Evaluation of DiaSorin Molecular Simplexa™ Bordetella Real-Time Sample-to-Result PCR Test on the LIAISON® MDX System

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Introduction

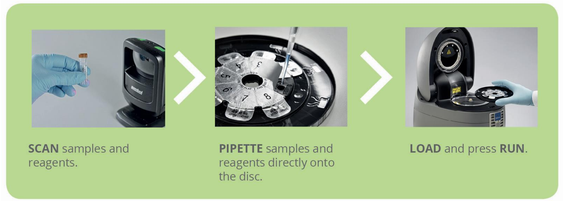
Bordetella pertussis (*B. pertussis*), the etiologic agent for whooping cough, remains a public health issue despite widespread vaccination of most children in the U.S. This is due to the failure of some parents to vaccinate their children, as well as waning immunity in the vaccinated population typically between the ages of 11 to 18 years. The very contagious nature of *B. pertussis* is responsible for localized community outbreaks of whooping cough requiring the need for a rapid, highly specific and sensitive test. DiaSorin Molecular has developed a simple to use "sample to answer" test for *Bordetella pertussis* and *Bordetella parapertussis,* the Simplexa™ Bordetella Direct kit, based on TaqMan PCR technology, for their LIAISON® MDX platform. The instrument utilizes an 8 well direct amplification disc into which the patient sample is added. Once placed into the LIAISON® MDX instrument, sample processing and PCR analysis are fully automated. The Simplexa test targets the repetitive element IS481, which is present as multiple copies per organism (50 - >100)

Objective: To determine the suitability of the LIAISON® MDX platform for detection of *B. pertussis/parapertussis* DNA directly from patient samples compared to our current method utilizing Luminex MultiCode® *B. pertussis/parapertussis* reagents on the Luminex ARIES® platform.

Materials and Methods

- Testing for *B. pertussis/parapertussis* on the LIAISON® MDX platform using the DiaSorin Molecular Simplexa™ Bordetella Direct Real-Time Sample-to-Result PCR
- Test was performed using 50 µL of patient sample (nasopharyngeal swabs in M4 transport media) as per the manufacturer's instructions. The patient samples are pipetted directly into the amplification disc (Figure 1). The disc is placed into the LIAISON® MDX instrument, and the operator presses "RUN" to start the analysis.

Figure 1. The LIAISON MDX® instrument



- . MultiCode® primers for *B. pertussis* and *B. parapertussis* PCR and ARIES® test cassettes were obtained from Luminex.
- Testing was performed using 200 µL of patient sample according to standard instrument settings supplied by Luminex using their proprietary SYNCT software.
- The MultiCode® primers are pipetted into the ARIES® MultiCode® Ready Mix tubes which are attached to the appropriate position of the test cassette.
- The patient sample is pipetted directly into the appropriate position of the test cassette which is placed into the ARIES® instrument for analysis.

Table 1. Samples tested on the ARIES ® and the LIAISON MDX®

Sample ID	IS481 Ct (FAM)	IS1001 Ct (CFR610)	Result	ARIES Result	Concordant
Bord Positive Control	17.7	19.1	BP/BPP Pos	BP/BPP Pos	Yes
610285	28.8		BP Pos	BP Pos	Yes
659882			ND	ND	Yes
661817	39.7		BP Pos	ND	No
315962	31.2		BP Pos	BP Pos	Yes
666167			ND	ND	Yes
666420			ND	ND	Yes
562103			ND	ND	Yes
347834	26.9		BP Pos	BP Pos	Yes
755778	25.4		BP Pos	BP Pos	Yes
552745			ND	ND	Yes
316845		25.7	BPP Pos	BPP Pos	Yes
572344	19.7		BP Pos	BP Pos	Yes
773996	20.0		BP Pos	BP Pos	Yes
495340	23.0		BP Pos	BP Pos	Yes
076053	19.6		BP Pos	BP Pos	Yes
405738	31.3		BP Pos	BP Pos	Yes
700859			ND	ND	Yes
710994			ND	ND	Yes
716338	32.8		BP Pos	BP Pos	Yes
682025			ND	ND	Yes
682061			ND	ND	Yes
681873			ND	ND	Yes
681852			ND	ND	Yes
558045			ND	ND	Yes
568510			ND	ND	Yes
569134			ND	ND	Yes
584961	37.7		BP Pos	BP Pos	Yes
581360			ND	ND	Yes
766287	18.1		BP Pos	BP Pos	Yes
628877			ND	ND	Yes
641451			ND	ND	Yes
642173			ND	ND	Yes
781018			ND	ND	Yes
786335			ND	ND	Yes
786347			ND	ND	Yes
845754			ND	ND	Yes
846643			ND	ND	Yes
854694			ND	ND	Yes
644733			ND	ND	Yes
675860			ND	ND	Yes
679246			ND	ND	Yes
973733	28.0		BP Pos	BP Pos	Yes
966848			ND	ND	Yes
965314	33.6		BP Pos	BP Pos	Yes

