To: Elizabeth Holmes[eholmes@theranos.com]
Cc: Brad Arington[barington@theranos.com]; Hobson, John (Peyton)[redacted] Feldblyum, Tamara[redacted] Scherf, Uwe[redacted]; Chan, Maria M[redacted]; Lias, Courtney H[redacted]; Gutierrez, Alberto[redacted]; Roth, Kristian[redacted]
From: Hojvat, Sally A
Sent: Fri 12/27/2013 1:30:46 PM
Importance: Normal
Subject: RE: December PreSub from Theranos
Received: Fri 12/27/2013 1:30:45 PM
001_Theranos Confidential PreSub Request for Lower Resp and Gen Chem Ass....pdf

Dear Elizabeth,

Thank you for the heads up on your latest pre-Submission package .

I have not seen the hard copy come in to the FDA tracking system yet but expect it will show up by the end of today or Monday.

The Microbiology Division will make sure that the Chemistry Division is updated on where we are with the review of the overall Theranos system .

I will be out of the Office for the next 2 weeks but will have blackberry e mail contact .

Peyton, Tamara and Uwe will be back in the Office at various times next week and will make sure that this pre-Sub. goes to the appropriate Branches for review.

Best wishes for a successful New Year to you and your staff.

Regards,

Sally

Sally A. Hojvat Ph.D., M.Sc.

Director, Division of microbiology Devices

OIR/CDRH/FDA

From: Elizabeth Holmes [mailto:eholmes@theranos.com]
Sent: Thursday, December 26, 2013 11:59 PM
To: Hojvat, Sally A; Hobson, John (Peyton)
Cc: Brad Arington
Subject: FW: December PreSub

Dear Sally and Peyton:

Please find attached our next pre-submission in accordance with our plan. Based on the calls we had the opportunity to have, we have updated the assay list for this pre-submission, as reflected in the test list attached, and the additional pre-submissions to follow this one in accordance with our conversations.

We will be following up with you separately on the updated month by month plan and with additional pre-submissions shortly.

This submission should arrive by hard copy tomorrow or by Monday at the latest.

With my best regards and very best wishes for a wonderful holiday season and new year,

Elizabeth

====================================
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Theranos, Inc., 1601 S. California Avenue, Palo Alto, CA, 94304
650-838-9292 www.theranos.com
====================================

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

December 26, 2013

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

Dear Sally,

Please find herewith our third 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 lower respiratory nucleic acid amplification ("***NAA***") and general chemistry assays for use with our CLIA-certified laboratory's automated sample processing and analysis system. As described in the prior Pre-Submissions (Q13199 and Q131542), 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. Just prior to the Informational Meeting (Q131148) on November 4th of this year, Theranos submitted a monthly plan for Pre-Submissions and regulatory approval and clearance submissions covering these assays. We appreciate that the various Divisions of the Office of *In Vitro* Diagnostics took the time to review this plan and provide feedback, including recommendations as to whether certain assays should be included in Pre-Submissions. We have noted that a Pre-Submission for the Clinical Chemistry assays that have been included in this Pre-Submission was not recommended. Since we have some questions regarding the 510(k) for these assays, as highlighted in the Specific Questions section, however, we felt that it would be of value to include them.

Pursuant to these goals and to this Pre-Submission, we have submitted our lower respiratory NAA and general chemistry assays 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 lower respiratory illnesses can simultaneously receive additional assay results providing insight into the patient's overall medical condition.

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For our pre-submission meeting, Sunny Balwani (Theranos' COO and President) and I will attend. Brad Arington (Theranos' Senior Regulatory Counsel) will attend as well, and others may join by telephone. We would like to reserve a room that has a conference phone and an LCD projector.

As always, we can make ourselves available to meet at any time that is convenient for you. With the logistics of traveling from the West Coast, Mondays are the most convenient for us if possible. However, 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

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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)*
12/26/2013		

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

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

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

Field	Value	Field	Value
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

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

- [ ] Location change:
  - [ ] Manufacturer
  - [ ] Sterilizer
  - [ ] Packager

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

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

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

- [ ] Response to FDA correspondence:

- [ ] 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

- [x] 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	CJW	3	KHS	4	CDT
5	JFM	6	LBS	7	KNK	8	

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	K132664	1	Advia Chemistry Albumin BCP	1	Siemens Healthcare Diagnostics, Inc.
2	K100289	2	Advia Chemistry Systems Carbon Dioxide Assay	2	Siemens Healthcare Diagnostics, Inc.
3	K133067	3	Advia Chemistry Triglycerides	3	Siemens Healthcare Diagnostics, Inc.
4	K063845	4	Advia Chemistry Total Bilirubin	4	Siemens Healthcare Diagnostics, Inc
5	K050632	5	Advia Direct HDL Cholesterol Assay	5	Siemens Healthcare Diagnostics, Inc.
6	K022096	6	Advia Uric Acid Assay	6	Siemens Healthcare Diagnostics, Inc.

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 Lower Respiratory Nucleic Acid Amplification Assays	1	NA
2	Theranos General Chemistry 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 Q131542	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 ☐ Class III ☐ Unclassified

Classification Panel

Microbiology and General Chemistry

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: 3010479366
☐ Manufacturer ☐ Contract Manufacturer ☐ Contract Sterilizer ☐ Repackager / Relabeler	
Company / Institution Name: Theranos, Inc.	Establishment Registration Number: 3010479366
Division Name *(if applicable)*: Palo Alto Facility	Phone Number *(including area code)*: (650) 856-7304
Street Address: 1601 S. California Ave.	FAX Number *(including area code)*:
City: Palo Alto	State / Province: CA; ZIP Code: 94304; 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:

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.					
	Standards No.	Standards Organization	Standards Title	Version	Date
1	MM3-A2	Clinical Laboratory Standards Institute (CLSI)	Molecular Diagnostic Methods for Infectious Diseases; Approved Guideline	Second Edition	03/10/2006
2	EP24-A2	CLSI	Assessment of the Diagnostic Accuracy of Laboratory Tests Using Receiver Operating Characteristic Curves; Approved Guideline	Second Edition	08/05/2013
3	EP05-A2	CLSI	Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline	Second Edition	10-31-2005
4	EP17-A2	CLSI	Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline	Second Edition	01/15/2013
5	IEC 60601	International Electrotechnical Commission (IEC)	Medical Electrical Equipment - Part 1: General Requirements for Basic Safety and Essential Performance	3.1	08/20/2012
6	IEC 62304	IEC	Medical Device Software -- Software Lifecycle Processes	1.0	05/09/2005
7	14971	International Organization for Standardization	Medical Devices -- Application of Risk Management to Medical Devices	2	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.

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ADDITIONAL SECTION I STANDARDS

	Standards No.	Standards Organization	Standards Title	Version	Date
8	EP09-A3E	CLSI	Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline	Third Edition	08/30/2013
9	EP12-A2	CLSI	User Protocol for Evaluation of Qualitative Test Performance; Approved Guideline	Second Edition	01/01/2008
10	I/LA18-A2	CLSI	Specifications for Immunological Testing for Infectious Diseases; Approved Guideline	Second Edition	09/20/2001

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Introduction

This document is a 510(k) Pre-Submission for Theranos, Inc.'s Clinical Laboratory Improvement Amendments ("***CLIA***")-certified laboratory's Lower Respiratory TNAA assays and General Chemistry assays for use with its automated sample processing and analysis system (the "***Theranos System***"). As described in prior Pre-Submissions (Q131199 and Q131542), 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 Lower Respiratory assay results and General Chemistry assay results.

The Lower Respiratory assays includes TNAA assays for the following pathogens: Methicillin-resistant staphylococcus aureus (MRSA), Methicillin-sensitive staphylococcus aureus (MSSA), Vancomycin-resistant staphylococcus aureus (VRSA), Klebsiella pneumoniae (Enterobacteriaceae spp), Klebsiella pneumoniae carbapenemase (KPC), Haemophilus influenzae (ampic R), Haemophilus influenzae (ampic S), Moraxella catarrhalis, Haemophilus parainfluenzae, Enterobacter cloacae (Enterobacteriaceae spp), Enterobacter aerogenes (Enterobacteriaceae spp), Serratia marcescens (Enterobacteriaceae spp), Acinetobacter baumanii, Legionella spp, Burkholderia cepacia, and Mycobacterium abscessus.

The General Chemistry assays includes assays for the following analytes: Albumin, Total Bilirubin, Direct Bilirubin, Calcium, Carbon Dioxide, Chloride, Total Cholesterol, Creatine kinase, Creatinine, Glucose, Gamma-glutamyl transpeptidase, Iron (non-heme), Low-density Lipoprotein, High-density Lipoprotein, Magnesium, Alkaline phosphatase, Phosphorus, Potassium, Total Protein, Sodium, Aspartate amino transferase (AST/SGOT), Alanine amino transferase (ALT/SGPT), Triglycerides, Urea nitrogen and Uric acid.

The TSPU is capable of automated extraction and processing of nucleic acids from multiple sample types, generating raw signals for transport to and analysis by the TLAS to determine the presence or absence of targeted nucleic acid sequences in the sample. The TSPU is also capable of automated processing of whole blood to generate raw signals that are transported to and analyzed by the TLAS to generate chemiluminescence intensity values for Alkaline Phosphatase assay and transmitted intensity spectra for all other General Chemistry assays.

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. Just prior to the Informational Meeting (Q131148) on November 4th of this year, Theranos submitted a monthly plan for Pre-Submissions and regulatory approval and clearance submissions covering these assays. We appreciate that the various Divisions of the Office of *In Vitro* Diagnostics took the time to review this plan and provide feedback, including recommendations as to whether certain assays should be included in Pre-Submissions. We have noted that a Pre-Submission for the Clinical Chemistry assays that have been included in this Pre-Submission was not

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recommended. Since we have some questions regarding the 510(k) for these assays, as highlighted in the Specific Questions section, however, we felt that it would be of value to include them.

Pursuant to these goals and to this Pre-Submission, we have submitted our lower respiratory NAA and General Chemistry assays 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 lower respiratory illnesses can simultaneously receive additional assay results providing insight into the patient's overall medical condition.

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Device Description

The Theranos System

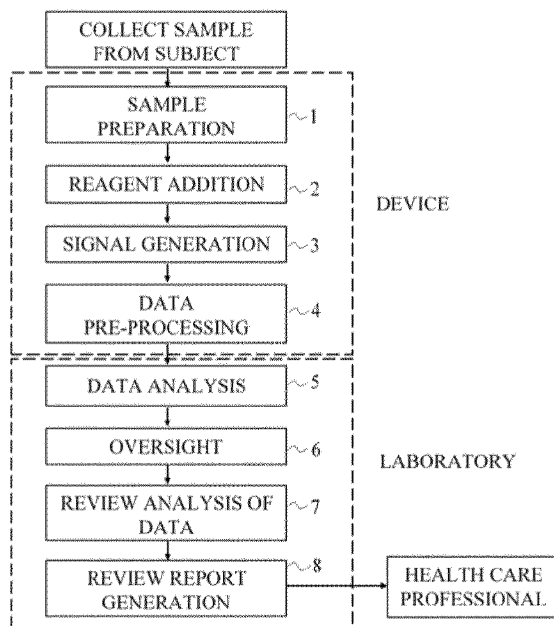

Figure 1. Outline of the Theranos system, depicting the pre-analytical (sample processing, performed by the TSPU as controlled by the TLAS), analytical (report generation, performed by the TLAS), and post-analytical (report transmission, performed by the TLAS) parts.

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 laboratory, running the following assays that are the subject of this Pre-Submission: the Microbiology assays described below through nucleic assay amplification and the Clinical Chemistry assays described below through the Theranos general chemistry protocols.

The primary applications of the nucleic acid amplification assays are to accurately detect the presence of the following pathogens that are commonly tested for patients with symptoms of lower respiratory infections ("***Lower Respiratory Assays***"): Methicillin-resistant staphylococcus aureus (MRSA), Methicillin-sensitive staphylococcus aureus (MSSA), Vancomycin-resistant staphylococcus aureus (VRSA), Klebsiella pneumoniae (Enterobacteriaceae spp), Klebsiella pneumoniae carbapenemase (KPC), Haemophilus influenzae (ampic R), Haemophilus influenzae (ampic S), Moraxella catarrhalis, Haemophilus parainfluenzae, Enterobacter cloacae (Enterobacteriaceae spp), Enterobacter aerogenes (Enterobacteriaceae spp), Serratia marcescens (Enterobacteriaceae spp), Acinetobacter baumanii, Legionella spp, Burkholderia cepacia, and

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Mycobacterium abscessus. The presence of the above mentioned pathogens is determined through nucleic acid amplification assays (Theranos Nucleic Acid Amplification assays, ("***TNAA***") assays).

The primary applications of the clinical chemistry assays are to accurately and precisely quantify the concentration of the following analytes: Albumin, Total Bilirubin, Direct Bilirubin, Calcium, Carbon Dioxide, Chloride, Total Cholesterol, Creatine kinase, Creatinine, Glucose, Gamma-glutamyl transpeptidase, Iron (non-heme), Low-density Lipoprotein, High-density Lipoprotein, Magnesium, Alkaline phosphatase, Phosphorus, Potassium, Total Protein, Sodium, Aspartate amino transferase (AST/SGOT), Alanine amino transferase (ALT/SGPT), Triglycerides, Urea nitrogen and Uric acid (Theranos "***General Chemistry***" assays).

Samples for the TNAA assays are collected in the form of throat swabs and sputum.

Sample for the Clinical Chemistry assays is plasma collected by a fingerstick or venipuncture as whole blood (processed in the TSPU into plasma). Patient samples for TNAA and Clinical Chemistry assays can be loaded together onto a Theranos Cartridge and be processed at the same time in the TSPU.

The swab, Lithium-Heparin anti-coagulated whole blood and 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 are 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

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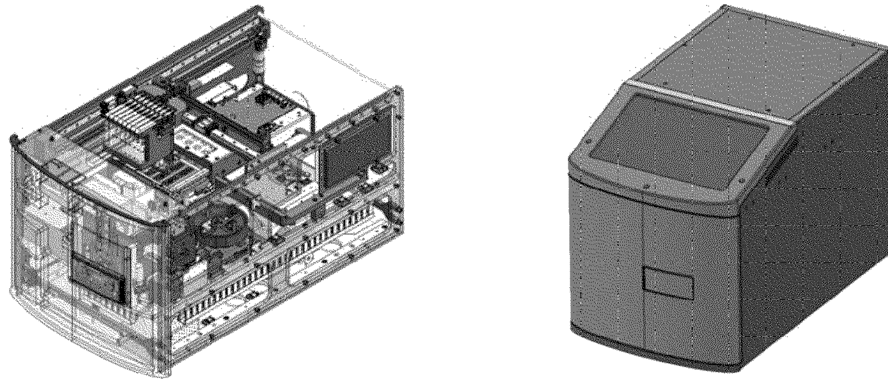

Figure 2. Theranos Sample Processing Unit, shown (L) without enclosure and (R) with enclosure

3. Sonicator Module
4. Magnet Tool
5. Detector 1 – Luminometer Module
6. Detector 2 – Fluorometer Module
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 TNAA and General Chemistry assays described above.

Modules 1-4, 7, 10 and 11 are used in connection with Theranos TNAA assays.

Modules 1, 2, 5 (for alkaline phosphatase only), 8 (for all others), 10 and 11 are used in connection with Theranos General Chemistry assays.

Modules 6 and 9 have been included here and briefly described for completeness. These modules are not used for Theranos TNAA or these General Chemistry assays.

The TSPU is composed of 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

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

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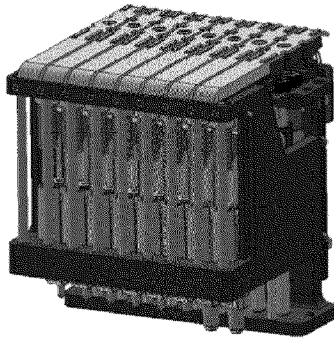

Figure 3. Liquid Handling Module

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

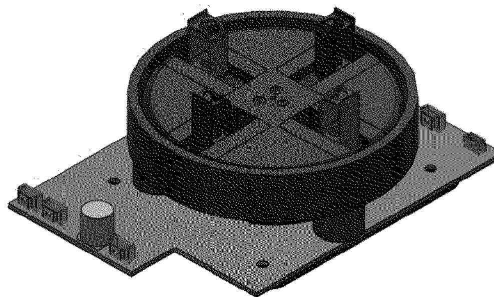

Figure 4. Centrifuge Module

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).

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3. Sonicator Module

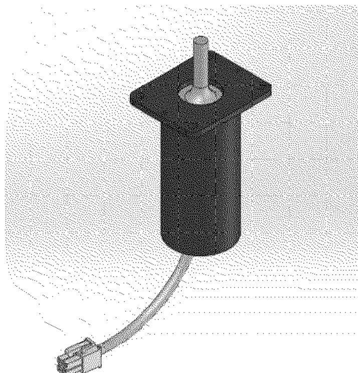

Figure 5. Sonicator module

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.

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4. Magnet Tool

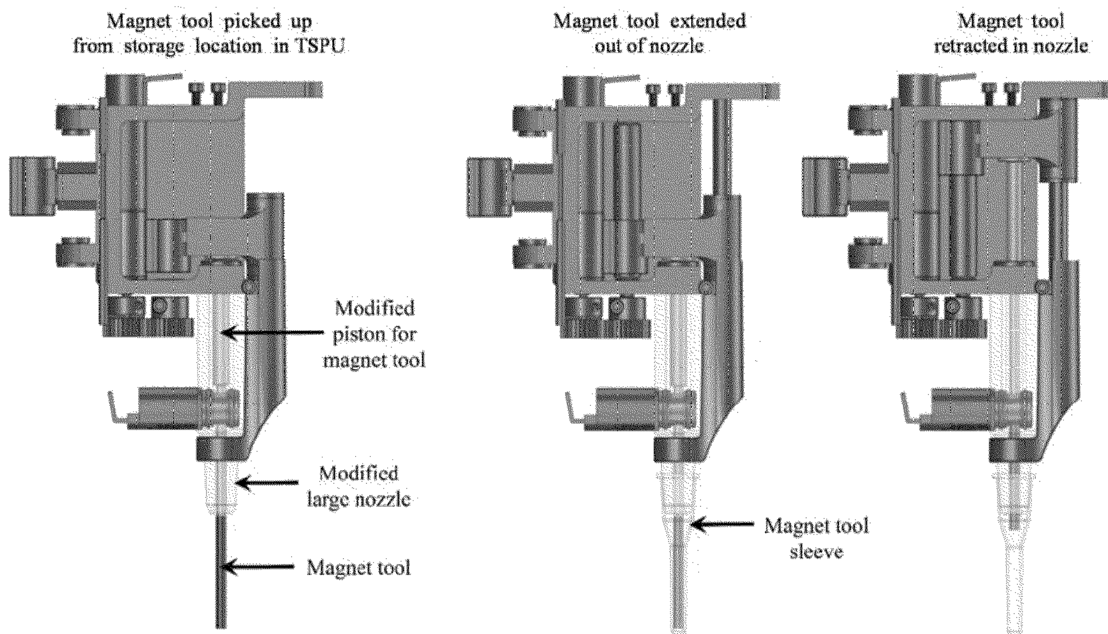

Figure 6. Magnet Tool

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.

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5. Detector 1 – Luminometer Module

The luminometer (Figure 7) is used for reading signals in ELISA and General Chemistry 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.

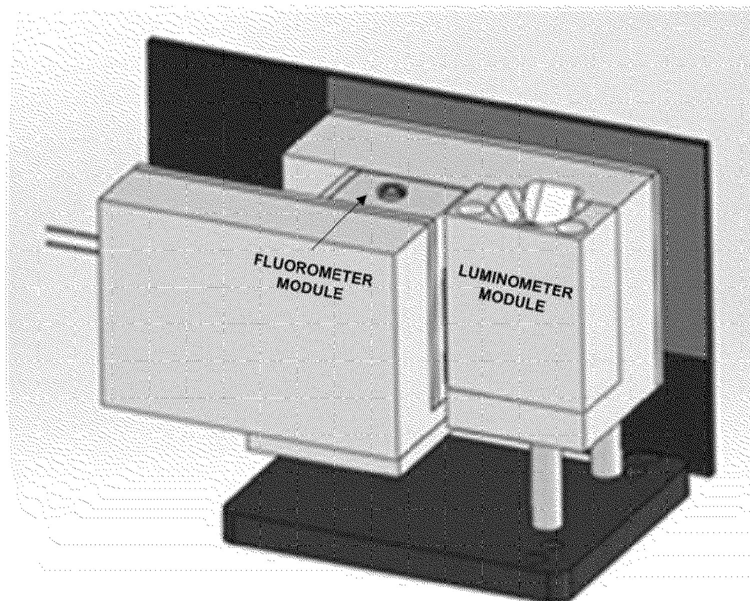

Figure 7. Luminometer and Fluorometer Modules

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.

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7. Detector 3 – Fluorometer/Turbidimeter Module

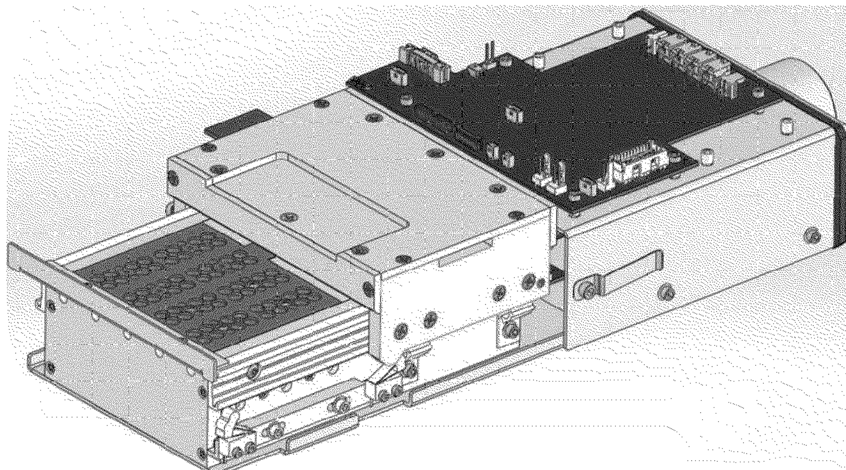

Figure 8. Fluorometer/Turbidimeter Module

Detector 3 (Figure 8) 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 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 detection in the TSPU ("fluorescence wells"), while the remaining 4 wells are used to process samples for turbidity detection in the TSPU ("turbidity wells"). Raw data are transmitted to the TLAS for analysis. 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 detecting fluorescence, the turbidity wells detect 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.

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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

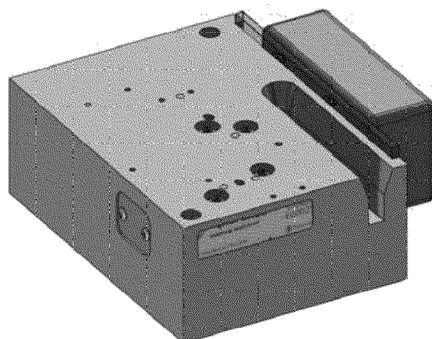

Figure 9. Spectrophotometer Module

The TSPU has spectrophotometric capabilities, which are provided by the Spectrophotometer Module (Figure 9), and may be used to detect 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 detection and a 90mW red laser diode used for fluorescence detection.

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

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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

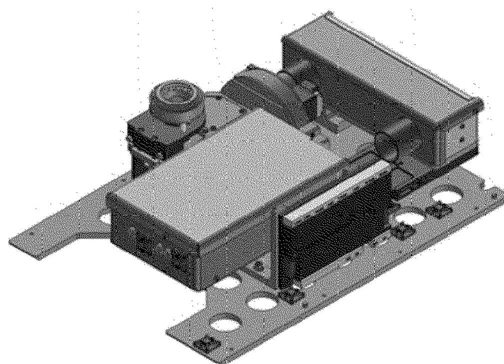

Figure 10. Microscopy Module

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.

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.

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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

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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 and 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 General Chemistry 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.

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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 coated polystyrene tips for transporting solutions; 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.

Sputum vessel – the polypropylene sputum collection vessel is designed to collect 200-400uL of sputum sample. After collection, the vessel is inserted into the Cartridge for subsequent processing.

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 detected in the spectrophotometer.

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

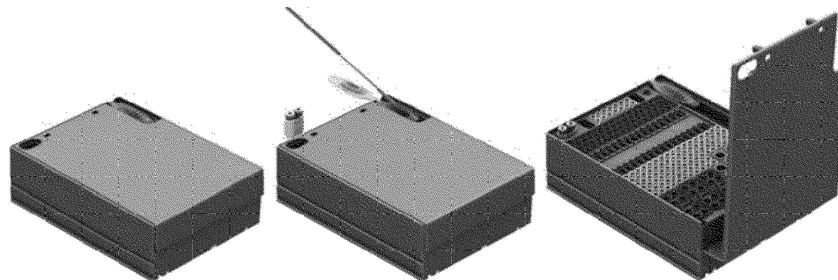

Figure 11. Cartridge in various configurations. Left: Closed cartridge. Center: Samples (Swab and Nanotainer) being introduced into cartridge. Right: Cartridge with lid open inside the TSPU.

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The Cartridge comes with a closed lid (Figure 11, Left), under which are all pre-populated consumables required for the TNAA and Clinical Chemistry. Regents and buffers required for the assays are pre-filled and sealed in Round vessels and Wash vessels. Similarly, consumables and reagents for cytometry assays and ELISA assays may be included with the TNAA assays and General Chemistry assays in the same Cartridge. 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 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.

Sputum samples are collected in sputum collection vessels. The sputum collection vessel is inserted into the Cartridge for subsequent processing.

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.

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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 transmitted light intensity spectrum for the General Chemistry assays) and signal detection by detectors (Detector 3 for TNAA and Detector 4 for the General Chemistry assays except for Detector 1 for Alkaline Phosphatase). The data are transmitted back to the TLAS, which performs

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analysis on these raw data and yields clinically relevant analyte reportables for CLIA laboratory staff to oversee and further analyze, as applicable.

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Theranos Nucleic Acid Amplification (TNAA) Assay for the Lower Respiratory Assays

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:

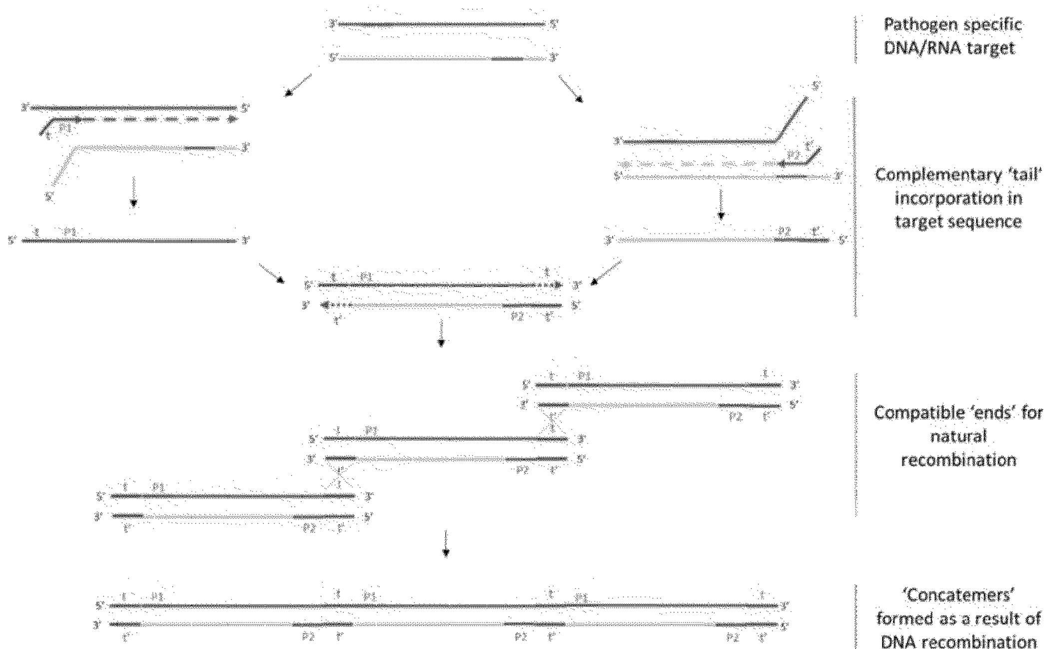

Figure 12. TNAA assay schematic

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)

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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 Lower Respiratory TNAA Assays

Pathogen nucleic acid extraction

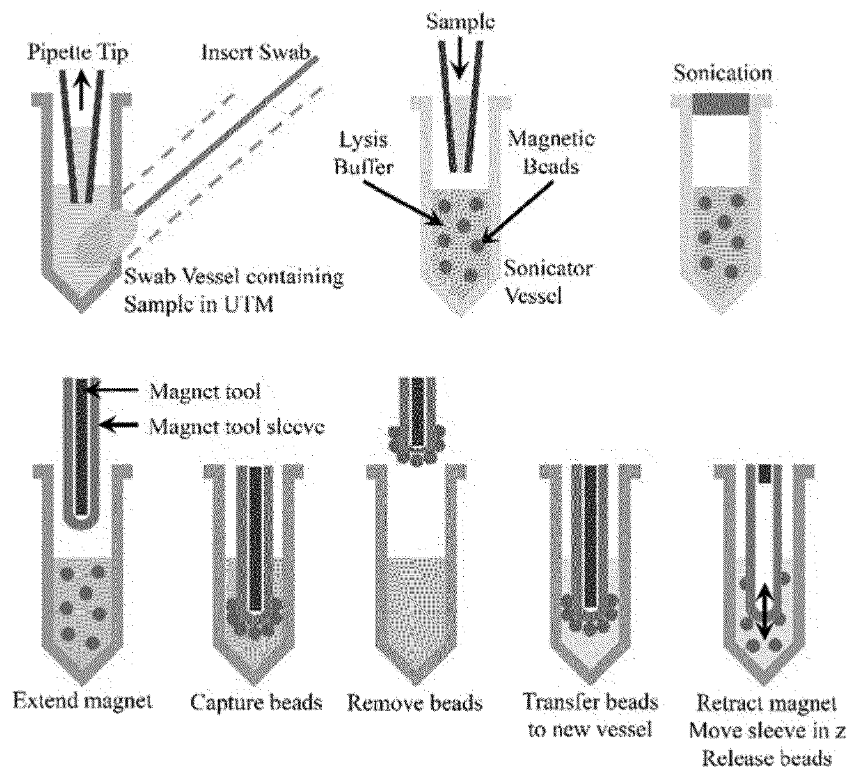

Figure 13. TNAA nucleic acid purification steps in the TSPU

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,

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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 Lower Respiratory 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 is 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.

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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.

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Theranos General Chemistry Assays – Chemistry Background, Principles and Protocol

General Chemistry Principle

Theranos General Chemistry Assays cover common clinical chemistry tests which include electrolytes, renal function tests, liver function tests, minerals, and metabolites. Theranos General Chemistry assays described in this pre-submission adopt the following methodology – Plasma is separated from whole blood by centrifugation inside the TSPU, and the plasma is diluted in either water or saline, and mixed with reagent(s). The reaction mixture(s) is incubated as required for each assay.

For the Alkaline Phosphatase assay, the reaction generates chemiluminescence that is detected in Detector 1. This raw signal is transmitted to and analyzed by the TLAS to generate the chemiluminescence intensity value, which is proportional to the concentration of Alkaline Phosphatase in the sample. For all other General Chemistry assays, the proceeding reaction results in a solution which absorbs light at a particular wavelength, which is detected on Detector 4. This raw signal is transmitted to and analyzed by the TLAS to generate the intensity spectra, which is proportional to the concentration of the analyte according to Beer-Lambert law.

1. Albumin Assay

a. Overview

Albumin is a carbohydrate-free protein made by the liver, which constitutes 55-65% of the total plasma protein pool. Albumin helps move small molecules through the bloodstream and it plays an important role in keeping the fluid from blood from leaking out into tissues in the body. It maintains oncotic plasma pressure, is involved in the transport and storage of a wide variety of ligands, and is a source of endogenous amino acids. The normal range of albumin in the human body is 3.4-5.4 g/dL. Low values of albumin (Hypoalbuminemia) may indicate ascites, kidney problems, liver disease, malabsorption syndrome, or malnutrition. High values of albumin would only indicate dehydration.

b. Method Principle

The Theranos Albumin assay in plasma determines the amount of protein that is in the clear liquid portion of the blood. Plasma albumin quantitatively binds to bromocresol green (BCG) to form an albumin-BCG complex. The absorption of this solution is detected by the TSPU at 620nm. This raw signal is transmitted to the TLAS, where it is analyzed to generate a concentration of Albumin. The assay has a reportable range of 0 - 8 g/dL for Albumin.

Reaction Equation

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2. Total Bilirubin Assay

a. Overview

Bilirubin is a metabolite of the heme portion of heme proteins, mainly hemoglobin. Normally it is excreted into the intestine and bile from the liver. The site of the catabolism of hemoglobin is the reticuloendothelial system (RES). Bilirubin is then released into the bloodstream where it binds tightly to albumin and is transported to the liver. Upon uptake by the liver, bilirubin is conjugated with glucuronic acid to form bilirubin mono and diglucuronide that are water soluble metabolites. The metabolites will react with aqueous diazo reagent and are commonly referred to as "direct bilirubin". Unconjugated bilirubin, which normally comprises 90% or more of total bilirubin, is insoluble in water.

Elevation of total plasma bilirubin may occur due to (1) excessive hemolysis or destruction of the red blood cells e.g. hemolytic disease of the newborn, (2) liver diseases e.g. hepatitis and cirrhosis or (3) obstruction of the biliary tract e.g., gallstones.

This assay is designed to determine Total Bilirubin in plasma (EDTA, Li-Heparin) and has a reportable range of 0.2 – 25 mg/dL.

b. Method Principle

Total (conjugated and unconjugated) bilirubin, in the presence of a solubilizing agent (here, dimetylsulfoxide), reacts with diazotized sulfanilic acid to produce azobilirubin, which has an absorbance maximum at 560 nm in the aqueous solution. The absorbance is detected by the TSPU, with the raw signal transmitted to the TLAS, where it is analyzed to generate a concentration of total bilirubin. The intensity of the color produced is directly proportional to the amount of total bilirubin concentration present in the sample.

3. Direct Bilirubin Assay

a. Overview

Bilirubin is a metabolite of the heme portion of heme proteins, mainly hemoglobin. Normally it is excreted into the intestine and bile from the liver. The site of the catabolism of hemoglobin is the reticuloendothelial system (RES). Bilirubin is then released into the bloodstream where it binds tightly to albumin and is transported to the liver. Upon uptake

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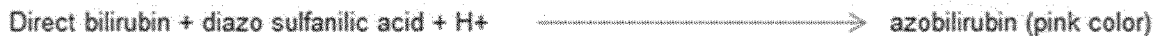
by the liver, bilirubin is conjugated with glucuronic acid to form bilirubin mono and diglucuronide that are water soluble metabolites. The metabolites will react with aqueous diazo reagent and are commonly referred to as "direct bilirubin". Elevation of total serum bilirubin may occur due to (a) excessive hemolysis or destruction of the red blood cells e.g. hemolytic disease of the newborn, (b) liver diseases e.g. hepatitis and cirrhosis (c) obstruction of the biliary tract e.g., gallstones. This assay is designed to determine Bilirubin in plasma (EDTA or Li-Heparin) with a reportable range of 0 – 3 mg/dL.

b. Method Principle

Bilirubin reacts with diazotized sulfanilic acid to produce azobilirubin, which has an absorbance maximum at 560 nm in the aqueous solution. The absorbance is detected by the TSPU, with the raw signal transmitted to the TLAS, where it is analyzed to generate a concentration of direct bilirubin. The intensity of the color produced is directly proportional to the amount of direct bilirubin concentration present in the sample.



4. Calcium Assay

a. Overview

Calcium is the most abundant mineral in the human body with 99% of total calcium deposited in the skeletal system. A higher proportion of non-skeletal calcium is present within cells than in extracellular fluids, and most of this intracellular calcium is bound to proteins in the cell membrane. Intracellular ionized calcium is physiologically active and functions as an intracellular messenger. Calcium ions affect the contractility of the heart and the skeletal musculature, and are essential for the function of the nervous system. In addition, calcium ions play an important role in blood clotting and bone mineralization.

Hypocalcemia is due to the absence or impaired function of the parathyroid glands or impaired vitamin-D synthesis. Chronic renal failure is also frequently associated with hypocalcemia due to decreased vitamin-D synthesis as well as hyperphosphatemia and skeletal resistance to the action of parathyroid hormone (PTH). A characteristic symptom of hypocalcemia is latent or manifest tetany and osteeomalacia.

Hypercalcemia is brought about by increased mobilization of calcium from the skeletal system or increased intestinal absorption. The majorities of cases are due to primary

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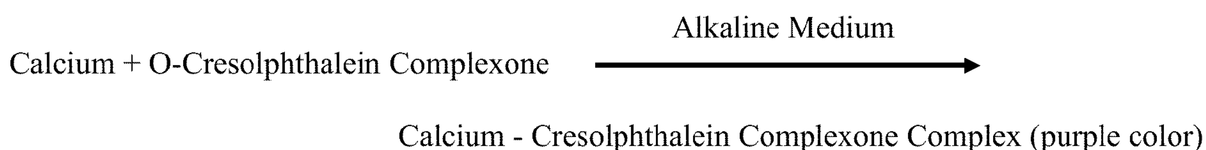
hyperparathyroidism (pHPT) or bone metastasis of carcinoma of the breast, prostate, thyroid gland, or lung. Patients who have photo and bone disease, renal stones or nephrocalcinosis, or other signs or symptoms are candidates for surgical removal of the parathyroid gland(s). Severe hypercalcemia may result in cardiac arrhythmia. This assay is designed to determine calcium in human lithium-heparin plasma. The analytical range is 2.5 – 22 mg/dL. It is not suited to EDTA plasma or whole blood. The reference range for plasma calcium is 8.7-10.4 mg/dL.

b. Method Principle

Calcium reacts with cresolphthalein complexone in 8-hydroxyquinoline to form a colored complex (purple color) that absorbs at 570 nm (550 – 580 nm). The absorbance is detected by the TSPU, with the raw signal transmitted to the TLAS, where it is analyzed to generate a concentration of calcium. The intensity of the color is proportional to the calcium concentration. Color intensifiers and a stabilizer are present to minimize interference by other metallic ions.



5. Carbon Dioxide Assay

a. Overview

Blood is composed of a bicarbonate buffering system which allows for the transport of carbon dioxide (CO_2) from around the body to the lungs where it is exhaled. In the plasma the dissolved CO_2 is hydrated and converted to bicarbonate spontaneously or by carbonic anhydrase. Since CO_2 is sparely soluble in plasma, the conversion to HCO_3 allows for high concentrations of dissolved CO_2/HCO_3 to be transported. Normally, the bicarbonate concentration of arterial blood is maintained between 22-26 mM and venous blood is maintained between 23-29 mM. Changes in ventilation can quickly affect blood pH as increased CO_2 from not breathing will result in a decrease in pH and decreased CO_2 concentrations from hyperventilating will result in increased pH. On a much slower time scale, the kidneys also regulate bicarbonate concentrations by reabsorbing it when concentrations are too high and excreting bicarbonate when concentrations are too low.

