Evaluation of DiaSorin Molecular Simplexa™ Bordetella Direct Kit for the Detection and Differentiation of *Bordetella pertussis* and *Bordetella parapertussis*

Trang Ton, MB(ASCP)[1], Mong-Diep Tran, MB(ASCP)[1], Erica Tam, MB(ASCP)[1], Keith Newton, MB(ASCP)[1], Renee Webb, MT (ASCP), James Dunn, PhD, D(ABMM)[1,2]

Department of Pathology[1], Texas Children's Hospital, Department of Pathology and Immunology[2], Baylor College of Medicine

ABSTRACT (revised)

INTRODUCTION

Whooping cough is a highly contagious respiratory tract infection caused mainly by *Bordetella pertussis* and, less frequently, by *Bordetella parapertussis*. The Simplexa™ Bordetella Direct assay (DiaSorin Molecular, Cypress, CA) is a qualitative, real-time PCR assay that combines direct amplification, detection and differentiation of *B. pertussis* (Bp) and *B. parapertussis* (Bpp) DNA from nasopharyngeal (NP) swabs. This study compares the performance of the Simplexa Bordetella Direct assay to the currently used lab-developed real-time PCR assay (LDT) at Texas Children's Hospital (TCH).

METHODS

A total of 90 NP swab samples collected using the ESwab Mini-tip Collection & Transport device (BD, Franklin Lakes, NJ) were tested: 82 prospective and retrospective patient specimens and 8 previously negative patient samples contrived by adding dilutions of Bpp from 1x10^3 to 2.5x10^5 cells/mL. For the LDT, 200µL of sample was extracted using the EZ1 DNA Tissue Kit on the EZ1 Advanced XL (Qiagen, Germantown, MD) with the Bacteria Card and eluted in 50µL. Real-time PCR targeting IS481 (Bp) and IS1001 (Bpp) was performed on the LightCycler 2.0 (Roche, Indianapolis, IN) using 5µL input DNA. ESwab samples were archived at -80˚ C after initial testing and were retrieved and retested with the Simplexa Bordetella Direct kit on the LIAISON® MDX instrument. Automated analysis was performed with the LIAISON® MDX Studio Software. Serial dilutions of bacterial DNA (ATCC, Manassas, VA) at defined concentrations were tested by both assays to compare the analytical sensitivity for both targets.

RESULTS

The clinical sensitivity for the Simplexa assay was 98% for Bp (49/50) and 100% for Bpp (10/10) compared to the LDT. Clinical specificity was 100% for both targets. The Simplexa assay demonstrated higher limits of detection (LOD) by 2 Log_{10} for Bp and 1 Log_{10} for Bpp compared to the LDT. Specifically, the Simplexa LOD was 3 fg/µL for Bp and10 fg/µL for Bpp, while the LDT detected 30 ag/µL and 1 fg/µL, respectively. Crossing thresholds for patient samples tested on the Simplexa assay were on average 7.6 and 7.8 cycles higher than those on the LDT for Bp and Bpp, respectively.

CONCLUSION

The overall agreement for patient samples was 98.9% between the LDT and Simplexa . With a total test time of 1.25 hours (compared to 2.5 hours for the LDT) and reduction in hands-on time by eliminating extraction and testing NP specimens directly, the Simplexa assay could be run on-demand multiple times per day; thus impacting the time to detection of organisms with public health implications and institutional infection control concerns for healthcare exposures.

INTRODUCTION

Pertussis or whooping cough is caused by small gram negative bacteria: *Bordetella pertussis* and *Bordetella parapertussis*. In the 20th century, pertussis was one of the most common childhood diseases and a major cause of childhood mortality in the United States. With the availability of pertussis vaccine in the 1940s, the infection has decreased more than 75% compared to the pre-vaccination era. Since the 1980, there has been an increase in the number of reported cases of pertussis. 2012 was the highest peak year that CDC reported since 1955 with 48,277 cases of pertussis total in which 4,994 cases (10.3%) from children younger than one year of age; 8,280 cases (17.2%) from children of 1-6 years of age; and 23,970 cases (49.7%) from children of 7-19 years of age. Thus it is important to accurately identify Bordetella infection in early stages for proper antibiotic treatment and exposure prevention.

Recently, the FDA has approved the Simplexa™ Bordetella Direct assay (DiaSorin Molecular) for use with frozen nasopharyngeal swab specimens in Remel M4, Remel M4RT, Remel M5, Remel M6, UTM, BD UVT and Liquid Aimes (Eswab) transport media. The Simplexa assay system is a real-time PCR assay that enables direct amplification, detection and differentiation of *Bordetella pertussis* and *Bordetella parapertussis* DNA from unprocessed nasopharyngeal specimens without nucleic acid extraction. Primers and fluorescent probes are used together to amplify and detect *Bordetella pertussis* (IS481), *Bordetella parapertussis* (IS1001), and internal control (which is used as an inhibition and PCR control) on the LIAISON® MDX Integrated Cycler (DiaSorin Molecular) with LIAISON® MDX Studio Software. This study compares the performance of the Simplexa assay to the currently used lab-developed real-time PCR assay (LDT) at Texas Children's Hospital (TCH) using the EZ1 DNA Tissue Kit on the EZ1 Advanced XL (Qiagen) with the Bacteria Card. Real-time PCR is with performed on LightCycler 2.0 (Roche).

