BioGX
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Manual

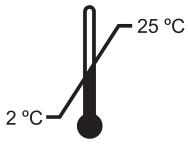
Bordetella Speciation Plus Toxin – *OSR for BD MAX*™

Version 08

REF 400-002-C-MAX

Σ 24 reactions

For *In Vitro* Diagnostic Use
For use with BD MAX™ Open System Reagents on the BD MAX™ System

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PROPRIETARY NAME

Bordetella Speciation Plus Toxin – OSR for BD MAX™

COMMON OR USUAL NAME

Bordetella Assay

INTENDED USE

The BioGX *Bordetella* Assay performed on the BD MAX™ System is an automated, multiplex real-time RT-PCR assay for the *in vitro* qualitative detection and differentiation of *Bordetella pertussis, Bordetella parapertussis,* and *Bordetella holmesii*. The BioGX *Bordetella* Assay uses nasophayryngeal (NP) swab or nasal wash specimens collected from patients with signs and symptoms of *Bordetella* in conjunction with clinical and epidemiological risk factors. The BioGX *Bordetella* Assay is intended as an aid in the diagnosis of *Bordetella*. Negative results do not preclude *Bordetella* and should not be used as the sole basis for treatment or other patient management decisions.

SUMMARY AND EXPLANATION

Pertussis, commonly referred to as “whooping cough”, is an infectious disease caused by the bacterium *Bordetella pertussis.* Before the availability of the pertussis vaccine in the 1940s, more than 200,000 cases of pertussis were reported annually. Since widespread use of the vaccine began, incidence has decreased more than 75% compared with the pre-vaccine era. In recent years, there has been an increase in the number of reported cases that can be attributed to waning immunity from vaccines as well as the use of more sensitive diagnostics such as polymerase chain reaction (PCR).

In addition to *Bordetella pertussis*, three other *Bordetella* species can cause disease in humans: *B. parapertussis, B. holmesii,* and *B. bronchiseptica. B. parapertussis* causes a pertussis-like illness that is generally milder than pertussis. Co-infection of *B. pertussis* and *B. parapertussis* is not unusual. Surveillance data using PCR assays for pertussis are used to assess the impact of the disease and develop control strategies. Due to the worldwide occurrences of these *Bordetella* species and varying severity of infection, species identification allows for an accurate diagnosis and treatment of pertussis and parapertussis-like disease in humans as well as public health tracking of outbreaks.

Among several chromosomal regions utilized for real-time PCR (RT-PCR) detection of *B. pertussis*, the multicopy insertion sequence (IS) *IS481* is often the target of choice because it is found in multiple copies in *B. pertussis* (50 to 238 copies per genome), making this assay highly sensitive. However, positive results with a single PCR assay targeting *IS481* could lead to a false diagnosis of pertussis because *IS481* is also found in *B. holmesii* (8 to 10 copies per genome), in animal isolates of *B. bronchiseptica*, and less frequently, in human isolates of *B. bronchiseptica*. Moreover, pseudo outbreaks due

to false-positive results of assays using *IS481* as a single PCR target have demonstrated the need for defined cutoff values based on analytical sensitivity and clinical relevance.

The BioGX *Bordetella* Speciation Plus Toxin – OSR for BD MAX™ is an automated *in vitro* diagnostic test reagent for the multiplex qualitative detection of DNA from *Bordetella pertussis*, *Bordetella parapertusis*, and *Bordetella holmesii,* and a DNA sample processing control (SPC).

Adapted from:

> Centers for Disease Control Website, www.cdc.gov, accessed October 12, 2016.
> "Novel Multitarget Real-Time PCR Assay for Rapid Detection of *Bordetella Species* in Clinical Specimens.," Kathleen M. Tatti,* Kansas N. Sparks, et al. (Centers for Disease Control and Prevention), J. Clin. Microbiol. 2011 Dec;49(12):4059-66.

