RANDOX

EDUCATIONAL GUIDE

Bordetella Detection and Species Identification

BOSCH vivalytic

In the post-pandemic era, cases of whooping cough are on the rise. In England, between January and March 2024, there were 2793 laboratory confirmed cases of whooping cough causing the deaths of 5 infants, compared with a total of 858 cases in 2023[1]. A rudimentary projection model estimates that without intervention, whooping cough cases in the England could total over 15,000 cases by the end of 2024 (Figure 1). Rising cases are not isolated to the UK - increased rates of diagnosis have also been reported in Denmark, Spain, and Croatia[2].

Whooping cough, or *pertussis*, which literally means *violent cough*, was first discovered in 1906, with the first vaccine deployed in the 1940s, until which *pertussis* was a major cause of morbidity and mortality. Nowadays, most healthy adults fully recover from *pertussis* after suffering often prolonged disease. However, in children, the elderly, and the immunosuppressed, *pertussis* can cause significant morbidity and mortality[3].

Bordetella species are responsible for *pertussis*, namely *Bordetella pertussis and Bordetella parapertussis*. These are commonly referred to as classical *Bordetella*. *Bordetella holmesii* is another species of bacteria which causes *pertussis*-like disease. These bacteria are difficult to culture due to their fastidious and slow-growing nature. *Bordetella* species cultures typically take around 3-7 days to provide a positive result and provide limited sensitivity, between 20% and 40% for classical *Bordetella*[3] and between 12% and 60% for B. *holmesii*[4].

To aid in the rapid and accurate detection of *pertussis* and the causative agents, a new test has been developed on the Vivalytic, for the Real-time PCR detection of B. *pertussis*, B. *parapertussis* and B. *holmesii*. The Vivalytic platform is a universal, fully automated, cartridge-based platform enabling high-plex and low-plex testing, providing an all-in-one solution for molecular diagnostics.

In this whitepaper, we will discuss the *Bordetella* genus, specifically the species mentioned above, the Vivalytic system and *Bordetella* cartridge, and a recent investigation into its utility, which showed a minimum of 97.7% concordance with a reference method.

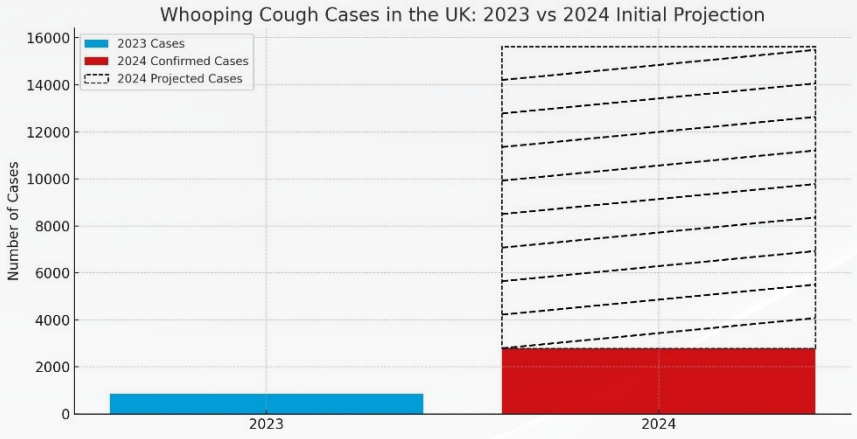

Figure 1. *Whooping Cough Cases in the UK: 2023 vs 2024 Initial Projection: This bar chart illustrates the total number of whooping cough cases in the UK for 2023 and the projected number of cases for 2024. The total number of cases in 2023 was 858. For 2024, the confirmed cases from January to March were 2,793. The projection for the remaining quarters of 2024 was based on historical seasonal trends observed from the years 2018, 2019, 2020, and 2023.*

Bordetella genus

Bacteria of the *Bordetella* genus are gram-negative coccobacilli[5] which are important pathogens in human medicine as they colonise the respiratory tract leading to a range of pulmonary and bronchial infections[6]. There are 3 main species associated with whooping cough: of B. *pertussis*, B. *parapertussis* (Classical *Bordetella*) and B. *holmesii* (*pertussis*-like disease pathogen). Classical *Bordetella* species share over 98% DNA sequence similarity and share many crucial virulence factors like toxins adenylate cyclase toxin (ACT), *pertussis* toxin (PXT), and dermonecrotic toxin[5] yet there are variations in potential hosts and disease. For example, B. *pertussis* is an exclusively human pathogen, whereas B. *parapertussis* can infect both humans and sheep[6].

