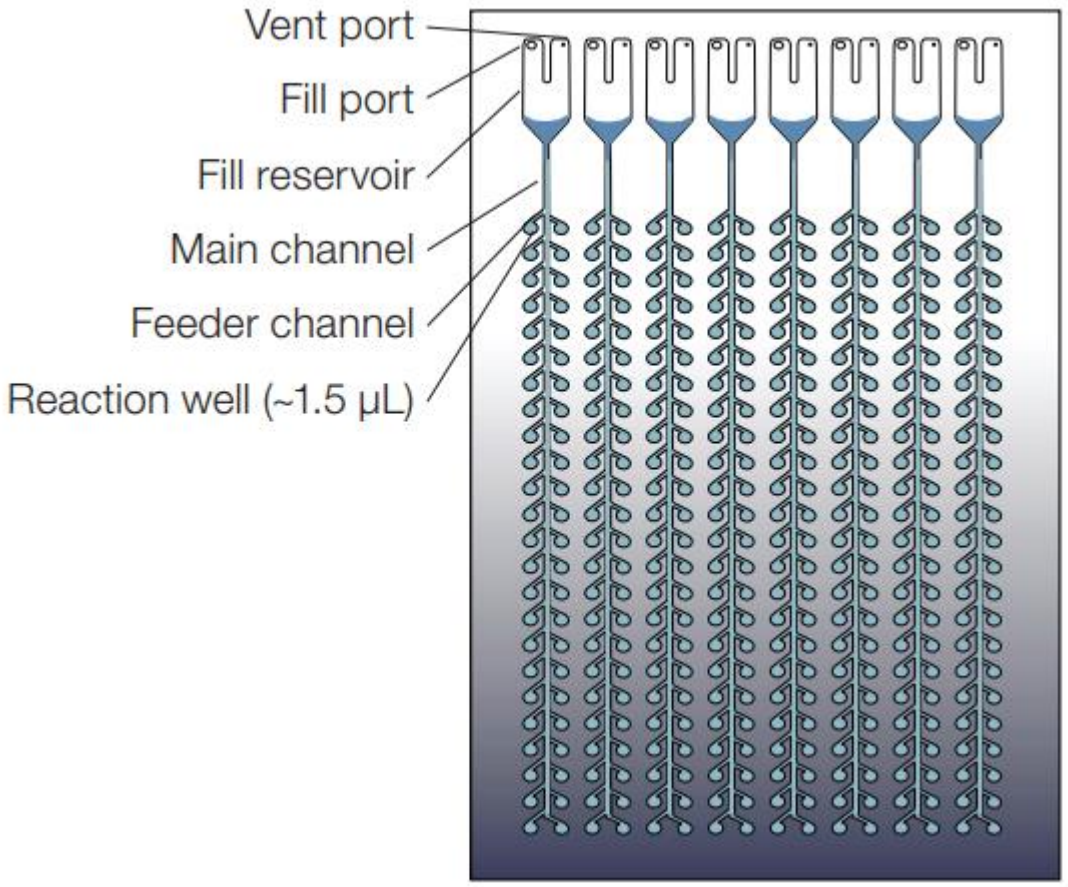
The traditional tools (i.e. culture) in these areas are inadequate, providing an opportunity for molecular solutions to address current pain points

Building a Throughput Continuum for Customizable Pathogen Detection



- Pre-spotted Arrays
- High-throughput
- Customization

Flexible Microfluidic Solution



Ease of Use
Fast and easy setup in a closed system

Coverage Flexibility
Choice of target aligned to need

Performance Proficiency
High Sensitivity and Specificity

Cost Effectiveness
Aligned to acceptable payment by users

TAC has over 50 peer reviewed publications on microbial detection covering clinical research and epidemiological studies

Groundbreaking ANISA Study Using Microfluidic Card (Saha et al, 2018)

