Appendix 1
National Microbiology Framework Agreement
Order Form – Reference C
Hologic Laboratories Limited

FROM

Authority:	The Secretary of State for Health and Social Care as part of the Crown acting through the UK Health Security Agency of Nobel House, 17 Smith Square, London, SW1P 3HX (the **"Authority"**)
Invoice address:	Post: The UK Health Security Agency, Nobel House, 17 Smith Square, London, SW1P 3JR Email: [redacted]
Contract Manager:	Name: [redacted] E-mail: [redacted]
Secondary Contact: business operational contact, project manager	Name: [redacted] E-mail: [redacted]
Procurement lead	Name: [redacted] E-mail: [redacted]
Name and address for notices:	Name: [redacted] Senior Commercial Manager E-mail: [redacted] Address: UK Health Security Agency, Windsor House, 50 Victoria Street, London, SW1H 0TL
Internal reference (if applicable):	UKHSA CRE-ID 6244

TO

Supplier:	Hologic Limited Heron House, Oaks Business Park, Crewe Road, Wythenshawe, Manchester, M23 9HZ
Contract Manager:	Name: [redacted] Phone: [redacted] E-mail: [redacted]
Secondary Contact:	Name: [redacted] Email [redacted] Phone: [redacted]
Name and address for notices:	Name: [redacted] Address: Hologic Limited Heron House, Oaks Business Park, Crewe Road, Wythenshawe, Manchester, M23 9HZ

Applicable terms and conditions

The following terms and conditions are applicable to the Contract for this Order:

Appendix A	Call-off Terms and Conditions for the Supply of Goods and the Provision of Services	**Applicable to this Contract**
Appendix B	Optional Additional Call-off Terms and Conditions for Installation and Commissioning Services	☐ **(only applicable if this box is checked)**
Appendix C	Optional Additional Call-off Terms and Conditions for Maintenance Services	☐ **(only applicable if this box is checked)**
Appendix D	Optional Additional Call-off Terms and Conditions for Bespoke Research, Development and Manufacturing Requirements	☐ **(only applicable if this box is checked and to the extent the applicable terms are included in Annex A (Order Specific Key Provisions))**
Appendix E	Optional Additional Call-off Terms and Conditions for Reagent Rental	☐ **(only applicable if this box is checked)**
Appendix F	Optional Additional Call-off Terms and Conditions for Managed Equipment Services	☐ **(only applicable if this box is checked)**
Appendix G	Optional Additional Call-off Terms and Conditions for Clinical Laboratory Diagnostic Testing Services	☐ **(only applicable if this box is checked and to the extent the applicable terms are included in Annex A (Order Specific Key Provisions))**
Appendix H	Further Optional Additional Call-off Terms and Conditions Each of the following clauses in Appendix H is only applicable to this Contract if the relevant box is checked:	**(only applicable if one or more boxes are checked)**

1. TUPE applies at the commencement of the provision of Services	☐
2. TUPE on exit	☐
3. Different levels and/or types of insurance	☐
4. Induction training for Services	☐
5. Further Authority obligations	☐
6. Assignment of Intellectual Property Rights in deliverables, materials and outputs of the Services	☐
7. Inclusion of a Change Control Process	☐
8. Authority step-in rights	☐

	9. Guarantee	☐	
	10. Termination for convenience	☒	
	11. Pre-Acquisition Questionnaire	☐	
	12. Time of the essence (Goods)	☐	
	13. Time of the essence (Services)	☐	
	14. Specific time periods for inspection	☐	
	15. Specific time periods for rights and remedies under Clause 3.6 of Schedule 2 of Appendix A	☐	
	16. Right to terminate following a specified number of material breaches	☐	
	17. Expert Determination	☐	
	18. Consigned Goods	☐	
	19. Improving visibility of Sub-contract opportunities available to Small and Medium Size Enterprises and Voluntary, Community and Social Enterprises	☐	
	20. Management Charges and Information	☐	
	21. COVID-19 related enhanced business continuity provisions	☐	
	22. Buffer stock requirements	☐	
	23. Modern slavery	☒	
	The additional Order Specific Key Provisions set out at Annex A (Order Specific Key Provisions) to this Order Form shall also apply to this Contract.	☒	

1. CONTRACT DETAILS
(1.1) Commencement Date: 24 April 2022
(1.2) Services Commencement Date (if applicable): N/A
(1.3) Contract Price ((i) breakdown and (ii) payment profile): 1.3.1 The total contract value shall be [redacted] (Excl. VAT) (the "**Total Contract Value**") 1.3.2 The Total Contract values comprises the supply for reagents for use in testing workstreams to support STI & Respiratory testing in UKHSA labs located in Bristol, Birmingham and Cambridge. (the "**Goods**") 1.3.3 Following execution of this Contract, the Authority shall submit to the Supplier a single purchase order that equates to the Contract Value (the "**Purchase Order**"). The Purchase Order shall be for the Goods specified in Appendix 1: 1.3.4 For the avoidance of doubt, the Authority is not committed to pay the Total Contract Value. 1.3.5 Only orders placed directly by the Authority are binding under this Contract. 1.3.6 See Appendix 1 - Goods Information and Pricing for the breakdown of Goods. 1.3.7 The Supplier shall comply with the invoicing process and associated terms see Section 2 of Annex A (Order Specific Key Provisions), including the provision of monthly consolidated invoices. 1.3.8 Payment terms are net 30 days in arrears from the date the Authority receives valid consolidated invoices in accordance with this Contract. 1.3.9 The Purchase orders issued by the Authority in respect of this Agreement do not form part of this Agreement.
(1.4) Term of Contract: 1.4.1 This Contract shall be deemed to have commenced on 24 April 2022 (the **"Commencement Date"**) and shall, unless terminated earlier, or extended, in accordance with its terms, expire on 31 March 2023 (the **"Term"**). 1.4.2 The Authority may terminate the Contract for convenience at any time pursuant to clause 10 (Termination for convenience) of Appendix H (Further Optional Additional Call-off Terms and Conditions) of this Contract provided the Authority gives the Supplier not less than 90 days' written notice.
(1.5) Term extension options: 1.5.1 The Authority may give notice of its intention to extend the contract for the period 1 April 2023 to 31 March 2024, or such shorter period as the Authority may specify in the notice, (the **"Extension Period"**) by giving the Supplier written notice no later than 31 August 2022.

2. GOODS AND/OR SERVICES REQUIREMENTS

(2.1) Description of the Goods:

2.1.1 The Authority may, but is not obliged to, order, and the Supplier shall provide, Panther reagents as specified in Appendix 2 (the "**Specification**") to be delivered and used within the UKHSa lab network over the Term (the "**Goods**").

2.1.2 Subject to Clauses of this Order Form, the Authority shall be entitled to order the Goods, and the Supplier shall provide the Goods.

Ordering Procedure:

2.1.3 The Authority may, but shall not be obliged to, provide the Supplier with call off orders for reagents up to, but not exceeding cumulatively the Contract Price.

2.1.4 The Parties agree that the period of 1 WEEKS is adequate notice.

2.1.5 Where the Authority provides the Supplier with a call off order pursuant to clause 2.1.1 above with notice that is not less than the period specified in clause 2.1.4 above then the Supplier shall fulfil such call off order.

2.1.6 Where the Authority provides the Supplier with a call off order pursuant to clause 2.1.1 above with notice that is less than the period specified in clause 2.1.4 above then the Supplier shall use its reasonable endeavours to fulfil such call off order in whole, and where the Supplier is not able to fulfil in whole in part, in the timeframe specified by the Authority.

2.1.7 Where the Authority's call off order made pursuant to clause 2.1.6 has been in the Supplier's possession for a period not less than that set out in clause 2.1.4 above the Supplier shall treat such call off order as if the Authority had submitted it pursuant to clause 2.1.3 accordingly.

2.1.8 The Supplier shall deliver the Goods according to the Specification ("**Specification**") and shall be defined as the Supplier description of the Goods being delivered which will contain any relevant technical information, quality standard, relevant testing and validation information and any relevant handling and storage information given, as set out in Appendix 2.

2.1.9 The supplier warrants that any Goods that are shown to fail this Specification within the expiry date required for the goods are either replaced or full credit given.

(2.2) Premises and Location(s) at which the Goods are to be delivered / provided:

2.2.1 The supplier shall deliver the goods to the Premises and Location(s) detailed in Appendix 3 – Delivery Locations or such other location as the Authority specifies from time to time.

2.2.2 The Supplier shall ensure that all products are labelled with product description, part number, volume, batch number, storage requirements and barcode.

2.2.2 All planned deliveries shall be pre-advised by the Supplier to the Authority's primary delivery contact stated below (individually or collectively be known as the "**Delivery Contact**") at least 48 hours prior to attendance:

2.2.3 Primary delivery contact: Detailed by site in Appendix 3.

2.2.4 The Supplier shall provide the following data when notifying the Delivery Contact:

- Supplier name;
- Authority's Order Number;
- Item reference, Supplier's part code, description and quantity;
- Full service detail at item level and any special instructions originally entered for Authority's Order (e.g. project).

2.2.5 The Delivery Contact will confirm:

- Booking reference number;
- Date and time of service (where applicable); and
- Delivery address.

2.2.6 Delivery of the Goods/Services shall be considered to have occurred when the Delivery Contact or other authorised representative of the Authority at the Authority's nominated location has signed the service / maintenance recording sheet.

2.2.7 The Supplier shall carry out deliveries within the ordinary working hours at the delivery location on the date specified.

(2.3) Key personnel of the Supplier to be involved in the Goods / Services:

Name: [redacted]

Phone: [redacted]

E-mail: [redacted]

(2.4) Performance standards:

- The Supplier shall ensure the goods conform and perform to the Specification.
- Timely delivery of the Services in accordance with section 2.6 below.
- Proof of delivery of the Services to be supplied with each monthly consolidated invoice.

(2.5) Quality Standards & Warranty:

- Unless expressly agreed otherwise the Supplier shall ensure that the Goods have an expiry date of at least 6 months following the date of delivery by the Supplier, to allow the laboratories sufficient time to use the kit.
- The Supplier warrants the Goods shall be fit for purpose and shall conform to the Specification for not less than six (6) months commencing from the date of delivery in accordance with Clause 10 of the Call-Off Terms and Conditions.
- In the event that Goods are deemed to be Defective Goods by the Authority, the Authority, at its sole discretion, shall provide a written notice to the Supplier in accordance with Schedule 2, clause 4.7 of the Call-Off Terms and Conditions.

(2.5.1) Return Conditions:

For Goods that do not meet the quality and performance standards The Return Conditions will be as follows:

- The Supplier is responsible for collecting the Goods.
- The Supplier is responsible for the costs of returning/collecting the Goods.
- Return Conditions shall be in accordance with Schedule 2 - clause 4 (Inspection, rejection, return and recall of the Goods) of the Call Off Terms and Conditions

(2.6) Contract monitoring arrangements:

The Authority Contract Manager (or their delegate) and the Supplier Contract Manager shall meet Monthly (or such other frequency as reasonably requested by the Authority) and no less than quarterly (unless otherwise notified by the Authority) to discuss the Supplier's performance and other matters connected to the delivery of the Contract.

The Supplier shall provide any management information required on a monthly basis to include:

2.6.1 Performance against below KPIs, delivery expectations, demand/call-off plan
2.6.2 Stock and deliveries against contract schedule and forecast
2.6.3 Compliance to processes: Delivery schedules and Invoicing
2.6.4 Overview of any Innovation, product performance/enhancement, service redesign, and horizon plans
2.6.5 Supplier input/issues on contract performance
2.6.6 The Supplier agrees to conform to the following key performance indicators (**KPI's**) during the Term of this Contract and shall be obliged to provide compliance reports at the request of the Authority:

- Quantity of delivery correct against the relevant Order (including deliveries in excess and shortfall of the Order quantity).
- Quality of delivery in accordance with the Framework Agreement and this Contract (including delivery presentation in accordance with the Framework Agreement and this Contract (the delivery must be presented in such a way that it can be unloaded safely and in a ready for use condition taking into consideration the Framework Agreement and this Contract requirements) and damaged Goods (the Goods must be in a condition that is new and ready to use).
- Timely and accurate administration (including booking/amending delivery times and Orders and invoices, delivery advice notes and labels being in accordance with the requirements of the Framework Agreement and this Contract).

(2.7) Management information and meetings:

2.7.1 At the Authority's request, within five (5) Working Days of such request, the Supplier shall provide such management information to the Authority as the Authority may reasonably request from time to time (including without limit any information about the Supplier's supply chain and its compliance in relation to sustainability requirements).

2.7.2 Performance and key performance indicators to be reported by the Supplier on a monthly basis include:

- Overall contract operational performance against service targets and/or service levels;
- Service failures and root cause analysis;
- Invoicing and billing;
- Establish any improvement action plans required;
- Corrective action notices;
- Process improvements / best industry practice;
- Review and discussion of a continuous improvement plan;
- Change control;
- Capacity management achievement of authority's objectives;
- Review of risks; and
- Objective reviews and scope for delivery of Goods and services.
- Commercial opportunity / strategic & executive review meetings shall discuss the following: Supplier's Business Continuity Plan including monitoring supply chain risks and issues
- Strategic development and the delivery of the Goods;
- Explore opportunities to increase value of business (added value, efficiency improvement, business development, cost improvement etc.) to the Authority and the Supplier.

3. CONFIDENTIAL INFORMATION (if applicable)

(3.1) The following information shall be deemed Confidential Information:

- Supplier pricing.
- Contact details including, but not limited to, email addresses, landline / mobile phone numbers, etc. of Supplier representatives
- Contact details including, but not limited to, email addresses, landline / mobile phone numbers, etc. of Authority's representatives

(3.2) Duration that the information shall be deemed Confidential Information:
For a period of three (3) years after the expiry or earlier termination of this Contract unless otherwise agreed in writing by the Parties.

4. DATA PROCESSING (if applicable)

(4.1) Personal Data to be processed by the Supplier:

In accordance with the Data Protection Protocol.

5. LEASE / LICENSE (if applicable)

(5.1) The Authority is granting the following lease or licence to the Supplier:

N/A

Signature:

For and on behalf of the Authority

DocuSigned by:
[redacted]
5CDAB3B7E533421...

Full Name: [redacted]
Job Title/Role: [redacted]
Date Signed: 26th August 2022

Signature:

For and on behalf of the Supplier

DocuSigned by:
[redacted]
1E1CD6B77C2B486...

Full Name: [redacted]
Job Title/Role: [redacted]
Date Signed: 26/08/2022

Annex A

Order Specific Key Provisions

1. Delivery and Risk:

1.1. The Supplier shall deliver the services to the location set out in Appendix 3 of this order form.

1.2. The Supplier will ensure that the provisions of goods are made in accordance with the terms of this Order Form including Annex A, Appendix 1 and the Call-Off Terms and Conditions.

2. Invoicing Process:

2.1 Payment terms are net 30 days from receipt of a valid monthly invoice.

2.2 Within 10 Business Days of receipt of the Supplier's countersigned copy of the Contract, the Authority will send a unique purchase order ("PO") number. The Supplier must be in receipt of a valid PO number before submitting an invoice.

2.3 Notwithstanding submission of the Purchase Order to the Supplier, the Authority is only committed to purchasing such quantities of the Goods as it orders in accordance with this paragraph 3; and submission of the Purchase Order to the Supplier shall not constitute commitment on behalf of the Authority to purchase Goods up to the full Contract Price.

2.4 At least once a week, the Parties operational teams will hold a call to determine the Authority's then current demand for the Goods. At such meetings, the Parties will:

(i) agree the volume of Goods to be delivered for the following week

(ii)review current inventory levels; and

(iii) discuss such other matters as the parties may consider appropriate.

(iv) Purchase obligations are limited to volumes specified in to manage against over ordering of stock. Time is of the essence where the Supplier will deliver to the order as requested and will deliver to the time slots with a plus or minus 30-minute tolerance.

(v) discuss such other matters as the parties may consider appropriate.

2.5 The Supplier shall provide a consolidated monthly invoice to the Authority for all Goods received and accepted by the Authority each month.

2.6 All invoices should be sent for approval and must include the proof of delivery to the Authority's designated finance mailbox e-mail: [redacted] and their agreed representative (to be confirmed at first Supplier meeting) before being submitted for payment.

2.7 The Supplier shall provide compliant invoices that include a valid PO number, PO line item number (if applicable), PO line description, and the details (name and telephone number) of the Authority's authorised representative. Non – compliant invoices will be sent back to the Supplier, which may lead to a delay in a payment.

2.8 In support of Goods being delivered the Supplier shall provide to the Authority a signed delivery note confirming receipt of the Goods by email to [redacted]

2.9 If you have a query regarding an outstanding payment, please contact our Accounts Payable section by email to: [redacted]

Appendix 1

Appendix 1 – Goods information and pricing

Panther Reagents and Consumables					
Product Code	Description	Annual Volume Commitment	Term		Offer Price per kit
			Start Date	End Date	
PRD-05576	Aptima Combo 2 Assay Panther 100 Test Kit	As Required	10/04/2022	31/03/2023	[redacted]
PRD-05571	Aptima Combo 2 Assay Panther 250 Test Kit	540			[redacted]
302925	Aptima CT Assay Panther 100 Test Kit	100			[redacted]
302927	Aptima GC Assay Panther 100 Test Kit	50			[redacted]
303209	Aptima TV Assay Panther 100 Test Kit	20			[redacted]
303163	Aptima TV Assay Panther 250 Test Kit	As Required			[redacted]
PRD-03919	Aptima Mgen Assay Panther 100 Test Kit	12			[redacted]
301040	Aptima Urine Specimen	1400			[redacted]

Hologic Ltd., Heron House, Oaks Business Park, Crewe Road, Wythenshawe, Manchester, M23 9HZ, UK
T: [redacted] | F: [redacted] | [redacted]
Registered in England and Wales No. 2722343

	Collection Kit (box of 50)				
301041	Aptima Unisex Swab Specimen Collection Kit (Box of 50)	As Required			[redacted]
PRD-03546	Aptima Multitest Swab Specimen Collection Kit (Box of 50)	1400			[redacted]
105575	Aptima Urine Tubes (bag 100)	As Required			[redacted]
903031	Tecan 1000ul filter tips (960)	764			[redacted]
303096	Panther run Kit (MTU's; Waste Bag; Waste Bin Covers; Assay Fluids; Auto Detect)	As Required			
104772-02	Multi-Tube Unit (MTU) Kit	750			[redacted]
902731	Panther Waste Bag Kit	70			[redacted]
504405	Panther Waste Bin Cover	70			[redacted]
303014	Panther Assay Fluids Kit	320			[redacted]
303013	Panther Auto Detect Kit	320			[redacted]
402950	Panther Advanced Cleaning Solution, 255ml	100			[redacted]
302807	Aptima TV Controls Kit (additional to those supplied in the test kit)	As Required			[redacted]
CL0041	Replacement Caps Amp & Probe Reagent 250TK + Amp & Probe, Enzyme reagent 100 Test Kit	36			[redacted]
CL0040	Replacement Caps for TCR and selection 250 Test Kit	36			[redacted]
501616	Replacement Caps for Enzyme 250 Test Kit	36			[redacted]
501604	Replacement Caps for TCR and Selection 100 Test Kit	36			[redacted]

Fusion Reagents and Consumables				
Product Code	Description	Annual Volume Commitment	Term	Offer Price per kit
PRD-04328	Panther Fusion Flu A/B/RSV Assay Cartridge (96 Tests)	104	10/04/2022 – 31/03/2023	[redacted]
PRD-04329	Panther Fusion Paraflu Assay Cartridge (96 Tests)	104		
PRD-04330	Panther Fusion AdV/hMPV/RV Assay Cartridge (96 tests)	104		
PRD-04868	Panther Fusion Bordetella Assay Cartridge (96 Tests)	3		
PRD-04303	Panther Fusion DNA/RNA Enzyme Cartridge (96 tests)	20		
PRD-04336	Panther Fusion Flu A/B/RSV Assay Controls (5 Controls per Unit)	3 sets of controls per UKHSA Laboratory		
PRD-04338	Panther Fusion ParaFlu Assay Controls (5 Controls per Unit)	3 sets of controls per UKHSA Laboratory		
PRD-04337	Panther Fusion AdV/hMPV/RV Controls (5 Controls Per Unit)	3 sets of controls per UKHSA Laboratory		
PRD-04869	Panther Fusion Bordetella Assay Controls (5 Controls Per Unit)	3 sets of controls per UKHSA Laboratory		
PRD-04339	Panther Fusion Lysis Tubes (100 tubes per bag)	100		
PRD-04306	IC DNA Primers (~2000 tests per unit)	1		
PRD-04308	IC DNA Probes (~2000 tests per unit)	1		

PRD-04307	IC RNA Primers (~2000 tests per unit)	1	Quote UKD	[redacted]
PRD-04309	IC RNA Probes (~2000 tests per unit)	1		
PRD-04000	Panther Fusion Tube Tray (1008 tubes per tray)	40		
PRD-04430	Panther Fusion Universal Fluids kit (1 x oil; 1 x Elution buffer)	As Required		
PRD-04331	Panther Fusion Assay Fluids (2 x Extraction reagent; 2 x IC; 1 x Recon Buffer)	As Required		
PRD-04331	Panther Fusion Extraction Reagent-S (960 tests)	40		
PRD-04332	Panther Fusion Internal Control- S (960 tests)	40		
PRD-04334	Panther Fusion Elution Buffer (2400 tests)	40		
PRD-04333	Panther Fusion Reconstitution Buffer (1920 tests)	25		
PRD-04335	Panther Fusion Oil Reagent (1920 Tests)	25		
PRD-04477	Panther Fusion Extraction Reagents-X (960 Tests)	As Required		
PRD-04476	Panther Fusion Internal Control- X (960 tests)	As Required		
PRD-04311	Panther Fusion Open Access PPR Tubes	As Required		
PRD-04312	Panther Fusion Open Access PPR Caps	As Required		

SARS-CoV-2/Flu A/B/RSV Assay (Panther Fusion™ System)

For *in vitro* diagnostic use.

For U.S. Export only.

CONTENTS

General Information

Intended Use

The Panther Fusion™ SARS-CoV-2/Flu A/B/RSV assay is a fully automated multiplexed real-time RT-PCR test intended for the qualitative detection and differentiation of RNA from SARS-CoV-2 virus, influenza A virus (Flu A), influenza B virus (Flu B) and respiratory syncytial virus (RSV) isolated and purified from nasopharyngeal (NP) swab specimens obtained from individuals exhibiting signs and symptoms of a respiratory tract infection. Clinical signs and symptoms of respiratory viral infection due to SARS-CoV-2, influenza and RSV can be similar. This assay is intended to aid in the differential diagnosis of SARS-CoV-2, influenza A virus, influenza B virus and RSV infections in humans and is not intended to detect influenza C virus infections.

Negative results do not preclude SARS-CoV-2, influenza A virus, influenza B virus or RSV infections and should not be used as the sole basis for treatment or other management decisions. This assay is designed for use on the Panther Fusion system.

Summary and Explanation of the Test

Respiratory viruses are responsible for a wide range of acute respiratory tract infections including the common cold, influenza (flu), RSV infection, COVID-19 and croup and represent the most common cause of acute illness in the United States. Some symptoms of COVID-19, flu and RSV are similar making diagnosis based on symptoms alone virtually impossible.[1,2]

Disease severity of flu and RSV can be especially high in the young, the immunocompromised, and elderly patients. Accurate and timely diagnosis of the cause of respiratory tract infections has many benefits. They include improved treatment of the patient by ensuring appropriate antiviral treatment (e.g. oseltamivir for influenza),[3] decreasing the overall cost of care, reducing the potential for further development of antimicrobial resistance due to excessive and inappropriate use of antibiotics,[4] assisting infection control personnel in providing appropriate measures to minimize nosocomial spread, and providing valued information to public health authorities regarding which viruses are circulating in the community.[5]

Influenza is an acute respiratory illness caused by infection with the influenza virus, primarily types A and B.[6] Influenza A viruses are further categorized into subtypes based on the two major surface protein antigens: hemagglutinin (H) and neuraminidase (N).[7] Influenza B viruses are not categorized into subtypes.[7] Influenza viruses continuously undergo genetic changes including drift (random mutation) and variation (genomic reassortment), generating new strains of virus each year, leaving the human population vulnerable to these seasonal changes. Epidemics occur yearly (typically in winter) and while both types A and B circulate in the population, type A is usually dominant. Transmission of influenza is primarily via airborne droplet (coughing or sneezing). Symptoms arise on average 1 to 2 days post-exposure and include fever, chills, headache, malaise, cough, and coryza.

