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PERFORMANCE EVALUATION OF RESPDIRECT™ SPECIMEN COLLECTION KIT IN THE PANTHER FUSION® SARS-CoV-2/Flu A/B/RSV ASSAY

[1]Beatriz Amro, [1]Youna Kang, [1]Gayani Dedduwa-Mudalige, [1]Anh Trieu, [1]Isai Garcia, [1]Denise Carigo, [1]Andrew Worlock, [1]Sangeetha Nair

[1] Hologic, Inc. 10210 Genetic Center Drive, San Diego, CA 92121, USA

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beatriz.amro@hologic.com

Abstract

Background: The RespDirect™ specimen collection kit ("RespDirect") is a new FDA cleared specimen collection kit for collection and testing of respiratory specimens. It uses the same swab for collection of nasal (NS) or nasopharyngeal (NP) specimens and an Enhanced Direct Load Tube containing eSTM media. These RespDirect collection kit can be loaded directly onto the Panther Fusion® system. The Panther Fusion® SARS-CoV-2/Flu A/B/RSV (Fusion) assay is a multiplex nucleic acid amplification test currently cleared for the detection of SARS-CoV-2, influenza A, influenza B, and respiratory syncytial virus (RSV) from NP specimens. This study evaluates the performance of respiratory samples collected with RespDirect in the Fusion assay. **Methods:** Limit of detection (95% LoD) was assessed for all 4 viruses by testing 30 replicates at 1x LoD over 3 days. Collection device equivalency (CDE) was performed by testing 20 replicates per concentration for all analytes. Samples were prepared using NP specimens collected with RespDirect versus NP swab collected in VTM commonly used as standard of care (SOC). Precision was evaluated by 2 operators, testing 2 concentrations in 3 RespDirect tube lots, with 3 reagent lots on 2 Panthers over 6 days. Potential interfering endogenous and exogenous substances were tested in the absence and presence of target analytes. Specimen stability was evaluated at multiple timepoints at 30°C, 4°C, -20°C and -70°C. For these studies, negatives were prepared using pooled negative clinical matrix. Positives were prepared by spiking all 4 viruses into the matrix to concentrations ranging from 1x-10x LoD. **Results:** The 95% LoD was 0.110, 0.030, 0.029, 0.053 TCID50/mL for Flu A, Flu B, RSVA and RSVB and 98.6 IU/mL for WHO SARS. There was no statistically significant difference in positivity between RespDirect and SOC collection device ($p > 0.05$) in CDE studies. The standard deviation in cycle threshold (Ct) was ≤1.11 at 2x LoD and above for all analytes tested. Potential interfering substances did not interfere with the assay. Specimens were stable at 30°C for 7 days, and at 4°C, -20°C and -70°C for 3 months. **Conclusion:** RespDirect collection kit demonstrated equivalent performance to SOC, while effectively stabilizing specimens for molecular testing. Its usage increases ease-of-use and traceability because a single collection kit, used for either NS or NP specimens, is directly loaded onto Panther for testing. Its workflow eliminates capping and uncapping, reduces hands on time and opportunity for repetitive stress injuries and exposure to respiratory pathogens. This makes RespDirect an attractive option to streamline collection and testing of respiratory specimens.

Introduction

RespDirect is an FDA cleared specimen collection kit for the collection of NS and NP specimen using the same swab for collection and testing of Respiratory Specimens. This study evaluates the performance of respiratory samples collected with RespDirect in the Fusion assay.

Methods

Limit of Detection: (95% LoD) this was verified for all 4 viruses by testing 30 replicates at 1x LoD established in viral transport media (VTM) by testing over 3 days.

Collection Device Equivalency: Collection Device Equivalency was performed by testing 20 replicates each for all analytes at 0.5x, 2x and 5x LoD. A negative panel was also tested. Samples were prepared using NP specimens collected with RespDirect versus NP swab collected in VTM commonly used as standard of care (SOC).

Precision: Precision was evaluated by 2 operators, testing panels at 2x and 3x LOD in 3 Lots of Enhanced Direct Load Tubes, with 3 lots of assay reagent on 2 Panthers over 6 days.

Interference: Potential endogenous and exogenous interfering substances were tested in the absence and presence of target analytes.

Stability: Specimen stability was evaluated at multiple timepoints at 30°C, 4°C, -20°C and -70°C. by testing RespDirect specimens spiked to 2x and 10x LoD.

For these studies, negatives were prepared using pooled negative clinical matrix. Positives were prepared by spiking all 4 viruses into the matrix.

Viruses used were First WHO International Standard for SARS-CoV-2 RNA, NIBSC code:20/146, Influenza A H3N2, Strain Kansas/14/17, Influenza B, Strain Washington/02/19, RSV-A, isolate 2006, RSV-B, Strain CH93(18)-18. All four strains were culture fluid from Zeptometrix.

