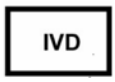
Prodesse ProPneumo™-1 Assay

For detection of *Chlamydophila pneumoniae* and *Mycoplasma pneumoniae.*

Contents



96

-70 °C

REF

P53SK96

Intended Use

ProPneumo™-1 Real-Time Assay is an in vitro diagnostic assay intended for use in a clinical laboratory setting by trained laboratory personnel to test patient respiratory specimens for the presence of *Chlamydophila pneumoniae* and *Mycoplasma pneumoniae* nucleic acids.

Principles of the Procedure

ProPneumo-1 Real-Time Assay enables detection of *Chlamydophila pneumoniae*, *Mycoplasma pneumoniae*, and Internal Control DNA and is based on two processes: nucleic acid extraction and Real-Time PCR amplification and detection of target DNA. The PCR Supermix contains primers complementary to highly conserved regions of genetic sequences for the bacteria and target-specific dual-labeled oligonucleotide probes: *Chlamydophila pneumoniae* (FAM, BHQ1), *Mycoplasma pneumoniae* (Cal Orange, BHQ1), and Internal Control (Q670, BHQ2).

Sample Preparation

Samples received without transport media should be mixed (1:1) with liquid viral transport media (i.e. Micro Test M4 media by Remel Inc.). Samples are stored at 4ºC if they are analyzed within 24 hours of collection, otherwise samples should be frozen at ≤ -20°C. A minimum of 400 µL of sample is required for analysis. Centrifuge samples at 500-1000 X g for 3 minutes at 4°C to remove cellular or particulate debris prior to analysis.

Nucleic Acid Extraction

Extraction of nucleic acids is performed with a Roche High Pure Viral Nucleic Acid Kit. Lysis is accomplished by incubation at 72°C in the presence of Proteinase K. Nucleic acids are then bound to glass fibers, washed several times to remove salts, proteins, and other impurities, and eluted in a low salt buffer.

Real-Time Polymerase Chain Reaction (Real-Time PCR)

Real-Time PCR is performed in a mix containing buffer, stabilizing additives, dNTPs, oligonucleotide primers, oligonucleotide probes labeled with fluorescent and quencher dyes, and Taq DNA Polymerase.

Internal DNA Control

An Internal Control is incorporated into every specimen sample and is carried through all steps of the procedure from Nucleic Acid Extraction through Real-Time PCR. The Internal Control is a non-infectious DNA fragment. The amplification of the Internal Control in spiked specimens is an indication that both extraction and PCR were successfully completed in the specimen sample.

Reagents

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

Materials Provided

ProPneumo-1 Real-Time Supermix (Catalog No. PSM53)

< 5 % Potassium chloride
< 5 % Tris (hydroxymethyl) aminomethane hydrochloride
< 1 % Magnesium chloride
< 1 % Bovine serum albumin
< 10 % Glycerol

ProPneumo-1 *C. pneumoniae* DNA Control (Catalog No. PCT31)

< 0.0005 % Non-infectious amplified DNA fragments of specific bacterial sequences

ProPneumo-1 *M. pneumoniae* DNA Control (Catalog No. PCT32)

< 0.0005 % Non-infectious amplified DNA fragments of specific bacterial sequences

Internal DNA Control II (Catalog No. GCT02)

< 0.0005 % Non-infectious amplified DNA fragments of specific bacterial sequences

Table 1. Summary of reagents provided

Component	P53SK96
	96 Reactions
0.370 mL ProPneumo-1 Real-Time Supermix	6 vials
30 µL ProPneumo-1 C. *pneumoniae* DNA Control	1 vial
30 µL ProPneumo-1 M. *pneumoniae* DNA Control	1 vial
30 µL Internal DNA Control II	1 vial

Table 2. Fluorescent real-time probes present in ProPneumo-1 Real-Time Supermix

Probe	Fluorophore	Absorbance Peak	Emission Peak	Gene Targeted
Mycoplasma pneumoniae	CAL Fluor Orange 560	540 nm	561 nm	16S-23S rRNA genes
Chlamydophila pneumoniae	FAM	495 nm	520 nm	OmpA gene
Internal Control	Quasar 670	651 nm	674 nm	N/A

Storage and Handling

- All reagents should be frozen upon arrival. If reagents are not frozen, contact Customer Service for assistance.
- Store all reagents (opened and unopened) at ≤ -70°C until the expiration date listed on the kit.
- Protect Real-Time Supermix from light.
- An internal study demonstrated that performance of Real-Time Supermix is not affected for up to 5 freeze-thaw cycles. Do not expose controls to more than two freeze-thaw cycles.