Complications due to influenza include pneumonia causing increased morbidity and mortality in pediatric, elderly and immunocompromised populations. Influenza occurs globally with an annual attack rate estimated at 5%–10% in adults and 20%–30% in children. Illnesses can result in hospitalization and death mainly among high-risk groups (the very young, elderly or chronically ill). Worldwide, these annual epidemics are estimated to result in about 3 to 5 million cases of severe illness, and about 250,000 to 500,000 deaths.[8]

Respiratory syncytial virus (RSV) is a leading cause of respiratory infections in infants and children. There are 2 types of RSV (A and B) based on antigenic and surface protein variations. Most yearly epidemics (typically during winter) contain a mix of type A and B viruses, but one subgroup can dominate during a season. RSV infection can cause severe respiratory illness among all ages but is more prevalent in pediatric, elderly and immunocompromised populations. Annually in the United States, RSV infection has been associated with an estimated 58,000 hospitalizations and 2.1 million outpatient visits among children younger than 5 years, and 177,000 hospitalizations and 14,000 deaths among adults older than 65 years.[9]

Coronaviruses are a large family of viruses which may cause illness in animals or humans. In humans, several coronaviruses are known to cause respiratory infections ranging from the common cold to more severe diseases such as Middle East Respiratory Syndrome (MERS) and Severe Acute Respiratory Syndrome (SARS). The most recently discovered coronavirus, SARS-CoV-2, causes the associated coronavirus disease COVID-19. This new virus and disease were unknown before outbreak in Wuhan, China, in December 2019.[9]

People with COVID-19 have had a wide range of symptoms reported, ranging from mild symptoms to severe illness. Symptoms may appear 2-14 days after exposure to the virus. People with COVID-19 may exhibit fever or chills, cough, shortness of breath or difficulty breathing, fatigue, muscle or body aches, headache, new loss of taste or smell, sore throat, congestion or runny nose, nausea or vomiting, and/or diarrhea.[10] On March 11, 2020, the COVID-19 outbreak was characterized as a pandemic by the World Health Organization (WHO).[11]

Principles of the Procedure

The Panther Fusion SARS-CoV-2/Flu A/B/RSV assay involves the following steps: sample lysis, nucleic acid capture and elution transfer, and multiplex RT-PCR when analytes are simultaneously amplified, detected and differentiated. Nucleic acid capture and elution takes place in a single tube on the Panther Fusion system. The eluate is transferred to the Panther Fusion system reaction tube containing the assay reagents. Multiplex RT-PCR is then performed for the eluted nucleic acid on the Panther Fusion system.

Nucleic acid capture and elution: Prior to processing and testing on the Panther Fusion system, specimens are transferred to a Specimen Lysis Tube containing specimen transport media (STM). STM lyses the cells, releases target nucleic acid, and protects them from degradation during storage.

The Internal Control-S (IC-S) is added to each test specimen and controls via the working Panther Fusion Capture Reagent-S (wFCR-S). The IC-S in the reagent monitors specimen processing, amplification and detection.

Capture oligonucleotides hybridize to nucleic acid in the test specimen. Hybridized nucleic acid is then separated from the specimen in a magnetic field.

Wash steps remove extraneous components from the reaction tube. The elution step elutes purified nucleic acid. During the nucleic acid capture and elution step, total nucleic acid is isolated from specimens.

Elution transfer and RT-PCR: During the elution transfer step, eluted nucleic acid is transferred to a Panther Fusion reaction tube already containing oil and reconstituted mastermix.

Target amplification occurs via RT-PCR. A reverse transcriptase generates a DNA copy of the target sequence. Target specific forward and reverse primers and probes then amplify targets while simultaneously detecting and discriminating multiple target types via multiplex RT-PCR.

The Panther Fusion system compares the fluorescence signal to a predetermined cut-off to produce a qualitative result for the presence or absence of the analyte.

The analytes and the channel used for their detection on the Panther Fusion system is summarized in the table below.

Analyte	Gene Targeted	Instrument Channel
Influenza A Virus	Matrix	FAM
Respiratory Syncytial Virus A/B	Matrix	HEX
SARS-CoV-2	ORF1ab	ROX
Influenza B Virus	Matrix	RED647
Internal Control	Not applicable	RED677

Warnings and Precautions

A. For *in vitro* diagnostic use. Carefully read this entire package insert and the *Panther*/*Panther Fusion System Operator's Manual*.

B. For professional use.

C. The Panther Fusion Enhancer Reagent-S (FER-S) is corrosive, harmful if swallowed and causes severe skin burns and eye damage.

D. Only personnel adequately trained on the use of this assay and in handling potentially infectious materials should perform these procedures. If a spill occurs, immediately disinfect using appropriate site procedures.

E. Handle all specimens as if infectious using safe laboratory procedures. Refer to Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with 2019-nCoV. https://www.cdc.gov/coronavirus/2019-ncov/lab/lab-biosafety-guidelines.html.

F. Specimens may be infectious. Use Universal Precautions when performing this assay. Proper handling and disposal methods should be established by the laboratory director. Only personnel adequately trained in handling infectious materials should be permitted to perform this diagnostic procedure.[7]

Note: *If infection with a novel influenza A virus is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, collect specimens with appropriate infection control precautions for novel virulent influenza viruses and send to state or local health department for testing. Do not attempt viral culture in these cases unless a BSL 3+ facility is available to receive and culture specimens.*

G. If infection with 2019-nCoV is suspected based on current clinical screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions.

H. Use appropriate personal protective equipment when collecting and handling specimens from individuals suspected of being infected with SARS-CoV-2 as outlined in the CDC Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with 2019 Novel Coronavirus (2019-nCoV).

I. Use only supplied or specified disposable laboratory ware.

J. Wear disposable, powderless gloves, protective eye wear, and laboratory coats when handling specimens and reagents. Wash hands thoroughly after handling specimens and reagents. Dispose of all material that has come into contact with specimens and reagents in accordance with applicable national, international, and regional regulations.

K. Expiration dates listed on the Panther Fusion Specimen Lysis Tubes pertain to the transfer of sample into the tube and not to testing of the sample. Specimens collected/transferred any time prior to these expiration dates are valid for testing provided they are transported and stored in accordance with the appropriate package insert, even if these expiration dates have passed.

L. Maintain proper storage conditions during specimen shipping to ensure the integrity of the specimen. Specimen stability under shipping conditions other than those recommended has not been evaluated.

M. Avoid cross-contamination during the specimen handling steps. Specimens can contain extremely high levels of virus or other organisms. Ensure that specimen containers do not come in contact with one another, and discard used materials without passing them over any open containers. Change gloves if they come in contact with specimens.

N. Do not use the reagents and controls after the expiration date.

O. Store assay components at the recommended storage condition. See *Reagent Storage and Handling Requirements (*page 7), and *Panther Fusion System Test Procedure* (page 12) for more information.

P. Do not combine any assay reagents or fluids. Do not top off reagents or fluids; the Panther Fusion system verifies reagent levels.

Q. Avoid microbial and ribonuclease contamination of reagents.

R. Quality control requirements must be performed in conformance with local, state, and/or federal regulations or accreditation requirements and your laboratory's standard quality control procedures.

S. Do not use the assay cartridge if the storage pouch has lost its seal or if the assay cartridge foil is not intact. Contact Hologic if either occurs.

T. Do not use the fluid packs if the foil seal is leaking. Contact Hologic if this occurs.

U. Handle the assay cartridges with care. Do not drop or invert assay cartridges. Avoid prolonged exposure to ambient light.

V. Do not use material that may contain Guanidinium thiocyanate or any guanidine-containing materials on the instrument. Highly reactive and/or toxic compounds may form if combined with sodium hypochlorite.

W. Some reagents in the kit are labeled with hazard information.

Note: *For information on any hazard and precautionary statements that may be associated with reagents, refer to the Safety Data Sheet Library at www.hologicsds.com.*

<table>
<tr>
<td>  </td>
<td>Panther Fusion Oil
Polydimethylsiloxane 95-100%

WARNING
H315 - Causes skin irritation
H319 - Causes serious eye irritation
P264 - Wash face, hands and any exposed skin thoroughly after handling
P280 - Wear protective gloves/protective clothing/eye protection/face protection</td>
</tr>
<tr>
<td> 
  </td>
<td>Panther Fusion Enhancer Reagent-S
Lithium Hydroxide, Monohydrate 5-10%

DANGER
H302 - Harmful if swallowed
H314 - Causes severe skin burns and eye damage
P260 - Do not breathe dust/fume/gas/mist/vapors/spray
P264 - Wash face, hands and any exposed skin thoroughly after handling
P270 - Do not eat, drink or smoke when using this product
P280 - Wear protective gloves/protective clothing/eye protection/face protection</td>
</tr>
</table>

Reagent Storage and Handling Requirements

A. The following table provides storage and handling requirements for this assay.

Reagent	Unopened Storage	On Board/ Open Stability[1]	Opened Storage
Panther Fusion SARS-CoV-2/Flu A/B/RSV Assay Cartridge	2°C to 8°C	60 days	2°C to 8°C [2]
Panther Fusion Capture Reagent-S (FCR-S)	15°C to 30°C	30 days	15°C to 30°C
Panther Fusion Enhancer Reagent-S (FER-S)	15°C to 30°C	30 days	15°C to 30°C
Panther Fusion Internal Control-S (IC-S)	2°C to 8°C	(In wFCR-S)	Not applicable
Panther Fusion Elution Buffer	15°C to 30°C	60 days	15°C to 30°C
Panther Fusion Oil	15°C to 30°C	60 days	15°C to 30°C
Panther Fusion Reconstitution Buffer I	15°C to 30°C	60 days	15°C to 30°C
Panther Fusion SARS-CoV-2/Flu A/B/RSV Positive Control	2°C to 8°C	Single use vial	Not applicable-single use
Panther Fusion Negative Control	2°C to 8°C	Single use vial	Not applicable-single use

When reagents are removed from the Panther Fusion system, return them immediately to their appropriate storage temperatures.

[1] On board stability starts at the time the reagent is placed on the Panther Fusion system for the Panther Fusion SARS-CoV-2/Flu A/B/RSV assay cartridge, FCR-S, FER-S and IC-S. On board stability starts for the Panther Fusion Reconstitution Buffer I, Panther Fusion Elution Buffer, and Panther Fusion Oil when the reagent pack is first used.

[2] If removed from the Panther Fusion system, store the assay cartridge in an air-tight container with desiccant at the recommended storage temperature.

B. Working Panther Fusion Capture Reagent-S and Panther Fusion Enhancer Reagent-S are stable for 60 days when capped and stored at 15ºC to 30ºC. Do not refrigerate.

C. Discard any unused reagents that have surpassed their on board stability.

D. Controls are stable until the date indicated on the vials.

E. Avoid cross-contamination during reagent handling and storage.

F. **Do not freeze reagents.**

Specimen Collection and Storage

Specimens - Clinical material collected from patient placed in an appropriate transport system. For the Panther Fusion SARS-CoV-2/Flu A/B/RSV assay, this includes NP swab specimens in viral transport medium (VTM), or universal transport medium (UTM).

Samples - Represents a more generic term to describe any material for testing on the Panther Fusion system including specimens, specimens transferred into a Panther Fusion Specimen Lysis Tube and controls.

Note: *Handle all specimens as if they contain potentially infectious agents. Use Universal Precautions.*

Note: *Take care to avoid cross-contamination during specimen handling steps. For example, discard used material without passing over open tubes.*

Specimen Collection

Collect NP swab specimens according to standard technique using a polyester-, rayon, or nylon-tipped swab. Immediately place the swab specimen into 3mL of VTM or UTM.

Note: *Do not use medium that may contain Guanidium thiocyanate or any guanidine-containing material.*

Specimen Processing

Specimen Processing with the Panther Fusion Specimen Lysis Tube

1. Prior to testing on the Panther Fusion system, transfer specimen* collected in UTM or VTM into a Panther Fusion Specimen Lysis Tube.
   - Transfer 500 µL of the specimen to a Panther Fusion Specimen Lysis Tube.

   Note: *When testing frozen specimen, allow specimen to reach room temperature prior to processing.*

Specimen Storage

Storing Specimens with the Panther Fusion Specimen Lysis Tube

1. After collection, specimens can be stored at 2°C to 8°C up to 96 hours before transferred to the Panther Fusion Specimen Lysis Tube. Remaining specimen volumes can be stored at ≤-70°C.
2. Samples (in the Panther Fusion Specimen Lysis Tube) can be stored under the following conditions:
   - 15°C to 30°C up to 6 days or
   - 2°C to 8°C, -20°C, and -70°C for up to 30 days
3. Previously tested samples should be covered with a new, clean plastic film or foil barrier.
4. If assayed samples need to be frozen or shipped, remove the penetrable cap and place a new non-penetrable cap on the specimen tubes. If samples need to be shipped for testing at another facility, recommended temperatures must be maintained. Prior to uncapping previously tested and recapped samples, specimen transport tubes must be centrifuged for 5 minutes at 420 Relative Centrifugal Force (RCF) to bring all of the liquid down to the bottom of the tube. Avoid splashing and cross-contamination.

Specimen Transport

Maintain specimen storage conditions as described in the *Specimen Collection and Storage section on* page 8.

Note: *Specimens must be shipped in accordance with applicable national, international, and regional transportation regulations.*

Panther Fusion System

The Panther Fusion System is an integrated nucleic acid testing system that fully automates all steps necessary to perform various Panther Fusion assays from sample processing through amplification, detection, and data reduction.

Reagents and Materials Provided for Panther Fusion SARS-CoV-2/Flu A/B/RSV Assay

Assay Packaging

Components[1]	Part No.	Storage
Panther Fusion SARS-CoV-2/Flu A/B/RSV Assay Cartridges 96 Tests Panther Fusion SARS-CoV-2/Flu A/B/RSV assay cartridge, 12 tests, 8 per box	PRD-07400	2°C to 8°C
Panther Fusion Internal Control-S 960 Tests Panther Fusion Internal Control-S tube, 4 per box	PRD-04332	2°C to 8°C
Panther Fusion SARS-CoV-2/Flu A/B/RSV Controls Panther Fusion SARS-CoV-2/Flu A/B/RSV Positive Controls tube, 5 per box Panther Fusion Negative Control tube, 5 per box	PRD-07401	2°C to 8°C
Panther Fusion Extraction Reagent-S 960 Tests Panther Fusion Capture Reagent-S bottle, 240 tests, 4 per box Panther Fusion Enhancer Reagent-S bottle, 240 tests, 4 per box	PRD-04331	15°C to 30°C
Panther Fusion Elution Buffer 2400 Tests Panther Fusion Elution Buffer pack, 1200 tests, 2 per box	PRD-04334	15°C to 30°C
Panther Fusion Reconstitution Buffer I 1920 Tests Panther Fusion Reconstitution Buffer I pack, 960 tests, 2 per box	PRD-04333	15°C to 30°C
Panther Fusion Oil 1920 Tests Panther Fusion Oil pack, 960 tests, 2 per box	PRD-04335	15°C to 30°C

[1] Components can also be ordered in the following bundles:
Panther Fusion Universal Fluids Kit, PRD-04430, contains 1 each Panther Fusion Oil and Panther Fusion Elution buffer.
Panther Fusion Assay Fluids I-S, PRD-04431, contains 2 Panther Fusion Extraction Reagents-S, 2 Panther Fusion Internal Control-S, and 1 Panther Fusion Reconstitution Buffer I.

Individually Packaged Items

Items	Part No.
Panther Fusion Specimen Lysis Tubes, 100 per bag	PRD-04339

Materials Required and Available Separately

Note: *Materials available from Hologic have catalog numbers listed, unless otherwise specified.*

Material	Cat. No.
Panther™ System	303095
Panther Fusion System	PRD-04172
Panther Fusion Module	PRD-04173
Aptima™ Assay Fluids Kit (Aptima Wash Solution, Aptima Buffer for Deactivation Fluid, and Aptima Oil Reagent)	303014 (1000 tests)
Multi-tube units (MTUs)	104772-02
Panther Waste Bag Kit	902731
Panther Waste Bin Cover	504405
Or Panther System Run Kit for Real Time Assays contains MTUs, waste bags, waste bin covers, and assay fluids	PRD-03455 (5000 tests)
Or Panther System Run Kit (when running TMA assays in parallel with real time-TMA assays) contains MTUs, waste bags, waste bin covers, auto detect*, and assay fluids	303096 (5000 tests)
Panther Fusion Tube Trays, 1008 tests, 18 trays per box	PRD-04000
Tips, µL, filtered, liquid-sensing, conductive, and disposable. Not all products are available in all regions. Contact your representative for region-specific information.	901121 (10612513 Tecan) 903031 (10612513 Tecan) MME-04134 (30180117 Tecan) MME-04128
Aptima penetrable caps (optional)	105668
Replacement non-penetrable caps (optional)	103036A
Replacement extraction reagent bottle caps	CL0040
P1000 pipettor and tips with hydrophobic plugs	-
Bleach, 5% to 8.25% (0.7 M to 1.16 M) sodium hypochlorite solution **Note**: Refer to the *Panther/Panther Fusion System Operator's Manual* for instructions on preparing diluted sodium hypochlorite solution.	-
Disposable powderless gloves	-

*Needed only for Panther Aptima TMA assays.

Panther Fusion System Test Procedure

Note: *Refer to the Panther/Panther Fusion System Operator's Manual for additional procedural information.*

A. Work Area Preparation

1. Wipe down work surfaces with 2.5% to 3.5% (0.35 M to 0.5 M) sodium hypochlorite solution. Allow the sodium hypochlorite solution to contact surfaces for at least 1 minute and follow with a deionized (DI) water rinse. Do not allow the sodium hypochlorite solution to dry. Cover the bench surface with clean, plastic-backed absorbent laboratory bench covers.
2. Clean a separate work surface where samples will be prepared using the procedure described in step A.1.

B. Reagent Preparation

1. Remove the bottles of IC-S, FCR-S and FER-S from storage.
2. Open the bottles of IC-S, FCR-S and FER-S, and discard the caps. Open the TCR door on the upper bay of the Panther Fusion system.
3. Place the IC-S, FCR-S and FER-S bottles in the appropriate positions on the TCR carousel.
4. Close the TCR door.

Note: *The Panther Fusion system adds the IC-S to the FCR-S. After the IC-S is added to the FCR-S, it is referred to as wFCR-S (working FCR-S). If the FCR-S and FER-S are removed from the system, use new caps and immediately store according to the proper storage conditions.*

C. Specimen Handling

Note: *Prepare specimens per the Specimen Processing instructions in the Specimen Collection and Storage section before loading specimens onto the Panther Fusion system.*

1. **Do not vortex samples.**
2. Inspect sample tubes before loading into the rack. If a sample tube contains bubbles or has a lower volume than is typically observed, gently tap the bottom of the tube to bring contents to the bottom.

Note: *To avoid a processing error, ensure adequate specimen volume is added to the Panther Fusion Specimen Lysis Tube. When 500 µL of NP swab specimen is added to the Panther Fusion Specimen Lysis Tube, there is sufficient volume to perform 3 nucleic acid extractions.*

D. System Preparation

For instructions on setting up the Panther Fusion system including loading samples, reagents, assay cartridges and universal fluids, refer to the *Panther/Panther Fusion System Operator's Manual.*

Procedural Notes

A. Controls

1. The Panther Fusion SARS-CoV-2/Flu A/B/RSV Positive Control and Panther Fusion Negative Control can be loaded in any rack position, in any Sample Bay lane on the Panther Fusion system.
2. Once the control tubes are pipetted and are processed for the Panther Fusion SARS-CoV-2/Flu A/B/RSV assay, they are active for up to 30 days (control frequency configured by an administrator) unless control results are invalid or a new assay cartridge lot is loaded.
3. Each control tube can be tested once.
4. Patient specimen pipetting begins when one of the following two conditions is met:
   a. Valid results for the controls are registered on the system.
   b. A pair of controls is currently in process on the system.

Quality Control

A run or specimen result may be invalidated by the Panther Fusion system if problems occur while performing the assay. Specimens with invalid results must be retested.

Negative and Positive Controls

To generate valid results, a set of assay controls must be tested. One replicate of the negative assay control and positive assay control must be tested each time a new lot of assay cartridges is loaded on the Panther Fusion system or when the current set of valid controls for an active cartridge lot have expired.

The Panther Fusion system is configured to require assay controls run at an administrator-specified interval of up to 30 days. Software on the Panther Fusion system alerts the operator when assay controls are required and does not start new tests until the assay controls are loaded and have started processing.

During processing, criteria for acceptance of the assay controls are automatically verified by the Panther Fusion system. To generate valid results, the assay controls must pass a series of validity checks performed by the Panther Fusion system.

If the assay controls pass all validity checks, they are considered valid for the administrator-specified time interval. When the time interval has passed, the assay controls are expired by the Panther Fusion system and requires a new set of assay controls be tested prior to starting any new samples.

If any one of the assay controls fails the validity checks, the Panther Fusion system automatically invalidates the affected samples and requires a new set of assay controls be tested prior to starting any new samples.

Internal Control

An internal control is added to each sample during the extraction process. During processing, the internal control acceptance criteria is automatically verified by the Panther Fusion system software. Detection of the internal control is not required for samples that are positive for SARS-CoV-2, Flu A, Flu B and/or RSV. The internal control must be detected in all samples that are negative for SARS-CoV-2, Flu A, Flu B, and RSV; samples that fail to meet that criteria will be reported as Invalid. Each sample with an Invalid result must be retested.

The Panther Fusion system is designed to accurately verify processes when procedures are performed following the instructions provided in this package insert and the *Panther*/*Panther Fusion System Operator's Manual.*

Interpretation of Results

The Panther Fusion system automatically determines the test results for samples and controls. Results for SARS-CoV-2, Flu A, Flu B, and RSV detection are reported separately. A test result may be negative, positive, or invalid.

Table 1 shows the possible results reported in a valid run with result interpretations.

Table 1: Result Interpretation

SARS-CoV-2 Result	Flu A Result	Flu B Result	RSV Result	IC Result	Interpretation
Neg	Neg	Neg	Neg	Valid	SARS-CoV-2, Flu A, Flu B, and RSV not detected.
Neg	POS	Neg	Neg	Valid	Flu A detected. SARS-CoV-2, Flu B, and RSV not detected.
Neg	Neg	POS	Neg	Valid	Flu B detected. SARS-CoV-2, Flu A, and RSV not detected.
Neg	Neg	Neg	POS	Valid	RSV detected. SARS-CoV-2, Flu A, and Flu B not detected.
POS	Neg	Neg	Neg	Valid	SARS-CoV-2 detected. Flu A, Flu B, and RSV not detected.
Neg	POS	POS	Neg	Valid	Flu A and Flu B detected. SARS-CoV-2 and RSV not detected.
Neg	Neg	POS	POS	Valid	Flu B and RSV detected. SARS-CoV-2 and Flu A not detected.
Neg	POS	Neg	POS	Valid	Flu A and RSV detected. SARS-CoV-2 and Flu B not detected.
POS	POS	Neg	Neg	Valid	SARS-CoV-2 and Flu A detected. Flu B and RSV not deteted
POS	Neg	POS	Neg	Valid	SARS-CoV-2 and Flu B detected. Flu A and RSV not detected.
POS	Neg	Neg	POS	Valid	SARS-CoV-2 and RSV detected. Flu A and Flu B not detected.
Neg	POS	POS	POS	Valid	Flu A, Flu B, and RSV detected. SARS-CoV-2 not detected Triple infections are rare. Retest to confirm result.
POS	Neg	POS	POS	Valid	SARS-CoV-2, Flu B, and RSV detected. Flu A not detected. Triple infections are rare. Retest to confirm result.
POS	POS	Neg	POS	Valid	SARS-CoV-2, Flu A, and RSV detected. Flu B not detected. Triple infections are rare. Retest to confirm result.
POS	POS	POS	Neg	Valid	SARS-CoV-2, Flu A, and Flu B detected. RSV not detected. Triple infections are rare. Retest to confirm result.
POS	POS	POS	POS	Valid	SARS-CoV-2, Flu A, Flu B, and RSV detected. Quadruple infections are rare. Retest to confirm result.
Invalid	Invalid	Invalid	Invalid	Invalid	Invalid. There was an error in the generation of the result; retest sample.