PRINCIPLES OF THE PROCEDURE

The BioGX *Bordetella* Assay is to be used with the BD MAX™ Open System for automated patient sample processing and molecular analysis. The BD MAX™ System uses a combination of lytic and extraction reagents to perform cell lysis and nucleic acid extraction. Following enzymatic cell lysis at elevated temperature, the released nucleic acids are captured by magnetic affinity beads. To control for extraction efficiency, a DNA Sample Processing Control is included in each BD MAX™ DNA Extraction Tube. The beads with bound nucleic acids are washed and the nucleic acids are eluted by heat in an elution buffer. The eluted nucleic acid is then mixed with the BioGX Rehydration Buffer, which is then transferred to the BioGX Sample-Ready™ lyophilized master mix tube in order to rehydrate the Sample-Ready™ lyophilized master mix. The rehydrated mix of amplification reagent and nucleic acid is then dispensed into the BD MAX™ PCR Cartridge. Microvalves in the BD MAX™ PCR Cartridge are sealed by the system prior to initiating PCR to prevent evaporation and amplicon contamination.

The amplified DNA targets are detected using hydrolysis probes labeled at one end with a fluorescent reporter dye (fluorophore) and at the other end with a quencher moiety. Probes labeled with different fluorophores are used to detect specific amplicons originating from *Bordetella pertussis, Bordetella parapertussis, Bordetella holmesii* and a Sample Processing Control in five different optical channels of the BD MAX™ System: *ptxS1* amplicons (present in *B. pertussis* and *B. parapertussis)* are detected in the 475/520 channel, *pIS1001* amplicons (present in *B.* parapertussis) are detected in the 530/565 channel, *IS481* amplicons (present in *B. pertussis* and *B. holmesii*) are detected in the 585/630 channel, *hIS1001* amplicons (present in *B. holmesii)* are detected in the 630/665 channel, and the DNA Sample Processing Control is detected in the 680/715 channel. When the probes are in their native state, the fluorescence of the fluorophore is quenched due to its proximity to the quencher. However, in the presence of their specific target cDNA, the probes hybridize to their complementary sequences and are hydrolyzed by the 5'-3' exonuclease activity of the DNA polymerase as it synthesizes the nascent strand along the DNA template. As a result, the fluorophores

are separated from their quencher molecules and fluorescence is emitted. The amount of fluorescence detected in the five optical channels used for the BioGX *Bordetella* Assay is directly proportional to the quantity of the corresponding probe that is hydrolyzed, and therefore proportional to the amount of synthesized target. The BD MAX™ System measures these signals at the end of each amplification cycle in real time, and interprets the data to provide a qualitative result for each of the above targets.

REAGENTS

Qty	REF	Contents	Tests
2	400-002-MAX	**BioGX *Bordetella* Speciation Plus Toxin - OSR for BD MAX™** Sample-Ready™ lyophilized PCR Master Mix containing polymerase, nucleotides, specific molecular primers and probes, Sample Processing Control-specific molecular primers and probe.	12 tests per pouch
1	800-028-C	**Rehydration Buffer Tube (C) Open System Reagents for BD MAX™** Reagent tube containing a rehydration buffer for use in Lyophilized PCR Master Mix rehydration.	24 tests per pouch

NOTE: Safety Data Sheets (SDS) are available at www.biogx.com/eu or by request.

EQUIPMENT AND MATERIALS REQUIRED BUT NOT PROVIDED

- BD MAX™ ExK™ DNA-1 (BD catalog no. 442818)
  Extraction Kits include Sample Buffer Tubes (SBT), Septum Caps, Extraction Tubes, and Unitized Reagent Strips sufficient for 24 tests.
- BD MAX™ PCR Cartridges (BD catalog no. 437519)
- Sterile Swab Collection Device appropriate for nasopharyngeal swab collection and storage in viral transport media (VTM), including but not limited to flocked (Nylon), Dacron, polyester, Sigma (foam), and rayon.
- Vortex Genie 2 Vortexer (VWR catalog no. 58815-234) or equivalent.
- Disposable nitrile gloves.

WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use.
- Treat all biological specimens, including used Extraction Kits and PCR Cartridges, as if capable of transmitting infectious agents in accordance with safe laboratory procedures such as those described in CLSI Document M29[3] and in Biosafety in Microbiological and biomedical Laboratories.[4]
- Performance characteristics of this test have been established only with the specimen types listed in “Intended Use” section. The performance of this assay with other specimen types or samples has not been evaluated.
- Do not use the reagents if the protective pouches are open or torn upon arrival.

