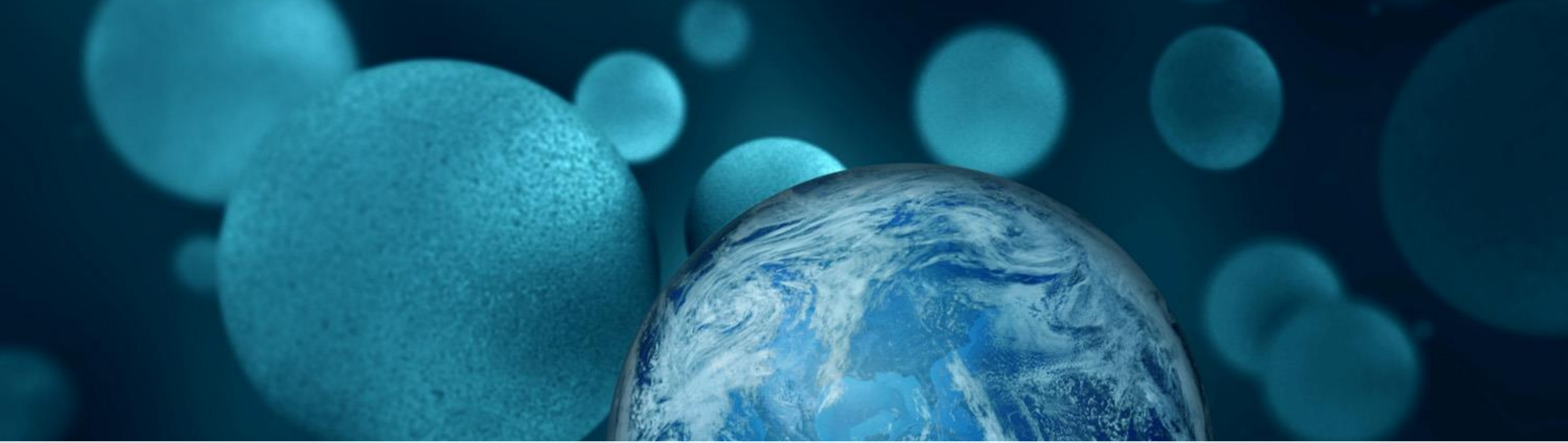

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Flexible Panel-Based Microbial Detection using Spatial Multiplexing on Nanofluidic qPCR Platform

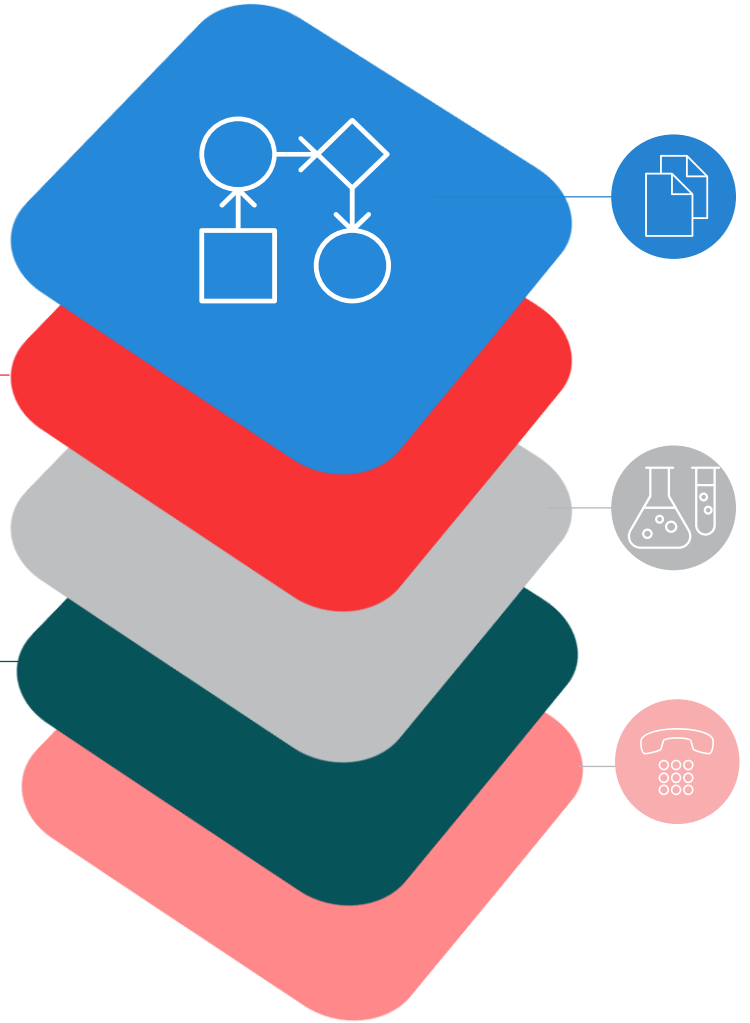
Nitin Puri, PhD
Associate Director, Infectious Disease
Oct 29th, 2018