Note: POS result will be accompanied by cycle threshold (Ct) values.

Note: Detection of internal control is not required for samples that are positive for SARS-CoV-2, Flu A, Flu B, and/or RSV.

Limitations

A. Use of this assay is limited to personnel who are trained in the procedure. Failure to follow these instructions may result in erroneous results.

B. Reliable results are dependent on adequate specimen collection, transport, storage, and processing.

C. Avoid contamination by adhering to good laboratory practices and to the procedures specified in this package insert.

D. Negative results do not preclude SARS-CoV-2, influenza A virus, influenza B virus, or RSV infections and should not be used as the sole basis for treatment or other management decisions.

E. This test does not differentiate influenza A subtypes (i.e. H1N1, H3N2) or RSV subgroups (i.e., A or B); additional testing is required to differentiate any specific influenza A subtypes or strains or specific RSV subgroups, in consultation with local public health departments.

F. A positive result indicates the detection of nucleic acid from the relevant virus. Nucleic acid may persist even after the virus is no longer viable.

SARS-CoV-2/Flu A/B/RSV Assay Performance

Analytical Sensitivity

The analytical sensitivity (limit of detection or LoD) of the Panther Fusion SARS-CoV-2/Flu A/B/RSV assay was determined by testing dilutions of pooled negative clinical nasopharyngeal (NP) swab VTM/UTM matrix spiked with either the WHO International Standard for SARS-CoV-2, NIBSC (20/146) or the following virus cultures: Influenza A (2 strains), Influenza B (2 strains), RSV A and RSV B (1 strain each). A minimum of 24 replicates were tested with each of three reagent lots for a combined total of a minimum of 72 replicates per dilution. Each target specific LoD concentration was confirmed by testing an additional 24 replicates in negative clinical NP swab VTM/UTM matrix with one reagent lot. The LoD for each was determined by Probit analysis and the highest value between three reagent lots is summarized in Table 2.

Table 2: Analytical Sensitivity

Viral Strain/Standard	LoD Concentration
WHO International Standard SARS-CoV-2, NIBSC (20/146)	47.20 IU/mL
SARS-CoV-2 USA-WA1/2020	0.03 $TCID_{50}$/mL
Influenza A/Brisbane/02/18 (H1N1)	0.06 $TCID_{50}$/mL
Influenza A/Kansas/14/17 (H3N2)	0.11 $TCID_{50}$/mL
Influenza B/Washington/02/19 (Victoria lineage)	0.03 $TCID_{50}$/mL
Influenza B/Phuket/3073/13 (Yamagata lineage)	0.002 $TCID_{50}$/mL
RSV A	0.02 $TCID_{50}$/mL
RSV B	0.03 $TCID_{50}$/mL

Reactivity

The reactivity of the Panther Fusion SARS-CoV-2/Flu A/B/RSV assay was determined by testing virus strains in negative clinical NP swab VTM/UTM matrix. Each strain was tested in triplicate with one reagent lot. Table 3 shows the lowest concentration of each strain in which 100% positivity was observed.

Table 3: Analytical Reactivity Summary for SARS-CoV-2, Flu A and Flu B and RSV Strains

Description	Subtype	Concentration	SARS-CoV-2	Flu A	Flu B	RSV
USA-WA1/2020*	SARS-CoV-2	0.09 $TCID_{50}$/mL	+	-	-	-
USA-CA1/2020	SARS-CoV-2	0.09 $TCID_{50}$/mL	+	-	-	-
USA-AZ1/2020	SARS-CoV-2	0.09 $TCID_{50}$/mL	+	-	-	-
USA-WI1/2020	SARS-CoV-2	0.09 $TCID_{50}$/mL	+	-	-	-
USA/OR-OHSU-PHL00037/ 2021|B.1.1.7	SARS-CoV-2	0.09 $TCID_{50}$/mL	+	-	-	-
Uganda/MUWRP-20200195568/ 2020|A.23.1	SARS-CoV-2	0.09 $TCID_{50}$/mL	+	-	-	-
USA/PHC658/2021|B.1.617.2	SARS-CoV-2	0.09 $TCID_{50}$/mL	+	-	-	-
USA/MD-HP05285/2021|B.1.617.2	SARS-CoV-2	0.09 $TCID_{50}$/mL	+	-	-	-
USA/CA/VRLC009/2021|B.1.427	SARS-CoV-2	0.09 $TCID_{50}$/mL	+	-	-	-
USA/CA/VRLC012/2021|P.2	SARS-CoV-2	0.3 $TCID_{50}$/mL	+	-	-	-
USA/MD-HP03056/2021|B.1.525	SARS-CoV-2	0.3 $TCID_{50}$/mL	+	-	-	-
USA/CA-Stanford-16_S02/ 2021|B.1.617.1	SARS-CoV-2	0.09 $TCID_{50}$/mL	+	-	-	-
Peru/un-CDC-2-4069945/ 2021|C.37	SARS-CoV-2	0.09 TCID50/mL	+	-	-	-
USA/MD-HP20874/2021|B.1.1.529	SARS-CoV-2	0.09 $TCID_{50}$/mL	+	-	-	-
USA/GA-EHC-2811C/ 2021|B.1.1.529	SARS-CoV-2	0.09 $TCID_{50}$/mL	+	-	-	-
A/Brisbane/02/18*	Flu A (H1N1)	0.18 $TCID_{50}$/mL	-	+	-	-
A/Michigan/45/2015	Flu A (H1N1)	0.18 $TCID_{50}$/mL	-	+	-	-
A/Christ Church/16/2010	Flu A (H1N1)	180 $TCID_{50}$/mL	-	+	-	-
A/Kentucky/2/06	Flu A (H1N1)	1.8 $TCID_{50}$/mL	-	+	-	-
A/Solomon Islands/03/06	Flu A (H1N1)	1.8 $TCID_{50}$/mL	-	+	-	-
A/Guangdong-maonan/1536/2019	Flu A (H1N1)	180 $TCID_{50}$/mL	-	+	-	-
A/Taiwan/42/2006	Flu A (H1N1)	1.8 $TCID_{50}$/mL	-	+	-	-
A/Henan/8/05	Flu A (H1N1)	1.8 $TCID_{50}$/mL	-	+	-	-
A/Hawaii/15/01	Flu A (H1N1)	18 $TCID_{50}$/mL	-	+	-	-
A/California/07/2009	Flu A (H1N1)	0.18 $TCID_{50}$/mL	-	+	-	-

Table 3: Analytical Reactivity Summary for SARS-CoV-2, Flu A and Flu B and RSV Strains (Continued)

Description	Subtype	Concentration	SARS-CoV-2	Flu A	Flu B	RSV
A/Hawaii/66/2019	Flu A (H1N1)	180 $CEID_{50}$/mL	-	+	-	-
A/Indiana/02/2020	Flu A (H1N1)	60 $CEID_{50}$/mL	-	+	-	-
A/Michigan/45/2015	Flu A (H1N1)	1.8 $TCID_{50}$/mL	-	+	-	-
A/Kansas/14/17*	Flu A (H3N2)	0.33 $TCID_{50}$/mL	-	+	-	-
A/Arizona/45/2018	Flu A (H3N2)	3.3 FFU/mL	-	+	-	-
A/New York/21/2020	Flu A (H3N2)	3.3 FFU/mL	-	+	-	-
A/Hong Kong/45/2019	Flu A (H3N2)	3.3 FFU/mL	-	+	-	-
A/Singapore/INFIMH-16-0019/ 2016	Flu A (H3N2)	110 $CEID_{50}$/mL	-	+	-	-
A/Hong Kong/2671/2019	Flu A (H3N2)	33 $TCID_{50}$/mL	-	+	-	-
A/Hiroshima/52/05	Flu A (H3N2)	3.3 $TCID_{50}$/mL	-	+	-	-
A/Costa Rica/07/99	Flu A (H3N2)	33 $TCID_{50}$/mL	-	+	-	-
A/Port Chalmers/1/73	Flu A (H3N2)	3.3 $TCID_{50}$/mL	-	+	-	-
A/Brazil/113/99	Flu A (H3N2)	3.3 $TCID_{50}$/mL	-	+	-	-
A/Perth/16/2009	Flu A (H3N2)	0.33 $TCID_{50}$/mL	-	+	-	-
A/Texas/50/2012	Flu A (H3N2)	0.33 $TCID_{50}$/mL	-	+	-	-
A/Hong Kong/4801/2014	Flu A (H3N2)	3.3 $TCID_{50}$/mL	-	+	-	-
A/Indiana/08/2011	Flu A (H3N2)	3.3 TCID50/mL	-	+	-	-
A/Hong Kong/486/97	Flu A (H5N1)	0.01 ng/mL	-	+	-	-
B/Washington/02/2019*	Flu B (Victoria)	0.09 $TCID_{50}$/mL	-	-	+	-
B/Colorado/06/2017	Flu B (Victoria)	0.09 $TCID_{50}$/mL	-	-	+	-
B/Florida/78/2015	Flu B (Victoria)	0.9 $TCID_{50}$/mL	-	-	+	-
B/Alabama/2/17	Flu B (Victoria)	0.09 $TCID_{50}$/mL	-	-	+	-
B/Ohio/1/2005	Flu B (Victoria)	0.9 $TCID_{50}$/mL	-	-	+	-
B/Michigan/09/2011	Flu B (Victoria)	3 $TCID_{50}$/mL	-	-	+	-
B/Hawaii/01/2018 (NA D197N)	Flu B (Victoria)	0.9 $TCID_{50}$/mL	-	-	+	-
B/Brisbane/33/08	Flu B (Victoria)	0.09 $TCID_{50}$/mL	-	-	+	-
B/Phuket/3073/2013*	Flu B (Yamagata)	0.006 $TCID_{50}$/mL	-	-	+	-
B/Wisconsin/1/2010	Flu B (Yamagata)	2 $TCID_{50}$/mL	-	-	+	-
B/Utah/9/14	Flu B (Yamagata)	0.006 $TCID_{50}$/mL	-	-	+	-
B/St. Petersburg/04/06	Flu B (Yamagata)	0.06 $TCID_{50}$/mL	-	-	+	-
B/Texas/81/2016	Flu B (Yamagata)	20 $TCID_{50}$/mL	-	-	+	-
B/Indiana/17/2017	Flu B (Yamagata)	0.6 $TCID_{50}$/mL	-	-	+	-

Table 3: Analytical Reactivity Summary for SARS-CoV-2, Flu A and Flu B and RSV Strains (Continued)

Description	Subtype	Concentration	SARS-CoV-2	Flu A	Flu B	RSV
B/Oklahoma/10/2018	Flu B (Yamagata)	2 $TCID_{50}$/mL	-	-	+	-
B/Massachusetts/02/2012	Flu B (Yamagata)	0.2 $TCID_{50}$/mL	-	-	+	-
B/Lee/40	Flu B	0.09 $TCID_{50}$/mL	-	-	+	-
RSV-A/2006 Isolate*	RSVA	0.06 $TCID_{50}$/mL	-	-	-	+
RSV A/4/2015 isolate #1	RSVA	0.06 $TCID_{50}$/mL	-	-	-	+
RSV A/A2	RSVA	0.06 $TCID_{50}$/mL	-	-	-	+
RSV A/12/2014 isolate #2	RSVA	0.06 $TCID_{50}$/mL	-	-	-	+
RSV-B/CH93(18)-18*	RSVB	0.3 $TCID_{50}$/mL	-	-	-	+
RSV B/3/2015 isolate #1	RSVB	0.09 $TCID_{50}$/mL	-	-	-	+
RSV B/9320	RSVB	0.09 $TCID_{50}$/mL	-	-	-	+

*Strain used to establish LoD.

Analytical Specificity and Microbial Interference

Analytical specificity (cross-reactivity) and microbial interference with the Panther Fusion SARS-CoV-2/Flu A/B/RSV assay was evaluated in the presence of closely related and non-targeted organisms. Panels consisting of 41 organisms (Table 4) were tested in pooled negative clinical NP swab VTM/UTM matrix in the absence or presence of 3x LoD SARS-CoV-2, Flu A, Flu B and RSV. Bacteria were tested at 10^6 CFU/mL and viruses were tested at 10^5 $TCID_{50}$/mL, except where noted. No cross-reactivity or microbial interference was observed for any of the 41 organisms tested in the Panther Fusion SARS-CoV-2/Flu A/B/RSV assay at the following concentrations.

Table 4: Cross Reactivity and Microbial Interference Microorganisms

Microorganism	Concentration[1]	Microorganism	Concentration[1]
Adenovirus 1	$1x10^5$ $TCID_{50}$/mL	*Bordetella pertussis*	$1x10^6$ CFU/mL
Adenovirus 7a	$1x10^5$ $TCID_{50}$/mL	*Candida albicans*	$1x10^6$ CFU/mL
CMV Strain AD 169	$1x10^4$ $TCID_{50}$/mL	*Chlamydophila pneumoniae*	$1x10^6$ IFU/mL
Human coronavirus 229E	$1x10^4$ $TCID_{50}$/mL	*Corynebacterium diphtheriae*	$1x10^6$ CFU/mL
Human coronavirus NL63	$1x10^4$ $TCID_{50}$/mL	*Escherichia coli*	$1x10^6$ CFU/mL
Human coronavirus OC43	$1x10^5$ $TCID_{50}$/mL	*Haemophilus influenzae*	$1x10^6$ CFU/mL
Epstein-Barr virus (EBV)	$1x10^6$ copies/mL	*Lactobacillus plantarum*	$1x10^6$ CFU/mL
Enterovirus (e.g. EV68)	$1x10^5$ $TCID_{50}$/mL	*Legionella pneumophila*	$1x10^6$ CFU/mL
Human coronavirus HKU1[2]	$1x10^6$ copies/mL	*Moraxella catarrhalis*	$1x10^5$ CFU/mL
Human Metapneumovirus (hMPV)	$1x10^5$ $TCID_{50}$/mL	*Mycobacterium tuberculosis*	$1x10^9$ rRNA copies/mL
HPIV-1	$1x10^5$ $TCID_{50}$/mL	*Mycoplasma pneumoniae*	$1x10^9$ rRNA copies/mL
HPIV-2	$1x10^5$ $TCID_{50}$/mL	*Neisseria spp*	$1x10^6$ CFU/mL
HPIV-3	$1x10^5$ $TCID_{50}$/mL	*Neisseria meningitides*	$1x10^6$ CFU/mL
HPIV-4	$1x10^4$ $TCID_{50}$/mL	*Neisseria mucosa*	$1x10^6$ CFU/mL
Measles	$1x10^4$ $TCID_{50}$/mL	*Pneumocystis jirovecii*	$1x10^6$ CFU/mL
MERS-Coronavirus	$5x10^4$ $TCID_{50}$/mL	*Pseudomonas aeruginosa*	$1x10^6$ CFU/mL
Mumps virus	$1x10^5$ $TCID_{50}$/mL	*Staphylococcus aureus*	$1x10^6$ CFU/mL
Rhinovirus 1A	$1x10^4$ $TCID_{50}$/mL	*Staphylococcus epidermidis*	$1x10^6$ CFU/mL
SARS coronavirus 1[2]	$1x10^6$ copies/mL	*Streptococcus pneumoniae*	$1x10^6$ CFU/mL
Varicella Zoster Virus	$1x10^3$ $TCID_{50}$/mL	*Streptococcus pyogenes*	$1x10^6$ CFU/mL
		Streptococcus salivarius	$1x10^6$ CFU/mL

[1]CFU = Colony Forming Units; IFU = Inclusion Forming Units; $TCID_{50}$ = Median Tissue Culture Infectious Dose

[2]Cultured virus and whole genome purified nucleic acid for Human HKU1 and SARS-coronavirus are not readily available. HKU1 and SARS-coronavirus *in vitro* transcript (IVT) corresponding to the ORF1a gene regions targeted by the assay were used to evaluate cross-reactivity and microbial interference.

Competitive Interference

Competitive interference in the Panther Fusion SARS-CoV-2/Flu A/B/RSV assay was evaluated using pairs of targeted viruses at low/high concentrations in pooled negative clinical NP swab VTM/UTM matrix. The low concentration was tested at 3x LoD, while the high concentration virus was tested at 1000x LoD. Results of the study are shown in Table 5. The presence of two viruses at varying concentrations had no effect on the analytical sensitivity of one target in the presence of high concentrations of the other target.

Table 5: Competitive Interference

Low Target		High Target		SARS-CoV-2 (detected)	Flu A (detected)	Flu B (detected)	RSV (detected)
Virus	3x LoD ($TCID_{50}$/mL)	Virus	1000x LoD ($TCID_{50}$/mL)				
SARS-CoV-2	0.09	Flu A	110	+	+	-	-
SARS-CoV-2	0.09	Flu B	30	+	-	+	-
SARS-CoV-2	0.09	RSV	30	+	-	-	+
Flu A	0.33	SARS-CoV-2	30	+	+	-	-
Flu A	0.33	Flu B	30	-	+	+	-
Flu A	0.33	RSV	30	-	+	-	+
Flu B	0.09	SARS-CoV-2	30	+	-	+	-
Flu B	0.09	Flu A	110	-	+	+	-
Flu B	0.09	RSV	30	-	-	+	+
RSV	0.09	SARS-CoV-2	30	+	-	-	+
RSV	0.09	Flu A	110	-	+	-	+
RSV	0.09	Flu B	30	-	-	+	+

Interference

Interfering endogenous and exogenous substances (mucin, whole blood, other potential medications and over-the-counter products) that may be present in a specimen were evaluated in the Panther Fusion SARS-CoV-2/Flu A/B/RSV assay. Clinically relevant concentrations of potentially interfering substances were added to pooled clinical negative NP swab VTM/UTM matrix and tested in the absence and presence of SARS-CoV-2, Flu A, Flu B and RSV cultured virus at their respective 3x LoD concentrations. The substances and concentrations are shown in Table 6.

No impact on the performance of the Panther Fusion SARS-CoV-2/Flu A/B/RSV assay was seen for any of the substances at the concentrations tested.

Table 6: Potentially Interfering Substances

Substance Type	Substance Name	Active Ingredient(s)	Concentration[1]
Endogenous	Mucin	Purified mucin protein	60 µg/mL
	Blood (human)	N/A	2% v/v
Nasal sprays or drops	Neo-Synephrine®	Phenylephrine	15% v/v
	Anefrin	Oxymetazoline	15% v/v
	Saline	Sodium chloride	15% v/v
	Ventolin HFA[2]	Albuterol	45 ng/mL
Nasal corticosteroids	QVAR® Beconase AQ[2]	Beclomethasone	15 ng/mL
	Dexacort[2]	Dexamethasone	12 µg/mL
	Nasacort	Triamcinolone	5% v/v
	Flonase	Fluticasone	5% v/v
	Rhinocort	Budesonide	5% v/v
	Nasonex[2]	Mometasone	0.5 ng/mL
	AEROSPAN®[2]	Flunisolide	10 µg/mL
Nasal gel	Zicam® (Allergy Relief)	Luffa opperculata, Galphimia, Glauca, Histaminum hydrochloricum, Sulfur	5% v/v
Throat lozenge	Cepacol Extra Strength	Benzocaine, Menthol	0.7 mg/mL
Anti-viral drug	Relenza®[2]	Zanamivir	3.3 mg/mL
	TamiFlu[2]	Oseltamivir	400 µg/mL
	Virazole[2]	Ribavirin	10.5 µg/mL
Antibiotic, nasal ointment	Bactroban cream[2]	Mupirocin	1.6 µg/mL
Antibiotic, systemic	Tobramycin	Tobramycin	33.1 µg/mL

[1] v/v: volume by volume

[2] Active ingredients tested

Assay Precision

Panther Fusion SARS-CoV-2/Flu A/B/RSV assay within-lab precision was evaluated with a 5-member panel consisting of virus in negative clinical NP swab VTM/UTM matrix. The panels were tested by two operators on two runs per day, using three reagent lots on three Panther Fusion systems over twelve days.

The panel members are described in Table 7, along with a summary of the agreement with the expected results and the Ct mean and variability analysis between reagent lots, operators, instruments, between and within runs, and overall (total).

Table 7: Signal Variability of the Panther Fusion SARS-CoV-2/Flu A/B/RSV Assay by Panel Member

Panel	Description	Analyte	N* Agreed/	ent (%) Agreem	Mean Ct	Between Lots		Between Instrument		Between Operators		Between Days		Between Runs		Within Run		Total	
						SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
1	Neg	Internal Control	95/ 96	99	33.7	0.19	0.57	0.08	0.23	0.00	0.00	0.00	0.00	0.21	0.62	0.29	0.86	0.42	1.23
2	SARS-CoV-2/ Flu A Low Pos	Flu A	96/ 96	100	35.1	0.33	0.93	0.06	0.17	0.00	0.00	0.00	0.00	0.30	0.85	0.56	1.59	0.72	2.04
		SARS-CoV-2	96/ 96	100	35.9	0.00	0.00	0.13	0.36	0.00	0.00	0.00	0.00	0.00	0.00	0.60	1.67	0.61	1.71
3	Flu B/ RSV Low Pos	Flu B	96/ 96	100	36.0	0.14	0.40	0.09	0.25	0.00	0.00	0.00	0.00	0.00	0.00	0.36	0.99	0.39	1.09
		RSV	96/ 96	100	36.1	0.12	0.33	0.28	0.77	0.00	0.00	0.00	0.00	0.37	1.04	0.53	1.46	0.71	1.97
4	SARS-CoV-2/ Flu A Mod Pos	Flu A	96/ 96	100	33.9	0.23	0.66	0.00	0.00	0.00	0.00	0.19	0.56	0.00	0.00	0.47	1.37	0.55	1.63
		SARS-CoV-2	96/ 96	100	34.7	0.21	0.62	0.16	0.45	0.06	0.17	0.00	0.00	0.00	0.00	0.45	1.30	0.52	1.51
5	Flu B/ RSV Mod Pos	Flu B	96/ 96	100	34.7	0.15	0.44	0.00	0.00	0.00	0.00	0.00	0.00	0.06	0.18	0.28	0.80	0.32	0.93
		RSV	96/ 96	100	34.5	0.10	0.30	0.18	0.51	0.00	0.00	0.00	0.00	0.00	0.00	0.40	1.15	0.44	1.29

*Agreement to expected panel positivity result.
Low Pos = Low positive 1-2x LoD.
Mod Pos = Moderate positive 3-5x LoD.
Note: Variability from some factors may be numerically negative, which can occur if the variability due to those factors is very small. When this occurs, SD=0 and CV=0%.

Clinical Performance

The clinical performance of the Panther Fusion SARS-CoV-2/Flu A/B/RSV assay was evaluated in comparison to an FDA Emergency Use Authorization (EUA) nucleic acid amplification test (NAAT) assay and an FDA-cleared Flu/RSV NAAT assay using individual remnant clinical NP specimens in VTM/UTM collected from patients with signs and symptoms of respiratory infection. For the evaluation, a combination of negative, SARS-CoV-2 positive, Flu A positive, Flu B positive, and RSV positive specimens were tested with each assay.

The Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) for SARS-CoV-2 was calculated in relation to the FDA EUA authorized NAAT assay as the reference result, as shown in Table 8. The assay showed positive and negative percent agreements of 98.1% and 98.5% respectively for SARS-CoV-2.

For Flu A, Flu B and RSV, the PPA and NPA were calculated in relation to the FDA-cleared Flu/RSV NAAT assay as the reference result, as shown in Table 9 for Flu A, Table 10 for Flu B and Table 11 for RSV. The assay showed positive and negative percent agreements of 100.0% and 99.6% respectively, for Flu A, 98.1% and 99.6% for Flu B and 98.1% and 100.0% for RSV.