Sample ID	IS481 Ct (FAM)	IS1001 Ct (CFR610)	Result	ARIES Result	Concordant
347202			ND	ND	Yes
882377			ND	ND	Yes
585296			ND	ND	Yes
097608			ND	ND	Yes
097946			ND	ND	Yes
038073			ND	ND	Yes
018342			ND	ND	Yes
007328			ND	ND	Yes
434458		24.3	BPP Pos	BPP Pos	Yes
151437			ND	ND	Yes
172426			ND	ND	Yes
182068			ND	ND	Yes
182104	34.5		BP Pos	BP Pos	Yes
198095			ND	ND	Yes
206575			ND	ND	Yes
294766			ND	ND	Yes
290089			ND	ND	Yes
290088			ND	ND	Yes
290043			ND	ND	Yes
294517			ND	ND	Yes
290218			ND	ND	Yes
243569			ND	ND	Yes
226455			ND	ND	Yes
221004			ND	ND	Yes
313980			ND	ND	Yes
307142			ND	ND	Yes
661817			ND	ND	Yes
359293			ND	ND	Yes
425285			ND	ND	Yes
501466			ND	ND	Yes
510907			ND	ND	Yes
515010			ND	ND	Yes
547223			ND	ND	Yes
566380			ND	ND	Yes
572942			ND	ND	Yes
565167			ND	ND	Yes
603885			ND	ND	Yes
623178			ND	ND	Yes
613452			ND	ND	Yes
621780			ND	ND	Yes
654481			ND	ND	Yes
768182			ND	ND	Yes
775376			ND	ND	Yes
809133			ND	ND	Yes
818918	35.7		BP Pos	BP Pos	Yes
839917			ND	ND	Yes
857984			ND	ND	Yes

Table 2. Agreement between samples that tested positive with the ARIES® platform and the LIAISON MDX® results.

	Bordetella pertussis		
	Luminex Pos	Luminex Neg	% Agreement
Diasorin Pos	17	1	94%
Diasorin Neg	0	73	100%

	Bordetella parapertussis		
	Luminex Pos	Luminex Neg	% Agreement
Diasorin Pos	2	0	100%
Diasorin Neg	0	89	100%

Results

We tested 91 nasopharyngeal samples collected in M4 transport media that were submitted to our laboratory for *B. pertussis/parapertussis* PCR testing.

- There was 100% agreement between those samples that tested positive with the ARIES® platform and the LIAISON® MDX results (17/91) (Table 2).
- All samples resulting as negative on the ARIES® agreed with the LIAISON® MDX results with one exception; a sample (661817), which was negative on the ARIES® for *B. pertussis* was positive on the LIAISON® MDX. This sample was weakly positive with a Ct value of 39.7 and tested negative when repeated. This is not unusual for samples that contain very low copy number of the target DNA.
- The cutoff threshold for both the Simplexa™ and MultiCode® *B. pertussis/parapertussis* assays is a Ct value of 40. There were only 2 patient samples that were positive for *B. parapertussis,* which tested positive by both methods.

Conclusions

- The LIAISON® MDX system is a robust, simple to use, sample to result platform.
- The performance of the Simplexa™ Bordetella Direct assay compared very favorably to results obtained using the MultiCode ARIES® Bordetella test.
- A major advantage of the LIAISON® MDX method is the elimination of all upfront sample processing steps, significantly decreasing hands-on time, and greatly enhancing workflow, resulting in decreased turnaround time to a final result.