

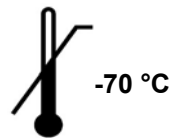
Prodesse[®] ProAdeno[™]+

Instructions for Use

For detection of human adenovirus




100



REF

H72VK00

	
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 Intended Use

The ProAdeno™+ Assay is a multiplex Real Time PCR *in vitro* diagnostic test for the qualitative detection of human Adenovirus (HAdV) DNA isolated and purified from nasopharyngeal (NP) swab specimens obtained from individuals exhibiting signs and symptoms of acute respiratory infection. This test is intended for use to aid in the diagnosis of HAdV infections in humans in conjunction with other clinical and laboratory findings. The test detects, but does not differentiate, serotypes 1-51.

Negative results do not preclude HAdV infection and should not be used as the sole basis for treatment or other patient management decisions.

 Summary and Explanation

Adenoviruses are medium sized, non-enveloped, icosahedral viruses that contain a linear double-stranded DNA genome that is 35-38kb. (1) There are 53 recognized serotypes of human adenoviruses belonging to the family *Adenoviridae* and the genus *Mastadenovirus*. (2) The serotypes are divided into seven species, A-G. The site of infection depends on the infecting serotype. Serotypes from species B, C, and E predominately infect the respiratory tract causing illnesses including upper and lower respiratory tract infections and acute respiratory disease. They can also cause hemorrhagic cystitis, conjunctivitis, and disseminated disease. Serotypes from species A and F typically cause gastroenteritis. While most serotypes from species D do not commonly cause disease, some can cause conjunctivitis. (3)

Adenoviruses are highly resistant to physical and chemical agents; as a result they can remain infectious for up to three weeks at room temperature. There have been reports of severe outbreaks and nosocomial infections. (3) Adenoviruses can be spread through direct contact, fecal-oral transmission, and waterborne transmission. (4) While infections can occur any time of year, respiratory outbreaks have been noted in late winter, spring, and early summer. (4) Prevalence has been shown to vary widely depending on time, location, and populations studied.

Principles of the Procedure

The ProAdeno+ Assay enables detection of human adenovirus and a Universal Internal Control.

An overview of the procedure is as follows:

1. Collect nasopharyngeal swab specimens from symptomatic patients using a polyester, rayon or nylon tipped swab and place into viral transport medium (refer to **Materials Required but not Provided section of this Instruction for Use**).
2. Add a Universal Internal Control (UIC) to every sample to monitor for inhibitors present in the specimens.
3. Perform isolation and purification of nucleic acids using a MagNA Pure LC System (Roche) and the MagNA Pure Total Nucleic Acid Isolation Kit (Roche) or a NucliSENS easyMAG System (bioMérieux) and the Automated Magnetic Extraction Reagents (bioMérieux).
4. Add purified nucleic acids to ProAdeno+ Supermix included in the ProAdeno+ Assay Kit. The ProAdeno+ Supermix contains oligonucleotide primers, target-specific oligonucleotide probes, and a Taq DNA polymerase. The primers are complementary to a highly conserved region of the HAdV hexon gene. The probes are dual-labeled with a reporter dye attached to the 5'-end and a quencher dye attached to the 3'-end (see table below).
5. Perform amplification of DNA in a Cepheid SmartCycler II instrument. In this process, the probe anneals specifically to the template followed by primer extension and amplification. The ProAdeno+ Assay is based on Taqman reagent chemistry, which utilizes the 5' – 3' exonuclease activity of the Taq polymerase to cleave the probe thus separating the reporter dye from the quencher. This generates an increase in fluorescent signal upon excitation from a light source. With each cycle, additional reporter dye molecules are cleaved from their respective probes, further increasing fluorescent signal. The amount of fluorescence at any given cycle is dependent on the amount of amplification products present at that time. Fluorescent intensity is monitored during each PCR cycle by the real-time instrument.

Analyte	Gene Targeted	Probe Fluorophore	Absorbance Peak	Emission Peak	Instrument Channel
Adenovirus	hexon	FAM	495 nm	520 nm	FAM
Universal Internal Control	NA	Quasar 670	647 nm	667 nm	Cy5

Materials Provided

ProAdeno+ Assay Kit (Cat. # H72VK00)

Reagents	Description	Quantity/ Tube	Cap Color	Cat. #	Reactions/ Tube
ProAdeno+ Supermix	<ul style="list-style-type: none"> ➤ Taq DNA polymerase ➤ Oligonucleotide primer pairs ➤ Oligonucleotide probes ➤ Buffer containing dNTPs (dATP, dCTP, dGTP, dTTP), MgCl₂ and stabilizers 	1030 µL	Brown	HSM72	50 (2 tubes provided)
RNase Inhibitor III	<ul style="list-style-type: none"> ➤ 40U/µL 	70 µL	Yellow	GLS37	100
Positive Control (PC) - Adenovirus DNA Control	<ul style="list-style-type: none"> ➤ Non-infectious DNA plasmid containing a portion of the targeted gene (<i>hexon</i>) 	500 µL	White	HCT72	25
Universal Internal Control (UIC)	<ul style="list-style-type: none"> ➤ Non-infectious double-stranded DNA (PCR product) ➤ Non-infectious <i>in vitro</i> transcribed RNA 	30 µL	Purple	GCT13	100

Materials Required But Not Provided
Plasticware and consumables

- Polyester, rayon or nylon tipped nasopharyngeal swabs
- RNase/DNase-free 1.5 mL polypropylene microcentrifuge tubes
- Sterile RNase/DNase-free filter or positive displacement micropipettor tips
- MagNA Pure LC System Disposables (Reagent Tubs, Reaction Tips, Tip Trays, Cartridges) or easyMAG System Disposables (Sample Strips and Tips)
- Biohit Pipette Tips for use with easyMAG System
- Greiner Break Four uncoated plates for use with easyMAG System
- Cepheid PCR reaction tubes, 25 μ L
- Parafilm M or MagNA Pure LC Cartridge Seals

Reagents

- †Roche MagNA Pure LC Total Nucleic Acid Isolation Kit (*Roche Cat. # 03038505001*) for 192 isolations or bioMérieux NucliSENS easyMAG reagents (*Buffer 1 Cat. # 280130, Buffer 2 Cat. # 280131, Buffer 3 Cat. # 280132, Magnetic Silica Cat. # 280133, and Lysis Buffer Cat. # 280134*)
- Micro Test M4 Viral Transport Medium (*Remel, Inc. Cat. No. R12500*), Micro Test M5 Viral Transport Medium (*Remel, Inc. Cat. No. R12515*), Micro Test M6 Viral Transport Medium (*Remel, Inc. Cat. No. R12530*), Micro Test M4RT Viral Transport Medium (*Remel, Inc. Cat. No. R12505*), Copan Universal Transport Medium (*Copan Diagnostics, Inc., Cat. No. 330C*), or BD Universal Viral Transport vial, 3mL (*Becton, Dickinson and Co. Cat. No. 220220*)
- Molecular Grade Water (RNase/DNase Free)
- Extraction Control (*e.g. previously characterized positive sample*)

† NOTE: Only qualified lots of the MagNA Pure LC Total Nucleic Acid Isolation Kit can be used with the ProAdeno+ Assay. Any lots not specifically qualified by Gen-Probe Prodesse, Inc. for use with the ProAdeno+ Assay are not verified for use with this assay, and may cause erroneous results.

A list of these qualified extraction reagents is available at www.gen-probe.com. Please notify the reagent manufacturer of issues with this ancillary reagent and Gen-Probe Prodesse, Inc. of the impact on the performance of the ProAdeno+ Assay.

