510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:

k121942

B. Purpose for Submission:

This is a 510(k) application for a new qualitative Real-Time Polymerase Chain Reaction (PCR) assay used with the Cepheid SmartCycler II instrument for the *in vitro* qualitative detection of adenovirus DNA in nasopharyngeal swabs (NPS) and nasopharyngeal wash/aspirates (NPA) from symptomatic human patients.

C. Measurand:

Target DNA sequences for a region of the hexon gene of Adenovirus species

D. Type of Test:

Real-Time Polymerase Chain Reaction assay for the qualitative detection of adenovirus DNA in NPS and NPA samples. A bi-functional fluorescent probe-primer is used together with a reverse primer to amplify a specific target for the analyte and the RNA internal control. Amplification and detection is performed on the Cepheid SmartCycler II with Dx software version 1.7b or 3.0.

E. Applicant:

Argene SA

F. Proprietary and Established Names:

Adenovirus R-gene® US

G. Regulatory Information:

1. Regulation section:

   21 CFR 866.3980 Respiratory viral panel multiplex nucleic acid assay

2. Classification:

   Class II

3. Product code:

   OCC

4. Panel:

   Microbiology (83)

H. Intended Use:

1. Intended use(s):

   Adenovirus R-gene® US assay is a Real Time PCR *in vitro* diagnostic test for the rapid and qualitative detection of Adenovirus viral DNA isolated and purified from nasopharyngeal swab or nasopharyngeal wash/aspirate specimens obtained from individuals exhibiting signs and symptoms of acute respiratory infection. The intended use for this test is to aid in the diagnosis of respiratory Adenovirus infections in humans in conjunction with other clinical and laboratory findings. The test detects, but does not differentiate, Adenovirus species (A, B, C, D, E, F and G). Negative results do not preclude Adenovirus infection and should not be used as the sole basis for treatment or other patient management decisions.

2. Indication(s) for use:

   Same as Intended Use

3. Special conditions for use statement(s):

   For prescription use only

4. Special instrument requirements:

   To be used with the Cepheid SmartCycler II utilizing Dx software version 1.7b or 3.0

I. Device Description:

The test is a Taqman-based real-time PCR amplification and detection assay for the detection of human adenovirus DNA in nasopharyngeal swab and nasopharyngeal aspirate/wash specimens. The assay uses the bioMérieux NucliSENS easyMAG nucleic acid extraction system to extract adenovirus DNA from swab or wash specimens obtained from patients with signs and symptoms of acute respiratory infection. Extracted adenovirus DNA is then amplified using the Adenovirus R-gene US assay components. Amplification is performed on the Cepheid SmartCycler II using Dx software versions 1.7b or 3.0.

The Adenovirus R-gene US assay contains the reaction mix (R^x10), internal control (IC2), molecular-grade water (W0), positive control (PC10), and Package Insert.

Adenovirus R-gene US reaction mix contents and probe info:

Reagents	Description	Composition	Quantity/tube	# of Reactions	Form
R^x10	Adenovirus and IC2 amplification pre-mix	Adenovirus and IC2 primers and probes, Tris, $MgCl_2$, dNTP's, Taq Polymerase, Rox Carboxy x Rhodamine, Ammonium Sulfide,	450 µL	90	Liquid
IC2	Internal Control 2	Native Phix bacteriophage, Phast media, Glycerol	1 mL	100	Liquid
WO	Water for extraction (molecular grade)	Water	1.8 mL	18	Liquid
PC10	Adenovirus Positive Control	Composite plasmid, Tris buffer, yeast tRNA	300 µL	30	Liquid

Materials required but not supplied: Polyester, rayon or nylon tipped nasopharyngeal swabs, Sterile suction catheter (#8) for washes/aspirates specimen, 1.5 mL polypropylene microcentrifuge tubes, Sterile filter or positive displacement micropipettor tips, EasyMAG™ System Disposables (Sample Vessels and Tips), Biohit Pipette Tips for use with easyMAG™ System, Greiner Break Four uncoated plates for use with easyMAG™ System, Cepheid 25 µL PCR reaction tubes, single use latex or similar gloves, bioMérieux NucliSENS® easyMAG™ reagents (Buffer 1 Cat.#280130, Buffer 2 Cat.#280131, Buffer 3 Cat.#280132, Magnetic Silica Cat. #280133, Lysis Buffer (bottles) Cat.# 280134, Universal Transport Medium from DHI or MicroTest™ M4RT Transport from Remel, Proteinase K Solution, 600 mAU/mL (Novagen – Merck – 71049-3/71049-4), Molecular grade water, Sterile physiologic buffer, -18°C/-22°C Freezer, bioMérieux NucliSENS® easyMAG™ System with Software version 2.0, Cepheid SmartCycler II instrument with Dx Software version 1.7b or 3.0, Micropipettors (range between 1-10 µL, 10-200µL and 100-1000µL), Mini-centrifuge with adapter for Cepheid Reaction Tubes, Cepheid cooling block, U.V. light, Workstation or Plexiglas screen for samples and premix distribution.

Assay Procedure:

1- Collect swab specimens from symptomatic patients using a polyester, rayon, nylon tipped swab or wash/aspirate specimens using a sterile suction catheter.
2- Add Internal Control (IC2) to every sample to monitor for the presence of inhibitors or lysis failure.
3- Perform isolation and purification of nucleic acids using the NucliSENS® easyMAG® System (bioMérieux) and the automated Magnetic Extraction Reagents (bioMérieux).
4- Perform real time amplification of extracted samples using the Adenovirus R-gene US kit on the Cepheid SmartCycler II instrument.
5- Interpretation.