After opening reagent vials, store individual components according to Table 3.

Table 3. Proper storage temperature

	≤ -70°C	≤ -20°C
Unopened Vials		
Supermix	■	
Controls	■	
Opened Vials		
Supermix		■
Controls	■	
Controls (aliquots)		■

Material Required but Not Provided

Plasticware and Consumables

- Individually wrapped sterile transfer pipettes (1 mL)
- RNase/DNase-free 1.5 mL polypropylene microcentrifuge tubes and racks
- RNase/DNase-free 0.2 mL thermocycler tubes and racks
- Micropipettes with aerosol barrier or positive displacement RNase/DNase-free tips (10-, 200-, and 1000 μL)
- Consumables and accessories (reaction tubes, microcentrifuge, reaction plates, and adhesive seal) for the ABI 7500, Cepheid SmartCycler II instrument, or Rotor-Gene 3000 Real-Time PCR System
- Disposable protective gloves

Reagents

- RNase/DNase Free Water (Cambrex Cat No. 51200 or equivalent)
- Platinum Taq DNA Polymerase (Invitrogen Cat No. 10966-034)
- Micro Test M4 Media (Remel, Inc. Cat No. 12500 or equivalent)
- Isopropyl Alcohol (99.5 % or higher) (EMD Chemicals Cat No. PX1835 or equivalent)
- Ethyl Alcohol, 200 proof (99.5 % or higher) (EMD Chemicals Cat No. EX0276 or equivalent)

Nucleic Acid Isolation

- Roche High Pure Viral Nucleic Acid Kit (Roche Diagnostics GmbH, Cat No. 11 858 874 001) for 100 isolations

Equipment Required but Not Provided

- -70°C Freezer
- -20°C Freezer
- Microcentrifuge capable of 8,000 X g
- Centrifuge capable of 1,000 X g and 4°C
- Real-Time PCR instrument such as the ABI 7500, Cepheid SmartCycler II instrument (Life Science or Dx software), or Rotor-Gene 3000 Real-Time PCR System
- Dry heat block capable of 72°C

Specimen Collection, Transportation, and Storage

Note: *Handle all specimens as if they are infectious material.*

A. **Specimen Collection**
Collect respiratory samples (nasopharyngeal swabs or washes, bronchoalveolar lavage specimens, or sputum). Mix swabs with liquid viral transport media (Remel M4 or equivalent). Mix liquid samples (ratio of 1:1) with viral transport media. A minimum of 400 μL of liquid is required. Do not use calcium alginate swabs for specimen collection as they have been shown to inhibit PCR[1]. Chemically fixed or preserved specimens are not acceptable.

B. **Specimen Transport**
Transportation of human respiratory specimens must comply with all applicable regulations for the transport of etiologic agents. Human respiratory specimens must be transported at ≤ 4°C.

C. **Specimen Storage**
Store specimens refrigerated (2-8°C) for up to 72 hours before processing. Store any leftover specimens at ≤-70°. Store specimens in polypropylene screw-top tubes. Avoid repeat freezing and thawing of specimens which may lead to degradation of nucleic acids and decreased sensitivity.

Warnings and Precautions

A. FOR *IN VITRO* DIAGNOSTIC USE.

B. Limit use of this product to personnel who are trained in the techniques of Real-Time PCR.

C. This assay is validated for use with nasopharyngeal swab, bronchoalveolar lavage, and sputum specimens.

D. Handle specimens as if infectious using safe laboratory procedures such as those outlined in CDC/NIH document *Biosafety in Microbiological and Biomedical Laboratories* and in 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.

E. Screw-cap tubes must be used for specimen collection and preparation to prevent splashing and potential cross-contamination of specimens.

F. Use pipettors with aerosol barrier or positive displacement tips for all procedures.

G. Proceed with laboratory workflow in a uni-directional 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. Dedicate supplies and equipment to each pre-amplification activity and not for other activities; do not move between areas. Do not use equipment and supplies for reagent preparation with specimen preparation activities or for pipetting or processing amplified DNA or other sources of target nucleic acid. Keep post-amplification supplies and equipment in the amplification/detection area at all times. Disposable gloves must be worn in each area and must be changed before leaving that area.