Bill & Melinda Gates Foundation funded study published in Lancet investigates incidence of community-acquired infections caused by specific organisms among neonates in 3rd world countries. More than half a million neonatal deaths per year results from possible serious bacterial infections (pSBIs) which is investigated in this study using blood cultures and TAC platform

Causes and incidence of community-acquired serious infections among young children in south Asia (ANISA): an observational cohort study

Samir K Saha*, Stephanie J Schrag*, Shams El Arifeen, Luke C Mullany, Mohammad Shahidul Islam, Nong Shang, Shamim A Qazi, Anita K M Zaidi, Zulfiqar A Bhutta, Anuradha Bose, Pinaki Panigrahi, Sajid B Soofi, Nicholas E Connor, Dipak K Mitra, Rita Isaac, Jonas M Winchell, Melissa L Arvay, Maksuda Islam, Yasir Shafiq, Imran Nisar, Benazir Baloch, Furqan Kabir, Murtaza Ali, Maureen H Diaz, Radhanath Satpathy, Pritish Nanda, Bijaya K Padhi, Sailajanandan Parida, Aneeta Hotwani, M Hasanuzzaman, Sheraz Ahmed, Mohammad Belal Hossain, Shabina Ariff, Imran Ahmed, Syed Mamun Ibne Moin, Arif Mahmud, Jessica L Waller, Iftekhar Rafiqullah, Mohammad A Quaiyum, Nazma Begum, Veeraraghavan Balaji, Jasmin Halen, A S M Nawshad Uddin Ahmed, Martin W Weber Davidson, H Hamer, Patricia L Hibberd, Qazi Sadeq-ur Rahman, Venkat Raghava Mogan, Tanvir Hossain, Lesley McGee, Shalini Anandan, Anran Liu, Kalpana Panigrahi, Asha Mary Abraham, Abdullah H Baqui

Summary

Background More than 500 000 neonatal deaths per year result from possible serious bacterial infections (pSBIs), but the causes are largely unknown. We investigated the incidence of community-acquired infections caused by specific organisms among neonates in south Asia.

Methods From 2011 to 2014, we identified babies through population-based pregnancy surveillance at five sites in Bangladesh, India, and Pakistan. Babies were visited at home by community health workers up to ten times from age 0 to 59 days. Illness meeting the WHO definition of pSBI and randomly selected healthy babies were referred to study physicians. The primary objective was to estimate proportions of specific infectious causes by blood culture and Custom TaqMan Array Cards molecular assay (Thermo Fisher, Bartlesville, OK, USA) of blood and respiratory samples.

Findings 6022 pSBI episodes were identified among 63 114 babies (95·4 per 1000 livebirths). Causes were attributed in 28% of episodes (16% bacterial and 12% viral). Mean incidence of bacterial infections was 13·2 (95% credible interval [CrI] 11·2–15·6) per 1000 livebirths and of viral infections was 10·1 (9·4–11·6) per 1000 livebirths. The leading pathogen was respiratory syncytial virus (5·4, 95% CrI 4·8–6·3 episodes per 1000 livebirths), followed by *Ureaplasma* spp (2·4, 1·6–3·2 episodes per 1000 livebirths). Among babies who died, causes were attributed to 46% of pSBI episodes, among which 92% were bacterial. 85 (83%) of 102 blood culture isolates were susceptible to penicillin, ampicillin, gentamicin, or a combination of these drugs.

Interpretation Non-attribution of a cause in a high proportion of patients suggests that a substantial proportion of pSBI episodes might not have been due to infection. The predominance of bacterial causes among babies who died, however, indicates that appropriate prevention measures and management could substantially affect neonatal mortality. Susceptibility of bacterial isolates to first-line antibiotics emphasises the need for prudent and limited use of newer-generation antibiotics. Furthermore, the predominance of atypical bacteria we found and high incidence of respiratory syncytial virus indicated that changes in management strategies for treatment and prevention are needed. Given the burden of disease, prevention of respiratory syncytial virus would have a notable effect on the overall health system and achievement of Sustainable Development Goal.

