Theranos Science & Technology: The Miniaturization of Laboratory Testing

Elizabeth Holmes

theranos

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Scientific Exchange

- Some of the medical device technologies discussed today are regulated by the FDA and are not yet cleared or approved.
- This presentation is not intended to promote Theranos devices or testing.
- Theranos technologies are not intended or offered for sale or commercial use at this time.
- The purpose of our presentation is to provide an exchange of scientific information about Theranos' inventions and technologies.

Our mission is to make actionable health information accessible at the time it matters

Theranos Technologies

Reagents and Assays for Small-Volume Samples

Collection Technologies

Sample Collection Device (SCD)

Nanotainer™ Tubes

High Throughput Platforms

Theranos Virtual Analyzer (TVA)

Theranos Sample Processing Unit (miniLab)

This technology has not been cleared or approved by the FDA and is not for sale in the United States.

Presentation Overview

I. Miniaturization of laboratory testing

II. miniLab results across detection methodologies

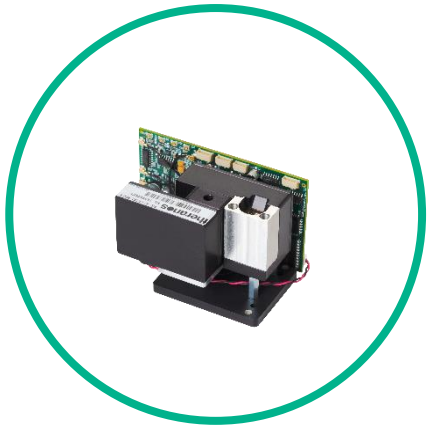
III. Small sample volumes: collection of capillary blood and analysis

Miniaturization and Integration of Detection Systems

Spectrophotometer

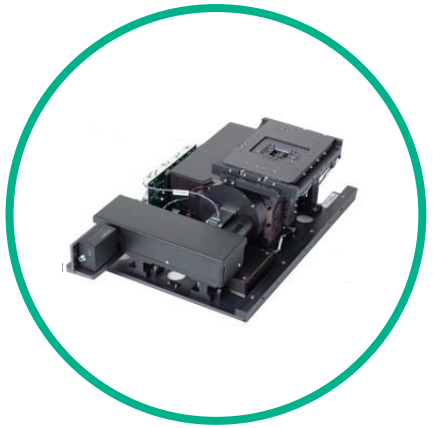
Clinical Chemistry

Luminometer & Fluorometer



Immunochemistry

Cytometer



Hematology & Immunology

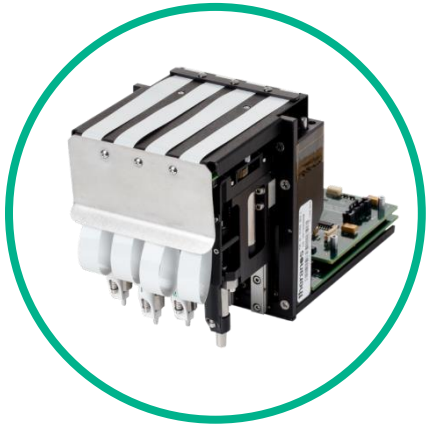
Fluorescence-based Isothermal Detector

Molecular Biology

Images not to scale

Miniaturization and Integration of Processing Modules

Camera

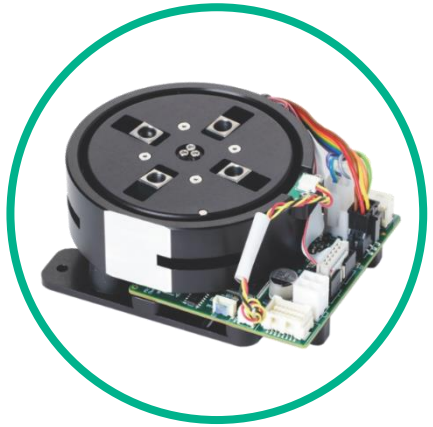


Material Handling Robot

Cartridge

Sonicator

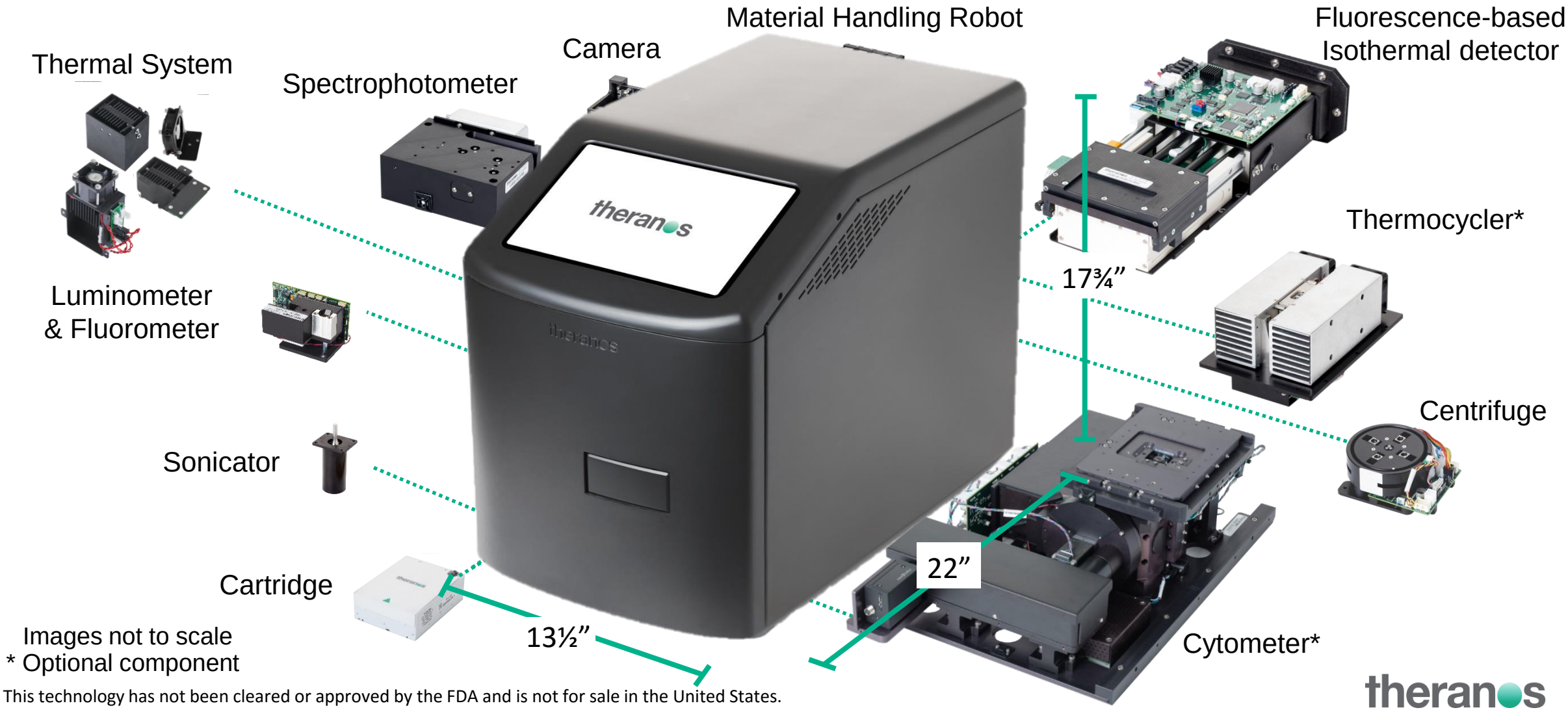
Thermocycler



Centrifuge

Images not to scale

Theranos Sample Processing Unit (miniLab)



Images not to scale
* Optional component

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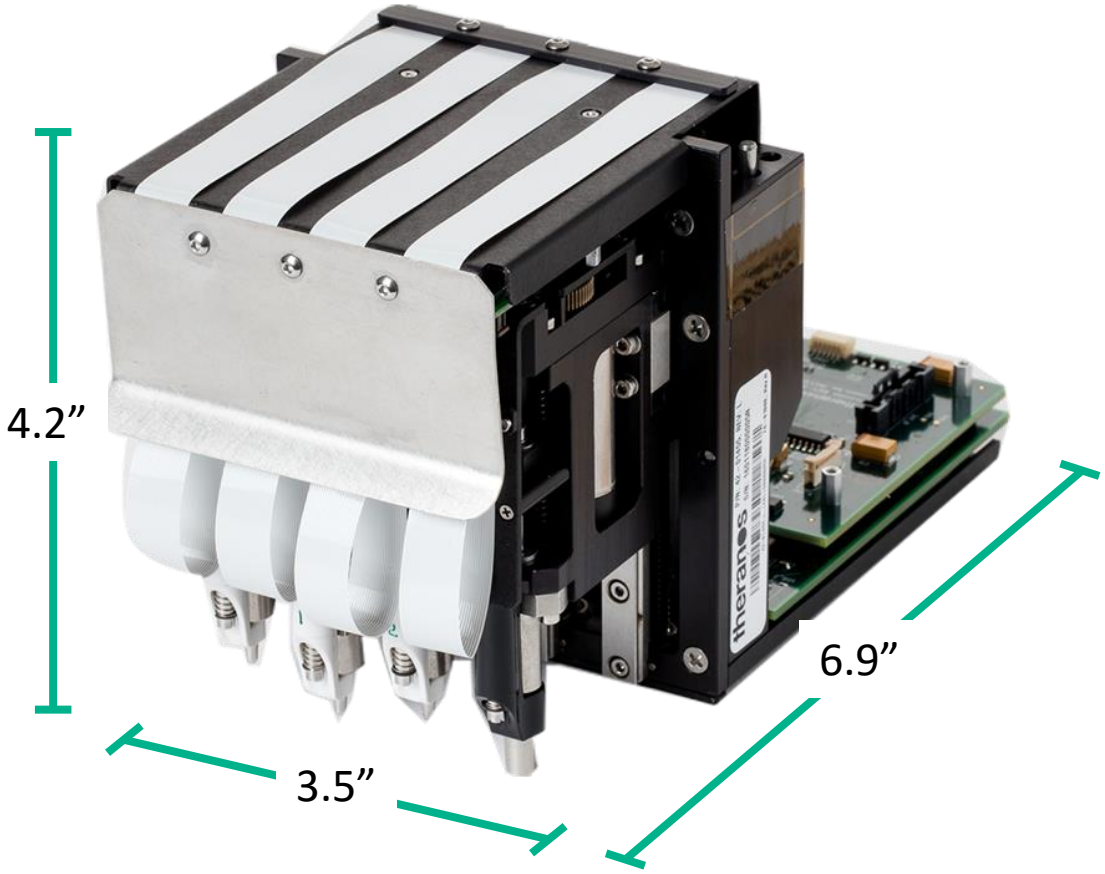
Multi-faceted Material Handling Robot Allows Versatility

- Multiple volumes simultaneously
- Transports consumables
- Transfers fluids
  - Precision: 2.75% at 2 µL
  - Accuracy: 2 µL ± 2.0%



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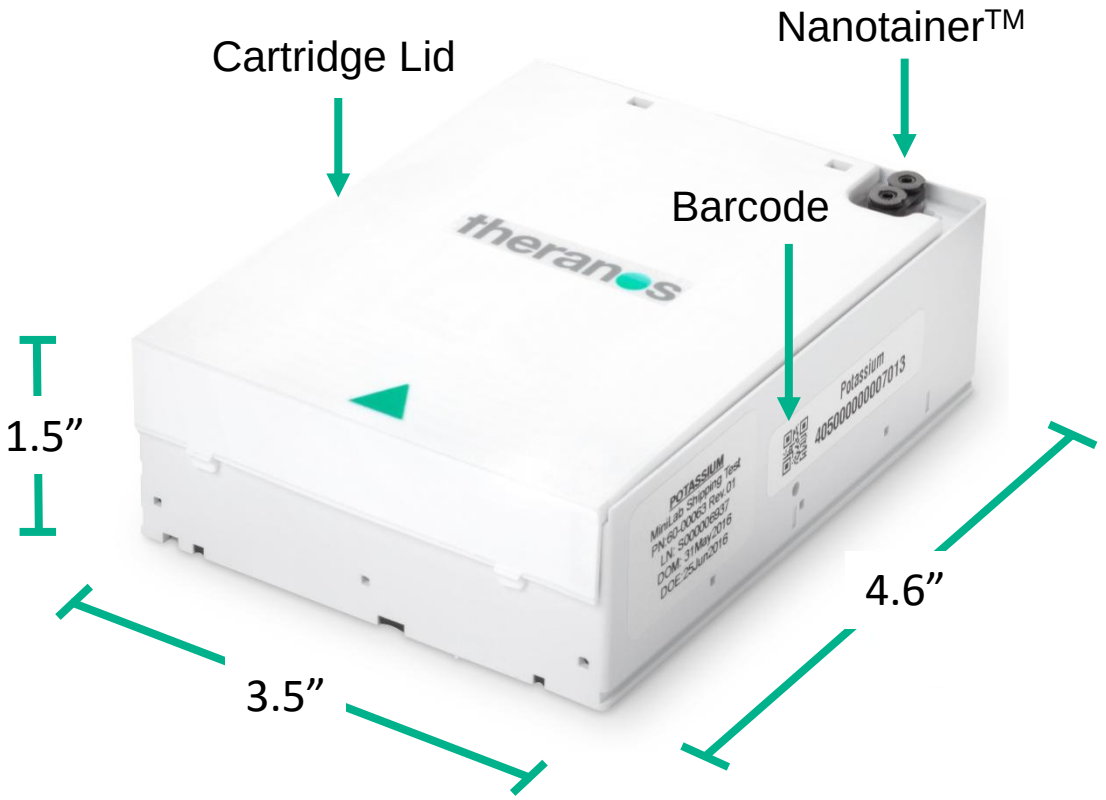
Cartridge Carries Sample and Reagents