Table 8: Clinical Performance for SARS-CoV-2

SARS-CoV-2		**FDA EUA Authorized NAAT Assay**		
		Positive	**Negative**	**Total**
Panther Fusion SARS/Flu A/B/RSV Assay	**Positive**	52	4	56
	Negative	1	256	257
	Total	53	260	313
Positive Agreement (95% CI)		98.1%	(90.1% - 99.7%)	
Negative Agreement (95% CI)		98.5%	(96.1% - 99.4%)	

Table 9: Clinical Performance for Flu A

Flu A		**FDA-Cleared Assay**		
		Positive	**Negative**	**Total**
Panther Fusion SARS/Flu A/B/RSV Assay	**Positive**	52	1	53
	Negative	0	260	260
	Total	52	261	313
Positive Agreement (95% CI)		100.0%	(93.1% - 100.0%)	
Negative Agreement (95% CI)		99.6%	(97.9% - 99.9%)	

Table 10: Clinical Performance for Flu B

Flu B	**FDA-Cleared Assay**

		Positive	Negative	Total
Panther Fusion SARS/Flu A/B/RSV Assay	**Positive**	52	1	53
	Negative	1	259	260
	Total	53	260	313
Positive Agreement (95% CI)		98.1%	(90.1% - 99.7%)	
Negative Agreement (95% CI)		99.6%	(97.9% - 99.9%)	

Table 11: Clinical Performance for RSV

RSV		**FDA-Cleared Assay**		
		Positive	**Negative**	**Total**
Panther Fusion SARS/Flu A/B/RSV Assay	**Positive**	52	0	52
	Negative	1	260	261
	Total	53	260	313
Positive Agreement (95% CI)		98.1%	(90.1% - 99.7%)	
Negative Agreement (95% CI)		100.0%	(98.5% - 100.0%)	

Aptima™ SARS-CoV-2/Flu Assay (Panther™ System)

For *in vitro* diagnostic use only

For U.S Export only

CONTENTS

General Information

Intended Use

The Aptima SARS-CoV-2/Flu assay is a target amplification nucleic acid probe *in vitro* diagnostic test intended for the qualitative detection and differentiation of RNA from SARS-CoV-2 virus, influenza A virus (Flu A) and influenza B virus (Flu B) isolated and purified from nasopharyngeal (NP), oropharyngeal (OP), nasal, and mid-turbinate swab or nasopharyngeal wash/aspirate and nasal aspirate specimens obtained from individuals with signs and symptoms of a respiratory tract infection or who meet COVID-19 clinical and/or epidemiological criteria. Clinical signs and symptoms of respiratory viral infections due to SARS-CoV-2 and influenza can be similar.

Results are for the identification of SARS-CoV-2, Flu A and Flu B RNA. SARS-CoV-2, Flu A and Flu B RNA are generally detectable in upper respiratory specimens during the acute phase of infection. Positive results are indicative of the presence of RNA from SARS-CoV-2, Flu A or Flu B; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease.

Negative results do not preclude SARS-CoV-2, Flu A, or Flu B infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

The Aptima SARS-CoV-2/Flu assay on the Panther™ and Panther Fusion™ system is intended for use by trained clinical laboratory personnel specifically instructed and trained in the operation of the Panther and Panther Fusion systems and *in vitro* diagnostic procedures.

Summary and Explanation of the Test

Influenza (flu) and COVID-19 are both contagious respiratory illnesses, but they are caused by different viruses. COVID-19 is caused by infection with a new coronavirus (called SARS-CoV-2) and flu is caused by infection with influenza viruses. Because some of the symptoms of flu and COVID-19 are similar, it may be hard to tell the difference between them based on symptoms alone.[1]

Flu is a contagious respiratory illness caused by influenza viruses. It can cause mild to severe illness. Serious outcomes of flu infection can result in hospitalization or death. Some people, such as older people, young children, and people with certain health conditions, are at high risk of serious flu complications. There are two main types of flu virus: types A and B. Flu A and B viruses that routinely spread in people (human influenza viruses) are responsible for seasonal flu epidemics each year.[2]

Flu signs and symptoms usually come on suddenly. People who are sick with flu may experience fever or feeling feverish/chills, cough, sore throat, runny or stuffy nose, muscle or body aches, headaches, fatigue, and some people may have vomiting and diarrhea, though this is more common in children than adults.[3]

Coronaviruses are a large family of viruses which may cause illness in animals or humans. In humans, several coronaviruses are known to cause respiratory infections ranging from the common cold to more severe diseases such as Middle East Respiratory Syndrome (MERS) and Severe Acute Respiratory Syndrome (SARS). The most recently discovered coronavirus, SARS-

CoV-2, causes the associated coronavirus disease COVID-19. This new virus and disease were unknown before outbreak in Wuhan, China, in December 2019.[3]

People with COVID-19 have had a wide range of symptoms reported, ranging from mild symptoms to severe illness. Symptoms may appear 2-14 days after exposure to the virus. People with COVID-19 may exhibit fever or chills, cough, shortness of breath or difficulty breathing, fatigue, muscle or body aches, headache, new loss of taste or smell, sore throat, congestion or runny nose, nausea or vomiting, and/or diarrhea.[5]

The virus that causes COVID-19 is infecting people and spreading easily from person to person. On March 11, 2020, the COVID-19 outbreak was characterized as a pandemic by the World Health Organization (WHO).[3,5]

Principles of the Procedure

The Aptima SARS-CoV-2/Flu assay combines the technologies of target capture, Real-Time Transcription Mediated Amplification (RT-TMA), and real time detection of amplicons using fluorescently labeled torches.

Specimens are collected and transferred into their respective specimen transport tubes. The transport solutions in these tubes release the RNA target and protect them from degradation during storage. When the Aptima SARS-CoV-2/Flu assay is performed in the laboratory on the Panther system, an Internal Control (IC) nucleic acid is added to each specimen reaction, and the IC along with the target RNA molecules are isolated from specimens by use of capture oligomers via target capture that utilizes magnetic microparticles. The capture oligomers contain sequences complementary to specific regions of the target molecules as well as a string of deoxyadenosine residues. A separate capture oligomer is used for each target. During the hybridization step, the sequence specific regions of the capture oligomers bind to specific regions of the target molecules. The capture oligomer:target complex is then captured out of solution by decreasing the temperature of the reaction to room temperature. This temperature reduction allows hybridization to occur between the deoxyadenosine region on the capture oligomer and the poly-deoxythymidine molecules that are covalently attached to the magnetic particles. The microparticles, including the captured target molecules bound to them, are pulled to the side of the reaction vessel using magnets and the supernatant is aspirated. The particles are washed to remove residual specimen matrix that may contain amplification reaction inhibitors. After the target capture steps are completed, the specimens are ready for amplification.

Target amplification assays are based on the ability of complementary oligonucleotide primers to specifically anneal and allow enzymatic amplification of the target and IC nucleic acid strands. The Aptima SARS-CoV-2/Flu assay replicates specific regions of the RNA from SARS-CoV-2, Flu A and Flu B via DNA intermediates. Detection is achieved using single-stranded nucleic acid torches that are present during the amplification of the target and that hybridize specifically to the amplicon in real-time. Each torch has a fluorophore and a quencher. When the torch is not hybridized to the amplicon, the quencher is in close proximity of the fluorophore and suppresses the fluorescence. When the torch binds to the amplicon, the quencher is moved farther away from the fluorophore and it will emit a signal at a specific wavelength when excited by a light source. As more torch hybridize to amplicon, a higher fluorescent signal is generated. The fluorophores associated with the viral targets and IC targets emit light at different wavelengths, thus allowing these targets to be distinguished from one another. The fluorescent signals

generated by the amplification are measured by fluorometers then used by the system to generate qualitative results.

The Aptima SARS-CoV-2/Flu assay amplifies and detects two conserved regions of the ORF1ab gene in the same reaction for SARS-CoV-2, one region of the Matrix gene for Flu A, and one region of the Matrix gene for Flu B. For detection, both SARS-CoV-2 gene targets are reported into the FAM fluorescent channel, the Flu A target is reported into the ROX fluorescent channel, and the Flu B target is reported into the HEX fluorescent channel of the Panther system. The two regions of the SARS-CoV-2 target are not differentiated, and amplification of either or both regions leads to RFU signal. The assay results for all targets are determined by fluorescence and emergence cut-offs.

Warnings and Precautions

A. For *in vitro* diagnostic use. Carefully read this entire package insert and the *Panther/Panther Fusion System Operator's Manual*.

B. Only personnel adequately trained on the use of this assay and in handling potentially infectious materials should perform these procedures. If a spill occurs, immediately disinfect using appropriate site procedures.

C. Handle and process all specimens as if infectious following laboratory practices and procedures that are basic to good microbiological practice and procedures (GMPP). Refer to World Health Organization's (WHO) Laboratory biosafety guidance related to coronavirus disease (COVID-19): interim guidance. https://www.who.int/publications/i/item/laboratory-biosafety-guidance-related-to-coronavirus-disease-(covid-19).

D. Specimens may be infectious. Use Universal Precautions when performing this assay. Proper handling and disposal methods should be established by the laboratory director. Only personnel adequately trained in handling infectious materials should be permitted to perform this diagnostic procedure.[6]

E. If infection with SARS-CoV-2, Flu A, and/or Flu B is suspected based on current clinical screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions.

F. Use only supplied or specified disposable laboratory ware.

G. Appropriate personal protective equipment (PPE), as determined by a detailed risk assessment, should be worn by all laboratory personnel collecting and handling specimens from individuals suspected of being infected with SARS-CoV-2, Flu A, and/or Flu B, as outlined in WHO's Laboratory biosafety guidance related to coronavirus disease (COVID-19): interim guidance.

H. Wear disposable, powderless gloves, protective eye wear, and laboratory coats when handling specimens and reagents. Wash hands thoroughly after handling specimens and reagents.

I. Dispose of all material that has come into contact with specimens and reagents in accordance with applicable national, international, and regional regulations.

J. Expiration dates listed on the Panther Fusion Specimen Lysis Tubes, Hologic Specimen Lysis Tubes, the Aptima Multitest Collection Kit, and the Hologic Direct Load Capture Cap Collection Kit, pertain to the transfer of sample into the tube and not to testing of the sample. Specimens collected/transferred any time prior to these expiration dates are valid for testing provided they are transported and stored in accordance with the appropriate package insert, even if these expiration dates have passed.

K. Maintain proper storage conditions during specimen shipping to ensure the integrity of the specimen. Specimen stability under shipping conditions other than those recommended has not been evaluated.

L. Avoid cross-contamination during the specimen handling steps. Specimens can contain extremely high levels of virus or other organisms. Ensure that specimen containers do not come in contact with one another, and discard used materials without passing them over any open containers. Change gloves if they come in contact with specimens.

M. Do not use the reagents and controls after the expiration date.

N. Store assay components at the recommended storage condition. See *Reagent Storage and Handling Requirements (*page 6), and *Panther System Test Procedure* (page 14) for more information.

O. Do not combine any assay reagents or fluids. Do not top off reagents or fluids; the Panther system verifies reagent levels.

P. Avoid microbial and ribonuclease contamination of reagents.

Q. Do not use material that may contain Guanidinium thiocyanate or any guanidine-containing materials on the instrument. Highly reactive and/or toxic compounds may form if combined with sodium hypochlorite.

R. Some reagents in this kit are labeled with risk and safety symbols.

Note: *Hazard Communication information reflects the EU Safety Data Sheets (SDS) classifications. For hazard communication information specific to your region, refer to the region specific SDS on the Safety Data Sheet Library at www.hologicsds.com.*

Target Capture Reagent ***EDTA 1-5%*** ***LITHIUM HYDROXIDE, MONOHYDRATE 1-5%*** H412 - Harmful to aquatic life with long lasting effects P273 - Avoid release to the environment P280 - Wear eye protection/face protection
Promoter Reagent ***MAGNESIUM CHLORIDE 35-40%*** H412 - Harmful to aquatic life with long lasting effects P273 - Avoid release to the environment P280 - Wear eye protection/face protection

Reagent Storage and Handling Requirements

A. The following reagents are stable when stored at 2°C to 8°C (refrigerated):

Aptima SARS-CoV-2/Flu Amplification Reagent

Aptima SARS-CoV-2/Flu Enzyme Reagent

Aptima SARS-CoV-2/Flu Promoter Reagent

Aptima SARS-CoV-2/Flu Internal Control

Aptima SARS-CoV-2/Flu Positive Control

Aptima SARS-CoV-2/Flu Negative Control

B. The following reagents are stable when stored at 2°C to 30°C:

Aptima SARS-CoV-2/Flu Amplification Reconstitution Solution

Aptima SARS-CoV-2/Flu Enzyme Reconstitution Solution

Aptima SARS-CoV-2/Flu Promoter Reconstitution Solution

C. The following reagents are stable when stored at 15°C to 30°C (room temperature):

Aptima SARS-CoV-2/Flu Target Capture Reagent

Aptima Wash Solution

Aptima Buffer for Deactivation Fluid

Aptima Oil Reagent

D. Working Target Capture Reagent (wTCR) is stable for 30 days when stored at 15°C to 30°C. Do not refrigerate.

E. After reconstitution, the Enzyme Reagent, Amplification Reagent, and Promoter Reagent are stable for 30 days when stored at 2°C to 8°C.

F. Discard any unused reconstituted reagents and wTCR after 30 days or after the Master Lot expiration date, whichever comes first.

G. Controls are stable until the date indicated on the vials.

H. Reagents stored on-board the Panther system have 72 hours of on-board stability. Reagents can be loaded onto the Panther system up to 5 times. The Panther system logs each time the reagents are loaded.

I. The Promoter Reagent and Reconstituted Promoter Reagent are photosensitive. Store the reagents protected from light. The specified reconstituted stability is based on 12 hours exposure of the Reconstituted Promoter Reagent to two 60W fluorescent bulbs, at a distance of 17 inches (43 cm), and temperature less than 30°C. Light exposure of the Reconstituted Promoter Reagent should be limited accordingly.

J. Upon warming to room temperature, some control tubes may appear cloudy or contain precipitates. Cloudiness or precipitation associated with controls does not affect control performance. The controls may be used whether they are clear or cloudy/precipitated. If clear controls are desired, solubilization may be expedited by incubating them at the upper end of the room temperature range (15°C to 30°C).

K. Do not freeze the reagents.

Specimen Collection and Storage

Specimens - Clinical material collected from patient placed in an appropriate transport system. For the Aptima SARS-CoV-2/Flu assay, this includes NP, OP, nasal, and mid-turbinate swab specimen, or nasopharyngeal wash/aspirate and nasal aspirate specimen collection in viral transport medium (VTM/UTM), saline, Liquid Amies, or specimen transport medium (STM).

Samples - Represents a more generic term to describe any material for testing on the Panther System including specimens, specimens transferred into a Panther Fusion Specimen Lysis Tube, Hologic Specimen Lysis Tube with solid cap, Custom Specimen Lysis Tube, Aptima Multitest Transport Tube, Hologic Direct Load Capture Cap Tube, and controls.

Note: *Handle all specimens as if they contain potentially infectious agents. Use Universal Precautions.*

Note: *Take care to avoid cross-contamination during specimen handling steps. For example, discard used material without passing over open tubes.*

Swab Specimen Collection

Collect NP, OP, nasal, and mid-turbinate swab specimens according to standard technique using a polyester-, rayon-, or nylon-tipped swab. Immediately place the swab specimen into 3mL of VTM or UTM. Swab specimens may alternatively be added to saline, Liquid Amies, or STM. The Aptima Multitest Swab Specimen Collection Kit may be used for the collection of OP, nasal, and mid-turbinate swab samples. The Hologic Direct Load Capture Cap Collection Kit - CLASSIQSwab is for the collection of OP and nasal swab samples. The Hologic Direct Load Capture Cap Collection Kit - FLOQSwab is for the collection of NP swab samples.

After collection, specimens collected in VTM/UTM, Liquid Amies, or saline can be stored at 2°C to 8°C up to 96 hours before transferring to the Specimen Lysis Tube (i.e., Panther Fusion Specimen Lysis Tube, Hologic Specimen Lysis Tube with solid cap, or Custom Specimen Lysis Tube) as described in the specimen processing section below. Remaining specimen volumes in VTM/UTM, Liquid Amies, or saline can be stored at ≤-70°C.

After collection, specimens in the Aptima Multitest Tube and the Hologic Direct Load Capture Cap Tube, may be stored at 2°C to 30°C up to 6 days.

Note: *It is recommended that specimens collected in the Aptima Multitest Tube and the Hologic Direct Load Capture Cap Tube are stored capped and upright in a rack.*

The following types of VTM/UTM can be used.

- Remel MicroTest M4, M4RT, M5 or M6 formulations
- Copan Universal Transport Medium
- BD Universal Viral Transport Medium

Note: *Do not use medium that may contain Guanidium thiocyanate or any guanidine-containing material.*

Nasopharyngeal Wash/aspirate and Nasal Aspirate Specimen Collection

Collect nasopharyngeal wash/aspirate and nasal aspirate specimens according to standard techniques.

Specimen Processing

Capped Workflow using Aptima SARS-CoV-2/Flu Assay Software

Specimen Processing using the Panther Fusion Specimen Lysis Tube

A. Prior to testing on the Panther system, transfer 500 µL of the collected specimen* to a Panther Fusion Specimen Lysis Tube.

***Note:** When testing frozen specimen, allow specimen to reach room temperature prior to processing.*

Specimen Processing for Specimen Collected with the Aptima Multitest Collection Kit

A. After placing the collected specimen* into the Aptima Multitest Tube using the Aptima Multitest Collection Kit, no further processing is required.

***Note:** When testing frozen specimen, allow specimen to reach room temperature prior to processing.*

Uncapped Workflow using Aptima SARS-CoV-2/Flu Uncapped Tube Assay Software

Specimen Processing using the Panther Fusion Specimen Lysis Tube

A. Uncap the Panther Fusion Specimen Lysis Tube with penetrable cap. The penetrable cap can be retained or a replacement solid cap can be used in the next step.

B. Prior to testing on the Panther system, transfer 500 µL of the specimen to the Panther Fusion Specimen Lysis Tube, with penetrable cap or replacement solid cap.

C. It is recommended to recap the tube and gently invert three times to ensure viral inactivation and a homogeneous mixture.

D. To avoid contact with the top of the tube, loosen the cap and place the sample tube into the sample rack.

E. Remove and discard the cap. To avoid contamination, do not pass the cap over any other sample racks or sample tube. Inspect the sample tube. If bubbles are present, carefully remove from the sample tube (for example, use the tip of a sterile swab or similar method).

***Note:** Failure to remove bubbles may affect assay processing and cause invalid results.*

F. Place the rack retainer on the sample rack and load the rack into the instrument.

Specimen Processing using the Hologic Specimen Lysis Tube with Solid Cap

A. Uncap the Hologic Specimen Lysis Tube with solid cap and retain the cap.

B. Prior to testing on the Panther system, transfer 500 µL of the specimen to the Hologic Specimen Lysis Tube with solid cap.

C. It is recommended to recap the tube and gently invert three times to ensure viral inactivation and a homogeneous mixture.

D. To avoid contact with the top of the tube, loosen the cap and place the sample tube into the sample rack.

E. Remove and discard the cap. To avoid contamination, do not pass the cap over any other sample racks or sample tube. Inspect the sample tube. If bubbles are present, carefully remove from the sample tube (for example, use the tip of a sterile swab or similar method).

Note: *Failure to remove bubbles may affect assay processing and cause invalid results.*

F. Place the rack retainer on the sample rack and load the rack into the instrument.

Specimen Processing for Specimen Collected with the Hologic Direct Load Capture Cap Collection Kit - CLASSIQSwabs and the Hologic Direct Load Capture Cap Collection Kit - FLOQSwabs

A. After placing the collected specimen* into the Hologic Direct Load Capture Cap Tube, no further processing is required.

***Note:** Allow specimen to reach room temperature prior to processing.*

B. To avoid contact with the top of the tube, loosen the cap and place the sample tube ino the sample rack.

C. Remove and discard the cap and swab. To avoid contamination, do not pass the cap over any other sample racks or sample tubes. Inspect the sample tube. If bubbles are present, carefully remove from the sample tube (for example, use the tip of a sterile swab or similar method).

Note: *If the swab wasn't captured by the cap, recap the tube to ensure that the swab is captured and removed from the tube. Direct Load Capture Cap tubes containing a swab should not be loaded into the Panther System.*

Note: *Failure to remove bubbles may affect assay processing and cause invalid results.*

D. Place the rack retainer on the sample rack and load the rack into the instrument.

Specimen Processing using a Custom Specimen Lysis Tube

A. Using a sterile, or non-sterile (unused) generic tube made of polypropylene plastic or similar material that is 12 mm to 13 mm in outer diameter and 75 mm to 100 mm in height, aliquot 0.78 mL ± 0.07 mL of bulk STM into the tube using a pipet or repeat pipettor.

Note: *This step should be conducted in an area where SARS-CoV-2, Flu A, and Flu B specimens are NOT processed.*

Note: *If tubes are prepared prior to use, recap the tube and store at* 15°C to 30°C until use in specimen processing.

Note: *When the filled Custom Specimen Lysis Tube is stored closed, if no contaminants were introduced during the filling of the Custom Specimen Lysis Tube, the STM should be stable until the expiration date provided for the STM.*

Note: *There may be an increased risk of contamination when using non-sterile (unused) tubes.*

B. Uncap the Custom Specimen Lysis Tube containing STM and retain the cap.

C. Prior to testing on the Panther system, transfer 500 µL of the specimen to the Custom Specimen Lysis Tube containing STM.

D. It is recommended to recap the sample tube and gently invert three times to ensure viral inactivation and a homogeneous mixture.

E. To avoid contact with the top of the tube, loosen the cap and place the sample tube into the sample rack.

F. Remove and discard the cap. To avoid contamination, do not pass the cap over any other sample racks or sample tube. Inspect the sample tube. If bubbles are present, carefully remove from the tube (for example, use the tip of a sterile swab or similar method).

Note: *Failure to remove bubbles may affect assay processing and cause invalid results.*

G. Place the rack retainer on the sample rack and load the rack into the instrument.

Specimen Processing for Specimens Collected with the Aptima Multitest Collection Kit

A. Obtain and follow instructions for Panther Fusion Specimen Lysis Tube (Step A), Hologic Specimen Lysis Tube with Solid Cap (Step A), or Custom Specimen Lysis Tube (Step A-B).

B. Prior to testing on the Panther system, transfer 500 µL of the collected specimen from the Aptima Multitest Tube to a Panther Fusion Specimen Lysis Tube, Hologic Specimen Lysis Tube or Custom Specimen Lysis Tube as described in the specimen processing sections above.

Sample Storage

A. Samples on board the Panther system may be archived for additional testing at a later time.

B. Storing samples before or after testing

1. Samples in the Aptima Multitest Tube, Panther Fusion Specimen Lysis Tube, Hologic Specimen Lysis Tube, or Custom Specimen Lysis Tube, or Hologic Direct Load Capture Cap Tube should be stored upright in the rack under the following condition:
    - 2°C to 30°C up to 6 days
2. For both capped and uncapped workflows, samples should be covered with a new, clean plastic film or foil barrier.
3. If assayed samples need to be frozen or shipped:
    - Capped workflows

      Remove the penetrable cap and place a new non-penetrable cap on the specimen tubes. If samples need to be shipped for testing at another facility, recommended temperatures must be maintained. Prior to uncapping, specimen transport tubes must be centrifuged for 5 minutes at 420 Relative Centrifugal Force (RCF) to bring all of the liquid down to the bottom of the tube. Avoid splashing and cross-contamination.

    - Uncapped workflows

      If samples need to be shipped for testing at another facility, place a new solid cap on the specimen lysis tube, and the recommended temperatures must be maintained. Prior to uncapping, specimen transport tubes must be centrifuged for 5 minutes at 420

Relative Centrifugal Force (RCF) to bring all of the liquid down to the bottom of the tube. Avoid splashing and cross-contamination.

Note: *Replacement tube closures and tube plugs should not be used to cover tubes when centrifuging, freezing, or shipping.*

Specimen Transport

Maintain specimen storage conditions as described in the *Specimen Collection and Storage section on* page 7.