Equipment

- 70°C Freezer
- Roche MagNA Pure LC System with software version 3.0.11 or bioMérieux NucliSENS easyMAG System with Software version 1.0.1 or 2.0
- Biohit multi-channel pipettor for use with easyMAG System
- Cepheid SmartCycler II Real Time Instrument with Dx Software version 1.7b, 3.0a, or 3.0b
- Micropipettors (range between 1-10 μ L, 10-200 μ L and 100-1000 μ L)
- Mini-centrifuge with adapter for Cepheid Reaction Tubes
- Cepheid cooling block
- Wet ice or microcentrifuge tube cooling block
- Biological safety cabinet

Warnings and Precautions

- For *in vitro* diagnostic use only.
- Use of this product should be limited to personnel who have been trained in the techniques of Real-Time PCR.
- **The ProAdeno+ Assay should be performed on the Cepheid SmartCycler II instrument only.**
- **Once the ProAdeno+ Supermix has been thawed, the run must be started within one hour.**
- Do not update the SmartCycler Dx Software beyond version 3.0b until Gen-Probe Prodesse communicates that the updated software version has been validated for use with the ProAdeno+ Assay.
- Performance characteristics of this assay have been determined only with nasopharyngeal swab specimens.
- Handle all specimens as if infectious using safe laboratory procedures such as those outlined in CDC/NIH *Biosafety in Microbiological and Biomedical Laboratories* and in the CLSI Document M29 *Protection of Laboratory Workers from Occupationally Acquired Infections*. Thoroughly clean and disinfect all surfaces with 10% bleach. Autoclave any equipment or materials that have contacted clinical specimens before discarding.
- Use micropipettes with aerosol barrier or positive displacement tips for all procedures.
- Always pre-plan, organize and segregate workflow. Workflow in the laboratory should proceed in a unidirectional manner, beginning in the Pre-Amplification Area and moving to the Amplification/Detection Area.
 - Begin pre-amplification activities with reagent preparation and proceed to specimen preparation.
 - Always dedicate supplies and equipment to a specified area; no cross-movement allowed between areas.
 - Do not use equipment and supplies used for reagent preparation for specimen preparation activities or for pipeting or processing other sources of target nucleic acid.
 - Keep all amplification supplies and equipment in the Amplification/Detection Area at all times.
 - Always wear disposable gloves in each area and change them before entering a different area.
 - Do not open sample tubes following PCR.
- Take care to preserve the purity of kit reagents. Avoid contamination from Positive Controls and specimens by following good laboratory practices.
- Do not use kit after its expiration date.
- Do not mix reagents with different lot numbers or substitute reagents from other manufacturers.
- Material Safety Data Sheets (MSDS) are available on Gen-Probe Prodesse's website at www.gen-probe.com from the **Package Inserts** tab.

Reagent Storage, Handling and Stability

- Store all reagents (opened and unopened) at $\leq -70^{\circ}\text{C}$ until the expiration date listed on the appropriate Assay kit.
- Always check the expiration date on the reagent tubes. For Intermediate stock of the Universal Internal Control, use the expiration date of the originating stock control tube. Do not expose controls to more than one (1) freeze-thaw cycle.
- ProAdeno+ Assay Kits are shipped frozen, should arrive frozen and should be stored frozen after receipt. If the kit contents are not frozen or the tubes are compromised, contact Gen-Probe Prodesse, Inc. for assistance.
- An internal study demonstrated that performance of ProAdeno+ Supermix and RNase Inhibitor III are not affected for up to 10 freeze-thaw cycles.
- Visually examine reagents for adequate reagent volume before beginning any test procedures.
- Protect the ProAdeno+ Supermix from light.
- **Controls and aliquots of controls must be thawed and kept on ice at all times during preparation and use.**



Aliquoting of kit components is recommended for labs with smaller batch sizes.

Recommendation



Specimen Collection, Handling and Storage

Collecting the Specimen

To obtain nasopharyngeal swab samples:

1. Insert a flexible-shaft polyester, rayon or nylon tipped swab containing a dry tip into one nostril and into the nasopharyngeal area.
2. Press the swab gently against the nasopharyngeal wall to allow the swab to absorb secretions.
3. Rotate the swab two to three times and withdraw it.
4. Place the swab into a tube containing 3 mL of viral transport medium (Remel M4, M4RT, M5, M6; Copan UTM; or BD UVT).



Note

Using a smaller volume of the viral transport medium may result in inhibition.

5. Break off the shaft of the swab and cap the tube.

Transporting Specimens

Ensure that when transporting human respiratory specimens, all applicable regulations for the transport of etiologic agents are met. Transport human respiratory specimens refrigerated at 2-8°C.

Storing Specimens

Store specimens refrigerated (2-8°C) for up to 72 hours before processing. Specimens may also be stored frozen at $\leq -70^{\circ}\text{C}$ before processing. Store any leftover specimens at $\leq -70^{\circ}\text{C}$. If retesting a frozen specimen, thaw specimen quickly (1 to 2 minutes) in a 37°C water bath and immediately place on ice or thaw specimen on ice.

Storing Purified Nucleic Acid

Store purified nucleic acids at $\leq -70^{\circ}\text{C}$. They should be tested after no more than one (1) freeze-thaw cycle.



Note

Inadequate or inappropriate specimen collection, storage and transport are likely to yield false negative results.



Recommendation

Training in specimen collection is highly recommended because of the importance of specimen quality.

Reagent and Control Preparation

Reagents



Note

Prepare reagents from the Roche MagNA Pure LC Total Nucleic Acid Isolation Kit or the bioMérieux easyMAG Automated Magnetic Extraction Reagents following the manufacturer's instructions.

Controls



Note

- ❖ For aliquots of the Positive Control and Intermediate stock of the Universal Internal Control, use the expiration date of the originating stock control tube.
- ❖ Controls and aliquots of controls must be thawed and kept on ice/cold block at all times during preparation and use. It is recommended to prepare controls in a sample prep area, such as a Biological Safety Cabinet.
- ❖ It is required to include the Positive Control and Negative Control in each PCR run. It is recommended to include an Extraction Control with each nucleic acid isolation run.

Positive Control (PC)

1. Thaw Positive Control (the white cap tube) on ice.
2. Make 25 aliquots of 20 μL , label and store at $\leq -70^{\circ}\text{C}$. **Ensure that aliquots do not undergo more than one (1) freeze-thaw cycle.**
3. Dilute the Positive Control just prior to setup of the PCR reaction (see [Step 4 \(a\)](#) of the [Assay Procedure](#)).



Note

Do not spike Positive Control with the Universal Internal Control. Do not take Positive Control through the nucleic acid isolation procedure.

Universal Internal Control (UIC)

1. Thaw Universal Internal Control (the purple cap tube) and RNase Inhibitor (the yellow cap tube) on ice.
2. Create Intermediate stock tubes of the Universal Internal Control using the following dilution scheme:

$$26 \mu\text{L Universal Internal Control} + 65 \mu\text{L RNase Inhibitor} + 2509 \mu\text{L molecular grade water} = 2600 \mu\text{L total volume}$$

3. Make aliquots of 110 μL , label, and store at $\leq -70^{\circ}\text{C}$ (this is enough volume to add to 5 samples at 20 μL per sample). Make aliquots of larger or smaller volumes based on the number of samples expected to be processed in a single run. **Ensure that aliquots do not undergo more than one (1) freeze-thaw cycle.**
4. Add the appropriate volume of Intermediate stock of the Universal Internal Control to each sample prior to nucleic acid isolation (see [Step 1](#) of the [Assay Procedure](#)).

Negative Control (NC)

1. Use viral transport medium as the Negative Control.
2. Add the appropriate volume of Intermediate stock of the Universal Internal Control to the Negative Control prior to nucleic acid isolation (see [Step 1](#) of the [Assay Procedure](#)).

Extraction Control

Good laboratory practice recommends including a positive extraction control (e.g. previously characterized positive sample) in each nucleic acid isolation run. The extraction control should be treated like a sample during assay performance and analysis. The extraction control should have a Ct between 13-40 cycles.

Assay Procedure

Assay Overview:

Get Ready: Create the Assay Protocol for the Cepheid SmartCycler instrument using the Dx Software (first time only).

1. Prepare the Samples, Extraction Control, and Negative Control.
2. Isolate the Nucleic Acid – MagNA Pure LC System using the Total Nucleic Acid Isolation (TNAI) Kit

OR

3. Isolate the Nucleic Acid – NucliSENS easyMAG System using the Automated Magnetic Extraction Reagents.
4. Set up the PCR Reaction.
5. Run the ProAdeno+ Assay.
6. Print report.



Note

- ❖ Instructions provided for the Cepheid SmartCycler II Real Time Instrument with Dx Software version 1.7b (Instructions for version 3.0a / 3.0b noted).
- ❖ Do NOT deviate from the protocol settings defined in this section.