- Single use
- On-board controls
- All reagents and waste onboard
- No tubing
- Barcode control



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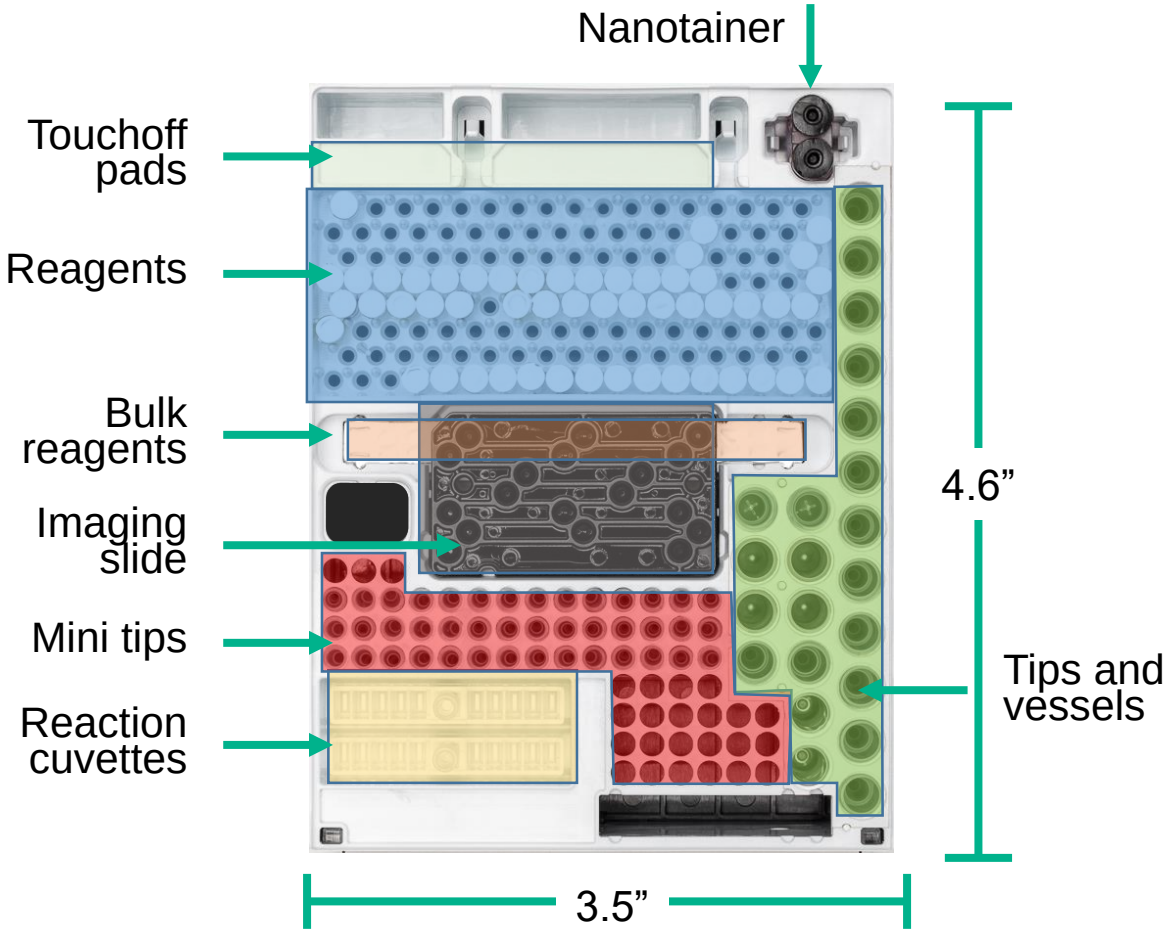
Cartridges: Customized to the Assays



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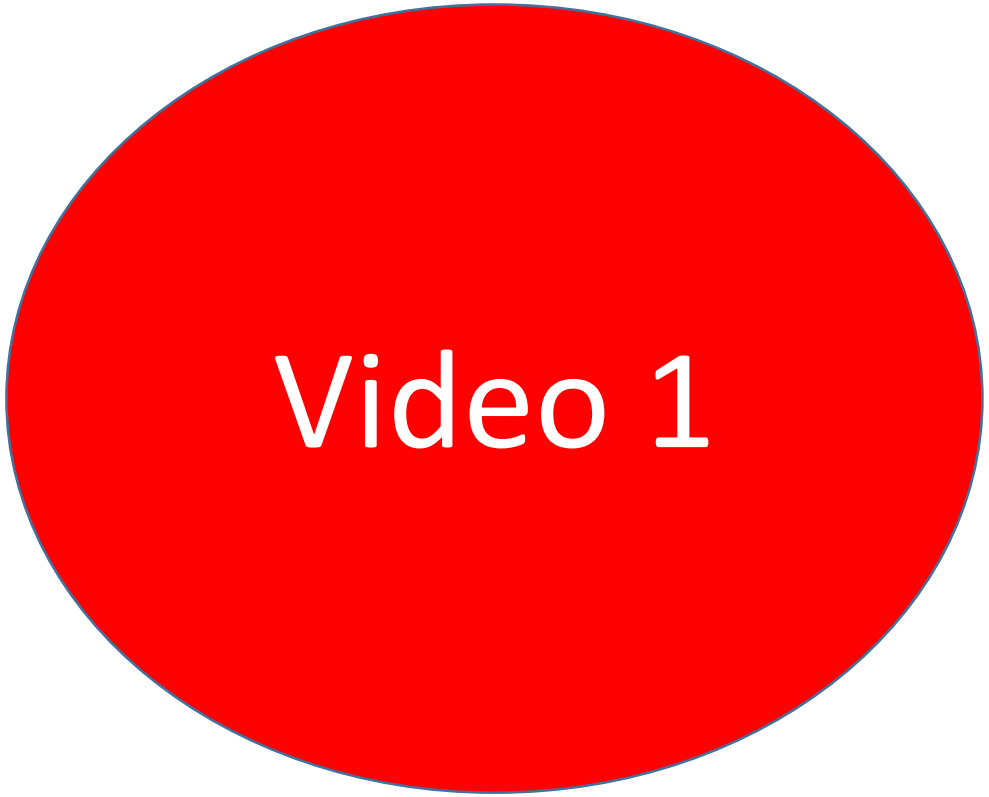
Video of Cartridge Components



Theranos Virtual Analyzer (TVA) Enables Remote Processing and Analysis

- Recognizes barcode on cartridge
- Facilitates two-way communication
- Dictates protocol for device
- Remote interpretation of digital images and results, reviewed and released through clinical lab
- Protocols remotely downloaded for novel analytes on existing systems

Video of miniLab

Presentation Overview

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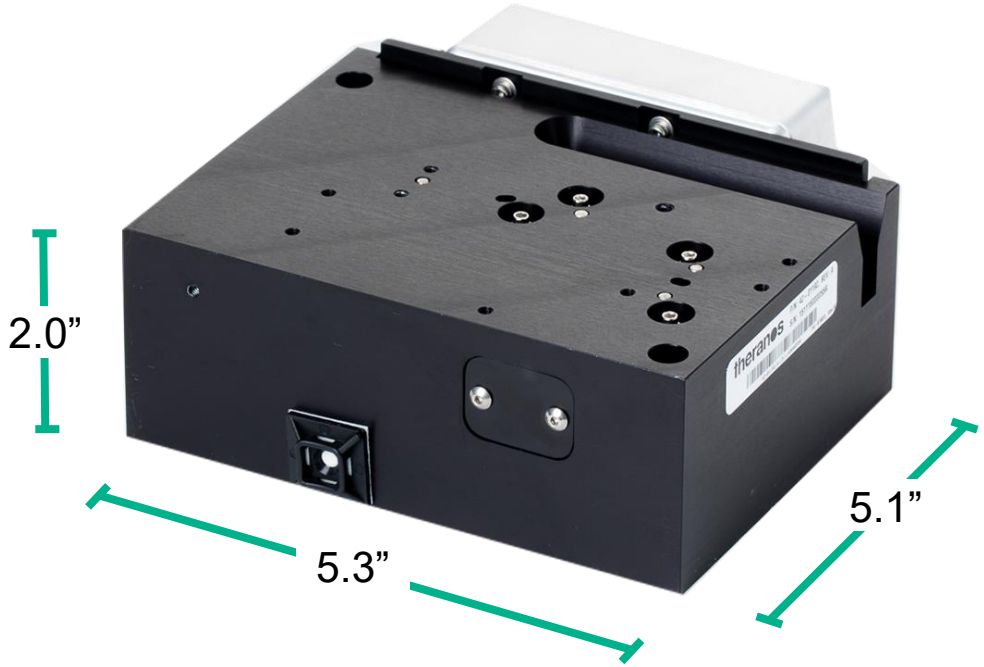
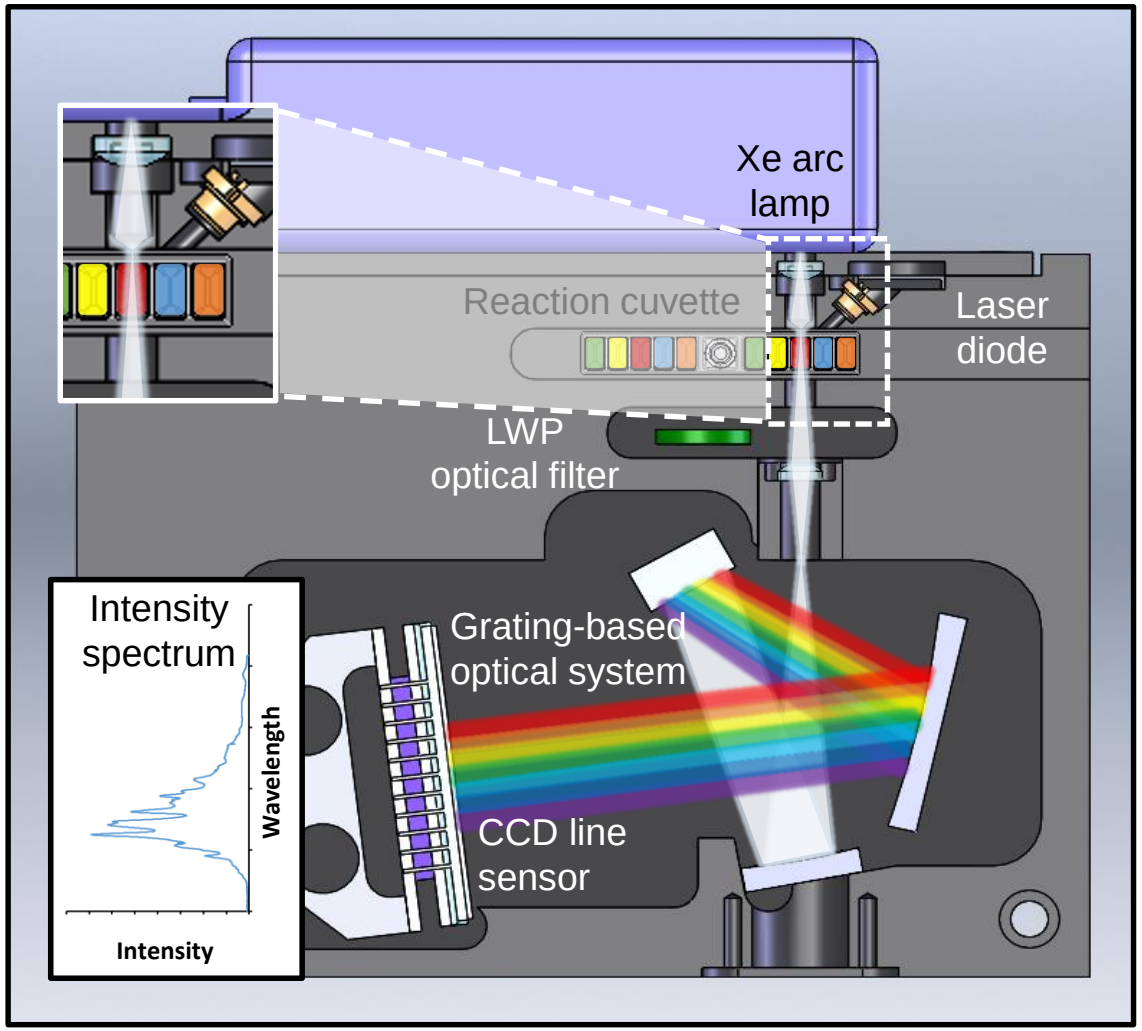
III. Small sample volumes: collection of capillary blood and analysis

