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NOTICE of CHANGE dated 07/02/2025

IMPORTANT COMMUNICATION FOR THE USERS OF PRODUCT:

«Bordetella ELITe MGB® Kit»
Ref. RTS140ING

This new revision of the Instruction for Use (IFU) contains the following changes:

Insertion of interpretative sentence relating to IS481 in paragraphs:

- *"Validation of Sample results"*
- *"Potential interfering markers (cross-reactivity)"*
- *"Potential interfering markers (inhibition)"*
- *"Procedure limitations"*

Composition, use and performance of the product remain unchanged.

PLEASE NOTE

	LA REVISIONE DI QUESTO IFU E' COMPATIBILE ANCHE CON LA VERSIONE PRECEDENTE DEL KIT
	THE REVIEW OF THIS IFU IS ALSO COMPATIBLE WITH THE PREVIOUS VERSION OF THE KIT
	CET IFU MIS A JOUR ANNULE ET REMPLACE ET EST PARFAITEMENT COMPATIBLE AVEC LA VERSION PRECEDENTE DU KIT
	LA REVISIÓN DE ESTE IFU ES COMPATIBLE TAMBIÉN CON LA VERSIÓN ANTERIOR DEL KIT
	A REVISÃO DO ESTE IFU ÉTAMBÉM COMPATÍVEL COM A VERSÃO ANTERIOR DO KIT
	DIESE FASSUNG DER GEBRAUCHSANLEITUNG IST KOMPATIBEL MIT DER VORHERIGEN VERSION DES TESTKITS

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Bordetella ELITe MGB® Kit

reagent for DNA Real Time amplification

REF RTS140ING CE -20°C

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INTENDED USE

The **«Bordetella ELITe MGB® Kit»** product is part of a qualitative multiplex nucleic acids amplification assay for the detection and identification of the DNA of ***Bordetella pertussis*** **(BP),** ***Bordetella parapertussis*** **(BPP)** and ***Bordetella holmesii*** **(BH)** in clinical samples.

The assay was validated in association with **ELITe InGenius®** system starting from nasopharyngeal aspirate.

The product is intended for use as an aid in the diagnosis of *Bordetella pertussis*, *Bordetella parapertussis* and *Bordetella holmesii* respiratory infections, in conjunction with the patient's clinical data and other laboratory test results.

ASSAY PRINCIPLES

The assay consists of a multiplex real time amplification reaction performed by **ELITe InGenius®**, an automated and integrated system for extraction, amplification and detection of nucleic acids and result interpretation.

Starting from DNA extracted from each sample under test, different amplification reactions are performed by the **BORD PCR Mix** in the PCR Cassette in order to amplify the following targets:

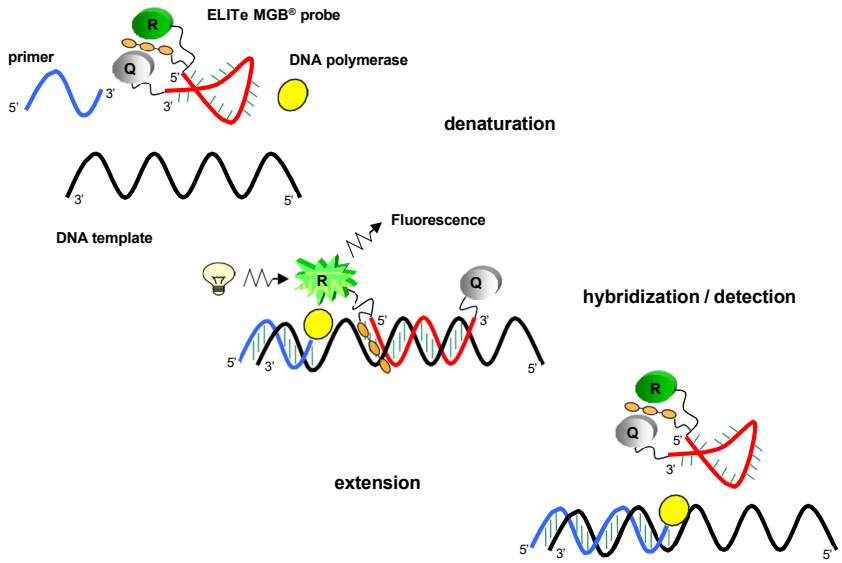
- IS481 repeated sequence of *B. pertussis* and *B. holmesii*, detected by the specific probe **IS481** (Channel 1),
- ptxA gene of *B. pertussis*, detected by the specific probe **BP** (Channel 5),
- recA gene of *B. holmesii*, detected by the specific probe **BH** (Channel 6),
- IS1001 repeated sequence of *B. parapertussis*, detected by the specific probe **BPP** (Channel 4).

The BORD PCR Mix also amplifies the extraction and inhibition Internal Control based on an artificial sequence (IC2) and detected by the specific probe **IC** (Channel 2).

The probes with ELITe MGB® technology are activated when they hybridize with the specific product of the amplification reaction. The fluorescence emission is measured and recorded by the instrument. At the end of amplification cycle, the fluorescence plots are analysed to identify the threshold cycles (Ct). The result interpretation allows to detect the presence of the pathogens of interest in the starting sample.

The assay has been validated with **ELITe InGenius**, an automated and integrated system for extraction, amplification and detection of nucleic acids and result interpretation.

In the following picture is shortly showed the mechanism of activation and fluorescence emission of ELITe MGB® technology probe. Note that the probe is not hydrolyzed during the amplification cycle.



PRODUCT DESCRIPTION

The **«Bordetella ELITe MGB® Kit»** product supplies a complete and ready to use mixture for Real Time amplification, **BORD PCR Mix**, aliquoted into eight test tubes. Each tube contains **280 µL** of solution, sufficient for **12 tests** in optimal reagent consumption conditions (at least 2 tests per session) when used with **ELITe InGenius** system.

The BORD PCR Mix contains the specific primers and probe for:

- the **IS481** repeated sequence of *B. pertussis* and *B. holmesii*. The probe **IS481** (Channel 1) is labelled with FAM fluorophore, stabilized by the MGB® group and quenched by a non-fluorescent moiety,
- the **ptxA** gene of *B. pertussis*. The probe **BP** (Channel 5) is labelled with AP639 fluorophore, stabilized by the MGB® group and quenched by a non-fluorescent moiety,
- the **recA** gene of *B. holmesii.* The probe **BH** (Channel 6) is labelled with AP690 fluorophore, stabilized by the MGB® group and quenched by a non-fluorescent moiety,
- the **IS1001** repeated sequence of *B. parapertussis*. The probe **BPP** (Channel 4) is labelled with AP593 fluorophore, stabilized by the MGB® group and quenched by a non-fluorescent moiety,
- the **IC2** artificial sequence of exogenous Internal Control (IC). The probe **IC** (Channel 2) is labelled with AP525 fluorophore, stabilized by the MGB® group and quenched by a non-fluorescent moiety.

The BORD PCR Mix contains the buffer, the magnesium chloride, the nucleotide triphosphates, the stabilizers and the enzyme Taq DNA polymerase with thermic activation (hot start).