Applied Biosystems - Microbial Detection Solutions

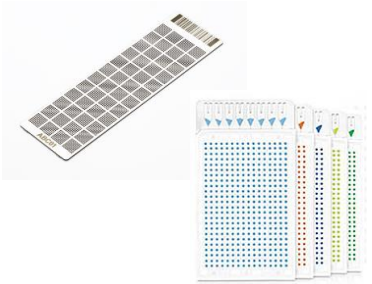
End to end solution approach for supporting pathogen testing

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

Applied Biosystems Solution for Real-Time PCR Detection of Microbiota

Taqman® **OpenArray® (OA)** Platform offers a a pan-molecular detection method for quantifiable and comparable results of all tested microbes, thereby reducing the variability associated with multiple conventional methods.

High Throughput
Up to 800 Samples a day

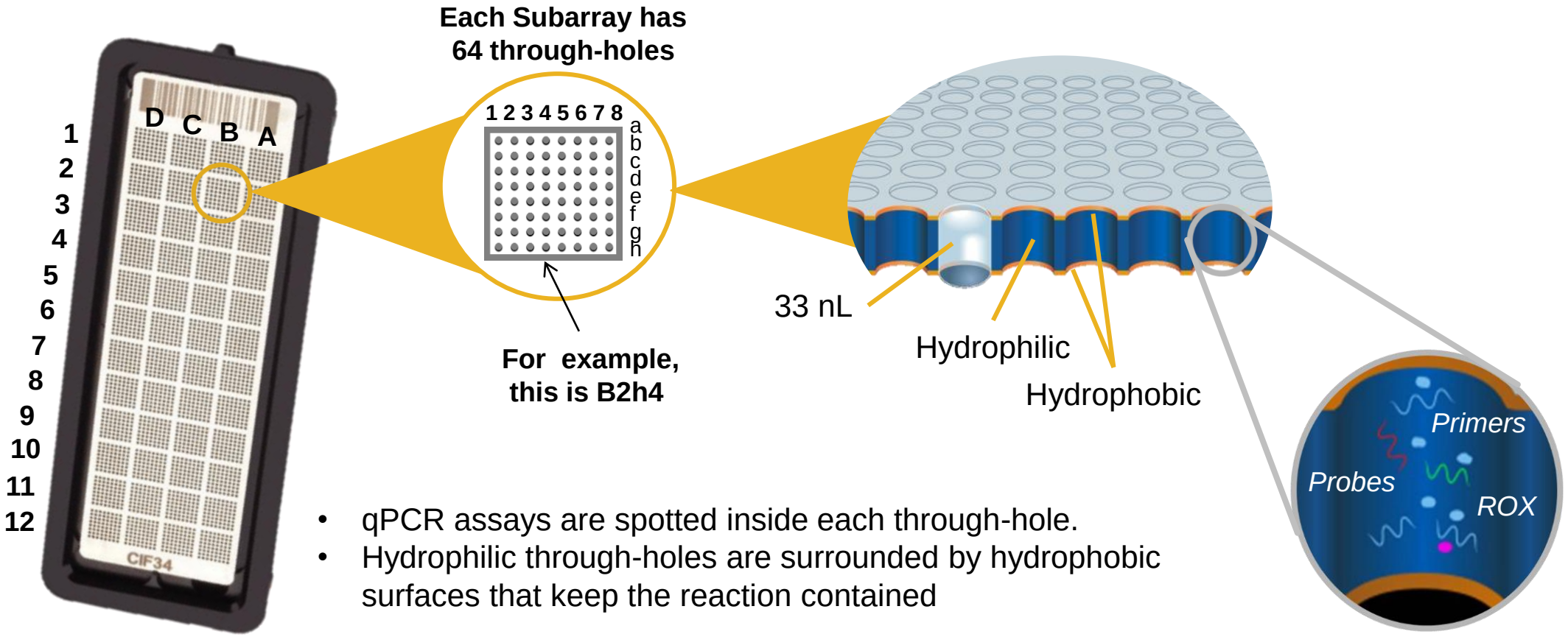
Performance Proficiency
High Sensitivity and Specificity