The diversity in this genus is thought to be the result of loss- and gain-of-function of numerous genes, including those encoding bacterial toxins and protein secretion systems along with other virulence factors. Genomic studies have shown that since their divergence from their ancestral species, loss of gene function has been more frequent and associated with the specialisation of several species in the *Bordetella* genus, including B. *pertussis*, B. *parapertussis* and B. *holmesii*[5].

Classical Bordetella – Pertussis

Pertussis is caused by 2 main pathogens: B. *pertussis* and B. *parapertussis*. These pathogens are spread through airborne droplets expelled during the characteristic cough and is highly contagious, infecting up to 100% of household contacts[3]. While vaccine deployment has largely contained the spread of *pertussis*, recent reports show prevalence is increasing around the world[2]. This is thought to be partially due to waning vaccine-derived immunity in adults and adolescents, which drops to 50% after 12 years of completing a vaccination course.

It is estimated that approximately 24 million cases are reported each year, resulting in over 160,000 deaths. *Pertussis* is a largely paediatric disease, with around 71% of cases reported in children under 5 years old. However, adults and adolescents can also become infected, albeit with lower morbidity and mortality associated with the infection[3]. Infants are at the highest risk of morbidity and mortality - the fatality rate among infants is estimated to be around 2%, making up 96% of *pertussis*-related deaths[3]. The elderly and immunocompromised are also at increased risk of morbidity and mortality due to alternative chronic medical conditions and complications.

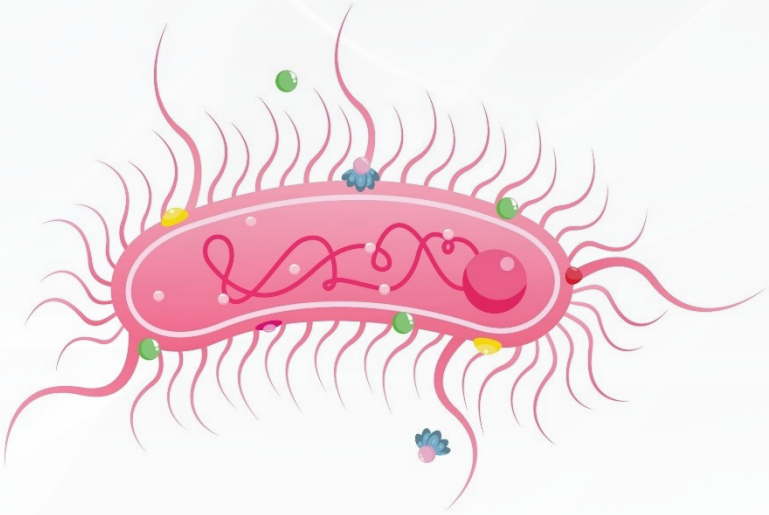

Figure 2. Illustration of *Bordetella pertussis*

Pertussis pathogens adhere to ciliated respiratory epithelial cells. Once infected with classical *Bordetella*, the pathogen reaches the mucosal lining of the respiratory tract, causing local inflammatory changes. These bacteria release a variety of toxins including PXT, ACT, dermonecrotic toxin and tracheal toxin, which act locally and systemically.

