Evaluation of the BioGX BD-Max PCR assay for detection of pathogenic *Bordetella*

Sir,

Pertussis (whooping cough) has experienced a resurgence despite high vaccine coverage against the main aetiological agent, *Bordetella pertussis*.[1] Recent reports of an increase in pertussis cases especially among adults and adolescents is also of concern, given this group can serve as a reservoir of infection for infants who have not completed their vaccination.[2–4] While *B. pertussis* remains the most common cause of human disease worldwide, significant contribution of two other species, *B. parapertussis* and *B. holmesii* to endemic activity and local outbreaks has been recognised.[4,5]

Polymerase chain reaction (PCR)-based assays have become the most commonly used laboratory method for reliable diagnosis of recent pertussis. Genomes of both *B. pertussis* and *B. holmesii* contain an insertion element (IS), *IS481*, which has been the preferred target for pertussis PCR assays. *Bordetella pertussis* can carry between 50 and 238 copies of *IS481* while *B. holmesii* contains 8−10 copies.[6,7] Therefore, if *B. holmesii* is present in the population it can potentially confound the results of *B. pertussis* prevalence.[4] *Bordetella parapertussis* can be detected by amplifying another type of IS, *IS1001*, which is present in approximately 20 copies. However, *B. holmesii* also contains 3−5 copies of *IS1001*; therefore, when referring to this IS in *B. parapertussis*, the nomenclature *pIS1001* is used and the *IS1001* in *B. holmesii* is referred to as *hIS1001*. The nucleic acid homology between *pIS1001* of *B. parapertussis* and *hIS1001* of *B. holmesii* is only 76−87%, enabling the differentiation of the two types of *IS1001*. Different sets of PCR primers have been designed and verified for detection and differentiation of *B. pertussis* from *B. holmesii* and *B. parapertussis* that target *IS481*, *pIS1001* and *hIS1001*.[8,9]

Automated real-time PCR (rtPCR) systems such as the BD Max (Becton Dickinson, USA) have been developed to diagnose *Bordetella* infection. Recently, rtPCR on the BD Max platform (Becton Dickinson) that employs the internally controlled, BioGX kit (BioGX, USA), has become available. The BD Max system has been evaluated using the Diagenode kit to detect *Bordetella*.[10] However, the Diagenode kit is not available in Australia and therefore the BioGX kit is used for this purpose instead. We evaluated the BioGX BD Max automated system for detection of pathogenic *Bordetella* and compared results to a widely used rtPCR format.

Five *B. pertussis* strains were included: L1048 (SNP cluster 14), L1660 (SNP cluster 13) and ATCC18323 (all pertactin positive), and L1663 (SNP cluster 13) and L1665 (SNP cluster 16), the latter two both lacking pertactin, to reflect the diversity of strains co-circulating in Australia.[11] All strains were grown on charcoal agar (CM119; Oxoid, UK) with 10% horse blood, 1% protease peptone No.3 (Difco, Becton Dickinson) and 40 µg/mL cephalexin. *Bordetella holmesii* (clinical strain CIDM4970), *B. parapertussis* (ATCC9305) and *B. bronchiseptica* (clinical strain CIDM 13678) were cultured on blood agar. A bacterial suspension was prepared in PBS corresponding to a 0.5 McFarland standard which was diluted between 1 in 100 to 1 in 10^9 and plated out to determine colony forming units (CFU) of the original sample. Serial dilutions (10^{-2}−10^{-10}) of each 0.5 McFarland solution was made and DNA extracted using the NucliSENS easyMAG nucleic acid extraction system (bioMérieux, France) and the BD Max system using the BD MAX ExK extraction kit (Becton Dickinson).

For the rtPCR, amplification and detection of targets was achieved using previously published primers and probes for *IS481*,[12] *pIS1001*[8] and *hIS1001*.[13] The PCR reaction mixture (20 µL) contained Qiagen HotStarTaq MasterMix (Qiagen, Germany), made up to final concentrations of 1.5 mM $MgCl_2$, 200 µM dNTPs and 1.23 U HotStarTaq DNA polymerase (Qiagen), 300-550 nM Primers (Sigma Aldrich, USA), 70−120 nM of TaqMan FAM Probe (Sigma Aldrich) and 5 µL of DNA extract. PCR conditions were: enzyme activation for 10 min at 95°C, 45 cycles of amplification (95°C for 10 s, 55°C for 30 s and 72°C for 15 s) and cooling at 40°C for 10 min on a Roche LightCycler 480 (Roche Diagnostics, Switzerland). Non-template-controls (PCR-grade water) were included in each run. An average C_T value of the duplicate rtPCR assays was calculated to give a final value.