Clinical Chemistry: Precision and Method Comparison

Clinical Chemistry: Spectrophotometer



- Measures absorbance across UV-visible spectrum
- Colorimetric and turbidimetric assays



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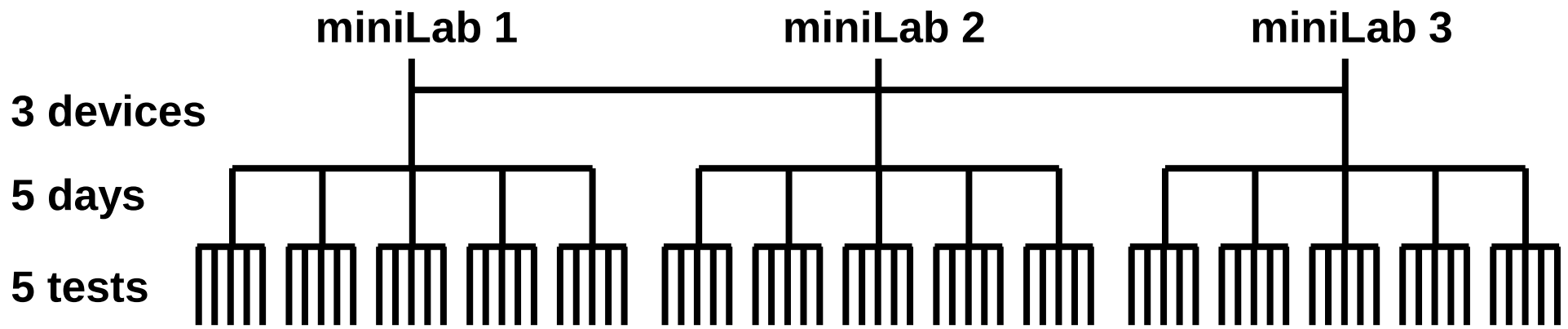
Clinical Chemistry: Precision Study Overview

Sample type and matrix	Li-Hep venous plasma
Control levels	Low analyte concentration High analyte concentration
Study Design	3 miniLabs x 5 Days x 5 Tests per day (CLSI EP05-A3)
Analysis	Analysis of variance to determine repeatability, within-lab variability, and reproducibility

Multi-miniLab Precision Study Design



Repeatability
(within-run/day)
one day-one device
baseline variance of
assay system

Within-laboratory
(within-miniLab)
intermediate precision,
includes day-to-day
variability

Reproducibility
encompasses
imprecision across
multiple devices or sites

CLSI EP05-A3

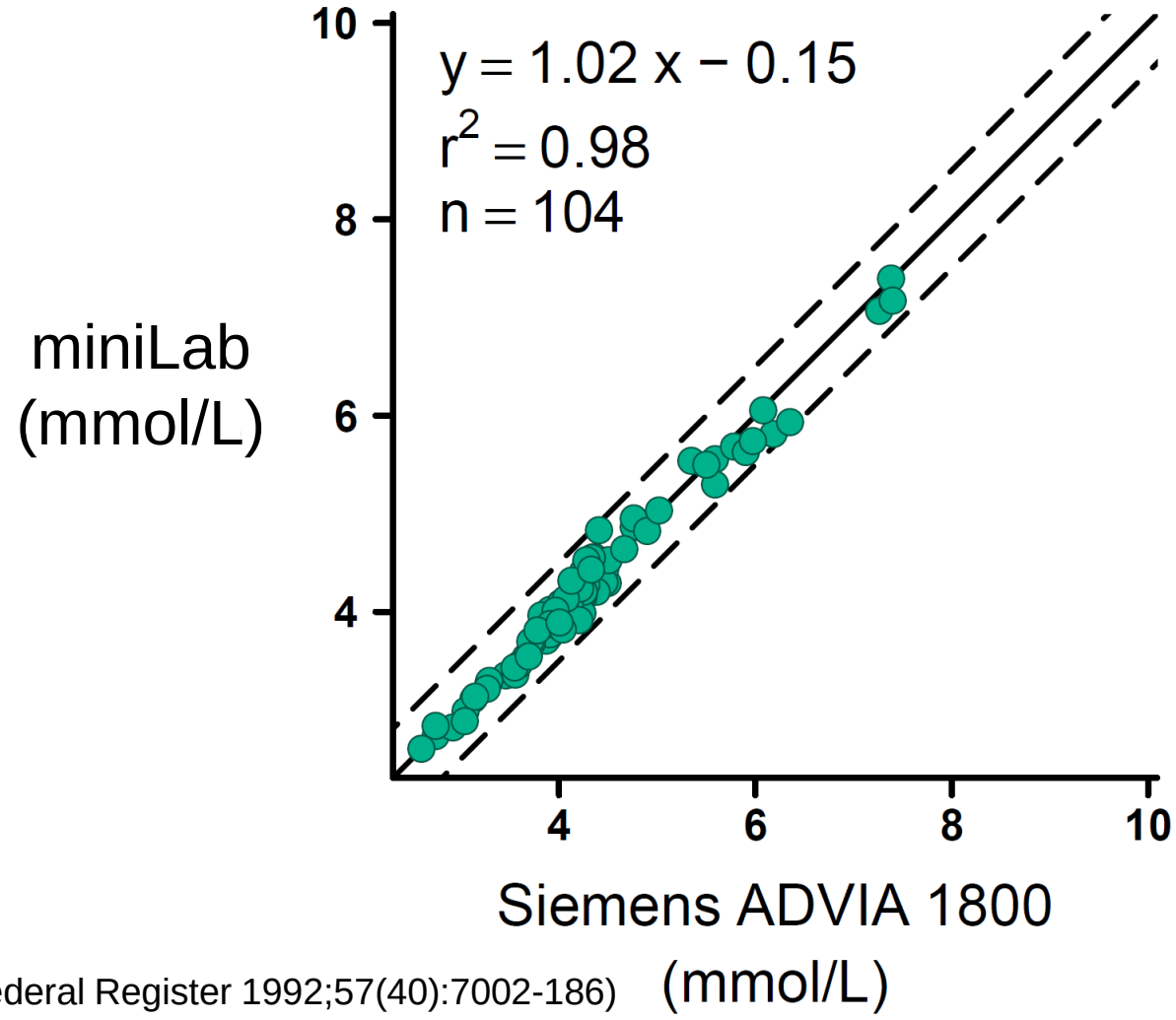
Clinical Chemistry: Precision Results Meet Performance Criteria

Analyte	Mean	Repeatability CV (Within-day)	Within-laboratory CV (Within-miniLab)	Reproducibility CV (Across 3 miniLabs)
Total Cholesterol	120 mg/dL	1.5%	1.6%	1.6%
	320 mg/dL	1.7%	1.8%	2.0%
Triglycerides	78 mg/dL	1.9%	1.9%	2.6%
	405 mg/dL	3.9%	4.4%	4.4%
LDL Cholesterol	71 mg/dL	3.6%	3.6%	3.7%
	203 mg/dL	2.2%	2.4%	2.4%
HDL Cholesterol	40 mg/dL	2.4%	2.4%	2.5%
	84 mg/dL	2.3%	2.5%	2.8%
Potassium	2.9 mmol/L	2.8%	3.4%	6.6%
	6.0 mmol/L	2.6%	2.7%	2.8%

Clinical Chemistry: Method Comparison Study Overview

Population	Apparently healthy subjects and archived samples
Sample type and matrix	Li-Hep venous plasma
Comparator	Siemens ADVIA 1800
Number of miniLabs	7 for potassium 9 for lipids
Study design	n > 100 subjects (per CLSI EP09-A3)
Analysis	Passing-Bablok regression analysis and calculate median bias

Potassium on miniLab Correlates to Comparator Method for Venous Plasma



Legend:

— Unity (y = x)

– – Allowable total error[1] = ±0.5 mmol/L

Points within ATE = 100%

Median bias = -0.08 mmol/L

[1] CLIA (Federal Register 1992;57(40):7002-186)

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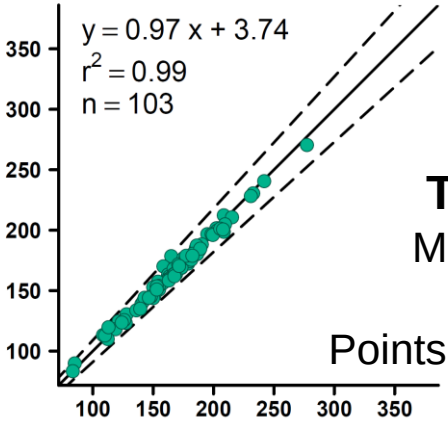
Lipids on miniLab Correlate to Comparator Method for Venous Plasma

Legend:
Unity (y = x)
Allowable total error

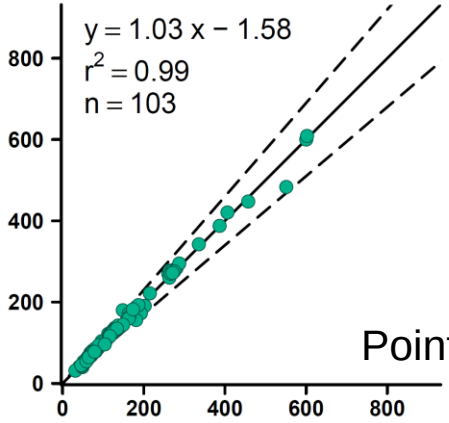
NCEP (Clin Chem 1988;34:193-201)
NCEP (Clin Chem 1997;43:2164-2168)



miniLab (mg/dL)

Siemens ADVIA 1800 (mg/dL)

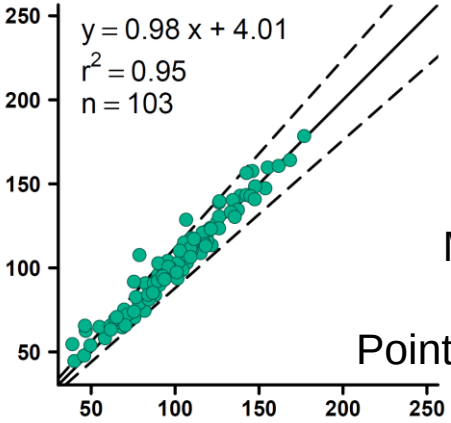
Total Cholesterol
Median bias = -0.9 %
ATE = ± 9%
Points within ATE = 100%



miniLab (mg/dL)

Siemens ADVIA 1800 (mg/dL)

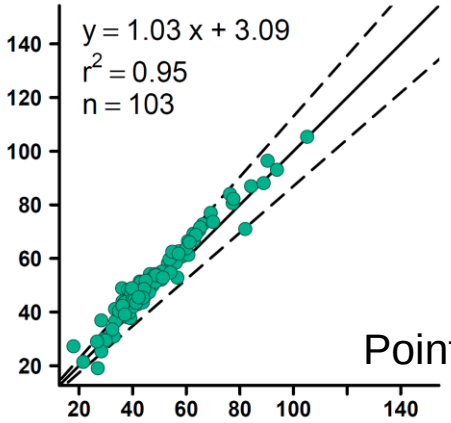
Triglycerides
Median bias = 0.6%
ATE = ± 15%
Points within ATE = 98%



miniLab (mg/dL)

Siemens ADVIA 1800 (mg/dL)

LDL Cholesterol
Median bias = 2.4%
ATE = ± 12%
Points within ATE = 92%



miniLab (mg/dL)

Siemens ADVIA 1800 (mg/dL)