The product is sufficient for **96 tests in association with ELITe InGenius** system, including controls.

MATERIALS PROVIDED IN THE PRODUCT

Component	Description	Quantity	Classification of hazards
BORD PCR Mix	Complete reaction mixture WHITE cap	**8 x 280 µL**	-

MATERIALS REQUIRED BUT NOT PROVIDED IN THE PRODUCT

- Laminar airflow hood.
- Disposable nitrile powder-free gloves or similar material.
- Vortex mixer.
- Bench microcentrifuge (12,000 - 14,000 RPM).
- Micropipettes and sterile tips with aerosol filter or sterile positive displacement tips (2-20 µL, 5-50 µL, 50-200 µL, 200-1000 µL).
- Molecular biology grade water.

OTHER PRODUCTS REQUIRED

The reagents for the extraction of DNA from the samples to be analyzed, the extraction and inhibition Internal Control, the amplification Positive Control and the consumables are **not** included in this product.

For automatic DNA extraction, Real Time PCR and result interpretation of samples, the **«ELITe InGenius»** instrument (ELITechGroup S.p.A., ref. INT030) and the following specific Assay Protocols (ELITechGroup S.p.A.) are required:

- parameters for positive control amplification **«BORD ELITe_PC»**,
- parameters for negative control amplification **«BORD ELITe_NC»**,
- parameters for nasopharyngeal aspirate samples to be analyzed **«BORD ELITe_NPA_200_100»**.

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With the instrument **«ELITe InGenius»** the following generic products are required:

- extraction cartridges **«ELITe InGenius® SP 200»** (ELITechGroup S.p.A., ref. INT032SP200),
- consumables for extraction **«ELITe InGenius® SP 200 Consumable Set»** (ELITechGroup S.p.A, ref. INT032CS),
- amplification cartridges **«ELITe InGenius® PCR Cassette»** (ELITechGroup S.p.A, ref. INT035PCR),
- tips **«300 µL Filter Tips Axygen»** (Axygen BioScience Inc., CA, ref. TF-350-L-R-S),
- boxes **«ELITe InGenius® Waste Box»** (ELITechGroup S.p.A, ref. F2102-000).

As template of extraction and inhibition Internal Control, the generic product **«CPE - Internal Control»** (ELITechGroup S.p.A., ref. CTRCPE), is required. This is a stabilised solution containing plasmid DNAs and phage genomic RNA.

As template of amplification positive control, the specifc product **«Bordetella - ELITe Positive Control»** (ELITechGroup S.p.A., ref. CTR140ING), is required. This is a stabilised solution containing plasmid DNAs.

WARNINGS AND PRECAUTIONS

This product is designed for *in-vitro* use.

General warnings and precautions

Handle and dispose of all biological samples as if they were able to transmit infective agents. Avoid direct contact with the biological samples. Avoid splashing or spraying. The materials that come into contact with the biological samples must be treated for at least 30 minutes with 3% sodium hypochlorite or autoclaved for one hour at 121 °C before disposal.

Handle and dispose of all reagents and all materials used to carry out the assay as if they were able to transmit infective agents. Avoid direct contact with the reagents. Avoid splashing or spraying. Waste must be handled and disposed of in compliance with adequate safety standards. Disposable combustible material must be incinerated. Liquid waste containing acids or bases must be neutralised before disposal.

Wear suitable protective clothes and gloves and protect eyes and face.
Never pipette solutions by mouth.
Do not eat, drink, smoke or apply cosmetic products in the work areas.
Carefully wash hands after handling samples and reagents.
Dispose of leftover reagents and waste in compliance with the regulations in force.
Carefully read all the instructions provided with the product before running the assay.
While running the assay, follow the instructions provided with the product.
Do not use the product after the indicated expiry date.
Only use the reagents provided with the product and those recommended by the manufacturer.
Do not use reagents from different batches.
Do not use reagents from other manufacturers.

Warnings and precautions for molecular biology

Molecular biology procedures require qualified and trained staff to avoid the risk of erroneous results, especially due to the degradation of nucleic acids contained in the samples or sample contamination by amplification products.

Laboratory coats, gloves and tools dedicated to work session setup are needed. It is necessary to have available separate areas for the molecular biology test and the microbiological culture test. Never handle the liquid or solid culture into the area designated for extraction / amplification reactions.

The samples must be suitable and, if possible, dedicated for this type of analysis. Samples must be handled under a laminar airflow hood. Pipettes used to handle samples must be exclusively used for this specific purpose. The pipettes must be of the positive displacement type or be used with aerosol filter tips. The tips used must be sterile, free from DNases and RNases and free from DNA and RNA.

The PCR Cassettes must be handled in such a way to reduce as much as possible amplification product diffusion into the environment in order to avoid sample and reagent contamination.

Warnings and precautions specific for the components

The **PCR Mix** must be stored at -20 °C in the dark.

The **PCR Mix** can be frozen and thawed for no more than **seven times**: further freezing / thawing cycles may cause a loss of product performance.

SAMPLES AND CONTROLS

Samples

This product must be used with the following clinical samples:

Nasopharyngeal aspirate

The nasopharyngeal aspirate samples for nucleic acid extraction must be collected according to laboratory guidelines, transported and stored at room temperature (+18 / +25 °C) for a maximum of two days or at +2 / +8 °C for a maximum of seven days, otherwise they must be frozen and stored at -20 °C for a maximum of one month or at -70 °C for longer periods.

It is recommended to split the samples to be frozen into aliquots in order to prevent repeated cycles of freezing and thawing. When using frozen samples, thaw the samples just immediately before the extraction in order to avoid possible nucleic acid degradation.

Before the analysis with this product 0.2 mL of sample has to be transferred in the Sonication Tube provided with «ELITe InGenius® SP 200 Consumable Set».

Note: To carry out the DNA extraction from nasopharyngeal aspirate by the ELITe InGenius system and ELITe InGenius Software version 1.3 (or later versions), use the Assay Protocol **BORD ELITe_NPA_200_100**. This protocol processes 200 µL of sample, adds the **CPE** (Internal Control) at 10 µL / extraction and elutes the nucleic acids in 100 µL.

Interfering substances

Data available concerning inhibition caused by drugs and other substances are reported in "Interfering substances" paragraph of "Performance characteristics" chapter.

Amplification controls

Before analysis of any sample, it is absolutely mandatory to generate and to approve the amplification controls for the amplification reagent lot that will be used in testing:

- as amplification Positive Control, use the **Bordetella – ELITe Positive Control** reagent (not provided with this kit) in association with Assay Protocol **BORD ELITe_PC**,
- as amplification Negative Control, use molecular biology grade water (not provided with this kit) in association with Assay Protocol **BORD ELITe_NC**.

Note: The **ELITe InGenius** system requires approved and valid results of amplification controls for each amplification reagent lot stored in its database.

The amplification control results, approved and stored in the database, will expire **after 15 days**. At the expiration date it is necessary to re-run the positive and negative controls in association with the amplification reagent lot in use.

Furthermore, the amplification controls must be re-run when:

- a new lot of amplification reagents is started,
- the results of quality controls (see following paragraph) are out of specification,
- any major maintenance service is performed on the **ELITe InGenius** instrument.

