TrueScience™ RespiFinder®
Identification Panels

Protocol
Disclaimer

The results obtained from these or any other diagnostic panels should be used and interpreted only in the context of the overall clinical picture. Applied Biosystems cannot accept responsibility for any clinical decisions that are made.

Applied Biosystems does not represent this guide as a comprehensive summary of all possible outcomes from using the TrueScience™ RespiFinder® 19 Pathogen Identification Panel and TrueScience™ RespiFinder® 15 Viral Identification Panel. This guide is intended for use solely as an aid to memory. It is not for use in any clinical interpretation of the results of the assay. Laboratories must interpret and report the results of the assay in accordance with their own locally developed procedures.

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Customer is responsible for validation of assays and compliance with regulatory requirements that pertain to their procedures and uses of the RespiFinder products.

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About This Guide

Purpose


Safety information

Note: For general safety information, see this section and Appendix C, “Safety” on page 39. When a hazard symbol and hazard type appear by a chemical name or instrument hazard, see the “Safety” Appendix for the complete alert on the chemical or instrument.

Safety alert words

Four safety alert words appear in Applied Biosystems user documentation at points in the document where you need to be aware of relevant hazards. Each alert word — IMPORTANT, CAUTION, WARNING, DANGER — implies a particular level of observation or action, as defined below:

IMPORTANT! – Indicates information that is necessary for proper instrument operation, accurate chemistry kit use, or safe use of a chemical.

CAUTION! – Indicates a potentially hazardous situation that, if not avoided, may result in minor or moderate injury. It may also be used to alert against unsafe practices.

WARNING! – Indicates a potentially hazardous situation that, if not avoided, could result in death or serious injury.

DANGER! – Indicates an imminently hazardous situation that, if not avoided, will result in death or serious injury. This signal word is to be limited to the most extreme situations.
SDSs

The SDSs for any chemicals supplied by Applied Biosystems or Ambion are available to you free 24 hours a day. For instructions on obtaining SDSs, see “SDSs” on page 41.

IMPORTANT! For the SDSs of chemicals not distributed by Applied Biosystems or Ambion contact the chemical manufacturer.

Regulatory information

Manufacturer

Manufactured by PathoFinder B.V., the Netherlands, for:
Applied Biosystems Ltd
7 Kingsland Grange
Woolston, Warrington, Cheshire, UK WA1 4SR
Telephone: 01925 282700
www.appliedbiosystems.com/respifinderlit
TrueScience™ RespiFinder® Identification Panels

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Product description and principles

TrueScience™ RespiFinder® Identification Panels use a multiplex PCR test to detect and differentiate among pathogens that can cause respiratory tract infections. The TrueScience™ RespiFinder® Identification Panels assay includes a combined reverse transcription/PCR step (to convert viral RNA into cDNA and to amplify the target DNA/cDNA), a probe hybridization step, and a probe ligation/amplification step. An Internal Amplification Control (IAC) is included in the assay to distinguish between true negative samples and false negatives caused by nucleic acid degradation, PCR inhibition, or handling errors. The targets can be detected through DNA sizing fragment analysis using capillary electrophoresis. See Figure 1 on page 8.

Table 1  Product information

<table>
<thead>
<tr>
<th>REF</th>
<th>Panel</th>
<th>Pathogens detected</th>
<th>Number of target probe sets</th>
<th>Probe size range</th>
</tr>
</thead>
<tbody>
<tr>
<td>4460381</td>
<td>TrueScience™ RespiFinder® 15 Viral Identification Panel</td>
<td>14 RNA viruses and 1 DNA virus</td>
<td>15 plus IAC</td>
<td>163 to 498 nucleotides</td>
</tr>
<tr>
<td>4460382</td>
<td>TrueScience™ RespiFinder® 19 Pathogen Identification Panel</td>
<td>14 RNA viruses, 1 DNA virus, and 4 bacteria</td>
<td>19 plus IAC</td>
<td></td>
</tr>
</tbody>
</table>
Figure 1  Overview of TrueScience™ RespiFinder® assay

Total nucleic acid + RT PCR mix

Reverse transcription and PCR (target amplification)

Diluted RT/PCR reaction + Probe Hybridization Mix

Probe hybridization

Probe hybridization reaction + TwoStep Mix

Probe ligation and PCR (probe amplification)

Diluted PCR product + Size Standard Mix

Capillary electrophoresis

Data analysis and interpretation

**Intended use**

The TrueScience™ RespiFinder® 19 Pathogen Identification Panel and TrueScience™ RespiFinder® 15 Viral Identification Panel are qualitative multi-parameter tests intended to simultaneously detect and identify common respiratory pathogens from purified total nucleic acids.
The input sample is total nucleic acids extracted and purified from nasopharyngeal swabs, nasal aspirates, sputum, and broncho-alveolar lavages (BAL) from patients suspected of respiratory tract infections. Preparation of clinical samples is a separate process from the scope of the panels; use suitable methods or products to handle specimens and extract and purify nucleic acids.

The TrueScience™ RespiFinder® Identification Panels aid in the diagnosis of respiratory tract infection when used in conjunction with other clinical and laboratory findings. Negative results do not necessarily indicate absence of viral or bacterial respiratory tract infection; negative results should not be used as the sole basis for diagnosis, therapy, or other treatment decisions. Positive results do not exclude co-infection with other pathogens. The pathogen(s) detected may not be the definite cause of disease. Other laboratory testing and assessment of clinical presentation must be included in the final diagnosis. Performance characteristics were established with validated EQA panels from www.qcmd.org. The product is for use by laboratory professionals, and it is intended for use with certain instruments and data analysis software from Applied Biosystems.

### Table 2  Target genes used for probe and primer design (TrueScience™ RespiFinder® 19 Pathogen Identification Panel and TrueScience™ RespiFinder® 15 Viral Identification Panel)

<table>
<thead>
<tr>
<th>Target Genes</th>
<th>Gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>Internal Amplification Control (IAC)</td>
<td>Polyprotein gene (PP) of encephalomyocarditis virus</td>
</tr>
<tr>
<td>Adenovirus</td>
<td>Hexon gene (H)</td>
</tr>
<tr>
<td>Rhinovirus</td>
<td>5' untranslated region polyprotein gene (PP)</td>
</tr>
<tr>
<td>Influenza A</td>
<td>Matrix protein gene (M1)</td>
</tr>
<tr>
<td>Influenza B</td>
<td></td>
</tr>
<tr>
<td>Influenza A H5N1</td>
<td></td>
</tr>
<tr>
<td>Parainfluenza 1</td>
<td>Haemagglutinin-neuraminidase gene (HN)</td>
</tr>
<tr>
<td>Parainfluenza 2</td>
<td></td>
</tr>
<tr>
<td>Parainfluenza 3</td>
<td></td>
</tr>
<tr>
<td>Parainfluenza 4</td>
<td>Major nucleocapsid protein gene (N)</td>
</tr>
<tr>
<td>RSV-A</td>
<td></td>
</tr>
<tr>
<td>RSV-B</td>
<td></td>
</tr>
<tr>
<td>Corona 229E</td>
<td>Nucleocapsid protein gene (NP)</td>
</tr>
<tr>
<td>Corona NL63</td>
<td></td>
</tr>
<tr>
<td>Corona OC43</td>
<td></td>
</tr>
<tr>
<td>hMPV</td>
<td></td>
</tr>
</tbody>
</table>

### Table 3  Additional target genes used for probe and primer design in the TrueScience™ RespiFinder® 19 Pathogen Identification Panel only

<table>
<thead>
<tr>
<th>Target Genes</th>
<th>Gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bordetella pertussis</td>
<td>Pertussis Toxin promoter (PT) gene</td>
</tr>
<tr>
<td>Chlamyaphila pneumoniae</td>
<td>Major outer membrane (ompA) gene</td>
</tr>
<tr>
<td>Legionella pneumophila</td>
<td>Macrophage inhibitor potentiator (Mip) gene</td>
</tr>
<tr>
<td>Mycoplasma pneumoniae</td>
<td>Cytadhesin protein gene (P1)</td>
</tr>
</tbody>
</table>