Get Ready: Create the Assay Protocol for the Cepheid SmartCycler instrument using the Dx Software (first time only)



Note

- ❖ The protocol is created prior to first time use; it does not need to be recreated with each sample run.
- ❖ Refer to SmartCycler Dx Software Operator Manual for assistance in defining assay protocols.
- ❖ To **Define** and **Edit** Assay protocols, the user must have administrative access rights. Otherwise, the fields will not be accessible for data entry and editing (they will be grayed out).
- ❖ Cepheid Dx Software interprets the data and reports the run as either **VALID** or **INVALID**, based on the results of the Negative Control. Enter the Positive Control as if it was a sample. The user must interpret the Positive Control result and determine if the PCR run is **VALID**.
- ❖ The user must interpret the Extraction Control result (if included) and determine if the extraction run is **VALID** or **INVALID**.
- ❖ All Control criteria must be met in order for the run to be **VALID** (see [Interpretation of Control Results](#)).

1. Create the ProAdeno+ Assay protocol:

- a. Launch the Cepheid Dx software application.
- b. Click on the **Define Assay** box at the top of the screen.
- c. Click on the **New Assay** box at the bottom of the screen.
- d. Enter **ProAdeno+ Assay** for the assay protocol in the window that opens.
- e. Click **OK**.
- f. In the middle of the **Define Assay** screen, select **FTTC25** for the **Dye Set**.
- g. Select **NC fails if: Any target criterion is positive** (Dx 3.0a / 3.0b: this is default setting in **Control Settings** Tab).
- h. Check the box to **Require Lot Number** (Dx 3.0a / 3.0b: click on **Advanced** Tab and check **Require Lot Number** box).
- i. Deselect the box to **Use Patient IDs** (Dx 3.0a / 3.0b: click on **Advanced** Tab, **Use Patient IDs** box is deselected by default).

- j. Enter **Thermocycler Parameter** in the Protocol section (bottom half of **Define Assay** screen).

Stage 1			Stage 2 Repeat 5 times			Stage 3 Repeat 40 times		
Hold			2- Temperature Cycle			2- Temperature Cycle		
Temp	Secs	Optics	Temp	Secs	Optics	Temp	Secs	Optics
95	600	OFF	95	30	OFF	95	10	OFF
			57	30	ON	57	30	ON

Stages 4 – 10 remain **UNUSED**

2. Enter information in **Analysis Settings** tab as follows:



Note

- ❖ Gray boxes are default settings.

Channel	Dye Name	Target	Usage	Curve Analysis	Thresh Setting	Manual Thresh	Auto Thresh	Auto Min. Cycle	Auto Max. Cycle	Valid Min. Cycle	Valid Max. Cycle	Bkgnd Sub	Bkgnd Min. Cycle	Bknd Max. Cycle	Boxcar Avg	EndPt Thresh	NC IC %	IC Delta
1	FAM*	Adeno	Assay**	Primary Curve	Manual Thresh	40	NA	5	10	13.0	45	On	5	45	0	40	10.0†	NA
2	TET*	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
3	TxR*	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
4	Cy5*	Universal Internal Control	Internal Control	Primary Curve	Manual Thresh	35	NA	5	10	13.0	45	On	5	45	0	35	10.0†	NA

* If the Dye Names are incorrect, check that FTTC25 Dye Set is being used.

** Dx 3.0a / 3.0b = Target

† Dx3.0a / 3.0b = NA

3. Enter information in the **Control Settings** tab.



Note

- ❖ Enter **Positive Control** and **Extraction Control** as samples. Do not use the **Positive Control Settings**. Enter **0** Replicates to inactivate **Positive Controls PC1-3**. A 3079 error (**Fluorescence Signal Too High**) in the **Positive Control** invalidates the run, this is avoided if the **Positive Control** and/or the **Extraction Control** is entered as a sample and the results for each of the target channels are individually evaluated.
- ❖ Use only one **Negative Control (NC1)**. Enter **0** Replicates to inactivate the **Negative Controls NC2** and **NC3**.
- ❖ Gray boxes are default settings.

Control ID	Control Name	Replicate	Adeno Valid Min Cycle	Adeno Valid Max Cycle	Adeno EndPt Thresh	UIC +/-	UIC Valid Min Cycle	UIC Valid Max Cycle	UIC EndPt Thresh
PC1	Pos Cntrl 1	0	13.0	45.0	10	+	13.0	45.0	10
PC2	Pos Cntrl 2	0	13.0	45.0	10	+	13.0	45.0	10
PC3	Pos Cntrl 3	0	13.0	45.0	10	+	13.0	45.0	10
NC1	Neg Cntrl 1	1	13	45	40	+	20	41	35
NC2	Neg Cntrl 2	0	13.0	45.0	10	+	13.0	45.0	10
NC3	Neg Cntrl 3	0	13.0	45.0	10	+	13.0	45.0	10

4. Confirm Probe Check Settings tab.

Note

The probe check is not used in the ProAdeno+ Assay protocol.

Channel	Dye Name	Prb1 Min	Prb1 Max	Prb2 Min	Prb2 Max	Prb3 Min	Prb3 Max	Delta 12 Min	Delta 12 Max	Delta 23 Min	Delta 23 Max	Delta 13 Min	Delta 13 Max
1	FAM	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
2	TET	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
3	TxR	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
4	Cy5	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

5. Select Save Assay.
1. Prepare the Samples, Extraction Control (if included), and Negative Control (Pre-Amplification Area)
a. Add Universal Internal Control to all samples and Extraction Control.

- i.* Thaw the appropriate number of aliquots of Intermediate stock of the Universal Internal Control (enough volume needed for each sample, the Extraction Control, and the Negative Control) on ice.
- ii.* Remove 180 µL of sample from the original sample tube and pipet into a labeled 1.5 mL microcentrifuge tube. Alternately, pipet 180 µL of sample directly into sample cartridge or sample vessel.
- iii.* Remove 180 µL of Extraction Control from the original sample tube and pipet into a labeled 1.5 mL microcentrifuge tube. Alternately, pipet 180 µL into directly into sample cartridge or sample vessel.
- iv.* Add 20 µL of Intermediate stock of the Universal Internal Control to each sample and Extraction Control. **Pipet up and down a minimum of 5 times to mix using a new pipet tip for each tube.**
- v.* Keep tubes on ice.
- vi.* Store any remaining sample at $\leq -70^{\circ}\text{C}$.

b. Add Universal Internal Control to the Negative Control.

- i.* Include one (1) Negative Control in each run.
- ii.* Add 180 µL of viral transport medium to a labeled 1.5 mL microcentrifuge tube. Alternately, pipet 180µL of viral transport medium directly into sample cartridge or sample vessel.
- iii.* Add 20 µL of Intermediate Stock of Universal Internal Control to the viral transport medium. **Pipet up and down a minimum of 5 times to mix.**
- iv.* Keep tube on ice.
- v.* Discard remaining volume of Universal Internal Control – DO NOT reuse.



Do not reuse Universal Internal Control.

2. Isolate the Nucleic Acid (*Pre-Amplification Area I*) - MagNA Pure LC System using the Total Nucleic Acid Isolation (TNAI) Kit

a. Start the instrument and software.

- i. Turn power on to the MagNA Pure LC instrument and then turn on the computer.
- ii. Start the MagNA Pure LC software.
- iii. From the *Main Menu* screen, select **Sample Ordering** and enter sample information in **Sample Name** column.
- iv. Select the **Total NA Variable_elution_volume.blk** protocol.
- v. Follow the software instructions and specify the number of samples.
- vi. Type in **200 µL** for the sample volume and verify that **50 µL** elution volume is selected.
- vii. Select **Stage Setup** and the software will automatically calculate the amount of each reagent that is required.

b. Fill the reagent tubs.

Before starting the isolation procedure, fill all reagent tubs outside the instrument with the required volume of each reagent listed on the **Start Information** screen.



Note

*Use only the reagent amount needed for the number of samples entered into the software. Reagents are not stable for long-term storage in tubs. Vortex Magnetic Glass Particles (MGPs) and load the exact amount of MGPs (as listed on the **Start Information** Screen) into the instrument just before the run starts.*

c. Load reagent tubs and disposables into the instrument.

Use the information on the **Start Information** screen to place all disposable plastics and reagent tubs necessary for the batch run on the Reagent/Sample Stage.



Recommendation

Use a colored "Positioning Frame" (provided with the TNAI kit) on the Reagent Tub Rack to help to correctly load reagents.

d. Load the samples, Extraction Control, and Negative Control into the MagNA Pure Sample Cartridge.