Quality controls

The planned validation of the extraction and amplification procedure is recommended. Tested samples or certified reference material can be used. External controls shall be used in accordance with local, state and federal accrediting organizations, as applicable.

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PROCEDURE

The procedure to use the **Bordetella ELITe MGB® Kit** with the **ELITe InGenius** system consists of three steps:

- Verification of the system readiness,
- Setup of the session,
- Review and export of results.

Verification of the system readiness

Before starting the session, referring to the instrument documentation, it is necessary to:

- switch on the **ELITe InGenius** instrument and select the login mode "**CLOSED**",
- verify that the amplification controls (Controls, BORD Positive Control, BORD Negative Control) were run, in association with the amplification reagent lot to be used and the results are approved and valid (Status). If there are not amplification control results approved or valid, generate them as described in the following paragraphs,
- choose the type of run, following the instructions on the Graphical User Interface (GUI) for the session setup and using the Assay Protocols provided by ELITechGroup S.p.A. These IVD protocols were specifically validated with ELITe MGB® kits, the **ELITe InGenius** instrument and the cited matrix.

The Assay Protocol available for sample testing with the product **Bordetella ELITe MGB® Kit** is described in the table below.

Assay Protocol for Bordetella ELITe MGB® Kit

Name	Matrix	Report	Characteristics
BORD ELITe_NPA_200_100	Nasopharyngeal aspirate	Positive / Negative	Extraction Input Volume: 200 µL Extraction Elute Volume: 100 µL Internal Control: 10 µL Sonication: NO Dilution Factor: 1 PCR Mix volume: 20 µL Sample PCR input volume: 20 µL

If the Assay Protocol of interest is not loaded in the system, contact your local ELITechGroup Customer Service.

Setup of the session

The product **Bordetella ELITe MGB® Kit** can be used with the **ELITe InGenius** system in order to perform:

A. Integrated run (Extract + PCR),
B. Amplification run, (PCR only),
C. Amplification run for Positive Control and Negative Control (PCR only),

All the parameters needed for the session are included in the Assay Protocol available on the instrument and are automatically recalled when the Assay Protocol is selected.

Note: The **ELITe InGenius** system can be linked to the "Location Information Server" (LIS) through which it is possible to load the work session information. Refer to the instrument user's manual for more details.

The main steps for the setup of the three types of run are described here below.

A. Integrated run

To setup an integrated run with sample extraction and amplification, carry out the following steps as per the GUI:

1. Thaw BORD PCR Mix tubes for the session. Each tube is sufficient for 12 reactions in optimal reagent consumption conditions (at least 2 tests per session). Mix gently, spin down the content for 5 seconds.

Note: Thaw BORD PCR Mix in the dark because this reagent is sensitive to the light.

2. Thaw the CPE tubes for the session. Each tube is sufficient for 12 extractions. Mix gently, spin down the content for 5 seconds.
3. Select “Perform Run” from the “Home” screen.
4. Ensure that the “Extraction Input Volume” is 200 µL and the “Extracted Elute Volume” is 100 µL.
5. For each Track of interest fill in the “Sample ID” (SID) by typing or by scanning the sample barcode.
6. Select the Assay Protocol to be used in the “Assay” column (e.g. BORD ELITe_NPA_200_100).
7. Ensure that the “Protocol” displayed is: “Extract + PCR”.
8. Select “Extraction Tube” in the “Sample Position” column.
9. Click “Next” to continue the setup.
10. Load CPE and BORD PCR Mix on the “Inventory Block” selected by following the GUI instruction. Click “Next” button to continue the setup.
11. Load and check the Tip Racks in the “Inventory Area” selected by following the GUI instruction. Click “Next” button to continue the setup.
12. Load the “PCR Cassettes”, the “ELITe InGenius SP 200” extraction cartridges, all the required consumables and the samples to be extracted in the positions specified in step 8, following the GUI instruction. Click “Next” to continue the setup.
13. Close the instrument door.
14. Press “Start” to start the run.

After process completion, the **ELITe InGenius** system allows users to view, approve, store the results and to print and save the report.

Note: At the end of the run the remaining extracted sample in the “Elution tube” must be removed from the instrument, capped, identified and stored at -20 °C for one month. Avoid spilling the Extracted Sample.

Note: At the end of the run the PCR Cassettes with the reaction products and the consumables must be removed from the instrument and disposed of without environmental contaminations. Avoid spilling the reaction products.

Note: The PCR Mix can be kept on board in the refrigerated block up to 7 work sessions of 3 hours each.

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B. Amplification run

To set up the amplification run starting from extracted DNA, carry out the following steps as per GUI:

1. Thaw BORD PCR Mix tubes for the session. Each tube is sufficient for 12 reactions in optimal reagent consumption conditions (at least 2 tests per session). Mix gently, spin down the content for 5 seconds.

Note: Thaw BORD PCR Mix in the dark because this reagent is sensitive to the light.

2. Select “Perform Run” from the “Home” screen.
3. Even if no extraction will be carried out, ensure that the “Extraction Input Volume” is 200 µL and the “Extracted Elute Volume” is 100 µL.
4. For each Track of interest fill in the SID by typing or by scanning the sample barcode.
5. Select the Assay Protocol to be used in the “Assay” column (e.g. BORD ELITe_NPA_200_100).
6. Select “PCR Only” in the “Protocol” column.
7. Ensure the sample loading position in the “Sample Position” column is “Elution Tube (bottom row)”. Click “Next” to continue the setup.
8. Load BORD PCR Mix on the “Inventory Block” selected by following the GUI instruction. Click “Next” to continue the setup.
9. Load and check the Tip Racks in the “Inventory Area” selected by following the GUI instruction. Click “Next” to continue the setup.
10. Load the “PCR Cassettes” and the extracted Nucleic Acid samples following the GUI instruction. Click “Next” to continue the setup.
11. Close the instrument door.
12. Press “Start” to start the run.

After process completion, the **ELITe InGenius** system allows users to view, approve, store the results and to print and save the report.

Note: At the end of the run the remaining extracted sample in the “Elution tube” must be removed from the instrument, capped and stored at -20 °C for one month. Avoid the spilling of the Extracted Sample.

Note: At the end of the run the PCR Cassettes with the reaction products and the consumables must be removed from the instrument and disposed of without environmental contaminations. Avoid any spilling of the reaction products.

Note: The PCR mix can be kept on board in the refrigerated block up to 7 work sessions of 3 hours each.

C. Amplification run for Positive Control and Negative Control

To setup the amplification run for Positive Control and Negative Control, carry out the following steps as per GUI:

1. Thaw BORD PCR Mix tubes for the session. Each tube is sufficient for preparing 12 reactions in optimal reagent consumption conditions (at least 2 tests per session). Mix gently, spin down the content for 5 seconds.

Note: Thaw BORD PCR Mix in the dark because this reagent is sensitive to the light.

