



New Microfluidic Platforms for the simultaneous Detection of Pathogens using quantitative PCR

Dr Astrid Ferlinz
Business Strategy & Application Development

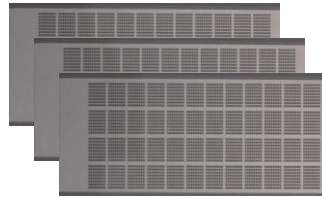
Adventitious Agents, New Technology and Risk
Assessment
Baltimore, May 2011

Agenda

- TaqMan[®] Array Cards



- OpenArray[®]



- Acrometrix Standards & Controls



Development of a TaqMan[®] Array Viral Screening Card

- Quality of stem cell lines is defined by
 - Authenticity
 - Stability
 - Purity

- Requirements from Stem Cell Laboratories for a pathogen screening tool to assess cell cultures in research labs
 - Sensitive
 - Cost Effective
 - Standardized & Reproducible
 - Accurate
 - Rapid & Easy to use



TaqMan® Array Cards

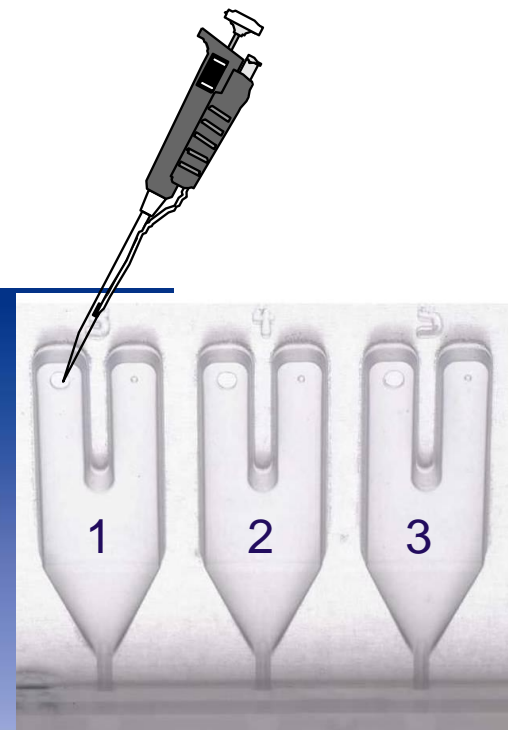
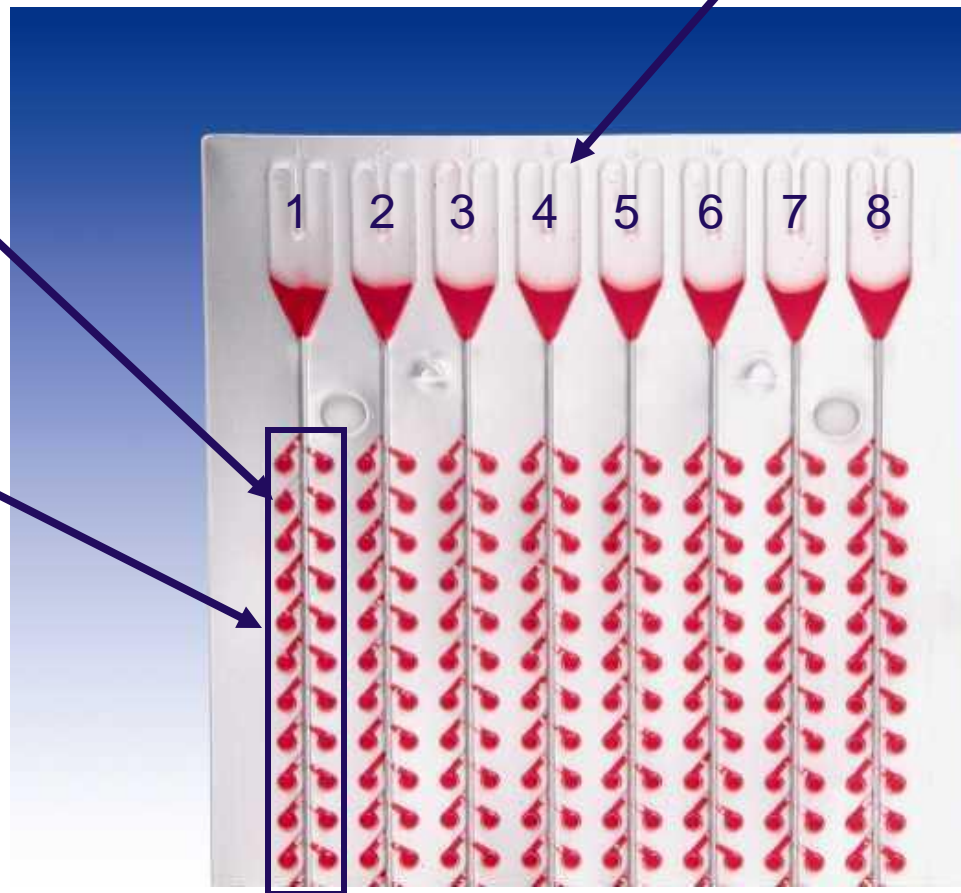