Funding Bill & Melinda Gates Foundation

Lancet 2018; 392: 145–59

Published Online July 6, 2018 http://dx.doi.org/10.1016/S0140-6736(18)31127-9

See Comment page 100

*Contributed equally

Department of Microbiology, Child Health Research Foundation, Dhaka Shishu Hospital, Sher-E-Bangla Nagar, Dhaka, Bangladesh (Prof S K Saha PhD, M Shahidul Islam MSPH, N E Connor MSc, M Islam BA, M Hasanuzzaman MSc, M Belal Hossain MSc, I Rafiqullah MSc, Prof A S M Nawshad Uddin Ahmed FCPS); Centers for Disease Control and Prevention, Respiratory Diseases Branch, Atlanta, GA, USA (S J Schrag DrPhil, N Shang PhD, J M Winchell PhD, M L Arvay PhD, M H Diaz PhD, J L Waller MSc, L McGee PhD, A Liu MSc); Maternal and Child Health Division, icddr,b, Dhaka, Bangladesh (S El Arifeen DrPh, D K Mitra PhD, M A Quaiyum MBBS, Q Sadeq-ur Rahman MSc, T Hossain MSc); Johns Hopkins Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD, USA (Prof [illegible] PhD,

***Lancet* 2018 v392: p145–59**

Microfluidic Card Workflow

Load

Dispense 100 µL of nucleic acid and master mix into each fill port

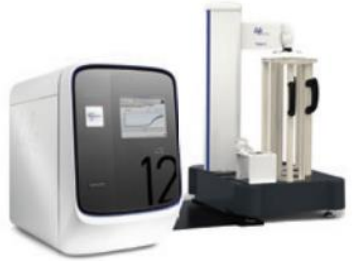
Spin

Centrifuge the TaqMan Array Cards for 2 min

Seal

Seal off the main channels with the sealer and cut the strip

Run



Perform amplification and analysis on a compatible instrument

Ready to run in ~10 min

Ease of use enabled through a close system

Targeted Species for Respiratory Tract Microbiota (RTM)

3rd Leading Cause of Mortality

The Leading Cause of Pediatric Hospital Admissions

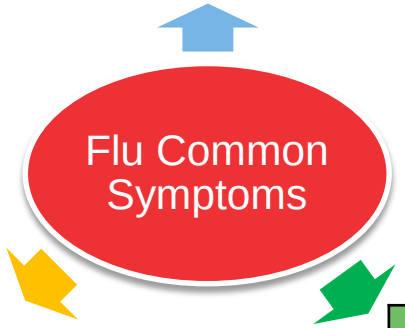
A Primary Danger for the Immuno-compromised

Multiple Pathogens Overlapping Symptoms

28 viral targets
Adenovirus
Bocavirus
Coronavirus 229E
Coronavirus HKU1
Coronavirus NL63
Coronavirus OC43
Enterovirus
HHV3 (Varicella Zoster Virus)
HHV4 (Epstein-Barr Virus)
HHV5 (Cytomegalovirus)
Human Herpesvirus 6
Human Metapneumovirus
Influenza A H1-2009
Influenza A H3
Influenza A pan
Influenza-B
Measles
MERS_CoV
Mumps
Parainfluenza virus 1
Parainfluenza virus 2
Parainfluenza virus 3
Parainfluenza virus 4
Parechovirus
Respiratory Syncytial Virus A
Respiratory Syncytial Virus B
Rhinovirus
SARS_CoV

12 Bacterial Targets
Bordetella (PAN)
Bordetella holmesii
Bordetella pertussis
Chlamydophila pneumoniae
Coxiella burnetii
Haemophilus Influenzae
Klebsiella pneumoniae
Legionella pneumoniae
Moraxella catarrhalis
Mycoplasma pneumoniae
Streptococcus pneumoniae
Staphylococcus aureus