Note: *Specimens must be shipped in accordance with applicable national, international, and regional transportation regulations.*

Panther System

Reagents for the Aptima SARS-CoV-2/Flu assay are listed below for the Panther System. Reagent Identification Symbols are also listed next to the reagent name.

Reagents and Materials Provided

Aptima SARS-CoV-2/Flu Assay Kit PRD-06815

250 tests (2 boxes)

Aptima SARS-CoV-2/Flu Refrigerated Box (Box 1 of 2)
(store at 2°C to 8°C upon receipt)

Symbol	Component	Quantity 250 test kit
A	**Aptima SARS-CoV-2/Flu Amplification Reagent** *Non-infectious nucleic acids dried in buffered solution.*	1 vial
E	**Aptima SARS-CoV-2/Flu Enzyme Reagent** *Reverse transcriptase and RNA polymerase dried in HEPES buffered solution.*	1 vial
PRO	**Aptima SARS-CoV-2/Flu Promoter Reagent** *Non-infectious nucleic acids dried in buffered solution.*	1 vial
IC	**Aptima SARS-CoV-2/Flu Internal Control** *Non-infectious RNA nucleic acids in buffered solution.*	1 vial

Aptima SARS-CoV-2/Flu Room Temperature Box (Box 2 of 2)
(store at 15°C to 30°C upon receipt)

Symbol	Component	Quantity 250 test kit
AR	**Aptima SARS-CoV-2/Flu Amplification Reconstitution Solution** *Aqueous solution containing preservatives.*	1 x 27.7 mL
ER	**Aptima SARS-CoV-2/Flu Enzyme Reconstitution Solution** *HEPES buffered solution containing a surfactant and glycerol.*	1 x 11.1 mL
PROR	**Aptima SARS-CoV-2/Flu Promoter Reconstitution Solution** *Aqueous solution containing preservatives.*	1 x 35.4 mL
TCR	**Aptima SARS-CoV-2/Flu Target Capture Reagent** *Buffered salt solution containing solid phase and nucleic acids.*	1 x 54 mL
	Reconstitution Collars	3
	Master Lot Barcode Sheet	1 sheet

Materials Required and Available Separately

Note: *Materials available from Hologic have catalog numbers listed, unless otherwise specified.*

	Cat. No.
Panther System	303095
Aptima Assay Fluids Kit *(Aptima Wash Solution, Aptima Buffer for Deactivation Fluid, and Aptima Oil Reagent)*	303014 (1000 tests)
Multi-tube units (MTUs)	104772-02
Panther Waste Bag Kit	902731
Panther Waste Bin Cover	504405
Or Panther Run Kit *contains MTUs, waste bags, waste bin covers, assay fluids, and auto detects*	303096 (5000 tests)
Tips, liquid handling (LiHa),1000 µL filtered, conductive, and disposable.	901121 (10612513 Tecan) 903031 (10612513 Tecan) MME-04134 (30180117 Tecan) MME-04128 MME-04110
Aptima SARS-CoV-2/Flu Controls Kit *PC - Aptima SARS-CoV-2/Flu Positive Control. Non-infectious nucleic acid in a buffered solution containing < 5% detergent. Quantity 5 x 1.7 mL* *NC - Aptima SARS-CoV-2/Flu Negative Control. A buffered solution containing < 5% detergent. Quantity 5 x 1.7 mL*	PRD-06816
Aptima Multitest Swab Specimen Collection Kit	PRD-03546
Hologic Direct Load Capture Cap Collection Kit - CLASSIQSwabs	PRD-06951
Hologic Direct Load Capture Cap Collection Kit - FLOQSwabs	PRD-06952
Aptima Unisex Swab Specimen Collection Kit for Endocervical and Male Urethral Swab Specimens* **used for lab contamination monitoring*	*testing as part of the uncapped workflow*
Panther Fusion Specimen Lysis Tubes, 100 per bag *tube contains 0.71 mL of STM with a penetrable cap*	
Hologic Specimen Lysis Tubes, 100 each *tube contains 0.71 mL of STM with a solid cap (for uncapped workflow)*	
Hologic Specimen Lysis Tubes, 1200 each *tube contains 0.71 mL of STM with a solid cap (for uncapped workflow)*	
Hologic Solid Cap for use with PRD-06554*, 100 caps per bag **a single-use cover for the Hologic Specimen Lysis Tube (PRD-06554 only) after testing as part of the uncapped workflow*	
Hologic Solid Cap for use with PRD-06660*, 1000 caps per bag **a single-use cover for the Hologic Specimen Lysis Tube (PRD-06660 only) after*	

301041

PRD-04339 PRD-06554 PRD-06660 PRD-06744

PRD-06723

	Cat. No.
Hologic Solid Cap for use with PRD-06951* and PRD-06952*, 100 caps per bag **a single-use cover for the Direct Load Capture Cap (PRD-06951 and PRD-06952) after testing as part of the uncapped workflow*	PRD-07028
Specimen Transport Medium, 1 bottle, 80 mL (for uncapped workflow)	PRD-04423
Bleach, 5% to 7% (0.7M to 1.0M) sodium hypochlorite solution	—
Disposable gloves	—
Fisherbrand VersaClosure Tube Closures*, 1000 per pack **a single-use tube cover for the Hologic Specimen Lysis Tube (PRD-06554 only) after testing as part of the uncapped workflow*	02-707
Replacement Caps for the 250-test kits *Amplification and Promoter reagent reconstitution solutions CL0041 (100 caps)* *Enzyme Reagent reconstitution solution 501616 (100 caps)* *TCR reagent CL0040 (100 caps)*	—

Optional Materials

	Cat. No.
Hologic Bleach Enhancer for Cleaning *for routine cleaning of surfaces and equipment*	302101
Generic Sample Tube (for Custom Specimen Lysis Tube) *Size: 12 x 75 mm to 13 x 100 mm (including 12 x 100 mm, 13 x 75 mm, and 13 x 82 mm)* *Material: Polypropylene plastic or similar material* *Non-sterile (unused) or sterile* *Round, flat bottom, or conical (skirted conical)*	—
Tube rocker	—

Panther System Test Procedure

Note: *Refer to the Panther/Panther System Operator's Manual for additional procedural information.*

A. Work Area Preparation

Clean work surfaces where reagents and samples will be prepared. Wipe down work surfaces with 2.5% to 3.5% (0.35M to 0.5M) sodium hypochlorite solution. Allow the sodium hypochlorite solution to contact surfaces for at least 1 minute and then follow with a water rinse. Do not allow the sodium hypochlorite solution to dry. Cover the bench surface on which the reagents and samples will be prepared with clean, plastic-backed absorbent laboratory bench covers.

B. Reagent Reconstitution/Preparation of a New Kit

Note: *Reagent reconstitution should be performed prior to beginning any work on the Panther System.*

1. To reconstitute Amplification, Enzyme, and Promoter Reagents, combine the bottles of lyophilized reagent with the reconstitution solution. If refrigerated, allow the reconstitution solutions to reach room temperature before use.
   a. Pair each reconstitution solution with its lyophilized reagent. Ensure that the reconstitution solution and reagent have matching label colors before attaching the reconstitution collar.
   b. Check the lot numbers on the Master Lot Barcode Sheet to ensure that the appropriate reagents are paired.
   c. Open the lyophilized reagent vial and firmly insert the notched end of the reconstitution collar into the vial opening (Figure 1, Step 1).
   d. Open the matching reconstitution solution, and set the cap on a clean, covered work surface.
   e. While holding the reconstitution solution bottle on the bench, firmly insert the other end of the reconstitution collar into the bottle opening (Figure 1, Step 2).
   f. Slowly invert the assembled bottles. Allow the solution to drain from the bottle into the glass vial (Figure 1, Step 3).
   g. Thoroughly mix the solution in the glass vial by swirling (Figure 1, Step 4).
   h. Wait for the lyophilized reagent to go into solution, then invert the assembled bottles again, tilting at a 45° angle to minimize foaming (Figure 1, Step 5). Allow all of the liquid to drain back into the plastic bottle.
   i. Remove the reconstitution collar and glass vial (Figure 1, Step 6).
   j. Recap the plastic bottle. Record operator initials and reconstitution date on the label (Figure 1, Step 7).
   k. Discard the reconstitution collar and glass vial (Figure 1, Step 8).

      Option: Additional mixing of the Amplification, Enzyme, and Promoter Reagents using a tube rocker is allowed. The reagents may be mixed by placing the recapped plastic bottle on a tube rocker set to 20 RPM (or equivalent) for a minimum of 5 minutes.

Warning: *Avoid creating foam when reconstituting reagents. Foam compromises the level-sensing in the Panther System.*

Warning: *Adequate mixing of the reagents is necessary to achieve expected assay results.*

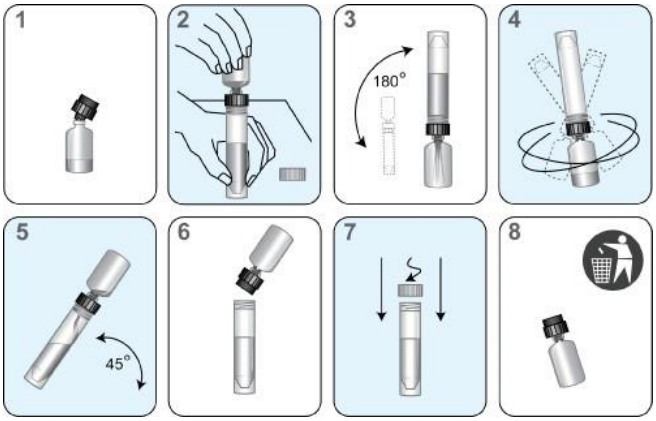

Figure 1. Panther System Reconstitution Process

2. Prepare Working Target Capture Reagent (wTCR)
   a. Pair the appropriate bottles of TCR and IC.
   b. Check the reagent lot numbers on the Master Lot Barcode Sheet to make sure that the appropriate reagents in the kit are paired.
   c. Open the bottle of TCR, and set the cap on a clean, covered work surface.
   d. Open the IC bottle and pour the entire contents into the bottle of TCR. Expect a small amount of liquid to remain in the IC bottle.
   e. Cap the bottle of TCR and gently swirl the solution to mix the contents. Avoid creating foam during this step.
   f. Record operator initials and the current date on the label.
   g. Discard the IC bottle and cap.

Note: *Thoroughly mix by gently inverting all reagents prior to loading on the system. Avoid creating foam during inversion of reagents.*

C. Reagent Preparation for Previously Reconstituted Reagents

1. Previously reconstituted Amplification, Enzyme, and Promoter Reagents must reach room temperature (15°C to 30°C) prior to the start of the assay.

   Option: The reagents may be brought to room temperature by placing the reconstituted Amplification, Enzyme, and Promoter Reagents on a tube rocker set to 20 RPM (or equivalent) for a minimum of 25 minutes.

2. If reconstituted Promoter Reagent contains precipitate that does not return to solution at room temperature, heat the capped bottle at a temperature that does not exceed 62°C for 1 to 2 minutes. After this heat step, the Promoter Reagent may be used even if residual precipitate remains. Mix Promoter Reagent by inversion, being careful not to induce foam, prior to loading onto the system.
3. Thoroughly mix each reagent by gently inverting prior to loading on the system. Avoid creating foam during inversion of reagents. This step is not required if reagents are loaded onto the system directly after mixing on the tube rocker.
4. Do not top off reagent bottles. The Panther System will recognize and reject bottles that have been topped off.

5. *Adequate mixing of the reagents is necessary to achieve expected assay results.*

D. Specimen Handling using Panther Fusion Specimen Lysis Tube

Note: *Prepare specimens per the Specimen Processing instructions in the Specimen Collection and Storage section before loading specimens onto the Panther system.*

1. Inspect sample tubes before loading into the rack. If a sample tube contains bubbles or has a lower volume than is typically observed, gently tap the bottom of the tube to bring contents to the bottom.

Note: *For samples transferred to the Panther Fusion Specimen Lysis Tube, to avoid a processing error, ensure adequate specimen volume is added to the tube. When adequate collected specimen is added to the tube, there is sufficient volume to perform 3 nucleic acid extractions.*

E. Specimen Handling using Hologic Specimen Lysis Tube with Solid Cap or Custom Specimen Lysis Tube

1. Prepare specimens per the specimen processing instructions in the *Specimen Collection and Storage* section.

Note: *For samples transferred to the Hologic Specimen Lysis Tube with solid cap or a Custom Specimen Lysis Tube, to avoid a processing error, ensure adequate specimen volume is added to the tube.*

Note: *When adequate collected specimen is added to the Hologic Specimen Lysis Tube (PRD-06554) or a custom Specimen Lysis Tube, there is sufficient volume to perform 2 nucleic acid extractions.*

Note: *When adequate collected specimen is added to the Hologic Specimen Lysis tube (PRD-06660), there is sufficient volume to perform 1 nucleic acid extraction.*

Note: *When using the Aptima SARS-CoV-2/Flu uncapped tube assay software, remove the cap from the Positive and Negative control before loading onto the Panther system.*

F. System Preparation

1. Set up the system according to the instructions in the *Panther/Panther Fusion System Operator's Manual* and *Procedural Notes*. Make sure that the appropriately sized reagent racks and TCR adapters are used.
2. Load samples.

Procedural Notes

A. Controls

1. To work properly with the Aptima Assay software for the Panther system, one pair of controls is required. The Aptima SARS-CoV-2/Flu positive and negative controls can be loaded in any rack position or in any Sample Bay Lane on the Panther system. Patient specimen pipetting will begin when one of the following two conditions has been met:
   a. A pair of controls is currently being processed by the system.
   b. Valid results for the controls are registered on the system.
2. Once the control tubes have been pipetted and are processing for a specific reagent kit, patient specimens can be run with the associated kit up to 24 hours unless:

a. Controls results are invalid.
b. The associated assay reagent kit is removed from the system.
c. The associated assay reagent kit has exceeded stability limits.

3. Each Aptima control tube can be tested once. Attempts to pipette more than once from the tube can lead to processing errors.
4. Patient specimen pipetting begins when one of the following two conditions is met:
   a. Valid results for the controls are registered on the system.
   b. A pair of controls is currently in process on the system.

B. Temperature

Room temperature is defined as 15°C to 30°C.

C. Glove Powder

As in any reagent system, excess powder on some gloves may cause contamination of opened tubes. Powderless gloves are recommended.

D. Lab Contamination Monitoring Protocol for the Panther System

There are many laboratory-specific factors that may contribute to contamination, including testing volume, workflow, disease prevalence and various other laboratory activities. These factors should be taken into consideration when contamination monitoring frequency is being established. Intervals for contamination monitoring should be established based on each laboratory's practices and procedures.

To monitor for laboratory contamination, the following procedure may be performed using the Aptima Unisex Swab Specimen Collection Kit for Endocervical and Male Urethral Swab Specimens:

1. Label swab transport tubes with numbers corresponding to the areas to be tested.
2. Remove the specimen collection swab (blue shaft swab with green printing) from its packaging, wet the swab in the specimen transport medium (STM), and swab the designated area using a circular motion.
3. Immediately insert the swab into transport tube.
4. Carefully break the swab shaft at the score line; use care to avoid splashing of the contents.
5. Recap the swab transport tube tightly.
6. Repeat Steps 2 to 5 for each area to be swabbed.

E. If the results are positive, see *Interpretation of Results*. For additional Panther system-specific contamination monitoring information, contact Hologic Technical Support.

Quality Control

A run or specimen result may be invalidated by the Panther system if problems occur while performing the assay. Specimens with invalid results must be retested.

Negative and Positive Controls

To generate valid results, a set of assay controls must be tested. One replicate of the negative assay control and positive assay control must be tested each time a new kit is loaded on the Panther system or when the current set of valid controls have expired.

The Panther system is configured to require assay controls run at an administrator-specified interval of up to 24 hours. Software on the Panther system alerts the operator when assay controls are required and does not start new tests until the assay controls are loaded and have started processing.

During processing, criteria for acceptance of the assay controls are automatically verified by the Panther system. To generate valid results, the assay controls must pass a series of validity checks performed by the Panther system.

If the assay controls pass all validity checks, they are considered valid for the administrator-specified time interval. When the time interval has passed, the assay controls are expired by the Panther system which requires a new set of assay controls be tested prior to starting any new samples.

If any one of the assay controls fails the validity checks, the Panther system automatically invalidates the affected samples and requires a new set of assay controls be tested prior to starting any new samples.

Internal Control

An internal control is added to each sample with the wTCR. During processing, the internal control acceptance criteria are automatically verified by the Panther system software. Detection of the internal control is not required for samples that are positive for SARS-CoV-2 and/or flu. The internal control must be detected in all samples that are negative for SARS-CoV-2 and flu targets; samples that fail to meet that criteria will be reported as Invalid. Each sample with an Invalid result must be retested.

The Panther system is designed to accurately verify processes when procedures are performed following the instructions provided in this package insert and the *Panther/Panther Fusion System Operator's Manual.*

Interpretation of Results

The Panther system automatically determines the test results for samples and controls. A test result may be Negative, Positive, No Test, or Invalid.

Table1 shows the possible results reported in a valid run with result interpretations.

Table 1: Aptima SARS-CoV-2/Flu Results Interpretation

SARS-CoV-2 Result	Flu A Result	Flu B Result	IC Result	Interpretation
Negative	Negative	Negative	Valid	SARS-CoV-2, Flu A, and Flu B not detected.
Positive	Negative	Negative	Valid	SARS-CoV-2 detected. Flu A and Flu B not detected.
Negative	Positive	Negative	Valid	Flu A detected. SARS-CoV-2 and Flu B not detected.
Negative	Negative	Positive	Valid	Flu B detected. SARS-CoV-2 and Flu A not detected.
Positive	Positive	Negative	Valid	SARS-CoV-2 and Flu A detected. Flu B not detected
Negative	Positive	Positive	Valid	Flu A and Flu B detected. SARS-CoV-2 not detected.
Positive	Negative	Positive	Valid	SARS-CoV-2 and Flu B detected. Flu A not detected.
Positive	Positive	Positive	Valid	SARS-CoV-2, Flu A and Flu B detected.
Invalid	Invalid	Invalid	Invalid	Invalid. There was an error in the generation of the result; retest sample.

Note: Positive result will be accompanied by TTime values.

Note: Detection of IC is not required for samples that are positive for SARS-CoV-2, Flu A, and/or Flu B.

Note: Users can only mask Flu A and/or Flu B results but not SARS-CoV-2 results. The result is shown as No Test if the analyte is masked in the software.

Note: If an invalid result due to an assay processing error (p flag) is observed with a sample collected directly into Specimen Transport Medium, consider vortexing the sample for a minimum of 5 minutes before repeating the test.

Limitations

A. Use of this assay is limited to personnel who are trained in the procedure. Failure to follow these instructions may result in erroneous results.

B. Reliable results are dependent on adequate specimen collection, transport, storage, and processing.

C. Avoid contamination by adhering to good laboratory practices and to the procedures specified in this package insert.

D. Negative results do not preclude SARS-CoV-2 infections and should not be used as the sole basis for treatment or other management decisions.

E. A positive result indicates the detection of nucleic acid from the relevant virus. Nucleic acid may persist even after the virus is no longer viable.

Panther SARS-CoV-2/Flu Assay Performance

Analytical Sensitivity

The analytical sensitivity (limit of detection or LoD) of the Aptima SARS-CoV-2/Flu assay was determined by testing serial dilutions of pooled negative clinical nasopharyngeal swab VTM/UTM specimens spiked with the following virus cultures: 1 SARS-CoV-2 strain, 2 Flu A strains, and 2 Flu B strains. Ten replicates of each serial dilution for each strain were evaluated using each of two assay reagent lots. The LoD is defined as the lowest concentration at which ≥95% of all replicates tested positive, as summarized in Table 2. Each target specific LoD was confirmed by testing an additional 20 replicates in negative clinical NP swab VTM/UTM matrix with one reagent lot. The LoD was also confirmed in negative clinical Multitest matrix, negative clinical saline matrix, specimen transport medium (STM) swab collection media and saline media.

Table 2: Analytical Sensitivity in Clinical VTM/UTM Matrix

Viral Strain	LoD Concentration
SARS-CoV-2 (USA-WA1/2020)	0.001 $TCID_{50}$/mL
Influenza A/California/07/2009 (H1N1)	0.03 $TCID_{50}$/mL
Influenza A/Switzerland/9715293/2015 (H3N2)	0.003 $TCID_{50}$/mL
Influenza B/Brisbane/33/08 (Victoria lineage)	0.01 $TCID_{50}$/mL
Influenza B/Massachusetts/02/2012 (Yamagata lineage)	0.3 $TCID_{50}$/mL

Reactivity

The reactivity of the Aptima SARS-CoV-2/Flu assay was evaluated against multiple strains of Flu A (H1N1 & H3N2) and multiple strains of Flu B (Victoria and Yamagata lineages). Viral strains were tested in triplicate with one reagent lot. Table 3 shows the lowest concentration of each strain in which 100% positivity was observed. Additionally, the 2020 CDC Human Influenza Panel was evaluated with the assay. Five-fold dilutions of each panel member were evaluated with a minimum of five replicates according to the CDC protocol. Table 4 shows the lowest concentration of each panel member in which at least one replicate yielded a positive result.

Table 3: Analytical Reactivity Summary for Flu A and Flu B Strains

Strain	Subtype	Concentration ($TCID_{50}$/mL)	Concentration Relative to LoD	SARS-CoV-2	Flu A	Flu B
Influenza A/Massachusetts/15/13	Flu A (H1N1)	0.09	3x LOD	-	+	-
A/Taiwan/42/2006	Flu A (H1N1)	0.09	3x LOD	-	+	-
A/Henan/8/05	Flu A (H1N1)	0.09	3x LOD	-	+	-
A/Kentucky/2/06	Flu A (H1N1)	0.3	10x LOD	-	+	-
A/Hawaii/15/01	Flu A (H1N1)	3	100x LOD	-	+	-
A/Brisbane/59/2007	Flu A (H1N1)	0.09	3x LOD	-	+	-
A/Solomon Islands/03/06	Flu A (H1N1)	0.09	3x LOD	-	+	-
A1/Mal/302/54	Flu A (H1N1)	0.09	3x LOD	-	+	-
A1/Denver/1/57	Flu A (H1N1)	0.9	30x LOD	-	+	-
Ohio/09SW1477/2009	Flu A (H1N2)	0.3	10x LOD	-	+	-
Michigan/45/2015	Flu A (H1N1)	0.09	3x LOD	-	+	-
A/Hiroshima/52/05	Flu A(H3N2)	0.009	3x LOD	-	+	-
A/Victoria/3/75	Flu A(H3N2)	9	3000x LOD	-	+	-
A/Brazil/1137/99	Flu A (H3N2)	0.09	30x LOD	-	+	-
A/Hong Kong/8/68	Flu A (H3N2)	0.9	300x LOD	-	+	-
A/Aichi/2/68	Flu A (H3N2)	0.3	100x LOD	-	+	-
Indiana/08/2011	Flu A (H3N2)	0.03	10x LOD	-	+	-
Perth/16/2009	Flu A (H3N2)	0.009	3x LOD	-	+	-
A/Costa Rica/07/99	Flu A (H3N2)	3	1000x LOD	-	+	-
Port Chalmers/1/73	Flu A (H3N2)	0.3	100x LOD	-	+	-
HongKong/4801/2014	Flu A (H3N2)	0.009	3x LOD	-	+	-
Texas/50/2012	Flu A (H3N2)	0.009	3x LOD	-	+	-
B/Ohio/1/2005	Flu B (Victoria)	0.03	3x LOD	-	-	+
Alabama/2/17	Flu B (Victoria)	0.03	3x LOD	-	-	+
Florida/78/2015	Flu B (Victoria)	0.03	3x LOD	-	-	+
Colorado/06/2017	Flu B (Victoria)	0.03	3x LOD	-	-	+
B/St. Petersburg/14/06	Flu B (Yamagata)	0.9	3x LOD	-	-	+
Utah/9/14	Flu B (Yamagata)	0.9	3x LOD	-	-	+
Wisconsin/1/2010	Flu B (Yamagata)	0.9	3x LOD	-	-	+
Phuket/3073/2013	Flu B (Yamagata)	0.9	3x LOD	-	-	+
B/Lee/40	Flu B	3	N/A	-	-	+

Table 4: 2020 CDC Human Influenza Panel

Virus	Strain	Minimum Reactive Concentration (EID_{50}/mL)
Influenza A	A/Perth/16/2009 (H3N2)	1.02E+01
	A/Hong Kong/2671/2019 (H3N2)	8.10E-01
	A/Christ Church/16/2010 (H1N1 pdm)	1.62E+01
	A/Guangdong-maonan/1536/2019 pdm)	1.29E+00
Influenza B	B/Michigan/09/2011	8.13E-03
	B/Washington/02/2019	1.62E+00
	B/Texas/81/2016	2.04E-01
	B/Phuket/3073/2013	8.13E+00

Inclusivity

The inclusivity of the Aptima SARS-CoV-2/Flu assay was evaluated using *in silico* analysis of the assay target capture oligos, amplification primers, and detection torches for the SARS-CoV-2, Flu A and Flu B target systems in relation to sequences available in the NCBI and GISAID gene databases as of September 30, 2020. Any sequence with missing or ambiguous sequence information was removed from the analysis for that target region.