HDL Cholesterol
Median bias = 9%
ATE = ± 13%
Points within ATE = 76%

miniLab HDL Assay Accuracy Confirmed With NIST Standard

Instrument	Replicates		Level 1	Level 2
NIST True Value	N/A	Assigned	41.0 mg/dL	64.9 mg/dL
miniLab	9	Mean	39.9 mg/dL	67.1 mg/dL
		Difference	-3%	3%
Comparator Method	25	Mean	36.7 mg/dL	63.9 mg/dL
		Difference	-10%	-2%

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Clinical Chemistry Assays on miniLab Meet Allowable Total Error Criteria

Analyte	miniLab Total Analytical Error	Allowable Total Error
Potassium (mmol/L)	0.28 mmol/L	< 0.5 mmol/L[1]
Total Cholesterol (mg/dL)	4.0%	< 9%[2]
Triglycerides (mg/dL)	4.5%	< 15%[3]
LDL Cholesterol (mg/dL)	9.5%	< 12%[3]
HDL Cholesterol (mg/dL)*	7.8%	< 13%[3]

Total analytical error = median bias + 2 * within-laboratory CV or SD
*HDL bias against NIST standard was used in this calculation
[1] CLIA (Federal Register 1992;57(40):7002-186)
[2] NCEP (Clin Chem 1988;34:193-201)
[3] NCEP (Clin Chem 1997;43:2164-2168)
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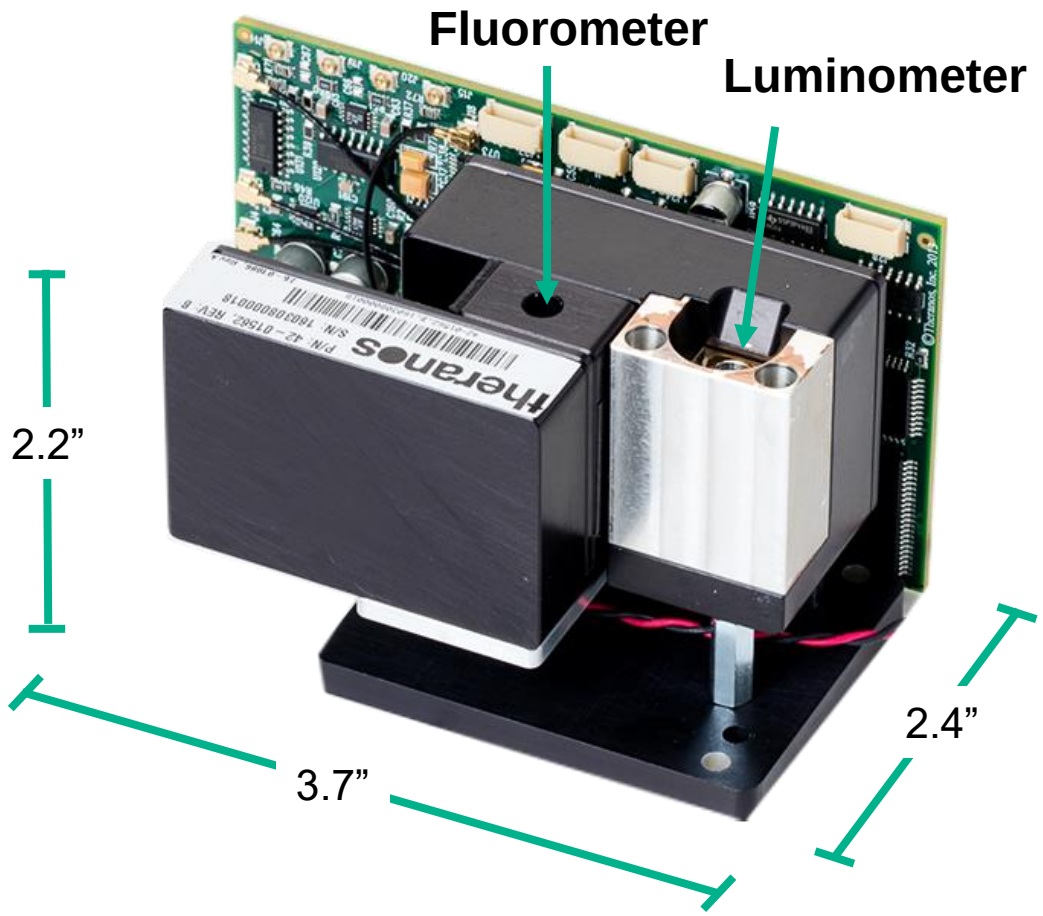
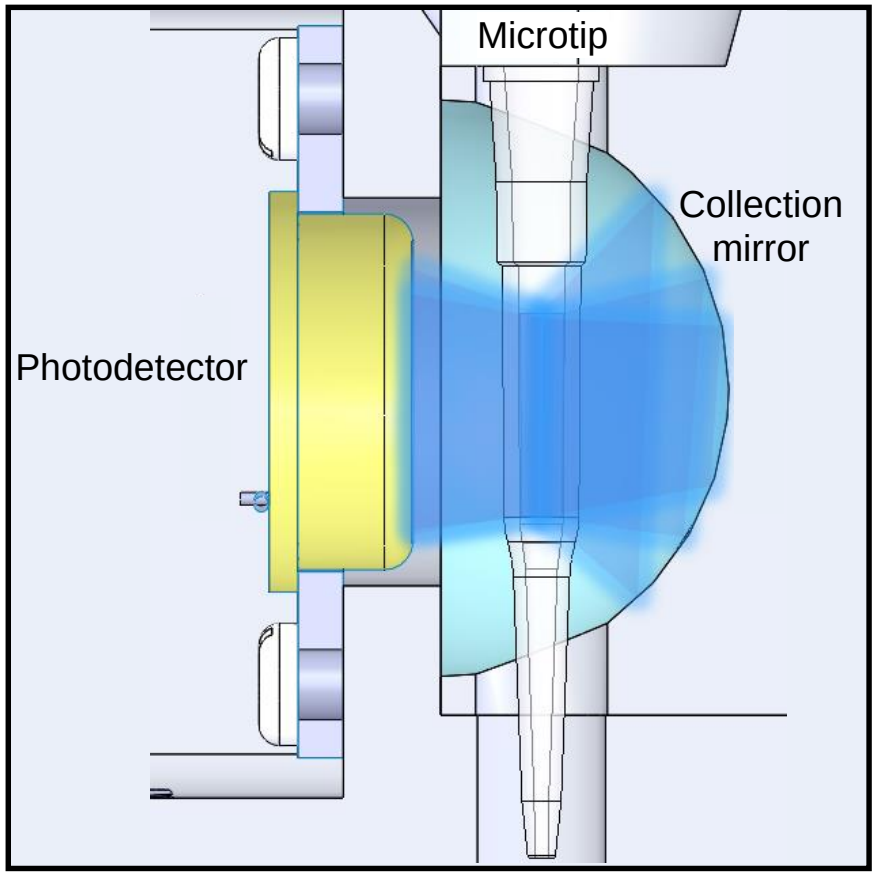
Immunochemistry: Precision and Method Comparison

Immunochemistry: Luminometer & Fluorometer



Luminometer



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Immunochemistry: Precision Study Overview

Sample type and matrix	Venous serum
Control levels	Low titre sample (High Negative) High titre sample (Low Positive)
Study Design	3 miniLabs x 5 Days x 5 Tests per day (CLSI EP05-A3)
Analysis	Analysis of variance to determine repeatability, within-lab variability, and reproducibility

Immunochemistry: HSV-2 IgG Precision Results Show Acceptable %CV

Analyte	Index Value	Repeatability CV (Within-day)	Within-laboratory CV (Within-miniLab)	Reproducibility CV (Across 3 miniLabs)
HSV-2	0.75 (L)	7.6%	7.6%	7.7%
	1.06 (H)	7.3%	7.5%	7.7%

(L): low titre sample; (H): high titre sample

Other industry standard assays approved by FDA: K120625, K090409, K081687

Immunochemistry: Method Comparison Study Overview

Population	At risk for Herpes (intended use population)
Samples type and matrix	Venous serum
Comparator	Focus HerpeSelect 1 & 2 Immunoblot
Number of miniLabs	7
Study design	CLSI EP12-A2
Analysis	Compute negative and positive percent agreement compared to comparative method

Immunochemistry: HSV-2 IgG Measurements are Consistent with Comparator Method

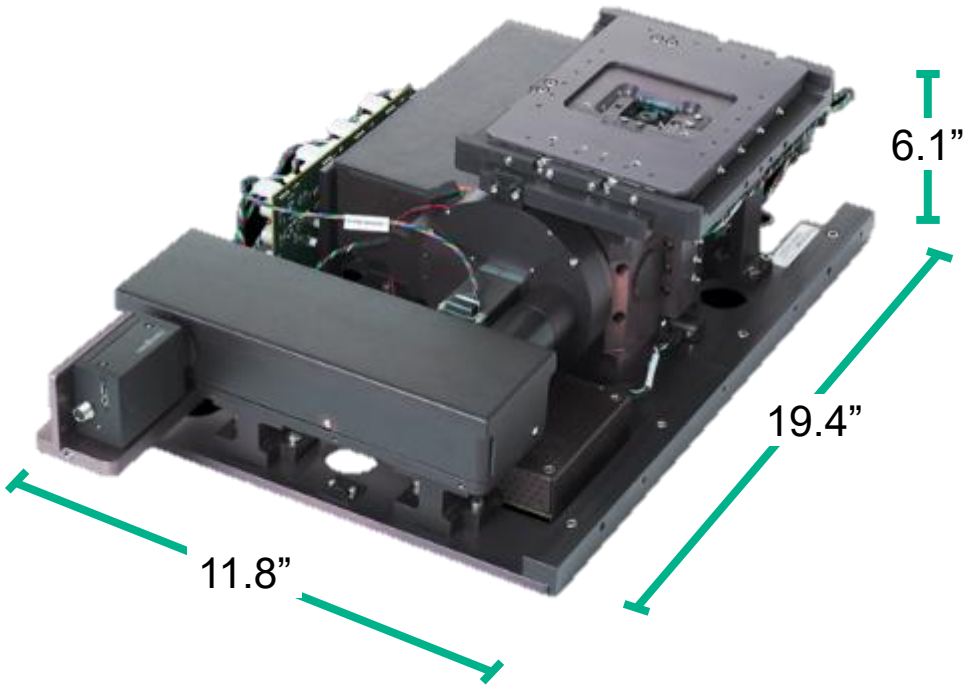
	Theranos/ Immunoblot	Percent Agreement	95% Confidence Interval
Negative percent agreement (specificity)	127 / 127	100%	(97.1%, 100%)
Positive percent agreement (sensitivity)	71 / 75	94.7%	(87.1%, 97.9%)

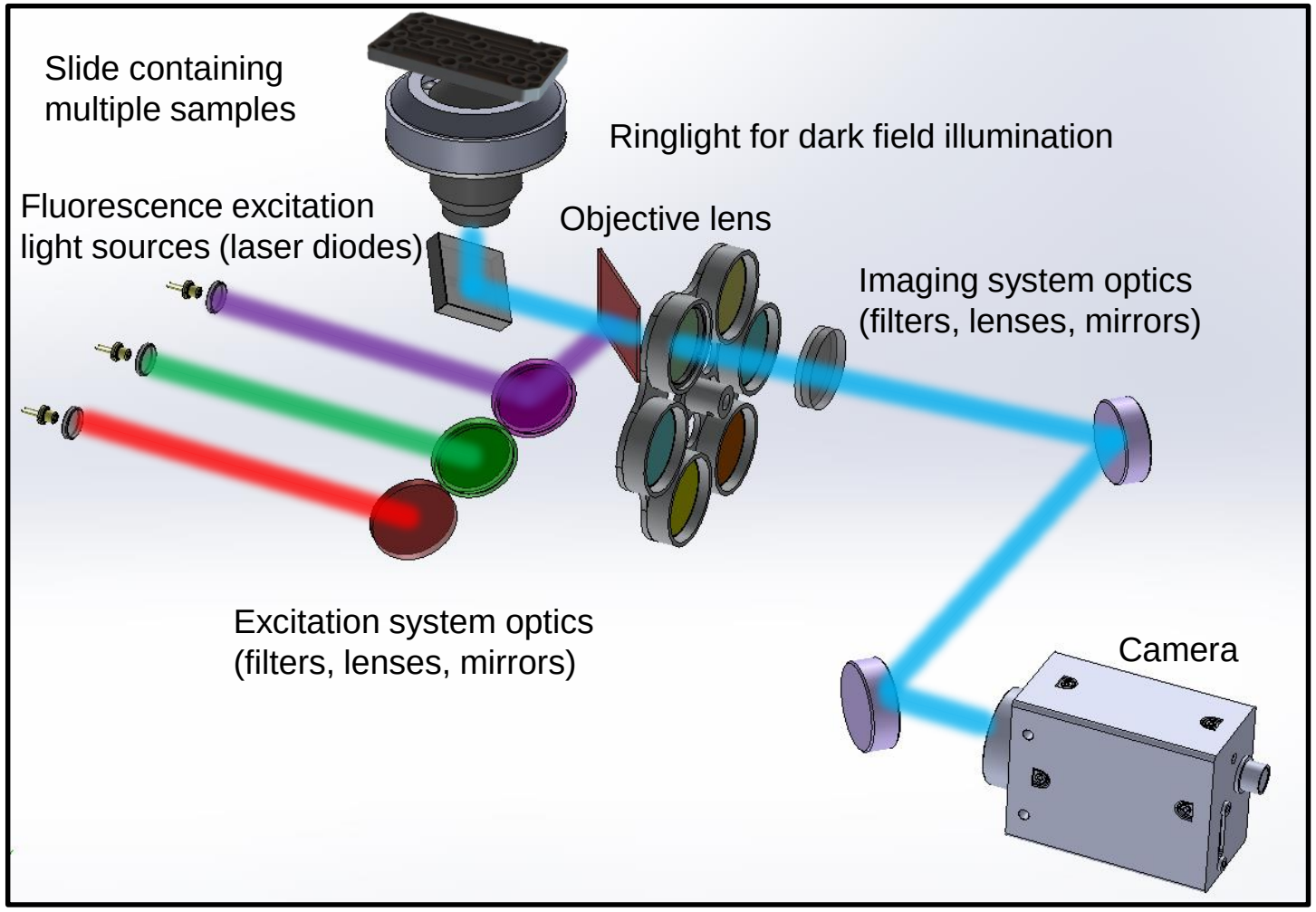
Other industry standard assays approved by FDA: K120625, K090409, K081687