RespDirect Specimen Collection Kit

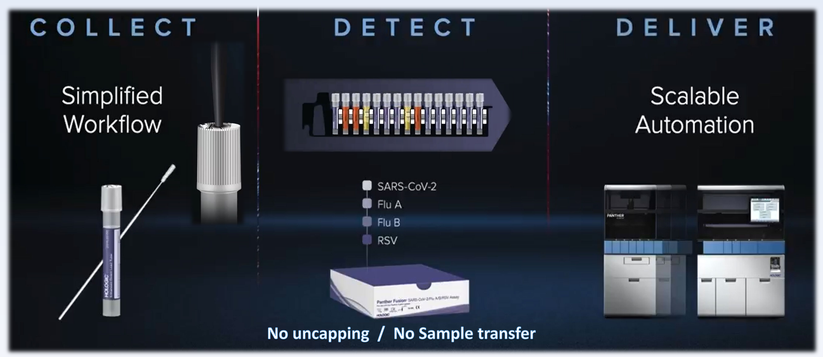

Figure 1. RespDirect Collection Kit workflow on the Panther Fusion system.

Results

Limit of Detection

Table 1. Limit of detection for Flu A, Flu B, RSV A, RSV B and WHO SARS; Table 1 shows that the LoD previously established with the VTM sample type for the Panther Fusion® SARS-CoV-2/Flu A/B/RSV assay was successfully verified with RespDirect.

Virus	LOD	Unit
Flu A	0.110	$TCID_{50}$/mL
Flu B	0.030	
RSV A	0.029	
RSV B	0.053	
WHO SARS	98.6	IU/mL

Collection Device Equivalency

There was no statistically significant difference in positivity between NP specimens collected with RespDirect and NP swab specimens collected in VTM (SOC) collection device ($p > 0.05$) on testing negative, 0.5X, 2X and 5X LoD panels.

Precision

Table 2. Precision at 2x and 5x LoD; Standard deviations were ≤ 1.11 in cycle threshold (Ct).

Virus	LoD	Contribution to Total Standard Deviation							
		Inter-Day	Inter-Operator	Inter-Instrument	Inter-Lot	Inter-RespDirect Lot	Inter-Run	Intra-Run	Total SD
Flu A	2x	0.09	0.00	0.20	0.27	0.00	0.14	0.92	1.00
	5x	0.21	0.03	0.30	0.09	0.12	0.00	0.50	0.64
Flu B	2x	0.00	0.00	0.00	0.00	0.04	0.00	0.86	0.86
	5x	0.12	0.00	0.13	0.11	0.09	0.00	0.44	0.50
RSV	2x	0.00	0.17	0.17	0.00	0.11	0.29	0.71	0.81
	5x	0.17	0.00	0.15	0.14	0.12	0.10	0.50	0.59
WHO SARS	2x	0.00	0.00	0.16	0.20	0.35	0.00	1.02	1.11
	5x	0.00	0.00	0.12	0.00	0.16	0.00	0.52	0.56

Interference

Table 3. Potential interfering substances tested using RespDirect; Potential interfering substances did not interfere with the assay.

Type	Substance	Percent Positivity Positive 3x LoD Pool (N=5)	Percent Negativity Negative Pool (N=5)
Endogenous	Mucin (60 µg/mL)	100%	100%
	Blood (human) (2 % v/v)	100%	100%
Nasal sprays or drops	Neo-Synephrine (15 % v/v)	100%	100%
	Anefrin (Afrin) (15 % v/v)	100%	100%
	Saline (15 % v/v)	100%	100%
	Ventolin HFA	100%	100%
Nasal corticosteroids	Nasacort (5 % v/v)	100%	100%
	Dexacort (12 µg/mL)	100%	100%
	QVAR, Beconase AQ (15 ng/mL)		
	Rhinocort (Nasal Spray) (5 % v/v)	100%	100%
	Flonase (5 % v/v)	100%	100%
	Nasonex (0.5 ng/mL)	100%	100%
	AEROSPAN (10 µg/mL)		
Nasal gel	Zicam (Allergy Relief) (5 % v/v)	100%	100%
Throat lozenges	Cepacol Extra Strength (0.7mg/mL)	100%	100%
Anti-viral drugs	Relenza (3.3 mg/mL)	100%	100%
	TamiFlu (400 ng/mL)	100%	100%
	Virazole (10.5 µg/mL)		
Antibiotic, nasal ointment	Bactroban cream (1.6 µg/mL)	100%	100%
Antibiotic, systemic	Tobramycin (33.1 µg/mL)	100%	100%
Analgesic	Acetaminophen (0.156 mg/mL)	100%	100%
Solvent Control	Water (5 % v/v)	100%	100%
	Dimethyl sulfoxide (5 % v/v)	100%	100%

Specimen Stability

Table 4. Specimen stability for specimen collected with RespDirect; RespDirect specimens at 3x and 10x LoD were stable at all storage conditions. 20 replicates per storage temperature per timepoint. The condition was stable when ≥95% of replicates were positive per panel per storage condition.

Storage Temperature (°C)	Stability
30 °C	7 Days
4 °C	3 Months
-20 °C	
-70 °C	

Conclusion

RespDirect showed equivalent performance to NP VTM (SOC). It is effective in stabilizing specimens, it is loaded directly onto Panther, eliminating capping and uncapping, making RespDirect an attractive option to streamline collection and testing of respiratory specimens.

Acknowledgment

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