Clinical Evaluation of the 3M™ Rapid Detection RSV Test in Nasopharyngeal Aspirate Specimens

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ABSTRACT

The 3M™ Rapid Detection RSV Test (3M Health Care, Saint Paul, MN), an investigational, in vitro diagnostic device, was compared with viral culture, direct immunofluorescence (DFA), BinaxNOW® RSV (Binax; Scarborough, ME), and Xpect®RSV (Remel; Lenexa, KS) for direct detection of respiratory syncytial virus (RSV) in predominantly nasopharyngeal (NP) aspirates from a pediatric population. This IRB-approved, 3M-sponsored, clinical trial was conducted from December 2008 to mid-January 2009. A total of 199 specimens were tested; 95% were NP aspirates, 5% were NP swabs. RSV prevalence was 55%. Fifty-two percent of specimens were from males. Eighty-one percent of subjects were ≤ 24 months old; 46% were ≤ 6 months of age. A true positive was defined as positive by either viral culture or DFA. Viral culture was performed using R-Mix shell vials (SV; Diagnostic Hybrids Inc., Athens, OH) which were stained with RSV DFA reagent (DHI) 2 days post-inoculation. DFA was performed on washed cells harvested by low speed centrifugation with subsequent application to microscope slides by cytocentrifugation. The same reagent used for SV culture was used for DFA of patient samples.

RESULTS

TEST	# POSITIVE	# NEGATIVE	# INDETERMINATE	NOT PERFORMED
DFA	108	85	6	0
Culture	96	102	0	1
DFA and culture	110	82	6	1
BinaxNOW® RSV	94	98	7	0
3M™ RSV	93	92	14	0
Xpect®RSV	69	123	5	2

As compared to either culture or DFA results, the performance characteristics of each test (excluding indeterminate results) were:

TEST	SENSITIVITY (%)	SPECIFICITY (%)	PPV (%)	NPV (%)
DFA	98	100	100	98%
Culture	87	100	100	85
BinaxNOW® RSV	80	92	93	76
3M™ RSV	90	94	95	88
Xpect®RSV	64	100	100	66

Thirty-two percent of 3M™ Rapid Detection RSV tests required at least one repetition and 7% were indeterminate even after specimen dilution. Rapid screening tests with high PPVs are used at CGCMC to allow reporting of positive results without confirmatory testing. BinaxNOW® RSV and the 3M™ Rapid Detection RSV Test demonstrated PPVs, of 93% and 95%, respectively. When RSV prevalence is low, for example, 10%, calculated PPVs of BinaxNOW® RSV and the 3M™ Rapid Detection RSV Test fall to 53% and 64%, respectively. Xpect®RSV, while the least sensitive assay, had a PPV of 100%. At CGCMC, all negative rapid screening tests undergo confirmatory testing to maximize RSV detection. For this purpose, DFA demonstrated excellent sensitivity and an acceptable turn-around-time and is the test of choice among non-amplification methods.

METHODS

STUDY DESIGN

This was an IRB-approved, multicenter, prospective clinical trial using leftover specimens. The 3M™ Rapid Detection RSV Test (3M Health Care, Saint Paul, MN) is an investigational in vitro diagnostic device for rapid detection of RSV. It was compared to viral culture, direct immunofluorescence, and the BinaxNOW® RSV (Binax, Scarborough, ME). Results of the Xpect® RSV test (Remel, Inc., Lenexa, KS), which is currently used at CGCMC, were also included but were not part of the clinical trial.

3M™ RAPID DETECTION RSV TEST

Principles of the Test

The 3M™ Rapid Detection RSV Test is a qualitative immunochromatographic test that utilizes the 3M™ Rapid Detection Reader for the identification of RSV F-protein antigens in nasal wash/aspirate, nasopharyngeal (NP) aspirate, and NP swab samples. Sample is added to the Sample Buffer. This sample is then mixed with the Assay Tip containing fluorescent-dyed particles conjugated to specific RSV antibodies that bind to RSV antigen if present **(Figure 1)**. Sample is then applied into the sample well of the Test Cartridge. The sample migrates along the strip. Fluorescent-dyed particles coated with anti-RSV antibodies bind to RSV F-protein antigens, if present in the sample. As the sample migrates along the strip, RSV-bound particles are captured by anti-RSV antibodies at the detection zone. Excess fluorescent-dyed particles are captured at the internal standard zone **(Figure 2)**. The Reader **(Figures 3 *and* 4)** then measures the amount of fluorescence emitted at the detection zone and at the internal standard zone. The instrument calculates a ratio (RAMP Ratio) of the detection zone fluorescence reading to the internal standard zone reading. The Reader then compares these ratios to pre-defined threshold limits to determine a positive or negative result for RSV in the tested sample.

