Evaluation of the Luminex ARIES® System for the Detection of *Bordetella Pertussis* DNA directly From Clinical Samples

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Introduction

Bordetella pertussis (*B. pertussis*), the etiologic agent for whooping cough is still a public health issue despite widespread vaccination of most children in the US. This is due to the failure of some parents to vaccinate their children, as well as waning immunity in the vaccinated population, typically those 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.

Recently, Luminex (Luminex Corporation, Austin, TX) has developed a new "sample to answer" automated instrument, the ARIES® (Figure 1), which is designed for their proprietary MultiCode® PCR technology. The instrument utilizes test cassettes into which the sample is added. The appropriate MultiCode® PCR primers are also manually added to a small master mix tube (Ready Mix) that clips on to the end of the cassettes. Once placed into the ARIES® instrument, nucleic acid extraction and PCR analysis are fully automated.

The purpose of this study was to determine the suitability of the ARIES® platform for detection of *B. pertussis* DNA directly from patient samples. The performance of the ARIES® *B. pertussis* method was compared to our current method utilizing MultiCode® *Bordetella pertussis* analyte-specific reagents on the Roche LightCycler® 2.0 (LC2) platform.

Materials and Methods

- 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 (nasopharyngeal swabs in M4 transport media) according to default instrument settings provided by Luminex in their proprietary SYNCT software.
- Our current method uses the same MultiCode® primers and reagents with analysis performed on the LC2.
- For the latter, DNA was extracted from 200 µL of patient sample (nasopharyngeal swabs in M4 universal transport medium) using the bioMerieux EasyMag, and eluted into 50 µL of EasyMag Buffer (bioMerieux Inc., Durham, NC).
- Five microliters of the DNA eluate was added to the master-mix containing the MultiCode® *B. pertussis* and *parapertussis* primers for a final volume of 25 µL.
- The PCR reaction also contained an internal amplification control.
- PCR amplification and melting point analysis were performed according to the manufacturer's recommendations.

Figure 1. The ARIES® "sample to answer" automated instrument workflow

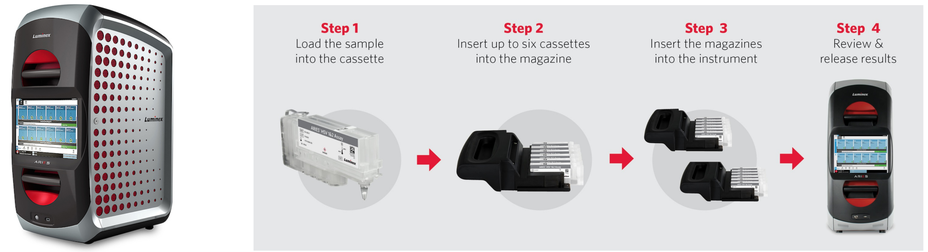

Table 1. Testing Results – Luminex Sample Panel

SAMPLE	ARIES Results				LightCycler		Expected Result	Concordant	Comment
	Ct (FAM)	Tm	Pertussis	ParaPertussis	Ct (FAM)	Tm			
1			Negative	Negative			Negative	Yes	
2	22.4	81.2	Positive	Negative			BP Pos	Yes	
3			Negative	Negative			Negative	Yes	
4	22.8	81.1	Positive	Negative			BP Pos	Yes	
5			Negative	Negative			Negative	Yes	
6	23.0	81.1	Positive	Negative			BP Pos	Yes	
7			Negative	Negative			Negative	Yes	
8	26.8	80.6	Positive	Negative			BP Pos	Yes	
9	26.7	80.6	Positive	Negative			BP Pos	Yes	
10	23.6	80.6	Positive	Negative			BP Pos	Yes	
11	18.9	80.6	Positive	Negative			BP Pos	Yes	
12	26.9	80.6	Positive	Negative			BP Pos	Yes	
13			Negative	Negative			Negative	Yes	
14	18.8	80.5	Positive	Negative			BP Pos	Yes	
15	31.1	80.8	Positive	Negative			BP Pos	Yes	
16	22.9	80.7	Positive	Negative			BP Pos	Yes	
17	22.3	80.6	Positive	Negative			BP Pos	Yes	
18	17.2	80.6	Positive	Negative			BP Pos	Yes	
Control			Negative	Negative			Neg Control	Yes	
Control	26.7	80.8 : 83.5	Positive	Positive			BP/BPP POS	Yes	
Control			Negative	Negative			Neg Control	Yes	
Control	26.9	80.7	Positive	Negative			BP Pos	Yes	
Control	30.2	83.3	Negative	Positive			BPP Pos	Yes	
Control	27.1	80.6 : 83.4	Positive	Positive			BP/BPP POS	Yes	
20			Negative	Negative			Negative	Yes	
21			Negative	Negative			Negative	Yes	
22			Negative	Negative			Negative	Yes	
23	38.4	83.2	Negative	Positive			Negative	Yes	Specimen from Luinex, only known result was neg BP.
24	31.5	83.4	Negative	Positive			BPP Pos	Yes	
25	30.4	83.4	Negative	Positive			BPP Pos	Yes	
26	35.6	83.3	Negative	Positive			BPP Pos	Yes	
27	30.1	80.8	Positive	Negative			BP Pos	Yes	
Control			Negative	Negative			Neg Control	Yes	
Control	28.4	80.5 : 83.4	Positive	Positive			BP/BPP POS	Yes	
28			Negative	Negative			Negative	Yes	
29	27.1	83.3	Negative	Positive			BPP Pos	Yes	
30			Negative	Negative			Negative	Yes	
31	27.2	83.2	Negative	Positive			BPP Pos	Yes	
32	19.7	83.3	Negative	Positive			BPP Pos	Yes	
33	20.4	83.3	Negative	Positive			BPP Pos	Yes	
34	23.7	83.4	Negative	Positive			BPP Pos	Yes	
35	24.4	83.2	Negative	Positive			BPP Pos	Yes	