Interpretation of Results:

There are five possible results reported by the SmartCycler II summarized in the table and below:

Assay Result	IC Result	Warning/Error Code	Adenovirus Result	Interpretation
Negative	Pass		Negative	Adenovirus nucleic acid not detected
Positive	NA*		Positive	Adenovirus nucleic acid detected
Unresolved	Fail		ND	Unresolved; PCR inhibition or reagent failure. Repeat testing with purified nucleic acid or obtain new sample.
ND	ND	3079	ND	Not Determined; error code 3079
Invalid		4098	ND	Not Determined; error code 4098

Positive: Human adenovirus DNA was detected in the sample
Negative: Human adenovirus DNA was not detected in the sample
Unresolved: PCR inhibition or reagent failure. Repeat testing from the purified nucleic acid or collect and test a new sample.
Invalid: Not determined; error code 4098
ND: (Not Determined) if the specimen result is unavailable because the run is in progress or an error or warning occurred during the run.

* Detection of Internal Control in the Cy3 channel is not required for a positive result. High viral load can lead to reduced or absent Internal Control signal.

Error Code 3079 can be observed with positives (Adenovirus Positive Control, Adenovirus positive swab/wash samples) when the fluorescence 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 ≥ 5 is reported in the Adenovirus Ct columns, the sample results can be recorded as positive for the specific analyte. There were no 3079 type errors reported among the 1576 prospective clinical study specimens analyzed.

Error Code 4098: An invalid assay run will display Error Code 4098. One of the controls has failed and repeat testing should be done starting from the purified nucleic acid and using a new aliquot of the Positive Control. If repeated 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. There were no 4098 type errors reported among the 1576 prospective clinical study specimens analyzed.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   Prodesse® ProAdeno™+

2. Predicate 510(k) number(s):

   k102952

3. Comparison with predicate:

Features		
Item	**Adenovirus R-gene US**	**Prodesse ProAdeno™+ Assay**
Intended Use	Adenovirus R-gene US is a Real Time PCR in vitro diagnostic test for the rapid and qualitative detection of Adenovirus viral DNA isolated and purified from nasopharyngeal specimens (swab or wash/aspirate) obtained from individuals exhibiting signs and symptoms of acute respiratory infection. The intended use for this test is to aid in the diagnosis of Adenovirus infections in humans. Negative results do not preclude Adenovirus infection and should not be used as the sole basis for treatment or other management decisions.	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 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.
510(k)	k121942	k102952
Regulation	21 CFR 866.3980	21 CFR 866.3980
Product Code	OCC	OCC

Features		
Item	**Adenovirus R-gene US**	**Prodesse ProAdeno™+ Assay**
Assay Targets	DNA from Human Adenovirus	DNA from Human Adenovirus
Sample Types	Nasopharyngeal swabs and Nasal aspirates/washes	Nasopharyngeal swabs
Assay Type	Real-Time PCR	Real-Time PCR
Assay Results	Qualitative	Qualitative
Detection	Instrument-based fluorogenic target-specific hybridization	Instrument-based fluorogenic target-specific hybridization
Gene Target	Hexon gene	Hexon gene
Collection and Transport Media	Universal Transport Medium (DHI), MicroTest™ M4RT Transport (Remel)	M4 and M5 Viral Transport Medium (Remel), UVT (Becton Dickinson), UTM (Copan)
Assay Instrumentation	Cepheid SmartCycler® II with Dx Software	Cepheid SmartCycler® II System
Nucleic Acid Isolation	NucliSENS® easyMAG™ (bioMérieux)	MagnaPure LC System (Roche) NucliSENS® easyMAG™ (bioMérieux)
Controls Included	Positive Control plasmid DNA Neg control (mol. grade water) Internal control (IC2) phage particle	Adenovirus positive DNA transcript control and Internal DNA/RNA control
Results	Positive Negative Unresolved	Positive Negative Unresolved

K. Standard/Guidance Document Referenced (if applicable):

None

L. Test Principle:

The real-time PCR process simultaneously amplifies and detects nucleic acid targets in a single closed-tube reaction. The Adenovirus R-gene US assay enables simultaneous detection of AdV and Internal Control DNA. The whole process is based on two steps: nucleic acid isolation and Real Time PCR amplification/detection. Human respiratory specimens (nasopharyngeal swabs, washes/aspirates) from symptomatic patients are processed initially to isolate and purify viral nucleic acid from the cellular specimen matrix. Each purified nucleic

acid sample is added to the Rx10 amplification premix (Ready to use, Taq polymerase included). The Rx10 amplification premix contains oligonucleotide primers complementary to a fragment of the Hexon gene region coding for the hexagonal capsomers for AdV and target-specific oligonucleotide probes dual-labeled with a reporter dye and a quencher dye. As the amplification proceeds, the probe anneals specifically to a region of the template between the forward and reverse primers. As primer extension and amplification occurs, the exonuclease activity of the Taq polymerase cleaves the probe separating the reporter dye away from the quencher. This generates an increase in fluorescent signal upon excitation from a LED light source of appropriate wavelength. With each cycle, additional reporter dye molecules are cleaved from their respective probes, yielding increased fluorescence signal. The amount of fluorescence at any given cycle is dependent on the amount of PCR product (amplicons) present at that time. Fluorescent intensity is monitored at each PCR cycle by fluorescent detection modules within the real-time instrument.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