Carbon dioxide is the anhydride of carbonic acid, which is generally unstable in aqueous solutions. In water, carbon dioxide tends to spontaneously interconvert between carbonic

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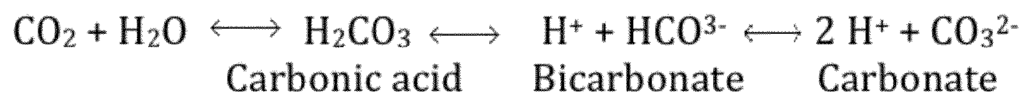
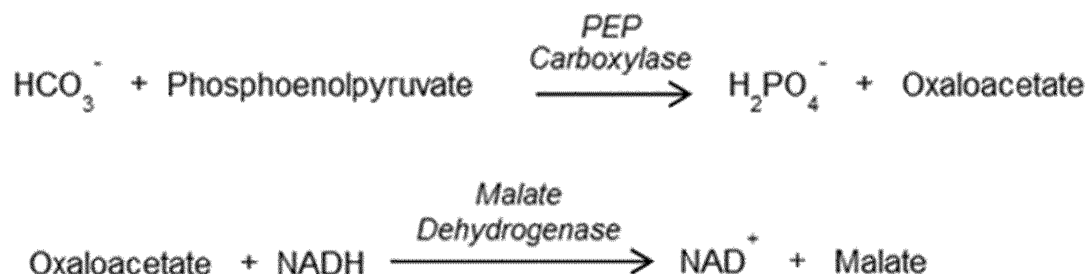
acid and CO_2. In organisms the enzyme carbonic anhydrase catalyzes the conversion of CO_2 to bicarbonate and protons. For reference, the carbon dioxide and carbonic acid equilibriums are shown in the equations below. Please note that the dissociation of carbonic acid to bicarbonate and H^+ has a pKa of 6.4 @ 25^0C and the dissociation of bicarbonate into carbonate and 2 H^+ has a pKa = 10.3 @ 25^0C. In the pH range from 9.0 > pH > 7.5, almost all dissolved CO_2 is in the bicarbonate form.



Lab testing for CO_2 is generally testing for bicarbonate. Carbon dioxide is often measured as part of an electrolyte panel and used as an indicator of blood acid/base condition and a marker for respiratory status. In metabolic acidosis, CO_2 levels are decreased and in metabolic and respiratory acidosis, the levels are increased. This assay is designed to determine the bicarbonate (HCO_3) concentration in Li-Heparin plasma across a range of 7.5-50 mM.

b. Method Principle

The NADH-Based Bicarbonate Assay uses two enzymatic reactions to convert HCO_3 to malate and β-NADH to β-NAD^+ which can be detected as a decrease in signal at 340 nm. In the first reaction, Phosphoenolpyruvate (PEP) Carboxylase simultaneously de-phosphorylates and carboxylates the enolpyruvate moiety to produce oxaloacetate. In the second reaction, oxaloacetate is reduced to malate while β-NADH is oxidized to β-NAD^+ by malate dehydrogenase. β-NADH absorbs at 340 nm and the decrease in concentration as it is oxidized to β-NAD^+ can be detected as a decrease in signal over time with a rate that is dependent on the original HCO_3 concentration. The absorbance is detected by the TSPU at certain intervals, with the raw signals transmitted to the TLAS, where they are analyzed to generate a concentration of HCO_3.



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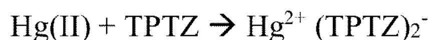
6. Chloride Assay

a. Overview

Chloride is a type of electrolyte. Chloride works with other electrolytes in the body to help keep fluid balance and acid-base balance in the body. The typical normal range for Chloride in the body is 96-106mM. Greater than normal levels of chloride, hyperchloremia can indicate kidney damage, dehydration, respiratory alkalosis, metabolic acidosis, or too much bromide in the body. Lower than normal level of chloride, hypochloremia, can indicate excessive sweating, heart failure, kidney disorders, overhydration, SIADH secretion, gastric suction, Addison's disease, burns, or chronic compensated respiratory acidosis. This assay is designed to detect Chloride in human plasma. The assay has a reportable range of 0-200mM of Chloride in plasma.

b. Method Principle

The Theranos chloride assay utilizes a colorimetric endpoint method. The increase in absorbance at 600 nm is proportional to the concentration of chloride in the sample. This absorbance is detected by the TSPU, with the raw signal transmitted to the TLAS, where it is analyzed to generate a concentration of chloride.



$$Hg(II) + TPTZ \rightarrow Hg^{2+} (TPTZ)_2^-$$

$$Hg^{2+} (TPTZ)_2^- + 2Cl^- \rightarrow HgCl_2 + 2TPTZ^-$$

$$TPTZ^- + Fe_2SO_4 \rightarrow Fe^+ TPTZ^- \text{ (Color product)}$$

7. Total Cholesterol Assay

a. Overview

Cholesterol is a steroid metabolite found in cell membranes and plasma of animals. It is essential because it helps establish proper membrane permeability and fluidity. Cholesterol is also an essential component the body uses to make bile acids, steroid hormones, and fat-soluble vitamins. It is synthesized in many types of tissue, but particularly in the liver and intestinal wall. Approximately 75% of cholesterol is newly synthesized and 25% originates from dietary intake. Normally, the cholesterol in the plasma or serum is 60%-80% esterified. Approximately 50-75% of the plasma cholesterol is transported by low density lipoproteins (LDL) and 15-40% by high density lipoproteins (HDL).

Although cholesterol is an essential steroid for the body, high levels of cholesterol in plasma/serum suggest a high risk for heart disease and other diseases. Serum cholesterol is elevated in hereditary hyperlipoproteinemias and in various other metabolic diseases.

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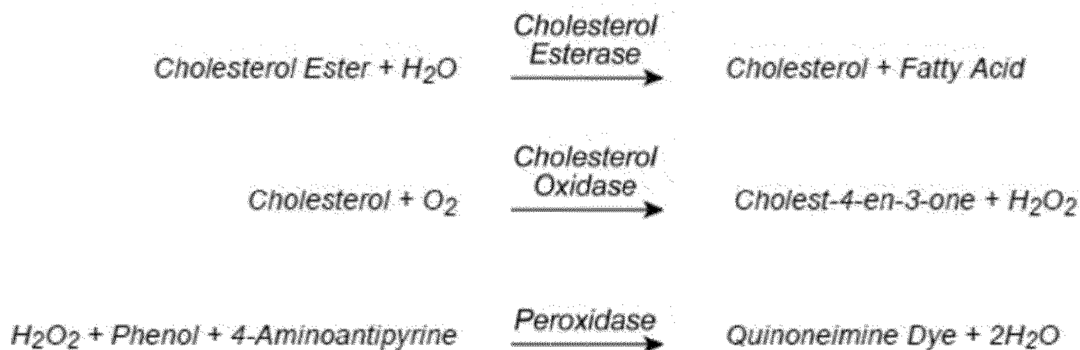
Moderate to elevated values are also seen in cholestatic liver disease. Hypercholesterolemia reflects an increase of lipoproteins of 1 or more specific classes (e.g., β-LDL, α-1 HDL, α-2 HDL, or LP-X). Hypercholesterolemia is a risk factor for cardiovascular disease. Low levels of cholesterol can be seen in disorders that include hyperthyroidism, malabsorption, and deficiencies of apolipoproteins.

Total cholesterol is determined as part of a lipoprotein profile. The total cholesterol concentration in plasma is a determination of LDL cholesterol, HDL cholesterol, and other lipid components. The recommended total cholesterol level to not be at risk for heart disease and other related diseases is below 200mg/dL. ULOQ and LLOQ in assay buffer are 300mg/dL and 50mg/dL respectively. The assay's reportable range is from 0-800mg/dL of total cholesterol.

b. Method Principle

The cholesterol esters are hydrolyzed by cholesterol esterase to cholesterol and free fatty acids. The cholesterol is converted to choles-4-en-3-one by cholesterol oxidase in the presence of oxygen to form hydrogen peroxide. A colored complex is formed from hydrogen peroxide, 4-aminoantipyrine and phenol under the catalytic influence of peroxidase. The absorbance of the complex is detected as an endpoint reaction at 500 nm by the TSPU, with the raw signal transmitted to the TLAS, where it is analyzed to generate a concentration of total cholesterol.



8. Creatine Kinase Assay

a. Overview

Creatine Kinase (CK) activity is greatest in striated muscle, heart tissue and brain. The determination of CK activity is a proven tool in the investigation of skeletal muscle disease (muscular dystrophy) and is also useful in the diagnosis of myocardial infarction (MI) and

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cerebrovascular accidents. Increased levels of CK also can be found in viral myositis, polymyositis, and hypothyroidism.

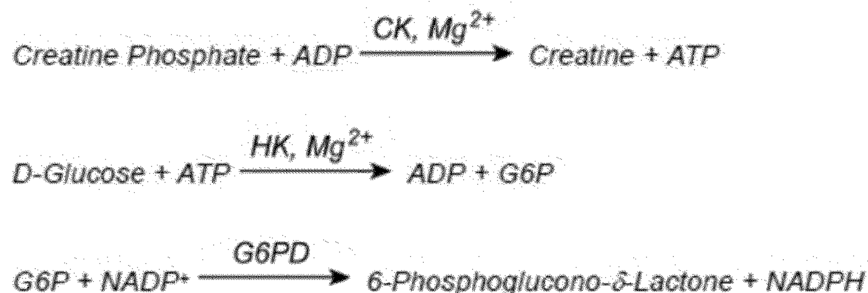
The assay is designed to determine CK in lithium heparin plasma. The assay has a reportable range of 0 to 2000 U/L. The assay has an LLOQ of 62.5 and ULOQ of 2000 U/L.

Following injury to the myocardium, such as occurs in acute MI, CK is released from the damaged myocardial cells. A rise in the CK activity can be found 4 to 8 hours after an infarction.

b. Method Principle

Creatine Kinase reacts with the creatine phosphate and ADP to form ATP which is coupled to the hexokinase-G6PD reaction, generating NADPH. The increase in absorbance at 340/410 nm is detected by the TSPU, with the raw signal transmitted to the TLAS, where it is analyzed to generate a concentration of NADPH, which is further analyzed to generate the final resulting concentration of CK.

Reaction Equation



$$\text{Creatine Phosphate} + \text{ADP} \xrightarrow{\text{CK}, Mg^{2+}} \text{Creatine} + \text{ATP}$$

$$\text{D-Glucose} + \text{ATP} \xrightarrow{\text{HK}, Mg^{2+}} \text{ADP} + \text{G6P}$$

$$\text{G6P} + \text{NADP}^{+} \xrightarrow{\text{G6PD}} \text{6-Phosphoglucono-}\delta\text{-Lactone} + \text{NADPH}$$

9. Creatinine Assay

a. Overview

Creatinine is a break-down product of creatine phosphate in muscle, and is usually produced at a fairly constant rate by the body (depending on muscle mass). In chemical terms, creatinine is a spontaneously formed cyclic derivative of creatine. Creatinine is chiefly filtered out of the blood by the kidneys (glomerular filtration and proximal tubular secretion). There is little-to-no tubular reabsorption of creatinine. If the filtering of the kidney is deficient, creatinine blood levels rise. Therefore, creatinine levels in blood and

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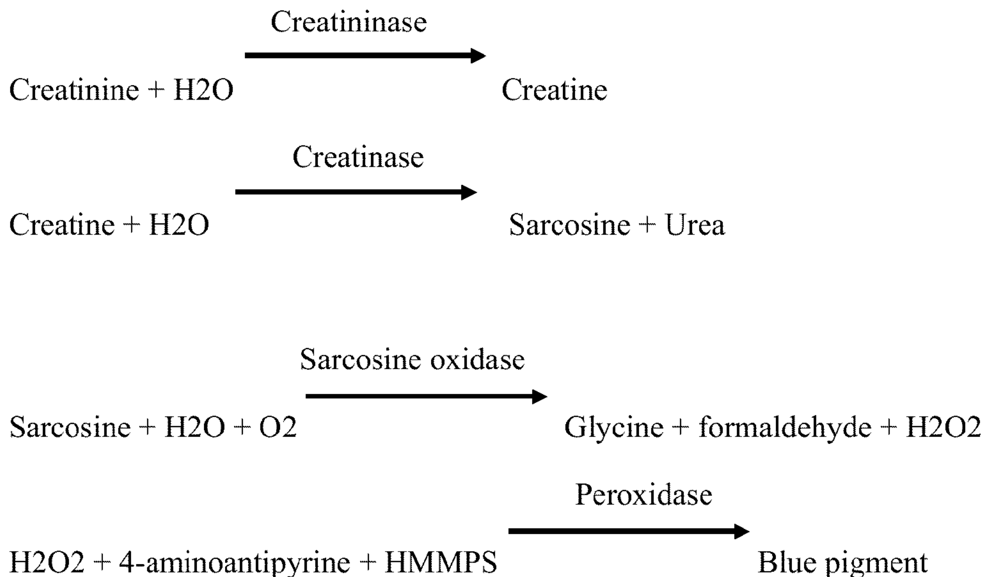
urine may be used to calculate the creatinine clearance (CrCl), which reflects the glomerular filtration rate (GFR).

The GFR is clinically important because it is a measurement of renal function. However, in cases of severe renal dysfunction, the creatinine clearance rate will be "overestimated" because the active secretion of creatinine will account for a larger fraction of the total creatinine cleared. Ketoacids, cimetidine and trimethoprim reduce creatinine tubular secretion and therefore increase the accuracy of the GFR estimate, particularly in severe renal dysfunction. (In the absence of secretion, creatinine behaves like insulin.)

Creatinine has an assay range from 0 to 8mg/dL in plasma. The reference ranges for males is 0.76 – 1.27 mg/dL and for females 0.57 – 1.0 mg/dL in plasma.

b. Method Principle

Creatinine is converted to creatine by the action of creatininase. The creatine formed is hydrolyzed by creatinase to produce sarcosine, which is decomposed by sarcosine oxidase to form glycine, formaldehyde, and hydrogen peroxide. In the presence of peroxidase, the hydrogen peroxide formed yields a blue pigment by quantitative oxidative condensation with N-(3-sulfopropyl)-3-methoxy-5-methylaniline (HMMPS) and 4-aminoantipyrine. The absorbance of the blue color at 561 nm is detected by the TSPU, with the raw signal transmitted to the TLAS, where it is analyzed to generate a concentration of creatinine. The absorbance of the color is proportional to the creatinine concentration. This assay has a reportable range of 0.4 – 8 mg/dL in human plasma (EDTA, Li-Heparin).



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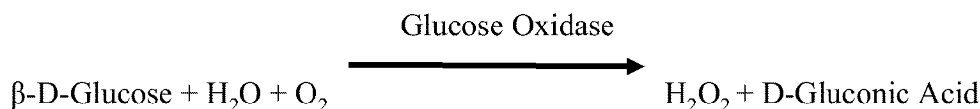
10. Glucose Assay

a. Overview

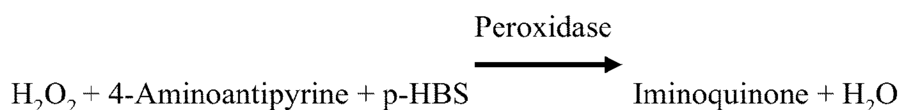
Glucose is the major carbohydrate present in the peripheral blood. The oxidation of glucose is the major source of cellular energy in the body. Glucose determinations are run primarily to aid in the diagnosis and treatment of diabetes mellitus. Elevated glucose levels may be associated with pancreatitis, pituitary or thyroid dysfunction, renal failure and liver disease, whereas low glucose levels may be associated with insulinoma, hypopituitaryism, neoplasms, or insulin-induced hypoglycemia. The assay has a reportable range of 0 - 300 mg/dL.

b. Method Principle

β-D-Glucose is oxidized by glucose oxidase to produce D-gluconic acid and hydrogen peroxide. The hydrogen peroxide is then oxidatively coupled with 4-aminoantipyrine and phenol substitute, pHBA, in the presence of peroxidase to yield a red quinoneimine dye. The amount of colored complex formed is proportional to glucose concentration and can be photometrically detected at 510nm. This raw signal transmitted to the TLAS, where it is analyzed to generate a concentration of glucose.



$$\beta\text{-D-Glucose} + H_2O + O_2 \xrightarrow{\text{Glucose Oxidase}} H_2O_2 + \text{D-Gluconic Acid}$$



$$H_2O_2 + \text{4-Aminoantipyrine} + \text{p-HBS} \xrightarrow{\text{Peroxidase}} \text{Iminoquinone} + H_2O$$

11. Gamma-glutamyl transpeptidase (GGT) Assay

a. Overview

Gamma-glutamyl transpeptidase (GGT) is primarily present in kidney, liver, and pancreatic cells. Small amounts are present in other tissues. Even though renal tissue has the highest level of GGT, the enzyme present in the plasma appears to originate primarily from the hepatobiliary system, and GGT activity is elevated in any and all forms of liver

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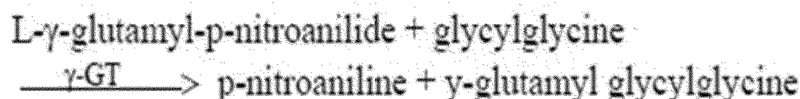
disease. It is highest in cases of intra- or post-hepatic biliary obstruction, reaching levels some 5 to 30 times normal. It is more sensitive than alkaline phosphatase (ALP), leucine aminopeptidase, aspartate amino transferase, and alanine amintotransferase in detecting obstructive jaundice, cholangitis and cholecystitis; its rise occurs earlier than with these other enzymes and persists longer. Only modest elevations (2-5 times normal) occur in infectious hepatitis, and in this condition GGT determinations are less useful diagnostically than are measurements of the amino transferases. High elevations of GGT are also observed in patients with either primary or secondary (metastatic) neoplasms. Elevated levels of GGT are noted not only in the sera of patients with alcoholic cirrhosis but also in the majority of sera from persons who are heavy drinkers. Studies have emphasized the value of serum GGT levels in detecting alcohol-induced liver disease. Elevated values are also seen in patients receiving drugs such as phenytoin and phenobarbital, and this is thought to reflect induction of new enzyme activity.

Abnormal values are observed in various muscle diseases and in renal failure. Abnormal values are also seen in cases of skeletal disease, children older than 1 year and in healthy pregnant women-conditions in which ALP is elevated.

This assay is designed to determine GGT in lithium heparin plasma. The assay has a reportable range of 0 to 1524 U/L. The assay has an LLOQ of 25 U/L and ULOQ of 1524 U/L.

b. Method Principle

In the reaction with the synthetic substrate (L-γ-glutamyl-p -nitroanilide), glycylglycine acts as an acceptor for the γ-glutamyl residue and p-nitroaniline is liberated. The liberated product has an absorption maximum near 405 nm. The rate of formation is detected spectrophotometrically at 405 nm in the TSPU, with the raw signal transmitted to the TLAS, where it is analyzed to generate a concentration of GGT.



12. Iron (non-heme) Assay

a. Overview

Ingested iron is absorbed primarily from the intestinal tract and is temporarily stored in the mucosal cells as Fe(III)-ferritin. Ferritin provides a soluble protein shell to encapsulate a

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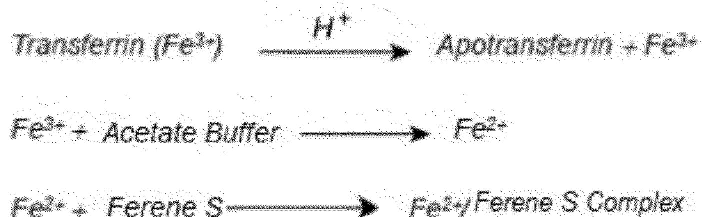
complex of insoluble ferric hydroxide-ferric phosphate. On demand, iron is released into the blood and transported as Fe(III)-transferrin.

Transferrin is the primary plasma iron transport protein, which binds iron strongly at physiological pH. Transferrin is generally only 25% to 30% saturated with iron. The additional amount of iron that can be bound is the unsaturated iron-binding capacity (UIBC). The total ion binding capacity (TIBC) can be indirectly determined using the sum of serum iron (Total Iron) and UIBC. Knowing the molecular weight of the transferrin and that each molecule of transferring can bind 2 atoms of iron, TIBC and transferrin concentration is interconvertible. The assay has a reportable range of 0-1000 µg/dL of plasma iron.

Percent saturation (100 x serum iron/TIBC) is usually normal or increased in persons who are iron deficient, pregnant, or are taking oral contraceptive medications. Persons with chronic inflammatory processes, hemochromatosis or malignancies generally display low transferrin.

b. Method Principle

Iron in human plasma is released from its carrier protein, transferrin, in an acid medium and simultaneously reduced to the ferrous form by a reducing agent. The ferrous ions chelate with the Ferene-S, a sensitive iron indicator forming a stable blue complex whose absorbance, spectrophotometrically detected at 590 nm in the TSPU. This raw signal is transmitted to the TLAS, where it is analyzed to generate a concentration of iron. The absorbance is proportional to the iron content.



13. Low-density lipoprotein (LDL) Assay

a. Overview

Cholesterol and fatty acid esters of cholesterol are essentially insoluble in aqueous solutions and must be transported in the blood in complex with various solubilizing proteins. These lipoprotein complexes (including other fatty acid esters, apolipoproteins, antibodies/antigens, enzymes, transporters and toxins) circulate in the blood in widely varied and inter-changing forms that have diverse sizes and densities. The major classes in

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human serum, from smallest to largest as established by density-gradient ultracentrifugation, are High Density Lipoproteins (HDL), Intermediate Density Lipoproteins (IDL), Low Density Lipoproteins (LDL), Very Low Density Lipoproteins (VLDL), and Chylomicrons.

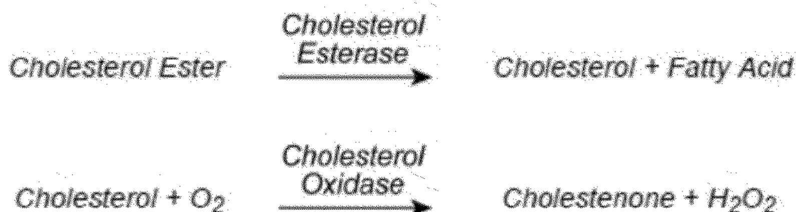
The concentration of cholesterol contained in low-density lipoproteins (LDL-C, “bad cholesterol”) correlates closely with the risk for developing or diagnosis of atherosclerosis and heart disease. Relative to serum cholesterol circulating in high-density lipoproteins (HDL-C, “good cholesterol”), the cholesterol contained in low-density lipoproteins is more easily oxidized. This oxidation can result in the lipoproteins binding to arterial walls and forming a recalcitrant plaque along the wall, narrowing blood vessels (atherosclerosis). Narrowing of the blood vessels can eventually cause severe restriction of blood flow through the arteries, resulting in chronic high blood pressure. Additionally, these plaques can degrade or rupture, liberating particles into the blood and resulting in a heart attack, stroke, peripheral vascular disease, and other circulatory ailments. Increased low-density lipoprotein (LDL) cholesterol is widely recognized as a risk factor for atherosclerotic disease, specifically coronary atherosclerosis. Diminished or absent LDL cholesterol may be a cause of polyneuropathy.

The normal range for LDL-C in adults is ~50-180 mg/dL, where >120 mg/dL is considered high risk. Extreme cases can exceed 200 mg/dL. The target range for a healthy adult is <100 mg/dL. The assay has a reportable range of 0 – 550 mg/dL.

b. Method Principle

The assay method consists of 2 distinct reaction steps:

1. Cholesterol esterase and cholesterol oxidase eliminate cholesterol, other than from low density lipoprotein. The action of catalase removes the peroxide produced by the oxidase.



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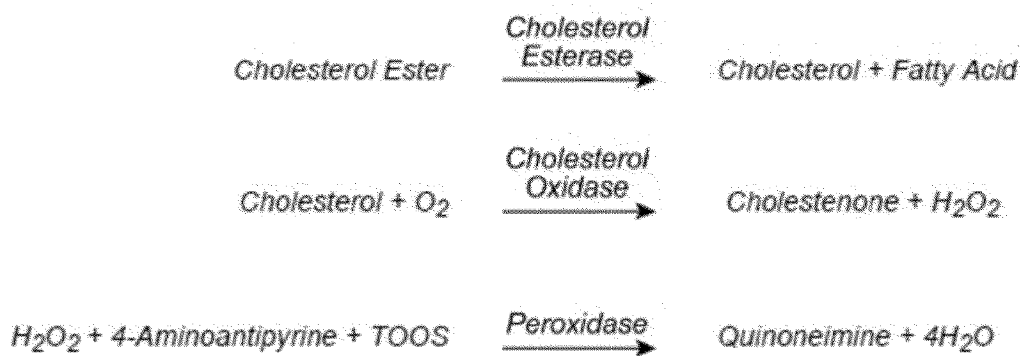
2. Specific detection of LDL Cholesterol is made after its release by detergent in Reagent 2. Catalase in step 1 is inhibited by sodium azide in Reagent 2. The absorbance is detected at 560 nm by the TSPU, with the raw signal transmitted to the TLAS, where it is analyzed to generate the concentration of LDL Cholesterol. The intensity of the quinoneimine produced in the Trinder reaction is directly proportional to the LDL Cholesterol concentration.



Where TOOS = N-ethyl-N-(2-hydroxy-3-sulfopropyl)-3-methylaniline

14. High-density lipoprotein (HDL) Assay

a. Overview

High density lipoprotein (HDL) is the smallest of the lipoprotein particles and comprises a complex family of lipoprotein particles that exist in a constant state of dynamic flux as the particles interact with other HDL particles and with low density lipoprotein (LDL) particles and very low density lipoprotein (VLDL) particles. HDL has the largest proportion of protein relative to lipid, compared to other lipoproteins (>50% protein). Total cholesterol levels have long been known to be related to coronary heart disease. HDL cholesterol is also an important tool used to assess an individual's risk of developing coronary heart disease, since a strong negative relationship between HDL cholesterol concentration and the incidence of coronary heart disease has been reported. In some individuals, exercise increases the HDL cholesterol level; those with more physical activity tend to have higher HDL cholesterol values. The assay has a reportable range of 0 - 120 mg/dL.

b. Method Principle

The assay method consists of 2 distinct reaction steps:

1. Elimination of chylomicrons, VLDL-Cholesterol, and LDL-Cholesterol by cholesterol esterase and cholesterol oxidase. The peroxides produced by the oxidase are removed by catalase.

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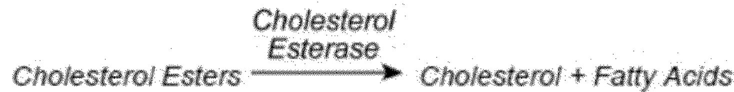
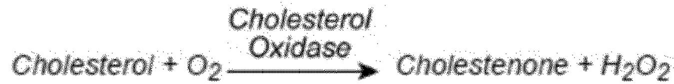
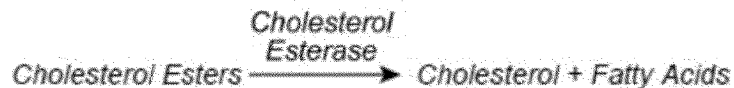
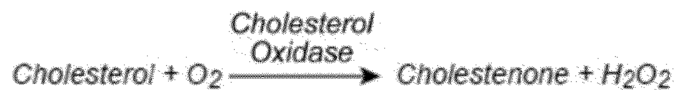




2. Specific detection of HDL-Cholesterol is made after its release by surfactant in Reagent 2. Catalase from step 1 is inhibited by sodium azide in reagent 2. The absorbance is detected at 561 nm by the TSPU, with the raw signal transmitted to the TLAS, where it is analyzed to generate the concentration of HDL Cholesterol. The intensity of the quinoneimine dye produced in the Trinder reaction is directly proporational to the HDL cholesterol concentration.





Where HDAOS = N-(2-hydroxy-2-sulfopropyl)-3,5-dimethoxyaniline.

15. Magnesium Assay

a. Overview

Magnesium, along with potassium, is a major intracellular cation. Magnesium is a cofactor of many enzyme systems. All adenosine triphosphate (ATP)-dependent enzymatic reactions require magnesium as a cofactor. Approximately 70% of magnesium ions are stored in bone. The remainder is involved in intermediary metabolic processes; about 70% is present in free form while the other 30% is bound to proteins (especially albumin), citrates, phosphate, and other complex formers. The serum magnesium level is kept constant within very narrow limits. Regulation takes place mainly via the kidneys, primarily via the ascending loop of Henle.

Conditions that interfere with glomerular filtration result in retention of magnesium and hence elevation of serum concentrations. Hypermagnesemia is found in acute and chronic renal failure, magnesium overload, and magnesium release from the intracellular space. Mild-to-moderate hypermagnesemia may prolong atrioventricular conduction time.

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Magnesium toxicity may result in central nervous system (CNS) depression, cardiac arrest, and respiratory arrest.

Numerous studies have shown a correlation between magnesium deficiency and changes in calcium-, potassium-, and phosphate-homeostasis which are associated with cardiac disorders such as ventricular arrhythmias that cannot be treated by conventional therapy, increased sensitivity to digoxin, coronary artery spasms, and sudden death. Additional concurrent symptoms include neuromuscular and neuropsychiatric disorders. Conditions that have been associated with hypomagnesemia include chronic alcoholism, childhood malnutrition, lactation, malabsorption, acute pancreatitis, hypothyroidism, chronic glomerulonephritis, aldosteronism, and prolonged intravenous feeding.

The Magnesium assay has a reportable range of 0.6 – 4.4 mg/dL of Magnesium.

b. Method Principle

Magnesium ions react with xylidyl blue in an alkaline medium to form a water-soluble purple-red complex. The increase in absorbance of xylidyl blue is detected at 505 nm by the TSPU, with the raw signal transmitted to the TLAS, where it is analyzed to generate the concentration of magnesium. This increase in absorbance of xylidyl blue is proportional to the concentration of magnesium in the sample. Calcium is excluded from the reaction by complexing with EGTA.

Reaction Equation

16. Alkaline Phosphatase Assay

a. Overview:

Alkaline phosphatase (ALP) catalyzes the hydrolysis of phosphate esters in alkaline buffer and produces an organic radical and inorganic phosphate. Changes in alkaline phosphatase level and activity are associated with various disease states in the liver and bone. The Theranos Alkaline Phosphatase Assay is a highly sensitive, simple, direct chemiluminescent assay designed to determine the ALP activity in plasma. The reportable range for this assay is about 2.6-420 U/L of alkaline phosphatase activity. The reference range for total alkaline phosphatase activity is 45-115 U/L in adult males, and 52-144 U/L in adult females. The assay has a reportable range of 2.6 to 840 U/L.

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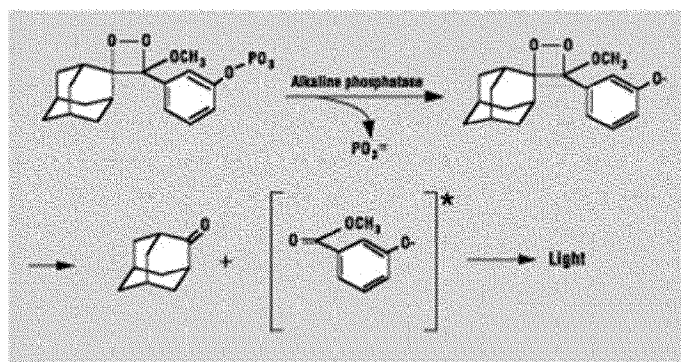
b. Method Principle

The Theranos plasma alkaline phosphatase assay utilizes the enzyme activity of alkaline phosphatase to dephosphorylate the chemiluminescent alkaline phosphatase substrate present in the assay reagent into an unstable dioxetane anion which decomposes and emits light. Hydrolytic dephosphorylation of adamantyl-1,2-dioxetane phosphate substrates by AP results in the formation of a metastable anion, which fragments further to form an excited-state anion that emits light. The dioxetane phenolate anion decomposes via a chemically initiated electron exchange luminescence (CIEEL) mechanism. Charge transfer from the phenolate to the dioxetane ring promotes cleavage of the cyclic peroxide, releasing about 100 kcal to chemically excite one of the resulting carbonyl fragments to a singlet electronic state. This excited species emits light at 477 nm wavelength as it reverts to the ground state. Light emission obtained from the AP-catalyzed dioxetane decomposition reaction is a steady-state glow, the intensity of which is detected using a photodiode-based luminometer. This raw signal is transmitted to and analyzed by the TLAS to generate the chemiluminescence intensity value, which is proportional to the concentration of AP in the sample.



17. Phosphorus Assay

a. Overview

Phosphorus is present in bones and other tissues in the form of phosphate. However, the Theranos assay expresses the analyte concentration as phosphorus in mg/dL. The body needs phosphate to build and repair bones, help nerves function and make muscles contract. Kidneys help control the amount of phosphate in the blood. Thus, a phosphate test may be ordered if a person has symptoms suggesting kidney, bone, or gastrointestinal disorders. Also, because phosphate levels affect calcium levels, phosphate tests are usually ordered along tests for calcium, parathyroid hormone, and vitamin D tests, or when calcium levels are found to be abnormal. The stated normal range for phosphorous in adults is 2.7-

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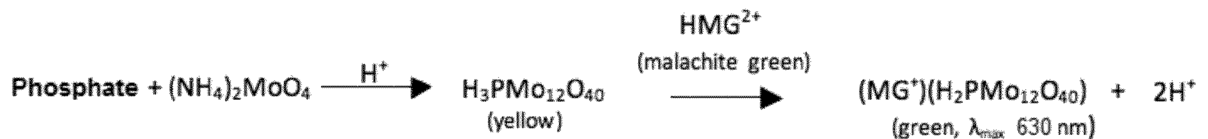
4.5mg/dL, 4.5-5.5 for children and 4.5-9.0 for newborns. This assay is designed to determine Phosphorus from 2-15mg/dL in human plasma.

b. Method Principle

The Theranos phosphorus assay utilizes an improved malachite green method that uses malachite green dye and molybdate, which forms a stable colored complex with inorganic phosphate. When Malachite green is added in acid the resulting phosphate-molybdate-malachite green complex gives a color in the visible range, which is detected at 630nm by the TSPU, with the raw signal transmitted to the TLAS, where it is analyzed to generate the concentration of phosphorus.



18. Potassium Assay

a. **Overview**

Potassium is the major cation of the intracellular fluid. It is an important mineral and electrolyte in the body. It is necessary for proper functioning of the heart, nerves, kidneys, muscles, and the digestive system. Disturbance of potassium homeostasis (intra- and extracellular) can cause serious health effects. Decreases in extracellular potassium are characterized by muscle weakness, irritability, and eventual paralysis. Cardiac effects include tachycardia, other cardiac conduction abnormalities that are apparent by electrocardiographic examination, and eventual cardiac arrest. Hypokalemia is common in vomiting, diarrhea, alcoholism, and folic acid deficiency. Additionally, >90% of hypertensive patients with aldosteronism have hypokalemia.

Abnormally high extracellular potassium levels produce symptoms of mental confusion; weakness, numbness and tingling of the extremities; weakness of the respiratory muscles; flaccid paralysis of the extremities; slowed heart rate; and eventually peripheral vascular collapse and cardiac arrest. Hyperkalemia may be seen in end-stage renal failure, hemolysis, trauma, Addison's disease, metabolic acidosis, acute starvation, dehydration, and with rapid potassium infusion.

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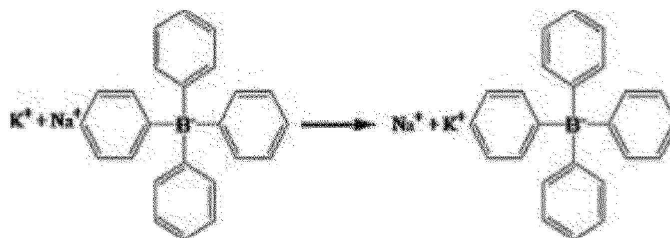
b. **Method Principle**

Potassium is normally maintained in levels from 3.5 to 5.5mM within the body and most test methods only read out to 8mM, making for a very narrow assay range. Potassium levels are often determined using ion selective electrodes or flame photometry. Theranos potassium assay uses a sodium tetraphenylborate method for the determination of potassium levels. In this method the compound Sodium Tetraphenylborate reacts with potassium from plasma. K^+ replaces Na^+ from Sodium Tetraphenylborate (NaTPB) to form potassium tetraphenylborate, a white precipitate. The precipitate remains in suspension with the help of some thickeners and stabilizers, causing an increase in turbidity. The resulting increase in turbidity is detected by the TSPU at 450nm. This raw signal is transmitted to the TLAS, where it is analyzed to generate the concentration of potassium. The assay has a reportable range of 0.1 – 7.5 mM for Potassium.



19. Total Protein Assay

a. **Overview**

Plasma proteins are synthesized predominantly in the liver; immunoglobulins are synthesized by mononuclear cells of lymph nodes, spleen and bone marrow. The two general causes for alterations of plasma total protein concentrations are a change in the volume of plasma water and/or a change in the concentration of one or more of the specific proteins in the plasma. Of the individual plasma proteins, albumin is present in such high concentrations that low levels of this protein alone may cause hypoproteinemia.

Hemoconcentration (decrease in the volume of plasma water) results in relative hyperproteinemia whereas hemodilution results in relative hypoproteinemia. In both situations, the relative concentrations of all the individual plasma proteins are affected to the same degree. Hyperproteinemia may be seen during dehydration, due to inadequate water intake, to excessive water loss (e.g., severe vomiting, diarrhea, Addison's disease and diabetic acidosis), or as a result of increased production of proteins. Increased polyclonal protein production is seen in reactive, inflammatory processes and increased monoclonal protein production is seen in some hematopoeitic neoplasms (e.g., multiple myeloma,

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Waldenstrom's macroglobulinemia, monoclonal gammopathy of undetermined significance).

This assay has a reportable range of 0 - 15 g/dL. The assay LLOQ is 2g/dL and ULOQ is 15g/dL.

b. Method Principle

Protein peptide bonds interact with the cupric ions to form a purple complex that is detected as an endpoint reaction at 540 nm by the TSPU, with this raw signal transmitted to the TLAS, where it is analyzed to generate the concentration of total protein.

20. Sodium Assay

a. Overview:

Sodium is an essential electrolyte and mineral in the body. It helps keeps fluids and electrolytes balanced in our body. When we find concentration of sodium in the blood, it is actually a reflection of the amount of sodium and the amount of water in the vascular space.

The amount of sodium in our body is partially controlled by aldosterone, a hormone made by our adrenal glands. Aldosterone levels in the body tell the kidneys how much sodium should be excreted and how much sodium should be retained in the body. Sodium is also found in a majority of our foods and medications. Yet, too much sodium intake may raise a person's blood pressure and put them at a greater risk for developing heart disease/heart failure, stroke, and kidney damages. Hypernatremia (low sodium in the body) is very rare, but most often occurs in people who take medications which causes increased urination. Sodium assays are important in assessing acid-base balance, water balance, water intoxication, and dehydration.

The Theranos Sodium assay is designed to detect Sodium in plasma with a reportable range of 90-200mM.

b. Method Principle

In the Theranos Sodium colorimetric assay, the sodium dependent enzyme β-galactosidase cleaves the substrate o-nitrophenyl-β-D-galactopyranose, yielding the product o-nitrophenol, a dye with color intensity at 405/420nm. This absorption is detected by the

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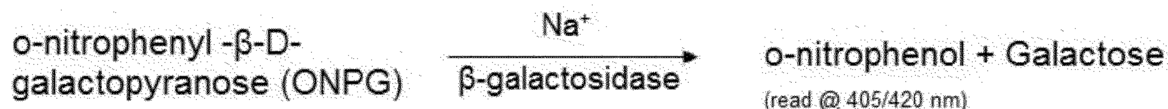
TSPU, with the raw signal transmitted to the TLAS, where it is analyzed to generate a concentration of sodium based on the rate at which o-nitrophenol is formed.



21. Aspartate amino transferase (AST/SGOT) Assay

a. Overview

Aspartate amino transferase (AST), previously known as serum glutamic oxaloacetic transaminase, is found in high concentrations in the liver, heart, skeletal muscle and kidneys. AST is present in both the cytoplasm and mitochondria of cells. In cases involving mild tissue injury, the predominant form of AST is the cytoplasmic form. Severe tissue damage results in increased release of the mitochondrial enzyme. High levels of AST can be found in cases such as myocardial infarction, acute liver cell damage, viral hepatitis, and carbon tetrachloride poisoning. Slight to moderate elevation of AST is seen in muscular dystrophy, dermatomyositis, acute pancreatitis and crushed muscle injuries. For diagnostic purposes, AST is often measured along with ALT and the ratio of the two enzymes (AST/ALT) compared since ALT is a more specific liver marker. The diagnostic condition of interest is elevated levels of the enzyme. The normal range for AST is roughly from 0 to 40 U/L. In disease states however, the enzyme can be greatly elevated and levels of 3,000 U/L or more have been reported. The assay is designed to determine the concentration of AST in lithium heparin plasma. The assay has a reportable range of 0 to 526 U/L. The assay has an LLOQ of 20 U/L and ULOQ of 526 U/L.

b. Method Principle

The Theranos aspartate amino transferase assay utilizes a kinetic colorimetric method. The rate of NADH consumption is proportional to the activity of AST in the sample. In the presence of L-aspartate and α-ketoglutarate, AST generates L-glutamate and oxaloacetate. The latter is decarboxylated by malate dehydrogenase coupled with the oxidation of NADH. The absorbance is detected by the TSPU at 340 nm at certain intervals, with the raw signals transmitted to the TSPU, which analyzes this data and determines the concentration of AST based on the rate of NADH consumption.