Hematology and Immunology: Precision and Method Comparison

Hematology and Immunology: Cytometer

Automated imaging cytometer
3 Lasers, 5 Emission Filters





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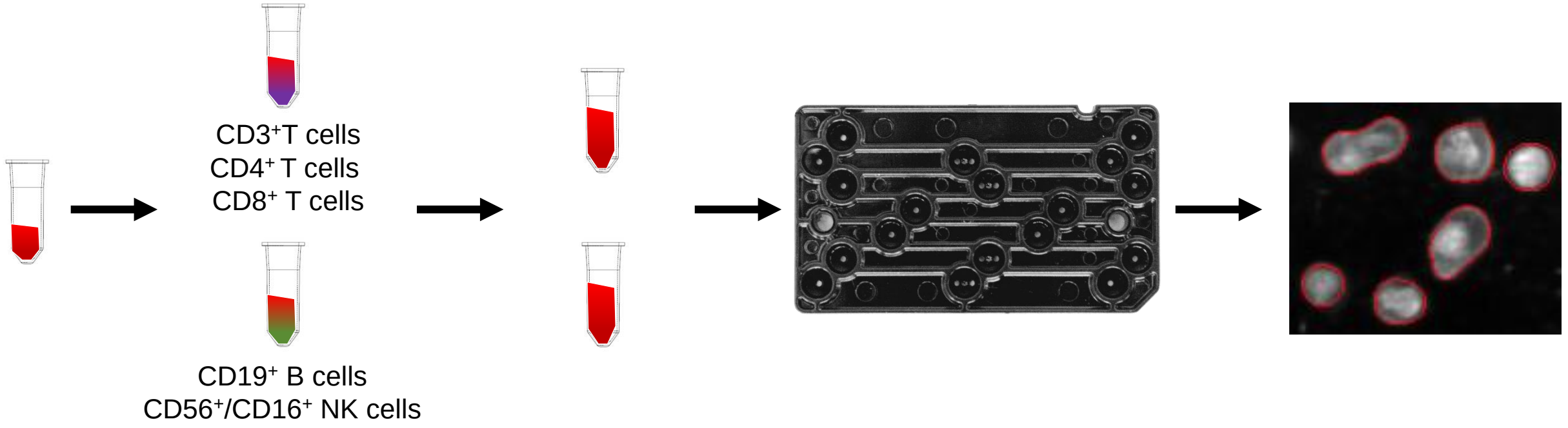
Hematology and Immunology: Assay Methodology



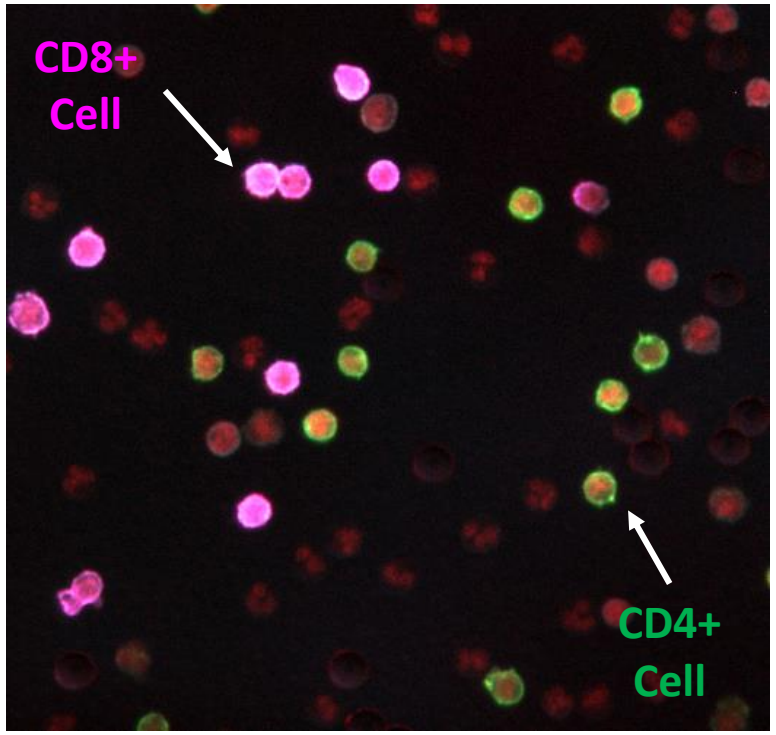
Two reaction tubes stained on-board

Erythrocytes lysed and leukocytes fixed

Samples are loaded into an imaging slide

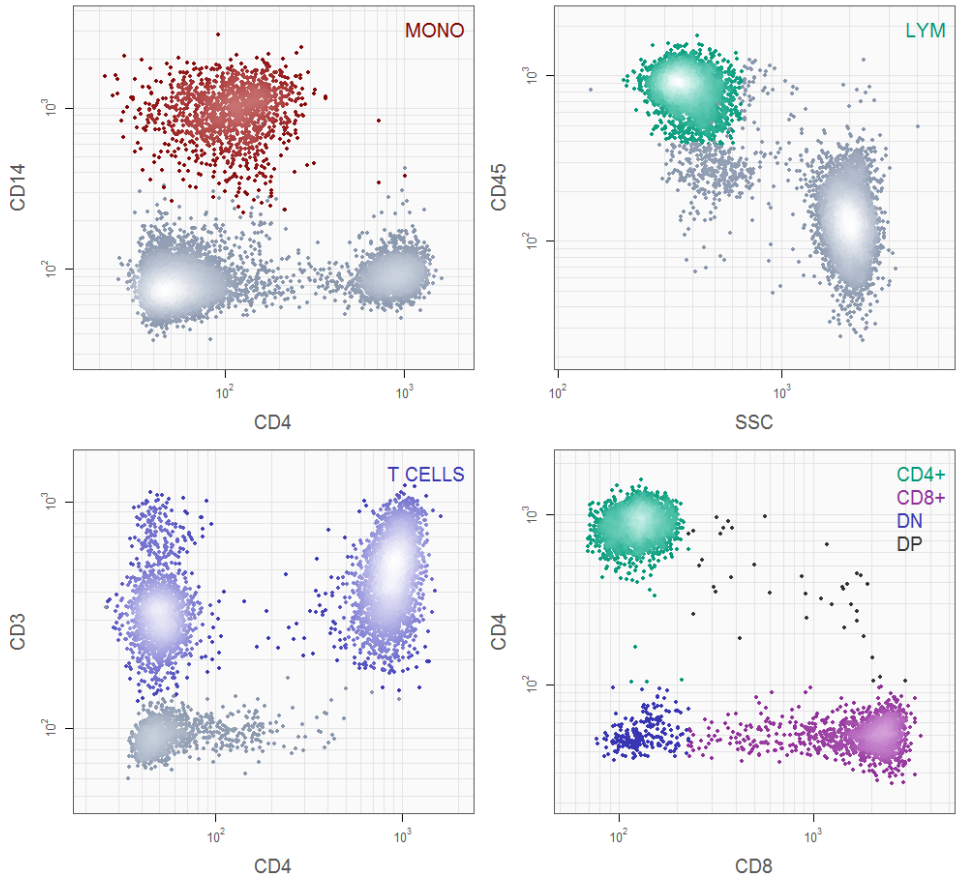
Images and cell properties analyzed using classification algorithms

Hematology and Immunology: Image Processing



Pseudocolor image of T-cell image with select staining shown. **CD4 in green**, **CD8 in magenta**, **CD3 in blue, Nucleus in Red.**

Image segmentation



Scatterplot reviewable by trained personnel

T Cell, B Cell, NK Cells (TBNK, Lymphocyte Subset): Precision Study Overview

Sample type and matrix	Quality control material (R&D Systems StatusFlow)
Control levels	CD4 Low (all other analytes normal) CD4 Normal (all other analytes normal)
Study Design	3 miniLabs x 5 Days x 5 Tests per day (CLSI EP05-A3)
Analysis	Analysis of variance to determine repeatability, within-lab variability, and reproducibility

Lymphocyte Subset: Precision Results Meet Performance Criteria

Analyte	Mean (cells/μL)	Repeatability CV (Within-day)	Within-laboratory CV (Within-miniLab)	Reproducibility CV (Across 3 miniLabs)
Total T cells	841	2.5%	3.2%	3.3%
$CD4^+$ T cells	153 (L)	4.6%	5.0%	5.3%
	838 (N)	2.7%	2.7%	2.8%
$CD8^+$ T cells	571	3.5%	3.9%	4.1%
B cells	357	4.5%	4.7%	4.7%
NK cells	308	5.3%	5.4%	6.1%
Lymphocytes	1533	2.7%	2.8%	3.1%

(L): low cell count; (N): normal cell count

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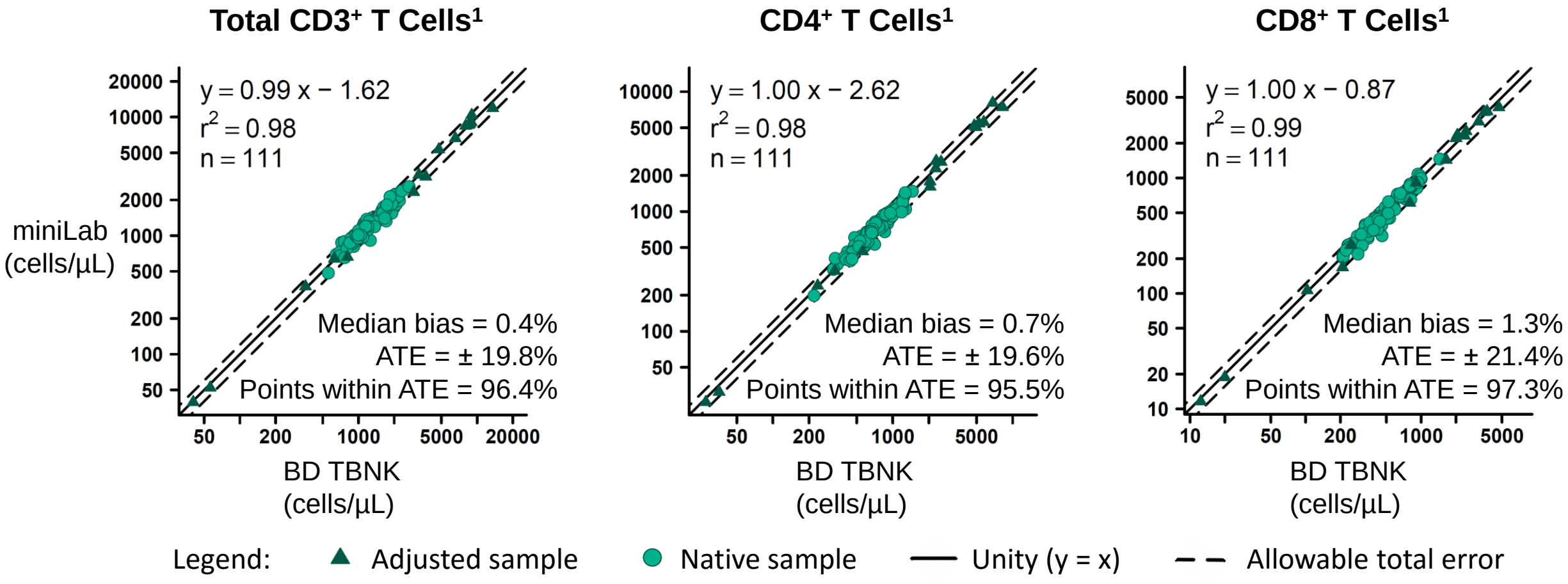
Lymphocyte Subset: Method Comparison Study Overview

Population	Apparently healthy subjects, and <15% adjusted to abnormal levels
Sample type and matrix	K_2-EDTA venous whole blood
Comparator	BD Multitest 6-color TBNK Reagent with Trucount Tubes on FACSCanto II (BD TBNK)
Number of miniLabs	6
Study design	n > 100 subjects (per CLSI EP09-A3) 1 replicate on miniLab, 2 replicates on comparator method
Analysis	Weighted Deming regression analysis and calculate median bias

Lymphocyte Subset: Counts Correlate with Comparator Method



[1] Clin Chim Acta, 2015;438:166-170. Ann Clin Biochem 1997;34:8-12.