The study evaluated the device's inter-laboratory, inter-assay, and intra-assay reproducibility for high negative (0.01x LoD), low positive (2x LoD), and moderately positive (10x LoD) specimens. The precision (repeatability and reproducibility) of the Adenovirus R-gene US assay was evaluated using human nasal matrix spiked with 3 viral inputs (moderate positive sample, low positive sample, high negative sample). A within-laboratory (repeatability) precision study was performed at an internal site (5 days, 2 operators, 1 instrument, 1 lot of reagent) and a between laboratory (reproducibility) precision study was performed at 3 external sites in the U.S. (5 days, 2 operators, 1 instrument at each site, 1 lot of reagent. Test panels consisted of 14 members (12 Adenovirus positive specimens plus two negative controls). Samples were extracted each day by both operators (5 days x 2 operators = 10 tests per sample) and tested in triplicate. For the moderate positive samples, 5 samples were present in the panel for a total of 150 tests (5 samples x 10 tests per sample x 3 replicates = 150) For low positive samples, 4 samples were present in the panel for a total of 120 tests (4 samples x 10 tests x 3 replicates). For high negative samples, there were 3 samples per panel for a total of 90 tests (3 samples x 10 tests x 3 replicates). Combined results for all sites and results stratified by site are presented in the tables below.

Reproducibility – Within Laboratory

Sample	% Agreement	95% CI	Mean Ct	SD	%CV
Moderate Positive	100% (150/150)	97.6-100%	28.73	0.47	1.6
Low Positive	95% (114/120)	89.4-98.1%	32.90	3.00	9.1
High Negative	100% (90/90)	96-100%	24.19*	0.17	0.70
Total Agreement	98.3% (354/360)	96.4-99.4%			

* Mean Ct calculated from Internal Control

Reproducibility – Between Laboratories

Sample	% Agreement			Total % Agreement and 95% CI	Mean Ct	SD	%CV
	Site 1	Site 2	Site 3				
Moderate Positive	100% (150/150)	100% (150/150)	100% (150/150)	100% (450/450) 99.2-100%	28.78	1.35	4.7
Low Positive	99.2% (119/120)	99.2% (119/120)	97.5% (117/120)	98.6% (355/360) 96.8-99.6%	32.0	2.17	6.8
High Negative	100% (90/90)	95.6% (86/90)	100% (90/90)	98.5% (266/270) 96.2-99.6%	24.12*	0.36	1.5
Total Agreement	99.7% (359/360)	98.6% (355/360)	99.2% (357/360)	99.2% (1071/1080) 98.4-99.6%			

* Mean Ct calculated from Internal Control

b. Linearity/assay reportable range:

Not applicable

c. Traceability, Stability, Expected values (controls, calibrators, or methods):

Adenovirus R-gene US Stability and Positive Control Inter-Lot Reproducibility

Argene assessed the reproducibility of three different lots of Positive Control (PC) by testing each lot of controls with one lot of reaction mix. The study yielded a total of seven replicates per lot of PC mix. Reproducibility was determined simultaneously with freeze/thaw stability for the PC mix. The table below shows consistent reproducibility between lots and also stability for up to 29 freeze/thaw cycles.

PC Freeze/Thaw Stability and Reproducibility

	PC10 R3424			PC10 R3642			PC10 R4070		
Freeze/Thaw Cycles	Ct	Mean Ct	SD	Ct	Mean Ct	SD	Ct	Mean Ct	SD
1	28.6	28.43	0.24	28.3	28.43	0.14	28.3	28.26	0.22
5	28.3			28.3			28.5		
10	28.3			28.4			28.3		
15	28.9			28.6			28.3		
20	28.4			28.3			28.4		
25	28.2			28.6			28.2		
30	28.3			28.5			27.8		
PC10	28.3	n/a	n/a	28.3	n/a	n/a	28.3	n/a	n/a
IC2W0	neg	n/a	n/a	neg	n/a	n/a	neg	n/a	n/a

The table below shows that reaction mix (R^x10) and Internal Control are stable for up to 10 freeze/thaw cycles.

Reaction Mix Freeze/Thaw Stability

		Reference 1 Thaw		11 Freeze/Thaw cycles		Delta CT after 11 cycles	
	Dilution	**AdV 530nm**	**IC2 560nm**	**AdV 530nm**	**IC2 560nm**	**AdV 530nm**	**IC2 560nm**
AdV12	10^{-4}	26.3	24.4	26.1	24.5	-0.2	0.1
AdV3	10^{-7}	29.2	24.3	29.1	24.4	-0.1	0.1
AdV11	10^{-4}	26.0	24.8	25.5	24.2	-0.5	-0.6
AdV5	10^{-5}	30.0	24.4	30.5	24.3	0.5	-0.1
AdV8	10^{-5}	27.2	24.3	27.0	24.1	-0.2	-0.2
AdV4	10^{-6}	31.7	24.1	32.9	24.3	1.2	0.2
AdV40	10^{-5}	29.9	24.1	29.7	24.1	-0.2	0.0
IC2W0	-	neg	24.6	neg	24.6		
PC10	-	28.0	neg	28.0	neg		

Adenovirus R-gene US Specimen Stability

Samples must be stored refrigerated at +2°C/+8°C for up to 24 hours prior to processing or stored at -18°C/-22°C for 4 days or -78°C/-82°C for longer period.

