theranos

Attn: Sally Hojvat
U.S. Food and Drug Administration
Center for Devices and Radiological Health Pre-Sub Document Mail Center – WO66-G609
10903 New Hampshire Avenue
Silver Spring, MD 20993

November 30, 2013

Re: Pre-Sub for 510(k) Requesting Pre-Submission Meeting Related to Theranos' Upper Respiratory NAA and Cytometry Assays

Dear Sally,

Please find herewith our second formal pre-submission meeting request per the submission plan previously provided to you.

For your reference, we have not yet been assigned a premarket submission number for this submission.

We hereby request an in-person Pre-Submission Meeting to solicit feedback from you and FDA on the submission of Theranos, Inc.'s viral versus bacterial nucleic acid amplification ("*NAA*") and cytometry assays for use with our CLIA-certified laboratory's automated sample processing and analysis system. As described in the prior Pre-Submission (Q13199), this system is composed of Theranos' Sample Processing Unit ("***TSPU***") (i.e. the device that we intend to use in Theranos' Patient Service Centers), and Theranos' Laboratory Automation System ("***TLAS***") (i.e. the software that conducts the analysis in Theranos' CLIA-certified laboratory).

As discussed previously, we anticipate that the 510(k) submission(s) corresponding to Pre-Submission Q131199 (the TSPU, TLAS and influenza NAA assays) will create a framework for FDA clearance for subsequent assays, including the assays under this Pre-Submission request. Theranos' ultimate goal is to convert all of its LDTs into FDA-cleared and FDA-approved assays, as applicable.

Pursuant to that goal and to this Pre-Submission, we are proposing to submit our viral versus bacterial NAA and cytometry panels together in one 510(k) submission for use with the Theranos system. These tests are being submitted together so that physicians ordering tests for patients demonstrating symptoms of viral or bacterial illnesses could simultaneously receive cell count results with the insight into the type of infection a patient has.

At the prior Pre-Submission Meeting (Q131191), Theranos and FDA discussed obtaining separate clearance for the TSPU for the intended use of the cleared influenza assays and device modules used therein. FDA explained that the TSPU will have a User Guide, which would be limited to data only for the functions for the cleared assays. At that meeting, FDA mentioned that functionalities related to subsequently cleared assays could be addressed with updates to the User Guide without a new 510(k) submission, noting that where there are significant

theranos

changes that would impact the cleared intended use, a new 510(k) submission for the TSPU may be required. We have included details of the TSPU in this Pre-Submission with the view that the corresponding 510(k) submission will be for clearance of the viral versus bacterial TNAA and cytometry assays with updates to the cleared TSPU User Guide, as applicable. We hope to discuss and further clarify this as relevant pursuant to this Pre-Submission.

For our pre-submission meeting, Sunny Balwani (Theranos' COO and President) and I will attend. Our regulatory counsel may attend as well. We would like to reserve a room that has a conference phone and an LCD projector.

As always, we are available to meet at any time that is convenient for you. Please feel free to provide us with dates that are convenient and we will work to make them happen on our end. We look forward to the opportunity to continue building a long term relationship with the FDA as we pursue the ongoing filings of our tests with you.

Enclosed please find two (2) paper copies of this Pre-Submission Meeting request and one (1) eCopy. The eCopy is an exact duplicate of the paper copy.

We request, in advance, that you treat this letter and our entire pre-submission package as proprietary and confidential information of Theranos in the event the FDA receives a public records request. Theranos considers the content of these materials highly confidential since the materials reveal trade secrets and other closely guarded information about the inner workings of Theranos' proprietary technology. This letter additionally contains Theranos' confidential information and future business plans.

We look forward to our coming meetings and to working with you on this filing and additional filings to come.

With my best regards,

Elizabeth Holmes, CEO
Theranos, Inc.
650-470-6111
eholmes@theranos.com

Previous Discussions or Submissions

In October 2012, Elizabeth Holmes (Theranos' CEO) and Sunny Balwani (Theranos' COO) and the FDA held a meeting. A teleconference was also held in August 2013 between Ms. Holmes and Sally Hojvat and John Hobson from the FDA.

During these meetings, Ms. Holmes relayed that Theranos' initial goal was to convert all of its LDTs into FDA-cleared assays, as appropriate. She also discussed how Theranos sought to gain FDA-clearance for the TSPU. During these discussions, the FDA indicated it would be possible to submit Theranos' NAA assays and ELISA assays together in an initial filing for the TSPU and TLAS. Ms. Holmes expressed Theranos' intent to use its first submission to create a framework for FDA clearance for its other assays. She indicated that Theranos would be submitting formal pre-submission request(s) accordingly.

During the meetings, Ms. Holmes also indicated that, until Theranos received clearance from the FDA, its devices would only be used in and by Theranos' Palo Alto-based CLIA-certified Laboratory. She provided that all samples would be physically transported to Theranos' Palo Alto-based CLIA-certified Laboratory to be run through Theranos' Laboratory Developed Tests, or where relevant, on FDA approved analyzers and tests in Theranos' Palo Alto-based CLIA-certified Laboratory. Ms. Holmes provided that, accordingly, in September 2013, Theranos intended to begin processing micro-samples and traditional phlebotomy draws collected by trained and certified phlebotomists qualified under the appropriate state laws and employed by Theranos in its CLIA-certified laboratory.

On September 5, 2013, Theranos submitted formal Informational Meeting Request Q131148 and referenced that it would be additionally filing a formal request for a Pre-Submission Meeting. On September 13, 2013, John Hobson, on behalf of the FDA, confirmed that (i) administrative review of Theranos' submission requesting FDA feedback was complete and (ii) its submission included sufficient information to enable feedback in the manner requested. Mr. Hobson was referenced as the lead reviewer assigned to Informational Meeting Request Q131148.

In Informational Meeting Request Q131148, Theranos provided that it would be configuring its devices to collect video of the inside of the devices and would be following with the pre-submission for the initial influenza NAA and ELISA assays. Ms. Holmes followed with an email to Sally Hojvat and John Hobson indicating availability of the video for viewing at their convenience. Informational Meeting Request Q131148 also confirmed Theranos' plans as previously conveyed to the FDA in the prior meetings, and further described its plans for collecting micro-samples and branding Patient Service Centers or Collection Sites as Theranos Wellness Centers. Informational Meeting Request Q131148 referenced the launch of Theranos' first Patient Service Center inside a Walgreens pharmacy store and its plans for opening 1-3 locations in Palo Alto, including one at Theranos' headquarters, in the month of September.

On November 4, 2013, Elizabeth Holmes (Theranos' CEO), Sunny Balwani (Theranos' COO) and legal counsel for Theranos attended the Informational Meeting (Q131148) with FDA and two representatives of CMS. On November 21, 2013, Theranos submitted draft minutes to FDA from that meeting for review.

On November 4, 2013, Elizabeth Holmes (Theranos' CEO) and Sunny Balwani (Theranos' COO) and others from Theranos attended a Pre-Submission Meeting (Q131191) to obtain feedback from FDA on 510(k) submission(s) for the TSPU, the associated software for analysis, the TLAS, and its influenza NAA and ELISA assays. On November 18, 2013, Theranos submitted draft minutes of that Pre-Submission Meeting to FDA for review.

DEPARTMENT OF HEALTH AND HUMAN SERVICES
FOOD AND DRUG ADMINISTRATION

CDRH PREMARKET REVIEW SUBMISSION COVER SHEET

Form Approval
OMB No. 0910-0120
Expiration Date: December 31, 2013
See PRA Statement on page 5.

Date of Submission	User Fee Payment ID Number	FDA Submission Document Number *(if known)*
11/30/2013		

SECTION A TYPE OF SUBMISSION

PMA	PMA & HDE Supplement	PDP	510(k)	Request for Feedback
☐ Original Submission	☐ Regular (180 day)	☐ Original PDP	☐ Original Submission:	☒ Pre-Submission
☐ Premarket Report	☐ Special	☐ Notice of Completion	☐ Traditional	☐ Informational Meeting
☐ Modular Submission	☐ Panel Track (PMA Only)	☐ Amendment to PDP	☐ Special	☐ Submision Issue Meeting
☐ Amendment	☐ 30-day Supplement		☐ Abbreviated (Complete section I, Page 5)	☐ Day 100 Meeting
☐ Report	☐ 30-day Notice		☐ Additional Information	☐ Agreement Meeting
☐ Report Amendment	☐ 135-day Supplement		☐ Third Party	☐ Determination Meeting
☐ Licensing Agreement	☐ Real-time Review			☐ Study Risk Determination
	☐ Amendment to PMA & HDE Supplement			☐ Other *(specify):*
	☐ Other			

IDE	Humanitarian Device Exemption (HDE)	Class II Exemption Petition	Evaluation of Automatic Class III Designation (De Novo)	Other Submission
☐ Original Submission	☐ Original Submission	☐ Original Submission	☐ Original Submission	☐ 513(g)
☐ Amendment	☐ Amendment	☐ Additional Information	☐ Additional Information	☐ Other *(describe submission):*
☐ Supplement	☐ Supplement			
	☐ Report			
	☐ Report Amendment			

Have you used or cited Standards in your submission? ☐ Yes ☐ No *(If Yes, please complete Section I, Page 5)*

SECTION B SUBMITTER, APPLICANT OR SPONSOR

Company / Institution Name	Establishment Registration Number *(if known)*		
Theranos, Inc.	Owner/Operator # 10041002		
Division Name *(if applicable)*	Phone Number *(including area code)*		
	650-856-7304		
Street Address	FAX Number *(including area code)*		
1601 S. California Avenue			
City	State / Province	ZIP/Postal Code	Country
Palo Alto	California	94304	USA
Contact Name			
Brad Arington			
Contact Title	Contact E-mail Address		
Senior Regulatory Counsel	barington@theranos.com		

SECTION C APPLICATION CORRESPONDENT (e.g., consultant, if different from above)

Company / Institution Name			
Division Name *(if applicable)*	Phone Number *(including area code)*		
Street Address	FAX Number *(including area code)*		
City	State / Province	ZIP Code	Country
Contact Name			
Contact Title	Contact E-mail Address		

PSC Publishing Services (301) 443-6740 EF

SECTION D1 REASON FOR APPLICATION - PMA, PDP, OR HDE

- ☐ New Device
- ☐ Withdrawal
- ☐ Additional or Expanded Indications
- ☐ Request for Extension
- ☐ Post-approval Study Protocol
- ☐ Request for Applicant Hold
- ☐ Request for Removal of Applicant Hold
- ☐ Request to Remove or Add Manufacturing Site

- ☐ Process change:
  - ☐ Manufacturing ☐ Packaging
  - ☐ Sterilization
  - ☐ Other *(specify below)*

- ☐ Response to FDA correspondence:

- ☐ Change in design, component, or specification:
  - ☐ Software/Hardware
  - ☐ Color Additive
  - ☐ Material
  - ☐ Specifications
  - ☐ Other *(specify below)*

- ☐ Labeling change:
  - ☐ Indications
  - ☐ Instructions
  - ☐ Performance Characteristics
  - ☐ Shelf Life
  - ☐ Trade Name
  - ☐ Other *(specify below)*

- ☐ Location change:
  - ☐ Manufacturer
  - ☐ Sterilizer
  - ☐ Packager

- ☐ Report Submission:
  - ☐ Annual or Periodic
  - ☐ Post-approval Study
  - ☐ Adverse Reaction
  - ☐ Device Defect
  - ☐ Amendment

- ☐ Change in Ownership
- ☐ Change in Correspondent
- ☐ Change of Applicant Address

☐ Other Reason *(specify)*:

SECTION D2 REASON FOR APPLICATION - IDE

- ☐ New Device
- ☐ New Indication
- ☐ Addition of Institution
- ☐ Expansion / Extension of Study
- ☐ IRB Certification
- ☐ Termination of Study
- ☐ Withdrawal of Application
- ☐ Unanticipated Adverse Effect
- ☐ Notification of Emergency Use
- ☐ Compassionate Use Request
- ☐ Treatment IDE
- ☐ Continued Access

- ☐ Change in:
  - ☐ Correspondent/Applicant
  - ☐ Design/Device
  - ☐ Informed Consent
  - ☐ Manufacturer
  - ☐ Manufacturing Process
  - ☐ Protocol - Feasibility
  - ☐ Protocol - Other
  - ☐ Sponsor

- ☐ Report submission:
  - ☐ Current Investigator
  - ☐ Annual Progress Report
  - ☐ Site Waiver Report
  - ☐ Final

- ☐ Response to FDA Letter Concerning:
  - ☐ Conditional Approval
  - ☐ Deemed Approved
  - ☐ Deficient Final Report
  - ☐ Deficient Progress Report
  - ☐ Deficient Investigator Report
  - ☐ Disapproval
  - ☐ Request Extension of Time to Respond to FDA
  - ☐ Request Meeting
  - ☐ Request Hearing

☐ Other Reason *(specify)*:

SECTION D3 REASON FOR SUBMISSION - 510(k)

- ☐ New Device
- ☐ Additional or Expanded Indications
- ☐ Change in Technology

☒ Other Reason *(specify)*:

Pre-Submission

SECTION E ADDITIONAL INFORMATION ON 510(K) SUBMISSIONS

Product codes of devices to which substantial equivalence is claimed

1	OCC	2	OEM	3	OOU	4	OOS
5	OZX	6	OZY	7	OZZ	8	GZK

Summary of, or statement concerning, safety and effectiveness information

☐ 510 (k) summary attached

☐ 510 (k) statement

Information on devices to which substantial equivalence is claimed *(if known)*

	510(k) Number		*Trade or Proprietary or Model Name*		*Manufacturer*
1	K110764	1	FilmArray Respiratory Panel (RP)	1	BioFire Diagnostics, Inc. (formerly Idaho Technology, Inc.)
2	K103776	2	Luminex Respiratory Viral Panel (RVP)	2	Luminex Molecular Diagnostics, Inc.
3	K122019	3	illumigene Group A Streptococcus DNA Amplification Assay	3	Meridian Bioscience, Inc.
4	K062087	4	BD Facscanto II Flow Cytometer	4	BD Biosciences
5	K090967	5	BD Multitest 6-Color TBNK Kit	5	BD Biosciences
6	K061667	6	Cell-Dyn Ruby System	6	Abbott Laboratories

SECTION F PRODUCT INFORMATION - APPLICATION TO ALL APPLICATIONS

Common or usual name or classification name

Theranos Assays

	Trade or Proprietary or Model Name for This Device		Model Number
1	Theranos Viral Versus Bacterial Nucleic Acid Amplification Assays	1	NA
2	Theranos Cytometry Assays	2	NA
3		3	
4		4	
5		5	

FDA document numbers of all prior related submissions *(regardless of outcome)*

1 Q131148	2 Q131199	3	4	5	6
7	8	9	10	11	12

Data Included in Submission

☐ Laboratory Testing ☐ Animal Trials ☐ Human Trials

SECTION G PRODUCT CLASSIFICATION - APPLICATION TO ALL APPLICATIONS

Product Code	C.F.R. Section *(if applicable)*	Device Class
OCC	21 CFR 866.3980	☐ Class I ☒ Class II
Classification Panel		☐ Class III ☐ Unclassified
Microbiology and Hematology		

Indications *(from labeling)*

TBD

Note: Submission of the information entered in Section H does not affect the need to submit device establishment registration.	FDA Document Number *(if known)*

SECTION H — MANUFACTURING / PACKAGING / STERILIZATION SITES RELATING TO A SUBMISSION

☒ Original ☐ Add ☐ Delete	Facility Establishment Identifier (FEI) Number: 3006231732	☒ Manufacturer ☐ Contract Manufacturer	☐ Contract Sterilizer ☐ Repackager / Relabeler
Company / Institution Name: Theranos, Inc.		Establishment Registration Number: 3006231732	
Division Name *(if applicable)*: Newark Facility		Phone Number *(including area code)*: (650) 856-7304	
Street Address: 7333 Gateway Blvd.		FAX Number *(including area code)*	
City: Newark	State / Province: CA	ZIP Code: 94560	Country: USA
Contact Name: Brad Arington	Contact Title: Senior Regulatory Counsel	Contact E-mail Address: barington@theranos.com	

☐ Original ☐ Add ☐ Delete	Facility Establishment Identifier (FEI) Number	☐ Manufacturer ☐ Contract Manufacturer	☐ Contract Sterilizer ☐ Repackager / Relabeler
Company / Institution Name		Establishment Registration Number	
Division Name *(if applicable)*		Phone Number *(including area code)*	
Street Address		FAX Number *(including area code)*	
City	State / Province	ZIP Code	Country
Contact Name	Contact Title	Contact E-mail Address	

☐ Original ☐ Add ☐ Delete	Facility Establishment Identifier (FEI) Number	☐ Manufacturer ☐ Contract Manufacturer	☐ Contract Sterilizer ☐ Repackager / Relabeler
Company / Institution Name		Establishment Registration Number	
Division Name *(if applicable)*		Phone Number *(including area code)*	
Street Address		FAX Number *(including area code)*	
City	State / Province	ZIP Code	Country
Contact Name	Contact Title	Contact E-mail Address	

SECTION I	UTILIZATION OF STANDARDS				
Note: Complete this section if your application or submission cites standards or includes a *"Declaration of Conformity to a Recognized Standard"* statement.					
1	Standards No. MM3-A2	Standards Organization Clinical Laboratory Standards Institute (CLSI)	Standards Title Molecular Diagnostic Methods for Infectious Diseases; Approved Guideline	Version Second Edition	Date 03/10/2006
2	Standards No. EP24-A2	Standards Organization CLSI	Standards Title Assessment of the Diagnostic Accuracy of Laboratory Tests Using Receiver Operating Characteristic Curves; Approved Guideline	Version Second Edition	Date 08/05/2013
3	Standards No. EP05-A2	Standards Organization CLSI	Standards Title Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline	Version Second Edition	Date 10-31-2005
4	Standards No. EP17-A2	Standards Organization CLSI	Standards Title Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline	Version Second Edition	Date 01/15/2013
5	Standards No. IEC 60601	Standards Organization International Electrotechnical Commission (IEC)	Standards Title Medical Electrical Equipment - Part 1: General Requirements for Basic Safety and Essential Performance	Version 3.1	Date 08/20/2012
6	Standards No. IEC 62304	Standards Organization IEC	Standards Title Medical Device Software -- Software Lifecycle Processes	Version 1.0	Date 05/09/2005
7	Standards No. 14971	Standards Organization International Organization for Standardization	Standards Title Medical Devices -- Application of Risk Management to Medical Devices	Version 2	Date 01/01/2007

Please include any additional standards to be cited on a separate page.

This section applies only to requirements of the Paperwork Reduction Act of 1995.

DO NOT SEND YOUR COMPLETED FORM TO THE PRA STAFF ADDRESS BELOW.

The burden time for this collection of information is estimated to average 0.5 hour per response, including the time to review instructions, search existing data sources, gather and maintain the data needed and complete and review the collection of information. Send comments regarding this burden estimate or any other aspect of this information collection, including suggestions for reducing this burden, to:

Department of Health and Human Services
Food and Drug Administration
Office of Chief Information Officer
Paperwork Reduction Act (PRA) Staff
1350 Piccard Drive, Room 400
Rockville, MD 20850

An agency may not conduct or sponsor, and a person is not required to respond to, a collection of information unless it displays a currently valid OMB control number.

ADDITIONAL SECTION I STANDARDS

	Standards No.	Standards Organization	Standards Title	Version	Date
8	H26-A2	CLSI	Validation, Verification, and Quality Assurance of Automated Hematology Analyzers; Approved Standard	Second Edition	01/01/2010
9	H44-A2	CLSI	Methods for Reticulocyte Counting (Flow Cytometry and Supravital Dyes); Approved Guideline	Second Edition	02/01/2004
10	H20-A2	CLSI	Reference Leukocyte (WBC) Differential Count (Proportional) and Evaluation of Instrumental Methods; Approved Standard	Second Edition	01/01/2007
11	EP09-A3E	CLSI	Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline	Third Edition	08/30/2013
12	EP12-A2	CLSI	User Protocol for Evaluation of Qualitative Test Performance; Approved Guideline	Second Edition	01/01/2008

Table of Contents

Introduction

This document is a 510(k) Pre-Submission for Theranos, Inc.'s Clinical Laboratory Improvement Amendments ("***CLIA***")-certified laboratory's Viral Versus Bacterial panel TNAA assays and cytometry assays for use with its automated sample processing and analysis system (the "***Theranos System***"). As described in Pre-Submission (Q131199), dated September 13, 2013, the Theranos System is composed of Theranos' Sample Processing Units ("***TSPUs***") and Theranos' Laboratory Automation System ("***TLAS***").

This pre-submission describes the use of the TSPU and TLAS in reporting nucleic acid-based Viral Versus Bacterial panel test results and microscopy-based CBC, reticulocyte, and lymphocyte subset (collectively: cytometry) test results. The Viral Versus Bacterial panel includes TNAA assays for the following pathogens: Bocavirus, Coronavirus (subtypes MERS, 229E, HKU1, NL63 & OC43), Human Metapneumovirus A & B, Parainfluenza (subtypes 1, 2, 3 & 4), Respiratory Syncytial Virus (subtypes A & B), Adenovirus (subtypes B, C & E), Rhinovirus, Streptococcus pyogenes, Bordetella parapertussis, Bordetella pertussis, Bordetella holmesii, Chlamydophila pneumoniae, Mycoplasma pneumoniae, Streptococcus pneumoniae and Penicillin-resistant Streptococcus pneumoniae. The TSPU is capable of automated extraction and processing of nucleic acids from multiple sample types, as well as automated processing of whole blood to label relevant cell types with fluorescent markers and image the sample using fluorescence microscopy. The TPSU generates signals that are transported to the TLAS for analysis.

Device Description

The Theranos System

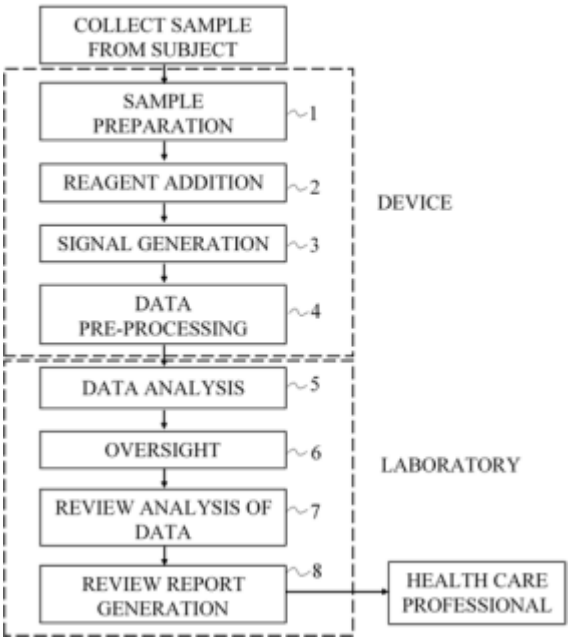


Figure 1. Outline of the Theranos system, depicting the pre-analytical (sample processing, performed by the TSPU as controlled by the TLAS), analytical (report generation, performed by the TLAS), and post-analytical (report transmission, performed by the TLAS) parts.

The system described in this document consists of the following components operating under oversight of the Theranos CLIA-certified laboratory: the Theranos Sample Processing Unit ("***TSPU***"), designed to be housed in a Theranos' Patient Service Center ("***TPSC***"), and a centralized Theranos Laboratory Automation System ("***TLAS***"), which is overseen by the Theranos' CLIA-certified labora*t*ory, running the Viral Versus Bacterial panel and cytometry assays through nucleic assay amplification and fluorescence microscopy-based protocols.

The primary applications of the nucleic acid amplification assays are to accurately detect the presence of Bocavirus, Coronavirus (subtypes MERS, 229E, HKU1, NL63 & OC43), Human Metapneumovirus A & B, Parainfluenza (subtypes 1, 2, 3 & 4), Respiratory Syncytial Virus (subtypes A & B), Adenovirus (subtypes B, C & E), Rhinovirus, Streptococcus pyogenes, Bordetella parapertussis, Bordetella pertussis, Bordetella holmesii, Chlamydophila pneumoniae, Mycoplasma pneumoniae, Streptococcus pneumoniae and Penicillin-resistant Streptococcus pneumoniae.

The presence of the above mentioned Viral Versus Bacterial panel pathogens is determined through nucleic acid amplification assays (Theranos Nucleic Acid Amplification assay, ("***TNAA***") assay).

The primary applications of the CBC, reticulocyte, and lymphocyte subset assays are to accurately measure the counts and properties of various cell types and platelets in peripheral blood.

Samples for the TNAA assays are collected in the form of nasopharyngeal swabs, nasopharyngeal aspirates, nasopharyngeal washes, and nasal swabs, except for the TNAA assay for Streptococcus pyogenes, for which the samples are collected in the form of throat swabs, and Streptococcus pneumoniae, for which the samples will be sputum. Sample for the cytometry assays is whole blood collected by a fingerstick or venipuncture. Patient samples for TNAA and cytometry assays can be processed at the same time in the TSPU.

The EDTA-anti- coagulated whole blood samples are collected and introduced into a disposable Cartridge at a TPSC and fed to the TSPU, where the samples undergo processing and reaction steps, and are eventually introduced to a detector to yield a set of signals. These signal sets are transferred to the TLAS where the raw data is processed and analyzed and oversight is provided, and the relevant reportables are generated.

Figure 1 shows a schematic diagram of the workflow of the Theranos system. Steps illustrated by boxes numbered from 1 to 4 represent pre-analytic steps. Pre-analytic steps include sample collection, sample processing, reagent addition, signal generation, and transmission. Steps illustrated by boxes numbered from 5 to 8 represent analytic steps. Analytic steps include analysis of data received from a device at a sample collection site, oversight, including analysis of controls, calibrations, replicates, outliers, device and sample identification and quality information, and generation of the reportable. Transmission of the report to the health care professional represents a post-analytic step. Post-analytic steps include further review of the analysis of data, and review of report generation and of the report generated for a particular test prior to sign off by CLIA-laboratory personnel and transmission to the physician who ordered a given test.

Theranos Sample Processing Unit (TSPU) and Materials

The TSPU is a modular hardware unit (Figure 2) utilized for performing the pre-analytic functions described in the "*Device Description – The Theranos System*" Section. The TSPU was designed to automatically replicate the processing systems used in the relevant traditional 'gold-standard' assay protocols. The TSPU is enclosed in a thermally insulated and light-tight sheet metal enclosure. It consists of the following components:

1. Liquid Handling Module
2. Centrifuge Module
3. Sonicator Module
4. Magnet Tool
5. Detector 1 – Luminometer Module
6. Detector 2 – Fluorometer Module

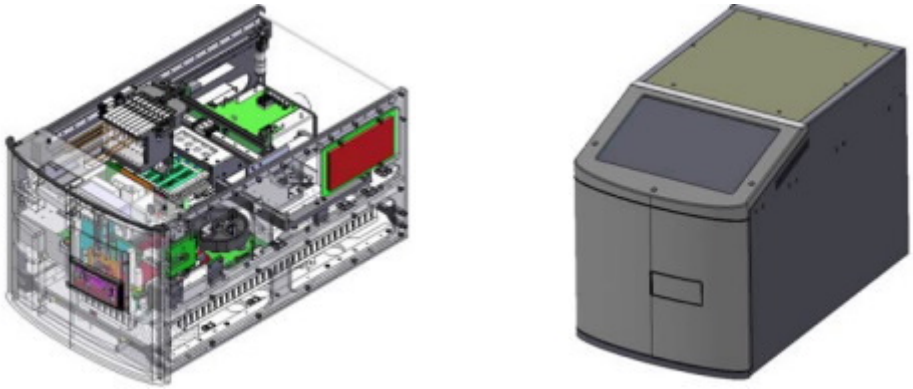


Figure 2. Theranos Sample Processing Unit, shown (L) without enclosure and (R) with enclosure

7. Detector 3 – Fluorometer/Turbidimeter Module
8. Detector 4 - Spectrophotometer Module
9. Detector 5 - Microscopy Module
10. Thermal Control System
11. Machine Vision System and Materials

This pre-submission covers Theranos' Viral Versus Bacterial panel TNAA assays and CBC, reticulocyte, lymphocyte subset cytometry assays.

Modules 1-4, 7, the Thermal Control System (10), and the Machine Vision System (11) are used in connection with our TNAA assays.

Modules 1, 2, and 8-11 are used in connection with our cytometry assays.

Modules 5 and 6 have been included here and briefly described for completeness. These modules are not used for Theranos TNAA or cytometry assays.

The TSPU is composed of both purchased components, machined parts, and molded parts. Most of the machined parts are made of aluminum, with stainless steel used in parts where greater tensile strength is required.

1. Liquid Handling Module

Figure 3. Liquid Handling Module

The automated liquid handling and processing module (Figure 3) is an electromechanical pipette-based assembly that resides within the TSPU. The pipette assembly is mounted onto a controllable gantry. This gantry moves the pipette assembly horizontally throughout the TSPU, allowing it to access different modules. The pipette assembly has individual pipette cards, each of which is independently actuated in the vertical direction. Each pipette card is capable of individually aspirating and dispensing fluids (including samples and reagents). The pipette assembly is used for engaging with vessels, for moving and mixing sample using pipette tips and vessels, and for moving those tips and vessels within the TSPU. One pipette card in the Liquid Handling Module is configured to actuate the Magnetic Tool for sample processing, including extraction and purification as described in the *"Device Description – Theranos Sample Processing Unit (TSPU) and Materials – 4. Magnet Tool"* Section.

2. Centrifuge Module

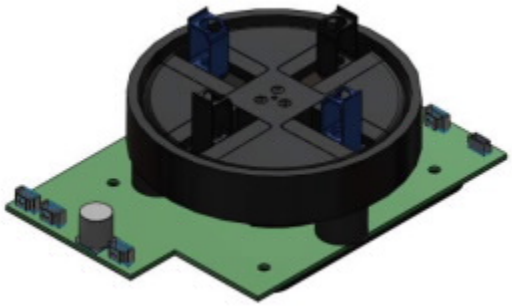


Figure 4. Centrifuge Module

The on-board centrifuge (Figure 4) is a horizontal centrifuge used for separations in the TSPU. The centrifuge can hold 4 centrifuge tubes, is powered by a DC motor with an optical encoder, and is capable of achieving speeds of up to 10,000 RPM (and thus providing up to 3300g of centrifugal acceleration).

3. Sonicator Module

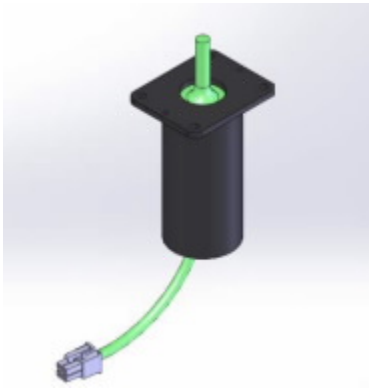


Figure 5. Sonicator module

The sonicator (Figure 5) is used during sample preparation steps to lyse cells, freeing the DNA for further processing and data generation. The sonicator assembly consists of a commercially available 40kHz sonotrode, housing, and power supply electronics. During sample purification, the sample is placed in a polystyrene vessel. This vessel is moved to the sonicator, using the automated liquid handling system. The handling system establishes contact between the vessel and

sonicator by pushing the vessel against the sonicator probe with a slight amount of force. Once contact has been firmly established, the sonicator is energized. This transmits mechanical energy into the vessel and sample, causing cavitation and eventual lysis within the sample. Sonication energy can be modulated in power and applied in a continuous or pulsed fashion. Once sonication is complete, the liquid handling system returns the sample vessel for further purification processing.

4. Magnet Tool

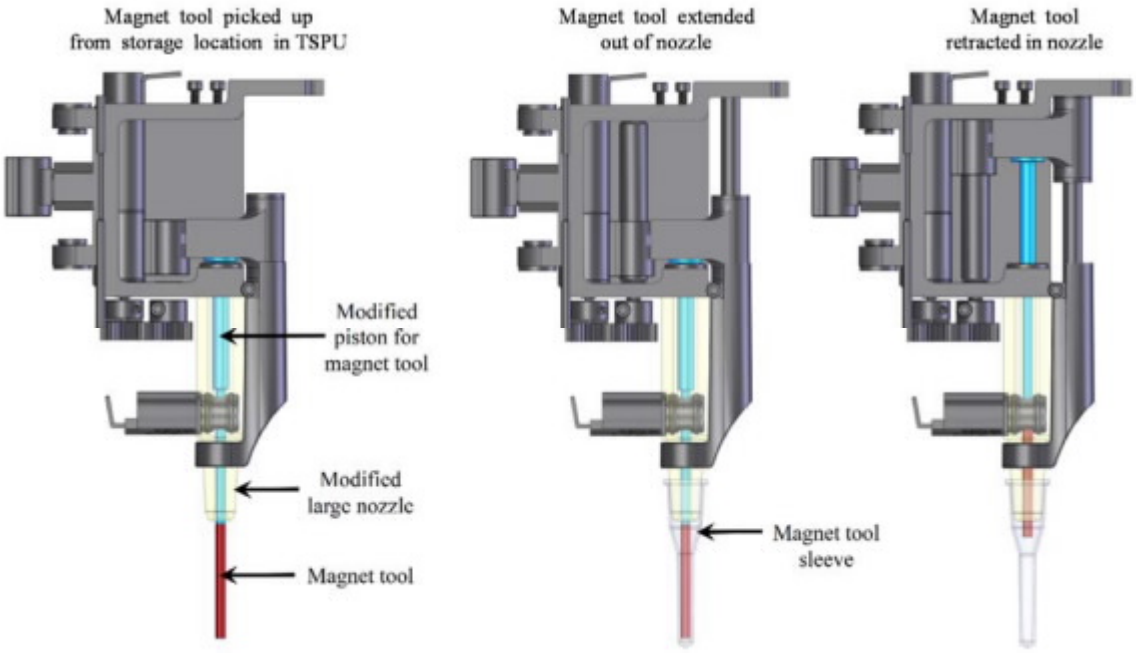


Figure 6. Magnet Tool

One of the pipette cards in the Liquid Handling Module has a modified piston assembly and nozzle to interface with the Magnet Tool. The Magnet Tool is a magnetized rod used for separation of magnetic beads during TNAA sample prep. Figure 6 shows a side view of the Magnet Tool operation. Left: The Magnet Tool is picked up from its resting location in the device when the piston is extended out of the nozzle. The Magnet Tool secures its position onto the piston and is retracted back into the nozzle prior to pickup of the Magnet Tool Sleeve from a location on the Cartridge; Center: The Magnet Tool is shown extended out of the nozzle into the Magnet Tool Sleeve, positioned for capture of magnetic beads onto the exterior wall of the Magnet Tool Sleeve; Right: The Magnet Tool is shown retracted in the nozzle and clear of the Magnet Tool Sleeve, used in conjunction with vertical movement of the nozzle for magnetic bead release.

5. Detector 1 – Luminometer Module

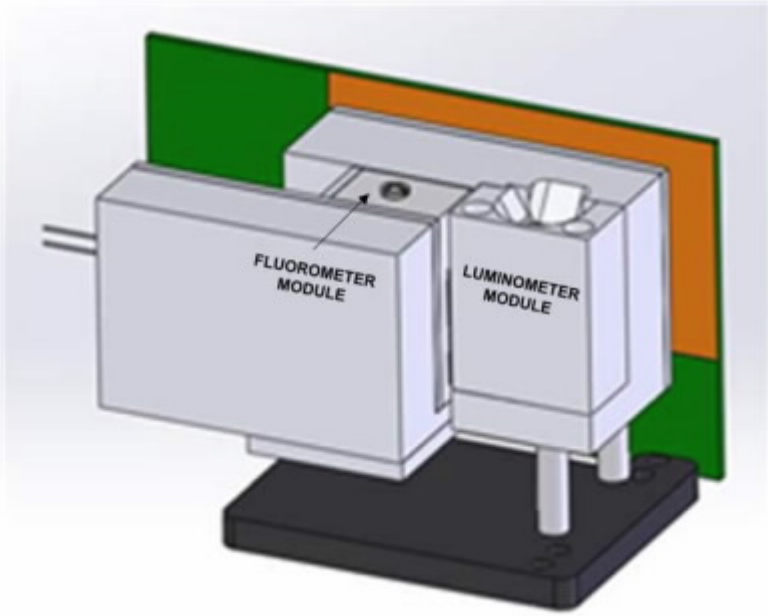


Figure 7. Luminometer/Fluorometer Module

The luminometer (Figure 7) is used for reading signals in ELISA assays. The luminometer consists of a high-gain photodiode, which has performance characteristics similar to photomultiplier tubes typically used in microtiter plate readers. The Luminometer Module includes an opening at its top through which a tip is inserted, and a detector which detects light generated as part of a chemiluminescence reaction in the tip. The luminometer can accurately detect emitted light intensity as low as ~100photons/s.

6. Detector 2 – Fluorometer Module

The Fluorometer Module (Figure 7) is specifically used for measuring fluorescence at an excitation wavelength band between 420 and 450nm, with an emission band between 570nm and 600nm. This is used for signal generation in determining the concentration of porphyrins in the TLAS (for example, Zinc Protoporphyrin (ZPP), an analyte requested for measurement in military combat applications) in red blood cells. The Fluorometer Module includes a laser diode, excitation filters, and emission filters. The detector is a high-sensitivity photodiode, similar to the one in the Luminometer Module. The Fluorometer Module shares the same processor as the Luminometer Module in the TSPU.

7. Detector 3 – Fluorometer/Turbidimeter Module

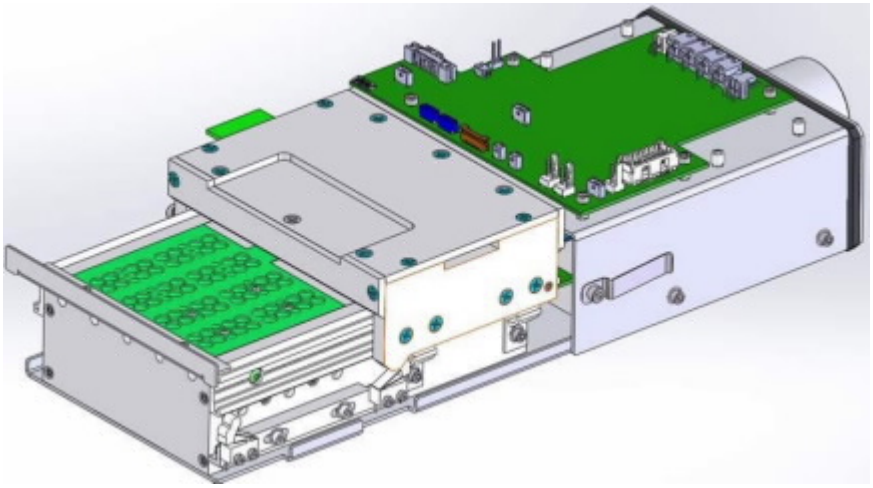


Figure 8. Fluorometer/Turbidimeter Module

Detector 3 (Figure 8) is an automated sample processing device that generates signals for assays using fluorescence and turbidity. The core functionality of the this module is the excitation and detection of emitted fluorescence from products from nucleic acid amplification reactions and the detection and measurement of light transmission through the sample for turbidity samples. The module is comprised of the following major segments: excitation signal emitter, emission signal detector, Thermal Control System, automated door, and local command printed circuit board. As part of the TSPU, the TSPU mechanically interacts with the module through the use of the pipette-based automated liquid handling system.

The circuitry and device architecture have been designed to process up to 64 discrete samples simultaneously, and to work with the automated Liquid Handling Module. 60 of the individual wells are used to process samples for fluorescence measurements in the TLAS ("fluorescence wells"), while the remaining 4 wells are used to process samples for turbidity measurements in the TLAS ("turbidity wells"). The same hardware is used with turbidity wells as is used with the fluorescence wells with the exception of omitting filters and changing the gain values on the detector. Instead of measuring fluorescence, the turbidity wells measure light transmission through the samples over time.

Reactions in the module are supported by a Thermal Control System that is local to the module. The Thermal Control System is comprised of a finned heat sink, DC Cartridge heaters, fan, temperature sensors, thermal block, and heat pipes. The discrete nucleic acid amplification samples are located in vessels that rest within the thermal block, which provides a means to maintain a desired temperature within the sample. The temperature of the thermal block is monitored via two digital temperature sensors.

The heat sink, heater, and fan system is located at the back of the device, remote to the heater block. The heat sink is connected to the heater block via water filled copper heat tubes as a means of transporting thermal energy to and away from the heater block. Air circulation is active only during cooling of the heat sink. The heat pipes allow for complete isolation of air between the thermal block where sample is present and the heat sink. This prevents contamination and aerosolization.

8. Detector 4 - Spectrophotometer Module

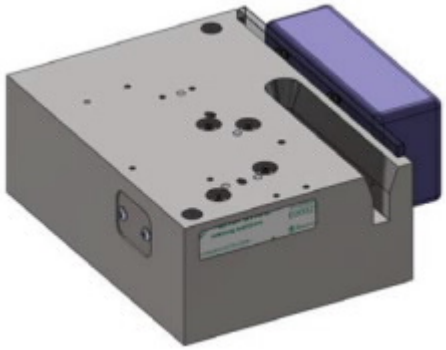


Figure 9. Spectrophotometer Module

The TSPU (Figure 9) has spectrophotometric capabilities, which are provided by the Spectrophotometer Module, and may be used to measure absorbance of small volumes (30uL) of fluid samples. The spectrophotometer is a compact, CCD-based spectrophotometer which can capture the complete intensity spectrum (32-bit intensity resolution, spectral range 300-800nm, 6nm spectral resolution) in a single capture. The output data from this module is an intensity spectrum, which is transmitted to the TLAS, where the absorbance spectrum is calculated. Based on the assay, the appropriate spectral range is chosen to compute the absorbance value. The spectrophotometer is equipped with two light sources: a broad-spectrum, 2W pulsed Xenon lamp used for absorbance measurements and a 90mW red laser diode used for fluorescence measurements.

In the CBC and lymphocyte subset assays, the spectrophotometer is used to detect the fluorescence of the cells in suspension after a nuclear stain has been added in order to determine an approximate cell concentration. This value is then used subsequently by the TLAS to automate the addition of the fluorescent binders to the cells in an appropriate concentration in the TSPU.

9. Detector 5 - Microscopy Module

The TPSU has a microscopy module (Figure 10) for imaging cells and other cellular and non-cellular components in samples. This is an epi-fluorescence microscopy module that uses an apochromatic objective lens to image objects with more than 10x magnification on a high

sensitivity CCD sensor. This optical design yields a field of view of roughly 0.2 sq mm. The module is fitted with three laser diode light sources (violet at 405nm, green at 532nm and red at 641nm wavelengths) which allow imaging of fluorescent samples in more than 5 independent spectral channels. In addition to this, there is a ringlight in the near infrared red wavelength region which enables imaging of light scattered by objects in the field of view. Sample is loaded on a cuvette by the automated liquid handling module. The cuvette is then placed onto the XY stage by the automated liquid handling module. The cuvette is translated across the objective lens by automated movement of the XY stage. At every location that is to be imaged, a mechanical actuator moves the objective lens relative to the sample in the Z direction to achieve best focus using a passive feedback mechanism. The cuvette dimensions allow for 64 fields of view to be imaged in each channel. In the CBC assay, the RBC/platelet sample utilizes one channel and the WBC sample utilizes one channel in the cuvette. There are two more channels available for the

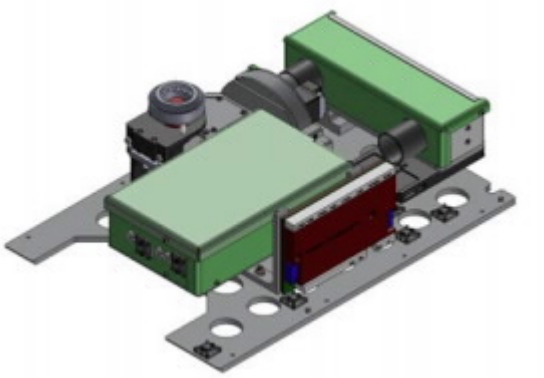


Figure 10. Microscopy Module

Lymphocyte subset assay and four more channels available for on-board controls.

10. Thermal Control System and Air Filter

The TSPU has precise temperature control to maintain the air temperature inside the TSPU at target temperature during preparatory and processing steps. The Thermal Control System consists of a forced-air convection heater and three temperature sensors which are placed in different parts of the TSPU. There is a closed-loop feedback controller which regulates the heater output based on temperature input from the sensors. The insulation around the TSPU ensures minimal thermal exchange with ambient air.

In addition to the ambient temperature control, the TSPU also has a localized heater used to bring the Cartridge up to operating temperature. The Cartridge (see section on consumables for a more detailed description) is stored at refrigerated temperature. The sample(s) are placed in the Cartridge, and the Cartridge is introduced into the TSPU. There are vents underneath the Cartridge which circulate hot air to bring the Cartridge up to the operating temperature (e.g. from refrigerated

temperature to operating temperature). The heater is regulated by a closed-loop temperature controller, the input to which is a temperature sensor which is adjacent to the Cartridge.

The Cartridge heater and the ambient heater, along with the temperature sensors and the temperature controllers, ensure that the Cartridge (containing the sample, reagents, buffers, etc.) are rapidly brought up to operating temperature and maintained at that temperature. The temperature sensors frequently record temperature across the TSPU and transmit information back to the TLAS for monitoring. Such environmental information is used by the TLAS for accessing integrity of the operation and control of the device, maintaining quality control of the operation and control of the device, and for reducing variation or error in the data collection and sample processing performed by the device.

The air circulated in the TSPU is continuously cleaned by running it through a HEPA filter for removing particles, debris, etc. The air circulation is facilitated by an induced draft fan. The induced draft fan also maintains a slight negative pressure inside the TSPU to contain the air inside the TSPU.

11. Machine Vision System

The TSPU is configured to include sensors within the enclosure for the TLAS to monitor device status and operation. The TSPU has a camera in the enclosure for capturing whether there are bubbles, particles, fibers, particulates, debris, precipitates, or other anomalies associated with any tips or vessels being handled within the TSPU which may affect readouts. The TSPU camera also captures images of components that can be used to determine whether the components are positioned properly, or where components are positioned. Imaging can be used to allow the TLAS to assess if a volume of sample, reagent, or other material falls within a desired range, or whether a sample, reagent, or other material is located in a desired location. This information is frequently communicated to the TLAS for error-checking, calibration, protocol execution, and quality control of the TSPU.

Software, Touchscreen, and Process Work Flow

The TSPU operates under the control of the TLAS. The TSPU is connected to the TLAS via a secure Internet or other data network connection, and the TSPU and TLAS are capable of two-way communication with each other. For example, the TLAS can send various commands and protocols to the processor of the TSPU, for execution by the TSPU. Similarly, the TSPU can send information obtained by the TSPU to the TLAS, such as data obtained from pre-analytic steps with a sample or information obtained from sensors within the TSPU (e.g. signal, image, temperature information). Information sent by the TSPU to the TLAS may be in response to a specific request for information from the TLAS to the TSPU, or it may be part of a standardized protocol. Upon completion of pre-analytic processing in the TSPU, the TLAS performs analysis and post-analytic processing.

Although a TSPU may be situated at a Theranos' CLIA-certified laboratory Patient Service Center location which is physically separate from the TLAS, complete control and oversight is extended from the central TLAS to the remote TSPU to ensure CLIA-oversight and certification of the tests

being reported. The TSPU serves as part of the Theranos CLIA-certified laboratory, and laboratory results generated from data analyzed in the TLAS and obtained from a sample processed on a TSPU are CLIA-certified.

There is a touch screen embedded in the TSPU for operation of the device. The touchscreen allows for detailed, user-oriented instructions, oversight, by ensuring a technician follows all appropriate steps before processing a sample, and two way communications. Operation of the TSPU at a Theranos PSC is performed by a Theranos- certified phlebotomist or other appropriately state-licensed technician; the technician is trained in the Theranos CLIA-certified laboratory and is managed by a Theranos laboratory director.

In accordance with FDA guidance, the TLAS allows the operation of the clinical laboratory process without operator intervention, including control of the TSPU through direct LAS interfacing, specimen manipulation, transportation of the specimen and related signals, result evaluation, repeat testing, reflex testing and quality assessment and results reporting.

The secure network infrastructure allows for CLIA-compliance for certified analysis and testing through the TLAS for determination of the presence or absence of various substances in the human body in Theranos' CLIA-certified laboratory while automating sample processing in field through the TSPU in TPSCs to minimize pre-analytic error and variability.

Consumables and Materials

The sample, as well as products of further processing and reaction are contained in disposable consumables inside the disposable reagent tray or Cartridge. All consumables are discrete such that reagents and reactions for each assay reside and occur, respectively, in physically separate locations to prevent cross-reactivity. The consumables contain all liquids or reagents such that no sample or reagent ever directly interacts with the device. All consumables for processing are contained in the Cartridge (and are not built into the TSPU) and are placed back into the Cartridge at the completion of processing for disposal.

The consumables used for the TNAA and Cytometry assays include the following:

Round vessels – 60uL capacity polypropylene vessels for storing reagents, dilutions, mixing, and reactions.

Wash vessels – 200uL capacity polypropylene vessels for storing wash buffers.

Centrifuge vessel 1 – narrow diameter 100uL capacity polystyrene vessels for centrifuging blood and efficiently removing supernatant

Centrifuge vessel 2 – 120uL capacity polypropylene vessels for centrifuging samples and efficiently mixing and transferring small volumes.

Mini tips – 10uL capacity polypropylene tips for transporting fluids; with silica filters for preventing cross-contamination.

Large tips – 40uL capacity polypropylene tips for transporting fluids; with silica filters for preventing cross-contamination.

Dynamic Dilution tips – 10uL capacity polystyrene tips for transporting fluids; with silica filters for preventing cross-contamination.

TNAA vessels – 60uL capacity polypropylene vessels which serve as reaction vessels for the amplification reaction. The final fluorescence signal (from the product generated in these vessels) is detected from these vessels.

TNAA trays – Trays which hold 8 TNAA vessels. The trays can also be picked up by the fluid handling module to transport the vessels between the Cartridge and the TNAA module.

Sonicator vessel – 350uL polystyrene vessel, used to contain sample during sonication.

Magnet Tool Sleeve – disposable polypropylene sleeve separates magnet from consumable to prevent contamination.

Swab vessel – 400uL capacity polypropylene vessel designed to contain the nasopharyngeal or throat swab, as applicable.

Cuvette – a zeonor slide with molded polystyrene top containing six channels that fluid can be introduced into for imaging on the microscopy module

Colorstrip – multiwell strip made from PMAA; this part contains multiple optically clear cavities into which colorimetric reactions are placed. The absorbance of the samples is then measured in the spectrophotometer.

Cartridge – Houses all consumables listed above. Secured by a lid to hold all consumables in place and prevent user interaction.

The Cartridge comes with a closed lid (Figure 11, Left), under which are all pre-populated consumables required for the TNAA and Cytometry Assays. Regents and buffers required for the assays are pre-filled and sealed in Round vessels and Wash vessels. The TNAA vessels come pre-filled with the master mix for the assay, followed by a protective wax layer on top.

Only the sample entry port(s) on the Cartridge are exposed to the certified phlebotomist or appropriately state licensed sample collection technician. Figure 11 Center shows a cartridge in which both the blood sample and the swab sample are introduced.

The nasopharyngeal or throat swab is placed directly inside the accessible Swab vessel on the Cartridge, which is pre-filled with a transfer medium. The lid to the swab vessel is closed after introduction of the swab.

Figure 11. Cartridge in various configurations. Left: Closed cartridge. Center: Samples (Swab and Nanotainer) being introduced into cartridge. Right: Cartridge with lid open inside the TSPU.

Once collected, blood samples are collected in Theranos' Nanotainer ™ tubes, which Theranos has separately registered with FDA as sample transport containers (Registration Number 3006231732).

The Nanotainer is then placed in the Cartridge (Figure 11, center). The Cartridge is then inserted into the TSPU, and the cartridge is drawn in, the door to the TSPU is closed, and the lid is opened by means of a mechanism inside the TSPU. This exposes all consumables (Figure 11, Right) inside the TSPU. Afterwards, the sample processing, reagent addition, and signal generation steps take place in the TSPU, as instructed by commands from TLAS.

After completion of the appropriate processing steps, a Cartridge is ejected with the lid closed, and can be appropriately disposed of in its entirety in the Theranos Patient Service Center.

Scientific Basis

The TSPU and TLAS were designed to automate the exact processing steps and protocols associated with the most precise and accurate CLIA-certified test methods. The TSPU is configured to automatically perform a wide range of standard laboratory sample processing steps, such as pipetting, sonicating, centrifuging, mixing, and heating. These steps may be automatically performed by the TSPU in accordance with a protocol executed by a processor on the TSPU that received commands from the TLAS. Automation of the laboratory sample processing steps permits the steps to be performed by the TSPU with very high accuracy and precision targeted to exceed that achieved by human technicians for the same sample processing steps. In addition, the TSPU is capable of performing customizable sample processing steps (e.g. variable pipetting volumes, sonication times, etc.), based on the specific instructions of a given protocol from the TLAS.

Device Manufacturing and Materials

The device is assembled in a GMP environment from a variety of commercially available components, fabricated electrical assemblies, cable assemblies, sheet metal structures, and machined mechanical parts. All component inventory is managed through Theranos' supply chain group using an ERP system. All parts, except for the commercially available components, are fabricated based on Theranos designs. Most machined parts are produced at Theranos' internal machine shop.

When building a module, components are kitted and transferred to the assembly group. The assembly technicians assemble the module per a Manufacturing Operating Procedure (MOP) document. MOPs are developed by manufacturing engineers, reviewed by design engineers, and officially released into a controlled system for revision management. Once assembly is complete, modules are subjected to a functional checkout to verify functionality.

Manufacturing and production are performed in accordance with QSR, following the Theranos Quality System, which is drafted for compliance with the applicable Code of Federal Regulations ("***CFR***") provisions.

Theranos Laboratory Automation System

The TLAS comprises at least one server configured to communicate with and control one or more TSPUs with an encrypted, certificate-based security system. The TLAS provides a number of functions, including sending test protocols to the TSPU based on the desired tests to be run on the sample and for maintaining oversight over the TSPUs. During processing, the TSPU and TLAS are communicating to validate the quality and integrity of the consumables, based on lot information tracked in the TLAS, execute the sample processing steps, and monitor and oversee the quality of the sample processing. After controlling sample processing in the TSPU, signal sets from the sample are transferred to the TLAS where the raw data is analyzed, the relevant reportables are generated for a Laboratory Information System, and post-analytic processing steps are performed.

The TLAS is run in and overseen by Theranos' CLIA-certified laboratory, and provides oversight and remote control of the TSPU. The consumables containing patient samples (Swab vessel for nasopharyngeal or throat swab and Theranos Nanotainer tube for blood sample) are placed in a Cartridge and introduced into the TSPU. The TSPU scans a barcode on the Cartridge, and the barcode value is transmitted to the TLAS, which securely de-codes the barcode value, and sends a sample processing protocol to the processor in the TSPU. The processor further distributes tasks received from the TLAS to various modules in the TSPU. The TSPU constantly feeds information back to the TLAS to ensure constant monitoring of the TSPU and its performance. The final steps of sample processing are signal generation (fluorescence light for the TNAA assays and fluorescence light and scattered white light for the cytometry assays) and signal detection by detectors (Detector 3 for TNAA and a CCD sensor for cytometry). The data are transmitted back to the TLAS, which performs analysis on these raw data and yields clinically relevant analyte reportables for CLIA laboratory staff to oversee and further analyze, as applicable.

Theranos Nucleic Acid Amplification (TNAA) Assay for Viral Versus Bacterial Panel Detection

TNAA Chemistry:
Background:

Theranos Nucleic Acid Amplification (TNAA) is an isothermal method that provides rapid qualitative detection and identification of pathogens from clinical samples.

TNAA Principle:

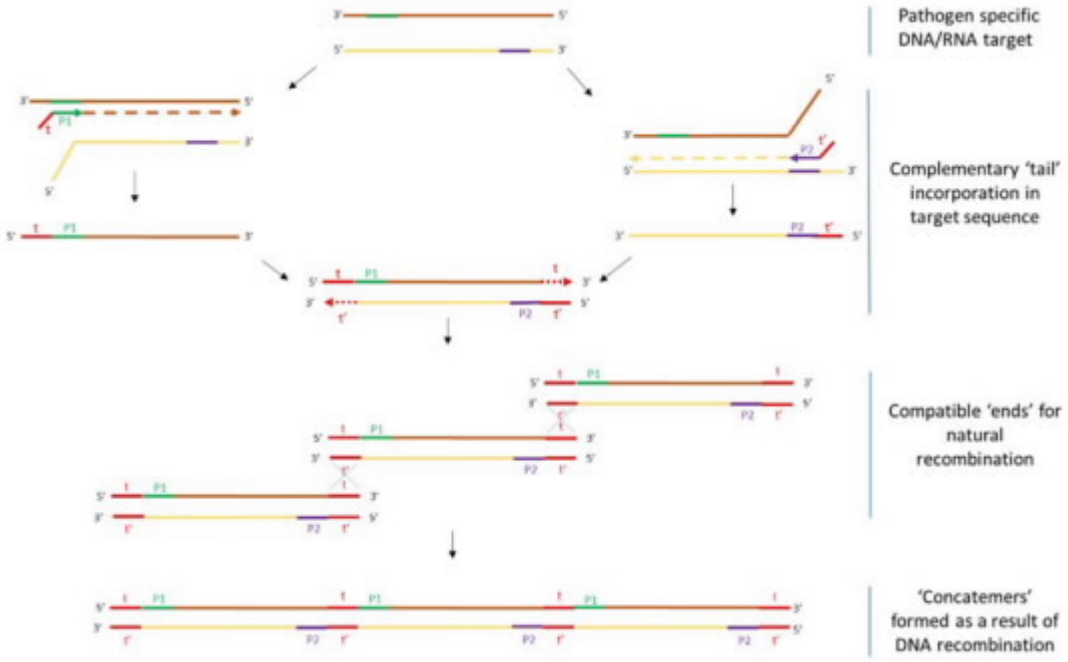


Figure 12. TNAA assay schematic

TNAA harnesses the power of DNA recombination which is facilitated through primer design during the course of Nucleic Acid Amplification. The TNAA reaction is of an exponential nature and can be observed using DNA intercalating fluorescent dyes, nucleic acid probes, etc. in real-time. The data can be interpreted to detect the presence or absence of pathogen-specific genetic material in a given sample. Figure 12 shows a schematic diagram of the TNAA method.

Amplification is done on a selected DNA/RNA target which is specific to the target pathogen. The brown and yellow lines represent the two strands of duplex DNA whereas the green and purple part is the selected region against which the primers are designed. The two primers (P1 and P2)

used in the amplification process are shown as green and purple arrows with red 'tails'. Tails (t and t') on the 5'end of the primers are complementary sequences of each other. During the initial amplification cycles, the tails t & t' are incorporated in the product DNA strands. This process generates duplex DNA molecules with homologous ends that can go through natural recombination (cross-over sites demonstrated by 'X' mark) as is seen in nature during DNA replication. Recombination results in the formation of 'concatemers' of DNA molecules that grow in molecular size with each cycle of amplification. The replication of DNA concatemers in the presence of primers results in amplification of target nucleic acid at an exponential rate, which can be observed in real-time using DNA intercalating fluorescent dyes.

Protocol for Viral Versus Bacterial panel TNAA Assays

Pathogen nucleic acid extraction

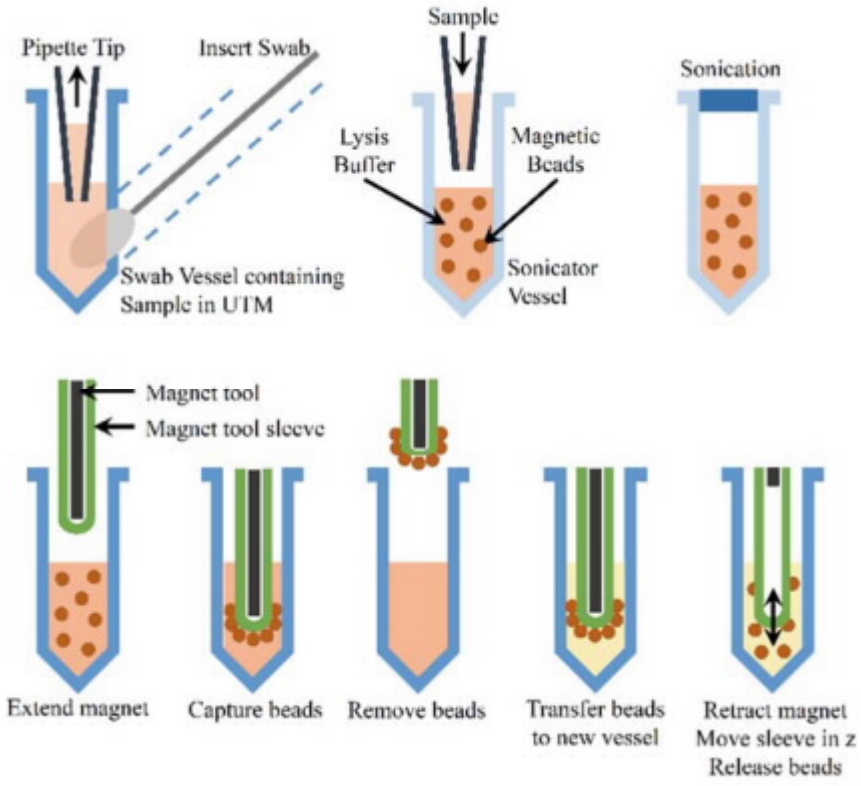


Figure 13. TNAA nucleic acid purification steps in the TSPU

A Theranos certified CLIA-laboratory (and appropriately state licensed sample collection) technician takes a Swab vessel which comes sealed with a lid at the swab port. The technician removes the lid, inserts the flocked swab sample into the Swab vessel embedded in the Cartridge,

which contains an aliquot of universal transport medium, separates the swab from the plastic handle at the defined breakpoint in the handle, and re-closes the lid. The majority of the sample on the swab immediately releases into the medium upon contact. The vessel containing the swab is then capped, and the Cartridge is ready for processing with the device. The Viral Versus Bacterial panel TNAA assays begin with sample being transferred from the Swab vessel by a Large tip into the Sonicator vessel.

The nucleic acid extraction implemented in the TSPU utilizes a magnetic-bead based methodology to isolate and purify nucleic acids from a sample matrix. A brief overview of the steps involved are as follows:

1. A Large tip is inserted into the access point on the Swab vessel, breaking through a foil seal barrier to access the sample. The sample is mixed by pipetting up and down for several cycles to ensure sample release from the swab through agitation of the surrounding fluid.

2. The sample is transferred from the Swab vessel to the Sonicator vessel by means of two large pipette tips, and lysis buffer and functionalized magnetic beads are added to the Sonicator vessel from other reagent storage locations on the Cartridge.

3. The Sonicator vessel is moved to the sonicator probe location, and the vessel is sonicated in order to lyse open the cells to release the nucleic acids. The sonication vessel is returned to its location on the Cartridge after this step.

4. Binding buffer, which helps the nucleic acids bind to the functionalized magnetic beads, is transferred from a reagent storage well to the Sonicator vessel and mixed by pipetting up and down.

5. The Magnet Tool that resides inside the TSPU is picked up using a large pipette nozzle. The Magnet Tool is retracted inside of the nozzle such that only 2-3mm is visible before that nozzle is used to pick up the Magnet Tool Sleeve in the consumable. This sleeve shields the Magnet Tool from the sample to prevent contamination.

6. The Magnet Tool is then extended into the tip sleeve and inserted into the Sonicator vessel to capture the magnet beads on the exterior of the sleeve.

7. The magnetic beads with captured nucleic acids aggregate on the tip of the sleeve and can be transported into a well containing wash buffer.

8. The Magnet Tool, covered by the Magnet Tool Sleeve, is retracted into the nozzle by moving the piston motor, and the nozzle is moved in a vertical direction multiple times to release the beads and mix them with the fluid. The Magnet Tool/Magnet Tool Sleeve is removed from the well by moving the nozzle.

9. The Magnet Tool is extended back into the tip sleeve and inserted into the wash buffer well to capture the washed magnet beads and transport them to the next step.

10. For each additional bead wash to purify the sample, steps 7 through 9 are repeated.

11. The Magnet Tool and its sleeve carrying captured magnetic beads with purified nucleic acid sample is inserted into the elution well.

12. The Magnet Tool is retracted into the nozzle, and the entire nozzle is moved in vertical directions for several cycles to release the beads and mixed by fluid displacement using a piston motion before retracting the nozzle such that the tip clears the entire well.

13. The released beads are allowed to incubate in the elution well for 1 minute.

14. The Magnet Tool is extended back into the Magnet Tool Sleeve and inserted into the elution buffer well to capture the magnetic beads. The Magnet Tool Sleeve is then discarded into its original location on the Cartridge and the Magnet Tool is returned to its resting location in the TSPU.

15. The elution buffer is ready to be distributed into the downstream TNAA assays.

TNAA Assay and Signal Generation

The elution buffer extracted from the steps above contains the extracted nucleic acid material. Detector 3 is brought up to 56 °C utilizing the module's thermal controller. The TNAA tray with the TNAA vessels is picked up by the Liquid Handling Module and transferred to Detector 3. The vessels contain the master mix for the TNAA assay, capped with a wax layer. This wax layer melts at the elevated (56 °C) temperature. 3uL of elution buffer is aspirated from the elution well on the Cartridge and transferred into the TNAA vessel using a Mini tip, ensuring that the tip penetrates past the molten wax layer. The sample is mixed with the master mix to ensure homogeneity. The tip is discarded back into the Cartridge. When the tip is moved away from the detector module, the lower temperature of the TSPU causes the molten wax around the tip to solidify, thereby forming a physical barrier around the tip opening and preventing any sample from leaking out of the tip. This protects against contaminating the TSPU. A new tip is picked up by the Liquid Handling Module, and 2uL of enzyme is transferred from a reagent well in the Cartridge to the NAA vessel, and is mixed with the sample and the master mix. The tip is retracted and returned back to its location on the Cartridge.

The reaction mixture in each NAA vessel is incubated for 5 minutes, after which the photodiode corresponding to each reaction vessel is used to capture the reaction signals of 30 samples sequentially, and capturing such signals of all 30 samples takes slightly over 10s. After that there is an approximately 10s pause before capturing the next set, ensuring that each sample is detected at a frequency of 1 detection every 20s. The data (in the form of counts) is transmitted in real time to the TLAS, where the fluorescence signal is recorded and analyzed in real-time. The analysis consists of identifying a change point to determine the inflection time of the assay.

Theranos Cytometry Assay Methodology

Cytometry Chemistry Perspective:

Theranos cytometry is a method for identification, classification and enumeration of cells and objects in clinical samples that is based on measurement/detection of specific molecular markers, optical properties and morphological properties of the cells and objects.

Cytometry Principle:

Theranos cytometry uses well established molecular markers to identify different cell types in biological samples. These markers or epitopes are tagged using fluorescently labeled binders (e.g. monoclonal antibodies) and visualized using fluorescence microscopy in the TSPU and TLAS. In addition, the TSPU also detects light scattered by cells over roughly 64-110°. For objects like crystals, synthetic beads, etc. where specific markers are not available, morphology can also be used for particle identification. TSPU acquires signals in different spectral channels. These images are combined and segmented using image analysis tools in the TLAS to detect presence of cells/objects, delineate the area of each cell/object and measure the intensity or brightness within that area for each spectral channel. Thus for each cell/object intensities in up to 7 spectral channels can be measured in addition to geometric properties. The data are transmitted to the TLAS where they are analyzed using automated classification algorithms under the oversight of the CLIA laboratory. The algorithm clusters cell populations and provides counts of each cell type. In addition to cells, there are beads present in the sample at a known concentration. Therefore cell/bead ratio in conjunction with the known bead concentration allows calculation of cell concentration.

- *CBC – RBC/PLT assay:*

  RBCs are labeled with a fluorescent anti-CD235a antibody and platelets are labeled with fluorescent anti-CD41/CD61 antibodies. This sample is mixed with 10um polystyrene beads at a known concentration. In addition to identifying RBCs as CD235a positive objects, the Theranos system also quantifies their intensity profile and fits a region of interest (ROI) around it. The RBCs are isovolumetrically sphered by the use of a zwitterionic-surfactant solution. Therefore the geometry of the ROI can directly be used to estimate diameter and hence the volume of the spherical RBC. Similarly, platelets are identified as CD41/61 positive events and the distribution of intensity around the centroid of the platelet is used to derive a measure of the mean platelet volume. Shown below are examples of the images collected by the TLAS and the analyzed data for the RBC/PLT assay.

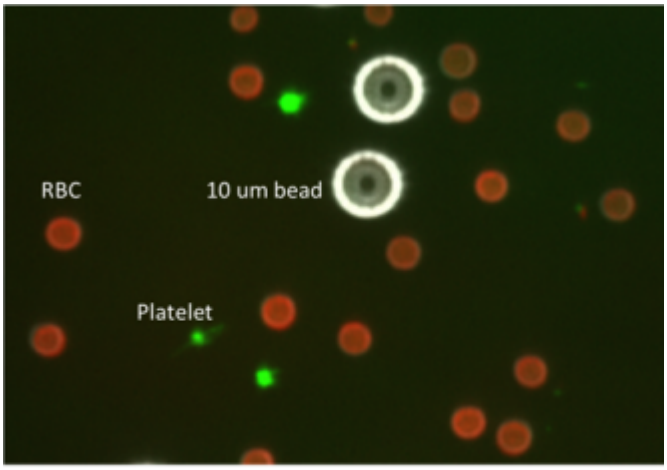


Figure 12. Composite image showing scatter of 10 um polystyrene beads and stained RBCs and PLTs.