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Reaction Equation

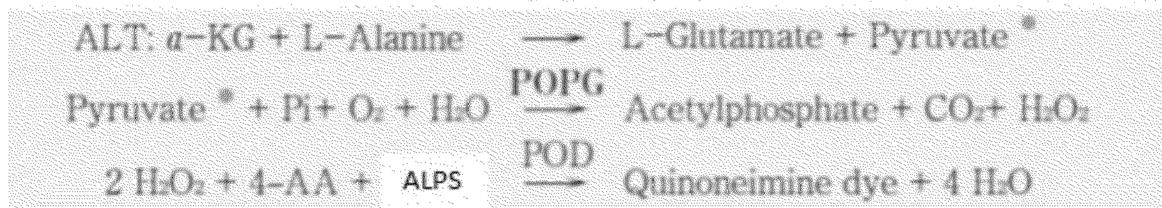
22. Alanine amino transferase (ALT/SGPT) Assay

a. Overview

Alanine amino transferase (ALT) is an enzyme that converts L-alanine and alpha-ketoglutarate or 2-oxoglutarate to pyruvate and L-glutamate via transamination. The enzyme is also known as serum glutamic pyruvic aminase (SGPT) or alanine aminotransferase (ALAT). It is found in several places in the body including the heart and liver. Most diagnostic tests currently on the market focus on the detection of the liver form of the enzyme as an indicator of liver damage. Serum levels of the enzyme can be elevated when there is liver damage, such as that caused by cirrhosis, hepatitis, or liver disease. The test for ALT is often ordered as part of a liver function panel which includes aspartate amino transferase (AST) and alkaline phosphatase (AP or ALP). The stated normal range varies depending on the reporting agency but the general range accepted as normal is 0 – 60U/L, with elevated enzyme levels being the condition of diagnostic interest. The assay has a reportable range of 0 to 600 U/L with an LLOQ of 24 U/L and ULOQ of 600 U/L.

b. Method Principle

ALT catalyzes the transfer of an amino group between L-alanine and α-ketoglutarate (α-KG). The pyruvate formed in the first reaction is then phosphorylated by pyruvate oxidase (POPG) in the presence of oxygen and water, forming peroxide in the process. The TSPU detects the absorbance of the color generated, at 561 nm when the perodide forms a quinoneimine dye in the presence of 4-aminoantipyrine (4-AA) and ALPS. This raw signal is transmitted to the TLAS, where it is analyzed and generates the concentration of ALT.



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23. Triglycerides Assay

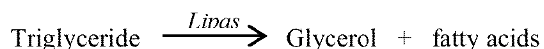
a. Overview

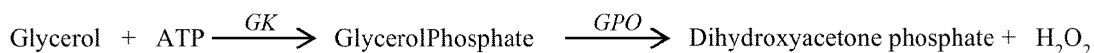
Triglycerides are a group of molecules containing a glycerol core covalently bound to 3 fatty acids which can vary in length and degree of saturation. Triglycerides containing saturated fatty acid are solid at room temperature and are considered unhealthy fats, while those containing saturated fatty acid chains (liquid at room temperature) are considered healthy. In plasma, triglycerides are the major components of very-low-density-lipoprotein (VLDL) and chylomicrons which play a role in energy transport. However, high levels of triglycerides have been linked to increased risk of atherosclerosis, heart disease, and stoke.

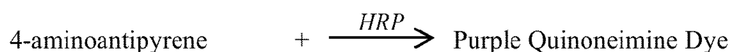
Increased plasma triglyceride levels are indicative of a metabolic abnormality and, along with elevated cholesterol, are considered a risk factor for atherosclerotic disease. Hyperlipidemia may be inherited or be associated with biliary obstruction, diabetes mellitus, nephrotic syndrome, renal failure, or metabolic disorders related to endocrinopathies. Increased triglycerides may also be medication-induced (e.g., prednisone). Healthy individuals are expected to have triglyceride values < 150 mg/dL, borderline levels are 150-200 mg/dL, high levels are 200-500 mg/dL, and very high triglyceride levels are >500 mg/dL. This assay is designed to determine the Triglycerides concentration in plasma (EDTA, Li-Heparin) in the clinical range 25-1000 mg/dL.

b. Method Principle

This assay uses an enzyme reaction cascade with results in the production of hydrogen peroxide (H_2O_2) which is used by Horse Radish Peroxidase (HRP) to produce a purple color product. The absorbance of the complex is detected by the TSPU as an endpoint reaction at 561 nm, with the raw signal transmitted to the TLAS, where it is analyzed to generate the level of Triglycerides.







GK = Glycerol Kinase

GPO = Glycerol 3-phosphate Oxidase

HPR = Horse Radish Peroxidase

ALPS = N-Ethyl-N-(3-sulfopropyl) aniline

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24. Urea nitrogen Assay

a. Overview

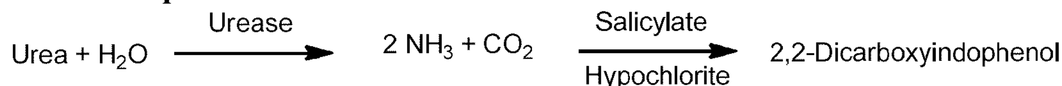
Urea is the final degradation product of protein and amino acid metabolism. During protein catabolism, the proteins are broken down to amino acids and deaminated. The ammonia formed by this process is used to synthesize urea in the liver. This is the most important catabolic pathway for eliminating excess nitrogen in the human body.

Increased blood urea nitrogen (BUN) may be due to pre-renal causes (cardiac decompensation, water depletion due to decreased intake and/or excessive loss, increased protein catabolism, and high protein diet), renal causes (acute glomerulonephritis, chronic nephritis, polycystic kidney disease, nephrosclerosis, and tubular necrosis), and post-renal causes (e.g., all types of obstruction of the urinary tract, such as stones, enlarged prostate gland, tumors). The assay has a reportable range of 0 – 25 mM urea.

b. Method Principle

The Theranos blood urea nitrogen assay utilizes a colorimetric endpoint method. The increase in absorbance at 630 nm is proportional to the concentration of urea nitrogen in the sample. The colorimetric signal is a result of the urease-mediated conversion of urea to ammonia, and the subsequent oxidative formation of 2,2-dicarboxyindophenol. The absorbance at 630nm is detected by the TLAS, with the raw signal transmitted to the TLAS where it is analyzed to generate the concentration of blood urea nitrogen.

Reaction Equation



25. Uric Acid Assay

a. Overview

Uric acid is produced as a byproduct of purine metabolism in humans and higher primates. Purines, compounds that are vital components of nucleic acids and coenzymes, may be synthesized in the body or they may be obtained by ingesting foods rich in nucleic material (eg, liver, sweetbreads, etc.). As the body breaks down the purines found in food, more uric acid is produced. Uric acid is toxic to the body and thus must be excreted to prevent build up. Most uric acid removal is done via the kidneys, with the waste passed out in the urine.

Several disease states are associated with elevated uric acid, with a notable one being gout. In patients with gout, uric acid precipitates in the body and forms crystalline deposits, often

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localized to joints and capillaries. These deposits cause swelling and severe pain. Patients with renal failure or other kidney problems also tend towards elevated uric acid because their kidneys are no longer able to properly dispose of the uric acid.

Asymptomatic hyperuricemia is frequently detected through biochemical screening. The major causes of hyperuricemia are increased purine synthesis, inherited metabolic disorder, excess dietary purine intake, increased nucleic acid turnover, malignancy, cytotoxic drugs, and decreased excretion due to chronic renal failure or increased renal reabsorption. Long-term follow-up of these patients is undertaken because many are at risk of renal disease that may develop; few of these patients ever develop the clinical syndrome of gout.

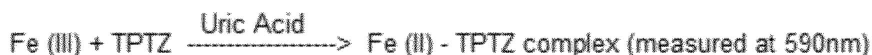
Hypouricemia, often defined as serum urate less than 2.0 mg/dL, is much less common than hyperuricemia. It may be secondary to severe hepatocellular disease with reduced purine synthesis; defective renal tubular reabsorption; overtreatment of hyperuricemia with allopurinol and well as some cancer therapies (eg, 6-mercaptopurine).

The often stated normal range for uric acid is 3 to 7mg/dL, but some sources report the normal range up to 8.5 mg/dL. For the Theranos assay, the normal range goes to 8.5mg/dL. Elevated levels of uric acid are the conditions of diagnostic interest.

This assay is designed to determine uric acid in serum and lithium heparin plasma. The assay has a reportable range of 0 to 54 mg/dL. The expected LLOQ is 2.37 mg/dL and the ULOQ is 54 mg/dL.

b. **Method Principle**

Theranos uric acid assay chemistry is based on the iron reduction method. For this method, ferric iron (iron III) is reduced to ferrous iron (iron II) which then reacts with TPTZ to form a colored product. The absorbance is detected at 590nm by the TSPU, with the raw signal transmitted to the TLAS where it is analyzed to generate the concentration of uric acid. The level of the resulting complex is directly proportional to the uric acid level of the sample.



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General Chemistry Assay Protocol

A typical General Chemistry assay starts with K2EDTA or Lithium-Heparin anticoagulated whole blood in Theranos Nanotainers. Details (sample type, diluent, dilution ratio, volume of sample/reagent, dilution ratio, number of reagents, incubation times, and read wavelengths) specific to each of the assays listed in "Theranos System" description (Page 3), are listed in the attached spreadsheet in Appendix A (General Chemistry Assay Details). All assays use Detector 4, except for Alkaline Phosphatase, which uses Detector 1. Two on-board controls per assay are included in each cartridge.

The following is the sequence of steps for a General Chemistry Assay:

Sample preparation for Whole Blood

1. A Large tip is used to mix Whole Blood in the Nanotainer by pipetting the sample up and down for several cycles.

2. The Whole Blood is transferred to Centrifuge Vessel 1. The Centrifuge Vessel is moved by the liquid handling module to the centrifuge and spun at 6000RPM for 5 minutes. The Centrifuge Vessel is returned to the cartridge by the liquid handling module.

3. A Large tip is picked up and used to transfer plasma from the Centrifuge Vessel to a Round Vessel.

4. Diluent is transferred from the Wash Well to three Round Vessels by the Liquid Handling Module using either a Mini tip (if diluent volume is <=10uL) or a Large tip (if diluent volume is > 10uL). The tip is returned back to its position in the cartridge.

5. Three Mini tips are picked up by the Liquid Handling Module and an assay-specific volume of sample is transferred to three Round Vessels with the diluent and mixed well by pipetting up and down. The Mini tips are returned back to the cartridge.

6. The Liquid Handling Module picks up 4 Mini tips. The 4 Mini tips are used to transfer assay-specific volume of sample, two on-board controls, and blank to 4 wells in the color strip. The Mini tips are returned back to the cartridge.

7. Four more Mini tips are picked up by the Liquid Handling Module and assay-specific volumes of Reagent 1 are aspirated into the 4 tips from 4 sealed Round Vessels. The contents of the 4 tips are dispensed into the 4 color strip wells (where previously sample, controls, and blank were dispensed), and mixed well by pipetting up and down.

8. Depending on the assay, the reaction mixture is incubated for the specified time.

9. Reagent 2 (if any) is transferred into the 4 color strip wells by the 4 Mini tips, followed by any specified incubation time.

10. Mini tips are returned back to the cartridge.

11. Reagent 3 (if any) is transferred into the 4 color strip wells by the 4 Mini tips, followed by any specified incubation time.

12. Mini tips are returned back to the cartridge.

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13a. For Alkaline Phosphatase, 4 Dynamic Dilution tips are picked up by the Liquid Handling Module, and 10uL of reaction mixture is aspirated into each of the 4 tips from the 4 color strip wells. The tips are transferred to Detector 1, where all 4 tips are processed sequentially to detect the corresponding chemiluminescence. The set of 4 raw signals are transferred to the TLAS where the data are analyzed and converted into analyte concentrations. The Dynamic Dilution tips are returned back to the cartridge.

13b. For all other assays, the Liquid Handling Module picks up with the Colorstrip and moves it to Detector 4. Each of the 4 wells is sequentially positioned in the beam path and an intensity spectrum is detected, with the raw data sent to the TLAS for further analysis. For endpoint assays, each well is read once. For kinetic assays, each well is processed twice, separated by a specified time interval. The color strip is returned back to the cartridge.

Data analysis

For each General Chemistry Assay, the TSPU detects signals (chemiluminescence intensity values for Alkaline Phosphatase assay; transmitted intensity spectra for all other assays) corresponding to the sample, blank, and two controls. The following steps are then performed in the TLAS to determine the analyte concentrations:

Alkaline Phosphatase

1. The chemiluminescence intensity corresponding to the blank is subtracted from the intensity values corresponding to the sample and the two controls.

2. Assay calibration is applied to the blank-subtracted intensity values and analyte concentrations for both the sample and the two controls are determined.

3. The analyte concentration for controls is compared against their expected range and a pass/fail decision is made by the TLAS. Both controls must pass for the sample results to be reportable.

Other General Chemistry assays

1. Intensity data sets are received by the TLAS from the TSPU. A wavelength calibration, specific to the TSPU, is applied to each intensity set, resulting in intensity as a function of wavelength.

2. Absorbance values for sample/controls are calculated using the intensity values for sample/controls and the intensity values for blank, at a wavelength specific to each assay.

3. Assay calibration is applied to the absorbance values to determine analyte concentrations for both sample and controls.

4. The analyte concentration for controls is compared against their expected range and a pass/fail decision is made by the TLAS. Both controls must pass for the sample results to be reportable.

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Performance Testing / Product Development

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) was attached in a validation template provided in Pre-Submission Q131199. 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.

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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 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 General Chemistry assays, the following on-board controls are run:
For each General Chemistry assay, a low and a high on-board control is processed in parallel to processing the sample on the TSPU. The data from these controls are sent to the TLAS for analysis. Both controls must be within pre-established ranges for the sample results to be reported.

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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 clinical chemistry tests described in this pre-submission will be submitted as IVDs and will be performed on individuals exhibiting signs and symptoms of lower respiratory infections. In addition, General Chemistry tests will be performed for indications for which such evaluations are known to aid in overall health assessment and detection of a wide range of diseases and disorders, including those described in the table for the General Chemistry assays starting on the next page.

Elements of Intended Use for NAA:

- The Lower Respiratory Assays will identify and detect the following pathogens: Methicillin-resistant staphylococcus aureus (MRSA), Methicillin-sensitive staphylococcus aureus (MSSA), Vancomycin-resistant staphylococcus aureus (VRSA), Klebsiella pneumoniae (Enterobacteriaceae spp), Klebsiella pneumoniae carbapenemase (KPC), Haemophilus influenzae (ampic R), Haemophilus influenzae (ampic S), Moraxella catarrhalis, Haemophilus parainfluenzae, Enterobacter cloacae (Enterobacteriaceae spp), Enterobacter aerogenes (Enterobacteriaceae spp), Serratia marcescens (Enterobacteriaceae spp), Acinetobacter baumanii, Legionella spp, Burkholderia cepacia, and Mycobacterium abscessus.

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- The assays will be qualitative in nature.
- The assays are performed on samples collected in the form of throat swabs ("***TS***") or sputum processed by the TSPU in Theranos' PSCs, and analyzed by the TLAS in Theranos' CLIA-certified laboratory.
- TS or sputum samples will be mixed automatically in the TSPU. When placed into the TSPU, the TS 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 Lower Respiratory Assays are for prescription use.
- The tests will be performed on individuals suspected of suffering from lower respiratory tract infections.
- The results will be reported as positive, equivocal, or negative for a given Lower Respiratory Assay 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 an applicable assay. 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 General Chemistry assays:

These assays will quantitatively measure the following measurands through the Theranos System:

Assay	**Regulation under 21 CFR**	**Specimen**	**Class**	**Intended Use**
Albumin	862.1035	Plasma	II	Albumin measurements are used in the diagnosis and treatment of numerous diseases involving primarily the liver or kidneys.
Total Bilirubin	862.1110	Plasma	II	Measurements of the levels of bilirubin, an organic compound formed during the normal and abnormal distruction of red blood cells, if used in the diagnosis and treatment of liver, hemolytic hematological, and metabolic disorders, including hepatitis and gall bladder block.

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Assay	Regulation under 21 CFR	Specimen	Class	Intended Use
Direct Bilirubin	862.1110	Plasma	II	Measurements of the levels of bilirubin, an organic compound formed during the normal and abnormal destruction of red blood cells, if used in the diagnosis and treatment of liver, hemolytic hematological, and metabolic disorders, including hepatitis and gall bladder block.
Calcium	862.1145	Plasma	II	Calcium measurements are used in the diagnosis and treatment of parathyroid disease, a variety of bone diseases, chronic renal disease and tetany (intermittent muscular contractions or spasms).
Carbon Dioxide	862.1160	Plasma	II	Bicarbonate/carbon dioxide measurements are used in the diagnosis and treatment of numerous potentially serious disorders associated with changes in body acid-base balance.
Chloride	862.1170	Plasma	II	Chloride measurements are used in the diagnosis and treatment of electrolyte and metabolic disorders such as cystic fibrosis and diabetic acidosis.
Total Cholesterol	862.1175	Plasma	I General controls Exempt Reserved	Cholesterol measurements are used in the diagnosis and treatment of disorders involving excess cholesterol in the blood and lipid and lipoprotein metabolism disorders.
Creatine kinase	862.1215	Plasma	II	Measurements of creatine phosphokinase and its isoenzymes are used in the diagnosis and treatment of myocardial infarction and muscle diseases such as progressive, Duchenne-type muscular dystrophy.
Creatinine	862.1225	Plasma	II	Creatinine measurements are used in the diagnosis and treatment of renal diseases, in monitoring renal dialysis, and as a calculation basis for measuring other urine analytes.

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Assay	Regulation under 21 CFR	Specimen	Class	Intended Use
Glucose	862.1345	Plasma	II	Glucose measurements are used in the diagnosis and treatment of carbohydrate metabolism disorders including diabetes mellitus, neonatal hypoglycemia, and idiopathic hypoglycemia, and of pancreatic islet cell carcinoma.
Gamma-glutamyl transpeptidase	862.1360	Plasma	I General controls Exempt	Gamma-glutamyl transpeptidase and isoenzymes measurements are used in the diagnosis and treatment of liver diseases such as alcoholic cirrhosis and primary and secondary liver tumors.
Iron (non-heme)	862.1410	Plasma	I Reserved	Iron (non-heme) measurements are used in the diagnosis and treatment of diseases such as iron deficiency anemia, hemochromatosis (a disease associated with widespread deposit in the tissues of two iron-containing pigments, hemosiderin and hemofuscin, and characterized by pigmentation of the skin), and chronic renal disease.
Low-density Lipoprotein	862.1475	Plasma	I General controls Exempt	Lipoprotein measurements are used in the diagnosis and treatment of lipid disorders (such as diabetes mellitus), atherosclerosis, and various liver and renal diseases.
High-density Lipoprotein	862.1475	Plasma	I General controls Exempt	Lipoprotein measurements are used in the diagnosis and treatment of lipid disorders (such as diabetes mellitus), atherosclerosis, and various liver and renal diseases.
Magnesium	862.1495	Plasma	I Reserved	Magnesium measurements are used in the diagnosis and treatment of hypomagnesemia (abnormally low plasma levels of magnesium)

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Assay	Regulation under 21 CFR	Specimen	Class	Intended Use
				and hypermagnesemia (abnormally high plasma levels of magnesium).
Alkaline phosphatase	862.1050	Plasma	II	An alkaline phosphatase or isoenzymes test system is a device intended to measure alkaline phosphatase or its isoenzymes (a group of enzymes with similar biological activity) in serum or plasma. Measurements of alkaline phosphatase or its isoenzymes are used in the diagnosis and treatment of liver, bone, parathyroid, and intestinal diseases
Phosphorus	862.1580	Plasma	I Reserved	Measurements of phosphorus (inorganic) are used in the diagnosis and treatment of various disorders, including parathyroid gland and kidney diseases, and vitamin D imbalance.
Potassium	862.1600	Plasma	II	A potassium test system is a device intended to measure potassium in serum, plasma, and urine. Measurements obtained by this device are used to monitor electrolyte balance in the diagnosis and treatment of diseases conditions characterized by low or high blood potassium levels.
Total Protein	862.1635	Plasma	II Special controls Exempt	Measurements obtained by this device are used in the diagnosis and treatment of a variety of diseases involving the liver, kidney, or bone marrow as well as other metabolic or nutritional disorders.
Sodium	862.1665	Plasma	II	Measurements obtained by this device are used in the diagnosis and treatment of aldosteronism (excessive secretion of the hormone aldosterone), diabetes insipidus (chronic excretion of large amounts of dilute urine, accompanied by extreme thirst), adrenal hypertension, Addison's disease (caused by destruction of the adrenal glands), dehydration,

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Assay	Regulation under 21 CFR	Specimen	Class	Intended Use
				inappropriate antidiuretic hormone secretion, or other diseases involving electrolyte imbalance.
Aspartate amino transferase (AST/SGOT)	862.1100	Plasma	II	Aspartate amino transferase measurements are used in the diagnosis and treatment of certain types of liver and heart disease.
Alanine amino transferase (ALT/SGPT)	862.1030	Plasma	I General controls Exempt	Alanine amino transferase measurements are used in the diagnosis and treatment of certain liver diseases (e.g., viral hepatitis and cirrhosis) and heart diseases.
Triglycerides	862.1705	Plasma	I General controls Exempt Reserved	Measurements obtained by this device are used in the diagnosis and treatment of patients with diabetes mellitus, nephrosis, liver obstruction, other diseases involving lipid metabolism, or various endocrine disorders.
Urea nitrogen	862.1770	Plasma	II	Measurements obtained by this device are used in the diagnosis and treatment of certain renal and metabolic diseases.
Uric acid	862.1775	Plasma	I Reserved	Measurements obtained by this device are used in the diagnosis and treatment of numerous renal and metabolic disorders, including renal failure, gout, leukemia, psoriasis, starvation or other wasting conditions, and of patients receiving cytotoxic drugs.

- 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.

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- The assays are performed on plasma processed in the TSPU from whole blood collected from fingerstick punctures or venipuncture.
- All the above assays are for prescription use, performed through Theranos' CLIA laboratory's PSC.
- All results will be reported as numeric values with appropriate units and reference ranges. Results outside of normal value ranges may reflect an underlying disease. Results should be interpreted in conjunction with the patient's clinical picture and appropriate additional testing performed.
- The above tests are routinely performed on individuals for the conditions described in the Indications in the table above.

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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 Lower Respiratory Assays 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 throat swab or sputum collection, 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 Lower Respiratory 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 General Chemistry Assays:

These samples will be collected at a TPSC from patients with a prescription for the relevant General Chemistry 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 TSPU performs the sample prep steps and processing, and transmits the raw data to the TLAS. The data will be analyzed by the TLAS to determine analyte concentrations and a report will be generated by Theranos' CLIA-certified laboratory. This information will be made available to the ordering doctor directly for their diagnostic decision making.

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Risk Analysis

Results obtained from these tests can be used for diagnosis of lower respiratory tract infection when used in conjunction with other clinical information. Positive results do not rule out co-infection with pathogens not tested. 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 controls are run with each TNAA test to reduce the chances 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.

Results obtained from the General Chemistry tests are intended for diagnostic use in the quantitative determination of analyte concentrations in human plasma. Such measurements are used in the diagnosis and treatment of a wide variety of disorders. Low and high assay controls for each General Chemistry test are run in parallel with each clinical sample to ensure that the TSPU/TLAS and reagents are performing as intended thereby reducing the chances of incorrect results. Normal results do not rule out adverse medical conditions 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.

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

1. **Method characterization:** 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 three study designs comprise 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 regarding each of the above studies are covered below.

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 bacterial count or 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/bacteria and for each dilution, and record positives and negatives.

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	1000 $TCID_{50}$	100 $TCID_{50}$	10 $TCID_{50}$
Staphylococcus aureus (MR)	#Pos: #Neg:	#Pos: #Neg:	#Pos: #Neg:
Staphylococcus aureus (MS)	#Pos: #Neg:	#Pos: #Neg:	#Pos: #Neg:
Klebsiella pneumoniae (Enterobacteriaceae spp)	#Pos: #Neg:	#Pos: #Neg:	#Pos: #Neg:
Klebsiella pneumoniae carbapenemase (KPC)	#Pos: #Neg:	#Pos: #Neg:	#Pos: #Neg:
Haemophilus influenzae (ampic R)	#Pos: #Neg:	#Pos: #Neg:	#Pos: #Neg:
Haemophilus influenzae (ampic S)	#Pos: #Neg:	#Pos: #Neg:	#Pos: #Neg:
Moraxella catarrhalis	#Pos: #Neg:	#Pos: #Neg:	#Pos: #Neg:
Haemophilus parainfluenzae	#Pos: #Neg:	#Pos: #Neg:	#Pos: #Neg:
Enterobacter cloacae (Enterobacteriaceae spp)	#Pos: #Neg:	#Pos: #Neg:	#Pos: #Neg:
Enterobacter aerogenes (Enterobacteriaceae spp)	#Pos: #Neg:	#Pos: #Neg:	#Pos: #Neg:
Serratia marcescens (Enterobacteriaceae spp)	#Pos: #Neg:	#Pos: #Neg:	#Pos: #Neg:
Acinetobacter baumanii	#Pos: #Neg:	#Pos: #Neg:	#Pos: #Neg:
Legionella spp	#Pos: #Neg:	#Pos: #Neg:	#Pos: #Neg:
Burkholderia cepacia	#Pos: #Neg:	#Pos: #Neg:	#Pos: #Neg:

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Mycobacterium abscessus	#Pos: #Neg:	#Pos: #Neg:	#Pos: #Neg:
Staphylococcus aureus (Vancomycin-resistant) VRSA	#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 from pathogens as shown below in the table. The nucleic acid concentration should reflect expected median viral loads in clinical specimens.

TNAA test	**Staphylococcus aureus (MR)**	**Staphylococcus aureus (MS)**	**Klebsiella pneumoniae (Enterobacteriaceae spp)**	**Klebsiella pneumoniae carbapenemase (KPC)**
Pathogen titer	Enterobacter cloacae	Adenovirus 4	Enterobacter cloacae	Adenovirus 4
	Streptococcus agalactiae	Candida albicans	Streptococcus agalactiae	Candida albicans
	Streptococcus pneumoniae	Klebsiella pneumoniae	Streptococcus pneumoniae	Escherichia coli
	Acinetobacter baumannii	Escherichia coli	Acinetobacter baumannii	5ng human genomic DNA
	Neisseria meningitidis	5ng human genomic DNA	Neisseria meningitidis	Bordetella pertussis
	Pseudomonas aeruginosa	Bordetella pertussis	Pseudomonas aeruginosa	A/California/7/2009 (H1N1 novel)

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	Escherichia coli	A/California/7/2009 (H1N1 novel)	Escherichia coli	Flu B/Mass/3/66
	Klebsiella pneumoniae	Flu B/Mass/3/66	Serratia marcescens	Pseudomonas aeruginosa
	Serratia marcescens	Pseudomonas aeruginosa	Klebsiella oxytoca	Staphylococcus aureus MSSA (DmecA)
	Klebsiella oxytoca			
TNAA test	**Haemophilus influenzae (ampic R)**	**Haemophilus influenzae (ampic S)**	**Moraxella catarrhalis**	**Haemophilus parainfluenzae**
Pathogen titer	*Enterobacter cloacae*	Adenovirus 4	Adenovirus 4	Adenovirus 4
	Streptococcus agalactiae	Candida albicans	Candida albicans	Candida albicans
	Streptococcus pneumoniae	Klebsiella pneumoniae	Klebsiella pneumoniae	Klebsiella pneumoniae
	Acinetobacter baumannii	Escherichia coli	Escherichia coli	Escherichia coli
	Neisseria meningitidis	5ng human genomic DNA	5ng human genomic DNA	5ng human genomic DNA
	Pseudomonas aeruginosa	Bordetella pertussis	Bordetella pertussis	Bordetella pertussis
	Escherichia coli	A/California/7/2009 (H1N1 novel)	A/California/7/2009 (H1N1 novel)	A/California/7/2009 (H1N1 novel)
	Klebsiella pneumoniae	Flu B/Mass/3/66	Flu B/Mass/3/66	Flu B/Mass/3/66
	Serratia marcescens	Pseudomonas aeruginosa	Pseudomonas aeruginosa	Pseudomonas aeruginosa

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	Klebsiella oxytoca	Staphylococcus aureus MSSA (DmecA)	Staphylococcus aureus MSSA (DmecA)	Staphylococcus aureus MSSA (DmecA)
	Enterobacter aerogenes	Enterobacter cloacae		
		Streptococcus agalactiae		
		Streptococcus pneumoniae		
		Acinetobacter baumannii		
		Neisseria meningitidis		
		Serratia marcescens		
		Klebsiella oxytoca		
TNAA test	**Enterobacter cloacae (Enterobacteriaceae spp)**	**Enterobacter aerogenes (Enterobacteriaceae spp)**	**Serratia marcescens (Enterobacteriaceae spp)**	**Acinetobacter baumanii**
Pathogen titer	Adenovirus 4	Adenovirus 4	Adenovirus 4	Adenovirus 4
	Candida albicans	Candida albicans	Candida albicans	Candida albicans
	Klebsiella pneumoniae	Klebsiella pneumoniae	Escherichia coli	Klebsiella pneumoniae
	Escherichia coli	Escherichia coli	5ng human genomic DNA	Escherichia coli
	5ng human genomic DNA	Bordetella pertussis	Bordetella pertussis	5ng human genomic DNA

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	Bordetella pertussis	Influenza A/California/7/2009 (H1N1 novel)	FluA (H1N1)	Bordetella pertussis
	A/California/7/2009 (H1N1 novel)	Influenza B/Russia/69	FluB	A/California/7/2009 (H1N1 novel)
	Flu B/Mass/3/66	Pseudomonas aeruginosa	*Pseudomonas aeruginosa*	Flu B/Mass/3/66
	Pseudomonas aeruginosa	Staphylococcus aureus MSSA (DmecA)	*Staphylococcus aureus*	Pseudomonas aeruginosa
	Staphylococcus aureus MSSA (DmecA)	Streptococcus pyogenes	*Enterobacter cloacae*	Staphylococcus aureus MSSA (DmecA)
	Streptococcus agalactiae	Enterobacter cloacae	*Streptococcus agalactiae*	Enterobacter cloacae
	Streptococcus pneumoniae	Streptococcus agalactiae	*Streptococcus pneumoniae*	Streptococcus agalactiae
	Acinetobacter baumannii	Streptococcus pneumoniae	*Acinetobacter baumannii*	Streptococcus pneumoniae
	Neisseria meningitidis	Acinetobacter baumannii	*Neisseria meningitidis*	Neisseria meningitidis
	Serratia marcescens	Neisseria meningitidis	*Klebsiella pneumoniae*	Serratia marcescens
	Klebsiella oxytoca	Serratia marcescens	*Klebsiella oxytoca*	Klebsiella oxytoca
	Enterobacter aerogenes	Klebsiella oxytoca		

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TNAA test	**Legionella spp**	**Burkholderia cepacia**	**Mycobacterium abscessus**	**Staphylococcus aureus(Vancomycin-resistant) VRSA**
Pathogen titer	Adenovirus 4	Adenovirus 4	Adenovirus 4	Adenovirus 4
	Candida albicans	*Candida albicans*	*Candida albicans*	*Candida albicans*
	Klebsiella pneumoniae	Klebsiella pneumoniae	Klebsiella pneumoniae	Klebsiella pneumoniae
	Escherichia coli	*Escherichia coli*	*Escherichia coli*	*Escherichia coli*
	5ng human genomic DNA	5ng human genomic DNA	Bordetella pertussis	Bordetella pertussis
	Bordetella pertussis	Bordetella pertussis	Influenza A/California/7/2009 (H1N1 novel)	Influenza A/California/7/2009 (H1N1 novel)
	A/California/7/2009 (H1N1 novel)	A/California/7/2009 (H1N1 novel)	Influenza B/Russia/69	Influenza B/Russia/69
	Flu B/Mass/3/66	Flu B/Mass/3/66	*Pseudomonas aeruginosa*	*Pseudomonas aeruginosa*
	Pseudomonas aeruginosa	*Pseudomonas aeruginosa*	*Staphylococcus aureus* MSSA (DmecA)	*Staphylococcus aureus* MSSA (DmecA)
	Staphylococcus aureus MSSA (DmecA)	*Staphylococcus aureus* MSSA (DmecA)	*Streptococcus pyogenes*	*Streptococcus pyogenes*
		Streptococcus pyogenes	Enterobacter cloacae	Enterobacter cloacae
		Enterobacter cloacae	Streptococcus agalactiae	Streptococcus agalactiae
		Streptococcus agalactiae	Streptococcus pneumoniae	Streptococcus pneumoniae
		Streptococcus pneumoniae	Acinetobacter baumannii	Acinetobacter baumannii

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		Acinetobacter baumannii	Neisseria meningitidis	Neisseria meningitidis
		Neisseria meningitidis	Serratia marcescens	Serratia marcescens
		Serratia marcescens	Klebsiella oxytoca	Klebsiella oxytoca
		Klebsiella oxytoca		
		Enterobacter aerogenes		

Carryover

- Set up a plate with Pathogen count as follows:

TNAA test	**Staphylococcus aureus (MR)**	**Staphylococcus aureus (MS)**	**Klebsiella pneumoniae (Enterobacteriaceae spp)**	**Klebsiella pneumoniae carbapenemase (KPC)**
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

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TNAA test	Haemophilus influenzae (ampic R)	Haemophilus influenzae (ampic S)	Moraxella catarrhalis	Haemophilus parainfluenzae
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	Enterobacter cloacae (Enterobacteriaceae spp)	Enterobacter aerogenes (Enterobacteriaceae spp)	Serratia marcescens (Enterobacteriaceae spp)	Acinetobacter baumanii
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	Legionella spp	Burkholderia cepacia	Mycobacterium abscessus	Staphylococcus aureus (Vancomycin-resistant) VRSA
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

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	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

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 will be evaluated.

The following interfering substances will be 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)

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	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 and Bacterial assays.