- i. Transfer all 200 µL of each sample to individual wells in the MagNA Pure Sample Cartridge.
- ii. Transfer all 200 µL of the Extraction Control and Negative Control to different wells in the MagNA Pure Sample Cartridge.
- iii. Cover cartridge with Parafilm or MagNA Pure LC Cartridge Seal and keep cartridge on ice until ready to load the instrument.

e. Load the samples.

Transfer the Sample Cartridge containing the samples, Extraction Control, and Negative Control into the MagNA Pure LC instrument.

f. Start the run.

- i. Start the Batch Run by confirming the correct placement of all disposable plastics and reagents by mouse-clicking the respective text boxes on the **Start Information** screen.
- ii. Select the **OK** button to start the automated isolation procedure. The instrument will automatically dispense all reagents and process the samples.

g. Store the eluted total nucleic acid.

After completing the run, place the Storage Cartridge containing the eluted nucleic acids immediately on ice or transfer eluted nucleic acid to 1.5 mL tubes and store for longer durations at $\leq -70^{\circ}\text{C}$.



Note


*Do not store purified nucleic acids in the Storage Cartridge on the Cooling Unit 1.
Do not use nucleic acids for PCR if the extraction instrument/run failed.*

3. Isolate the Nucleic Acid (*Pre-Amplification Area I*) - NucliSENS easyMAG System using the Automated Magnetic Extraction Reagents

a. Start Instrument and Software.



Turn power on to the easyMAG instrument and once the LED on the instrument turns green, turn on the computer and log into the software.

b. Prepare the software for a run.



To prepare for a run, touch the “Settings”  icon in the main toolbar which defaults to the “Application Settings” icon and choose the following run settings:

Default Request: Generic 1.0.6 or 2.0.1 (for software version 1.0.1 or 2.0, respectively)
Run Name Prefix: N/A (leave as default)
Sample ID prefix: N/A (leave as default)
Sample Type: Primary (on-board lysis)
Default On-board Lysis Dispensing: Yes
Default On-board Lysis Incubation: Yes
Sample Addition Guidance: Off
Reagent Tracking: Off


c. Input buffer information.

Touch the “Instrument”  icon to default to the “Reagent Inventory”  icon and input the buffer barcodes by first scanning the instrument position (A, B, C, or D) and then its corresponding buffer. For example, scan position A and then scan the bottle of Lysis buffer in that position and then move on to position B and its corresponding bottle.




d. Create a worklist.



i. Touch the “Daily Use”  icon which will default to the “Define Extraction Request”  icon and select the following settings:

Sample ID: Manually enter the sample name.
Matrix: Other
Request: Generic 1.0.6 or 2.0.1 (for software version 1.0.1 or 2.0, respectively)
Volume (mL): 0.200 (input volume of sample)
Euate (µL): 55
Type: Primary
Priority: Normal

ii. Press **Enter** on the keyboard or touch the “New Extraction Request”  icon after each manual sample addition. The settings above will remain as the default settings for each subsequent entry as long as you do not navigate to other pages.

e. Create a run and add samples from the worklist.


Touch the “Organize Runs”  icon and then the “Create Run”  icon which will bring up the *New Run Window*. In this screen, name the run appropriately and verify that the **auto-number** box is left unchecked (NOT SELECTED) and that **Yes** is selected for both the On-Board Lysis Dispensing and On-Board Lysis Incubation options. Touch **OK** and the *New Run Window* will close and you will be in the “Organize Runs” screen. Assign samples to run with the positioning (arrow)  icons. Touch the

“Load Run”  icon and select the run. Print the worklist with the “Print worklist”  icon. This worklist will aid in keeping track of the order of the samples to be loaded into the sample vessels.

f. Load the samples and tips and barcode the sample strip(s).

Add all of the 200 μ L from each sample into the proper vessels of the sample strips(s) as noted in the worklist. Insert tips into sample vessel(s) in the correct order as noted in the worklist and scan the sample strip(s) position on the instrument and then the sample strip itself. For example, scan position A and then the sample strip in that position, then B and then C, if necessary. After scanning the sample strip(s), the indicator will change from red to green on the screen.

g. On-board lysis dispensing.


Once the samples and tips are loaded and the strip(s) scanned, close the lid and touch the “**Dispense Lysis**”  icon. The instrument will dispense 2 mL of Lysis Buffer to each sample and incubate for 10 minutes.

h. Prepare the magnetic silica to add to the sample vessels.

During the 10-minute lysis incubation, use the Biohit multi-channel pipettor to prepare the magnetic silica. This procedure will need to be performed for each sample vessel used in the run (1, 2, or 3 times).

- i.** Set the pipettor to **Program 1** and place a Biohit pipette tip on position 1. Program 1 provides the means to aspirate and dispense 550 μ L of liquid. The magnetic silica is prepared in a 1:1 ratio of Molecular Biology Grade Water to Magnetic Silica.
- ii.** Using Program 1 of the pipettor, press the **start** button to aspirate and then again to dispense 550 μ L of water into a microcentrifuge tube. Vortex the tube of magnetic silica briefly to mix and use Program 1 of the pipettor to aspirate and then dispense 550 μ L of magnetic silica into the same microcentrifuge tube as the water. Eject the tip, cap the tube and vortex to mix.
- iii.** Set the pipettor to **Program 2** and place a Biohit pipette tip on position 1. Program 2 will transfer 8 volumes of the previous mix to the 8 wells of a strip on an ELISA plate (1 ELISA strip/sample strip). Press the **start** button to aspirate the mix. Press the **start** button again to dispense the remaining mixture back into the tube containing the mix to reset the pipette.
- iv.** Press the **start** button 8 separate times to dispense the remaining mix in each of 8 wells of an ELISA plate strip and eject the tip.
- v.** After the 10 minute lysis incubation is done, set the pipettor to **Program 3** and place 8 Biohit pipette tips on the multichannel pipettor (or however many samples are present in the specific sample strip). Make sure that the filter tips are very well connected with the multichannel pipettor to prevent leakage errors. Program 3 first mixes the magnetic silica mixture in the ELISA plate and then aspirates it to be delivered to the vessels of the sample strip where it will be mixed. Press the **start** button once and the pipette will mix the silica in the ELISA plate and then aspirate it for addition to the sample vessel. Verify that each tip has the same volume of silica mix before placing in the sample vessel. Place the pipettor over the sample vessel strip so the tips are below the liquid level of each sample and press the **start** button again, which will then aspirate 800 μ L out of each sample vessel and perform 3 mix cycles with 1000 μ L. At this point be sure to hold the pipette steady below the liquid/air interface as it is mixing so as not to introduce bubbles to the sample.
- vi.** Repeat for each additional sample strip in the run.

i. Start the run.

Touch the “**Start**”  icon to begin the run. The instrument will perform 5 washes and heat and elute. The purified nucleic acids need to be transferred to appropriate storage tubes (1.5 mL microcentrifuge tubes) on ice within 30 minutes of extraction completion to avoid contamination by the magnetic silica stuck to the front wall of the sample vessel(s). Use immediately or store at ≤ -70 °C.



Note

Do not use nucleic acids for PCR if the extraction instrument/run failed.

4. Set up the PCR Reaction (Pre-Amplification Area II)



Start the SmartCycler ProAdeno+ Assay run within 1 hour of thawing the ProAdeno+ Supermix.

Note

a. Dilute the Positive Control.

- i.* Include the Positive Control with each run. Thaw one (1) aliquot of Positive Control on ice.
- ii.* Add 45 μL of molecular grade water to a 1.5 mL microcentrifuge tube.
- iii.* Transfer 5 μL from the Positive Control aliquot to the appropriate tube. **Pipet up and down a minimum of 5 times to mix.**
- iv.* Keep tube on ice.
- v.* Discard remaining volume of Positive Control aliquot – DO NOT reuse.



Do not reuse Positive Control.



Keep the cold blocks on ice while adding reagents to the SmartCycler tubes.

Recommendation

b. Thaw the ProAdeno+ Supermix and add 20 μL to the SmartCycler tubes.