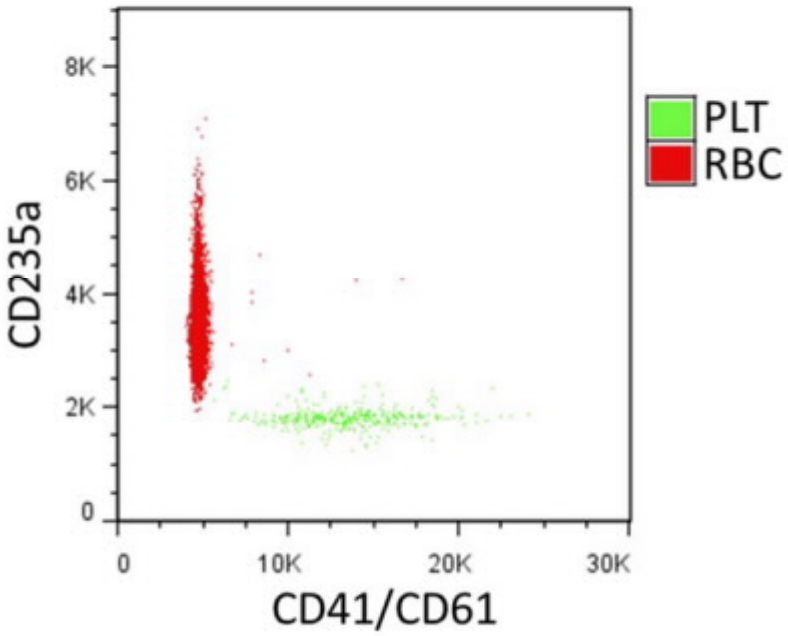


Figure 13. Scatterplot showing fluorescence intensity data for RBC/PLT sample. RBCs are shown as red dots, and PLTs are shown as green dots.

- *CBC – WBC and WBC differential assay:*

  WBCs are labeled with DRAQ5, a DNA stain, in addition to CD14 (monocyte marker), CD16 (neutrophil marker), CD123 (basophil marker) and CD45 (pan-leukocyte marker). Each of these markers is visualized in a different spectral channel. The sample also has 10um polystyrene beads at a known concentration. Shown below is a composite image demonstrating the different cell types identified in the WBC assay, as well as the gating scheme that is used to determine the WBC differential.

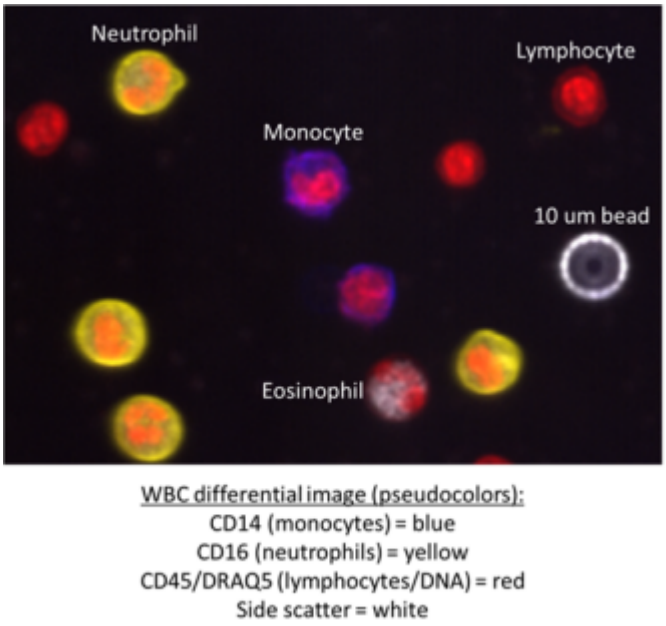


Figure 14. Composite image showing WBC differential sample. CD14 labeling is shown in blue, CD16 labeling is shown in yellow, CD45 and DRAQ5 are shown in red, and side scatter is shown in white.

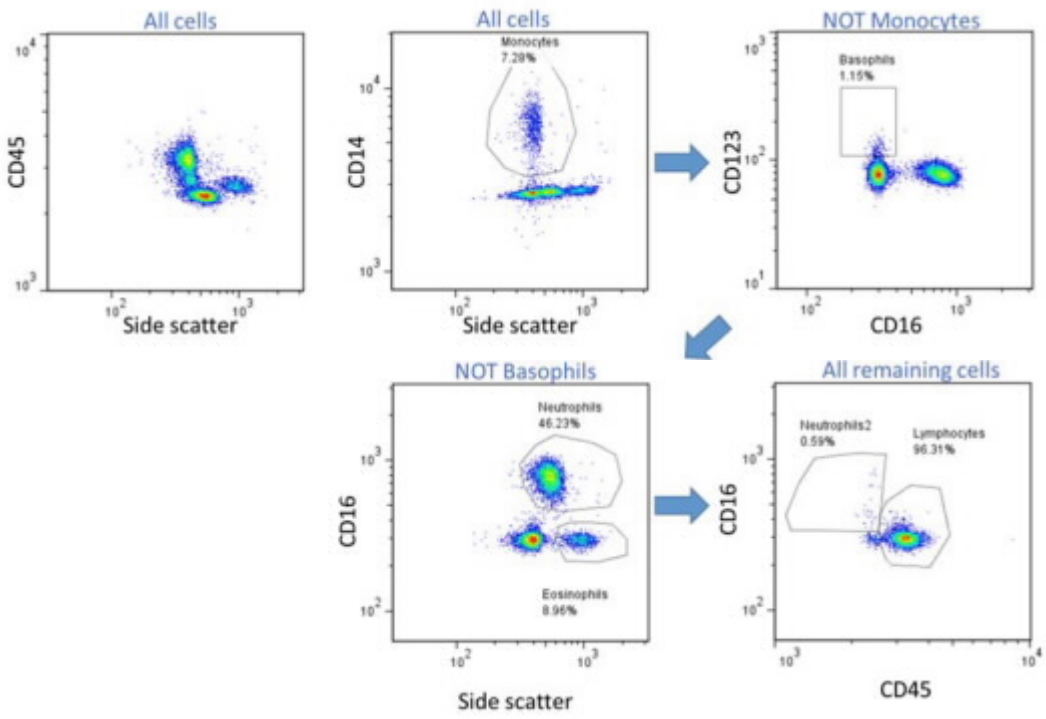


Figure 15. Scattergrams showing data classification for WBC differential. Neutrophils, Lymphocytes, Monocytes, Eosinophils, and Basophils are all identified by multiple markers.

- *CBC – Hemoglobin Assay:*

  Whole blood is osmotically lysed in reagent-grade water, releasing the hemoglobin into solution. This lysed blood is then mixed in a known proportion with Hemoglobin Reagent, which is based on Drabkin's reagent to oxidize all the hemoglobin in the sample to cyanmethemoglobin. The sample is taken in a color strip well and transferred by the pipette to the spectrophotometer module. The intensity spectrum of light passing through this sample is detected by the spectrophotometer module and transferred to the TLAS. The TLAS uses this and a blank intensity spectrum and computes the absorbance of the sample at 540nm, which is proportional to the concentration of hemoglobin in the original sample.

- *CBC – Hematocrit Assay:*

  Whole blood is transferred to a centrifuge vessel and centrifuged to separate the blood cells and the plasma. The vessel is imaged, and the proportion of the volume that is occupied

by the red blood cell pellet is calculated by the TLAS using image analysis. These volumes are used by the TLAS to calculate the hematocrit.

- *Reticulocyte Assay:*

  This assay is designed to enumerate reticulocytes in whole blood. Reticulocytes are reported as percentage of erythrocyte count. RBCs are labeled with fluorescent anti-CD235a antibody and reticulocytes are additionally labelled with Thiazole Orange. This is followed by isovolumetric sphering of red blood cells using a zwitterionic surfactant based buffer, and subsequent fixation by 0.5% paraformaldehyde. Stained, sphered and fixed cell suspensions are analyzed by the TLAS for positive staining for CD235a and cell morphology based on imaging of light scattered by the cells. Cells that are positive for both CD235a and Thiazole Orange are classified as reticulocytes.

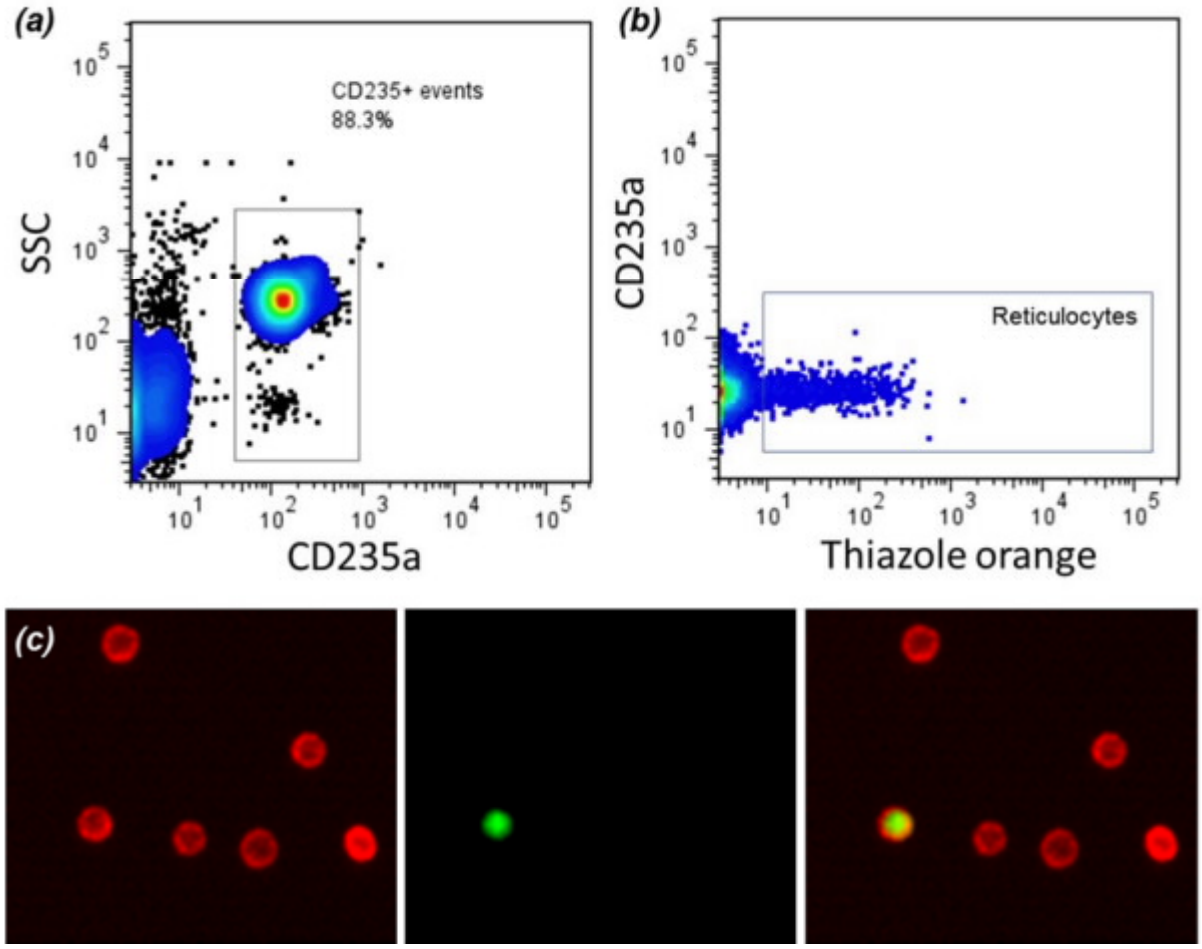


Figure 16 Staining of Reticulocytes. Whole blood stained with anti-CD235a and thiazole orange. Scattergrams shown are from a commercial flow cytometer, used for assay development. All erythrocytic CD235a positive events are gated in (a) and examined for TO fluorescence (b). The reticulocyte gate is drawn based upon a known negative population to account for

background and autofluorescence. Panel (c) shows confirmatory fluorescence micrographs. From left to right: CD235 positive cells, TO positive reticulocytes and the merged image. The fact that TO staining is internal to the cell is supported by the merged image. Images are presented in pseudocolor for visual clarity.

- *Lymphocyte subset assay:*

  WBCs are labeled with DRAQ5, a DNA stain, in addition to CD45 (pan-leukocyte marker that is most highly expressed on lymphocytes), CD3 (pan-T cell marker), CD4 (T-helper marker), CD8 (T-suppressor/cytotoxic marker), CD19 (B-cell marker), CD16+CD56 (NK cell markers). These markers are divided into two subsets (T-cell subset panel and T/B/NK panel). Within each panel the markers for each cell type are visualized in a different spectral channel. The samples also have 10 um polystyrene beads at a known concentration.

Protocol for Theranos cytometry assays

CBC – Protocol for Cytometry WBC and Hematocrit

The CBC protocol uses whole blood as the starting sample. The following is the sequence of steps for the WBC and hematocrit assays.

1. A Large tip used to mix whole blood in the Nanotainer by pipetting the blood up and down for several cycles.
2. The blood is transferred to centrifuge vessel 1. The centrifuge vessel is moved by the liquid handling module to the centrifuge and spun. After spinning it is imaged with a camera for calculating hematocrit concentration in the TLAS, and then returned to the cartridge by the liquid handling module.
3. A Large tip is picked up and used to transfer plasma from centrifuge vessel to a round vessel.
4. A Large tip is used to mix and transfer plasma substitution buffer containing a known concentration of beads to centrifuge vessel 1. The blood is mixed by pipetting and then mixed with an antibody cocktail. The mixture is mixed well by pipetting the fluid back and forth.
5. Following the predefined incubation time with antibody cocktail, a lysis buffer containing fixative is mixed with the blood to lyse red blood cells in Centrifuge Vessel type 2.
6. Following lysis, the Centrifuge Vessel type 2 is moved to the centrifuge and spun. A large tip is used to remove the supernatant following spinning.
7. The cell pellet is resuspended and a portion of the sample is moved to the spectrometer for dynamic dilution measurements. The fluorescence intensity, from the nucleic acid stain, is used by the TLAS to compute an approximate cell count. Based on this cell count, additional beads in buffer are added to dilute the cells.

8. Additional antibody cocktail is then added to the cells and mixed well by pipetting up and down.
9. The WBCs are then immediately transferred to the cuvette with a large tip. A mini tip is used to transfer oil to cover the ports of the cuvette to prevent evaporation.
10. The cells are allowed to incubate and stain with antibody cocktail and settle to the bottom of the cuvette.
11. The cuvette is transferred to the cytometer stage, where imaging takes place as described in the microscopy module section.
12. Following imaging, the cuvette is transferred back to the cartridge.

Once acquired, images are transferred to TLAS where they are analyzed using automated classification algorithms.

CBC – Protocol for Cytometry RBC and Platelet Assays

The following is the sequence of steps for the RBC and Platelet assays. This protocol can be multiplexed to occur in parallel with the WBC and hematocrit assays.

1. A Large tip used to mix whole blood in the Nanotainer by pipetting the blood up and down for several cycles.
2. Blood is transferred to a dilution buffer containing a known concentration of beads and mixed by pipetting up and down.
3. The diluted blood is then mixed with antibody cocktail by pipetting up and down and allowed to incubate.
4. The stained blood is then mixed by pipetting and transferred to a vessel containing sphering buffer and allowed to incubate.
5. The sphered blood is then mixed and transferred to a vessel containing a fixative.
6. The solution is mixed well by pipetting and then transferred to the cuvette with a Large tip. A mini tip is used to transfer oil to cover the ports of the cuvette to prevent evaporation.
7. The cells are allowed to incubate to finish fixing and settle to the bottom of the cuvette.
8. The cuvette is transferred to the cytometer stage by the liquid handling module, where imaging takes place as described in the microscopy module section.
9. Following imaging, the cuvette is transferred back to the cartridge.

Once acquired, images are transferred to TLAS where they are analyzed using automated classification algorithms.

CBC – Protocol for the Hemoglobin Assay

The following is the sequence of steps for the Hemoglobin (***"HGB"***) Assay.

1. A large tip is used to mix whole blood in the Nanotainer by pipetting the blood up and down for several cycles.

2. A mini tip is used to transfer an aliquot of the blood to a vessel containing reagent-grade water, which lyses all the RBCs.
3. The diluted/lysed blood is mixed with a large tip to ensure homogeneity.
4. An aliquot of the diluted/lysed blood is added to a vessel containing Hemoglobin Reagent.
5. The sample is incubated with the Hemoglobin Reagent for a predetermined amount of time, which allows all the hemoglobin to become oxidized.
6. The fully reacted sample is transferred to a well on the Colorstrip, which is then moved to the spectrophotometer. The Xenon lamp is fired and the spectrophotometer module captured an intensity spectrum which passes through the sample. This is transferred to the TLAS, where the absorbance at 540nm is calculated.
7. The Colorstrip is transferred back to the cartridge for disposal.

Protocol for Cytometry Reticulocyte Assay

The following is the sequence of steps for the Reticulocyte assay:

1. A Large tip used to mix whole blood in the Nanotainer by pipetting the blood up and down for several cycles.
2. Blood is transferred to a dilution buffer containing a known concentration of beads and mixed by pipetting up and down.
3. The diluted blood is then mixed with either (A) anti-CD235a antibody alone or (B) anti-CD235a antibody and Thiazole Orange cocktail by pipetting up and down and allowed to incubate for a pre-determined length of time.
4. The stained blood is then mixed by pipetting and transferred to separate vessels containing sphering buffer and allowed to incubate for a pre-determined length of time.
5. The sphered blood is then mixed and transferred to separate vessels containing fixative.
6. The solutions are mixed well by pipetting and then transferred to the cuvette with a Large tip. A mini tip is used to transfer oil to cover the ports of the cuvette to prevent evaporation.
7. The cells are allowed to incubate to finish fixing and settle to the bottom of the cuvette.
8. The cuvette is transferred to the cytometer stage, where imaging takes place as described in the microscopy module section. The anti-CD235a only and anti-CD235a + Thiazole Orange samples are imaged separately.
9. Following imaging, the cuvette is transferred back to the cartridge.

Once acquired, images are transferred to TLAS where they are analyzed using automated classification algorithms. The algorithm uses the anti-CD235a only sample to establish the gate for the anti-CD235a + Thiazole Orange sample.

Protocol for Cytometry Lymphocyte Subset Assay

The following is the sequence of steps for the Lymphocyte Subset Assay:

1. A Large tip used to mix whole blood in the Nanotainer by pipetting the blood up and down for several cycles.
2. The blood is transferred to centrifuge vessel 1. The centrifuge vessel is moved to the centrifuge and spun. After spinning it is returned to the cartridge.
3. A Large tip is used to mix and transfer a buffer containing a known concentration of beads to centrifuge vessel 1. The blood is mixed by pipetting.
4. Aliquots of the blood + beads mixture are added to two round vessels containing antibody cocktails. Round vessel A contains cocktail to identify T cells, B cells, and NK cells. Round vessel B contains cocktail to identify the T cell subset (CD3, CD4, and CD8). The mixtures are each mixed well by pipetting the fluid back and forth.
5. Following a pre-determined incubation time with antibody cocktails, samples are transferred to Centrifuge Vessel type 2 (A) and Centrifuge Vessel type 2 (B). A lysis and fixation buffer is mixed with the blood to lyse red blood cells and fix the WBCs in these vessels.
6. Following lysis, the Centrifuge Vessels type 2 are moved to the centrifuge and spun. Large tips are used to remove the supernatant following spinning.
7. The cell pellets are resuspended and a portion of each sample is moved to the spectrometer for dynamic dilution. The fluorescence intensity, from the nucleic acid stain, is used to compute an approximate cell count. Based on this cell count, additional beads in buffer are added to dilute the cells.
8. Additional antibody cocktail is then added to the cells and mixed well by pipetting up and down.
9. The samples are then immediately transferred to the cuvette with a large tip. A mini tip is used to transfer oil to cover the ports of the cuvette to prevent evaporation.
10. The cells are allowed to incubate and stain with antibody cocktail and settle to the bottom of the cuvette.
11. The cuvette is transferred to the cytometer stage, where imaging takes place as described in the microscopy module section.
12. Following imaging, the cuvette is transferred back to the cartridge.

Once acquired, images are transferred to TLAS where they are analyzed using automated classification algorithms.

Performance Testing / Product Development

For reference, the Appendix includes Theranos'clinical data along with Theranos' template for system validation.

Performance Testing

In addition to the assembly-level and system-level tests which are done on every TSPU that is manufactured, a set of 25 TSPUs will all undergo an extensive device validation exercise. The device validation exercise is meant to quantify the key performance metrics of the device (hardware and software internal to the device). An example of a device validation plan, detailing validation tests for selected modules (which are involved in the application discussed in this document) is attached (see document titled TSPU_validation_template). The validation tests describe the metric to be tested, description of the test, number of replicates, and passing criteria for ensuring that the device meets all requirements.

Product Development

Functional TSPUs have been built and undergone extensive system tests. The TSPU has been completely designed and assembled by Theranos in its facilities in California. Most machined parts are fabricated in-house using state-of-the art CNC machine lines. All machined components, sub-assemblies, and key assemblies go through a thorough Co-ordinate Mapping Machine (CMM) inspection to ensure dimensional accuracy. Method of Procedure (MOP) for fabrication and assembly of various modules has been established in accordance with Good Manufacturing Practice (GMP) requirements.

The individual modules in the TSPU go through tests to quantify precision, as well as hardware-level calibration. For instance, the microscopy module is tested for laser alignment and uniform intensity across devices. The luminometer is tested for precision in responding to a constant-intensity light source, and calibrated such that the same light source yields the same total counts across all Luminometer Modules. Each spectrometer module is tested and calibrated to ensure dynamic range and spectral concordance across all devices. Similarly, the Fluorometer/Turbidimeter Module is characterized for temperature precision and accuracy. The Liquid Handling Module is independently characterized for volumetric precision, calibrated for accuracy, and re-tested for post-calibration bias. After assembly, there are system-level tests to ensure the device meets overall device accuracy.

Safety

TSPU will comply with IEC 60601, IEC 62304, and ISO 14971 standards for Medical Devices. The electronics in the TSPU have been designed to be in compliance with IEC standards.

Quality Control

The TSPU is designed and constructed for high accuracy and precision. Quality control ("***QC***") and calibration starts with individual modules in the device. All modules, including the Liquid Handling Module and detectors are independently qualified for precision, and independently calibrated for accuracy. This is followed by a complete system level QC check, which quantifies both accuracy and precision of the hardware. This approach is rather unique to the Theranos system, since most devices only go through an overall system level QC check and calibration.

The TSPU goes through several stages of QC checks to ensure high degree of accuracy and precision. As described above, the individual modules as well as the complete device is tested for accuracy and precision by running several QC protocols with onboard controls.

The two assay methods also have on-board controls to ensure that the system performs adequately. For The Influenza TNAA assays, the following on-board controls are run:

- Sample collection and transfer control: Each patient sample will carry varying amounts of human specific nucleic acid. For each sample processed, a control human RNaseP assay is run to verify appropriate sample collection.

- Sample Prep control: A non-natural/synthetic target in the form of DNA or RNA is automatically spiked into the sample. This is used as an internal calibrator to QC sample prep and amplification. This test also checks the general performance of the chemistry and the device.

- No Template Control (NTC): For each assay, a NTC test will be run simultaneously to QC for background signal and contamination.

For Cytometry assays, the following on-board controls are run:

- Reagent activity: Stabilized materials have been developed to control for the binding of fluorescent reagents and dyes to cell-surface markers and cellular components. Since commercial hematology controls are made with fixed blood cells from different animals, these controls are not suited for assays that require the integrity of human-specific epitopes to be maintained. Special controls have been formulated that preserve epitopes on blood cells. These controls will also demonstrate the effectiveness of both the reagents (fluorescent antibodies) and fluorescent dyes as well as the auxiliary reagents (RBC sphering buffer and RBC fixative buffer) in each run. The stability of these controls and the results generated with them will be demonstrated over the time. The controls will be included in each cartridge and will be prepared with the appropriate reagents during each sample run. Expiration dates for control lots will be assigned during manufacturing, thus maintaining strict quality control.

- Liquid handling system accuracy: This will be evaluated in each run by monitoring the number of beads that appear in each field of view.

- Imaging system accuracy: This will be evaluated in each run by using fluorescent beads. The fluorescence intensity of the beads will be characterized before release.

Elements of Intended Use

In accordance with FDA guidance, in vitro diagnostic products ("***IVDs***") are those reagents, instruments, and systems intended for use in diagnosis of disease or other conditions, including a determination of the state of health, in order to cure, mitigate, treat, or prevent disease or its sequelae. Such products are intended for use in the collection, preparation, and examination of specimens taken from the human body.

The Theranos system is designed to be used in accordance with CLIA for CLIA-certified laboratory testing.

All analysis will be done in Theranos' CLIA-certified laboratory, applying Theranos' intellectual property and associated technology in the TLAS and TSPU, and helping to minimize pre-analytic processing errors and variability in order to generate results of the highest quality.

All tests are physician directed and reported back to ordering physicians directly through Theranos' CLIA-certified laboratory. The Theranos SPU is intended to be used in TPSCs.

The TSPU will be overseen by Theranos' CLIA laboratory personnel in the TPSC and Theranos' CLIA-certified laboratory. Samples will be collected in the TPSC by an appropriately certified phlebotomist or state licensed sample collection technician or nurse. The only actions for the Theranos TPSC personnel to perform once the sample has been obtained are to place the sample into the Cartridge, press the touchscreen as prompted to open the TSPU, place the Cartridge into the TSPU, and press the touch screen as prompted to close the TSPU and initiate processing. All sample preparatory steps are automated in the TSPU and overseen by the TLAS and Theranos' CLIA-certified laboratory, and all analysis is done by the TLAS in and overseen by Theranos' CLIA-certified laboratory.

The NAA and cytometry tests described in this pre-submission will be submitted as IVDs and will be performed on individuals exhibiting signs and symptoms of respiratory infections. In addition, cytometry tests will be performed for indications for which hematology evaluation are known to aid in overall health assessment and detection of a wide range of disorders, including primary disorders such as anemia, leukemia, polycythemia, thrombocytosis and thrombocytopenia and conditions that secondarily affect hematology parameters, including reaction to inflammation and infections, coagulopathies, neoplasms and exposure to toxic substances.

Elements of Intended Use for NAA:

- The Viral Versus Bacterial panel assays will identify and detect the following pathogens: Bocavirus, Coronavirus (subtypes MERS, 229E, HKU1, NL63 & OC43), Human Metapneumovirus A & B, Parainfluenza (subtype, 1, 2, 3 & 4), Respiratory Syncytial Virus (subtype A & B), Adenovirus (subtype B, C & E), Rhinovirus, Streptococcus pyogenes, Bordetella parapertussis, Bordetella Pertussis, Bordetella holmesii, Chlamydophila pneumoniae, Mycoplasma pneumoniae, Streptococcus pneumoniae and Penicilling resistant Streptococcus pneumoniae.

- The assays will be qualitative in nature.

- The assays are performed on samples collected in the form of nasopharyngeal swabs ("***NPS***"), nasopharyngeal aspirate ("***NPA***"), nasopharyngeal wash ("***NPW***"), nasal swabs ("***NS***"), throat swabs ("***TS***") or sputum processed by the TSPU in Theranos' PSCs, and analyzed by the TLAS in Theranos' CLIA-certified laboratory.

- NPS, NPA, NPW, NS, TS, or sputum samples will be mixed automatically in the TSPU. When placed into the TSPU, the sample is automatically mixed by several cycles of pipetting to ensure maximal sample release from the swab so that no manual processing of the sample is required.

- The Viral Versus Bacterial panel testing is for prescription use.

- The test will be performed on individuals suspected of suffering from respiratory tract infections.

- The results will be reported as positive, equivocal, or negative for a given Viral Versus Bacterial panel pathogen. This information can be for diagnosis of respiratory infection when used in conjunction with other clinical information. Positive results do not rule out co-infection with pathogens not tested on this panel. Negative results do not rule out respiratory infection and should not be used as the only basis for diagnosis or treatment.

Elements of Intended Use for Theranos cytometry assays:

- These assays will quantitatively measure the following measurands in whole blood:
    1. RBC count (RBC#)
    2. Hemoglobin (HGB)
    3. Mean cell volume (MCV)
    4. Red cell distribution width (RDW)
    5. Hematocrit (HCT)
    6. Platelet count (PLT#)
    7. Mean platelet volume (MPV)
    8. WBC count (WBC#)
    9. Neutrophil count (NEU#)
    10. Lymphocyte count (LYM#)
    11. Monocyte count (MONO#)
    12. Eosinophil count (EOS#)
    13. Basophil count (BASO#)
    14. Reticulocytes (% of RBC) (RETIC%)
    15. T cell count (T#)
    16. T-helper count (CD4#)

17. T-suppressor/cytotoxic count (CD8#)
18. B cell count (B#)
19. NK cell count (NK#)

In addition, the following metrics/indices will be calculated from the measurands listed above:

- Mean corpuscular hemoglobin (MCH)
- Mean corpuscular hemoglobin concentration (MCHC)
- Proportional WBC differential (Neu%, Lym%, Mono%, Eos%, Baso%)
- Proportional T, B, and NK cell subsets (T%, B%, NK%)
- Proportional T cell subset (CD4%, CD8%)

- All the above assays will be quantitative in nature.
- All the above assays are single-platform; that is, they do not require measurements from another device.
- The assays are performed on whole blood collected from fingerstick punctures or venipuncture.
- All the above assays are for prescription use, performed through Theranos' CLIA laboratory's PSC.
- The above tests will be performed on individuals for the following reasons:
    - Complete blood count with differential (tests 1 through 13):
        - As part of routine testing to assess overall health and wellness
        - To diagnose a medical condition with specific or non-specific symptoms such as fatigue, fever, bruising, bleeding etc
        - To monitor conditions such as anemia, leukemia, polycythemia vera etc where blood counts provide the physician an assessment of disease progression
        - To monitor medical treatments that affect any cell counts in blood, e.g. EPO
    - Reticulocytes (test 14): To monitor erythropoetic bone marrow activity in anemia and other hematologic conditions
    - Lymphocyte subset and T cell subset (tests 15-19):
        - For evaluating patients with immunodeficiencies, where quantitative decreases in T cells can lead to reduced functional antigen and/or mitogen responses.
        - Serial monitoring of CD4+ T-cell count in HIV-positive patients
        - Follow-up and diagnostic evaluation of primary immunodeficiencies, including severe combined immunodeficiency
        - Immune monitoring following immunosuppressive therapy for transplantation, autoimmunity, and other immunological conditions where such treatment is utilized

    - Assessment of immune reconstitution post hematopoietic cell transplantation
    - Early screening of gross quantitative anomalies in lymphocyte subsets in infection or malignancies
    - Absolute quantitation of circulating B cells for diagnosis of chronic lymphocytic leukemia patients as indicated in the 2008 IWCLL guidelines

- All results will be reported as numeric values with appropriate units and reference ranges (see appendix A). Results outside of normal value ranges may reflect a primary disorder of the cell-producing organs or an underlying disease. Results should be interpreted in conjunction with the patient's clinical picture and appropriate additional testing performed.

Description of How the Device is Planned to be Used in a Real-Life Setting

Real-Life Setting for TNAA:

These samples will be collected at a TPSC from patients with a prescription for the relevant Viral Versus Bacterial panel tests from their doctor. Samples will be collected in the TPSC by an appropriately certified phlebotomist or state licensed sample collection technician or nurse using a nasopharyngeal swab, nasopharyngeal aspirate, nasopharyngeal wash, nasal swab, throat swab and sputum, as applicable. The TSPU performs sample preparatory steps and processing, during which it will extract and purify nucleic acid from the patient sample. The purified sample is then used in the TNAA Viral Versus Bacterial panel assays. The data will be analyzed by the TLAS for pathogen detection and a report will be generated by Theranos' CLIA-certified laboratory suggesting positive or negative identification of specific pathogens. This information will be made available to the ordering doctor directly for their diagnostic decision making.

Real-Life Setting for Theranos Cytometry Assays:

These samples will be collected at a TPSC from patients with a prescription for the relevant CBC, reticulocyte, or lymphocyte subset test from their doctor. Samples will be collected in the TPSC by an appropriately certified phlebotomist or state licensed sample collection technician or nurse via venous or fingerstick methods. The TPSU performs sample preparatory steps during which it will label cells with fluorescently tagged antibodies. For the CBC, the spun whole blood sample will be imaged to determine the hematocrit in the TLAS. Hemoglobin will be detected through the spectrophotometer for the TLAS. For all cytometry assays, cells will be loaded onto the cuvette and imaged on the TSPU microscopy station. The data will be analyzed by the TLAS and a report will be generated by Theranos' CLIA-certified laboratory with cell counts, proportions, dimensions, and other clinically relevant information as described under "Elements of Intended Use" above. This information will be made available to the ordering doctor for their diagnostic decision making.

Risk Analysis

Results obtained from these tests can be used for diagnosis of respiratory infection when used in conjunction with other clinical information. Positive results do not rule out co-infection with pathogens not tested on this panel. Negative results do not rule out respiratory infection and should not be used as the only basis for diagnosis or treatment. Additional patient risk factors such as the patient age and medical history should be considered for the basis of treatment.

The following on-board controls are run with each TNAA test to reduce the changes of incorrect results:

- Sample collection and transfer control: Each patient sample will carry varying amounts of human specific nucleic acid. For each sample processed, a control human RNaseP assay is run to verify appropriate sample collection.

- Sample Prep control: A non-natural/synthetic target in the form of DNA or RNA is spiked in the sample. This will be used as an internal calibrator to QC sample prep and amplification. This also checks the general performance of the chemistry and the device.

- No Template Control (NTC): For each assay, a NTC test will be run simultaneously to QC for background signal and contamination.

The cytometry assays included in this submission are key elements of clinical investigation of many common symptoms and diseases. False results may confound diagnosis—for example a falsely high hemoglobin measurement may lead to missing diagnosis of anemia. In the case of Lymphocyte subset assays, false results may confound monitoring of therapy that the patient is undergoing.

- Theranos cytometry assays use multiple positive and negative markers to identify each relevant cell type. This prevents cells that may be positive for a single marker from being categorized with other, different, cells, because the anomalous cell does not match the entire profile of the main cell population.
- The Theranos cytometry assays have multiple in-built controls to validate the process during each run. If any of these are out of range, the sample is flagged for additional analysis.
    - Cells should generally have fluorescence intensities that fall within specific ranges. Samples with fluorescence intensities out of range will be flagged.
    - Each sample should have an identifiable pattern of cells when the scattergrams are reviewed. Samples with abnormal patterns will be flagged.
    - Cells and beads should be evenly distributed throughout the cuvette channel. Samples with uneven distribution will be flagged.

    - Samples should have a certain number of beads per field of view. Samples with more or fewer beads per field of view than expected will be flagged.
    - Samples with cell counts outside the reference range will also be flagged.
- For any flagged samples, the recorded images and data from that sample can be reviewed by personnel in Theranos' CLIA-certified laboratory. These images and data can provide more in depth information about the sample and provide clues as to why it may have been flagged. Listed below are a few potential reasons samples may be flagged:
    - Cell aggregation
    - Altered cell morphology (incomplete sphering; cell membrane blebbing, antibody capping, etc.)
    - Inappropriate cell lysis
- By having a disposable cartridge, there is no chance for carryover or contamination between samples, which reduces risk of potentially altered results.

Risk	Impact	Mitigation
Cell type misidentification from single markers	Incorrect enumeration or classification of cells; incorrect test results reported to physician	Multiple literature-validated markers and physical properties identify each cell type
Imaging or analyte-specific reagent abnormalities	Incorrect enumeration or classification of cells; incorrect test results	On-board control materials would fail the control criteria. Sample would be flagged for review and follow-up by the CLIA lab.
Liquid handling system inaccuracy	Cell counts could be incorrect; incorrect test results	Numbers of beads per field of view are enumerated and must fall within pre-determined limits. Samples with bead numbers outside these values will be flagged for review and follow-up by the CLIA lab.
Irregular sample behavior (such as cell aggregation, altered cell morphology, inappropriate cell lysis)	Incorrect cell counts or differential; incorrect test results	Image segmentation can distinguish aggregated cells as single cells. Images are available for review and clarification of results.

Carryover from one sample to the next	Incorrect values of measurands; incorrect test results	Disposable cartridges mean there is no chance for carryover.

TNAA Proposed Study Design(s), Predicate, and Analytical and Pre-Clinical Performance

1. **Method characterization:** Basic analytical performance of the method in terms of limit of detection, specificity, carry-over, interference, and inclusivity/exclusivity will be established in this phase.

2. **Determination of amplification cut-offs:** This phase will consist of a pre-clinical study, where samples will be analyzed to establish infection time cutoffs. In combination with the precision of the method itself, these data will be used to establish cut-offs for negative and positive classification. Precision of the method at points near the cut-offs will then be established.

3. **Method comparison:** The final phase of pre-clinical demonstrations will compare the Theranos method as characterized in (1) and (2) above with a reference method and diagnosis.

The study design comprises of the following:

1. Sensitivity and Limit of Detection (LOD)

2. Specificity

3. Carryover study

4. Interference substances

5. Inclusivity/Exclusivity

6. Determination of cut-off criteria

7. Reproducibility (precision)

Further details on each of the above categories:

Sensitivity and Limit of Detection (LOD)

Purpose: *The purpose of this study is to determine the limit of detection (LOD) for the Theranos TNAA assay. The LOD_{95} is the viral titer at which >95% of known positive samples test positive using the TNAA assay.*

- Make serial 10-fold dilutions of each virus from 10^6 to 10^1 organisms, or serial 10-fold dilutions ranging from 1000 $TCID_{50}$, to 10 $TCID_{50}$.

- Perform each assay 20 times for each virus and for each dilution, and record positives and negatives.

	1000 $TCID_{50}$	100 $TCID_{50}$	10 $TCID_{50}$
Bocavirus	#Pos: #Neg:	#Pos: #Neg:	#Pos: #Neg:
Coronavirus MERS	#Pos: #Neg:	#Pos: #Neg:	#Pos: #Neg:
Coronavirus 229E	#Pos: #Neg:	#Pos: #Neg:	#Pos: #Neg:
Coronavirus HKU1	#Pos: #Neg:	#Pos: #Neg:	#Pos: #Neg:
Coronavirus NL63	#Pos: #Neg:	#Pos: #Neg:	#Pos: #Neg:
Coronavirus OC43	#Pos: #Neg:	#Pos: #Neg:	#Pos: #Neg:
Human Metapneumovirus A	#Pos: #Neg:	#Pos: #Neg:	#Pos: #Neg:
Human Metapneumovirus B	#Pos: #Neg:	#Pos: #Neg:	#Pos: #Neg:
Parainfluenza 1	#Pos: #Neg:	#Pos: #Neg:	#Pos: #Neg:
Parainfluenza 2	#Pos: #Neg:	#Pos: #Neg:	#Pos: #Neg:
Parainfluenza 3	#Pos: #Neg:	#Pos: #Neg:	#Pos: #Neg:
Parainfluenza 4	#Pos: #Neg:	#Pos: #Neg:	#Pos: #Neg:
Respiratory Syncytial Virus A	#Pos: #Neg:	#Pos: #Neg:	#Pos: #Neg:
Respiratory Syncytial Virus B	#Pos: #Neg:	#Pos: #Neg:	#Pos: #Neg:

Adenovirus B	#Pos: #Neg:	#Pos: #Neg:	#Pos: #Neg:
Adenovirus C	#Pos: #Neg:	#Pos: #Neg:	#Pos: #Neg:
Adenovirus E	#Pos: #Neg:	#Pos: #Neg:	#Pos: #Neg:
Rinovirus	#Pos: #Neg:	#Pos: #Neg:	#Pos: #Neg:
Streptococcus pyogenes	#Pos: #Neg:	#Pos: #Neg:	#Pos: #Neg:
Bordetella parapertussis	#Pos: #Neg:	#Pos: #Neg:	#Pos: #Neg:
Bordetella Pertussis	#Pos: #Neg:	#Pos: #Neg:	#Pos: #Neg:
Bordetella holmesii	#Pos: #Neg:	#Pos: #Neg:	#Pos: #Neg:
Chlamydophila pneumoniae	#Pos: #Neg:	#Pos: #Neg:	#Pos: #Neg:
Mycoplasma pneumoniae	#Pos: #Neg:	#Pos: #Neg:	#Pos: #Neg:
Streptococcus pneumoniae	#Pos: #Neg:	#Pos: #Neg:	#Pos: #Neg:
Penicilling-resistant Streptococcus pneumoniae	#Pos: #Neg:	#Pos: #Neg:	#Pos: #Neg:

Acceptance Criteria:

- If 1 or fewer known positive samples scores as negative then the LOD_{95} has been confirmed for that titer.
- If >1 known positive samples scores as negative then the LOD_{95} has been rejected for that titer.

Specificity

- Test the lysates from clinical samples spiked with nucleic acid Adenovirus 4, Candida albicans, Klebsiella pneumoniae, Escherichia coli, 5ng human genomic DNA, Bordetella pertussis, A/Solomon Islands/3/2006 (H1N1), Influenza B/Russia/69, Pseudomonas aeruginosa, Staphylococcus aureus MSSA (DmecA). The nucleic acid concentration should reflect expected median viral loads in clinical specimens.

Carryover

- Set up a plate with Pathogen count as follows:

TNAA test	Bocavirus	Coronavirus MERS	Coronavirus 229E	Coronavirus HKU1
Pathogen titer	1.00E+05	1.00E+05	1.00E+05	1.00E+05
	0	0	0	0
	1.00E+05	1.00E+05	1.00E+05	1.00E+05
	0	0	0	0
	1.00E+02	1.00E+02	1.00E+02	1.00E+02
	0	0	0	0
	1.00E+02	1.00E+02	1.00E+02	1.00E+02
	0	0	0	0
	0	0	0	0
	0	0	0	0
	+ control	+control	+control	+control

TNAA test	Coronavirus NL63	Coronavirus OC43	Human Metapneumovirus A	Human Metapneumovirus B
Pathogen titer	1.00E+05	1.00E+05	1.00E+05	1.00E+05
	0	0	0	0
	1.00E+05	1.00E+05	1.00E+05	1.00E+05
	0	0	0	0
	1.00E+02	1.00E+02	1.00E+02	1.00E+02
	0	0	0	0
	1.00E+02	1.00E+02	1.00E+02	1.00E+02
	0	0	0	0
	0	0	0	0

	0	0	0	0
	+ control	+control	+control	+control

TNAA test	Parainfluenza 1	Parainfluenza 2	Parainfluenza 3	Parainfluenza 4
Pathogen titer	1.00E+05	1.00E+05	1.00E+05	1.00E+05
	0	0	0	0
	1.00E+05	1.00E+05	1.00E+05	1.00E+05
	0	0	0	0
	1.00E+02	1.00E+02	1.00E+02	1.00E+02
	0	0	0	0
	1.00E+02	1.00E+02	1.00E+02	1.00E+02
	0	0	0	0
	0	0	0	0
	0	0	0	0
	+control	+control	+control	+control

TNAA test	Respiratory Syncytial Virus A	Respiratory Syncytial Virus B	Adenovirus B	Adenovirus C
Pathogen titer	1.00E+05	1.00E+05	1.00E+05	1.00E+05
	0	0	0	0
	1.00E+05	1.00E+05	1.00E+05	1.00E+05
	0	0	0	0
	1.00E+02	1.00E+02	1.00E+02	1.00E+02
	0	0	0	0
	1.00E+02	1.00E+02	1.00E+02	1.00E+02
	0	0	0	0
	0	0	0	0
	0	0	0	0
	+control	+control	+control	+control

TNAA test	Adenovirus E	Rinovirus	Streptococcus pyogenes	Bordetella parapertussis
Pathogen titer	1.00E+05	1.00E+05	1.00E+05	1.00E+05
	0	0	0	0

	1.00E+05	1.00E+05	1.00E+05	1.00E+05
	0	0	0	0
	1.00E+02	1.00E+02	1.00E+02	1.00E+02
	0	0	0	0
	1.00E+02	1.00E+02	1.00E+02	1.00E+02
	0	0	0	0
	0	0	0	0
	0	0	0	0
	+control	+control	+control	+control

TNAA test	Bordetella Pertussis	Bordetella holmesii	Chlamydophila pneumoniae	Mycoplasma pneumoniae
Pathogen titer	1.00E+05	1.00E+05	1.00E+05	1.00E+05
	0	0	0	0
	1.00E+05	1.00E+05	1.00E+05	1.00E+05
	0	0	0	0
	1.00E+02	1.00E+02	1.00E+02	1.00E+02
	0	0	0	0
	1.00E+02	1.00E+02	1.00E+02	1.00E+02
	0	0	0	0
	0	0	0	0
	0	0	0	0
	+control	+control	+control	+control

TNAA test	Streptococcus pneumoniae	Penicilling resistant Streptococcus pneumoniae
Pathogen titer	1.00E+05	1.00E+05
	0	0
	1.00E+05	1.00E+05
	0	0
	1.00E+02	1.00E+02
	0	0
	1.00E+02	1.00E+02
	0	0

	0	0
	0	0
	+control	+control

Acceptance Criteria:

- Compare relative time to detection or relative cycle number at which signal crosses threshold (C_T) in zero wells that are adjacent to 1.00E+05 and 1.00E+02 wells versus 0 wells adjacent to other 0 wells.
- TNAA assay run in negative wells exceeds maximum number of cycles or cycle time for the assay.

Interfering Substances

Performance of the TNAA assay in the presence of interfering substances has been evaluated.

The following interfering substances have been tested:

Endogenous Substances:	**Competitive Interfering Microorganisms:**	**Exogenous Substances:**	**Laboratory Reagents:**
Human Blood (with Na Citrate)	Respiratory Syncytial Virus A	Bactroban nasal	Bleach (1%, 2%/15,% v/v)
Mucin (bovine submaxillary gland)	Human Rhinovirus	Flonase	Disinfecting wipes
Human Genomic DNA	Influenza A2009 HINI	Nasonex	Ethanol (7% v/v)
	Staphylococcus aureus	Astelin	DNAzap (I% v/v)

	Nleisseria meningitis	Anefrin Nasal Spray	RNaseOut (I% v/v)
	Corynebacterium diphtheria	NeoSynephrine	
		Vicks VapoRub cough suppressant	
		ZiCam Allergy Relief Nasal Gel	
		UTM	

Acceptance Criteria:

Mean time to detection of triplicates for positives without interfering substances shall not be different from mean time to detection of positives in the presence of interfering substances using the student's T-test.

Inclusivity/Exclusivity

The following tables summarize the inclusivity and exclusivity pathogens to be evaluated for each of the Viral Versus Bacterial panel assays.

Bocavirus		
Inclusivity	**Exclusivity**	**Cross-reactivity**
Bocavirus 1	Corona 229E	Adenovirus 4
Bocavirus 2	HMPV 27A2	Candida albicans
Bocavirus 3	Parainfluenza Virus 3	Klebsiella pneumoniae
Bocavirus 4	RSV-B	Escherichia coli
	Rhinovirus 1B (Type A)	5ng human genomic DNA

	Chlamydophila pneumoniae	Bordetella pertussis
	Haemophilus influenzae	A/California/7/2009 (H1N1 novel)
	Moraxella catarrhalis	Flu B/Mass/3/66
	Mycoplasma pneumoniae	Pseudomonas aeruginosa
		Staphylococcus aureus MSSA (DmecA)

Coronavirus MERS		
Inclusivity	**Exclusivity**	**Cross-reactivity**
Coronavirus MERS	Human Coronavirus 229E	Adenovirus 4
	Human Coronavirus OC43	*Candida albicans*
	Human Coronavirus NL63	Klebsiella pneumoniae
	Metapneumovirus 27 Type A2	*Escherichia coli*
	Human Parainfluenza Virus 3	5ng human genomic DNA
	Respiratory Syncytial Virus B	Bordetella pertussis
	Chlamydophila pneumoniae	A/Solomon Islands/3/2006 (H1N1)
	Haemophilus influenzae	B/Russia/69
	Moraxella catarrhalis	*Pseudomonas aeruginosa*
	Mycoplasma pneumoniae	*Staphylococcus aureus* MSSA (DmecA)
	Human Coronavirus HKU1 synth	

Coronavirus 229E		
Inclusivity	**Exclusivity**	**Cross-reactivity**
Human Coronavirus 229E	Human Coronavirus MERS	Adenovirus 4
	Human Coronavirus OC43	*Candida albicans*
	Human Coronavirus NL63	Klebsiella pneumoniae
	Metapneumovirus 27 Type A2	*Escherichia coli*
	Human Parainfluenza Virus 3	5ng human genomic DNA
	Respiratory Syncytial Virus B	Bordetella pertussis
	Chlamydophila pneumoniae	A/Solomon Islands/3/2006 (H1N1)
	Haemophilus influenzae	B/Russia/69
	Moraxella catarrhalis	*Pseudomonas aeruginosa*
	Mycoplasma pneumoniae	*Staphylococcus aureus* MSSA (DmecA)
	Human Coronavirus HKU1 synth	

Coronavirus HKU1		
Inclusivity	**Exclusivity**	**Cross-reactivity**
Human Coronavirus HKU1	Human Coronavirus MERS	Adenovirus 4
	Human Coronavirus OC43	*Candida albicans*
	Human Coronavirus NL63	Klebsiella pneumoniae
	Metapneumovirus 27 Type A2	*Escherichia coli*

	Human Parainfluenza Virus 3	5ng human genomic DNA
	Respiratory Syncytial Virus B	Bordetella pertussis
	Chlamydophila pneumoniae	A/Solomon Islands/3/2006 (H1N1)
	Haemophilus influenzae	B/Russia/69
	Moraxella catarrhalis	*Pseudomonas aeruginosa*
	Mycoplasma pneumoniae	*Staphylococcus aureus* MSSA (DmecA)
	Human Coronavirus229E	

Human Metapneumovirus A		
Inclusivity	**Exclusivity**	**Cross-reactivity**
Metapneumovirus 9 Type A1	Human Coronavirus	Adenovirus 4
Metapneumovirus 16 Type A1	Metapneumovirus 3 Type B1	Candida albicans
Metapneumovirus 27 Type A2	Human Parainfluenza Virus 3	Klebsiella pneumoniae
	Respiratory Syncytial Virus A	Escherichia coli
	Respiratory Syncytial Virus B	Bordetella pertussis
	Rhinovirus 1B (type A)	Influenza A/California/7/2009 (H1N1 novel)
	Chlamydophila pneumoniae	Influenza B/Russia/69
	Haemophilus influenzae	Pseudomonas aeruginosa

	Moraxella catarrhalis	Staphylococcus aureus MSSA (DmecA)
	Mycoplasma pneumoniae	Streptococcus pyogenes
	Metapneumovirus 4 Type B2	
	Metapneumovirus 5 Type B1	
	Metapneumovirus 8 Type B2	
	Metapneumovirus 18 Type B2	

Human Metapneumovirus B		
Inclusivity	**Exclusivity**	**Cross-reactivity**
Metapneumovirus 20 Type B*	Metapneumovirus 9 Type A1	Adenovirus 4
Metapneumovirus 3 Type B1	Metapneumovirus 16 Type A1	Candida albicans
Metapneumovirus 4 Type B2	Metapneumovirus 27 Type A2	Klebsiella pneumoniae
Metapneumovirus 5 Type B1	Human Coronavirus	Escherichia coli
Metapneumovirus 8 Type B2	Respiratory Syncytial Virus A	Bordetella pertussis
Metapneumovirus 18 Type B2	Respiratory Syncytial Virus B	Influenza A/California/7/2009 (H1N1 novel)
	Rhinovirus 1B (type A)	Influenza B/Russia/69
	Chlamydophila pneumoniae	Pseudomonas aeruginosa
	Haemophilus influenzae	Staphylococcus aureus MSSA (DmecA)

	Moraxella catarrhalis	Streptococcus pyogenes
	Mycoplasma pneumoniae	

Parainfluenza 1		
Inclusivity	**Exclusivity**	**Cross-reactivity**
Parainfluenza virus 1	Parainfluenza virus 2	Adenovirus 4
	Human Parainfluenza Virus 3	Candida albicans
	Parainfluenza virus 4a	Klebsiella pneumoniae
	Parainfluenza virus 4b	Escherichia coli
	Human Coronavirus	5ng human genomic DNA
	Metapneumovirus 27 Type A2	Bordetella pertussis
	RSV B	A/California/7/2009 (H1N1 novel)
	Rhinovirus 1B (type A)	Flu B/Mass/3/66
	Chlamydophila pneumoniae	Pseudomonas aeruginosa
	Haemophilus influenzae	Staphylococcus aureus MSSA (DmecA)

Parainfluenza 2		
Inclusivity	**Exclusivity**	**Cross-reactivity**
Parainfluenza virus 2	Parainfluenza virus 1	Adenovirus 4
	Human Parainfluenza Virus 3	Candida albicans

	Parainfluenza virus 4a	Klebsiella pneumoniae
	Parainfluenza virus 4b	Escherichia coli
	Human Coronavirus	5ng human genomic DNA
	Metapneumovirus 27 Type A2	Bordetella pertussis
	RSV B	A/California/7/2009 (H1N1 novel)
	Rhinovirus 1B (type A)	Flu B/Mass/3/66
	Chlamydophila pneumoniae	Pseudomonas aeruginosa
	Haemophilus influenzae	Staphylococcus aureus MSSA (DmecA)

Parainfluenza 3		
Inclusivity	**Exclusivity**	**Cross-reactivity**
Human Parainfluenza Virus 3	Parainfluenza virus 1	Adenovirus 4
	Parainfluenza virus 2	Candida albicans
	Parainfluenza virus 4a	Klebsiella pneumoniae
	Parainfluenza virus 4b	Escherichia coli
	Human Coronavirus	5ng human genomic DNA
	Metapneumovirus 27 Type A2	Bordetella pertussis
	RSV B	A/California/7/2009 (H1N1 novel)
	Rhinovirus 1B (type A)	Flu B/Mass/3/66
	Chlamydophila pneumoniae	Pseudomonas aeruginosa
	Haemophilus influenzae	Staphylococcus aureus MSSA (DmecA)

Parainfluenza 4		
Inclusivity	**Exclusivity**	**Cross-reactivity**
Parainfluenza virus 4a	Parainfluenza virus 1	Adenovirus 4
Parainfluenza virus 4b	Parainfluenza virus 2	Candida albicans
	Human Parainfluenza Virus 3	Klebsiella pneumoniae
	Human Coronavirus	Escherichia coli
	Metapneumovirus 27 Type A2	5ng human genomic DNA
	RSV B	Bordetella pertussis
	Rhinovirus 1B (type A)	A/California/7/2009 (H1N1 novel)
	Chlamydophila pneumoniae	Flu B/Mass/3/66
	Haemophilus influenzae	Pseudomonas aeruginosa
		Staphylococcus aureus MSSA (DmecA)

Respiratory Syncytial Virus A		
Inclusivity	**Exclusivity**	**Cross-reactivity**
Respiratory Syncytial Virus A A2	Human Coronavirus	Adenovirus 4
Respiratory Syncytial Virus A Long	Metapneumovirus 27 Type A2	Candida albicans
	Metapneumovirus 3 Type B1	Klebsiella pneumoniae

	Human Parainfluenza Virus 3	Escherichia coli
	Respiratory Syncytial Virus B	5ng human genomic DNA
	Rhinovirus 1B (type A)	Bordetella pertussis
	Chlamydophila pneumoniae	A/California/7/2009 (H1N1 novel)
	Haemophilus influenzae	Flu B/Mass/3/66
	Moraxella catarrhalis	Pseudomonas aeruginosa
	Mycoplasma pneumoniae	Staphylococcus aureus MSSA (DmecA)

Respiratory Syncytial Virus B		
Inclusivity	**Exclusivity**	**Cross-reactivity**
Respiratory Syncytial Virus B B/WV/14617/85	Human Coronavirus	Adenovirus 4
Human Respiratory Syncytial Virus B 9320	Metapneumovirus 27 Type A2	Candida albicans
Human Respiratory Syncytial Virus B 18537	Metapneumovirus 3 Type B1	Klebsiella pneumoniae
	Human Parainfluenza Virus 3	Escherichia coli
	Respiratory Syncytial Virus A	5ng human genomic DNA
	Rhinovirus 1B (type A)	Bordetella pertussis
	Chlamydophila pneumoniae	A/California/7/2009 (H1N1 novel)
	Haemophilus influenzae	Flu B/Mass/3/66
	Moraxella catarrhalis	Pseudomonas aeruginosa
	Mycoplasma pneumoniae	Staphylococcus aureus MSSA (DmecA)

Adenovirus B		
Inclusivity	**Exclusivity**	**Cross-reactivity**
Adeno7B	Adeno12 A	Adenovirus 4
AdenoB 3B	Adeno 1C	Candida albicans
Adeno7a B	Adeno5C	Klebsiella pneumoniae
Adeno 21B	Adeno 2C	Escherichia coli
Adeno11B	Adeno37D	5ng human genomic DNA
adeno 14B	Adeno 51D	Bordetella pertussis
Adeno35B	Adeno 4E	A/California/7/2009 (H1N1 novel)
Adeno 50B	Adeno 40F	Flu B/Mass/3/66
	Adeno 41F	Pseudomonas aeruginosa
		Staphylococcus aureus MSSA (DmecA)

Adenovirus C		
Inclusivity	**Exclusivity**	**Cross-reactivity**
Adenovirus 1 C	Adenovirus 12 A	Adenovirus 4
Adenovirus 5 C	Adenovirus 7 B	Candida albicans
Adenovirus 2 C	Adenovirus 50 B	Klebsiella pneumoniae
	Adenovirus 37 D	Escherichia coli

	Adenovirus 51 D	5ng human genomic DNA
	Adenovirus 4 E	Bordetella pertussis
	Adenovirus 40 F	A/California/7/2009 (H1N1 novel)
	Adenovirus 41 F	Flu B/Mass/3/66
		Pseudomonas aeruginosa
		Staphylococcus aureus MSSA (DmecA)

Adenovirus E		
Inclusivity	**Exclusivity**	**Cross-reactivity**
Adenovirus 4 E	Adenovirus 1 C	Adenovirus 4
	Adenovirus 5 C	Candida albicans
	Adenovirus 2 C	Klebsiella pneumoniae
	Adenovirus 12 A	Escherichia coli
	Adenovirus 7 B	5ng human genomic DNA
	Adenovirus 50 B	Bordetella pertussis
	Adenovirus 37 D	A/California/7/2009 (H1N1 novel)

	Adenovirus 51 D	Flu B/Mass/3/66
	Adenovirus 40 F	Pseudomonas aeruginosa
	Adenovirus 41 F	Staphylococcus aureus MSSA (DmecA)

Rhinovirus		
Inclusivity	**Exclusivity**	**Cross Reactivity**
Rhinovirus 1A (type A)	Coronavirus OC43	Adenovirus 4
Rhinovirus 1B (type A)	Metapneumovirus 27 Type A2	Candida albicans
Rhinovirus 2 (type A)	Respiratory Syncytial Virus B	Klebsiella pneumoniae
Rhinovirus 7 (type A)	Human Parainfluenza Virus 3	Escherichia coli
Rhinovirus 16 (type A)	Chlamydophila pneumoniae	5ng human genomic DNA
Rhinovirus 34 (type A)	Haemophilus influenzae	Bordetella pertussis
Rhinovirus 57 (type A)	Moraxella catarrhalis	A/California/7/2009 (H1N1 novel)
Rhinovirus 77 (type A)	*Mycoplasma pneumoniae*	Flu B/Mass/3/66
Rhinovirus 85 (type A)		Pseudomonas aeruginosa
Rhinovirus 1B (type A)		Staphylococcus aureus MSSA (DmecA)

Rhinovirus 14 (type B)		
Rhinovirus C		

Streptococcus pyogenes		
Inclusivity	**Exclusivity**	**Cross Reactivity**
Streptococcus pyogenes MGAS 315	Streptococcus agalactiae (B)	Adenovirus 4
Streptococcus pyogenes QC A62	Streptococcus pneumomoniae	Candida albicans
Streptococcus pyogenes NZ131	Streptococcus mutans	Klebsiella pneumoniae
Streptococcus pyogenes SF370; M1 GAS	Streptococcus salivarus	Escherichia coli
Streptococcus pyogenes CDC-SS-1095 [Cairo 2]		5ng human genomic DNA
Streptococcus pyogenes MGAS 10270		Bordetella pertussis
		A/California/7/2009 (H1N1 novel)
		Flu B/Mass/3/66
		Pseudomonas aeruginosa
		Staphylococcus aureus MSSA (DmecA)

Bordetella parapertussis		
Inclusivity	**Exclusivity**	**Cross Reactivity**
Bordetella parapertussis NCTC 5952	Bordetella bronchiseptica	Adenovirus 4
Bordetella parapertussis 12822	Bordetella pertussis	Candida albicans

Bordetella parapertussis PT28G	Bordetella Holmesii	Klebsiella pneumoniae
Bordetella parapertussis 509 and 609	Bordetella Avium	Escherichia coli
Bordetella parapertussis 508 and 344		5ng human genomic DNA
Bordetella parapertussis 517		Bordetella pertussis
		FluA/Solomon Islands/3/2006 (H1N1)
		FluB/Russia/69
		Pseudomonas aeruginosa
		Staphylococcus aureus MSSA (DmecA)
		Streptococcus pyogenes

Bordetella Pertussis		
Inclusivity	**Exclusivity**	**Cross Reactivity**
Bordetella pertussis F	Bordetella bronchiseptica	Adenovirus 4
Bordetella pertussis Tohama 1	Bordetella parapertussis	Candida albicans
Bordetella pertussis MN2531	Bordetella Holmesii	Klebsiella pneumoniae
Bordetella pertussis CNCTC Hp 12/63 [623]	Bordetella Avium	Escherichia coli
Bordetella pertussis 40103		5ng human genomic DNA
Bordetella pertussis 10-536		FluA/WS/33 (H1N1)
Bordetella pertussis 589		FluB/Hubei-Wujiagang/158/2009
Bordetella pertussis 5 [17921]		Pseudomonas aeruginosa

Bordetella pertussis PT9/28G [W28]		Staphylococcus aureus MSSA (DmecA)
Bordetella pertussis 5374 [3747]		Streptococcus pyogenes

Bordetella holmesii		
Inclusivity	**Exclusivity**	**Cross Reactivity**
Bordetella holmesii L0571	Bordetella parapertussis L0519	Adenovirus 4
Bordetella holmesii L0639	Bordetella pertussis L0522	*Candida albicans*
Bordetella holmesii L0797	Bordetella avium L0572	*Klebsiella pneumoniae*
Bordetella holmesii L0798	Bordetella bronchiseptica L0116	*Escherichia coli*
Bordetella holmesii L0788		*Bordetella pertussis*
		Influenza A/California/7/2009 (H1N1 novel)
		Influenza B/Russia/69
		Pseudomonas aeruginosa
		Staphylococcus aureus MSSA (DmecA)
		Streptococcus pyogenes
		Enterobacter cloacae
		Streptococcus agalactiae
		Streptococcus pneumoniae
		Acinetobacter baumannii
		Neisseria meningitidis

		Serratia marcescens
		Klebsiella oxytoca
		Enterobacter aerogenes

Chlamydophila pneumoniae		
Inclusivity	**Exclusivity**	**Cross Reactivity**
Chlamydophila pneumoniae AR-39	Chlamydia trachomatis BOUR	Adenovirus 4
Chlamydophila pneumoniae CM-1	Chlamydia trachomatis UW-36/Cx	Candida albicans
Chlamydophila pneumoniae CWL-029		Klebsiella pneumoniae
Chlamydophila pneumoniae TW-183		Escherichia coli
Chlamydophila pneumoniae 2023		5ng human genomic DNA
Chlamydophila pneumoniae AO3		Bordetella pertussis
Chlamydophila pneumoniae J-21		Flu A (H1N1)
		Flu B
		Pseudomonas aeruginosa
		Staphylococcus aureus
		Streptococcus pyogenes

Mycoplasma pneumoniae		
Inclusivity	**Exclusivity**	**Cross Reactivity**

Mycoplasma pneumoniae M129-B7	Mycoplasma hominis PG21	Adenovirus 4
Mycoplasma pneumoniae FH	Mycoplasma arginini G230	Candida albicans
	Mycoplasma genitalium G37	Klebsiella pneumoniae
		Escherichia coli
		5ng human genomic DNA
		Bordetella pertussis
		FluA (H1N1)
		FluB
		Pseudomonas aeruginosa
		Staphylococcus aureus
		Streptococcus pyogenes

Streptococcus pneumoniae		
Inclusivity	**Exclusivity**	**Cross Reactivity**
Streptococcus pneumoniae England 14-9 [PN93/872/B] (penicillin S)	Streptococcus pyogenes (A)	Adenovirus 4
Streptococcus pneumoniae Colombia 5-19 (penS)	Streptococcus agalactiae (B)	Candida albicans
Streptococcus pneumoniae GR890 [Greece 6B-22] (penicillin S)	Streptococcus mutans	Escherichia coli

Streptococcus pneumoniae DCC98 [Portugal 19F-21] (penicillin S)	Streptococcus salivarus	5ng human genomic DNA
Streptococcus pneumoniae S. Africa 6B-8 [50803] (penicillin S)		Bordetella pertussis
Streptococcus pneumoniae TW17 [Taiwan23F-15] (penicillin S)		FluA (H1N1)
Streptococcus pneumoniae DCC1476 [Sweden 15A-25] (penicillin S)		FluB
Streptococcus pneumoniae 178 [Poland 23F-16] (penicillin R)		Pseudomonas aeruginosa
Streptococcus pneumoniae TW31 [Taiwan19F-14] (penicillin R)		Staphylococcus aureus
Streptococcus pneumoniae Hungary 19A-6 [HUN663] (penicillin R)		Streptococcus pyogenes
Streptococcus pneumoniae 97-1177 [North Carolina 6A-23] (penicillin R)		Enterobacter cloacae
Streptococcus pneumoniae Spain 23F-1 [Sp264] (penicillin R)		Streptococcus agalactiae
		Acinetobacter baumannii
		Neisseria meningitidis
		Klebsiella pneumoniae
		Serratia marcescens
		Klebsiella oxytoca
		Enterobacter aerogenes

Penicillin sensitive Streptococcus pneumoniae		
Inclusivity	**Exclusivity**	**Cross Reactivity**
Streptococcus pneumoniae England 14-9 [PN93/872/B] (penicillin S)	Streptococcus pneumoniae 178 [Poland 23F-16] (penicillin R)	Adenovirus 4
Streptococcus pneumoniae SPN1439-106 [Colombia 5-19](penicillin S)	Streptococcus pneumoniae TW31 [Taiwan19F-14] (penicillin R)	*Candida albicans*
Streptococcus pneumoniae GR890 [Greece 6B-22] (penicillin S)	Streptococcus pneumoniae Hungary 19A-6 [HUN663] (penicillin R)	Klebsiella pneumoniae
Streptococcus pneumoniae DCC98 [Portugal 19F-21] (penicillin S)	Streptococcus pneumoniae 97-1177 [North Carolina 6A-23] (penicillin R)	*Escherichia coli*
Streptococcus pneumoniae S. Africa 6B-8 [50803] (penicillin S)	Streptococcus pneumoniae Spain 23F-1 [Sp264] (penicillin R)	5ng human genomic DNA
Streptococcus pneumoniae TW17 [Taiwan23F-15] (penicillin S)		Bordetella pertussis
Streptococcus pneumoniae DCC1476 [Sweden 15A-25] (penicillin S)		A/Solomon Islands/3/2006 (H1N1)
		B/Russia/69
		Pseudomonas aeruginosa
		Staphylococcus aureus MSSA (DmecA)
		Streptococcus pyogenes
		Enterococcus fecalis
		Streptococcus agalactiae
		Acinetobacter baumannii

		Neisseria meningitidis
		Serratia marcescens

- Mean time to detection of negatives shall be at minimum 10 minutes greater than the mean time to detection of positives.
- All 3 replicates obey the above criteria.
- Mean time to detection of negatives statistically different from mean time to positives using the students T-test.

Acceptance Criteria: Inclusivity

- For each pathogen specific assay test, a mean range of time to detection will be assessed across all inclusive strains and substrains, which shall not exceed 5 minutes.
- The %CV for time to detection of all positives and negatives shall be <15%.

Determination of Assay Cutoffs

The isothermal amplification process generates fluorescence upon incorporation of the double-stranded-DNA intercalating dye, and the relative fluorescence units (RFUs) detected once per minute over a 30 minute reaction. An amplification signal is detected if a statistical changepoint is found, with two populations of measurements, the latter of which has a mean RFU at least 1.5 times greater than the population of earlier measurements. The changepoint, the measurement that divides the samples into two populations, is used then to interpolate the time at which the amplification curve of the observed RFUs exceed the mean of the first population of measurements.

Cut-off times for making positive/equivocal/negative calls will be determined for each target empirically. A set of experiments for each target, repeated over five days, will be conducted that included 8 replicates each of three dilutions (LoD, 10X LoD, and 100X LoD), as well as 8 NTCs, using the target primers for amplification. These data will be processed using a receiver-operator character (ROC) analysis as per CLSI guidance document MM3-A2 and EP24-A2. The best threshold detection time for distinguishing positives and negatives determined using the Youden test statistic as implemented by the R package, pROC.