TrueScience™ RespiFinder® Identification Panels Protocol
Contents and storage

Contents

Each of the TrueScience™ RespiFinder® Identification Panels includes a product insert with safety information and the following components:

Table 4 Components for TrueScience™ RespiFinder® 19 Pathogen Identification Panel and TrueScience™ RespiFinder® 15 Viral Identification Panel

<table>
<thead>
<tr>
<th>Component†</th>
<th>Minimum volume in tube (µL)</th>
<th>Tube cap color</th>
</tr>
</thead>
<tbody>
<tr>
<td>5X RT-PCR Buffer</td>
<td>250</td>
<td>Transparent (A)</td>
</tr>
<tr>
<td>dNTP Mix</td>
<td>50</td>
<td>Transparent (B)</td>
</tr>
<tr>
<td>RT-PCR Enzyme Mix</td>
<td>50</td>
<td>Transparent (C)</td>
</tr>
<tr>
<td>Internal Amplification Control (IAC)</td>
<td>300</td>
<td>Black</td>
</tr>
<tr>
<td>Pre-Amplification Primer Mix</td>
<td>125</td>
<td>White</td>
</tr>
<tr>
<td>Hybridization Buffer</td>
<td>75</td>
<td>Yellow</td>
</tr>
<tr>
<td>Probe Mix</td>
<td>75</td>
<td>Red</td>
</tr>
<tr>
<td>TwoStep Buffer</td>
<td>1550</td>
<td>Green (in amber tube)</td>
</tr>
<tr>
<td>Ligase Enzyme</td>
<td>50</td>
<td>Brown</td>
</tr>
<tr>
<td>Taq Polymerase</td>
<td>20</td>
<td>Orange</td>
</tr>
<tr>
<td>Dilution Buffer</td>
<td>1500</td>
<td>Transparent</td>
</tr>
<tr>
<td>Reference Marker (FAM™)‡</td>
<td>10</td>
<td>Purple (in amber tube)</td>
</tr>
</tbody>
</table>

† Read SDS.
‡ Volume sufficient for 10 capillary electrophoresis runs.

More information on the panels is available at www.appliedbiosystems.com/respifinderlit.

Storage

- Store the TrueScience™ RespiFinder® Identification Panels protected from light at -15 °C to -25 °C
- Avoid repeated thawing and freezing (limit to no more than 10 thaw/freeze cycles)

Expiration dates are shown on the individual component labels.

Materials and equipment supplied by the user

Use of the TrueScience™ RespiFinder® 19 Pathogen Identification Panel and TrueScience™ RespiFinder® 15 Viral Identification Panel requires materials such as a thermal cycler, a genetic analysis system, and standard laboratory equipment to be provided by the user or test site. For more information, see “Materials and equipment supplied by the user” on page 29.
## Workflow

<table>
<thead>
<tr>
<th>Before you begin: one-time procedures</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prepare work areas</td>
</tr>
<tr>
<td>Program the thermal cycler</td>
</tr>
<tr>
<td>Set up the data collection software</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Prepare clinical specimens</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collect samples</td>
</tr>
<tr>
<td>Extract total nucleic acids and add Internal Amplification Control (IAC) to the lysed samples</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Perform assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prepare and pipet the RT/PCR Mix</td>
</tr>
<tr>
<td>Prepare and pipet the Probe Hybridization Mix</td>
</tr>
<tr>
<td>Set up and run reverse transcription and PCR</td>
</tr>
<tr>
<td>Set up and run probe hybridization</td>
</tr>
<tr>
<td>(During probe hybridization) Prepare the TwoStep Mix</td>
</tr>
<tr>
<td>(Immediately after probe hybridization) Set up and run ligation and PCR</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Set up samples for capillary electrophoresis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Combine size standard, formamide, and PCR product</td>
</tr>
<tr>
<td>Run the sample plate</td>
</tr>
</tbody>
</table>

| Analyze data                                 |

| Interpret results                            |

**IMPORTANT!** The results obtained from these or any other diagnostic kits should be used and interpreted only in the context of the overall clinical picture. Applied Biosystems cannot accept responsibility for any clinical decisions that are made. For more information, see “Limitations and disclaimer” on page 36. For best practice guidelines, refer to *Molecular Diagnostics of Infectious Diseases* (Harald H. Kessler, ed. 2010. Berlin; New York: De Gruyter.)
Before you begin: one-time procedures

Prepare work areas

To prevent contamination, it is strongly recommend that you perform the experimental activities in separate areas:

- Area 1: Prepare mixes
- Area 2: Add sample total nucleic acids template to the mix
- Area 3:
  - Perform reverse transcription and PCR
  - Dilute the reactions
  - Perform the probe hybridization and ligation and PCR reactions
- Area 4:
  - Open reaction tubes after the ligation and PCR reaction
  - Perform fragment analysis

If it is not possible to create four different areas, combine the activities of area 3 and 4 in one area. When combining areas 3 and 4, try to physically separate the proceedings of areas 3 and 4 as much as possible, for example, by using different bench spaces or using a PCR workstation.

Program the thermal cycler

Use a thermal cycler with a heated lid such as a Veriti® Thermal Cycler or GeneAmp® PCR System 9700.

Before performing the assay, program the thermal cycler with the following three programs:

<table>
<thead>
<tr>
<th>1. Reverse transcription and PCR program</th>
<th>2. Probe hybridization program</th>
<th>3. Ligation and PCR program</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Stage</strong></td>
<td><strong>Temp</strong></td>
<td><strong>Time (mm:ss)</strong></td>
</tr>
<tr>
<td>Reverse transcription</td>
<td>50 °C</td>
<td>30:00</td>
</tr>
<tr>
<td>Activate hot-start Taq</td>
<td>95 °C</td>
<td>15:00</td>
</tr>
<tr>
<td>Cycling: 30 cycles</td>
<td>94 °C</td>
<td>00:30</td>
</tr>
<tr>
<td>55 °C</td>
<td>00:30</td>
<td></td>
</tr>
<tr>
<td>72 °C</td>
<td>01:00</td>
<td></td>
</tr>
<tr>
<td>Hold</td>
<td>20 °C</td>
<td>Continue directly to next step or store†</td>
</tr>
</tbody>
</table>

† Store at 4 °C for up to 1 week or at −20 °C for longer periods.
Prepare clinical specimens

Collect samples

Sample types

A variety of clinical specimens is suitable for the diagnosis of viral and/or bacterial infections of the respiratory tract:

- Nasal swab
- Nasopharyngeal swab
- Nasopharyngeal aspirate
- Nasal wash
- Throat swab
- Transtracheal aspirate
- Bronchoalveolar lavage (BAL)
- Sputum

Validation of the TrueScience™ RespiFinder® Identification Panels was performed on respiratory secretions such as nasopharyngeal/throat swabs/washes and bronchoalveolar lavages (BAL).

Sample collection guidelines

Respiratory pathogen diagnosis depends on the collection of high-quality specimens, their rapid transport to the laboratory, and appropriate storage before laboratory testing.

- Read “Biological hazard safety” on page 43 for information about handling clinical specimens.
- Conduct internal validation studies to ensure that your laboratory’s sample collection devices and DNA preparation methods are compatible with this test.
- Use your sample collection device according to the manufacturer’s instructions.
- Transport clinical specimens to the laboratory as soon as possible, then aliquot and process the specimens as soon as possible.
- Store the specimens at 4 °C. If specimens cannot be processed within 48 hours, store the specimens frozen at or below -70 °C (preferred) or at -20 °C.
- If clinical specimens are viscous (for example, sputum), or contain solid components, it is recommended that you perform sample pretreatment before you perform RNA/DNA extraction (see step 1 on page 14).
Extract total nucleic acids and add Internal Amplification Control (IAC) to the lysed samples

About the Internal Amplification Control (IAC)

The IAC is a SP6 RNA transcript of the polyprotein gene (PP) from the encephalomyocarditis (EMC) virus and is supplied as a control for the RNA/DNA extraction, a control for the TrueScience™ RespiFinder® Identification Panels assay, and a check for possible PCR inhibitors.