Classical *Bordetella* causes disease which progresses through 3 distinct phases:

1. Catarrhal phase - Clinical presentation is like other upper respiratory tract infections (URTIs), with fever, fatigue, rhinorrhoea (runny nose) and conjunctival infection. This is the most infective stage, lasting between 1-2 weeks[3].
2. Paroxysmal phase – This phase is characterised by paroxysms (sudden, recurrent attacks) of a cough and the resolution of fever. The cough is typically repetitive and followed by a forceful inspiration, providing the characteristic *whoop*. Between coughing fits, patients appear non-toxic, but during paroxysms, they may exhibit cyanosis (bluish-purple colouration of the skin), diaphoresis (excessive sweating) or apnoea (temporary cessation of breathing)[3].
3. Convalescent phase – A persistent cough, lasting for weeks to months, often triggered by exposure to another URTI[3].

Clinical presentation in infants is commonly atypical and non-specific. Fever may not occur. Indications may instead include tachypnoea (abnormally rapid breathing), apnoea, cyanosis, and episodic bradycardia (slower than normal heart rate)[3].

Compared with B. *pertussis*, B. *parapertussis* causes a similar disease, often with milder symptoms which resolve more quickly. Due to high rates of concomitant infections of B. *pertussis* and B. *parapertussis*, the incidence of the later is thought to be underestimated[7].

Strict isolation is essential during the most infective stages of *pertussis*, namely through the catarrhal phase and for the first 3 weeks of the paroxysmal please. Treatment for *pertussis* is mostly supportive, ensuring patients receive sufficient oxygen, hydration and the avoid respiratory irritants[3]. In cases of *pertussis*-related pneumonia, hypoxia, central nervous system (CNS) complications, or for those who cannot take food or water orally, patients require hospitalisation. Patients under the age of 1 years should be admitted to hospital regardless of their symptoms due to the high risks associated with this cohort[3]. Finally, neonates require intensive care admission as life-threatening cardiopulmonary complications and arrest are known to occur spontaneously[3].

Antibiotics have been shown to been effective during the catarrhal phase, but there is limited data on their efficacy during the other phases of *pertussis*. The main goal of antibiotic administration is to limit the carriage and spread of the disease. Therefore, individuals who have been in close contact with an infected person are generally treated with azithromycin or erythromycin[3].

Vaccination has been historically successful in limiting the spread of Classical *Bordetella*. It is recommended that at the ages of 2, 4, 6, 15-18 months, and between the ages of 4 and 6, children should receive an acellular vaccine. The Centre for Disease Control and Prevention (CDC) further recommends that adults receive a single dose of Tdap (Diphtheria, tetanus, and acellular *pertussis* booster vaccine) to help reduce transmission to children[3].

Bordetella holmesii – Pertussis-like disease

B. *holmesii* is another species of *Bordetella* which cases *pertussis*-like disease, discovered more recently that classical *Bordetella* species. The 16S rRNA sequence of B. *holmesii* shares 99.5% sequence similarity with B. *pertussis*. However, B. *holmesii* lacks many of the virulence factors associated with Classical *Bordetella* species. Instead, the B. *holmesii* genome contains around 400 genes not previously characterised in *Bordetella* species, many of which are thought to be involved in its pathogenicity through transport and detoxification of organic compounds and antibiotics[4].

Due to a lack of data, the true prevalence of B. *holmesii* infection is unclear and likely to be underestimated. The studies which are available suggest B. *holmesii* is the pathogen responsible for between 0-29% of patients suffering from *pertussis*-like symptoms[8]. The highest rates were reported following the 2010 Ohio *pertussis* outbreak, during which B. *holmesii* was the cause of around 33% of all cases, and 45% of cases in children between 11-18 years old[9].

Studies have shown that many laboratories struggle to differentiate between B. *holmesii* and other *Bordetella* species, with only 4% of European[10] and 7% of Australian[11] laboratories achieving success. Furthermore, like other *Bordetella* species, culture of B. *holmesii* provides low analytical sensitivity, between 12-60%[4].