Adenovirus R-gene US Quality Control Ranges

Quality control ranges have been established as indicated in the table below. If the controls are not within these parameters, patient results should be considered invalid and the assay repeated. Each laboratory should establish its own Quality Control ranges and frequency of QC testing based on applicable local laws, regulations, and standard good laboratory practice.

Adenovirus R-gene US Expected Control Results

	IC2[a] + W0		IC2 [a] + sample		PC10 [b]	
	Detected (+)	**Not Detected (-)**	**Detected (+)**	**Not Detected (-)**	**Detected (+)**	**Not Detected (-)**
Cy3	Pass	Fail	Pass or error 3079	Fail or error 4098 or n/a*	n/a	n/a
FAM	Invalid; error 4098	Valid	Positive or error 3079	Negative or error 4098 or Unresolved	Valid or error 3079	Invalid; error 4098

[a] Typical Ct values for the Internal Control (IC2) range between 13.0 and 45.0 cycles
[b] Typical Ct values for the Positive Control range between 26.0 and 33.0 cycles

Error Code 3079 can be observed with positives (Adenovirus Positive Control, Adenovirus positive NP swab samples) when the fluorescence 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 ≥ 5 is reported in the Adenovirus Ct columns, the sample results can be recorded as positive for the specific analyte.

* Detection of Internal Control is not required in the Cy3 channel for a positive Adenovirus result. High viral load can lead to reduced or absent Internal Control signal. Detection of IC2 is required for a valid negative Adenovirus result.

d. Detection limit

Prior to the LoD determination, a sequence homology study was performed to classify the 60 known Human Adenoviruses into 21 different groups. Homology groups were established according to the number and the position of base mismatches with the best primer (forward and reverse) and the best probe which comprise the Adenovirus R-gene US amplification/detection premix.
One Human Adenovirus (AdV) type was chosen from each group of homologies to represent each of the 21 different sequences. The LoD study was set up so that each virus was tested using 4, 5, or 6 different dilutions near the estimated LoD and 15 replicates per dilution. Each replicate was taken through the DNA extraction step to amplification and detection. A total of 22 LoD's were determined comprising the 21 selected Human Adenoviruses, plus Adenovirus serotype 2 (a member of adenovirus species C). The homology groups and Adenovirus subtypes/species are displayed in the table below:

Homology Group	HAdV Type	Species
1	AdV 12	A
2	AdV 18	
3	AdV 31	
4	AdV 3	B1
5	AdV 7	
6	AdV 11	B2
7	AdV 1	C
8	AdV 2,5	
9	AdV 6	
10	AdV 39	D
11	AdV 15	
12	AdV 9	
13	AdV 8	
14	AdV 17	
15	AdV 25	
16	AdV 28	
17	AdV 42	
18	AdV 4	E
19	AdV 40	F
20	AdV 41	
21	AdV 52	G

The Limit of Detection (LoD) was determined for the Adenovirus R-gene US assay by limiting dilution analysis of representative Adenoviruses from each of the 21 homology groups listed above as well as Adenovirus serotype 2. Fifteen replicates of viral dilutions were tested for Adenovirus positivity over a wide range of dilutions that surround the empirical LoD. The LoD was established by selecting the highest dilution of virus which could be detected at least 95% of the time.

Adenovirus R-gene US Limit of Detection Summary

Homology Group	HAdV Species	HAdV Type	LoD 95% $TCID_{50}$/ml
1	A	HAdV 12	.000416
2		HAdV 18	18
3		HAdV 31	10
4	B1	HAdV 3	0.00316
5		HAdV 7	0.0445
6	B2	HAdV 11	889
7	C	HAdV 1	0.0209
8-1		HAdV 2	0.00625
8-2		HAdV 5	0.044
9		HAdV 6	0.00512
10	D	HAdV 39	0.0158
11		HAdV 15	0.316
12		HAdV 9	0.1
13		HAdV 8	0.000812
14		HAdV 17	158
15		HAdV 25	0.05
16		HAdV 28	28.1
17		HAdV 42	0.05
18	E	HAdV 4	0.183
19	F	HAdV 40	0.0104
20		HAdV 41	0.158
21	G	HAdV 52	na*

* HAdV 52 was not available via organism collections and therefore the LoD was determined using plasmid DNA. The LoD was calculated to be 5000 copies/ml by qPCR.

e. Analytical reactivity

Cross-Reactivity

A panel of 61 potentially cross-reacting microorganisms (34 viral samples and 27 bacterial samples), representing respiratory pathogens or flora commonly present in the nasopharynx, was evaluated for cross-reactivity. Each potential cross-reactant was individually spiked into an Adenovirus negative nasal wash/aspirate at a challenging concentration. Adenovirus positive samples at 100 and 3 times the limit of detection (100x LoD and 2x LoD) were also tested. The Adenovirus R-gene US assay did not cross-react with any of the cross-reactants tested.