For samples that are high-titer and/or contain multiple infections, the IAC fragment may not be visible in the electropherogram, because high amounts of pathogenic nucleic acids consumed most of the reagents in the assay. Consequently, if the IAC signal is absent in the presence of one or more specific fragments indicating an infection, the assay is still valid.

Procedure

Extract total nucleic acids (RNA and DNA) using an appropriate extraction method and following the manufacturer’s instructions. For most extraction methods, it is strongly recommended to use 200 µL of sample material and to elute the purified nucleic acids in 100 µL of elution buffer.

**IMPORTANT!** Do not overconcentrate the nucleic acids; high concentrations may increase the risk of inhibition of the RT-PCR enzymes.

1. Recommended sample pretreatment for viscous or non-homogeneous specimens:
   Pre-treat samples if specimens are viscous and/or contain solid cellular components. Liquefy samples by performing a pretreatment with dithiotreitol (DTT). Use the protocol below for common clinical specimen types.
   a. Pipet the clinical specimen into a sterile tube (see table for amounts).

<table>
<thead>
<tr>
<th>Clinical specimen</th>
<th>Sample volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nasopharyngeal aspirate</td>
<td>200 µL</td>
</tr>
<tr>
<td>Sputum</td>
<td>50 µL</td>
</tr>
<tr>
<td>Bronchoalveolar lavage</td>
<td>200 µL</td>
</tr>
<tr>
<td>Swab (throat, nose)</td>
<td>200 µL†</td>
</tr>
</tbody>
</table>

   † Dispersed in 300 µL of PBS.

   b. Add 10 µL of dithiotreitol (DTT) (10% v/v), then incubate for 15 minutes at 37 °C. During incubation, mix regularly to stimulate sample liquefaction.

   c. Spin down the solid components for 5 minutes at 8000 rpm.

2. Transfer the sample (or pre-treated sample supernatant from step 1) to the lysis buffer, mix well, then incubate as described by the manufacturer’s instructions.

**Note:** It is recommended to add lysis buffer to the clinical sample in a laminar airflow cabinet. The extraction procedure can be continued on a bench after the clinical sample is lysed. After use, it is recommended to decontaminate the laminar airflow cabinet with a decontamination solution (for example, 1000 ppm bleach) followed by UV light for 30 minutes.
3. After incubation of the sample with the lysis buffer, add the Internal Amplification Control (IAC), then mix well. Add 5 µL of IAC for every 100 µL of elution buffer.

**IMPORTANT!** For all extraction protocols, add the IAC after you finish incubating the sample with the lysis buffer. If you add the IAC to the sample/lysis buffer mixture before incubation is complete, the RNases and DNases in the sample can break down the IAC, resulting in false negatives. The lysis buffer inhibits RNases and DNases and prevents the degradation of the IAC.

4. Proceed with the RNA/DNA extraction protocol as described in the manufacturer’s instructions.

Avoid freeze-thaw cycles as much as possible. After extraction, you can store samples at 4 °C for up to 2 days, or at -20 °C or -70 °C for longer periods.

**Perform the assay**

**General recommendations**

Take the following precautions to avoid contamination and to allow optimal reproducibility of the assays:

- This molecular diagnostic assay should only be performed by qualified laboratory personnel.
- Wear disposable gloves when performing the assay.
- Use disposable tips containing hydrophobic filters to prevent cross-contamination.
- Use disposable low-retention tips containing hydrophobic filters when handling the RT-PCR enzyme mix.
- Use RNase/DNase-free PCR vials.
- Thaw RNA/DNA samples on ice, then keep them on ice or on a cooling block.
- Keep enzymes on ice or on a cooling block when they are not in the freezer.
- Handle enzymes with care and mix very gently.
- Open the 0.2-mL PCR vials with care to avoid aerosols.
Perform the assay

**Prepare and pipet the RT/PCR Mix**

1. Thaw the following items, then keep the items on ice.
   - Total nucleic acid (RNA and DNA) template
   - Dilution Buffer
   - 5X RT-PCR Buffer
   - Pre-Amplification Primer Mix
   - dNTP Mix

2. Prepare enough RT/PCR Mix for the number of samples that you are processing plus additional volume for losses that occur during pipetting. When you prepare the mix, follow these reagent handling guidelines:
   - For the RT-PCR Enzyme Mix: Remove the mix from storage immediately before pipetting. Centrifuge the mix for 5 seconds, then mix by gently pipetting up and down. Use low-retention pipette tips when handling the mix. Return the mix to storage immediately after use.
   - For all other reagents and for the total nucleic acid template: Immediately before use, vortex and briefly centrifuge the tubes.

<table>
<thead>
<tr>
<th>RT/PCR Mix component</th>
<th>Volume per reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dilution Buffer</td>
<td>5.5 µL</td>
</tr>
<tr>
<td>5X RT-PCR Buffer</td>
<td>5.0 µL</td>
</tr>
<tr>
<td>Pre-Amplification Primer Mix</td>
<td>2.5 µL</td>
</tr>
<tr>
<td>dNTP Mix</td>
<td>1.0 µL</td>
</tr>
<tr>
<td>RT-PCR Enzyme Mix</td>
<td>1.0 µL</td>
</tr>
<tr>
<td><strong>Total volume RT/PCR Mix per reaction</strong></td>
<td><strong>15 µL</strong></td>
</tr>
</tbody>
</table>

3. Briefly vortex the RT/PCR Mix, dispense 15 µL into 0.2-mL PCR tubes, then close the tubes. Keep the PCR tubes on ice.

**Prepare and pipet the Probe Hybridization Mix**

1. Thaw the Dilution Buffer, Hybridization Buffer, and Probe Mix, then keep the items on ice.

2. Prepare enough Probe Hybridization Mix for the number of samples that you are processing plus additional volume for losses that occur during pipetting.

   **Note:** Immediately before use, vortex and briefly centrifuge each reagent.

<table>
<thead>
<tr>
<th>Probe Hybridization Mix component</th>
<th>Volume per reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dilution Buffer</td>
<td>3.0 µL</td>
</tr>
<tr>
<td>Hybridization Buffer</td>
<td>1.5 µL</td>
</tr>
<tr>
<td>Probe Mix</td>
<td>1.5 µL</td>
</tr>
<tr>
<td><strong>Total volume Probe Hybridization Mix per reaction</strong></td>
<td><strong>6.0 µL</strong></td>
</tr>
</tbody>
</table>

3. Pipet 6 µL of Probe Hybridization Mix into new 0.2-mL PCR tubes, close the tubes, then keep the PCR tubes at 4 °C until use.
Set up and run reverse transcription and PCR

1. Add 10 µL of total nucleic acid template to each PCR tube that contains 15 µL of RT/PCR Mix. Mix the contents well by gently pipetting up and down, then briefly centrifuge.

   Keep the PCR tubes on ice until the thermal cycler is pre-heated.

   Note: If your internal laboratory procedures require that you run a negative control, add 0.25 µL of Internal Amplification Control to 10 µL of DNase/RNase-free water, then add this mixture to a PCR tube that contains 15 µL of RT/PCR Mix.

2. Start the Reverse Transcription/PCR thermal cycler program. When the temperature reaches 50 °C, place the PCR tubes in the thermal cycler.

Set up and run probe hybridization

1. Add 100 µL sterile water (not provided) to each reverse transcription/PCR reaction, then mix well by gently pipetting up and down.

2. Add 2 µL of the diluted reverse transcription/PCR reaction to each tube that contains 6 µL of Probe Hybridization Mix, then briefly centrifuge.

3. Place the PCR tubes in the thermal cycler, then run the Probe Hybridization program.

**IMPORTANT!** During probe hybridization, prepare the TwoStep Mix. After probe hybridization, proceed immediately with the ligation and PCR run. Keep the PCR tubes in the thermal cycler at 60 °C until you are ready to proceed with the ligation and PCR run.