Table 2. Testing Results – SJP Clinical Sample Panel

SAMPLE	ARIES Results				LightCycler		Expected Result	Concordant	Δ Ct
	Ct (FAM)	Tm	Pertussis	ParaPertussis	Ct (FAM)	Tm			
1	36.5	80.5	Positive	Negative	39.0	80.3	BP Pos	Yes	-2.5
2			Negative	Negative			Negative	Yes	
3	33.2	80.6	Positive	Negative	36.9	80.1	BP Pos	Yes	-3.7
4	30.2	80.6	Positive	Negative	35.4	80.2	BP Pos	Yes	-5.2
5	21.1	80.7	Positive	Negative	24.4	80.2	BP Pos	Yes	-3.3
6	32.6	80.7	Positive	Negative	36.8	80.4	BP Pos	Yes	-4.2
7	32.8	80.6	Positive	Negative	36.0	81.1	BP Pos	Yes	-3.2
8	27.8	83.3	Negative	Positive	32.0	83.8	BPP Pos	Yes	-4.2
9	34.8	80.7	Positive	Negative	38.4	80.3	BP Pos	Yes	-3.6
10	40.2	80.3	Positive	Negative			BP Neg	NO	
11			Negative	Negative			Negative	Yes	
12			Negative	Negative			Negative	Yes	
13			Negative	Negative			Negative	Yes	
14			Negative	Negative			Negative	Yes	
15			Negative	Negative			Negative	Yes	
16			Negative	Negative			Negative	Yes	
17			Negative	Negative			Negative	Yes	
18			Negative	Negative			Negative	Yes	
19			Negative	Negative			Negative	Yes	
20			Negative	Negative			Negative	Yes	
21			Negative	Negative			Negative	Yes	
22			Negative	Negative			Negative	Yes	
23			Negative	Negative			Negative	Yes	
24			Negative	Negative			Negative	Yes	
25			Negative	Negative			Negative	Yes	
26			Negative	Negative			Negative	Yes	
27	35.7	80.5	Positive	Negative	40.2	80.3	BP Pos	Yes	-4.5
28	39.2	80.5	Positive	Negative	40.0	80.7	BP Pos	Yes	-0.8
29	38.4	80.6	Positive	Negative	39.2	80.6	BP Pos	Yes	-0.8
30	35.0	80.5	Positive	Negative	35.8	80.2	BP Pos	Yes	-0.8
31			Negative	Negative	42.5	80	BP Pos	NO	
32	38.5	80.6	Positive	Negative	38.2	79.9	BP Pos	Yes	0.3
33	38.8	80.7	Positive	Negative	39.8	79.9	BP Pos	Yes	-1.0
34	38.3	80.6	Positive	Negative	42.9	80.2	BP Pos	Yes	-4.6
35	39.1	80.6	Positive	Negative	43.0	80.2	BP Pos	Yes	-3.9
36			Negative	Negative	42.8	80	BP Pos	NO	
37			Negative	Negative	41.4	80.1	BP Pos	NO	
38			Negative	Negative			BP Pos	NO	
39			Negative	Negative			BP Pos	NO	
40			Negative	Negative			BP Pos	NO	
41	39.9	80.4	Positive	Negative	42.8	80.1	BP Pos	Yes	-2.9
42	32.8	83.4	Negative	Positive	41.2	83.9	BPP Pos	Yes	-8.4

Results and Discussion

- Original testing to compare the performance of the ARIES® and LC2 was performed using a panel of clinical samples provided by Luminex. The panel consisted of 34 samples; the results are shown in Table 1. Correlation between both the LC2 and ARIES® results was 100%.
- We next tested 42 clinical samples submitted to our laboratory for *B. pertussis* and *parapertussis* PCR testing. All samples that tested negative with the LC2 platform were in agreement with the ARIES® results with one exception; a sample that was negative on the LightCycler 2.0 for both *Bordetella* species was positive for *B. pertussis* on the ARIES®. The sample was weakly positive with a Ct value of 40.2. This sample is most likely a low copy number positive sample. Since the ARIES® platform uses significantly more sample DNA in the PCR reaction than the LC2-based test such a result is not unexpected. In fact, the Ct values for all positive samples but one were lower when tested by the ARIES® platform when compared to the LC2. ARIES® Ct values were lower than those obtained by the LC2 by an average of 3.2 cycles.
- There was 100% correlation of *B. parapertussis* positive results between the LC2 and the ARIES®. Three samples that were determined to be positive for *B. pertussis* using the Light Cycler® 2.0 were resulted as not detected using the ARIES®. Each of these samples was submitted to an independent reference laboratory for retesting. Two of the three were resulted as not detected and one was reported as equivocal. It was noted that all three samples had very high Ct values with the LC2, indicating that the target copy number was extremely low. These samples were stored long term in the -70°C freezer and may have undergone some degree of deterioration.

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Conclusions

- The ARIES® system is a robust, simple to use, sample to result platform.
- The performance of the ARIES® *B. pertussis/parapertussis* assay compared very favorably to results obtained using the Light Cycler® 2.0
- The major advantage of the ARIES® method is the elimination of all up front sample processing steps resulting in:
  - significantly decreased hands-on time
  - greatly simplified workflow
  - decreased turnaround time to a final result
  - Increased cost effectiveness by elimination of up front sample processing and the need for batch testing.