Staphylococcus aureus (MR)		
Inclusivity	**Exclusivity**	**Cross-reactivity**
MRSA NYBK2464	*S. aureus* Wood 46	Enterobacter cloacae
MRSA HDE288	*S. aureus* FDA 209	Streptococcus agalactiae
MRSA HFH-30364	*S. aureus* PCI 1158	Streptococcus pneumoniae

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MRSA M10/0148	*S. aureus* TCH959	Acinetobacter baumannii
MRSA Mu50 [NRS1]	*S. aureus* NCTC 8530 [S11]	Neisseria meningitidis
	S. aureus Rose	Pseudomonas aeruginosa
		Escherichia coli
		Klebsiella pneumoniae
		Serratia marcescens
		Klebsiella oxytoca
Staphylococcus aureus (MS)		
Inclusivity	**Exclusivity**	**Cross-reactivity**
S. aureus Wood 46	MRSA NYBK2464	Adenovirus 4
S. aureus FDA 209	MRSA HDE288	Candida albicans
S. aureus PCI 1158	MRSA HFH-30364	Klebsiella pneumoniae
S. aureus TCH959	MRSA M10/0148	Escherichia coli
S. aureus NCTC 8530 [S11]	MRSA Mu50 [NRS1]	5ng human genomic DNA
S. aureus Rose		Bordetella pertussis
		A/California/7/2009 (H1N1 novel)
		Flu B/Mass/3/66
		Pseudomonas aeruginosa
Klebsiella pneumoniae (Enterobacteriaceae spp)		
Inclusivity	**Exclusivity**	**Cross-reactivity**
K. pneumoniae 1002565	Klebsiella oxytoca	
K. pneumoniae 1100975		Enterobacter cloacae

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K. pneumoniae ATCC BAA-1898		Streptococcus agalactiae
K. pneumoniae ATCC BAA-1899		Streptococcus pneumoniae
K. pneumoniae K6 [CCUG 45421, LMG 20218, MCV37]		Acinetobacter baumannii
K. pneumoniae AIS 2007023 [6179]		Neisseria meningitidis
K. pneumoniae ART 2008133 [D-05, 1338]		Pseudomonas aeruginosa
K. pneumoniae ATCC BAA-1903		Escherichia coli
K. pneumoniae ATCC BAA-1905		Serratia marcescens
		Klebsiella oxytoca
Klebsiella pneumoniae carbapenemase (KPC)		
Inclusivity	**Exclusivity**	**Cross-reactivity**
K. pneumoniae ATCC BAA-1898	K. pneumoniae 1002565	Adenovirus 4
K. pneumoniae ATCC BAA-1899	K. pneumoniae 1100975	Candida albicans
K. pneumoniae ART 2008133 [D-05, 1338]	K. pneumoniae K6 [CCUG 45421, LMG 20218, MCV37]	Escherichia coli
K. pneumoniae ATCC BAA-1903	K. pneumoniae AIS 2007023 [6179]	5ng human genomic DNA
K. pneumoniae ATCC BAA-1905		Bordetella pertussis
		A/California/7/2009 (H1N1 novel)
		Flu B/Mass/3/66
		Pseudomonas aeruginosa
		Staphylococcus aureus MSSA (DmecA)

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Haemophilus influenzae (ampic R)		
Inclusivity	**Exclusivity**	**Cross-reactivity**
Haemophilus influenzae (ROB-1+) T2494		*Enterobacter cloacae*
E. coli 1532 blaTEM+ 1532		*Streptococcus agalactiae*
Serratia marcescens blaTEM+ 1532		*Streptococcus pneumoniae*
		Acinetobacter baumannii
		Neisseria meningitidis
		Pseudomonas aeruginosa
		Escherichia coli
		Klebsiella pneumoniae
		Serratia marcescens
		Klebsiella oxytoca
		Enterobacter aerogenes

Haemophilus influenzae (ampic S)		
Inclusivity	**Exclusivity**	**Cross-reactivity**
Haemophilus influenzae type a	Haemophilus parainfluenzae	Adenovirus 4
Haemophilus influenzae type b (ampS)	Haemophilus haemolyticus	Candida albicans
Haemophilus influenzae type c	Aggregatibacter segnis (deposited as Haemophilus Segnis)	Klebsiella pneumoniae
Haemophilus influenzae type d		Escherichia coli
Haemophilus influenzae type e		5ng human genomic DNA
Haemophilus influenzae type f		Bordetella pertussis

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Haemophilus influenzae (ROB-1+)		A/California/7/2009 (H1N1 novel)
Haemophilus influenzae (ROB-1+)		Flu B/Mass/3/66
		Pseudomonas aeruginosa
		Staphylococcus aureus MSSA (DmecA)
		Enterobacter cloacae
		Streptococcus agalactiae
		Streptococcus pneumoniae
		Acinetobacter baumannii
		Neisseria meningitidis
		Serratia marcescens
		Klebsiella oxytoca

Moraxella catarrhalis		
Inclusivity	**Exclusivity**	**Cross-reactivity**
Moraxella catarrhalis Bc-30	Acinetobacter baumannii	Adenovirus 4
Moraxella catarrhalis Ne 11	Neisseria meningitidis	Candida albicans
Moraxella catarrhalis 20	Kingella kingae	Klebsiella pneumoniae
Moraxella catarrhalis N9	Haemophilus influenzae	Escherichia coli
Moraxella catarrhalis 1908	Streptococcus pneumoniae	5ng human genomic DNA
Moraxella catarrhalis mMS 116		Bordetella pertussis
Moraxella catarrhalis 57135		A/California/7/2009 (H1N1 novel)
Moraxella catarrhalis 59632		Flu B/Mass/3/66
		Pseudomonas aeruginosa

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		Staphylococcus aureus MSSA (DmecA)

Haemophilus parainfluenzae		
Inclusivity	**Exclusivity**	**Cross-reactivity**
Haemophilus parainfluenzae NCTC 7857	Haemophilus parainfluenzae NCTC 7857	Adenovirus 4
Haemophilus parainfluenzae 655	Haemophilus parainfluenzae 655	Candida albicans
Haemophilus parainfluenzae P142	Haemophilus parainfluenzae P142	Klebsiella pneumoniae
Haemophilus parainfluenzae P110	Haemophilus parainfluenzae P110	Escherichia coli
Haemophilus parainfluenzae H30	Haemophilus parainfluenzae H30	5ng human genomic DNA
	Haemophilus influenzae type b (ampS)	Bordetella pertussis
	Haemophilus influenzae (ROB-1+)	A/California/7/2009 (H1N1 novel)
	Haemophilus haemolyticus	Flu B/Mass/3/66
	Aggregatibacter segnis (deposited as Haemophilus Segnis)	Pseudomonas aeruginosa
		Staphylococcus aureus MSSA (DmecA)
Enterobacter cloacae (Enterobacteriaceae spp)		
Inclusivity	**Exclusivity**	**Cross-reactivity**
Enterobacter cloacae NCDC 279-56	Enterobacter aerogenes	Adenovirus 4

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Enterobacter cloacae 1101152		Candida albicans
Enterobacter cloacae 1101177		Klebsiella pneumoniae
Enterobacter cloacae 1000654		Escherichia coli
Enterobacter cloacae [ATCC BAA-2080]		5ng human genomic DNA
Enterobacter hormaechei		Bordetella pertussis
		A/California/7/2009 (H1N1 novel)
		Flu B/Mass/3/66
		Pseudomonas aeruginosa
		Staphylococcus aureus MSSA (DmecA)
		Streptococcus agalactiae
		Streptococcus pneumoniae
		Acinetobacter baumannii
		Neisseria meningitidis
		Serratia marcescens
		Klebsiella oxytoca
		Enterobacter aerogenes
Enterobacter aerogenes (Enterobacteriaceae spp)		
Inclusivity	**Exclusivity**	**Cross-reactivity**
Enterobacter aerogenes NCDC 819-56	Enterobacter cloacae	Adenovirus 4
Enterobacter aerogenes IFO 12010	Enterobacter hormaechei	Candida albicans
Enterobacter aerogenes 1101206		Klebsiella pneumoniae

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Enterobacter aerogenes AmMS 264		Escherichia coli
Enterobacter aerogenes 1101481		Bordetella pertussis
Enterobacter aerogenes MULB-250		Influenza A/California/7/2009 (H1N1 novel)
Enterobacter aerogenes CDC 120-75		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
Serratia marcescens (Enterobacteriaceae spp)		
Inclusivity	**Exclusivity**	**Cross-reactivity**
Serratia marcescens ATCC 10759	Enterobacter cloacae	Adenovirus 4
Serratia marcescens CDC 4385-74	Streptococcus agalactiae	*Candida albicans*
Serratia marcescens CDC 3100-71	Streptococcus pneumoniae	*Escherichia coli*
Serratia marcescens CDC 4340-74	Acinetobacter baumannii	5ng human genomic DNA
Serratia marcescens CY918	Neisseria meningitidis	*Bordetella pertussis*
Serratia marcescens CY429	Pseudomonas aeruginosa	FluA (H1N1)

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	Escherichia coli	FluB
	Klebsiella pneumoniae	*Pseudomonas aeruginosa*
	Serratia marcescens	*Staphylococcus aureus*
	Klebsiella oxytoca	*Enterobacter cloacae*
	Enterobacter aerogenes	*Streptococcus agalactiae*
		Streptococcus pneumoniae
		Acinetobacter baumannii
		Neisseria meningitidis
		Klebsiella pneumoniae
		Klebsiella oxytoca
Acinetobacter baumanii		
Inclusivity	**Exclusivity**	**Cross-reactivity**
Acinetobacter baumanii CLIA strain	Acinetobacter lwoffiI	Adenovirus 4
Acinetobacter baumanii B5W71	Acinetobacter johnsonii	Candida albicans
Acinetobacter baumanii 5377	Acinetobacter ursingii	Klebsiella pneumoniae
Acinetobacter baumanii AYE	Acinetobacter schindleri	Escherichia coli
Acinetobacter baumanii 2208	Acinetobacter calcaoaceticus	5ng human genomic DNA
Acinetobacter baumanii BAA-1605		Bordetella pertussis
		A/California/7/2009 (H1N1 novel)
		Flu B/Mass/3/66
		Pseudomonas aeruginosa
		Staphylococcus aureus MSSA (DmecA)

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		Enterobacter cloacae
		Streptococcus agalactiae
		Streptococcus pneumoniae
		Neisseria meningitidis
		Serratia marcescens
		Klebsiella oxytoca
Legionella spp		
Inclusivity	**Exclusivity**	**Cross-reactivity**
L. pneumo. subsp *pneumo.* (Philadelphia-1)	Legionella micdadei	Adenovirus 4
L. pneumo. subsp pneumo. (Knoxville-1)	Legionella longbeachae	Candida albicans
L. pneumo. subsp pneumo. (Leiden 1)	Legionella israeliensis	Klebsiella pneumoniae
L. pneumo. subsp pneumo. (570-CO-H)		Escherichia coli
L. pneumo. subsp pneumo. (1169-MN-H)		5ng human genomic DNA
L. pneumo. subsp pneumo. (Togus-1)		Bordetella pertussis
L. pneumo. subsp pneumo. (Bloomington-2)		A/California/7/2009 (H1N1 novel)
L. pneumo. subsp fraseri(Los Angeles-1)		Flu B/Mass/3/66
L. pneumo. subsp pneumo. (Chicago 2)		Pseudomonas aeruginosa
L. pneumophila (Chicago 8)		Staphylococcus aureus MSSA (DmecA)

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L. pneumo. subsp pneumo. (IN-23-G1-C2)		
L. pneumo. subsp. pascullei (MICU B)		
Burkholderia cepacia		
Inclusivity	**Exclusivity**	**Cross-reactivity**
Burkholderia cepacia 249 [S-4-An]	N/A	Adenovirus 4
Burkholderia cepacia UCB 717		*Candida albicans*
Burkholderia cepacia NCDC A977		Klebsiella pneumoniae
Burkholderia cepacia 105597		*Escherichia coli*
Burkholderia multivorans LMG 13010		5ng human genomic DNA
Burkholderia cenocepacia LMG 16656		Bordetella pertussis
Burkholderia stabilis LMG 14294		A/California/7/2009 (H1N1 novel)
Burkholderia vietnamiensis IFF-8296C		Flu B/Mass/3/66
Burkholderia pyrrocinia 2327		*Pseudomonas aeruginosa*
Burkholderia glathei N15		*Staphylococcus aureus* MSSA (DmecA)
		Streptococcus pyogenes
		Enterobacter cloacae
		Streptococcus agalactiae
		Streptococcus pneumoniae
		Acinetobacter baumannii
		Neisseria meningitidis

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		Serratia marcescens
		Klebsiella oxytoca
		Enterobacter aerogenes
Mycobacterium abscessus		
Inclusivity	**Exclusivity**	**Cross-reactivity**
M. abscessus [TMC 1543] gDNA	Mycobacterium bovis	Adenovirus 4
M. abscessus MC1549 [K.K.]	Mycobacterium gastri	*Candida albicans*
M. abscessus [SSC 210]	Mycobacterium tuberculosis	Klebsiella pneumoniae
M. abscessus [TMC 1543]	Mycobacterium avium	*Escherichia coli*
M. abscessus MC1549 [K.K.]		Bordetella pertussis
M. abscessus [SSC 210]		Influenza A/California/7/2009 (H1N1 novel)
M. abscessus [TMC 1543]		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

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Staphylococcus aureus (Vancomycin-resistant) VRSA		
Inclusivity	**Exclusivity**	**Cross Reactivity**
None currently available	*S. aureus* Wood 46	Adenovirus 4
	S. aureus FDA 209	*Candida albicans*
	S. aureus PCI 1158	Klebsiella pneumoniae
	S. aureus TCH959	*Escherichia coli*
	S. aureus NCTC 8530 [S11]	Bordetella pertussis
	S. aureus Rose	Influenza A/California/7/2009 (H1N1 novel)
	MRSA NYBK2464	Influenza B/Russia/69
	MRSA HDE288	*Pseudomonas aeruginosa*
	MRSA HFH-30364	*Staphylococcus aureus* MSSA (DmecA)
	MRSA M10/0148	*Streptococcus pyogenes*
	MRSA Mu50 [NRS1]	Enterobacter cloacae
		Streptococcus agalactiae
		Streptococcus pneumoniae
		Acinetobacter baumannii
		Neisseria meningitidis
		Serratia marcescens
		Klebsiella oxytoca

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- 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.

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- 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: As there are no cleared comparators available for TNAA tests for the Lower Respiratory Assays in this Pre-Submission, two Theranos in-house PCR tests with bi-directional sequence confirmation will be used. A predetermine algorithm will be 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.

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

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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 retrospective samples each day using TNAA and Theranos PCR methods over 20 days using retrospective samples from demographically diverse population covering gender and age:

Target	Positive	Negative
Staphylococcus aureus (MR)	50	100
Staphylococcus aureus (RS)	50	100
Klebsiella pneumoniae (Enterobacteriaceae spp)	50	100
Klebsiella pneumoniae carbapenemase (KPC)	50	100
Haemophilus influenzae (ampic R)	50	100
Haemophilus influenzae (ampic S)	50	100
Moraxella catarrhalis	50	100
Haemophilus parainfluenzae	50	100
Enterobacter cloacae (Enterobacteriaceae spp)	50	100
Enterobacter aerogenes (Enterobacteriaceae spp)	50	100
Serratia marcescens (Enterobacteriaceae spp)	50	100
Acinetobacter baumanii	50	100
Legionella spp	50	100
Burkholderia cepacia	50	100
Mycobacterium abscessus	50	100
Staphylococcus aureus (Vancomycin-resistant) VRSA	50	100

- Carefully document positives and negatives for each pathogen on each method.

- Calculate the percent concordance and percent discordance of TNAA versus comparator RT-PCR assays.

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

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Anticipated Predicate Device

As the Theranos System, including the TSPU and TLAS, will have been cleared for running the influenza TNAA assays pursuant to the 510(k) submission corresponding to the Pre-Submission Q131199, Theranos plans to use that Theranos System as the predicate for the Lower Respiratory Assays under this submission with the Theranos in-house PCR tests as comparators as described above.

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 for Theranos Lower Respriatory TNAA assays and the previously cleared TSPU for Theranos Influenza TNAA assays		
Element	TSPU	Cleared TSPU
Analyte	RNA or DNA	Same
Technological Principles	Nucleic acid amplification	Same
Technological Principles	Recombination based isothermal method (See the "*Device Description – Theranos Nucleic Acid Amplification (TNAA) Assay*	Same
Test to result	<45mins	Same
Sample Preparation Method	Sample processing is automated in Theranos TSPU	Same
Reagent Storage	Reagents stored at 4 °C	Same
User Complexity (for instrument)	Low	Same
Test Interpretation	Automated test interpretation and report generation by TLAS. TSPU operator cannot access raw data.	Same

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Differences between TSPU and FilmArray RP		
Element	TSPU	Cleared TSPU
Organisms Detected	Staphylococcus aureus (MR) Staphylococcus aureus (RS) Klebsiella pneumoniae (Enterobacteriaceae spp) Klebsiella pneumoniae carbapenemase (KPC) Haemophilus influenzae (ampic R) Haemophilus influenzae (ampic S) Enterobacter cloacae (Enterobacteriaceae spp) Serratia marcescens (Enterobacteriaceae spp) Acinetobacter baumanii Staphylococcus aureus (Vancomycin-resistant) VRSA Moraxella catarrhalis Haemophilus parainfluenzae Enterobacter aerogenes (Enterobacteriaceae spp) Legionella spp Burkholderia cepacia Mycobacterium abscessus	Influenza Influenza A/H1 Influenza A/H3 Influenza A/H1-2009 Influenza A/H5N1 Influenza A/N7N9 Influenza B
Specimen Types	Throat swabs, Sputum	Nasopharyngeal swabs, aspirates and washes, and nasal swabs

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General Chemistry Proposed Study Design(s), Predicate, and Analytical and Pre-Clinical Performance:

Specific Performance Characteristics

The study design comprises the following:

<u>Pre-Clinical/Laboratory/In-vitro Studies</u>

1. Sensitivity and Limit of Detection (LOD)

The limit of detection (LoD) will be established with a minimum of sixty (60) measurements with both blank and low level samples. The LoD will be determined from both the measure limit of blank (LoB) and test replicates of the low level sample (CLSI EP17-A). The mean and standard deviation (SD) of the low concentration sample is then calculated according to

$LoD = LoB + 1.645\ (SD_{\text{Low concentration}})$, where $LoB = mean_{blank} + 1.645\ (SD_{blank})$

2. Reproducibility (Precision)

A minimum of two control samples (low and high) spanning the measurable range and preferably close to a medical decision level will each be tested in duplicate in two runs a day for 20 days for a total of 80 data points (CLSI EP5-A2). With-in run, between-run, total, mean, SD & calculated CV's will be determined using standard statistical analysis.

3. Specificity/Interference

Endogenous Interferences such as hemolysis, lipemia and icterus will be evaluated at high levels to quantify interference. One to three levels of the test analyte will be tested at levels close to concentrations where medical decision are made in replicate of five (5). For control the test analyte will be tested (in replicates of 5) without the interferent (CLSI EP7-A2). Non-interference is defined as bias of less than or equal to 10% of the results from samples with interferent compared to the mean results of the control samples.

4. Linearity/Assay Reportable Range

In order to determine the assay reportable range, a low concentration sample (ideally near and within the lower limit) and high concentration sample (the highest concentration tested) will be used to generate a minimum of 5- 8 levels equally spaced and including appropriate medical decision limits using defined ratios of high and low samples (CLSI EP6-A). Each level will be

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tested in triplicate. Criteria for allowable non-linearity (+/- 10%) and recovery (+/-10%) will be applied.

5. Method Comparison (venous)

A minimum of 40 clinical samples consisting of both clinical and spiked samples spanning the reportable assay range, including the medical decision levels will be tested and compared with a comparator method (CLSI EP09-A2). Duplicate measurement will be obtained for the comparator and the Theranos test methods. Standard statistical technique will be applied to determine method comparability including slope, intercept, range of values, correlation, estimation of standard error and bias. Based on the results of the data examined, either a simple linear regression or alternative procedure will be used to estimate expected (average) bias and the confidence interval of expected bias at the desired medical decision level (s) as per CLSI EP0A9-A2 guidance. The acceptable bias at each medical decision level will be determined based on the total allowable error (TEa) minus the measured precision at the level closest to that decision level for each assay. If the confidence interval for the predicted bias includes the defined acceptable bias or if the acceptable bias is greater than the higher limit of the confidence interval of the predicted bias, then the data do not show that the bias of the Theranos method is different from the acceptable bias or there is a high probability (97%) that the predicated bias is acceptable, respectively. Exclusion criteria for outliers will be applied if samples show mean absolute difference greater than 4 between the Theranos and comparator methods.

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1. **Albumin Assay**

Parameter	**Theranos Albumin**	**Siemens Advia 1800 Albumin**
Specimen Type	Human plasma	Human serum and plasma
Test Principle	The Theranos Albumin assay in plasma determines the amount of protein that is in the clear liquid portion of the blood. Plasma albumin quantitatively binds to bromocresol green (BCG) to form an albumin-BCG complex that is detected as an endpoint reaction spectrophotometrically.	Same
Intended Use	Quantitative determination of Albumin	Same
Controls	Bio-Rad or other commercial controls	Bio-Rad or other commercial controls
Reagent Storage	Reagent stored at 4^0 C	Reagent stored at 4^0 C
Testing Environment	Clinical Lab	Clinical Lab
Reaction Type	Endpoint	Endpoint
Detection Wavelength	620 nm	596/694 nm
Calibrators	Theranos General Chemistry calibrators	Siemens calibrators
Instrumentation	TSPU and TLAS	Advia 1800
User Complexity	Low	Moderate/Low

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2. **Total Bilirubin Assay**

Parameter	**Theranos Total Bilirubin**	**Siemens Advia 1800 Total Bilirubin**
Specimen Type	Human plasma	Human serum and plasma
Test Principle	Total (conjugated and unconjugated) bilirubin, in the presence of a solubilizing agent (here, dimetylsulfoxide), reacts with diazotized sulfanilic acid to produce azobilirubin, in the aqueous solution, a colored product. The intensity of this product is directly proportional to the amount of total bilirubin concentration present in the sample.	The bilirubin is oxidized by vanadate at about pH 2.9 to produce biliverdin. In the presence of the detergent and the vanadate, both conjugated (direct) and unconjugated bilirubin are oxidized. This oxidation reaction causes the decrease in the optical density of the yellow color,which is proportional to the total bilirubin concentration in the sample.
Intended Use	Quantitative determination of Bilirubin	Same
Controls	Bio-Rad or other commercial controls	Bio-Rad or other commercial controls
Reagent Storage	Reagent stored at 4^0 C	Reagent stored at 4^0 C
Testing Environment	Clinical Lab	Clinical Lab
Reaction Type	Endpoint	Endpoint
Detection Wavelength	560 nm	451/545 nm
Calibrators	Theranos General Chemistry calibrators	Siemens calibrators
Instrumentation	TSPU and TLAS	Advia 1800
User Complexity	Low	Moderate/Low

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3. **Direct Bilirubin Assay**

Parameter	**Theranos Direct Bilirubin**	**Siemens Advia 1800 Direct Bilirubin**
Specimen Type	Human plasma	Human serum and plasma
Test Principle	Bilirubin reacts with diazotized sulfanilic acid to produce azobilirubin. The intensity of the color produced is detected spectrophotometrically and is directly proportional to the amount of direct bilirubin concentration present in the sample.	The bilirubin is oxidized by vanadate at about pH 3 to produce biliverdin. In the presence of the detergent and the vanadate, both conjugated (direct) is oxidized. This oxidation reaction causes the decrease in the optical density of the yellow color, which is specific to bilirubin. The decrease in optical density is proportional to the total bilirubin concentration in the sample and detected as an endpoint reaction specrophotometrically.
Intended Use	Quantitative determination of Direct Bilirubin	Same
Controls	Bio-Rad or other commercial controls	Bio-Rad or other commercial controls
Reagent Storage	Reagent stored at 4^0 C	Reagent stored at 4^0 C
Testing Environment	Clinical Lab	Clinical Lab
Reaction Type	Endpoint	Endpoint
Detection Wavelength	560 nm	451/545 nm
Calibrators	Theranos General Chemistry calibrators	Siemens calibrators
Instrumentation	TSPU and TLAS	Advia 1800
User Complexity	Low	Moderate/Low

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4. **Calcium Assay**

Parameter	**Theranos Calcium**	**Siemens Advia 1800 Calcium**
Specimen Type	Human plasma	Human serum and plasma
Test Principle	Calcium reacts with cresolphthalein complexone in 8-hydroxyquinoline to form a colored complex (purple color) whose intensity is detected spectrophotometrically. The intensity of the color is proportional to the calcium concentration in the sample. Color intensifiers and a stabilizer are present to minimize interference by other metallic ions.	Calcium ions form a colored complex with Arsenazo III, which is detected spectrophotometrically. The amount of calcium present in the sample is directly proportional to the intensity of the colored complex formed.
Intended Use	Quantitative determination of Calcium	Same
Controls	Bio-Rad or other commercial controls	Bio-Rad or other commercial controls
Reagent Storage	Reagent stored at 4^0 C	Reagent stored at 4^0 C
Testing Environment	Clinical Lab	Clinical Lab
Reaction Type	Endpoint	Endpoint
Detection Wavelength	570 nm	658/694 nm
Calibrators	Theranos General Chemistry calibrators	Siemens calibrators
Instrumentation	TSPU and TLAS	Advia 1800
User Complexity	Low	Moderate/Low

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5. **Carbon Dioxide Assay**

Parameter	**Theranos Carbon Dioxide**	**Siemens Advia 1800 Carbon Dioxide**
Specimen Type	Human plasma	Human serum and plasma
Test Principle	The NADH-Based Bicarbonate Assay uses two enzymatic reactions to convert HCO3 to malate and β-NADH to β-NAD+ which can be detected spectrophotometrically as a decrease in signal over time with a rate that is dependent on the original HCO3 concentration.	PEPC catalyzes the first reaction involving HCO3 from the sample which generates oxaloacetate. In the presence of MDH, the NADH analog is oxidized by oxaloacetate to NAD+ analog. The oxidation of NADH analogue is detected by the decreased absorbance spectrophotometrically, which is proportional to the amount of CO2 in the sample.
Intended Use	Quantitative determination of Carbon dioxide/bicarbonate	Same
Controls	Bio-Rad or other commercial controls	Bio-Rad or other commercial controls
Reagent Storage	Reagent stored at 4^0 C	Reagent stored at 4^0 C
Testing Environment	Clinical Lab	Clinical Lab
Reaction Type	Kinetic	Kinetic
Detection Wavelength	340 nm	410/478 nm
Calibrators	Theranos General Chemistry calibrators	Siemens calibrators
Instrumentation	TSPU and TLAS	Advia 1800
User Complexity	Low	Moderate/Low

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6. **Chloride Assay**

Parameter	Theranos Chloride	Siemens Advia 1800 Chloride
Specimen Type	Human plasma	Human serum and plasma
Test Principle	Chloride from the sample replaces Hg from Hg^{2+} $(TPTZ)_2$ complex using Fe_2SO_4 from the reagent. The color intensity of resulting complex Fe^{+} $TPTZ^{-}$ is detected spectrophotometrically and is proportional to the chloride concentration in the sample.	The sample is mixed with ISE buffer, thereby providing a constant pH and a constant ionic strength solution. As the buffered sample is moved through the ISE, changes in the electrical potential take place. These electrical potential changes are detected against the potential of a reference electrode to derive the correct analog value for the sample.
Intended Use	Quantitative determination of Chloride	Same
Controls	Bio-Rad or other commercial controls	Bio-Rad or other commercial controls
Reagent Storage	Reagent stored at 4^0 C	Reagent stored at 4^0 C
Testing Environment	Clinical Lab	Clinical Lab
Reaction Type	Endpoint	Potentiometric
Detection Wavelength	600 nm	N/A
Calibrators	Theranos General Chemistry calibrators	Siemens calibrators
Instrumentation	TSPU and TLAS	Advia 1800
User Complexity	Low	Moderate/Low

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7. **Total Cholesterol Assay**

Parameter	**Theranos Total Cholesterol**	**Siemens Advia 1800 Total Cholesterol**
Specimen Type	Human plasma	Human serum and plasma
Test Principle	The cholesterol esters are hydrolyzed by cholesterol esterase to cholesterol and free fatty acids. The cholesterol is converted to choles-4-en-3-one by cholesterol oxidase in the presence of oxygen to form hydrogen peroxide. A colored complex is formed from hydrogen peroxide, 4-aminoantipyrine and phenol under the catalytic influence of peroxidase. The absorbance of the complex is detected as an endpoint reaction spectrophotometrically.	Same
Intended Use	Quantitative determination of Total Cholesterol	Same
Controls	Bio-Rad or other commercial controls	Bio-Rad or other commercial controls
Reagent Storage	Reagent stored at 4^0 C	Reagent stored at 4^0 C
Testing Environment	Clinical Lab	Clinical Lab
Reaction Type	Endpoint	Endpoint
Detection Wavelength	505/694 nm	505/694 nm
Calibrators	Theranos General Chemistry calibrators	Siemens calibrators
Instrumentation	TSPU and TLAS	Advia 1800
User Complexity	Low	Moderate/Low

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8. **Creatine Kinase Assay**

Parameter	Theranos Creatine Kinase	Siemens Advia 1800 Creatine Kinase
Specimen Type	Human plasma	Human serum and plasma
Test Principle	Creatine Kinase reacts with the creatine phosphate and ADP to form ATP which is coupled to the hexokinase-G6PD reaction, generating NADPH. The concentration of NADPH is detected spectrophotometrically.	Same
Intended Use	Quantitative determination of Creatine Kinase	Same
Controls	Bio-Rad or other commercial controls	Bio-Rad or other commercial controls
Reagent Storage	Reagent stored at 4^0 C	Reagent stored at 4^0 C
Testing Environment	Clinical Lab	Clinical Lab
Reaction Type	Kinetic	Kinetic
Detection Wavelength	340 nm	340/410 nm
Calibrators	Theranos General Chemistry calibrators	Siemens calibrators
Instrumentation	TSPU and TLAS	Advia 1800
User Complexity	Low	Moderate/Low

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9. **Creatinine Assay**

Parameter	**Theranos Creatinine**	**Siemens Advia 1800 Creatinine**
Specimen Type	Human plasma	Human serum and plasma
Test Principle	Creatinine is converted to creatine by the action of creatininase. The creatine formed is hydrolyzed by creatinase to produce sarcosine, which is decomposed by sarcosine oxidase to form glycine, formaldehyde, and hydrogen peroxide. In the presence of peroxidase, the hydrogen peroxide formed yields a blue pigment by quantitative oxidative condensation with HMMPS and 4-aminoantipyrine. The creatinine concentration is obtained by detecting the absorbance of the blue color spectrophotometrically.	Same
Intended Use	Quantitative determination of Creatinine	Same
Controls	Bio-Rad or other commercial controls	Bio-Rad or other commercial controls
Reagent Storage	Reagent stored at 4^0 C	Reagent stored at 4^0 C
Testing Environment	Clinical Lab	Clinical Lab
Reaction Type	Kinetic	Endpoint
Detection Wavelength	561 nm	596/694 nm
Calibrators	Theranos General Chemistry calibrators	Siemens calibrators
Instrumentation	TSPU and TLAS	Advia 1800
User Complexity	Low	Moderate/Low

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10. **Glucose Assay**

Parameter	**Theranos Glucose**	**Siemens Advia 1800 Glucose**
Specimen Type	Human plasma	Human serum and plasma
Test Principle	β-D-Glucose is oxidized by glucose oxidase to produce D-gluconic acid and hydrogen peroxide. The hydrogen peroxide is then oxidatively coupled with 4-aminoantipyrine and phenol substitute, pHBA, in the presence of peroxidase to yield a red quinoneimine dye. The amount of colored complex formed is proportional to glucose concentration and can be detected spectrophotometrically.	Glucose is phosphorylated by adenosine triphosphate (ATP) in the presence of hexokinase. The glucose-6-phosphate that forms is oxidized in the presence of glucose-6-phosphate dehydrogenase causing reduction of NAD to NADH. The absorbance of NADH is detected as an endpoint reaction spectrophotometrically.
Intended Use	Quantitative determination of Glucose	Same
Controls	Bio-Rad or other commercial controls	Bio-Rad or other commercial controls
Reagent Storage	Reagent stored at 4^0 C	Reagent stored at 4^0 C
Testing Environment	Clinical Lab	Clinical Lab
Reaction Type	Endpoint	Endpoint
Detection Wavelength	510 nm	340/410 nm
Calibrators	Theranos General Chemistry calibrators	Siemens calibrators
Instrumentation	TSPU and TLAS	Advia 1800
User Complexity	Low	Moderate/Low

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11. **Gamma-glutamyl transpeptidase (GGT) Assay**

Parameter	**Theranos GGT**	**Siemens Advia 1800 GGT**
Specimen Type	Human plasma	Human serum and plasma
Test Principle	In the reaction with the synthetic substrate (L-γ-glutamyl-p -nitroanilide), glycylglycine acts as an acceptor for the γ-glutamyl residue and p-nitroaniline is liberated. The color intensity of liberated product is detected spectrophotometrically.	Same
Intended Use	Quantitative determination of GGT	Same
Controls	Bio-Rad or other commercial controls	Bio-Rad or other commercial controls
Reagent Storage	Reagent stored at 4^0 C	Reagent stored at 4^0 C
Testing Environment	Clinical Lab	Clinical Lab
Reaction Type	Kinetic	Kinetic
Detection Wavelength	405 nm	410/478 nm
Calibrators	Theranos General Chemistry calibrators	Siemens calibrators
Instrumentation	TSPU and TLAS	Advia 1800
User Complexity	Low	Moderate/Low

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12. **Iron (non-heme) Assay**

Parameter	**Theranos Iron**	**Siemens Advia 1800 Iron**
Specimen Type	Human plasma	Human serum and plasma
Test Principle	Iron in human plasma is released from its carrier protein, transferrin, in an acid medium and simultaneously reduced to the ferrous form by a reducing agent. The ferrous ions chelate with the Ferene-S, a sensitive iron indicator forming a stable blue complex whose absorbance, spectrophotometrically detected, is proportional to the iron content.	Ferric iron is dissociated from its carrier protein, transferrin, in an acid medium and simultaneously reduced to the ferrous form. The ferrous iron is complexed with ferrozine, a sensitive iron indicator, to produce a colored chromophore, whose absorbance is read spectrophotometrically.
Intended Use	Quantitative determination of Iron	Same
Controls	Bio-Rad or other commercial controls	Bio-Rad or other commercial controls
Reagent Storage	Reagent stored at 4^0 C	Reagent stored at 4^0 C
Testing Environment	Clinical Lab	Clinical Lab
Reaction Type	Endpoint	Endpoint
Detection Wavelength	590 nm	571/658 nm
Calibrators	Theranos General Chemistry calibrators	Siemens calibrators
Instrumentation	TSPU and TLAS	Advia 1800
User Complexity	Low	Moderate/Low

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13. **Low-density lipoprotein (LDL) Assay**

Parameter	**Theranos LDL**	**Siemens Advia 1800 LDL**
Specimen Type	Human plasma	Human serum and plasma
Test Principle	Cholesterol esterase and cholesterol oxidase eliminate cholesterol, other than from low density lipoprotein. The action of catalase removes the peroxide produced by the oxidase. Specific detection of LDL Cholesterol is made after its release by detergent. The intensity of the quinoneimine produced in the Trinder reaction is directly proportional to the cholesterol concentration when detected spectrophotometrically.	Same
Intended Use	Quantitative determination of LDL	Same
Controls	Bio-Rad or other commercial controls	Bio-Rad or other commercial controls
Reagent Storage	Reagent stored at 4^0 C	Reagent stored at 4^0 C
Testing Environment	Clinical Lab	Clinical Lab
Reaction Type	Endpoint	Endpoint
Detection Wavelength	561 / 694 nm	596/694 nm
Calibrators	Theranos General Chemistry calibrators	Siemens calibrators
Instrumentation	TSPU and TLAS	Advia 1800
User Complexity	Low	Moderate/Low

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14. **High-density lipoprotein (HDL) Assay**

Parameter	**Theranos HDL**	**Siemens Advia 1800 HDL**
Specimen Type	Human plasma	Human serum and plasma
Test Principle	Elimination of chylomicrons, VLDL-Cholesterol, and LDL-Cholesterol takes place by PEG-Cholesterol esterase and cholesterol oxidase. The peroxide produced by the oxidase is removed by catalase. Specific detection of HDL-Cholesterol is made after release of HDL-Cholesterol by surfactant. The intensity of the quinoneimine dye produced in the Trinder reaction is directly proportional to the HDL cholesterol concentration when detected spectrophotometrically.	Same
Intended Use	Quantitative determination of HDL	Same
Controls	Bio-Rad or other commercial controls	Bio-Rad or other commercial controls
Reagent Storage	Reagent stored at 4^0 C	Reagent stored at 4^0 C
Testing Environment	Clinical Lab	Clinical Lab
Reaction Type	Endpoint	2-Point Kinetic
Detection Wavelength	561/ 694 nm	596/694 nm
Calibrators	Theranos General Chemistry calibrators	Siemens calibrators
Instrumentation	TSPU and TLAS	Advia 1800
User Complexity	Low	Moderate/Low

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15. **Magnesium Assay**

Parameter	Theranos Magnesium	Siemens Advia 1800 Magnesium
Specimen Type	Human plasma	Human serum and plasma
Test Principle	Under basic conditions, deprotonated Xylidyl Blue selectively complexes with Mg^{2+}, shifting the color of the chromophore from deep purple to bright pink. The concentration of Magnesium can be determined by detecting the absorbance of resulting color complex.	Magnesium ions react with Xylidyl blue in an alkaline medium to form a water-soluble purple-red complex. The increase in absorbance is proportional to the concentration of magnesium in the sample.
Intended Use	Quantitative determination of Magnesium	Same
Controls	Bio-Rad or other commercial controls	Bio-Rad or other commercial controls
Reagent Storage	Reagent stored at 4^0 C	Reagent stored at 4^0 C
Testing Environment	Clinical Lab	Clinical Lab
Reaction Type	Endpoint	Endpoint
Detection Wavelength	597 nm	505/694 nm
Calibrators	Theranos General Chemistry calibrators	Siemens calibrators
Instrumentation	TSPU and TLAS	Advia 1800
User Complexity	Low	Moderate/Low

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16. **Alkaline Phosphatase (ALP) Assay**

Parameter	**Theranos Alkaline Phosphatase**	**Siemens Advia 1800 Alkaline Phosphatase**
Specimen Type	Human plasma	Human serum and plasma
Test Principle	This assay uses the enzyme activity of alkaline phosphatase to dephosphorylate the chemiluminescent alkaline phosphatase substrate present in the assay reagent into an unstable dioxetane anion which decomposes and emits light. Charge transfer from the phenolate to the dioxetane ring promotes cleavage of the cyclic peroxide, releasing about 100 kcal to chemically excite one of the resulting carbonyl fragments to a singlet electronic state. Light emission obtained from the AP-catalyzed dioxetane decomposition reaction is a steady-state glow, the intensity of which is detected using a photodiode-based luminometer.	Alkaline phosphatase hydrolyzes the p-nitrophenyl substrate to form p-nitrophenol. The reaction is followed by the colorimetric detection of the rate of formation of the p-nitrophenol, which is proportional to the alkaline phosphatase activity.
Intended Use	Quantitative determination of ALP	Same
Controls	Bio-Rad or other commercial controls	Bio-Rad or other commercial controls
Reagent Storage	Reagent stored at 4^0 C	Reagent stored at 4^0 C
Testing Environment	Clinical Lab	Clinical Lab
Reaction Type	Kinetic	Kinetic
Detection Wavelength	Chemiluminescence	410/478 nm
Calibrators	Theranos General Chemistry calibrators	Siemens calibrators
Instrumentation	TSPU and TLAS	Advia 1800
User Complexity	Low	Moderate/Low

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17. **Phosphorus Assay**

Parameter	Theranos Phosphorus	Siemens Advia 1800 Phosphorus
Specimen Type	Human plasma	Human serum and plasma
Test Principle	The Theranos phosphorus assay utilizes an improved malachite green method that uses malachite green dye and molybdate, which forms a stable colored complex with inorganic phosphate. The resulting phosphate-molybdate-malachite green complex gives a color in the visible range, which can be detected spectrophotometrically.	Inorganic phosphorus reacts with ammonium molybdate in the presence of sulfuric acid to form an unreduced phosphomolybdate complex, which is detected spectrophotometrically as an endpoint reaction.
Intended Use	Quantitative determination of Phosphorus	Same
Controls	Bio-Rad or other commercial controls	Bio-Rad or other commercial controls
Reagent Storage	Reagent stored at 4^0 C	Reagent stored at 4^0 C
Testing Environment	Clinical Lab	Clinical Lab
Reaction Type	Endpoint	Endpoint
Detection Wavelength	630 nm	340/658 nm
Calibrators	Theranos General Chemistry calibrators	Siemens calibrators
Instrumentation	TSPU and TLAS	Advia 1800
User Complexity	Low	Moderate/Low

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18. **Potassium Assay**

Parameter	Theranos Potassium	Siemens Advia 1800 Potassium
Specimen Type	Human plasma	Human serum and plasma
Test Principle	In this method the compound Sodium Tetraphenylborate reacts with potassium from plasma. K^+ replaces Na^+ from Sodium Tetraphenylborate (NaTPB) to form potassium tetraphenylborate, a white precipitate. The turbidity caused by the precipitate is detected spectrophotometrically.	The sample is mixed with ISE buffer, thereby providing a constant pH and a constant ionic strength solution. As the buffered sample is moved through the ion selective electrode, changes in the electrical potential are measured against the potential of a reference electrode to derive the correct analog value for that sample.
Intended Use	Quantitative determination of Potassium	Same
Controls	Bio-Rad or other commercial controls	Bio-Rad or other commercial controls
Reagent Storage	Reagent stored at 4^0 C	Reagent stored at 4^0 C
Testing Environment	Clinical Lab	Clinical Lab
Reaction Type	Endpoint	Potentiometric
Detection Wavelength	450 nm	N/A
Calibrators	Theranos General Chemistry calibrators	Siemens calibrators
Instrumentation	TSPU and TLAS	Advia 1800
User Complexity	Low	Moderate/Low

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19. **Total Protein Assay**

Parameter	**Theranos Total Protein**	**Siemens Advia 1800 Total Protein**
Specimen Type	Human plasma	Human serum and plasma
Test Principle	Protein peptide bonds interact with the cupric ions to form a purple complex that is detected spectrophotometrically to quantitatively detect Total Protein in human plasma.	Protein peptide bonds interact with the cupric ions to form a purple complex that is detected spectrophotometrically to quantitatively detect Total Protein in human plasma.
Intended Use	Quantitative determination of Total Protein	Same
Controls	Bio-Rad or other commercial controls	Bio-Rad or other commercial controls
Reagent Storage	Reagent stored at 4^0 C	Reagent stored at 4^0 C
Testing Environment	Clinical Lab	Clinical Lab
Reaction Type	Endpoint	Endpoint
Detection Wavelength	540 nm	545 nm
Calibrators	Theranos General Chemistry calibrators	Siemens calibrators
Instrumentation	TSPU and TLAS	Advia 1800
User Complexity	Low	Moderate/Low

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20. **Sodium Assay**

Parameter	Theranos Sodium	Siemens Advia 1800 Sodium
Specimen Type	Human plasma	Human serum and plasma
Test Principle	In the Theranos Sodium colorimetric assay, sodium is determined enzymatically using the sodium dependent enzyme β-galactosidase which cleaves the substrate o-nitrophenyl-β-D-galactopyranose, yielding the product o-nitrophenol, a dye of which color intensity is detected spectrophotometrically.	The sample is mixed with ISE buffer, thereby providing a constant pH and a constant ionic strength solution. As the buffered sample is moved through the ion selective electrode, changes in the electrical potential are measured against the potential of a reference electrode in order to derive the correct analog value for that sample.
Intended Use	Quantitative determination of Sodium	Same
Controls	Bio-Rad or other commercial controls	Bio-Rad or other commercial controls
Reagent Storage	Reagent stored at 4^0 C	Reagent stored at 4^0 C
Testing Environment	Clinical Lab	Clinical Lab
Reaction Type	Kinetic	Potentiometric
Detection Wavelength	405/420 nm	N/A
Calibrators	Theranos General Chemistry calibrators	Siemens calibrators
Instrumentation	TSPU and TLAS	Advia 1800
User Complexity	Low	Moderate/Low

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21. **Aspartate amino transferase (AST/SGOT) Assay**

Parameter	**Theranos AST**	**Siemens Advia 1800 AST**
Specimen Type	Human plasma	Human serum and plasma
Test Principle	In the presence of L-aspartate and α-ketoglutarate, AST generates L-glutamate and oxaloacetate. The latter is decarboxylated by malate dehydrogenase coupled with the oxidation of NADH. The Theranos aspartate amino transferase assay utilizes a kinetic colorimetric method. The rate of NADH consumption detected is proportional to the activity of AST in the sample.	Same
Intended Use	Quantitative determination of AST	Same
Controls	Bio-Rad or other commercial controls	Bio-Rad or other commercial controls
Reagent Storage	Reagent stored at 4^0 C	Reagent stored at 4^0 C
Testing Environment	Clinical Lab	Clinical Lab
Reaction Type	Endpoint	Kinetic
Detection Wavelength	340 nm	340/410 nm
Calibrators	Theranos General Chemistry calibrators	Siemens calibrators
Instrumentation	TSPU and TLAS	Advia 1800
User Complexity	Low	Moderate/Low