TaqMan®
Assays pre-
spotted

48 wells
per
channel

12 to 384
different
assays

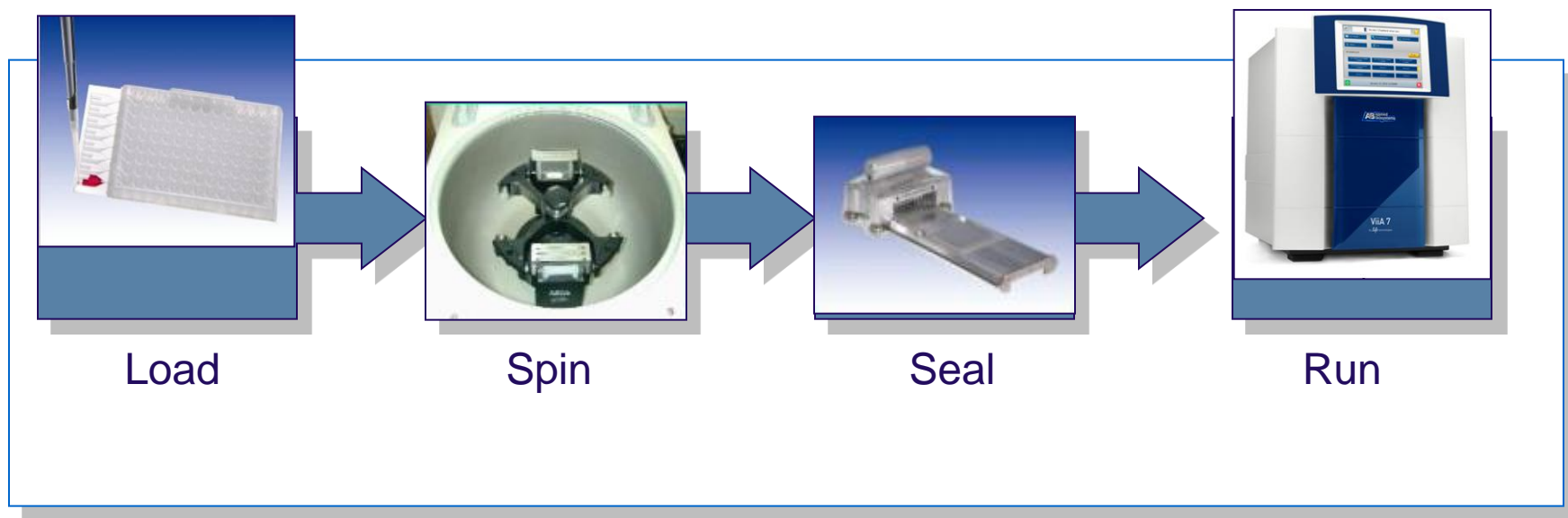
1 to 8 samples



384 wells (1µl reaction volume)



TaqMan® Array Cards



5 - 10 minutes



Validation Workflow

Design of specific TaqMan® Assays using Life Technologies Bioinformatic Pipeline

Combined DNA & RNA Isolation from Stem Cells

One-step RT-PCR and Detection by Real-time PCR

Performance Testing using Artificial Templates

Performance Testing using infected Stem Cell Lines

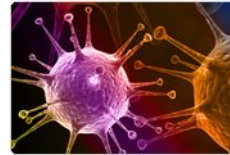
Done with Single Tubes/Plates & TaqMan® Arrays



TaqMan® Pathogen Detection Assays

- HPV16
 - Gene E1
- HSV-1
 - Gene UL41
- EBV
 - Gene IR1
- CMV
 - Gene UL132
- HHV-7
 - Gene U48
- BKV
 - Gene VP2
- JCV
 - Gene VP2
- SV40
 - Gene VP2
- HCV
 - 5'UTR
- HBV
 - Gene P,S
 - Gene P,X
- HTLV-1
 - Gene rex, tax
- HTLV-2
 - Gene LTR
- HIV-1
 - Gene LTR
- HIV-2
 - Gene LTR
- Human Adenovirus
 - Gene L4
 - Gene L3

Pathogen Detection & Infectious Disease Research



Infectious diseases can be caused by bacterial, viral, fungal, or parasitic microorganisms and contributes to millions of deaths each year. TaqMan® Assays provide one of the most powerful, sensitive, and fast techniques available for the detection of disease-causing pathogens that threaten our global health.



TaqMan® Array Viral Pathogen Screening Cards



TaqMan® Assays pre-spotted

48 wells with

16 assays in triplicate reactions

8 different samples



	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	
A	HPV16	HTLV-I	HIV-4, EBV	18S rRNA	HIV-5, CMV	BKV	JCV	SV-40																	
B	HIV-7	HCV	HBV	HTLV-2	HIV-1	HAdV-C	HIV-2	HSV-1	1																
C	HPV16	HTLV-I	HIV-4, EBV	18S rRNA	HIV-5, CMV	BKV	JCV	SV-40																	
D	HIV-7	HCV	HBV	HTLV-2	HIV-1	HAdV-C	HIV-2	HSV-1	2																
E	HPV16	HTLV-I	HIV-4, EBV	18S rRNA	HIV-5, CMV	BKV	JCV	SV-40																	
F	HIV-7	HCV	HBV	HTLV-2	HIV-1	HAdV-C	HIV-2	HSV-1	3																
G	HPV16	HTLV-I	HIV-4, EBV	18S rRNA	HIV-5, CMV	BKV	JCV	SV-40																	
H	HIV-7	HCV	HBV	HTLV-2	HIV-1	HAdV-C	HIV-2	HSV-1	4																
I	HPV16	HTLV-I	HIV-4, EBV	18S rRNA	HIV-5, CMV	BKV	JCV	SV-40																	
J	HIV-7	HCV	HBV	HTLV-2	HIV-1	HAdV-C	HIV-2	HSV-1	5																
K	HPV16	HTLV-I	HIV-4, EBV	18S rRNA	HIV-5, CMV	BKV	JCV	SV-40																	
L	HIV-7	HCV	HBV	HTLV-2	HIV-1	HAdV-C	HIV-2	HSV-1	6																
M	HPV16	HTLV-I	HIV-4, EBV	18S rRNA	HIV-5, CMV	BKV	JCV	SV-40																	
N	HIV-7	HCV	HBV	HTLV-2	HIV-1	HAdV-C	HIV-2	HSV-1	7																
O	HPV16	HTLV-I	HIV-4, EBV	18S rRNA	HIV-5, CMV	BKV	JCV	SV-40																	
P	HIV-7	HCV	HBV	HTLV-2	HIV-1	HAdV-C	HIV-2	HSV-1	8																