Adenovirus R-gene US Cross-Reactivity Results

		Adenovirus R-gene US	
Virus	**Concentration tested**	**530nm – ADV Ct (cycles)**	**560nm – IC2 Ct (cycles)**
Adenovirus 3	100x LoD	27.9	26.6
	2x LoD	34.1	25.4
Cytomegalovirus	10^4 $TCID_{50}$/mL	negative	26.0
Epstein Barr Virus	10^7 cp/mL	negative	25.5
BK Virus	10^4 $TCID_{50}$/mL	negative	26.6
Herpes Simplex Virus 1	10^4 $TCID_{50}$/mL	negative	26.3
Herpes Simplex Virus 2	10^4 $TCID_{50}$/mL	negative	26.5
Varicella Zoster Virus	10^3 $TCID_{50}$/mL	negative	26.5
Human Herpes Virus 6	$>10^3$ $TCID_{50}$/mL	negative	25.9
Human Herpes Virus 7	10^4 cp/mL	negative	25.9
Human Herpes Virus 8	10^4 cp/mL	negative	25.6
Influenza A Virus	10^5 $TCID_{50}$/mL	negative	26.3
Influenza B Virus	10^4 $TCID_{50}$/mL	negative	25.9
Respiratory Syncytial Virus A	10^4 $TCID_{50}$/mL	negative	25.8
Respiratory Syncytial Virus B	10^4 $TCID_{50}$/mL	negative	25.3
Human Metapneumovirus A	10^4 $TCID_{50}$/mL	negative	25.3
Human Metapneumovirus B	10^4 $TCID_{50}$/mL	negative	26.1
Human Bocavirus 1	10^6 cp/mL	negative	26.2
Parainfluenza Virus 1	10^4 $TCID_{50}$/mL	negative	25.9
Parainfluenza Virus 2	10^5 $TCID_{50}$/mL	negative	26.2
Parainfluenza Virus 3	10^6 $TCID_{50}$/mL	negative	26.1
Parainfluenza Virus 4	10^4 $TCID_{50}$/mL	negative	26.3
Coronavirus NL63	10^4 $TCID_{50}$/mL	negative	26.1
Rhinovirus 14	10^3 $TCID_{50}$/mL	negative	26.3
Rhinovirus 87	10^3 $TCID_{50}$/mL	negative	26.4
Rhinovirus 1B	10^3 $TCID_{50}$/mL	negative	26.2
Echovirus 25	10^5 $TCID_{50}$/mL	negative	26.8
Coxackie B2	10^5 $TCID_{50}$/mL	negative	26.2
Coxsackie A9	10^5 $TCID_{50}$/mL	negative	25.6
Echovirus 9	10^5 $TCID_{50}$/mL	negative	26.4
Poliovirus S3	10^4 $TCID_{50}$/mL	negative	25.6
Echovirus 30	10^4 $TCID_{50}$/mL	negative	25.9
Parechovirus 1	10^4 $TCID_{50}$/mL	negative	25.3
Parechovirus 2	10^4 $TCID_{50}$/mL	negative	26.1
Measles	10^4 pfu/mL	negative	25.9
Mumps	10^4 pfu/mL	negative	26.6

		Adenovirus R-gene US	
Bacteria	**Concentration tested**	**530nm – ADV Ct (cycles)**	**560nm – IC2 Ct (cycles)**
Bordetella pertussis	10^6 cfu/mL	negative	26.0
Bordetella parapertussis	10^6 cfu/mL	negative	26.2
Mycoplasma pneumoniae	10^6 ccu/mL	negative	26.3
Chlamydophila pneumoniae	10^3 $TCID_{50}$/mL	negative	26.2
Legionella pneumophila	10^6 cfu/mL	negative	26.4
Bordetella bronchiseptica	10^6 cfu/mL	negative	26.3
Escherichia coli	10^6 cfu/mL	negative	26.2
Staphylococcus epidermidis	10^6 cfu/mL	negative	26.3
Klebsiella pneumoniae	10^6 cfu/mL	negative	26.6
Haemophilus influenzae	10^6 cfu/mL	negative	26.4
Serratia marcescens	10^6 cfu/mL	negative	26.4
Proteus mirabilis	10^6 cfu/mL	negative	26.8
Pseudomonas aeruginosa	10^6 cfu/mL	negative	26.5
Stenotrophomonas maltophila	10^6 cfu/mL	negative	26.1
Citrobacter freundii	10^6 cfu/mL	negative	26.4
Citrobacter koseri	10^6 cfu/mL	negative	26.0
Enterobacter cloacae	10^6 cfu/mL	negative	26.1
Acinobacter baumannii	10^6 cfu/mL	negative	26.5
Streptococcus agalactiae	10^6 cfu/mL	negative	26.5
Staphylococcus aureus	10^6 cfu/mL	negative	25.5
Klebsiella oxytoca	10^6 cfu/mL	negative	25.8
Enterobacter kobei	10^6 cfu/mL	negative	25.5
Morganella morganii	10^6 cfu/mL	negative	26.0
Streptococcus constellatus	10^6 cfu/mL	negative	25.6
Raoultella ornithinolytica	10^6 cfu/mL	negative	25.8
Haemophilus parainfluenza	10^6 cfu/mL	negative	25.6
Branhamella catarrhalis	10^6 cfu/mL	negative	25.8

Reactivity

No additional strains beyond the 22 tested for LoD were tested for reactivity in analytical studies.

f. Interference studies:

Interfering Substances

Potentially interfering or inhibitory substances that may be present in nasopharyngeal swabs or interfere with the PCR reaction were evaluated for the viral strains indicated below. To determine the potential interference of endogenous and exogenous chemical PCR inhibitors on Adenovirus detection, clinically relevant amounts of interfering substances were added to nasopharyngeal samples spiked with Adenovirus at 2x LoD and 10x LoD. A reference sample with no interfering substance was included at both 10x and 2x LoD.