FIGURE 1: 3M RAPID DETECTION RSV TEST STEPS

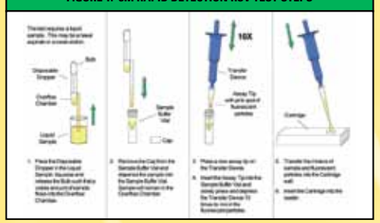


FIGURE 2: CARTRIDGE REACTION FOR 3M™ RAPID DETECTION TEST

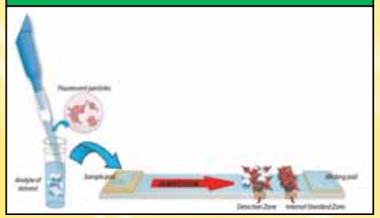


FIGURE 3: 3M™ RAPID DETECTION READER

FIGURE 4: 3M™ RAPID DETECTION READER

The Reader measures fluorescence, calculates the RAMP ratio, and determines a positive, negative, or indeterminate result.

3M™ RAPID DETECTION RSV TEST PROCEDURE

Specimens, collected in M4 viral transport medium, were held at room temperature for at least 15 minutes prior to testing. 150µl was transferred into the Sample Buffer Vial. The Assay Tip, attached to the Transfer Device, was inserted into the Sample Buffer Vial. The sample with buffer were mixed with the Assay Tip's fluorescent particles by slowly pressing and releasing the Transfer Device plunger 10 times. 75µl of mixed sample was dispensed into the sample well of the Test Cartridge. The Test Cartridge was inserted into the 3M™ Rapid Detection Reader. Test results were complete approximately 15 minutes from Test Cartridge insertion. Results at each trial site were displayed as fluorescence emission units. Specimens were considered indeterminate if the Reader aborted the test. Indeterminate results were retested. If the repeat was indeterminate, the sample was diluted 1:2 in viral transport medium and retested. If no result was obtained after specimen dilution, the final result was recorded as indeterminate.

After the completion of the study, 3M determined the "RAMP Ratio" denoting a positive or negative result based on data from all sites. The RAMP ratio is calculated by the Reader and is a ratio of the detection zone fluorescence reading to the internal standard zone fluorescence reading.

BinaxNOW® RSV Test: The BinaxNOW® RSV Test was performed according to manufacturer's instructions. Specimens were held at room temperature for at least 15 minutes prior to testing. Specimens that did not flow through the membrane or for which the control line did not appear were repeated. If a valid test result was not obtained after repetition, the specimen was considered indeterminate.

Xpect®RSV Test: The Xpect®RSV Test was performed according to the manufacturer's instructions. Specimens were usually transported on ice or were refrigerated prior to testing. Specimens were not deliberately held at room temperature for a specified amount of time prior to testing. Specimens that did not flow through the membrane or for which the control line did not appear were repeated. If a valid test result was not obtained after repetition, the specimen was considered indeterminate.

Direct immunofluorescence (DFA): 0.5ml of specimen was mixed with 5.0ml phosphate-buffered saline and centrifuged at 700x g for 10 minutes. Supernatant fluid was removed and 0.025ml of sediment was applied to a microscope slide by cytocentrifugation. After fixation, slides were stained with RSV DFA reagent from Diagnostic Hybrids, Inc. (Athens, Ohio). Slides were read with a fluorescence microscope for granular cytoplasmic fluorescence. Negative smears containing fewer than 25 columnar epithelial cells were considered indeterminate.

Viral culture: 0.2ml of specimen was inoculated to one R-Mix shell vial (Diagnostic Hybrids, Inc.) which was centrifuged at 3000 x g for 60 minutes at room temperature. After incubation at 35°C for 42-48 hrs, coverslips were fixed and stained with RSV DFA reagent (Diagnostic Hybrids, Inc). Coverslips were examined with a fluorescence microscope for granular cytoplasmic fluorescence.