Flexibility & freedom
Selecting only what you want to test for to suit your needs

Extensive Coverage
Range of commensal and pathogenic microbes

Unrivaled support
Validation services & 24/7 support available

OpenArray™ Anatomy



- qPCR assays are spotted inside each through-hole.
- Hydrophilic through-holes are surrounded by hydrophobic surfaces that keep the reaction contained

Flexibility to customize your own panels for number of samples and targets

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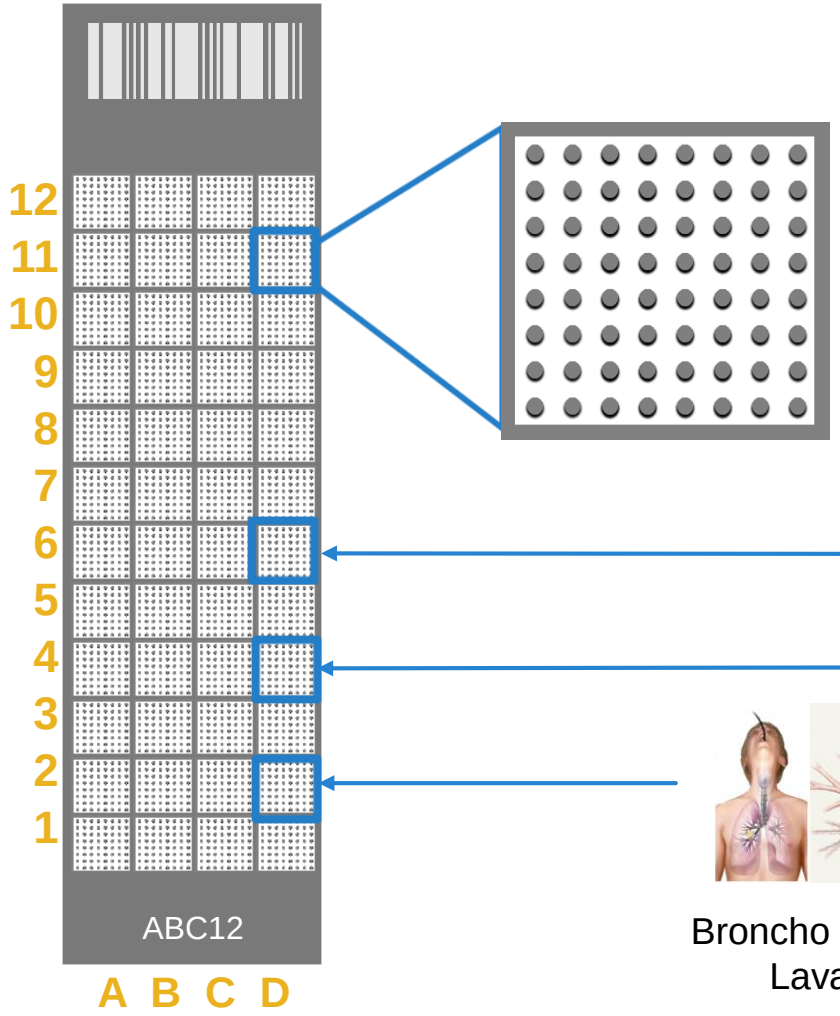
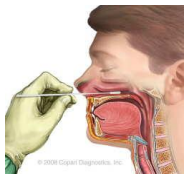
Flexibility for Customization for Targets & Samples