- i.* Keep the ProAdeno+ Supermix on ice and protected from light before adding to SmartCycler tubes.
- ii.* Load the required number of tubes into the Cepheid Cooling Block.
- iii.* Pipet 20 μL of the ProAdeno+ Supermix into the upper part of the SmartCycler tubes.
- iv.* Store any remaining ProAdeno+ Supermix at $\leq -70^{\circ}\text{C}$.

c. Add 5 μL of each sample's nucleic acid to individual SmartCycler tubes containing the ProAdeno+ Supermix.

- i.* After adding the sample's nucleic acid to the SmartCycler tube, **pipet up and down 2 to 3 times in the upper part of the tube.**
- ii.* Close the tube. Use a new pipette tip for each sample.

d. Add 5 μL of diluted Positive Control to a separate SmartCycler tube containing the ProAdeno+ Supermix.

- i.* After adding the diluted Positive Control to the SmartCycler tube, **pipet up and down 2 to 3 times in the upper part of the tube.**
- ii.* Close the tube.
- iii.* Discard remaining volume of diluted Positive Control – DO NOT reuse.



Do not reuse diluted Positive Control.

e. Add 5 μL of the Extraction Control nucleic acid to a separate SmartCycler tube containing the ProAdeno+ Supermix.

- i.* After adding the Extraction Control nucleic acid to the SmartCycler tube, **pipet up and down 2 to 3 times in the upper part of the tube.**
- ii.* Close the tube.

f. Add 5 μL of the Negative Control nucleic acid to the last SmartCycler tube containing the ProAdeno+ Supermix.

- i.* After adding the Negative Control nucleic acid to the SmartCycler tube, **pipet up and down 2 to 3 times in the upper part of the tube.**
- ii.* Close the tube.

g. Centrifuge all tubes.

- i.* Appropriately label the SmartCycler tubes on the caps.
- ii.* Centrifuge all tubes for 5 to 10 seconds using the Cepheid microcentrifuge specially adapted to fit the SmartCycler tubes.
- iii.* Return tubes to the cooling block.

h. Keep the tubes on the Cooling Block before loading them into the SmartCycler instrument.

5. Run the ProAdeno+ Assay (Amplification/Detection Area)

- a.** Create a new run by clicking on the **Create Run** icon at the top of the screen. This will open the *Create Run* screen.
- b.** Under **Run Name** in the left panel of the *Create Run* screen, enter a unique run identifier.
- c.** Click on the **Assay** arrow in the left panel of the *Create Run* screen and select the **ProAdeno+ Assay** protocol from the drop-down menu.
- d.** Under **Assay Information** in the left panel of the *Create Run* screen, enter the **Lot Number** and **Expiration Date** of the ProAdeno+ Kit.
- e.** In the left panel of the *Create Run* screen, enter the number of specimens (including the Positive and the Extraction Controls, but excluding the Negative Control) and click on **Apply**. This will display the **Site Table** and the Smartcycler Dx Software will automatically select the **I-Core** sites.
- f.** In the **Site Table** under the **Sample ID** column, enter the Sample Identifier, Positive Control Identifier or Extraction Control Identifier for the appropriate I-Core sites.
- g.** Insert each reaction tube into an I-Core site of the SmartCycler by pressing down firmly on all tubes and close each lid. Verify that the Negative Control (NC1) is loaded into the correct I-Core site specified by the software.
- h.** Select the **Start Run** button located at the bottom left corner of the screen. Verify that the LED is on for the appropriate I-Core sites.

6. Print Report

- a.** Click on **Report** at bottom of screen to open the **Report Preview** screen.
- b.** Click on the **Print Icon** at the top of the screen.

Interpretation of Control Results

Validation of Run



Note

The user must interpret the Positive Control (PC) and the Extraction Control (if included) results to determine whether the run is VALID and that results may be reported; the SmartCycler Dx software will automatically interpret the Negative Control result.

For a VALID Extraction run, the following conditions must be met:

Sample ID ¹	Assay Result	UIC Result	Warning / Error Code	Sample Type	UIC Ct	Adeno Result	Adeno Ct
Extraction Control	Positive	NA	**	SPEC	NA	POS	13-40
Neg Cntrl	Valid ²	Pass		NC1	20-41	Valid	0

¹ Columns and data not used for interpretation are not included.

² (Typical) an Invalid assay will display Error Code 4098.

** Error Code 3079: Warning/Error Code 3079 is periodically observed with Adenovirus positives (Positive Control, Extraction Control, Adenovirus positive NP swab samples). Warning/Error Code 3079 occurs when the fluorescence (RFU) signal is too high. In this case, all results for the sample are reported by the Dx software as ND (Not Determined). When this code is observed for the Extraction Control, extraction run validity can be determined based on Ct values of the Extraction Control. The Extraction Control must have a Ct value between 13-40 in the Adeno Ct column to be considered VALID.

Invalid Extraction Run

If the conditions for a Valid Extraction run are not met (i.e., the Extraction Control is not positive or the Negative Control is Invalid), start from original sample(s) using a new Extraction Control and a new Negative Control (starting at [Step 1](#) of the [Assay Procedure](#)). If the Negative Control gives a positive Ct result in the Adeno channel, it is recommended to investigate potential sources of contamination.

For a VALID PCR run, the conditions in the table below must be met. If the run is valid, specimens should be interpreted using the next section [Interpretation of Specimen Results](#).

Sample ID ¹	Assay Result	UIC Result	Warning / Error Code	Sample Type	UIC Ct	Adeno Result	Adeno Ct
Adeno PC	Positive	NA	**	SPEC	0	POS	20-41
Neg Control	Valid ²	Pass		NC1	20-41	Valid	0

¹ Columns and data not used for interpretation are not included.

² (Typical) An Invalid assay will display Error Code 4098.

** Error Code 3079: Warning/Error Code 3079 is periodically observed with Adenovirus positives (Adenovirus Positive Control, Adenovirus positive NP swab samples). Warning/Error Code 3079 occurs when the fluorescence (RFU) signal is too high. In this case, all results for that sample are reported by the Dx software as ND (Not Determined). If a Ct value between 20-41 is reported in the "Adenovirus Ct" column for the Adenovirus Positive DNA Control, results can be recorded as positive and the run considered VALID.

Invalid PCR Run

If the Positive Control is not positive within the specified Ct range but the Negative Control is valid, prepare all new reactions using remaining purified nucleic acids and a new Positive Control (starting with PCR at [Step 4](#) of the [Assay Procedure](#)).

If the Negative Control is invalid because the UIC Ct is outside the specified range, prepare all new extractions starting from original sample(s) using a new Extraction Control and a new Negative Control (starting at [Step 4](#) of the [Assay Procedure](#)). If the Negative Control gives a positive Ct result in the Adeno channel, it is recommended to investigate potential sources of contamination.

Interpretation of Specimen Results

The SmartCycler Dx software automatically determines the specimen results. The interpretation of the assay specimen results is as follows:

Sample ID ¹	Assay Result	UIC Result	Warning/Error Code	Adeno Result	Interpretation of Results
Sample ID	Negative	Pass		NEG	Adenovirus nucleic acid not detected
Sample ID	Positive	NA*		POS	Adenovirus nucleic acid detected
Sample ID	Unresolved	Fail		NEG	Unresolved – PCR inhibition or reagent failure. Repeat testing from the purified nucleic acid, re-test from original sample, or collect and test a new sample.
Sample ID	ND ²	ND	3079 ²	ND	Not Determined – error code 3079 ²
Sample ID	ND	ND	4098 ³	ND	Not Determined – error code 4098

¹ Columns and data not used for interpretation are not included

² Error Code 3079: Warning/Error Code 3079 is periodically observed with Adenovirus positives (Adenovirus Positive Control, Adenovirus positive NP swab samples). Warning/Error Code 3079 occurs when the fluorescence (RFU) signal is too high. In this case, all results for that sample are reported by the Dx software as ND (Not Determined). If a Ct value ≥ 13 is reported in the **Adeno Ct** columns, the sample results can be recorded as POS for Adenovirus nucleic acid.

³ An Invalid assay run will display Error Code 4098

* Detection of the Universal Internal Control in the Cy5 detection channel is not required for positive result. High viral load can lead to reduced or absent Universal Internal Control signal.

Not Determined Samples

If an assay result of **ND** (Not Determined) is reported with an instrument failure other than Warning/Error Code 3079, repeat testing from the purified nucleic acids (see **Step 4 (a)** of the **Assay Procedure**). Refer to the Cepheid Dx Software Operator Manual for interpretation of Warning Codes.