MATERIALS & METHODS

Nasopharyngeal specimens collected using BD Eswab Minitip Collection & Transport System were submitted to the Molecular Microbiology laboratory at Texas Children's Hospital for *Bordetella pertussis* and *Bordetella parapertussis* detection by the lab-developed test. These samples were retested prospectively and retrospectively by the Simplexa™ Bordetella Direct assay for evaluation. The LDT assay involves nucleic extraction from 200µL of sample using the EZ1 DNA Tissue Kit on the EZ1 Advanced XL (**Figure 1A**) with the Bacteria Card. Sample is eluted in 50µL final volume. Real-time PCR detection is performed on the LightCycler 2.0 (**Figure 1B**) using 5µL of nucleic acid and 15µL of master mix (**Table 1**).

LDT Reaction Component	µL/Rxn
Nuclease-Free Water	3.3
LC FastStart DNA Master Hybridization Probes	2.0
$MgCl_2$ (25mM)	3.2
BP-F Primer (10µM)	1.0
BP-R Primer (10µM)	1.0
BPa-F Primer (10µM)	1.0
BPa-R Primer (10µM)	1.0
BP 640 Probe (10µM)	0.4
BP FL Probe (10µM)	0.4
Bpa FL Probe (10µM)	0.4
Bpa 705 Probe (10µM)	0.8
AmpErase Uracil N-glycosylase	0.5
Total Volume	**15.0**

Table 1: Master Mix Preparation for TCH LDT Assay

The Simplexa Bordetella Direct assay contains Reaction Mix with contents: DNA polymerase, buffer, dNTPs, Internal Control DNA with Q670-labeled fluorescent probe to target DNA Internal Control, FAM-labeled fluorescent probe to target IS481 gene, CFR610-labeled fluorescent probe to target IS1001 gene, and primers specific for detection of *Bordetella pertussis, Bordetella parapertussis* and DNA Internal Control. The assay was performed on the LIAISON® MDX (**Figure 1C**) using the Direct Amplification Disc (DAD, **Figure 1D**) by loading 50µL of Reaction Mix into the Reaction (R) well and 50µL of sample into the Sample (S) well (**Figure 1E**). Results were analyzed using LIAISON® MDX Studio Software.

Analytical sensitivity studies were performed using commercial bacterial DNA purchased from ATCC for *Bordetella pertussis* (BAA-589DQ) and *Bordetella parapertussis* (BAA-587D-5). Dilutions were prepared using ESwab media and tested by both assays (**Table 3**).

FIGURES

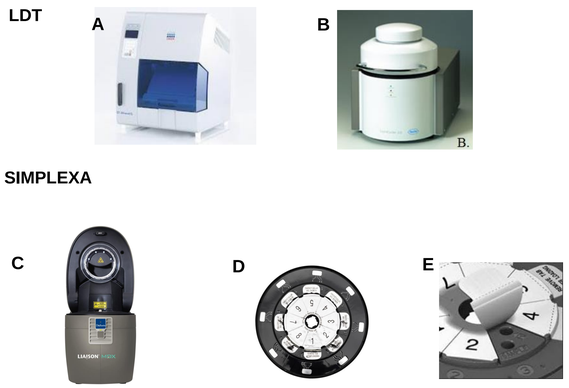

Figure 1. (**A**) EZ1 Advanced XL, (**B**) LightCycler 2.0, (**C**) LIAISON MDX, (**D**) Direct Amplification Disc (DAD), (**E**) Sample & Reagent wells on the DAD

RESULTS

A total of 90 NP swab samples were evaluated by the Simplexa Bordetella Direct assay: 82 prospective and retrospective patient specimens and 8 previously negative patient samples contrived by adding dilutions of Bpp from 1x10^3 to 2.5x10^5 cells/mL. Using the TCH LDT assay as a comparison method, clinical sensitivity and specificity results were as below (**Table 2**):

		Simplexa Bordetella Direct					
		BP			Bpp		
		POS	NEG	TOTAL	POS	NEG	TOTAL
TCH LDT	POS	49	1	50	10	0	10
	NEG	0	40	40	0	80	80
	TOTAL	49	41	90	10	80	90
Clinical Sensitivity		98%			100%		
Clinical Specificity		100%			100%		
Positive Predictive Value (PPV)		100%			100%		
Negative Predictive Value (NPV)		97.6%			100%		
Overall Agreement		98.9%					

Table 2: Clinical Sensitivity and Specificity Results

One sample originally testing positive for Bp (Ct = 29.85 with slightly shifted melting temperature at 57.98ºC) by the LDT tested negative for Bp on one replicate and EC500-Data Quality (data processing error due to noise, weak or late amplification in the signal) on the other by the Simplexa. Upon repeat in duplicate using 10µL of nucleic acid (extracted from the EZ1 Advanced XL) diluted in 40µL of PBS, 1 out of 2 replicate yielded weakly positive for Bp (Ct = 34.9) on the Simplexa and the other replicate was flagged as EC500 error. This is likely due to slight difference in sensitivities of the two assays. Therefore, weak positive samples near the lower limits of detection will be not detected occasionally. Crossing thresholds for patient samples tested on the Simplexa assay were on average of 7.6 and 7.8 cycles higher than those on the LDT for Bp and Bpp, respectively.

