Diagnostic **HYBRIDS**
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Suggested Metapneumovirus Training Panel Protocol

MPV Panel, 20 samples (0.6-mL fill volume)

for use with REF: 02-478020

For *Training* Use

Please contact Diagnostic HYBRIDS Technical Services for technical assistance regarding this procedure.

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DIRECT SPECIMEN VALIDATION

1. Retrieve a full Metapneumovirus Training Panel (MPV panel) from the freezer and transport in dry ice directly to the lab for processing.
2. Thaw vials in a 37°C water bath just long enough to melt thoroughly.
3. Immediately after thawing, transfer the entire volume of the vials into individual labeled 1.7-mL eppendorf tubes. (DO NOT VORTEX)
4. Centrifuge at 700 xg for 5 minutes.
5. Carefully remove the supernatants from each vial by aspiration and transfer to another labeled vial to be used later for Culture Confirmation Validation if necessary.
6. Add 0.6 mL PBS to the vials and mix gently using the pipette. DO NOT VORTEX.
7. Centrifuge again at 700 xg for 5 minutes.
8. Remove the PBS and add an additional 0.6 mL PBS.
9. Mix gently using the pipette. DO NOT VORTEX.
10. Spot a two-well slide with 25-uL of cell suspension per well and allow to air dry. Do not use a heat source of any kind above 37°C.
11. Fix slides in 100% acetone for 5 minutes.
12. Allow to fully air dry.
13. Cover wells with one drop each of Metapneumovirus DFA Reagent (01-035005) and place slides into a 37°C humidified incubator (cover slides to avoid drying) for 15 minutes.
14. Remove from incubator and rinse slides gently using a squirt bottle of PBS. Do not allow the stream to hit the wells directly. Aim above the well and allow it to run over the well gently.
15. Tap the edge of the slides on an absorbent towel to remove excess PBS.
16. Add one drop of Mounting Fluid (01-002007b) to each well and coverslip.
17. Read slides for typical fluorescence.

CULTURE CONFIRMATION VALIDATION

1. Prepare R-Mix™ shell-vials or MWP (multi-well plates) by removing shipping media by aspiration and replacing with 1-mL pre-warmed RM-03T R-Mix™ Refeed Medium (10-330100).
2. Remove the medium from the R-Mix™ shell-vials or wells and inoculate each with 200-uL of supernatant collected in step 6. Alternatively, 200-uL of whole sample mixed by inversion may my inoculated directly to shell-vials or wells if Direct Specimen Validation is not being performed.
3. Recap and centrifuge at 700 xg for 60 minutes. After centrifugation, add 1-mL RM-03T R-Mix™ Refeed Medium to all wells and incubate overnight at 37°C.
4. After R-Mix™ shell-vials or wells have incubated overnight, remove medium by aspiration and wash twice with PBS.
5. Add 1-mL of 100% acetone (80% for MWP) to each well and fix for 5 minutes at room temperature (20° to 25°C).
6. Remove acetone and wash with PBS.
7. Remove PBS and add 4 drops of Metapneumovirus DFA Reagent (01-035005) to each well.
8. Incubate at 37°C for 15 minutes.
9. Remove reagent and wash twice with PBS.
10. For shell vials add 0.5-mL demineralized water and pull the coverslips to mount on slides. For MWP, add 3- to 4-drops of Mounting Fluid (01-002007b) to each well.
11. Read wells for typical fluorescence.

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