B. *holmesii* infection is designated as a *pertussis*-like disease as it causes similar symptoms such as fever, paroxysmal cough, inspiratory whoop and post-tussive vomiting. However, it is believed to produce less severe illness and it is still unclear whether disease caused by this species progresses through the same 3 phases and therefore if disease duration is the same[4]. For example, it has been shown that 70% patients infected with B. *holmesii* were cough-free 80 days after antibiotic administration, compared with only 30% of patients infected with B. *pertussis*[9]. This is potentially a result of the inability of B. *holmesii* to secrete the toxins typically associated with classical *Bordetella* species. B. *holmesii* is also known to cause bacteriaemia and was the first *Bordetella* species reported to do so, mainly in the immunocompromised. The disease course is non-specific – symptoms may include mild fever with headache, chills and/or vomiting, commonly with accompanying respiratory symptoms[4].

Complications

Pertussis and *pertussis*-like diseases are associated with a range of complications. Pneumonia, either secondary to, or superimposed with *Bordetella* infection may occur. A fever that persists beyond the catarrhal phase may be a warning sign of the development of pneumonia[4]. CNS complications, including seizures or encephalopathy, can occur in less than 2% of cases. These complications are often secondary to factors such as hypoxia, hypoglycaemia, toxins, secondary infections, or cerebral bleeding caused by increased pressure during coughing[4]. PXT, secreted by classical *Bordetella* species, can increase histamine sensitivity and insulin secretion. Finally, infants are particularly prone to bradycardia, hypotension and cardiac arrest following *Bordetella* infection.

Bordetella Detection and Identification

The accurate identification of *Bordetella* species is crucial for several reasons. Firstly, the aggressive case investigation and public health interventions needed to control B. *pertussis* outbreaks are not typically required for cases of B. *holmesii*. Secondly, prophylactic antibiotics may be indicated for immunosuppressed or asplenic patients, or those in contact with them, in cases of B. *holmesii* respiratory infection. Finally, if B. *holmesii* respiratory infections are misdiagnosed as B. *pertussis* in vaccinated individuals, this can lead to incorrect evaluations of vaccine efficacy.

For these reasons, and due to the increasing prevalence of *Bordetella* infections, Randox, in partnership with Bosch, are proud to introduce the Vivalytic *Bordetella* cartridge for use on the Vivalytic platform.

Vivalytic

The Vivalytic platform is a versatile, fully automated solution for molecular diagnostics, developed with Bosch. This cartridge-based system supports both high-plex and low-plex testing, integrating extraction, PCR amplification, and detection into a compact benchtop device.

Figure 3. The Vivalytic

Powered by Randox's multiplex Biochip Technology, Vivalytic delivers multiple results from a single patient sample. Randox's patented Biochip Technology allows for simultaneous detection of multiple targets in one sample using a chemiluminescent signal. Each biochip has discrete testing regions (DTRs), each representing an individual test and customisable with specific oligonucleotides. This high-plex capability reduces the need for multiple, time-consuming assays.

The Vivalytic workflow is designed to be user-friendly and efficient, consisting of just four steps for optimal simplicity.

1. *Scan/Input Sample Code:* Begin by scanning or manually entering the sample information into the Vivalytic system.
2. *Scan/Input Cartridge Code:* Scan the cartridge code into the embedded Vivalytic software to ensure the correct test is being performed.
3. *Insert Sample:* Add the patient sample into the dedicated slot on the cartridge, close the cover, and insert the cartridge into the Vivalytic analyser.
4. *Run Test and Display Results:* The analyser will automatically run the test, with the touchscreen display counting down the time remaining to test completion. Once finished, the results will be displayed on the screen.