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22. **Alanine amino transferase (ALT/SGPT) Assay**

Parameter	**Theranos ALT**	**Siemens Advia 1800 ALT**
Specimen Type	Human plasma	Human serum and plasma
Test Principle	ALT catalyzes the transfer of an amino group between L-alanine and α-ketoglutarate (α-KG). The pyruvate formed in the first reaction is then phosphorylated by pyruvate oxidase (POPG) in the presence of oxygen and water, forming peroxide in the process. ALT activity is determined by detecting the absorbance of the color generated, at 561 nm when the perodide forms a quinoneimine dye in the presence of 4-aminoantipyrine (4-AA) and ALPS.	ALT catalyzes the transfer of an amino group between L-alanine and α-ketoglutarate (α-KG). The pyruvate formed in the first reaction converts NADH to NAD in presence of lactate dehydrogenase. Concentration of the NADH is detected spectrophotometrically as the rate of absorbance decrease which proportional to the alanine aminotransferase (ALT) activity.
Intended Use	Quantitative determination of ALT	Same
Controls	Bio-Rad or other commercial controls	Bio-Rad or other commercial controls
Reagent Storage	Reagent stored at 4^0 C	Reagent stored at 4^0 C
Testing Environment	Clinical Lab	Clinical Lab
Reaction Type	Endpoint	Kinetic (rate)
Detection Wavelength	561nm	340/410 nm
Calibrators	Theranos General Chemistry calibrators	Siemens calibrators
Instrumentation	TSPU and TLAS	Advia 1800
User Complexity	Low	Moderate/Low

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23. **Triglycerides Assay**

Parameter	**Theranos Triglycerides**	**Siemens Advia 1800 Triglycerides**
Specimen Type	Human plasma	Human serum and plasma
Test Principle	This assay uses an enzyme reaction cascade with results in the production of hydrogen peroxide (H_2O_2) which is used by Horse Radish Peroxidase (HRP) to produce a purple color product. The absorbance of the complex is detected as an endpoint reaction spectrophotometrically.	The triglycerides are converted to glycerol and free fatty acids by lipase. The glycerol is then converted to glycerol-3-phosphate by glycerol kinase followed by its conversion to glycerol-3-phosphate-oxidase to hydrogen peroxide. A colored complex is formed from hydrogen peroxide, 4-aminophenazone and 4-chlorophenol under the catalytic influence of peroxidase. The absorbance of the complex is detected as an endpoint reaction spectrophotometrically.
Intended Use	Quantitative determination of triglycerides	Same
Controls	Bio-Rad or other commercial controls	Bio-Rad or other commercial controls
Reagent Storage	Reagent stored at 4^0 C	Reagent stored at 4^0 C
Testing Environment	Clinical Lab	Clinical Lab
Reaction Type	Kinetic	Endpoint
Detection Wavelength	561 nm	505/694 nm
Calibrators	Theranos General Chemistry calibrators	Siemens calibrators
Instrumentation	TSPU and TLAS	Advia 1800
User Complexity	Low	Moderate/Low

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24. **Urea Nitrogen (BUN) Assay**

Parameter	**Theranos BUN**	**Siemens Advia 1800 BUN**
Specimen Type	Human plasma	Human serum and plasma
Test Principle	The Theranos blood urea nitrogen assay utilizes a colorimetric endpoint method. The colorimetric signal is a result of the urease-mediated conversion of urea to ammonia, and the subsequent oxidative formation of 2,2-dicarboxyindophenol.	Urea is hydrolyzed in the presence of water and urease to produce ammonia and carbon dioxide. The ammonia reacts with 2-oxoglutarate in the presence of glutamate dehydrogenase and NADH. The oxidation of NADH to NAD is detected as an inverse rate reaction.
Intended Use	Quantitative determination of BUN	Same
Controls	Bio-Rad or other commercial controls	Bio-Rad or other commercial controls
Reagent Storage	Reagent stored at 4^0 C	Reagent stored at 4^0 C
Testing Environment	Clinical Lab	Clinical Lab
Reaction Type	Endpoint	Kinetic (rate)
Detection Wavelength	630 nm	340/410 nm
Calibrators	Theranos General Chemistry calibrators	Siemens calibrators
Instrumentation	TSPU and TLAS	Advia 1800
User Complexity	Low	Moderate/Low

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25. **Uric Acid**

Parameter	**Theranos Uric Acid**	**Siemens Advia 1800 Uric Acid**
Specimen Type	Human plasma	Human serum and plasma
Test Principle	Theranos uric acid assay chemistry is based on the iron reduction method. For this method, ferric iron (iron III) is reduced to ferrous iron (iron II) which then reacts with TPTZ to form a colored product that can be detected spectrophotometrically. The level of the resulting complex is directly proportional to the uric acid level of the sample.	The Uric Acid is converted by uricase to allantoin and hydrogen peroxide. A colored complex is formed from hydrogen peroxide, 4-aminophenazone, and TOOS [N-ethyl-N-(2-hydroxy-3-sulfopropyl)-3-methylaniline] under catalytic influence of peroxidase. The absorbance of the complex is detected spectrophotometrically. The level of the resulting complex is directly proportional to the uric acid level of the sample.
Intended Use	Quantitative determination of Uric Acid	Same
Controls	Bio-Rad or other commercial controls	Bio-Rad or other commercial controls
Reagent Storage	Reagent stored at 4^0 C	Reagent stored at 4^0 C
Testing Environment	Clinical Lab	Clinical Lab
Reaction Type	Endpoint	Endpoint
Detection Wavelength	590 nm	545/694 nm
Calibrators	Theranos General Chemistry calibrators	Siemens calibrators
Instrumentation	TSPU and TLAS	Advia 1800
User Complexity	Low	Moderate/Low

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Specimen Information

Specimen Information for TNAA:

The TNAA assays are performed on samples collected in the form of throat swabs (“***TS***”) or sputum samples from individuals suspected of suffering from a lower respiratory infection. To collect a sample from a throat swab that has been applied to a subject’s throat passage, the throat swab 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. Sputum samples are collected in sputum collection vessels. The sputum collection vessel is inserted into the Cartridge for subsequent processing. Samples will typically be processed and analyzed within several hours of their acquisition; thus, fresh samples will typically be used.

Specimen Information for General Chemistries

General Chemistry assays will be performed on human plasma. Whole blood collected by fingerstick or venipuncture will be introduced to the TSPU in the cartridge for General Chemistry testing. The TSPU separates the plasma from the cells for subsequent assay testing. Samples will be processed and analyzed within several hours of their collection.

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Clinical Performance and Study Design Elements
Prospective Clinical Study for the Lower Respiratory TNAA Assays

Background and Study Goals:

The primary goal of this prospective study is to supplement existing clinical data to characterize the accuracy of the TNAA assays run on the Theranos SPU and TLAS for the diagnosis of lower respiratory tract infections in patients suspected of infection. The General Chemistry assays serve an important role in clinical assessment of these patients, as well as in the assessment of other patient types exhibiting other signs and symptoms. Enabling highly sensitive TNAA tests with concurrent General Chemistry testing will help aid in the rapid clinical assessment and diagnosis of these patients.

The traditional testing approaches for lower respiratory tract infection include culturing, followed in some cases by confirmatory/subtyping via PCR testing. However, such testing approaches are typically expensive with slow turnaround times, leading to delays in treatment and possibly inappropriate treatment due to delays in test reporting.

The Theranos SPU and TLAS enable more convenient and rapid testing for both highly sensitive and specific TNAA tests as well as General Chemistry, hematology, and serological testing. This comprehensive approach yields very high accuracy (sensitivity and specificity), overall physiologic conditions, 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. It can also facilitate assessment of infectivity. Simultaneous testing in two or more modalities, such as gene-based TNAA testing, general chemistry, hematology, and immune/serologic testing, provides orthogonal tests which should enhance overall performance and clinical decision making. For example, early infections may be detected by TNAA methods but not by serologic tests. Conversely, poor recovery of sputum or low viral loads in lower respiratory samples could result in false negative results by nucleic acid testing alone, while blood based testing may still reveal medical conditions that need urgent care.

The Theranos SPU and TLAS enable CLIA oversight of sample processing with subsequent analysis in and under the oversight of the Theranos CLIA laboratory. This approach has clear advantages compared to traditional error-prone practices that include manual sample preparation with minimal oversight and shipping of samples to laboratories for analysis.

Throat swabs or sputum specimens are commonly used for the detection of lower respiratory tract infections. The relative ease of such sample collection should enable increased patient participation and help improve overall healthcare response and surveillance.

Study Plan:

The clinical performance of the Theranos SPU will be evaluated in a prospective study. Throat swab and sputum samples will be collected from subjects exhibiting symptoms of lower respiratory tract infection at 3 or more U.S. sites. The samples will be collected by trained technicians and

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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 comparator 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 500 patients, for a total of 1,500 subjects. Subjects will be screened for exhibiting signs and symptoms of lower respiratory infection including but not limited to shortness of breath, weakness, high fever, coughing and fatigue. The subjects may or may not have had lower respiratory tract infection testing ordered by a physician. 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; and 3) current medications (self-reported and/or collected from medical records).

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.

Two sample types (throat swab or sputum samples) will be collected across 1500 of the subjects. When collected, two throat swabs will be collected from each subject. One swab will be used for testing on the Theranos System, and the second swab will be used for testing on the comparator method. As there are no cleared comparator methods available for these TNAA tests, two Theranos in-house PCR tests with bi-directional sequence confirmation will be used. A predetermine algorithm will be 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 are considered as any sample that tested negative by both of the comparator PCR assays. Any conflicting results will be further investigated by culture and sequencing.

Statistical Analysis Plan for Clinical Performance Study

The main outcome of this study will be the determination of the diagnostic accuracy of the TNAA lower respiratory tract tests. To this end, 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 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. Comparator outcomes will serve as the presumed truth. Each discrepant classification will be investigated and resolved (included by culture).

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As described, a total of 1500 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. 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.

Clinical Study for the General Chemistry Assays

The predicate test system (the Advia 1800) and the corresponding cleared assays did not require clinical data according to the published Decision Summaries. In the course of developing its Laboratory Developed Tests (LDTs), including the General Chemistry assays, Theranos has generated data from its development, validation, and various Theranos clinical programs relating to these LDTs. Given that the similar type of data that were the basis for clearance for the proposed predicate device, Theranos is not planning any additional clinical study for its General Chemistry Assays.

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Previous Discussions or Submissions

In October 2012, Elizabeth Holmes (Theranos' CEO) and Sunny Balwani (Theranos' President and 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' President and COO) and legal counsel for Theranos attended the Informational Meeting (Q131148) with

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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' President and 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. FDA confirmed that these minutes are final.

On November 30, 2013, Theranos submitted a Pre-Submission Meeting Request (Q131542) for Viral Versus Bacterial TNAA Assays and Cytometry Assays. This meeting has not yet been scheduled.

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Specific Questions

1. As discussed in our prior Pre-Submissions (Q131199 and Q131542), 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 for the Lower Respiratory TNAA assays included in Appendix B) would likely be sufficient for successful 510(k) submissions.

2. We would like to get FDA's guidance as to whether analytical and pre-clinical data will be adequate to support a successful 510(k) submission for the Theranos General Chemistry assays without additional clinical trial of prospective or retrospective clinical samples given that the predicate test system (the Advia 1800) and the corresponding cleared assays did not require such clinical data according to the published Decision Summaries.

3. We would like to get FDA's guidance as to whether the proposed study in the "*Clinical Performance and Study Design Elements*" Section of this Pre-Submission adequately addresses the Intended Use objectives for the Lower Respiratory TNAA assays and FDA's requirements for successful and expeditious 510(k) clearance.

4. As pointed out in the section on the anticipated predicate device for the TNAA assays, the Theranos System, including the TSPU and TLAS, will have been cleared for running the influenza TNAA assays pursuant to the 510(k) submission corresponding to the Pre-Submission Q131199 and Theranos plans to use that Theranos System as the predicate for the Lower Respiratory Assays with the Theranos in-house PCR tests as comparators. We would like to confirm with FDA that this is the best approach for obtaining 510(k) clearance of the Theranos Lower Respiratory TNAA assays included in this Pre-Submission.

5. We anticipate that there will low prevalence for some of the Lower Respiratory pathogens for the TNAA assays 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.

6. We anticipate that for at least one of the Lower Respiratory pathogens for the TNAA assays (e.g., Vancomycin-resistant Staphylococcus aureus), there will likely be no prevalence in the prospective clinical study and would like FDA's feedback on the best approach clinical validation of the assay for such pathogens.

7. 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 General Chemistry 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 General Chemistry assays?

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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.

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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 Clinical Chemistry will be reviewing information related to the Theranos system and assays for the first time.

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References

Clinical Laboratory Standards Institute (CLSI), MM3-A2, Molecular Diagnostic Methods for Infectious Diseases; Approved Guideline; Second Edition

CLSI, EP24-A2, Assessment of the Diagnostic Accuracy of Laboratory Tests Using Receiver Operating Characteristic Curves; Approved Guideline; Second Edition

CLSI, EP09-A3E, Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline; Third Edition

CLSI, EP12-A2, User Protocol for Evaluation of Qualitative Test Performance; Approved Guideline; Second Edition

CLSI, I/LA18-A2, Specifications for Immunological Testing for Infectious Diseases; Approved Guideline; Second Edition

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TS-0536238

Appendix A -- General Chemistry Assay Details

	A	B	C
1	**Assay**	**Dilution**	**Sample**
2	Albumin	30	Plasma*
3	Total Bilirubin	15	Plasma
4	Direct Bilirubin	15	Plasma
5	Calcium	15	Plasma
6	Carbon dioxide	50	Plasma
7	Chloride	300	Plasma
8	Cholesterol (Total)	30	Plasma
9	Creatine Kinase	15	Plasma
10	Creatinine	3	Plasma
11	Glucose	30	Plasma
12	GGT (Gamma Glutamyltranspeptidase)	15	Plasma
13	Total Iron	6	Plasma
14	LDL-Cholesterol	30	Plasma
15	HDL-Cholesterol	30	Plasma
16	Magnesium	15	Plasma
17	ALP	30	Plasma
18	Phosphorus	30	Plasma
19	Potassium	15	Plasma
20	Total Protein	30	Plasma
21	Sodium	15	Plasma
22	AST	15	Plasma
23	ALT	15	Plasma
24	Triglycerides	30	Plasma
25	Urea Nitrogen	100	Plasma
26	Uric Acid	30	Plasma
27			
28			*Sample for the General Chemistry assays is plasma collected by a fingerstick as whole blood (processed in the TSPU into plasma)

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	D	E	F
1	**Anticoagulant (plasma/whole blood)**	**Diluent**	**Type**
2	edta	water	endpoint
3	edta	water	endpoint
4	edta	water	endpoint
5	heparin	water	endpoint
6	heparin	water	kinetic
7	Heparin	water	endpoint
8	edta	0.9% Saline	endpoint
9	Heparin	water	kinetic
10	edta	water	kinetic
11	heparin	water	endpoint
12	Heparin	water	kinetic
13	Serum	water	endpoint
14	edta	0.9% Saline	endpoint
15	edta	0.9% Saline	endpoint
16	Heparin	water	endpoint
17	heparin	water	endpoint
18	heparin	water	Endpoint
19	heparin	water	endpoint
20	edta	water	endpoint
21	Heparin	water	kinetic
22	Heparin	water	kinetic
23	edta	water	endpoint
24	Heparin	water	endpoint
25	edta	water	endpoint
26	Heparin	water	endpoint
27			
28			

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	G	H	I
1	**Num_Reagents**	**Diluted Sample Volume**	**Reagent 1 Volume**
2	1	10	20
3	2	15	10
4	2	15	10
5	2	10	10
6	1	10	20
7	1	10	20
8	1	10	20
9	1	15	15
10	2	10	10
11	2	10	10
12	1	15	15
13	2	10	10
14	2	10	10
15	2	10	10
16	2	10	10
17	2	10	10
18	1	10	20
19	1	15	15
20	1	10	20
21	2	10	10
22	1	15	15
23	3	7.5	7.5
24	2	10	10
25	2	10	10
26	2	10	10
27			
28			

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	J	K	L
1	**Reagent 1 Mix**	**Reagent 2 Volume**	**Reagent 2 Mix**
2	yes	NA	
3	yes	5	yes
4	yes	5	yes
5	yes	10	yes
6	yes	NA	
7	Yes	NA	
8	yes	NA	
9	Yes	NA	
10	yes	10	yes
11	no	10	yes
12	Yes	N/A	N/A
13	Yes	10	Yes
14	Yes	10	Yes
15	Yes	10	Yes
16	No	10	Yes
17	No	10	Yes
18	yes	NA	
19	yes	NA	
20	yes	NA	
21	no	10	yes
22	Yes	NA	
23	Yes	7.5	Yes
24	no	10	yes
25	No	10	Yes
26	no	10	yes
27			
28			

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	M	N	O
1	**Reagent 3 Volume**	**Reagent 3 Mix**	**Incubation 1**
2	NA		10
3	NA		0
4	NA		0
5	NA		0
6	NA		0
7	NA		10
8	NA		10
9	NA		0
10	NA		5
11	NA		0
12	N/A		0
13	N/A		10
14	NA		5
15	NA		5
16	NA		0
17	NA		0
18	NA		20
19	NA		10
20	NA		10
21	NA		0
22	NA		0
23	7.5	Yes	10
24	NA		0
25	NA		5
26	NA		0
27			
28			

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	P	Q	R
1	**Incubation 2**	**Incubation 3**	**Kinetic Detection Interval**
2	NA	NA	
3	5	NA	
4	5	NA	NA
5	5	NA	
6	NA	NA	5
7	NA	NA	
8	NA	NA	
9	NA	NA	15
10	0	NA	15
11	10	NA	
12	NA	NA	5
13	10	N/A	N/A
14	5	NA	
15	10	NA	
16	1	NA	
17	10	NA	
18	NA	NA	
19	NA	NA	
20	NA	NA	
21	0	NA	9
22	NA	NA	10
23	5	0	
24	10	NA	
25	5	NA	
26	10	NA	NA
27			
28			

	S	T	U
1	**Reagent addition scheme**	**Detector**	**Wavelength**
2	Add reagents one at a time	Detector 4	620
3	(reag_1 + reag_2)	Detector 4	560
4	(reag_1 + reag_2)	Detector 4	560
5	Add reagents one at a time	Detector 4	570
6	Add reagents one at a time	Detector 4	340
7	Add reagents one at a time	Detector 4	600
8	Add reagents one at a time	Detector 4	500-700
9	Add reagents one at a time	Detector 4	340
10	Add reagents one at a time	Detector 4	340
11	Add reagents one at a time	Detector 4	510
12	Add reagents one at a time	Detector 4	340
13	Add reagents one at a time	Detector 4	590
14	Add reagents one at a time	Detector 4	560-700
15	Add reagents one at a time	Detector 4	560-700
16	Add reagents one at a time	Detector 4	582
17	Add reagents one at a time	Detector 1	NA
18	Add reagents one at a time	Detector 4	630
19	Add reagents one at a time	Detector 4	450
20	Add reagents one at a time	Detector 4	540
21	Add reagents one at a time	Detector 4	420
22	Add reagents one at a time	Detector 4	340
23	Add reagents one at a time	Detector 4	561
24	Add reagents one at a time	Detector 4	560
25	Add reagents one at a time	Detector 4	630
26	Add reagents one at a time	Detector 4	590
27			
28			

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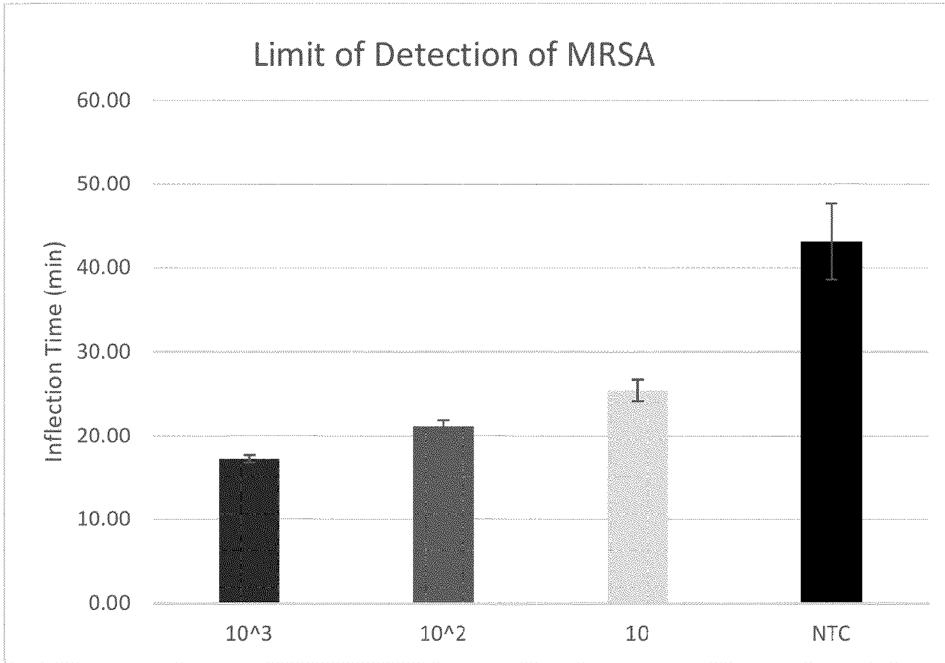
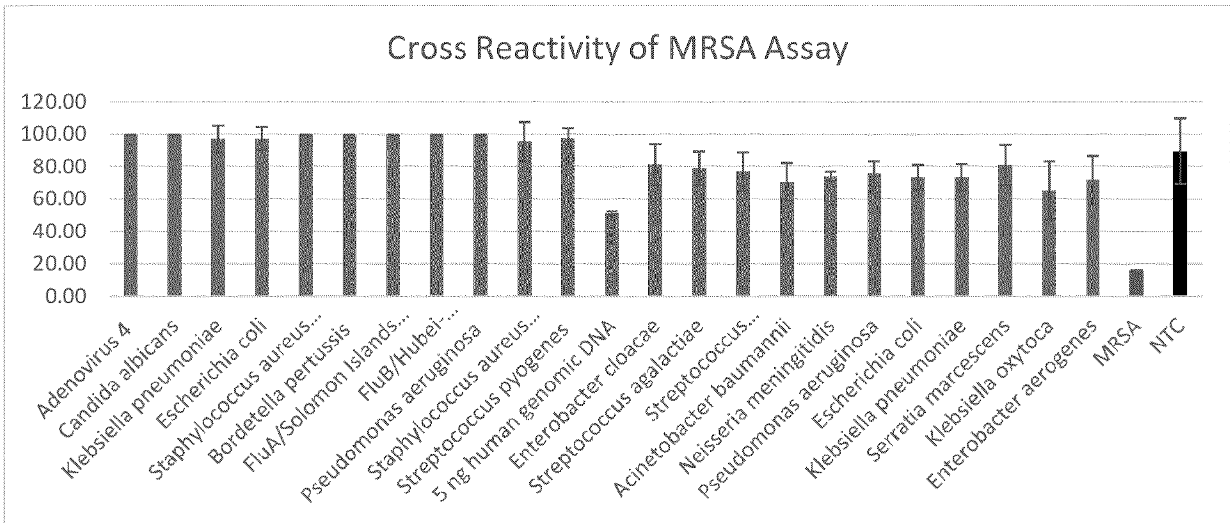
Appendix B: Representative Data for TNAA Assays

Methicillin-resistant Staphylococcus aureus





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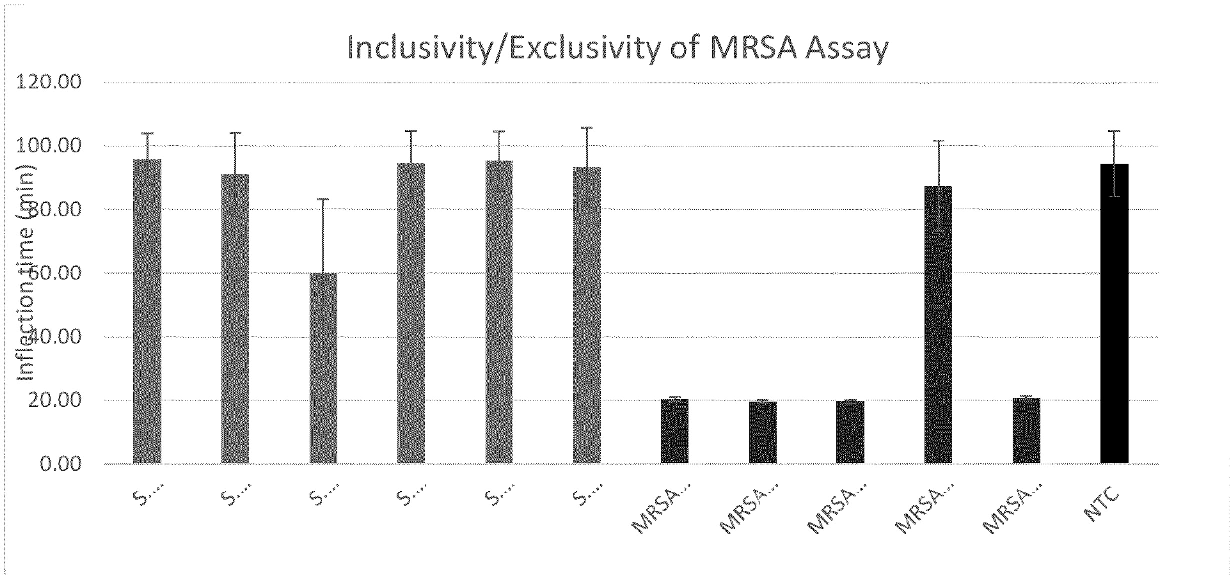

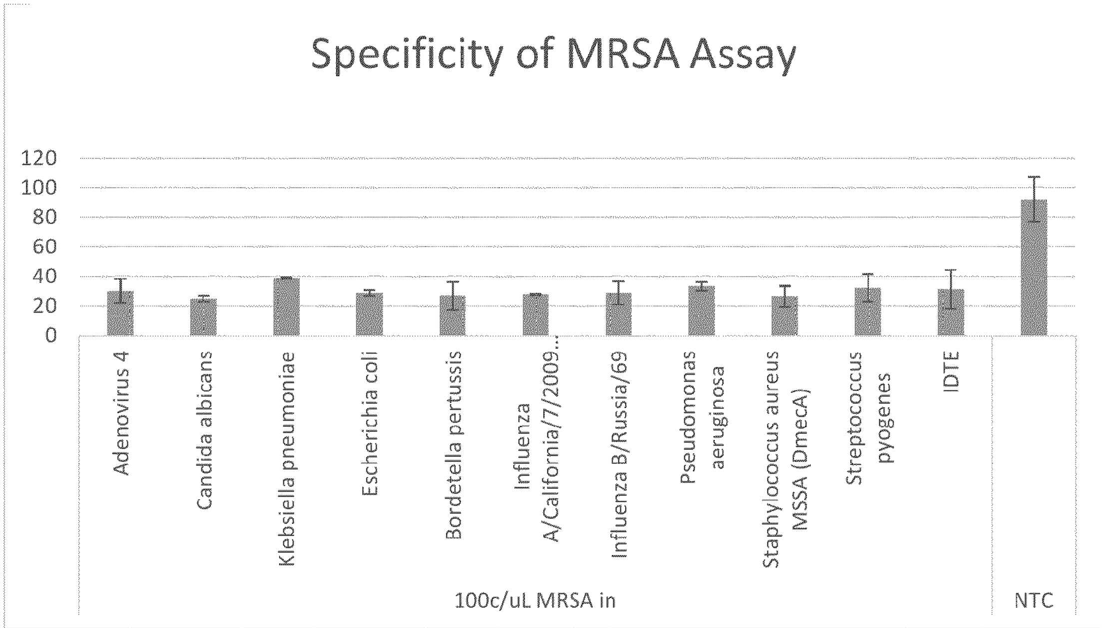

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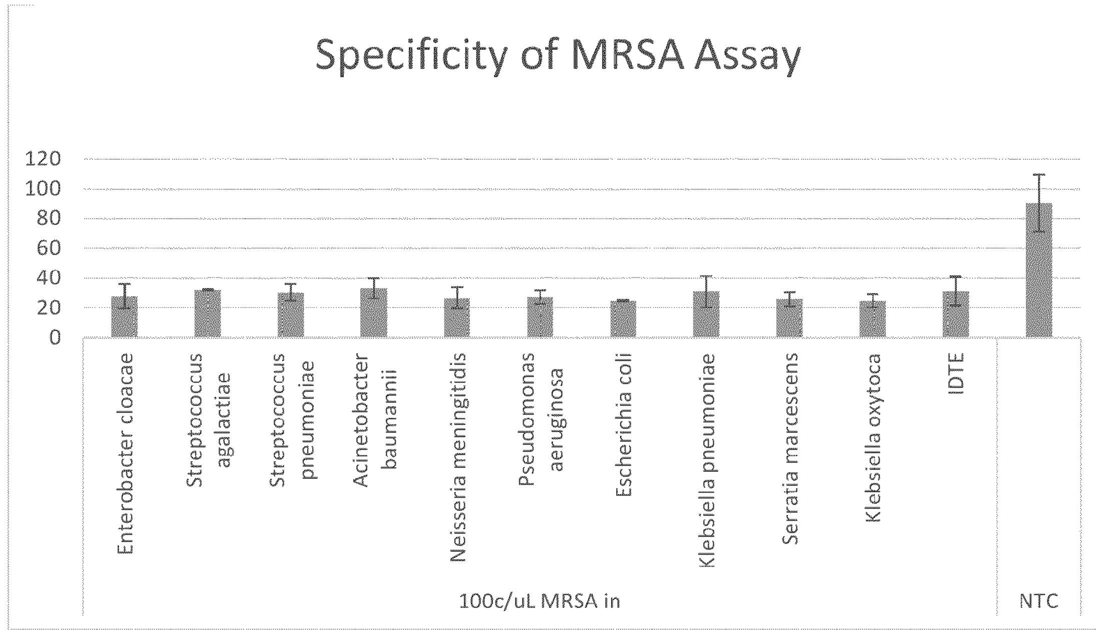
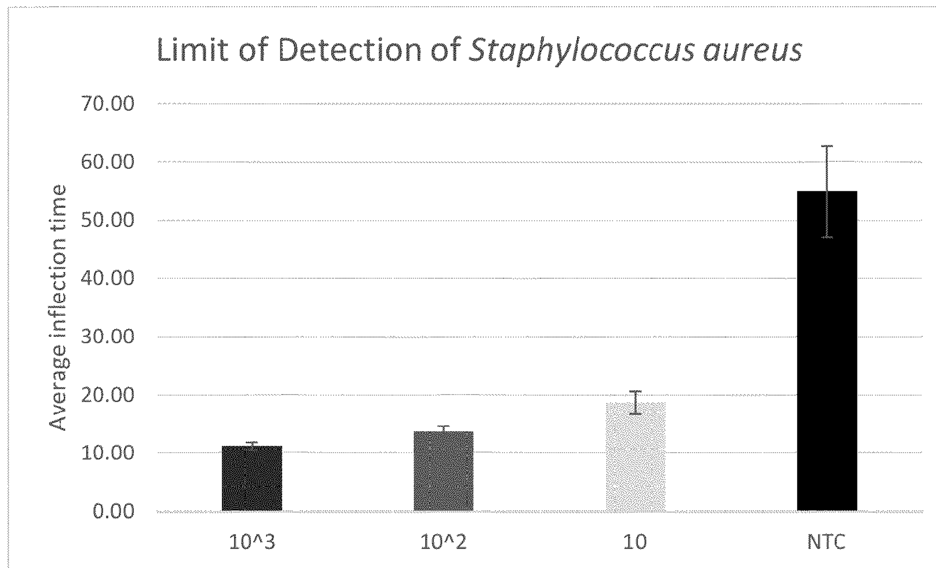
Appendix B: Representative Data for TNAA Assays



Staphylococcus aureus



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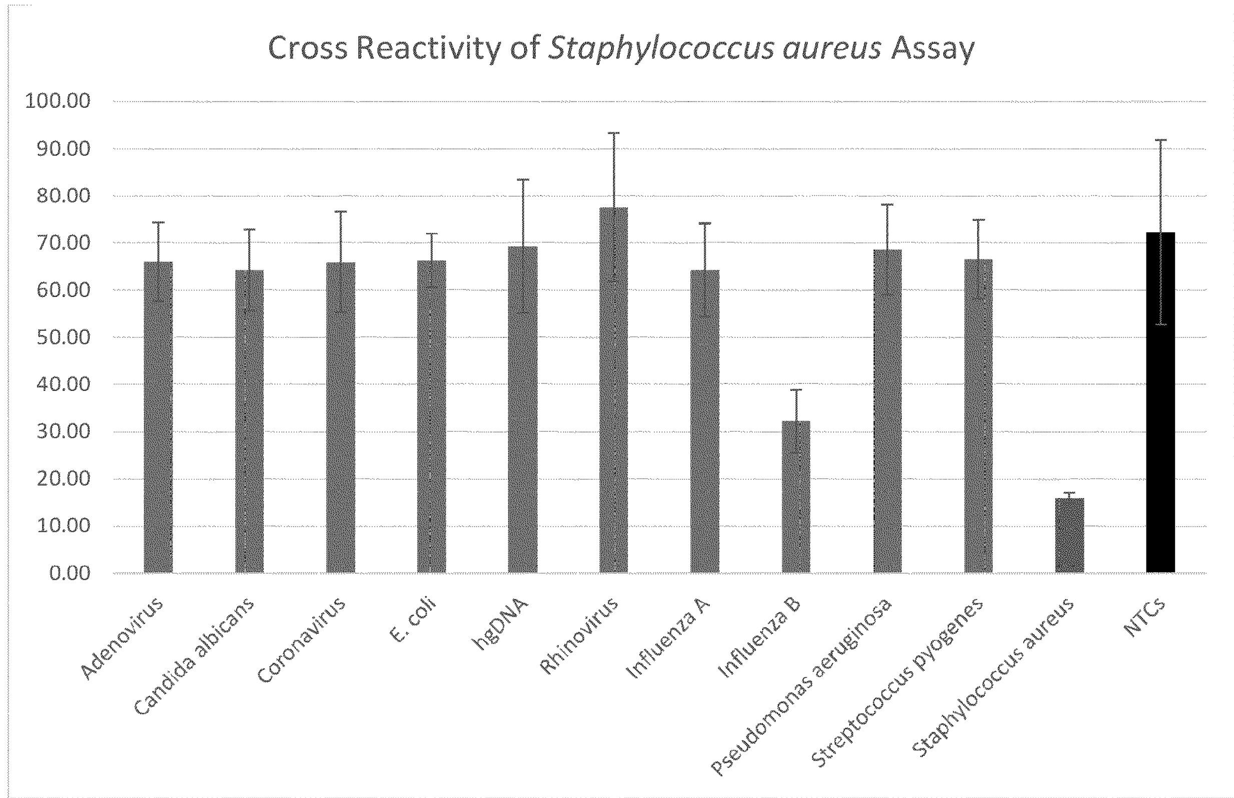

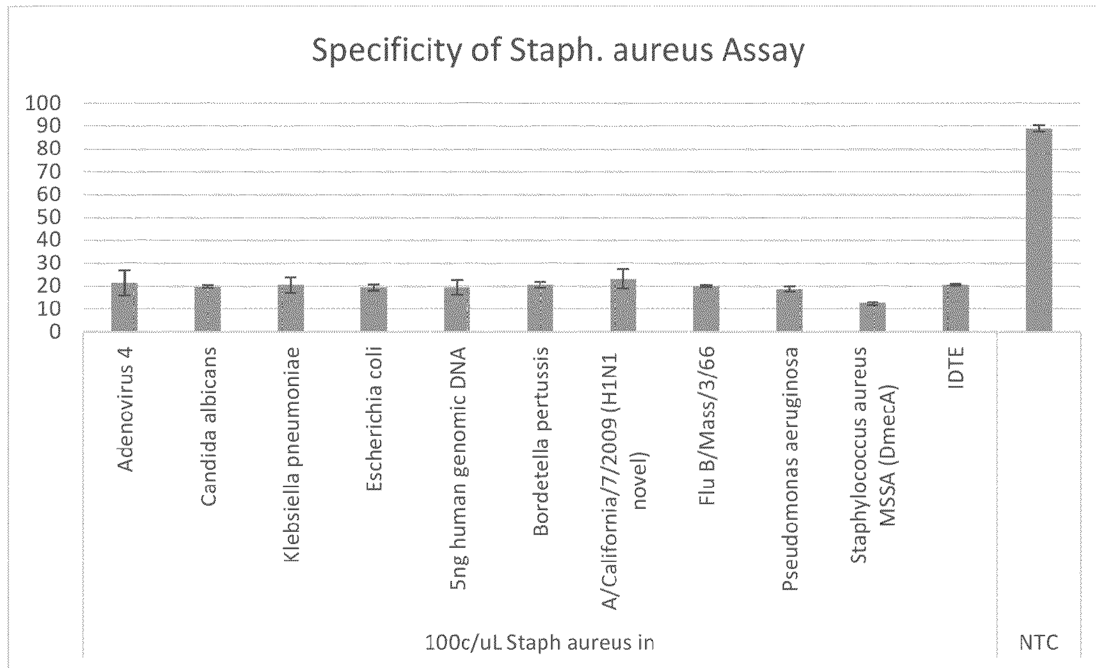

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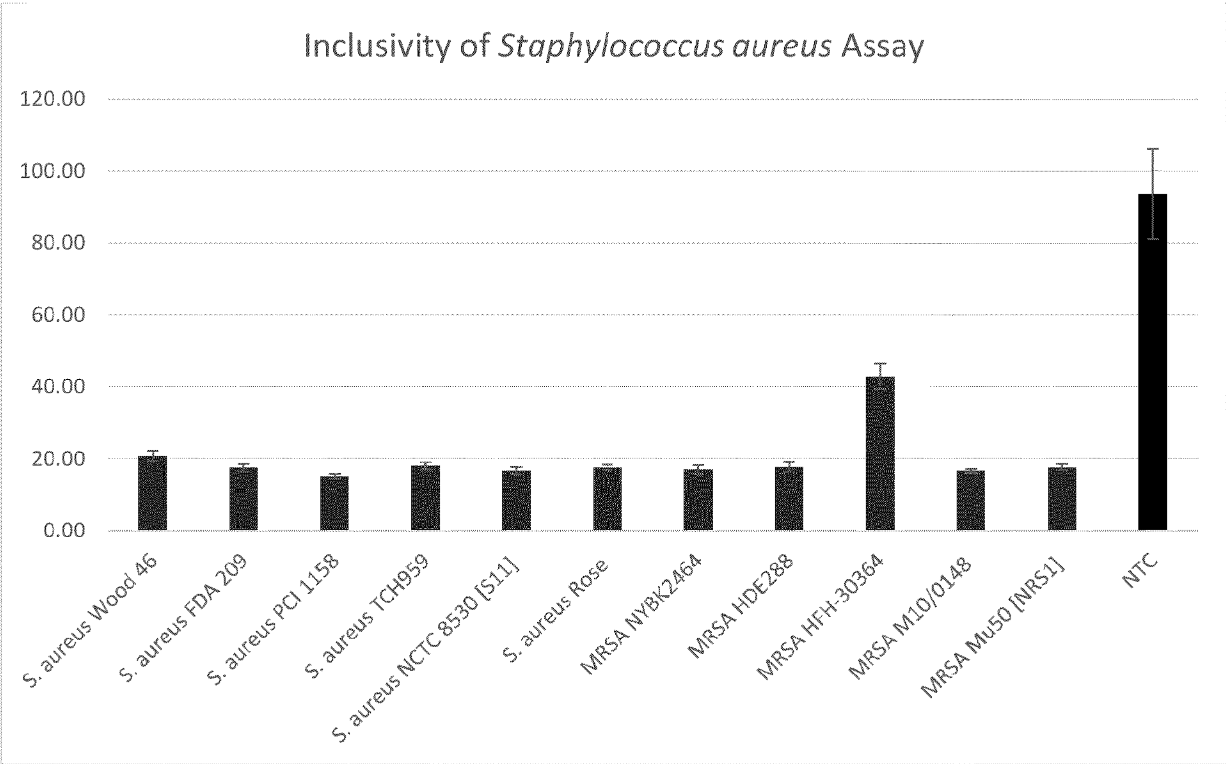
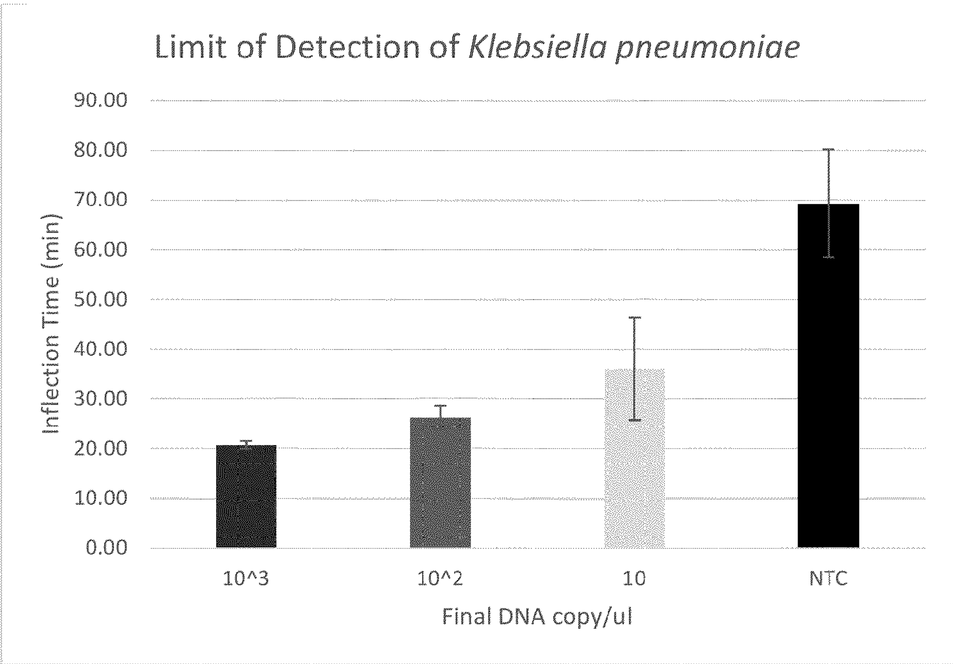