- HPV16
- HSV1
- CMV
- HHV7
- EBV
- BKV
- JCV
- HBV
- HCV
- SV40
- Adenovirus
- HTLV1
- HTLV2
- HIV1
- HIV2
- (18S)

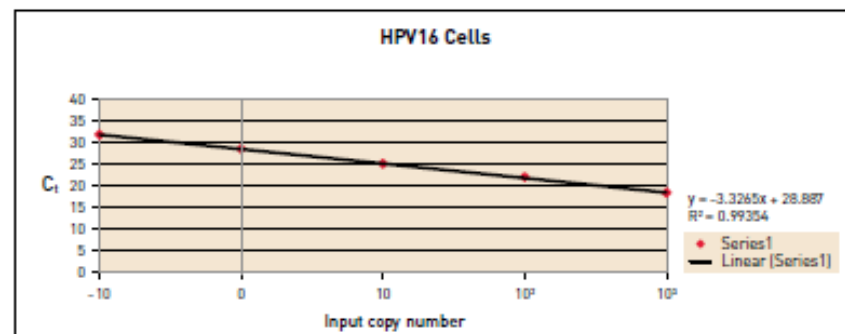
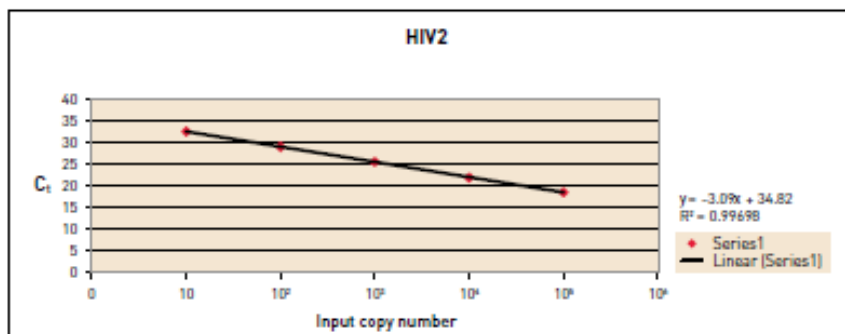
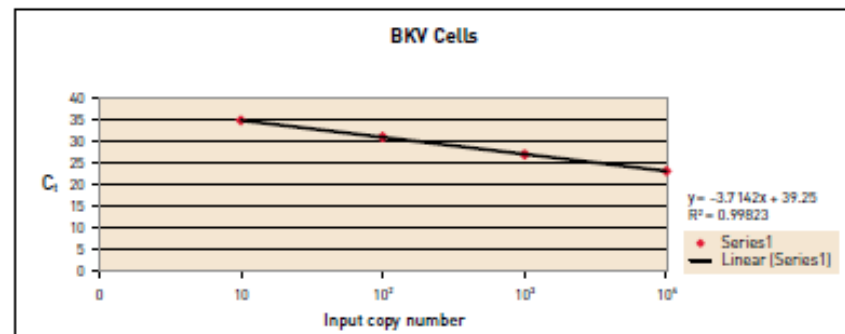
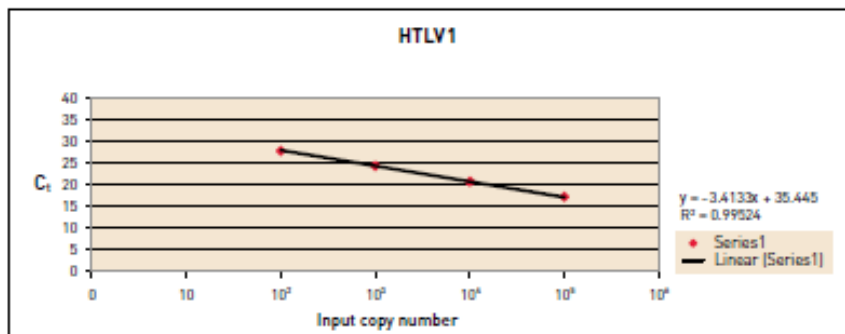


Data summary

- Using Artificial templates & **single tubes** (100,000 copies to 1 copy)
 - Single copies were all detected (Ct values 33.5-37.5)
 - Efficiencies of reactions 92-105%
- Total nucleic acid isolated from infected stem cell lines & **single tubes** (200 ng to 0.001ng)
 - Ct values in the range of 34-39 cycles were observed at the lowest concentration across all cell lines
 - Efficiencies from 96-108%
- Using Artificial templates & **cards** (100,000 to 1 copy),
 - Single copies were detected by 4 assays
 - > Dynamic range: 3-5 logs
 - Efficiencies of reactions 97-110% (R2 >0.995)
- Using nucleic acid isolated from infected stem cell lines & **cards**,
 - Efficiencies of reactions 86-110%
 - Highest dilution detected by 2 assays