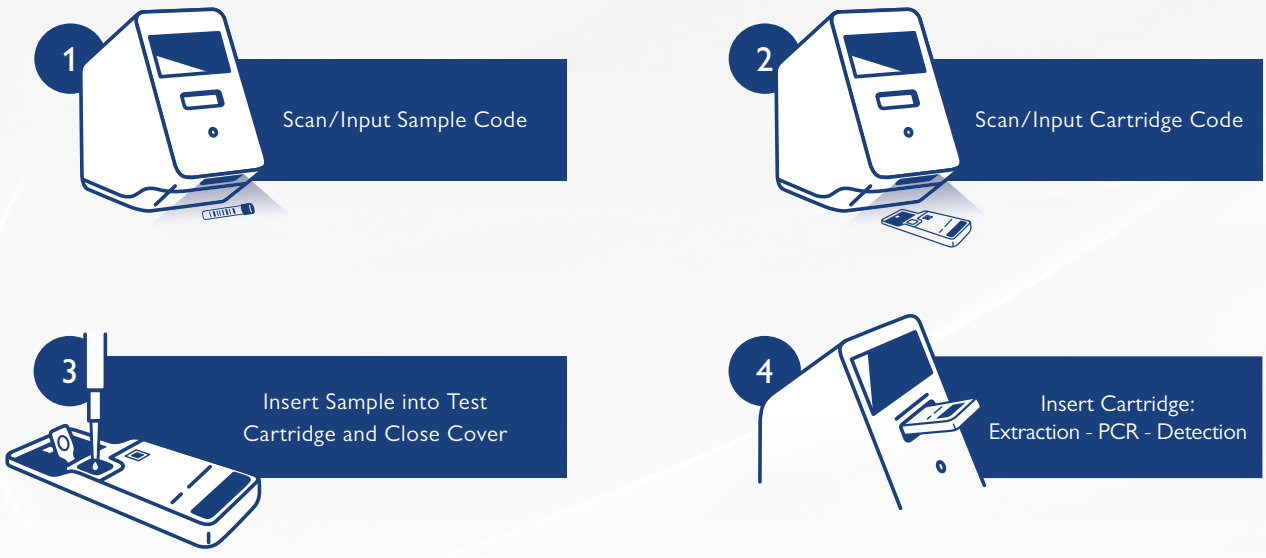

Figure 4. Vivalytic 4 step test process

Vivalytic Bordetella Cartridge

To enhance the detection and species identification of *Bordetella*, Randox introduces the Vivalytic *Bordetella* cartridge. This user-friendly assay is designed to detect B. *pertussis*, B. *parapertussis*, and B. *holmesii* from a single nasopharyngeal swab or aspirate sample. Utilising Real-time PCR, it enables rapid and accurate detection up to four weeks after symptom onset, differentiating between human pathogenic *Bordetella* species. With a time to result of just 47 minutes, this assay is invaluable for patient diagnosis and the containment of *Bordetella*, helping to reduce aerogenic transmission.

Figure 5. Vivalytic Bordetella Cartridge

Summary of Benefits

- Sample Volume - 300µl.
- Sample Type – Nasopharyngeal swab sample or aspirates.
- Real-time PCR detection.
- Time to result - ~47 minutes.
- Detection of B. *pertussis*, B. *parapertussis*, and B. *holmesii*.

Rapid and Accurate Detection of Whooping Cough in Clinical Samples

Zimmerman, 2024

Data presented at the annual congress of the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) 2024, illustrate the excellent features of the Vivalytic *Bordetella* array[12]. The following is a summary of the findings reported in the poster by Zimmerman (2024).

Initially, 411 nasopharyngeal samples were collected from symptomatic patients and pre-characterised using a sensitive reference test, the *Bordetella* Speciation Plus Toxin-OSR (BioGX, Amsterdam, NL), before being analysed on the Vivalytic system. Additionally, 150 spiked samples were tested, comprising 50 samples of each *Bordetella* species spiked at three different inoculation levels: low, medium, and high. Discrepant results were confirmed by RIDA Gene *Bordetella*[12].

The Vivalytic system demonstrated a high concordance of 97.7% with the reference method, achieving a positive percent agreement (PPA) of 97.9%. Due to low levels of positive results, an additional 11 positive samples were tested, 100% of which were correctly identified by the Vivalytic. For the spiked samples, the Vivalytic achieved a PPA of >98% across all samples, and an impressive PPA of >95% for the more challenging low inoculation samples. Additionally, the Vivalytic system had an invalid results rate of only 0.6%, compared to the BioGX assay's rate of 2.9%. The comparative sensitivity and number of invalid results are shown in Figure 6.