2. Thaw the BORD Positive Control tube for the session. Each tube is sufficient for 4 sessions. Mix gently, spin down the content for 5 seconds.
3. Transfer at least 50 µL of molecular biology grade water to an "Elution tube", provided with the ELITe InGenius SP 200 Consumable Set.
4. Select "Perform Run" from the "Home" screen.
5. Even if no extraction will be carried out, ensure that the "Extraction Input Volume" is 200 µL and the "Extracted Elute Volume" is 100 µL.
6. In the Track of interest, select the Assay Protocol to be used in the "Assay" column.
7. For the positive control, select BORD ELITe_PC in the "Assay" column and fill in the lot number and expiry date of BORD Positive Control,
8. For the negative control, select BORD ELITe_NC and fill in the lot number and expiry date of the molecular biology grade water.
9. Click "Next" to continue the setup.
10. Load BORD PCR Mix on the "Inventory Block" selected by following the GUI instruction. Click "Next" to continue the setup.
11. Load / check the Tip Racks in the "Inventory Area" selected by following the GUI instruction. Click "Next" to continue the setup.
12. Load the "PCR Cassettes", the BORD Positive Control tube and the negative control tube following the GUI instruction. Click "Next" to continue the setup.
13. Close the instrument door.
14. Press "Start" to start the run.

After process completion, the **ELITe InGenius®** system allows users to view, approve, store the results and to print and save the report.

Note: At the end of the run the remaining Positive Control must be removed from the instrument, capped, identified and stored at -20 °C. Avoid spilling the control. The remaining Negative Control must be disposed.

Note: At the end of the run the PCR Cassettes with the reaction products and consumables must be removed from the instrument and disposed of without environmental contaminations. Avoid spilling the reaction products.

Note: The PCR mix can be kept on board in the refrigerated block up to 7 work sessions of 3 hours each.

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Review and approval of results

At the end of the run, the "Results Display" screen is automatically shown. In this screen the sample / Control results and the information regarding the run are shown. From this screen is possible to approve the result, print or save the reports ("Sample Report" or "Track Report"). Refer to the instrument user's manual for more details.

Note: The **ELITe InGenius** system can be linked to the "Location Information Server" (LIS) through which it is possible send the work session results to the laboratory data centre. Refer to the instrument user's manual for more details.

The **ELITe InGenius** system generates the results with the product **Bordetella ELITe MGB® Kit** through the following procedure:

A. Validation of amplification Positive Control and Negative Control results,
B. Validation of sample results,
C. Sample results reporting.

A. Validation of amplification Positive Control and Negative Control results

The fluorescence signals emitted by the probes of pathogen genes (channels **IS481**, **BP**, **BH** and **BPP**) in the Positive Control and Negative Control amplification reaction are analysed automatically and interpreted by the instrument software with the parameters included in the Assay Protocols "BORD ELITe_PC" and "BORD ELITe_NC".

The amplification Positive Control and Negative Control results, specific for the lot of amplification reagent used, are recorded in the database (Controls). They can be viewed and approved by personnel qualified as "Administrator" or "Analyst", following the GUI instructions.

The amplification Positive Control and Negative Control results, specific for the amplification reagent lot, will expire **after 15 days**.

The results of Positive Control and Negative Control amplification runs are used by the instrument software to setup the "Control Charts" monitoring the amplification step performances. Refer to the instrument user's manual for more details.

Note: If the amplification Positive Control or Negative Control result does not meet the acceptance criteria, the "Failed" message is shown on the "Controls" screen and it is not possible to approve it. In this case, the amplification Positive Control or Negative Control reaction has to be repeated.

Note: If the Positive Control or Negative Control is run together with samples to be tested and its result is invalid, the entire session is invalid. In this case, the amplification of all samples must be repeated too.

B. Validation of Sample results

The fluorescence signals emitted by the probes of pathogen genes (channels **IS481**, **BP**, **BH** and **BPP**) and by the probe of Internal Control (channel **IC**) in the sample amplification reactions are analysed automatically and interpreted by the instrument software with the parameters included in the Assay Protocol BORD ELITe_NPA_200_100.

Results are shown in the reports generated by the instrument ("Result Display").
The sample run can be approved when the two conditions reported in the table below are met.

1) Positive Control	**Status**
BORD Positive Control	APPROVED
2) Negative Control	**Status**
BORD Negative Control	APPROVED

For each sample, the assay result is automatically interpreted by the system as established by the **ELITe InGenius® Software** algorithm and the Assay Protocol parameters.

The possible result messages are listed in the table below. For each sample the system reports a combination of the following messages specifying if the pathogen DNAs are either detected or not detected.

Result of sample run	Interpretation
IS481: DNA detected.	The **DNA of *B. pertussis* or *B. holmesii* (IS481) was detected** in the sample. **Note:** when IS481 DNA is detected, *B. pertussis* (BP) or *B. holmesii* (BH) DNA could also be typed.
IS481: DNA detected other related species.	**IS481-like DNA from an unintended organism was detected** in the sample.
IS481: DNA not detected or below LoD.	The **DNA of *B. pertussis* and *B. holmesii* (IS481) was not detected** in the sample. The sample is negative for these pathogens or their concentration is below the Limit of Detection of the assay.
BP: typing positive.	The **DNA of *B. pertussis* was detected** in the sample. **Note:** when the BP target is detected, the IS481 target must also be detected.
BP: typing not feasible.	This target specific for ***B. pertussis* was not detected** in the sample. Please, check also the IS481 target results.
BH: typing positive.	The **DNA of *B. holmesii* was detected** in the sample. **Note:** when the BH target is detected, the IS481 target must also be detected.
BH: typing not feasible.	This target specific for ***B. holmesii* was not detected** in the sample. Please, check also the IS481 target results.
BPP: DNA detected.	The **DNA of *B. parapertussis* was detected** in the sample.
BPP: DNA not detected or below LoD.	The **DNA of *B. parapertussis* was not detected** in the sample. The sample is negative for this pathogen or its concentration is below the Limit of Detection of the assay.
Invalid - Retest Sample.	**Invalid assay result** caused by Internal Control failure due to incorrect extraction, inhibitors carry-over. The test should be repeated.

Samples reported as “Invalid - Retest Sample” by the **ELITe InGenius Software** are not suitable for result interpretation. In this case, the Internal Control DNA was not efficiently detected due to problems in the amplification or extraction step (degradation of DNA, loss of DNA during the extraction or inhibitor carry-over in the eluate), which may cause incorrect results.

When the eluate volume is sufficient, the extracted sample can be retested via an amplification run in “PCR Only” mode. In the case of a second invalid result, the sample must be retested starting from extraction of a new aliquot using “Extract + PCR” mode.

Samples reported as "IS481: DNA not detected or below LoD" or "BPP DNA Not Detected or below the LoD" are suitable for analysis but it was not possible to detect the targets DNA. In this case it cannot be excluded that the target DNAs are present at a concentration below the limit of detection of the assay (see “Performance characteristics”).

Samples reported as “IS481: DNA detected other related species” are suitable for analysis and the IS481-like DNA from an unintended organism was detected in the sample. In this case, the sample is reported positive for IS481 target but *B. holmesii* specific target DNA (BH) or *B. pertussis* specific target DNA (BP) were not detected in the sample.