Dilution series using TaqMan® Array Viral Pathogen Screening Cards



TaqMan® Pathogen Assays & TaqMan® Array Viral Pathogen Screening Cards

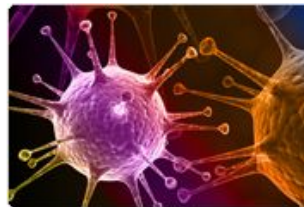
- Validation process finished
- Single Tube Assays released since 2009
- Cards released now
- Currently, we are collaborating with key-sites in Europe on the development of new application-specific panels



Clinical & Diagnostic Applications

Transplant, IVD, Cell Isolation, Pathology, Cytogenetics, Environmental, Biodefense, Cell Therapy Systems

Pathogen Detection & Infectious Disease Research



Infectious diseases can be caused by bacterial, viral, fungal, or parasitic microorganisms and contributes to millions of deaths each year. TaqMan® Assays provide one of the most powerful, sensitive, and fast techniques available for the detection of disease-causing pathogens that threaten our global health.



Just published: Detection of Respiratory Pathogens

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J Clin Microbiol April 2011
 doi:10.1128/JCM.02270-10

1 Application of TaqMan® Low Density Arrays for Simultaneous

2 Detection of Multiple Respiratory Pathogens

3

4 Maja Kodani¹, Genyan Yang¹, Laura M. Conklin¹, Tatiana C. Travis¹, Cynthia

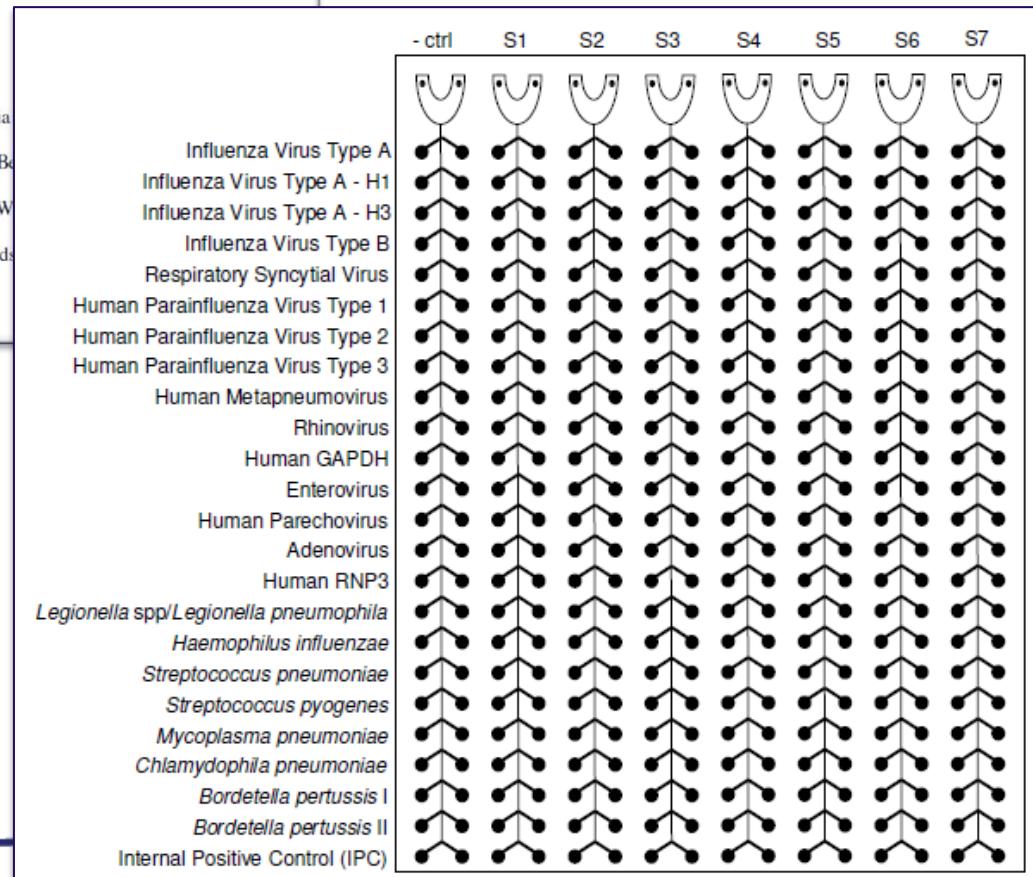
5 Whitney¹, Larry J. Anderson², Stephanie J. Schrag¹, Thomas H. Taylor, Jr.¹, Be