Klebsiella pneumoniae



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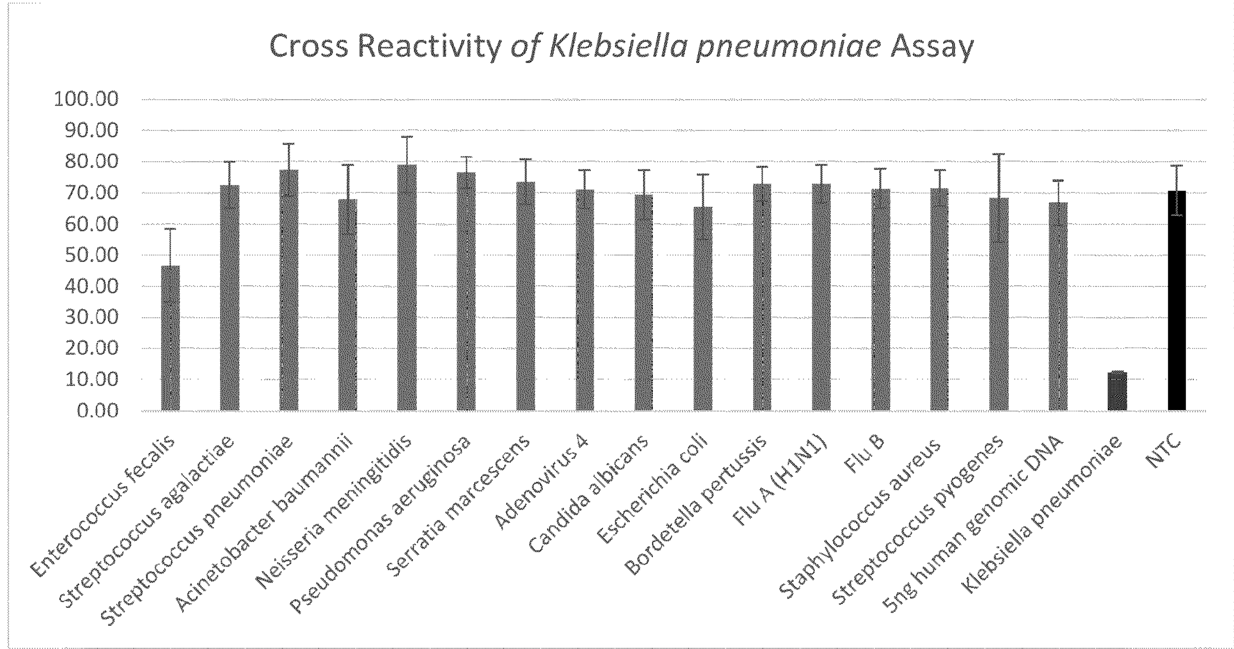

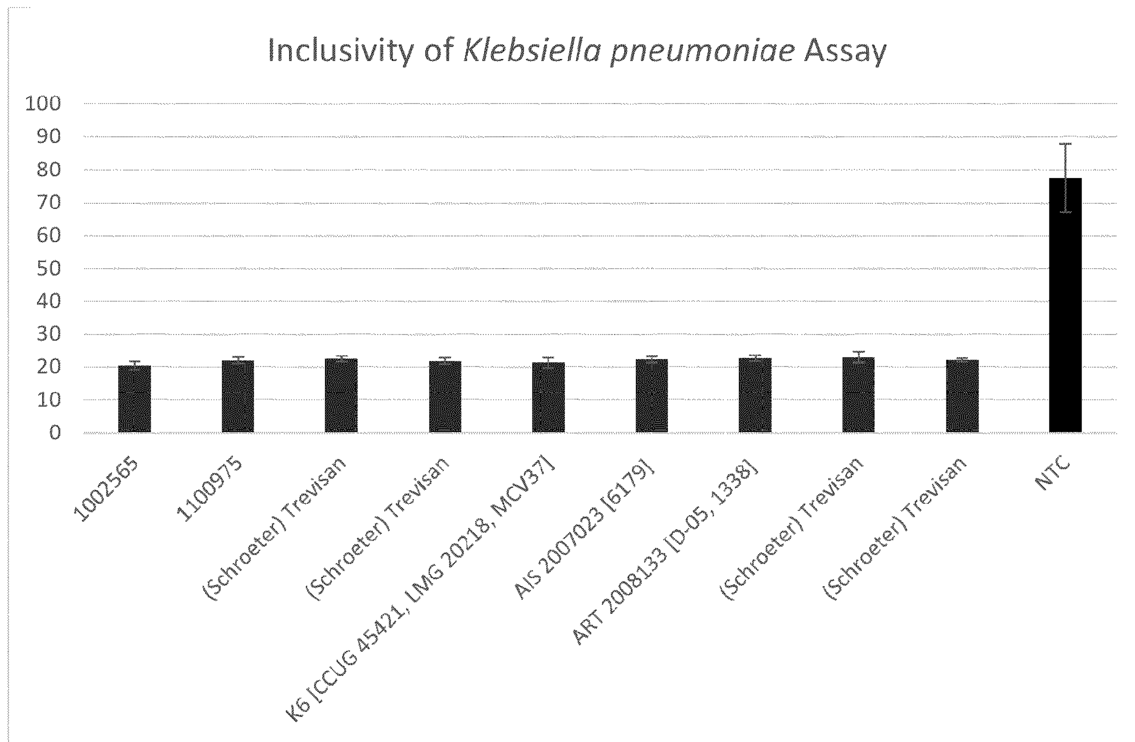

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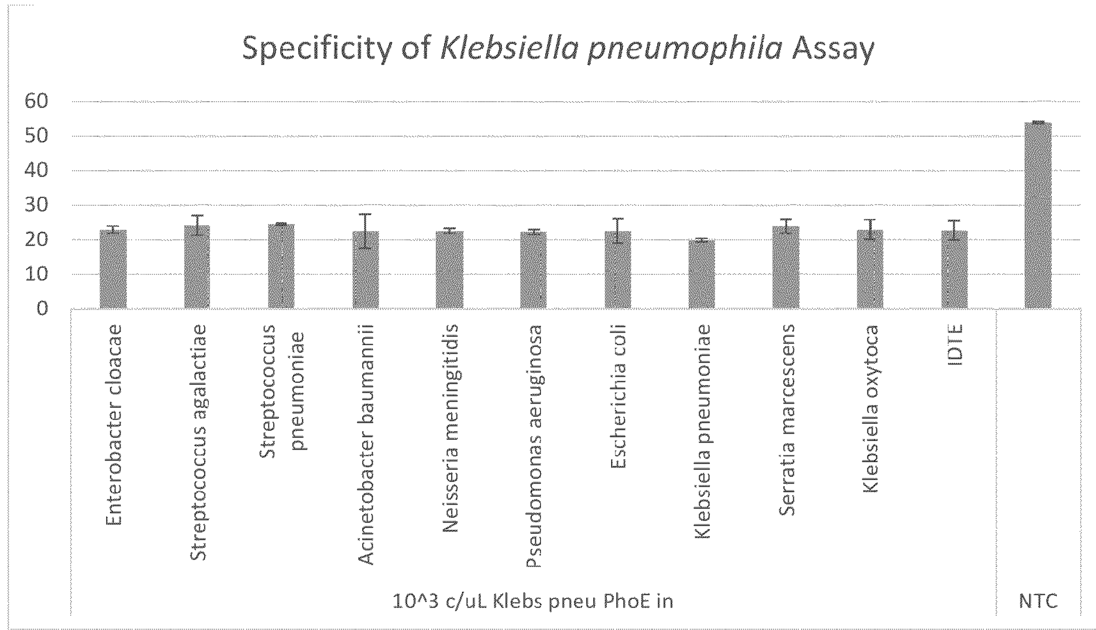

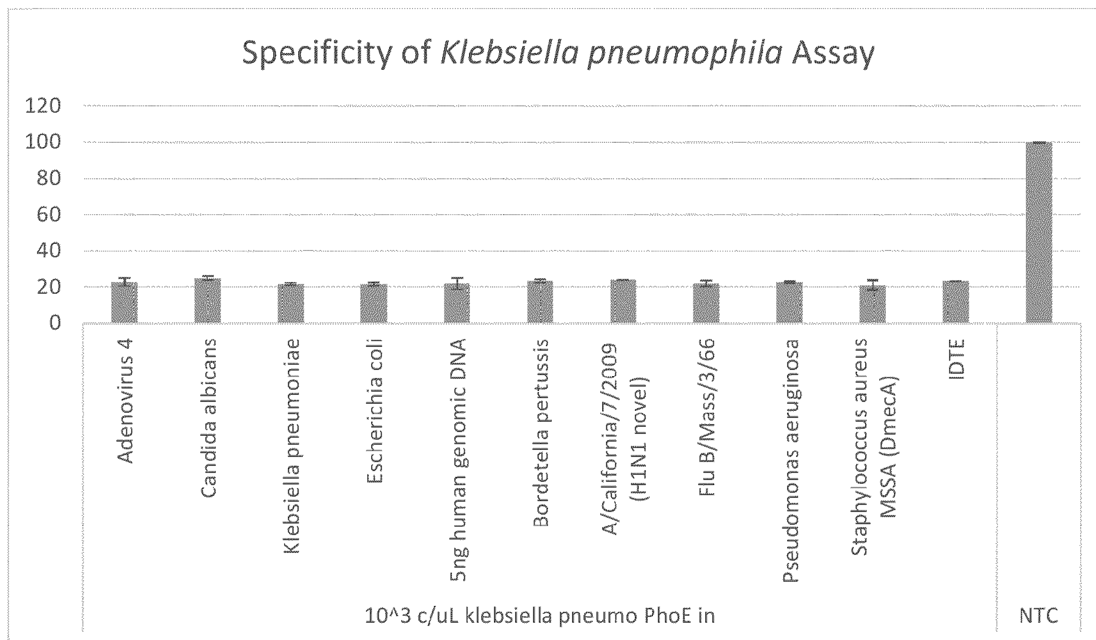

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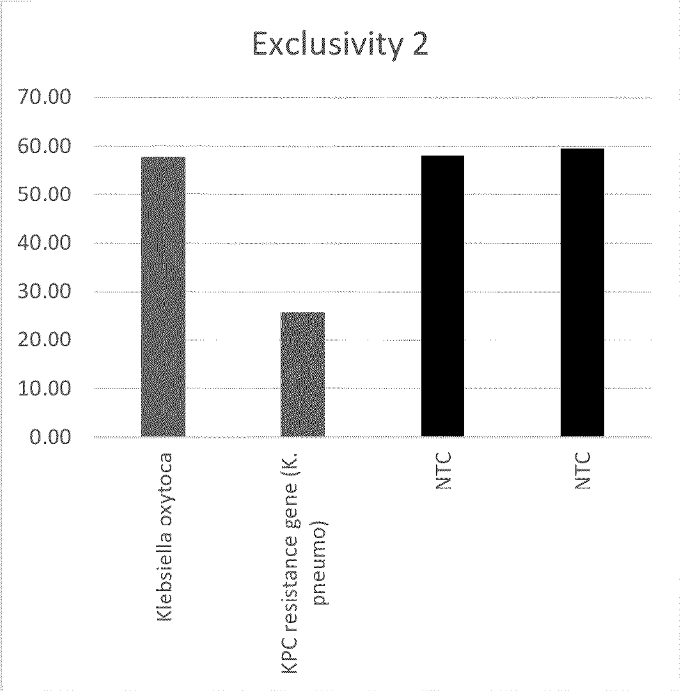
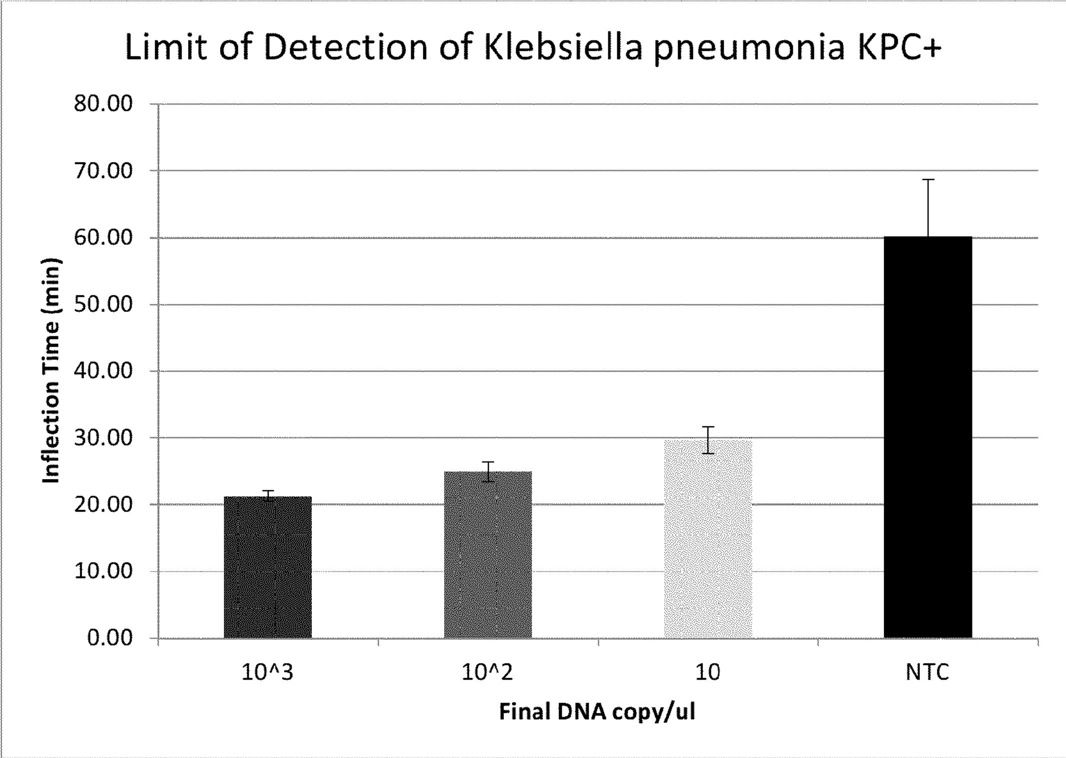


Klebsiella pneumoniae carbapenemase (KPC)



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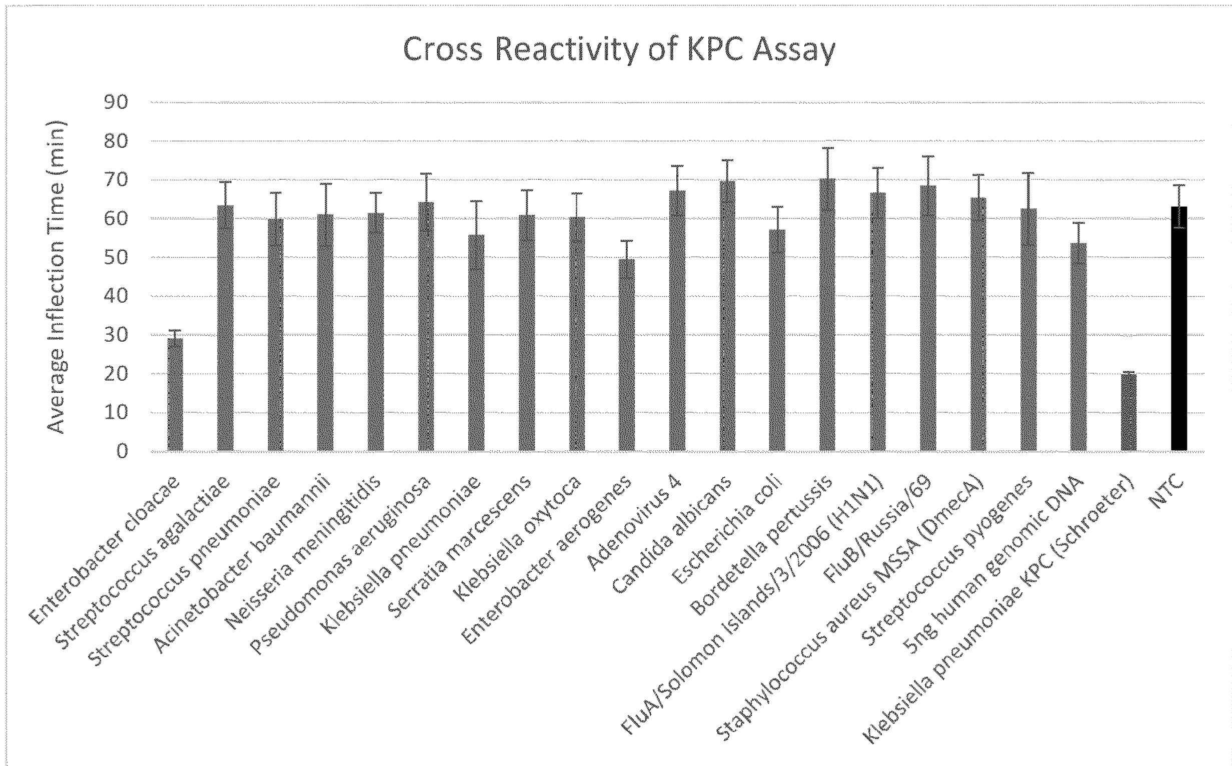

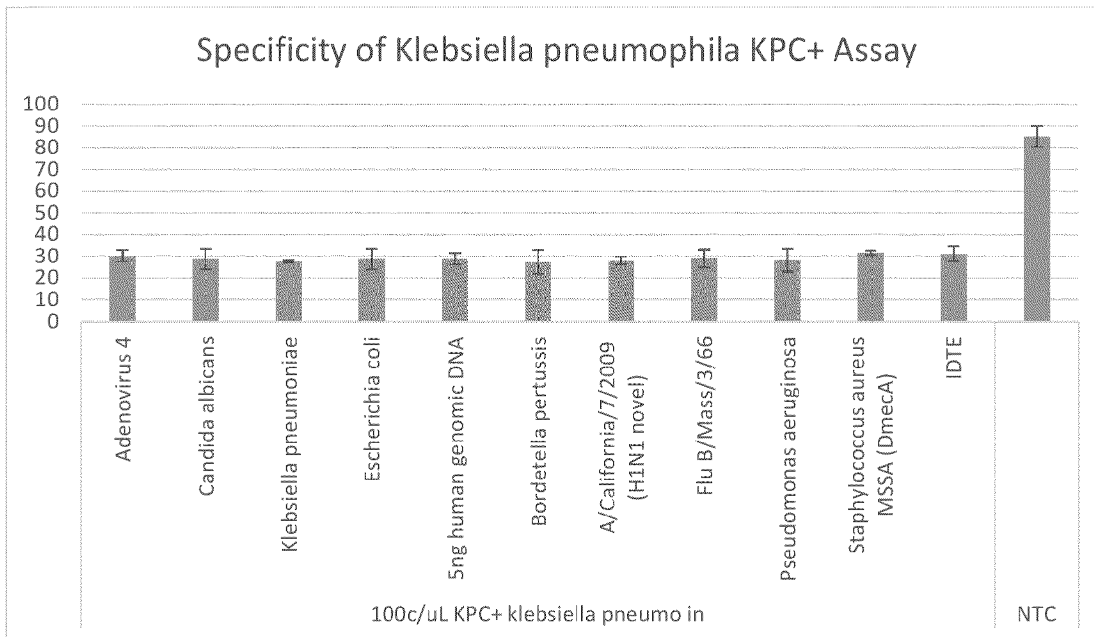

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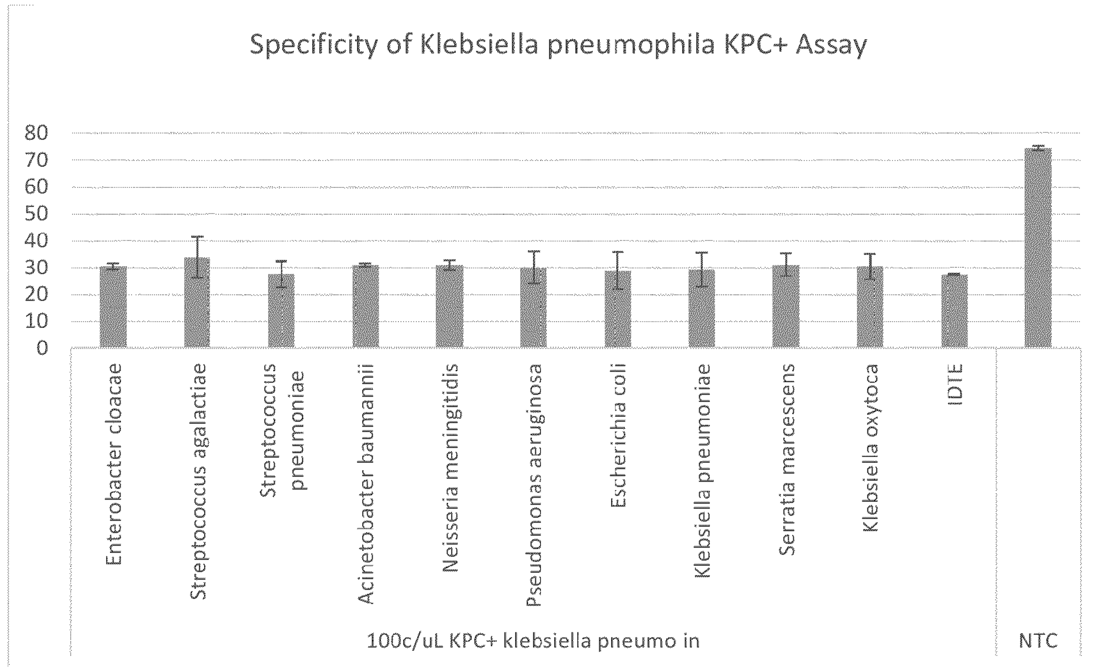

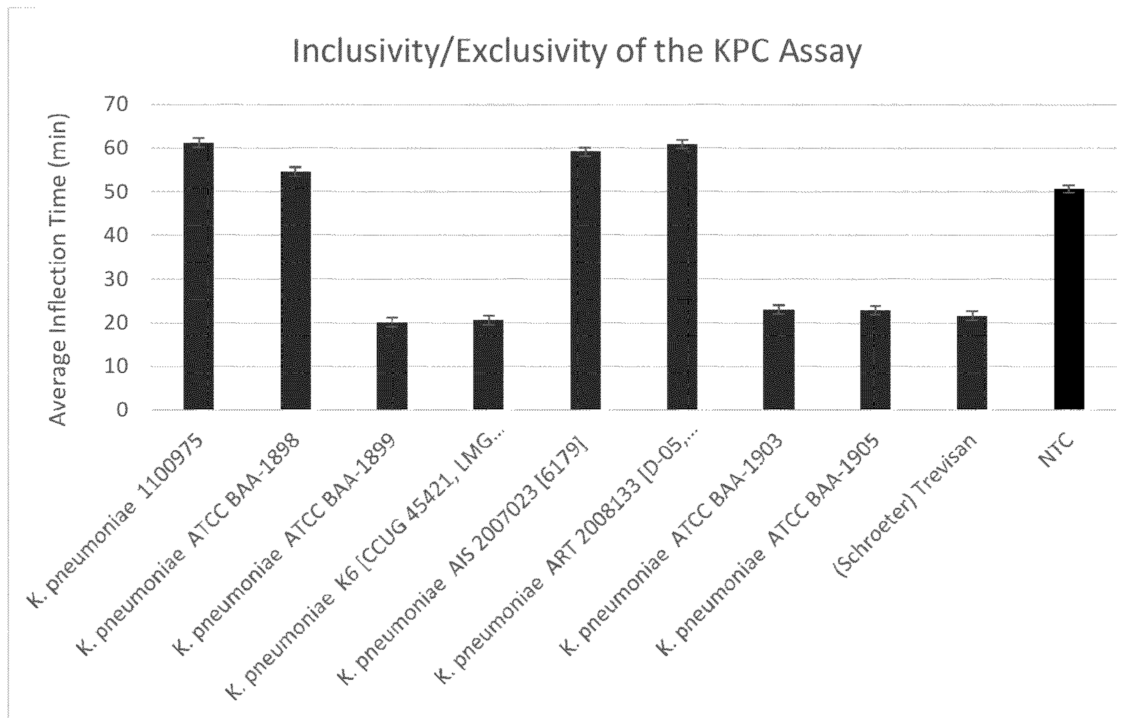

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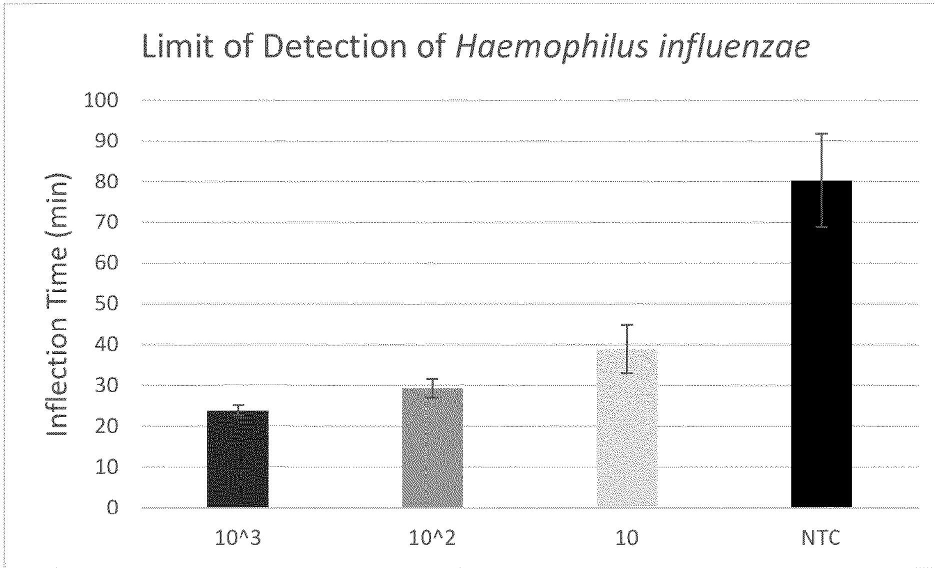
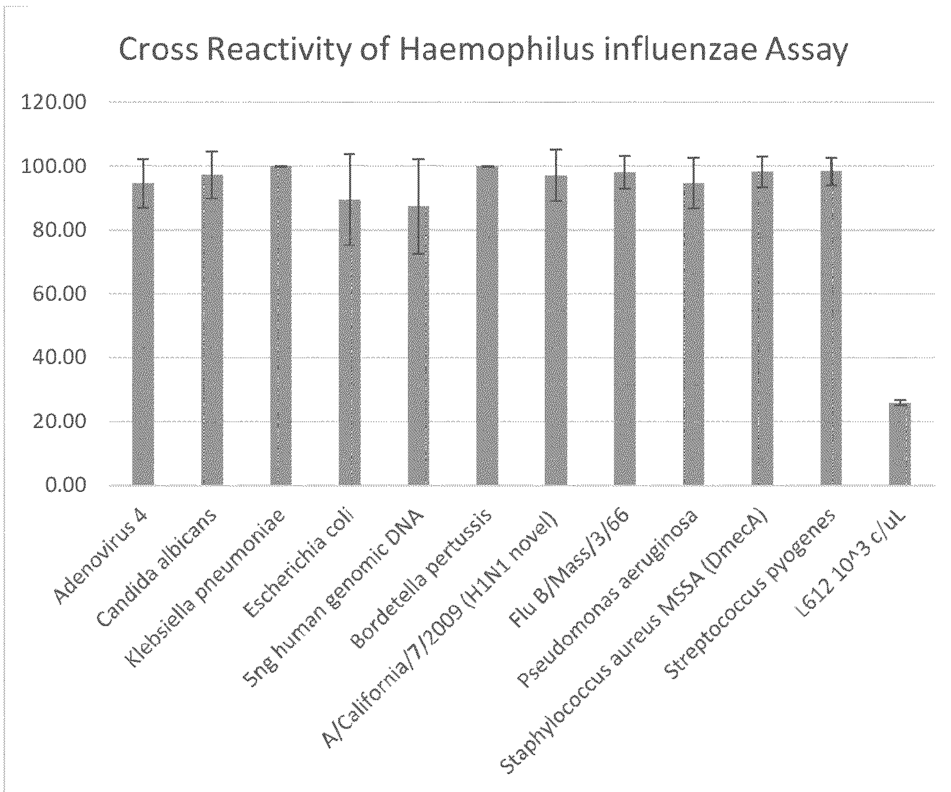
Appendix B: Representative Data for TNAA Assays

Haemophilus influenza (ampic S)





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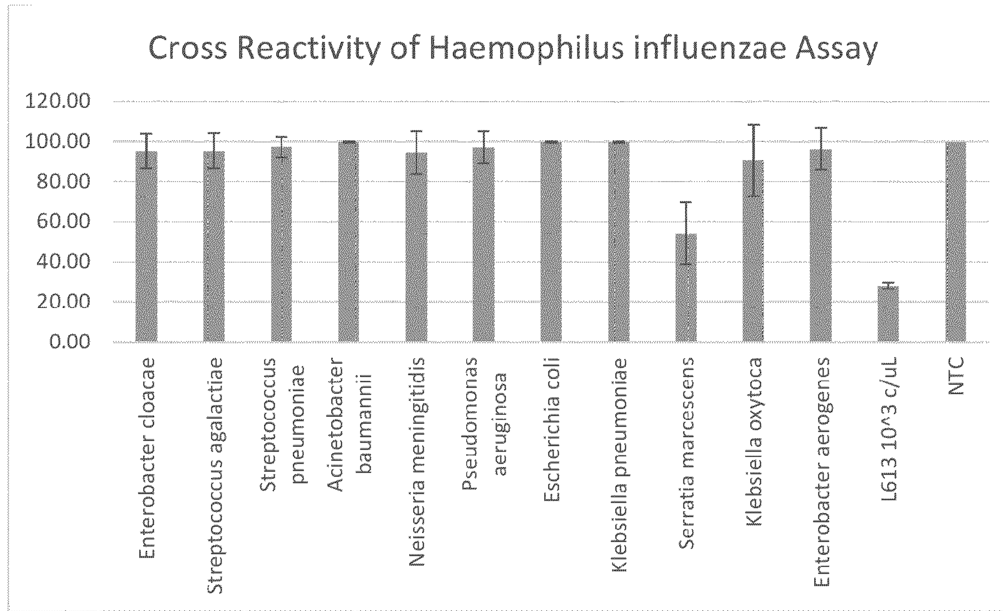
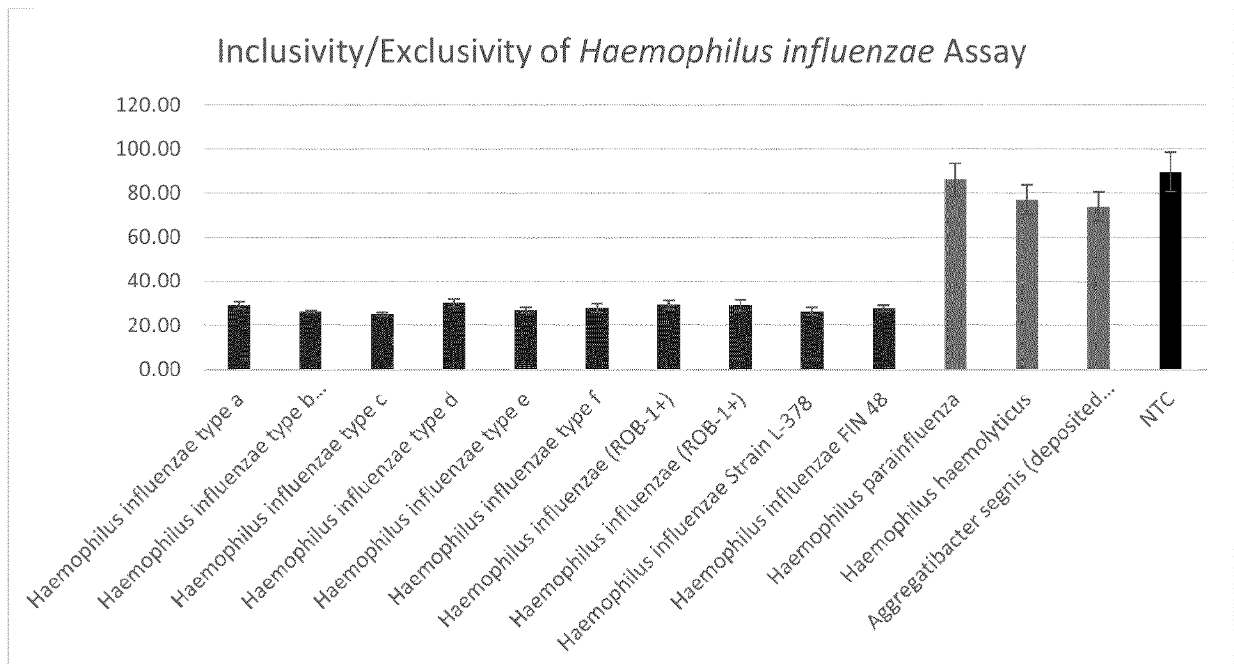
Appendix B: Representative Data for TNAA Assays





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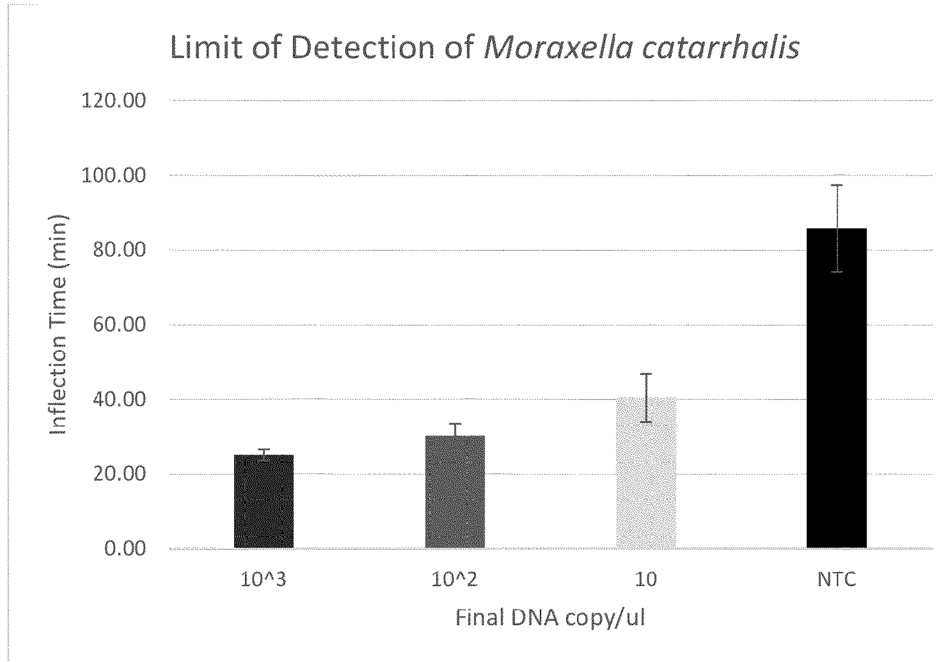
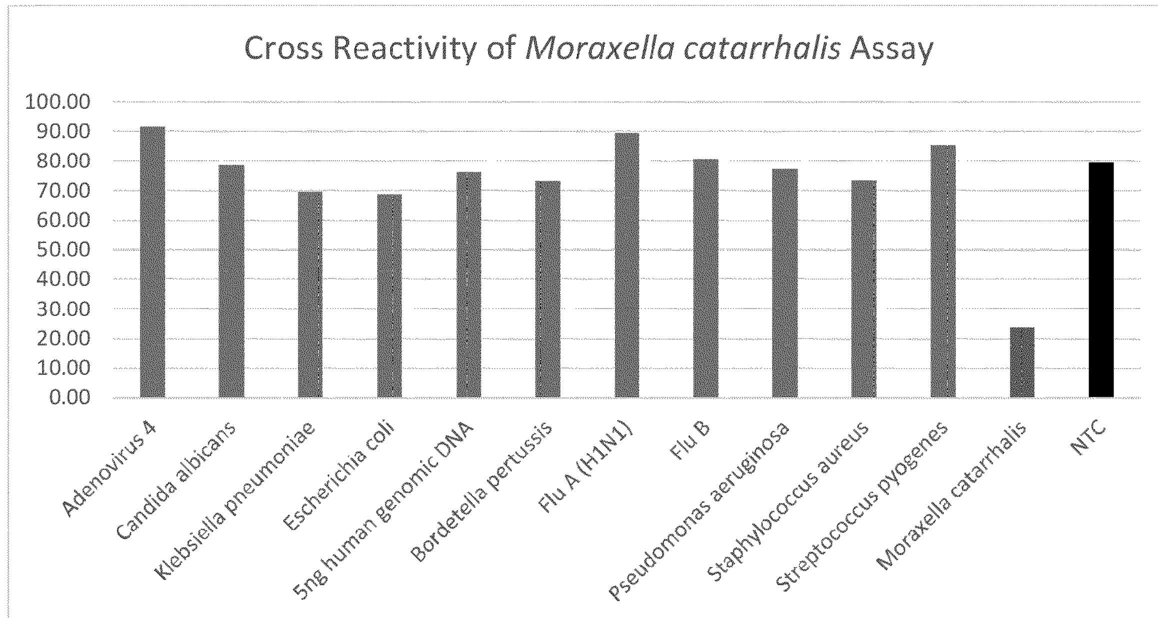
Appendix B: Representative Data for TNAA Assays