H. Due to the high sensitivity of this test, take extreme care to preserve the purity of kit reagents and amplification mixtures. Contamination from Positive Controls and specimens can be avoided only by good laboratory practices and careful adherence to the procedures specified in this Package Insert. Discard any reagents that may be suspect.

I. Do not use the kit after its expiration date.

J. Do not mix reagents with different lot numbers.

K. Dispose of unused reagents and waste in accordance with all applicable regulations.

L. Safety data sheets (SDS) are available on the manufacturer's website: www.hologic.com/sds.

ProPneumo -1 Test Procedure

Special Notes

- The number of samples processed per kit will vary based on run size and control strategy. For a run size of 12 samples plus controls, a 96 Reaction Kit will yield 96 'reportable results.'
- DNA Controls must be diluted prior to the start of the ProPneumo-1 Real-Time Assay. Aliquots containing
  40 µL of Control DNA may be stored at ≤ -20°C until use.
- Handle reagents according to their recommended temperature. Visually examine reagents for sufficient reagent volume before beginning the test procedure.
- Human respiratory specimens, DNA Controls, Internal Control, and Negative Controls must be stored on ice before use.
- Turn on the Real-Time PCR Instrument at least 30 minutes prior to beginning amplification.

Reagent Preparation

Performed in Pre-Amplification Area

A. **Reagents**

Prepare reagents from the Roche High Pure Viral Nucleic Acid Kit following manufacturer's instructions.

B. **Controls**

1. **DNA Controls** (ProPneumo-1 *C. pneumoniae* DNA Control, ProPneumo-1 *M. pneumoniae* DNA Control) are amplified DNA fragments containing the bacterial sequence of interest and are not intact bacteria. DNA Controls can be used for verification of assay performance. DNA Controls should not be spiked with Internal DNA Control.

a. Make a **1:100 dilution**: 5 µL Undiluted DNA Control + 495 µL RNase/DNase Free Water.

b. Make 12 aliquots (40 µL each). Store at ≤ -20°C until use.

c. Immediately before using, thaw one 40 µL aliquot and make a **1:10 dilution** by adding 360 µL RNase/DNase Free Water. Controls do not need to be extracted prior to use.

2. **Internal DNA Control** (IC) is provided for verification of successful extraction and PCR. Prior to extraction, **every sample should be spiked with Internal DNA Control**.

a. Make a **1:100 dilution**: 5 µL Undiluted IC + 495 µL RNase/DNase Free Water.

b. Make 12 aliquots (40 µL each). Store at ≤ -20°C until use.

c. Immediately before using, thaw one 40 µL aliquot and make a **1:10 dilution** by adding 360 µL RNase/DNase Free Water. Each diluted aliquot yields enough Internal DNA Control to spike 9 samples.

Sample and Control Preparation

Performed in Pre-Amplification Area

Note: *Store all specimens and controls on ice until nucleic acid extraction.*

A. Spin sputum or other clinical samples containing significant debris at 500-1000 X g for 3 minutes at 4°C to remove cellular or particulate debris.

B. Combine **360 µL of sample** and **40 µL of Diluted Internal DNA Control** (from Reagent PreparationB.2.c. above).

C. Prepare a Negative Control containing 360 µL of transport media or RNase/DNase Free Water and 40 µL Diluted Internal DNA Control (from Reagent Preparation B.2.c. above). A Negative Control must be included with every run starting at Nucleic Acid Isolation to check for contamination and Internal Control amplification.

D. Prepare one positive DNA control (ProPneumo-1 *C. pneumoniae* DNA Control, ProPneumo-1 *M. pneumoniae* DNA Control) as outlined in Reagent Preparation B.1.c. above. Do not extract this control prior to use; add it directly to a Real-Time PCR reaction.