Reproducibility (Precision)

Precision: Mean Time to Detection (Cycles or Minutes)

- Evaluate relative time to detection (ΔT) for each pathogen at 0, 1X10^2, 1X10^4 and 1X10^6 copies or 0 $TCID_{50}$, 10 $TCID_{50}$, 100 $TCID_{50}$, 1000 $TCID_{50}$.

- ΔT is defined as the time to detection of a zero template reaction minus the time to detection of a reaction containing target nucleic acid.

- Continue over 5 days to obtain 20 data points.

- Calculate ΔT mean, SD and %CV.Δ

Acceptance criteria: ΔT CV<20%

Precision: % positive, equivocal and negative at 3 detection limits

Prepare nucleic acid from high negative (LOD/10), low-positive (LOD) and high positive (3X LOD)

- Perform the above 4 times and record positive, negative and equivocal results, as well as mean relative time to detection for each.

- Continue over 5 days to obtain 20 data points.

- Calculate ΔT mean, SD and %CV.

Acceptance criteria:

- LOD/10: ΔT CV <20%

- LOD ΔT CV <15%

- 3XLOD ΔT CV <15%

Method Comparison

Purpose: *The purpose of this method comparison is to estimate the sensitivity, specificity, PPV and NPV of the TNAA assays using Filmarray as the comparator (predicate device). Additionally CDC Real-Time RT-PCR kits for Coronavirus MERS and Streptococcus pyogeneson ABI 7500 Fast Dx Real-Time PCR instrument will be used as predicate method. Two Theranos in-house PCR tests with bi-directional sequence confirmation will be used as predicate method[1] for Streptococcus pneumoniae and Penicillin sensitive Streptococcus pneumoniae.*

[1] Where there are no cleared comparators available for TNAA tests, two Theranos in-house PCR tests with bi-directional sequence confirmation will be used. A predetermine algorithm will be

For each clinical sample, each test will be run in duplicate, except for the NTC, which will be run for each primer pair. A positive determination is made for a sample when amplified products are detected prior to the assay cutoff time in both templated replicates, if the later of the two is no more than 20% later. A run with no positive calls for any of the assays is only valid if both the RnaseP and spike-in controls are positive by these metrics.

The following will invalidate a run and could necessitate retesting:

1. Detection of product in any of the NTC wells when a positive call is made for that target

2. Negative results for any of the pathogens, along with negative results in the Rnase P and spike-in controls

3. A negative result in the spike-in control, regardless of other results (indicates lack of template, so observed products are likely non-templated)

A positive result for a pathogen along with a negative result for Rnase P is still valid, as a high level infection could overcome poor sample collection.

Run samples each day using TNAA and Filmarray RP/RT-PCR/Theranos PCR methods over 20 days using retrospective samples from demographically diverse population covering gender and age:

Target	Positive	Negative
Coronavirus 229E	50	100
Coronavirus HKU1	50	100
Coronavirus NL63	50	100
Coronavirus OC43	50	100
Human Metapneumovirus A	50	100
Human Metapneumovirus B	50	100
Parainfluenza 1	50	100
Parainfluenza 2	50	100
Parainfluenza 3	50	100

used with these two analytically validated PCR tests. The comparator assays are designed to amplify a different sequence from that amplified from the respective TNAA test. "True" positives are considered as any sample that has at least one bi-directional sequencing data meeting pre-defined quality acceptance criteria. "True" negatives were considered as any sample that tested negative by both of the comparator PCR assays. Any conflicting results will be further investigated by culture.

Parainfluenza 4	50	100
Respiratory Syncytial Virus A	50	100
Respiratory Syncytial Virus B	50	100
Adenovirus B	50	100
Adenovirus C	50	100
Adenovirus E	50	100
Rinovirus	50	100
Bordetella Pertussis	50	100
Chlamydophila pneumoniae	50	100
Mycoplasma pneumoniae	50	100
Bocavirus	50	100
Coronavirus MERS	50	100
Streptococcus pyogenes	50	100
Bordetella parapertussis	50	100
Bordetella holmesii	50	100
Streptococcus pneumoniae	50	100
Penicillin sensitive Streptococcus pneumoniae	50	100

- Carefully document positives and negatives for each pathogen on each method.
- Calculate the sensitivity, specificity, PPV and NPV of the TNAA assay and Filmarray assays.
- Calculate the percent concordance and percent discordance of TNAA versus Filmarray assay and CDC approved RT-PCR assays.

Acceptance C*riteria:* Concordance >95%, Sensitivity >90%, Specificity >95%, PPV>80%, NPV>80%.

CBC Proposed Study Design(s)

The analytical performance of the device and the assay will be evaluated in accordance with CLSI and FDA guidelines. The study is divided into two phases:

A. **Method characterization:** Basic analytical performance of the method in terms of precision, specificity, limit of detection and other metrics will be established in this phase.
B. **Method comparison:** The second phase will compare the Theranos method as characterized in (A) above with the predicate method.

A. CBC: Method characterization

- **Specimen information:** Whole blood collected by fingerstick or venipuncture may be used for Theranos cytometry assays. Such samples will typically be processed and analyzed within several hours of their collection; thus, the samples used will be fresh samples. However, sample stability is tested by maintenance of test samples for various times under typical storage conditions. During storage, the whole blood samples will be stored in vacutainers or Nanotainer tubes, as applicable, and stored at 2-8 °C with EDTA anti-coagulant.

- **Development and use of controls:**
  Three key aspects of the TPSU that will be addressed using on-board controls:
  1. Reagent activity
  2. Liquid handling system accuracy
  3. Imaging system accuracy

  To address the first point, reagent activity, stabilized materials have been developed to control for the binding of fluorescent analyte-specific reagents and dyes to cell-surface markers and cellular components. Since commercial hematology controls are made with fixed blood cells from different animals, these controls are not suited for assays that require the integrity of human-specific epitopes to be maintained. Special controls have been formulated that preserve epitopes on blood cells. These controls will also demonstrate the effectiveness of both the analyte-specific reagents (fluorescent antibodies) and fluorescent dyes as well as the auxiliary reagents (hemoglobin reagent, RBC sphering buffer, RBC fixative buffer, and RBC lysis buffer) in each run. The stability of these controls and the results generated with them will be demonstrated over the time. The controls will be included in each cartridge and will be prepared with the appropriate reagents during each sample run. Expiration dates for control lots will be assigned during manufacturing, thus maintaining strict quality control.

  The second point, liquid handling system accuracy, will be evaluated in each run by monitoring the number of beads that appear in each field of view.

The third point, imaging system accuracy, will be evaluated in each run by using fluorescent beads. The fluorescence intensity of the beads will be characterized before release.

- For a control run to be valid, each of the above values must fall within pre-defined limits.
- During the course of the study, all failed control runs will be noted and documented.

Sample Source	**Measurement**	**Purpose of Control Measurement**	**Action if control is out of range**
Stabilized WBC and RBC/PLT control materials	Analyte-specific reagent fluorescence intensity	Verify activity of fluorescent antibodies and stains	Images and data will be reviewed and interpreted by members of Theranos' CLIA-certified laboratory. Results may not be reported pending this review. Cause of failure will be investigated by the CLIA laboratory.
Stabilized WBC and RBC/PLT control materials	RBC sphering, RBC lysis	Verify activity of sphering buffer and lysis buffer	CBC results will not be reported. Cause of failure will be investigated by the CLIA laboratory.
Stabilized RBC/PLT control materials	Hemoglobin concentration	Verify activity of hemoglobin reagent	Hemoglobin result will not be reported. Cause of failure will be investigated by the CLIA laboratory.
Beads added to clinical sample when performing WBC and RBC/PLT tests	Number of beads in each field of view	Accuracy of liquid handling system	Cell counts will not be reported. Cause of failure will be investigated by the CLIA laboratory.
Beads added to clinical sample when	Fluorescence intensity of beads	Accuracy of imaging system (laser performance	Images and data will be reviewed by members of Theranos' CLIA-certified

performing WBC and RBC/PLT tests		and detection of emitted fluorescence)	laboratory. Cause of failure will be investigated by the CLIA laboratory.

- **Invalid results:**
  - Hemoglobin is the only quantitative measurement in this case which falls in the category of calibrated assays. Any HGB measurements where the absorbance value falls outside of the analytical range of the assay will be considered as invalid.
  - For the cell enumeration assays, an invalid result is apparent when the distribution of fluorescence intensities and scatter of the cells is significantly different from what is expected; for example if there are no nucleated cells that are CD45 positive, it points to an obvious fault with the CD45-binder or preparation of the sample. In this case, the result can be tagged as invalid.
  - Further, the use of beads also forces there to be roughly the same absolute number of beads in each field of view regardless of what the composition of the sample is. If this number is beyond two standard deviations of the expected mean value, it points to a fault in the preparation of the sample or the loading of the sample on the cuvette. This result will be regarded as invalid.
  - During the course of the study, all invalid measurements will be documented and investigated by the CLIA laboratory.

- **Precision:** Precision (reproducibility or repeatability) of the method will be characterized at three different concentration levels spanning the analytical range. Note that this measurement of precision refers to the quantitative precision in terms of %CV.
  - Manipulated whole blood samples with high, medium and low levels of measurands will be selected based on the medical decision limits of the individual measurand. For example, the medical decision limit for WBC count is between 0-2 x 10^9/L. The low level formulated for this purpose will be in this range. Of the other two levels, one will be in the middle of the typical reference range and the third one on the higher end of a typical reference range.
  - Three different precision studies will be performed with a totality of 304 data points:
    - Multiple runs (n=31) on the same device: between run precision
    - Multiple runs (n=31) on three different devices: between device precision
    - Multiple runs (n=12 each) on three devices for 5 different days: between day precision
  - Precision for these conditions will be reported as %CV.

- Acceptance criteria: all CVs should satisfy the following conditions. Note that in all enumeration assays, the imprecision is expected to increase as the number of cells counted decreases (following Poisson distribution). The acceptance criteria are imposed on all of the three different CVs discussed above.

Assay	*CV in mid range*	*CV for high level*	*CV at medical decision (low) level*
RBC	<=3%	<=3%	<=6%
HGB	<=3.5%	<=3.5%	<=6%
HCT	<=3%	<=3%	<=6%
PLT	<=12.5%	<=12.5%	<=15%
WBC	<=7.5%	<=7.5%	<=7.5%
MCV	<=5%	<=5%	<=5%
MPV	<=5%	<=5%	<=5%
RDW	<=5%	<=5%	<=5%

- **Analytical specificity—Cross-reactivity**: The Theranos test is designed so as to provide a clear separation of cell populations depending upon fluorescence intensity and/or scatter and/or size of the cells. Any condition where the separation of cell population becomes ambiguous is a potential effect of cross-reactivity and would be excluded during the assay development phase. The monoclonal antibodies/binders selected for the Theranos cytometry assays recognize specific antigens that are expressed by unique cell types. These markers have been certified and recognized by a panel of experts from the international flow cytometry community (Zola et al., 2007; International Council for Standardization in Haematology Expert Panel on Cytometry and International Society of Laboratory Hematology Task Force on Platelet Counting, 2001). In addition, all antibodies/binders used for these assays have been validated extensively in house to show that they reliably label cells allowing for appropriate classification of samples from hundreds of individual donors.

  If a sample contains cells that do not react with the appropriate antibodies (i.e. due to sample age or condition, or pathological conditions), cells may be incorrectly classified. These samples are flagged by the TLAS for additional analysis by the CLIA lab and collected images from the TSPU are reviewed due to irregular scattergram patterns.

- **Analytical specificity—Interference**: In cell enumeration assays, interference can be caused by many agents by one or more of the following mechanisms:
    - Cells of abnormal size:
        - Giant platelets or megakaryocytes can be often be mistaken for RBCs in traditional hematology analyzers leading to spuriously high RBC count and spuriously low PLT counts. In the Theranos system, platelets are identified by specific staining of CD41/CD61. In this case, samples with known existence of giant platelets will be used to demonstrate the accuracy of the Theranos platform. The existence of giant platelets is confirmed using blood smear in Theranos' certified CLIA laboratory.
        - Small RBCs or microcytes can be often be mistaken for PLTs in traditional hematology analyzers. In the Theranos system, RBCs are identified by specific staining of CD235a. In this case, samples with known existence of microcytes will be used to demonstrate the accuracy of the Theranos test. The existence of microcytes is confirmed using blood smear in Theranos' certified CLIA laboratory.
    - Cells that do not express normal markers used in this assay:
        - In the United States, roughly 1 in 10,000 people have neutrophils that do not express CD16. Since CD16 is used for positive identification of neutrophils, such samples will not return correct results based on CD16 expression alone. In this situation, the neutrophils move to the "eosinophil gate". Instead of returning a spuriously high eosinophil count, the correct course of action is to flag the sample. This will be demonstrated by presenting historical data.
    - Aggregated cells:
        - In lymphocytic leukemias, lymphocytes often aggregate and lead to an incorrect WBC count. In a few conditions such as elevated IgM and hepatic disorders, neutrophils have also been observed to aggregate in-vitro leading to spurious leukocytopenia. In the Theranos system, images of cells are acquired. This provides a valuable and effective way to not only confirm the existence of aggregated lymphocytes (which has clinical value in itself) but also allows the enumeration to not be affected by aggregation. This capability will be demonstrated as part of this study.
        - In rare cases (~0.1-0.2%), platelets show EDTA-dependent aggregation and form large clumps. This leads to spurious thrombocytopenia. In the Theranos system, platelet clumps are apparent as large and CD41/CD61 bright objects in cell images. The subsequent course of action is to flag the sample rather than report thrombocytopenia.
        - Platelet satellitism, satellitosis or resetting is aggregation of platelets with WBCs (especially neutrophils) in EDTA-anticoagulated samples. Again,

the Theranos platform provides images to confirm the existence of satellitism and flag the sample.

- Fragmented cells:
  - RBCs can get fragmented in patients with osmotically and mechanically fragile RBCs. Fragments of RBCs are often mistaken for platelets due to their small size. However, the specific positive identification of platelets using CD41/CD61 in the Theranos system will obviate the possibility of getting spurious thrombocytosis. This will be demonstrated in contrived as well as real samples.
  - Cytoplasmic fragments of nucleated cells can also be mistaken for platelets. Lack of interference due to such fragments will be demonstrated using contrived as well as real samples.
- Microorganisms:
  - Cases of bacteremia or fungemia, especially in immune-compromised patients can lead to spuriously high platelet counts since these organisms are the same size range as platelets. Lack of interference from such sources will be demonstrated using contrived samples.
- Presence of abnormal cell populations in peripheral blood:
  - Nucleated RBCs: Nucleated RBCs can potentially lead to spuriously high WBC counts. However, in the Theranos test, positive identification of WBC subtypes is based on marker expression, scatter and size. Based on these parameters, nucleated RBCs will not be identified as WBCs. This will be demonstrated with samples with known existence (confirmed on a blood smear) of nucleated RBCs.
  - In neoplastic leukemias, CD45 expression on lymphocytes can be reduced or increased significantly. In neoplastic diseases of mature B-cells, CD14 expression has also been reported on B-cells. These abnormalities will lead to formation of populations that may not be normally observed. Some examples of such abnormal cells will be collected and investigated as part of this study.
  - Furthermore, in hematological disorders, immature cells can also be present in significant numbers in peripheral blood, e.g. immature granulocytes, promyelocytes etc. In many cases, these cells form a separate, identifiable population such as immature granulocytes which express low levels of CD16 can be seen in the intermediate CD16 and high scatter region of a CD16-Scatter plot. In other cases, when separate populations cannot be identified, the samples may be flagged. A number of examples of such diseases will be collected and documented during the course of this study.
- Factors that interfere with the assay method:

- Lipids: Excessive lipemia can potentially affect binding of a labeled antibody or binder to cell surface markers. Samples will be contrived with different levels of lipemia and assayed to establish an acceptability limit.
- Lysis-resistant RBCs: If a patient has lysis resistant RBCs, the field of view in WBC images will be too crowded. Such abnormalities can be detected easily by virtue of the fact that images are available for post-assay review. Such samples will be flagged.

For hemoglobin, the sources of interference are as follows:

- Lipids
- Bilirubin
- Cryoglobulins
- High WBC counts
- Immunoglobulins
- Carboxyhemoglobin
- Coagulation within sample
- Sulfahemoglobin

  - For each interferent, typically expected low, medium and high levels will be used for testing. If the interferent sample cannot be contrived (e.g. giant platelets), at least 2 samples with similar conditions will be used.
  - Six samples, each with a medium-positive level of the measurands, will be spiked with interferent. Each sample will be analyzed in quadruplicate. This sample set is powered to distinguish interference of the following extents for different assays with 95% confidence:
    - HGB: 4%
    - RBC: 3%
    - WBC: 8%
    - PLT: 13%
    - %Neu, %Lym, %Mon, %Eos: <10%
    - %Baso: 25%

  - Any interferent found to have a significant effect on recovery will be investigated further over a wider range of interferent concentrations. The results will be documented and called out in the labeling.

- **Matrix comparison:**
  - Two sample matrices are proposed for use in this study: EDTA anti-coagulated whole blood from venipuncture and fingerstick samples respectively. These will each be considered as a separate sample.

- At least 40 paired venipuncture and venous samples, spanning the low to high levels of different measurands will be used for analysis.
- Measurements on these samples will be analyzed for correlation using regression—Deming or Passing-Bablok. Slope, intercept and 95% confidence intervals on these will serve as estimators of correlation and bias.
- Precision will be characterized at three levels of interest and repeatability of measurement will be compared for the 2 matrices.
- Acceptance criteria: linear regression correlation coefficient between venous and fingerstick samples should be greater than 0.9 for the following measurands: WBC#, RBC#, PLT#, HGB, MCV, NEU#, LYM#, MONO#, EOS#, NEU%, LYM%, MONO%, EOS%.

- **Linearity: (Following CLSI Guidance H26AE)** For cell enumeration assays, the variance of the measurement is governed by the Poisson distribution. As the number of cells enumerated decreases, the coefficient of variation increases, adversely affecting the statistical significance of the result. It is therefore important to establish "linearity" or correlation of the method with predicate over a wide range of measurand concentration. In the case of RBC and HGB, near-zero concentrations of the measurands are not compatible with life and do not need to be tested at these levels.
  - In the Theranos system, the WBC assay includes a rough measurement of WBC concentration in the sample which allows the system to dynamically adjust the dilution of the sample based on an approximate WBC count. Consequently, low WBC samples are diluted proportionally less to maintain close to constant concentration of WBCs in the final suspension that is processed and analyzed. The overall number of WBCs that are enumerated depends on the capacity of the cuvette channel. Therefore for very low concentrations of WBC, the CV is expected to be higher. For each assay, linearity will be demonstrated by analyzing 8-12 levels over the ranges specified below. Each level will be measured in quadruplicate.
    - WBC: 0-100 x 10^9 cells/L
    - RBC: 1- 8 x 10^{12} cells/L
    - PLT: 0 – 1000 x 10^9 cells/L
    - HGB: 5-19 g/dL
  - In addition to the cell counts, another important metric in an antibody labeling assay is the sufficiency of antibody or binder titer at high concentrations of cells. This will be demonstrated by showing that even at high cell concentrations, the intensity of fluorescent labeling of cells is sufficient for unambiguous classification of cell populations.

    This part of the study established the analytical measurement interval for the above assays.

- **Limit of Blank (LoB) (Following CLSI Guidance EP17-A2):** The Theranos system is designed such that there is no contact of the sample with any non-consumable part of the system. Each cartridge is an independent unit and provides all the necessary and sufficient vessels required for preparing the sample including imaging it. Carryover is therefore mitigated in this system. The limit of blank is therefore solely determined by spurious identification of particles/objects as cells.
    - Eight blank samples will be analyzed to get the mean value and imprecision around the expected limit of blank. This is the limit of blank without any carryover.
    - Eight high and 8 blank samples will be analyzed alternately to obtain the same value with the possibility of carryover.

- **Limit of detection (LoD): (Following CLSI Guidance EP17-A2**) The limit of detection (LoD) is defined as the measurand quantity value, obtained by a given measurement procedure, for which the probability of falsely claiming the absence of a measurand in a material is β, given a probability α of falsely claiming its presence. For the purpose of this validation program, α and β will be selected to be 0.05. Guidelines from Section 5.3.3 will be used for designing the experiments and analyzing data. Briefly, three low level samples will contrived from fresh whole blood at concentrations ranges spanning the desired LoD. These samples will be analyzed in quadruplicate along with blanks. From these data the LoD will be computed:

  J = number of low level samples run = 3 (consider only the lowest here)

  L = number of results from these J samples = 12 (quadruplicate)

  SD_L = standard deviation of all the L low level samples

$$\text{LoD} = \text{LoB} + 1.645 \times SD_L \times \sqrt{1 + \frac{1}{\text{number of low level samples}}}$$

Limit of Quantification (LoQ): (Following CLSI Guidance EP17-A2): The limit of quantification (LoQ) is defined as the lowest amount of measurand in a material that can be quantitatively determined with stated accuracy under stated experimental conditions. Accuracy goals therefore need to be stated *a priori*. Then trial value of LoQ is picked based on the desired value to be claimed. Based on the analytical measuring interval established above, an appropriate value will be selected as the target LoQ.

Accuracy goal will be stated using the Westgard model:

$$\text{Accuracy goal} = \text{LoQ} \pm 2 \times SD_L$$

where, TE = the total error, bias is calculated as difference of measured value from the reference value of the sample at LoQ and SD is the standard deviation of measured values. The total error goals for this assay will be defined as follows:

- WBC: 15%
- PLT: 30%
- HGB: 10%
- RBC: 10%

- **Reagent and sample stability studies:** Reagent stability will be demonstrated over a period that is desired to be claimed as the life of a cartridge—e.g. at least 3 months and life of the sample e.g. 48 hours.
    - Sample stability:
        - Whole blood samples from 6 donors will be used for the temporal precision study. Samples will be aliquotted into 24 single-use amounts immediately after collection so as to prevent repeated thermal cycles during the course of the study.
        - Samples will be stored at 2-8 deg C.
        - Samples will be tested on at least 6 different time points over the duration of the study. For example: 0, 6, 12, 24, 36, 48 hours. Samples will be tested in quadruplicate at each time point.
        - This study is powered to distinguish a change in the mean value of the measurand by the following extents at 95% confidence level by student's t-test:
            - HGB: 4%
            - RBC: 3%
            - WBC: 8%
            - PLT: 13%
            - %Neu, %Lym, %Mon, %Eos: <10%
            - %Baso: 25%
        - Further, this study is powered to distinguish a change in the CV of the measurement between 2 different time points by the following extents at 95% confidence interval using ANOVA:
            - HGB: 2.6%
            - RBC: 2.5%
            - WBC: 6%
            - PLT: 10%
            - %Neu, %Lym, %Mon, %Eos: <10%
            - %Baso: 20%

        - Any significant effects measured in this study will be used to determine cut-offs for sample stability.
    - Reagent stability:
        - For assessment of reagent stability, a batch of 24 x 8 (192) cartridges will be prepared using single batch reagents, using processes established in manufacturing.
        - Cartridges will be stored individually at 2-8 deg C
        - Twenty-four cartridges will be used to test fresh venous whole blood samples for which a CLIA-certified value of each measurand is known. Tests will be performed at 8 time points such as 0, 15, 30, 60, 90, 120, 150, 180 days.
        - The study is powered the same as the sample stability study detailed above.

Reticulocyte assay: Method Characterization

- **Specimen information:** Whole blood collected by fingerstick or venipuncture may be used for Theranos cytometry assays. Such samples will typically be processed and analyzed within several hours of their collection; thus, the samples used will be fresh samples. However, sample stability is tested by maintenance of test samples for various times under typical storage conditions. During transport, the whole blood samples will be stored in vacutainers or Nanotainers, as applicable, and stored at 2-8 °C with EDTA anti-coagulant.

- **Development and use of controls:**
  Three key aspects of the TPSU that will be addressed using on-board controls:
    - Reagent activity
    - Liquid handling system accuracy
    - Imaging system accuracy

  To address the first point, reagent activity, stabilized materials have been developed to control for the binding of fluorescent analyte-specific reagents and dyes to cell-surface markers and cellular components. Since commercial hematology controls are made with fixed blood cells from different animals, these controls are not suited for assays that require the integrity of human-specific epitopes to be maintained. Special controls have been formulated that preserve epitopes on blood cells. These controls will also demonstrate the effectiveness of both the analyte-specific reagents (fluorescent antibodies) and fluorescent dyes as well as the auxiliary reagents (RBC sphering buffer and RBC fixative buffer) in each run. The stability of these controls and the results generated with them will be demonstrated over the time. The controls will be included in each cartridge and will be prepared with the appropriate reagents during each sample run. Expiration dates for control lots will be assigned during manufacturing, thus maintaining strict quality control.

The second point, liquid handling system accuracy, will be evaluated in each run by monitoring the number of beads that appear in each field of view.

The third point, imaging system accuracy, will be evaluated in each run by using fluorescent beads. The fluorescence intensity of the beads will be characterized before release.

- For a control run to be valid, each of the above values must fall within pre-defined limits.
- During the course of the study, all failed control runs will be noted and documented.

Sample Source	Measurement	Purpose of Control Measurement	Action if control is out of range
Stabilized RBC and Reticulocyte control materials	Analyte-specific reagent fluorescence intensity	Verify activity of fluorescent antibodies and stains	Images and data will be reviewed and interpreted by members of Theranos' CLIA-certified laboratory. Results may not be reported pending this review. Cause of failure will be investigated by the CLIA lab.
Stabilized RBC and Reticulocyte control materials	RBC sphering	Verify activity of sphering buffer	Images and data will be reviewed and interpreted by members of Theranos' CLIA-certified laboratory. Results may not be reported pending this review. Cause of failure will be investigated by the CLIA lab.
Beads added to patient sample when preforming RBC and Reticulocyte tests	Number of beads in each field of view	Accuracy of liquid handling system	Images and data will be reviewed and interpreted by members of Theranos' CLIA-certified laboratory. Results may not be reported pending this review. Cause of failure will be investigated by the CLIA lab.
Beads added to patient sample when	Fluorescence intensity of beads	Accuracy of imaging system (laser performance and detection of	Images and data will be reviewed and interpreted by members of Theranos' CLIA-certified laboratory.

preforming RBC and Reticulocyte tests		emitted fluorescence)	Results may not be reported pending this review. Cause of failure will be investigated by the CLIA lab.

- **Invalid results:**
  - For the cell enumeration assays, an invalid result is apparent when the distribution of fluorescence intensities and scatter of the cells is significantly different from what is expected; for example if there are no cells that are CD235 positive, it points to an obvious fault with the CD235-binder or preparation of the sample. In this case, the result will be tagged as invalid.
  - Further, the use of beads also ensures roughly the same absolute number of beads in each field of view regardless of what the composition of the sample is. If this number is beyond two standard deviations of the expected mean value, it points to a fault in the preparation of the sample or the loading of the sample on the cuvette. This result will be regarded as invalid.
  - During the course of the study, all invalid measurements will be documented and investigated by the CLIA lab.

- **Precision:** Precision (reproducibility or repeatability) of the method will be characterized at three different concentration levels spanning the analytical range. High, medium and low levels will be selected based on the medical decision limits of the individual measurand. For the three levels of reticulocytes, low ($<10 \times 10^9$ / L), normal ($\sim 60 \times 10^9$ / L), and high ($>200 \times 10^9$ / L) concentrations of reticulocyte will be tested.
  - Three different precision studies will be performed with a totality of 330 data points:
    - Precision will be tested as recommended by NCCLS Guideline H44-A2 by determining the absolute differences between duplicate samples in each range on the same device. A minimum of 10 donor samples in each range will be run on the same device in duplicate to calculate between run precision.
    - Multiple runs (n=10 unique donors in each range for venipuncture samples) on three different devices: between device precision.
    - Multiple runs (n=12 each) on three devices for 5 different days: between day precision.
  - Precision for these conditions will be reported as %CV.
  - Acceptance criteria: all CVs should satisfy the following conditions. Note that in all enumeration assays, the imprecision is expected to increase as the number of

cells counted decreases (following Poisson distribution). The acceptance criteria are imposed on all of the three different CVs discussed above.

Assay	*CV in mid range*	*CV for high level*	*CV at medical decision (low) level*
Reticulocytes	<=10%	<=10%	<=15%

- **Analytical specificity—Cross-reactivity**: The Theranos test is designed to provide a clear separation of cell populations depending upon fluorescence intensity and/or scatter and/or size of the cells. Any condition where the separation of a cell population becomes ambiguous is a potential effect of cross-reactivity and would be excluded during the assay development phase. The monoclonal antibodies/binders selected for the Theranos cytometry assays recognize specific antigens that are expressed by unique cell types. These markers have been certified and recognized by a panel of experts from the international flow cytometry community (NCCLS document H44-A2 and Linda G. Lee, C.-H. C. a. L. A. C., 1986. Thiazole Orange: A New Dye for Reticulocyte Analysis. Cytometry, Volume 7, p. 518). In addition, all antibodies/binders used for these assays have been validated extensively in house to show that they reliably label cells to allow for appropriate classification of samples from hundreds of individual donors. If a sample contains cells that do not react with the appropriate antibodies (i.e. due to sample age or condition, or pathological conditions), cells may be incorrectly classified. These samples would get flagged by the CLIA lab for additional analysis and image review due to irregular scattergram patterns.

- **Analytical specificity—Interference**: In cell enumeration assays, interference can be caused by many agents by one or more of the following mechanisms:
    - Cellular Elements:
        - Giant platelets, platelet clumps, or megakaryocytes can be often be mistaken for RBCs in traditional hematology analyzers leading to spuriously high RBC count and spuriously low PLT counts. In the Theranos system, platelets are identified by specific staining of CD41/CD61. In this case, samples with known existence of giant platelets will be used to demonstrate the accuracy of the Theranos platform. The existence of giant platelets will be confirmed using blood smear in Theranos' certified CLIA laboratory.

        - Abnormal, elevated, or fragmented WBCs may interfere with RBC in certain automated reticulocyte counts. In the Theranos system, RBC's are positively identified with CD235a marker and will not be confused with WBCs.
        - Nucleated RBCs have been reported to disturb reticulocyte count, but are generally not counted as reticulocytes in hematology analyzers. Scatter of the RBCs will be used to identify nucleated RBCs and flag the sample for manual review of the images that are collected. This will be demonstrated with samples with known existence (confirmed on a blood smear) of nucleated RBCs.
    - Cellular Inclusions:
        - Cytoplasmic particles that can also be stained with supravital dyes, including Howell-Jolly bodies, Pappenheimer bodies, and Heinz Bodies. Intraerythrocytic parasites may also interfere with the value for reticulocyte count. Lack of interference from such sources will be demonstrated using contrived as well as real samples.
    - Miscellaneous
        - Platelet and RBC coincidence events may interfere with the reticulocyte assay. Lack of interference from such sources will be demonstrated using contrived as well as real samples.
        - Additional studies have seen spurious reticulocyte counts due to agglutinated cells in the presence of cold agglutinins, autofluorescence of RBC due to cause such as porphyria and rugs, diagnostic intravenous fluorescent dyes, high amounts of paraproteins, and hemolysis. Lack of interference from such sources will be demonstrated using contrived as well as real samples.
    - Factors that interfere with the assay method:
        - Lipids: Excessive lipemia can potentially affect binding of a labeled antibody or binder to cell surface markers. Samples will be contrived with different levels of lipemia and assayed to establish an acceptability limit.
    - For each interferent, typically expected low, medium and high levels will be used for testing. If the interferent sample cannot be contrived (e.g. giant platelets), at least 2 samples with similar conditions will be used.
    - Six samples, each with a medium-positive level of the measurands, will be spiked with interferent. Each sample will be analyzed in quadruplicate. This sample set is powered to distinguish interference of the following extents for different assays with 95% confidence:
        - RETIC% 11%
    - Any interferent found to have a significant effect on recovery will be investigated further over a wider range of interferent concentrations. The results will be documented and called out in the labeling.

- **Matrix comparison:**
    - Two sample matrices are proposed for use in this study: EDTA anti-coagulated whole blood from venipuncture and fingerstick samples respectively. These will each be considered as a separate sample.
    - At least 40 paired venipuncture and venous samples, spanning the low to high levels of different measurands will be used for analysis.
    - Measurements on these samples will be analyzed for correlation using regression—Deming or Passing-Bablok. Slope, intercept and 95% confidence intervals on these will serve as estimators of correlation and bias.
    - Precision will be characterized at three levels of interest and repeatability of measurement will be compared for the 2 matrices.
    - Acceptance criteria: linear regression correlation coefficient between venous and fingerstick samples should be greater than 0.9.

- **Linearity: (Following CLSI Guidance H44-A2)** For cell enumeration assays, the variance of the measurement is governed by the Poisson distribution. As the number of cells enumerated decreases, the coefficient of variation increases, adversely affecting the statistical significance of the result. It is therefore important to establish "linearity" or correlation of the method with predicate over a wide range of measurand concentration.
    - Linearity will be demonstrated by analyzing 8-12 levels over the ranges specified below. Each level will be measured in quadruplicate.
        - Reticulocytes 1000 x 10^9 / L
    - In addition to the cell counts, another important metric in an antibody labeling assay is the sufficiency of antibody or binder titer at high concentrations of cells. This will be demonstrated by showing that even at high cell concentrations, the intensity of fluorescent labeling of cells is sufficient for unambiguous classification of cell populations.

      This part of the study established the analytical measurement interval for the above assays.

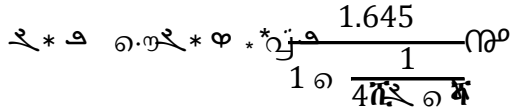
- **Limit of Blank (LoB) (Following CLSI Guidance EP17-A2):** The Theranos system is designed such that there is no contact of the sample with any non-consumable part of the system. Each cartridge is an independent unit and provides all the necessary and sufficient vessels required for preparing the sample including imaging it. Carryover is therefore highly mitigated. The limit of blank is therefore solely determined by spurious identification of particles/objects as cells.
    - Eight blank samples will be analyzed to get the mean value and imprecision around the expected limit of blank. This is the limit of blank without any carryover.
    - Eight high and 8 blank samples will be analyzed alternately to obtain the same value with the possibility of carryover.

- **Limit of detection (LoD): (Following CLSI Guidance EP17-A2**) The limit of detection (LoD) is defined as the measurand quantity value, obtained by a given measurement procedure, for which the probability of falsely claiming the absence of a measurand in a material is β, given a probability α of falsely claiming its presence. For the purpose of this validation program, α and β will be selected to be 0.05. Guidelines from section 5.3.3 will be used for designing the experiments and analyzing data. Briefly, three low level samples will contrived from fresh whole blood at concentrations ranges spanning the desired LoD. These samples will be analyzed in quadruplicate along with blanks. From these data the LoD will be computed:

  J = number of low level samples run = 3 (consider only the lowest here)

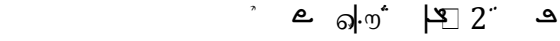
  L = number of results from these J samples = 12 (quadruplicate)

  SD_L = standard deviation of all the L low level samples



Limit of Quantification (LoQ): (Following CLSI Guidance EP17-A2): The limit of quantification (LoQ) is defined as the lowest amount of measurand in a material that can be quantitatively determined with stated accuracy under stated experimental conditions. Accuracy goals therefore need to be stated *a priori*. Then trial value of LoQ is picked based on the desired value to be claimed. Based on the analytical measuring interval established above, an appropriate value will be selected as the target LoQ.

Accuracy goal will be stated using the Westgard model:



where, TE = the total error, bias is calculated as difference of measured value from the reference value of the sample at LoQ and SD is the standard deviation of measured values. The total error goals for this assay will be defined as follows:

- Reticulocyte: 30%

- **Reagent and sample stability studies:** Reagent stability will be demonstrated over a period that is desired to be claimed as the life of a cartridge—e.g. at least 3 months and life of the sample e.g. 48 hours.
  - Sample stability:

        - Fingerstick and venous whole blood samples from 6 donors will be used for the temporal precision study. Samples will be aliquoted into 24 single use amounts immediately after collection so as to prevent repeated thermal cycles during the course of the study.
        - Samples will be stored at 2-8 deg C.
        - Samples will be tested on at least 6 different time points over the duration of the study. For example: 0, 6, 12, 24, 36, 48 hours. Samples will be tested in quadruplicate at each time point.
        - This study is powered to distinguish a change in the mean value of the measurand by the following extents at 95% confidence level by student's t-test:
            - RETIC%: 11%
        - Further, this study is powered to distinguish a change in the CV of the measurement between 2 different time points by the following extents at 95% confidence interval using ANOVA:
            - RETIC%: 11%
        - Any significant effects measured in this study will be used to determine cut-offs for sample stability.
    - Reagent stability:
        - For assessment of reagent stability, a batch of 24 x 8 (192) cartridges will be prepared using single batch reagents, using processes established in manufacturing.
        - Cartridges will be stored individually at 2-8 deg C
        - Twenty-four cartridges will be used to test fresh venous whole blood samples for which a CLIA-certified value of each measurand is known. Tests will be performed at 8 time points such as 0, 15, 30, 60, 90, 120, 150, 180 days.
        - The study is powered the same as the sample stability study detailed above.

Lymphocyte subset: Method Characterization

- **Specimen information:** Whole blood collected by fingerstick or venipuncture may be used for Theranos cytometry assays. Such samples will typically be processed and analyzed within several hours of their collection; thus, the samples used will be fresh samples. However, sample stability is tested by maintenance of test samples for various times under typical storage conditions. During transport, the whole blood samples will be stored in vacutainers or Nanotainer tubes, as applicable, and stored at 4 °C with EDTA anti-coagulant.

- **Development and use of controls:**

Three key aspects of the TPSU that will be addressed using on-board controls:

- Reagent activity
- Liquid handling system accuracy
- Imaging system accuracy

To address the first point, reagent activity, stabilized materials have been developed to control for the binding of fluorescent analyte-specific reagents and dyes to cell-surface markers and cellular components. Since commercial hematology controls are made with fixed blood cells from different animals, these controls are not suited for assays that require the integrity of human-specific epitopes to be maintained. Special controls have been formulated that preserve epitopes on blood cells. These controls will also demonstrate the effectiveness of both the analyte-specific reagents (fluorescent antibodies) and fluorescent dyes as well as the auxiliary reagents (RBC lysis buffer and WBC fixative) in each run. The stability of these controls and the results generated with them will be demonstrated over the time. The controls will be included in each cartridge and will be prepared with the appropriate reagents during each sample run. Expiration dates for control lots will be assigned during manufacturing, thus maintaining strict quality control.

The second point, liquid handling system accuracy, will be evaluated in each run by monitoring the number of beads that appear in each field of view.

The third point, imaging system accuracy, will be evaluated in each run by using fluorescent beads. The fluorescence intensity of the beads will be characterized before release.

- For a control run to be valid, each of the above values must fall within pre-defined limits.
- During the course of the study, all failed control runs will be noted and documented.

Sample Source	Measurement	Purpose of Control Measurement	Action if control is out of range
Stabilized lymphocyte subset control materials	Analyte-specific reagent fluorescence intensity	Verify activity of fluorescent antibodies and stains	Images and data will be reviewed and interpreted by members of Theranos' CLIA-certified laboratory. Results may not be reported pending this review. Cause of failure will be investigated by the CLIA lab.
Stabilized lymphocyte	RBC lysis	Verify activity of lysis buffer	Results will not be reported. Cause of failure

subset control materials			will be investigated by the CLIA lab.
Beads added to patient sample when performing lymphocyte subset tests	Number of beads in each field of view	Accuracy of liquid handling system	Cell counts will not be reported. Cause of failure will be investigated by the CLIA lab.
Beads added to patient sample when performing lymphocyte subset tests	Fluorescence intensity of beads	Accuracy of imaging system (laser performance and detection of emitted fluorescence)	Images and data will be reviewed and interpreted by members of Theranos' CLIA-certified laboratory. Results may not be reported pending this review. Cause of failure will be investigated by the CLIA lab.

- **Invalid results:**
  - An invalid result is apparent when the distribution of fluorescence intensities and scatter of the cells is significantly different from what is expected; for example if there are no nucleated cells that are CD45 positive, it points to an obvious fault with the CD45-binder or preparation of the sample. In this case, the result will be tagged as invalid.
  - Further, the use of beads also forces there to be roughly the same absolute number of beads in each field of view regardless of what the composition of the sample is. If this number is beyond two standard deviations of the expected mean value, it points to a fault in the preparation of the sample or the loading of the sample on the cuvette. This result will be regarded as invalid.
  - During the course of the study, all invalid measurements will be documented and investigated by the CLIA lab.

- **Precision:** Precision (reproducibility or repeatability) of the method will be characterized at two different concentration levels (low CD4+ T cells and normal CD4+ T cells). The low level of CD4+ T cells will be at the medical decision limit (200 CD4+ T cells/uL of whole blood). The two levels of stabilized control will be run twice daily on each of three devices for 20 operating days. Note that this measurement of precision refers to the quantitative precision in terms of %CV.
  - Precision for these conditions will be reported as %CV.
  - Acceptance criteria: all CVs should satisfy the following conditions. Note that in all enumeration assays, the imprecision is expected to increase as the number of

cells counted decreases (following Poisson distribution). The acceptance criteria are imposed on all of the three different CVs discussed above.

Assay	*CV in mid range for all measurands*	*CV at CD4+ T cells medical decision (low) level*
T#	<=7.5%	<=7.5%
B#	<=10%	<=10%
NK#	<=10%	<=10%
CD4#	<=7.5%	<=10%
CD8#	<=7.5%	<=7.5%

- **Analytical specificity—Cross-reactivity**: Not applicable.

- **Matrix comparison:**
  - Two sample matrices are proposed for use in this study: EDTA anti-coagulated whole blood from venipuncture and fingerstick samples respectively. These will each be considered as a separate sample.
  - At least 40 paired venipuncture and venous samples, spanning the low to high levels of different measurands will be used for analysis.
  - Measurements on these samples will be analyzed for correlation using regression – Deming or Passing-Bablok. Slope, intercept and 95% confidence intervals on these will serve as estimators of correlation and bias.
  - Precision will be characterized at three levels of interest and repeatability of measurement will be compared for the 2 matrices.
  - Acceptance criteria: linear regression correlation coefficient between venous and fingerstick samples should be greater than 0.9 for each measurand (T#, B#, NK#, CD4#, CD8#).

- **Linearity: (Following CLSI Guidance H26AE)** For cell enumeration assays, the variance of the measurement is governed by the Poisson distribution. As the number of cells enumerated decreases, the coefficient of variation increases, adversely affecting the statistical significance of the result. It is therefore important to establish "linearity" or correlation of the method with predicate over a wide range of measurand concentration.
  - In the Theranos system, the lymphocyte subset assay includes a rough measurement of total WBC concentration in the sample which allows the system to dynamically adjust the dilution of the sample based on an approximate WBC count. Consequently, low WBC samples are diluted proportionally less to maintain close to constant concentration of WBCs in the final suspension that is analyzed by the TLAS. The overall number of WBCs that are enumerated depend on the capacity of the cuvette channel. Therefore for very low concentrations of WBC, the CV is expected to be higher. For each assay, linearity will be demonstrated by analyzing

8-12 levels over the ranges specified below. Each level will be measured in quadruplicate.

- Lymphocytes: 0-50 x 10^9 cells/L

- In addition to the cell counts, another important metric in an antibody labeling assay is the sufficiency of antibody or binder titer at high concentrations of cells. This will be demonstrated by showing that even at high cell concentrations, the intensity of fluorescent labeling of cells is sufficient for unambiguous classification of cell populations.

  This part of the study established the analytical measurement interval for the above assays.

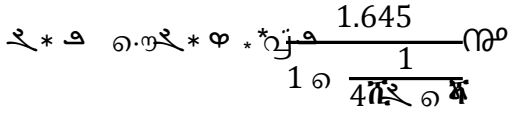
- **Limit of Blank (LoB) (Following CLSI Guidance EP17-A2):** The Theranos system is designed such that there is no contact of the sample with any non-consumable part of the system. Each cartridge is an independent unit and provides all the necessary and sufficient vessels required for preparing the sample including imaging it. Carryover is therefore not a relevant metric for this system. The limit of blank is therefore solely determined by spurious identification of particles/objects as cells.
  - Eight blank samples will be analyzed to get the mean value and imprecision around the expected limit of blank. This is the limit of blank without any carryover.
  - Eight high and 8 blank samples will be analyzed alternately to obtain the same value with the possibility of carryover.

- **Limit of detection (LoD): (Following CLSI Guidance EP17-A2**) The limit of detection (LoD) is defined as the measurand quantity value, obtained by a given measurement procedure, for which the probability of falsely claiming the absence of a measurand in a material is β, given a probability α of falsely claiming its presence. For the purpose of this validation program, α and β will be selected to be 0.05. Guidelines from section 5.3.3 will be used for designing the experiments and analyzing data. Briefly, three low level samples will contrived from fresh whole blood at concentrations ranges spanning the desired LoD. These samples will be analyzed in quadruplicate along with blanks. From these data the LoD will be computed:

  J = number of low level samples run = 3 (consider only the lowest here)

  L = number of results from these J samples = 12 (quadruplicate)

  SD_L = standard deviation of all the L low level samples



Limit of Quantification (LoQ): (Following CLSI Guidance EP17-A2): The limit of quantification (LoQ) is defined as the lowest amount of measurand in a material that can be quantitatively determined with stated accuracy under stated experimental conditions. Accuracy goals therefore need to be stated *a priori*. Then trial value of LoQ is picked based on the desired value to be claimed. Based on the analytical measuring interval established above, an appropriate value will be selected as the target LoQ.

Accuracy goal will be stated using the Westgard model:

where, TE = the total error, bias is calculated as difference of measured value from the reference value of the sample at LoQ and SD is the standard deviation of measured values. The total error goals for this assay will be defined as follows:

- T#: 15%
- CD4#: 20%
- CD8#: 15%
- B#: 20%
- NK#: 20%

- **Reagent and sample stability studies:** Reagent stability will be demonstrated over a period that is desired to be claimed as the life of a cartridge—e.g. at least 3 months and life of the sample e.g. 48 hours.
    - Sample stability:
        - Fingerstick and venous whole blood samples from 6 donors will be used for the temporal precision study. Venous samples will be aliquoted into 24 single-use amounts immediately after collection so as to prevent repeated thermal cycles during the course of the study. Samples will be stored at 2-8 deg C.
        - Venous samples will be tested on at least 6 different time points over the duration of the study. For example: 0, 6, 12, 24, 36, 48 hours. Samples will be tested in quadruplicate at each time point.
        - This study is powered to distinguish a change in the mean value of the measurand by the following extents at 95% confidence level by student's t-test:
            - T#, CD8#: 8%
            - CD4#, NK#, B#: 9%

        - Further, this study is powered to distinguish a change in the CV of the measurement between 2 different time points by the following extents at 95% confidence interval using ANOVA:
            - Absolute lymphocyte subset counts: %
            - T%, CD8%: <10%
            - CD4%, NK%, B%: <15%
        - Any significant effects measured in this study will be used to determine cut-offs for sample stability.
    - Reagent stability:
        - For assessment of reagent stability, a batch of 24 x 8 (192) cartridges will be prepared using single batch reagents, using processes established in manufacturing.
        - Cartridges will be stored individually at 2-8 deg C
        - Twenty-four cartridges will be used to test fresh venous whole blood samples for which a CLIA-certified value of each measurand is known. Tests will be performed at 8 time points such as 0, 15, 30, 60, 90, 120, 150, 180 days.
        - The study is powered the same as the sample stability study detailed above.

B. Method comparison:

Theranos' system is a versatile platform designed for routine blood counts, leukocyte differential as well more specialized assays such as reticulocyte counts, T-cell subsets and lymphocyte subset. Method comparison for CBC will be completed as part of the Clinical Study, outlined in the next section. Therefore, multiple predicates will be selected to cover this wide range of assays. Provisionally, the following predicates have been selected:

Assay/Reportable	**Predicate/Reference**	**Rationale for selection**
RBC count	Abbott Cell Dyn Ruby	This is a 510(k) approved automated hematology analyzer (510(k) number K061667)
HGB		
MCV		
MCH		
MCHC		

PLT		
MPV		
WBC count		
Reticulocytes		
WBC Differential	Manual blood smear 400 cell differential	This is the "gold standard" widely used in clinical laboratories for investigation and confirmation of automated differentials. Theranos technology is based on identification of WBC subtypes by biochemical interrogation as opposed to optical interrogation as used by the Abbott Ruby device. Reference Leukocyte (WBC) Differential Count (Proportional) and Evaluation of Instrument Methods. Approved Standard – Second Edition. CLSI document H20-A2.
Lymphocyte subset (T, B, NK, T cell subset)	BD FACS Canto or BD FACS Canto II with BD Multitest 6-color TBNK assay kit	This is a 510(k) approved device and accompanying kit specifically for the assays mentioned here. (BD Multitest 6-color TBNK kit 510(k) number K060375; BD FACS Canto 510(k) number K041074; BD FACS Canto II 510(k) number K062087)

Similarities between TSPU and Predicate method for CBC		
Element	*TSPU*	*Abbott Cell-Dyn Ruby*
Measurands quantified	HCT, HGB, RBC#, MCV, MPV, RDW, MCH, MCHC, WBC#, NEU#, LYM#, MONO#, EOS#, BASO#, NEU%, LYM%, MONO%, EOS%, BASO%	Same

Matrix	EDTA-anticoagulated whole blood	Same
Reagent Storage	Reagents stored at 2-8°C	Same
Sample preparation	Sample processing is automated in the TSPU	Sample preparation (i.e., mixing) required before sample introduction
Platforms	Single platform	Instrument and Console and separate systems for aliquoting sample where required
User Complexity	Low	High
Differences between TSPU and Predicate method		
Element	*TSPU*	*Abbott Cell-Dyn Ruby*
Technological Principles	Detection of cell type-specific epitopes with fluorescent binders and automated fluorescence microscopy	Enumeration and classification of cells into different cell types based on their size and optical scatter properties: Size (0°), complexity (10°), lobularity (90° polarized) and granulatiry (90° depolarized)
Specimen Type	Venous or fingerstick (capillary) blood	Venous blood
Matrix	EDTA-anticoagulated plasma-replaced blood	EDTA-anticoagulated whole blood
Instrumentation	TSPU and TLAS	Abbott Cell-Dyn Ruby
Test Interpretation	Automated test interpretation and report generation. TSPU operator cannot access raw data.	Automatic data analysis and report generation.

Reticulocyte Assay Proposed Method Comparison

Per CLSI guideline EP09A2IR, a study of at least 100 patient samples spanning the expected range of values (50% in normal range, 25% below, 25% above) will be conducted. Samples will be collected at two clinical evaluation sites and analyzed on the predicate method (Abbott Cell-Dyn Ruby) and the TPSU. The reticulocyte percent (RETIC%) will be evaluated on both platforms. Concordance (Pearson's r) will be calculated including 95% confidence intervals. Further regression fits using ordinary linear regression and Deming regression will be calculated. Slope and intercept of these fits will be presented with their respective 95% confidence intervals. For the study to pass, the correlation coefficients for all reportables shall be greater than 0.9.

Similarities between TSPU and Predicate method for Reticulocytes		
Element	*TSPU*	*Abbott Cell-Dyn Ruby*
Measurands quantified	Reticulocyte % and number	Same
Matrix	EDTA-anticoagulated whole blood	Same
Reagent Storage	Cartridges (with reagents) are stored at 2-8°C	Reagents are stored on board the device as well as separately
Sample preparation	Sample processing is automated in the TSPU	Sample preparation (i.e., mixing) required before sample introduction
Platforms	Single platform	Instrument and Console and separate systems for aliquoting sample where required
User Complexity	Low	High
Differences between TSPU and Predicate method		
Element	*TSPU*	*Abbott Cell-Dyn Ruby*
Technological Principles	Detection of cell type-specific epitopes with fluorescent binders and automated fluorescence microscopy. Detection of reticulocytes based on RNA binding stain (Thiazole Orange)	Enumeration and classification of cells into different cell types based on their size and optical scatter properties: Size (0°), complexity (10°), lobularity (90° polarized) and granularity (90° depolarized). Detection for reticulocyte based on RNA binding stain (New Methylene Blue)
Specimen Type	Venous or fingerstick (capillary) blood	Venous blood

Matrix	EDTA-anticoagulated plasma-replaced blood	EDTA-anticoagulated whole blood
Instrumentation	TSPU and TLAS	Abbott Cell-Dyn Ruby
Test Interpretation	Automated test interpretation and report generation. TSPU operator cannot access raw data.	Automatic data analysis and report generation.

Lymphocyte Subset Assay Proposed Method Comparison

Per CLSI guideline EP09A2IR, a study of at least 100 patient samples spanning the expected range of values (50% in normal range, 25% below, 25% above) will be conducted. Samples will be collected at two clinical evaluation sites and analyzed on the predicate method (BD Multitest 6-color TBNK kit on BD FACS Canto or BD FACS Canto II) and the TPSU. The lymphocyte subset measurands (T#, B#, NK#, CD4#, CD8#, T%, B%, NK%, CD4%, CD8%) will be evaluated on both platforms. Concordance (Pearson's r) will be calculated including 95% confidence intervals. Further regression fits using ordinary linear regression and Deming regression will be calculated. Slope and intercept of these fits will be presented with their respective 95% confidence intervals. For the study to pass, the correlation coefficients for all reportables shall be greater than 0.9.

Similarities between TSPU and Predicate method for Lymphocyte Subset		
Element	*TSPU*	*BD Multitest 6-color TBNK kit with BD FACS Canto or BD FACS Canto II flow cytometer*
Measurands quantified	T cell count, B cell count, NK cell count, CD4+ T cell count, CD8+ T cell count	Same
Matrix	EDTA-anticoagulated whole blood	Same
Technological Principles	Detection of cell type-specific epitopes with fluorescent binders	Same
Reagent Storage	Cartridges (with reagents) are stored at 2-8°C	Same
Differences between TSPU and Predicate method for Lymphocyte Subset		
Element	*TSPU*	*BD Multitest 6-color TBNK kit with BD FACS Canto or BD FACS Canto II flow cytometer*

Sample preparation	Sample processing is automated in TSPU.	Laboratory staff manually prepare the samples.
Technological Principles	Automated fluorescence microscopy	Flow cytometry
Specimen Type	Venous or fingerstick (capillary) blood	Venous blood
Matrix	EDTA-anticoagulated plasma-replaced blood	EDTA-anticoagulated whole blood
Instrumentation	TSPU and TLAS	BD FACS Canto or BD FACS Canto II flow cytometer
Test Interpretation	Automated test interpretation and report generation. TSPU operator cannot access raw data.	Automatic data analysis with option for user intervention.
Platforms	Single platform	Single or dual platform
User Complexity	Low	High

Specimen Information

Specimen Information for TNAA:

The TNAA assays are performed on samples collected in the form of nasopharyngeal swabs ("***NPS***"), nasopharyngeal aspirate ("***NPA***"), nasopharyngeal wash ("***NPW***"), nasal swabs ("***NS***"), throat swabs ("***TS"***), or sputum samples from individuals suspected of suffering from a respiratory infection. To collect a sample from an NPS that has been applied to a subject's nasal passage, the NPS is placed inside a Swab vessel embedded in the Cartridge which is pre-filled with a transfer medium. The swab handle may be broken-off, and the vessel capped to preserve sample integrity. Most of the sample on the swab is released on contact into the transfer medium. When processed, the sample is automatically mixed by several cycles of pipetting to ensure maximal sample release from the swab so no human processing is required. NPA, NPW, and sputum samples are transferred directly into the Cartridge for subsequent processing identically to swab samples. Samples will typically be processed and analyzed within several hours of their acquisition; thus, fresh samples will typically be used. However, sample stability is tested by maintenance of test samples for various times under typical storage conditions. During transport, the samples are stored in Universal transport media (UTM) which is formulated for maximum sample stability and pathogen viability. Furthermore, samples are transported in a frozen state (on dry ice) to further increase stability.

Specimen Information for Cytometry:

Whole blood collected by fingerstick or venipuncture will be used for cytometry testing. Such samples will typically be processed and analyzed within several hours of their collection; thus, the samples used will be fresh samples. However, sample stability is tested by maintenance of test samples for various times under typical storage conditions. During transport, the whole blood samples will be stored in vessels or Nanotainer tubes, as applicable, and stored at 4 °C with EDTA anti-coagulant. Tests can be performed on whole blood samples.

Method Comparison

Anticipated Predicate Device

Theranos plans to use the FilmArray as the predicate for TNAA. *Additionally CDC Real-Time RT-PCR kits for Coronavirus MERS and Streptococcus pyogeneson ABI 7500 Fast Dx Real-Time PCR instrument will be used as predicate method. Two Theranos in-house PCR tests with bi-directional sequence confirmation will be used as predicate method for Streptococcus pneumoniae and Penicillin sensitive Streptococcus pneumoniae*. Information on these predicate devices is below:

Biofire FilmArray:

- Trade Name: FilmArray® Respiratory Panel (RP)
- Regulation Number: 21 CFR 866.3980
- Classification Name: Respiratory Viral Panel Multiplex Nucleic Acid Assay

Coronavirus MERS:

- CDC: NCV-2012 rRT-PCR kit, Catalog # KT0136
- Device run on: ABI 7500 Fast Dx Real-Time PCR instrument with Sequence Detection Software version 1.4.
- Proprietary and Established Names: CDC Novel Coronavirus 2012 Real-Time RT PCR Assay

Bocavirus:

- Trade Name: Luminex® Respiratory Viral Panel (RVP)
- Regulation Number: 21 CFR 866.3980
- Classification Name: xTAG Respiratory Viral Panel (RVP) FAST v2

Streptococcus pyogenes:

- 510(k) number: K122019
- Device run on: ABI 7500 Fast Dx Real-Time PCR instrument with Sequence Detection Software version 1.4.
- Proprietary and Established Names: illumigene® Group A Streptococcus (GBS) DNA Amplification Assay
    -

Bordetella parapertussis and Bordetella holmesii:

- R-biopharm: Art. No. PG2505
- Device run on: ABI 7500 Fast Dx Real-Time PCR instrument with Sequence Detection Software version 1.4.
- Proprietary and Established Names: RIDA®GENE Bordetella

Streptococcus pneumoniae and Penicillin resistant Streptococcus pneumoniae:

- Two Theranos in-house PCR tests with bi-directional sequence confirmation.

Comparison to the Subject Device

See the "*Performance Testing / Product Development – Performance Testing*" Section for more information regarding comparisons to the predicate device.

TNAA:

Similarities between TSPU and FilmArray RP		
Element	TSPU	FilmArray RP Test system
Organisms Detected	Coronavirus 229E Coronavirus HKU1 Coronavirus NL63 Coronavirus OC43 Human Metapneumovirus A Human Metapneumovirus B Parainfluenza 1 Parainfluenza 2 Parainfluenza 3 Parainfluenza 4 Respiratory Syncytial Virus A Respiratory Syncytial Virus B Adenovirus B	Same, see below for differences

	Adenovirus C Adenovirus E Rinovirus Bordetella Pertussis Chlamydophila pneumoniae Mycoplasma pneumoniae Bocavirus Coronavirus MERS Streptococcus pyogenes Bordetella parapertussis Bordetella holmesii Streptococcus pneumoniae Penicillin sensitive Streptococcus pneumoniae	
Analyte	RNA or DNA	Same
Technological Principles	Multiplex Nucleic Acid	Same, see below for difference
Specimen Types	Nasopharyngeal swabs, nasopharyngeal aspirate, and nasopharyngeal wash, throat swabs	Same, see below for difference
Test Interpretation	Automated test interpretation and report generation. TSPU operator cannot access raw data.	Automated analysis with raw data available to operator.
Differences between TSPU and FilmArray RP		
Element	TSPU	FilmArray RP Test system

Additional Organisms Detected	Coronavirus MERS Streptococcus pyogenes Bordetella parapertussis Bordetella holmesii Streptococcus pneumoniae Penicillin sensitive Streptococcus pneumoniae	Film Array does not provide these tests.
Technological Principles	Recombination based isothermal method (See the "*Device Description – Theranos Nucleic Acid Amplification (TNAA) Assay*	Nested multiplex RT-PCR followed by high resolution melting analysis to confirm identity of amplified product
Specimen Type	Nasal swab	
Instrumentation	TSPU and TLAS	FilmArray Instrument
Test to result	<50mins	65mins
Sample Preparation Method	Sample processing is automated in Theranos TSPU	Sample processing is automated in FilmArray instrument
Additionally	ELISAs can be performed simultaneously	
Reagent Storage	Reagents stored at 4 °C	Reagents stored at RT
User Complexity	Low	Moderate/Low
Similarities between TNAA and RT-PCR kits for Coroniavirus MERS, Streptococcus pyogenes, Bordetella parapertussis and Bordetella holmesii.		
Analyte	RNA or DNA	Same
Technological Principles	Multiplex Nucleic Acid, see below for difference	RT-PCR
Specimen Types	Nasopharyngeal swabs, nasopharyngeal aspirate, throat swabs and nasopharyngeal wash	Same
Differences between TNAA and RT-PCR kits for Coroniavirus MERS, Streptococcus pyogenes, Bordetella parapertussis and Bordetella holmesii.		
Element	TNAA	FlimArray RP Test system

Technological Principles	Recombination based isothermal method (more info in Principle section)	RT-PCR
Instrumentation	TSPU and TLAS	ABI 7500 Fast Dx Real-Time PCR instrument
Test to result	45 mins	2 hours
Sample Preparation Method	Sample processing is automated in Theranos instrument.	Sample processing is manual.
Reagent Storage	Reagents stored at 4 °C	Reagents stored at RT
User Complexity	Low	High
Test Interpretation	Automated test interpretation and report generation. TSPU operator cannot access raw data.	Automated analysis with raw data available to operator.

Clinical Performance

Clinical Study Design Elements

Prospective Clinical Study

Background and Study Goals:

The primary goal of this prospective study is to supplement existing clinical data to characterize the accuracy of the TNAA and cytometry assays run on the Theranos SPU and TLAS for the diagnosis of respiratory infections in patients exhibiting signs and symptoms of infection. Combining highly sensitive TNAA tests with hematologic measures is believed to provide advantages over traditional methods as detailed below. In addition, cytometry tests will be evaluated in patients for routine testing, diagnosis and monitoring of numerous health conditions. Use of cell surface markers or epitope-based identification of cells using imaging is believed to have many advantages over traditional hematology analyzers and flow cytometers as detailed below.

The traditional infectious disease testing approach includes rapid testing methods, followed in some cases by confirmatory testing (*e.g.,* RT-PCR or Theranos PCR tests of patient specimen with bidirectional sequence confirmation). The problem with this approach is that rapid test methods generally have poor accuracy. Product insert information and research publications indicate that rapid testing only achieves sensitivities of 50-70% and specificities of 90-95%. This can yield a very high frequency of false negatives. Confirmatory testing is traditionally expensive with slow turnaround times, leading to delays in treatment and possibly inappropriate treatment. Moreover, rapid test methods do not distinguish between pathogen subtypes. Such subtyping can be critical for risk assessment, treatment, vaccine planning, and surveillance.

The Theranos SPU and TLAS enable one to easily and rapidly perform both highly sensitive and specific TNAA tests as well as hematologic testing. This approach yields both very high accuracy (sensitivity and specificity) and also provides information concerning the stage of the infection and state of the immune response. This timely and rich information can facilitate medical care, such as the decision to treat with an antiviral medication or not. In addition, testing for common viral and bacterial infections can improve decisions regarding which treatments to administer, thereby preventing unnecessary and ineffective anti-viral or anti-bacterial prescriptions. It can also facilitate assessment of infectivity.

The traditional hematology analyzers use physical properties of cells such as size, light scattering properties, conductivity, light polarization or non-specific biochemical properties such as presence of granules, affinity to basic or acidic dyes etc. as the basis of identification of different cell types. This has resulted in rapid methods that have significant limitations. These methods are more susceptible to abnormalities in the sample such as presence of cell fragments or presence of giant platelets. Many results are therefore flagged by these analyzers and require confirmatory blood smears to be performed. These tests are time consuming and expensive. Moreover, typically more

than 50% of the confirmatory blood smears that are performed are due to false positive flags raised by these instruments. In routine applications, 20-25% of all CBC assays require a confirmatory blood smear to be performed.

The Theranos SPU and TLAS enable one to easily and rapidly perform routine hematology assays that combine the speed and statistical power of an automated assay with the depth of information provided by specific identification of cell types using antibodies to cell surface markers. The reliability of the CD markers used in Theranos tests has been exhaustively validated in literature and their correlation with different diseases remains an active area of research even today. An epitope-based assay therefore allows for future improvement in terms of diagnostic value.

The Theranos SPU and TLAS enable CLIA oversight of sample processing with subsequent analysis in and under the oversight of Theranos' CLIA laboratory. The images from each test are available for quick review by the CLIA laboratory for resolution of any abnormal flags. This approach has clear advantages compared to traditional hematology analyzers.

Nasopharyngeal aspirates or nasal wash specimens are commonly used for the detection of respiratory viruses. However these procedures are generally unpleasant. Obtaining an aspirate requires a suction device, a feature which makes it unfeasible for widespread use in clinical practice. Less invasive testing with nasal or throat swabs, which will be evaluated in this study alone, in comparison to aspirates and wash specimens, and in combination with blood samples, should enable increased patient participation and help improve overall healthcare response and surveillance.

Venipuncture samples are generally used for routine hematology. However, these procedures are generally painful and especially in patients requiring repeated testing, these procedures can become a serious detriment to the quality of life. Less invasive and more patient friendly testing with fingerstick samples, which will be evaluated in this study, should enable increased patient participation, compliance, and comfort and improve overall healthcare response.

Study Plan:

The clinical performance of the Theranos SPU will be evaluated in a prospective study. Nasopharyngeal swab, nasopharyngeal aspirate, nasopharyngeal wash, nasal swab, throat, or sputum samples and simultaneous whole blood samples will be collected from subjects exhibiting symptoms of respiration infection at 3 U.S. sites during the 2013/2014 respiratory season. Fingerstick and venipuncture whole blood samples will be collected. The samples will be collected by trained technicians and processed using the Theranos SPU at Theranos' Patient Service Center ("***TPSC***"). Each site will have 4 Theranos SPU's. Analysis will be done through the TLAS. Testing accuracy and performance will be determined by comparison to predicate methods, including traditional CLIA-certified laboratory processing and analysis.

Clinical study sites are selected based on the desired geographic/demographic variation of the potential subject populations. Subjects will be selected from diverse demographic groups. Each site will enroll 360 patients, for a total of 1080 subjects. The goal of subject recruitment will be to

obtain symptomatic patients with at least a third of the subjects having abnormal or out of typical reference range values. These subjects could be individuals expressing symptoms of respiratory infection, subjects with known conditions like anemia, thrombocytopenia, leukemia or other hematological disorders. Further, an approximately 50-50 gender split will be desired in this test population. Written informed consent will be obtained from each subject and/or their parent/guardian (if under 18) at the time of enrollment into the study. To de-identify specimens, each subject will be assigned a Volunteer Identification Number (VIN) to track the sample. At the time of enrollment the following information will be recorded on the Case Report Form (CRF): 1) age; 2) sex; 3) information about their suspected respiratory infection, *i.e.,* signs and symptoms, date of onset; 3) current medications (self-reported and/or collected from medical records); 4) information about their suspected respiratory infection, *i.e.,* signs and symptoms, date of onset; 5) any hematological disorders or conditions (such as anemia, coagulation disorders, autoimmune conditions, leukemias or other cancers, bone marrow transplant); and 6) other conditions that may affect blood cell counts such as HIV, mononucleosis, recent blood donor, and pregnancy.

Technicians at the TPSC will be trained on both the sample collection process as well as the basic operations of the Theranos SPU according to the intended use and associated instructions for use.

Four sample types (nasopharyngeal swab, nasal swab, nasopharyngeal aspirate, or nasopharyngeal wash) will be collected across all subjects. One sample type will be collected per subject and two swabs will be collected from each subject (one from each nostril). For those subjects exhibiting streptococcus symptoms, throat swabs or sputum samples will be collected. One swab will be used for testing on the Theranos System, and the second swab will be used for testing on the predicate method (Biofire, Luminex for Bocavirus, RIDAGENE for Bordetella parapertussis and Bordatella holmesii and CDC RT-PRC kits for Coronavirus MERS). Any conflicting results will be further investigated by viral culture and sequencing.

Of the two whole blood sample types collected per subject, the fingerstick sample will be used for testing on the TPSU, and the venous sample will be used for testing on the predicate method (Abbott Cell-Dyn Ruby/Reference LDA). Any conflicting results will be further investigated by retest and blood smears.

Statistical Analysis Plan for CBC Clinical Performance Study

The main outcome of this study will be the determination of the diagnostic accuracy and analytical accuracy of the TSPU and TLAS for TNAA and hematology assays, respectively.

TNAA Analysis Plan:

To this end, for TNAA, concordance, sensitivity, specificity, positive predictive value ("***PPV***"), and negative predictive values ("***NPV***") will be calculated including 95% confidence intervals (CLSI guidance document EP12-A2).

These performance metrics will be calculated across all sites for each TNAA diagnostic outcome. The analytic cut-off values will be based on the pre-clinical study results. Performance metrics will be calculated as per CLSI guidance document I/LA18-A2, MM3-A2, and EP12-A2. Predicate outcomes will serve as the presumed truth. Each discrepant classification will be investigated and resolved (included by viral culture).