When IS481 multicopy gene DNA is detected, in low positive samples the *B. pertussis* specific target DNA (BP) or the *B. holmesii* specific target DNA (BH) may not be detected due to differences specific target genes copy number (e. g. promoter of ptxA gene and recA gene are present in single copy in BP and BH, respectively). However, the sample is positive for *B. pertussis* or for *B. holmesii,* but identification will not be possible.

Note: The results obtained with this assay must be interpreted taking into account all the clinical data and the other laboratory test outcomes concerning the patient.

The sample run results are stored in the database and, if valid, can be approved (Result Display) by personnel qualified as “Administrator” or “Analyst”, following the GUI instruction. From the “Result Display” window it is possible to print and save the Sample run results as “Sample Report” and “Track Report”.

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C. Sample result export

The sample results are stored in the database and can be exported as “Sample Report” and “Track Report”.

The “Sample Report” shows the details of a work session sorted by selected sample (SID).

The “Track Report” shows the details of a work session by selected Track.

The "Sample Report" and "Track Report" can be printed and signed by authorized personnel.

PERFORMANCE CHARACTERISTICS

Limit of Blank (LoB)

The Limit of Blank (LoB) of the Bordetella ELITe MGB® Kit was defined in association with Nasopharyngeal Aspirate (NPA) samples and ELITe InGenius system.

The LoB was verified by testing a panel of 60 NPA clinical samples tested negative for the IS481 target (*B. pertussis* and *B. holmesii*) and for IS1001 (*B. parapertussis*) by cultural method and a CE IVD commercial assay at the external laboratory. Each sample was processed on ELITe InGenius system in "Extract + PCR" mode. Due to high analytical sensitivity, the LoB was defined by applying a Ct Cut-off equal to 40 for the IS481 target (*B. pertussis* and *B. holmesii*) and for BPP target (IS1001, *B. parapertussis*) in order to obtain the 95% of negative calls.

The final results are reported in the following table.

***B. pertussis* / *B. holmesii* (IS481 target)**				
Matrix	**N**	**Positive**	**Negative**	**% negativity**
Negative Nasopharyngeal aspirates	60	3	57	95
***B. parapertussis* (BPP target)**				
Matrix	**N**	**Positive**	**Negative**	**% negativity**
Negative Nasopharyngeal aspirates	60	1	59	98

The samples that gave a positive result in association with the Bordetella ELITe MGB® Kit and ELITe InGenius system showed high Ct values (between 37 and 40) and so they were at very low concentration.

Limit of Detection (LoD)

The Limit of Detection (LoD) of the Bordetella ELITe MGB® Kit was defined in association with Nasopharyngeal Aspirate (NPA) samples and ELITe InGenius system.

The LoD was calculated by testing a panel of negative NPA samples spiked with a reference material of *B. pertussis, B. parapertussis* and *B. holmesii* (DMSZ, Germany) at known titre. Six levels of dilutions were prepared starting from a concentration higher than the expected LoD value. Each dilution level was processed in 12 replicates on ELITe InGenius system in "Extract + PCR" mode. The LoD was estimated by Probit regression analysis of the data as the concentration corresponding to 95% probability of a positive call.

The final results are reported in the following table.

Target	**LoD (CFU / mL)**	**95% confidence interval limits**	
		Lower limit	**Upper limit**
B. pertussis (IS481 target)	12	7	58
B. parapertussis (IS1001 target)	11	6	19
B. holmesii (IS481 target)	12	5	34

Efficiency of detection (inclusivity)

The efficiency of detection of different strains or isolates of *B. pertussis, B. parapertussis* and *B. holmesii* of the product Bordetella ELITe MGB® Kit was evaluated by *in silico* analysis of sequences available in the EBI ENA nucleotide database.

The regions chosen for the hybridisation of the primers and the fluorescent probes were checked on the alignment of the sequences for the IS481 repeated sequence (*B. pertussis* and *B. holmesii*), the ptxA gene (*B. pertussis*), the recA gene (*B. holmesii*), IS1001 repeated sequence (*B. parapertussis*). The hybridisation regions showed sequence conservation and absence of significant mutations, so an efficient amplification of all the organisms analysed is expected.

The efficiency of detection of different strains or isolates of *B. pertussis, B. parapertussis* and *B. holmesii* was also evaluated through the analysis of a panel of certified materials tested at low concentration (about 100 copies / reaction).

Certified genomic DNA samples from Vircell Microbiologists (Spain), DSMZ (Germany) and ATCC (USA), were diluted and analyzed in triplicate in association with ELITe InGenius system in "PCR Only" mode.

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The final results are reported in the following table.

Inclusivity		
Organisms	**Strain**	**Outcome**
B. pertussis	Vircell, F-strain	IS481 detected, BP typing positive
B. parapertussis	Vircell, CDC F5101	BPP detected
B. holmesii	Vircell, clinical isolate	IS481 detected, BH typing positive
B. pertussis	DSMZ, clinical isolate	IS481 detected, BP typing positive
B. parapertussis	DSMZ, clinical isolate	BPP detected
B. holmesii	DSMZ, clinical isolate	IS481 detected, BH typing positive
B. pertussis	ATCC, Tohama I	IS481 detected, BP typing positive
B. parapertussis	ATCC, 12822	BPP detected

All the tested samples were detected as positive for the correct pathogen by the Bordetella ELITe MGB® Kit.

Potential interfering markers (cross-reactivity)

The potential cross-reactivity with other unintended organisms found in respiratory samples of the product Bordetella ELITe MGB® Kit was evaluated by *in silico* analysis of sequences available in the EBI ENA nucleotide database.

The analysis showed no significant homology for targets with the main part of unintended organisms (viruses, bacteria, protozoa and fungi), whereby no cross-reactivity is expected. However, significant homologies and potential interference were observed with some strains of *B. hinzii*, *B. bronchialis* and *B. bronchiseptica* for the IS481 detection, with some strains of *B. petrii* for the recA detection and with *Achromobacter denitrificans* for the IS1001 detection.

The cross-reactivity with other organisms found in respiratory samples was also verified by testing a panel of certified genomic DNA and RNA from ATCC (USA).

Genomic DNA samples were analysed in triplicate for each potentially interfering marker in association with ELITe InGenius system in "PCR Only" mode.

The final results are reported in the following table.