6 Beall¹, Robert F. Breiman⁴, Daniel R. Feikin⁶, M. Kariuki Njenga⁵, Leonard W

7 M. Steven Oberste², Maria Lucia C. Tondella¹, Jonas Winchell¹, Stephen Linds

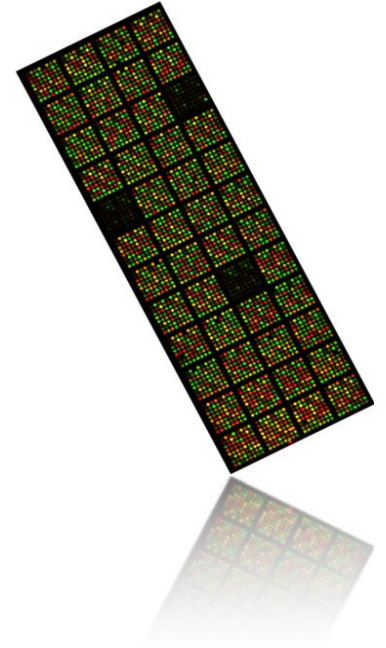
8 Dean D. Erdman² and Barry S. Fields¹

Centers for Disease Control
 & Prevention, Atlanta

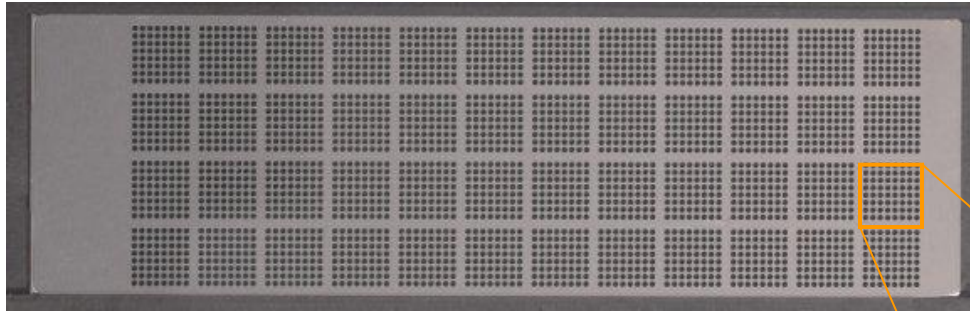


The OpenArray[®] Solution

- High-density format
- Simple and reliable workflow from passive nanofluidics
- Quantification & Identification of pathogens
- Short time to answer from rapid, parallel processing



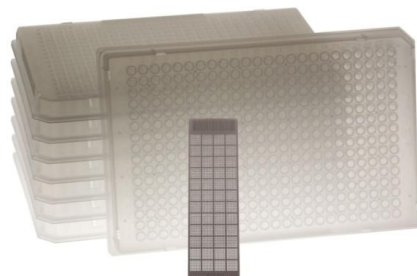
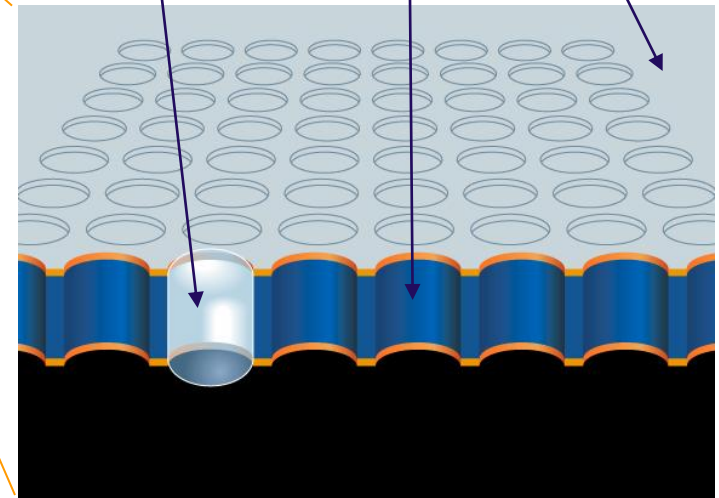
OpenArray[®] Anatomy



- Coatings enable reagents to load into and stay within the bottomless through-holes via passive capillary action.

33 nL volume

Hydrophobic
Hydrophilic

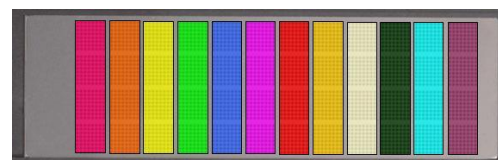
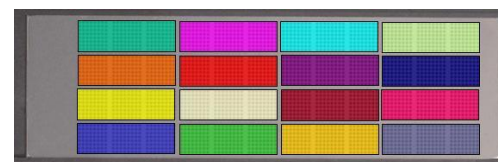
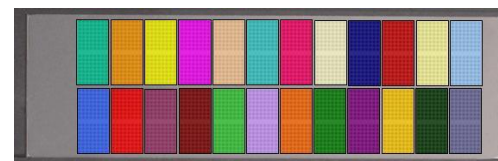


Flexible Custom Layouts

- OpenArray® Real-Time qPCR Plate, 18 (48 samples)
- OpenArray® Real-Time qPCR Plate, 56 (48 samples)
- OpenArray® Real-Time qPCR Plate, 112 (24 samples)
- OpenArray® Real-Time qPCR Plate, 168 (16 samples)
- OpenArray® Real-Time qPCR Plate, 224 (12 samples)

Subarray:

A1	1	2	3	4	5	6	7	8
a	1	2	3	4	5	6	7	8
b	9	10	11	12	13	14	15	16
c	17	18	19	20	21	22	23	24
d	25	26	27	28	29	30	31	32
e	33	34	35	36	37	38	39	40
f	41	42	43	44	45	46	47	48
g	49	50	51	52	53	54	55	56
h	57	58	59	60	61	62	63	64



Standards & Controls



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Business Development

AcroMetrix offers a range of high quality molecular products designed to meet customer needs. This product range includes controls and standards to assist in the implementation and monitoring of diagnostic assays for clinical laboratories, blood centers and manufacturers.

