EVALUATION OF A COST-EFFECTIVE SCRAPING PROCEDURE OF D³ DIRECT FLUORESCENT ANTIBODY (DFA) METAPNEUMOVIRUS IDENTIFICATION KIT

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BACKGROUND

- Human metapneumovirus (hMPV)
  - a respiratory viral pathogen
  - causes a spectrum of illnesses ranging from. asymptomatic infection to severe bronchiolitis.
  - ubiquitous worldwide distribution.
- D³ DFA diagnostic kit (Diagnostic Hybrids, Athens, OH) is available for the detection of hMPV antigens in shell vials.
- Objective is to compare performance of the shell vial cell culture testing procedure recommended by the manufacturer to a modified scraping procedure.

MATERIALS & METHODS

- A 20-sample hMPV training panel (Diagnostic Hybrids) were inoculated into 40 R-mix shell vials (Diagnostic Hybrids) and incubated at 37°C for two days.
- After two days, the following procedures were followed.

Manufacturer's Procedure	Modified Scraping Procedure
1. Wash with 1mL 1X PBS twice	
2. Fix in cold acetone	2. Suspend in 0.5mL 1X PBS
3. Add 0.5mL 1X PBS	3. Scrape the vial thoroughly
4. Add 4 drops of hMPV stain and rock to ensure complete coverage of the cell monolayer by the stain	4. Spot 80µL cell suspension onto a Heavy Teflon® Coating Super Cured 8-Well Slide (Thermo Fisher Scientific, Portsmouth, NH)
5. Incubate stoppered vials at 37°C for 30min	5. Allow to air-dry in bio-safety cabinet
6. Wash with 1mL 1X PBS twice	6. Fix slide in cold acetone
7. Wash with 1mL de-mineralized water once	7. Incubate in humid chamber at 37°C for 30 min
8. Transfer and mount cover slip onto a standard Superfrost® Disposable Microscope Slide (Thermo Fisher Scientific)	8. Wash slide with 1X PBS and de-mineralized water
9. Examine slide using a fluorescence microscope	

RESULTS

Table 1. Comparison of DFA hMPV Identification Results

Panel Number	Expected Result	Manufacturer Procedure	Modified Scraping Procedure
1	Negative	Negative	Negative
2	Positive	Positive	Positive
3	Positive	Positive	Positive
4	Negative	Negative	Negative
5	Negative	Negative	Negative
6	Positive	Positive	Positive
7	Negative	Negative	Negative
8	Positive	Positive	Positive
9	Negative	Negative	Negative
10	Positive	Positive	Positive
11	Positive	Positive	Positive
12	Negative	Negative	Negative
13	Positive	Positive	Positive
14	Positive	Positive	Positive
15	Negative	Negative	Negative
16	Negative	Negative	Negative
17	Positive	Positive	Positive
18	Negative	Negative	Negative
19	Positive	Positive	Positive
20	Negative	Negative	Negative

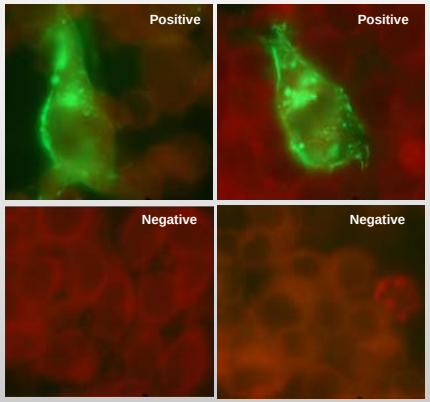

Figure 1. Representative Fluorescence Images (100x) Obtained from Following the Manufacturer's Procedure

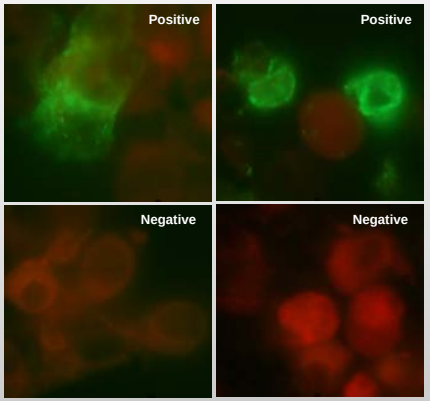

Figure 2. Representative Fluorescence Images (100x) Obtained from Following the Modified Scraping Procedure

Table 2. Cost and Work-flow Analysis of DFA hMPV Tests Following the Manufacturer's Procedure Compared to the Modified Scraping Procedure

Materials	Manufacturer's Procedure	Modified Scraping Procedure
No. of shell vials	20	
Cost of shell vials[1]	$3.39 x 20 = $67.80	
Volume of Re-feed	1mL x 20 = 20mL	
Cost of Re-feed[1]	$33.81 per 100mL x 20 mL = $6.76	
Volume of stain	240µL x 20 = 4.8mL	60µL x 20 = 1.2mL
Cost of stain[1]	$523.60 per 5mL x 4.8mL = $502.67	$523.60 per 5mL x 1.2mL = $125.66
No. of slides	10	3
Cost of slides[2]	$0.508 per slide x 10 = $5.08	$0.377 per slide x 3 = $1.13
Cost of Materials	**$582.31**	**$201.35**
Accessioning	5min	5min
Specimen set-up	15min	15min
Preparation for staining	5min	15min[3]
Incubation	30min	30min
After incubation	15min	5min
Microscope examination	30min	30min
Hands-on Time	**100 min**	**100min**
Turn-around Time[4]	**25.7hrs**	**27.7hrs**

1. Price quotes obtained from Diagnostic Hybrids on 07/08/2009.
2. Price quotes obtained from Thermo Fisher Scientific on 11/10/2009.
3. The 2hr air-dry time was not counted as part of hands-on time.
4. Includes the 2-day incubation (in both procedures) and air-dry time (in the scraping procedure).

CONCLUSIONS

Although the turn-around-time for the scraping procedure was slightly longer than the shell vial procedure recommended by the manufacturer, the scraping procedure was a cost-effective method for the detection of hMPV antigens in the hMPV training panel. However, it may be possible to obtain false negative results using the scraping procedure since only a small number of cells from a cell monolayer were represented on the spotted slide. Therefore, further investigation using cell culture amplified specimens is needed to determine the specificity, sensitivity and the limit of detection of the scraping procedure.

REFERENCES

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- D³ DFA Metapneumovirus Identification Kit Package Insert (Ref: 01-030000) and Metapneumovirus Training Panel Package Insert (Ref: 02-478020). Diagnostic Hybrids, Athens, OH.

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DISCLAIMER

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