(During probe hybridization)
Prepare the TwoStep Mix

During the probe hybridization run, prepare the TwoStep Mix:

1. Thaw the TwoStep Buffer, then keep it on ice.

2. Prepare enough TwoStep Mix for the number of samples that you are processing plus additional volume for losses that occur during pipetting. Keep the mix on ice until use. When preparing the mix, follow these reagent handling guidelines:

   - For the Ligase Enzyme and Taq Polymerase: Remove each reagent from storage immediately before pipetting. Centrifuge each reagent for 5 seconds, then mix each reagent by gently pipetting up and down. Use low-retention tips when handling these reagents. Return the reagents to storage immediately after use.
   - Vortex and briefly centrifuge the TwoStep Buffer before pipetting.

<table>
<thead>
<tr>
<th>TwoStep Mix component</th>
<th>Volume per reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>TwoStep Buffer</td>
<td>30.6 µL</td>
</tr>
<tr>
<td>Ligase Enzyme</td>
<td>1.0 µL</td>
</tr>
<tr>
<td>Taq Polymerase</td>
<td>0.4 µL</td>
</tr>
<tr>
<td><strong>Total volume TwoStep Mix per reaction</strong></td>
<td><strong>32.0 µL</strong></td>
</tr>
</tbody>
</table>
Immediately after the probe hybridization run:

1. Stop the Probe Hybridization program and immediately start the Ligation and PCR program.

2. When the thermal cycler temperature reaches 54 °C, press Pause to keep the thermal cycler block temperature at 54 °C.

3. Prepare the probe hybridization reactions for ligation and PCR:
   a. Remove the probe hybridization reactions from the thermal cycler.
   b. Add 32 µL of TwoStep Mix to each 8 µL probe hybridization reaction.
   c. Place the tubes back in the thermal cycler.

**IMPORTANT!** When you perform step 3, do not keep the probe hybridization reactions at room temperature for longer than 5 minutes.

**Note:** If you are running a large number of reactions, perform step 3 in batches. For example, if you are using 8-strip tubes, remove 8 reactions, add the TwoStep Mix, then replace the tubes in the thermal cycler before removing the next 8 reactions.

4. Immediately restart the paused Ligation and PCR program.

After the program completes, you can store the PCR products at 4 °C for up to 1 week or at -20 °C for longer periods.
Set up samples for capillary electrophoresis

The following Applied Biosystems® genetic analyzers and software are supported for use with the TrueScience™ RespiFinder® Identification Panels:

<table>
<thead>
<tr>
<th>Genetic Analyzer</th>
<th>Data Collection Software</th>
<th>Data Analysis Software</th>
</tr>
</thead>
<tbody>
<tr>
<td>3130 Genetic Analyzer or 3130xl Genetic Analyzer</td>
<td>Data Collection Software v3.0</td>
<td>GeneMapper® Software v4.0 or v4.1†</td>
</tr>
<tr>
<td>3500 Genetic Analyzer or 3500xL Genetic Analyzer</td>
<td>3500 Data Collection Software v1.0</td>
<td>GeneMapper® Software v4.1</td>
</tr>
</tbody>
</table>

† GeneMapper® Software v 4.1 cannot be installed on a Data Collection Software v3.0 computer.

When performing capillary electrophoresis, use the genetic analyzer according to the directions in the appropriate instrument user guide.

Combine size standard, formamide, and PCR product

1. Combine the following and mix thoroughly. Prepare enough volume for the number of samples that you are processing plus additional volume for losses that occur during pipetting:

<table>
<thead>
<tr>
<th>Size Standard Mix component</th>
<th>Volume per reaction (PCR product or Reference marker)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GeneScan™ 600 LIZ™ Size Standard [PN 4366589 or PN 4408399]</td>
<td>0.5 µL</td>
</tr>
<tr>
<td>Hi-Di™ Formamide [PN 4311320 or 4440753]</td>
<td>10.0 µL</td>
</tr>
<tr>
<td>Total volume Size Standard Mix per reaction</td>
<td>10.5 µL</td>
</tr>
</tbody>
</table>

2. In a 96-well optical plate, pipet 10.5 µL of the Size Standard Mix into each of the required number of wells.

3. (Recommended) Pipet 1 µL of Reference Marker (FAM™) into one of the wells containing the Size Standard Mix. Use the pipette to mix.

**IMPORTANT!** To facilitate identification of target fragments, it is recommended to include at least one reference marker for each plate. The TrueScience™ RespiFinder® Identification Panels contain enough Reference Marker (FAM™) for 10 capillary electrophoresis runs.
4. For each sample PCR product:
   - Dilute 5 µL of sample PCR product in 45 µL of deionized water.
   - Pipet 1 µL of the diluted sample PCR product into a well containing the Size Standard Mix, then use the pipette to mix.

   **Note:** In some cases (for example, high viral load), it may be necessary to further dilute the PCR product to avoid off-scale peaks. See “Troubleshooting” on page 27 for more information.

5. Cover the plate with a 96-well plate septa.

**Run the sample plate**

1. Centrifuge the plate at 1000 × g for 30 seconds to bring liquid to the bottom of the wells and remove air bubbles.

2. Prepare the plate assembly.

3. Load the plate assembly into the genetic analyzer, set up the data collection software as described in the *TrueScience™ RespiFinder® Identification Panels Software Setup and Data Analysis User Guide*, then link the plate and start the run.

**Analyze data**

Perform identification of pathogen candidate status by analyzing data using the recommended analysis settings, panels, and binsets. For instructions, refer to the *TrueScience™ RespiFinder® Identification Panels Software Setup and Data Analysis User Guide* (PN 4460428) at [www.appliedbiosystems.com/respifinderlit](http://www.appliedbiosystems.com/respifinderlit).

Applied Biosystems recommends that each laboratory develop its own interpretation and reporting procedures and criteria.
Interpret Results

**IMPORTANT!** Before continuing, read “Limitations and disclaimer” on page 36.

- Review the electropherogram to identify the specific pathogen(s) that are present in a sample. A positive sample has one or more significant peaks that fall within the size intervals defined by the known probe fragments in the reference marker.
- Include the reference marker at least once in each capillary electrophoresis plate to establish the exact size intervals for each probe. The size interval is the measured length, plus or minus two nucleotides, for each probe within the reference marker.
- A true negative sample has no peaks that fall within the size intervals defined by the reference marker, and always contains a significant IAC peak. A missing IAC may be the result of nucleic acid degradation, PCR inhibition, handling errors, or other failure during the reaction. If no specific fragments are present, and the IAC fragment is absent as well, the result is not valid.
- During validation of the kit, confirm that the default bin settings result in accurate peak labelling and adjust the bin settings if necessary.
- For each run, review the sizing quality values and the size standard to verify that the GeneMapper® Software has correctly size-called the sample fragments. Due to variations between instruments and between runs on the same instrument, small differences between the observed and actual fragment sizes (shown in Table 5 on page 22) are possible. Refer to the TrueScience™ RespiFinder® Identification Panels Software Setup and Data Analysis User Guide for information on reviewing the sizing quality values and the size standard.
- Note that peak heights may differ from instrument to instrument and from run to run.
- Electropherograms should not show electrophoretic spikes (sharp peaks present in more than one dye). If spikes are present, check for bubbles in the polymer, then re-inject the samples.
- Examine off-scale peaks. In the case of high pathogen input, a corresponding peak of very high height may occur, which may exceed the detection scale of the system. In this case, the GeneMapper® Software flags the peak as “off-scale” (labeled as "OS" in the software) which does not necessarily disqualify or invalidate the finding; it just indicates that a very strong signal for a given target was detected.
- Examine the electropherogram for peaks that are in the target bins but below the detection threshold. These peaks may indicate the presence of a low abundance or additional pathogen in the patient sample and require follow-up investigation.
- Note that in some cases, an infection with novel 2009 InfA H1N1v can also result in a peak at the H5N1 position.
### Table 5  Overview of TrueScience™ RespiFinder® Identification Panels fragment sizes and intervals