With our combination of well characterized, consistent quality control products and internet-based solutions for interpreting and managing results, we strive to make it easy for laboratories to:

- ▶ **validate new assays with the new EZValidation™ Online Tool**
- ▶ train laboratory technologists, and
- ▶ set the standard when creating noteworthy quality control programs

OptiQuant® CMVtc Calibration Panel

First traceable and commutable panel that mimics a true patient sample



Acrometrix Controls

- Nucleic Acid Controls as well as Serological Diagnostic Control Products
- OptiQual, OptiQuant, Valiquant Products are designed for hospital laboratories
 - Transplant Centers – CMV, EBV, BKV
 - Drug monitoring, blood borne viruses – HIV, HBV, HCV
 - > Eg OptiQuant HBV DNA Quantification Panel
 - Hospital acquired infections – MRSA, C diff
- PeliSpy Multi-Marker and PeliSpy Pro Products are designed for blood centers
 - PeliSpy Multi-Marker: assessing performance of ImmunoAssay procedures for qualitative determination of eg anti-HIV, anti-HCV etc
 - PeliSpy Pro: estimates precision & reproducibility of a NA based test (resembles donor sample)
- H1N1 2009 Positive Control



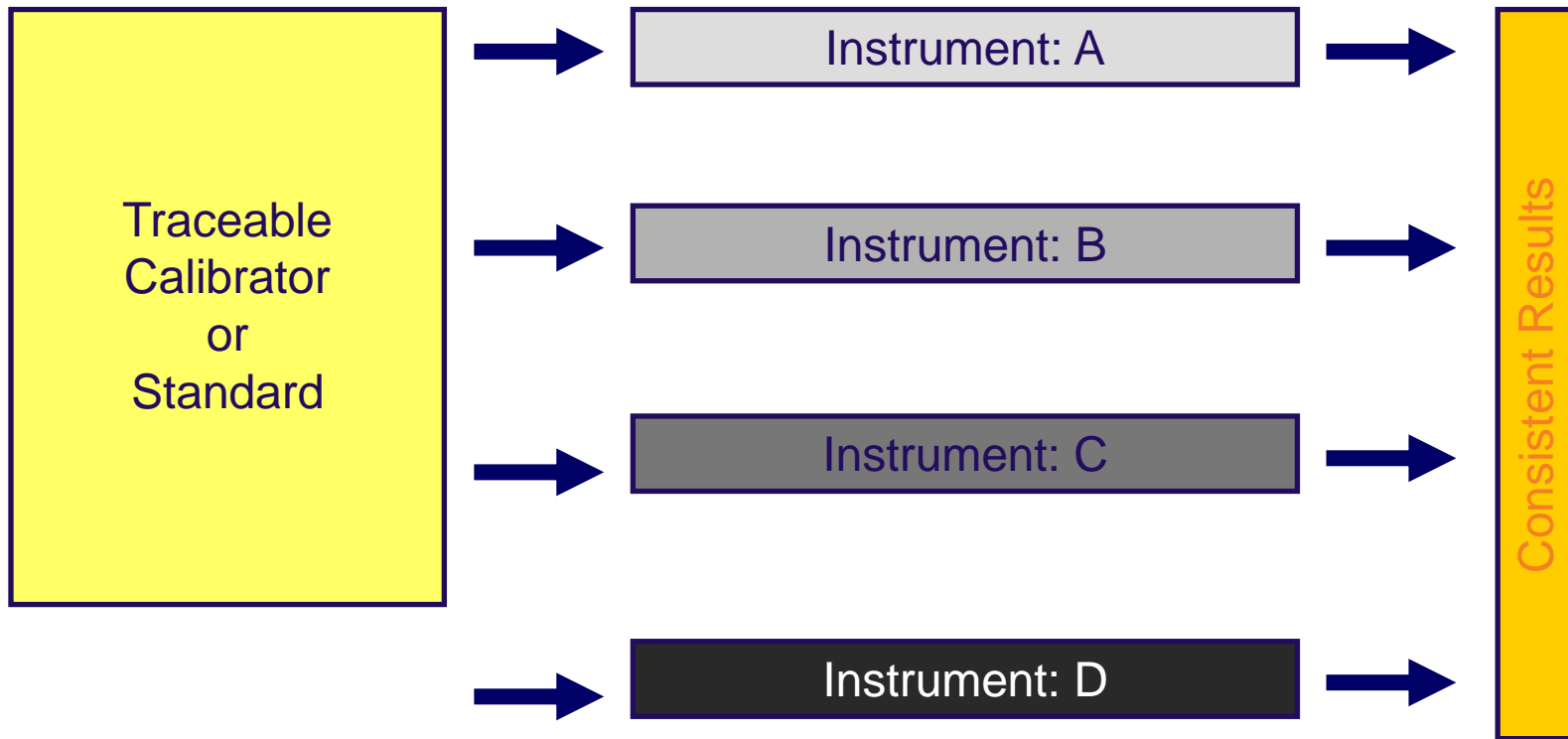
Standardisation

“A fundamental goal of laboratory medicine is that results for patients’ samples will be **comparable independent** of the medical laboratory that produced the results.*“