For SARS-CoV-2, there were 111,055 sequences evaluated for the first target region, 110,932 sequences evaluated for the second target region, and 110,784 sequences with complete information for both regions. The *in silico* analysis showed 100% homology to the assay oligos of both target systems for 96,883 (87.5%) of the evaluated sequences and 100% homology to the assay oligos of at least one target system for 110,743 (99.96%) of the sequences. There were no evaluated sequences with identified mismatches predicted to impact binding or assay performance.

For Flu A and Flu B, there were 79,898 and 28,146 sequences, respectively, since January 01, 2015 with information corresponding to the oligos for the target regions of the assay. Of the available sequences for Flu A, 38,700 (48.4%) showed 100% homology to all oligos of the target region. Of the remaining 41,198 sequences, oligo binding is predicted for all but 687 for an overall inclusivity of 99.1% for the evaluated sequences. Of the available sequences for Flu B, 5,867 (20.8%) showed 100% homology to all oligos of the target region. Of the remaining 22,279 sequences, oligo binding is predicted for all but 22 for an overall inclusivity of 99.9% for the evaluated sequences.

Analytical Specificity and Microbial Interference

The analytical specificity of the Aptima SARS-CoV-2/Flu assay was evaluated by testing 37 microorganisms representing common respiratory pathogens or closely related species (Table 5). Bacteria were tested at 10^6 CFU/mL and viruses were tested at 10^5 $TCID_{50}$/mL, except where noted. Microorganisms were tested with and without the presence of SARS-CoV-2, Flu A (H1N1) and Flu B (Victoria lineage) cultured virus at 3x LoD concentrations. Analytical specificity of the Aptima SARS-CoV-2/Flu assay was 100% with no evidence of microbial interference from non-

target microorganisms. In addition to microorganism testing, *in silico* BLAST analysis was performed to assess the specificity of the assay in relation to the microorganisms listed in Table 5. The *in silico* analysis showed no probable cross reactivity to any of the 202 GenBank sequences evaluated.

Table 5: Analytical Specificity and Microbial Interference Microorganisms

Microorganism	Concentration	Microorganism	Concentration
Adenovirus	1.0E+06 $TCID_{50}$/mL	*Legionella pneumophila*	1.0E+06 CFU/mL
Enterovirus (e.g. EV68)	1.0E+04 $TCID_{50}$/mL	*Mycobacterium tuberculosis*	1.0E+08 $TCID_{50}$/mL
Rhinovirus	1.0E+04 $TCID_{50}$/mL	*Mycoplasma pneumoniae*	1.0E+05 CFU/mL
Human coronavirus 229E	1.0E+06 $TCID_{50}$/mL	*Pneumocystis jirovecii* (PJP)	1.0E+06 nuc/mL
Human coronavirus HKU1	1.0E+06 c/mL	*Pseudomonas aeruginosa*	1.0E+06 CFU/mL
Human coronavirus[1] NL63	1.0E+03 $TCID_{50}$/mL	*Staphylococcus epidermidis*	1.0E+06 CFU/mL
Human coronavirus OC43	1.0E+04 $TCID_{50}$/mL	*Streptococcus pneumonia*	1.0E+04 CFU/mL
MERS-coronavirus	1.0E+03 $TCID_{50}$/mL	*Streptococcus pyogenes*	1.0E+06 CFU/mL
SARS-coronavirus[1]	1.0E+06 c/mL	*Streptococcus salivarius*	1.0E+06 CFU/mL
Parainfluenza virus 1	1.0E+05 $TCID_{50}$/mL	Influenza A[3]	1.0E+05 $TCID_{50}$/mL
Parainfluenza virus 2	1.0E+03 $TCID_{50}$/mL	Influenza B[3]	1.0E+04 $TCID_{50}$/mL
Parainfluenza virus 3	1.0E+05 $TCID_{50}$/mL	*Neisseria meningitides*	1.0E+06 CFU/mL
Parainfluenza virus 4a	1.0E+05 $TCID_{50}$/mL	*Neisseria gonorrhea*	1.0E+06 CFU/mL
Human Metapneumovirus (hMPV)	1.0E+05 $TCID_{50}$/mL	*Moraxella catarrhalis*	1.0E+06 CFU/mL
Respiratory syncytial virus	1.0E+04 $TCID_{50}$/mL	*Lactobacillus plantarum*	1.0E+06 CFU/mL
Bordetella pertussis	1.0E+06 CFU/mL	*Corynebacterium diphtheria*	1.0E+06 CFU/mL
Candida albicans	1.0E+06 CFU/mL	*Escherichia coli*	1.0E+06 CFU/mL
Chlamydia pneumonia	1.0E+05 CFU/mL	SARS-CoV-2[3]	1.0E+05 $TCID_{50}$/mL
Haemophilus influenzae	1.0E+06 CFU/mL	30 Individual negative clinical NP swab VTM/UTM specimens[2]	N/A

[1] Cultured virus and whole genome purified nucleic acid for Human coronavirus HKU1 and SARS-coronavirus are not readily available. HKU1 and SARS-coronavirus IVTs corresponding to the ORF1ab gene regions targeted by the assay were used to evaluate cross-reactivity and microbial interference.

[2] In place of evaluating pooled human nasal wash, 30 individual negative clinical NP swab specimens were tested in triplicate to represent diverse microbial flora in the human respiratory tract.

[3] SARS-CoV-2, Influenza A, and Influenza B are targets of the assay. Analysis of cross-reactivity was only performed for the other targets.

Competitive Interference

Competitive interference of the Aptima SARS-CoV-2/Flu assay was evaluated using pairs of target viruses at low/high concentrations in negative clinical NP swab VTM/UTM matrix. The low concentration virus was tested at 3x LoD, while the high concentration virus was tested at the maximum allowable concentration based on the stock titer. Testing was performed using one SARS-CoV-2, one Flu A (H1N1), and one Flu B (Victoria lineage) virus strain. The presence of two viruses at varying low/high concentrations in a single sample had no effect on the analytical sensitivity (100% detection for both targets) at the concentrations noted in Table 6.

Table 6: Competitive Interference

Condition	Target 1		Target 2		SARS-CoV-2	Flu A	Flu B
	3x LoD		High				
	Virus	Concentration ($TCID_{50}$/mL)	Virus	Concentration ($TCID_{50}$/mL)			
1	SARS-CoV-2	0.003	Flu A	3.16e4	+	+	-
2	SARS-CoV-2	0.003	Flu B	1.17e4	+	-	+
3	Flu A	0.09	SARS-CoV-2	1.4e1	+	+	-
4	Flu A	0.09	Flu B	1.17e1	-	+	+
5	Flu B	0.03	SARS-CoV-2	1.4e4	+	-	+
6	Flu B	0.03	Flu A	3.16e3	-	+	+

Clinical Performance

The clinical performance of the Aptima SARS-CoV-2/Flu assay was evaluated in comparison to the Panther Fusion SARS-CoV-2 assay (Hologic, Inc) and the Panther Fusion Flu A/B/RSV assay (Hologic, Inc.) using a panel of remnant clinical nasopharyngeal specimens collected from patients with signs and symptoms of respiratory infection. For the evaluation, a combination of negative, SARS-CoV-2 positive, Flu A positive, and Flu B positive specimens were tested with each assay.

The Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) for SARS-CoV-2 was calculated in relation to the Panther Fusion SARS-CoV-2 assay as the reference result, as shown in Table 7. The assay showed positive and negative percent agreements of 96.1% and 99.6%, respectively for SARS-CoV-2.

For Flu A and Flu B, the PPA and NPA were calculated in relation to the Panther Fusion Flu A/B/RSV assay as the reference result, as shown in Table 8 for Flu A and Table 9 for Flu B. The assay showed positive and negative percent agreements of 100% and 99.2%, respectively, for Flu A and of 100% and 100%, respectively, for Flu B.

Table 7: Clinical Performance Results for SARS-CoV-2

SARS-CoV-2		Panther Fusion Result		
		Positive	**Negative**	**Total**
Aptima SARS/Flu Result	**Positive**	49	1	50
	Negative	2	247	249
	Total	51	248	299
Positive Agreement		96.1%	(86.8% - 98.9%)	
Negative Agreement		99.6%	(97.8% - 99.9%)	

Table 8: Clinical Performance Results for Flu A

Flu A		Panther Fusion Result		
		Positive	**Negative**	**Total**
Aptima SARS/Flu Result	**Positive**	48	2	50
	Negative	0	249	249
	Total	48	251	299
Positive Agreement		100%	(92.6% - 100%)	
Negative Agreement		99.2%	(97.1% - 99.8%)	

Table 9: Clinical Performance Results for Flu B

Flu B		Panther Fusion Result		
		Positive	**Negative**	**Total**
Aptima SARS/Flu Result	**Positive**	49	0	49
	Negative	0	250	250
	Total	49	250	299
Positive Agreement		100%	(92.7% - 100%)	
Negative Agreement		100%	(98.5% - 100%)	

Bibliography

1. **Centers for Disease Control and Prevention.** https://www.cdc.gov/coronavirus/2019-ncov/symptoms-testing/symptoms.html. Accessed October 7, 2020.
2. **Centers for Disease Control and Prevention.** https://www.cdc.gov/flu/about/index.html. Accessed October 7, 2020.
3. **Centers for Disease Control and Prevention.** https://www.cdc.gov/flu/symptoms/index.html. Accessed October 7, 2020.

4. **World Health Organization.** Q&A on coronaviruses (COVID-19). http://www.emro.who.int/health-topics/corona-virus/questions-and- answers.html. Accessed October 7, 2020.
5. **Centers for Disease Control and Prevention.** https://www.cdc.gov/coronavirus/2019-ncov/symptoms-testing/symptoms.html. Accessed October 7, 2020.
6. **Clinical & Laboratory Standards Institute.** Document M29 Protection of Laboratory Workers from Occupationally Acquired Infections. CLSI Web site https://clsi.org/standards/products/microbiology/documents/m29/. Accessed September 2017.

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Aptima™ SARS-CoV-2 Assay (Panther™ System)

For i*n vitro* diagnostic use.

For U.S. Export only.

CONTENTS

General Information

Intended Use

The Aptima™ SARS-CoV-2 assay is a nucleic acid amplification *in vitro* diagnostic test intended for the qualitative detection of RNA from SARS-CoV-2 isolated and purified from nasopharyngeal (NP), nasal, mid-turbinate and oropharyngeal (OP) swab specimens, nasopharyngeal wash/ aspirate, nasal aspirates, or saliva obtained from individuals meeting COVID-19 clinical and/or epidemiological criteria including from individuals without symptoms or other reasons to suspect COVID-19 infection.

This test is also for the qualitative detection of nucleic acid from the SARS-CoV-2 in pooled samples containing up to 5 individual upper respiratory swab specimens (i.e. nasopharyngeal, nasal, mid-turbinate, or oropharyngeal swabs), where each specimen is collected under observation or by a healthcare provider using individual vials containing transport media. Negative results from pooled testing should not be treated as definitive. If a patient's clinical signs and symptoms are inconsistent with a negative result and if results are necessary for patient management, then the patient should be considered for individual testing. Specimens included in pools with a positive or invalid result must be tested individually prior to reporting a result. Specimens with low viral loads may not be detected in sample pools due to the decreased sensitivity of pooled testing. For specific patients, whose specimen(s) were the subject of pooling, a notice that pooling was used during testing must be included when reporting the result to the healthcare provider.

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in upper respiratory specimens during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 RNA, clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses.

Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

The Aptima SARS-CoV-2 assay on the Panther™ and Panther Fusion™ system is intended for use by clinical laboratory personnel specifically instructed and trained in the operation of the Panther and Panther Fusion systems and in vitro diagnostic procedures.

Summary and Explanation of the Test

Coronaviruses are a large family of viruses which may cause illness in animals or humans. In humans, several coronaviruses are known to cause respiratory infections ranging from the common cold to more severe diseases such as Middle East Respiratory Syndrome (MERS) and Severe Acute Respiratory Syndrome (SARS). The most recently discovered coronavirus, SARS-CoV-2, causes the associated coronavirus disease COVID-19. This new virus and disease were unknown before the outbreak began in Wuhan, China, in December 2019.[1]

The most common symptoms of COVID-19 are fever, tiredness, and dry cough. Some patients may have aches and pains, nasal congestion, runny nose, sore throat, new loss of taste or smell, or diarrhea. These symptoms are usually mild and begin gradually. Some people become infected but don't develop any symptoms and don't feel unwell. The disease can spread through

respiratory droplets produced when an infected person coughs or sneezes. These droplets can land in the mouths or noses of people who are nearby or possibly be inhaled into the lungs.[2] These droplets also can land on objects and surfaces around the person. Other people may acquire SARS-CoV-2 by touching these objects or surfaces, then touching their eyes, nose, or mouth.

The virus that causes COVID-19 is infecting people and spreading easily from person to person.[3] On March 11, 2020, the COVID-19 outbreak was characterized as a pandemic by the World Health Organization (WHO).[4,5]

Principles of the Procedure

The Aptima SARS-CoV-2 assay combines the technologies of target capture, Transcription Mediated Amplification (TMA), and Dual Kinetic Assay (DKA).

Specimens are collected and transferred into their respective specimen transport tubes. The transport solutions in these tubes release the RNA target and protect them from degradation during storage. When the Aptima SARS-CoV-2 assay is performed in the laboratory, the target RNA molecules are isolated from specimens by use of capture oligomers via target capture that utilizes magnetic microparticles. The capture oligomers contain sequences complementary to specific regions of the target molecules as well as a string of deoxyadenosine residues. A separate capture oligomer is used for each target. During the hybridization step, the sequence specific regions of the capture oligomers bind to specific regions of the target molecules. The capture oligomer:target complex is then captured out of solution by decreasing the temperature of the reaction to room temperature. This temperature reduction allows hybridization to occur between the deoxyadenosine region on the capture oligomer and the poly-deoxythymidine molecules that are covalently attached to the magnetic particles. The microparticles, including the captured target molecules bound to them, are pulled to the side of the reaction vessel using magnets and the supernatant is aspirated. The particles are washed to remove residual specimen matrix that may contain amplification reaction inhibitors. After the target capture steps are completed, the specimens are ready for amplification.

Target amplification assays are based on the ability of complementary oligonucleotide primers to specifically anneal and allow enzymatic amplification of the target nucleic acid strands. The Aptima SARS-CoV-2 assay replicates specific regions of the RNA from SARS-CoV-2 virus. Detection of the RNA amplification product sequences (amplicon) is achieved using nucleic acid hybridization. Single-stranded chemiluminescent nucleic acid probes, which are unique and complementary to a region of each target amplicon and Internal Control (IC) amplicon, are labeled with different acridinium ester (AE) molecules. The AE labeled probes combine with amplicon to form stable hybrids. The Selection Reagent differentiates hybridized from unhybridized probe, eliminating the generation of signal from unhybridized probe. During the detection step, light emitted from the labeled hybrids is measured as photon signals in a luminometer, and are reported as Relative Light Units (RLU). In DKA, differences in the kinetic profiles of the labeled probes allow for the differentiation of signal; kinetic profiles are derived from measurements of photon output during the detection read time. The chemiluminescent detection reaction for the IC signal has very rapid kinetics and has the “flasher” kinetic type. The chemiluminescent detection reaction for the SARS-CoV-2 signal is relatively slower and has the “glower” kinetic type. Assay results are determined by a cut-off based on the total RLU and the kinetic curve type.

The Aptima SARS-CoV-2 assay amplifies and detects two conserved regions of the ORF1ab gene in the same reaction, using the same "glower" kinetic type. The two regions are not differentiated and amplification of either or both regions leads to RLU signal. The assay results are determined by a cut-off based on the total RLU and the kinetic curve type.

Warnings and Precautions

A. For *in vitro* diagnostic use. Carefully read this entire package insert and the *Panther/Panther Fusion System Operator's Manual*.

B. Only personnel adequately trained on the use of this assay and in handling potentially infectious materials should perform these procedures. If a spill occurs, immediately disinfect using appropriate site procedures.

C. Handle and process all specimens as if infectious following laboratory practices and procedures that are basic to good microbiological practice and procedures (GMPP). Refer to World Health Organization's (WHO) Laboratory biosafety guidance related to coronavirus disease (COVID-19): interim guidance. https://www.who.int/publications/i/item/laboratory-biosafety-guidance-related-to-coronavirus-disease-(covid-19).

D. Specimens may be infectious. Use Universal Precautions when performing this assay. Proper handling and disposal methods should be established by the laboratory director. Only personnel adequately trained in handling infectious materials should be permitted to perform this diagnostic procedure.[6]

E. If infection with SARS-CoV-2 is suspected based on current clinical screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions.

F. Use only supplied or specified disposable laboratory ware.

G. Use appropriate personal protective equipment when collecting and handling specimens from individuals suspected of being infected with SARS-CoV-2 as outlined in CDC Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with 2019 Novel Coronavirus (2019-nCoV).

H. Wear disposable, powderless gloves, protective eye wear, and laboratory coats when handling specimens and reagents. Wash hands thoroughly after handling specimens and reagents.

I. Dispose of all material that has come into contact with specimens and reagents in accordance with applicable national, international, and regional regulations.

J. Expiration dates listed on the Panther Fusion Specimen Lysis Tubes, Hologic Specimen Lysis Tubes, the Aptima Multitest Collection Kit, the Aptima Unisex Swab Specimen Collection Kit, the Aptima Specimen Transfer Kit, and the Hologic Direct Load Capture Cap Collection Kit pertain to the transfer of sample into the tube and not to testing of the sample. Specimens collected/transferred any time prior to these expiration dates are valid for testing provided they are transported and stored in accordance with the appropriate package insert, even if these expiration dates have passed.

K. Maintain proper storage conditions during specimen shipping to ensure the integrity of the specimen. Specimen stability under shipping conditions other than those recommended has not been evaluated.

L. Testing of a saliva specimen that has been stored outside the conditions specified may lead to a higher risk of an invalid result.

M. Avoid cross-contamination during the specimen handling steps. Specimens can contain extremely high levels of virus or other organisms. Ensure that specimen containers do not come in contact with one another, and discard used materials without passing them over any open containers. Change gloves if they come in contact with specimens.

N. Do not use the reagents and controls after the expiration date.

O. Store assay components at the recommended storage condition. See *Reagent Storage and Handling Requirements (*page 6), and *Panther System Test Procedure* (page 14) for more information.

P. Do not combine any assay reagents or fluids. Do not top off reagents or fluids; the Panther system verifies reagent levels.

Q. Avoid microbial and ribonuclease contamination of reagents.

R. Do not use material that may contain Guanidinium thiocyanate or any guanidine-containing materials on the instrument. Highly reactive and/or toxic compounds may form if combined with sodium hypochlorite.

S. A reagent in this kit is labeled with risk and safety symbols.

Note: Hazard Communication reflects the EU Safety Data Sheets (SDS) classification. For hazard communication information specific to your region, refer to the region specific SDS on the Safety Data Sheet Library at www.hologicsds.com.

Selection Reagent
BORIC ACID 1-5%
WARNING
H315 - Causes skin irritation

Target Capture Reagent
EDTA 1-5%
LITHIUM HYDROXIDE, MONOHYDRATE 1-5%
H412 - Harmful to aquatic life with long lasting effects
H402 - Harmful to aquatic life

Reagent Storage and Handling Requirements

A. The following reagents are stable when stored at 2°C to 8°C (refrigerated):

Aptima SARS-CoV-2 Amplification Reagent

Aptima SARS-CoV-2 Enzyme Reagent

Aptima SARS-CoV-2 Probe Reagent

Aptima SARS-CoV-2 Internal Control

Aptima SARS-CoV-2 Positive Control

Aptima SARS-CoV-2 Negative Control

B. The following reagents are stable when stored at 2°C to 30°C:

Aptima SARS-CoV-2 Amplification Reconstitution Solution

Aptima SARS-CoV-2 Enzyme Reconstitution Solution

Aptima SARS-CoV-2 Probe Reconstitution Solution

Aptima SARS-CoV-2 Selection Reagent

C. The following reagents are stable when stored at 15°C to 30°C (room temperature):

Aptima SARS-CoV-2 Target Capture Reagent

Aptima Wash Solution

Aptima Buffer for Deactivation Fluid

Aptima Oil Reagent

D. Working Target Capture Reagent (wTCR) is stable for 30 days when stored at 15°C to 30°C. Do not refrigerate.

E. After reconstitution, the Enzyme Reagent, Amplification Reagent, and Probe Reagent are stable for 30 days when stored at 2°C to 8°C.

F. Discard any unused reconstituted reagents and wTCR after 30 days or after the Master Lot expiration date, whichever comes first.

G. Controls are stable until the date indicated on the vials.

H. Reagents stored on-board the Panther System have ~~72~~120 hours of on-board stability.

I. The Probe Reagent and Reconstituted Probe Reagent are photosensitive. Store the reagents protected from light. The specified reconstituted stability is based on 12 hours exposure of the Reconstituted Probe Reagent to two 60W fluorescent bulbs, at a distance of 17 inches (43 cm), and temperature less than 30°C. Light exposure of the Reconstituted Probe Reagent should be limited accordingly.

J. Upon warming to room temperature, some control tubes may appear cloudy or contain precipitates. Cloudiness or precipitation associated with controls does not affect control performance. The controls may be used whether they are clear or cloudy/precipitated. If clear controls are desired, solubilization may be expedited by incubating them at the upper end of the room temperature range (15°C to 30°C).

K. Do not freeze the reagents.

Specimen Collection and Storage

Specimens - Clinical material collected from patient placed in an appropriate transport system. For the Aptima SARS-CoV-2 assay, this includes NP, nasal, mid-turbinate and OP swab specimens, or nasopharyngeal wash/aspirate and nasal aspirate specimen collection in viral transport medium (VTM/UTM), saline, Liquid Amies, or specimen transport medium (STM). Additionally, saliva may be collected for use with the assay.

Samples - Represents a more generic term to describe any material for testing on the Panther System including specimens, specimens transferred into a Panther Fusion Specimen Lysis Tube, Hologic Specimen Lysis Tube with solid cap, Aptima Specimen Transfer Tube, Aptima Multitest Transport Tube, Hologic Direct Load Capture Cap Tube, and controls.

Note: *Handle all specimens as if they contain potentially infectious agents. Use Universal Precautions.*

Note: *Take care to avoid cross-contamination during specimen handling steps. For example, discard used material without passing over open tubes.*

Swab Specimen Collection

Collect NP swab, nasal swab, and OP swab specimens according to standard technique using a polyester-, rayon-, or nylon-tipped swab. Immediately place the swab specimen into 3mL of VTM or UTM. Swab specimens may alternatively be added to saline, Liquid Amies or STM. The Aptima Multitest Swab Specimen Collection Kit and the Hologic Direct Load Capture Cap Collection Kit may be used for the collection of OP and nasal swab samples. The Hologic Direct Load Capture Cap Collection Kit - CLASSIQSwab is for the collection of OP and nasal swab samples. The Hologic Direct Load Capture Cap Collection Kit - FLOQSwab is for the collection of mid-turbinate and NP swab samples.

After collection, specimens collected in VTM/UTM can be stored at 2°C to 8°C up to 96 hours before transferring to the Specimen Lysis Tube or transfer tubes as described in the specimen processing section below. Remaining specimen volumes can be stored at ≤-70°C.