<table>
<thead>
<tr>
<th>TrueScience™ RespiFinder® Identification Panels fragment sizes and intervals</th>
<th>Target</th>
<th>Actual size (bp)</th>
<th>Observed size† (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>500</td>
<td>IAC</td>
<td>498</td>
<td>496.8</td>
</tr>
<tr>
<td></td>
<td>Rhinovirus</td>
<td>454</td>
<td>451.7</td>
</tr>
<tr>
<td></td>
<td>Chlamyphila pneumoniae</td>
<td>443</td>
<td>442.3</td>
</tr>
<tr>
<td>400</td>
<td>Influenza A (incl. 2009 H1N1v)</td>
<td>415</td>
<td>413.2</td>
</tr>
<tr>
<td></td>
<td>Influenza B</td>
<td>403</td>
<td>404.2</td>
</tr>
<tr>
<td></td>
<td>Influenza H5N1</td>
<td>394</td>
<td>393.0</td>
</tr>
<tr>
<td></td>
<td>Mycoplasma pneumoniae</td>
<td>385</td>
<td>382.2</td>
</tr>
<tr>
<td></td>
<td>Human metapneumovirus</td>
<td>364</td>
<td>361.1</td>
</tr>
<tr>
<td></td>
<td>Adenovirus</td>
<td>345</td>
<td>341.8</td>
</tr>
<tr>
<td></td>
<td>Parainfluenza 1</td>
<td>325</td>
<td>323.2</td>
</tr>
<tr>
<td></td>
<td>Parainfluenza 2</td>
<td>316</td>
<td>314.8</td>
</tr>
<tr>
<td></td>
<td>Parainfluenza 3</td>
<td>307</td>
<td>304.8</td>
</tr>
<tr>
<td></td>
<td>Parainfluenza 4</td>
<td>298</td>
<td>294.9</td>
</tr>
<tr>
<td></td>
<td>Legionella pneumophila</td>
<td>280</td>
<td>275.1</td>
</tr>
<tr>
<td>300</td>
<td>RSV-A</td>
<td>256</td>
<td>253.9</td>
</tr>
<tr>
<td></td>
<td>RSV-B</td>
<td>247</td>
<td>244.7</td>
</tr>
<tr>
<td></td>
<td>Bordetella pertussis</td>
<td>235</td>
<td>231.9</td>
</tr>
<tr>
<td></td>
<td>Coronavirus NL63</td>
<td>187</td>
<td>183.7</td>
</tr>
<tr>
<td></td>
<td>Coronavirus OC43</td>
<td>176</td>
<td>175.1</td>
</tr>
<tr>
<td></td>
<td>Coronavirus 229E</td>
<td>163</td>
<td>160.0</td>
</tr>
</tbody>
</table>

† Typically observed fragment size when run with POP-7™ Polymer and GeneScan™ 600 LIZ™ Size Standard.
As a guideline for interpreting the results, examples of typical results for the following are shown:

- “Positive samples” on page 23
- “Positive sample – Adenovirus” on page 23
- “True negative samples and failed samples” on page 24
- “Partially inhibited sample” on page 25
- “Sample with increased background” on page 26

Positive samples

A positive sample has one or more significant peaks that fall within the size intervals defined by the known probe fragments in the reference marker. Depending on the concentration of the infectious agent, the IAC signal may be absent or present. In samples with highly-concentrated infectious agent(s), the IAC signal is usually low or absent. In samples with a low concentration of the infectious agent(s), the IAC signal is normally present. Typical examples are shown in figures 2 and 3 below.

**Figure 2**  Positive sample with IAC signal

**Figure 3**  Positive sample without IAC signal

Positive sample – Adenovirus

The Adenovirus probe consists of two related probes designed to cover all six subgenera. Most Adeno species are detected by both probes, resulting in a typical peak profile with two peaks as shown in Figure 4 on page 24. A small number of Adeno strains react with only one of the probes, resulting in a peak profile with only one peak. These signals should also be considered to be an adenovirus positive sample.
**Figure 4** Sample positive for Adenovirus

A true negative sample has no peaks that fall within the size intervals defined by the reference marker. A true negative sample always contains a significant IAC peak (see Figure 5).

A failed sample does not contain a substantial signal at any probe position and does not include an IAC peak. Failed samples are not valid (see Figure 6 on page 25).

A failed sample may be the result of nucleic acid degradation, PCR inhibition, handling errors, or other failure during the reaction. If no specific fragments are present, and the IAC fragment is absent as well, the result is not valid. See “Troubleshooting” on page 27 for recommended actions.

**Figure 5** True negative sample
Partially inhibited sample

A partially inhibited sample is defined as a sample that shows lower than normal peak heights, for example:

- A sample with an IAC peak height lower than typical IAC peak heights for other samples processed in the same plate, and having no other peaks (Figure 7)
- A positive sample that contains unusually low peak heights compared to other samples processed in the same batch (Figure 8 on page 26).

No result can be claimed regarding these samples. See “Troubleshooting” on page 27 for recommended actions.
Figure 8 Example of a partially-inhibited sample

Sample with increased background

Increased background is defined as numerous peaks outside the size intervals defined by the reference marker (Figure 9). These patterns can be the result of the presence of high concentration of human DNA in the sample. No result can be claimed regarding this sample. See “Troubleshooting” on page 27 for recommended actions.

Figure 9 Sample with increased background
## Troubleshooting

<table>
<thead>
<tr>
<th>Observation</th>
<th>Possible cause</th>
<th>Recommended action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Off-scale peaks</td>
<td>High-titer sample.</td>
<td>Further dilute the sample PCR product, then repeat capillary electrophoresis.</td>
</tr>
<tr>
<td>No specific peaks are visible, and the IAC peak not visible</td>
<td>Programming error(s) in the thermal cycler program.</td>
<td>Correct errors in the thermal cycler program (see page 12), then repeat the assay.</td>
</tr>
<tr>
<td></td>
<td>Pipetting error or missing reagent(s).</td>
<td>Repeat the assay.</td>
</tr>
<tr>
<td></td>
<td>• IAC was not added.</td>
<td>Repeat the total nucleic acids extraction and assay.</td>
</tr>
<tr>
<td></td>
<td>• IAC was added directly to the clinical sample, or added before incubation</td>
<td></td>
</tr>
<tr>
<td></td>
<td>of the sample with the lysis buffer, resulting in degradation of the IAC.</td>
<td></td>
</tr>
<tr>
<td>Inhibitors present in the clinical sample.</td>
<td>Either:</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Repeat the assay on a 10-fold dilution of the extracted total nucleic acids.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Repeat the total nucleic acids extraction with additional sample pretreatment (DTT) (see step 1 on page 14), then repeat the assay.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Repeat the total nucleic acids extraction with 10 times less clinical specimen input and with additional sample pretreatment (DTT) (see step 1 on page 14), then repeat the assay.</td>
<td></td>
</tr>
<tr>
<td>No IAC fragment is visible in the presence of pathogen-specific fragment[s]</td>
<td>Strong infection and/or multiple infection. The IAC has been out-competed in the assay. The result is still valid.</td>
<td>No action.</td>
</tr>
<tr>
<td>Spurious fragments are shown</td>
<td>High background or presence of inhibitors.</td>
<td>• Make sure the reaction steps are performed in separate rooms or dedicated areas to prevent cross-contamination, then repeat the assay. See “Prepare work areas” on page 12.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Check the cycling program and make sure all handling steps are performed as described in the protocol, including working on ice, then repeat the assay.</td>
</tr>
</tbody>
</table>

---
### Troubleshooting

<table>
<thead>
<tr>
<th>Observation</th>
<th>Possible cause</th>
<th>Recommended action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak height(s) of target peak(s) (including IAC peak) is low compared to</td>
<td>Partial inhibition, due to high concentration of input nucleic acid, carryover of inhibitors from clinical specimens, or sample preparation chemistry.</td>
<td>To confirm these results are due to partial inhibition, rather than to issues with electrophoresis, re-run electrophoresis for the sample(s) in question.</td>
</tr>
<tr>
<td>typical peak height(s) for other samples in the same plate</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Ordering Information

How to order

To locate your nearest Applied Biosystems representative, go to:

www.appliedbiosystems.com/respifinderlit

TrueScience™ RespiFinder® Identification Panels

TrueScience™ RespiFinder® Identification Panels are available in the formats listed below. More information on the kits is available at www.appliedbiosystems.com/respifinderlit.