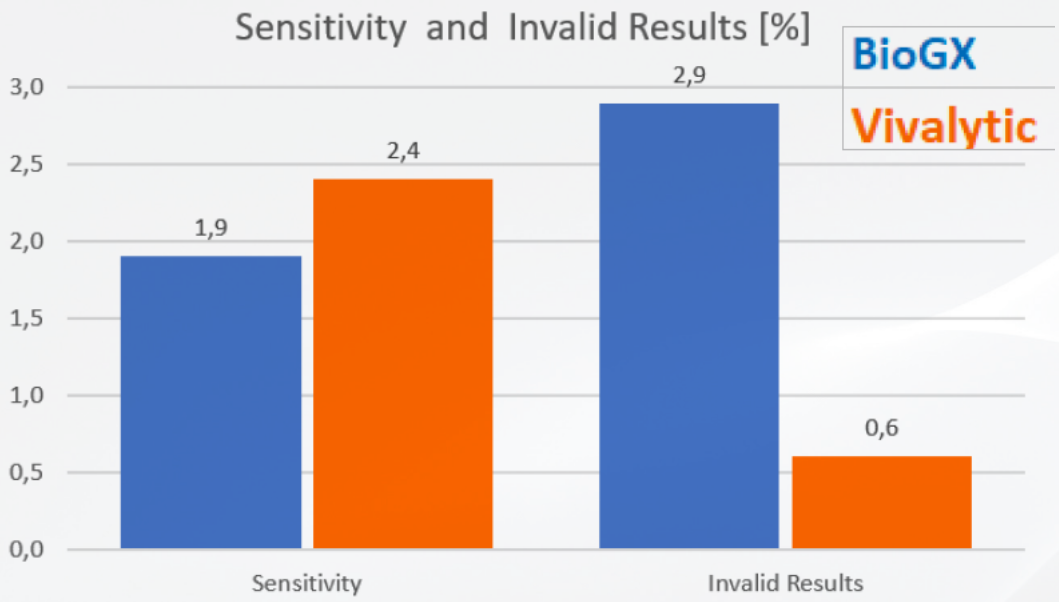

Figure 6. Sensitivity and invalid result rate of the Vivalytic Bordetella assay compared with BioGX Bordetella Speciation Plus Toxin-OSR

The conclusions drawn from this investigation are as follows:

- The Vivalytic *Bordetella* cartridge provided excellent concordance with a sensitive reference method and delivered fast and accurate results.
- This assay is ideal for both hospital laboratories and outpatient settings, thanks to its user-friendly design and quick turnaround times.
- Early identification of infected patients will aid in preventing the spread of re-emerging whooping cough epidemics.

Conclusions

The Vivalytic *Bordetella* cartridge by Randox, developed in partnership with Bosch, represents a significant advancement in the detection and species identification of *Bordetella* infections. Its high concordance with a sensitive reference method and rapid turnaround time makes it an invaluable tool for both hospital laboratories and outpatient settings. The ability to accurately identify B. *pertussis*, B. *parapertussis*, and B. *holmesii* from a single nasopharyngeal sample within 47 minutes enhances patient diagnosis and supports timely public health interventions, crucial in controlling the spread of whooping cough.

The data presented at the annual ESCMID congress highlights the robustness of the Vivalytic system, demonstrating a PPA of over 98% for all samples and over 95% for low inoculation samples, along with a low rate of invalid results. These attributes underscore the Vivalytic platform's reliability and efficiency, contributing to more effective management of *Bordetella* infections.

In an era where whooping cough is re-emerging, early and accurate identification of *Bordetella* species is essential. The Vivalytic *Bordetella* cartridge not only improves diagnostic capabilities but also plays a crucial role in public health by aiding in the containment of *Bordetella* transmission. This innovative solution reflects Randox's commitment to advancing molecular diagnostics and enhancing patient care through technological excellence.

Conclusions

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