Samples extracted and amplified on the BD Max system were prepared by aliquoting 500 µL of bacterial suspension to the sample buffer tube and extracted according to the manufacturer's protocol. The BioGX PCR protocol included the multiplex detection of *B. pertussis* (*IS481*), *B. holmesii* (*hIS1001*) and *B. parapertussis* (*pIS1001*), pertussis toxin gene (*ptxS1*) and a *Drosophila* sample processing control (SPC). The BioGX PCR assay had the following thermoprofile: 2 min at 99°C (1 cycle) and a cycle program (45 cycles) of Step 1, 12 s at 99°C, and Step 2, 60 s at 60°C (plus detection). BioGX BD-Max and rtPCR assay findings were compared and their reproducibility was assessed by quintuplicate testing of samples containing 1000 CFU/mL in order to determine inter-assay and intra-assay variability and the coefficient of variation. The analytical sensitivity of each assay was evaluated by testing ten-fold dilutions of each bacterial sample. We used C_T cut-offs with a value $\leq$40 considered as positive and over 40 considered as negative.[8]

While sensitivity of the rtPCR and BioGx BD-Max assays for the detection of *B. pertussis* and *B. holmseii* was similar, they were less sensitive in picking up *B. parapertussis* DNA. A limit of detection for *B. parapertussis* was higher in both assays which may reflect the lower copy number of the PCR targets in this species. These results are in line with previous reports that have found the sensitivity of the *B. pertussis* assay to be as much as five times higher than the detection of other Bordetella.[9] *Bordetella pertussis* and *B. holmesii* rtPCR assays could detect 0.01 CFU/mL while *B. parapertussis* was detected down to 10 CFU/mL (Table 1). The BD-Max assay performed well with the *IS481* assay directed at *B. pertussis* and could detect 0.01 CFU/mL. It retained this sensitivity when detecting *B. holmesii*; however, it also produced a positive *IS481* result down to 0.1 CFU/mL for *B. parapertussis*. Therefore, a positive *IS481* finding needed to be interpreted in conjunction with the *hIS1001* and *pIS1001* markers targeting *B. holmesii* and *B. parapertussis,* respectively. The inter- and intra-assay variability for the *B. pertussis* rtPCR was 5 and 5.04, respectively, which was less than the BioGX BD-Max assay (6.91 and 6.93, respectively) (Table 1). For the BioGX BD-Max assay, the inter-run variation was 1.89 and the intra-assay variability was 6.91.

Table 1 Mean C_T values and precision data of rtPCR and BD-Max assays

PCR target	Platform	Mean C_T at each dilution (CFU/mL)						Intra-assay		Inter-assay	
		10^8	10^6	10^3	10	0.1	0.01	SD	CV	SD	CV
B. pertussis IS481	rtPCR	12.97	22.62	25.53	33.69	34.51	35.28	1.38	5	3.74	5.04
	BD-Max	10.3	18.7	24.2	21.4	29.3	31	1.68	6.91	1.89	6.93
B. holmesii IS481	rtPCR	12.43	17.98	19.9	28.82	30.16	36.62	ND	ND	ND	ND
	BD-Max	16.4	29.4	24.9	29.35	36	33.4	ND	ND	ND	ND
B. holmesii hIS1001	rtPCR	11.54	17.66	23.76	36.84	37.87	37.03	0.02	0.1	0.02	0.09
	BD-Max	12.6	20.4	23.8	29.7	36.7	0	1.81	7.61	1.8	3.83
B. parapertussis pIS1001	rtPCR	33.23	36.5	37.84	38.29	40	0	1.19	3.07	1.09	2.81
	BD-Max	15.8	21.2	25.3	30.5	30.9	0	1.2	4.74	0.25	2.37
B. bronchiseptica IS481	rtPCR	12.07	18.75	22.6	26.92	28.05	35.46	ND	ND	ND	ND
	BD-Max	22.3	32.1	32.8	28.6	32.95	36.1	ND	ND	ND	ND

CFU, colony forming units; CV, co-efficient of variation (%); ND, not performed as these assays were not compared given low diagnostic value; SD, standard deviation.

This measure was higher than the intra- and inter-assay variability of the rtPCR.

Our findings demonstrated comparable performance of the rtPCR and BioGX BD-Max assays for the detection of *B. pertussis*, *B. holmesii* and *B. parapertussis*. Both assays were very sensitive in detecting *B. pertussis* and *B. holmesii.* The BioGX BD-Max assay was efficient and easy to use, however the rtPCR demonstrated higher specificity when detecting other *Bordetella* species. Further, the difference in cost per reportable analysis between the two assays was $21.30 for the BD-Max and $15.60 for the rtPCR which is an important consideration. These assays enable differentiation between relevant pathogenic species of *Bordetella* and improve the accuracy of public health notifications for pertussis.