- Close reagent protective pouches promptly with the zip seal after each use. Remove any excess air in the pouches prior to sealing and store at 2-25 °C.
- Do not remove desiccant from the PCR Master Mix pouches.
- Do not use Master Mix if the desiccant is not present or is broken inside the Master Mix pouches.
- Do not use reagent tubes if the foil seal has been opened or damaged.
- Do not mix reagents from different pouches and/or kits and/or lots.
- Do not use expired reagents and/or materials.
- Each Master Mix and Rehydration Buffer tube is used to process a single sample. Do not reuse Master Mix or Rehydration Buffer tubes.

- Refer to BD MAX™ ExK™ DNA-1 Extraction Kit Instructions for information about proper handling, cautions, and proper waste disposal.

- Do not mix septum caps between Sample Buffer Tubes or re-use septum caps as contamination may occur and compromise test results.
- Check BD Unitized Reagent Strips for proper liquid fills (ensure that the liquids are at the bottom of the tubes).
- Do not pipette by mouth.
- Do not smoke, drink, or eat in areas where specimens or kits are being handled.
- Dispose of unused reagents and waste in accordance with country, federal, provincial, state, and local regulations.
- Use clean gloves when handling extraction kit components and PCR reagents and buffer tubes.

STORAGE AND STABILITY

- BioGX recommends long-term storage at 2-25°C.

- Reagents have been tested to demonstrate optimal performance when stored properly and consumed by the Expiration Date. Long-term stability studies are ongoing and the Expiration Date will be amended as additional data is available.

- Avoid exposing the reagents (lyophilized or rehydrated) to direct sunlight or long-term ambient lighting.
- Tightly reseal the pouch with unused reactions and immediately store the pouch in a dry location after opening.

- Avoid exposure to moisture and use the entire contents of the opened pouch within 1 month.

INSTRUCTIONS FOR USE

Install the BioGX Electronic User Defined Protocol on the BD MAX™

It will be necessary to import an Electronic User Defined Protocol (eUDP) onto the BD MAX™. The most current eUDP is available for download on **www.biogx.com/eu** by clicking on "Product Documentation" and selecting the appropriate platform and product name. eUDPs can also be obtained by emailing BioGX at **eu@biogx.com**. Please refer to the BD MAX™ user manual for uploading instructions**.**

NOTE: eUDPs are specific to extraction kit type and are programmed to be used with 3-snap strips unless otherwise indicated. If a 4-snap strip is used it is necessary to modify the eUDP to a 4-snap program by opening the eUDP in "Test Editor" and selecting the corresponding 4-snap extraction strip type in the "Extraction Type" drop down.

Specimen Collection/Transport

Nasal specimens should be collected, transported, and stored according to institutional and laboratory standard operating procedures.

Specimen Preparation

Nasopharyngeal Swab (Copan ESwab™)

Pipette 50 µL of specimen into the Sample Buffer Tube (SBT), aseptically place the BD™ septum cap on each SBT . Pulse vortex the SBT for 1-3 seconds, and load the SBT into the extraction tray.

Nasopharyngeal Swab (Copan UTM™)

Pipette 100 µL of specimen into the Sample Buffer Tube (SBT), aseptically place the BD™ septum cap on each SBT. Pulse vortex the SBT for 1-3 seconds, and load the SBT into the extraction tray.

Nasal Wash

Pipette 50 µL of nasal wash specimen and 700 µL UTM into the Sample Buffer Tube (SBT),, aseptically place the BD™ septum cap on each SBT. Pulse vortex the SBT for 1-3 seconds, and load the SBT into the extraction tray.

Other Sample Types

⚠ Please contact BioGX for processing suggestions if collecting specimen types other than NP swabs in VTM, as some specimen types can be inhibitory to PCR or disrupt extraction without appropriate Guardrail and processing volume adjustments.

Setting up the Unitized Reagent Strip on the BD MAX™

1. Wear nitrile gloves when handling Sample-Ready™ lyophilized reagents to reduce the generation of static charges. DO NOT use latex gloves.
2. ⚠ Use only BD MAX™ ExK™ DNA-1 extraction kits with the BioGX *Bordetella* Assay. DO NOT use BD MAX™ mastermix or the blank 0.3 mL conical tubes from the BD MAX™ ExK™ DNA-1 extraction kit.
3. Load one extraction cartridge into the extraction tray per specimen to be tested.
4. Snap one BD MAX™ ExK™ DNA-1 Extraction Tube into position 1 (Snap-in 1) of each Unitized Reagent Strip. (See Figure 1)