Moraxella catarrhalis





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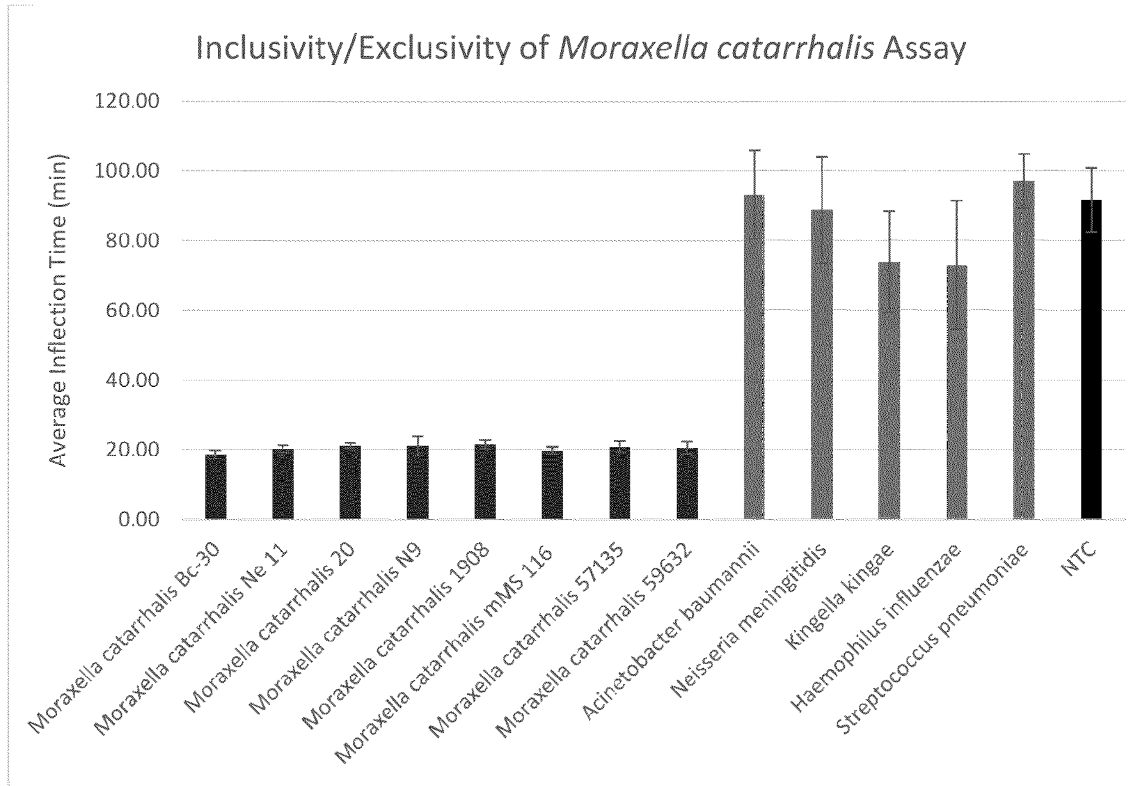

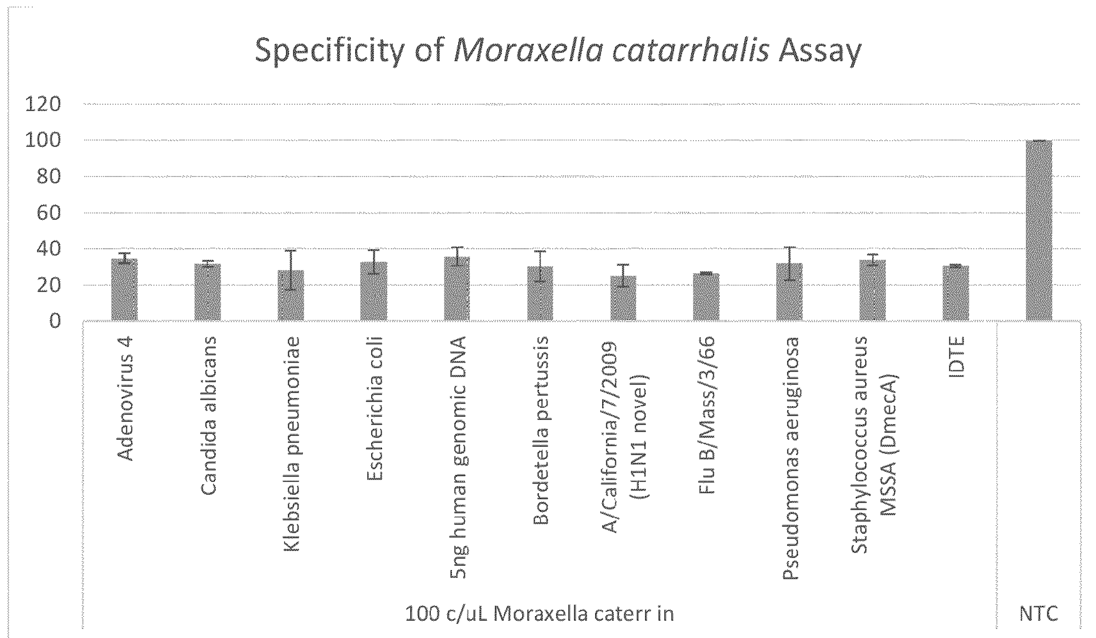

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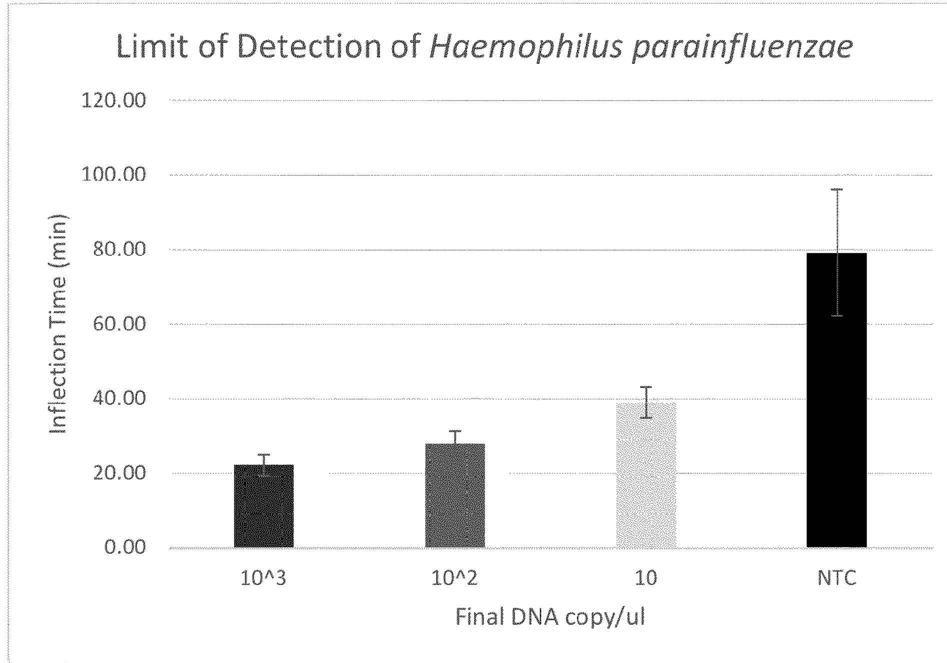
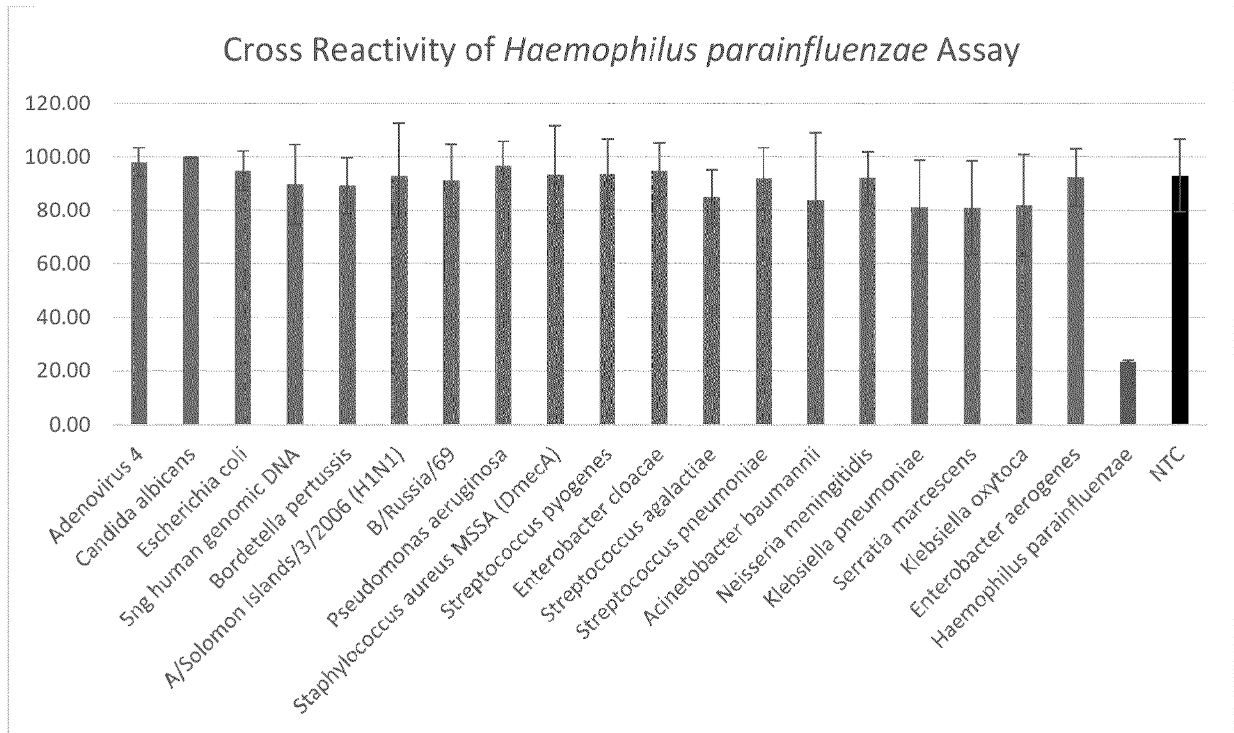
Appendix B: Representative Data for TNAA Assays

Haemophilus parainfluenza





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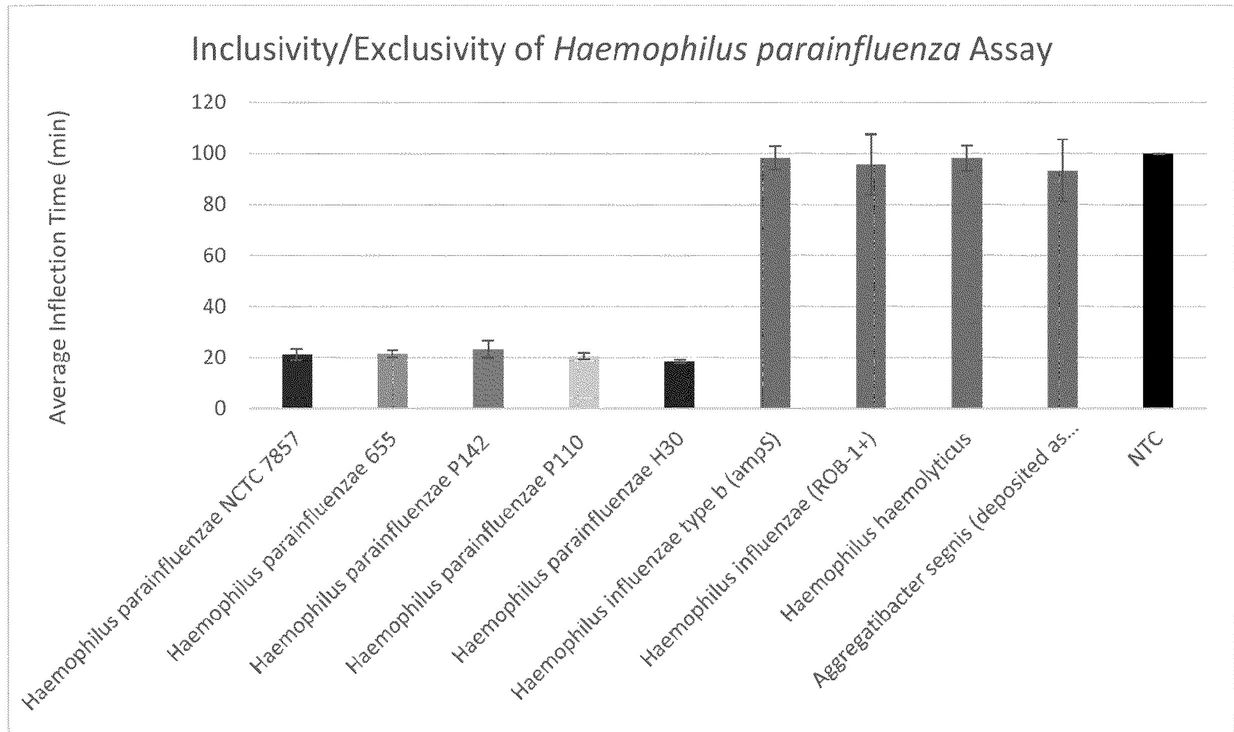
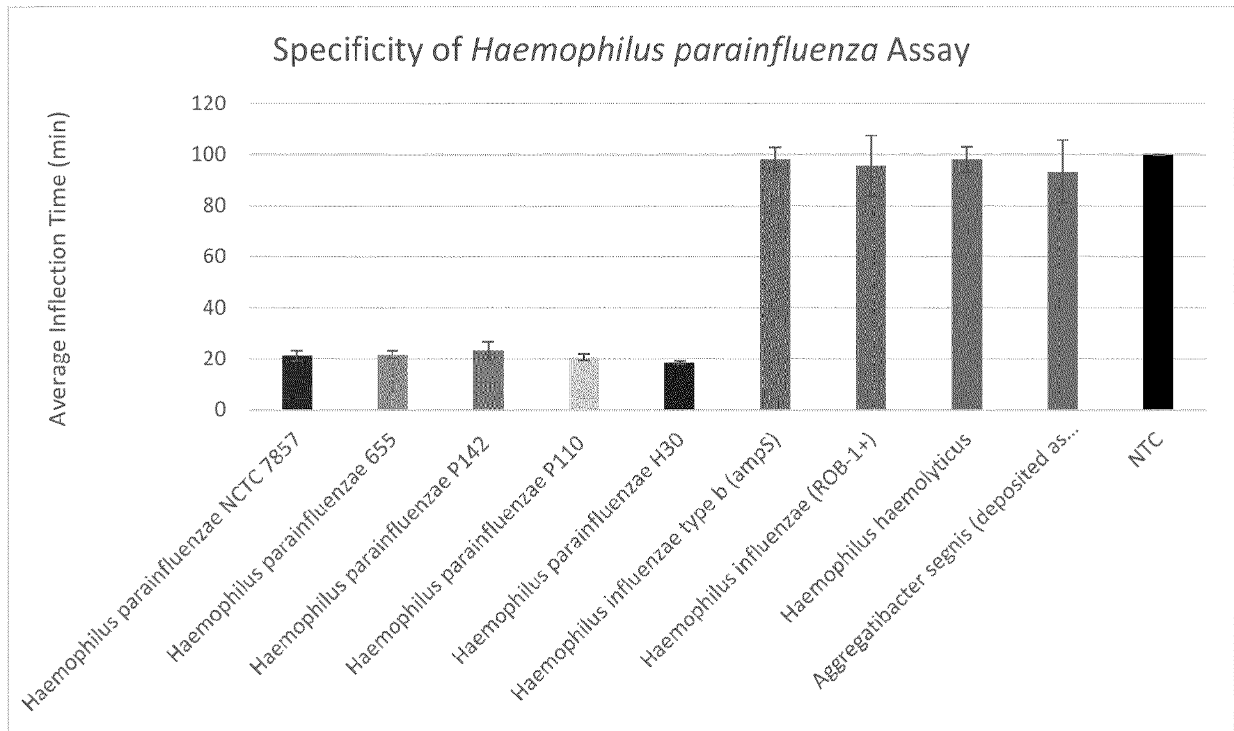
Appendix B: Representative Data for TNAA Assays





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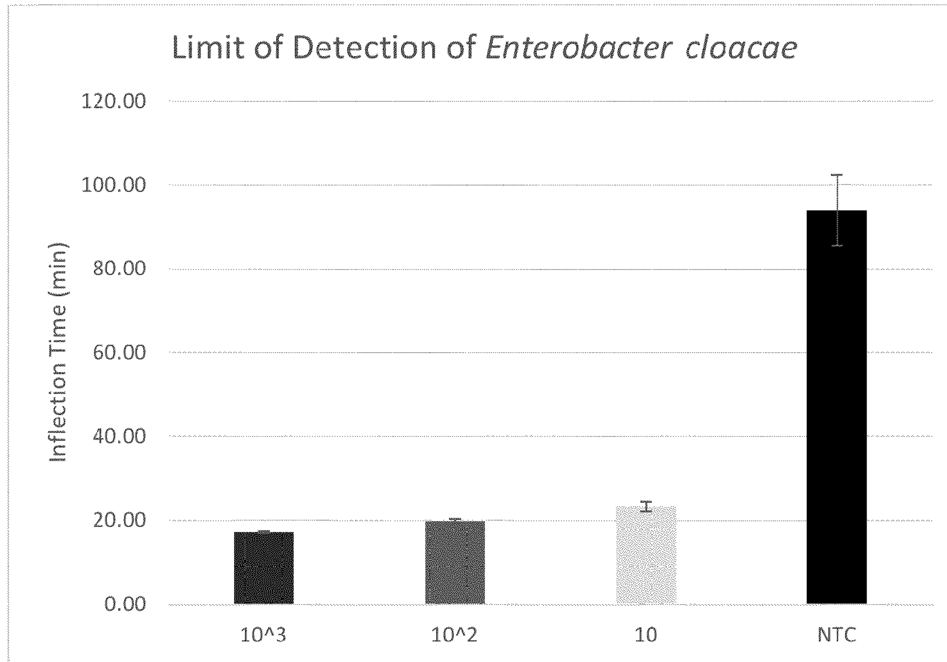
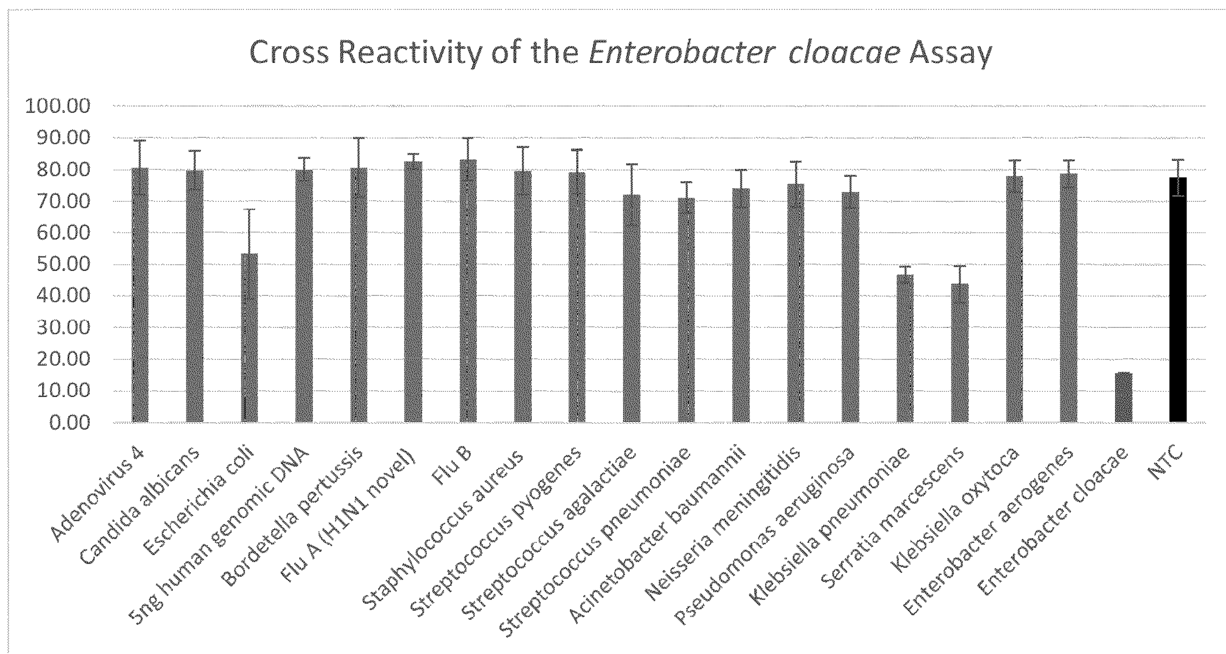
Appendix B: Representative Data for TNAA Assays

Enterobacter cloacae





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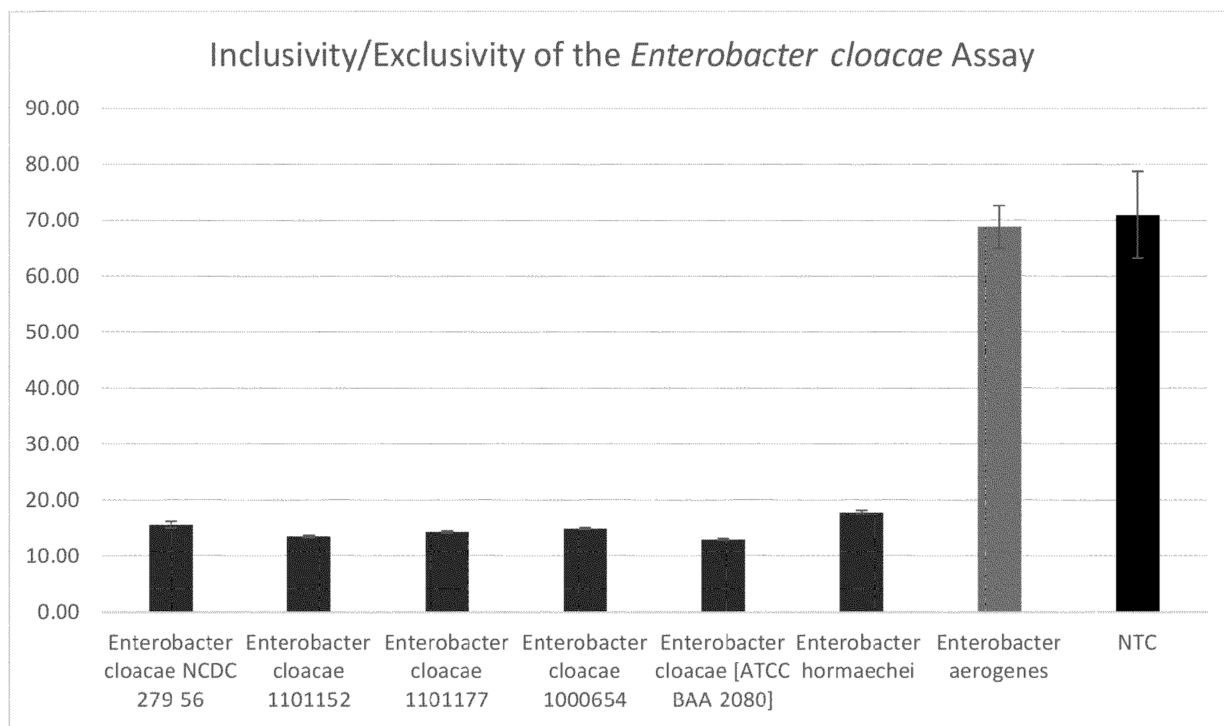
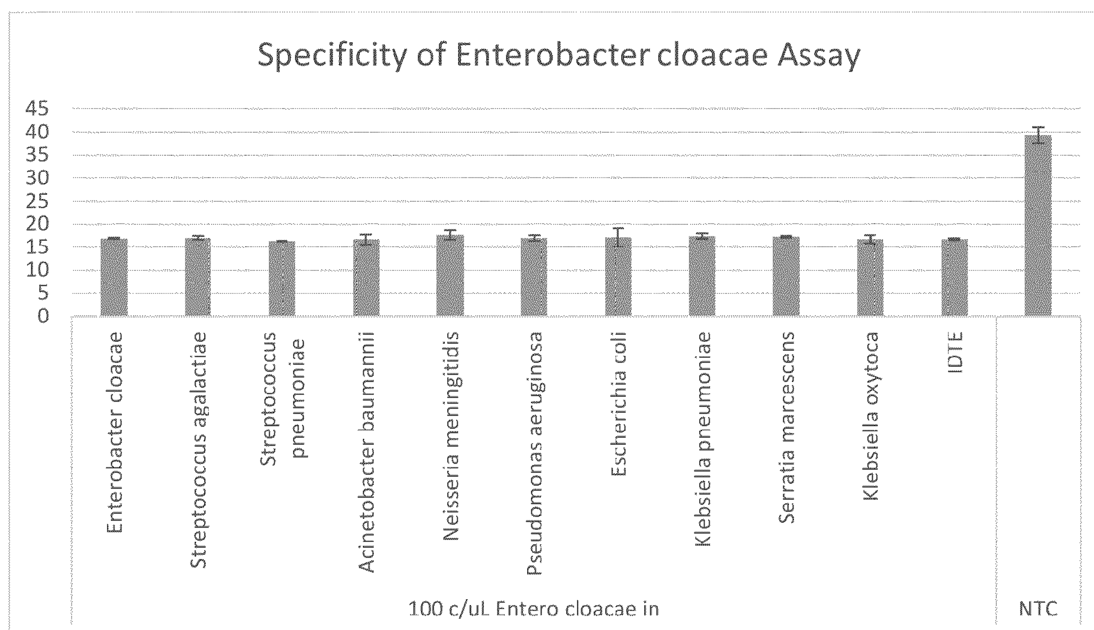
Appendix B: Representative Data for TNAA Assays





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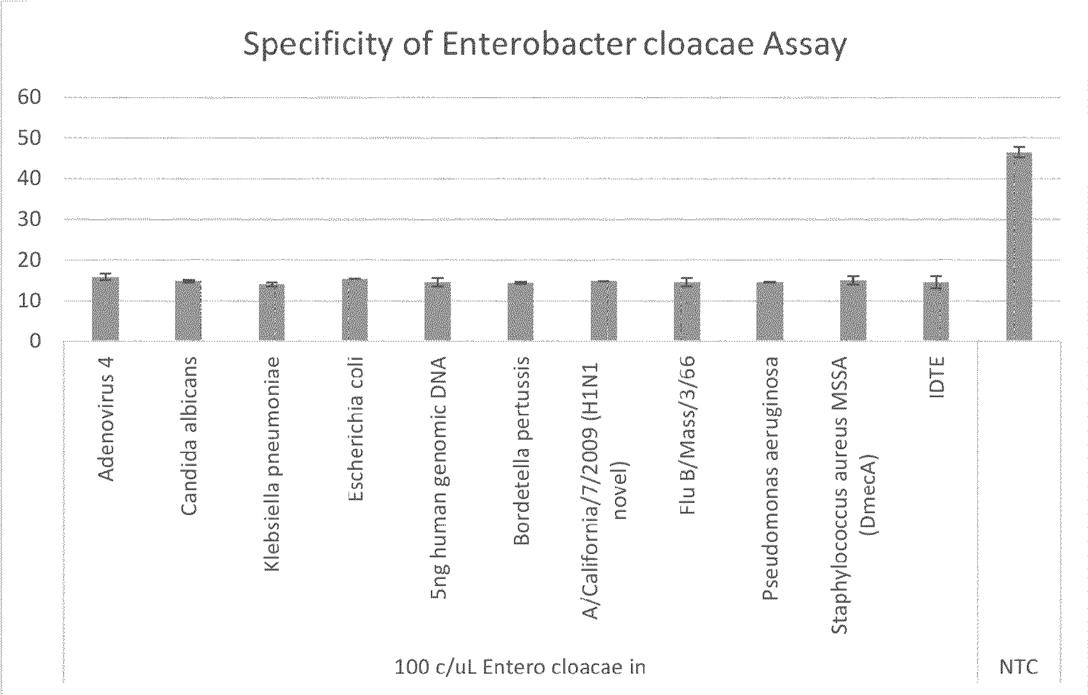

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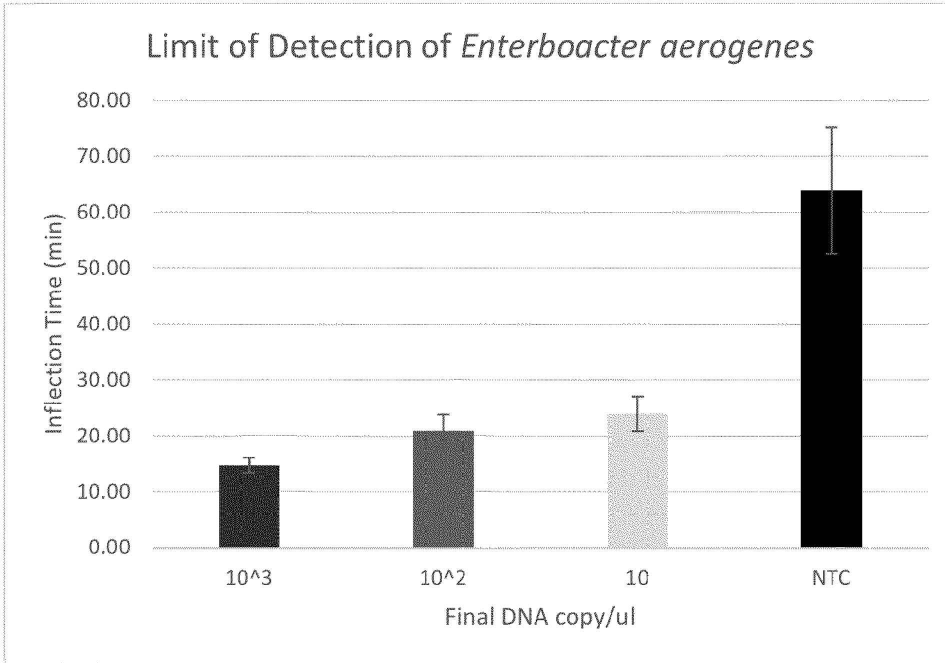
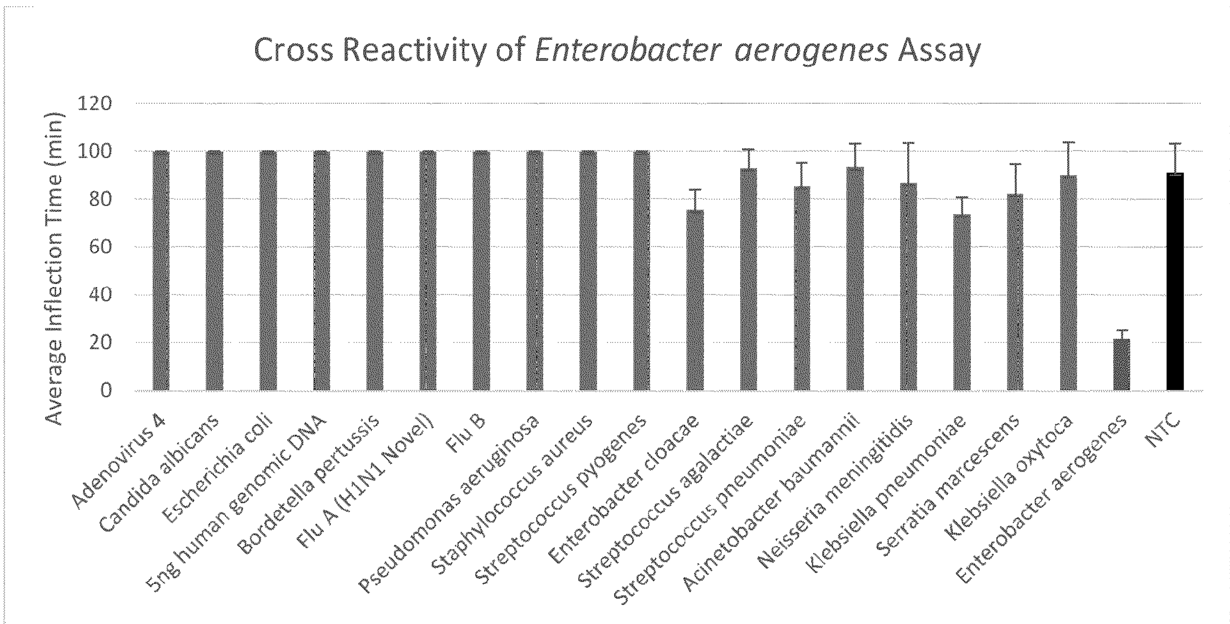
Appendix B: Representative Data for TNAA Assays

Enterobacter aerogenes





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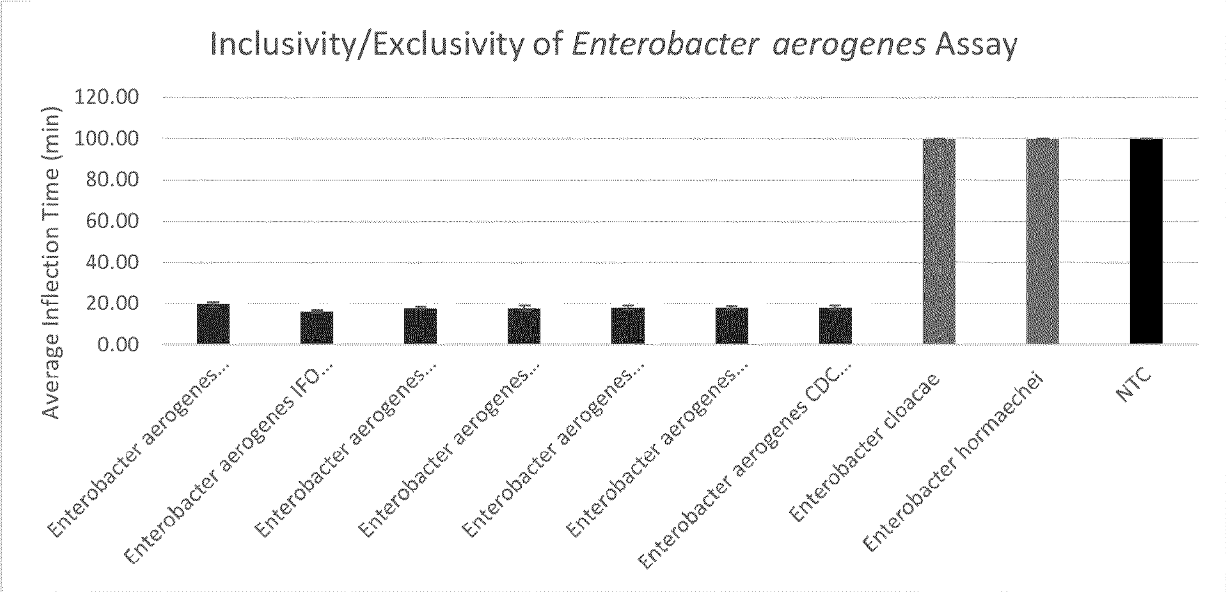
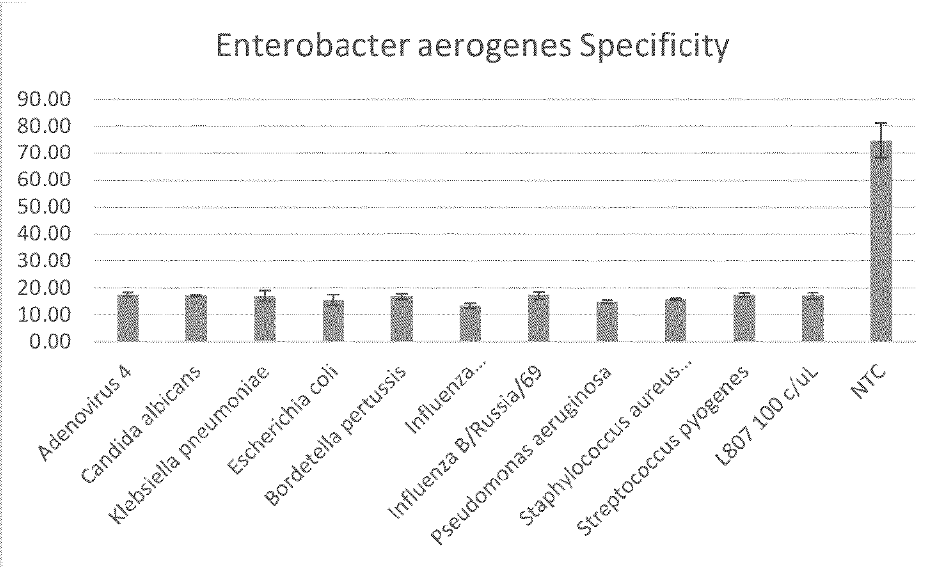
Appendix B: Representative Data for TNAA Assays





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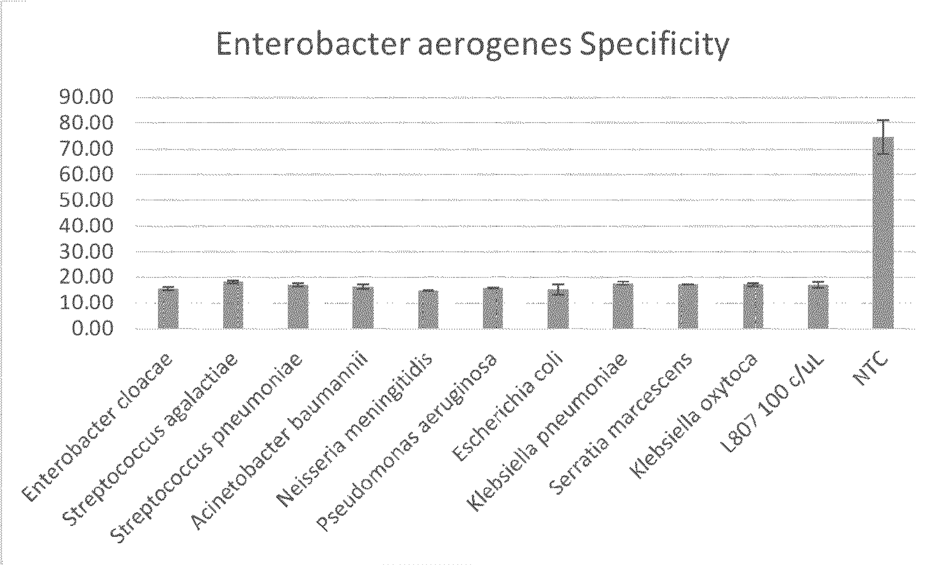
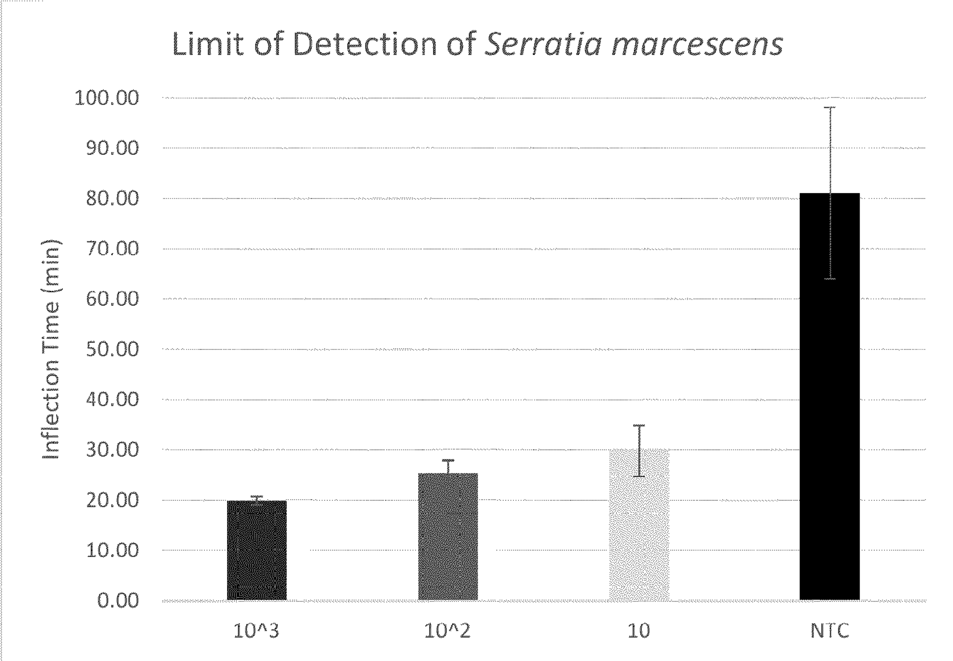


