DIAGNOSTIC HYBRIDS
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D³ FastPoint® Respiratory Virus Training Panel

Respiratory Viruses Panel, 12 samples (0.5-mL fill volume)

REF: 02-125012

For *Training* or *Validation* Use

Please contact Diagnostic Hybrids Technical Support for technical assistance regarding this procedure.

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Symbols Lexicon/Glossary

	Temperature limit	LOT	Batch code/lot number
	Consult instructions for use	REF	Catalog number
	Consult e-labeling instructions for use		Use by YYYY-MON-DD
	Manufacturer		

A. Intended Use:

For Training or Validation use.

The D³ FastPoint® Respiratory Viruses Training Panel is comprised of 12 frozen 0.5-mL liquid aliquots. Ten (10) of these aliquots have been tested and confirmed to contain viable respiratory viruses. The remaining 2 aliquots have been tested and confirmed as negative. The panel has been designed for use with the D³ FastPoint® L-DFA™ Respiratory Virus Identification Kit.

B. Principle:

Cell monolayers have been infected with known respiratory viruses. The infected monolayers have been harvested, and diluted with non-infected cells to achieve a known infected cell density (i.e., 4%). This cell suspension has been placed in a cryopreservative and frozen at -70°C. The suspensions will be thawed and stained according to the package insert of the D³ FastPoint L-DFA Respiratory Virus Identification Kit or seeded to respiratory virus-negative samples to produce contrived positives for validation protocols.

C. Storage and handling instructions:

The Respiratory Viruses Training Panel should be transferred from the shipping container to a -70°C or lower freezer immediately upon receipt. Once the panel has been thawed it should not be re-frozen.

D. Warnings and Precautions:

1. Personnel working with this panel must be properly trained in the use of the D³ FastPoint L-DFA Respiratory Virus Identification Kit and safe handling techniques.[1]
2. Manipulations which present potential personnel hazards should be conducted in a Class II hood with gloves worn at all times and in accordance with the guidelines presented in the CDC-NIH Manual.

E. Virus Handling and Usage Instructions for Direct Testing:

1. Remove the components of the D³ FastPoint L-DFA Respiratory Virus Identification Kit that are stored in the refrigerator and allow them to warm to room temperature for 15 to 30 minutes prior to use.
2. Retrieve training panel specimens to be run from the freezer and transport in dry ice directly to the lab for processing.
3. Thaw the panel aliquots rapidly in a 35° to 37°C water bath at the time of use.
4. Label each of 12 blue, orange and yellow centrifuge vials with 1 through 12 and place in a rack.
   NOTE: The "color coding" of the specimen processing vials is optional but recommended as a procedural aid for Steps 13, 14, 15, and 16, below.
5. Label each of the 12 clear centrifuge vials with 1 through 12 and place in a second rack.
6. Vortex gently each of the training panel specimens for 1 to 2-seconds.
7. Remove the contents from each specimen vial using a transfer pipette, and transfer to the corresponding labeled clear centrifuge vial in Step 5 above.
   NOTE: A new transfer pipette should be used for each specimen.
8. Place the centrifuge vials in the rotor, with the cap hinges facing outward and spin for 2-minutes at 2000xg. This will allow the pellet to form on the same side as the hinge for easy break up in step 10.
9. Decant the supernatant gently from each centrifuge vial into a waste container. Blot excess supernatant from each centrifuge vial onto an absorbent paper towel by lightly tapping the vial.
10. Add 0.5-mL of 1X PBS to each centrifuge vial, using a transfer pipette, using the 0.5-mL gradient mark as a guide.
11. Break up the cell pellet by pipetting up and down 5 to 10 times with a fixed volume 20-µL pipette or an adjustable pipette set to 20-µL.
12. Vortex each centrifuge vial for 1- to 2-seconds.
13. Add 3-drops (~70-µL) of the cell suspension, using a fine tip transfer pipette, to each of the three colored centrifuge vials (blue, orange and yellow) from Step 4 above with the corresponding specimen number.
    NOTE: A new transfer pipette should be used for each specimen.
14. Add 2-drops of the Influenza A/Influenza B Reagent to the blue centrifuge vials.
15. Add 2-drops of the RSV/MPV Reagent to the orange centrifuge vials.
16. Add 2-drops of the PIV/Adenovirus Reagent to the yellow centrifuge vials.
17. Cap all of the centrifuge vials and gently tap the rack to mix. Incubate in a heat block at 35° to 37° C for 5-minutes.
18. Fill each vial to the 1.5-mL gradient with 1X PBS using the squeeze bottle.
19. Place the centrifuge vials in the rotor, with the cap hinges facing outward and spin for 2-minutes at 2000xg. This will allow the pellet to form on the same side as the hinge for easy break up in step 22.
20. Decant the 1X PBS gently from each centrifuge vial into a waste container. Blot excess PBS from each centrifuge vial onto an absorbent paper towel by lightly tapping the vial.
21. Add 1-drop (~20-µL) of Re-suspension Buffer to each vial.

[1] Biosafety in Microbiological and Biomedical Laboratories (BMBL), 5th edition, 2007, CDC-NIH manual. http://www.cdc.gov/od/ohs/biosfty/bmbl5/bmbl5toc.htm]

22. Break up the cell pellet by pipetting up and down 5 to 10 times with a fixed volume 20-µL pipette or an adjustable pipette set to 20-µL.
23. Label specimen slides 1 through 12.
24. Add 20-µL of the cell suspension from the blue vial to the A/B labeled channel of the slide with the corresponding number using the 20-µL pipette.
25. Add 20-µL of the cell suspension from the orange vial to the R/M labeled channel of the slide with the corresponding number using the 20-µL pipette.
26. Add 20-µL of the cell suspension from the yellow vial to the P/Ad labeled channel of the slide with the corresponding number using the 20-µL pipette.
27. Repeat steps 22 through 26 for each panel specimen.
28. Examine each channel for the presence and color of fluorescent cells at 200X magnification with a fluorescent microscope.

F. Virus Handling and Usage Instructions for Seeding of Respiratory-Virus Negative Samples:

1. Retrieve training panel specimens to be run from the freezer and transport in dry ice directly to the lab for processing.
2. Thaw the panel aliquots rapidly in a 35° to 37°C water bath at the time of use.
3. Label each of 12 respiratory virus-negative samples with numbers from 1 to 12.
4. Vortex gently each of the training panel specimens for 1- to 2-seconds.
5. Remove the contents from each specimen vial using a transfer pipette, and transfer to the corresponding respiratory virus-negative samples in Step 3 above. NOTE: A new transfer pipette should be used for each specimen.
6. Vortex each sample for 5- to 10-seconds.
7. If the specimen tubes contain glass beads or swabs, transfer the vortexed samples into new 15-mL conical tubes or microcentrifuge vials.
8. Process samples using standard laboratory protocol.
9. Proceed with D³ FastPoint L-DFA Respiratory Virus Identification Kit

D³ FastPoint Respiratory Virus Training Panel Key	
Panel Member	Expected Result
1	Influenza A
2	Parainfluenza virus
3	RSV
4	Negative
5	*Influenza B and RSV*
6	Metapneumovirus
7	*Adenovirus and Parainfluenza*
8	Influenza B
9	*Influenza A and Influenza B*
10	Negative
11	Adenovirus
12	Influenza A

G Limitations:

Clinical respiratory samples chosen for method validation should be respiratory-virus negative prior to selection. Virus positive results that are additional to the expected results listed in the D³ FastPoint Respiratory Virus Training Panel Key may be due to virus present in the original clinical sample.

Warranty Statement

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