Nucleic Acid Isolation (Roche High Pure Viral Nucleic Acid Kit)

Performed in Pre-Amplification Area

A. Follow manufacturer's instructions with the following recommendations:
   1. Use a **400 µL** volume (**360 µL sample** + **40 µL Diluted Internal DNA Control**)
   2. Add 100 µL isopropanol instead of binding buffer after incubation for 10 min at 72°C
   3. Discard collection tube and replace with a clean collection tube between centrifugation steps
   4. Pre-heat Elution Buffer to 72°C prior to eluting Nucleic Acid
   5. Add 50 µL of pre-heated Elution Buffer, then incubate for 1 minute at 72°C prior to centrifugation

B. After elution, place purified Nucleic Acid immediately on ice.

Real-Time PCR Amplification

Performed in Pre-Amplification Area

A. Prepare the Real-Time PCR Master mix by combining the following reagents:

	per reaction
Real-Time Supermix	19.8 µL
Platinum Taq DNA Polymerase (5U/µL)	0.2 µL
	20.0 µL

To ensure sufficient volume, prepare PCR Master mix for one or two additional reactions.

Note: *Supermix is thawed at room temperature just prior to use. Protect from light. Platinum Taq DNA Polymerase must remain at proper storage temperature and is added just prior to Real-Time PCR Master mix use.*

B. Add 20 µL of the Real-Time PCR Master mix to a Real-Time thermocycler tube.

C. Add 5 µL DNA to the appropriate Real-Time thermocycler tube. Pipet to mix. Store tubes on ice or in a cold block until ready to start Real-Time PCR.

Note*: For performing the assay on the Cepheid SmartCycler II instrument, centrifuge for 10-15 seconds in the Cepheid mini-centrifuge to remove bubbles from the detection region (see manufacturer's instructions).*

Real-Time PCR Instrument Set Up

A. For the Cepheid SmartCycler II instrument, follow these instructions.

1. Define the following protocol:

Step No.	Description
1	2 minute hold at 95°C
2	Cycle Program (40 Cycles): 2a) 10 seconds at 95°C (optics OFF) 2b) 30 seconds at 60°C (optics ON)

2. Click Create Run and select the FTTC25 Dye Set.
3. Select sites using the protocol defined in the above table.
4. **PLACE TUBES IN THERMOCYCLER BLOCK.**
5. Click Start Run.

B. For the Rotor-Gene 3000 instrument, follow these instructions.

1. Start a new run using the new run wizard.
2. Under the Advanced tab, select Dual Labeled Probe and click New.
3. Select the 36-well rotor and No Domed Lid box. Click Next.
4. Select a 25 µL reaction volume. Click Next.
5. Click Edit Profile and create the following profile:

Cycle	Description
Hold	5 minute hold at 95°C
Cycling	Cycle Program (40 Cycles): 1st Timed Step: 1 second at 95°C (Not Acquiring) 2nd Timed Step: 5 seconds at 60°C (Acquiring to Cycling A on Cy5, FAM/Sybr, JOE)

6. Save the profile and click OK.
7. **PLACE TUBES IN THE ROTOR.**
8. Click Calibrate. The Auto Gain Calibration Window will open.
   a. In the new window, click Calibrate Acquiring. The FAM/Sybr, JOE, and Cy5 channels should appear in the Channel Setting box. Set the temperature for calibration to 60°C.
   b. Click Start. The instrument will heat to 60°C and select the appropriate gain settings for each channel.
   c. Click Close.
   d. Close the Auto Gain Calibration Window.
9. Click Start Run.
10. Enter Sample information and click Finish.

C. For the ABI 7500 Real-Time PCR System, follow these instructions.
1. Open a New File.
2. Select the following and click Next.
   a. Assay: Absolute
   b. Container: 96-well plate
   c. Template: Blank
   d. Run Mode: 9600 emulation
3. Select Detectors. If necessary, create new detectors with the reporter and quencher settings shown below.

Detector Name	Reporter	Quencher
Q670	CY5	(none)
CalO	JOE	(none)
FAM	FAM	(none)

4. Set Passive Reference Dye to none. Click Next.
5. Select wells and add all three detectors to the wells used in the run. Click Next.
6. Check the settings using the Well Inspector in the Set Up tab. Verify that the reporters and quenchers are set correctly (see above) and that the Passive Dye is set to none.

7. Select the Instrument tab and define the Thermal Profile as follows:

Stage	Description
1	5 minutes at 95°C (1 cycle)
2	Cycle Program (40 Cycles): Step 1: 10 seconds at 95°C Step 2: 45 seconds at 60°C

8. Set Sample Volume to 25 µL.
9. Set Data Collection to Stage 2, Step 2 (60.0°C for 0:45)
10. Set Ramp Rates to 100%. Click Next.
11. Save the file as a template. This template can be used for future ProPneumo-1 assay runs.
12. Enter sample information in the Set Up tab.
13. Save file as an sds file and close the file.
14. **PLACE PLATE IN HEATING BLOCK.**
15. Reopen the sds file. The instrument should initialize.
16. Click Start under the Instrument tab.