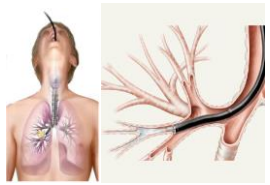


- Spatial Multiplexing allows for 56 Molecular targets per subarray
- Selection of assays for commensal and pathogenic microbes, host factors, antibiotic resistance genes



Nasopharyngeal Swab

Nasopharyngeal Aspirate



Broncho alveolar Lavage

ThermoFisher SCIENTIFIC

Blood-borne pathogen study using OA (Grigorenko et al, 2017)

Study from Robert Duncan et. al. at Center for Biologics Evaluation and Research, FDA, simultaneous detection and discrimination of **17 viral pathogens** in human plasma samples and **13 bacterial and protozoan pathogens** in human blood samples on the **OpenArray platform**. The custom plate was tested for specificity and analytical sensitivity with purified nucleic acids from each pathogen and with pathogen-spiked human blood and plasma samples.

The Journal of Molecular Diagnostics, Vol. 19, No. 4, July 2017

ELSEVIER

the Journal of Molecular Diagnostics
jmd.amjpathol.org

Highly Multiplex Real-Time PCR—Based Screening for Blood-Borne Pathogens on an OpenArray Platform

Elena Grigorenko,* Carolyn Fisher,† Sunali Patel,† Valerie Winkelman,‡ Phillip Williamson,¶ Caren Chancey,† Germán Añez,† Maria Rios,† Victoria Majam,† Sanjai Kumar,† and Robert Duncan†

From the Department of Research and Development, Diatherix Laboratories, LLC, Huntsville, Alabama; the Division of Emerging and Transfusion Transmitted Diseases,† Center for Biologics Evaluation and Research, Food and Drug Administration, Silver Spring, Maryland; the Genetic Sciences Division,‡ Thermo Fisher Scientific, South San Francisco, California; and Research and Development Support§ and Operations and Scientific Affairs,¶ Creative Testing Solutions, Tempe, Arizona*

Accepted for publication March 28, 2017.

Address correspondence to Robert Duncan, Ph.D., 10903 New Hampshire Ave., Silver Spring, MD 20993. E-mail: robert.duncan@fda.hhs.gov.

Molecular diagnostics are increasingly used in the blood bank industry. A device that can combine simultaneous detection of multiple targets with the flexibility of inclusion of emerging pathogens is desirable for testing blood products. A highly multiplexed blood-borne pathogen panel (BBPP) using dual-label probe chemistry (TaqMan assays) was developed for simultaneous detection and discrimination of 17 viral pathogens in human plasma samples and 13 bacterial and protozoan pathogens in human blood samples on the OpenArray platform. The custom BBPP OpenArray plate was tested for specificity and analytical sensitivity with purified nucleic acids from each pathogen and with pathogen-spiked human blood and plasma samples. The results of analytical validation of known samples yielded decision trees for identification of coded samples: pathogens spiked in human plasma or whole blood. Results from coded samples demonstrated no false positives among the plasma or whole blood specimens. Samples not detected were at the lower limit of the detectible range or qualified for retesting as indeterminate. Further demonstration of the performance of the BBPP OpenArray was achieved with clinical samples from a blood donor testing organization. Ninety-five percent of virus-positive samples were correctly identified. These results show that a high-throughput OpenArray PCR platform can be expanded and adapted for higher discrimination and newly emerging agents, enabling consideration for development as a next-generation device for testing blood products. *(J Mol Diagn 2017, 19: 549–560; http://dx.doi.org/10.1016/j.jmoldx.2017.03.004)*

The quality and safety of blood products used in transfusions are of significant public concern. The number of emerging pathogens that affect blood safety has steadily increased in the recent decades and has triggered a rapid development of molecular tests for detection of blood-borne pathogens such as West Nile virus,[1] dengue virus (DENV),[2] and *Babesia microti*[3] to name a few. Some highly virulent pathogens may have a low prevalence rate and/or be restricted seasonally or geographically, suggesting they may not be of concern for blood safety. However, the impact of transfusion-transmitted infection of such agents can have fatal consequences in highly vulnerable populations such as

Supported by the Food and Drug Administration Medical Countermeasures Initiative (R.D.).