The following table shows the interfering substances used for this study. Ten percent of the recommended dose was added to the sample unless otherwise stated in the table. The substances consisted of liquid nasal sprays, ingestible pills and lozenges, and endogenous substances.

Interfering Substances

Substance Name	Active Ingredient	Concentration Tested	Justification
Mucin (Bovine submaxillarygland type I-S)	Purified mucin protein	60 µg/mL	1000x maximum level present in serum
Blood (human) EDTA anticoagulated	n/a	2% (v/v)	Same concentration tested for similar IVD's
Tamiflu® 45 mg	Oseltamivir	7.5 mg/mL	10% of total recommended dose
Afrin nasal spray	Oxymetazoline HCl	7.45% (v/v)	10% of total recommended dose
Tobrex 0.3%	Tobramycin	9% (v/v) = 0.27 mg/mL	10% of total recommended dose
Synagis 50 mg	Palivizumab	81.8% (v/v) = 18 mg	10% of total recommended dose
Relenza	Zanamivir	45% (v/v) = 1 mg	10% of total recommended dose

Detailed chemical interference results are presented in the following table. Note that the study was performed in two separate runs with half of the potential interferents tested in run 1 and the other half in run 2. "Reference run 1" is the Adenovirus only control for the first run. "Reference run 2" is the Adenovirus only control for the second run. The two Adenovirus only reference samples were tested at concentrations of 10x LoD and 2x LoD. The data in the column indicating delta Ct values were calculated by taking the difference in average Ct cycles between the sample containing the potential chemical interferent and the "reference run" associated with that test.

Adenovirus R-gene US Interfering Substances

Substance Name	AdV 3 Concentration	AdV 3 Ct (avg cycles)	AdV Delta Ct (cycles)	Internal Control 2 Ct (cycles)	Internal Control 2 Delta Ct (cycles)
Reference run 1	**10x LoD**	**30.77**	**n/a**	**26.17**	**n/a**
	2x LoD	**33.90**	**n/a**	**25.97**	**n/a**
Mucin	10x LoD	31.77	1.00	26.27	0.10
	2x LoD	33.65	-0.25	25.97	0.00
Blood	10x LoD	32.00	1.23	26.23	0.07
	2x LoD	33.63	-0.27	25.87	-0.10
Tamiflu®	10x LoD	33.33	2.57	26.90	0.73
	2x LoD	36.90	3.00	27.03	1.07
Afrin Nasal Spray	10x LoD	32.30	1.53	26.33	0.17
	2x LoD	34.40	0.50	26.40	0.43
Tobrex 0.3%	10x LoD	31.53	0.77	26.27	0.10
	2x LoD	34.27	0.37	25.97	0.00
Synagis 50 mg	10x LoD	31.55	0.78	26.13	0.03
	2x LoD	33.27	-0.63	25.93	-0.03
Substance Name	**AdV 3 Concentration**	**AdV 3 Ct (avg cycles)**	**AdV Delta Ct (cycles)**	**Internal Control 2 Ct (cycles)**	**Internal Control 2 Delta Ct (cycles)**
Reference run 2	**10x LoD**	**30.87**	**n/a**	**25.30**	**n/a**
	2x LoD	**32.80**	**n/a**	**25.17**	**n/a**
Relenza	10x LoD	30.50	-0.37	25.60	0.30
	2x LoD	34.40	1.60	25.13	-0.03
FluMist®	10x LoD	30.77	-0.10	25.37	0.07
	2x LoD	34.15	1.35	25.07	-0.10
NeoSynephrine®	10x LoD	31.50	0.63	25.83	0.53
	2x LoD	34.10	1.30	25.57	0.40
Walgreens Original Anefrin Nasal Spray	10x LoD	31.50	0.63	26.50	1.20
	2x LoD	34.20	1.40	25.67	0.50
Zicam Homeopathic Nasal Gel	10x LoD	30.70	-0.17	26.03	0.73
	2x LoD	34.47	1.67	25.57	0.40
Walgreens Saline Nasal Spray	10x LoD	32.03	1.17	26.13	0.83
	2x LoD	35.73	2.93	25.63	0.47
Chloraseptic® Sore Throat Lozenges	10x LoD	30.37	-0.50	25.27	-0.03
	2x LoD	34.47	1.67	25.80	0.63

Microbial Interference

To determine the potential interference of common nasal microorganisms on qualitative Adenovirus detection, a panel of microorganisms was spiked into Adenovirus positive NP washes/aspirates containing Adenovirus at two times the limit of detection (2x LoD). Crossing threshold (Ct) values were compared with a reference sample with no additional microorganism.

Potential Interferents Tested for Microbial Interference

Microorganism	Source	Concentration Tested
Adenovirus 3 (reference)	ATCC VR-3 virus cultured on MRC5 cells	~2x LoD (0.0066 $TCID_{50}$/mL)
Staphylococcus aureus	ATCC 33497	10^6 cfu/mL
Bordetella pertussis	ATCC 9797	10^6 cfu/mL
Respiratory Syncytial Virus A	ATCC VR-26 virus cultured on MRC5 cells	10^4 $TCID_{50}$/mL
Escherichia coli	Zeptometrix, ref0801517	10^6 cfu/mL
Cytomegalovirus	ATCC VR-977	10^4 $TCID_{50}$/mL
Parainfluenza 3	ATCC VR-93 virus cultured on MK2 cells	10^6 $TCID_{50}$/mL
Influenza A	A/PR/8/34 ATCC VR-1469 virus cultured on MDCK cells	10^5 $TCID_{50}$/mL
Influenza B	Strain and source not specified; cultured on MDCK cells	10^4 $TCID_{50}$/mL
Rhinovirus 1B	ATCC VR-1645 strain B632 virus cultured on MRC5 cells	10^3 $TCID_{50}$/mL
Coronavirus NL63	Source not specified; cultured on MK2 cells	10^4 $TCID_{50}$/mL
Measles	NCPV ref0809213v strain Mvi/Nottingham/GBR/18.04	10^3 pfu/mL
Mumps	NCPV, ref0809281v strain 20082	10^4 pfu/mL

As shown in the table below, Adenovirus was consistently detected with no significant change in the Adenovirus Ct count regardless of whether an additional microorganism was present; thus indicating that there was no interference caused by the microorganisms tested.