<table>
<thead>
<tr>
<th>REF</th>
<th>Panel</th>
<th>Pathogens detected</th>
<th>Number of target probe sets</th>
<th>Probe size range</th>
</tr>
</thead>
<tbody>
<tr>
<td>4460381</td>
<td>TrueScience™ RespiFinder® 15 Viral Identification Panel</td>
<td>14 RNA viruses and 1 DNA virus</td>
<td>15 plus IAC</td>
<td>163 to 498 nucleotides</td>
</tr>
<tr>
<td>4460382</td>
<td>TrueScience™ RespiFinder® 19 Pathogen Identification Panel</td>
<td>14 RNA viruses, 1 DNA virus, and 4 bacteria</td>
<td>19 plus IAC</td>
<td></td>
</tr>
</tbody>
</table>

Materials and equipment supplied by the user

In addition to the contents of the TrueScience™ RespiFinder® Identification Panels, the following materials and equipment are provided by the user or test site:

<table>
<thead>
<tr>
<th>Item</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disposable pipette tips containing hydrophobic filters</td>
<td>MLS†</td>
</tr>
<tr>
<td>Disposable low-retention pipette tips containing hydrophobic filters</td>
<td>MLS</td>
</tr>
<tr>
<td>Disposable gloves</td>
<td>MLS</td>
</tr>
<tr>
<td>Sterile, deionized water</td>
<td>MLS</td>
</tr>
<tr>
<td>(Optional) Dithiothreitol (DTT)</td>
<td>MLS</td>
</tr>
<tr>
<td>Sterile RNase- and DNase-free 1.5-mL tubes</td>
<td>MLS</td>
</tr>
<tr>
<td>Sterile RNase- and DNase-free 0.2-mL PCR tubes</td>
<td>MLS</td>
</tr>
<tr>
<td><strong>Note:</strong> We recommend the use of single tubes with caps or strip tubes with individual caps.</td>
<td></td>
</tr>
<tr>
<td>Cooling block or ice</td>
<td>MLS</td>
</tr>
</tbody>
</table>
### Laboratory equipment

<table>
<thead>
<tr>
<th>Item</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Precision pipettes (preferably positive displacement pipettes):</td>
<td>MLS</td>
</tr>
<tr>
<td>• 1 set for pre-amplification</td>
<td></td>
</tr>
<tr>
<td>• 1 set for post-amplification handling</td>
<td></td>
</tr>
<tr>
<td>Adjustable pipettes: 0.1-2 µL, 2-20 µL, and 20-200 µL</td>
<td>MLS</td>
</tr>
<tr>
<td>Multichannel adjustable pipette 1-10 µL (optional)</td>
<td>MLS</td>
</tr>
<tr>
<td>Protective clothing</td>
<td>MLS</td>
</tr>
<tr>
<td>Vortexer</td>
<td>MLS</td>
</tr>
<tr>
<td>Benchtop centrifuge with a rotor for 2-mL reaction tubes</td>
<td>MLS</td>
</tr>
<tr>
<td>Centrifuge for 0.2-mL reaction tubes</td>
<td>MLS</td>
</tr>
<tr>
<td>Laminar airflow cabinet</td>
<td>MLS</td>
</tr>
</tbody>
</table>

### RNA/DNA extraction and preparation

<table>
<thead>
<tr>
<th>Item</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>RNA/DNA extraction kit</td>
<td>MLS</td>
</tr>
</tbody>
</table>

### PCR amplification

<table>
<thead>
<tr>
<th>Item</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thermal cycler with a 0.2-mL tube block such as:</td>
<td>Applied Biosystems PN 4375305</td>
</tr>
<tr>
<td>• Veriti® 96-Well Thermal Cycler</td>
<td>Applied Biosystems PN N8050200</td>
</tr>
<tr>
<td>• 96-Well GeneAmp® PCR System 9700</td>
<td>Applied Biosystems PN N8010560</td>
</tr>
<tr>
<td>MicroAmp® Optical 96-Well Reaction Plate</td>
<td>Applied Biosystems PN 4366589</td>
</tr>
</tbody>
</table>

### Capillary electrophoresis

<table>
<thead>
<tr>
<th>Item</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>GeneScan™ 600 LiZ™ Size Standard</td>
<td>Applied Biosystems PN 4345833</td>
</tr>
<tr>
<td>or</td>
<td>Applied Biosystems PN 4352759</td>
</tr>
<tr>
<td>DS-33 (Dye Set G5) Matrix Standard</td>
<td>Applied Biosystems PN N8010560</td>
</tr>
<tr>
<td>POP-7™ Polymer</td>
<td></td>
</tr>
<tr>
<td>MicroAmp® Optical 96-Well Reaction Plate</td>
<td></td>
</tr>
<tr>
<td>96-Well Retainer &amp; Base Set [Standard] for 3500/3500xL Genetic Analyzers</td>
<td></td>
</tr>
<tr>
<td>96-Well Plate Septa:</td>
<td></td>
</tr>
<tr>
<td>• For 3130 Series instruments</td>
<td>Applied Biosystems PN N8010560</td>
</tr>
<tr>
<td>• For 3500 Series instruments</td>
<td>Applied Biosystems PN 4315933</td>
</tr>
<tr>
<td>Instrument-appropriate electrophoresis buffer</td>
<td>Applied Biosystems PN 4440753 or 4311320</td>
</tr>
<tr>
<td>Hi-Di™ formamide</td>
<td>Applied Biosystems PN 4345833 or 4352759</td>
</tr>
<tr>
<td>Applied Biosystems 3500 Series Genetic Analyzer</td>
<td>Contact your Applied Biosystems representative or go to <a href="http://www.appliedbiosystems.com">www.appliedbiosystems.com</a></td>
</tr>
</tbody>
</table>
### Analysis

<table>
<thead>
<tr>
<th>Item</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>GeneMapper® Software v4.1 or GeneMapper® Software v4.0‡</td>
<td>Applied Biosystems PN 4366925</td>
</tr>
<tr>
<td></td>
<td>Applied Biosystems PN 4440915</td>
</tr>
</tbody>
</table>

† Major Laboratory Supplier. For the SDS of any chemical not distributed by Applied Biosystems, contact the chemical manufacturer. Before handling any chemicals, refer to the SDS provided by the manufacturer, and observe all relevant precautions.

‡ For use with Data Collection Software v3.0 on 3130 Series Genetic Analyzers only.
Appendix A  Ordering Information

Materials and equipment supplied by the user
Supplementary Information

IMPORTANT! Before continuing, read “Limitations and Disclaimers” on page 36.

This section covers:

- Product background ...................... 33
- Recommended total nucleic acid extraction methods ......................... 34
- Internal validation ......................... 35
- Limitations and disclaimer ................ 36
- PCR good laboratory practices ............ 37

Product background

Acute respiratory tract infection is the most widespread type of acute infection in adults and children, and it is a significant cause of disease in immunocompromised patients. Respiratory tract infections (RTI) are commonly divided into upper respiratory tract infections (URTI) and lower respiratory tract infections (LRTI). The URTI include rhinorrhea, conjunctivitis, pharyngitis, otis media and sinusitis. LRTI include pneumoniae, brochiolitis and bronchitis. Both viruses and bacteria can cause acute RTI. The large, diverse number of causative agents provide a great challenge for diagnostics.