Background subtraction and threshold values

A. For the Cepheid SmartCycler II System, follow these instructions.

Analysis is performed in two steps;

1. After the run is complete, select the following analysis settings:

Background Subtraction:	ON	Auto Min Cycle: 5
Curve Analysis:	Primary Curve	Auto Max Cycle: 10
Threshold:	Run-based Auto	Valid Min Cycle: 3
Auto-Threshold # SDs:	15	Valid Max Cycle: 40

These settings automatically subtract background and set the threshold values for all detection channels.

Note the threshold displayed on the graph.

2. In the Thresh Setting column, select Manual. In the Manual Thresh Fluor Units column, enter the threshold calculated using the Run-based Auto settings. Push Update Analysis.

Refer to Section F, Interpretation of Results for further information on data analysis.

B. For the Rotor-Gene 3000 instrument, follow these instructions.

After the run is complete, select the following analysis settings for each channel:

Analysis Mode:	Quantitation
NTC Threshold:	10%
Reaction Efficiency Threshold:	Enabled at 0%
Threshold Position:	FAM - 0.05
	JOE - 0.05
	Cy5 - 0.05
Dynamic Tube:	ON

Slope Correct: ON
Ignore First: Ignore first 5 cycles

Note that each channel must be analyzed individually. Refer to Section F, Interpretation of Results for further information on data analysis.

Interpretation of Results

A. For a valid run, the following Control conditions must be met:

	Signal in FAM detection channel	**Signal in TET/JOE/HEX detection channel**	**Signal in Cy5 detection channel**
ProPneumo-1 *C. pneumoniae* DNA Control	**Crosses** threshold between 17 and 40 cycles	**Below** the threshold	**Below** the threshold
ProPneumo-1 *M. pneumoniae* DNA Control	**Below** the threshold	**Crosses** threshold between 17 and 40 cycles	**Below** the threshold
Negative Control (containing Internal DNA Control)	**Below** the threshold	**Below** the threshold	**Crosses** threshold between 19 and 40 cycles

B. **Sample** results are interpreted as follows:

Signal in FAM detection channel	**Signal in TET/JOE/HEX detection channel**	**Signal in Cy5 detection channel**	**Interpretation**
Signal is detected **above** the threshold		N/A*	**The sample is positive for *Chlamydophila pneumoniae*.**
	Signal is detected **above** the threshold	N/A*	**The sample is positive for *Mycoplasma pneumoniae* (Figure 1).**
Signal is **below** the threshold	Signal is **below** the threshold	Signal is detected **above** the threshold	**The sample is negative.** There is no *Chlamydophila pneumoniae* or *Mycoplasma pneumoniae* in the sample or the sample is below the detection limit of the assay (Figure 2).
Signal is **below** the threshold	Signal is **below** the threshold	Signal is **below** the threshold	**The sample is invalid.** Failure to detect a positive sample or the Internal DNA Control indicates that PCR failed in the sample. PCR inhibition is one possible cause. See the Troubleshooting Guide.

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

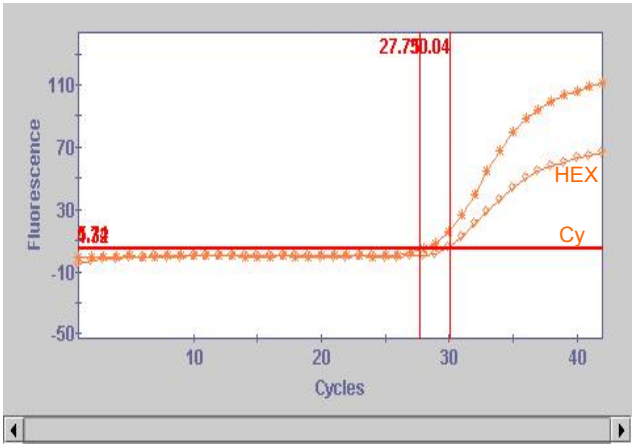


Figure 1. Typical positive clinical result for M. pneumoniae run on Cepheid SmartCycler II instrument.