As described, a total of 1080 subjects are planned to be enrolled in the prospective study. This sample size will provide sufficient subjects for the more common pathogens yielding low margins of error with a 95% confidence level for each performance metric. It is anticipated that certain strains will be encountered with limited frequency in the U.S. Retrospective samples may be used to supplement the prospective study if insufficient samples are obtained for certain test outcomes. In general, if incidence rates are <3.5%, retrospectives and/or contrived samples will be included.

Cytomery Analysis Plan:

For hematology assays assessment, concordance (Pearson's r) will be calculated including 95% confidence intervals. Further regression fits using ordinary linear regression and Deming regression will be calculated. Slope and intercept of these fits will be presented with their respective 95% confidence intervals.

This study is powered to detect "small" effect sizes or differences in the correlation between Theranos system and predicate device. (Cohen, J. (1988). Statistical power analysis for the behavioral sciences (2nd ed.). Hillsdale,NJ: Lawrence Erlbaum.) These performance metrics will be calculated across all sites for each assay. Performance metrics will be calculated as per CLSI guidance document EP09A2 (Method comparison). Predicate outcomes will serve as the presumed truth. Each result that differs from predicate by more than 2 standard deviations of the assay as established in the pre-clinical studies will be investigated.

The success criteria are correlation coefficients for all reportables greater than 0.9. Basophils, MPV and RDW, due to the narrow expected range for these reportables in the patient population, are deemed acceptable with lower correlation coefficients. Also, monocytes, due to the differences in detection method are deemed acceptable with lower correlation coefficients. In these cases, bias estimates must meet CLIA total error requirements.

Previous Discussions or Submissions

In October 2012, Elizabeth Holmes (Theranos' CEO) and Sunny Balwani (Theranos' COO) and the FDA held a meeting. A teleconference was also held in August 2013 between Ms. Holmes and Sally Hojvat and John Hobson from the FDA.

During these meetings, Ms. Holmes relayed that Theranos' initial goal was to convert all of its LDTs into FDA-cleared assays, as appropriate. She also discussed how Theranos sought to gain FDA-clearance for the TSPU. During these discussions, the FDA indicated it would be possible to submit Theranos' NAA assays and ELISA assays together in an initial filing for the TSPU and TLAS. Ms. Holmes expressed Theranos' intent to use its first submission to create a framework for FDA clearance for its other assays. She indicated that Theranos would be submitting formal pre-submission request(s) accordingly.

During the meetings, Ms. Holmes also indicated that, until Theranos received clearance from the FDA, its devices would only be used in and by Theranos' Palo Alto-based CLIA-certified Laboratory. She provided that all samples would be physically transported to Theranos' Palo Alto-based CLIA-certified Laboratory to be run through Theranos' Laboratory Developed Tests, or where relevant, on FDA approved analyzers and tests in Theranos' Palo Alto-based CLIA-certified Laboratory. Ms. Holmes provided that, accordingly, in September 2013, Theranos intended to begin processing micro-samples and traditional phlebotomy draws collected by trained and certified phlebotomists qualified under the appropriate state laws and employed by Theranos in its CLIA-certified laboratory.

On September 5, 2013, Theranos submitted formal Informational Meeting Request Q131148 and referenced that it would be additionally filing a formal request for a Pre-Submission Meeting. On September 13, 2013, John Hobson, on behalf of the FDA, confirmed that (i) administrative review of Theranos' submission requesting FDA feedback was complete and (ii) its submission included sufficient information to enable feedback in the manner requested. Mr. Hobson was referenced as the lead reviewer assigned to Informational Meeting Request Q131148.

In Informational Meeting Request Q131148, Theranos provided that it would be configuring its devices to collect video of the inside of the devices and would be following with the pre-submission for the initial influenza NAA and ELISA assays. Ms. Holmes followed with an email to Sally Hojvat and John Hobson indicating availability of the video for viewing at their convenience. Informational Meeting Request Q131148 also confirmed Theranos' plans as previously conveyed to the FDA in the prior meetings, and further described its plans for collecting micro-samples and branding Patient Service Centers or Collection Sites as Theranos Wellness Centers. Informational Meeting Request Q131148 referenced the launch of Theranos' first Patient Service Center inside a Walgreens pharmacy store and its plans for opening 1-3 locations in Palo Alto, including one at Theranos' headquarters, in the month of September.

On November 4, 2013, Elizabeth Holmes (Theranos' CEO), Sunny Balwani (Theranos' COO) and legal counsel for Theranos attended the Informational Meeting (Q131148) with FDA and two

representatives of CMS. On November 21, 2013, Theranos submitted draft minutes to FDA from that meeting for review.

On November 4, 2013, Elizabeth Holmes (Theranos' CEO) and Sunny Balwani (Theranos' COO) and others from Theranos attended a Pre-Submission Meeting (Q131191) to obtain feedback from FDA on 510(k) submission(s) for the TSPU, the associated software for analysis, the TLAS, and its influenza NAA and ELISA assays. On November 18, 2013, Theranos submitted draft minutes of that Pre-Submission Meeting to FDA for review.

Specific Questions

1. One of our primary objectives is to prepare ongoing 510(k) submissions for Laboratory Developed Tests (LDTs) that we develop or have developed which operate on our TSPU, TLAS system. We would like to get the FDA's advice as to whether the data we have already generated relating to these LDTs in our development, validation, and various Theranos clinical programs (representative data included in the Appendix) would likely be sufficient for successful 510(k) submissions.

2. If additional data is required, we would like to get the FDA's guidance as to whether the study delineated in the "*Clinical Performance and Study Design Elements*" Section of this pre-submission adequately addresses the Intended Use objectives and FDA's requirements for successful and expeditious 510(k) clearance.

3. We have used the Abbott Cell-Dyn Ruby as a predicate for blood cell counts in this submission. The Ruby does not use the same technology for analysis as the Theranos system. However, the two devices provide the same clinical information. There is no 510(k) approved device that we are aware of that uses epitope-based identification of cells coupled with imaging for counting blood cells. We would like the FDA's guidance on this selection.

4. We specified the Reference Leukocyte Differential as defined by CLSI as our reference in this document for the WBC differential part of the CBC assays. This choice reflects our understanding that this reference assay represents the gold standard and hence the presumed truth. Since hematology analyzers use physical properties of cells for classification and not biochemical properties, there may be differences between results of automated analyzers and the LDA. We do not want our comparison to be biased by such differences. Flow cytometry based reference WBC differential methods have also been published and extensively evaluated in the literature. For the purposes of comparison, a flow-cytometry based method would be the best choice for Theranos--this method would count similar or even larger number of events and thus provide a statistically robust reference point. We would like the FDA's guidance on the best reference method for Theranos to select for this assay.

5. The predicate method for the lymphocyte subset assay, BD Multitest 6-color TBNK kit, states that analytical specificity, clinical sensitivity and clinical specificity studies are not applicable. We are using the same cluster of differentiation epitopes to identify the cells in the lymphocyte subset, which have been well-established in the literature to be specific for the cells they identify. Therefore we have not proposed an additional large-scale clinical study for that. Please advise as to whether the Method characterization and method comparison studies that have been included are sufficient to demonstrate the substantial equivalence of this assay in accordance with the predicate method.

6. We've included a detailed description of the TSPU in the proposed application, including all modules of the TSPU, even though not all modules of the TSPU are required for the TNAA and cytometry assays described in the application. Is this the proper level of description of the TSPU for our 510(k) submission, or should we not describe modules of the TSPU which are not used in the TNAA and cytometry assays?

7. At the prior Pre-Submission Meeting (Q131191), Theranos and FDA discussed obtaining separate clearance for the TSPU for the intended use of the cleared Influenza Assays and device modules used therein. FDA explained that the TSPU will have a User Guide, which would be limited to data only for the functions for the cleared assays. At that meeting, FDA mentioned that functionalities related to subsequently cleared assays could be addressed with updates to the User Guide without a new 510(k) submission, noting that where there are significant changes that would impact the cleared intended use, a new 510(k) submission for the TSPU may be required. We have included details of the TSPU in this Pre-Submission with the view that the corresponding 510(k) submission will be for clearance of the Viral Versus Bacterial TNAA and cytometry assays with updates to the cleared TSPU User Guide, as applicable. We would like further feedback on this approach in accordance with the contents of this Pre-Submission for the bacterial versus viral and cytometry assays.

8. Does our proposed application content meet FDA's expectations to support an expeditious handling of our final 510(k) submission? For example, Appendix F to the Draft Guidance for Industry and FDA Staff, entitled "Medical Devices: The Pre-Submission Program and Meetings with FDA Staff" sets forth specific recommendations for IVD submissions. We would like the FDA's advice as to whether we are sufficiently addressing these topics.

9. Prospective samples of the human Coronavirus MERS are difficult to acquire and culturing requires BSL3 facilities. Is it sufficient to conduct assay development and validation work on synthetic material or are live clinical samples required at this stage? Is there an alternative to clinical samples that the FDA recommends be used for validation studies of hCoV MERS assay?

10. We anticipate that there will low prevalence for some of the TNAA strains in the prospective clinical study and are considering two options to supplement low prospective samples: 1) retrospective samples (as noted in the study plan) and 2) synthetic samples. We would like FDA's feedback on these two approaches.

Mechanism for Feedback

Theranos hereby requests an in-person Pre-Submission Meeting to solicit feedback on this Pre-Submission and the associated upcoming filing. Although we met in person with the Division of Microbiology Devices previously, we feel that in-person meeting would still be useful, especially given that the Division of Immunology and Hematology Devices will be reviewing information related to the Theranos system and assays for the first time.

References

510(k) Substantial Equivalence Determination Decision Summary, Reference Number K111507 http://www.accessdata.fda.gov/cdrh_docs/reviews/K111507.pdf

Title: Leukocyte and Stromal Cell Molecules: The CD Markers
Authors: Heddy Zola, Bernadette Swart, Ian Nicholson, Elena Voss
Published: John Wiley & Sons, 2007.
Published under the auspices of HCDM (Human Cell Differentiation Molecules), the international organization responsible for HLDA (Human Leukocyte Differentiation Antigens) Workshops and CD molecules.

Titile: Platelet Counting by the RBC/Platelet Ratio Method: A Reference Method
Authors: International Council for Standardization in Haematology Expert Panel on Cytometry and International Society of Laboratory Hematology Task Force on Platelet Counting
Published: Am J Clin Pathol, 2001;115:460-464

NCCLS document H44-A2 and Linda G. Lee, C.-H. C. a. L. A. C., 1986. Thiazole Orange: A New Dye for Reticulocyte Analysis. Cytometry, Volume 7

Title: Toward a reference method for leukocyte differential counts in blood: Comparison of three flow cytometric candidate methods
Authors: Mikael Rousse,Bruce H. Davis, Thierry Fest, Brent L. Wood5 on behalf of the International Council for Standardization in Hematology (ICSH).
Published: Cytometry Part A, Volume 81A, Issue 11, pages 973–982, November 2012

Appendix A

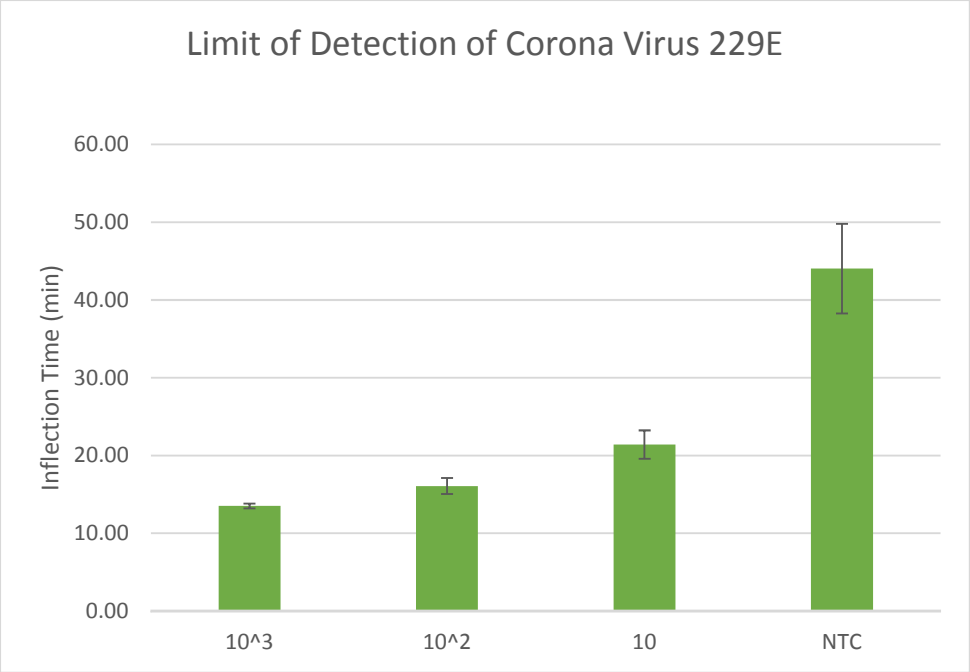
Representative Bench Data
TNAA assay validation data:

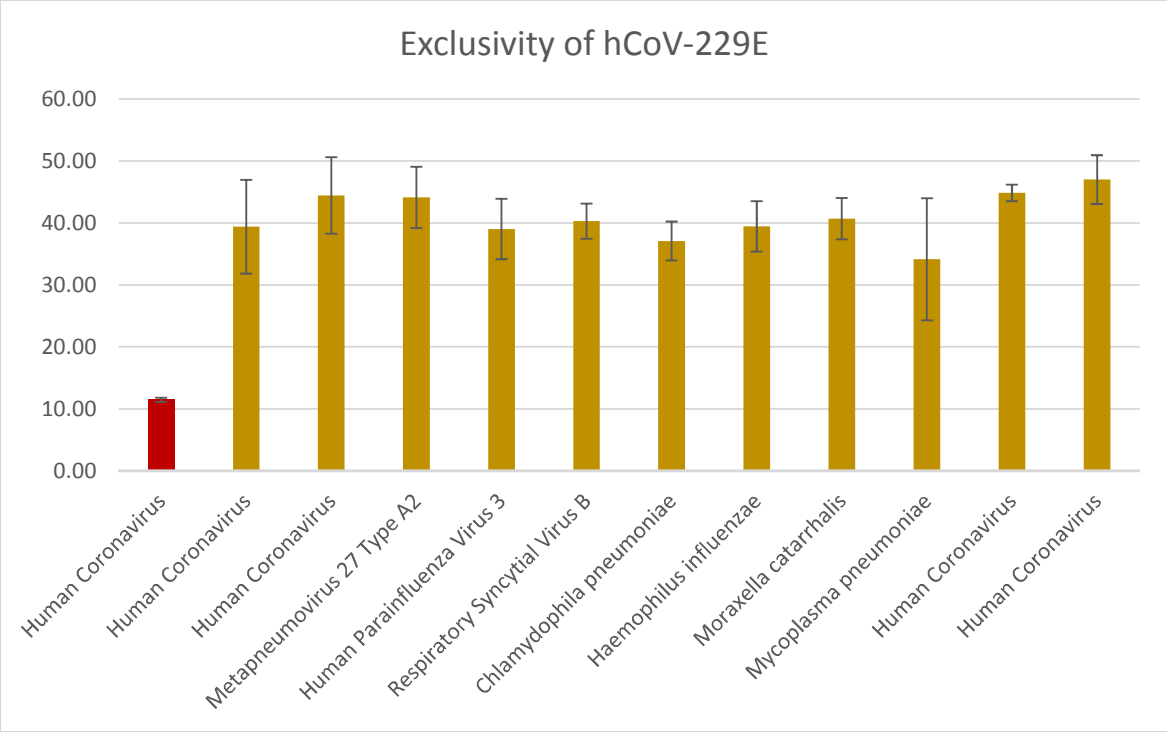
The data presented below was collected during TNAA assay development and validation and includes Limit of Detection (LOD), inclusivity, exclusivity and specificity. The TNAA assay methodology was validated on commercially available Biorad Real-Time PCR Detection Systems.

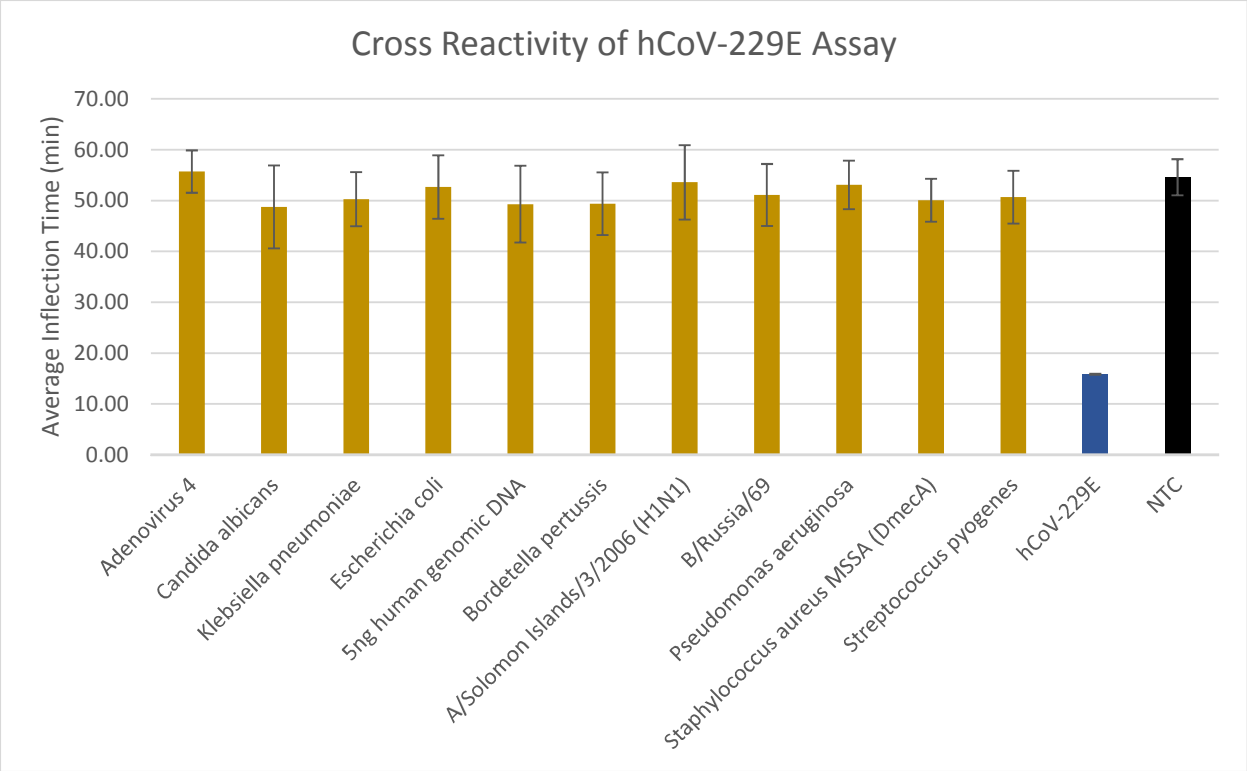
The Y-axis for each graph represents detection time in minutes unless otherwise labeled. These data were further analyzed using a statistical model before a positive/negative value was assigned. Background information about this model is not included here but is described the "*NAA Proposed Study Design(s), Predicate, and Analytical and Pre-Clinical Performance - Determination of Assay Cutoffs*" Section in accordance with the Draft Guidance for Industry and FDA Staff: "*Medical Devices: The Pre-Submission Program and Meetings with FDA Staff*." Each bar in the bar graphs below represents average values of at least eight replicates and variation is reflected by the error bars.

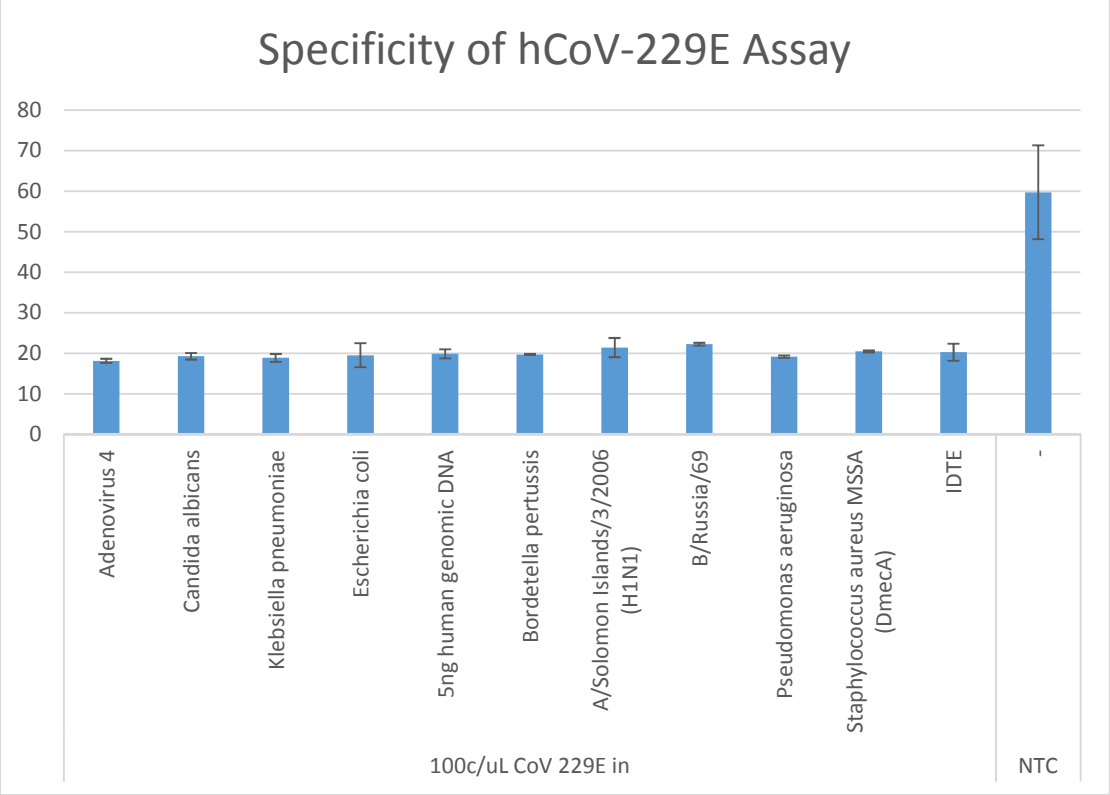
The data shown below is for the following assays:

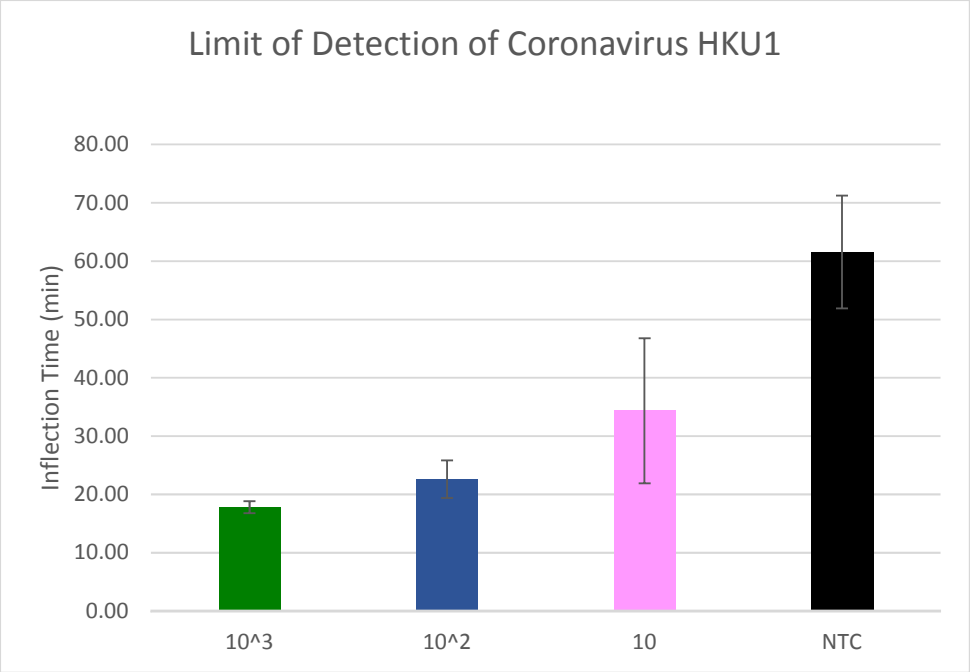
- Adenovirus B
- Adenovirus C
- Adenovirus E
- Bocavirus
- Bordetella holmesii
- Bordetella parapertussis
- Bordetella Pertussis
- Chlamydophila pneumoniae
- Coronavirus 229E
- Coronavirus HKU1
- Coronavirus MERS
- Coronavirus NL63
- Coronavirus OC43
- Human Metapneumovirus
- Mycoplasma pneumoniae
- Parainfluenza 1
- Parainfluenza 2
- Parainfluenza 3
- Parainfluenza 4
- Respiratory Syncytial Virus
- Streptococcus pneumoniae
- Streptococcus pyogenes

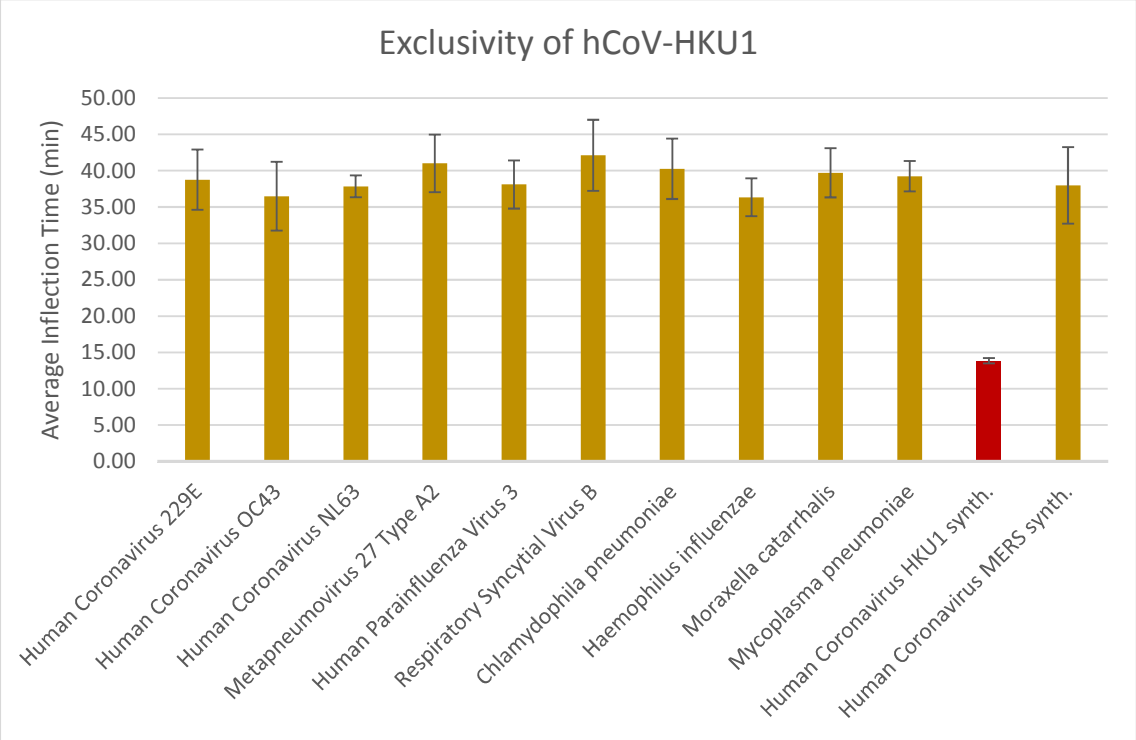

Limit of Detection of Corona Virus 229E
Inflection Time (min)
60.00
50.00
40.00
30.00
20.00
10.00
0.00
10^3
10^2
10
NTC

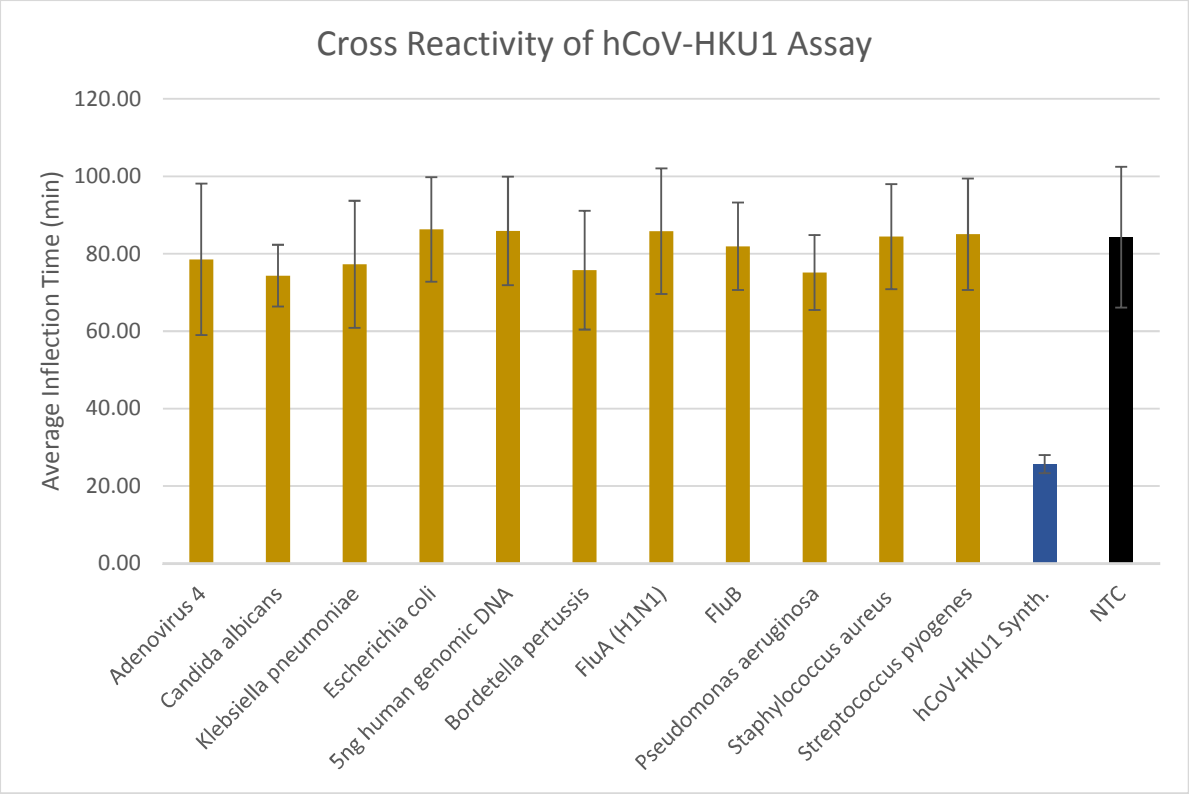

Exclusivity of hCoV-229E
60.00
50.00
40.00
30.00
20.00
10.00
0.00
Human Coronavirus
Human Coronavirus
Human Coronavirus
Metapneumovirus 27 Type A2
Human Parainfluenza Virus 3
Respiratory Syncytial Virus B
Chlamydophila pneumoniae
Haemophilus influenzae
Moraxella catarrhalis
Mycoplasma pneumoniae
Human Coronavirus
Human Coronavirus

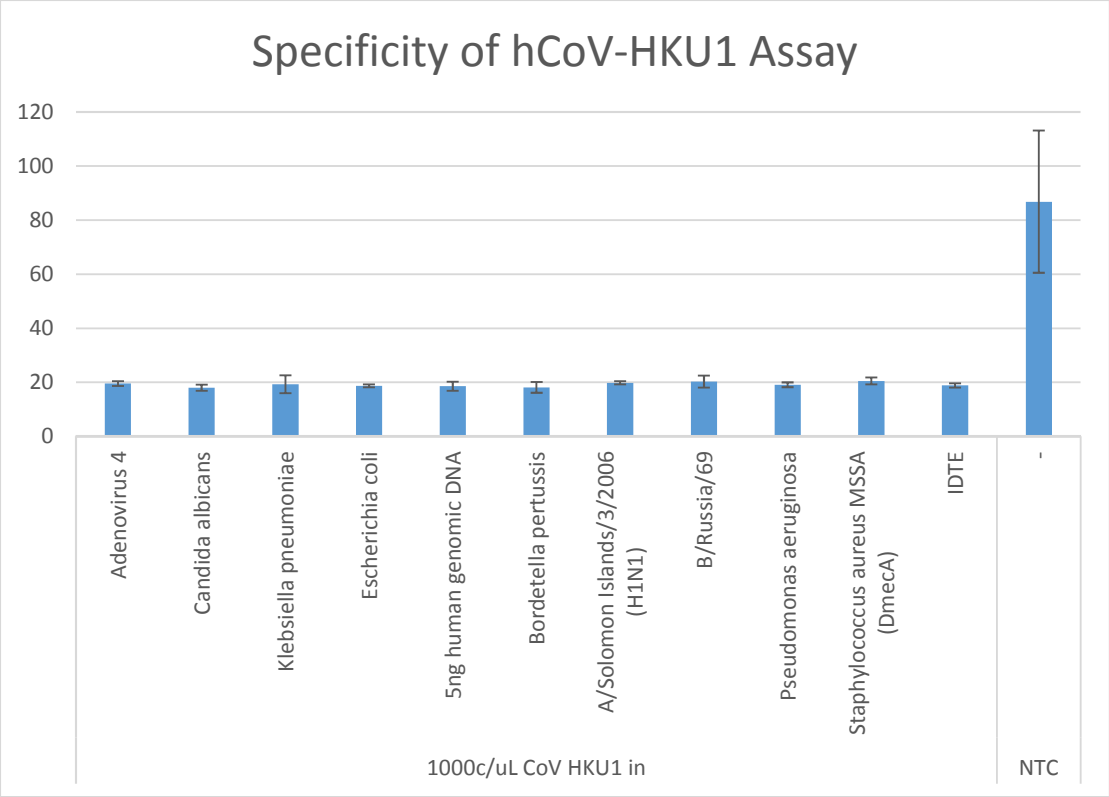

Cross Reactivity of hCoV-229E Assay
Average Inflection Time (min)
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
Adenovirus 4
Candida albicans
Klebsiella pneumoniae
Escherichia coli
5ng human genomic DNA
Bordetella pertussis
A/Solomon Islands/3/2006 (H1N1)
B/Russia/69
Pseudomonas aeruginosa
Staphylococcus aureus MSSA (DmecA)
Streptococcus pyogenes
hCoV-229E
NTC

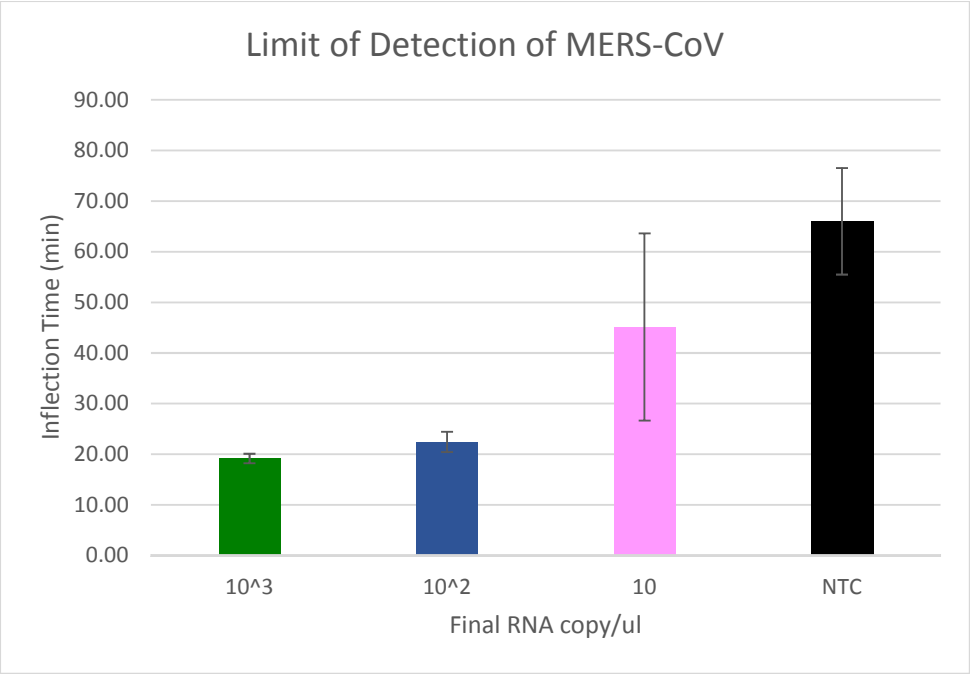

Specificity of hCoV-229E Assay
80
70
60
50
40
30
20
10
0
Adenovirus 4
Candida albicans
Klebsiella pneumoniae
Escherichia coli
5ng human genomic DNA
Bordetella pertussis
A/Solomon Islands/3/2006 (H1N1)
B/Russia/69
Pseudomonas aeruginosa
Staphylococcus aureus MSSA (DmecA)
IDTE
-
100c/uL CoV 229E in
NTC

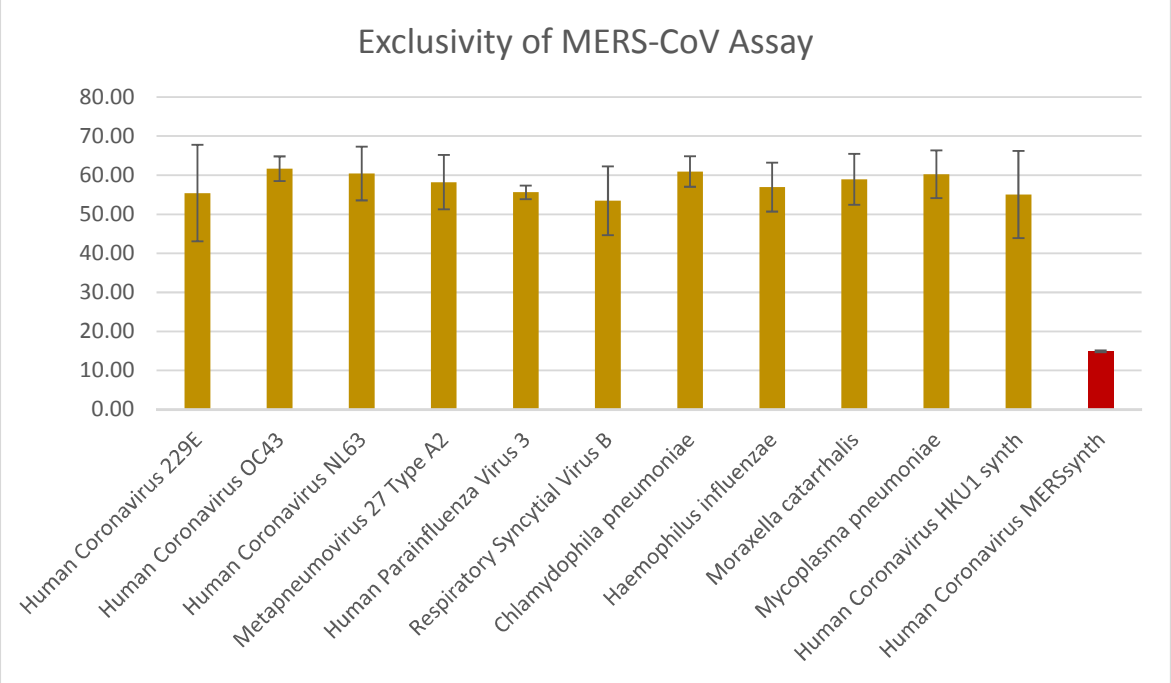

Limit of Detection of Coronavirus HKU1
Inflection Time (min)
80.00
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
10^3
10^2
10
NTC

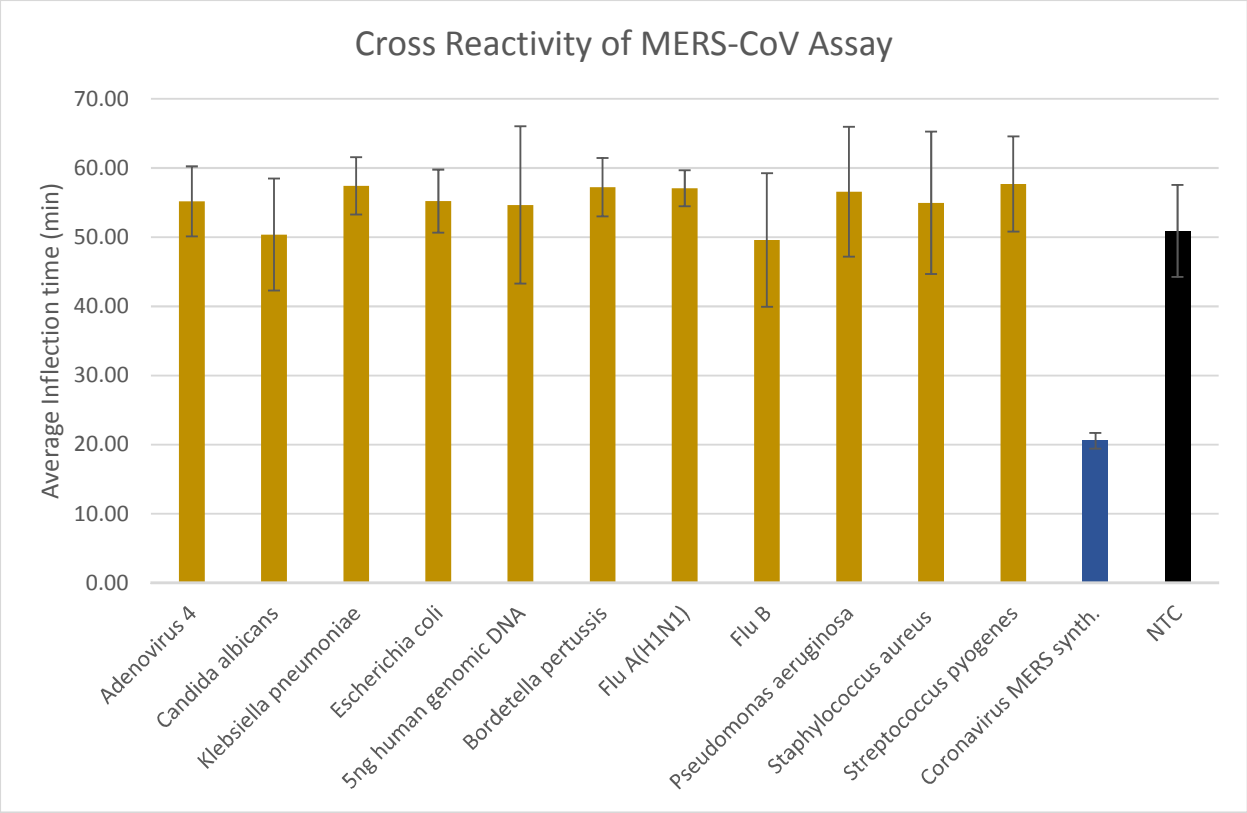

Exclusivity of hCoV-HKU1
Average Inflection Time (min)
50.00
45.00
40.00
35.00
30.00
25.00
20.00
15.00
10.00
5.00
0.00
Human Coronavirus 229E
Human Coronavirus OC43
Human Coronavirus NL63
Metapneumovirus 27 Type A2
Human Parainfluenza Virus 3
Respiratory Syncytial Virus B
Chlamydophila pneumoniae
Haemophilus influenzae
Moraxella catarrhalis
Mycoplasma pneumoniae
Human Coronavirus HKU1 synth.
Human Coronavirus MERS synth.

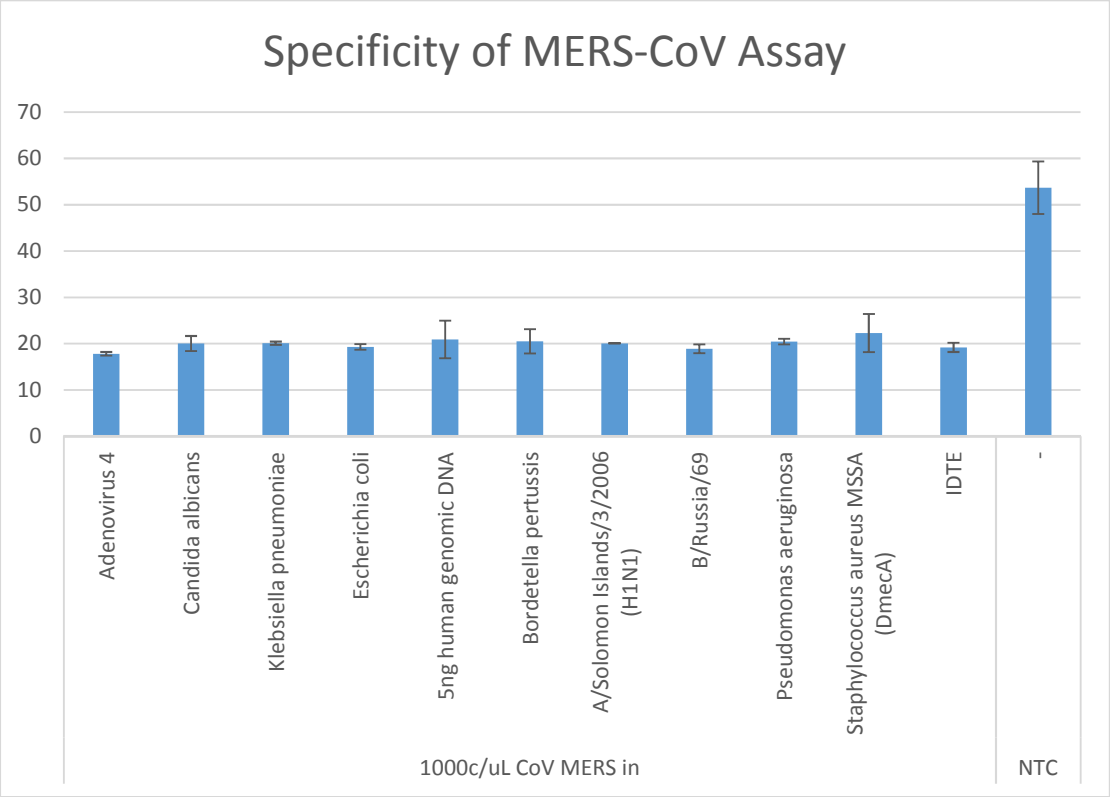

Cross Reactivity of hCoV-HKU1 Assay
Average Inflection Time (min)
120.00
100.00
80.00
60.00
40.00
20.00
0.00
Adenovirus 4
Candida albicans
Klebsiella pneumoniae
Escherichia coli
5ng human genomic DNA
Bordetella pertussis
FluA (H1N1)
FluB
Pseudomonas aeruginosa
Staphylococcus aureus
Streptococcus pyogenes
hCoV-HKU1 Synth.
NTC

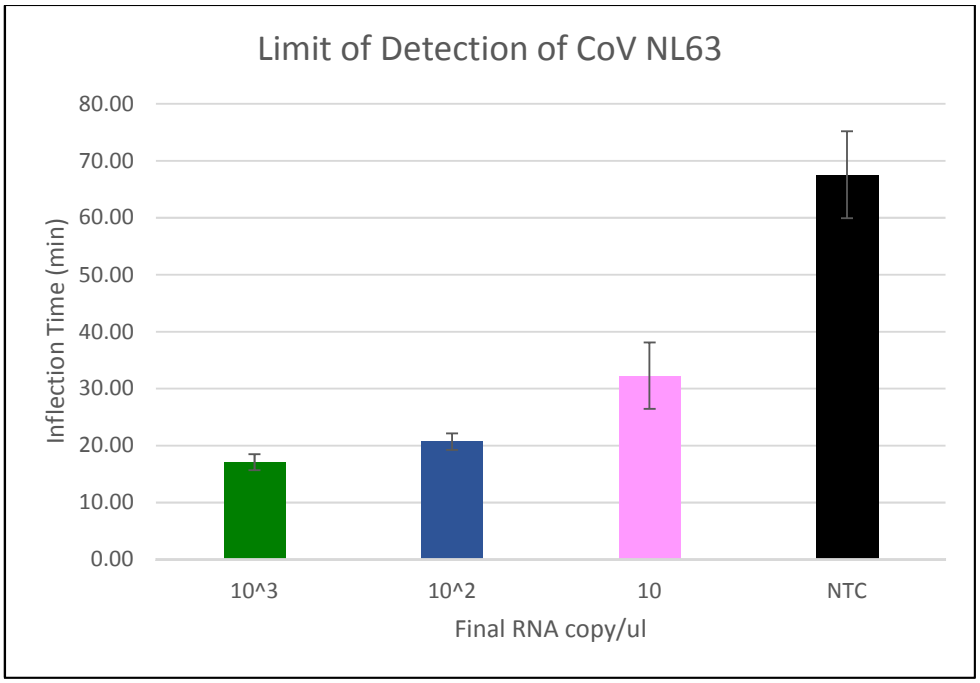

Specificity of hCoV-HKU1 Assay
120
100
80
60
40
20
0
Adenovirus 4
Candida albicans
Klebsiella pneumoniae
Escherichia coli
5ng human genomic DNA
Bordetella pertussis
A/Solomon Islands/3/2006 (H1N1)
B/Russia/69
Pseudomonas aeruginosa
Staphylococcus aureus MSSA (DmecA)
IDTE
-
1000c/uL CoV HKU1 in
NTC

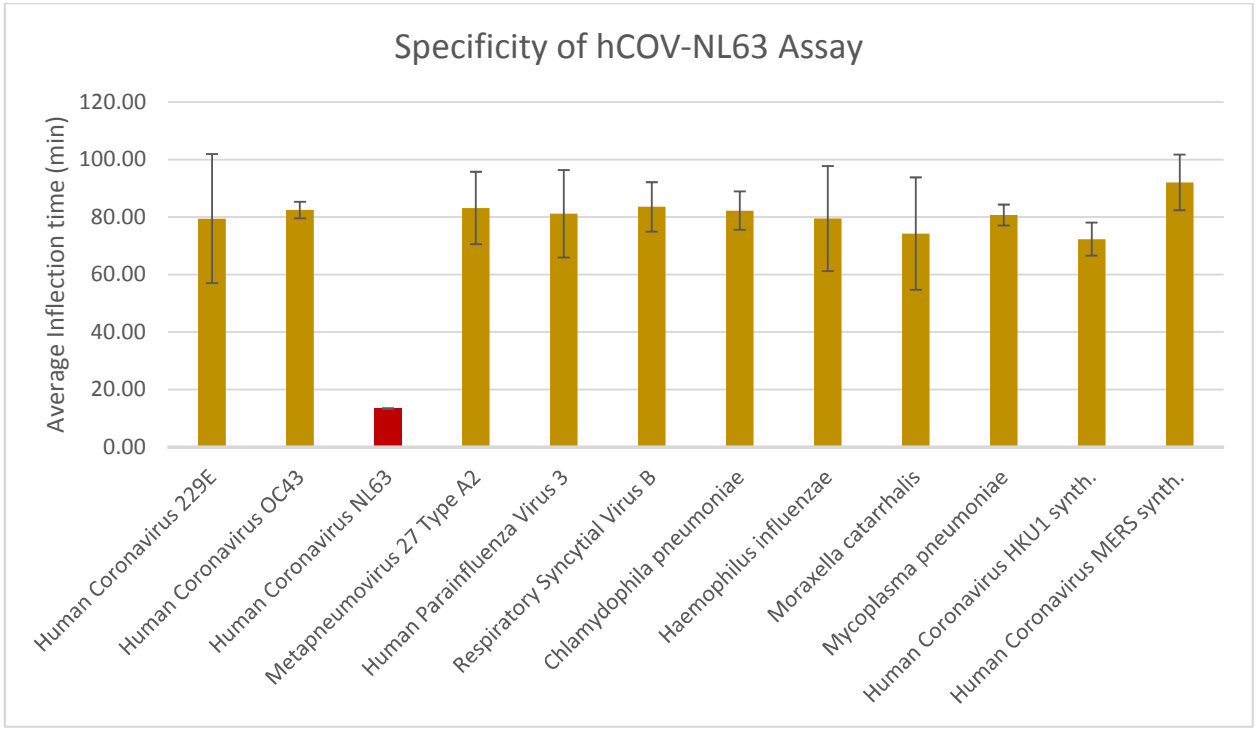

Limit of Detection of MERS-CoV
90.00
80.00
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
Inflection Time (min)
10^3
10^2
10
NTC
Final RNA copy/ul

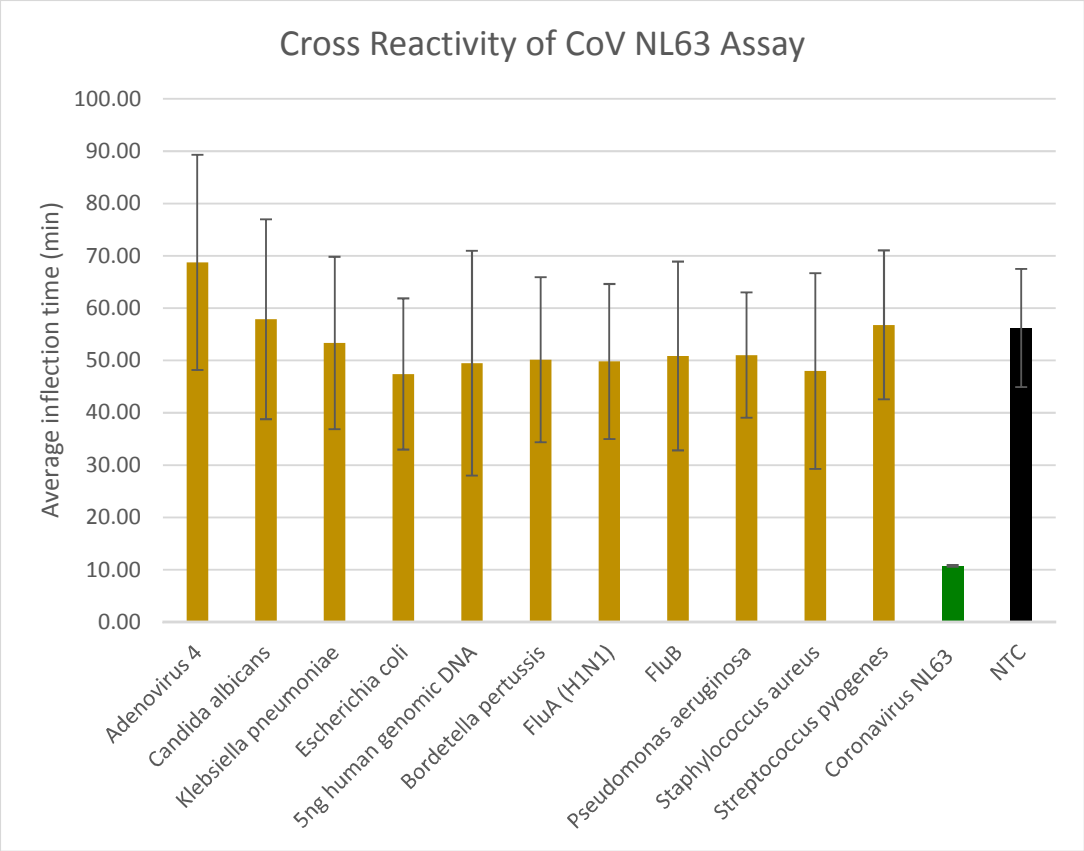

Exclusivity of MERS-CoV Assay
80.00
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
Human Coronavirus 229E
Human Coronavirus OC43
Human Coronavirus NL63
Metapneumovirus 27 Type A2
Human Parainfluenza Virus 3
Respiratory Syncytial Virus B
Chlamydophila pneumoniae
Haemophilus influenzae
Moraxella catarrhalis
Mycoplasma pneumoniae
Human Coronavirus HKU1 synth
Human Coronavirus MERSsynth

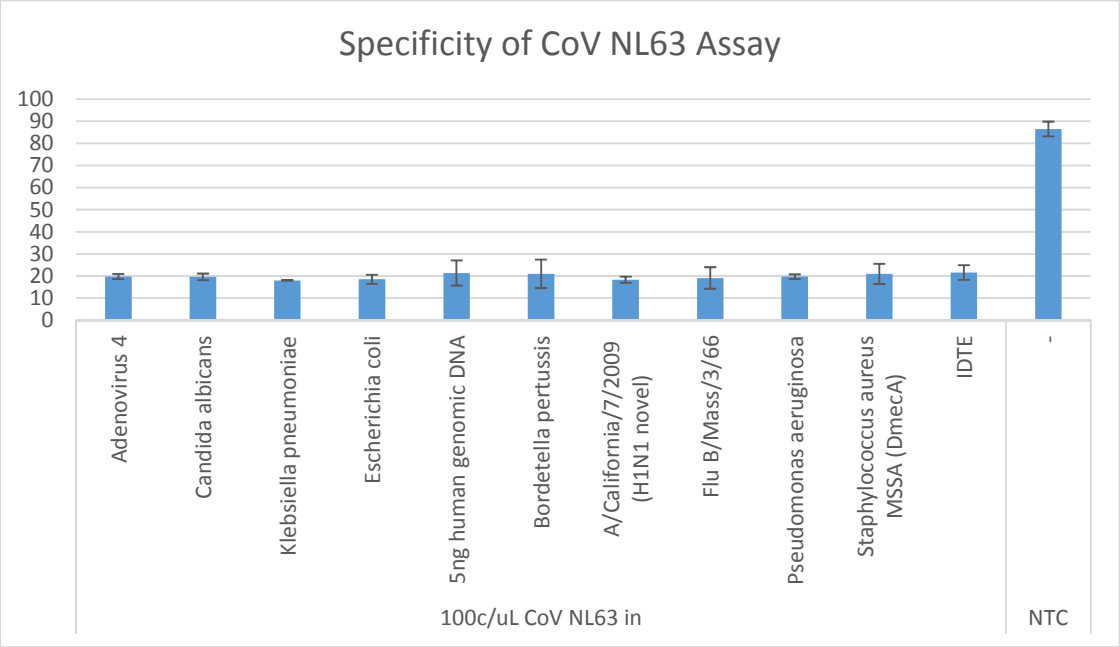

Cross Reactivity of MERS-CoV Assay
Average Inflection time (min)
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
Adenovirus 4
Candida albicans
Klebsiella pneumoniae
Escherichia coli
5ng human genomic DNA
Bordetella pertussis
Flu A(H1N1)
Flu B
Pseudomonas aeruginosa
Staphylococcus aureus
Streptococcus pyogenes
Coronavirus MERS synth.
NTC

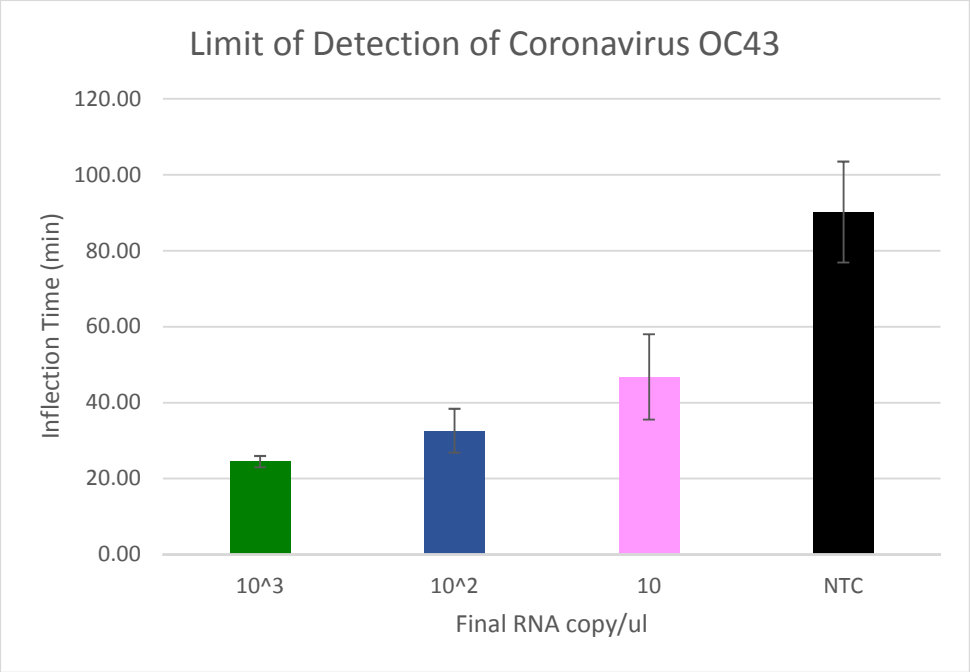

Specificity of MERS-CoV Assay
70
60
50
40
30
20
10
0
Adenovirus 4
Candida albicans
Klebsiella pneumoniae
Escherichia coli
5ng human genomic DNA
Bordetella pertussis
A/Solomon Islands/3/2006 (H1N1)
B/Russia/69
Pseudomonas aeruginosa
Staphylococcus aureus MSSA (DmecA)
IDTE
-
1000c/uL CoV MERS in
NTC

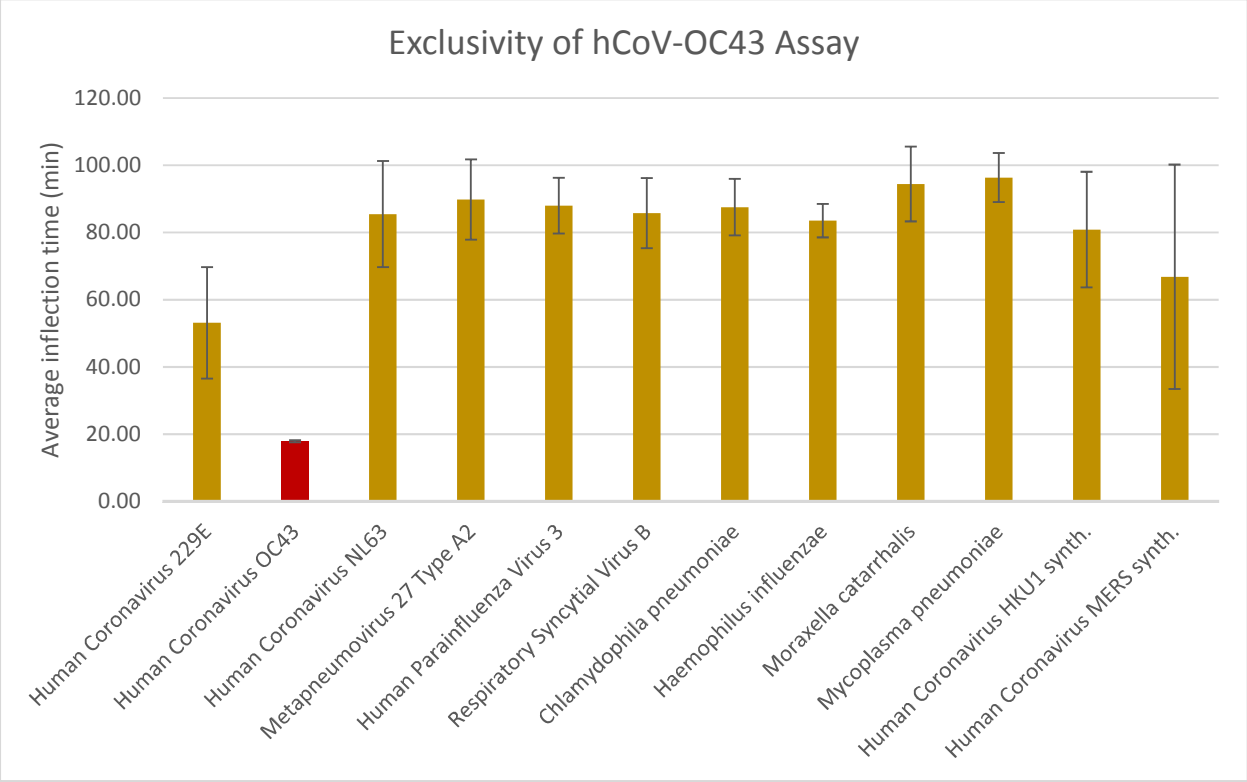

Limit of Detection of CoV NL63
Inflection Time (min)
80.00
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
10^3
10^2
10
NTC
Final RNA copy/ul

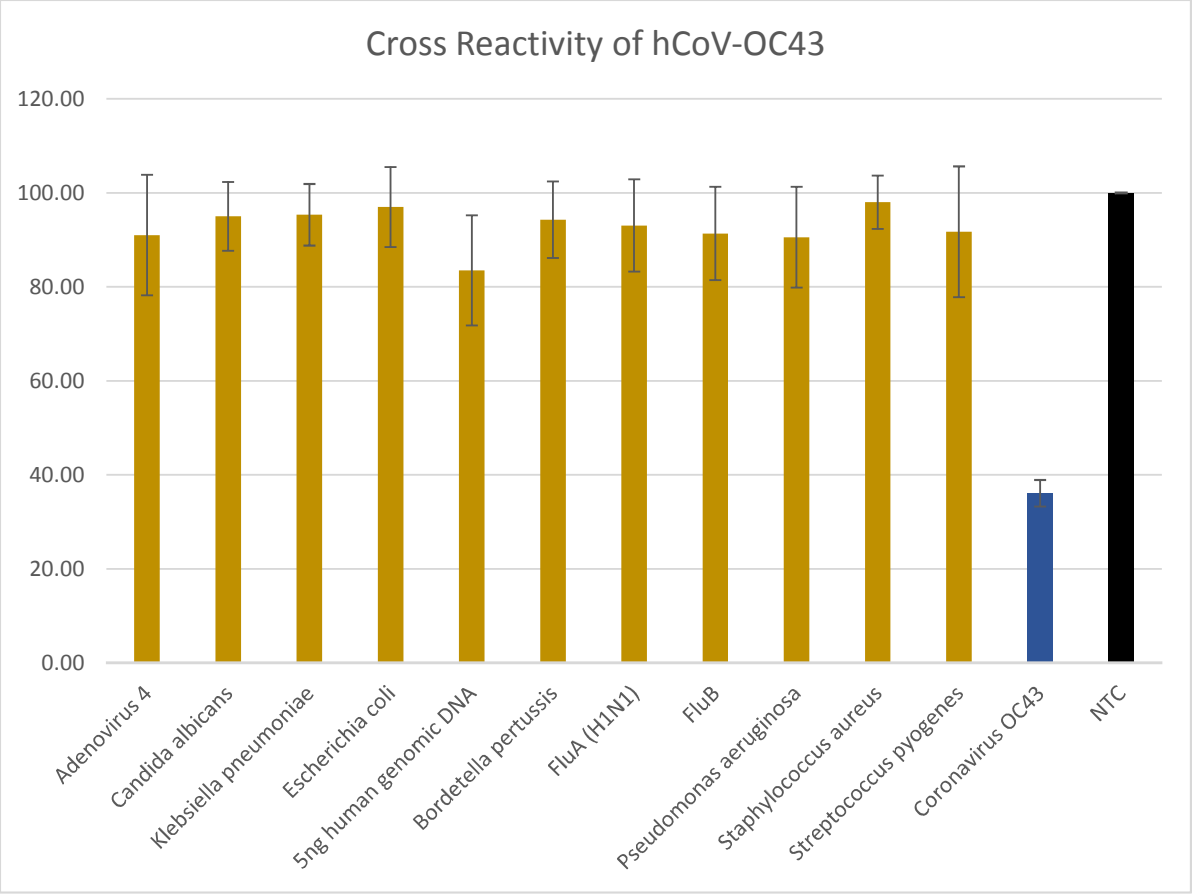

Specificity of hCOV-NL63 Assay
Average Inflection time (min)
120.00
100.00
80.00
60.00
40.00
20.00
0.00
Human Coronavirus 229E
Human Coronavirus OC43
Human Coronavirus NL63
Metapneumovirus 27 Type A2
Human Parainfluenza Virus 3
Respiratory Syncytial Virus B
Chlamydophila pneumoniae
Haemophilus influenzae
Moraxella catarrhalis
Mycoplasma pneumoniae
Human Coronavirus HKU1 synth.
Human Coronavirus MERS synth.