Quality Control

- 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. It is recommended that the user refer to CLSI document C24-A3, *Statistical Quality Control for Quantitative Measurements: Principles and Definitions*: [Approved Guideline – Third Edition] or other published guidelines for general quality control recommendations. For further guidance on appropriate quality control practices, refer to 42 CFR 493.1202(c).
- Quality control procedures are intended to monitor reagent and assay performance.

Control Type	Used to Monitor
Positive	Substantial reagent failure including primer and probe integrity
Negative	Reagent, environmental, or carry-over contamination
Extraction	Failure in lysis and extraction procedure
Internal	PCR inhibition in individual samples, reagent failure or process error

- Dilute and test the Positive Control and Universal Internal Control prior to running samples with each new kit lot to ensure all reagents and kit components are working properly.
- Good laboratory practice recommends including a positive Extraction Control and a Negative Control in each nucleic acid isolation run. The Extraction Control should be treated as a sample.
- Never run the Positive Control through nucleic acid isolation.

- Always include one Negative Control (containing Universal Internal Control) and one Positive Control in each amplification/detection run performed.
- Failure of Controls (Positive, Negative and/or Extraction) invalidates the run and results should not be reported.
- If the Positive Control is not positive within the specified Ct range but the Negative Control is valid, repeat testing should be done starting from the purified nucleic acid and using a new aliquot of the Positive Control. If repeat results are still invalid, results should not be reported and testing should be repeated from the original sample or a new sample should be collected and tested.
- If the Negative Control is invalid, repeat testing should be done starting from the original sample using a new Negative Control. If the Negative Control is invalid because of signal in the Adeno Channel, care should be taken to eliminate contamination. If repeat results are still invalid, results should not be reported and a new sample should be collected and tested.
- If the Extraction Control is not positive within the specified Ct range (13-40 cycles), repeat testing should be done starting from the original sample using a new Extraction Control. If repeat results are still invalid, results should not be reported and a new sample should be collected and tested.

Limitations

- **Once the ProAdeno+ Supermix has been thawed, the run must be started within one hour.**
- Negative results do not preclude infection and should not be used as the sole basis for diagnosis, treatment or other management decisions.
- Results from this test must be correlated with the clinical history, epidemiological data and other data available to the clinician evaluating the patient.
- The detection of viral nucleic acid is dependent upon proper specimen collection, handling, transportation, storage, and preparation (including extraction). Failure to observe proper procedures in any one of these steps can lead to incorrect results. There is a risk of false negative values resulting from improperly collected, transported, or handled specimens.
- There is a risk of false negative values due to the presence of sequence variants in the viral targets of the assay, procedural errors, amplification inhibitors in specimens, or inadequate numbers of organisms for amplification.
- Positive and negative predictive values are dependent on prevalence.
- The Universal Internal Control is included in the ProAdeno+ assay to aid in the identification of specimens that contain inhibitors to PCR amplification. The Universal Internal Control does not indicate whether or not nucleic acid has been lost due to inadequate collection, transport or storage of specimens.
- A specimen yielding a negative result may contain respiratory viruses other than Adenovirus.
- There is a risk of false positive values resulting from cross-contamination by target organisms, their nucleic acids or amplified product, or from non-specific signals in the assay.
- Analyte targets (viral nucleic acid) may persist *in vivo*, independent of virus viability. Detection of analyte target does not imply that the virus is infectious, or is the causative agent for clinical symptoms.
- This test is a qualitative test and does not provide the quantitative value of detected organism present.
- The performance of this test has been evaluated for human specimen material only.
- The performance of this test has not been evaluated for sample types other than nasopharyngeal (NP) swab specimens.
- The performance of the ProAdeno+ Assay has not been established in immunocompromised patients.
- The performance of this test has not been established for patients without symptoms of an acute respiratory infection.
- The performance of this test has not been established for screening of blood or blood products for the presence of Adenovirus.

Expected Values

The prevalence of adenovirus varies widely mainly depending on the patient population and the detection method utilized. Other factors that can impact the reported prevalence include the efficiency and timing of specimen collection and the handling and transport of the specimen. According to data from the National Respiratory and Enteric Viruses Surveillance System, from July 2009-November 2010, the percent positive rate for respiratory adenovirus in the United States ranged from ~0.5 to 3.0% with virus isolation and antigen detection (5) but there have been reports that reported prevalence may be higher using a molecular method. (6,7). The number and percentage of adenovirus positive cases determined by the ProAdeno+ Assay during this study (October 2009-August 2010), stratified by patient age group, are presented in the following table:

Age Group	Prospective		
	Total (N)	Total # Positive by ProAdeno+ Assay	Observed Prevalence
< 2 years	485	58	12.0%
2-5 years	184	18	9.8%
6-11 years	101	7	6.9%
12-18 years	67	3	4.5%
19-64 years	240	2	0.8%
> 65 years	89	0	0%
Total	1166	88	7.5%

Performance Characteristics

Clinical Performance

Performance characteristics of the ProAdeno+ Assay were established during a prospective study at 4 U.S. clinical laboratories from October 2009 – August 2010. Samples used for this study were nasopharyngeal (NP) swab specimens that were collected for routine respiratory viral testing by each site.

The reference method was rapid culture (shell vial) followed by direct fluorescent antibody (DFA) screening and identification.

A total of 1167 NP swab samples were tested with the ProAdeno+ Assay and by culture. One sample that initially gave unresolved results remained unresolved upon retesting with the ProAdeno+ Assay and is not included in the analysis below. The sample was culture negative.

Analysis of samples where ProAdeno+ Assay and culture results were in disagreement was performed using PCR primers obtained from literature (8,9) followed by sequencing.

Results from Prospective Study
Adenovirus Comparison Results

		Reference Method		Total	Comments
		Positive	Negative		
ProAdeno+ Assay	Positive	39	49 ^a	88	Sensitivity 97.5% (87.1% - 94.3%) 95% CI
	Negative	1 ^b	1077	1078	Specificity 95.6% (94.3% - 96.7%) 95% CI
	Total	40	1126	1166	

^a35 samples positive for HAdV by bi-directional sequence analysis, 14 samples negative for HAdV by bi-directional sequence analysis.

^b1 sample negative for HAdV by bi-directional sequence analysis

Reproducibility

The reproducibility of the ProAdeno+ Assay was evaluated at 3 laboratory sites. Reproducibility was assessed using a panel of 12 simulated clinical samples that included two adenovirus serotypes at medium and low positive levels (near the assay limit of detection, $\geq 95\%$ positive) and two high negative samples (high negative-1 at 0.1xLoD; high negative-2 at 0.001xLoD). Panels and controls were tested at each site by 2 operators for 5 days (12 samples and 3 controls X 2 operators X 5 days X 3 sites = 450). The overall percent agreement for the ProAdeno+ Assay was 99.2%.

	Panel Member ID	HAdV-3 high negative-2 ^a	HAdV-3 high negative-1	HAdV-3 low positive	HAdV-3 medium positive	HAdV-31 high negative-2 ^a	HAdV-31 high negative-1	HAdV-31 low positive	HAdV-31 medium positive	Extraction Control	Adenovirus DNA Control	Negative Control ^a	Total % Agreement
		Concentration	0.001X LoD	0.1X LoD	2X LoD	10X LoD	0.001X LoD	0.1X LoD	2X LoD	10X LoD	N/A	N/A	
Site 1 ^e	Agreement with Expected Result	15/15	5/15 ^b	15/15	15/15	15/15	10/15 ^b	15/15	15/15	10/10	10/10	10/10	120/120 ^d (100%)
	Mean C _T Value	36.9	40.4 ^c	35.8	33.9	37.2	38.6 ^c	33.8	29.3	31.8	32.5	36.7	
	% CV	2.5	2.8 ^c	1.2	1.0	2.2	6.2 ^c	1.7	1.4	0.5	0.8	1.0	
Site 2 ^f	Agreement with Expected Result	14/14	3/16 ^b	16/16	14/14	15/16	6/14 ^b	14/14	16/16	11/11	11/11	11/11	122/123 ^d (99.2%)
	Mean C _T Value	36.5	39.8 ^c	36.9	34.9	36.9	39.6 ^c	35.1	30.9	34.9	33.3	36.5	
	% CV	1.0	3.5 ^c	1.3	0.9	2.0	6.6 ^c	2.8	3.4	2.2	1.2	1.1	
Site 3 ^f	Agreement with Expected Result	15/15	2/15 ^b	15/15	15/15	13/15	3/15 ^b	15/15	15/15	10/10	10/10	10/10	118/120 ^d (98.3%)
	Mean C _T Value	36.5	40.1 ^c	36.7	34.6	36.3	39.2 ^c	34.9	30.8	32.3	32.5	36.5	
	% CV	1.1	5.3 ^c	2.6	1.1	1.3	6.1 ^c	1.3	1.1	1.0	1.5	1.1	
Total	Total Agreement with Expected Result	44/44	10/46 ^b	46/46	44/44	43/46	19/44 ^b	44/44	46/46	31/31	31/31	31/31	360/363 ^d (99.2%)
	95% CI	92.0-100%	N/A	92.3-100	92.0-100%	82.1-98.6%	N/A	92.0-100%	92.3-100	88.8-100%	88.8-100%	88.8-100%	98.0-99.9%
	Overall Mean C _T Value	36.6	40.2 ^c	36.5	34.4	36.8	39.0 ^c	34.6	30.3	33.1	32.8	36.6	
	Overall % CV	1.8	3.1 ^c	2.2	1.6	2.1	6.1 ^c	2.6	3.3	4.5	1.7	1.1	