Conflicts of interest and sources of funding: The authors state that there are no conflicts of interest to disclose.

Verlaine J. Timms[1,2], Winkie Fong[2], Neisha J. Jeoffreys[3], Vitali Sintchenko[1,2,3]

[1]Centre for Infectious Diseases and Microbiology — Public Health, Westmead Hospital, NSW, Australia; [2]Sydney Medical School, The University of Sydney, NSW, Australia; [3]Centre for Infectious Diseases and Microbiology Laboratory Services, NSW Health Pathology, Sydney, NSW, Australia

Contact Verlaine Timms, PhD.
E-mail: Verlaine.Timms@health.nsw.gov.au

1. Melvin JA, Scheller EV, Miller JF, *et al*. *Bordetella pertussis* pathogenesis: current and future challenges. *Nat Rev Microbiol* 2014; 12: 274–88.
2. Long SS, Lischner HW, Deforest A, *et al*. Serologic evidence of subclinical pertussis in immunized children. *Pediatr Infect Dis J* 1990; 9: 700–5.
3. Long SS, Welkon CJ, Clark JL. Widespread silent transmission of pertussis in families: antibody correlates of infection and symptomatology. *J Infect Dis* 1990; 161: 480–6.
4. Fong W, Timms V, Holmes N, *et al*. Detection and incidence of *Bordetella holmesii* in respiratory specimens from patients with pertussis-like symptoms in New South Wales, Australia. *Pathology* 2018; 50: 322–6.
5. Mattoo S, Cherry JD. Molecular pathogenesis, epidemiology, and clinical manifestations of respiratory infections due to *Bordetella pertussis* and other Bordetella subspecies. *Clin Microbiol Rev* 2005; 18: 326–82.
6. Glare EM, Paton JC, Premier RR, *et al*. Analysis of a repetitive DNA sequence from *Bordetella pertussis* and its application to the diagnosis of pertussis using the polymerase chain reaction. *J Clin Microbiol* 1990; 28: 1982–7.
7. Loeffelholz MJ, Thompson CJ, Long KS, *et al*. Detection of *Bordetella holmesii* using *Bordetella pertussis IS481* PCR assay. *J Clin Microbiol* 2000; 38: 467.
8. Tatti KM, Sparks KN, Boney KO, *et al*. Novel multitarget real-time PCR assay for rapid detection of Bordetella species in clinical specimens. *J Clin Microbiol* 2011; 49: 4059–66.
9. Cloud JL, Hymas WC, Turlak A, *et al*. Description of a multiplex *Bordetella pertussis* and *Bordetella parapertussis* LightCycler PCR assay with inhibition control. *Diagn Microbiol Infect Dis* 2003; 46: 189–95.
10. Kenicer J, Hardie A, Hamilton F, *et al*. Comparative evaluation of the Diagenode multiplex PCR assay on the BD max system versus a routine in-house assay for detection of *Bordetella pertussis*. *J Clin Microbiol* 2014; 52: 2668–70.
11. Octavia S, Sintchenko V, Gilbert GL, *et al*. Newly emerging clones of Bordetella pertussis carrying prn2 and ptxP3 alleles implicated in Australian pertussis epidemic in 2008-2010. *J Infect Dis* 2012; 205: 1220–4.
12. Poddar SK. Detection and discrimination of *B pertussis* and *B holmesii* by real-time PCR targeting *IS481* using a beacon probe and probe-target melting analysis. *Mol Cell Probes* 2003; 17: 91–8.
13. Rodgers L, Martin SW, Cohn A, *et al*. Epidemiologic and laboratory features of a large outbreak of pertussis-like illnesses associated with cocirculating *Bordetella holmesii* and *Bordetella pertussis*–Ohio, 2010-2011. *Clin Infect Dis* 2013; 56: 322–31.

DOI: https://doi.org/10.1016/j.pathol.2018.10.018

Malignant gastrointestinal neuroectodermal tumour (GNET): neural mesenchymal tumours of the gastrointestinal tract with striking histology and *EWSR1* gene rearrangement

Sir,

Malignant gastrointestinal neuroectodermal tumours (GNET) are rare primitive mesenchymal neoplasms of the tubular gut previously considered to be gastrointestinal manifestations of soft tissue clear cell sarcomas (CCS). While both entities share a recurrent balanced translocation profile involving the