One patient sample originally testing negative for both Bp & Bpp targets by the LDT tested EC500 on the first run, but negative for both targets upon repeat by the Simplexa.

Analytical sensitivity (LOD) studies were performed using dilutions of *Bordetella pertussis* and *Bordetella parapertussis* DNA (purchased from ATCC) in ESwab media. The Simplexa assay demonstrated higher limits of detection by 2 Log_{10} for *Bordetella pertussis* and 1 Log_{10} for *Bordetella parapertussis* compared to the LDT. Refer to **Table 3** for details.

Target	Dilution	Hit Rate	
		TCH LDT	Simplexa
Bp	30 pg/µL	1/1	1/1
	3 pg/µL	1/1	1/1
	300 fg/µL	3/3	3/3
	30 fg/µL	3/3	3/3
	3 fg/µL	3/3	3/3
	300 ag/µL	3/3	1/3
	30 ag/µL	3/3	0/3
	3 ag/µL	1/3	0/3
	300 zg/µL	0/3	0/3
Bpp	10 pg/µL	1/1	1/1
	1 pg/µL	3/3	3/3
	100 fg/µL	3/3	3/3
	10 fg/µL	3/3	3/3
	1 fg/µL	3/3	1/3
	100 ag/µL	0/3	0/3
	10 ag/µL	0/3	n/a

Table 3: Analytical Sensitivity Results

Analytical Specificity

Analytical specificity studies performed at TCH included testing *Streptococcus pneumoniae*, *Streptococcus mitis*, *Haemophilus influenzae*, *Staphylococcus aureus*, *Neisseria sicca*, *Moraxella catarrhalis*, *Staphylococcus epidermidis*, and Corynebacterium spp.

RESULTS cont'd

Bacteria suspensions from 1:1,000 dilution of ~0.5 McFarland were tested for each organism. No cross reactivity was found for all 8 bacterial samples.

Reproducibility

Reproducibility studies performed at TCH included testing a panel of 3 samples (1 positive Bp & Bpp sample obtained from Simplexa™ Bordetella Positive Control Pack MOL2760 , 1 previously tested Bp positive patient sample, and 1 previously tested Bp & Bpp negative patient sample) by 6 different technologists on different days. The results demonstrated that the Simplexa was 100% in agreement with the expected results among all operators.

External Control Study

To validate the use of the Simplexa DNA Internal Control (DNA IC) which processed in each reaction within the DAD as the daily run control, a panel included a positive Bp & Bpp sample obtained from Simplexa™ Bordetella Positive Control Pack MOL2760 , a previously tested Bp positive patient sample, and a previously tested Bp &Bpp negative patient sample were alternately tested daily for 20 consecutive test days with the exception of day 20 being delayed due to shortness of reagents. Day 20 testing was performed as soon as reagents were received. All runs yielded valid Internal Control.

CONCLUSIONS

- The Simplexa assay was easy to use with total set up time to result for a disc with 8 samples at ~ 1.25 hours compared to the in-house LDT assay (~ 2.5 hours).
- The Simplexa involved fewer steps with less hands-on time, thus reducing processing errors and the risks of contamination.
- With no extraction step required, the Simplexa assay could be run on-demand multiple times per day compared to the current practice at TCH with 1 testing batch per day/3 days of testing per week. This could also be an advantage to Infection Control as the earlier the organism detected, the earlier the treatment executed, and the quicker the patient to proper isolation unit, thus improving hospital exposure rate.
- The Simplexa was slightly less sensitive in detecting both Bp and Bpp targets than the LDT. The overall positive/negative agreement for all patient samples was 98.9%.
- The Simplexa demonstrated less inhibition/better PCR performance. Internal Control was valid with no failure identified for all samples tested during this study.

REFERENCES

1. Simplexa™ Bordetella Direct IFUK.US.MOL2750; Rev. 01; 07 August 2018
2. www.cdc.gov/pertussis/clinical/disease.specifics.html
3. www.cdc/2012 Final Pertussis Surveillance Report
4. LightCycler Bordetella Detection by Real-Time PCR (Lucidoc #12958)
5. EZ1 Advanced XL: EZ1 DNA Tissue Kit (Lucidoc #17799)

ACKNOWLEDGMENTS

The authors would like to thank DiaSorin Molecular for providing evaluation reagents, technical and analytical support for this study.