^aMean C_T calculated from Universal Internal Control

^bNumber of positive samples

^cAverage and %CV based on number of positive samples

^dDoes not include high negative-1 samples as those are at a concentration that is not reproducible

^ePerformed study using the bioMérieux NucliSENS easyMAG

^fPerformed study using the Roche MagNA Pure

Analytical Sensitivity

The analytical sensitivity (limit of detection or LoD) of the ProAdeno+ Assay was determined using quantified (TCID₅₀/mL) cultures of 6 adenovirus serotypes, one from each species A-F. Each serotype was serially diluted in nasopharyngeal clinical matrix and extracted on the Roche MagNA Pure LC and the bioMérieux NucliSENS easyMAG and tested in replicates of 20 per serotype per extraction instrument. Extractor Equivalency was also determined during this study (see *Extractor Equivalency* section).

Analytical sensitivity (LoD) as defined as the lowest concentration at which ≥ 95% of all replicates tested positive, ranged from 10⁻² – 10⁰ TCID₅₀/mL.

Serotype	Species	LoD Concentration
Adenovirus 1	C	10 ⁰ TCID ₅₀ /mL
Adenovirus 3	B	10 ⁰ TCID ₅₀ /mL
Adenovirus 4	E	10 ⁻¹ TCID ₅₀ /mL
Adenovirus 19	D	10 ⁰ TCID ₅₀ /mL
Adenovirus 31	A	10 ⁻² TCID ₅₀ /mL
Adenovirus 41	F	10 ^{-1.5} TCID ₅₀ /mL

Extractor Equivalency

Equivalency of the bioMérieux NucliSENS easyMAG and the Roche MagNA Pure LC instruments for nucleic acid extraction was evaluated during the Analytical Sensitivity Study by determining limits of detection on both extraction instruments and by analysis of clinical trial data.

Limits of Detection were determined on six serotypes and were within 1 log across both instruments for each serotype. The clinical trial ProAdeno+ positive samples were analyzed with respect to Ct values and the mean Ct values were not statistically different (at p<0.05) between the two instruments. The ProAdeno+ Assay demonstrated comparable performance on the bioMérieux NucliSens easyMAG and Roche MagNA Pure LC instruments.

Reactivity

The reactivity of the ProAdeno+ Assay was evaluated against 54 strains of adenovirus. The strains included in the panel, in combination with strains tested in the Analytical Sensitivity Study, represent 51 of 53 known human adenovirus serotypes. Additional strains with genomic changes that do not result in serological changes were also included in the reactivity study. Each viral strain was extracted using the bioMérieux NucliSens easyMAG instrument and tested in triplicate. All strains included in the panel were reactive in all three replicates with the ProAdeno+ Assay.

Viral Strain	Concentration	Adenovirus (FAM)
HAdV-2	3 x 10 ⁻¹ TCID ₅₀ /mL	+
HAdV-3a2	3 x 10 ² TCID ₅₀ /mL	+
HAdV-4a	3 x 10 ¹ TCID ₅₀ /mL	+
HAdV-4p3	3 x 10 ¹ TCID ₅₀ /mL	+
HAdV-5	3 x 10 ⁰ TCID ₅₀ /mL	+
HAdV-6	3 x 10 ² TCID ₅₀ /mL	+
HAdV-7	3 x 10 ¹ TCID ₅₀ /mL	+
HAdV-7a	3 x 10 ⁻¹ TCID ₅₀ /mL	+
HAdV-7d4	3 x 10 ² TCID ₅₀ /mL	+
HAdV-7h1	3 x 10 ² TCID ₅₀ /mL	+
HAdV-8	3 x 10 ¹ TCID ₅₀ /mL	+
HAdV-9	3 x 10 ⁻¹ TCID ₅₀ /mL	+
HAdV-10	3 x 10 ² TCID ₅₀ /mL	+
HAdV-11	3 x 10 ⁻¹ TCID ₅₀ /mL	+
HAdV-12	3 x 10 ⁻¹ TCID ₅₀ /mL	+
HAdV-13	3 x 10 ⁻¹ TCID ₅₀ /mL	+
HAdV-14	3 x 10 ⁰ TCID ₅₀ /mL	+
HAdV-14-4	3 x 10 ² TCID ₅₀ /mL	+
HAdV-15	3 x 10 ⁻¹ TCID ₅₀ /mL	+
HAdV-16	3 x 10 ⁰ TCID ₅₀ /mL	+
HAdV-17	3 x 10 ⁻¹ TCID ₅₀ /mL	+
HAdV-18	3 x 10 ² TCID ₅₀ /mL	+
HAdV-20	3 x 10 ⁻² TCID ₅₀ /mL	+
HAdV-21	3 x 10 ⁰ TCID ₅₀ /mL	+
HAdV-21a	3 x 10 ¹ TCID ₅₀ /mL	+
HAdV-21c	3 x 10 ¹ TCID ₅₀ /mL	+
HAdV-22	3 x 10 ⁻¹ TCID ₅₀ /mL	+
HAdV-23	3 x 10 ⁻¹ TCID ₅₀ /mL	+
HAdV-24	3 x 10 ⁰ TCID ₅₀ /mL	+
HAdV-25	3 x 10 ¹ TCID ₅₀ /mL	+
HAdV-26	3 x 10 ⁻² TCID ₅₀ /mL	+
HAdV-27	3 x 10 ⁻² TCID ₅₀ /mL	+
HAdV-28	3 x 10 ⁰ TCID ₅₀ /mL	+
HAdV-29	3 x 10 ⁰ TCID ₅₀ /mL	+
HAdV-30	3 x 10 ⁻² TCID ₅₀ /mL	+
HAdV-32	3 x 10 ⁻² TCID ₅₀ /mL	+
HAdV-33	3 x 10 ⁰ TCID ₅₀ /mL	+
HAdV-34	3 x 10 ⁰ TCID ₅₀ /mL	+
HAdV-35	3 x 10 ⁰ TCID ₅₀ /mL	+
HAdV-36	3 x 10 ⁻² TCID ₅₀ /mL	+
HAdV-37	3 x 10 ⁻² TCID ₅₀ /mL	+
HAdV-38	3 x 10 ⁰ TCID ₅₀ /mL	+
HAdV-39	3 x 10 ⁻² TCID ₅₀ /mL	+
HAdV-40	3 x 10 ⁻¹ TCID ₅₀ /mL	+
HAdV-42	3 x 10 ⁻² TCID ₅₀ /mL	+
HAdV-43	3 x 10 ⁻² TCID ₅₀ /mL	+
HAdV-44	3 x 10 ⁻² TCID ₅₀ /mL	+
HAdV-45	3 x 10 ⁻¹ TCID ₅₀ /mL	+
HAdV-46	3 x 10 ⁻² TCID ₅₀ /mL	+
HAdV-47	3 x 10 ⁻³ TCID ₅₀ /mL	+
HAdV-48	3 x 10 ⁻¹ TCID ₅₀ /mL	+
HAdV-49	3 x 10 ⁰ TCID ₅₀ /mL	+
HAdV-50	3 x 10 ⁻¹ TCID ₅₀ /mL	+
HAdV-51	3 x 10 ⁰ TCID ₅₀ /mL	+

Analytical Specificity

The analytical specificity of the ProAdeno+ Assay was evaluated by testing a panel of 56 cultures consisting of 29 viral, 26 bacterial, and 1 yeast strain representing common respiratory pathogens or flora commonly present in nasopharynx. Bacteria and yeast were tested at concentrations of 10^6 to 10^7 CFU/mL or 10^3 to 10^4 TCID₅₀/mL. Viruses were tested at concentrations of 10^3 to 10^6 TCID₅₀/mL or 10^8 copies/mL. Samples were extracted using the bioMérieux NucliSENS easyMAG instrument and tested in triplicate. Analytical specificity of the ProAdeno+ Assay was 100%.