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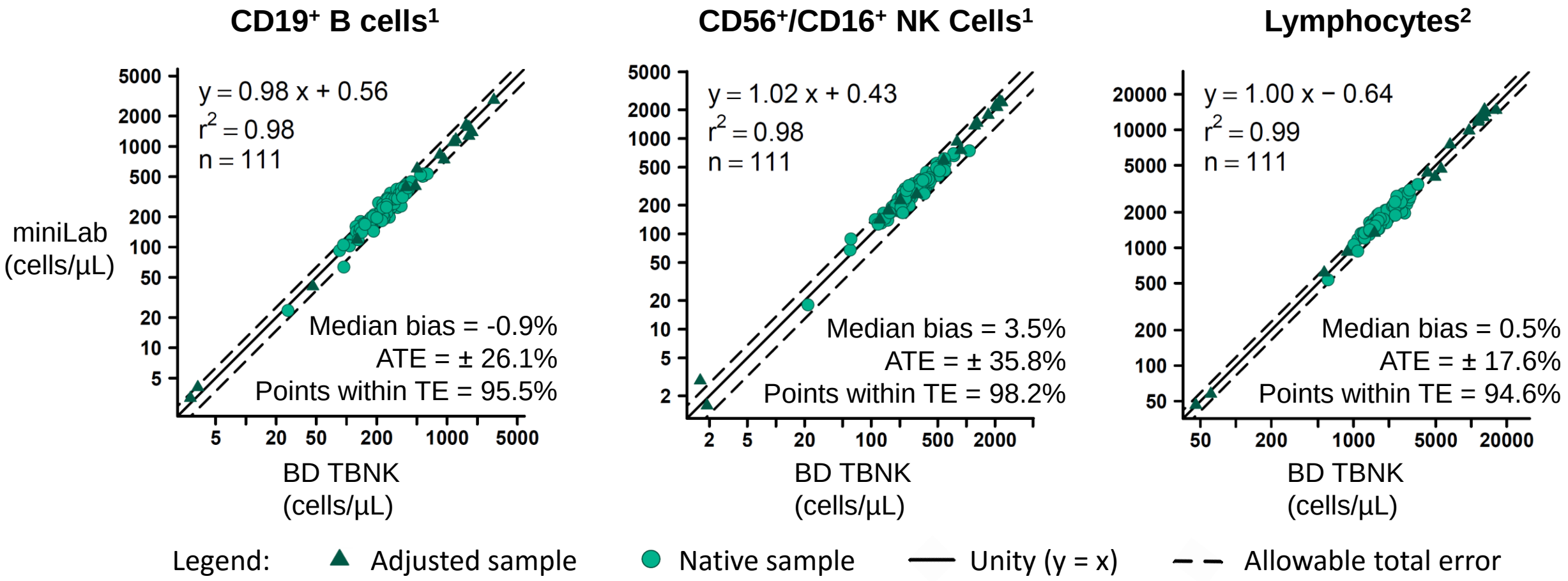
Lymphocyte Subset: Counts Correlate with Comparator Method



[1] Clin Chim Acta, 2015;438:166-170. Ann Clin Biochem 1997;34:8-12

[2] Ricos specifications available from www.westgard.com/biodatabase1.htm

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Lymphocyte Subset: Counts Meet Allowable Total Error Criteria

Analyte (cells/µL)	miniLab Total Analytical Error	Allowable Total Error[1]
Total T cells	6.7%	< 19.8%
$CD4^+$ T cells*	10.8%	< 19.6%
$CD8^+$ T cells	9.2%	< 21.4%
B cells	10.3%	< 26.1%
NK cells	14.3%	< 35.8%
Lymphocytes	6.1%	< 17.6%

Total analytical error = median bias + 2 * within-laboratory CV

*CV of Low CD4 cell count was used in this calculation

[1] Clin Chim Acta, 2015;438:166-170; Ann Clin Biochem 1997;34:8-12

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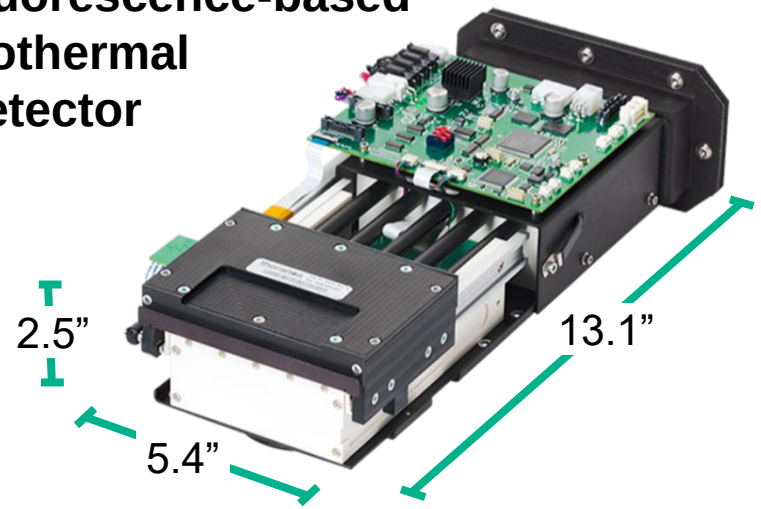
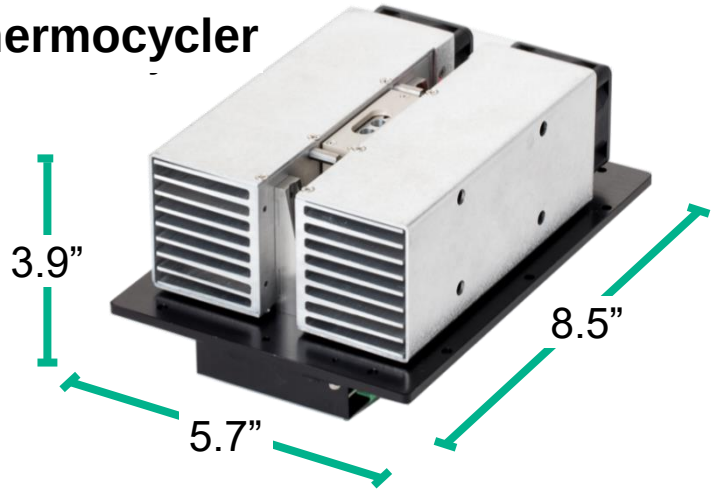
Molecular Biology: Nucleic Acid Amplification (NAA) Zika Assay in Venous Serum on miniLab – Performance Characteristics

Nucleic Acid Amplification (NAA): Fluorescence-based Isothermal Detector and Thermocycler

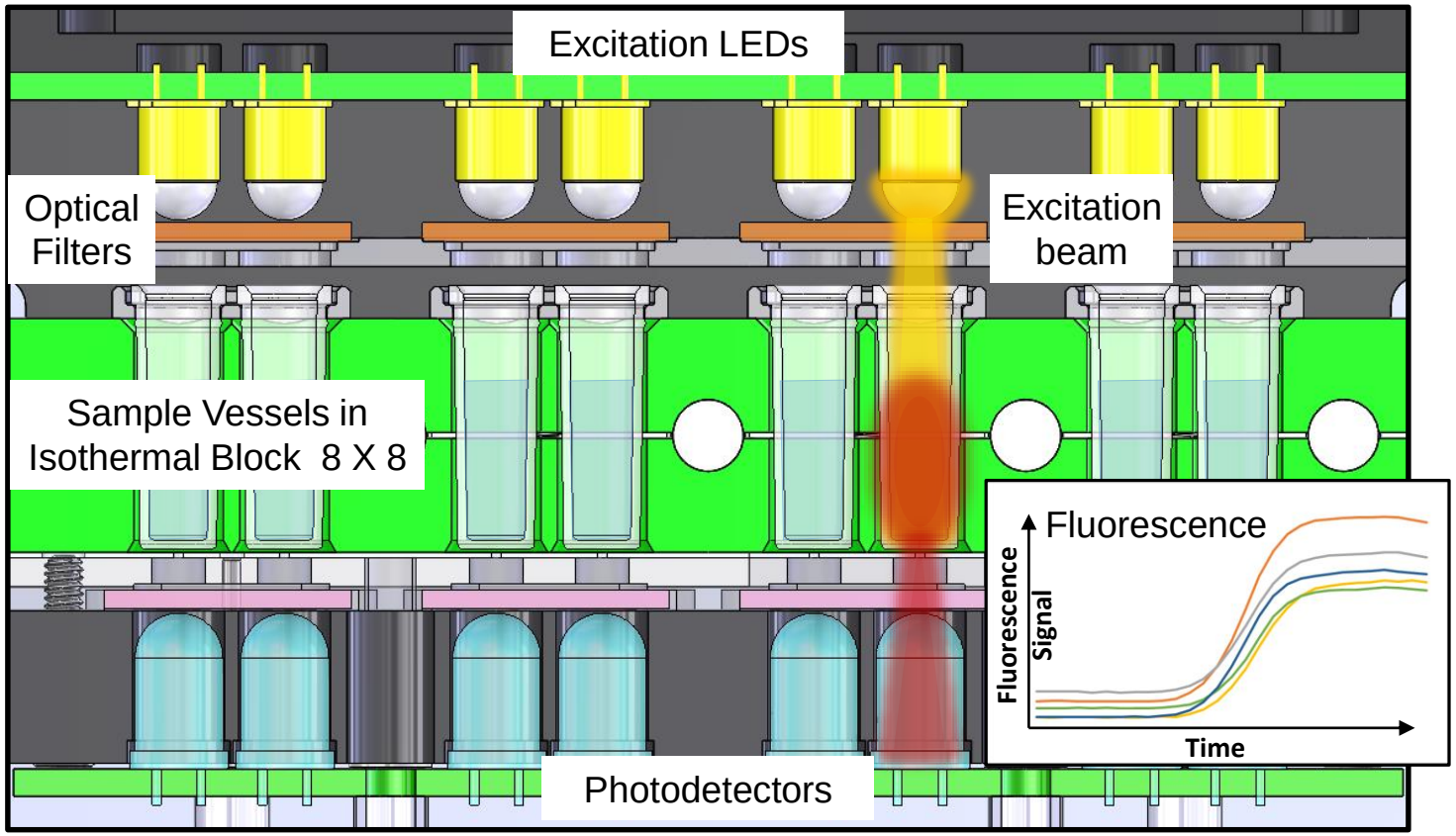
Fluorescence-based Isothermal Detector



Thermocycler



Fluorescence-based Isothermal Detector



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miniLab NAA Assays – Methodology

- Magnetic bead-based extraction of nucleic acids
- Isothermal amplification and detection
- RT-PCR based pre-amplification using thermocycler module
- High sensitivity achieved through integrated on-board sample extraction, amplification, and detection
- Primers designed using multisequence gene alignment

Molecular Biology: NAA Zika Assay is Sensitive for the Target Gene

Zika Concentration (copies/mL)	N (Positive) / N (Replicates)	% Positive
1920	6/6	100%
960	6/6	100%
480 (LoD)	25/26	96%
160	4/6	67%
32	2/6	33%
0	0/6	0%

LoD for CDC Zika test = 930 copies/mL
Emerg Infect Dis 2008;14:1232-1239

Molecular Biology: NAA Zika Assay Does Not Cross-React or Show Interference with Pathogens