Nucleic acid amplification tests have proven to be a rapid, sensitive, and specific alternative in either monoplex or multiplex format. Multiplex assays allow the co-amplification of more than one target, thus providing insight into the significance of mixed infections and into the prognostic and recrudescence of the respiratory disease.
Recommended total nucleic acid extraction methods

Check www.appliedbiosystems.com/respifinderlit for updates to the following list:

Manual extraction:
- QIAamp® MinElute® Virus Spin Kit (#57704)
- NucliSENS® miniMAC® (bioMérieux):
  - NucliSENS® Magnetic Extraction Reagent (#200 293)
  - NucliSENS® Lysis Buffer (2 mL) (#200 292)
  - Micro-tubes (1.5 mL with cap) (#200 294)

Automated extraction systems:
- QIAGEN® QIASymphony®
  - QIASymphony® Virus/Bacteria Midi Kit (96) (#931055)
  - QIASymphony® Virus/Bacteria Mini Kit (192) (#931036)
- QIAGEN® BioRobot® EZ1® Workstation
  - EZ1® Virus Mini Kit v2.0 (48) (#955134)
- MagNA Pure (Roche)
  - Total Nucleic Acid isolation kit (#03038505001)
- NucliSENS® easyMAG® (bioMérieux)
Internal validation

Table 6 shows the performance study data for the TrueScience™ RespiFinder® 19 Pathogen Identification Panel on QCMD samples. The data shown is a compilation of 184 samples from the 2008, 2009, and 2010 proficiency panels for respiratory pathogens (www.qcmd.org).

- For Adenovirus, the false negatives were samples with only 1 or 2 copies of Adenovirus per RespiFinder® assay.
- Coronavirus 229E positive samples were only validated using the TrueScience™ RespiFinder® 15 Viral Identification Panel.
- The Bordetella pertussis probe also detects B. parapertussis and B. bronchiseptica.
- The 2010 Legionella QCMD panel contained 5 samples with a triple infection of Legionella pneumophila together with high viral titers of Parainfluenza and Coronavirus. The high viral titers prevented the detection of lower concentrations of Legionella pneumophila.

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Correct positive</th>
<th>Correct negative</th>
<th>False positive</th>
<th>False negative</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Influenza A</td>
<td>14</td>
<td>169</td>
<td>0</td>
<td>1</td>
<td>93.3%</td>
<td>100.0%</td>
</tr>
<tr>
<td>Influenza B</td>
<td>5</td>
<td>179</td>
<td>0</td>
<td>0</td>
<td>100.0%</td>
<td>100.0%</td>
</tr>
<tr>
<td>Influenza H5N1</td>
<td>0</td>
<td>184</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RSV-A</td>
<td>5</td>
<td>178</td>
<td>0</td>
<td>1</td>
<td>83.3%</td>
<td>100.0%</td>
</tr>
<tr>
<td>RSV-B</td>
<td>6</td>
<td>178</td>
<td>0</td>
<td>0</td>
<td>100.0%</td>
<td>100.0%</td>
</tr>
<tr>
<td>Human metapneumovirus</td>
<td>8</td>
<td>174</td>
<td>0</td>
<td>2</td>
<td>80.0%</td>
<td>100.0%</td>
</tr>
<tr>
<td>Adenovirus</td>
<td>12</td>
<td>166</td>
<td>0</td>
<td>1</td>
<td>66.7%</td>
<td>100.0%</td>
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<tr>
<td>Rhinovirus</td>
<td>11</td>
<td>172</td>
<td>0</td>
<td>1</td>
<td>91.7%</td>
<td>100.0%</td>
</tr>
<tr>
<td>Coronavirus 229E</td>
<td>2</td>
<td>184</td>
<td>0</td>
<td>0</td>
<td>100.0%</td>
<td>100.0%</td>
</tr>
<tr>
<td>Coronavirus NL63</td>
<td>6</td>
<td>178</td>
<td>0</td>
<td>0</td>
<td>100.0%</td>
<td>100.0%</td>
</tr>
<tr>
<td>Coronavirus OC43</td>
<td>4</td>
<td>180</td>
<td>0</td>
<td>0</td>
<td>100.0%</td>
<td>100.0%</td>
</tr>
<tr>
<td>Parainfluenza 1</td>
<td>7</td>
<td>176</td>
<td>0</td>
<td>1</td>
<td>87.5%</td>
<td>100.0%</td>
</tr>
<tr>
<td>Parainfluenza 2</td>
<td>2</td>
<td>182</td>
<td>0</td>
<td>0</td>
<td>100.0%</td>
<td>100.0%</td>
</tr>
<tr>
<td>Parainfluenza 3</td>
<td>2</td>
<td>182</td>
<td>0</td>
<td>0</td>
<td>100.0%</td>
<td>100.0%</td>
</tr>
<tr>
<td>Parainfluenza 4</td>
<td>4</td>
<td>178</td>
<td>0</td>
<td>2</td>
<td>66.7%</td>
<td>100.0%</td>
</tr>
<tr>
<td>Bordetella pertussis</td>
<td>4</td>
<td>176</td>
<td>3</td>
<td>1</td>
<td>80.0%</td>
<td>98.3%</td>
</tr>
<tr>
<td>Chlamydia pneumoniae</td>
<td>18</td>
<td>166</td>
<td>0</td>
<td>0</td>
<td>100.0%</td>
<td>100.0%</td>
</tr>
<tr>
<td>Mycoplasma pneumoniae</td>
<td>13</td>
<td>169</td>
<td>0</td>
<td>2</td>
<td>86.7%</td>
<td>100.0%</td>
</tr>
<tr>
<td>Legionella pneumophila</td>
<td>11</td>
<td>168</td>
<td>0</td>
<td>5</td>
<td>68.8%</td>
<td>100.0%</td>
</tr>
</tbody>
</table>
Limitations and disclaimer

Limitations

The results obtained from these or any other diagnostic panels should be used and interpreted only in the context of the overall clinical picture. Applied Biosystems cannot accept responsibility for any clinical decisions that are made.

The TrueScience™ RespiFinder® 19 Pathogen Identification Panel and TrueScience™ RespiFinder® 15 Viral Identification Panel are qualitative multi-parameter tests intended to simultaneously detect and identify common respiratory pathogens from purified total nucleic acids.

The input sample is total nucleic acids extracted and purified from nasopharyngeal swabs, nasal aspirates, sputum, and broncho-alveolar lavages (BAL) from patients suspected of respiratory tract infections. Preparation of clinical samples is a separate process from the scope of the panels; use suitable methods or products to handle specimens and extract and purify nucleic acids.

The TrueScience™ RespiFinder® Identification Panels aid in the diagnosis of respiratory tract infection when used in conjunction with other clinical and laboratory findings. Negative results do not necessarily indicate absence of viral or bacterial respiratory tract infection; negative results should not be used as the sole basis for diagnosis, therapy, or other treatment decisions. Positive results do not exclude co-infection with other pathogens. The pathogen(s) detected may not be the definite cause of disease. Other laboratory testing and assessment of clinical presentation must be included in the final diagnosis. Performance characteristics were established with validated EQA panels from www.qcmd.org. The product is for use by laboratory professionals, and it is intended for use with certain instruments and data analysis software from Applied Biosystems.

Disclaimer

Applied Biosystems recommends that each laboratory develop its own interpretation and reporting procedures and criteria. The results obtained from these or any other diagnostic panels should be used and interpreted only in the context of the overall clinical picture. Applied Biosystems cannot accept responsibility for any clinical decisions that are made.

Applied Biosystems does not represent this guide as a comprehensive summary of all possible outcomes from using the TrueScience™ RespiFinder® 19 Pathogen Identification Panel and TrueScience™ RespiFinder® 15 Viral Identification Panel. This guide is intended for use solely as an aid to memory. It is not for use in any clinical interpretation of the results of the assay. Laboratories must interpret and report the results of the assay in accordance with their own locally developed procedures.
PCR good laboratory practices

Laboratories should process their own internal QC samples of known type in each assay, so that the validity of the procedure can be assessed.