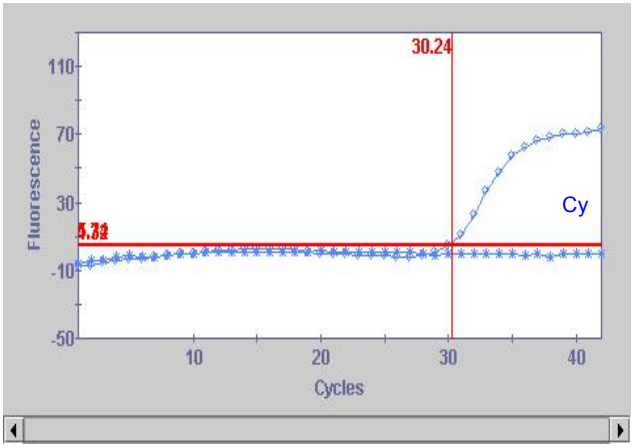


Figure 2. Typical negative clinical result run on Cepheid SmartCycler II instrument.

Quality Control

One Negative Control (containing Internal DNA Control) must be processed and included with each run performed.

- Fluorescence for positive DNA Controls must be above the threshold. If the positive DNA Controls do not consistently yield fluorescence above the background, contact the manufacturer for technical assistance.
- Fluorescence for the Negative Control (containing Internal DNA Control) must be above the threshold in the Cy5 detection channel and below the threshold in all other detection channels. If the Internal DNA Control does not consistently yield fluorescence above background, contact the manufacturer for technical assistance.

Troubleshooting Guide

Nucleic Acid Isolation Troubleshooting

Refer to Manufacturer's Product Insert

Positive controls are negative	Comments and suggestions
a) Platinum Taq DNA Polymerase was not added to the supermix	Ensure that Platinum Taq DNA Polymerase (Invitrogen) has been added to the supermix and rerun from extracted DNA or start with new sample.
b) Thermocycler did not complete the required number of cycles	Repeat amplification from extracted DNA or start with new sample.
c) Nucleic acid eluate contained significant amounts of residual wash buffer due to deficient duration or low speed spin	Be sure to incorporate dry spin steps in Nucleic Acid Isolation. Repeat entire procedure with a new sample/control.
d) Original sample or positive control was not stored properly	Storage at elevated temperature and multiple freeze/thaw cycles should be avoided. Repeat entire procedure with a new sample/control.
e) Improper Real-Time Instrument set up	Check all cycling and analysis parameters for the specific instrument being used. Check instrument calibration and function according to manufacturer's directions. Repeat testing from extracted DNA or start with new sample.
f) One or more of the kit components was stored improperly	Store all components according to manufacturer's recommendation. Repeat entire procedure with a new sample/control with properly stored reagents and materials.

Negative controls are positive	**Comments and suggestions**
a) Contamination of the reaction with previously amplified PCR product or Contamination of nucleic acid from concurrently processed samples	• Change tips after addition of each reagent to each tube. • Use aerosol resistant tips or positive displacement pipettors for specimen preparation. • Use dedicated and separate equipment and dedicated rooms for Pre and Post Amplification areas. • Use UV light on the equipment and area where Pre-Amplification is performed. • Change gloves as often as necessary. • Handle material/reagents carefully. • Avoid contact with the inner surface of tubes and caps. • Separate the reaction tubes in the centrifuge and racks with blank spaces when applicable. • Keep lab area clean and free of airborne contaminants. Repeat entire procedure with a new sample/control.

Lower result than expected	**Comments and suggestions**
a) DNA was left on ice for extended periods of time	Process samples at a consistent speed and with no interruption. Process DNA immediately after extraction.
b) Specimen preparation was performed slowly and interrupted	Process samples at a consistent speed and with no interruption. Repeat entire procedure with a new sample/control.

Performance Characteristics

Analytical Sensitivity

The sensitivity of the ProPneumo-1 Real-Time Assay was determined by analyzing 10-fold serial dilutions of quanitified stocks of *Chlamydophila pneumoniae* and unquantified stocks of *Mycoplasma pneumoniae* obtained from ATCC (Manassas, VA). The two bacterial strains were serially diluted in M4 viral transport medium and extracted using the Roche High Pure Viral Nucleic Acid Kit and tested on the Cepheid SmartCycler II, and Rotor-Gene 3000 Real-Time PCR instruments. Positive DNA controls were diluted in molecular grade water and added directly to the PCR reaction without extraction. The limit of detection was defined as the concentration that was detected in ≥95% of the reactions on each Real-Time PCR instrument.