Adenovirus R-gene US Microbial Interference

Microorganism	AdV Ct (avg cycles)	AdV Delta Ct (cycles)	Internal Control 2 Ct (cycles)	Internal Control 2 Delta Ct (cycles)
AdV 3 2x LoD (Run 1)	32.17	n/a	26.20	n/a
AdV 3 + E. coli	31.73	-0.43	26.13	-0.07
AdV 3 + CMV	32.33	0.17	26.23	0.03

AdV 3 + Parainfluenza 3	31.97	-0.20	26.30	0.10
AdV 3 + Influenza A	32.23	0.07	25.97	-0.23
AdV 3 + Influenza B	33.03	0.87	25.80	-0.40
AdV 3 + Rhinovirus 1B	31.60	-0.57	25.80	-0.40
AdV 3 + Coronavirus NL63	32.07	-0.10	26.40	0.20
AdV 3 + Measles virus	32.90	0.73	26.07	-0.13
AdV 3 + Mumps virus	32.53	0.37	25.97	-0.23
AdV 3 2x LoD (Run 2)	34.07	n/a	25.37	n/a
AdV 3 + Staph. aureus	32.90	-1.17	25.37	0.00
AdV 3 + B. pertussis	32.77	-1.30	25.53	0.17
AdV 3 + RSV A	34.93	0.87	25.67	0.30

g. Assay cut-off:

The cut-off determination was performed through testing of 218 samples: 98 negative, 67 high negative, and 53 positive. Of the 98 negative samples, all but four were below 20 AFU, four were between 20 and 30 AFU and none were over 30. Of the 67 high negative samples, 31 were below 20 AFU and 36 were between 20 and 30 AFU. Of the 53 positive samples, all were over 40 AFU. No fluorescence values were obtained between 30 and 40 AFU among the 218 samples tested. The threshold default value (30 AFU) of the SmartCycler II was confirmed to be the correct cut-off value for the Adenovirus R-gene US assay.

This cut-off value was further confirmed in a preliminary clinical study, performed at Caen Hospital (France), on 184 clinical samples, prior to US clinical studies. The cut-off was then confirmed by analysis of the U.S. clinical studies results at the 3 sites, New York, Chicago and Memphis. The 30 AFU value was confirmed as the cut-off to achieve the best sensitivity and specificity.

h. Carryover Contamination:

This study was performed to examine potential carry-over/cross-contamination with the Adenovirus R-gene US assay by testing simulated human adenovirus (HAdV) high positive samples run in series with HAdV negative samples. The high positive samples consisted of negative nasal washes/aspirates spiked with HAdV type 4 at four logs above the Limit of Detection (LoD). The samples were processed and extracted in a high positive/negative alternating fashion on the bioMérieux NucliSENS® easyMAG® extraction system and likewise processed and run on the Cepheid SmartCycler II

instrument in an alternating fashion. Eleven high positive and eleven negative samples and a negative control were extracted per run on the easyMAG for a total of 55 high positive samples and 55 negative samples over 5 extraction runs and 4 amplification runs. When tested with high positive samples, no carry-over contamination was seen for the Adenovirus R-gene US assay.

The data from the study are summarized in the table below.

EasyMag NucliSENS Run	**Run 1**		**Run 2**		**Run 3**		**Run 4**	**Run 5**	
SmartCycler II Run	**Run 1**		**Run 2**		**Run 3**			**Run 4**	**Total**
High Positive sample	11/11	4/4	7/7	8/8	3/3	11/11	1/1	10/10	55/55 (100%)
Mean Ct	19.4		19.5		19.3			19.6	
Negative sample	11/11	4/4	7/7	8/8	3/3	11/11	1/1	10/10	55/55 (100%)

2. Comparison studies:

   a. *Method comparison with predicate device:*

      Not applicable

   b. *Matrix comparison:*

      Not applicable

   c. *Transport media compatibility :*

      Both types of Viral Transport Media (Universal Transport Medium from DHI, MicroTest™ M4RT Transport from Remel) claimed in the package insert were used in the prospective clinical study and were compatible with the Adenovirus R-gene assay.