Disclosures: E.G. is an employee of Diatherix Laboratories; S.P. is an employee of Thermo Fisher Scientific, the manufacturer of the OpenArray; V.W. and P.W. are employees of Creative Testing Solutions, the source of blood donor specimens.

The comments in this publication are an informal communication and represent the authors' own best judgments. These comments do not bind or obligate the Food and Drug Administration (FDA). The devices described in this publication are for research use only and have not been formally cleared or approved by FDA for the uses discussed herein.

All samples and Thermo Fisher Scientific (Applied Biosystems) reagents and materials are for research use only, not for use in diagnostic procedures.

Current address of G.A., Sanofi Pasteur, Swiftwater, PA.

Table 3 Analytical Sensitivity Study Results of Whole Blood Panel

		Assay 1				Assay 2			
Pathogen	Cells/mL	Name	Mean Cq	95% CI	TH Pos/Total	Name	Mean Cq	95% CI	TH Pos/Total
Babesia microti	1000	BAB-1	22.90	22.67–23.13	18/18				
B. microti	100	BAB-1	26.31	25.8–26.82	18/18				
Leishmania braziliensis	1000	LCHAG	26.31	25.66–26.96	18/18	LEI-1	23.58	±0.29	18/18
L. braziliensis	100	LCHAG	24.08	23.81–24.35	18/18	LEI-1	21.84	±0.24	18/18
L. donovani	1000	LINF	23.21	22.77–23.65	18/18	LTRO	22.18	±0.40	18/18
L. donovani	100	LINF	26.70	26.12–27.28	18/18	LTRO	25.82	±0.49	18/18
L. infantum	1000	LCHAG	21.39	21.15–21.63	18/18	LINF	24.70	±0.36	18/18
L. infantum	100	LCHAG	25.06	24.59–25.53	18/18	LINF	28.68	±0.46	18/18
L. major	1000	LMAJ	26.29	25.71–26.87	18/18	LTRO	28.58	±0.57	18/18
L. major	100	LMAJ	25.78	25.4–26.16	18/18	LTRO	28.62	±0.45	18/18
L. mexicana	1000	LMEX	24.18	24.01–24.35	18/18	LMEX-1	27.49	±0.48	17/18
L. mexicana	100	LMEX	28.25	27.96–28.54	12/18	LEI-1	26.03	±0.41	11/18
L. tropica	1000	LTRO	23.97	23.67–24.27	18/18	LEI-2	24.56	±0.31	18/18
L. tropica	100	LTRO	26.90	26.37–27.43	18/18	LEI-2	28.48	±0.50	17/18
Plasmodium falciparum	1000	PLA-3	21.58	21.19–21.97	17/18	PLA-2	19.95	±0.31	18/18
P. falciparum	100	PLA-3	25.12	24.5–25.74	18/18	PLA-2	23.06	±0.35	18/18
Plasmodium vivax	1000	PLA-4	23.49	23.11–23.87	18/18	PLA-2	18.88	±0.36	18/18
P. vivax	100	PLA-4	25.58	24.99–26.17	18/18	PLA-2	21.37	±0.32	18/18
Trypanosoma cruzi	1000	TCF-1	13.47	12.95–13.99	18/18	TCF-2	13.40	±0.53	18/18
T. cruzi	100	TCF-1	17.35	16.86–17.84	18/18	TCF-2	17.20	±0.46	18/18
Escherichia coli	1000	GNEG-1	24.39	24.1–24.68	17/18	GNEG-2	24.89	±0.36	16/18
E. coli	100	GNEG-1	27.31	26.81–27.81	18/18	GNEG-2	27.40	±0.36	18/18
Yersinia enterocolitica	1000	GNEG-1	25.21	24.87–25.55	18/18	GNEG-2	25.71	±0.27	18/18
Y. enterocolitica	100	GNEG-1	28.98	28.43–29.53	15/18	GNEG-2	29.24	±0.56	16/18
Staphylococcus aureus	10,000	STAU	25.11	24.73–25.49	18/18				
S. aureus	1000	STAU	27.67	26.91–28.43	5/18				
Neg control blood		LEI-1	28.66	28.26–29.06	15/27	GNEG-1	29.53	±0.37	4/27
No-template control		GNEG-1	29.38	29.03–29.73	10/36	GNEG-2	29.64	±0.45	9/36