Table 4. Analytical sensitivity of ProPneumo-1 Real-Time Assay for bacteria and DNA controls

Organism	Limit of Detection Cepheid SmartCycler	Limit of Detection Rotorgene 3000
Chlamydophila pneumoniae (ATCC #2822)	10^{-1} $TCID_{50}$/mL	10^{-1} $TCID_{50}$/mL
Mycoplasma pneumoniae (ATCC #15293)	10^{-6} dilution	10^{-5} dilution
C. pneumoniae Control DNA	5 copies/rxn	5 copies/rxn
M. pneumoniae Control DNA	50 copies/rxn	50 copies/rxn

Analytical Specificity

The specificity of the ProPneumo-1 Real-Time Assay was evaluated by testing a panel of viruses and bacteria commonly found in the respiratory tract. Each organism was extracted using the Roche High Pure Viral Nucleic Acid Kit and tested on the Cepheid SmartCycler II and Real-Time PCR instrument.

Table 5. Analytical specificity of ProPneumo-1 Real-Time Assay tested against common respiratory microorganisms

Microorganisms	Concentration	*C. pneumoniae* Result	*M. pneumoniae* Result	IC Result
Chlamydophila pneumoniae	10^{-1} $TCID_{50}$/mL	+	-	-
Mycoplasma pneumoniae	10^{-5} dilution	-	+	-
Adenovirus A	10^{1} $TCID_{50}$/mL	-	-	-
Adenovirus D	10^{1} $TCID_{50}$/mL	-	-	-
Bordetella parapertussis	10^{-3} dilution	-	-	-
Bordetella pertussis	10^{-3} dilution	-	-	-
Legionella pneumophila	10^{-3} dilution	-	-	-
Legionella micdadei	10^{-3} dilution	-	-	-
Legionella birminghamensis	10^{-3} dilution	-	-	-
Legionella maceachernii	10^{-3} dilution	-	-	-
Mycoplasma fermentans	10^{-3} dilution	-	-	-
Mycoplasma hominis	10^{-3} dilution	-	-	-
Mycoplasma genitalium	10^{-3} dilution	-	+	-
Mycoplasma genitalium	10^{-6} dilution	-	+	-
Haemophila influenza	10^{-6} dilution	-	-	-
Influenza A	10^{1} $TCID_{50}$/mL	-	-	-
Influenza B	10^{1} $TCID_{50}$/mL	-	-	-
Parainfluenza II	10^{1} $TCID_{50}$/mL	-	-	-
Parainfluenza III	10^{1} $TCID_{50}$/mL	-	-	-
RSV A	10^{1} $TCID_{50}$/mL	-	-	-

Clinical Performance

Clinical performance of the ProPneumo-1 Real Time Assay was evaluated by analyzing 30 contrived *C. pneumoniae* positive, 30 contrived *M. pneumoniae* positive, and 12 negative clinical specimens with three unique lots of reagents, resulting in 216 test results. Samples were evenly split between the following sample types; sputum (n = 24), bronchoalveolar lavage (n = 24), and nasopharyngeal swab specimens (n = 24). *C. pneumoniae* contrived specimens were spiked at concentrations of 10^{-1}, 10^{0}, and 10^{1} $TCID_{50}$/mL. *M. pneumoniae* contrived specimens were spiked at concentrations of 10^{-5}, 10^{-4}, and 10^{-3} dilutions of an ATCC stock culture. All positive and negative samples were spiked with Internal Control. Samples were extracted with the Roche High Pure Viral Nucleic Acid Kit and tested on the Cepheid SmartCycler II and Rotorgene 3000 instruments. Percent Positive Agreement was calculated by dividing the number of true positive samples detected by the sum of the true positives and false negatives for each organism. Percent Negative Agreement was calculated by dividing the number of true negative samples by the sum of true negatives and false positives for each organism. There were no invalid results (invalid results occur when the sample is negative for all target detections and the Internal Control, indicating potentially PCR-inhibited samples) for this study.