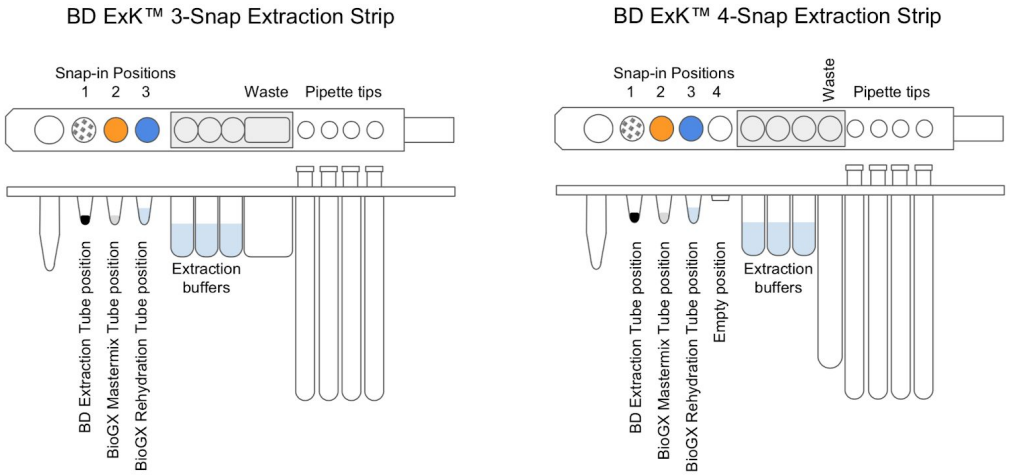

Figure 1 – Diagram of BD MAX™ ExK™ 3-snap and 4-snap Unitized Reagent Strips

5. Snap one BioGX Sample-Ready™ lyophilized PCR Master Mix reagent tube into position 2 (Snap-in 2) of each Unitized Reagent Strip. Check to make sure the Sample-Ready™ lyophilized cake is at bottom of tube prior to inserting into Unitized Reagent Strip. The funnel-shaped cake may be in any orientation (v, >, ^, <) in the ***bottom*** of the tube.
6. Snap one BioGX Rehydration Buffer tube into position 3 (Snap-in 3) of each Unitized Reagent Strip. Check to make sure the buffer is at bottom of tube prior to inserting into Unitized Reagent Strip.

7. Lift the tray and briefly examine the bottom of each Unitized Reagent Strip to ensure all reagents are at the bottom of each tube.
8. Proceed with worklist generation and sample loading per BD MAX™ operating instructions. Select the appropriate User Defined Protocol (eUDP) provided by BioGX. eUDPs are specific to extraction kit type and are programmed to be used with 3-snap strips unless otherwise indicated. If a 4-snap strip is used it is necessary to modify the eUDP to a 4-snap program by opening the eUDP in "Test Editor" and selecting the corresponding 4-snap extraction strip type in the "Extraction Type" drop down.
9. Load the extraction tray and, if necessary, a new PCR card into the instrument, close the door, and click "Start Run."

NOTE: Always first insert all Snap 1 tubes, then all Snap 2 tubes, then all Snap 3 tubes into the Unitized Reagent Strip.

NOTE: If using a 4-snap extraction strip, snap-in position 4 will remain empty.

QUALITY CONTROL

CONTROL

Each BioGX Bordetella Assay includes molecular primers and probes specific for the detection of the DNA sample processing control (SPC) present in the BD MAX™ ExK™ DNA-1 Extraction Kit. No external addition of SPC is required. The SPC serves as both a sample extraction control and a PCR internal amplification control (IAC).

RESULTS INTERPRETATION

Results are available on the *Results* tab in the *Results* window on the BD MAX™ System monitor. The BD MAX™ System software automatically interprets the test result when the BioGX eUDP is used. Possible results for each target are shown in Table 1. Presence of one or more of the targets is possible, and will result in multiple targets being positive at once.

Results	Interpretation
***ptxS1* POSITIVE** (Pertussis Toxin)	• The *ptxS1* target has a Ct within the valid range and endpoint above the minimum setting.
***IS481* POSITIVE** (B. pertussis, B. holmesii)	• The *IS481* target has a Ct within the valid range and endpoint above the minimum setting.
***hIS1001* POSITIVE** (B. holmesii)	• The *hIS1001* target has a Ct within the valid range and endpoint above the minimum setting.