J Mol Diagn 2017 v19: p549-560

Sample-to-Answer Workflow for UTM

Up to 3 runs per day

Total Turnaround Time

5 Hours / Run

Service and support available throughout the entire process

Sample type: urine

Sample prep

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

Sample loading

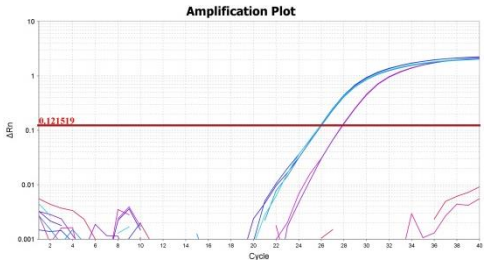
Applied Biosystems™ OpenArray™ AccuFill™ System for sample loading

Run real-time PCR

4 plates x 12 per day

UTI Pathogen Detection Assays

Analysis



Reporting

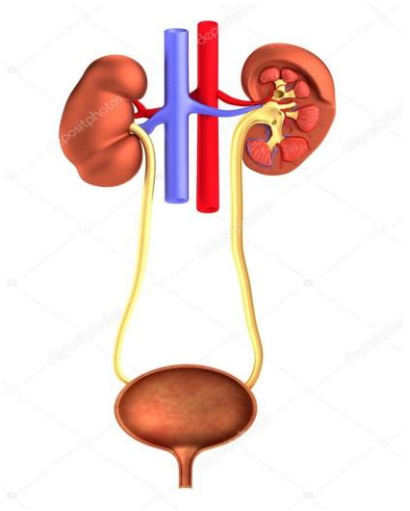
Data Analysis
In-house LIS or CLS

Coriell Life SCIENCES

ThermoFisher SCIENTIFIC

Microbiome Applications Areas in use on OpenArray