Virus/Bacteria/Parasite	Concentration (copies/mL)	Cross-Reactivity (0 copies/mL Zika) % Positive	Interference (recovery of 960 copies/mL Zika) % Positive
P. falciparum	1×10^6	0%	100%
Dengue Virus Types 1-4	1×10^6	0%	100%
West Nile Virus Types 1 & 2	1×10^6	0%	100%
Chikungunya Virus	5×10^3	0%	100%
Yellow Fever Virus	1×10^4	0%	100%
Parvovirus	1×10^6 IU/mL	0%	100%
Mayaro Virus	1×10^6	0%	100%

n = 3 replicates per test

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Molecular Biology: NAA Zika Assay Does Not Cross-React or Show Interference with Substances

Interfering Substance	Concentration (copies/mL)	Cross-Reactivity (0 copies/mL Zika) % Positive	Interference (recovery of 960 copies/mL Zika) % Positive
Bilirubin	342 µM	0%	100%
Cholesterol	13 mM	0%	100%
EDTA, pH 8·0	6.2 mM	0%	100%
Gamma Globulin	5 mg/mL	0%	100%
Hemoglobin	5 mg/mL	0%	100%
Heparin Lithium Salt	19 U/mL	0%	100%
Human Genomic DNA	4 µg/mL	0%	100%
Triglyceride Mixture (C2-C10)	37 mM	0%	100%
Sodium Citrate	0.25% (w/v)	0%	100%

n = 3 replicates per test

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Molecular Biology: NAA Zika Assay Inclusive for Other Zika Strains

Zika Virus Strain	Zika Concentration (copies/mL)	N Positive/N Replicates
DakArD 41662	960	3/3
MR 766	960	3/3

Current outbreak strain: PRVABC59 (Asian)

Molecular Biology: NAA Zika Assay Shows No Carryover

Round	Zika Concentration (copies/mL)	N Positive/N Replicates	% positive
1	1.3×10^7	5/5	100%
	0	0/5	0%
2	1.3×10^7	5/5	100%
	0	0/5	0%
3	1.3×10^7	5/5	100%
	0	0/5	0%
4	1.3×10^7	5/5	100%
	0	0/5	0%
5	1.3×10^7	5/5	100%
	0	0/5	0%

NAA Zika Assay – Clinical Study Overview

Population	Healthy, febrile, or Zika symptomatic pathologicals
Sample type and matrix	Venous serum
Analyzers & Assays	24 miniLabs CDC RT-PCR assay altona RealStar® (EUA Authorized Method)
Study design	FDA EUA guidance
Analysis	Compute negative and positive percent agreement compared to the comparative methods

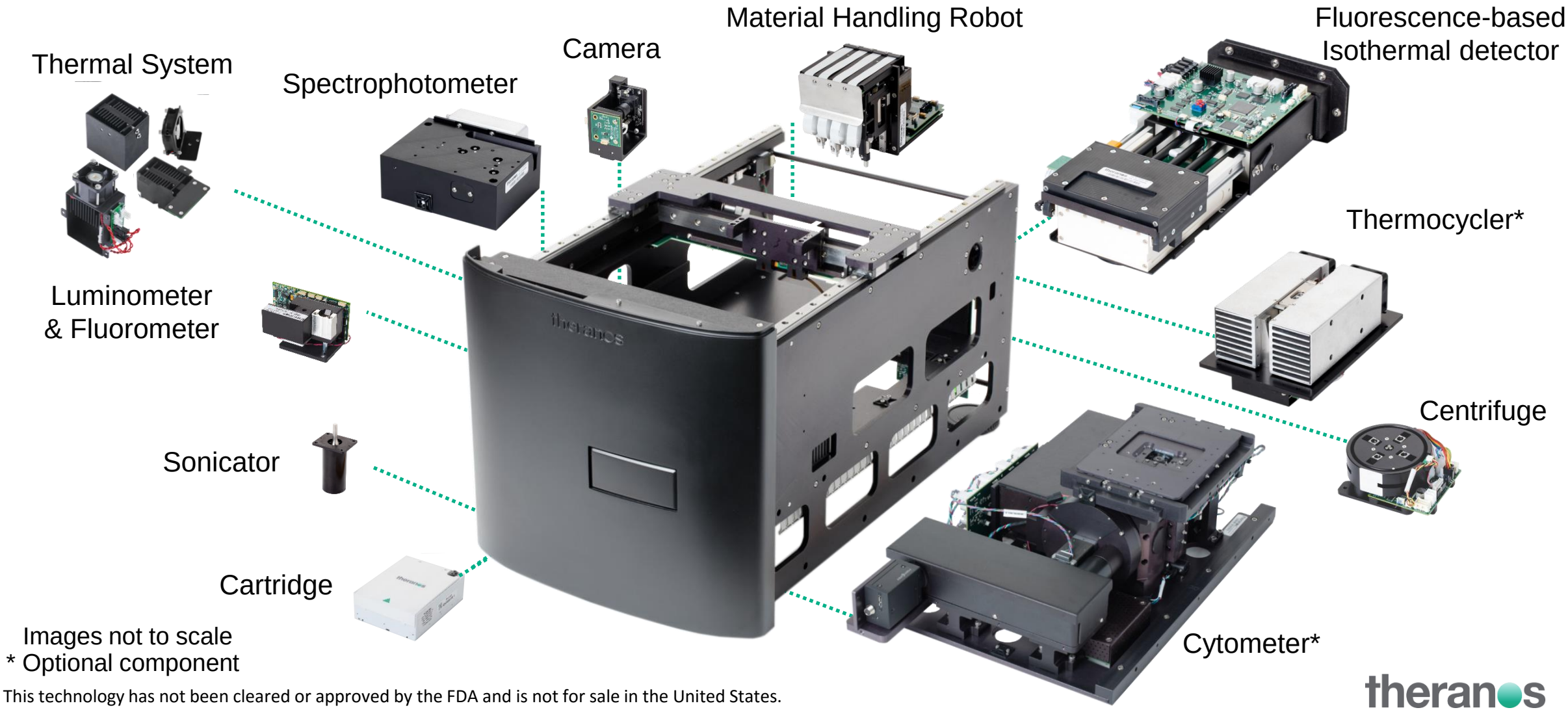
Molecular Biology: NAA Zika Assay with Venous Serum is Consistent with Comparators

	Theranos/ Comparator	Percent Agreement	95% Confidence Interval
Negative percent agreement	108 / 113	95.6%	(90.1%, 98.1%)
Positive percent agreement	67 / 67	100%	(94.6%, 100.0%)

Venous Serum (N=180)

- 78 from US (healthy and febrile subjects)
- 102 from Dominican Republic and Colombia (Zika symptomatic)

Theranos Sample Processing Unit (miniLab)



Images not to scale
* Optional component

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Presentation Overview

I. Miniaturization of laboratory testing

II. miniLab results across detection methodologies

III. Small sample volumes: collection of capillary blood and analysis

Capillary Collection
Optimization of Collection Variables

- Sample site preparation (detergent-based wipes and alcohol dry time)
- Wiping away first drop(s)
- Finger-stick techniques (minimize milking)
- Lancet selection (gauge and depth)
- Arterialization of capillary blood

Theranos Sample Collection Device

Sample Collection Device (SCD)

Nanotainer™ Tubes

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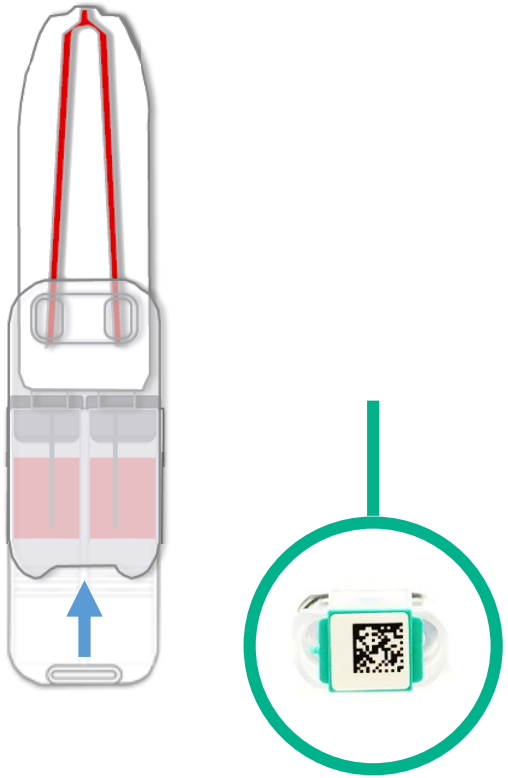
Sample Collection Device Design

Challenges	Theranos Design Considerations
Hemolysis	• Capillary tubes • Shape and diameter of capillary tubes and needles • Anti-coagulant coating
Clotting	• Anti-coagulant concentration • Rapid mixing of sample with anti-coagulant • Fill volume indicators
Altered cell morphology	• Optimized anti-coagulant concentration

Collection and Activation



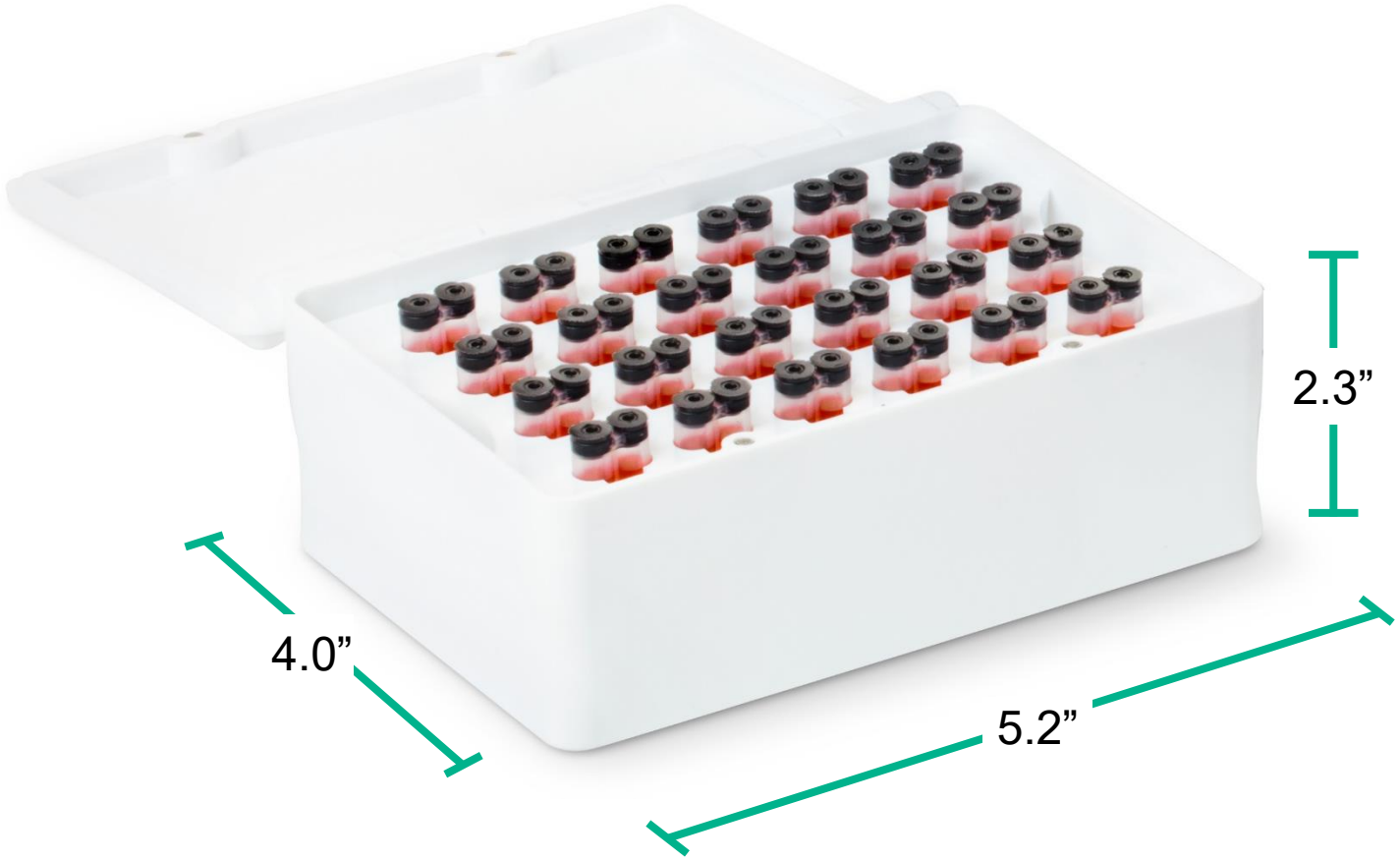
1. Blood drawn from lanced fingertip via capillary action