Potential interfering markers: cross-reactivity		
Organisms	**Strain**	**Outcome**
Aspergillus fumigatus	ATCC, 118	Negative, no cross-reactivity
Candida albicans	ATCC, 3147	Negative, no cross-reactivity
Staphylococcus aureus	ATCC, Rosenbach	Negative, no cross-reactivity
Escherichia coli	ATCC, H10407	Negative, no cross-reactivity
Bordetella bronchiseptica	ATCC, RB50	Negative, no cross-reactivity
Haemophilus influenzae	ATCC, Rd	Negative, no cross-reactivity
Streptococcus pneumoniae	ATCC, R6	Negative, no cross-reactivity
Legionella pneumophila	ATCC, Philadelphia-1	Negative, no cross-reactivity
Mycoplasma pneumoniae	ATCC, FH	Negative, no cross-reactivity
Chlamydophila pneumoniae	ATCC, AR-39	Negative, no cross-reactivity
Mycobacterium tuberculosis	clinical isolate	Negative, no cross-reactivity
CMV	ATCC, AD-169	Negative, no cross-reactivity
Enterovirus	ATCC, Pesascek	Negative, no cross-reactivity
ADV	ATCC, Adenoid 6	Negative, no cross-reactivity
FluA	ATCC, A/PR/8/34	Negative, no cross-reactivity
FluB	ATCC, B/Florida/4/2006	Negative, no cross-reactivity
RSV	ATCC, A2	Negative, no cross-reactivity
Bordetella petrii	DSMZ, DSM 12804	Negative, no cross-reactivity*
Bordetella petrii	clinical isolate, REF504	Negative, no cross-reactivity**
Bordetella petrii	clinical isolate, REF505	Negative, no cross-reactivity*
Bordetella petrii	clinical isolate, BORD1836	Negative, no cross-reactivity**
Bordetella petrii	clinical isolate, BUR-15-132	Negative, no cross-reactivity**
Bordetella petrii	clinical isolate, BUR-19-174	Negative, no cross-reactivity**

The main of potential interfering organisms tested showed no cross-reactivity for the targets using the Bordetella ELITe MGB® Kit.

As per sequence analysis, some strains of *Bordetella petrii (*)* were positive for BH target. However, due to the different melting Temperature (Tm) between the recA of *B. holmesii* and recA of other *Bordetella* species, the Assay Protocol calls "Typing not feasible".

Furthermore, other strains of *B. petrii* (**) have a IS481-like sequence and result positive for IS481 target. However, due to the different Tm between the IS481 of *B. pertussis* and *B. holmesii* and IS481-like of other *Bordetella* species, the Assay Protocol calls "other related species".

Potential interfering markers (inhibition)

The absence of inhibition due to other unintended organisms found in respiratory samples was verified by testing a panel of certified genomic DNA and RNA from ATCC.

Genomic DNA and RNA samples at high concentration (about 100,000 copies per reaction) were spiked by genomic DNA of *B. pertussis* or *B. parapertussis* or *B. holmesii* (DSMZ) at low concentration (about 100 copies per reaction) and analysed in triplicate for each potentially interfering marker in association with ELITe InGenius system in "PCR Only" mode.

The final results are reported in the following table.

Potential interfering markers: inhibition		
Organisms	**Strain**	**Outcome**
Aspergillus fumigatus	ATCC, 118	Positive, no inhibition
Candida albicans	ATCC, 3147	Positive, no inhibition
Staphylococcus aureus	ATCC, Rosenbach	Positive, no inhibition
Escherichia coli	ATCC, H10407	Positive, no inhibition
Bordetella bronchiseptica	ATCC, RB50	Positive, no inhibition
Haemophilus influenzae	ATCC, Rd	Positive, no inhibition
Streptococcus pneumoniae	ATCC, R6	Positive, no inhibition
Legionella pneumophila	ATCC, Philadelphia-1	Positive, no inhibition
Mycoplasma pneumoniae	ATCC, FH	Positive, no inhibition
Chlamydophila pneumoniae	ATCC, AR-39	Positive, no inhibition
Mycobacterium tuberculosis	clinical isolate	Positive, no inhibition
CMV	ATCC, AD-169	Positive, no inhibition
Enterovirus	ATCC, Pesascek	Positive, no inhibition
ADV	ATCC, Adenoid 6	Positive, no inhibition
FluA	ATCC, A/PR/8/34	Positive, no inhibition
FluB	ATCC, B/Florida/4/2006	Positive, no inhibition
RSV	ATCC, A2	Positive, no inhibition
Bordetella petrii	DSMZ, DSM 12804	Positive, no inhibition
Bordetella petrii	clinical isolate, REF504	Positive, no inhibition
Bordetella petrii	clinical isolate, REF505	Positive, no inhibition
Bordetella petrii	clinical isolate, BORD1836	Positive, no inhibition
Bordetella petrii	clinical isolate, BUR-15-132	Positive, no inhibition
Bordetella petrii	clinical isolate, BUR-19-174	Positive, no inhibition

All the pathogens of interest were correctly detected in presence of the potential interfering organisms listed above when tested by the Bordetella ELITe MGB® Kit.

Potential interference among targets

The potential interference among targets of the product Bordetella ELITe MGB® Kit was evaluated by a test of co-amplification of reference materials of *B. pertussis, B. parapertussis* (ATCC) and *B. holmesii* (DMSZ).

The panel included samples with genomic DNAs for *B. pertussis* or *B. parapertussis* or *B. holmesii* at high concentration (10^5 copies / reaction) and the other pathogens of interest at low concentration levels (10^3, 10^2, 10 copies / reaction).

Each condition was analysed in triplicate in association with ELITe InGenius system in "PCR Only" mode.

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The final results are reported in the following table.

Potential interference among targets			
Target under test	**Interfering target (~10^5 copies / reaction)**		
	B. pertussis	*B. parapertussis*	*B. holmesii*
B. pertussis (IS481)	-	10 c. / reaction	-
B. parapertussis (IS1001)	10 c. / reaction	-	100 c. / reaction
B. holmesii (IS481)	-	10 c. / reaction	-

B. pertussis, *B. parapertussis* and *B. holmesii* were detected by the Bordetella ELITe MGB® Kit at a concentration of at least 10 copies / reaction even in presence of other pathogens of interest at high concentration. In case of 10^5 copies / reaction of *B. holmesii*, *B. parapertussis* was detected at a concentration of at least 100 copies / reaction

B. pertussis was typed by the Bordetella ELITe MGB® Kit at a concentration of at least 10 copies / reaction in presence of *B. parapertussis* at high concentration and at a concentration of at least 2,000 copies / reaction in presence of *B. holmesii* at high concentration.

B. holmesii was typed by the Bordetella ELITe MGB® Kit at a concentration of at least 10 copies / reaction in presence of *B. parapertussis* at high concentration and at a concentration of at least 1,000 copies / reaction in presence of *B. pertussis* at high concentration.

Interfering substances

A panel of potentially interfering substances at their highest relevant concentrations was tested with the product Bordetella ELITe MGB® Kit. The substances tested were Mucin, Human Whole Blood, antibiotic Azithromycin, corticosteroid Beclometasone, antihistaminic Ebastine and mucolytic Ambroxol hydrochloride.

The substances were individually added to Nasopharyngeal Aspirate samples spiked by the reference materials of *B. pertussis*, *B. parapertussis* or *B. holmesii* (DSMZ) at concentration of 3x LoD. Samples were processed in 3 replicates on ELITe InGenius® system in "Extraction + PCR" mode.

The results are reported in the following table.

Interfering substances				
Substance	**Concentration**	**Pos. / Rep.**		
		B. pertussis	***B. parapertussis***	***B. holmesii***
Mucin	1% w/v (10 mg/mL)	3 / 3	3 / 3	3 / 3
Whole Blood	10% v/v	3 / 3	3 / 3	3 / 3
Azithromycin	0.2 µg/mL	3 / 3	3 / 3	3 / 3
Beclometasone	64 ng/mL	3 / 3	3 / 3	3 / 3
Ebastine	0.4 µg/mL	3 / 3	3 / 3	3 / 3
Ambroxol	0.6 µg/mL	3 / 3	3 / 3	3 / 3

None of the tested substances at the tested concentrations were found to interfere with the Bordetella ELITe MGB® Kit.