***pIS1001* POSITIVE** (B. parapertussis)	• The *pIS1001* target has a Ct within the valid range and endpoint above the minimum setting.
***ptxS1* NEGATIVE,** ***IS481* NEGATIVE,** ***hIS1001* NEGATIVE,** OR ***pIS1001* NEGATIVE**	• The respective target did not amplify and the SPC has a Ct within the valid range and endpoint above the minimum setting.
UNR	• Unresolved Result. No target amplification; No SPC amplification.
IND	• Indeterminate due to BD MAX™ System failure (with Warning or Error Codes*)
INC	• Incomplete Run (with Warning or Error Codes*)

*Refer to the "Troubleshooting section of the BD MAX™ System User's Manual for interpretation of warning and error codes.

NOTE: In the presence of a high concentration positive result for any target, the SPC may or may not amplify. This is normal.

Multiplex PCR Results Interpretation per Tatti et al. (see Reference below)

POS = Positive, NEG = Negative, UNR = Unresolved

ptxS1	*IS481*	*hIS1001*	*pIS1001*	SPC	Sample Positive for
POS or NEG*	POS	NEG	NEG	POS or UNR	*B. pertussis* POSITIVE
POS or NEG*	NEG	NEG	POS	POS or UNR	*B. parapertussis* POSITIVE
NEG	POS	POS	NEG	POS or UNR	*B. holmesii* POSITIVE
POS	POS	NEG	POS	POS or UNR	*B. pertussis* POSITIVE, *B. parapertussis* POSITIVE
POS	POS	POS	NEG	POS or UNR	*B. pertussis* POSITIVE, *B. holmesii* POSITIVE
POS	POS	POS	POS	POS or UNR	*B. parapertussis* POSITIVE, *B. holmesii* POSITIVE (possible *B. pertussis* POSITIVE)**
POS	NEG	NEG	NEG	POS or UNR	Presumed *B. bronchiseptica****
NEG	NEG	NEG	NEG	POS	NEGATIVE
NEG or UNR	NEG or UNR	NEG or UNR	NEG or UNR	UNR	UNR

Tatti et al. reported that a specimen positive for *pIS1001* may be considered to most probably contain *B. parapertussis*, but the possibility that it is positive for *B. bronchiseptica* cannot be totally excluded.

*Samples that are positive for *IS481* and are not positive for *ptxS1* are presumed to be *B. pertussis* and samples that are positive for *pIS1001* and not positive for *ptxS1* are presumed to be *B. parapertussis*.

**Samples that are positive for all targets: *ptxS1, IS481, hIS1001, and pIS1001* can be reported as positive for *B. parapertussis* and *B. holmesii* however this result does not rule out the possibility of coinfection with *B. pertussis* as well.

***Samples that are positive for only *ptxS1* (pertussis toxin) are presumed to be positive for *B. bronchiseptica*, however this assay is not designed for species identification of *B. bronchiseptica* and BioGX recommends additional biochemical and molecular testing of samples with this result to confirm diagnosis.

REPEAT TEST PROCEDURE

In case of instrument failure, repeat testing can be performed by setting up a new run using the original sample/specimen and a fresh SBT as described above in the Specimen Preparation section.

LIMITATIONS OF THE PROCEDURE

- This product is intended for use with nasal swab specimens collected using specimen collection and transport devices listed in the “Equipment and Materials Required But Not Provided” section.
- This product should only be used with BD MAX™ Open System Reagents on the BD MAX™ System.
- Incorrect test results may occur from improper specimen collection, handling or storage, technical error, sample mix-up or because the number of organisms in the specimen is below the analytical sensitivity of the test. Careful compliance with the package insert instructions and the BD MAX™ System User’s Manual are necessary to avoid erroneous results.
- Good laboratory technique is essential for the proper performance of this assay. Due to the high analytical sensitivity of this test, extreme care should be taken to preserve the purity of all materials and reagents.
- A positive test result does not necessarily indicate the presence of viable infectious organisms. A positive result is indicative of the presence of target nucleic acid.
- As with all PCR-based *in vitro* diagnostic tests, extremely low levels of target below the limit of detection of the assay may be detected, but results may not be reproducible.
- False negative results may occur due to loss of nucleic acid from inadequate collection, transport or storage of specimens, or due to a inadequate cell lysis and/or extraction. The Sample Processing Control has been added to the test to aid in the identification of specimens that contain inhibitors to PCR amplification and as a control for reagent integrity and of the assay system as a whole. The Sample Processing Control does not indicate if nucleic acid has been lost due to

inadequate collection, transport or storage of specimens, or if cells have been adequately lysed.