3 Controls
IC Bacillus Atropheus
IC Rnase P
IC Xeno



1 Fungal Target
Pneumocystis jiroveci

Example of Microfluidic Card Layout

Adenovirus 1of2	1		25	Mumps
Adenovirus 2of2	2		26	MERS_CoV
Bocavirus	3		27	SARS_CoV
HHV3 (VZV)	4		28	Enterovirus (pan)
HHV4 (EBV)	5		29	Enterovirus_D68
HHV5 (CMV)	6		30	Rhinovirus 1of2
HHV6	7		31	Rhinovirus 2of2
Influenza A virus (pan)	8		32	Parechovirus
Influenza A H1	9		33	IC Xeno
Influenza A H3	10		34	Bordetella (pan)
IC 18S rRNA	11		35	B_holmesii
Influenza_B	12		36	B_pertussis
hPIV1	13		37	C_burnetii
hPIV2	14		38	C_pneumoniae
hPIV3	15		39	H_influenzae
hPIV4	16		40	K_ pneumoniae
RSVA	17		41	L_pneumophila
RSVB	18		42	M_catarrhalis
hMPV	19		43	M_pneumoniae
Measles	20		44	S_aureus
Coronavirus_229E	21		45	S_pneumoniae
Coronavirus_HKU1	22		46	P_jirovecii
Coronavirus_NL63	23		47	IC B_atropheus
Coronavirus_OC43	24		48	IC RNase P