Repeatability

The Repeatability of results obtained by the product Bordetella ELITe MGB® Kit in association with the ELITe InGenius system was tested by performing the analysis of a panel of three Nasopharyngeal Aspirate samples spiked by the reference materials of *B. pertussis*, *B. parapertussis* or *B. holmesii* (DSMZ) at concentration of 3x LoD.

The Repeatability results were obtained through the analysis in three replicates in two runs per day with the same lot of product, on the same instrument, by the same operator. The results were analysed for the same lot of product (Intra-session Repeatability) and for the three different lots of product (Inter-batch Repeatability). Samples were processed on ELITe InGenius system in "Extract + PCR" mode.

The Ct values of each target and Internal Control target (IC2) were used to calculate the percentage Coefficient of Variability (%CV) in order to evaluate the Repeatability as imprecision.

A summary of results is shown in the table below.

Intra-session Repeatability				
Target	**N**	**Mean Ct**	**Dev std**	**%CV**
B. pertussis (IS481)	6	36.14	0.34	0.95
B. parapertussis (IS1001)	6	34.70	0.37	1.08
B. holmesii (IS481)	6	36.02	0.27	0.75
Internal Control	24	28.77	0.25	0.88

Inter-batch Repeatability				
Target	**N**	**Mean Ct**	**Dev std**	**%CV**
B. pertussis (IS481)	18	36.66	0.95	2.60
B. parapertussis (IS1001)	18	34.89	0.64	1.82
B. holmesii (IS481)	18	36.23	0.75	2.08
Internal Control	72	28.58	0.33	1.15

The Repeatability of the product Bordetella ELITe MGB® Kit for each target showed a %CV lower than 3%.

Reproducibility

The Reproducibility of results obtained by the product Bordetella ELITe MGB® Kit in association with the ELITe InGenius system was tested by performing the analysis of a panel of three Nasopharyngeal Aspirate samples spiked by the reference material of *B. pertussis*, *B. parapertussis* and *B. holmesii* (DSMZ) at concentration of 3x LoD.

The Reproducibility results were analysed in three replicates in two runs per day. Three different lots of product were tested in three different days, on three different instruments and by three different operators.

Samples were processed on ELITe InGenius system in “Extraction + PCR” mode.

The Ct values of each target and Internal Control target (IC2) were used to calculate the percentage Coefficient of Variability (%CV) in order to evaluate the Reproducibility as imprecision.

A summary of results is shown below.

Inter-Instrument Reproducibility				
Target	**N**	**Mean Ct**	**Dev std**	**%CV**
B. pertussis (IS481)	18	36.48	0.86	2.35
B. parapertussis (IS1001)	18	34.80	0.68	1.95
B. holmesii (IS481)	18	36.23	0.85	2.34
Internal Control	72	28.06	0.48	1.72

The Reproducibility of the product Bordetella ELITe MGB® Kit for each target showed a %CV lower than 3%.

Diagnostic Sensitivity: confirmation of positive samples

The Diagnostic Sensitivity of the assay, as confirmation of positive clinical samples, was evaluated by analysing Nasopharyngeal Asprirate clinical samples positive for *B. pertussis* and spiked by certified material of *B. parapertussis* or *B. holmesii* (DSMZ). The positive samples and the negative samples used for the spiking were certified by cultural method and a CE IVD commercial assay at the external laboratory. Contrived samples for *B. parapertussis* and *B. holmesii* were spiked at a concentration of 30x, 10x, 3x LoD.

Samples were collected as described in “Samples and Controls” and tested by Bordetella ELITe MGB® Kit and ELITe InGenius system in “Extraction + PCR” mode.

The results are summarized in the following table.

Samples	N	Positive	Negative	Invalid	% Diagnostic Sensitivity
B. pertussis positive Nasopharyngeal Aspirate	26	24	0	2	100%
B. parapertussis spiked Nasopharyngeal Aspirate	30	30	0	0	100%
B. holmesii spiked Nasopharyngeal Aspirate	30	30	0	0	100%

In the test, 24 out of 26 *B. pertussis* positive Nasopharyngeal Aspirate samples were confirmed positive. 2 samples resulted invalid and were excluded from the analysis.

In the test, all the *B. parapertussis and B. holmesii* spiked Nasopharyngeal Aspirate samples were confirmed positive.

In this test, the assay Diagnostic Sensitivity for *B. pertussis B. parapertussis* and *B. holmesii* was equal to 100 % in association with Nasopharyngeal Aspirate samples.

Diagnostic Specificity: confirmation of negative samples

The Diagnostic Specificity of the assay, as confirmation of negative clinical samples, was evaluated by analysing Nasopharyngeal Aspirate clinical samples certified negative for *B. pertussis*, *B. parapertussis* and *B. holmesii* by cultural method and a CE IVD commercial assay at the external laboratory.

Samples were collected as described in “Samples and Controls” then tested with Bordetella ELITe MGB® Kit and ELITe InGenius system in “Extraction + PCR” mode.

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The results are summarized in the following table.

Samples	N	Positive	Negative	Invalid	% Diagnostic Specificity
B. pertussis / *B. holmesii* negative Nasopharyngeal Aspirate	60	0	59	1	100%
B. parapertussis negative Nasopharyngeal Aspirate	86	1	82	3	98.8%

In the test, 59 out of 60 *B. pertussis* / *B. holmesii* negative Nasopharyngeal Aspirate samples were confirmed valid and negative. 1 sample resulted invalid and was excluded from the analysis.

In the test, 82 out of 86 *B. parapertussis* negative Nasopharyngeal Aspirate samples were confirmed valid and negative. 3 samples resulted invalid and were excluded from the analysis. 1 sample resulted discrepant positive.

In this test, the assay Diagnostic Specificity for *B. pertussis* / *B. holmesii* was equal to 100 % in association with Nasopharyngeal Aspirate samples.

In this test, the assay diagnostic specificity for *B. parapertussis* was equal to 98.8 % in association with Nasopharyngeal Aspirate samples.

Note: The complete data and results of the tests carried out to evaluate the product performance characteristics with matrices and instrument are recorded in the Product Technical File "Bordetella ELITe MGB® Kit", FTP 140ING.

REFERENCES

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N. K. Fry et al. (2009) J Med Microbiol. 58: 1023-9.
A. Van der Zee (1993) J Bacteriol. 175: 141-7.
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PROCEDURE LIMITATIONS

This product is exclusively designed for *in-vitro* use.

Use this product only with clinical samples of nasopharyngeal aspirate.

At the moment there are no data available concerning product performance with the following clinical samples: Nasopharyngeal Swab, Sputum, Broncho-alveolar lavage (BAL), Broncho aspirate (BA).

The results obtained with this product depend on an adequate identification, collection, transport storage and processing of the samples. To avoid incorrect results, it is therefore necessary to take care during these steps and to carefully follow the instructions for use provided with the products for nucleic acid extraction.