Strains	Concentration	Adenovirus (FAM)
Adenovirus 3	10^1 TCID ₅₀ /mL	+
Adenovirus 4	10^0 TCID ₅₀ /mL	+
Coronavirus 229E	10^6 TCID ₅₀ /mL	-
Coxsackie B4	10^4 TCID ₅₀ /mL	-
Coxsackie B5/10/2006	10^5 TCID ₅₀ /mL	-
Cytomegalovirus	10^4 TCID ₅₀ /mL	-
Echovirus 2	10^6 TCID ₅₀ /mL	-
Echovirus 3	10^5 TCID ₅₀ /mL	-
Echovirus 6	10^5 TCID ₅₀ /mL	-
Echovirus 11	10^6 TCID ₅₀ /mL	-
Enterovirus 68	10^3 TCID ₅₀ /mL	-
Enterovirus 70	10^3 TCID ₅₀ /mL	-
Epstein Barr Virus	10^8 copies/mL	-
HSV Type 1 MacIntyre Strain	10^5 TCID ₅₀ /mL	-
HSV Type 2 G strain	10^5 TCID ₅₀ /mL	-
Human Metapneumovirus A2	10^4 TCID ₅₀ /mL	-
Human Rhinovirus 1A	10^3 TCID ₅₀ /mL	-
Human Rhinovirus	10^3 TCID ₅₀ /mL	-
Influenza A/Port Chalmers	10^4 TCID ₅₀ /mL	-
Influenza A/2009 H1N1	10^5 TCID ₅₀ /mL	-
Influenza B/Wisconsin	10^4 TCID ₅₀ /mL	-
Measles/7/2000	10^4 TCID ₅₀ /mL	-
Mumps Virus	10^4 TCID ₅₀ /mL	-
Parainfluenza Type 1	10^4 TCID ₅₀ /mL	-
Parainfluenza Type 2	10^6 TCID ₅₀ /mL	-
Parainfluenza Type 3	10^6 TCID ₅₀ /mL	-
Parainfluenza Type 4	10^4 TCID ₅₀ /mL	-
Poliovirus 1	10^6 TCID ₅₀ /mL	-
RSV A Strain Long	10^4 TCID ₅₀ /mL	-
RSV B Strain Wash	10^4 TCID ₅₀ /mL	-
Varicella Zoster Virus	10^4 TCID ₅₀ /mL	-
<i>Bordetella pertussis</i>	10^7 CFU/mL	-
<i>Bordetella bronchiseptica</i>	10^7 CFU/mL	-
<i>Chlamydia pneumoniae</i>	10^3 TCID ₅₀ /mL	-
<i>Chlamydia trachomatis</i>	10^4 TCID ₅₀ /mL	-
<i>Legionella micdadei</i>	10^6 CFU/mL	-
<i>Legionella pneumophila</i>	10^6 CFU/mL	-
<i>Mycobacterium intracellulare</i>	10^7 CFU/mL	-
<i>Mycobacterium tuberculosis</i>	10^6 CFU/mL	-
<i>Mycoplasma pneumoniae</i>	10^6 CFU/mL	-
<i>Haemophilus influenza</i>	10^7 CFU/mL	-
<i>Pseudomonas aeruginosa</i>	10^7 CFU/mL	-

Strains	Concentration	Adenovirus (FAM)
<i>Proteus vulgaris</i>	10 ⁷ CFU/mL	-
<i>Proteus mirabilis</i>	10 ⁷ CFU/mL	-
<i>Neisseria gonorrhoeae</i>	10 ⁷ CFU/mL	-
<i>Neisseria meningitidis</i>	10 ⁷ CFU/mL	-
<i>Neisseria mucosa</i>	10 ⁷ CFU/mL	-
<i>Klebsiella pneumoniae</i>	10 ⁷ CFU/mL	-
<i>Escherichia coli</i>	10 ⁷ CFU/mL	-
<i>Moraxella catarrhalis</i>	10 ⁷ CFU/mL	-
<i>Corynebacterium diphtheriae</i>	10 ⁶ CFU/mL	-
<i>Lactobacillus plantarum</i>	10 ⁶ CFU/mL	-
<i>Streptococcus pneumoniae</i>	10 ⁷ CFU/mL	-
<i>Streptococcus pyogenes</i>	10 ⁷ CFU/mL	-
<i>Streptococcus salivarius</i>	10 ⁷ CFU/mL	-
<i>Staphylococcus epidermidis</i>	10 ⁷ CFU/mL	-
<i>Staphylococcus aureus</i>	10 ⁷ CFU/mL	-
<i>Candida albicans</i>	10 ⁷ CFU/mL	-

Interference

Mucin, whole blood and a number of other potentially interfering exogenous substances (medications and over the counter (OTC) products) that may be present in the nasopharynx were evaluated for interference with the ProAdeno+ Assay. Human adenovirus 1 was spiked into negative NP pools at 2X LoD. Clinically relevant amounts of the potential interfering substances were added to spiked samples. A Universal Internal Control (UIC) was also added to each sample and extracted on the bioMérieux NucliSENS easyMAG. The ProAdeno+ Assay was performed in triplicate reactions for each sample. The following table shows the potential interfering substances used for this study. The substances consisted of nasal sprays (liquid and powder), ingestible pills and lozenges, injectables, and endogenous substances:

Substance Name	Active Ingredient	Concentration Tested
Mucin (Bovine Submaxillary Gland, type I-S)	Purified mucin protein	60µg/mL
Blood (human), EDTA anticoagulated	N/A	2% (volume/volume)
Neo-Syneprine®	Phenylephrine HCl	15% (volume/volume)
Walgreens Original Anefrin Nasal Spray	Oxymetazoline Hydrochloride	15% (volume/volume)
Zicam Homeopathic Non-Drowsy Allergy Relief No Drip Liquid Nasal gel	Luffa Operculata, Galphimia Glauca, Histaminum Hydrochloricum, Sulphur	5% (volume/volume)
Walgreens Saline Nasal Spray	Sodium chloride with preservatives	15% (volume/volume) of dose
Beconase AQ®	Beclomethasone dipropionate	5% volume/volume
Walgreens Sore Throat Lozenges, Cherry	Oral anesthetic/analgesic	0.62mg/mL; 1/20 drop, crushed; active ingredients: 1.0mg/mL benzocaine, 1.7mg/mL menthol
Relenza®	Zanamivir	5mg/mL
Tobramycin	Tobramycin	4.0µg/mL
Bactroban Nasal Ointment	Mupirocin	10mg/mL
TamiFlu	Oseltamivir	7.5mg/mL

The ProAdeno+ assay was not affected by the presence of a panel of endogenous and exogenous potential PCR Inhibitors.

Carry-over/Contamination

To evaluate the degree of carry-over/cross-contamination that occurs with the use of the ProAdeno+ Assay in association with nucleic acid extraction on the Roche MagNA Pure LC and the bioMérieux NucliSens easyMAG instruments and PCR on the Cepheid SmartCycler II thermocycler, an internal Carry-Over study was performed. Simulated adenovirus (Adeno) high positive samples were run in series alternating with Adeno negative samples. The Adeno high positive samples targeted a low Ct value that represented the higher end of the “clinical range” of the ProAdeno+ Assay as seen in development and clinical testing. The results of this study demonstrate no evidence of carryover/cross-contamination over a 5 day course of processing high positive samples alongside negative samples using the ProAdeno+ Assay.

Disposal

Dispose of hazardous or biologically contaminated materials according to the practices of your institution.

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