Xeno RNA can be used as a process control

Bacillus atropheus can be used as an end to end workflow control

Available for early access

Sample-to-Answer Workflow for RTM

Up to 6 runs per day

Total Turnaround Time

5.5 Hours / Run

Service and support available throughout the entire process

Sample type: NT swabs

Sample prep

<2 Hrs

Thermo Scientific™ KingFisher™ Flex system with Applied Biosystems™ MagMAX kits

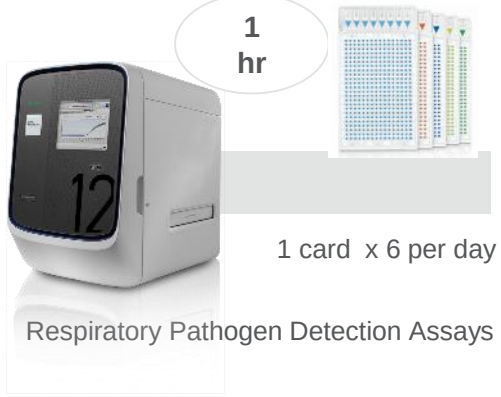
RT & Sample Processing

1.5 hr

Applied Biosystems™ ProFlex™ 96-well PCR System for RT

Run real-time PCR

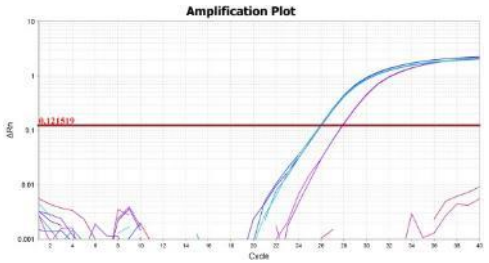
1 hr



1 card x 6 per day

Respiratory Pathogen Detection Assays

Analysis



Reporting

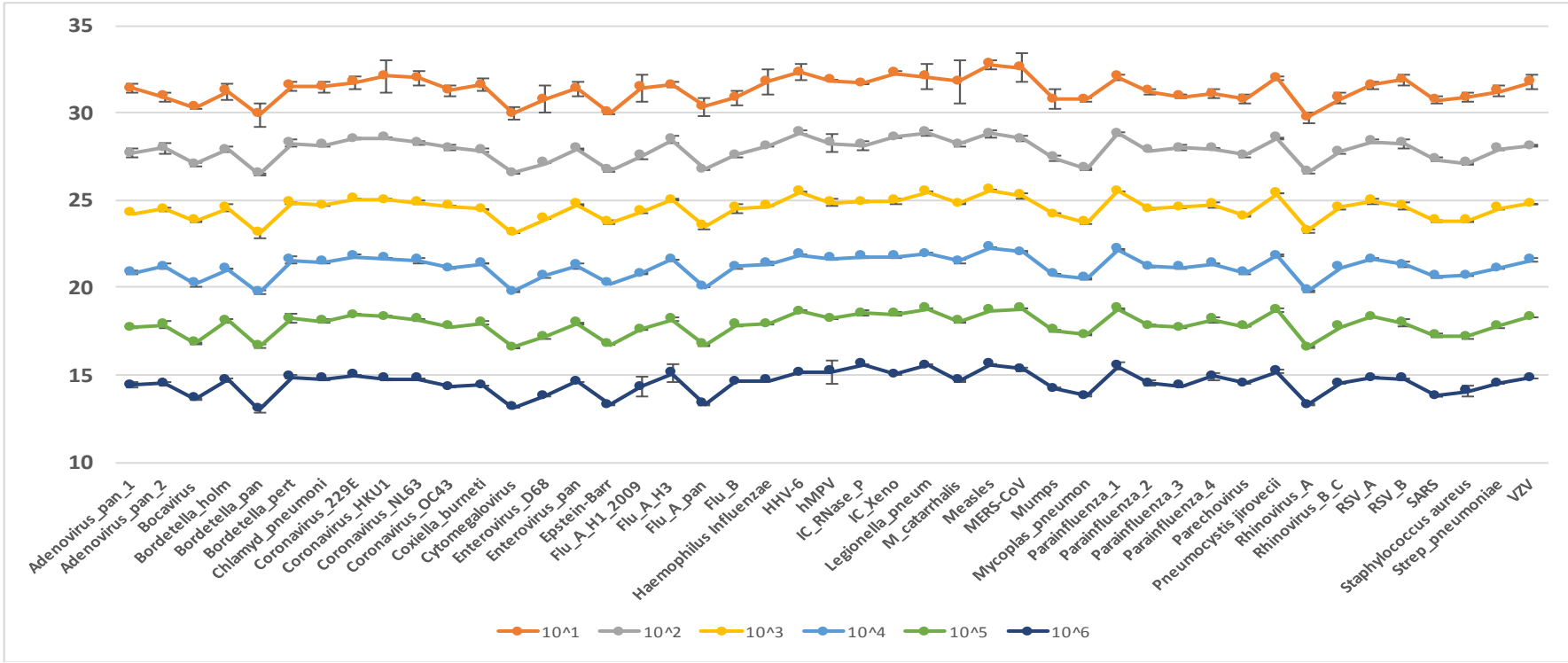
Data Analysis
In-house LIMS or CLS

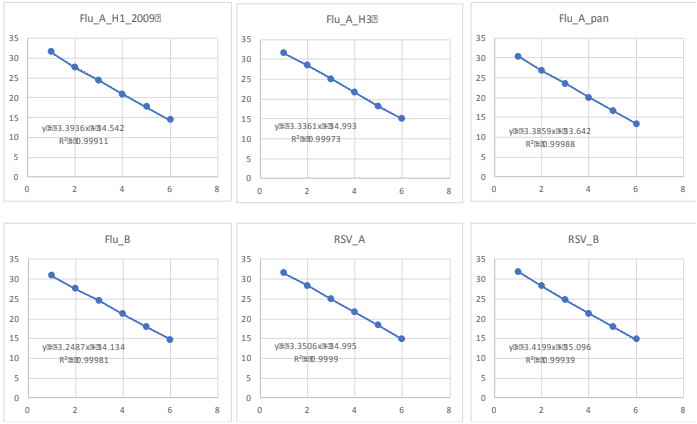
Coriell Life Sciences

ThermoFisher SCIENTIFIC

Analytical Sensitivity for RTM Assays Using Synthetic Template on TAC

Assays	R^2	Slope	PCR Efficiency
Adenovirus_pan_1	0.9993	-3.37	98.1%
Adenovirus_pan_2	0.9996	-3.30	100.8%
Bocavirus	0.9997	-3.36	98.4%
Bordetella_holm	0.9997	-3.29	101.3%
Bordetella_pan	0.9998	-3.35	99.0%
Bordetella_pert	1.0000	-3.33	99.6%
Chlamyd_pneumoniae	1.0000	-3.34	99.3%
Coronavirus_229E	0.9999	-3.35	98.8%
Coronavirus_HKU1	0.9999	-3.44	95.2%
Coronavirus_NL63	0.9997	-3.42	96.2%
Coronavirus_OC43	0.9999	-3.40	97.0%
Coxiella_burneti	0.9993	-3.39	97.3%
Cytomegalovirus	0.9999	-3.35	98.9%
Enterovirus_D68	0.9997	-3.37	98.1%
Enterovirus_pan	0.9999	-3.35	98.9%
Epstein-Barr	0.9993	-3.34	99.3%
Flu_A_H1_2009	0.9991	-3.39	97.1%
Flu_A_H3	0.9997	-3.34	99.4%
Flu_A_pan	0.9999	-3.39	97.4%
Flu_B	0.9998	-3.25	103.1%
Haemophilus Influenzae	0.9996	-3.41	96.4%
HHV-6	0.9999	-3.43	95.7%
hMPV	0.9994	-3.33	99.5%
Legionella_pneumoniae	0.9997	-3.32	100.0%
M_catarrhalis	0.9999	-3.41	96.5%
Measles	0.9989	-3.41	96.3%
MERS-CoV	0.9987	-3.39	97.3%
Mumps	0.9999	-3.31	100.6%
Mycoplas_pneumoniae	0.9988	-3.33	99.7%
Parainfluenza_1	1.0000	-3.31	100.4%
Parainfluenza_2	1.0000	-3.34	99.4%
Parainfluenza_3	0.9994	-3.35	99.0%
Parainfluenza_4	0.9999	-3.24	103.7%
Parechovirus	0.9998	-3.25	102.9%
Pneumocystis jirovecii	0.9998	-3.35	99.0%
Rhinovirus_A	0.9998	-3.30	100.8%
Rhinovirus_B_C	0.9996	-3.29	101.5%
RSV_A	0.9999	-3.35	98.8%
RSV_B	0.9994	-3.42	96.1%
SARS	0.9999	-3.37	98.1%
Staphylococcus aureus	0.9993	-3.34	99.4%
Strep_pneumoniae	1.0000	-3.35	98.7%
VZV	0.9996	-3.35	98.9%





- Serial dilution was done w/ synthetic control