*W. Greg Miller et al *Clinical Chemistry*. 2006;52:553-554



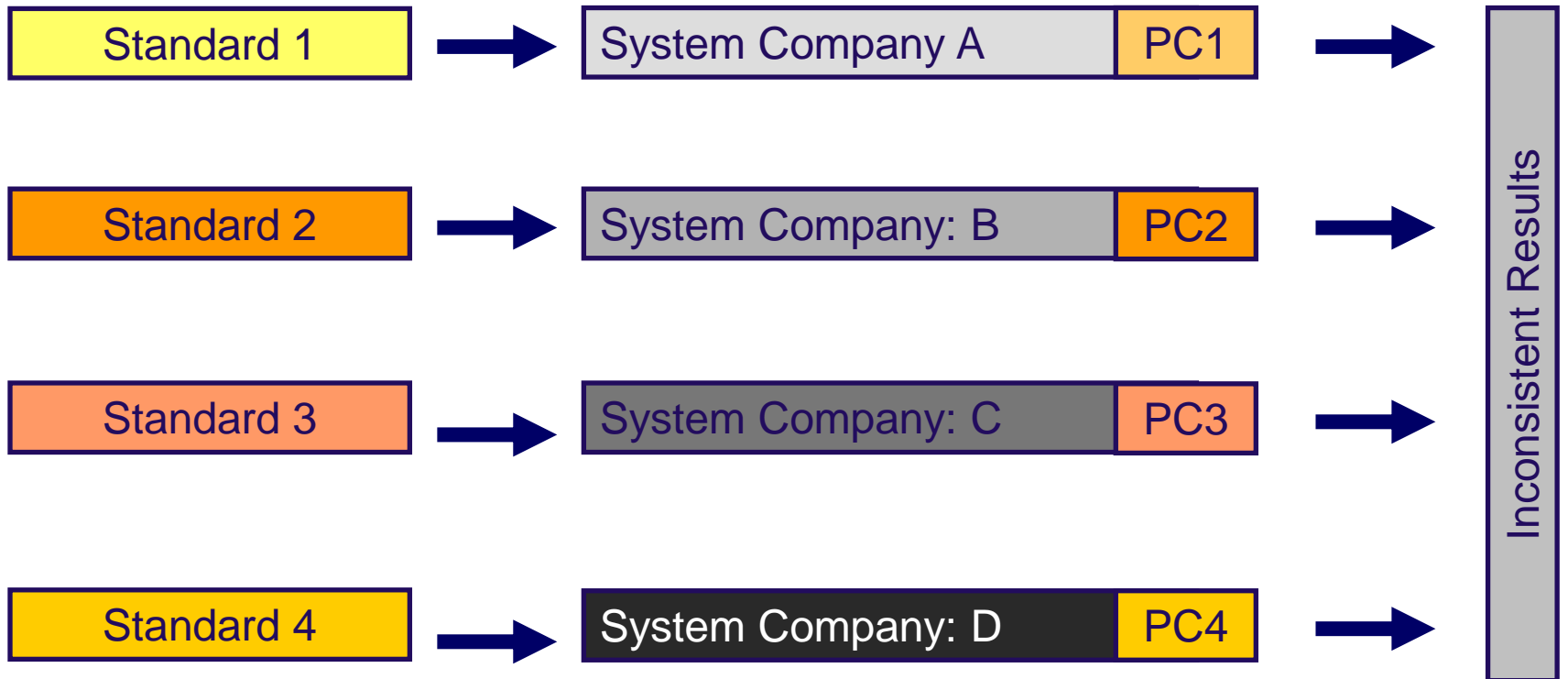
Standardised Calibration



Scenario for established measurement systems (e.g. scale)