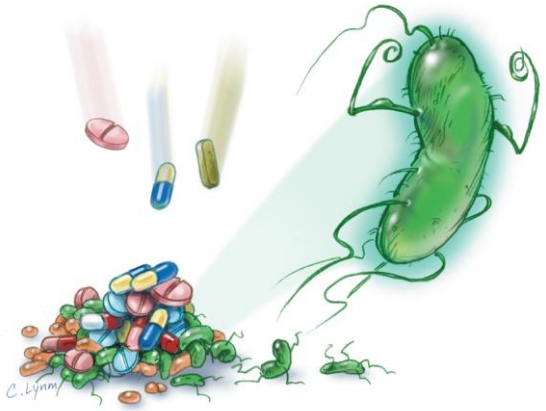
Urogenital



Gastrointestinal

Respiratory

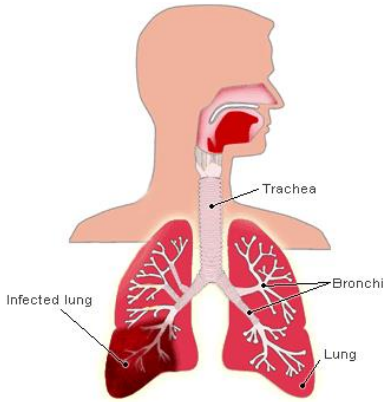
Antibiotic Resistance



Wound

Foot Fungus

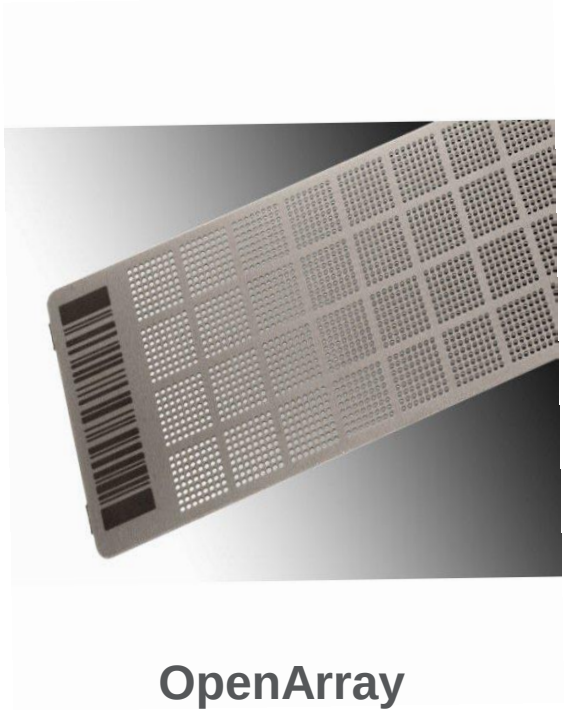
Tuberculosis



CNS

Summary

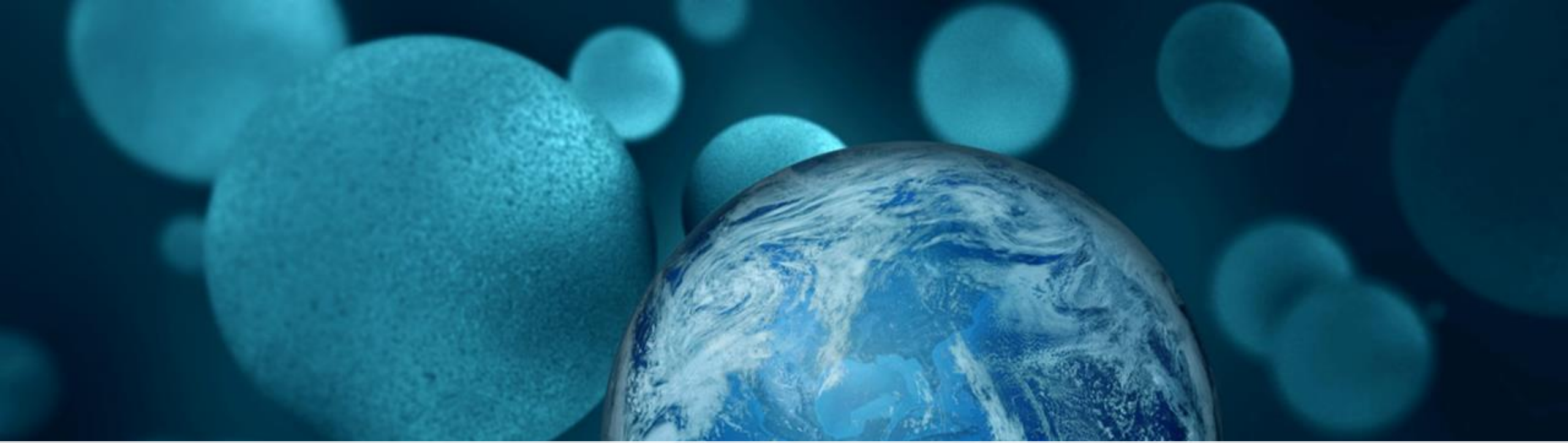
QuantStudio



OpenArray

- OpenArray® and QuantStudio offer a low cost, customized and proficient microbial detection solution system
- Applied Biosystems offers a tool box with supporting reagents including sample prep and customized controls’

Reach out to nitin.puri@thermofisher.com for potential interest / collaborations



Thank you

ThermoFisher SCIENTIFIC