- Similar results were obtained from Synthetic RNA and ATCC gDNA / gRNA controls

RTM Assay Specificity with ATCC gDNA and gRNA Controls

Assays/Controls	Human adenovirus 2	Human adenovirus 7	Human adenovirus 12	Bordetella bronchisepetica	Bordetella parapertussis	Bordetella holmesii	Bordetella pertussis	Chlamydophila pneumoniae	Chlamydophila pneumoniae	Human coronavirus 229E	Betacoronavirus 1 strain OC43	Human Herpesvirus 5 HCMV	Enterovirus D68 (US/KY/14-18953)	Enterovirus D68 (US/MO/14-18947)	Haemophilus influenzae	Influenza A virus (H1N1) strain A/	Influenza A virus (H3N2)	Influenza B virus	Klebsiella pneumoniae	Legionella pneumophila	Measles virus	Moraxella catarrhalis	Mumps virus	Mycoplasma pneumoniae	Parainfluenza PIV-1	Parainfluenza PIV-2	Parainfluenza PIV-3	Parainfluenza PIV-4	Human respiratory Syncytial Virus	Human respiratory Syncytial Virus	Human rhinovirus 17	Staphylococcus aureus	Streptococcus pneumoniae	Human herpesvirus 3
Adenovirus_pan_1	21.8	20.4																															
Adenovirus_pan_2		20.7	21.0																														
Bordetella_pan				23.9	22.7		22.4																										
B_holmesii						22.6																											
B_pertussis							22.6																										
C_pneumoniae								22.3	20.2																								
Coronavirus_229E										22.6																							
Coronavirus_OC43											21.7																						
HHV5_CMV												20.1																					
Enterovirus_D68													23.6	23.6																			
Enterovirus_pan														28.4																			
H_influenzae															19.7																		
Influenza_A_H1																23.2																	
Influenza_A_pan																20.7	22.6																
Influenza_B																		21.6															
K_pneumoniae																			20.1														
L_pneumophila																				20.8													
Measles																					21.6												
M_catarrhalis																						20.9											
Mumps																							23.0										
M_pneumoniae																								20.7									
PIV_1																									22.0								
PIV_2																										22.1							
PIV_3																											21.4						
PIV_4																												20.4					
RSVA																													20.0				
RSVB																													28.5	21.8			
Rhinovirus_pan_1																															25.5		
S_aureus																																20.1	
S_pneumoniae																																	19.0
HHV3_VZV																																	20.7