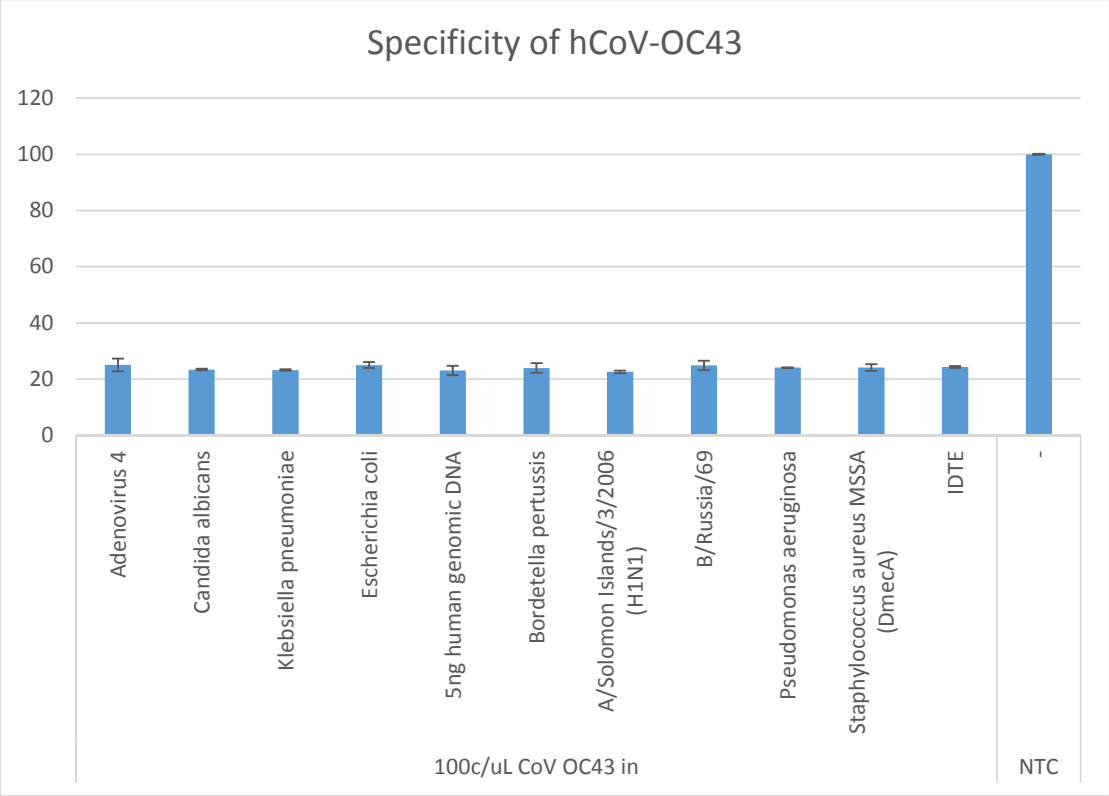

Cross Reactivity of CoV NL63 Assay
Average inflection time (min)
100.00
90.00
80.00
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
Adenovirus 4
Candida albicans
Klebsiella pneumoniae
Escherichia coli
5ng human genomic DNA
Bordetella pertussis
FluA (H1N1)
FluB
Pseudomonas aeruginosa
Staphylococcus aureus
Streptococcus pyogenes
Coronavirus NL63
NTC

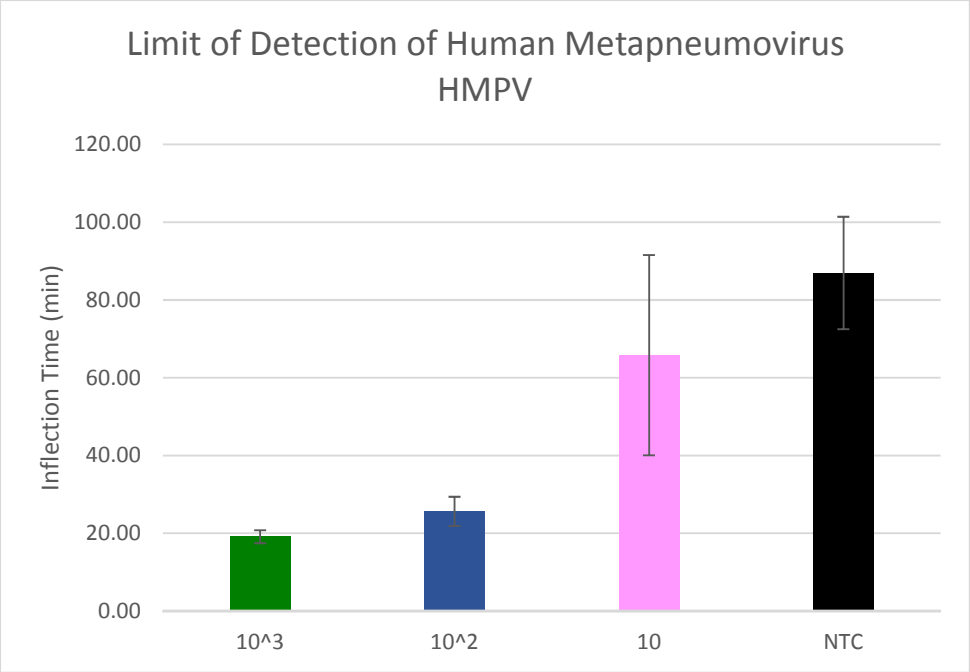

Specificity of CoV NL63 Assay
100
90
80
70
60
50
40
30
20
10
0
Adenovirus 4
Candida albicans
Klebsiella pneumoniae
Escherichia coli
5ng human genomic DNA
Bordetella pertussis
A/California/7/2009 (H1N1 novel)
Flu B/Mass/3/66
Pseudomonas aeruginosa
Staphylococcus aureus MSSA (DmecA)
IDTE
-
100c/uL CoV NL63 in
NTC

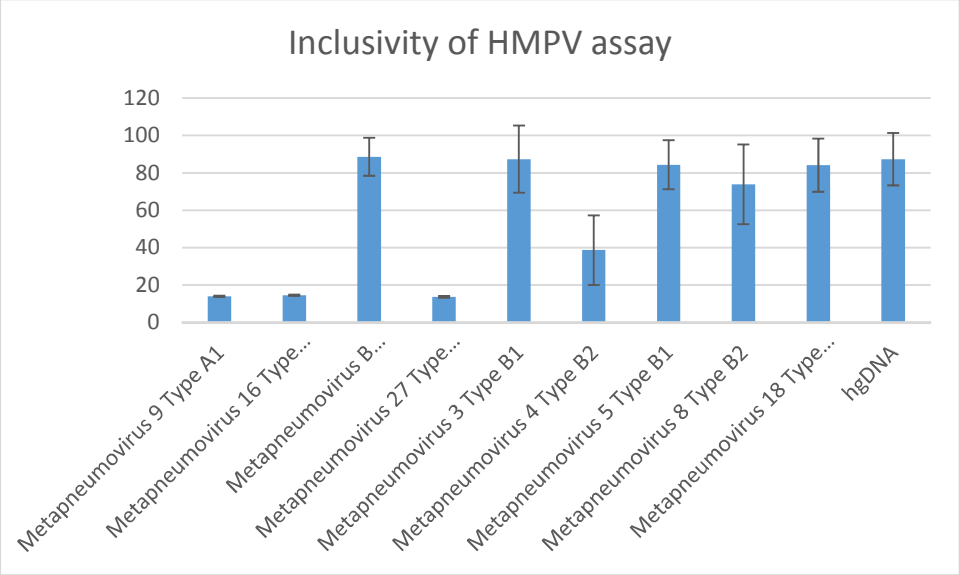

Limit of Detection of Coronavirus OC43
120.00
100.00
80.00
60.00
40.00
20.00
0.00
Inflection Time (min)
10^3
10^2
10
NTC
Final RNA copy/ul

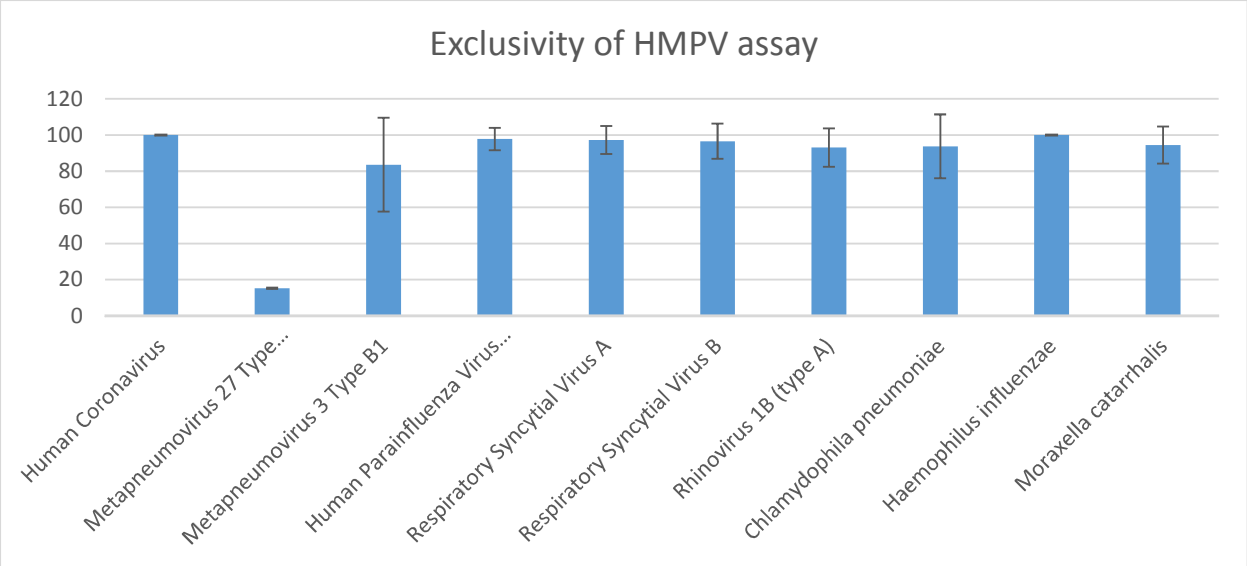

Exclusivity of hCoV-OC43 Assay
120.00
100.00
80.00
60.00
40.00
20.00
0.00
Average inflection time (min)
Human Coronavirus 229E
Human Coronavirus OC43
Human Coronavirus NL63
Metapneumovirus 27 Type A2
Human Parainfluenza Virus 3
Respiratory Syncytial Virus B
Chlamydophila pneumoniae
Haemophilus influenzae
Moraxella catarrhalis
Mycoplasma pneumoniae
Human Coronavirus HKU1 synth.
Human Coronavirus MERS synth.

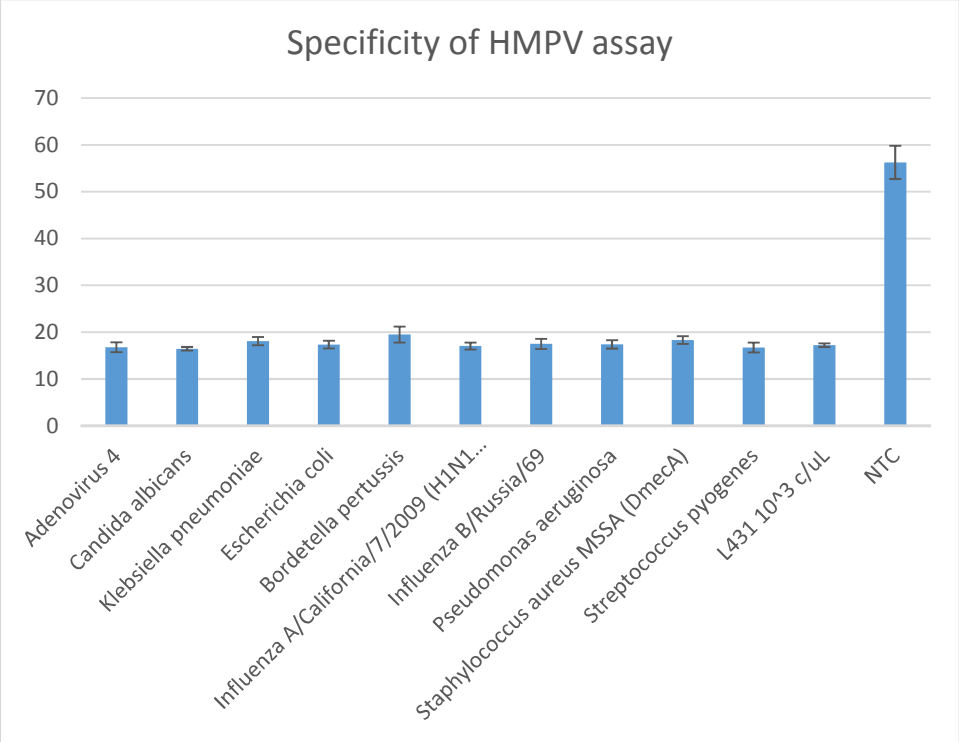

Cross Reactivity of hCoV-OC43
120.00
100.00
80.00
60.00
40.00
20.00
0.00
Adenovirus 4
Candida albicans
Klebsiella pneumoniae
Escherichia coli
5ng human genomic DNA
Bordetella pertussis
FluA (H1N1)
FluB
Pseudomonas aeruginosa
Staphylococcus aureus
Streptococcus pyogenes
Coronavirus OC43
NTC

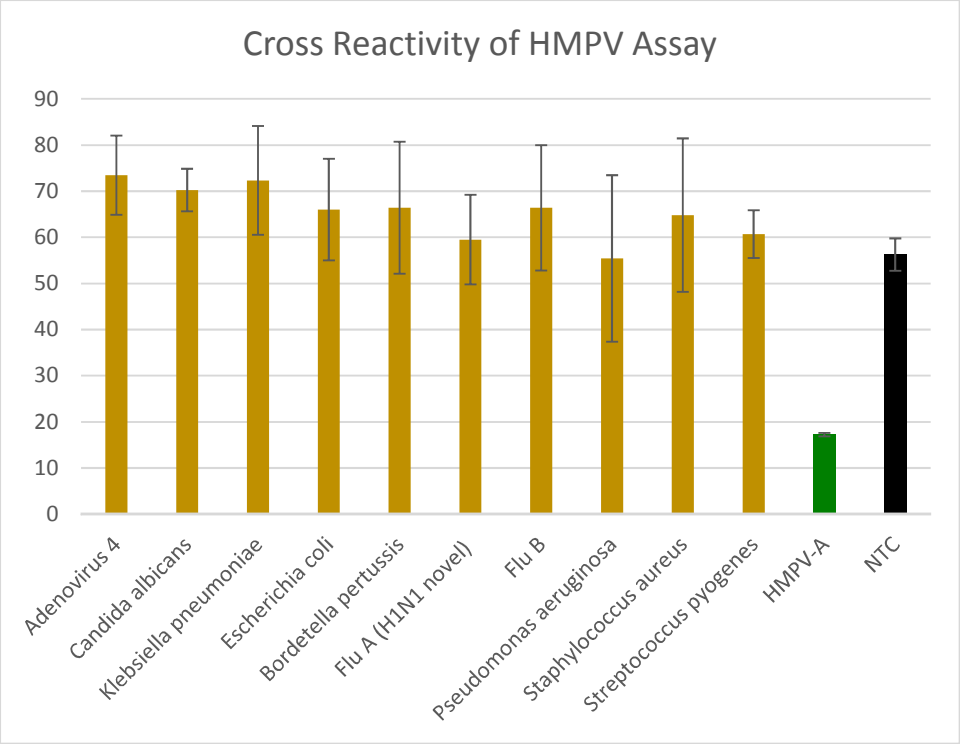

Specificity of hCoV-OC43
120
100
80
60
40
20
0
Adenovirus 4
Candida albicans
Klebsiella pneumoniae
Escherichia coli
5ng human genomic DNA
Bordetella pertussis
A/Solomon Islands/3/2006 (H1N1)
B/Russia/69
Pseudomonas aeruginosa
Staphylococcus aureus MSSA (DmecA)
IDTE
-
100c/uL CoV OC43 in
NTC

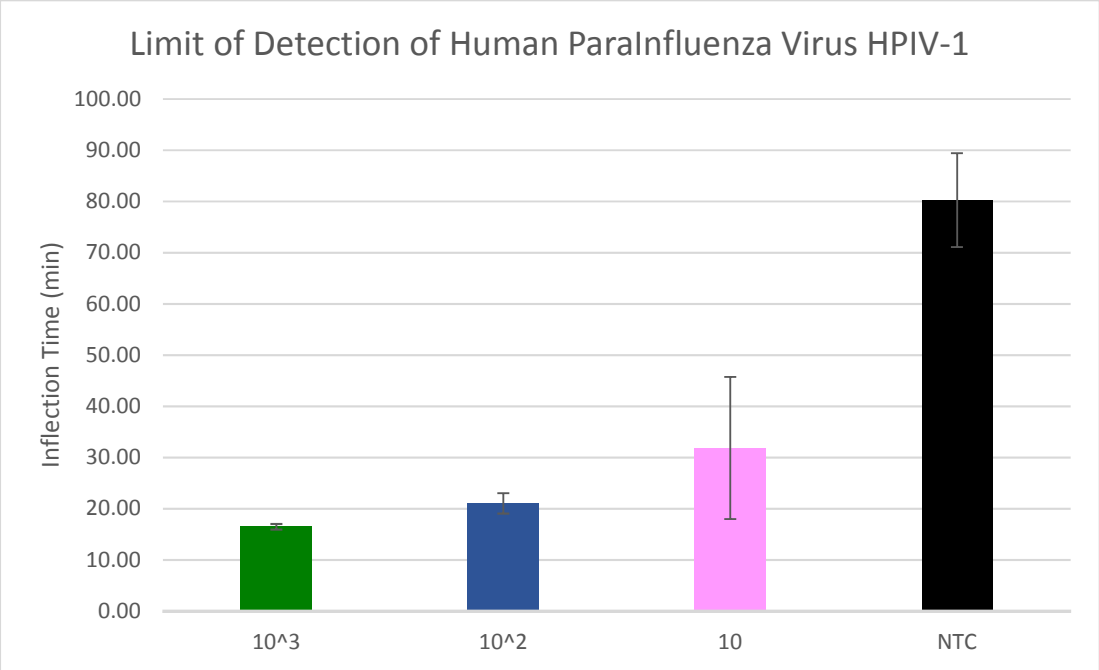

Limit of Detection of Human Metapneumovirus HMPV
Inflection Time (min)
120.00
100.00
80.00
60.00
40.00
20.00
0.00
10^3
10^2
10
NTC

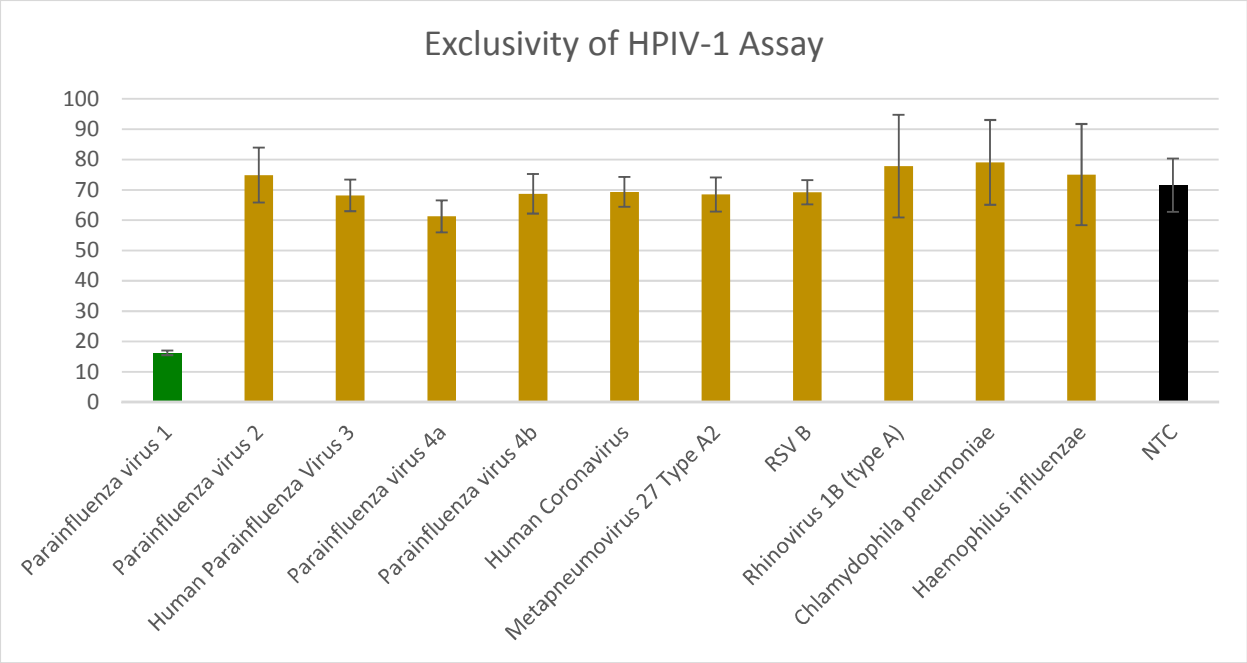

Inclusivity of HMPV assay
120
100
80
60
40
20
0
Metapneumovirus 9 Type A1
Metapneumovirus 16 Type...
Metapneumovirus B...
Metapneumovirus 27 Type...
Metapneumovirus 3 Type B1
Metapneumovirus 4 Type B2
Metapneumovirus 5 Type B1
Metapneumovirus 8 Type B2
Metapneumovirus 18 Type...
hgDNA

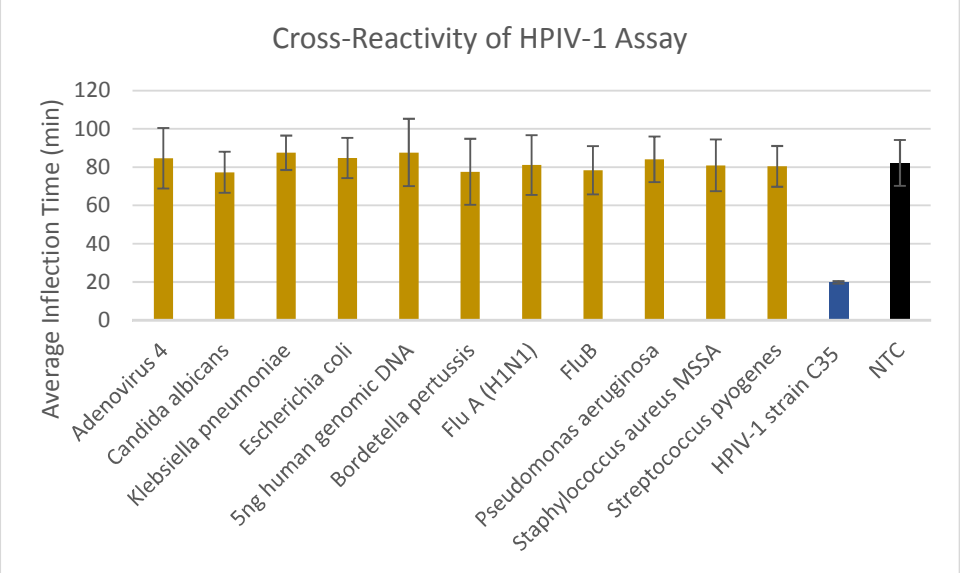

Exclusivity of HMPV assay
120
100
80
60
40
20
0
Human Coronavirus
Metapneumovirus 27 Type...
Metapneumovirus 3 Type B1
Human Parainfluenza Virus...
Respiratory Syncytial Virus A
Respiratory Syncytial Virus B
Rhinovirus 1B (type A)
Chlamydophila pneumoniae
Haemophilus influenzae
Moraxella catarrhalis

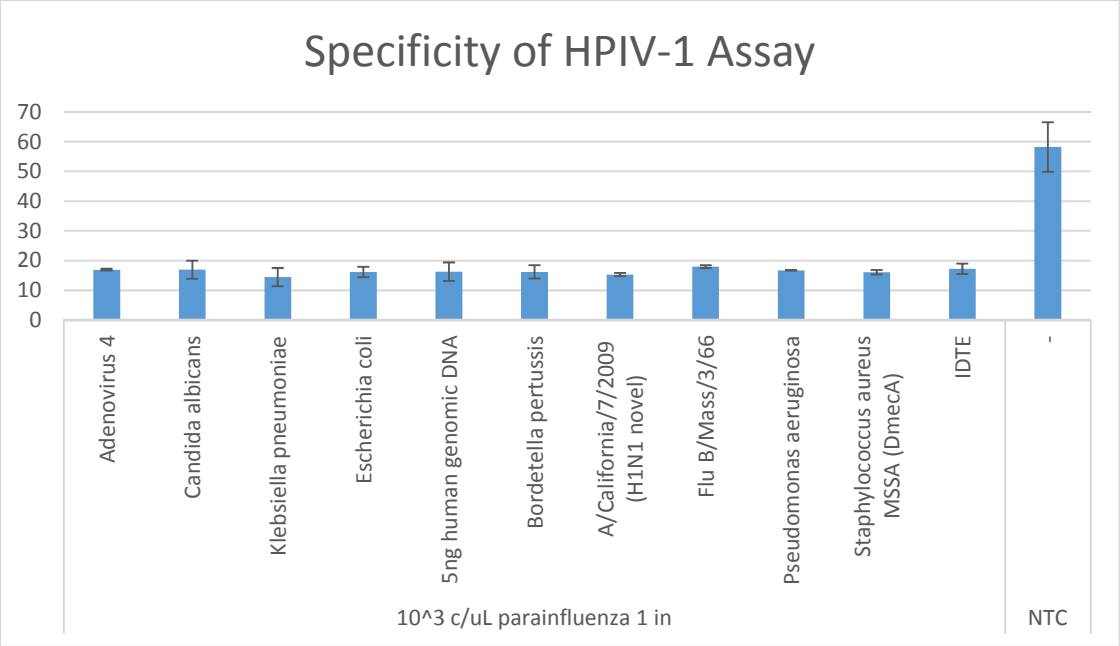

Specificity of HMPV assay
70
60
50
40
30
20
10
0
Adenovirus 4
Candida albicans
Klebsiella pneumoniae
Escherichia coli
Bordetella pertussis
Influenza A/California/7/2009 (H1N1...
Influenza B/Russia/69
Pseudomonas aeruginosa
Staphylococcus aureus MSSA (DmecA)
Streptococcus pyogenes
L431 10^3 c/uL
NTC

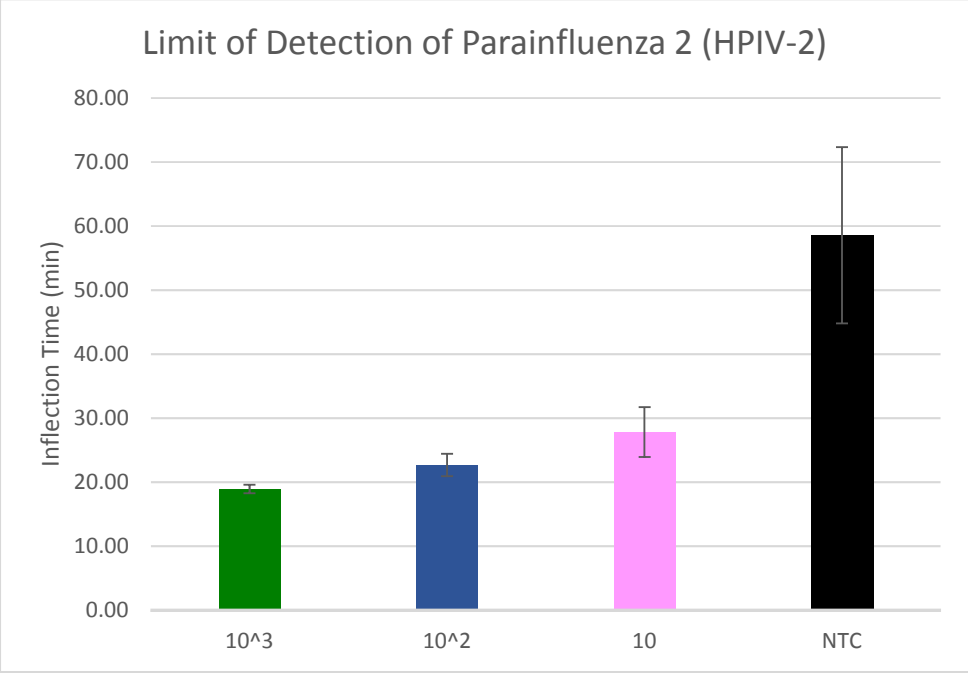

Cross Reactivity of HMPV Assay
90
80
70
60
50
40
30
20
10
0
Adenovirus 4
Candida albicans
Klebsiella pneumoniae
Escherichia coli
Bordetella pertussis
Flu A (H1N1 novel)
Flu B
Pseudomonas aeruginosa
Staphylococcus aureus
Streptococcus pyogenes
HMPV-A
NTC

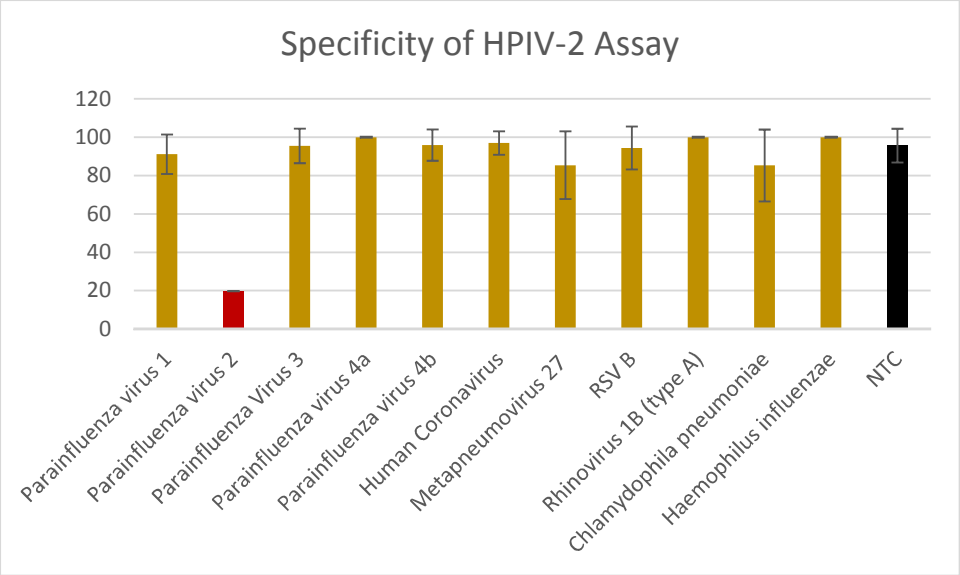

Limit of Detection of Human ParaInfluenza Virus HPIV-1
Inflection Time (min)
100.00
90.00
80.00
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
10^3
10^2
10
NTC

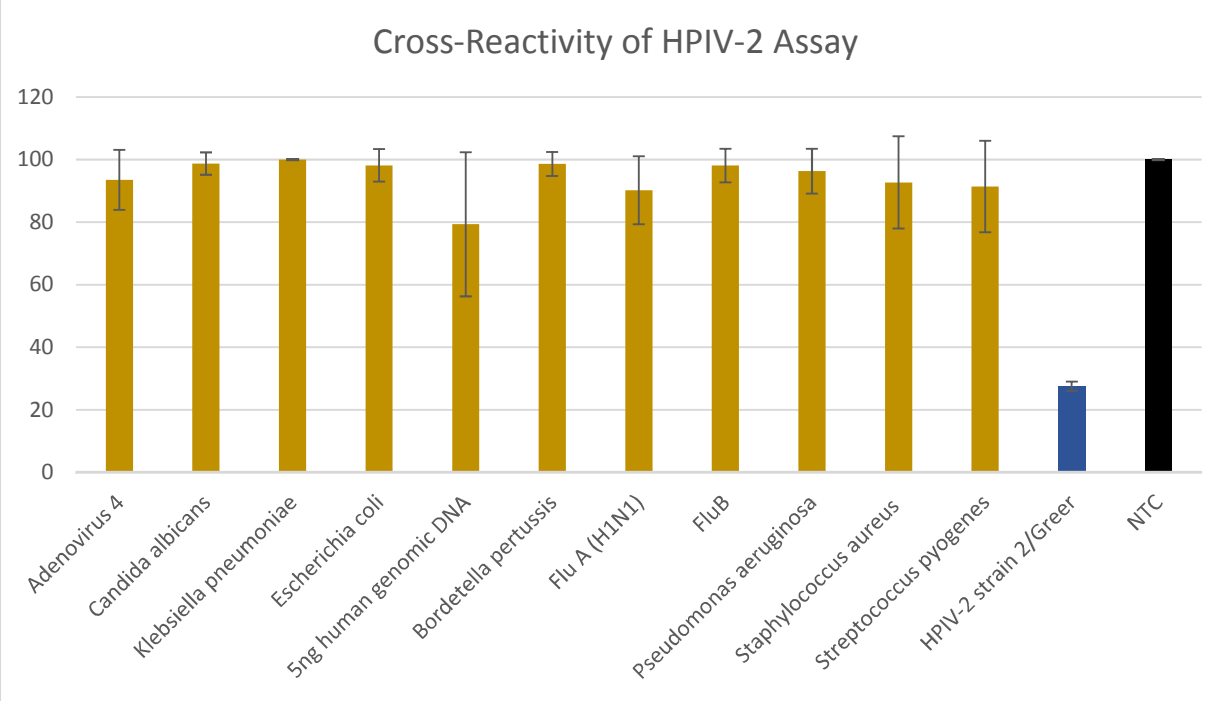

Exclusivity of HPIV-1 Assay
100
90
80
70
60
50
40
30
20
10
0
Parainfluenza virus 1
Parainfluenza virus 2
Human Parainfluenza Virus 3
Parainfluenza virus 4a
Parainfluenza virus 4b
Human Coronavirus
Metapneumovirus 27 Type A2
RSV B
Rhinovirus 1B (type A)
Chlamydophila pneumoniae
Haemophilus influenzae
NTC

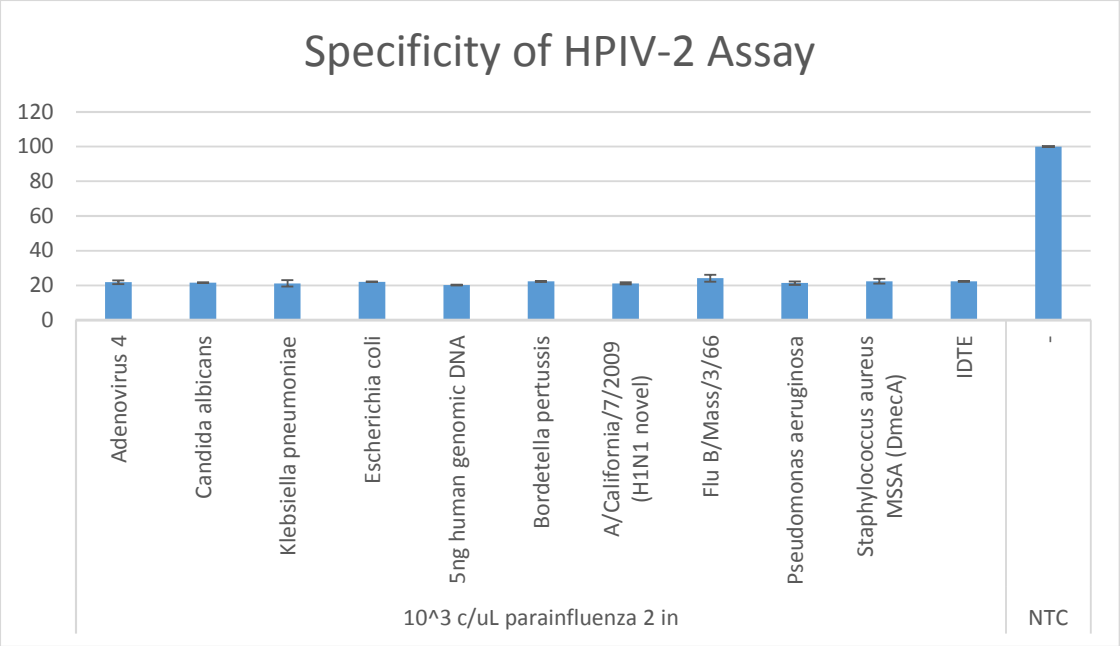

Cross-Reactivity of HPIV-1 Assay
Average Inflection Time (min)
120
100
80
60
40
20
0
Adenovirus 4
Candida albicans
Klebsiella pneumoniae
Escherichia coli
5ng human genomic DNA
Bordetella pertussis
Flu A (H1N1)
FluB
Pseudomonas aeruginosa
Staphylococcus aureus MSSA
Streptococcus pyogenes
HPIV-1 strain C35
NTC

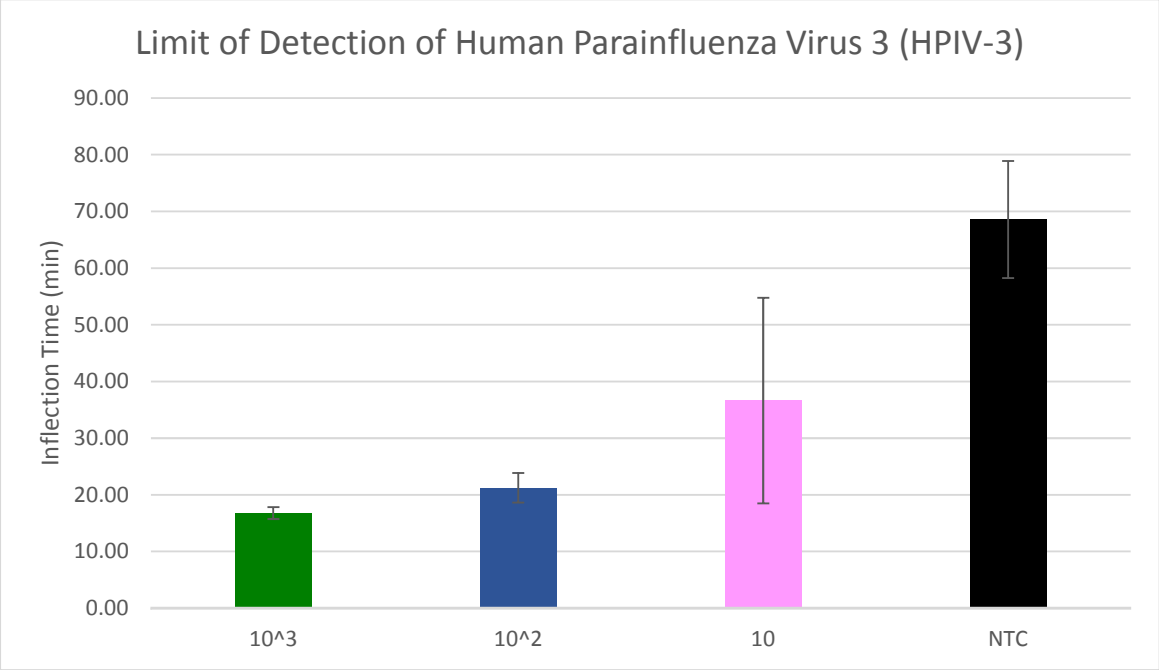

Specificity of HPIV-1 Assay
70
60
50
40
30
20
10
0
Adenovirus 4
Candida albicans
Klebsiella pneumoniae
Escherichia coli
5ng human genomic DNA
Bordetella pertussis
A/California/7/2009 (H1N1 novel)
Flu B/Mass/3/66
Pseudomonas aeruginosa
Staphylococcus aureus MSSA (DmecA)
IDTE
-
10^3 c/uL parainfluenza 1 in
NTC

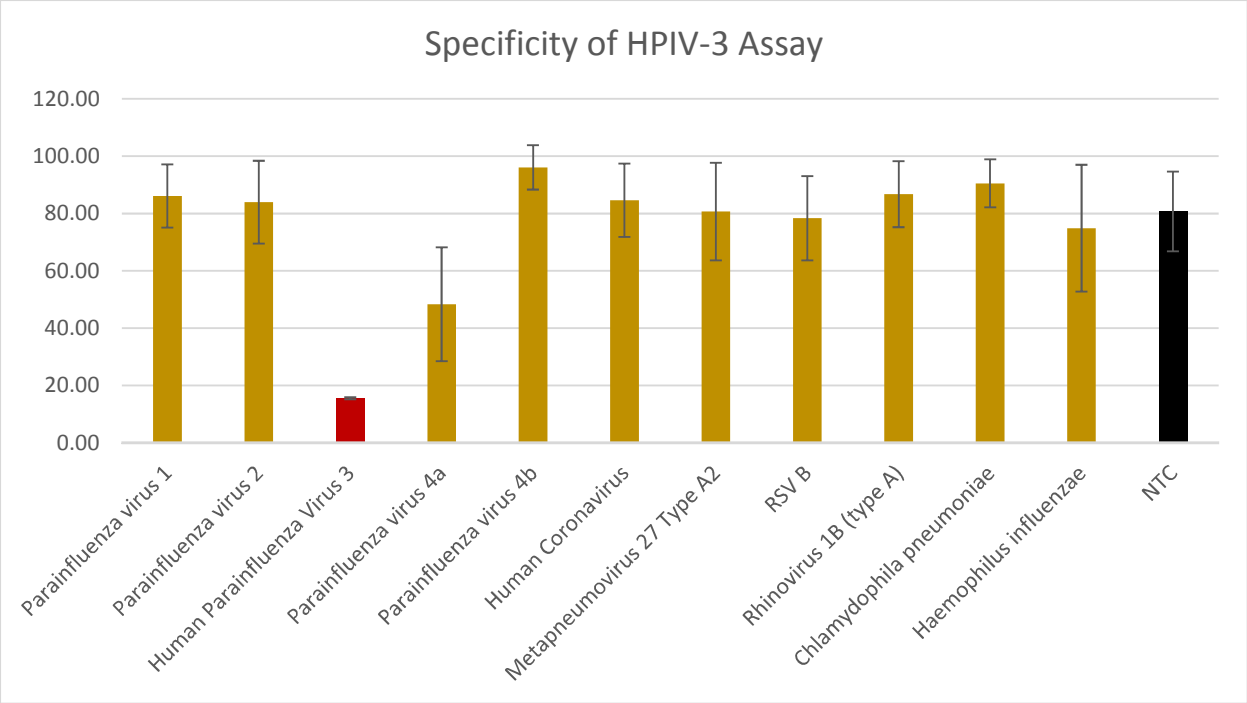

Limit of Detection of Parainfluenza 2 (HPIV-2)
Inflection Time (min)
80.00
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
10^3
10^2
10
NTC

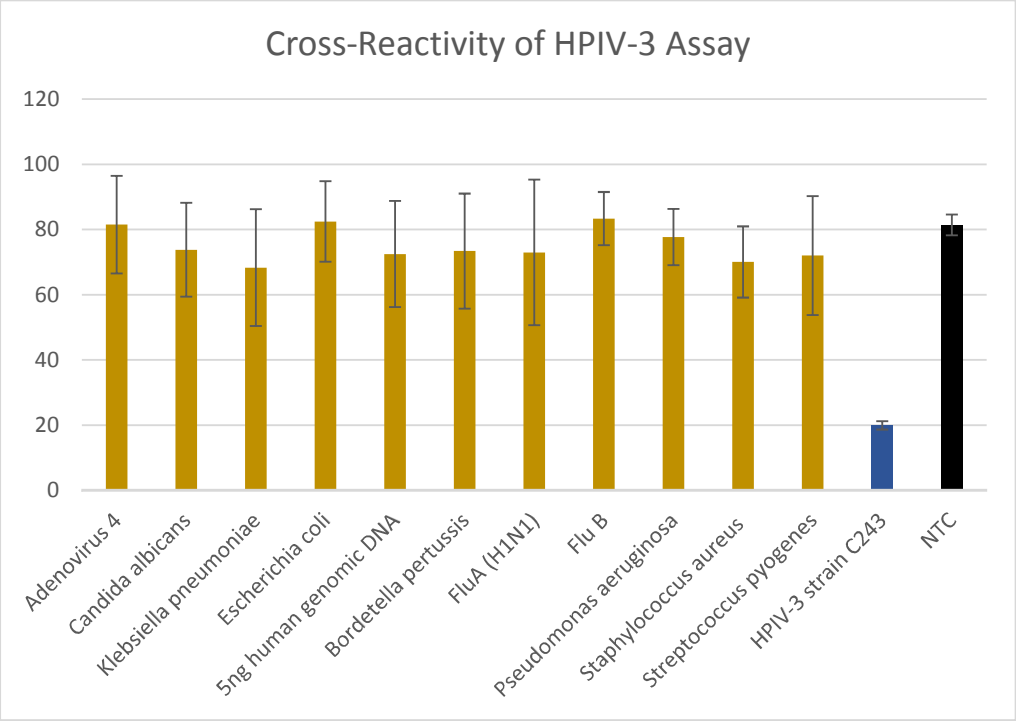

Specificity of HPIV-2 Assay
120
100
80
60
40
20
0
Parainfluenza virus 1
Parainfluenza virus 2
Parainfluenza Virus 3
Parainfluenza virus 4a
Parainfluenza virus 4b
Human Coronavirus
Metapneumovirus 27
RSV B
Rhinovirus 1B (type A)
Chlamydophila pneumoniae
Haemophilus influenzae
NTC

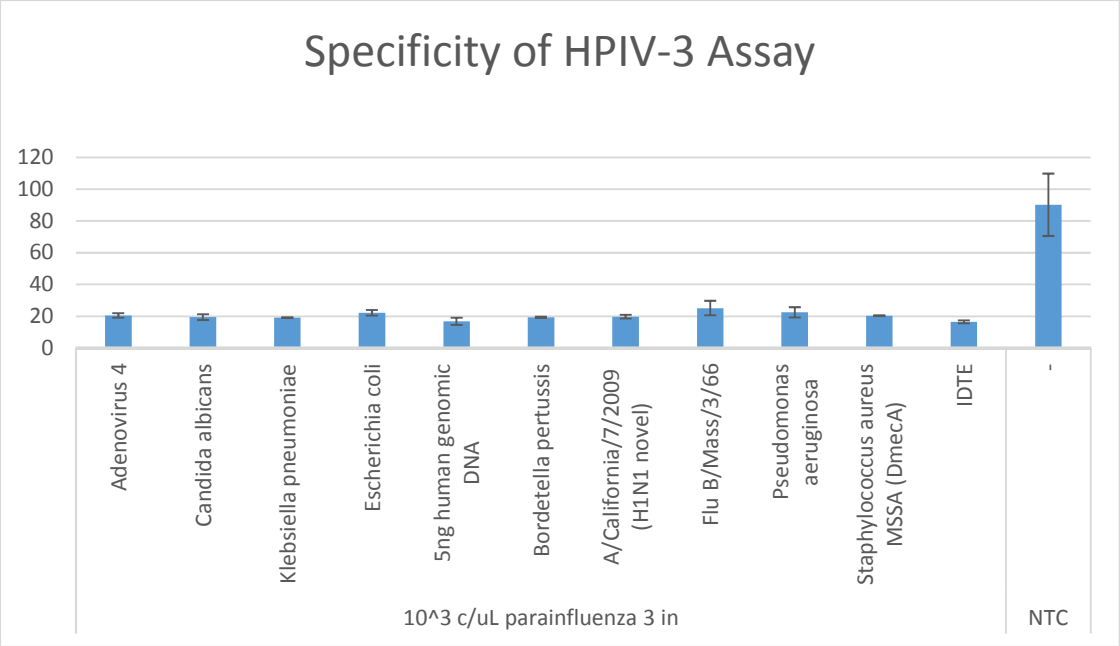

Cross-Reactivity of HPIV-2 Assay
120
100
80
60
40
20
0
Adenovirus 4
Candida albicans
Klebsiella pneumoniae
Escherichia coli
5ng human genomic DNA
Bordetella pertussis
Flu A (H1N1)
FluB
Pseudomonas aeruginosa
Staphylococcus aureus
Streptococcus pyogenes
HPIV-2 strain 2/Greer
NTC

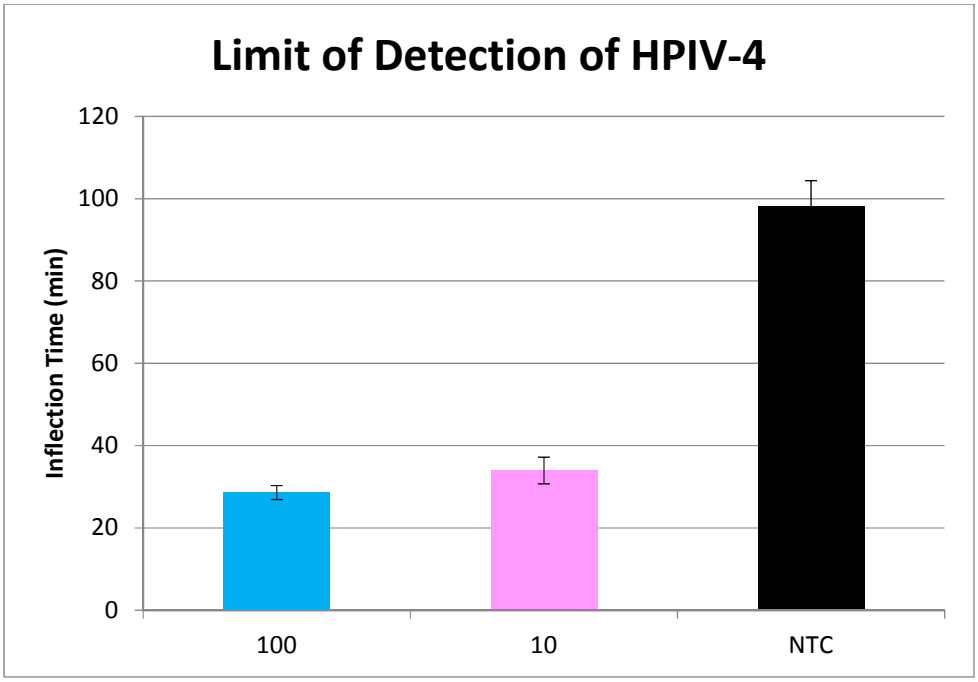

Specificity of HPIV-2 Assay
120
100
80
60
40
20
0
Adenovirus 4
Candida albicans
Klebsiella pneumoniae
Escherichia coli
5ng human genomic DNA
Bordetella pertussis
A/California/7/2009 (H1N1 novel)
Flu B/Mass/3/66
Pseudomonas aeruginosa
Staphylococcus aureus MSSA (DmecA)
IDTE
-
10^3 c/uL parainfluenza 2 in
NTC

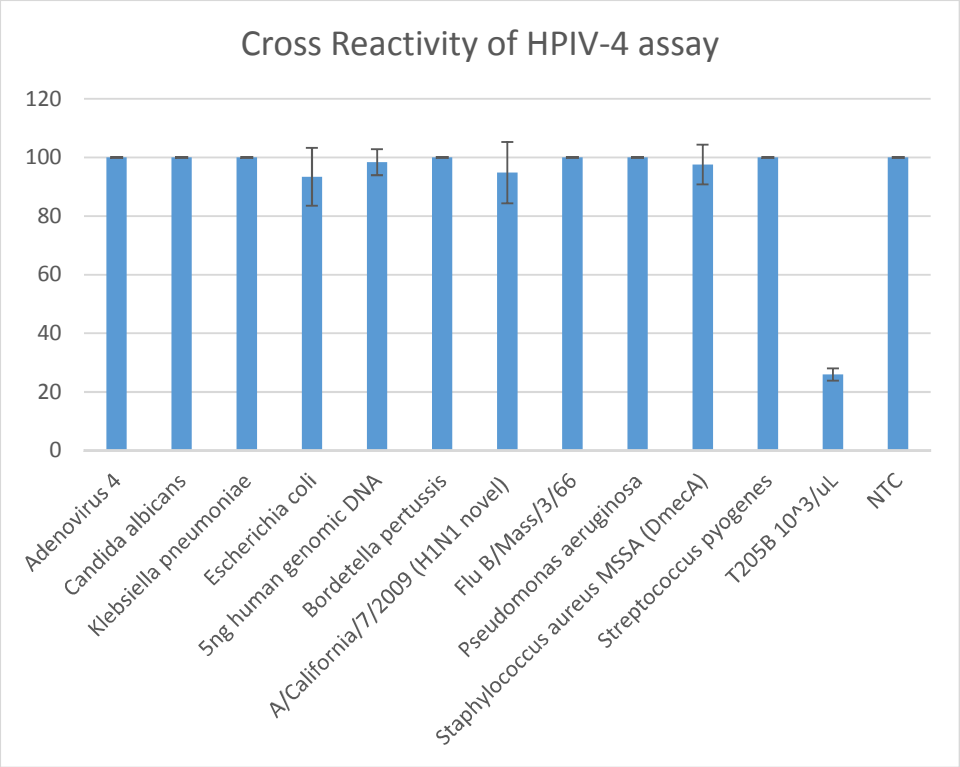

Limit of Detection of Human Parainfluenza Virus 3 (HPIV-3)
Inflection Time (min)
90.00
80.00
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
10^3
10^2
10
NTC

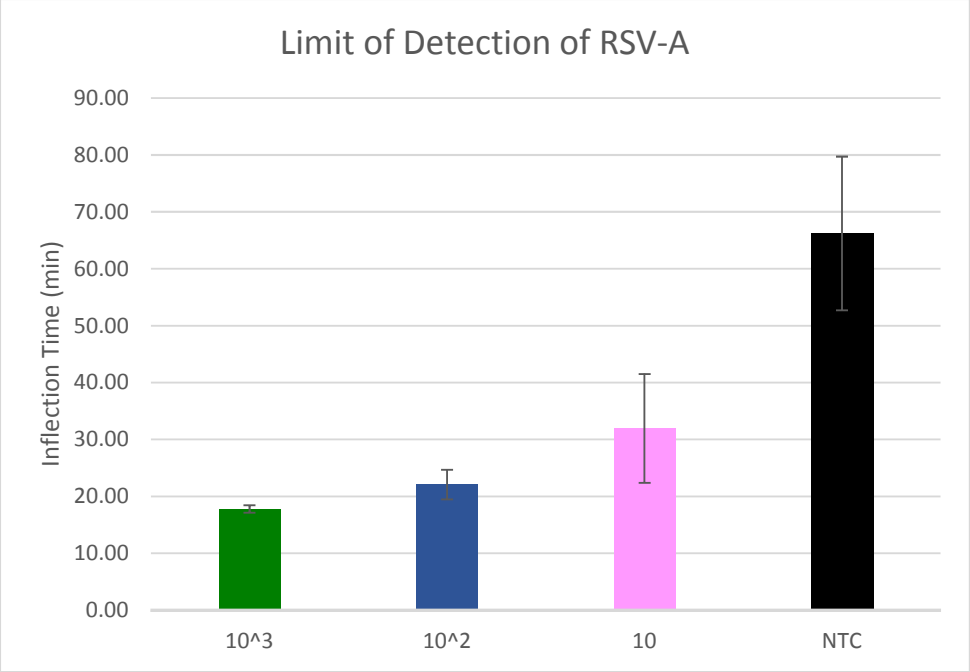

Specificity of HPIV-3 Assay
120.00
100.00
80.00
60.00
40.00
20.00
0.00
Parainfluenza virus 1
Parainfluenza virus 2
Human Parainfluenza Virus 3
Parainfluenza virus 4a
Parainfluenza virus 4b
Human Coronavirus
Metapneumovirus 27 Type A2
RSV B
Rhinovirus 1B (type A)
Chlamydophila pneumoniae
Haemophilus influenzae
NTC

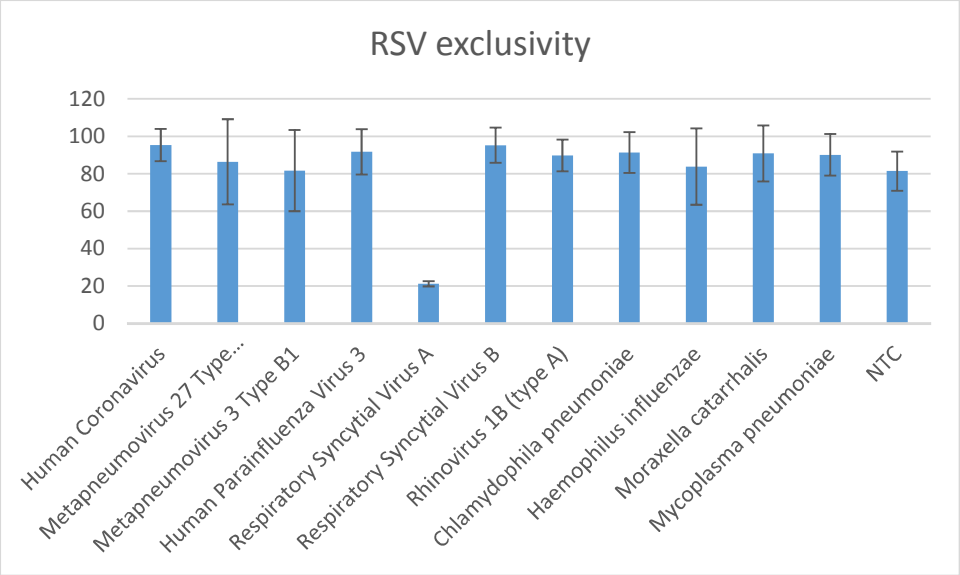

Cross-Reactivity of HPIV-3 Assay
120
100
80
60
40
20
0
Adenovirus 4
Candida albicans
Klebsiella pneumoniae
Escherichia coli
5ng human genomic DNA
Bordetella pertussis
FluA (H1N1)
Flu B
Pseudomonas aeruginosa
Staphylococcus aureus
Streptococcus pyogenes
HPIV-3 strain C243
NTC

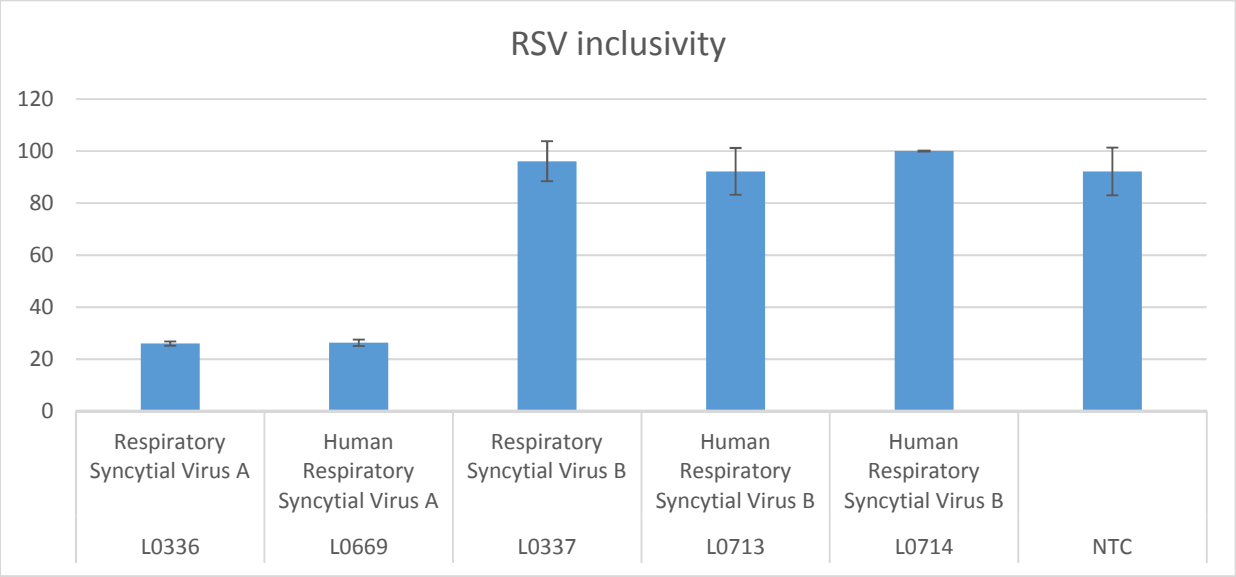

Specificity of HPIV-3 Assay
120
100
80
60
40
20
0
Adenovirus 4
Candida albicans
Klebsiella pneumoniae
Escherichia coli
5ng human genomic DNA
Bordetella pertussis
A/California/7/2009 (H1N1 novel)
Flu B/Mass/3/66
Pseudomonas aeruginosa
Staphylococcus aureus MSSA (DmecA)
IDTE
-
10^3 c/uL parainfluenza 3 in
NTC

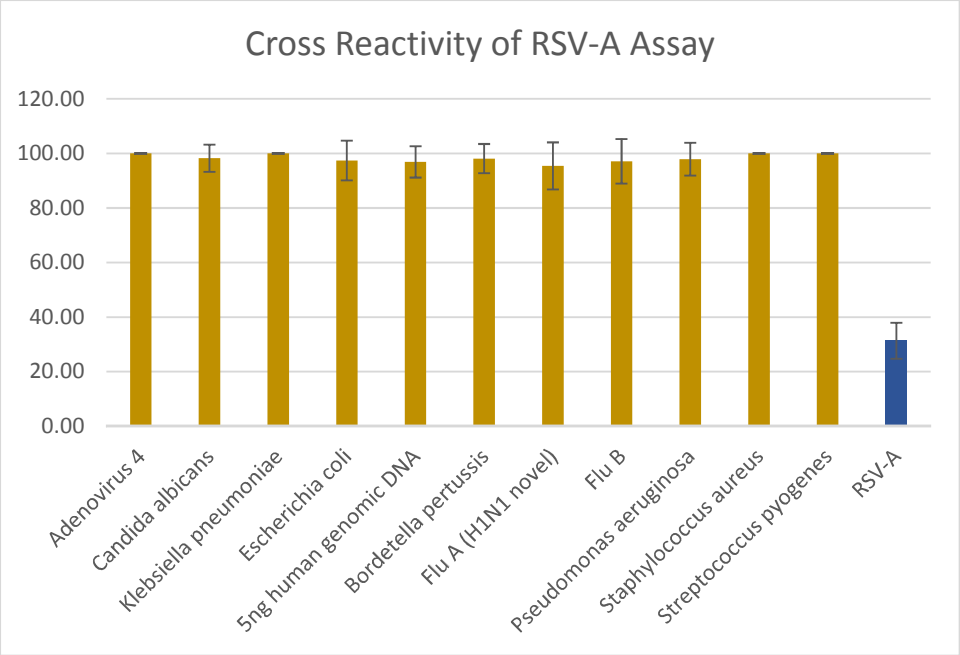

Limit of Detection of HPIV-4
Inflection Time (min)
120
100
80
60
40
20
0
100
10
NTC

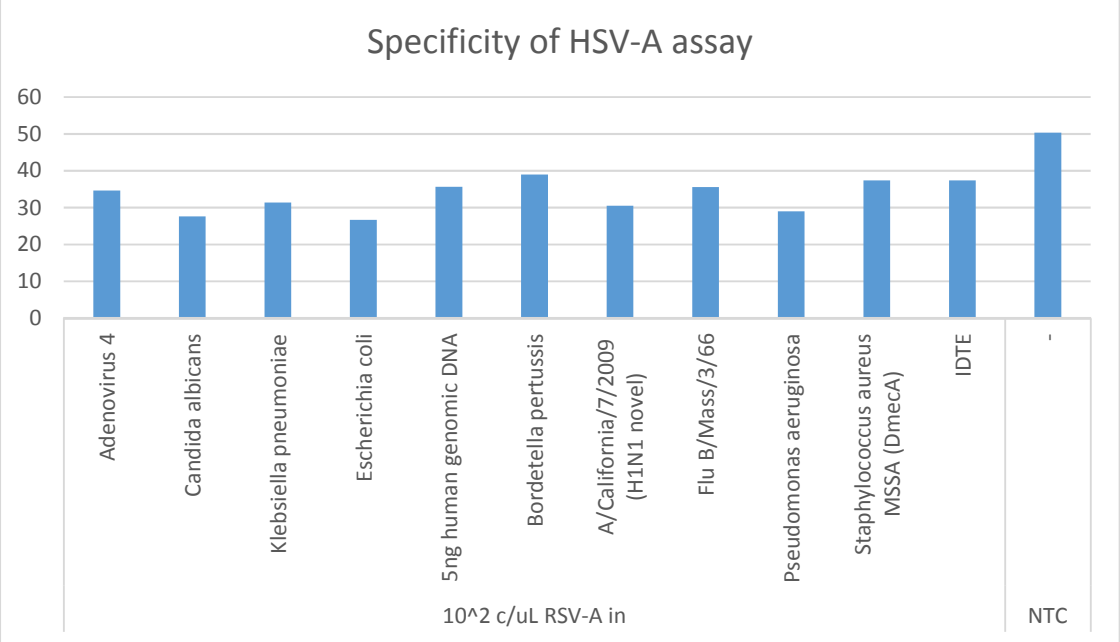

Cross Reactivity of HPIV-4 assay
120
100
80
60
40
20
0
Adenovirus 4
Candida albicans
Klebsiella pneumoniae
Escherichia coli
5ng human genomic DNA
Bordetella pertussis
A/California/7/2009 (H1N1 novel)
Flu B/Mass/3/66
Pseudomonas aeruginosa
Staphylococcus aureus MSSA (DmecA)
Streptococcus pyogenes
T205B 10^3/uL
NTC

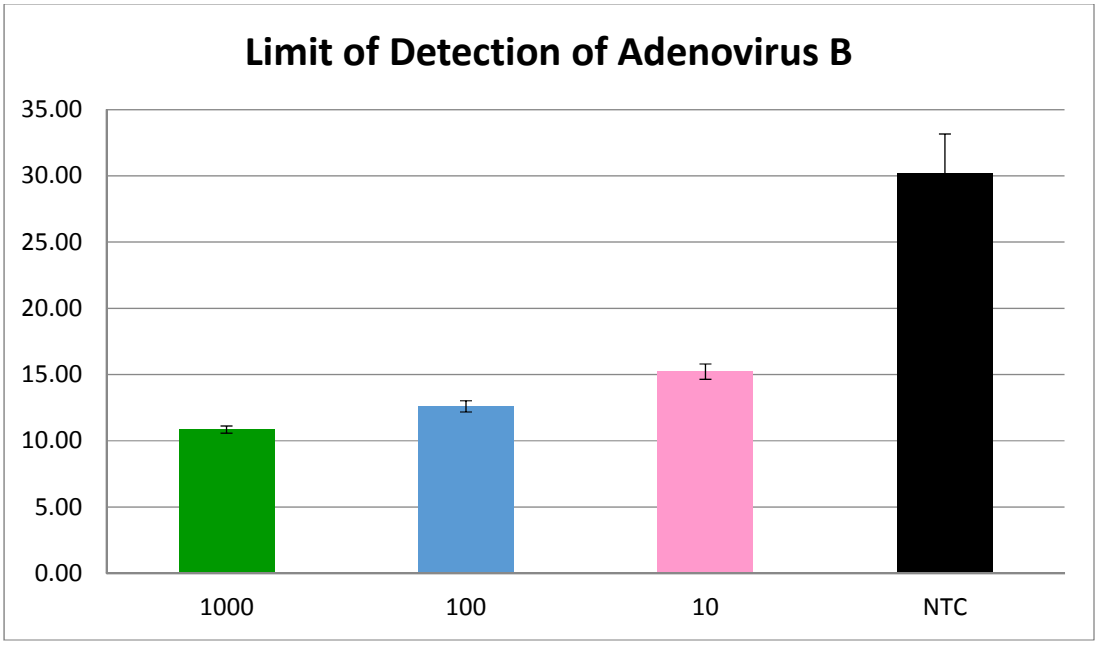

Limit of Detection of RSV-A
Inflection Time (min)
90.00
80.00
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
10^3
10^2
10
NTC

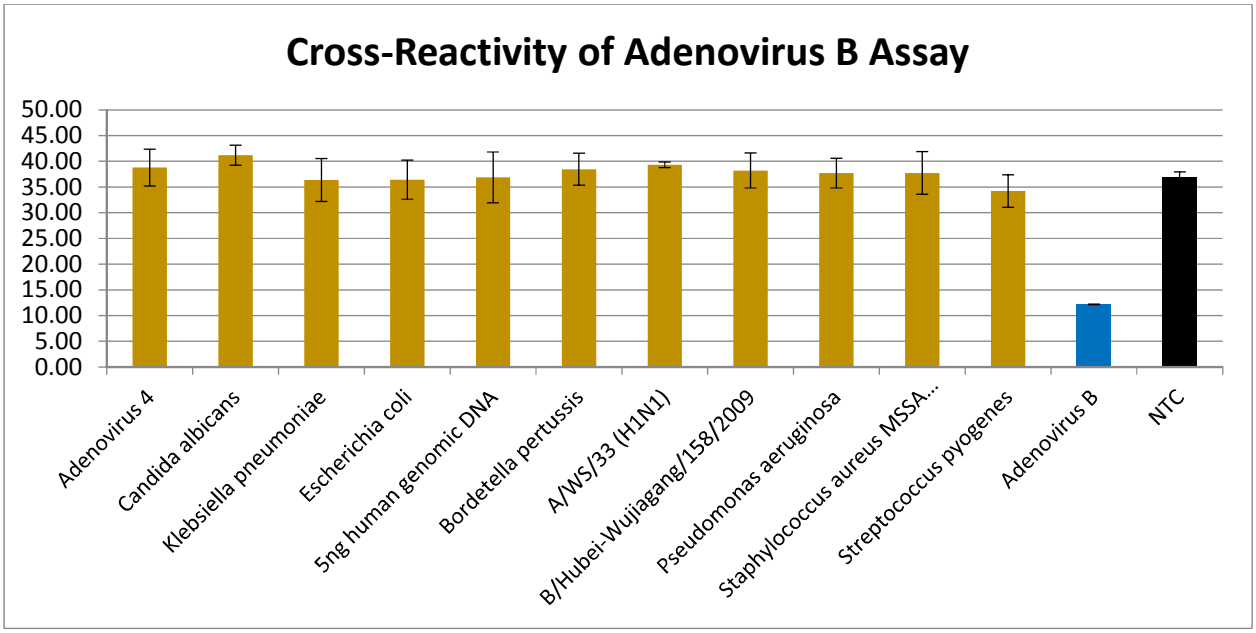

RSV exclusivity
120
100
80
60
40
20
0
Human Coronavirus
Metapneumovirus 27 Type...
Metapneumovirus 3 Type B1
Human Parainfluenza Virus 3
Respiratory Syncytial Virus A
Respiratory Syncytial Virus B
Rhinovirus 1B (type A)
Chlamydophila pneumoniae
Haemophilus influenzae
Moraxella catarrhalis
Mycoplasma pneumoniae
NTC