When preparing samples for PCR amplification:

- Use a positive-displacement pipette or aerosol-resistant pipette tips.
- Follow proper pipette-dispensing techniques to prevent aerosols.
- Wear clean gloves and a clean lab coat (not previously worn while handling amplified PCR products or used during sample preparation).
- Change gloves whenever you suspect that they are contaminated.
- Maintain separate areas and dedicated equipment and supplies for:
  - Sample preparation
  - PCR setup
  - PCR amplification
  - Analysis of PCR products
- Never bring amplified PCR products into the PCR setup area.
- Open and close all sample tubes carefully. Centrifuge tubes before opening. Try not to splash or spray PCR samples.
- Keep reactions and components capped as much as possible.
- Clean lab benches and equipment periodically with 10% bleach solution. Use DNAZap™ Solution (PN AM9890).
Appendix B  Supplementary Information

PCR good laboratory practices
This appendix covers:

- Symbols used on labels and packaging .................................................. 39
- Chemical safety .......................................................... 40
  - General chemical safety ............................................... 40
  - SDSs .................................................................. 41
  - Chemical waste safety ................................................. 41
  - Biological hazard safety .................................................. 43
- Safety alerts ................................................................. 43
  - General alerts for all chemicals ........................................... 43

Symbols used on labels and packaging

The symbols used on all labels and packaging conform to the harmonized standard EN980.

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
</table>
| ![Manufacturer Symbol](image) | MANUFACTURER  
The name and the address of the manufacturer will appear next to this symbol. |
| ![Number of Tests Symbol](image) | NUMBER OF TESTS |
| ![Consult Instructions Symbol](image) | CONSULT INSTRUCTIONS FOR USE |
| ![Store at Temperature Symbol](image) | STORE AT TEMPERATURE SHOWN |
| ![Use Before Date Symbol](image) | USE BEFORE DATE SHOWN |
| ![Catalogue Code Symbol](image) | CATALOGUE CODE |
| ![Lot or Batch Number Symbol](image) | LOT OR BATCH NUMBER |
Chemical safety

General chemical safety

Chemical hazard warning

⚠️ **WARNING! CHEMICAL HAZARD.** Before handling any chemicals, refer to the Safety Data Sheet (SDS) provided by the manufacturer, and observe all relevant precautions.

⚠️ **WARNING! CHEMICAL HAZARD.** All chemicals in the instrument, including liquid in the lines, are potentially hazardous. Always determine what chemicals have been used in the instrument before changing reagents or instrument components. Wear appropriate eyewear, protective clothing, and gloves when working on the instrument.

⚠️ **WARNING! CHEMICAL HAZARD.** Four-liter reagent and waste bottles can crack and leak. Each 4-liter bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position. Wear appropriate eyewear, clothing, and gloves when handling reagent and waste bottles.

⚠️ **WARNING! CHEMICAL STORAGE HAZARD.** Never collect or store waste in a glass container because of the risk of breaking or shattering. Reagent and waste bottles can crack and leak. Each waste bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position. Wear appropriate eyewear, clothing, and gloves when handling reagent and waste bottles.

Chemical safety guidelines

To minimize the hazards of chemicals:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. (See “About SDSs” on page 41.)
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the SDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood). For additional safety guidelines, consult the SDS.
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer’s cleanup procedures as recommended in the SDS.
- Comply with all local, state/provincial, or national laws and regulations related to chemical storage, handling, and disposal.


SDSs

About SDSs

Chemical manufacturers supply current Safety Data Sheets (SDSs) with shipments of hazardous chemicals to new customers. They also provide SDSs with the first shipment of a hazardous chemical to a customer after an SDS has been updated. SDSs provide the safety information you need to store, handle, transport, and dispose of the chemicals safely.

Each time you receive a new SDS packaged with a hazardous chemical, be sure to replace the appropriate SDS in your files.

Obtaining SDSs

The SDS for any chemical supplied by Applied Biosystems is available to you free 24 hours a day. To obtain SDSs:

1. Go to www.appliedbiosystems.com, click Support, then select SDS.

2. In the Keyword Search field, enter the chemical name, product name, SDS part number, or other information that appears in the SDS of interest. Select the language of your choice, then click Search.

3. Find the document of interest, right-click the document title, then select any of the following:
   - Open – To view the document
   - Print Target – To print the document
   - Save Target As – To download a PDF version of the document to a destination that you choose

Note: For the SDSs of chemicals not distributed by Applied Biosystems, contact the chemical manufacturer.

Chemical waste safety

Chemical waste hazards

⚠️ CAUTION! HAZARDOUS WASTE. Refer to Safety Data Sheets and local regulations for handling and disposal.

⚠️ WARNING! CHEMICAL WASTE HAZARD. Wastes produced by Applied Biosystems instruments are potentially hazardous and can cause injury, illness, or death.

⚠️ WARNING! CHEMICAL STORAGE HAZARD. Never collect or store waste in a glass container because of the risk of breaking or shattering. Reagent and waste bottles can crack and leak. Each waste bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position. Wear appropriate eyewear, clothing, and gloves when handling reagent and waste bottles.
Chemical waste safety guidelines

To minimize the hazards of chemical waste:

- Read and understand the Safety Data Sheets (SDSs) provided by the manufacturers of the chemicals in the waste container before you store, handle, or dispose of chemical waste.
- Provide primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the SDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood). For additional safety guidelines, consult the SDS.
- Handle chemical wastes in a fume hood.
- After emptying a waste container, seal it with the cap provided.
- Dispose of the contents of the waste tray and waste bottle in accordance with good laboratory practices and local, state/provincial, or national environmental and health regulations.

Waste disposal

If potentially hazardous waste is generated when you operate the instrument, you must:

- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure the health and safety of all personnel in your laboratory.
- Ensure that the instrument waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.

IMPORTANT! Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.
Biological hazard safety

**General biohazard**

**WARNING! BIOHAZARD.** Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Follow all applicable local, state/provincial, and/or national regulations. Wear appropriate protective equipment, which includes but is not limited to: protective eyewear, face shield, clothing/lab coat, and gloves. All work should be conducted in properly equipped facilities using the appropriate safety equipment (for example, physical containment devices). Individuals should be trained according to applicable regulatory and company/institution requirements before working with potentially infectious materials. Read and follow the applicable guidelines and/or regulatory requirements in the following:

- Local guidelines and legislation on biohazard and biosafety precautions.
- Your company's/institution's Biosafety Program protocols for working with/handling potentially infectious materials.

For additional information, refer to the following:

- Occupational Safety and Health Standards, Bloodborne Pathogens (29 CFR§1910.1030; [www.access.gpo.gov/nara/cfr/waisidx_01/29cfr1910a_01.html](http://www.access.gpo.gov/nara/cfr/waisidx_01/29cfr1910a_01.html)).

**Safety alerts**

For the definitions of the alert words **IMPORTANT, CAUTION, WARNING, and DANGER**, see “Safety alert words” on page 5.

**General alerts for all chemicals**

Avoid contact with skin, eyes, or clothing. Read the SDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
Documentation and Support

Related documentation

The following documents are available online at: www.appliedbiosystems.com/respifinderlit

<table>
<thead>
<tr>
<th>Document</th>
<th>Part number</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Software Setup and Data Analysis User Guide</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TrueScience™ RespiFinder® Identification Panels</td>
<td>4460430</td>
<td>Provides abbreviated procedures for performing the TrueScience™ RespiFinder® Identification Panels assay. The quick reference card does not represent a comprehensive summary of all possible outcomes and is intended for use solely as an aid to memory.</td>
</tr>
<tr>
<td>Quick Reference Card</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: To open the user documentation available online, use the Adobe® Acrobat® Reader® software available from www.adobe.com

Obtaining support

For the latest services and support information for all locations, go to:

www.appliedbiosystems.com

At the Applied Biosystems web site, you can:

- Access worldwide telephone and fax numbers to contact Applied Biosystems Technical Support and Sales facilities.
- Search through frequently asked questions (FAQs).
- Submit a question directly to Technical Support.
- Order Applied Biosystems user documents, SDSs, certificates of analysis, and other related documents.
- Download PDF documents.
- Obtain information about customer training.
- Download software updates and patches.
Documentation and Support

Obtaining support

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