After collection, specimens in the Aptima Multitest Tube and the Hologic Direct Load Capture Cap Tube may be stored at 2°C to 30°C up to 6 days.

Note: *It is recommended that specimens collected in the Aptima Multitest Tube and the Hologic Direct Load Capture Cap Tube, are stored capped and upright in a rack.*

Nasopharyngeal Wash/aspirate and Nasal Aspirate Specimen Collection

Collect nasopharyngeal wash/aspirate and nasal aspirate specimens according to standard techniques.

Saliva Specimen Collection

Collect 1 mL +/- 0.2 mL of saliva in a standard collection tube with a 1 mL mark. Instruct subjects to salivate and swirl the saliva around their mouth for at least 30s prior to spitting into the collection tube. Collected saliva can be stored at 15°C to 30°C up to 12 hours before adding 4 mL +/- 0.4 mL of Minimum Essential Media (MEM) to dilute and mix the saliva sample. Samples diluted in MEM can be stored at 15°C to 30°C up to 2 hours before transferring 500 µL

of diluted saliva to the Specimen Lysis Tube or transfer tubes as described in the specimen processing section below. Processed specimens can be stored at 2°C to 30°C up to 6 days.

Specimen Processing

Capped Workflow using Aptima SARS-CoV-2 Assay Software

Specimen Processing using the Panther Fusion Specimen Lysis Tube

A. Prior to testing on the Panther system, transfer 500 µL of the collected specimen* to a Panther Fusion Specimen Lysis Tube.

***Note:** When testing frozen specimen, allow specimen to reach room temperature prior to processing.*

Specimen Processing using the Aptima Specimen Transfer Tube

A. Prior to testing on the Panther system, transfer 1 mL of the collected specimen* to an Aptima Specimen Transfer Tube**.

***Note:** When testing frozen specimen, allow specimen to reach room temperature prior to processing.*

****Note:** Alternatively, an unused Aptima Multitest Tube or Aptima Unisex Tube can be used.*

B. Recap the Aptima Specimen Transfer Tube tightly.

C. Gently invert the tube 2 to 3 times to ensure complete mixture of the specimen.

Specimen Processing for Specimen Collected with Aptima Multitest Collection Kit

A. After placing the collected specimen* into the Aptima Multitest Tube using the Aptima Multitest Collection Kit, no further processing is required.

***Note:** When testing frozen specimen, allow specimen to reach room temperature prior to processing.*

Uncapped Workflow using Aptima SARS-CoV-2 Assay Software

Specimen Processing using the Panther Fusion Specimen Lysis Tube

A. Uncap the Panther Fusion Specimen Lysis Tube with penetrable cap. The penetrable cap can be retained or a replacement solid cap can be used in the next step.

B. Prior to testing on the Panther system, transfer 500 µL of the specimen to the Panther Fusion Specimen Lysis Tube, with penetrable cap or replacement solid cap.

C. To avoid contact with the top of the tube, loosen the cap and place the sample tube into the sample rack.

D. Remove and discard the cap. To avoid contamination, do not pass the cap over any other sample racks or sample tubes. Inspect the sample tube. If bubbles are present, carefully remove from the sample tube (for example, use the tip of a sterile swab or similar method).

Note: *Failure to remove bubbles may affect assay processing and cause invalid results.*

E. Place the rack retainer on the sample rack and load the rack into the instrument.

Specimen Processing using the Hologic Specimen Lysis Tube with Solid Cap

A. Uncap the Hologic Specimen Lysis Tube with solid cap and retain the cap.

B. Prior to testing on the Panther system, transfer 500 µL of the specimen to the Hologic Specimen Lysis Tube with solid cap.

C. It is recommended to recap the tube and gently invert three times to ensure viral inactivation and a homogeneous mixture.

D. To avoid contact with the top of the tube, loosen the cap and place the sample tube into the sample rack.

E. Remove and discard the cap. To avoid contamination, do not pass the cap over any other sample racks or sample tubes. Inspect the sample tube. If bubbles are present, carefully remove from the sample tube (for example, use the tip of a sterile swab or similar method).

Note: *Failure to remove bubbles may affect assay processing and cause invalid results.*

F. Place the rack retainer on the sample rack and load the rack into the instrument.

Specimen Processing for Specimen Collected with Hologic Direct Load Capture Cap Collection Kit

A. After placing the collected specimen* into the Hologic Direct Load Capture Cap Tube, no further processing is required.

Note: *When testing frozen specimen, allow specimen to reach room temperature prior to processing.*

B. To avoid contact with the top of the tube, loosen the cap and place the sample tube into the sample rack.

C. Remove and discard the cap and swab. To avoid contamination, do not pass the cap over any other sample racks or sample tubes. Inspect the sample tube. If bubbles are present,

carefully remove from the sample tube (for example, use the tip of a sterile swab or similar method).

Note: *If the swab wasn't captured by the cap, recap the tube to ensure that the swab is captured and removed from the tube. Direct Load Capture Cap tubes containing a swab should not be loaded into the Panther System.*

Note: *Failure to remove bubbles may affect assay processing and cause invalid results.*

D. Place the rack retainer on the sample rack and load the rack into the instrument.

Specimen Processing for Specimen Collected with Aptima Multitest Collection Kit

A. Obtain and follow instructions for Panther Fusion Specimen Lysis Tube (Step A), or Hologic Specimen Lysis Tube with Solid Cap (Step A).

B. Prior to testing on the Panther system, transfer 500 µL of the collected specimen from the Aptima Multitest Tube to a Panther Fusion Specimen Lysis Tube, or Hologic Specimen Lysis Tube as described in the specimen processing sections above.

Sample Storage

A. Samples on board the Panther system may be archived for additional testing at a later time.

B. Storing samples before or after testing

1. Samples in the Aptima Multitest Tube, Aptima Specimen Tube, Hologic Direct Load Capture Cap Tube, or Specimen Lysis Tube should be stored upright in the rack under the following condition:
    - 2°C to 30°C up to 6 days
2. The samples should be covered with a new, clean plastic film or foil barrier.
3. If assayed samples need to be frozen or shipped, remove the penetrable cap and place a new non-penetrable cap on the specimen tubes. If samples need to be shipped for testing at another facility, recommended temperatures must be maintained. Prior to uncapping, specimen transport tubes must be centrifuged for 5 minutes at 420 Relative Centrifugal Force (RCF) to bring all of the liquid down to the bottom of the tube. Avoid splashing and cross-contamination.

Specimen Transport

Maintain specimen storage conditions as described in the *Specimen Collection and Storage section on* page 7.

Note: *Specimens must be shipped in accordance with applicable national, international, and regional transportation regulations.*

Specimen Pooling - Determining Appropriate Strategy for Implementation and Monitoring

When considering specimen pooling, laboratories should evaluate the appropriateness of a pooling strategy based on the positivity rate in the testing population and the efficiency of the pooling workflow.

Preparing Samples for Pooling

The following upper respiratory tract specimens are validated for use with the Aptima SARS-CoV-2 assay and may be tested with sample pooling: nasopharyngeal, oropharyngeal, mid-turbinate, and nasal swab specimens collected into specimen transport media (STM). Each sample pool must be comprised of neat STM prepared specimens. The recommended sample pooling workflow is provided below.

Specimens to be Collected in Collection Tubes Containing 2.9 mL of STM

Specimen Preparation Instructions for Samples Pooled Directly into a Generic Tube

Perform the following procedure when pooling specimens collected in 2.9 mL of STM by transferring individual specimens directly into an empty tube per specifications in the *Panther or Panther Fusion System Operators Manual*.

A. Obtain a Panther system compatible empty tube.

B. Determine the appropriate volume required from each individual specimen based on the pool size being implemented. Specimens collected in 2.9 mL of STM do not require additional dilution with STM prior to testing.

   Note: *The recommended combined volume of each individual specimen is dependent upon the dimensions of tube being utilized. A Hologic representative can provide recommendations on minimum volume requirements for processing on the Panther system.*

C. Prior to testing on the Panther system, carefully transfer the determined volume of each individual specimen from the tubes containing 2.9 mL of STM to the empty tube.

D. Ensure homogeneous mixing of each prepared sample pool.

E. Retain the individual specimens for additional testing if required.

Panther System

Reagents for the Aptima SARS-CoV-2 assay are listed below for the Panther System. Reagent Identification Symbols are also listed next to the reagent name.

Reagents and Materials Provided

Aptima SARS-CoV-2 Assay Kit PRD-06419

250 tests (2 boxes)

Aptima SARS-CoV-2 Refrigerated Box (Box 1 of 2)
(store at 2°C to 8°C upon receipt)

Symbol	Component	Quantity 250 test kit
A	**Aptima SARS-CoV-2 Amplification Reagent** *Non-infectious nucleic acids dried in buffered solution containing < 5% bulking agent.*	1 vial
E	**Aptima SARS-CoV-2 Enzyme Reagent** *Reverse transcriptase and RNA polymerase dried in HEPES buffered solution containing < 10% bulking reagent.*	1 vial
P	**Aptima SARS-CoV-2 Probe Reagent** *Non-infectious chemiluminescent DNA probes dried in succinate buffered solution containing < 5% detergent.*	1 vial
IC	**Aptima SARS-CoV-2 Internal Control**	1 vial

Aptima SARS-CoV-2 Room Temperature Box (Box 2 of 2)
(store at 15°C to 30°C upon receipt)

Symbol	Component	Quantity 250 test kit
AR	**Aptima SARS-CoV-2 Amplification Reconstitution Solution** *Aqueous solution containing preservatives.*	1 x 27.7 mL
ER	**Aptima SARS-CoV-2 Enzyme Reconstitution Solution** *HEPES buffered solution containing a surfactant and glycerol.*	1 x 11.1 mL
PR	**Aptima SARS-CoV-2 Probe Reconstitution Solution** *Succinate buffered solution containing < 5% detergent.*	1 x 35.4 mL
S	**Aptima SARS-CoV-2 Selection Reagent** *600 mM borate buffered solution containing surfactant.*	1 x 108 mL
TCR	**Aptima SARS-CoV-2 Target Capture Reagent** *Buffered salt solution containing solid phase and capture oligomers.*	1 x 54 mL
	Reconstitution Collars	3
	Master Lot Barcode Sheet	1 sheet

Materials Required and Available Separately

Note: *Materials available from Hologic have catalog numbers listed, unless otherwise specified.*

	Cat. No.
Panther System	303095
Aptima Assay Fluids Kit *(Aptima Wash Solution, Aptima Buffer for Deactivation Fluid, and Aptima Oil Reagent)*	303014 (1000 tests)
Aptima Auto Detect Kit	303013 (1000 tests)
Multi-tube units (MTUs)	104772-02
Panther Waste Bag Kit	902731
Panther Waste Bin Cover	504405
Or Panther Run Kit *contains MTUs, waste bags, waste bin covers, assay fluids, and auto detects*	303096 (5000 tests)
Tips, 1000 µL, filtered, liquid-sensing, conductive, and disposable Not all products are available in all regions. Contact your representative for region-specific information	901121 (10612513 Tecan) 903031 (10612513 Tecan) MME-04134 (30180117 Tecan) MME-04128
Aptima SARS-CoV-2 Controls Kit *PC - Aptima SARS-CoV-2 Positive Control. Non-infectious nucleic acid in a buffered solution containing < 5% detergent. Quantity 5 x 1.7 mL* *NC - Aptima SARS-CoV-2 Negative Control. A buffered solution containing <5% detergent. Quantity 5 x 1.7 mL*	PRD-06420
Aptima Multitest Swab Specimen Collection Kit	PRD-03546
Hologic Direct Load Capture Cap Collection Kit - CLASSIQSwabs	PRD-06951
Hologic Direct Load Capture Cap Collection Kit - FLOQSwabs	PRD-06952
Aptima Specimen Transfer Kit	301154C
Aptima Specimen Transfer Kit - printable	PRD-05110
Aptima Unisex Swab Specimen Collection Kit for Endocervical and Male Urethral Swab Specimens	301041
Panther Fusion Specimen Lysis Tubes, 100 per bag *tube contains 0.71 mL of STM with a penetrable cap*	PRD-04339
Hologic Specimen Lysis Tubes, 100 each *tube contains 0.71 mL of STM with a solid cap*	PRD-06554
Bleach, 5% to 7% (0.7M to 1.0M) sodium hypochlorite solution	—
Disposable gloves	—
Replacement non-penetrable caps	504415
Hologic Solid Cap for use with PRD-06951* and PRD-06952*, 100 caps per bag	**a single-use cover for the Hologic Direct Load Capture Cap (PRD-06951 and PRD- 06952) after*

	Cat. No.
Replacement Caps for the 250-test kits	—
Amplification and Probe reagent reconstitution solutions	*CL0041 (100 caps)*
Enzyme Reagent reconstitution solution	*501616 (100 caps)*
TCR and Selection reagent	*CL0040 (100 caps)*

Optional Materials

	Cat. No.
Hologic Bleach Enhancer for Cleaning *for routine cleaning of surfaces and equipment*	302101
Tube rocker	—

Panther System Test Procedure

Note: *Refer to the Panther/Panther System Operator's Manual for additional procedural information.*

A. Work Area Preparation

Clean work surfaces where reagents and samples will be prepared. Wipe down work surfaces with 2.5% to 3.5% (0.35M to 0.5M) sodium hypochlorite solution. Allow the sodium hypochlorite solution to contact surfaces for at least 1 minute and then follow with a water rinse. Do not allow the sodium hypochlorite solution to dry. Cover the bench surface on which the reagents and samples will be prepared with clean, plastic-backed absorbent laboratory bench covers.

B. Reagent Reconstitution/Preparation of a New Kit

Note: *Reagent reconstitution should be performed prior to beginning any work on the Panther System.*

1. To reconstitute Amplification, Enzyme, and Probe Reagents, combine the bottles of lyophilized reagent with the reconstitution solution. If refrigerated, allow the reconstitution solutions to reach room temperature before use.
   a. Pair each reconstitution solution with its lyophilized reagent. Ensure that the reconstitution solution and reagent have matching label colors before attaching the reconstitution collar.
   b. Check the lot numbers on the Master Lot Barcode Sheet to ensure that the appropriate reagents are paired.
   c. Open the lyophilized reagent vial and firmly insert the notched end of the reconstitution collar into the vial opening (Figure 1, Step 1).
   d. Open the matching reconstitution solution, and set the cap on a clean, covered work surface.
   e. While holding the reconstitution solution bottle on the bench, firmly insert the other end of the reconstitution collar into the bottle opening (Figure 1, Step 2).

f. Slowly invert the assembled bottles. Allow the solution to drain from the bottle into the glass vial (Figure 1, Step 3).

g. Thoroughly mix the solution in the glass vial by swirling (Figure 1, Step 4).

h. Wait for the lyophilized reagent to go into solution, then invert the assembled bottles again, tilting at a 45° angle to minimize foaming (Figure 1, Step 5). Allow all of the liquid to drain back into the plastic bottle.

i. Remove the reconstitution collar and glass vial (Figure 1, Step 6).

j. Recap the plastic bottle. Record operator initials and reconstitution date on the label (Figure 1, Step 7).

k. Discard the reconstitution collar and glass vial (Figure 1, Step 8).

Option: Additional mixing of the Amplification, Enzyme, and Probe Reagents using a tube rocker is allowed. The reagents may be mixed by placing the recapped plastic bottle on a tube rocker set to 20 RPM (or equivalent) for a minimum of 5 minutes.

Warning: *Avoid creating foam when reconstituting reagents. Foam compromises the level-sensing in the Panther System.*

Warning: *Adequate mixing of the reagents is necessary to achieve expected assay results.*

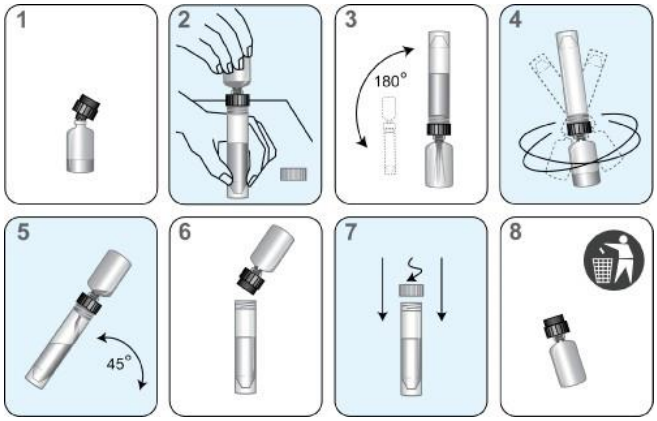

Figure 1. Panther System Reconstitution Process

2. Prepare Working Target Capture Reagent (wTCR)

a. Pair the appropriate bottles of TCR and IC.

b. Check the reagent lot numbers on the Master Lot Barcode Sheet to make sure that the appropriate reagents in the kit are paired.

c. Open the bottle of TCR, and set the cap on a clean, covered work surface.

d. Open the IC bottle and pour the entire contents into the bottle of TCR. Expect a small amount of liquid to remain in the IC bottle.

e. Cap the bottle of TCR and gently swirl the solution to mix the contents. Avoid creating foam during this step.

f. Record operator initials and the current date on the label.

g. Discard the IC bottle and cap.

3. Prepare Selection Reagent
   a. Check the lot number on the reagent bottle to make sure it matches the lot number on the Master Lot Barcode Sheet.
   b. Record operator initials and the current date on the label.

Note: *Thoroughly mix by gently inverting all reagents prior to loading on the system. Avoid creating foam during inversion of reagents.*

C. Reagent Preparation for Previously Reconstituted Reagents

1. Previously reconstituted Amplification, Enzyme, and Probe Reagents must reach room temperature (15°C to 30°C) prior to the start of the assay.

   Option: The reagents may be brought to room temperature by placing the reconstituted Amplification, Enzyme, and Probe Reagents on a tube rocker set to 20 RPM (or equivalent) for a minimum of 25 minutes.

2. If reconstituted Probe Reagent contains precipitate that does not return to solution at room temperature, heat the capped bottle at a temperature that does not exceed 62°C for 1 to 2 minutes. After this heat step, the Probe Reagent may be used even if residual precipitate remains. Mix Probe Reagent by inversion, being careful not to induce foam, prior to loading onto the system.
3. Thoroughly mix each reagent by gently inverting prior to loading on the system. Avoid creating foam during inversion of reagents. This step is not required if reagents are loaded onto the system directly after mixing on the tube rocker.
4. Do not top off reagent bottles. The Panther System will recognize and reject bottles that have been topped off.
5. *Adequate mixing of the reagents is necessary to achieve expected assay results.*

D. Specimen Handling using Panther Fusion Specimen Lysis Tube or Aptima Specimen Transfer Tube

Note: *Prepare specimens per the Specimen Processing instructions in the Specimen Collection and Storage section before loading specimens onto the Panther system.*

1. Inspect sample tubes before loading into the rack. If a sample tube contains bubbles or has a lower volume than is typically observed, gently tap the bottom of the tube to bring contents to the bottom.

Note: *For samples transferred to the Panther Fusion Specimen Lysis Tube or the Aptima Specimen Transfer Tube, to avoid a processing error, ensure adequate specimen volume is added to the tube. When adequate collected specimen is added to the tube, there is sufficient volume to perform 3 nucleic acid extractions.*

E. Specimen Handling using Hologic Specimen Lysis Tube

1. Prepare specimens per the specimen processing instructions in the *Specimen Collection and Storage* section.

Note: *For samples transferred to the Hologic Specimen Lysis Tube, to avoid a processing error, ensure adequate specimen volume is added to the tube.*

Note: *When adequate collected specimen is added to the Hologic Specimen Lysis Tube (PRD-06554), there is sufficient volume to perform 2 nucleic acid extractions.*

Note: *When using the Aptima SARS-CoV-2 uncapped tube assay software, remove the cap from the Positive and Negative control before loading onto the Panther system.*

F. System Preparation

1. Set up the system according to the instructions in the *Panther/Panther Fusion System Operator's Manual* and *Procedural Notes*. Make sure that the appropriately sized reagent racks and TCR adapters are used.
2. Load samples.

Procedural Notes

A. Controls

1. To work properly with the Aptima Assay software for the Panther system, one pair of controls is required. The Aptima SARS-CoV-2 positive and negative controls can be loaded in any rack position or in any Sample Bay Lane on the Panther system. Patient specimen pipetting will begin when one of the following two conditions has been met:
   a. A pair of controls is currently being processed by the system.
   b. Valid results for the controls are registered on the system.
2. Once the control tubes have been pipetted and are processing for a specific reagent kit, patient specimens can be run with the associated kit up to 24 hours unless:
   a. Controls results are invalid.
   b. The associated assay reagent kit is removed from the system.
   c. The associated assay reagent kit has exceeded stability limits.
3. Each Aptima control tube can be tested once. Attempts to pipette more than once from the tube can lead to processing errors.
4. Patient specimen pipetting begins when one of the following two conditions is met:
   a. Valid results for the controls are registered on the system.
   b. A pair of controls is currently in process on the system.

B. Temperature

Room temperature is defined as 15°C to 30°C.

C. Glove Powder

As in any reagent system, excess powder on some gloves may cause contamination of opened tubes. Powderless gloves are recommended.

D. Lab Contamination Monitoring Protocol for the Panther System

There are many laboratory-specific factors that may contribute to contamination, including testing volume, workflow, disease prevalence and various other laboratory activities. These factors should be taken into consideration when contamination monitoring frequency is being established. Intervals for contamination monitoring should be established based on each laboratory's practices and procedures.

To monitor for laboratory contamination, the following procedure may be performed using the Aptima Unisex Swab Specimen Collection Kit for Endocervical and Male Urethral Swab Specimens:

1. Label swab transport tubes with numbers corresponding to the areas to be tested.
2. Remove the specimen collection swab (blue shaft swab with green printing) from its packaging, wet the swab in the specimen transport medium (STM), and swab the designated area using a circular motion.
3. Immediately insert the swab into transport tube.
4. Carefully break the swab shaft at the score line; use care to avoid splashing of the contents.
5. Recap the swab transport tube tightly.
6. Repeat Steps 2 to 5 for each area to be swabbed.

E. If the results are positive, see *Interpretation of Results*. For additional Panther system-specific contamination monitoring information, contact Hologic Technical Support.

Quality Control

A run or specimen result may be invalidated by the Panther system if problems occur while performing the assay. Specimens with invalid results must be retested.

Negative and Positive Controls

To generate valid results, a set of assay controls must be tested. One replicate of the negative assay control and positive assay control must be tested each time a new kit is loaded on the Panther system or when the current set of valid controls have expired.

The Panther system is configured to require assay controls run at an administrator-specified interval of up to 24 hours. Software on the Panther system alerts the operator when assay controls are required and does not start new tests until the assay controls are loaded and have started processing.

During processing, criteria for acceptance of the assay controls are automatically verified by the Panther system. To generate valid results, the assay controls must pass a series of validity checks performed by the Panther system.

If the assay controls pass all validity checks, they are considered valid for the administrator-specified time interval. When the time interval has passed, the assay controls are expired by the Panther system which requires a new set of assay controls be tested prior to starting any new samples.

If any one of the assay controls fails the validity checks, the Panther system automatically invalidates the affected samples and requires a new set of assay controls be tested prior to starting any new samples.

Internal Control

An internal control is added to each sample with the wTCR. During processing, the internal control acceptance criteria are automatically verified by the Panther system software. Detection of the internal control is not required for samples that are positive for SARS-CoV-2. The internal control must be detected in all samples that are negative for SARS-CoV-2 targets; samples that fail to meet that criteria will be reported as Invalid. Each sample with an Invalid result must be retested.

The Panther system is designed to accurately verify processes when procedures are performed following the instructions provided in this package insert and the *Panther/Panther Fusion System Operator's Manual.*

Interpretation of Results

The Panther system automatically determines the test results for samples and controls. A test result may be negative, positive, or invalid.

Table1 shows the possible results reported in a valid run with result interpretations.

Table 1: Result Interpretation

SARS-CoV-2 Result	IC Result	Interpretation
Neg	Valid	SARS-CoV-2 not detected.
POS	Valid	SARS-CoV-2 detected.
Invalid	Invalid	Invalid. There was an error in the generation of the result; retest sample.