Table 6. Clinical Performance Summary – Cepheid SmartCycler II

C. pneumoniae		***Expected Result***			
		Positive	**Negative**	**Total**	
ProPneumo-1 Assay Result	Positive	79	0	79	**Percent Positive Agreement 87.8% (79.4% - 93.0%) 95% CI**
	Negative	11	126	137	**Percent Negative Agreement 100.0% (97.0% - 100.0%) 95% CI**
	Total	**90**	**126**	**216**	

M. pneumoniae		***Expected Result***			
		Positive	**Negative**	**Total**	
ProPneumo-1 Assay Result	Positive	89	0	89	**Percent Positive Agreement 98.9% (94.0% - 99.8%) 95% CI**
	Negative	1	126	127	**Percent Negative Agreement 100.0% (97.0% - 100.0%) 95% CI**
	Total	**90**	**126**	**216**	

Table 7. Clinical Performance Summary – RotorGene 3000

C. pneumoniae		***Expected Result***			
		Positive	**Negative**	**Total**	
ProPneumo-1 Assay Result	Positive	86	6	92	**Percent Positive Agreement 95.6% (89.1% - 98.3%) 95% CI**
	Negative	4	120	124	**Percent Negative Agreement 95.2% (90.0% - 97.8%) 95% CI**
	Total	**90**	**126**	**216**	

M. pneumoniae		***Expected Result***			
		Positive	**Negative**	**Total**	
ProPneumo-1 Assay Result	Positive	84	0	84	**Percent Positive Agreement 93.3% (86.2% - 96.9%) 95% CI**
	Negative	6	126	132	**Percent Negative Agreement 100.0% (97.0% - 100.0%) 95% CI**
	Total	**90**	**126**	**216**	

Procedural Limitations

1. Optimal assay performance required strict adherence to the assay procedure described in the insert.
2. This test is validated for use with nasopharyngeal swabs and washes, bronchoalveolar lavage specimens, and sputum. This test has not been validated with chemically fixed samples or other sample types.
3. Reliable results are dependent on adequate specimen collection, transport, storage and processing procedures.
4. Sensitivity and other performance characteristics have not been determined for all possible genotypes.
5. False negative results may occur due to loss of nucleic acid. The Internal Control has been added to the test to aid in the identification of specimens that contain inhibitors to PCR amplification. The Internal Control does not indicate whether or not nucleic acid has been lost due to inadequate collection, transport, or storage of specimens.
6. This test is validated for use with the Cepheid SmartCycler II and Rotor-Gene 3000 Real-Time PCR instruments. This test is also verified for use with the ABI 7500 Real-Time PCR System.
7. As with any diagnostic test, interpret results from this test while considering all clinical and laboratory findings.

Abbreviations

PCR	polymerase chain reaction
DNA	deoxyribonucleic acid
$TCID_{50}$	50% tissue culture infective dose
Ct	cycle threshold

Symbols

REF	Reference Number or Catalog Number
	Batch code or Lot number
	Use By Date or Expiration Date
	Upper Storage Temperature Limitation
	Number of Reactions Included

References

1. Cloud, J.L. et al. Impact of nasopharyngeal swab types on detection of *Bordetella pertussis* by PCR and culture. 2002. J Clin Microbiol 40: 3838-3840.

Notice to Purchaser

The procedure outlined in this protocol contains proprietary information. By purchasing this product, the user is granted a limited license by Hologic, Inc. to use this information as described. The user of this product agrees not to use any of these proprietary methods in any other application and agrees not to communicate (either orally or in writing) these proprietary methods to any other person or institution.

THE PURCHASE OF THIS PRODUCT GRANTS THE PURCHASER RIGHTS UNDER CERTAIN ROCHE PATENTS TO USE IT SOLELY FOR PROVIDING HUMAN IN VITRO DIAGNOSTIC SERVICES. NO GENERAL PATENT OR OTHER LICENSE OF ANY KIND OTHER THAN THIS SPECIFIC RIGHT OF USE FROM PURCHASE IS GRANTED HEREBY.

504370EN Rev. 005
2019-04

Hologic, Inc.
10210 Genetic Center Drive
San Diego, CA 92121 USA

Customer Support: +1 800 442 9892
customersupport@hologic.com
Technical Support: +1 888 484 4747
molecularsupport@hologic.com

For more contact information visit www.hologic.com.

EC REP
Hologic BVBA
Da Vincilaan 5
1930 Zaventem
Belgium