3. Clinical studies:

   Prospective clinical study:

   Performance characteristics of the Adenovirus R-gene US assay were determined in a multi-center prospective investigational study employing 3 geographically diverse institutions in the US. The study began with recruitment and testing commencing on September 28, 2010. Specimens used in the study represent excess nasal specimens (NPS and NPA) that were collected from symptomatic men and women of all ages suspected of respiratory infection and submitted for routine care or analysis. Age ranges for patients providing specimens in the study ranged from 1 month to 101 years. The database was frozen on November 21, 2011 after testing 1186 swab and 395 NP wash/aspirate specimens and this dataset was used in the analysis. Two NP aspirate/wash specimens and three NPS were excluded from the analysis due to

protocol deviations. The Adenovirus R-gene US assay was compared with rapid culture (shell vial) followed by direct fluorescent antibody (DFA) screening and identification using the D3Ultra™ Direct Fluorescent Antibody (DFA) Respiratory Virus screening & ID kit from Diagnostic Hybrids (DHI). Each US site analyzed around 280-650 samples (swabs and washes/aspirates), for a total of 1576 samples. The studies were performed on the remnant of the specimen collected from children and adults after the routine clinical care.

Each sample was tested by Adenovirus R-gene US assay and rapid culture (shell vial) followed by DFA screening and identification. The Argene and DHI test were performed according to the instructions provided by the manufacturer. The shell vial culture was performed following standard laboratory procedures. The chosen sites were hospital virology laboratories experienced in DFA, cell culture and real time PCR. Performances (sensitivity and specificity) of the Adenovirus R-gene US assay were calculated as compared to the method of viral culture followed by DFA staining with DHI test.

Clinical Study Results – Swab Specimens

		Culture Result			
		Detected	**Not Detected**	**Total**	
Adenovirus R-gene US	**Detected**	44	43[a]	87	Sensitivity: 91.7% (80.0%-97.7%) 95%CI
	Not Detected	4[b]	1092	1096	Specificity: 96.2% (94.9%-97.2%) 95%CI
		48	1135	**1183**	

[a] 42/43 samples confirmed as Adenovirus positive by quantitative PCR
[b] 1/4 samples confirmed as Adenovirus positive by quantitative PCR

Clinical Study Results – Wash/Aspirate Specimens

		Culture Result			
		Detected	**Not Detected**	**Total**	
Adenovirus R-gene US	**Detected**	21	21[a]	42	Sensitivity: 100% (86.7%-100%) 95%CI
	Not Detected	0	351	351	Specificity: 94.4% (91.5%-96.5%) 95%CI
		21	372	**393**	

[a] 19/21 samples confirmed as Adenovirus positive by quantitative PCR

4. Clinical cut-off**:**

   Not applicable

5. Expected values/Reference range:
   The general demographic data for all eligible prospective specimens are presented in the two following tables:

   Prevalence (positives as determined by reference method) and Expected Value (positives as determined by Adenovirus R-gene US assay) observed during the clinical study in swab specimens

Swab Prospective Study					
Subject Age (years)	**Number**	**Total Adeno R-gene US positive**	**Expected Value**	**Total DHI positive**	**Observed Prevalence**
< 2	519	45	8.7%	25	4.8%
2-5	280	26	9.3%	14	5.0%
6-19	229	11	4.8%	5	2.2%
19-64	112	5	4.5%	4	3.6%
>65	43	0	ND	0	ND
Total	1183	87	7.35%	48	4.06%

   Prevalence (positives as determined by reference method) and Expected Value (positives as determined by Adenovirus R-gene US assay) observed during the clinical study in wash/aspirate specimens

Wash/Aspirate Prospective Study					
Subject Age (years)	**Number**	**Total Adeno R-gene US positive**	**Expected Value**	**Total DHI positive**	**Observed Prevalence**
< 2	275	33	12.0%	17	6.2%
2-5	57	7	12.3%	2	3.5%
6-19	55	2	3.6%	2	3.6%
19-64	5	0	0.0%	0	0.0%
>65	1	0	ND	0	ND
Total	393	42	10.69%	21	5.34%

N. Instrument Name:

Cepheid SmartCycler II utilizing Dx software version 1.7b or 3.0

O. System Descriptions:

1. Modes of Operation:
   The bioMérieux NucliSens easyMAG is an automated nucleic acid isolation and purification system that is based upon the silica extraction technology. The easyMAG is capable of processing a total of 24 reactions with variable sample types, sample volumes, and elution volumes within a single run. Nucleic acid is purified within a single cartridge by several steps that include lysis and binding of nucleic acid to high affinity magnetic silica beads, a series of wash steps and heated elution of purified nucleic acid from the silica beads.

   The Cepheid SmartCycler II® Real Time instrument with Dx software version 1.7b or 3.0a is used to perform real time PCR amplification and detection of nucleic acid. The Cepheid SmartCycler II® instrument is an integrated nucleic acid amplification and detection instrument system based on Cepheid's proprietary microprocessor-controlled I-CORE module. For purified DNA samples, the SmartCycler II® instrument enables polymerase chain reaction (PCR) for the amplification of DNA, and hybridization of fluorogenic target-specific probes for the detection of the amplified DNA.

2. Software:

   FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types:

   Yes ___X____ or No _____

3. Specimen Identification:

   Specimens ID's are manually entered into the user interface by the user.

4. Specimen Sampling and Handling:

   Liquid samples from nasopharyngeal swabs and/or nasal wash/aspirates are manually transferred collected and transferred into tubes for nucleic acid extraction and purification.

5. Calibration:

   Calibration is not recommended.

6. Quality Control:

   The positive control plasmid enables the experiment to be properly validated. This positive control is amplified with the same primers as the viral DNA of any Adenovirus present in patient samples. A bacteriophage Internal Control (IC2) is added to every sample to detect the presence of inhibitors or lysis failure.

P. Other Supportive Instrument Performance Characteristics Data Not Covered In The "Performance Characteristics" Section above:

Not applicable

Q. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

R. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.