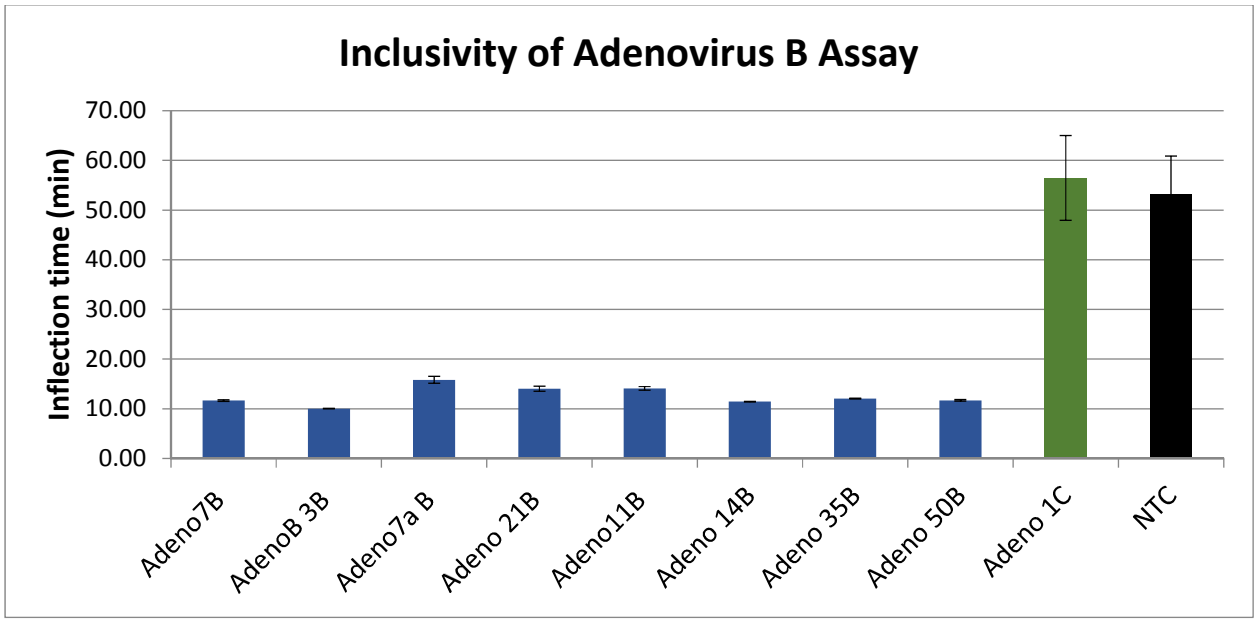

RSV inclusivity
120
100
80
60
40
20
0
Respiratory Syncytial Virus A
L0336
Human Respiratory Syncytial Virus A
L0669
Respiratory Syncytial Virus B
L0337
Human Respiratory Syncytial Virus B
L0713
Human Respiratory Syncytial Virus B
L0714
NTC

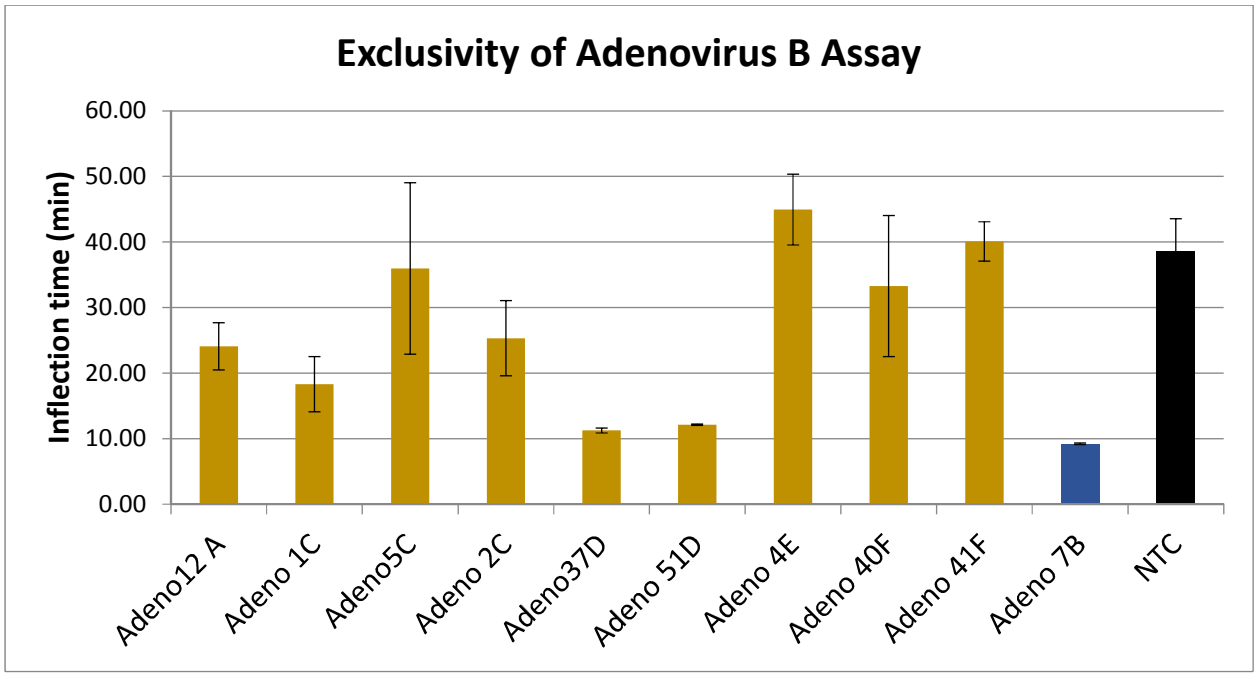

Cross Reactivity of RSV-A Assay
120.00
100.00
80.00
60.00
40.00
20.00
0.00
Adenovirus 4
Candida albicans
Klebsiella pneumoniae
Escherichia coli
5ng human genomic DNA
Bordetella pertussis
Flu A (H1N1 novel)
Flu B
Pseudomonas aeruginosa
Staphylococcus aureus
Streptococcus pyogenes
RSV-A

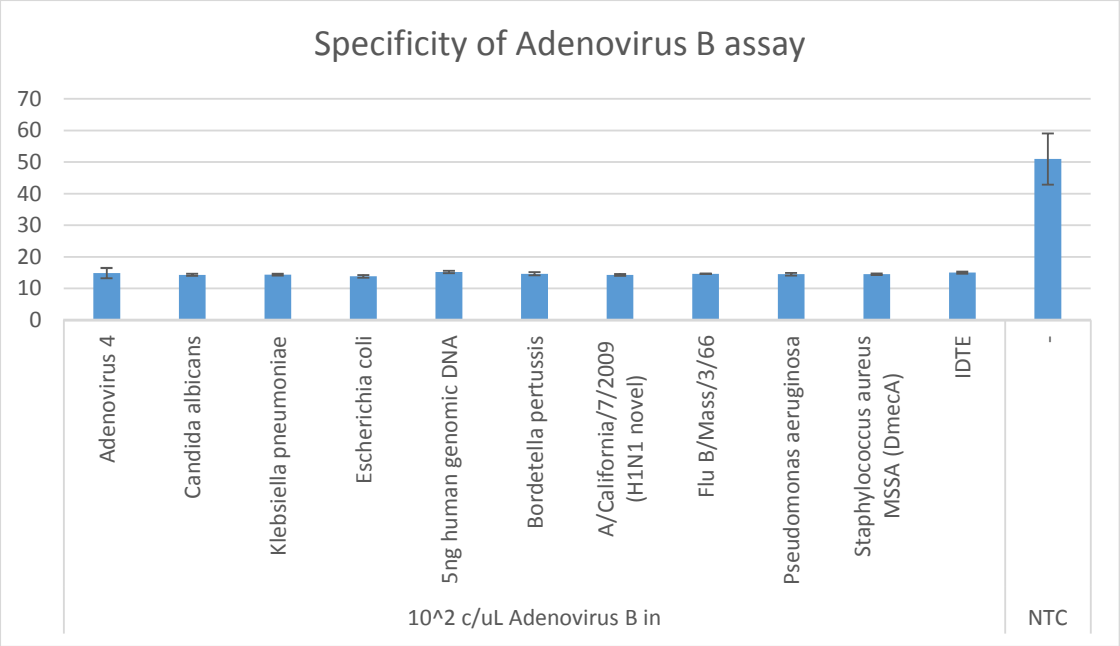

Specificity of HSV-A assay
60
50
40
30
20
10
0
Adenovirus 4
Candida albicans
Klebsiella pneumoniae
Escherichia coli
5ng human genomic DNA
Bordetella pertussis
A/California/7/2009 (H1N1 novel)
Flu B/Mass/3/66
Pseudomonas aeruginosa
Staphylococcus aureus MSSA (DmecA)
IDTE
-
10^2 c/uL RSV-A in
NTC

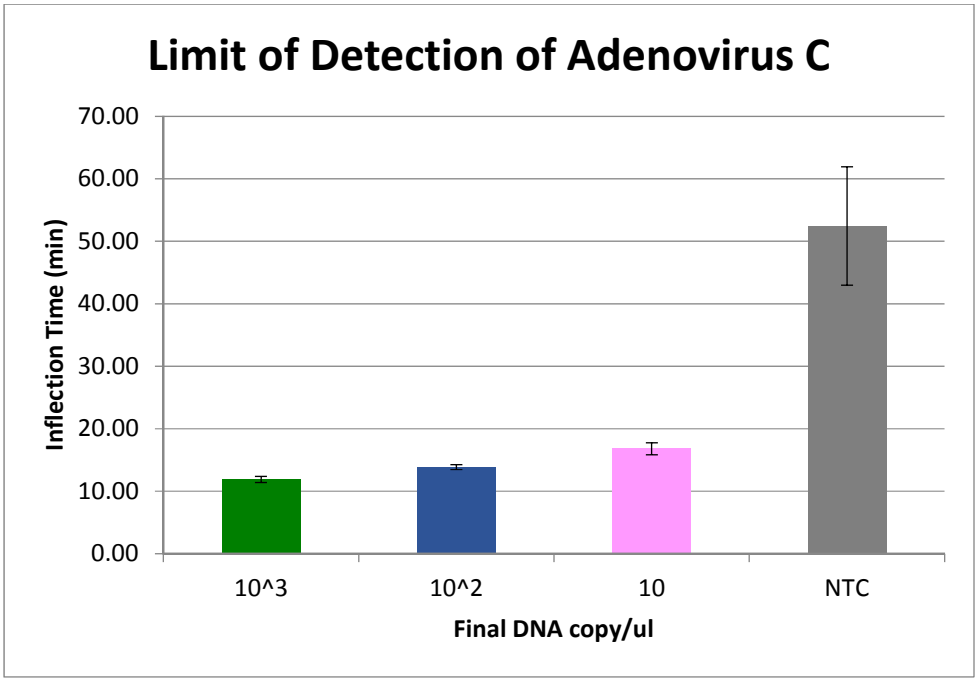

Limit of Detection of Adenovirus B
35.00
30.00
25.00
20.00
15.00
10.00
5.00
0.00
1000
100
10
NTC

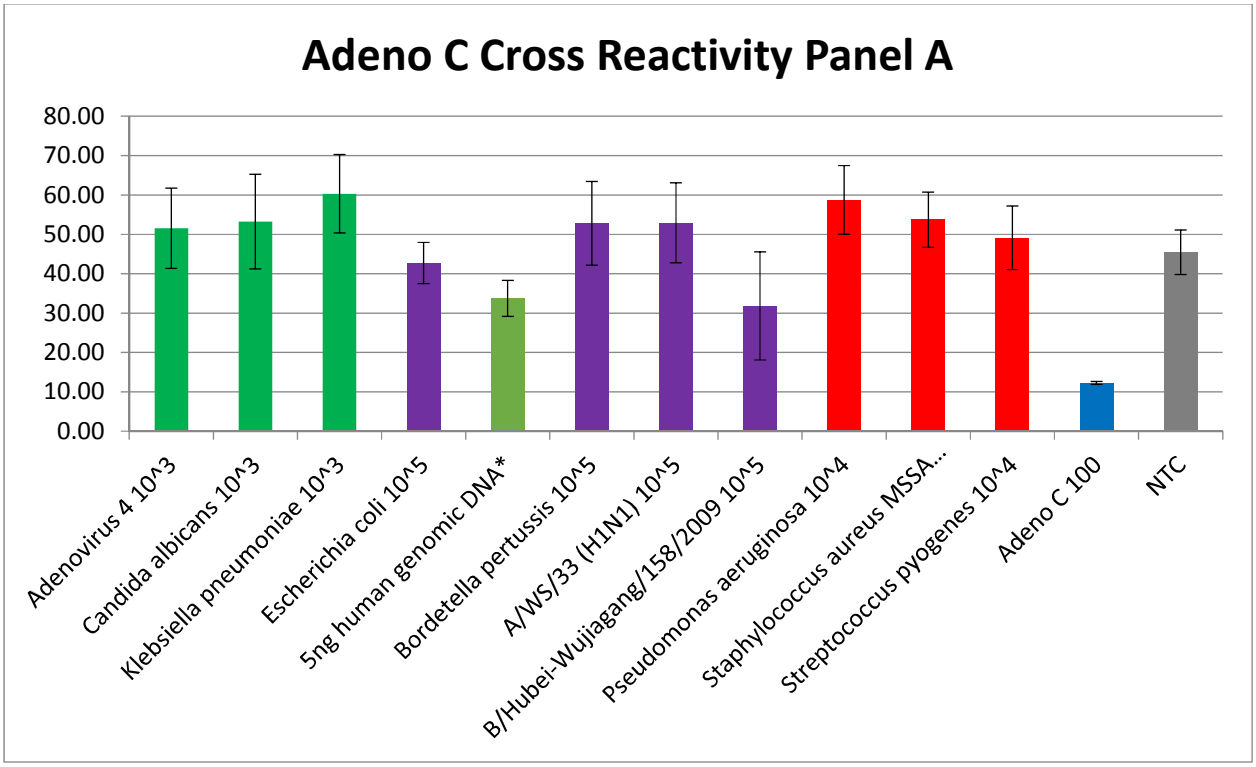

Cross-Reactivity of Adenovirus B Assay
50.00
45.00
40.00
35.00
30.00
25.00
20.00
15.00
10.00
5.00
0.00
Adenovirus 4
Candida albicans
Klebsiella pneumoniae
Escherichia coli
5ng human genomic DNA
Bordetella pertussis
A/WS/33 (H1N1)
B/Hubei-Wujiagang/158/2009
Pseudomonas aeruginosa
Staphylococcus aureus MSSA...
Streptococcus pyogenes
Adenovirus B
NTC

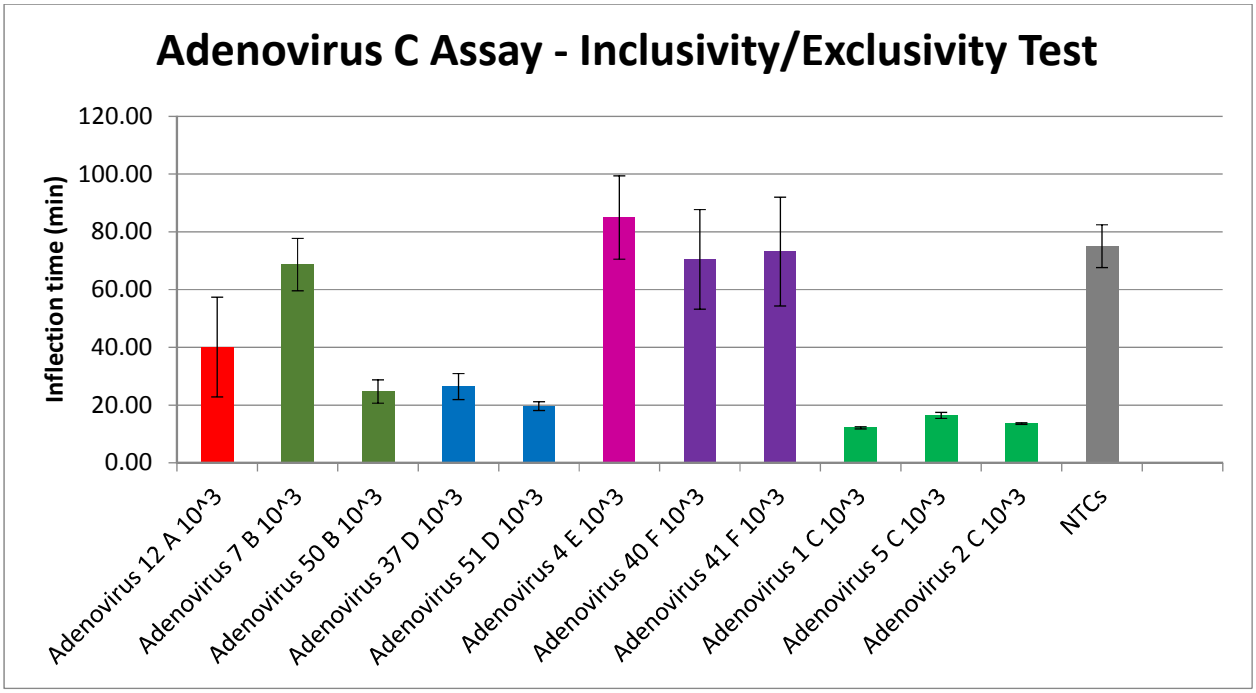

Inclusivity of Adenovirus B Assay
Inflection time (min)
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
Adeno7B
AdenoB 3B
Adeno7a B
Adeno 21B
Adeno11B
Adeno 14B
Adeno 35B
Adeno 50B
Adeno 1C
NTC

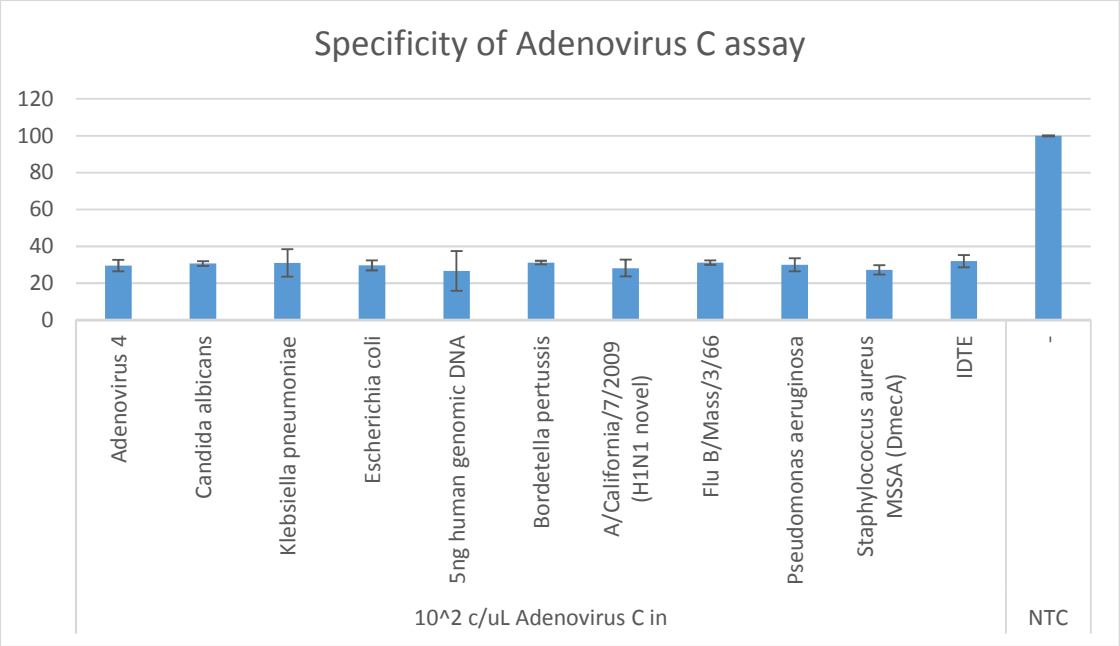

Exclusivity of Adenovirus B Assay
Inflection time (min)
60.00
50.00
40.00
30.00
20.00
10.00
0.00
Adeno12 A
Adeno 1C
Adeno5C
Adeno 2C
Adeno37D
Adeno 51D
Adeno 4E
Adeno 40F
Adeno 41F
Adeno 7B
NTC

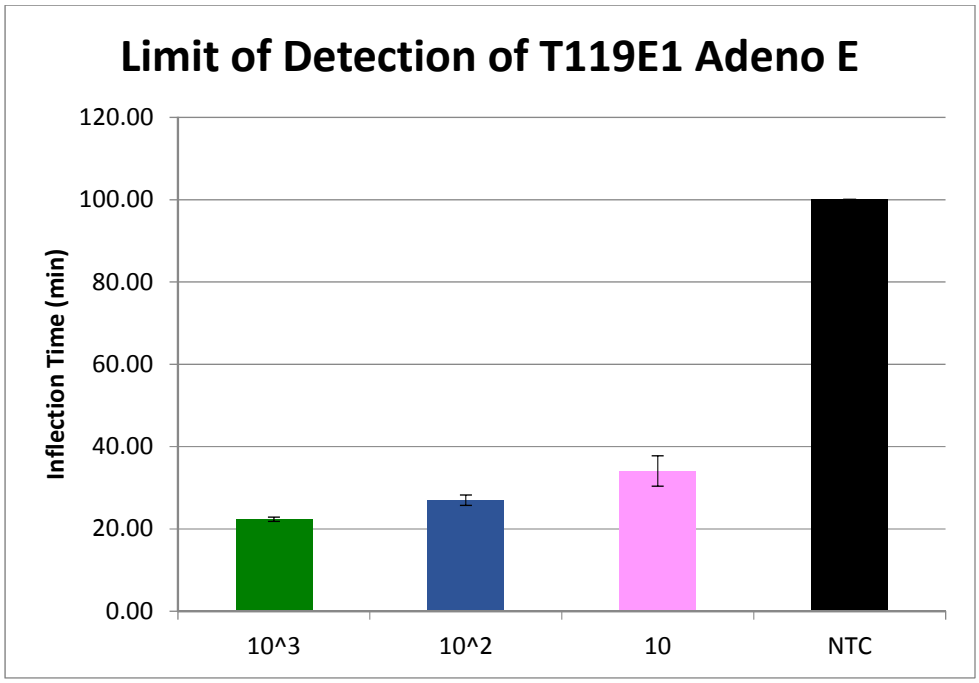

Specificity of Adenovirus B assay
70
60
50
40
30
20
10
0
Adenovirus 4
Candida albicans
Klebsiella pneumoniae
Escherichia coli
5ng human genomic DNA
Bordetella pertussis
A/California/7/2009 (H1N1 novel)
Flu B/Mass/3/66
Pseudomonas aeruginosa
Staphylococcus aureus MSSA (DmecA)
IDTE
-
10^2 c/uL Adenovirus B in
NTC

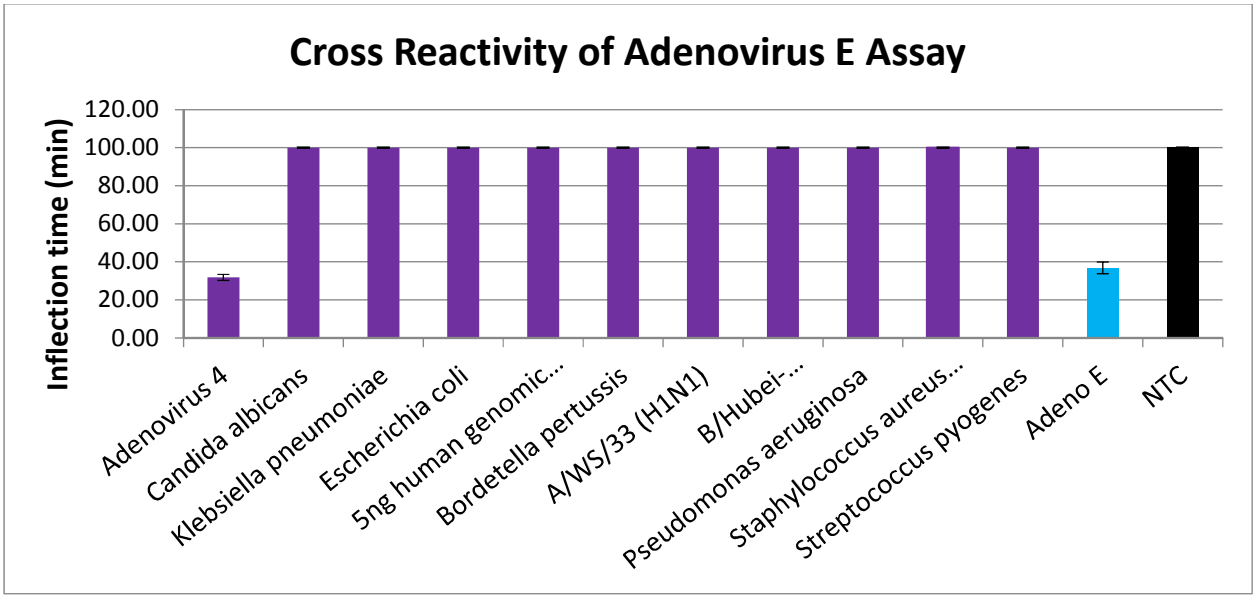

Limit of Detection of Adenovirus C
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
Inflection Time (min)
10^3
10^2
10
NTC
Final DNA copy/ul

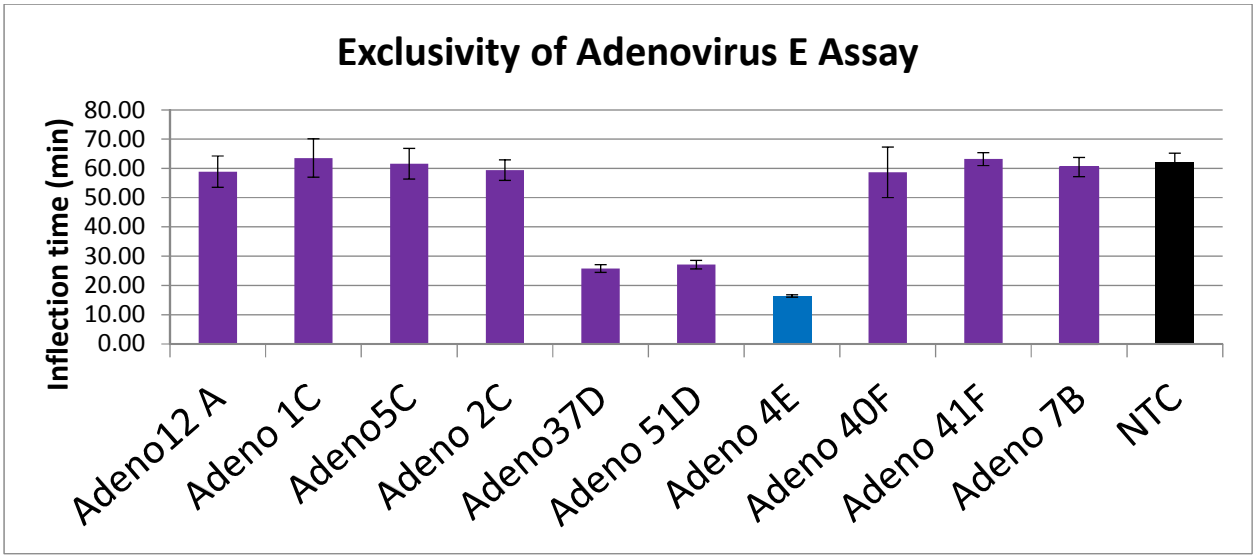

Adeno C Cross Reactivity Panel A
80.00
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
Adenovirus 4 10^3
Candida albicans 10^3
Klebsiella pneumoniae 10^3
Escherichia coli 10^5
5ng human genomic DNA*
Bordetella pertussis 10^5
A/WS/33 (H1N1) 10^5
B/Hubei-Wujiagang/158/2009 10^5
Pseudomonas aeruginosa 10^4
Staphylococcus aureus MSSA...
Streptococcus pyogenes 10^4
Adeno C 100
NTC

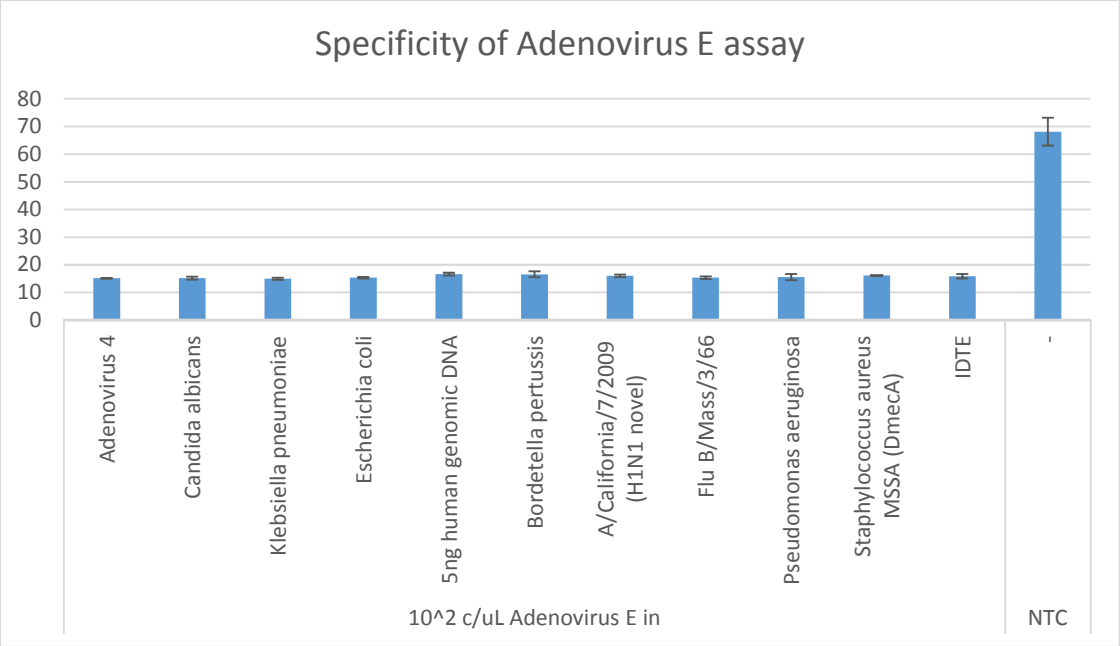

Adenovirus C Assay - Inclusivity/Exclusivity Test
Inflection time (min)
120.00
100.00
80.00
60.00
40.00
20.00
0.00
Adenovirus 12 A 10^3
Adenovirus 7 B 10^3
Adenovirus 50 B 10^3
Adenovirus 37 D 10^3
Adenovirus 51 D 10^3
Adenovirus 4 E 10^3
Adenovirus 40 F 10^3
Adenovirus 41 F 10^3
Adenovirus 1 C 10^3
Adenovirus 5 C 10^3
Adenovirus 2 C 10^3
NTCs

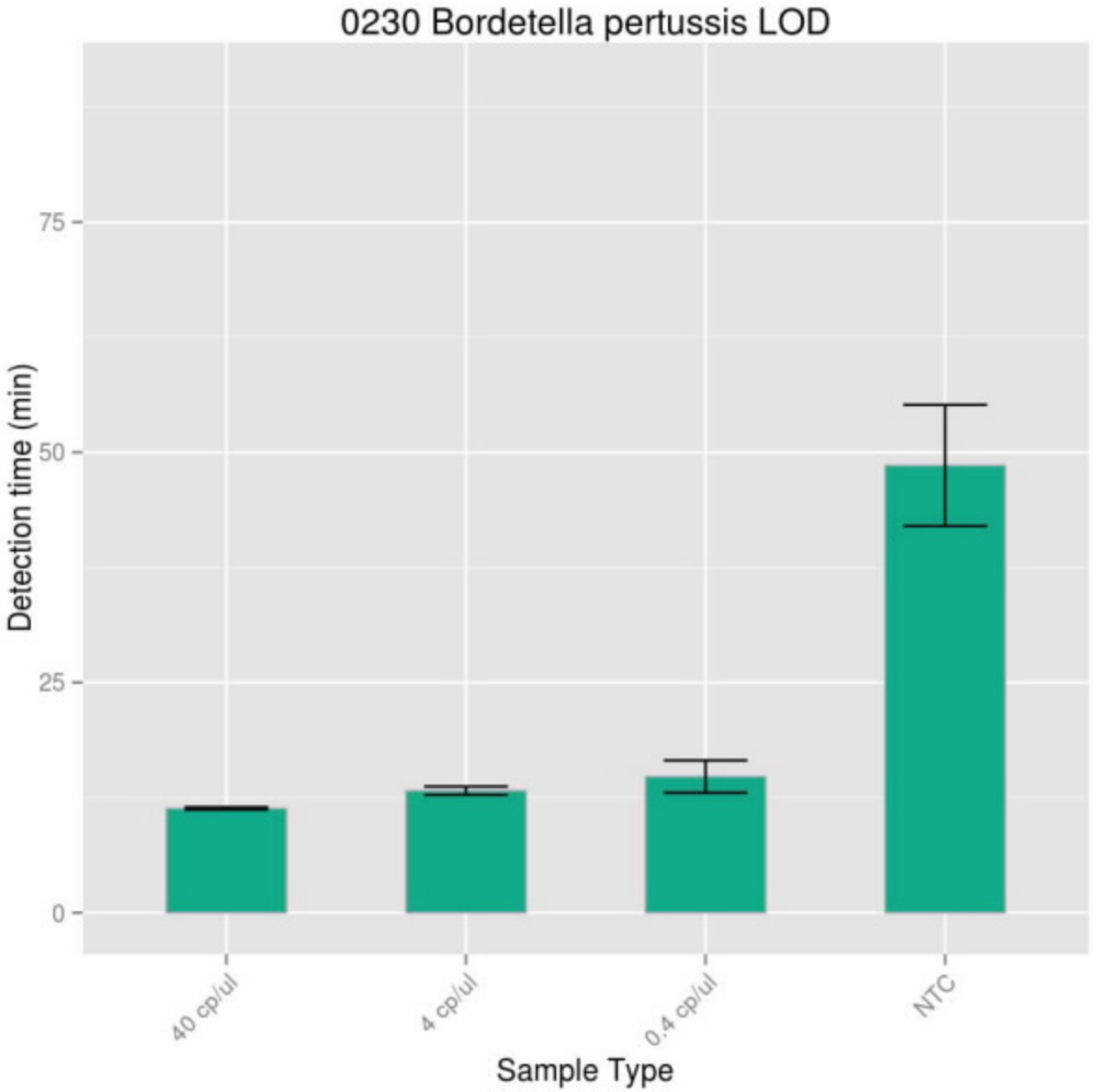

Specificity of Adenovirus C assay
120
100
80
60
40
20
0
Adenovirus 4
Candida albicans
Klebsiella pneumoniae
Escherichia coli
5ng human genomic DNA
Bordetella pertussis
A/California/7/2009 (H1N1 novel)
Flu B/Mass/3/66
Pseudomonas aeruginosa
Staphylococcus aureus MSSA (DmecA)
IDTE
-
10^2 c/uL Adenovirus C in
NTC

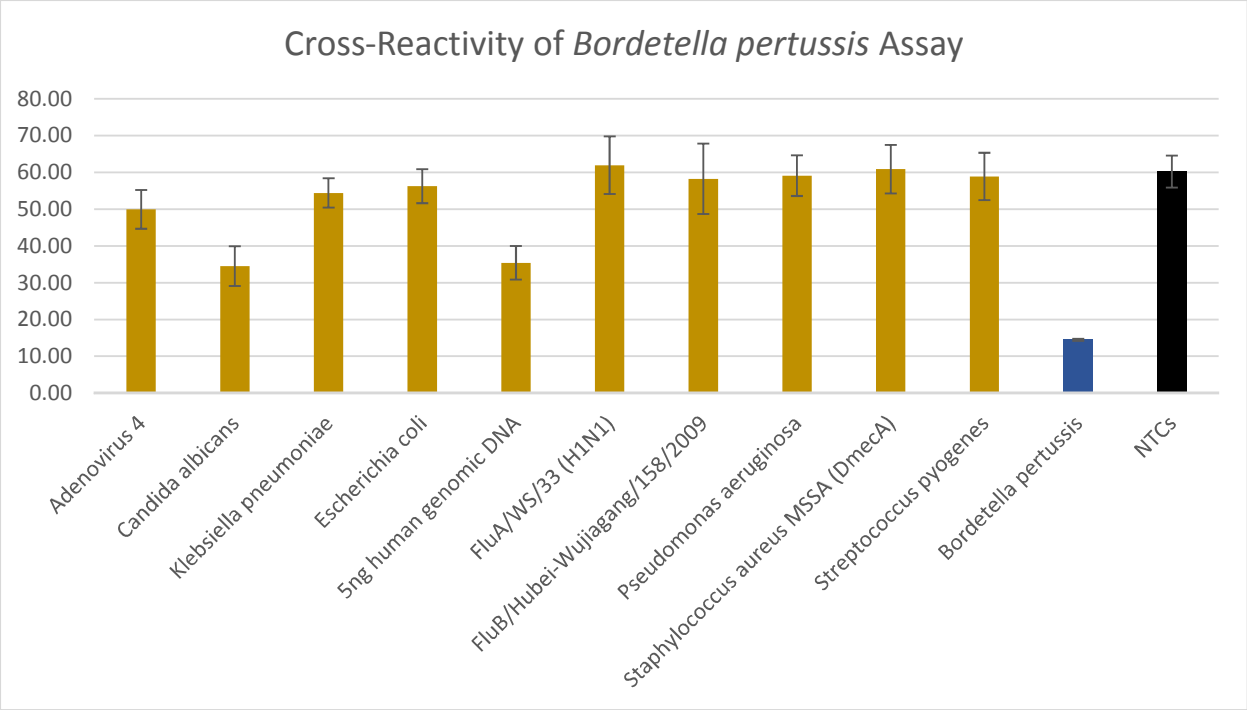

Limit of Detection of T119E1 Adeno E
Inflection Time (min)
120.00
100.00
80.00
60.00
40.00
20.00
0.00
10^3
10^2
10
NTC

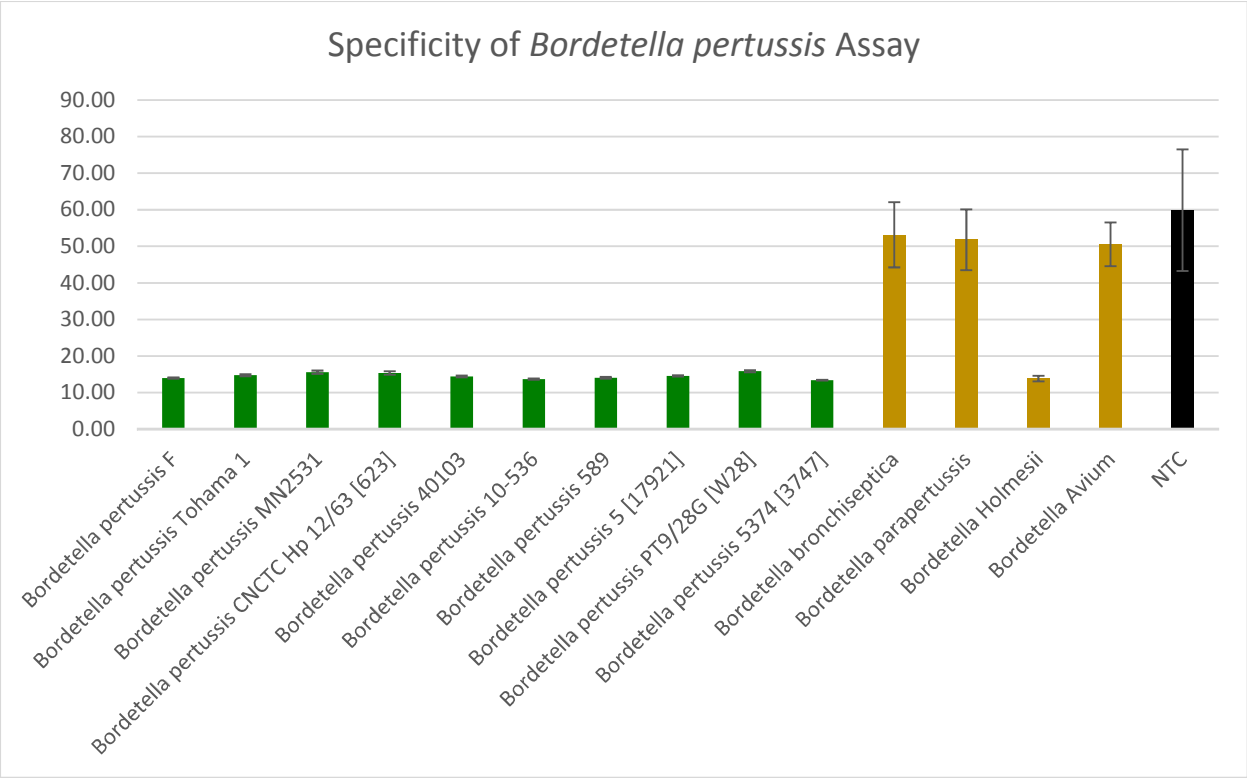

Cross Reactivity of Adenovirus E Assay
Inflection time (min)
120.00
100.00
80.00
60.00
40.00
20.00
0.00
Adenovirus 4
Candida albicans
Klebsiella pneumoniae
Escherichia coli
5ng human genomic...
Bordetella pertussis
A/WS/33 (H1N1)
B/Hubei-...
Pseudomonas aeruginosa
Staphylococcus aureus...
Streptococcus pyogenes
Adeno E
NTC

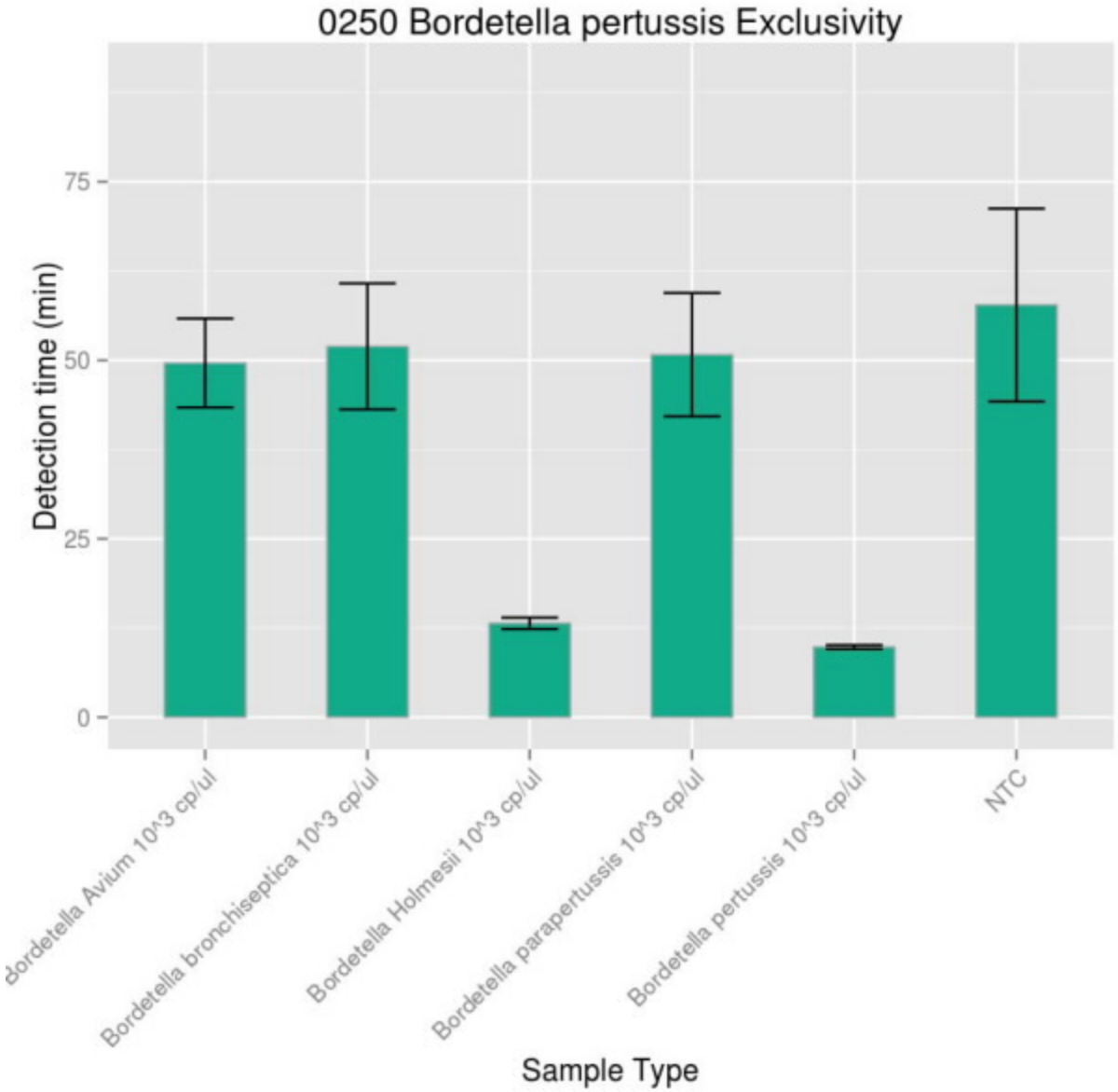

Exclusivity of Adenovirus E Assay
Inflection time (min)
80.00
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
Adeno12 A
Adeno 1C
Adeno5C
Adeno 2C
Adeno37D
Adeno 51D
Adeno 4E
Adeno 40F
Adeno 41F
Adeno 7B
NTC

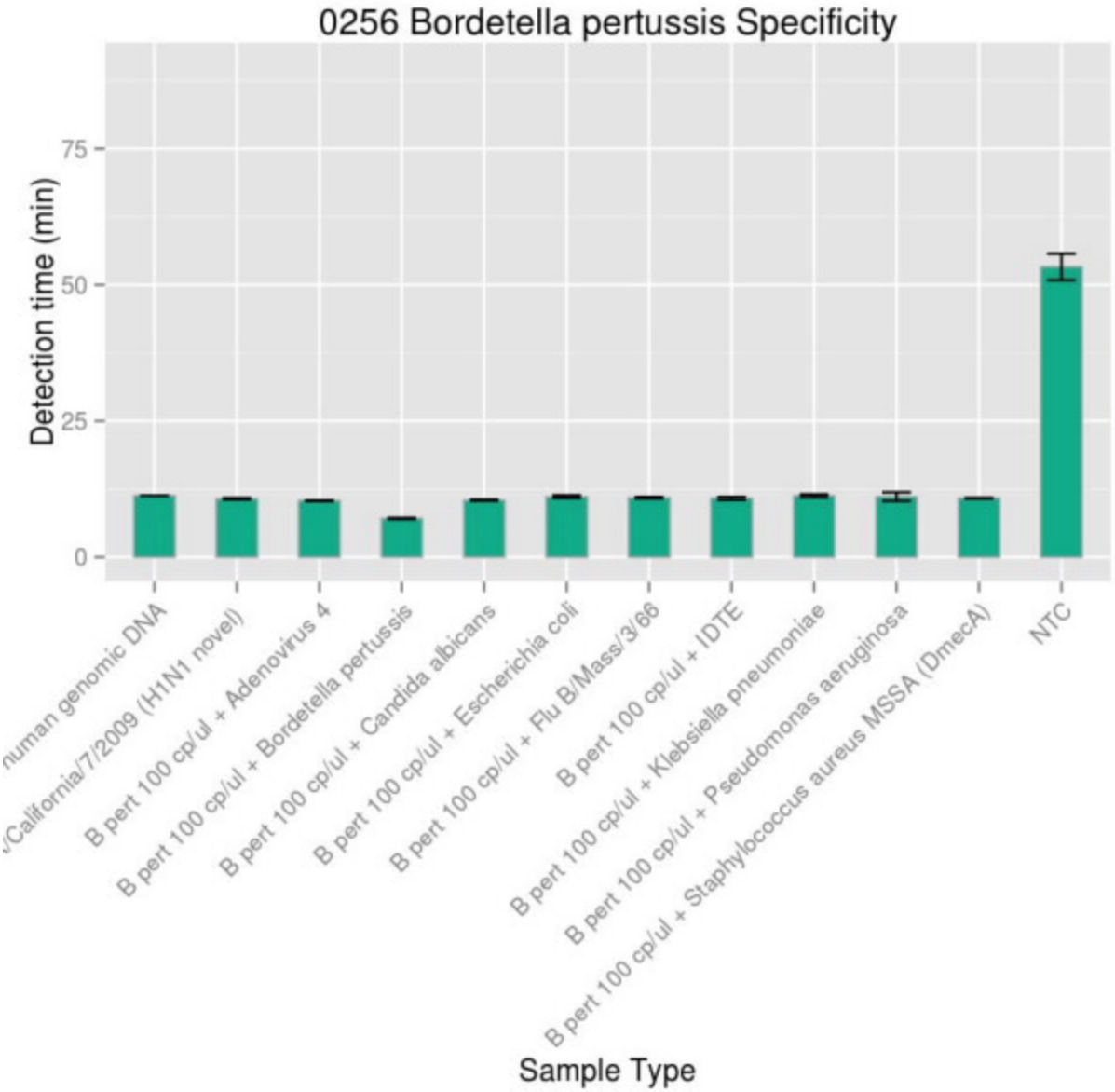

Specificity of Adenovirus E assay
80
70
60
50
40
30
20
10
0
Adenovirus 4
Candida albicans
Klebsiella pneumoniae
Escherichia coli
5ng human genomic DNA
Bordetella pertussis
A/California/7/2009 (H1N1 novel)
Flu B/Mass/3/66
Pseudomonas aeruginosa
Staphylococcus aureus MSSA (DmecA)
IDTE
-
10^2 c/uL Adenovirus E in
NTC

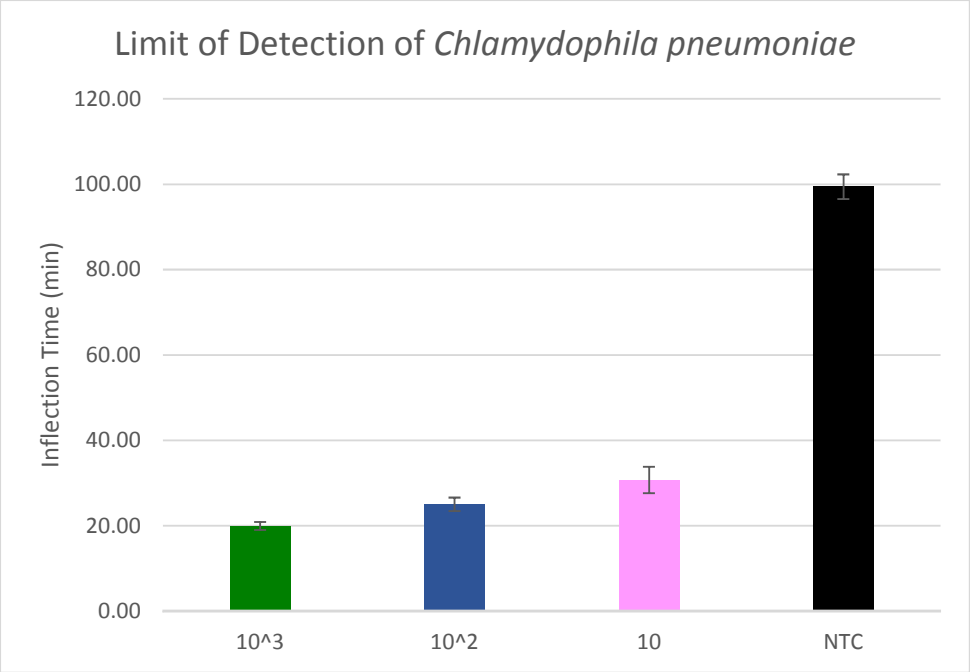

0230 Bordetella pertussis LOD
Detection time (min)
75
50
25
0
40 cp/ul
4 cp/ul
0.4 cp/ul
NTC
Sample Type

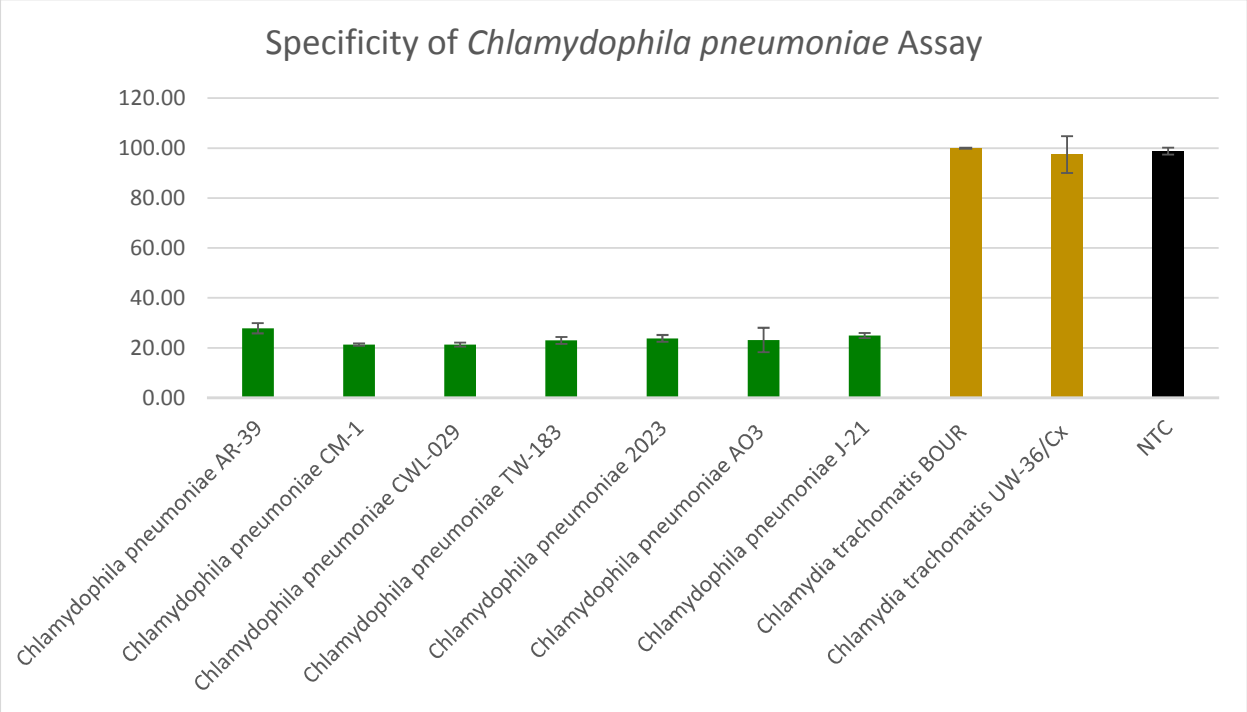

Cross-Reactivity of *Bordetella pertussis* Assay
80.00
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
Adenovirus 4
Candida albicans
Klebsiella pneumoniae
Escherichia coli
5ng human genomic DNA
FluA/WS/33 (H1N1)
FluB/Hubei-Wujiagang/158/2009
Pseudomonas aeruginosa
Staphylococcus aureus MSSA (DmecA)
Streptococcus pyogenes
Bordetella pertussis
NTCs

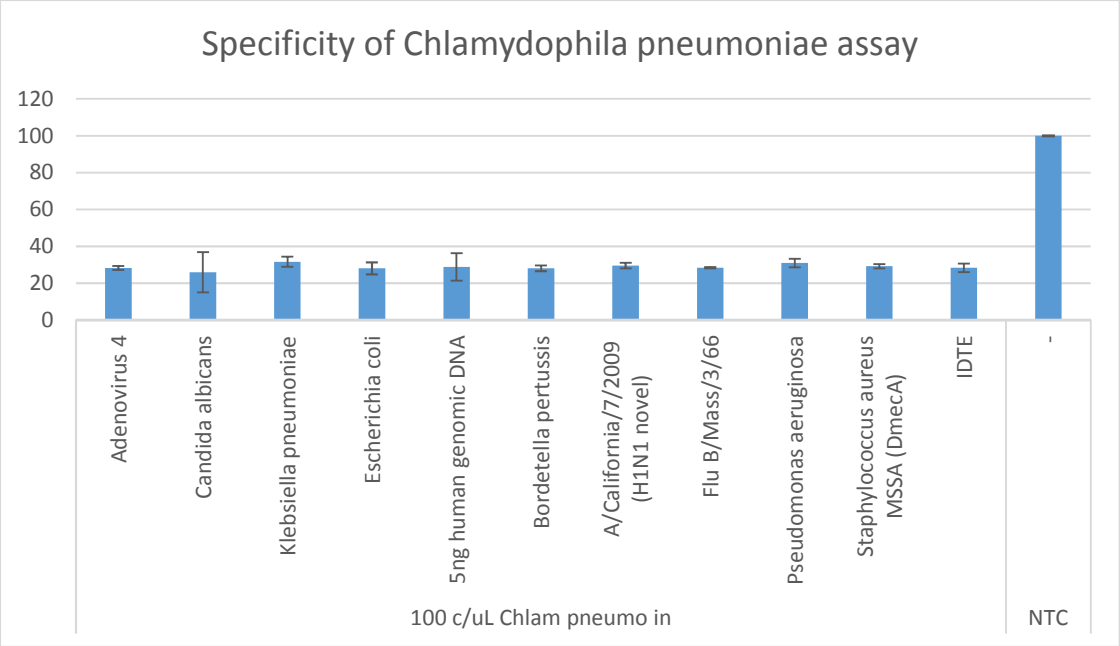

Specificity of *Bordetella pertussis* Assay
90.00
80.00
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
Bordetella pertussis F
Bordetella pertussis Tohama 1
Bordetella pertussis MN2531
Bordetella pertussis CNCTC Hp 12/63 [623]
Bordetella pertussis 40103
Bordetella pertussis 10-536
Bordetella pertussis 589
Bordetella pertussis 5 [17921]
Bordetella pertussis PT9/28G [W28]
Bordetella pertussis 5374 [3747]
Bordetella bronchiseptica
Bordetella parapertussis
Bordetella Holmesii
Bordetella Avium
NTC

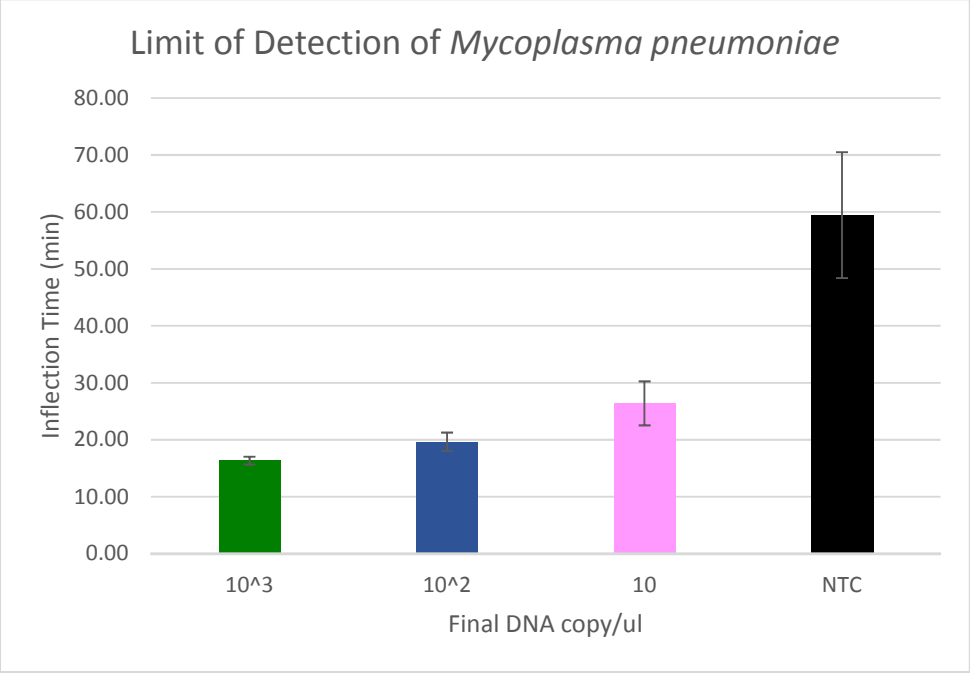

0250 Bordetella pertussis Exclusivity
Detection time (min)
75
50
25
0
Bordetella Avium 10^3 cp/ul
Bordetella bronchiseptica 10^3 cp/ul
Bordetella Holmesii 10^3 cp/ul
Bordetella parapertussis 10^3 cp/ul
Bordetella pertussis 10^3 cp/ul
NTC
Sample Type

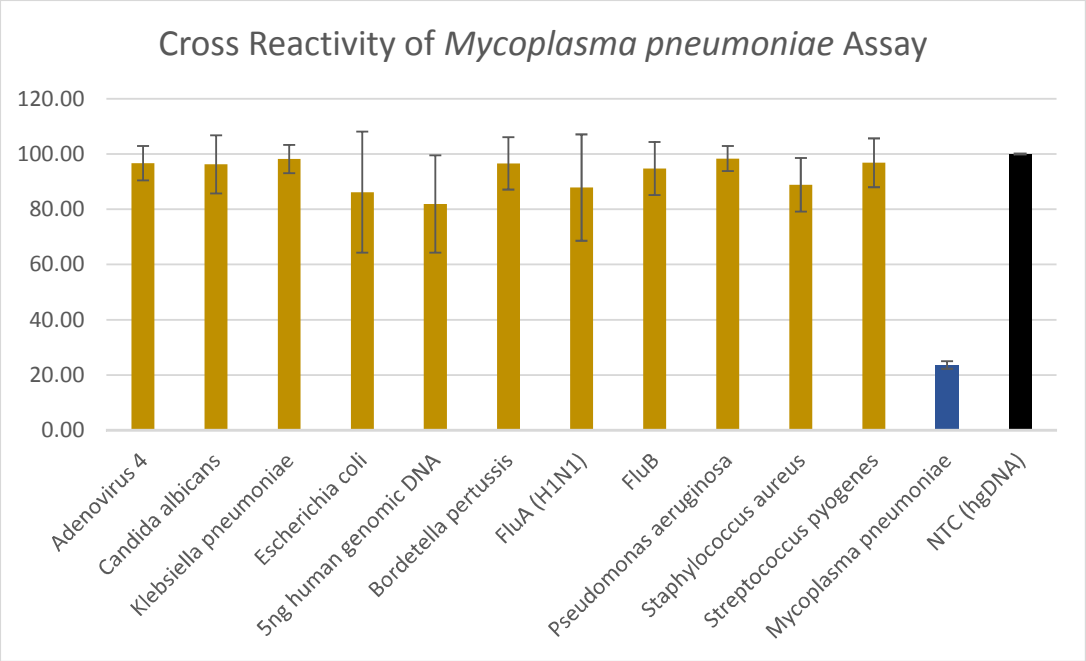

0256 Bordetella pertussis Specificity
Detection time (min)
75
50
25
0
human genomic DNA
/California/7/2009 (H1N1 novel)
B pert 100 cp/ul + Adenovirus 4
B pert 100 cp/ul + Bordetella pertussis
B pert 100 cp/ul + Candida albicans
B pert 100 cp/ul + Escherichia coli
B pert 100 cp/ul + Flu B/Mass/3/66
B pert 100 cp/ul + IDTE
B pert 100 cp/ul + Klebsiella pneumoniae
B pert 100 cp/ul + Pseudomonas aeruginosa
B pert 100 cp/ul + Staphylococcus aureus MSSA (DmecA)
NTC
Sample Type

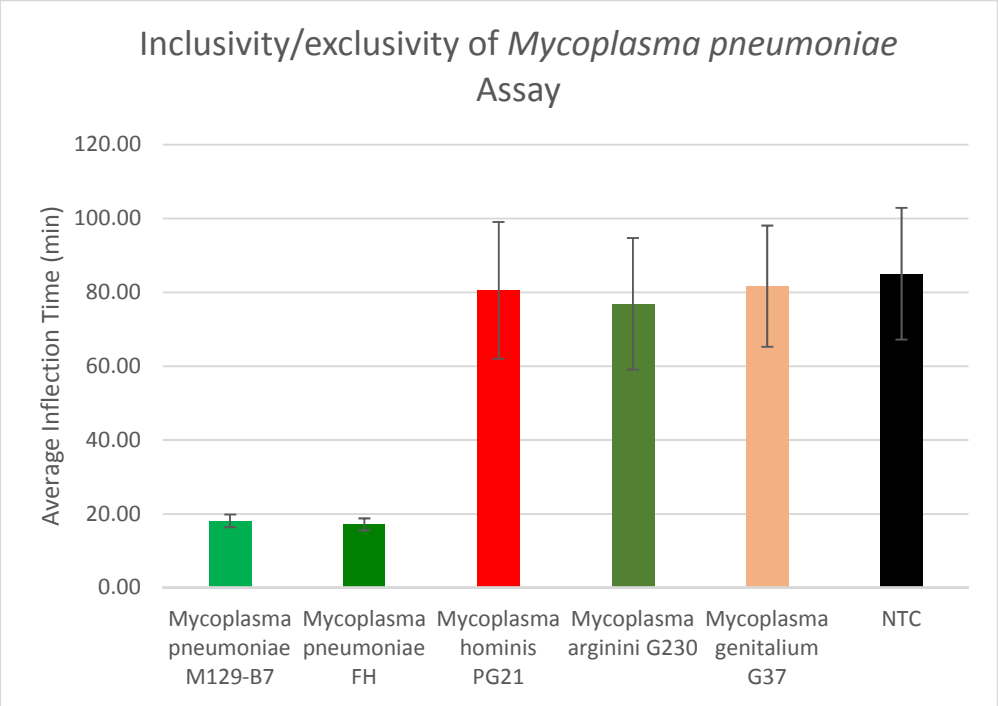

Limit of Detection of *Chlamydophila pneumoniae*
120.00
100.00
80.00
60.00
40.00
20.00
0.00
Inflection Time (min)
10^3
10^2
10
NTC

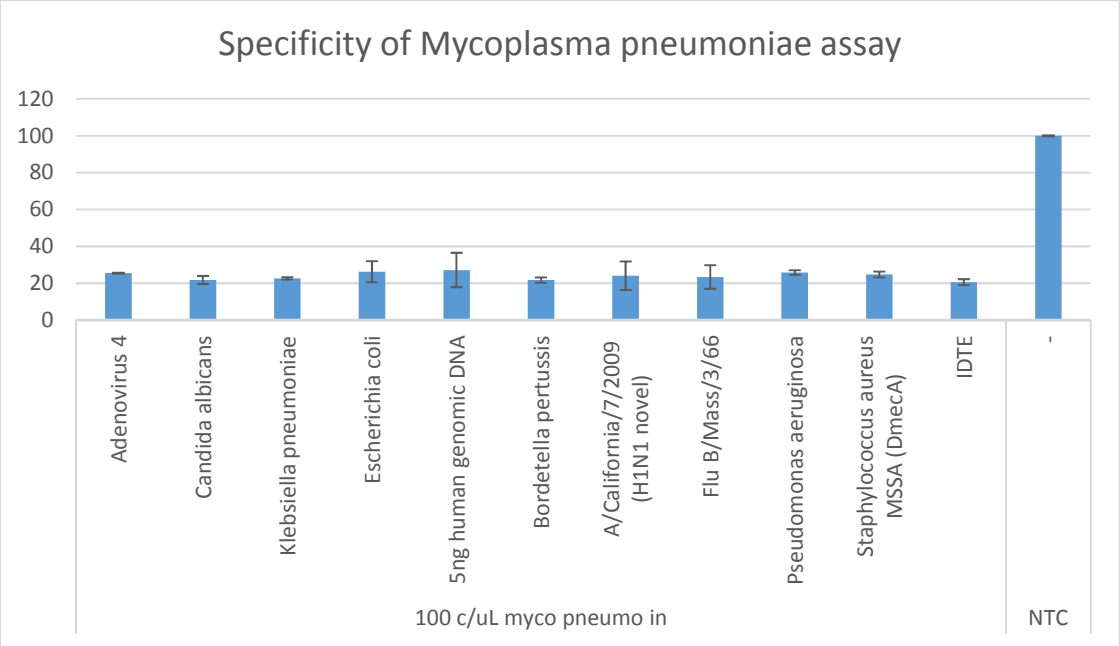

Specificity of *Chlamydophila pneumoniae* Assay
120.00
100.00
80.00
60.00
40.00
20.00
0.00
Chlamydophila pneumoniae AR-39
Chlamydophila pneumoniae CM-1
Chlamydophila pneumoniae CWL-029
Chlamydophila pneumoniae TW-183
Chlamydophila pneumoniae 2023
Chlamydophila pneumoniae AO3
Chlamydophila pneumoniae J-21
Chlamydia trachomatis BOUR
Chlamydia trachomatis UW-36/Cx
NTC