2. Collection unit pressed into housing. Needles puncture through nanotainer caps, sliding plungers downward, like the action of a syringe. Specimen is drawn into each nanotainer (170 µL blood total) and simultaneously mixed with anti-coagulant (EDTA and/or Li-Hep)

3. Nanotainer tubes removed for storage, transport, and processing. 2D barcode used to maintain traceability

Video of Capillary Collection Process

Sample Container Box for Shipping



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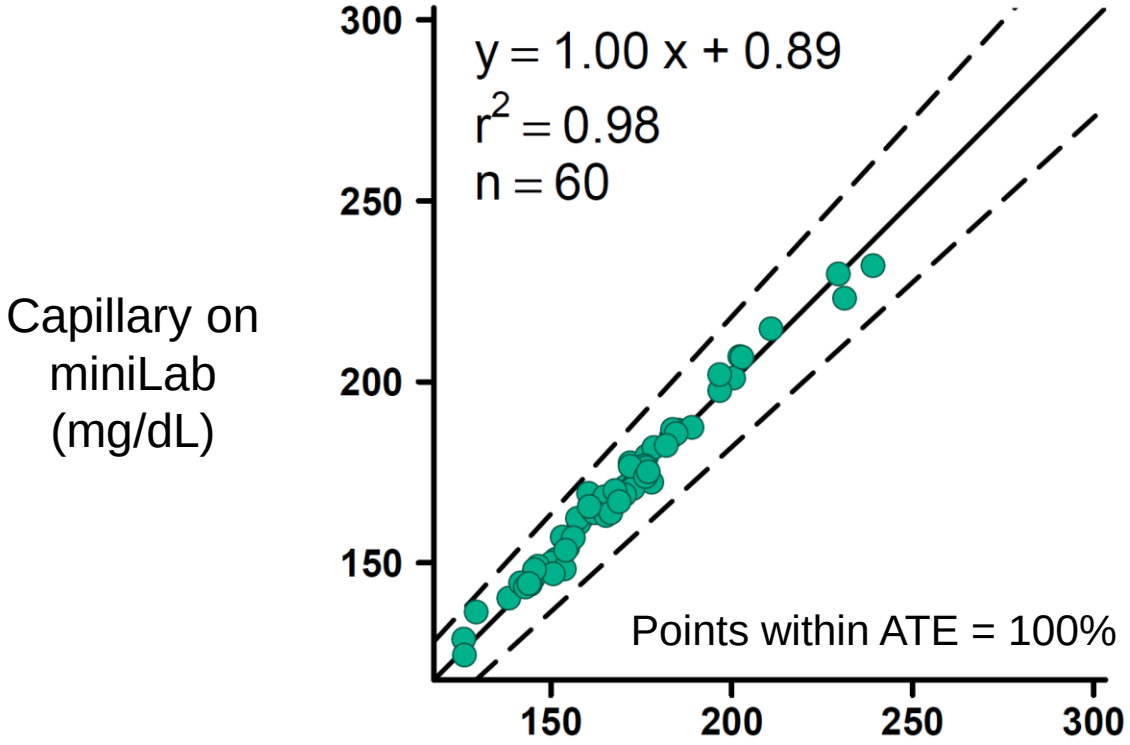
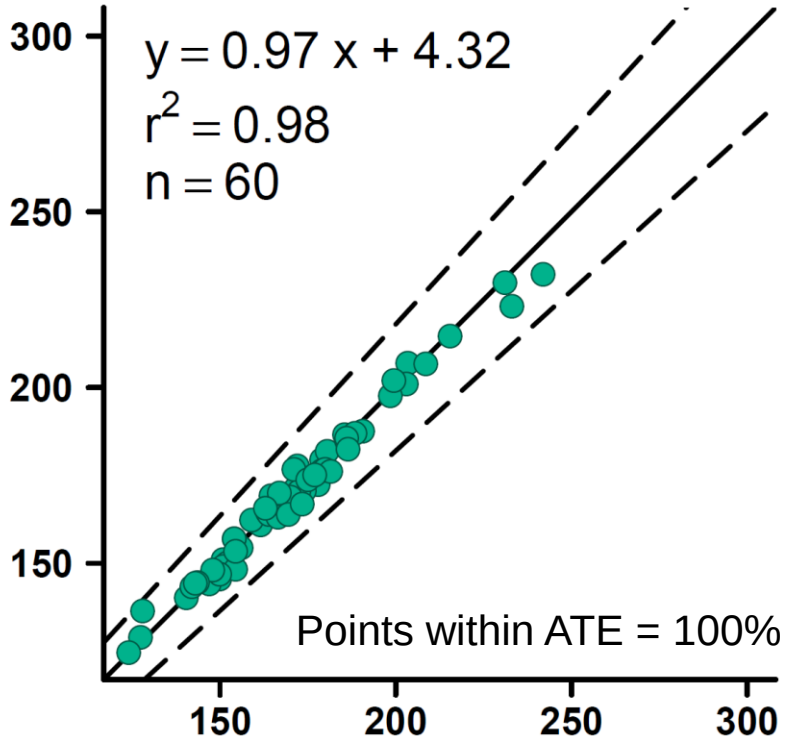
Matrix Comparison on miniLab

Lipid Panel: Matrix Comparison Study Overview

Population	Apparently healthy subjects
Sample type and matrix	Li-Hep capillary whole blood and venous plasma
Analyzers	8 miniLabs Siemens ADVIA 1800
Study design	2 replicates each matrix on miniLab 2 replicates for venous on comparator method
Analysis	Passing-Bablok regression analysis and calculate median bias

Capillary Total Cholesterol Correlates to Venous





Legend:
- Unity (y = x)
- Allowable total error[1] = ±9%

[1] NCEP (Clin Chem 1988;34:193-201)

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Lipid Panel: Capillary Bias Summary

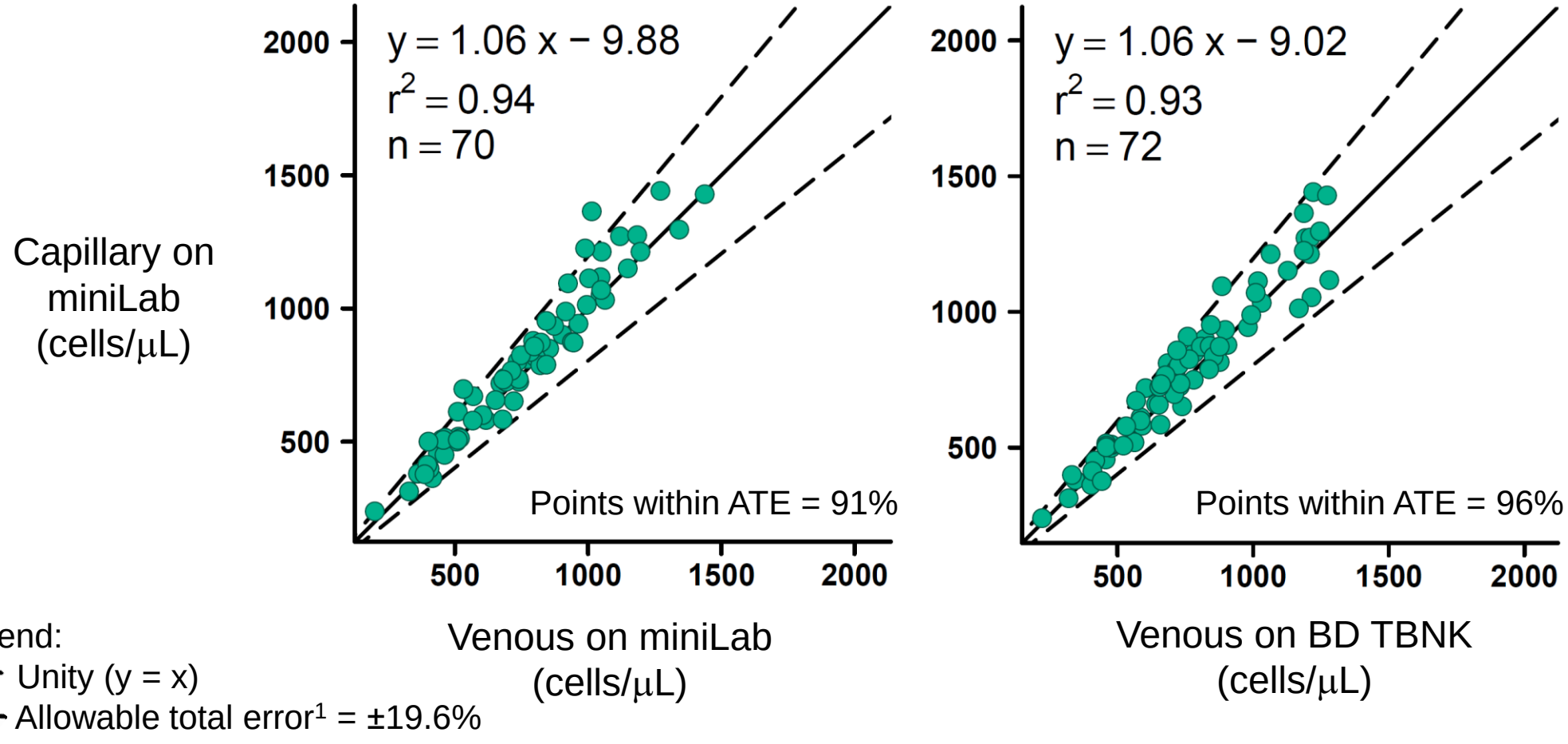
Analyte	Capillary miniLab vs Venous miniLab		Capillary miniLab vs Venous ADVIA	
	R^2	Median Bias	R^2	Median Bias
Total Cholesterol (mg/dL)	0.98	0.6%	0.98	-0.5%
HDL Cholesterol (mg/dL)	0.96	1.9%	0.96	11.6%
LDL Cholesterol (mg/dL)	0.97	0.6%	0.95	1.4%
Triglycerides (mg/dL)	1.00	3.7%	1.00	5.5%

Passing-Bablok regression

Lymphocyte Subset: Matrix Comparison Study Overview

Population	Apparently healthy subjects
Sample type and matrix	K_2-EDTA capillary and venous whole blood
Analyzers	6 miniLab TBNK on Becton Dickinson FACSCanto II
Study design	1 replicate each matrix on miniLab 2 replicates venous on comparator method
Analysis	Passing-Bablok regression analysis and calculate median bias

Capillary CD4+ T cell Count Correlates to Venous



Legend:
- Unity (y = x)
- Allowable total error[1] = ±19.6%

[1] Clin Chim Acta, 2015;438:166-170; Ann Clin Biochem 1997;34:8-12

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Lymphocyte Subset: Capillary Bias Summary

	Capillary miniLab vs Venous miniLab		Capillary miniLab vs Venous BD TBNK	
Analyte	R^2	Median Bias	R^2	Median Bias
Total T cells	0.93	3.7%	0.92	4.9%
$CD4^+$ T cells	0.94	4.2%	0.93	4.6%
$CD8^+$ T cells	0.92	5.9%	0.94	6.4%
B cells	0.93	6.1%	0.92	5.6%
NK cells	0.84	9.6%	0.90	8.9%
Lymphocytes	0.88	4.3%	0.89	6.8%

Passing-Bablok regression

NAA Zika Assay: Clinical Study Overview

Population	Healthy or Zika symptomatic
Sample type and matrix	Capillary whole blood, venous serum, and urine
Analyzers & assays	20 miniLabs CDC RT-PCR assay altona RealStar® (EUA Authorized Method)
Study design	FDA EUA guidance
Analysis	Compute negative and positive percent agreement compared to the comparative methods

Molecular Biology: NAA Zika Assay with Capillary Whole Blood is Consistent with Comparators

	NAA/ CDC & altona	Percent Agreement	95% Confidence Interval
Negative percent agreement	56 / 56	100%	[93.6, 100.0]%
Positive percent agreement	50 / 51	98%	[89.7, 99.7]%

Capillary whole blood, venous serum, and urine (n = 107)

- 77 from US (apparently healthy)
- 30 from the Dominican Republic (Zika symptomatic)

Capillary LoD = 320 copies/mL

Theranos Technologies

Reagents and Assays for Small-Volume Samples

Collection Technologies

Sample Collection Device (SCD)

Nanotainer™ Tubes

High Throughput Platforms

Theranos Virtual Analyzer (TVA)

Theranos Sample Processing Unit (miniLab)

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Manufacturing Video

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Theranos Science & Technology: The Miniaturization of Laboratory Testing

Elizabeth Holmes

theranos

August 1, 2016