Note: Detection of internal control is not required for samples that are positive for SARS-CoV-2.

Interpretation of Results for Pooled Samples

Negative: Negative results from pooled sample testing should not be treated as definitive. If the patient's clinical signs and symptoms are inconsistent with a negative result and results are necessary for patient management, then the patient should be considered for individual testing. The utilization of sample pooling should be indicated for any specimens with reported negative results.

Positive: Specimens with a positive sample pool result must be tested individually prior to reporting a result. Specimens with low viral loads may not be detected in sample pools due to the decreased sensitivity of pooled testing.

Invalid: Specimens with an invalid result must be tested individually prior to reporting a result. However, in instances of an invalid run, repeat testing of pooled specimens may be appropriate depending on the laboratory workflow and required result reporting time.

Limitations

A. Use of this assay is limited to personnel who are trained in the procedure. Failure to follow these instructions may result in erroneous results.

B. Reliable results are dependent on adequate specimen collection, transport, storage, and processing.

C. Avoid contamination by adhering to good laboratory practices and to the procedures specified in this package insert.

D. A positive result indicates the detection of nucleic acid from the relevant virus. Nucleic acid may persist even after the virus is no longer viable.

E. Use of the Aptima SARS-CoV-2 assay in a general, asymptomatic screening population is intended to be used as part of an infection control plan, that may include additional preventative measures, such as a predefined serial testing plan or directed testing of high-risk individuals. Negative results should be considered presumptive and do not preclude current or future infection obtained through community transmission or other exposures. Negative results must be considered in the context of an individual's recent exposures, history, and presence of clinical signs and symptoms consistent with COVID-19.

F. Asymptomatic individuals infected with COVID-19 may not shed enough virus to reach the limit of detection of the test, giving a false negative result.

G. In the absence of symptoms, it is difficult to determine if asymptomatic individuals have been tested too late or too early. Therefore, negative results in asymptomatic individuals may include individuals who were tested too early and may become positive later, individuals who were tested too late and may have serological evidence of infection, or individuals who were never infected.

H. The following types of VTM/UTM have been validated.

- Remel MicroTest M4, M4RT, M5 or M6 formulations
- Copan Universal Transport Medium
- BD Universal Viral Transport Medium

Note: *Do not use medium that may contain Guanidium thiocyanate or any guanidine-containing material.*

Panther SARS-CoV-2 Assay Performance

Analytical Sensitivity

The analytical sensitivity (limit of detection or LoD) of the Aptima SARS-CoV-2 assay was determined by testing serial dilutions of pooled negative clinical nasopharyngeal swab specimens spiked with inactivated cultured SARS-CoV-2 virus (USA-WA1/2020; BEI Resources; NR-52281). Ten replicates of each serial dilution were evaluated using each of two assay reagent lots across two Panther systems. The LoD was determined to be 0.01 $TCID_{50}$/mL in the test sample and verified by testing an additional 20 replicates with one assay reagent lot. The LoD was also confirmed using saline, Liquid Amies and specimen transport medium (STM) swab collection media.

A similarly designed study was performed to determine the analytical sensitivity of the Aptima SARS-CoV-2 assay using saliva specimens. Pooled negative clinical saliva specimen matrix was spiked with inactivated cultured SARS-CoV-2 virus (USA-WA1/2020; BEI Resources: NR-52281). The LoD was determined to be 0.01 $TCID_{50}$/mL in the test sample, corresponding to a concentration of 0.13 $TCID_{50}$/mL in the collected saliva specimen.

The analytical sensitivity of the Aptima SARS-CoV-2 assay was additionally evaluated using reference material from three commercial vendors. Serial dilutions of the reference material were made in STM and 20 or more replicates at each level were tested using each of two assay reagent lots across two Panther systems. The reference materials and the lowest dilution levels resulting in ≥ 95% detection are listed in Table 2.

Table 2: Analytical Sensitivity Evaluation of Commercial Reference Material

Vendor	Name	Reference #	Lot #	Analytical Sensitivity
ZeptoMetrix	SARS-CoV-2 External Run control	NATSARS(COV2)-ERC	324332	83 Copies/mL
SeraCare	AccuPlex SARS-Cov-2 Reference Material	0505-0126	10483977	83 Copies/mL
Exact Diagnostic	SARS-CoV-2 Standard	COV019	20033001	83 Copies/mL

Analytical Sensitivity with the Aptima Specimen Transfer Tube Workflow

The determined 0.01 $TCID_{50}$/mL analytical sensitivity (limit of detection) of the Aptima SARS-CoV-2 assay was confirmed using the Aptima Specimen Transfer tube specimen preparation workflow. Confirmation was performed using inactivated cultured SARS-CoV-2 virus (USA-QA1/2020; BEI Resources; NR-52281) in negative clinical nasopharyngeal (NP) swab, saline, Liquid Amies and specimen transport medium (STM) swab collection media by testing 20 replicates with one reagent lot (Table 3).

Table 3: LoD Confirmation with the Aptima Specimen Transfer Workflow

Target	Matrix	N Valid	N Positive	% Positive	Avg kRLU	StdDev kRLU	%CV
Inactivated SARS-CoV-2 virus	NP Swab	20	20	100%	1063	61	5.8%
	STM	20	20	100%	1064	116	10.9%
	Saline	20	20	100%	1102	60	5.4%
	Liquid Amies	20	20	100%	1101	51	4.7%

Inclusivity

The inclusivity of the Aptima SARS-CoV-2 assay was evaluated using *in silico* analysis of the assay target capture oligos, amplification primers, and detection probes in relation to 9,896 SARS-CoV-2 sequences available in the NCBI and GISAID gene databases. Any sequence with missing or ambiguous sequence information was removed from the analysis, resulting in 9,879 sequences evaluated for the first target region of the assay and 9,880 for the second target region. The *in silico* analysis showed 100% homology to the assay oligos of both target systems for 9,749 (98.5%) of the evaluated sequences and 100% homology to the assay oligos of at least one target system for all 9,896 sequences. There were no evaluated sequences with identified mismatches predicted to impact binding or performance of both target systems.

Analytical Specificity and Microbial Interference

The analytical specificity of the Aptima SARS-CoV-2 assay was evaluated by testing 30 microorganisms representing common respiratory pathogens or closely related species (Table 4). Bacteria were tested at 10^6 CFU/mL and viruses were tested at 10^5 $TCID_{50}$/mL, except where noted. Microorganisms were tested with and without the presence of SARS-CoV-2 inactivated virus at 3x LoD. Analytical specificity of the Aptima SARS-CoV-2 assay was 100% with no evidence of microbial interference.

In addition to microorganism testing, *in silico* analysis was performed to assess the specificity of the assay in relation to the microorganisms listed in Table 4. The *in silico* analysis showed no probable cross reactivity to any of the 112 GenBank sequences evaluated.

Table 4: Aptima SARS-CoV-2 Analytical Specificity and Microbial Interference Microorganisms

Microorganism	Concentration	Microorganism	Concentration
Human coronavirus 229E	1E+5 $TCID_{50}$/mL	Parainfluenza virus 1	1E+5 $TCID_{50}$/mL
Human coronavirus OC43	1E+5 $TCID_{50}$/mL	Parainfluenza virus 2	1E+5 $TCID_{50}$/mL
Human coronavirus HKU1[1]	1E+6 copies/mL	Parainfluenza virus 3	1E+5 $TCID_{50}$/mL
Human coronavirus NL63	1E+4 $TCID_{50}$/mL	Parainfluenza virus 4	1E+3 $TCID_{50}$/mL
SARS-coronavirus[1]	1E+6 copies/mL	Influenza A	1E+5 $TCID_{50}$/mL
MERS-coronavirus	1E+4 $TCID_{50}$/mL	Influenza B	2E+3 $TCID_{50}$/mL
Adenovirus (e.g. C1 Ad. 71)	1E+5 $TCID_{50}$/mL	Enterovirus (e.g. EV68)	1E+5 $TCID_{50}$/mL
Human Metapneumovirus (hMPV)	1E+6 $TCID_{50}$/mL	Rhinovirus	1E+4 $TCID_{50}$/mL
Respiratory syncytial virus	1E+5 $TCID_{50}$/mL	*Legionella pneumophila*	1E+6 CFU/mL
Chlamydia pneumoniae	1E+6 IFU/mL	*Mycobacterium tuberculosis*	1E+6 $TCID_{50}$/mL
Haemophilus influenzae	1E+6 CFU/mL	*Streptococcus pneumoniae*	1E+6 CFU/mL
Bordetella pertussis	1E+6 CFU/mL	*Streptococcus pyogenes*	1E+6 CFU/mL
Pneumocystis jirovecii (PJP)	1E+6 nuc/mL	*Streptococcus salivarius*	1E+6 CFU/mL
Candida albicans	1E+6 CFU/mL	*Mycoplasma pneumoniae*	1E+6 CFU/mL
Staphylococcus epidermidis	1E+6 CFU/mL	*Pseudomonas aeruginosa*	1E+6 CFU/mL
Pooled human nasal wash[2] - *to represent diverse microbial flora in human respiratory tract*	N/A		

[1] Cultured virus and whole genome purified nucleic acid for Human coronavirus HKU1 and SARS-coronavirus are not readily available. HKU1 and SARS-coronavirus IVTs corresponding to the ORF1ab gene regions targeted by the assay were used to evaluate cross-reactivity and microbial interference.

[2] In place of evaluating pooled human nasal wash, testing of 30 individual negative clinical NP swab specimens was performed to represent diverse microbial flora in the human respiratory tract.

Clinical Performance

The clinical performance of the Aptima SARS-CoV-2 assay was evaluated in comparison to the Panther Fusion SARS-CoV-2 assay (Hologic, Inc.) using a panel of remnant clinical specimens. For the study, remnant clinical nasopharyngeal specimens were collected from US patients with signs and symptoms of respiratory infection.

The Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) was calculated in relation to the Panther Fusion assay as the reference result, as shown in Table 5. The Aptima SARS-CoV-2 assay showed positive and negative agreements of 100% and 98.2%, respectively.

Nasopharyngeal wash/aspirate, nasal aspirates, nasal swabs and mid-turbinate nasal swabs are acceptable specimens to test for viral respiratory infections. However, performance with these specimen types has not been specifically evaluated with the Aptima SARS-CoV-2 assay.

Table 5: Aptima SARS-CoV-2 Clinical Agreement

		Panther Fusion SARS-CoV-2 Assay	
		Positive	Negative
Aptima SARS-CoV-2 Assay	Positive	50	1
	Negative	0	54

Positive Percent Agreement: (95% CI): 100% (92.9% – 100%)
Negative Percent Agreement: (95% CI): 98.2% (90.4% – 99.7%)
Overall Agreement: (95% CI): 99.0% (94.8% – 99.8%)

Clinical Performance with Contrived Panel

The clinical performance of the Aptima SARS-CoV-2 assay using the Aptima Specimen Transfer tube specimen preparation workflow was evaluated in comparison to a panel of contrived specimens. For the study, a panel of 115 remnant clinical nasopharyngeal specimens was tested using both the Panther Fusion Specimen Lysis Tube (Specimen Lysis Tube) and Aptima Specimen Transfer tube workflows. All specimens were collected from US patients with signs and symptoms of respiratory infection. The panel consisted of 65 SARS-CoV-2 positive and 50 SARS-CoV-2 negative specimens. Of the 65 positive specimens, 40 were at concentrations 0.5-2x LoD and 25 were at concentrations 3-5x LoD using inactivated cultured SARS-CoV-2 virus (USA-QA1/2020; BEI Resources; NR-52281) as the target.

The Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) for both specimen preparation workflows were calculated in relation to the expected result of the contrived specimen panel, as shown in Table 6 for the Aptima Specimen Transfer Tube and Table 7 for the Specimen Lysis Tube. Detection characteristics for the contrived specimens were calculated by target concentration, as shown in Table 8. Both specimen preparation workflows showed 100% agreement for the evaluated panels.

Table 6: Performance of the Aptima Specimen Transfer Tube Workflow Relative to Expected Results

		Expected Result		
		Positive	Negative	Total
Aptima Specimen Transfer Result	Positive	65	0	65
	Negative	0	50	50
	Total	65	50	115

Overall Agreement: 100% (96.8% – 100%)
Positive Agreement: 100% (94.4% – 100%)
Negative Agreement: 100% (92.9% – 100%)

Table 7: Performance of the Specimen Lysis Tube Workflow Relative to Expected Results

		Expected Result		
		Positive	Negative	Total
Specimen Lysis Tube Result	Positive	65	0	65
	Negative	0	50	50
	Total	65	50	115

Overall Agreement: 100% (96.8% – 100%)
Positive Agreement: 100% (94.4% – 100%)
Negative Agreement: 100% (92.9% – 100%)

Table 8: Detection Characteristics for Contrived Nasopharyngeal Swab Specimens

	Aptima Specimen Transfer Sample Workflow						Specimen Lysis Tube Sample Workflow					
Target Conc.	**n Valid**	**n Positive**	**% Positive**	**Average kRLU**	**St Dev kRLU**	**%CV**	**n Valid**	**n Positive**	**% Positive**	**Average kRLU**	**St Dev kRLU**	**%CV**
Neg	50	0	0	299	9.7	3.2	50	0	0	300	9.3	3.1
0.5x LoD	10	10	100	1050	208.5	19.9	10	10	100	1153	113.0	9.8
1.0x LoD	10	10	100	1176	102.1	8.7	10	10	100	1205	24.3	2.0
1.5x LoD	10	10	100	1222	31.6	2.6	10	10	100	1223	21.9	1.8
2.0x LoD	10	10	100	1225	22.6	1.8	10	10	100	1237	26.0	2.1
3.0x LoD	10	10	100	1228	13.6	1.1	10	10	100	1215	25.5	2.1
4.0x LoD	5	5	100	1238	16.7	1.4	5	5	100	1212	12.5	1.0
5.0x LoD	10	10	100	1237	18.2	1.5	10	10	100	1246	28.3	2.3

Clinical Performance with Naturally Infected Positive Specimens

The clinical performance of the Aptima SARS-CoV-2 assay using the Aptima Specimen Transfer tube specimen preparation workflow was evaluated in comparison to the Specimen Lysis Tube workflow tested with both the Aptima and Panther Fusion SARS-CoV-2 assays. For the study, three dilutions of 15 unique SARS-CoV-2 positive nasopharyngeal swab specimens were prepared and processed using both workflows. SARS-CoV-2 samples were previously determined to be positive using a non-Hologic molecular assay.

The positive percent agreement between the Aptima SARS-CoV-2 Assay using the Aptima Specimen Transfer Tube and the Specimen Lysis Tube workflows were 97.5% (87.1% – 99.6%) and 100% (91.0% – 100%), respectively, when compared to the Panther Fusion SARS-CoV-2 assay using the Specimen Lysis Tube workflow as reference. The positive percent agreement of the Aptima Specimen Transfer tube workflow was 95.0% (83.5% – 98.6%) when compared to the Specimen Lysis Tube workflow as reference.

Clinical Performance with Saliva Specimens

The clinical performance of the Aptima SARS-CoV-2 assay with saliva specimens was evaluated in comparison to NP swab specimens in 303 subjects who were tested simultaneously. The 303 subjects included 160 (52.8%) who were mildly symptomatic and 143 (47.2%) who were

asymptomatic at the time of testing. The Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) for saliva specimens was calculated in relation to NP swab specimens as the reference result, as shown in Table 9. The Aptima SARS-CoV-2 assay showed positive and negative agreements of 87.0% and 99.2% between the specimen types, respectively.

Table 9: Aptima SARS-CoV-2 Clinical Agreement between Saliva and NP Swab Specimens

		NP Swab	
		Positive	Negative
Saliva	Positive	47	2
	Negative	7	245

Note: 2 specimens gave invalid results.

Positive Percent Agreement: (95% CI): 87.0% (83.0% - 96.0%)
Negative Percent Agreement: (95% CI): 99.2% (97.1% - 99.9%)

Clinical Performance in Asymptomatic Individuals

The clinical performance of the Aptima SARS-CoV-2 assay in individuals without signs and symptoms of respiratory infection (asymptomatic individuals) was evaluated in comparison to an EUA molecular assay. Prospectively collected nasopharyngeal swab specimens from US patients were assessed, including 45 specimens positive for SARS-CoV-2 and 315 specimens negative for SARS-CoV-2 using the EUA comparator assay. The PPA and NPA were calculated in relation to the EUA comparator assay results. The PPA and NPA were 100% and 96.5%, respectively, for the Aptima SARS-CoV-2 assay in asymptomatic individuals, as shown in Table 10.

Table 10: Clinical Agreement in NP Swab Specimens from Asymptomatic Individuals

		EUA Assay	
		Positive	Negative
Aptima SARS-CoV-2 Assy	Positive	45	11
	Negative	0	304

Positive Percent Agreement (PPA): 100% (92.1% - 100%)
Negative Percent Agreement (NPA): 96.5% (93.9% - 98.0%)

Six (6) of the 11 NP swab specimens with false positive results were confirmed positive following retesting with the comparator EUA assay. Ct values for these 6 samples ranged between 35.5 and 38.9, suggestive of low viral load.

Clinical Performance of Pooling up to 5 Specimens Prior to Testing

The clinical performance of the Aptima SARS-CoV-2 assay was evaluated in pools consisting of up to 5 specimens. For the study, a pool size of 5 specimens was evaluated and included positive and negative specimen pools. Each positive specimen pool consisted of one positive specimen with the remaining specimens being negative, whereas the negative specimen pools consisted only of negative specimens. For the study, 50 positive and 20 negative specimen pools were evaluated. The positive specimens used in the study covered the detectable range of

the assay and included 20% low positive specimens. Specimens for inclusion in the clinical performance of pooling study were chosen based on Ct results obtained with the Panther Fusion SARS-CoV-2 assay. The Panther Fusion SARS-CoV-2 assay was used for this purpose because the Panther Fusion SARS-CoV-2 and Aptima SARS-CoV-2 assays have the same LoD when evaluated with the FDA reference panel (i.e., 600 NDU/mL). Low positive specimens included in the study were defined as having a Ct value within 1-2 Ct of the LoD of the Panther Fusion SARS-CoV-2 assay. Both the pooled and individual specimens were evaluated with the Aptima SARS-CoV-2 assay.

The Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) were calculated in relation to the expected (individual) result, as shown in Table 11. All evaluated positive specimens yielded a positive result in the pool. Since the kRLU values for the Aptima assay do not correspond to target concentration, signal and in silico sensitivity analysis was not performed.

Table 11: Individual and Pooled Specimen Agreement with a Pool Size of 5

		Individual Specimen Result		
		Positive	Negative	Total
Pool of 5 Result	Positive	50	0	50
	Negative	0	20	20
	Total	50	20	70

Overall Agreement: 100% (94.8% – 100.0%)
Positive Agreement: 100% (92.9% – 100.0%)
Negative Agreement: 100% (83.9% – 100.0%)

Clinical Performance of Pooling up to 5 Asymptomatic Patient Specimens Prior to Testing

The clinical performance of the Aptima SARS-CoV-2 assay was evaluated in specimen pools with specimens collected from asymptomatic patients. Pool sizes of up to 5 specimens were evaluated with both positive and negative asymptomatic patient specimens. Each positive specimen pool consisted of one positive specimen with the remaining specimens being negative, whereas the negative specimen pools consisted only of negative specimens. For a pool size of three, 32 positive and 32 negative specimen pools were evaluated. For a pool size of four, 36 positive and 31 negative specimen pools were evaluated. For a pool size of five, 36 positive and 30 negative specimen pools were evaluated. The positive specimens used in the study covered the detectable range of the assay and each pool size included 25% low positive specimens. Specimens included in the clinical performance study were chosen based on Ct results obtained with the Panther Fusion SARS-CoV-2 assay. The Panther Fusion SARS-Cov-2 assay was used for this purpose because the Panther Fusion SARS-CoV-2 and the Aptima SARS-CoV-2 assays have the same LoD when evaluated with the FDA reference panel (i.e. 600 NDU/mL). Low positive specimens included in the study were defined as having a Ct value within 1-2 Ct of the LoD of the Panther Fusion SARS-CoV-2 assay. Both the pooled and individual specimens were evaluated with the Aptima SARS-CoV-2 assay.

The Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) were calculated in relation to the expected (individual) result for each evaluated pool size, as shown in Table 12, Table 13, and Table 14. With a pool size of three, one of the eight specimens evaluated with a target concentration at or near the LoD of the assay yielded an individual positive result but was not detected as part of a specimen pool. With a pool size of four, all evaluated positive

specimens yielded a positive result when tested pooled. With a pool size of five, five of the nine specimens evaluated with target concentrations at or near the LoD of the assay yielded an individual positive result but were not detected as part of a specimen pool. Since the kRLU values for the Aptima assay do not correspond to target concentrations, signal and *in silico* sensitivity analysis was not performed.

Table 12: Asymptomatic Individual and Pooled Specimen Agreement with a Pool Size of 3

		Individual Specimen Result		
		Positive	Negative	Total
Pool of 3 Result	Positive	31	0	31
	Negative	1	32	33
	Total	32	32	64

Overall Agreement: 98.4% (91.7% - 99.7%)
Positive Agreement: 96.9% (84.3% - 99.4%)
Negative Agreement: 100% (89.3% - 100%)

Table 13: Asymptomatic Individual and Pooled Specimen Agreement with a Pool Size of 4

		Individual Specimen Result		
		Positive	Negative	Total
Pool of 4 Result	Positive	36	0	36
	Negative	0	31	31
	Total	36	31	67

Overall Agreement: 100% (94.6% - 100%)
Positive Agreement: 100% (90.4% - 100%)
Negative Agreement: 100% (89.0% - 100%)

Table 14: Asymptomatic Individual and Pooled Specimen Agreement with a Pool Size of 5

		Individual Specimen Result		
		Positive	Negative	Total
Pool of 5 Result	Positive	31	0	31
	Negative	5	30	35
	Total	36	30	66

Overall Agreement: 92.4% (83.5% - 96.7%)
Positive Agreement: 86.1% (71.3% - 93.9%)
Negative Agreement: 100% (88.6% - 100%)

Bibliography

1. **World Health Organization.** Q&A on coronaviruses (COVID-19). March 9, 2020. World Health Organization Web site https://www.who.int/ news-room/q-a-detail/q-a-coronaviruses. Accessed March 10, 2020.
2. **Centers for Disease Control and Prevention.** https://www.cdc.gov/coronavirus/2019-ncov/prevent-getting-sick/how-covid-spreads.html. Accessed June 17, 2020.
3. **Centers for Disease Control and Prevention.** Coronavirus Disease 2019-(COVID-19) in the U.S. Updated March 10, 2020. Centers for Disease Control and Prevention Web site https://www.cdc.gov/coronavirus/2019-ncov/cases-in-us.html. Accessed March 10, 2020.
4. **Centers for Disease Control and Prevention.** Coronavirus Disease 2019 Information for Travel. Page last reviewed March 8, 2020. Centers for Disease Control and Prevention Web site https://www.cdc.gov/coronavirus/2019-ncov/travelers/index.html. Accessed March 10, 2020.
5. **Centers for Disease Control and Prevention.** Coronavirus Disease 2019-(COVID-19) Situation Summary. Updated March 9, 2020. Centers for Disease Control and Prevention Web site https://www.cdc.gov/coronavirus/2019-ncov/summary.html. Accessed March 10, 2020.
6. **Clinical & Laboratory Standards Institute.** Document M29 Protection of Laboratory Workers from Occupationally Acquired Infections. CLSI Web site https://clsi.org/standards/products/microbiology/documents/m29/. Accessed September 2017.

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Appendix 3 – Initial Delivery Locations

Site	Address	**Postcode**	Delivery contact	**Email and contact number**
Bristol	UKHSA Pathology Building (Phase 2) Southmead Hospital Bristol	BS10 5NB	[redacted]	[redacted]
Birmingham	UKHSA Birmingham Pathology Heartlands Hospital Bordesley Green East Birmingham	B9 5SS	[redacted]	[redacted]
Cambridge	UKHSA Cambridge Box 236 Cambridge University Hospitals NHS Foundation Trust Hills Road Cambridge	CB2 0QQ	[redacted]	[redacted]