Serratia marcescens



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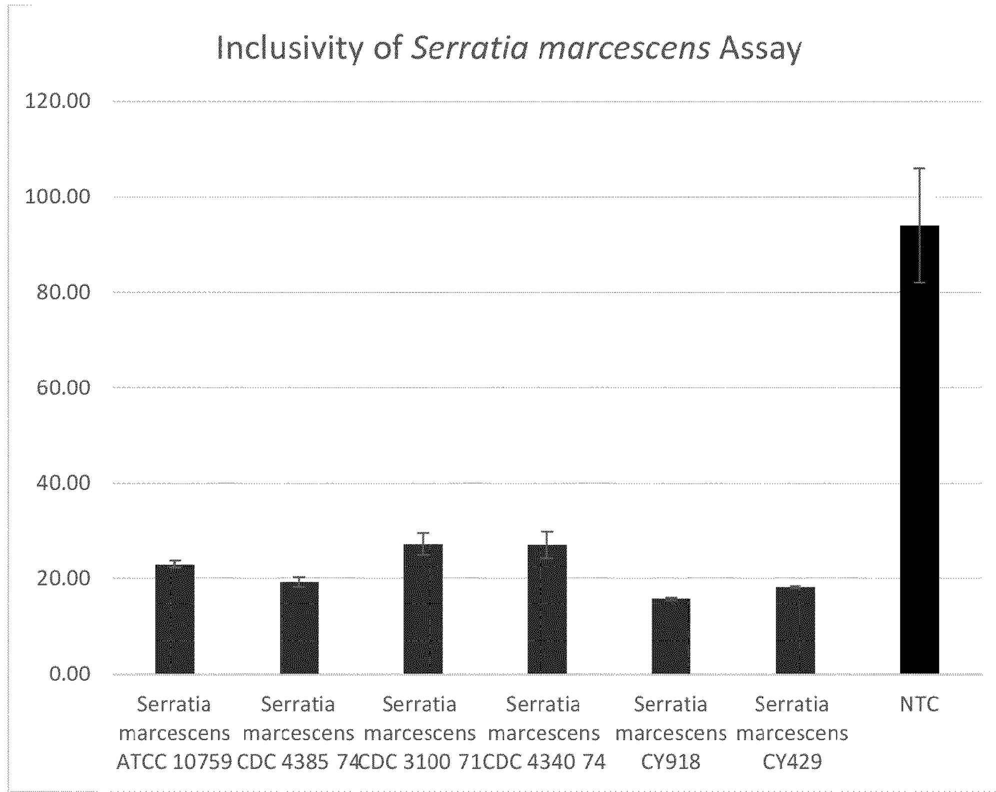

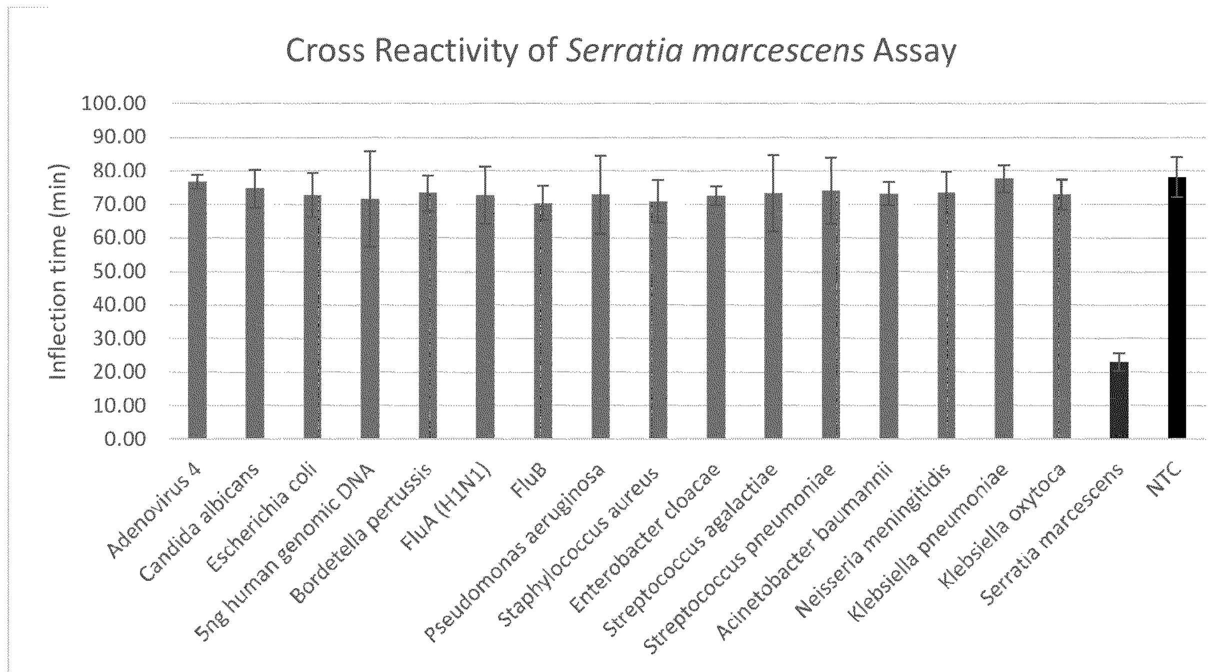

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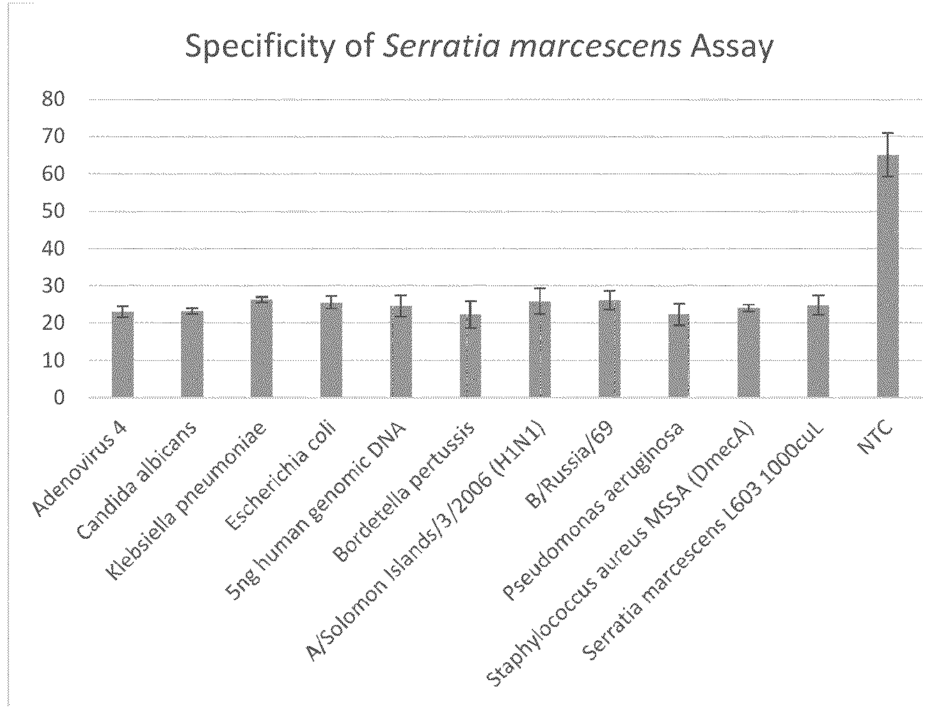

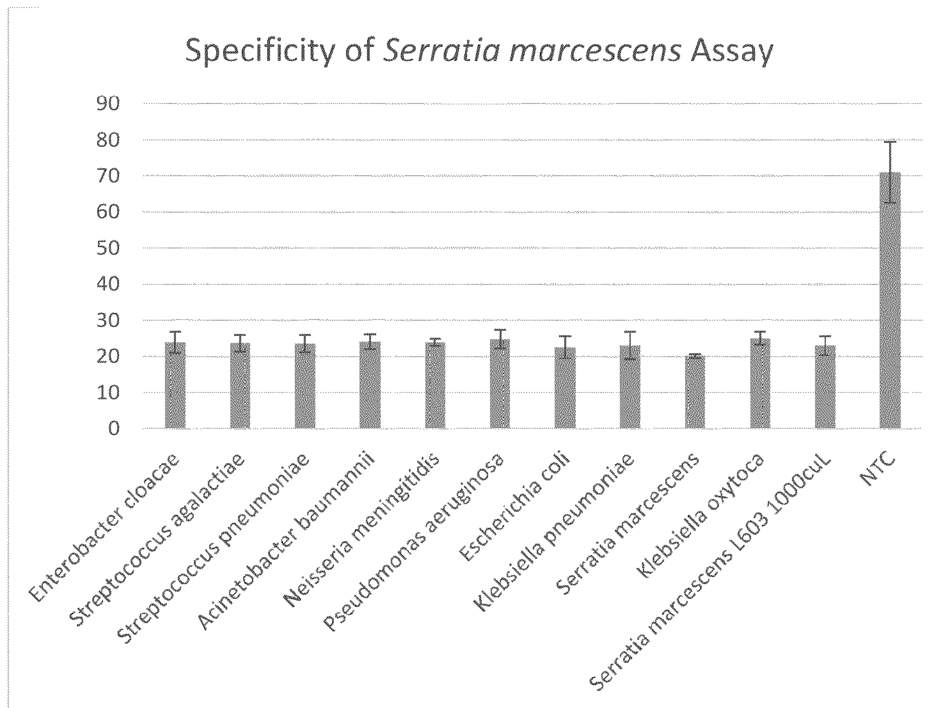

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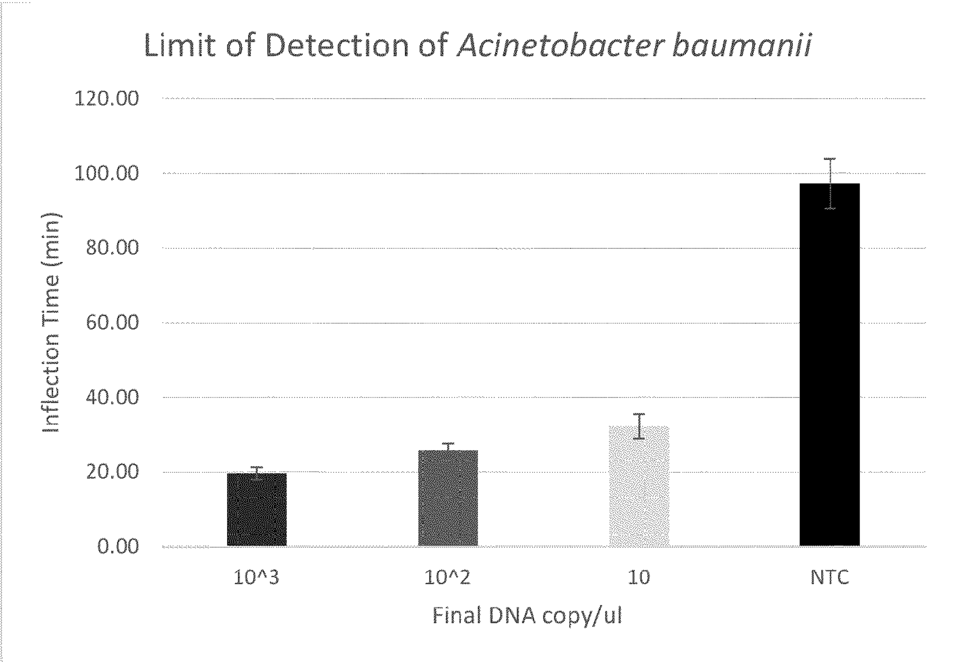
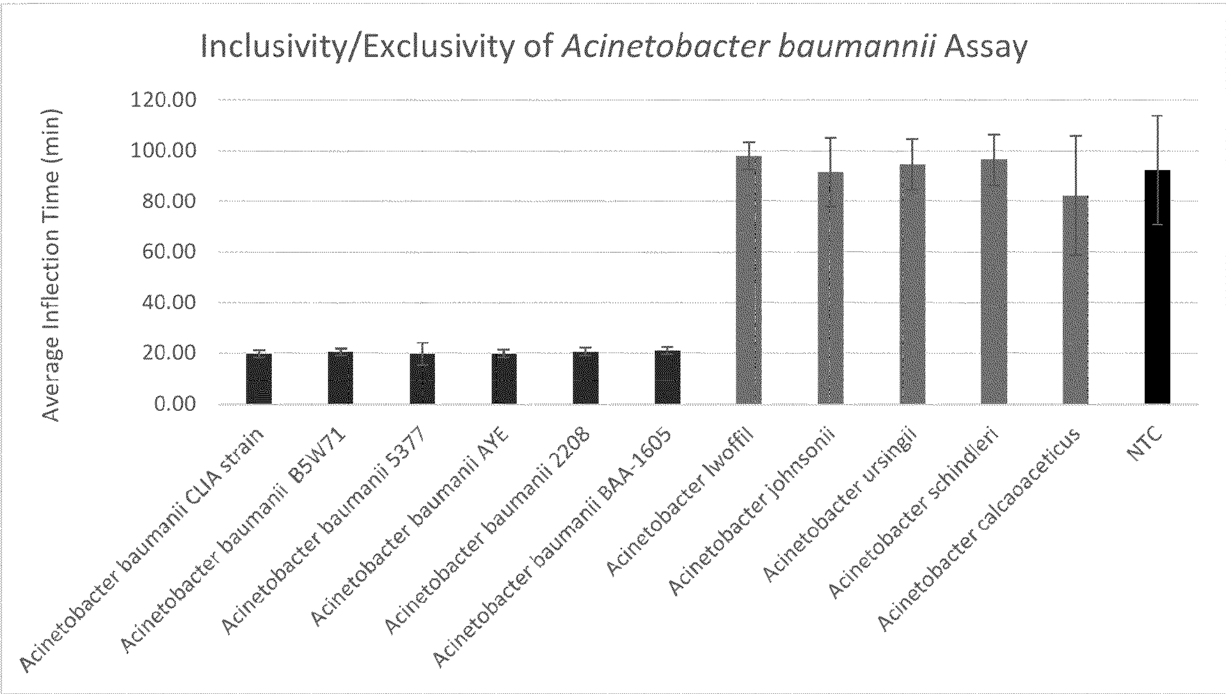
Appendix B: Representative Data for TNAA Assays

Acinetobacter baumannii





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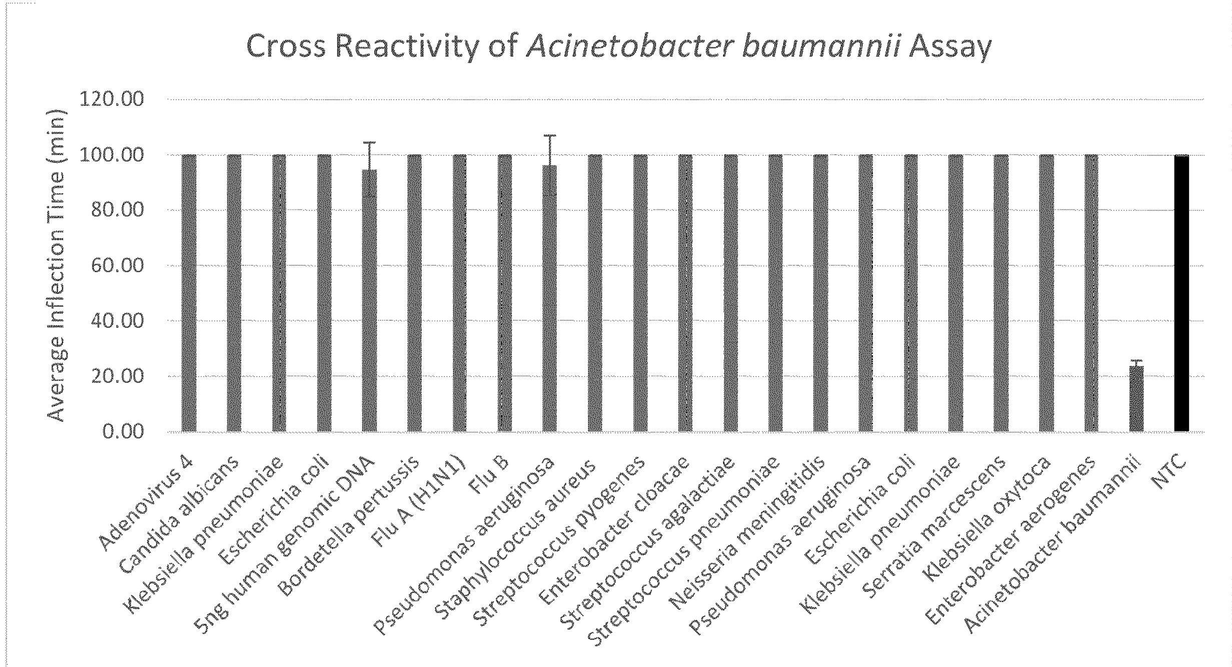

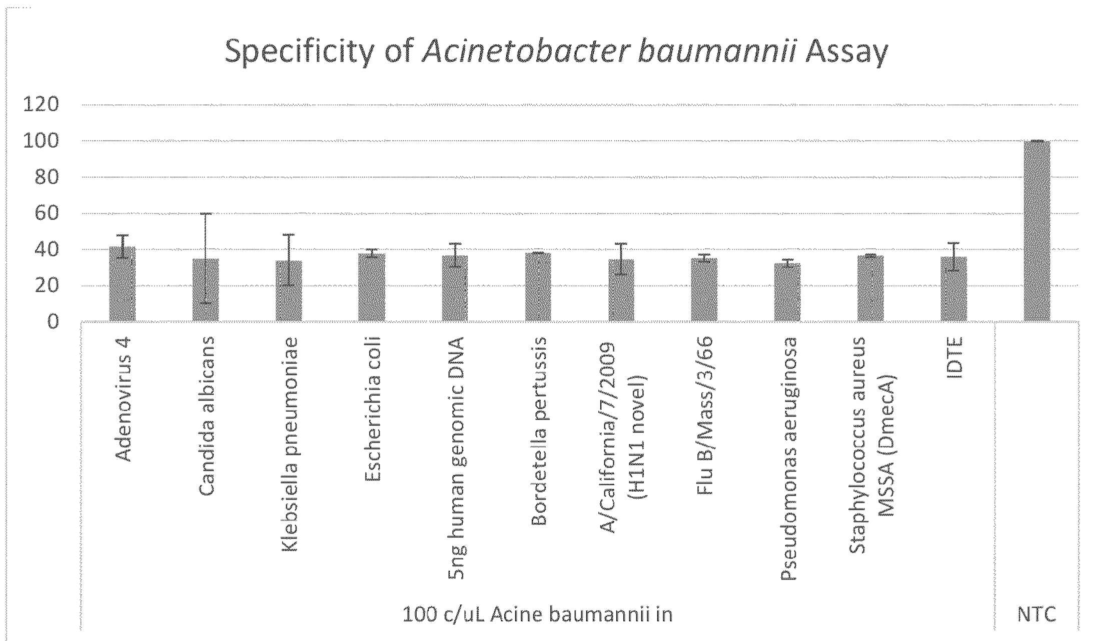

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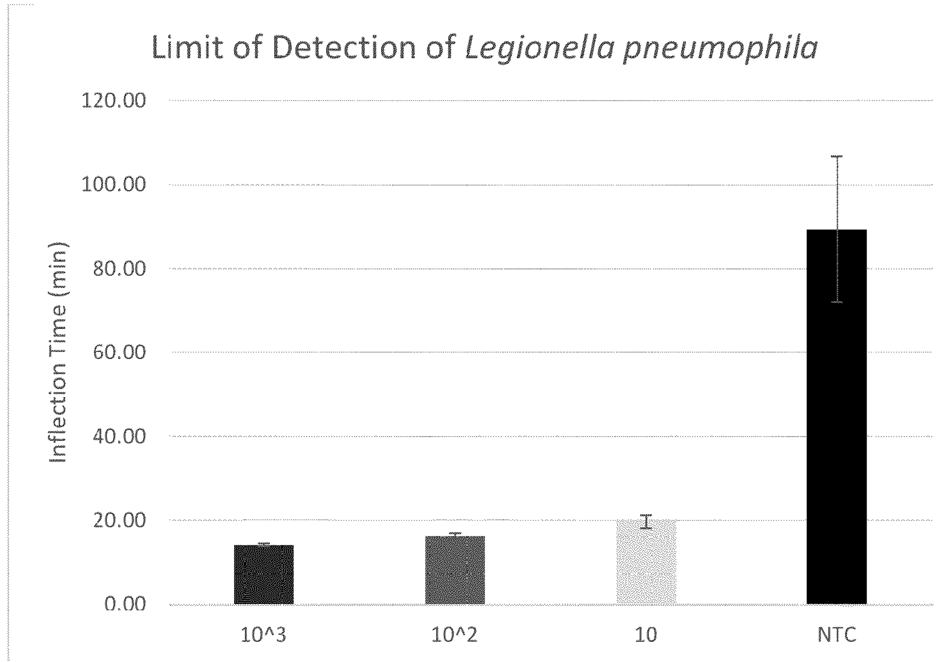
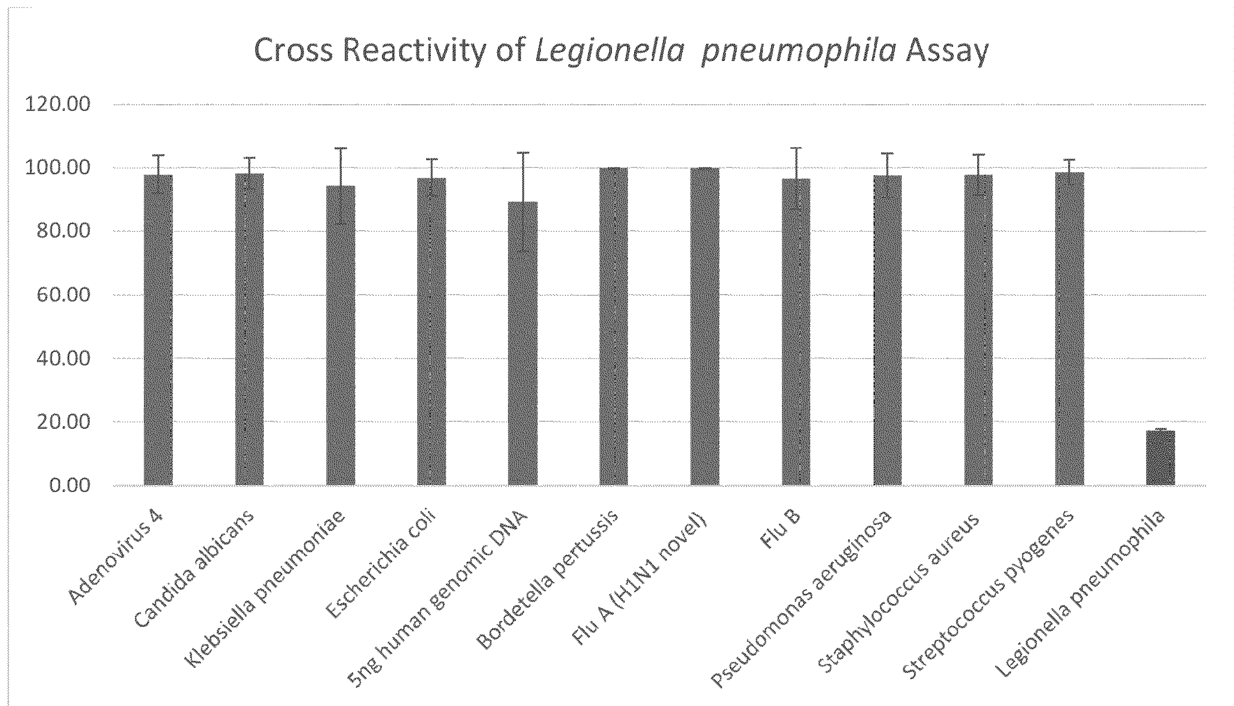
Appendix B: Representative Data for TNAA Assays

Legionella pneumophila





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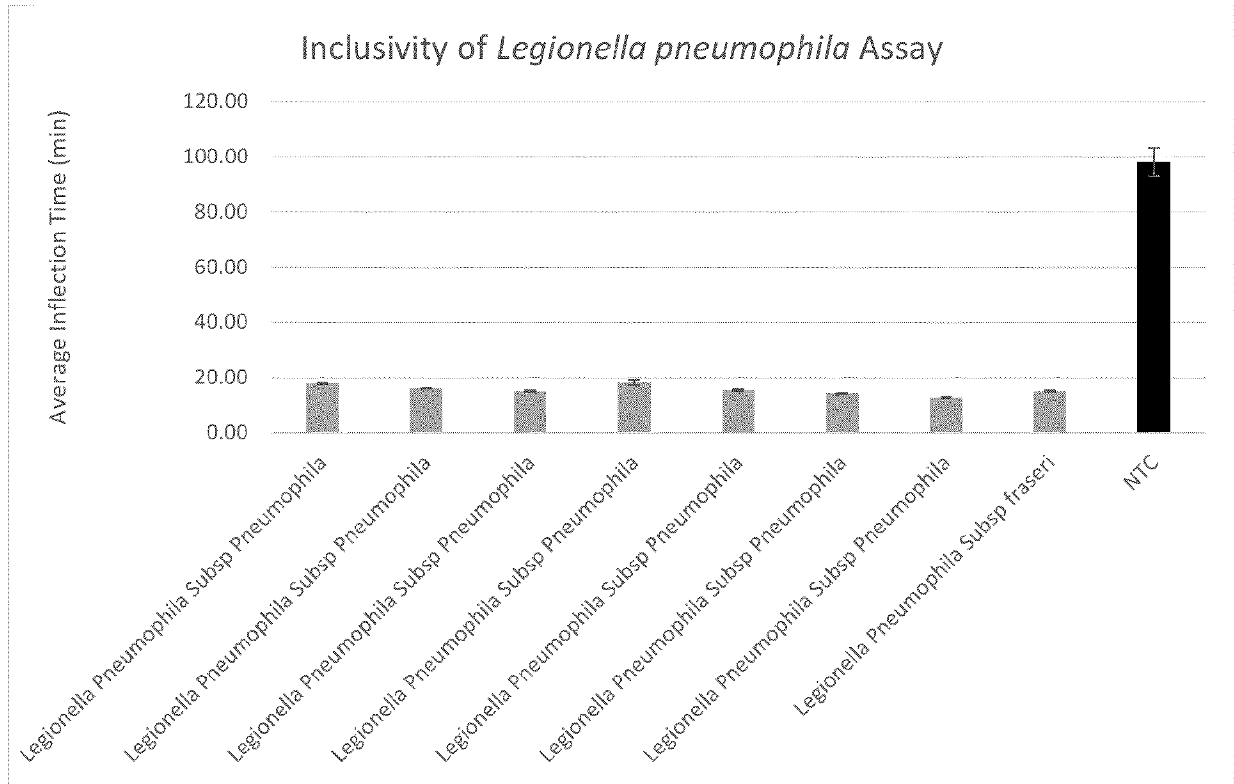
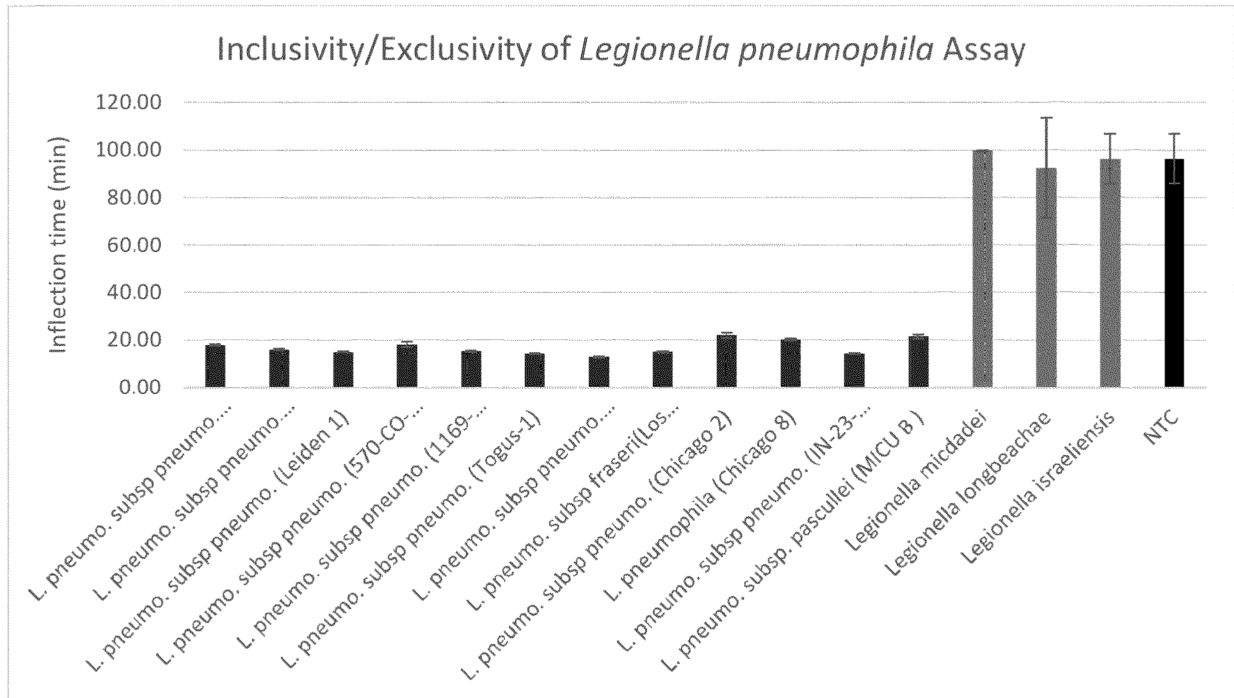
Appendix B: Representative Data for TNAA Assays





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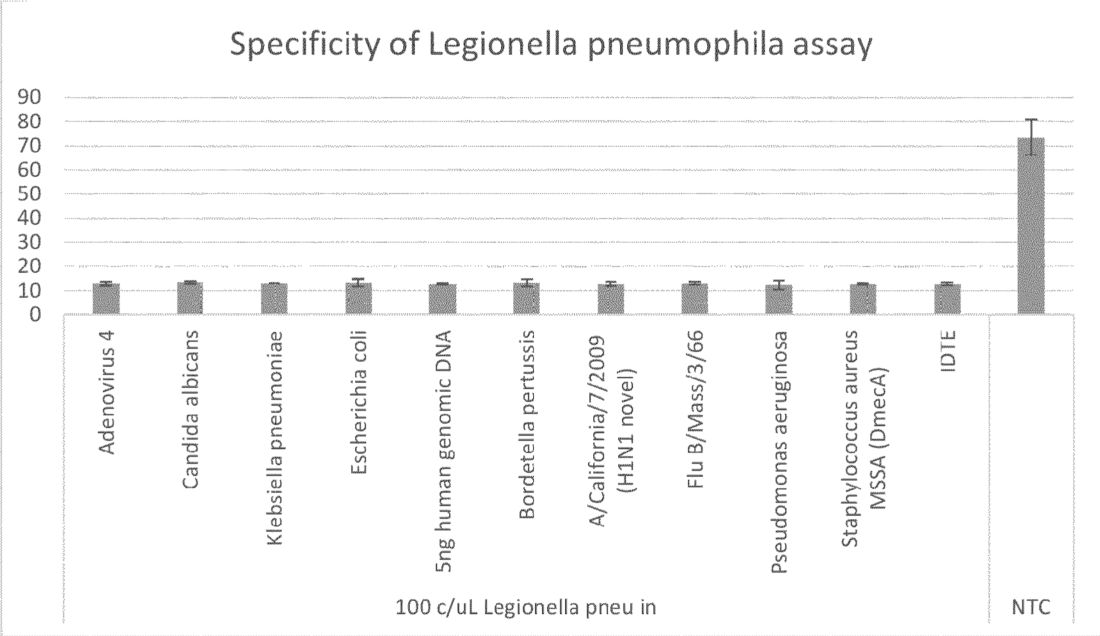

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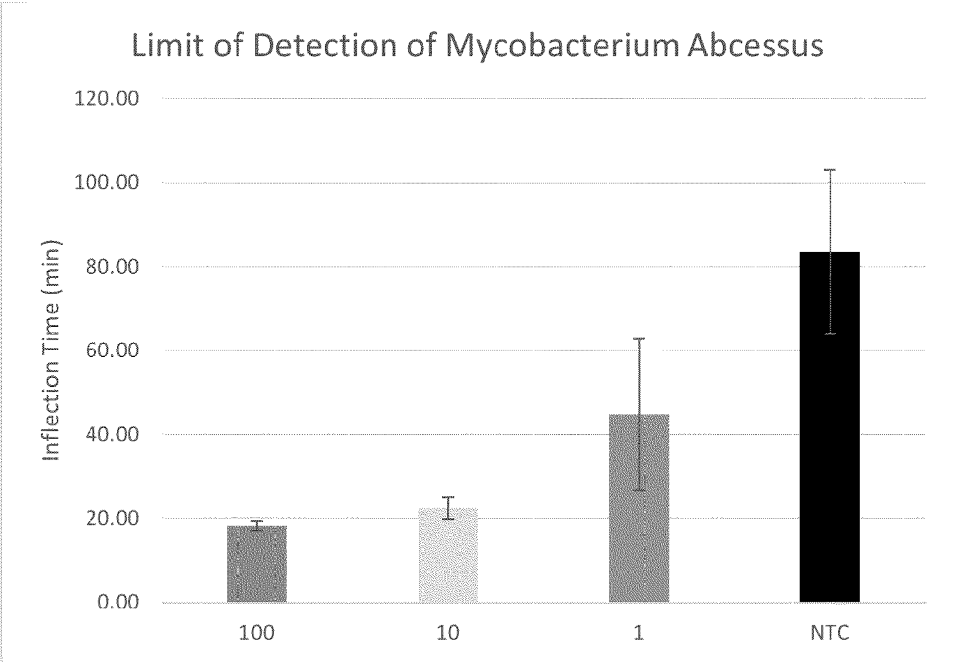
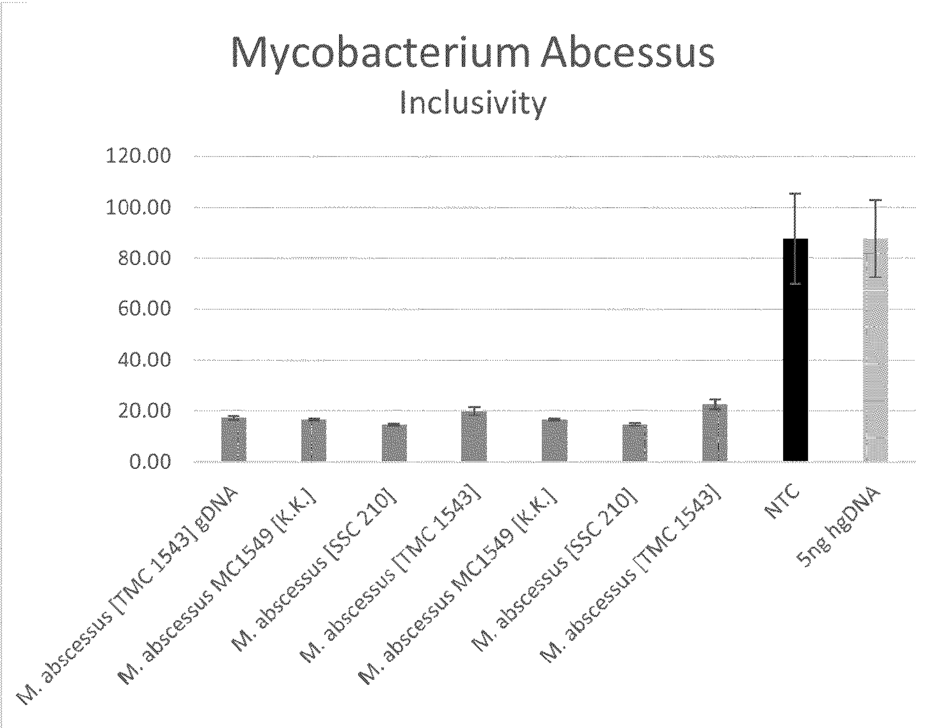
Appendix B: Representative Data for TNAA Assays

Mycobacterium abcessus





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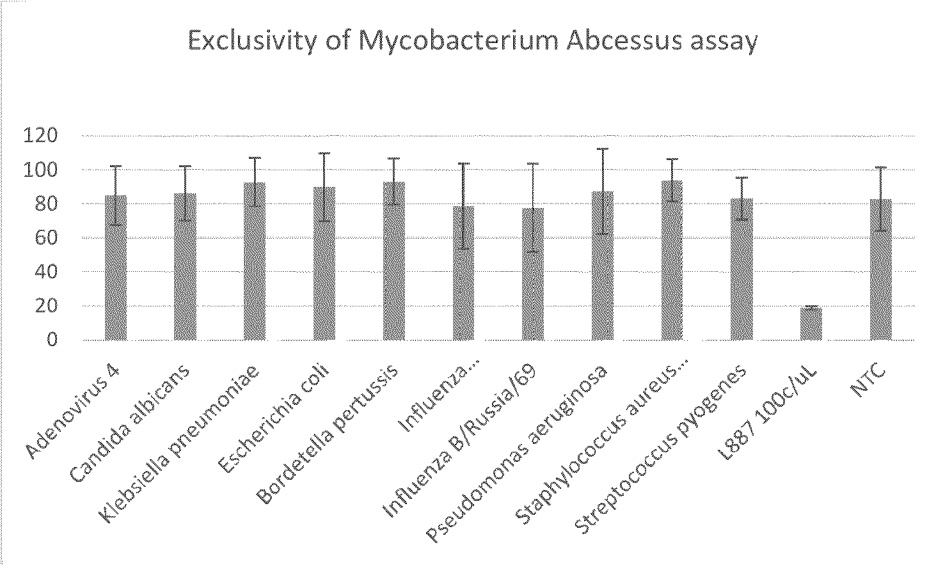

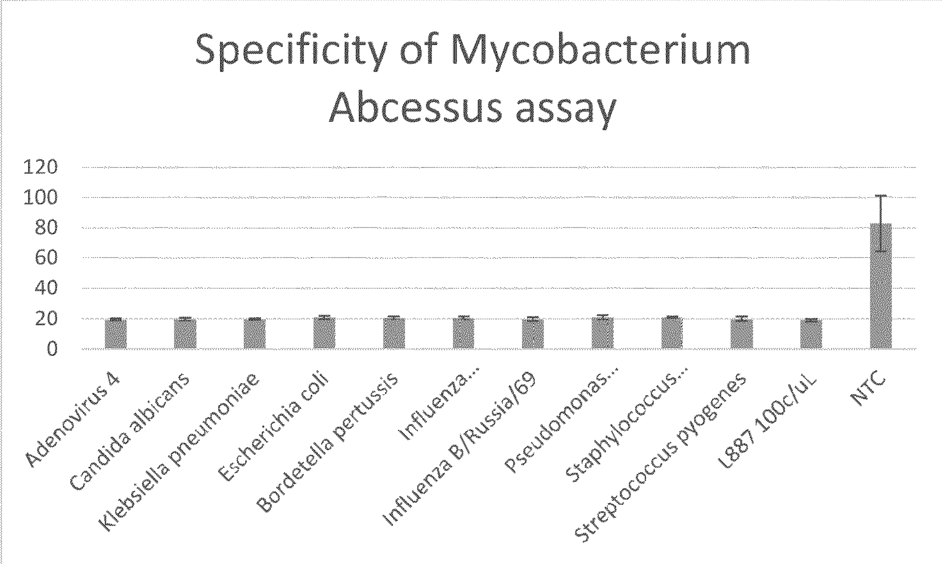

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