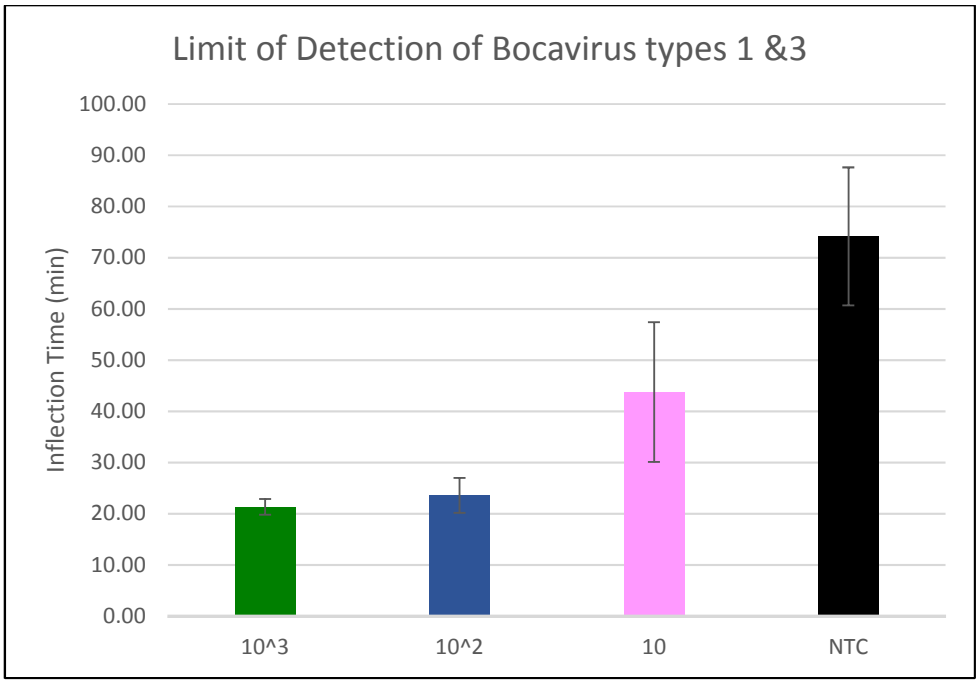

Specificity of Chlamydophila pneumoniae assay
120
100
80
60
40
20
0
Adenovirus 4
Candida albicans
Klebsiella pneumoniae
Escherichia coli
5ng human genomic DNA
Bordetella pertussis
A/California/7/2009 (H1N1 novel)
Flu B/Mass/3/66
Pseudomonas aeruginosa
Staphylococcus aureus MSSA (DmecA)
IDTE
-
100 c/uL Chlam pneumo in
NTC

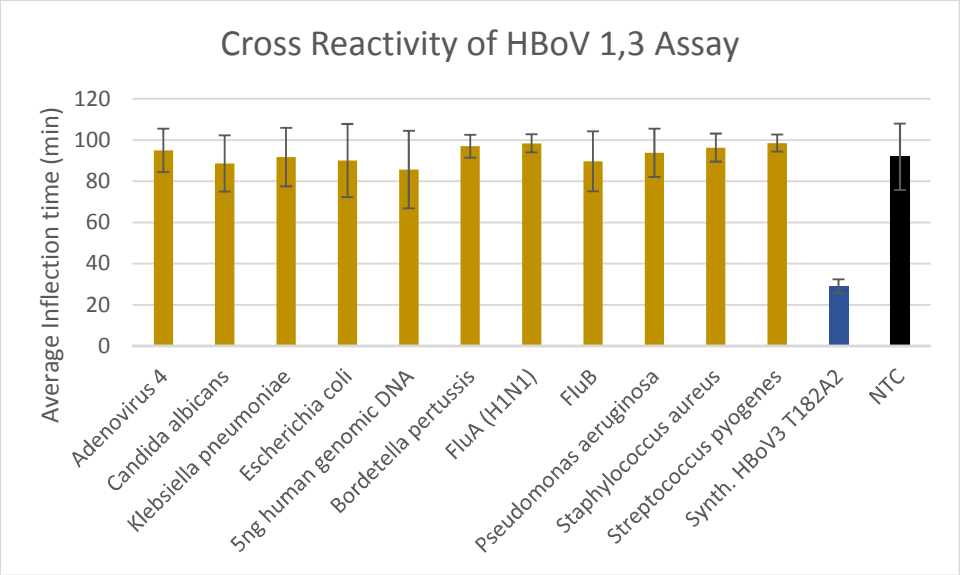

Limit of Detection of Mycoplasma pneumoniae
Inflection Time (min)
80.00
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
10^3
10^2
10
NTC
Final DNA copy/ul

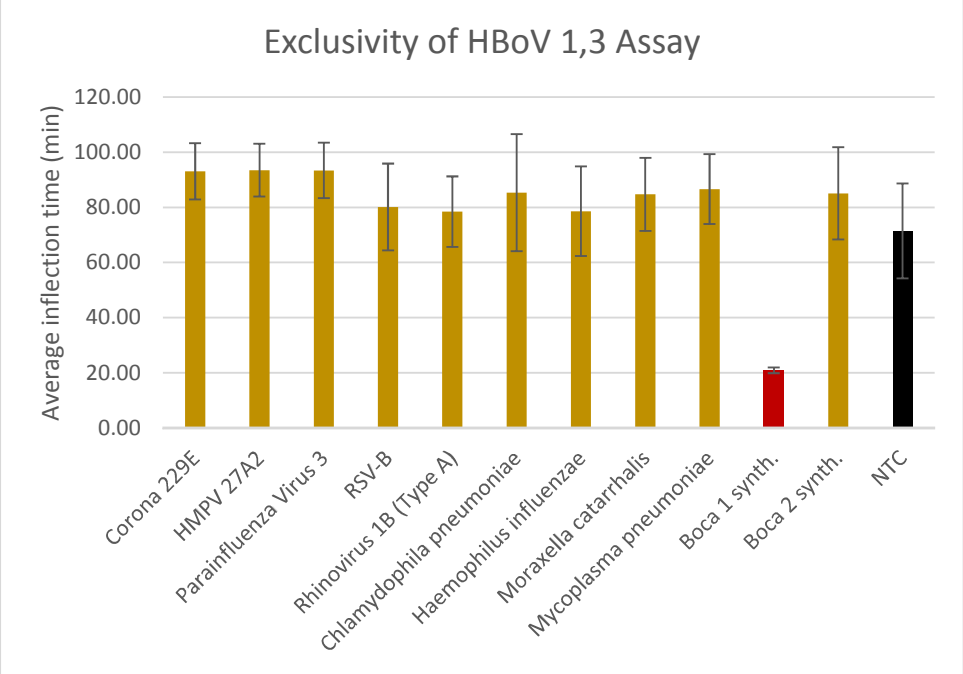

Cross Reactivity of *Mycoplasma pneumoniae* Assay
120.00
100.00
80.00
60.00
40.00
20.00
0.00
Adenovirus 4
Candida albicans
Klebsiella pneumoniae
Escherichia coli
5ng human genomic DNA
Bordetella pertussis
FluA (H1N1)
FluB
Pseudomonas aeruginosa
Staphylococcus aureus
Streptococcus pyogenes
Mycoplasma pneumoniae
NTC (hgDNA)

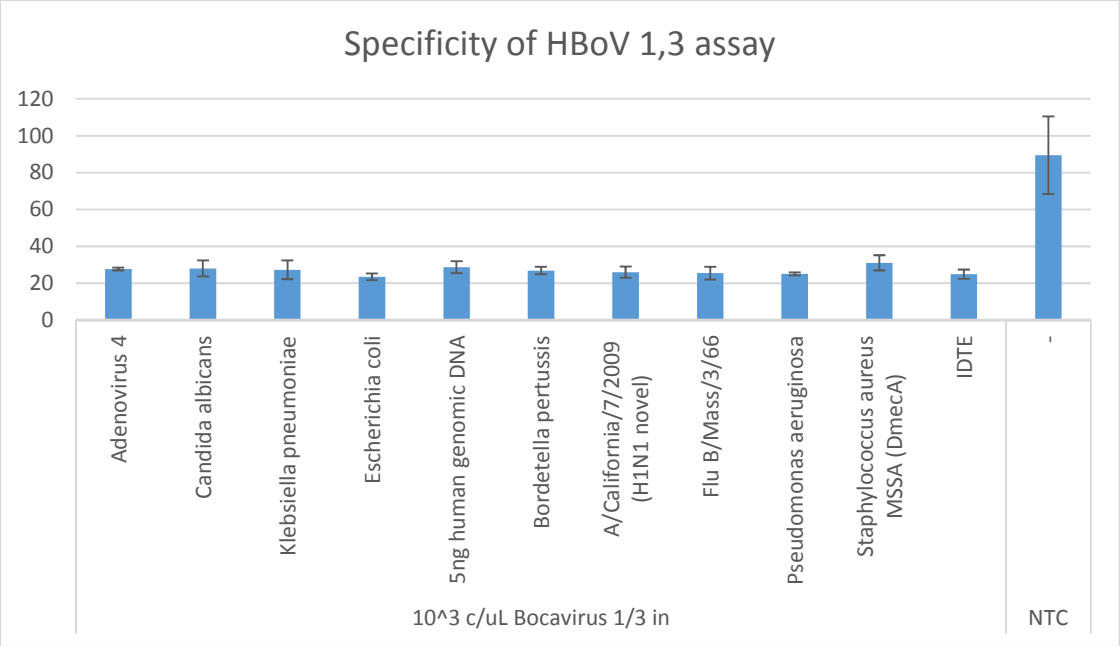

Inclusivity/exclusivity of *Mycoplasma pneumoniae* Assay
Average Inflection Time (min)
120.00
100.00
80.00
60.00
40.00
20.00
0.00
Mycoplasma pneumoniae M129-B7
Mycoplasma pneumoniae FH
Mycoplasma hominis PG21
Mycoplasma arginini G230
Mycoplasma genitalium G37
NTC

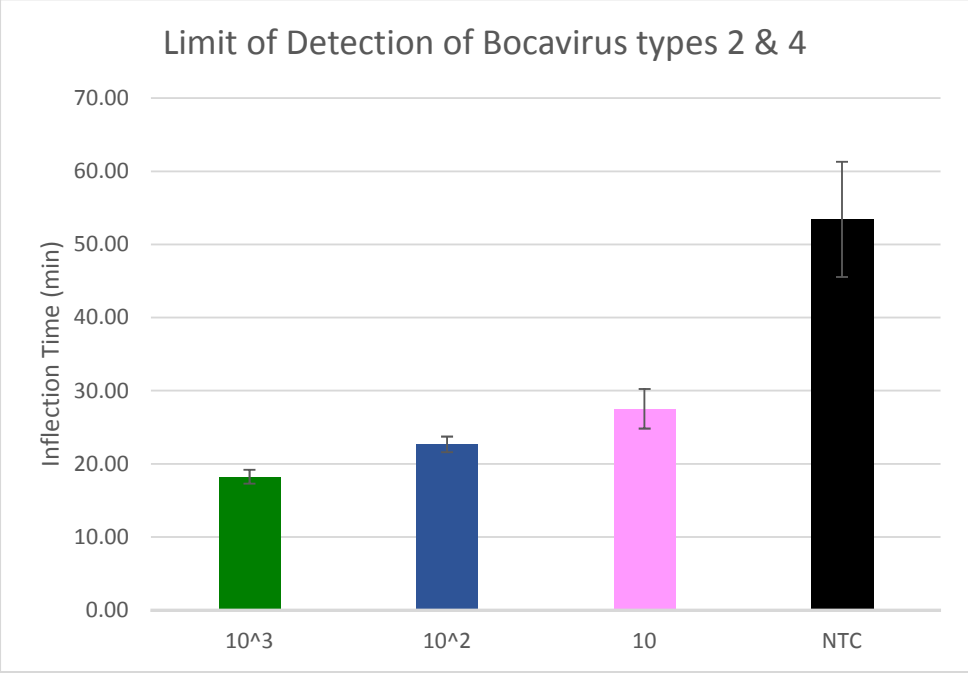

Specificity of Mycoplasma pneumoniae assay
120
100
80
60
40
20
0
Adenovirus 4
Candida albicans
Klebsiella pneumoniae
Escherichia coli
5ng human genomic DNA
Bordetella pertussis
A/California/7/2009 (H1N1 novel)
Flu B/Mass/3/66
Pseudomonas aeruginosa
Staphylococcus aureus MSSA (DmecA)
IDTE
-
100 c/uL myco pneumo in
NTC

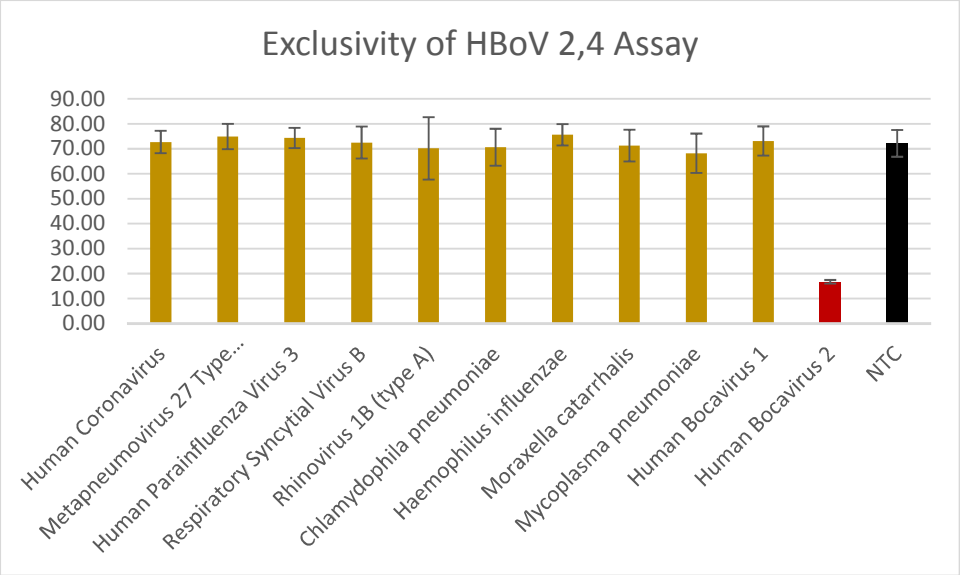

Limit of Detection of Bocavirus types 1 &3
Inflection Time (min)
100.00
90.00
80.00
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
10^3
10^2
10
NTC

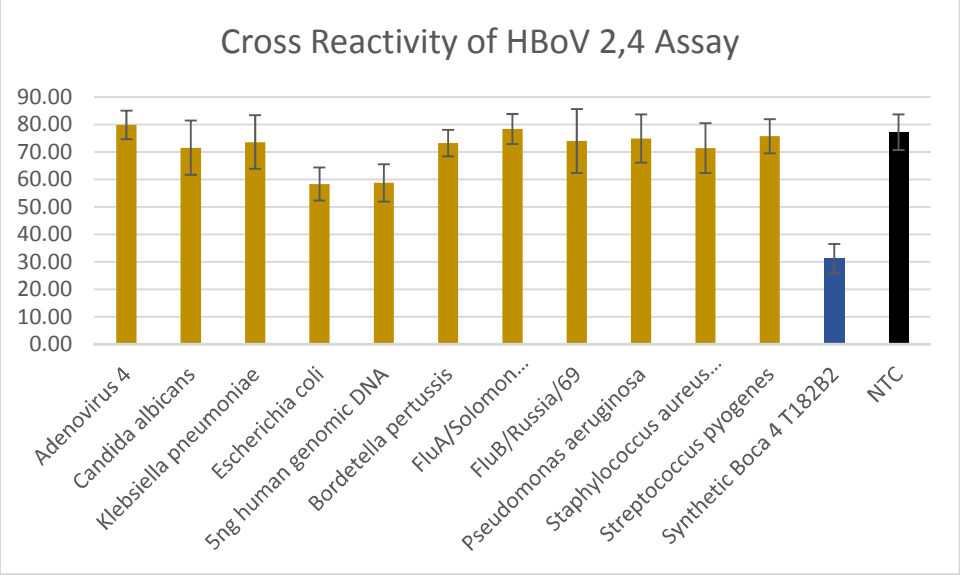

Cross Reactivity of HBoV 1,3 Assay
Average Inflection time (min)
120
100
80
60
40
20
0
Adenovirus 4
Candida albicans
Klebsiella pneumoniae
Escherichia coli
5ng human genomic DNA
Bordetella pertussis
FluA (H1N1)
FluB
Pseudomonas aeruginosa
Staphylococcus aureus
Streptococcus pyogenes
Synth. HBoV3 T182A2
NTC

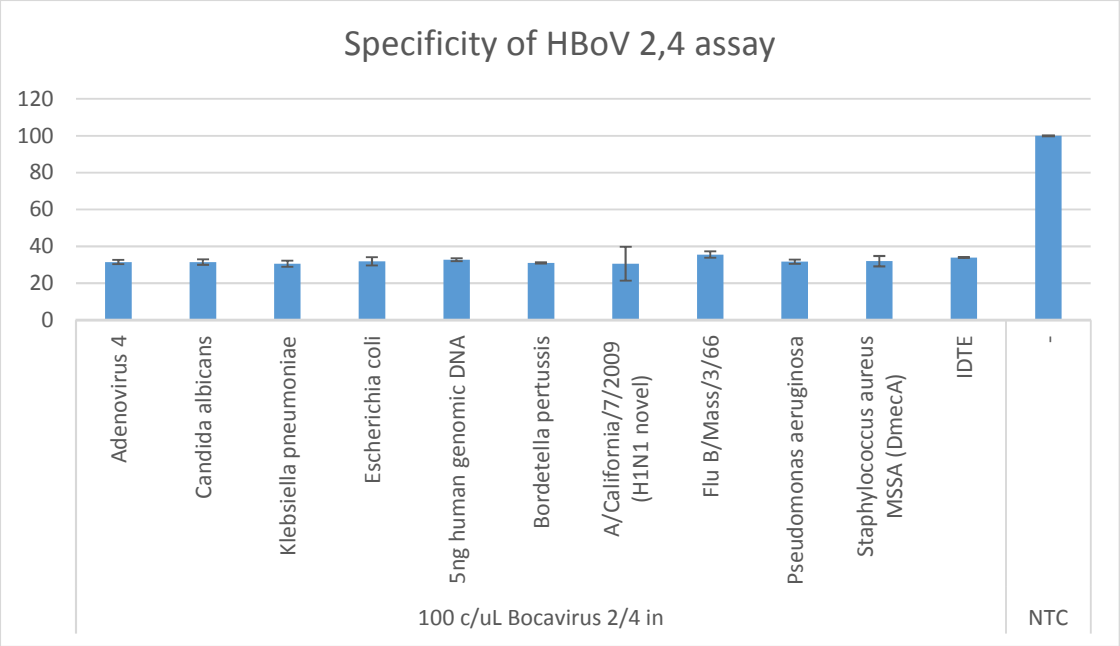

Exclusivity of HBoV 1,3 Assay
Average inflection time (min)
120.00
100.00
80.00
60.00
40.00
20.00
0.00
Corona 229E
HMPV 27A2
Parainfluenza Virus 3
RSV-B
Rhinovirus 1B (Type A)
Chlamydophila pneumoniae
Haemophilus influenzae
Moraxella catarrhalis
Mycoplasma pneumoniae
Boca 1 synth.
Boca 2 synth.
NTC

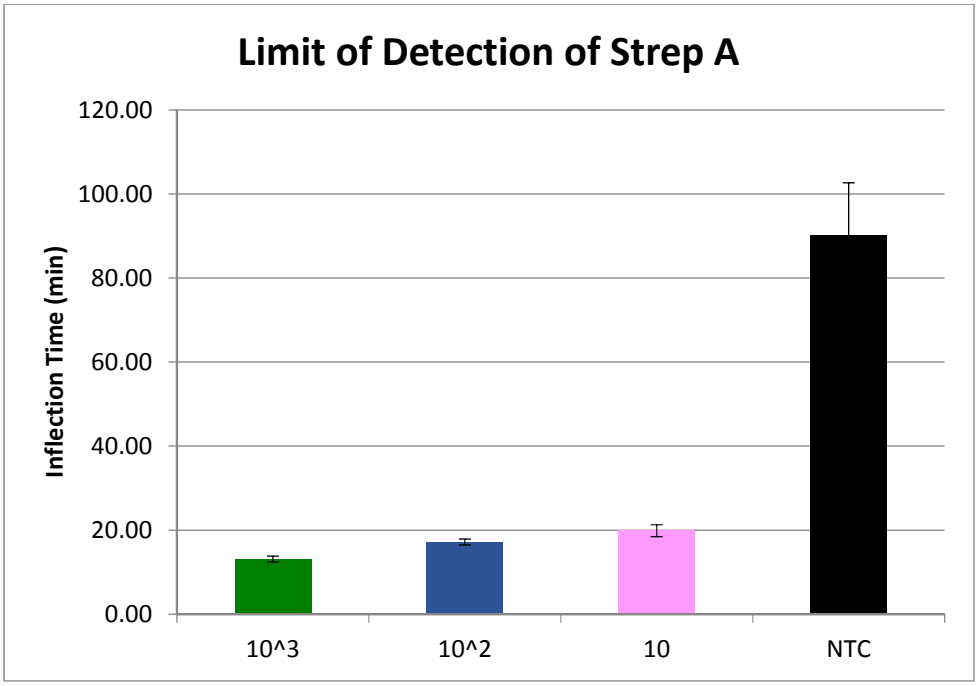

Specificity of HBoV 1,3 assay
120
100
80
60
40
20
0
Adenovirus 4
Candida albicans
Klebsiella pneumoniae
Escherichia coli
5ng human genomic DNA
Bordetella pertussis
A/California/7/2009 (H1N1 novel)
Flu B/Mass/3/66
Pseudomonas aeruginosa
Staphylococcus aureus MSSA (DmecA)
IDTE
-
10^3 c/uL Bocavirus 1/3 in
NTC

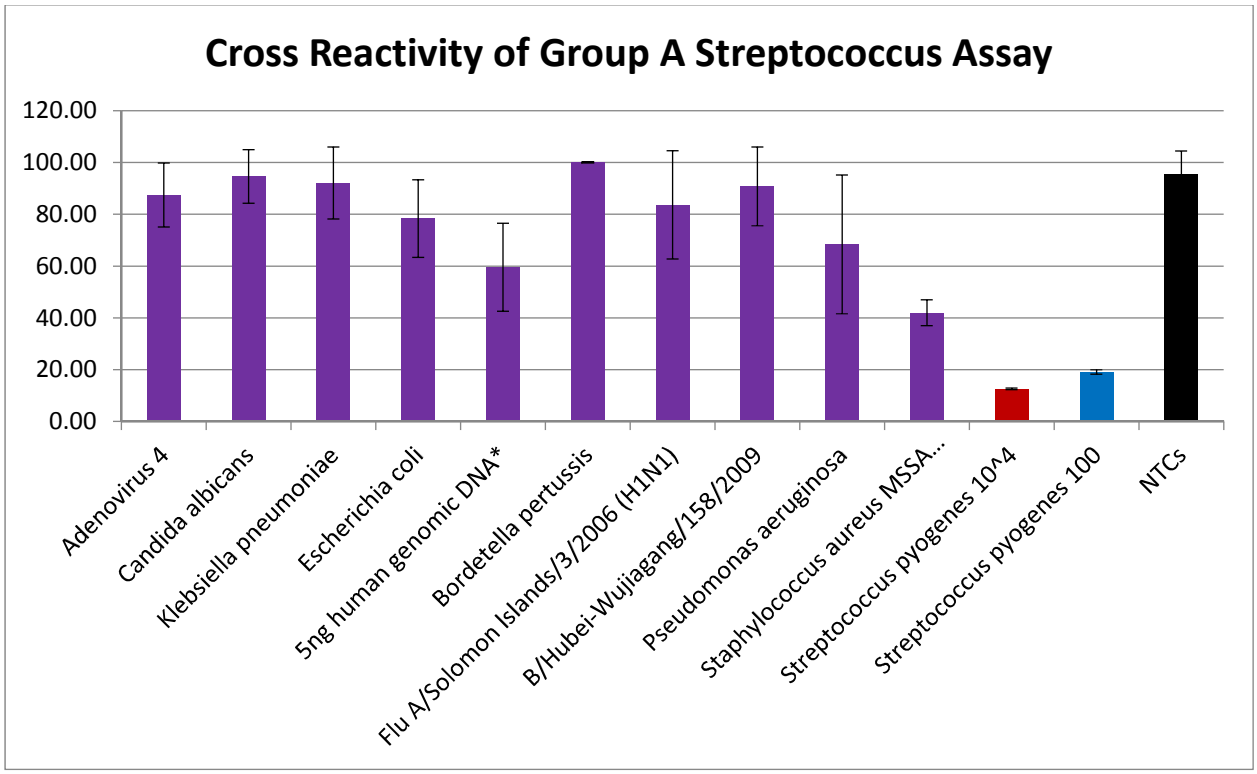

Limit of Detection of Bocavirus types 2 & 4
Inflection Time (min)
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
10^3
10^2
10
NTC

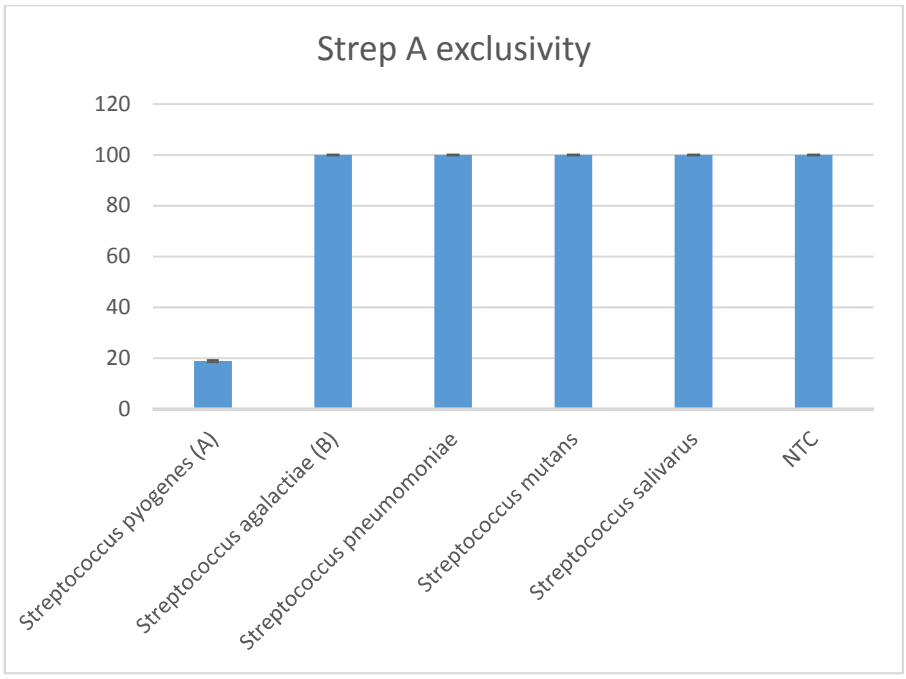

Exclusivity of HBoV 2,4 Assay
90.00
80.00
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
Human Coronavirus
Metapneumovirus 27 Type...
Human Parainfluenza Virus 3
Respiratory Syncytial Virus B
Rhinovirus 1B (type A)
Chlamydophila pneumoniae
Haemophilus influenzae
Moraxella catarrhalis
Mycoplasma pneumoniae
Human Bocavirus 1
Human Bocavirus 2
NTC

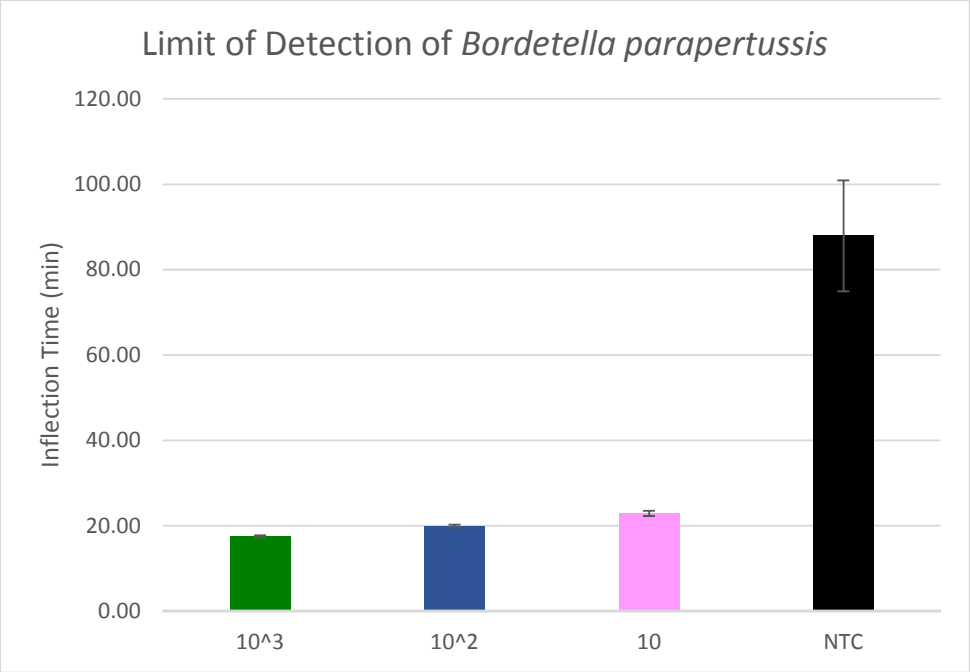

Cross Reactivity of HBoV 2,4 Assay
90.00
80.00
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
Adenovirus 4
Candida albicans
Klebsiella pneumoniae
Escherichia coli
5ng human genomic DNA
Bordetella pertussis
FluA/Solomon...
FluB/Russia/69
Pseudomonas aeruginosa
Staphylococcus aureus...
Streptococcus pyogenes
Synthetic Boca 4 T182B2
NTC

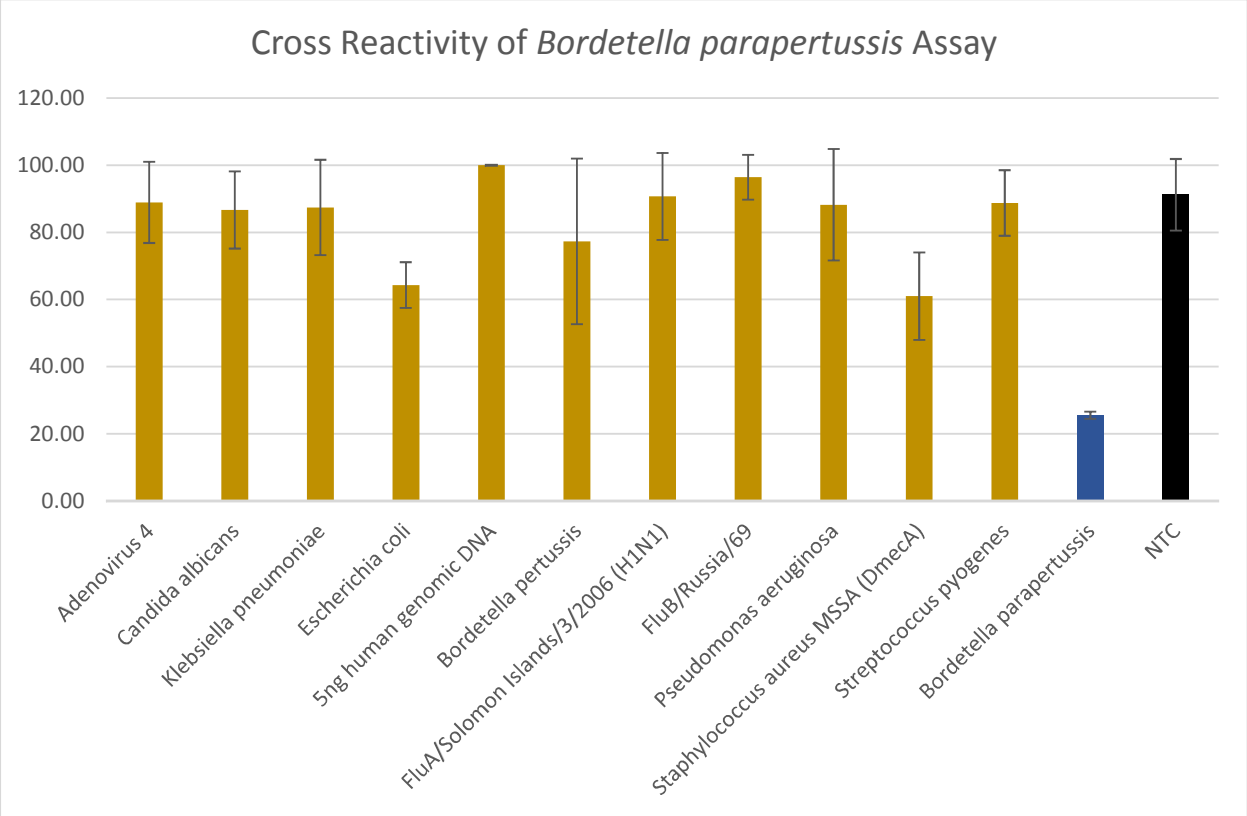

Specificity of HBoV 2,4 assay
120
100
80
60
40
20
0
Adenovirus 4
Candida albicans
Klebsiella pneumoniae
Escherichia coli
5ng human genomic DNA
Bordetella pertussis
A/California/7/2009 (H1N1 novel)
Flu B/Mass/3/66
Pseudomonas aeruginosa
Staphylococcus aureus MSSA (DmecA)
IDTE
-
100 c/uL Bocavirus 2/4 in
NTC

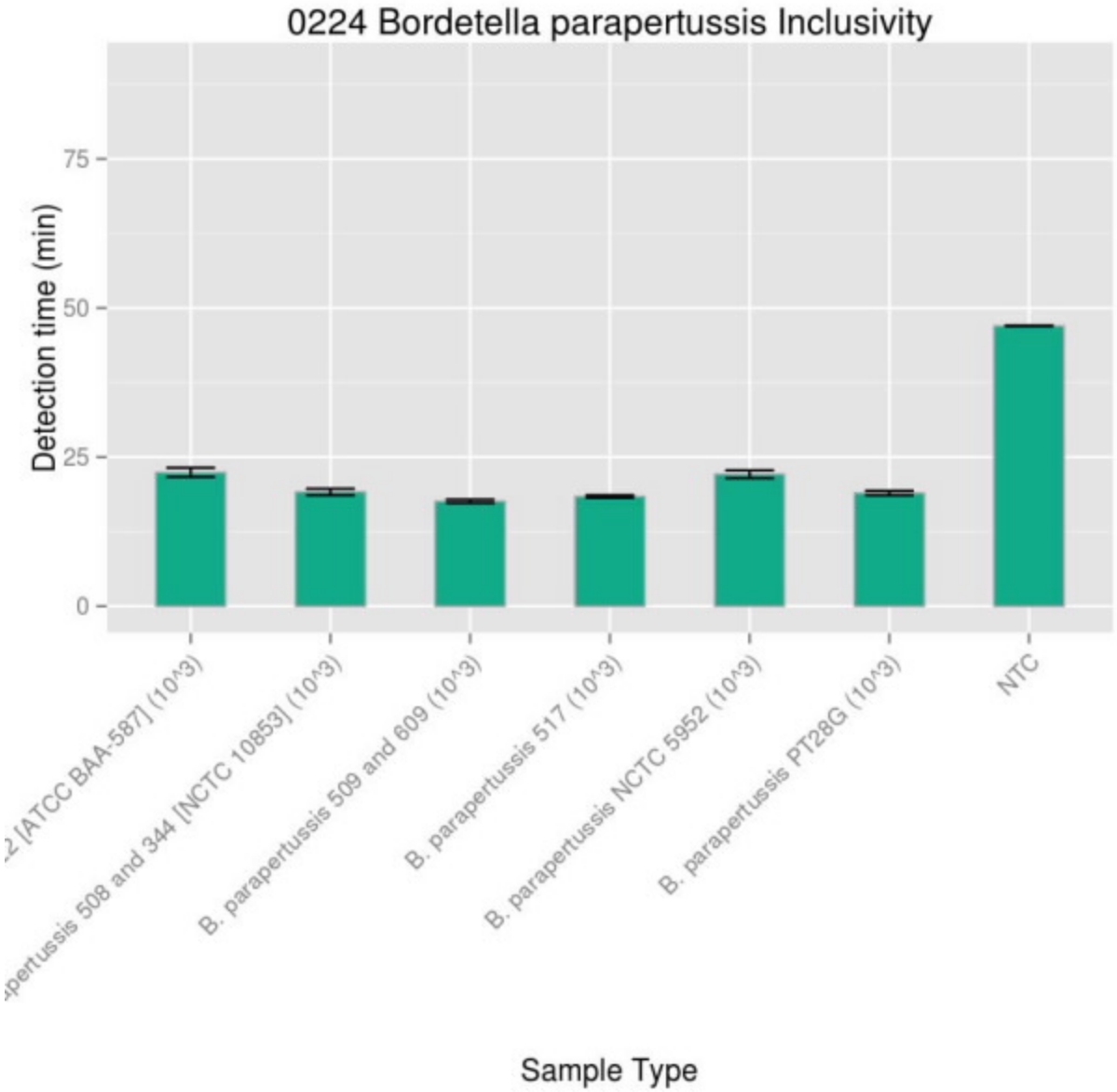

Limit of Detection of Strep A
Inflection Time (min)
120.00
100.00
80.00
60.00
40.00
20.00
0.00
10^3
10^2
10
NTC

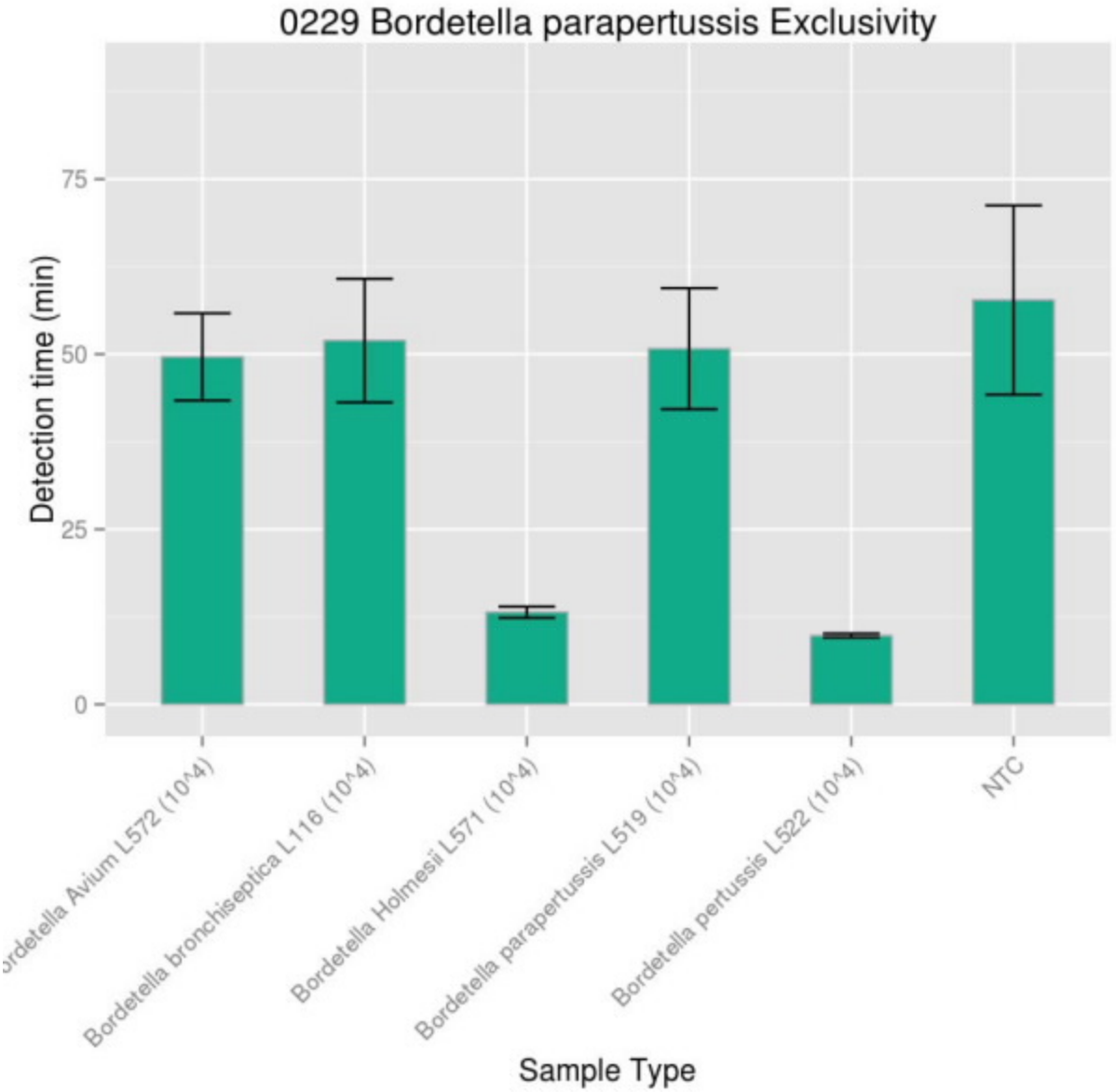

Cross Reactivity of Group A Streptococcus Assay
120.00
100.00
80.00
60.00
40.00
20.00
0.00
Adenovirus 4
Candida albicans
Klebsiella pneumoniae
Escherichia coli
5ng human genomic DNA*
Bordetella pertussis
Flu A/Solomon Islands/3/2006 (H1N1)
B/Hubei-Wujiagang/158/2009
Pseudomonas aeruginosa
Staphylococcus aureus MSSA...
Streptococcus pyogenes 10^4
Streptococcus pyogenes 100
NTCs

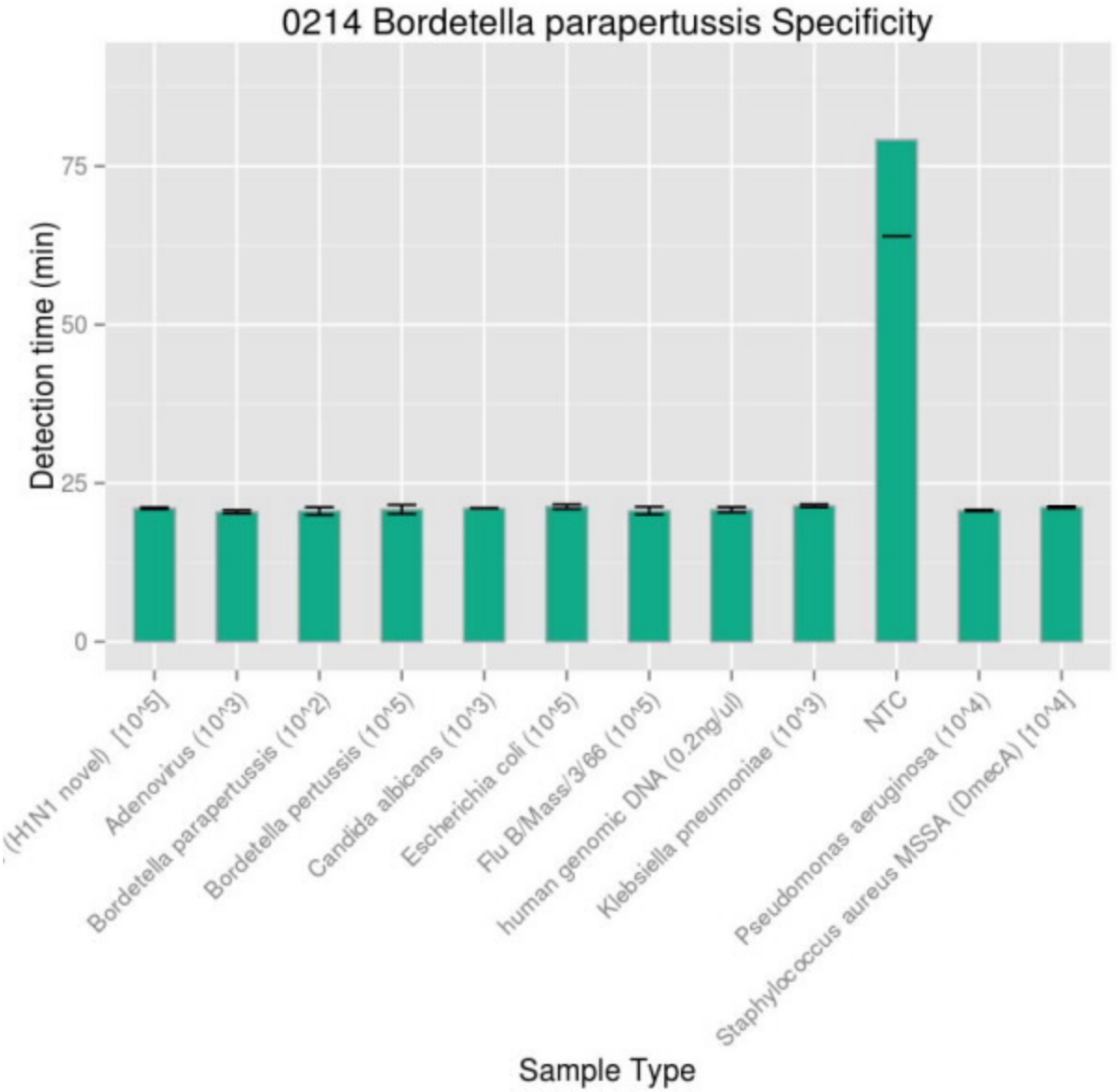

Strep A exclusivity
120
100
80
60
40
20
0
Streptococcus pyogenes (A)
Streptococcus agalactiae (B)
Streptococcus pneumomoniae
Streptococcus mutans
Streptococcus salivarus
NTC

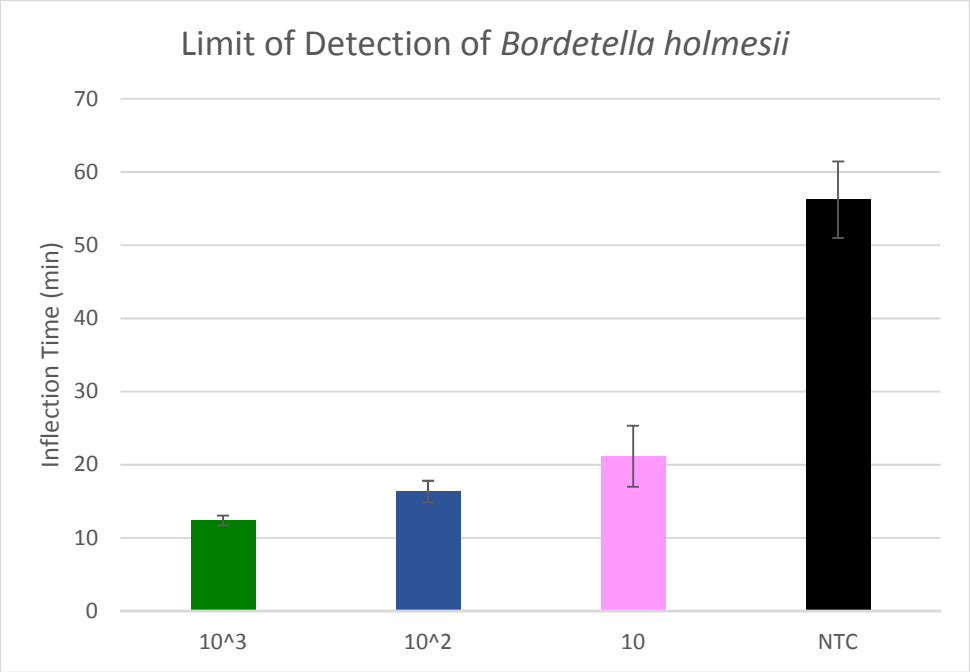

Limit of Detection of *Bordetella parapertussis*
120.00
100.00
80.00
60.00
40.00
20.00
0.00
Inflection Time (min)
10^3
10^2
10
NTC

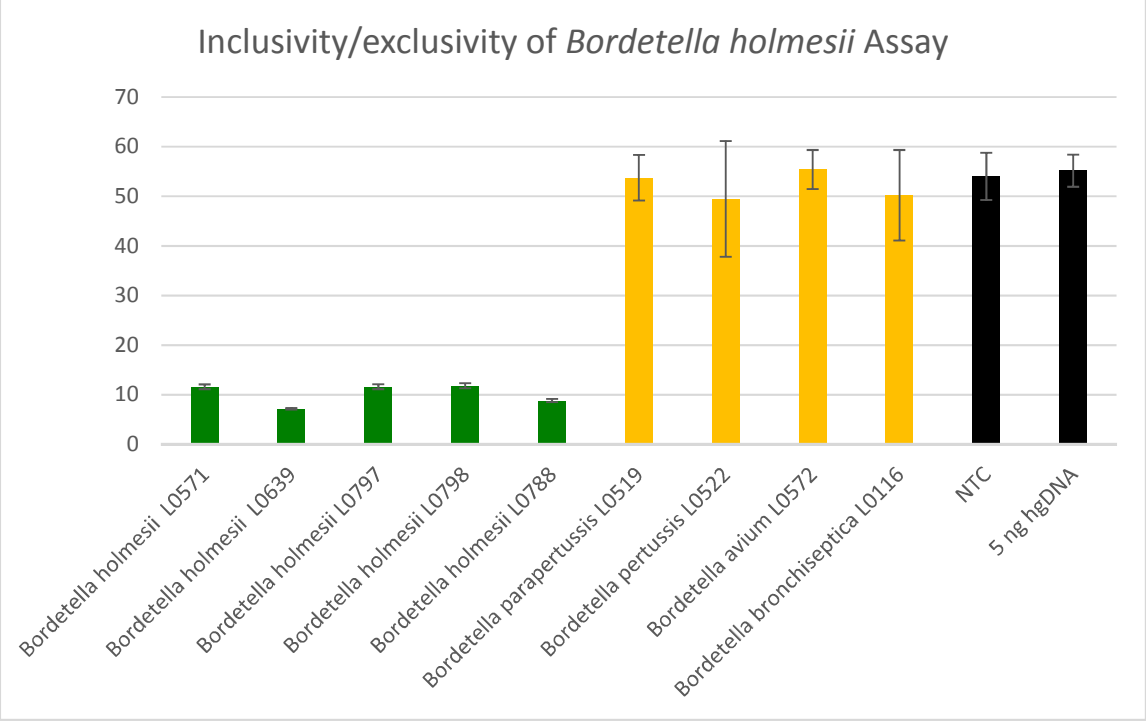

Cross Reactivity of *Bordetella parapertussis* Assay
120.00
100.00
80.00
60.00
40.00
20.00
0.00
Adenovirus 4
Candida albicans
Klebsiella pneumoniae
Escherichia coli
5ng human genomic DNA
Bordetella pertussis
FluA/Solomon Islands/3/2006 (H1N1)
FluB/Russia/69
Pseudomonas aeruginosa
Staphylococcus aureus MSSA (DmecA)
Streptococcus pyogenes
Bordetella parapertussis
NTC

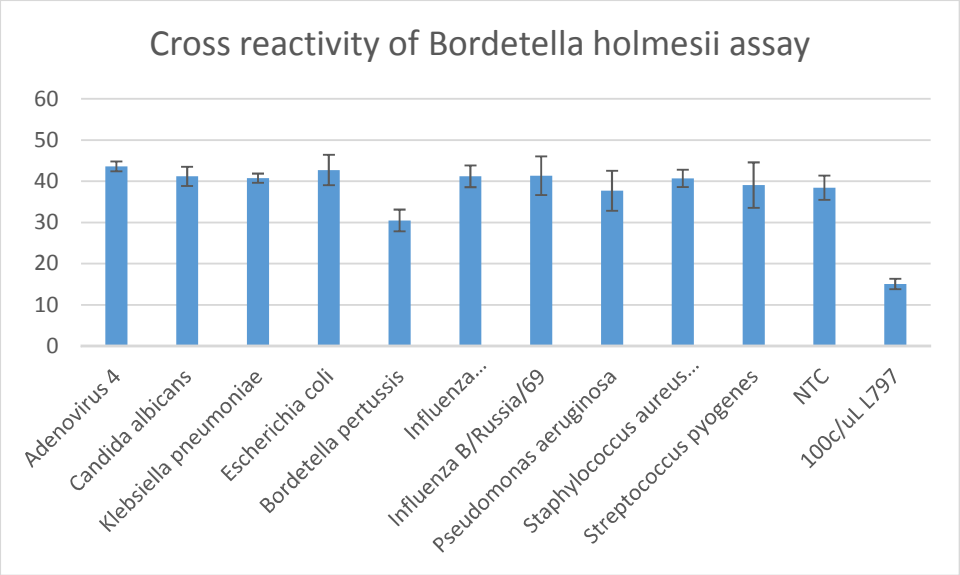

0224 Bordetella parapertussis Inclusivity
Detection time (min)
75
50
25
0
2 [ATCC BAA-587] (10^3)
pertussis 508 and 344 [NCTC 10853] (10^3)
B. parapertussis 509 and 609 (10^3)
B. parapertussis 517 (10^3)
B. parapertussis NCTC 5952 (10^3)
B. parapertussis PT28G (10^3)
NTC
Sample Type

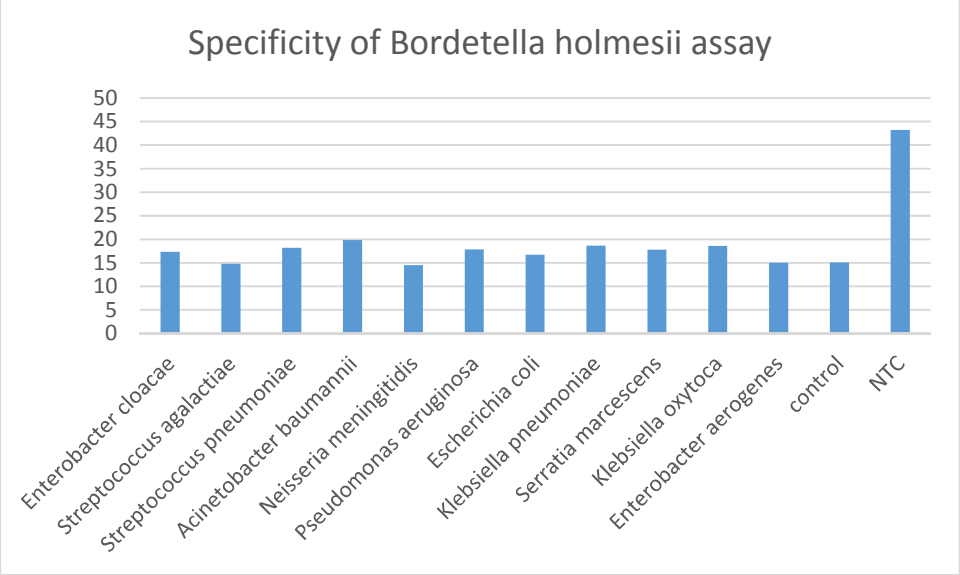

0229 Bordetella parapertussis Exclusivity
Detection time (min)
75
50
25
0
ordetella Avium L572 (10^4)
Bordetella bronchiseptica L116 (10^4)
Bordetella Holmesii L571 (10^4)
Bordetella parapertussis L519 (10^4)
Bordetella pertussis L522 (10^4)
NTC
Sample Type

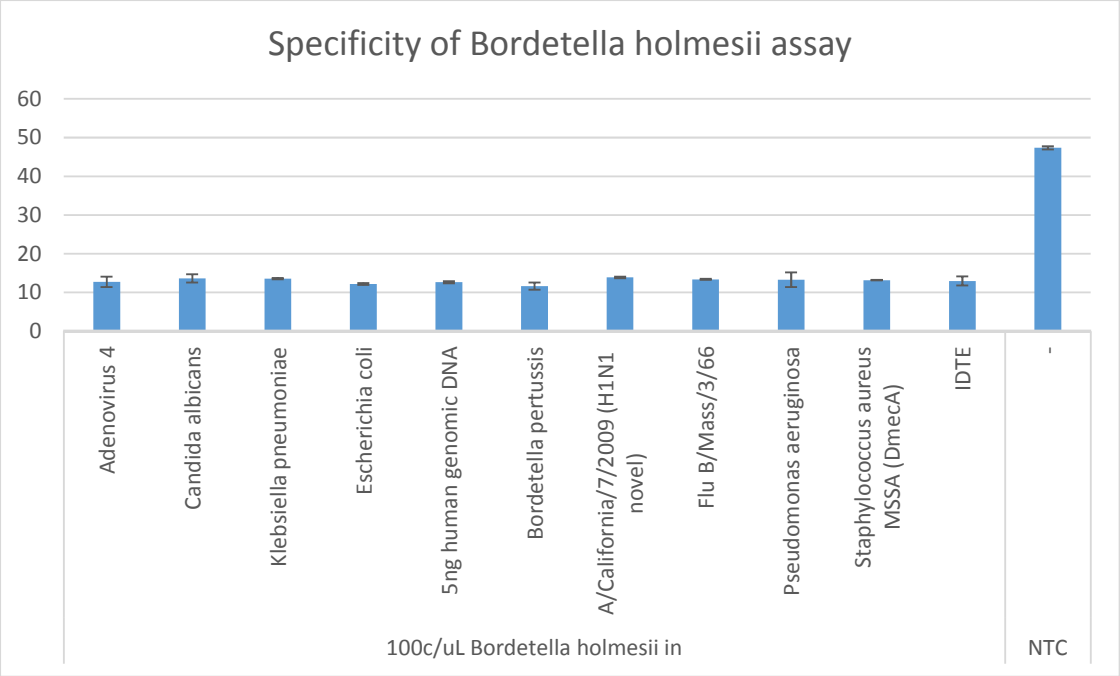

0214 Bordetella parapertussis Specificity
Detection time (min)
75
50
25
0
(H1N1 novel) [10^5]
Adenovirus (10^3)
Bordetella parapertussis (10^2)
Bordetella pertussis (10^5)
Candida albicans (10^3)
Escherichia coli (10^5)
Flu B/Mass/3/66 (10^5)
human genomic DNA (0.2ng/ul)
Klebsiella pneumoniae (10^3)
NTC
Pseudomonas aeruginosa (10^4)
Staphylococcus aureus MSSA (DmecA) [10^4]
Sample Type

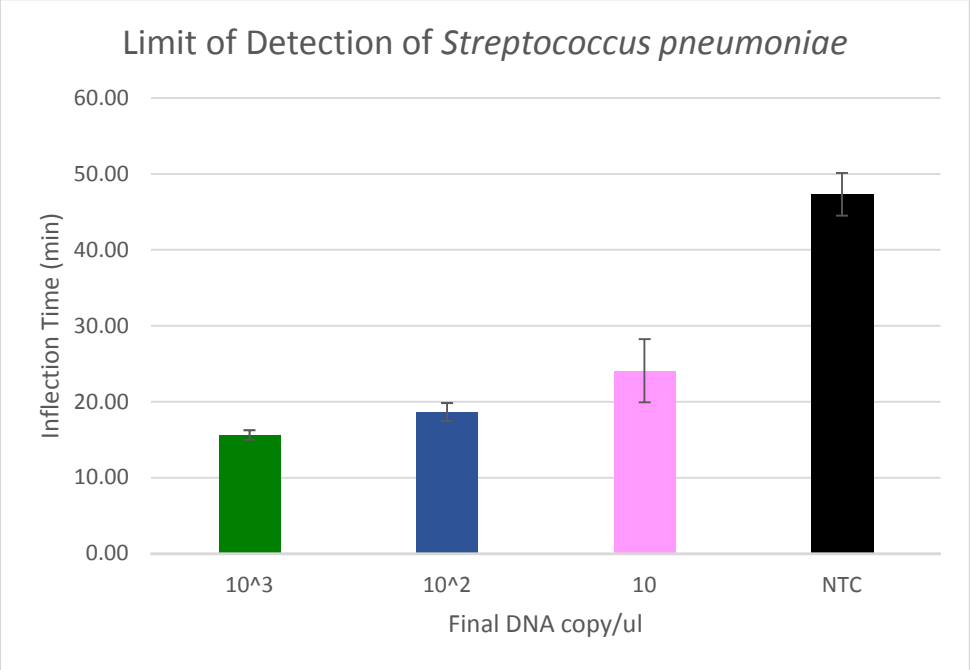

Limit of Detection of *Bordetella holmesii*
Inflection Time (min)
70
60
50
40
30
20
10
0
10^3
10^2
10
NTC

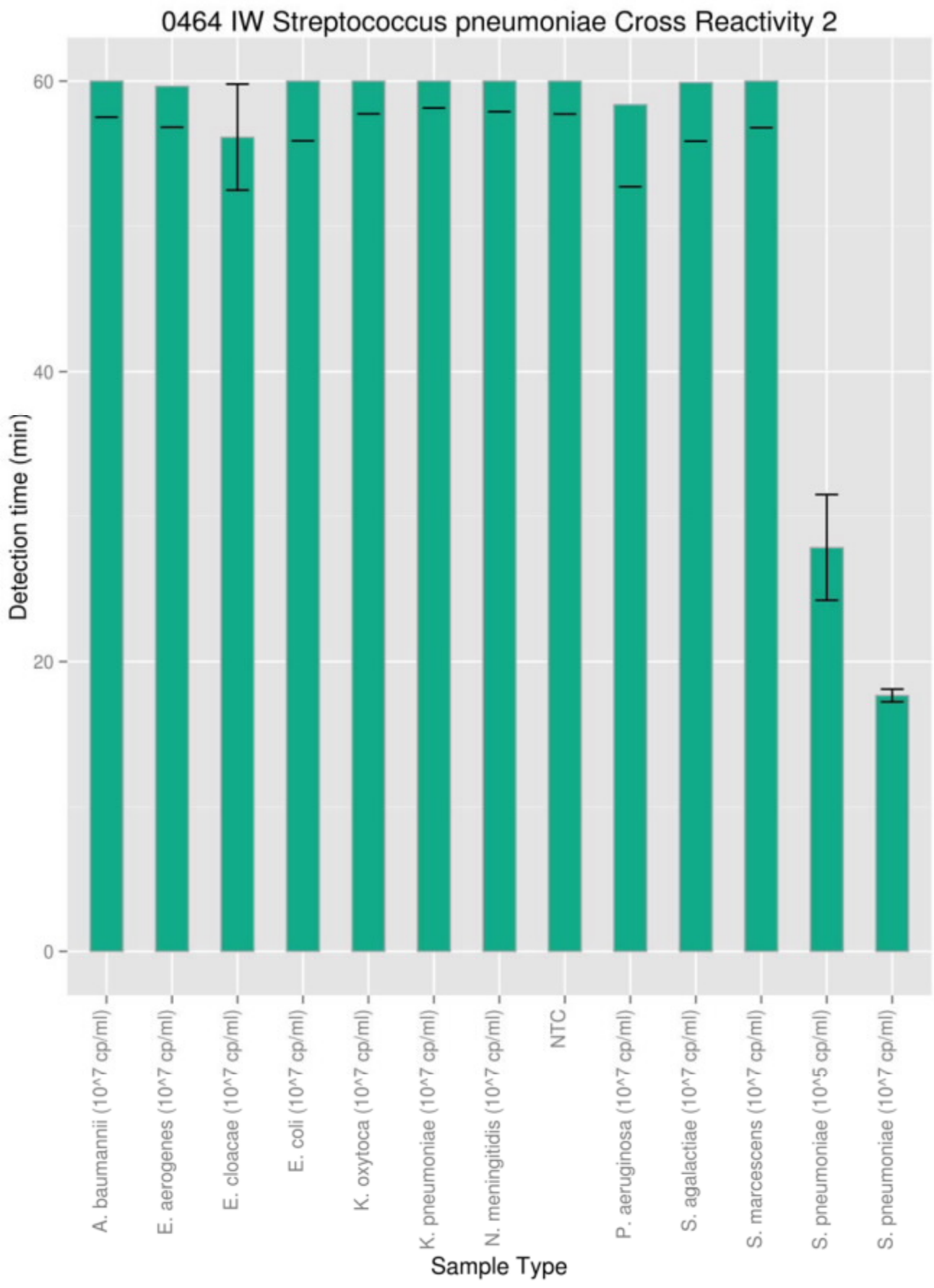

Inclusivity/exclusivity of *Bordetella holmesii* Assay
70
60
50
40
30
20
10
0
Bordetella holmesii L0571
Bordetella holmesii L0639
Bordetella holmesii L0797
Bordetella holmesii L0798
Bordetella holmesii L0788
Bordetella parapertussis L0519
Bordetella pertussis L0522
Bordetella avium L0572
Bordetella bronchiseptica L0116
NTC
5 ng hgDNA

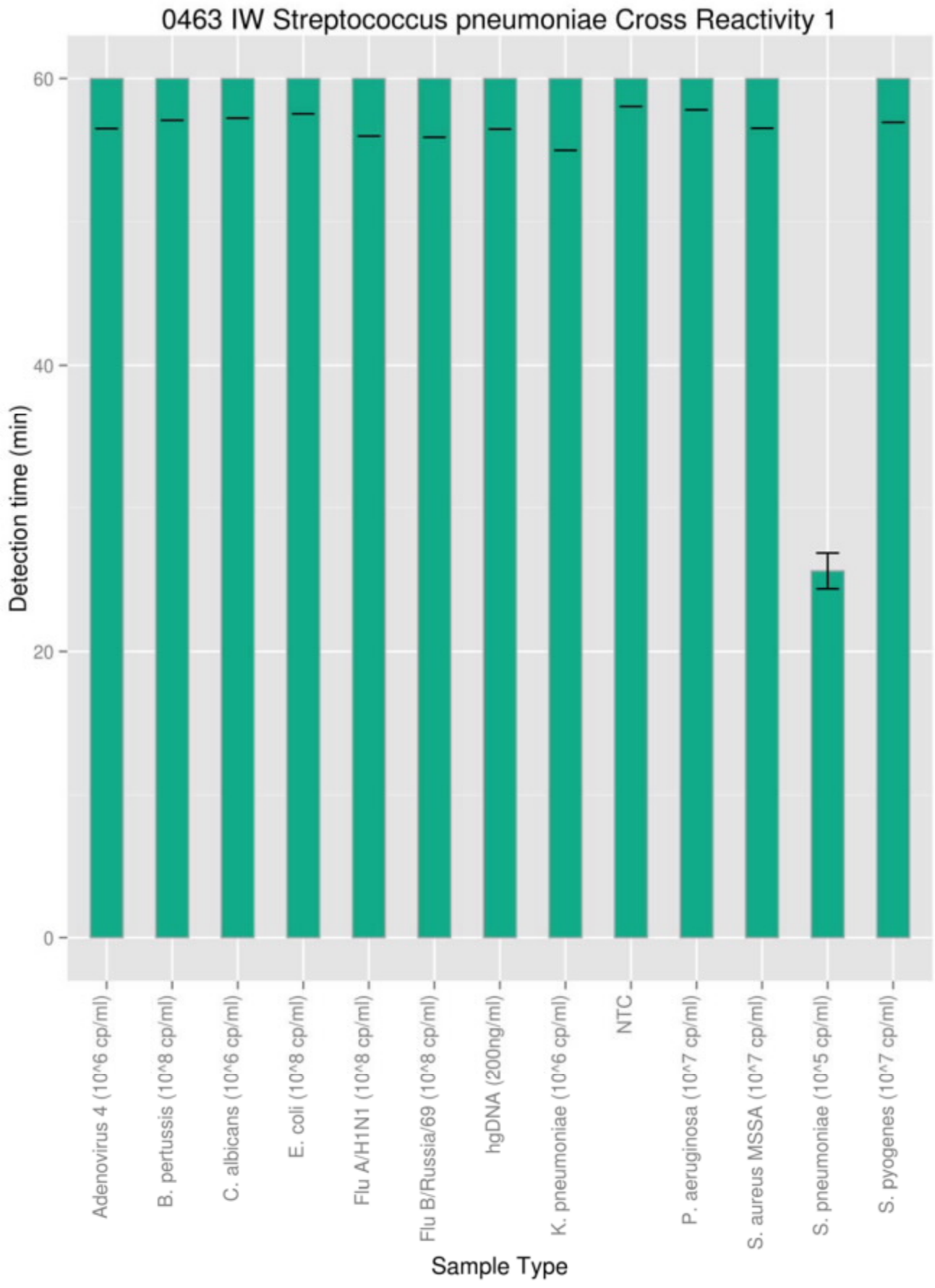

Cross reactivity of Bordetella holmesii assay
60
50
40
30
20
10
0
Adenovirus 4
Candida albicans
Klebsiella pneumoniae
Escherichia coli
Bordetella pertussis
Influenza...
Influenza B/Russia/69
Pseudomonas aeruginosa
Staphylococcus aureus...
Streptococcus pyogenes
NTC
100c/uL L797