RESULTS

STUDY PARAMETERS

Study Period	December 2008 to mid-January 2009	
Number of Specimens	199	
Nasopharyngeal Aspirates	191	
Nasopharyngeal Swabs	8	
Sex	Male: 52%	Female: 48%
Age Distribution	≤ 6 months: 46%	≤ 24 months old: 81%
RSV Prevalence	55%	

3M™ RAPID RSV vs. DFA AND CULTURE* N=178

		DFA or Culture POS	DFA or Culture NEG
3M™	POS	88	5
	NEG	10	75

Sensitivity = 90% PPV = 95%
Specificity = 94% NPV = 88%

9/10 DFA or culture POS/3M NEG specimens were positive for RSV by the ProFlu+™ Assay (Prodesse).

5/5 DFA and culture NEG/3M POS specimens were negative for RSV by the ProFlu+™ Assay; 2 were positive for influenza B by the ProFlu+™ Assay

BinaxNOW® RSV vs. DFA AND CULTURE* N=185

		DFA or Culture POS	DFA or Culture NEG
Binax	POS	86	6
	NEG	22	71

Sensitivity = 80% PPV = 93%
Specificity = 92% NPV = 76%

*including only specimens for which both DFA and culture results were available and excluding indeterminates

XPECT® vs. DFA AND CULTURE* N=185

		DFA or Culture POS	DFA or Culture NEG
3M™	POS	69	0
	NEG	39	77

Sensitivity = 64% PPV = 100%
Specificity = 100% NPV = 66%

DFA vs. DFA AND CULTURE* N=152

		DFA or Culture POS	DFA or Culture NEG
Binax	POS	108	0
	NEG	2	82

Sensitivity = 98% PPV = 100%
Specificity = 100% NPV = 98%

*including only specimens for which both DFA and culture results were available and excluding indeterminates

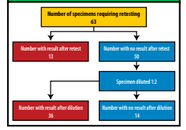
CULTURE vs. DFA AND CULTURE* N=192

		DFA or Culture POS	DFA or Culture NEG
3M™	POS	96	0
	NEG	14	82

Sensitivity = 87% PPV = 100%
Specificity = 100% NPV = 85%

*including only specimens for which both DFA and culture results were available and excluding indeterminates

ANALYSIS OF SPECIMENS REQUIRING RETESTING BY 3M™ RAPID DETECTION TEST



PERFORMANCE CHARACTERISTICS FOR ALL 5 TESTS PERFORMED SIMULTANEOUSLY N=165

TEST	SENSITIVITY (%)	SPECIFICITY (%)	PPV (%)	NPV (%)
DFA*	99% (93/94)	100% (71/71)	100% (93/93)	99% (71/72)
Culture	86% (81/94)	100% (71/71)	100% (81/81)	85% (71/84)
BinaxNOW® RSV	79% (74/94)	92% (65/71)	93% (74/80)	76% (65/85)
Xpect® RSV	63% (59/94)	100% (71/71)	100% (59/59)	66% (71/106)
3M™ RSV**	90% (85/94)	94% (67/71)	96% (85/89)	88% (67/76)

*DFA was significantly more sensitive than all other tests by Fisher's Exact Test
**3M™ Rapid Detection RSV was significantly more sensitive than BinaxNOW® RSV and Xpect® RSV by Fisher's Exact Test

RESULTS & DISCUSSION

1. DFA was the most sensitive test among the 5 test methodologies compared.
2. The 3M™ Rapid Detection RSV Test was more sensitive than BinaxNOW® RSV and Xpect® RSV.
3. Xpect® RSV had the lowest sensitivity among rapid immunochromatographic methods but the highest PPV (100%). During periods of low prevalence of RSV, false-positive results should not occur.
4. At CGCMC, negative rapid immunochromatographic results are tested by a more sensitive confirmatory assay. For this purpose, DFA demonstrated excellent sensitivity and an acceptable turn-around-time and is the test of choice among non-nucleic acid amplification methods for confirmation of negative rapid RSV antigen tests.
5. NP aspirate specimens, due to their viscosity, were problematic for all rapid membrane tests but particularly for the 3M™ Rapid Detection RSV Test; 32% of specimens required at least one repetition and 7% were indeterminate even after specimen dilution.

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