- The BioGX *Bordetella* Assay results may sometimes be Unresolved due to an invalid Sample Processing Control, or be Indeterminate or Incomplete due to instrument failure, and require retesting that can lead to a delay obtaining final results.
- Mutations or polymorphisms in primer- or probe-binding regions may affect detection of new or unknown *Bordetella pertussis, Bordetella parapertusis,* and *Bordetella holmesii* resulting in a false negative result with the BioGX *Bordetella* Assay.
- The BioGX *Bordetella* Assay requires the use of five (5) optical channels from the BD MAX™ System: 475/520 channel, 530/565 channel, 585/630 channel, 630/665 channel, and 680/715 channel.

PERFORMANCE CHARACTERISTICS

Analytical Performance

The QCMD 2014 and 2015 Bordetella Panels (N=12 and 10, respectively) were tested on the BioGX *Bordetella* Assay. Samples were spiked into BD MAX SBT tubes and subjected to full extraction mode utilizing BD MAX ExK DNA-1 Unitized Reagent Strips.

Qnostics 2014 B. pertussis Panel with DNA-1

Target	Result
B. pertussis (N = 6)	100% concordant
B. parapertussis (N = 1)	100% concordant
B. holmesii (N =1)	100% concordant
Bordetella Negative (N = 2)	100% concordant
*B. bronchiseptica*** (N = 2)	100% concordant

Qnostics 2015 B. pertussis Panel with DNA-1

Target	Result
B. pertussis (N = 5)	100% concordant
B. parapertussis (N = 1)	100% concordant
B. holmesii (N= 1)	100% concordant
Bordetella Negative (N = 2)	100% concordant
*B. bronchiseptica*** (N = 1)	100% concordant

**Samples that are positive for only *ptxS1* (pertussis toxin) are presumed to be positive for *B. bronchiseptica*, however this assay is not designed for species identification of *B. bronchiseptica* and BioGX recommends additional biochemical and molecular testing of samples with this result to confirm diagnosis.

Bordetella Speciation Plus Toxin - OSR for BD MAX™ was tested against the QCMD 2016 Bordetella pertussis DNA EQA Programme. All core samples were concordant with the expected result except for *H. influenzae* sample which was presumed to be environmental contamination.

QCMD 2016 Bordetella pertussis DNA EQA Programme Results

Sample	Expected Result	Result
B. pertussis CORE	IS481+, ptxS1+	100% concordant
B. bronchiseptica (IS481+) EDUCATIONAL	IS481+, ptxS1+	ptxS1 positive, no detection of IS481+
B. pertussis CORE	IS481+, ptxS1+	100% concordant
B. parapertussis CORE	pIS1001+, ptxS1+	100% concordant
B. pertussis EDUCATIONAL	IS481+, ptxS1+	100% concordant
B. holmesii (IS481+) EDUCATIONAL	IS481+, hIS1001+	hIS1001 positive, missed IS481 in one of two replicates
Negative CORE	Negative	100% concordant
H. influenzae CORE	Negative	pIS1001 positive in one of two replicates*
B. pertussis CORE	IS481+, ptxS1+	100% concordant
B. pertussis CORE	IS481+, ptxS1+	100% concordant
B. pertussis CORE	IS481+, ptxS1+	100% concordant
B. pertussis CORE	IS481+, ptxS1+	100% concordant

*pIS1001 amplification was recorded at Ct of 39.8. The late amplification in the pIS1001 optical channel is beyond the Limit of Detection for this target.

Analytical Sensitivity

The analytical sensitivity for the BioGX *Bordetella* Assay was determined as follows: Dilution series of positive synthetic DNA samples for each target were added to the SBT in duplicate. Analytical sensitivity (Limit of Detection, LoD) was defined as the lowest concentration at which 95% of all replicates tested positive.