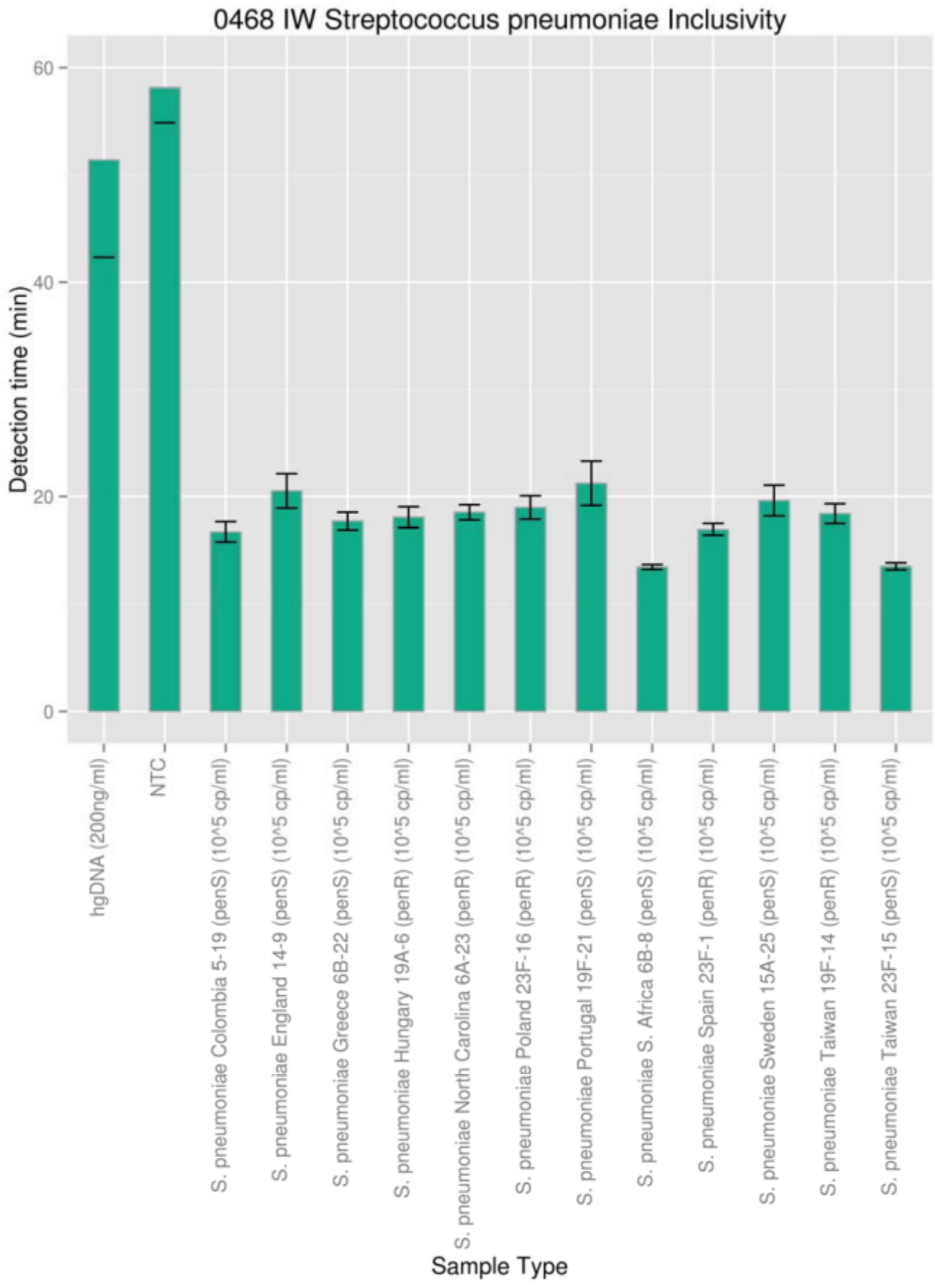

Specificity of Bordetella holmesii assay
50
45
40
35
30
25
20
15
10
5
0
Enterobacter cloacae
Streptococcus agalactiae
Streptococcus pneumoniae
Acinetobacter baumannii
Neisseria meningitidis
Pseudomonas aeruginosa
Escherichia coli
Klebsiella pneumoniae
Serratia marcescens
Klebsiella oxytoca
Enterobacter aerogenes
control
NTC

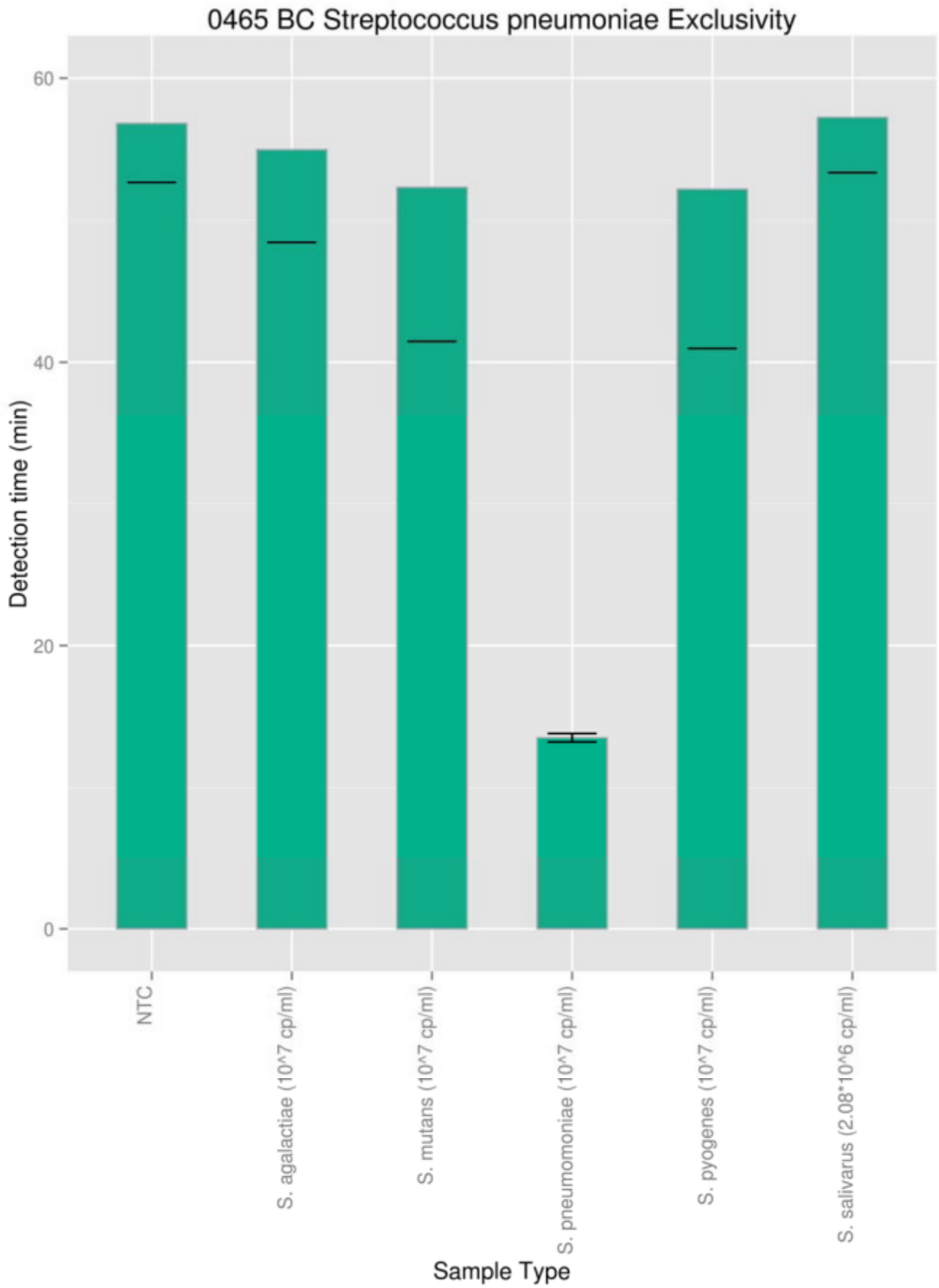

Specificity of Bordetella holmesii assay
60
50
40
30
20
10
0
Adenovirus 4
Candida albicans
Klebsiella pneumoniae
Escherichia coli
5ng human genomic DNA
Bordetella pertussis
A/California/7/2009 (H1N1 novel)
Flu B/Mass/3/66
Pseudomonas aeruginosa
Staphylococcus aureus MSSA (DmecA)
IDTE
-
100c/uL Bordetella holmesii in
NTC

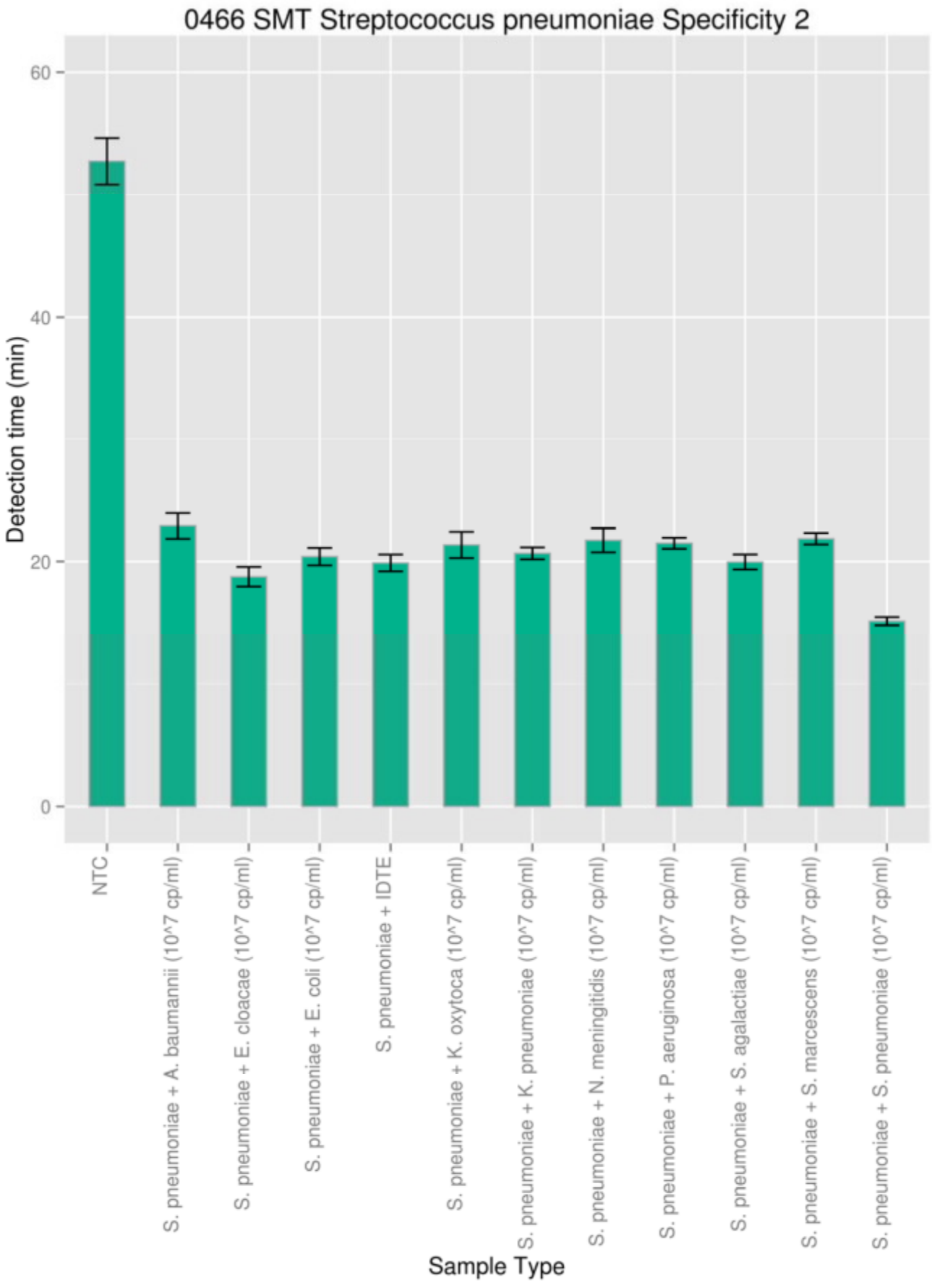

Limit of Detection of *Streptococcus pneumoniae*
Inflection Time (min)
60.00
50.00
40.00
30.00
20.00
10.00
0.00
10^3
10^2
10
NTC
Final DNA copy/ul

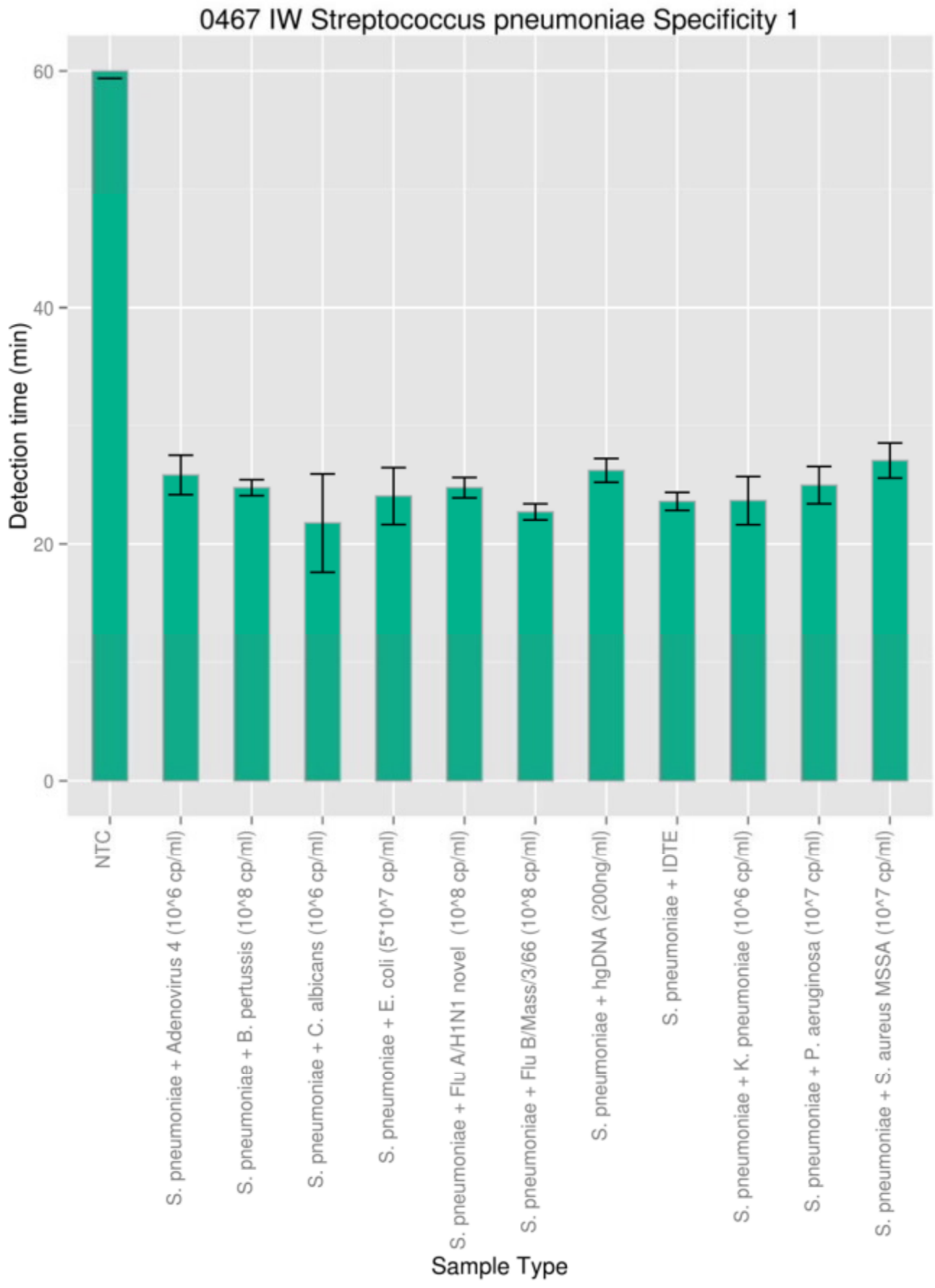

0464 IW Streptococcus pneumoniae Cross Reactivity 2
Detection time (min)
60
40
20
0
A. baumannii (10^7 cp/ml)
E. aerogenes (10^7 cp/ml)
E. cloacae (10^7 cp/ml)
E. coli (10^7 cp/ml)
K. oxytoca (10^7 cp/ml)
K. pneumoniae (10^7 cp/ml)
N. meningitidis (10^7 cp/ml)
NTC
P. aeruginosa (10^7 cp/ml)
S. agalactiae (10^7 cp/ml)
S. marcescens (10^7 cp/ml)
S. pneumoniae (10^5 cp/ml)
S. pneumoniae (10^7 cp/ml)
Sample Type


0463 IW Streptococcus pneumoniae Cross Reactivity 1
Detection time (min)
60
40
20
0
Adenovirus 4 (10^6 cp/ml)
B. pertussis (10^8 cp/ml)
C. albicans (10^6 cp/ml)
E. coli (10^8 cp/ml)
Flu A/H1N1 (10^8 cp/ml)
Flu B/Russia/69 (10^8 cp/ml)
hgDNA (200ng/ml)
K. pneumoniae (10^6 cp/ml)
NTC
P. aeruginosa (10^7 cp/ml)
S. aureus MSSA (10^7 cp/ml)
S. pneumoniae (10^5 cp/ml)
S. pyogenes (10^7 cp/ml)
Sample Type


0468 IW Streptococcus pneumoniae Inclusivity
Detection time (min)
60
40
20
0
hgDNA (200ng/ml)
NTC
S. pneumoniae Colombia 5-19 (penS) (10^5 cp/ml)
S. pneumoniae England 14-9 (penS) (10^5 cp/ml)
S. pneumoniae Greece 6B-22 (penS) (10^5 cp/ml)
S. pneumoniae Hungary 19A-6 (penR) (10^5 cp/ml)
S. pneumoniae North Carolina 6A-23 (penR) (10^5 cp/ml)
S. pneumoniae Poland 23F-16 (penR) (10^5 cp/ml)
S. pneumoniae Portugal 19F-21 (penS) (10^5 cp/ml)
S. pneumoniae S. Africa 6B-8 (penS) (10^5 cp/ml)
S. pneumoniae Spain 23F-1 (penR) (10^5 cp/ml)
S. pneumoniae Sweden 15A-25 (penS) (10^5 cp/ml)
S. pneumoniae Taiwan 19F-14 (penR) (10^5 cp/ml)
S. pneumoniae Taiwan 23F-15 (penS) (10^5 cp/ml)
Sample Type


0465 BC Streptococcus pneumoniae Exclusivity
Detection time (min)
60
40
20
0
NTC
S. agalactiae (10^7 cp/ml)
S. mutans (10^7 cp/ml)
S. pneumomoniae (10^7 cp/ml)
S. pyogenes (10^7 cp/ml)
S. salivarus (2.08*10^6 cp/ml)
Sample Type


0466 SMT Streptococcus pneumoniae Specificity 2
Detection time (min)
60
40
20
0
NTC
S. pneumoniae + A. baumannii (10^7 cp/ml)
S. pneumoniae + E. cloacae (10^7 cp/ml)
S. pneumoniae + E. coli (10^7 cp/ml)
S. pneumoniae + IDTE
S. pneumoniae + K. oxytoca (10^7 cp/ml)
S. pneumoniae + K. pneumoniae (10^7 cp/ml)
S. pneumoniae + N. meningitidis (10^7 cp/ml)
S. pneumoniae + P. aeruginosa (10^7 cp/ml)
S. pneumoniae + S. agalactiae (10^7 cp/ml)
S. pneumoniae + S. marcescens (10^7 cp/ml)
S. pneumoniae + S. pneumoniae (10^7 cp/ml)
Sample Type


0467 IW Streptococcus pneumoniae Specificity 1
Detection time (min)
60
40
20
0
NTC
S. pneumoniae + Adenovirus 4 (10^6 cp/ml)
S. pneumoniae + B. pertussis (10^8 cp/ml)
S. pneumoniae + C. albicans (10^6 cp/ml)
S. pneumoniae + E. coli (5*10^7 cp/ml)
S. pneumoniae + Flu A/H1N1 novel (10^8 cp/ml)
S. pneumoniae + Flu B/Mass/3/66 (10^8 cp/ml)
S. pneumoniae + hgDNA (200ng/ml)
S. pneumoniae + IDTE
S. pneumoniae + K. pneumoniae (10^6 cp/ml)
S. pneumoniae + P. aeruginosa (10^7 cp/ml)
S. pneumoniae + S. aureus MSSA (10^7 cp/ml)
Sample Type

Appendix B: Representative Clinical Data for CBC Assay

CBC assay validation data:

The data below characterizes several aspects of the CBC assay including assay Linearity, Method Comparison, and Matrix Comparison. The CBC assay used Theranos-developed chemistry and measurements were obtained on a commercial flow cytometer (for cellular measurands) or spectrophotometer (for hemoglobin).

The data shown below is for the following measurands:

- RBC count
- Platelet count
- WBC count
- NEU%
- MONO%
- LYM%
- EOS%
- BASO%
- Hemoglobin

RBC Count

1. Linearity

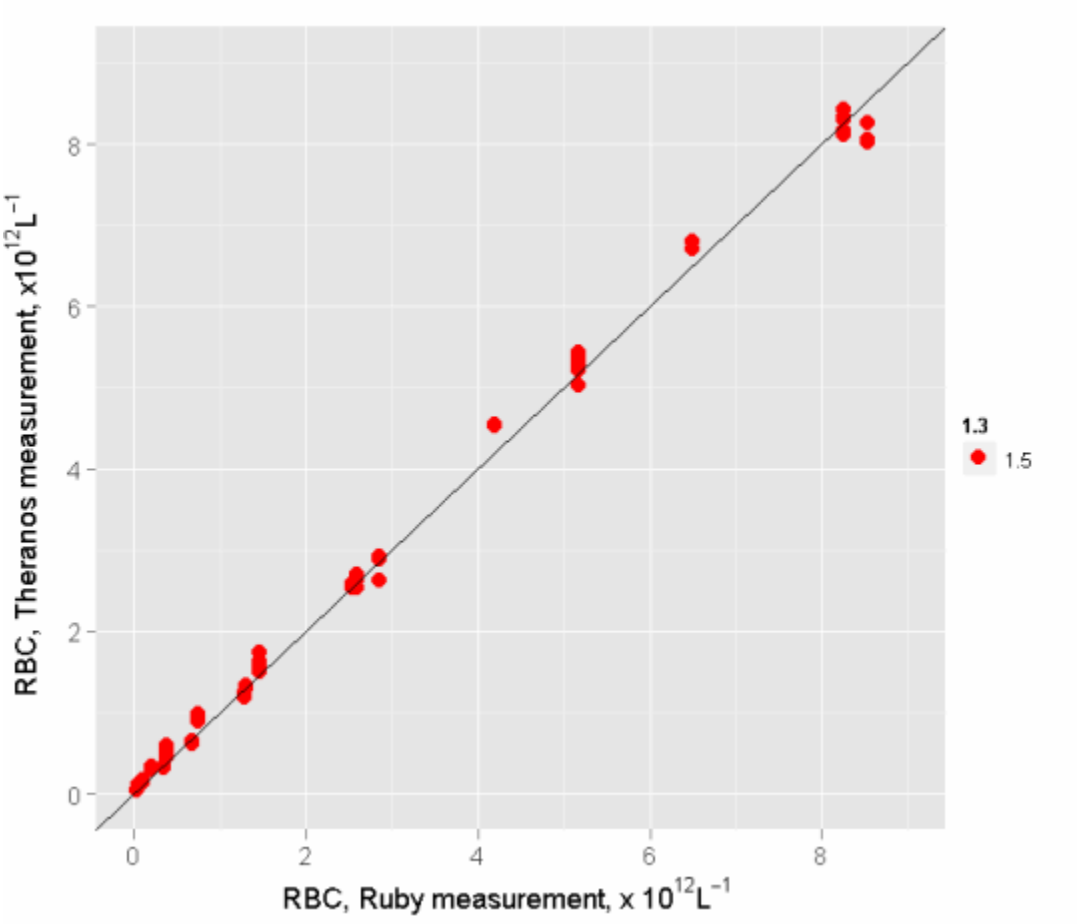


Figure 1 Concordance between Theranos and Abbott Cell-Dyn Ruby measurements showing linearity over the analytical range.

Parameter	*Value*
Slope , [95% C.I.]	0.988, [0.975, 1.000]
Intercept , [95% C.I.]	0.077, [0.028, 0.128]
R^2	0.997

2. Method Comparison

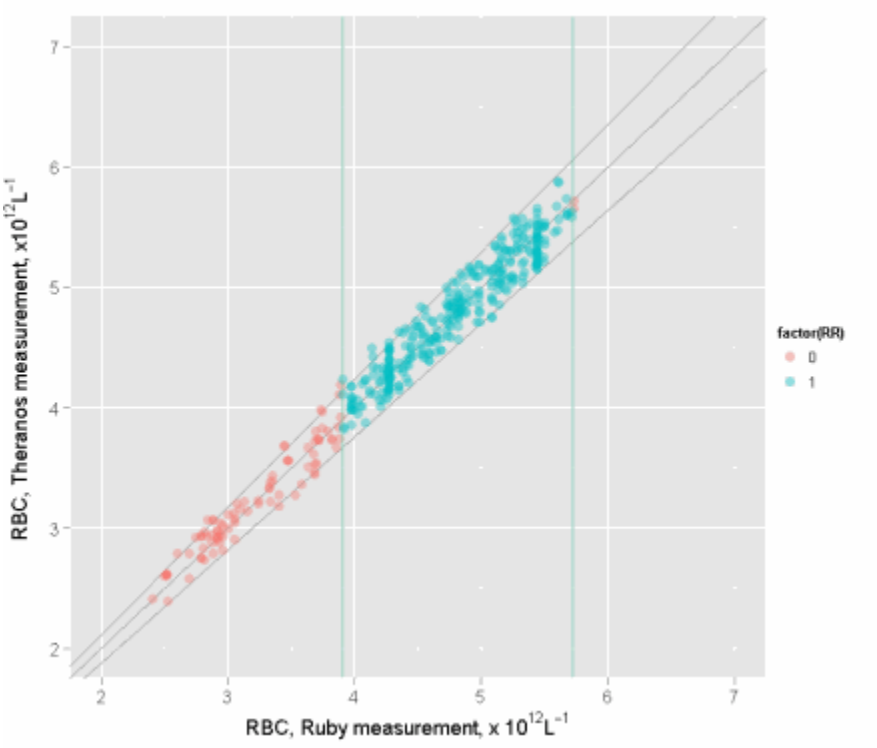


Figure 2 Concordance data showing accuracy of Theranos measurements with respect to Abbott Cell-Dyn Ruby for samples in the normal range (Blue) and pathological out of range samples (Red). Green vertical lines indicated the normal range for this measurand in normal healthy subjects.

Parameter	*Value*
Number of data points, unique samples	421, 195
Slope , [95% C.I.]	0.999, [0.982, 1.016]
Intercept , [95% C.I.]	-0.004, [-0.082, 0.074]
R^2	0.969
Mean bias (%)	0.211
t-test on mean bias, 95% CI	[0.02, 0.63]
p-value	0.04
Total allowable error, % (from CLIA 1988)	±6
Precision, (%CV)	2.3
Total error, %	0.211 + 2 x 2.3 = 4.81
% points with more than 6% total error	2.9

The total error in measurement with respect to Cell-Dyn Ruby is 4.81%, well within the 6% total allowable error.

3. Matrix comparison: fingerstick samples (Theranos system) vs. Venous sample (predicate method)

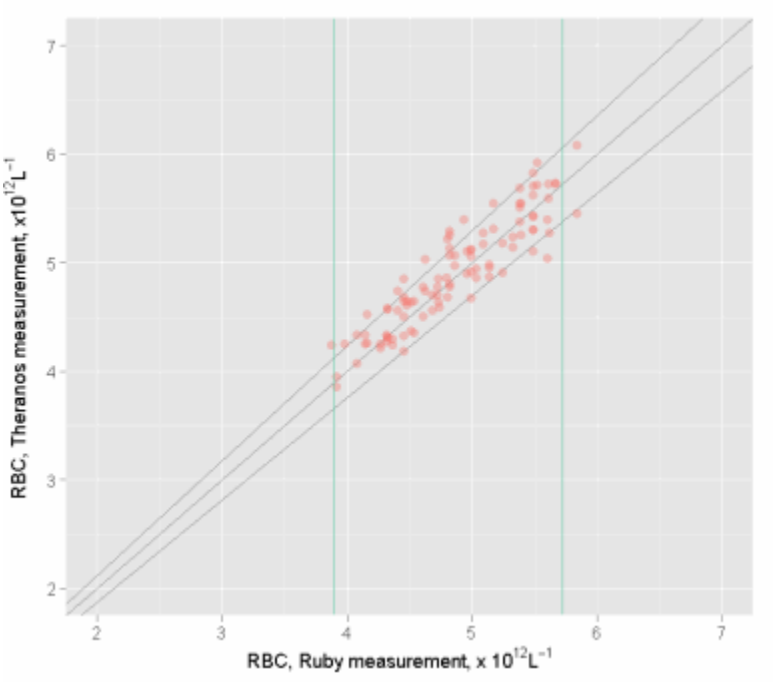


Figure 3. Concordance between fingerstick samples (measured on the Theranos method) and venous samples (measured on the predicate method Cell-Dyn Ruby). Green vertical lines indicated the normal range for this measurand in normal healthy subjects.

Parameter	*Value*
Number of data points, unique samples	102, 55
Slope , [95% C.I.]	0.93, [0.85, 1.03]
Intercept , [95% C.I.]	0.386, [0.009, 0.76]
R^2	0.85
Mean bias (%)	0.64
t-test on mean bias, 95% CI	[-0.17, 1.44]
p-value	0.1
Total allowable error, % (from CLIA 1988)	±6
Precision, (%CV)	2.3
Total error, %	0.64 + 2 x 2.3 = 5.24

Platelet Count

1. Linearity

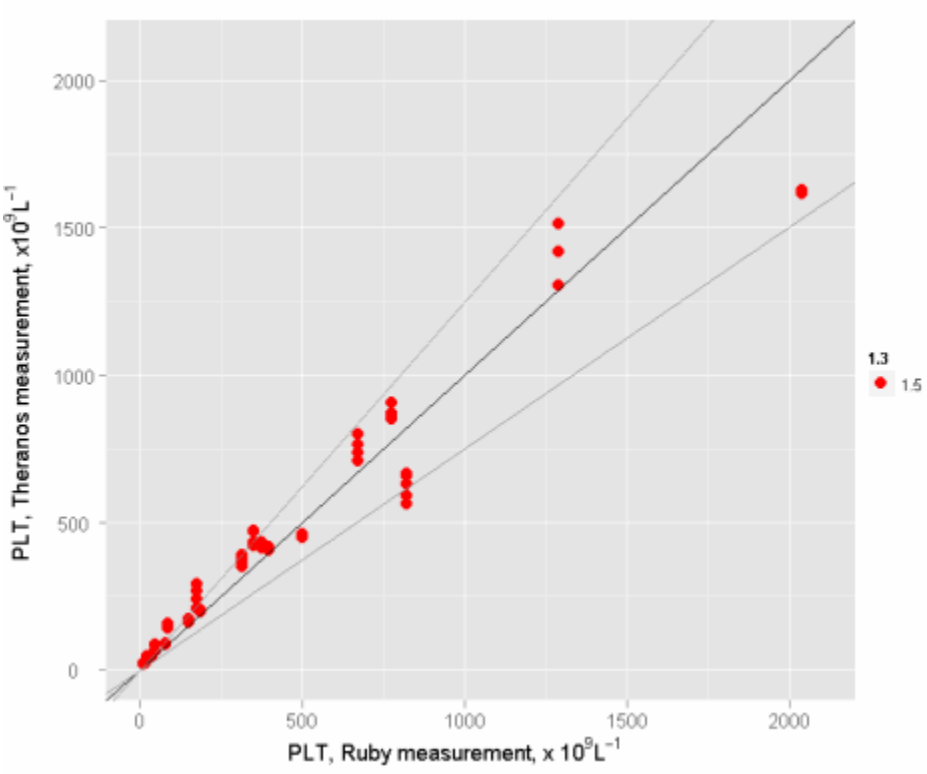


Figure 4. Concordance between Theranos method and Cell-Dyn Ruby showing linearity over the analytical measuring range.

Parameter	*Value*
Slope , [95% C.I.]	1.045, [0.992, 1.106]
Intercept , [95% C.I.]	-32.83, [-64.76, -0.887]
R^2	0.946

2. Method Comparison

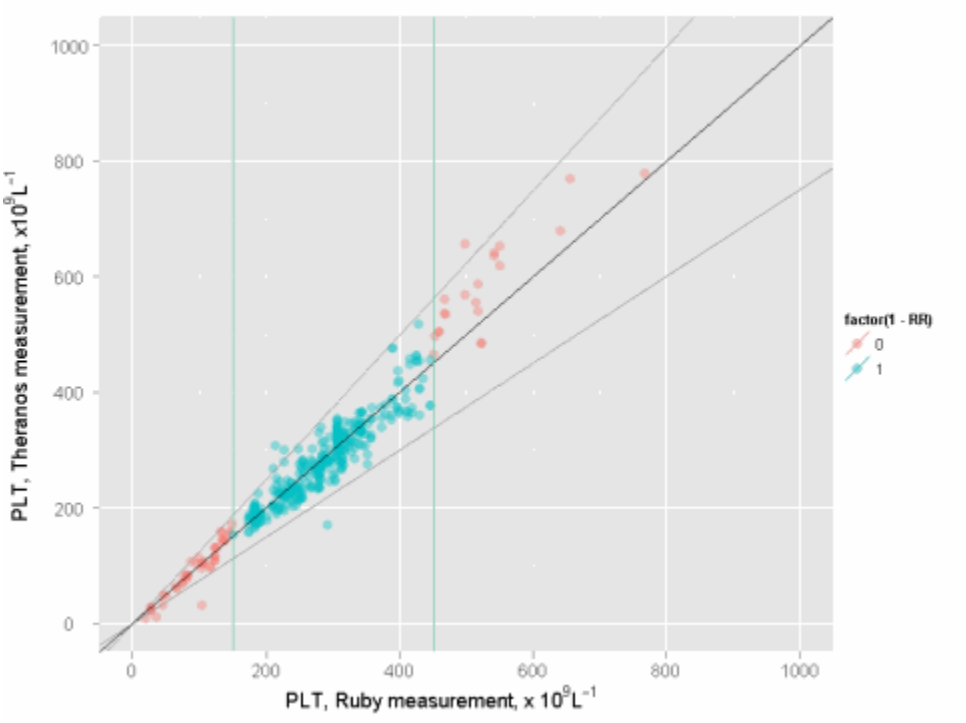


Figure 5. Concordance data showing accuracy of Theranos measurements with respect to Abbott Cell-Dyn Ruby for samples in the normal range (Blue) and pathological out of range samples (Red). Green vertical lines indicated the normal range for this measurand in normal healthy subjects.

Parameter	*Value*
Number of data points, unique samples	421, 195
Slope , [95% C.I.]	1.05, [1.024, 1.078]
Intercept , [95% C.I.]	-13.41, [-21.3, -5.52]
R^2	0.93
Mean bias (%)	-0.42
t-test on mean bias, 95% CI	[-1.49, 0.64]
p-value	0.04
Total allowable error, % (from CLIA 1988)	±25
Precision, (%CV)	4.5
Total error, %	0.42 + 2 x 4.5 = 9.42

The total error in measurement with respect to Cell-Dyn Ruby is 9.42%, well within the 25% total allowable error.

3. Matrix comparison

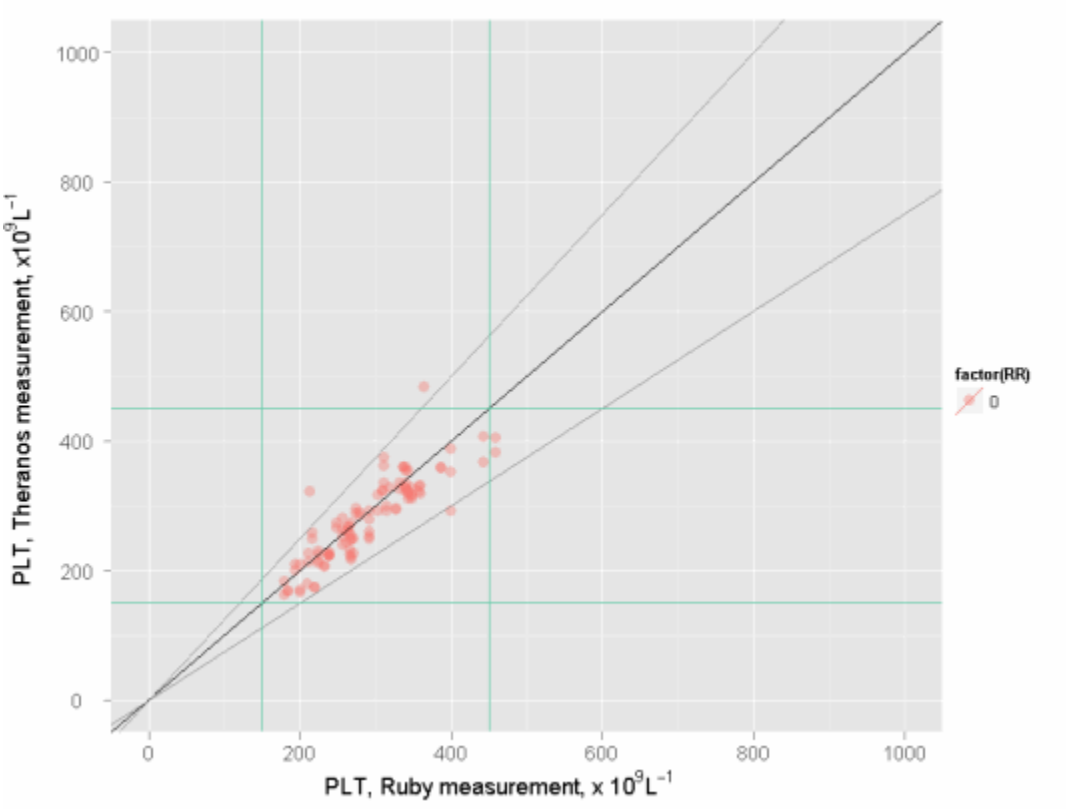


Figure 6 Concordance between fingerstick samples (measured on the Theranos method) and venous samples (measured on the predicate method Cell-Dyn Ruby). Green lines indicated the normal range for this measurand in normal healthy subjects.

Parameter	*Value*
Number of data points, unique samples	102, 55
Slope , [95% C.I.]	0.88, [0.8, 0.95]
Intercept , [95% C.I.]	24.36, [1.99, 46.74]
R^2	0.85
Mean bias (%)	-2.73
t-test on mean bias, 95% CI	[-4.9, -0.55]
p-value	0.01
Total allowable error, % (from CLIA 1988)	±25
Precision, (%CV)	4.5
Total error, %	2.73 + 2 x 4.5 = 11.73
% points with more than 25% total error	2.9

WBC count and differential

1. Linearity

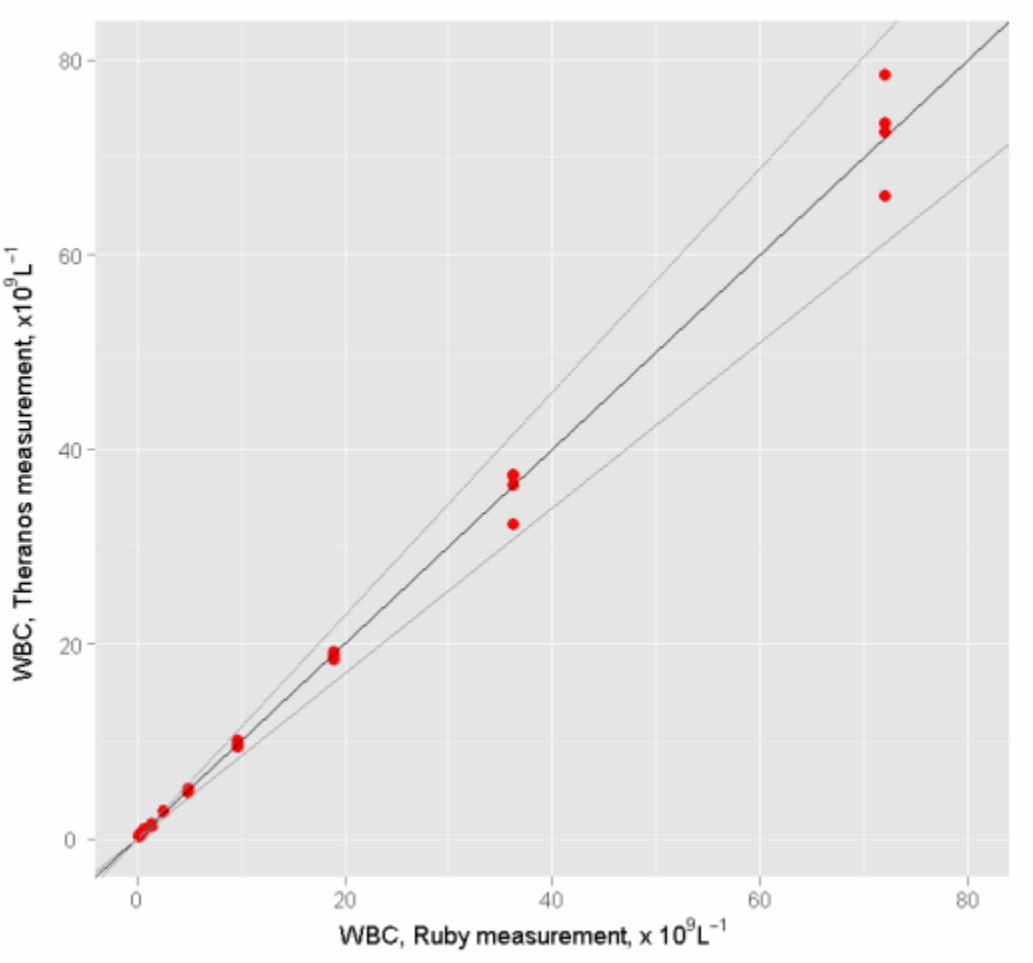


Figure 7 Concordance between Theranos method and Abbott Cell-Dyn Ruby showing linearity over the analytical measuring range.

Parameter	*Value*
Slope , [95% C.I.]	1.005, [0.984, 1.026]
Intercept , [95% C.I.]	0.024, [-0.485, 0.531]
R^2	0.99

2. Method comparison

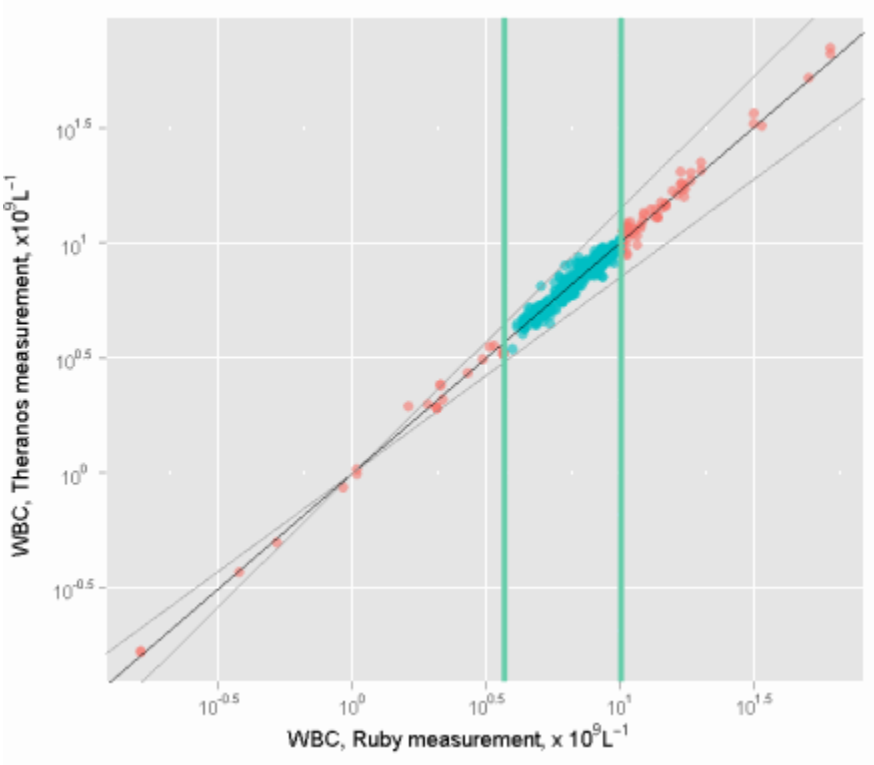


Figure 8 Concordance data showing accuracy of Theranos measurements with respect to Abbott Cell-Dyn Ruby for samples in the normal range (Blue) and pathological out of range samples (Red). Green vertical lines indicated the normal range for this measurand in normal healthy subjects.

Parameter	*Value*
Number of data points, unique samples	628, 244
Slope , [95% C.I.]	1.073, [1.063, 1.083]
Intercept , [95% C.I.]	-0.507, [-0.596, -0.418]
R^2	0.986
Mean bias (%)	0.35
t-test on mean bias, 95% CI	[-0.14, 0.85]
p-value	0.16
Total allowable error, % (from CLIA 1988)	±15
Precision, (%CV)	3.2
Total error, %	0.35 + 2 x 3.2 = 6.75

The total error in measurement with respect to Cell-Dyn Ruby is 6.75%, well within the 15% total allowable error.

3. Method comparison for WBC differential:
    a. Neutrophils

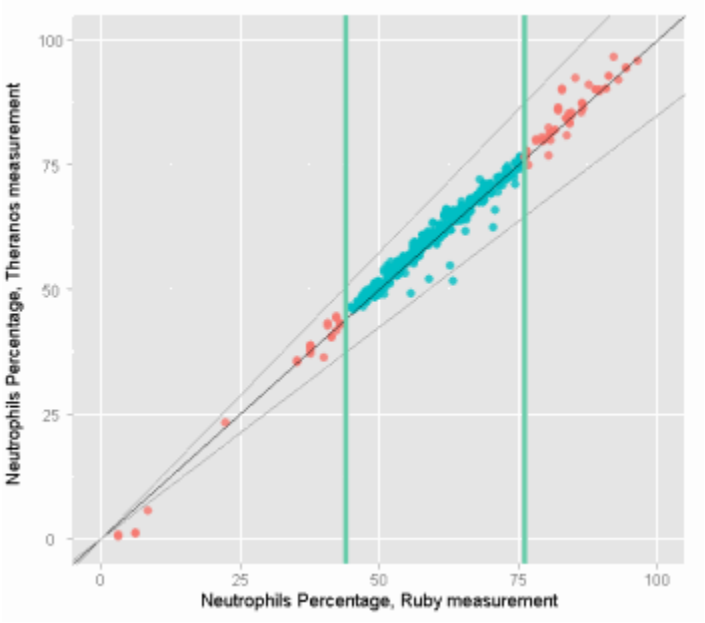


Figure 9. Concordance data showing accuracy of Theranos measurements with respect to Abbott Cell-Dyn Ruby for samples in the normal range (Blue) and pathological out of range samples (Red). Green vertical lines indicated the normal range for this measurand in normal healthy subjects.

Parameter	*Value*
Number of data points, unique samples	628, 244
Slope , [95% C.I.]	1.011, [1.001, 1.020]
Intercept , [95% C.I.]	0.147, [-0.421,0.716]
R^2	0.987
Mean bias (%)	0.84
t-test on mean bias, 95% CI	[0.29, 1.39]
p-value	0
Total allowable error, % (from CLIA 1988)	NA
Precision, (%CV)	1
Total error, %	0.84 + 2 x 1 = 2.84

b. Lymphocytes

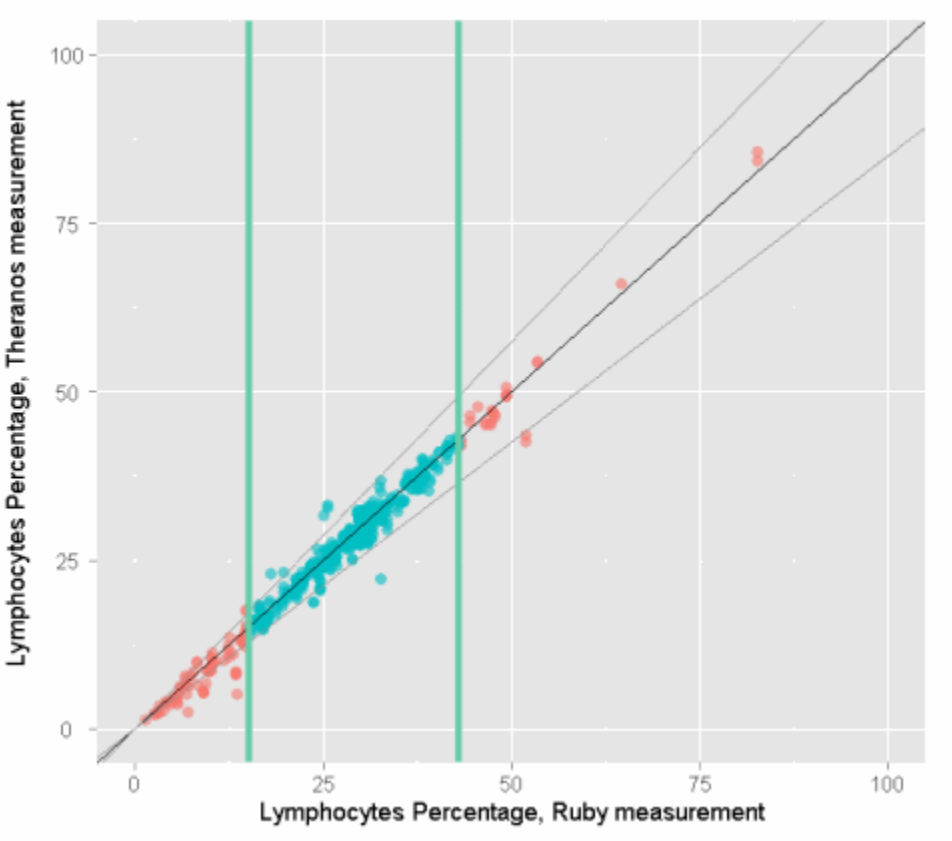


Figure 10. Concordance data showing accuracy of Theranos measurements with respect to Abbott Cell-Dyn Ruby for samples in the normal range (Blue) and pathological out of range samples (Red). Green vertical lines indicated the normal range for this measurand in normal healthy subjects.

Parameter	*Value*
Number of data points, unique samples	628, 244
Slope , [95% C.I.]	1.005, [0.993,1.017]
Intercept , [95% C.I.]	-0.511, [-0.871,-0.151]
R^2	0.977
Mean bias (%)	-2.19
t-test on mean bias, 95% CI	[-2.85,-1.525]
p-value	0
Total allowable error, % (from CLIA 1988)	NA
Precision, (%CV)	1.3
Total error, %	2.19 + 2 x 1.3 = 4.8

c. Monocytes

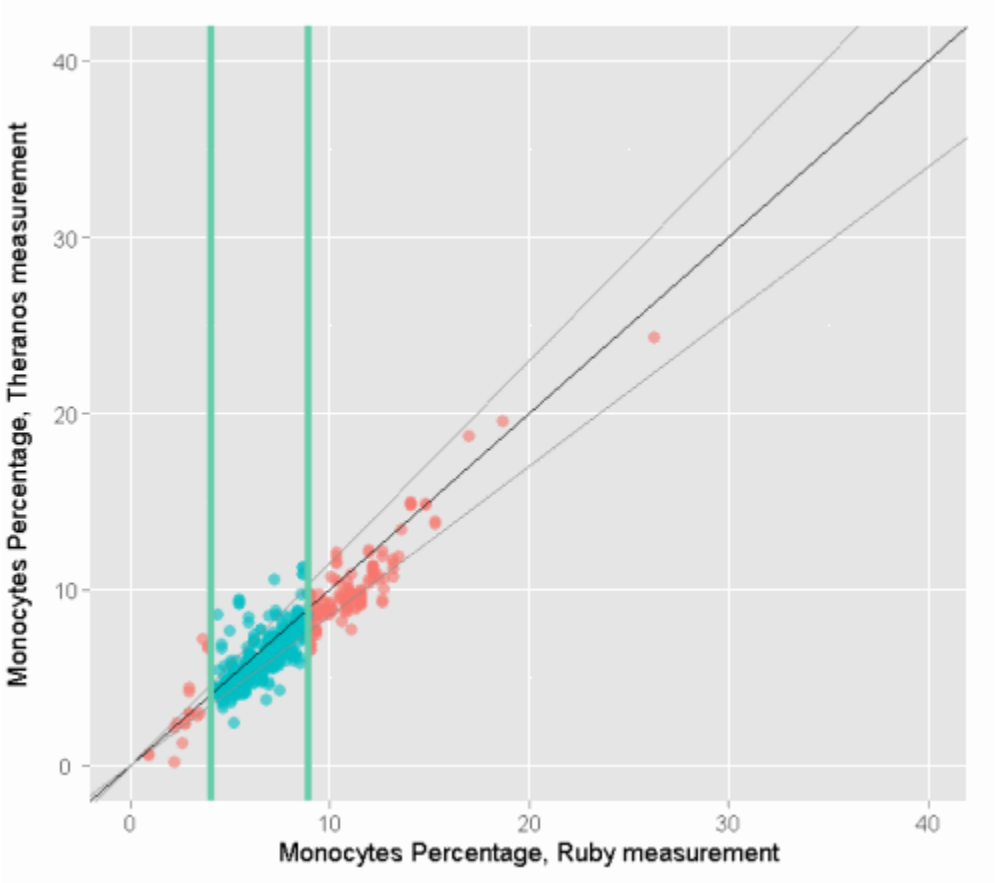


Figure 11. Concordance data showing accuracy of Theranos measurements with respect to Abbott Cell-Dyn Ruby for samples in the normal range (Blue) and pathological out of range samples (Red). Green vertical lines indicated the normal range for this measurand in normal healthy subjects.

Parameter	*Value*
Number of data points, unique samples	628, 244
Slope , [95% C.I.]	0.867, [0.838,0.897]
Intercept , [95% C.I.]	0.439, [0.197,0.681]
R^2	0.842
Mean bias (%)	-6.75
t-test on mean bias, 95% CI	[-7.99,-5.5]
p-value	0
Total allowable error, % (from CLIA 1988)	NA
Precision, (%CV)	2.5
Total error, %	6.75 + 2 x 2.5 = 11.75

d. Eosinophils

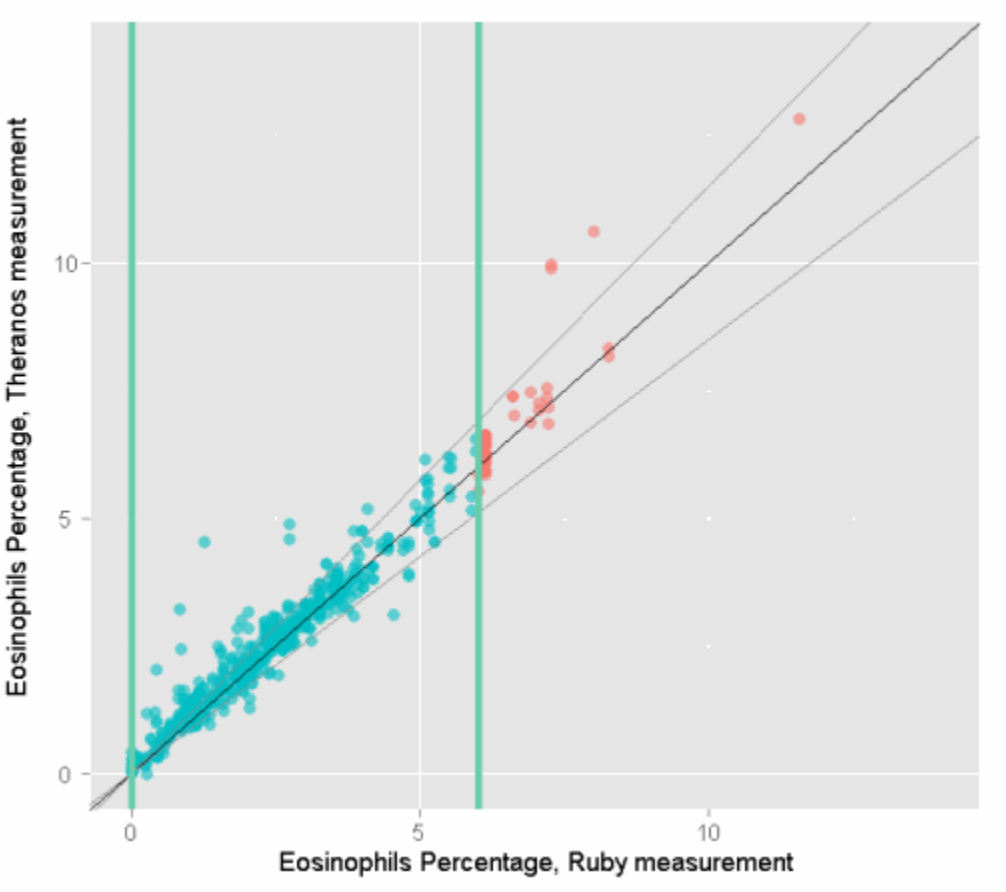


Figure 12. Concordance data showing accuracy of Theranos measurements with respect to Abbott Cell-Dyn Ruby for samples in the normal range (Blue) and pathological out of range samples (Red). Green vertical lines indicated the normal range for this measurand in normal healthy subjects.

Parameter	*Value*
Number of data points, unique samples	628, 244
Slope , [95% C.I.]	1.013, [0.993,1.033]
Intercept , [95% C.I.]	0.134, [0.068,0.2]
R^2	0.942
Mean bias (%)	4.63
t-test on mean bias, 95% CI	[NA]
p-value	0
Total allowable error, % (from CLIA 1988)	NA
Precision, (%CV)	3.9
Total error, %	4.63 + 2 x 3.9 = 12.43

e. Basophils

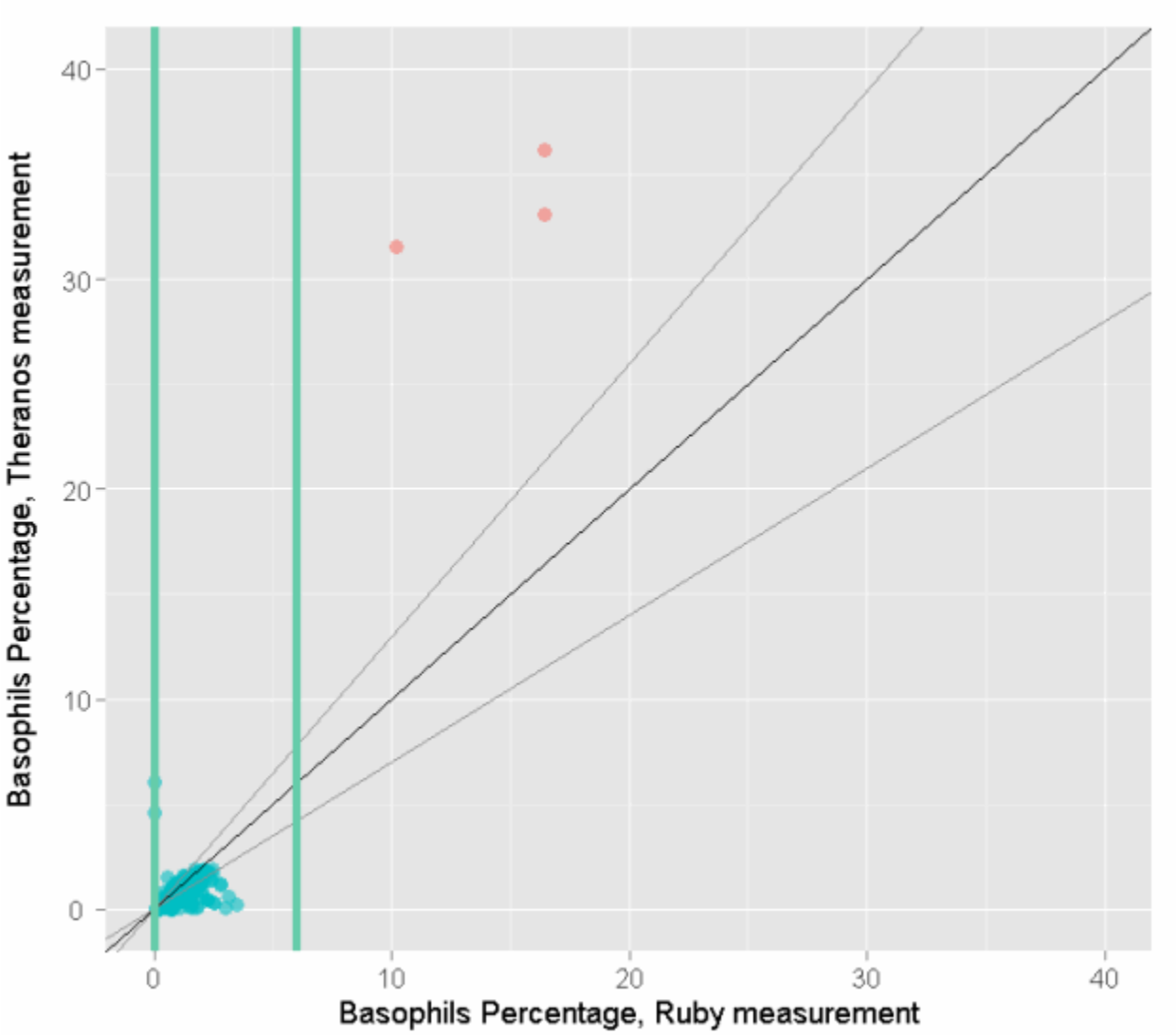


Figure 13. Concordance data showing accuracy of Theranos measurements with respect to Abbott Cell-Dyn Ruby for samples in the normal range (Blue) and pathological out of range samples (Red). Green vertical lines indicated the normal range for this measurand in normal healthy subjects.

4. Matrix comparison

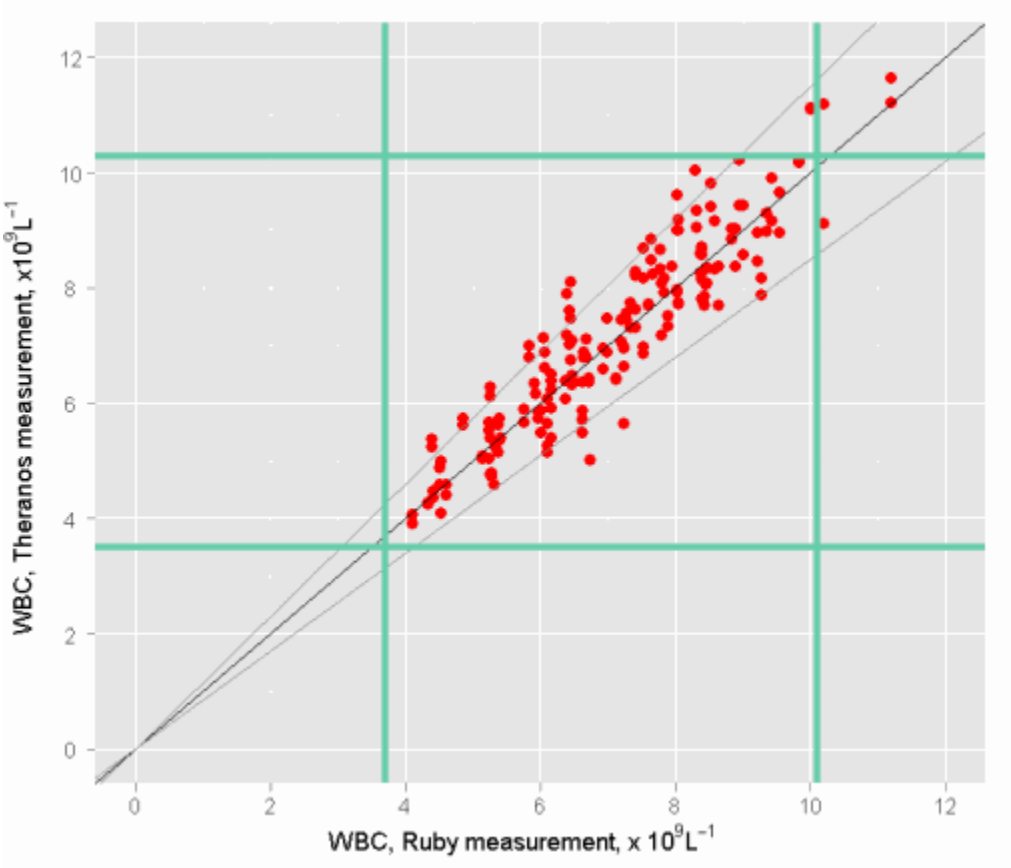


Figure 14. Concordance between fingerstick samples (measured on the Theranos method) and venous samples (measured on the predicate method Cell-Dyn Ruby). Green lines indicated the normal range for this measurand in normal healthy subjects.

Parameter	*Value*
Number of data points, unique samples	181, 93
Slope , [95% C.I.]	0.997, [0.937, 1.037]
Intercept , [95% C.I.]	0.141, [-0.293, 0.575]
R^2	0.857
Mean bias (%)	1.8
t-test on mean bias, 95% CI	[0.46,3.13]
p-value	0.01
Total allowable error, % (from CLIA 1988)	±15
Precision, (%CV)	3.2
Total error, %	1.8 + 2 x 3.2 = 8.2

Hemoglobin

1. Method comparison

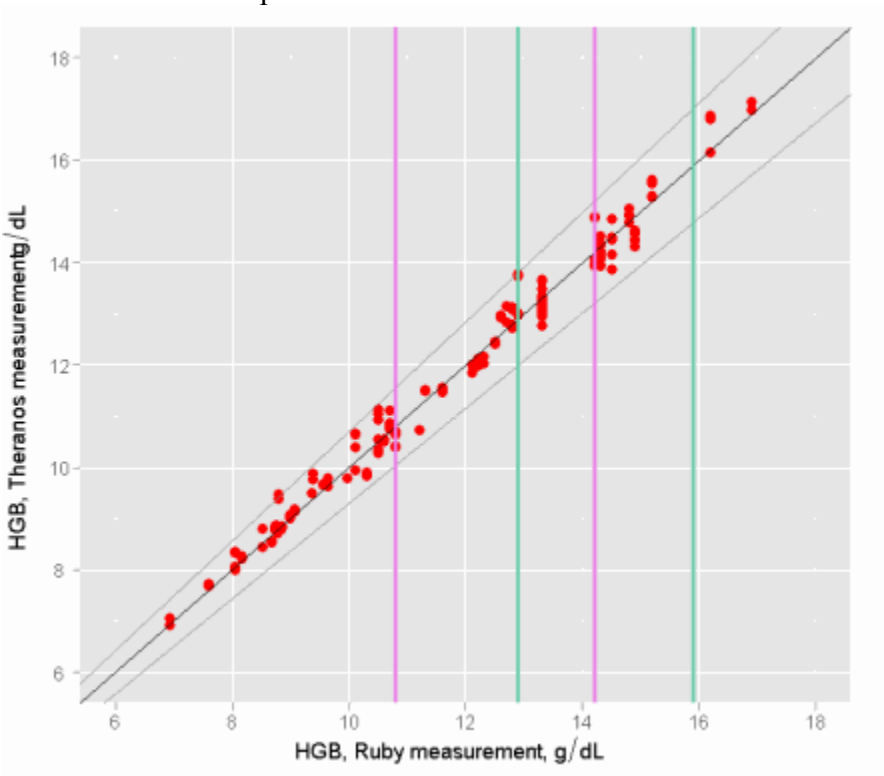


Figure 15. Concordance data showing accuracy of Theranos measurements with respect to Cell-Dyn Ruby. Purple lines show normal range for females and green lines show normal range for males.

Parameter	*Value*
Number of data points, unique samples	172, 62
Slope , [95% C.I.]	0.985, [0.959,1..011]
Intercept , [95% C.I.]	0.205, [-0.1122,0.522]
R^2	0.987
Mean bias (%)	0.31
t-test on mean bias, 95% CI	[-0.08, 0.69]
p-value	0.12
Total allowable error, % (from CLIA 1988)	±7
Precision, (%CV)	2.1
Total error, %	0.31 + 2 x 2.1 = 4.51
% points with more than 7% total error	0

The total error in measurement with respect to Cell-Dyn Ruby is 4.5%, significantly less than the CLIA mandated 7%.

2. Matrix comparison

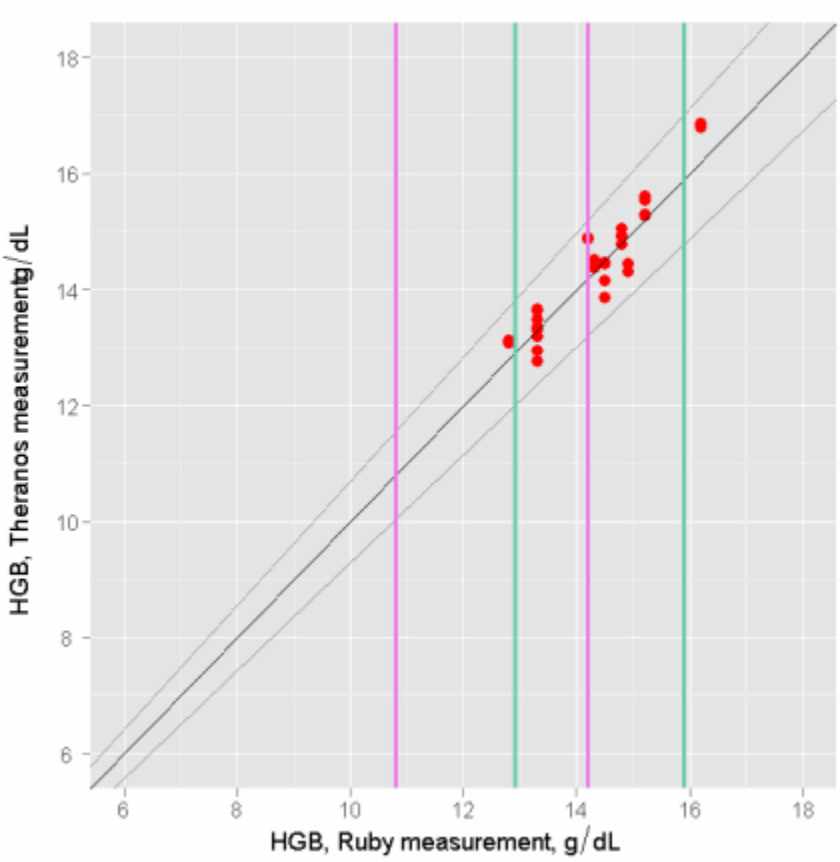


Figure 16. Concordance between fingerstick samples (measured on the Theranos method) and venous samples (measured on the predicate method Cell-Dyn Ruby). Purple lines show normal range for females and green lines show normal range for males.

Parameter	*Value*
Number of data points, unique samples	30, 15
Slope , [95% C.I.]	0.989, [0.8, 0.95]
Intercept , [95% C.I.]	0.213, [0.012, 0.413]
R^2	0.89