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

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ABSTRACT (revised)

Nasopharyngeal specimens collected using BD Eswab Minipin Collection & Transport device (BD, Franklin Lakes, NJ) were tested prospectively and retrospectively by the Simplexa™ Bordetella Direct assay for detection. 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 Bacteroides Card. Sample is eluted in 50µL final volume. Real-time PCR detection is performed on the LightCycler 2.0 (Figure 2B) using 5µL of nucleic acid and 15µL of master mix (Table 1).

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 specimens contributed by adding dilutions of BP (n=7) and Bp (n=1) to the LDT RNA control. Using the TCH LDT assay as a comparison method, clinical sensitivity and specificity results were as follows (Table 2):

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 & Bp sample obtained from Simplexa™ Bordetella Positive Control Pac MDX7010, 1 previously tested Bp positive patient sample, and 1 previously tested Bp & Bp 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 provides 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 of “daily DNA IC” with the LDT. This saves 95 days of testing per year. 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 demonstrated negative in detecting both Bp and Bp targets than the LDT. The overall positive/negative agreement for all patient samples was 98.9%.

REFERENCES

1. Simplexa™ Bordetella Direct IFUK.US.MDL750; Rev. 01; 07 August 2018
4. LightCycler® Bordetella Detection by Real-Time PCR (Ludwig et al. 2010)
5. EZ1 Advanced XL: EZ1 DNA Tissue Kit (Ludwig #17799)

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

INTRODUCTION

Whorping cough is a highly contagious respiratory tract infection caused mainly by Bordetella pertussis and less commonly, by Bordetella parapertussis. Simplexa™ Bordetella Direct assay (DiaSorin Molecular: Cress, CA) is a qualitative, real-time PCR assay that 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 Minipin Collection & Transport device (BD, Franklin Lakes, NJ) were tested prospectively and retrospectively by the Simplexa™ Bordetella Direct assay for detection. 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 Bacteroides Card. Sample is eluted in 50µL final volume. Real-time PCR detection is performed on the LightCycler 2.0 (Figure 2B) using 5µL of nucleic acid and 15µL of master mix (Table 1).

TABLE 1: Master Mix Preparation for TCH LDT Assay

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