Non-Standardised Calibration



Current scenario for many analytes
except HIV-1, HBV and HCV

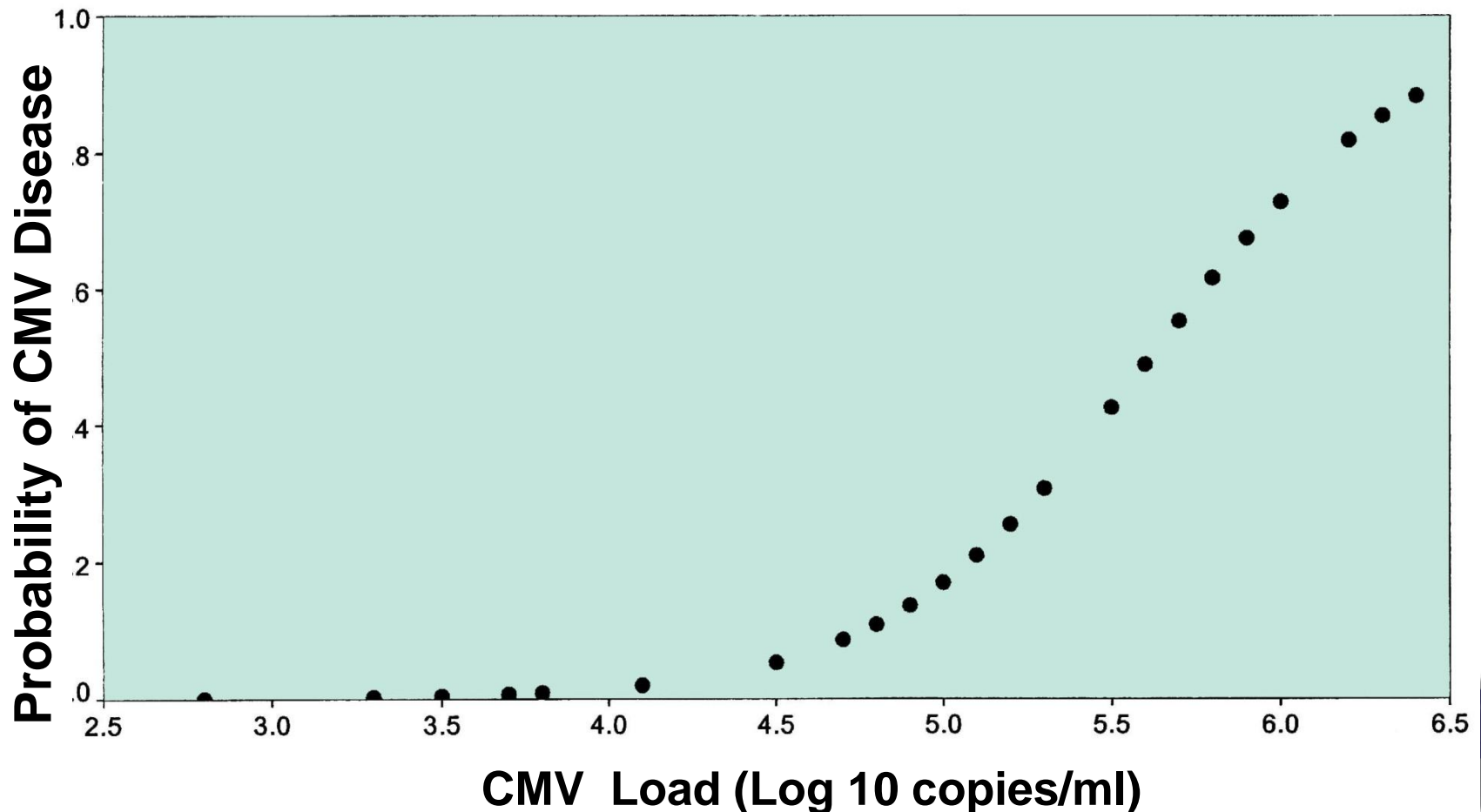


Is an “assayed control” acceptable?



“CMV Conclusion”

- Lack of defined units and calibration material results in “difficult to establish broadly applicable, clinically relevant guidelines”



Limitations of Current NAT Control Materials

- Not metrological based
- Often based on specific assay system
- Units not clearly defined
- Not independent (assayed & kit controls)
- Not useful for Standardisation



Acrometrix controls: Scientific Aspects

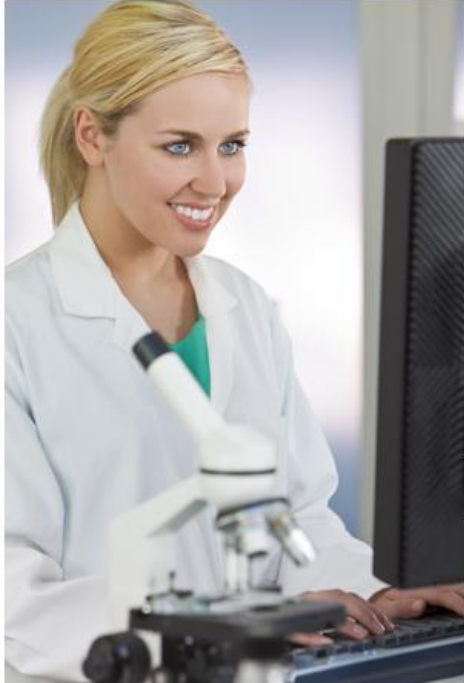
- Standardisation
 - Products are calibrated to WHO International Standards if available and value assigned in IU/ml
- Metrological Traceability
 - ISO 17511
- Commutability (behaving like patient sample)
- Full process control
- Reproducible
- Unlimited supply
- Ready to use



AcroMetrix' Control Development

- AcroMetrix Quality System
 - ISO 13485:2003 certified
 - FDA 21CFR Part 820 Quality System Regulations (QSR)
 - cGMP
 - Calibrators and Controls values assigned following ISO 17511:2003
 - > In vitro diagnostic medical devices — Measurement of quantities in biological samples — Metrological traceability of values assigned to calibrators and control materials





Validation and Verification

Welcome to the EZValidation™ Online Tool. This online tool is designed to aid molecular diagnostic laboratories in the verification or validation of a molecular test.

The EZValidation™ Online Tool is a tool intended to assist laboratories in meeting their verification and validation requirements. Each laboratory is responsible for ensuring compliance with applicable international, national and local clinical laboratory regulations and other specific accreditations requirements.

This tool will guide you through the validation and verification of your molecular assay.

- Choose guidelines applicable to your lab (CAP/CLIA/New York State or I 15189)
- Design additional custom guidelines to meet your laboratory's needs
- Set acceptance criteria for your study
- Print a final report of your validation or verification

Start Designing a New Study →

Need to validate your own assay?



Journal of Clinical Virology 40 (2007) 93–98

JOURNAL OF CLINICAL VIROLOGY

www.elsevier.com/locate/jcv

Review

Verification and validation of diagnostic laboratory tests in clinical virology

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Abstract

This review summarizes major issues of verification and validation procedures and describes minimum requirements for verification and validation of diagnostic assays in clinical virology including instructions for CE/IVD-labeled as well as for self-developed (“home-brewed”) tests or test systems. It covers techniques useful for detection of virus specific antibodies, for detection of viral antigens, for detection of viral nucleic acids, and for isolation of viruses on cell cultures in the routine virology laboratory.
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Keywords: Verification; Validation; Diagnostics; Virology; IVD; CE

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1. Introduction

Routine viral diagnostics includes techniques for indirect and those for direct detection of viruses. Indirect detection of viruses is performed by serological studies. Techniques

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