Target	LoD (copies per SBT)	LoD (copies per reaction*)
IS481	182	12.7
hIS1001	181	12.6

ptxS1	115	8.00
pIS1001	364	25.3

*Assuming 100% extraction efficiency on the BD MAX™

Analytical Inclusivity
An *in situ* analytical inclusivity study was performed using a variety of *Bordetella* strains. The BioGX *Bordetella* Speciation Plus Toxin - OSR for BD MAX™ detected the following: *Bordetella pertussis, Bordetella parapertussis, Bordetella holmesii,* and *Bordetella bronchioseptica.*

Analytical Specificity
The BioGX *Bordetella* Assay was performed against samples containing high levels of non-target organisms, using the BD MAX™ System, to demonstrate the specificity of the assay for the detection of *Bordetella pertussis, Bordetella parapertussis, Bordetella holmesii,* and pertussis toxin. Testing against the following targets yielded negative results on the BioGX *Bordetella* Assay:

Adenovirus, *Atopobium vaginae, Campylobacter jejuni, Campylobacter lari*, *Campylobacter upsaliensis*, *Campylobacter ureolyticus, Candida albicans, Candida glabrata*, *Candida krusei*, *Candida parapsilosis, Candida tropicalis*, *Citrobacter freundii*, *Cryptosporidium spp*., *Cyclospora cayatanensis, Dientamoeba fragilis*, Echovirus, *Entamoeba histoliticus, Enterobacter aerogenes, Escherichia coli, Gardnerella vaginitis*, *Giardia intestinalis*, Group A Streptococcus spp., Group B Streptococcus spp., HSV-1, HSV-2, *Klebsiella oxytoca*, *Klebsiella pnuemoniae*, Listeria spp., Norovirus GI, Norovirus GII, Rotavirus, *Salmonella spp., Shigella spp., vanA, vanB.*

Reproducibility
The reproducibility study was performed on pertussis toxin synthetic target template by three separate technicians independently on two BD MAX™ instruments. Using one lot of reagents, a series dilution of DNA template was run between 100,000X LoD and 10^{-1} LoD dilutions of the stock template. All samples from 1X LoD to 100,000X LoD were concordant positive between samples and technologists. All samples run at 10^{-1} LoD were concordant negative, as expected.

Manufacturing Reproducibility
Five independent lots were manufactured and were found to be equivalent based on internally established QC acceptance procedures. The lots included two test lots: #016-223-268 and #016 277-342 as well as a three validation lots #016-298-382, #016-309-405, and #016-356-499.

REFERENCES

1. Clinical and Laboratory Standards Institute. Protection of laboratory workers from occupationally acquired infections; Approved Guideline. Document M29 (Refer to the latest edition).
2. Centers for Disease Control and Prevention and National Institutes of Health. Biosafety in

microbiological and biomedical laboratories. Choosewood L.C. and Wislon D.E. (eds) (2009). HHS Publication No. (CDC) 21-1112.

3. Centers for Disease Control Website, www.cdc.gov, accessed October 12, 2016.
4. "Novel Multitarget Real-Time PCR Assay for Rapid Detection of *Bordetella Species* in Clinical Specimens.," Kathleen M. Tatti,* Kansas N. Sparks, et al. (Centers for Disease Control and Prevention), J. Clin. Microbiol. 2011 Dec;49(12):4059-66.
5. Clinical and Laboratory Standards Institute. Protection of laboratory workers from occupationally acquired infections; Approved Guideline. Document M29 (Refer to the latest edition).
6. Centers for Disease Control and Prevention and National Institutes of Health. Biosafety in microbiological and biomedical laboratories. Choosewood L.C. and Wislon D.E. (eds) (2009). HHS Publication No. (CDC) 21-1112.

REVISION HISTORY

Version	Date	Description of Change
08	01FEB2019	Updated storage recommendations from 2-8°C. to 2-25°C.
07	09NOV2018	Updated results interpretation. Added use of BD ExK 4-snap.
06	30 AUG 2018	Updated reagent section to reflect new packaging, added new performance data, and updated recommended specimen processing guidelines.
05	19 JUN 2018	Updated open pouch stability
04	22 FEB 2018	Transfer of product to BioGX EU
03	19 OCT 2017	Updated Summary and Explanation
02	07 SEP 2017	Updated Results Interpretation
01	29 MAR 2017	Initial Release

SYMBOLS

Symbol	Meaning
	Catalog number
	*In vitro* diagnostic medical device
	Do not reuse
	Batch code
	Caution
	Consult instructions for use
	Manufacturer
	Contains sufficient for <n> tests
	Authorized Representative in the European Community
	Temperature limitation
	Keep dry
	Keep away from sunlight
	Expiration date
	Biological Risks
	Control

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