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# Simplexa *Bordetella pertussis/parapertussis* PCR Background and Principle

## BACKGROUND

Pertussis (whooping cough) is an acute infectious illness of the respiratory tract caused by *Bordetella pertussis* (Bp). Pertussis-like symptoms have been attributed to related species with less severity including *B. parapertussis* (Bpp), *B. holmseii* and *B. bronchiseptica*. The illness occurs in all age groups but primarily in young children, finding the most serious in unvaccinated infants. In the last 15 years, reported cases of pertussis have increased despite vaccination programs. Some cases have been associated with waning immunity when the acellular vaccines were introduced as well as more sensitive tests such as PCR for detection of *Bordetella pertussis*. The acellular vaccine is made up of *Bordetella pertussis* inactivated virulence components including pertactin. Pertactin was included in the vaccine because of its role in mediating adherence to the cilia of respiratory epithelial cells resulting in stasis and difficulty clearing mucus secretions that affect transmission of *B. pertussis.* More recent studies suggest that *B. pertussis* has adapted to selective vaccine pressure as a pertactin-negative variant, eliminating pertactin expression [1, 2]. The loss of pertactin does not seem to affect the virulence of *Bordetella pertussis* possibly because of autotransporters within the organism that can compensate for the role of pertactin. The studies suggest that disease caused by pertactin-negative variants are greater in vaccinated individuals contributing to the increased rates of pertussis[3, 4].

In the past, laboratory diagnosis was traditionally based on culture which is considered the gold standard. Although culture is highly specific, Tilley P.A., et al.[5], found the sensitivity of culture to be only 36%. With the continuing resurgence of pertussis, PCR is being used more frequently for the detection of *Bordetella pertussis* and *Bordetella parapertussis* with a noticeable improvement in diagnostic accuracy and turn-around-time. Two target sequences, IS*481* and IS*1001,* were used for *B. pertussis* and *B. parapertussis*. The *B. pertussis* genome contains 50 – 200 copies of the IS*481* sequence making the IS*481* target very sensitive but not species specific. This same sequence is present in *B. holmseii* (8 – 10 copies) and occasionally *B. bronchiseptica* causing cross reactivity to occur. The *B. parapertussis* genome contains 20 – 22 copies of the IS*1001*target sequence that is used for the detection of *B. parapertussis.* The IS1001target sequence can occasionally be found in *B. bronchiseptica* [5, 6, 7].

## PRINCIPLE

The Simplexa ASR *Bordetella pertussis/parapertussis* assay is a real time PCR assay that utilizes two bi-functional fluorescent probe-primers for detection. These are referred to as Scorpion primers in which the primer is covalently linked to the probe. A hairpin loop containing the Scorpion probe is linked to the 5' end of the primer. The primer sequence contains a PCR blocker at the start of the hairpin loop to prevent the Taq DNA polymerase from reading through the Scorpion primer and copying the probe region. Such read-through would lead to the detection of non-specific PCR products, e.g. primer dimers or mispriming. The probe has a self-complementary stem sequence with a fluorophore at one end and a quencher at the other end. The loop of the probe includes a sequence that is complementary to an internal portion of the target sequence, *Bordetella pertussis* insertion sequence IS481 or *B. parapertussis* IS1001*.* After extension of the primer during PCR amplification, the specific probe sequence is able to bind to its complement within the same strand of DNA. The hybridization causes the hairpin loop to open so that the fluorescence is no longer quenched and an increase in signal is observed. The resulting signal is proportional to the amount of amplified product in the sample (Fig. 2)[6, 9]. An internal control (IC) is included in the assay that is amplified at the same time to detect PCR inhibition and to confirm that the reagents are working properly

Since the Scorpion probe and primer are physically linked, the probe reaction kinetics are extremely fast. The unimolecular reaction allows the Scorpion probes to provide stronger signals, shorter reaction times and better discrimination than other conventional bi-molecular techniques[8, 9].

**Figure 1: Gene target**

| Analyte | Gene Targeted | Probe Fluorophore | Excitation | Emission |
|---|---|---|---|---|
| *Bordetella pertussis* | IS*481* | FAM | 495 nm | 520 nm |
| *Bordetella parapertussis* | IS*1001* | CFR610 | 590 nm | 610 nm |
| Internal control | NA | Q670 | 644 nm | 670 nm |

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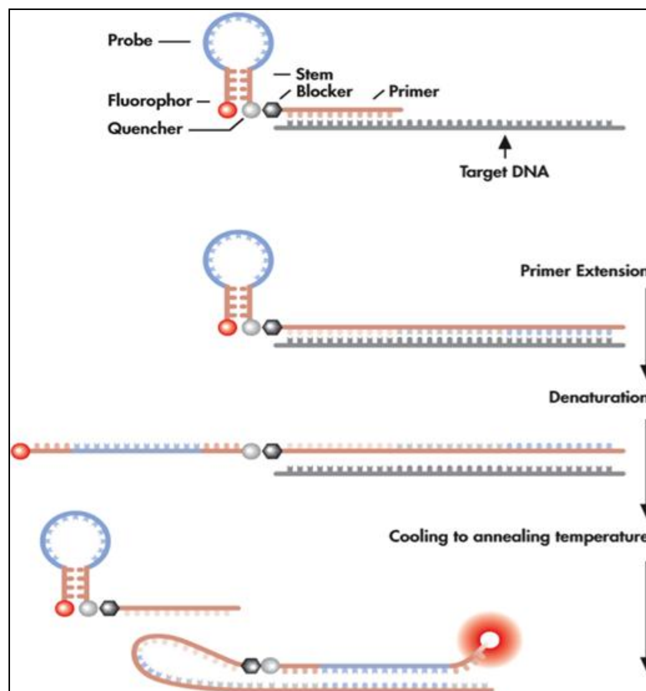
**Figure 2: Scorpion Primer Function**




1. The Scorpion primer acts as a primer and a probe. The probe forms a hairpin loop with a self-complimentary stem sequence so that the quenched reporter does not fluoresce. The primer is linked to the probe at the start of the hairpin loop.
2. During the annealing, the primer binds to the template and is extended.
3. The probe part of the Scorpion is complementary to the extension product of the attached primer. When the complementary strands are separated in the denaturation step of the next PCR step, the reporter separates from the quencher and opens the loop. When cooled to annealing temperature, the probe sequence binds to the internal target sequence. The reporter and the quencher are now far enough apart to generate detectable fluorescence.

## REFERENCES

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**Historical Record**

| Version | Written/Revised by: | Effective Date: | Summary of Revisions |
|---|---|---|---|
| 1 | P. Ackerman | 1.23.16 | Initial Version |
| 2 | P. Ackerman | 07.19.16 | Reformatted for CMS upload |