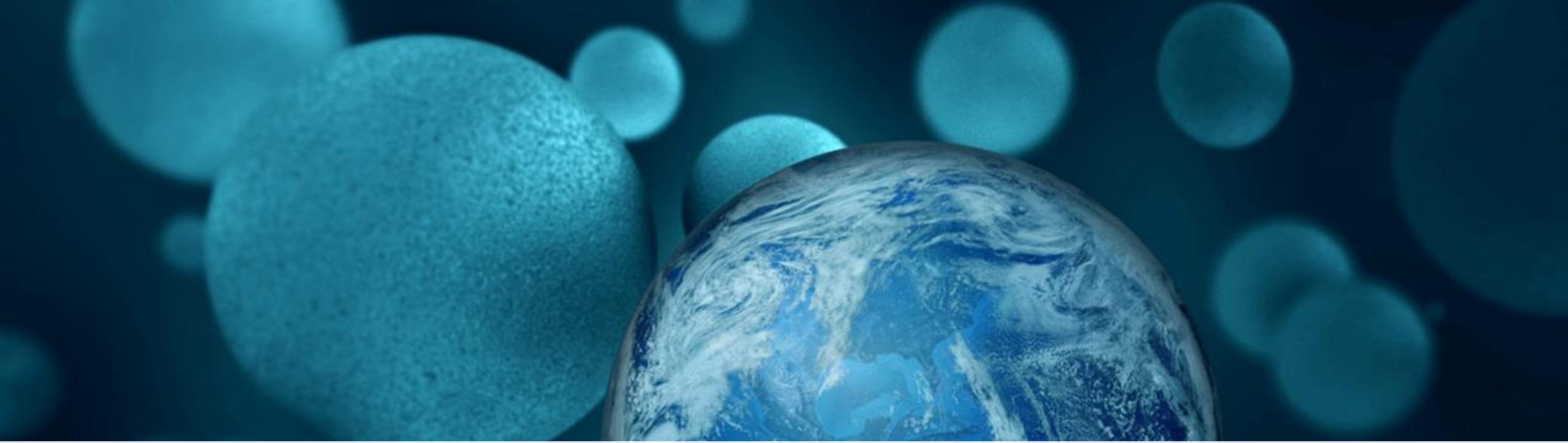
No significant off-target or cross-species activity was observed.

Summary

Nanofluidics platform

Microfluidics platform

- Applied Biosystems offers an end to end solution for supporting pathogen testing
- We currently offer microbial detection solutions for uro-genital pathogens on nanofluidics platform
- Microbial detection solutions for respiratory tract microbiota on microfluidics platform allows customization of both the size and content of the panel.
- The RTM panel demonstrates high sensitivity and specificity
- Our solution offers a simple workflow, fast turnaround time and high throughput with flexible sample/target combinations.



Thank you

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