Owing to its high analytical sensitivity, the real time amplification method used in this product is sensitive to cross-contaminations from the positive samples, the positive controls and the same amplification products. Cross-contaminations cause false positive results. The product format is able to limit cross-contaminations. However, cross-contaminations can be avoided only by good laboratory practices and following these instructions for use.

This product must be handled by qualified personnel trained in the processing of potentially infective biological samples and chemical preparations classified as dangerous to prevent accidents with potentially serious consequences for the user and other persons.

This product requires the use of work clothes and areas that are suitable for the processing of potentially infective biological samples and chemical preparations classified as dangerous to prevent accidents with potentially serious consequences for the user and other persons.

This product requires the use of separate areas for the molecular biology test and the microbiological culture test to avoid false positive results.

This product requires the use of special laboratory clothing and instruments dedicated to work session setup to avoid false positive results.

Due to inherent differences between technologies, it is recommended that users perform method correlation studies to estimate technology differences prior to switching to a new technology.

A negative result obtained with this product means that the target DNA is not detected in the DNA extracted from the sample. It cannot be excluded that the target DNA has a lower titre than the product detection limit (see Performance Characteristics). In this case the result could be a false negative.

In case of co-infections, the sensitivity for one target can be affected by the amplification of a second target (see Performance Characteristics).

In some cases, *B. bronchiseptica, B. hinzii and B. bronchialis* can harbour the IS481 repeated sequences and so it can generate positive results for this target.

In some cases, *B. bronchiseptica and Achromobacter denitrificans* can harbour the IS1001 repeated sequences and so it can generate positive results for the BPP target.

Results obtained with this product may sometimes be invalid due to failure of Internal Control. In this case the sample shall be retested, starting from extraction, which can lead to a delay in obtaining final results.

Possible polymorphisms within the region of the target DNA covered by the product primers and probes may impair detection of target DNA.

As with any other diagnostic medical device, the results obtained with this product must be interpreted taking into consideration all the clinical data and other laboratory tests done on the patient.

As with any other diagnostic medical device, there is a residual risk of invalid, false positive and false negative results obtained with this product. This residual risk cannot be eliminated or further reduced. In some cases, this residual risk could contribute to wrong decisions with potentially dangerous effects for the patient.

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TROUBLESHOOTING

Invalid Positive Control reaction	
Possible Causes	**Solutions**
Instrument setting error.	Check the position of PCR Mix and positive control. Check the volumes of PCR Mix and positive control.
Positive control degradation.	Use a new aliquot of positive control.
PCR Mix degradation.	Use a new aliquot of PCR Mix.
Instrument error.	Contact ELITechGroup Technical Service.

Invalid Negative Control reaction	
Possible Causes	**Solutions**
Instrument setting error.	Check the position of PCR Mix and negative control. Check the volumes of PCR Mix and negative control.
Contamination of the negative control	Use a new aliquot of molecular biology grade water.
Contamination of the PCR Mix.	Use a new aliquot of PCR Mix.
Contamination of the extraction area, of Racks or of Inventory Block.	Clean surfaces with aqueous detergents, wash lab coats, replace test tubes and tips in use.
Instrument error.	Contact ELITechGroup Technical Service.

Invalid Sample reaction	
Possible Causes	**Solutions**
Session setup error.	Check the position of PCR Mix and sample. Check the volumes of PCR Mix and sample.
Internal Control degradation.	Use new aliquots of Internal Control.
PCR Mix degradation.	Use a new aliquot of PCR Mix.
Inhibition due to sample interfering substances.	Repeat the amplification with a 1:2 dilution in molecular biology grade water of eluted sample in a “PCR only” session. Repeat the extraction with a 1:2 dilution in molecular biology grade water of primary the sample in a “Extract + PCR” session.
Instrument error.	Contact ELITechGroup Technical Service.

Abnormal high rate of positive results within the same session (reactions with similar late Ct values) **Possible Causes Solutions**	
Possible Causes	**Solutions**
Sample-to-sample contamination during the pre-analytical steps	Avoid any contact between micropipette and tube wall. Clean the micropipette with fresh 3% sodium hypochlorite solution or DNA/RNA cleaner after pipetting each sample. Do not use Pasteur pipettes. The pipettes must be of the positive displacement type or used with aerosol filter tips. Introduce samples in the last positions of the instruments, as indicated by the ELITe InGenius GUI. Follow the loading sequence indicated by the software
Laboratory environmental contamination	Clean all surfaces in contact with the operator and samples (including the pipettes) with fresh 3% sodium hypochlorite solution or DNA/RNA cleaner. Perform an U.V. decontamination cycle. Use a new tube of PCR Mix.

Error 30103	
Possible Causes	**Solutions**
Too high concentration of target in the sample.	If significant amplification is observed in PCR plot: - selected the track related to the sample and approve manually the result. If a Ct value is required: - repeat the amplification with a 1:10 dilution in molecular biology grade water of eluted sample in a “PCR only” session or - repeat the extraction with a 1:10 dilution in molecular biology grade water of the primary sample in a “Extract + PCR” session.

TH Error	
Possible Causes	**Solutions**
Sample with anomalous plot shape.	If significant amplification is observed in PCR plot with negative baseline: - repeat the amplification with a 1:10 dilution in molecular biology grade water of eluted sample in a “PCR only” session or - repeat the extraction with a 1:10 dilution in molecular biology grade water of the primary sample in a “Extract + PCR” session.

SYMBOLS

REF Catalogue Number.

Upper limit of temperature.

LOT Batch code.

Use by (last day of month).

IVD *in vitro* diagnostics medical device.

CE Fulfilling the requirements of the European Directive 98\79\EC for *in vitro* diagnostic medical device.

Σ Contains sufficient for "N" tests.

Attention, consult instructions for use.

CONT Contents.

Keep away from sunlight.

Manufacturer.

Bordetella ELITe MGB® Kit
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NOTICE TO PURCHASER: LIMITED LICENSE

This product contains reagents manufactured by Life Technologies Corporation and are sold under licensing arrangements between ELITechGroup S.p.A. and its Affiliates and Life Technologies Corporation. The purchase price of this product includes limited, non-transferable rights to use only this amount of the product solely for activities of the purchaser which are directly related to human diagnostics. For information on purchasing a license to this product for purposes other than those stated above, contact Licensing Department, Life Technologies Corporation, 5781 Van Allen Way, Carlsbad, CA 92008. Phone: +1(760)603-7200. Fax: +1(760)602-6500. Email: outlicensing@thermofisher.com.

ELITe MGB® detection reagents are covered by one or more of U.S. Patent numbers 6,127,121, 6,485,906, 6,660,845, 6,699,975, 6,727,356, 6,790,945, 6,949,367, 6,972,328, 7,045,610, 7,319,022, 7,368,549, 7,381,818, 7,662,942, 7,671,218, 7,715,989, 7,723,038, 7,759,126, 7,767,834, 7,897,736, 8,008,522, 8,067,177, 8,163,910, 8,389,745, 8,969,003, 8,980,855, 9,056,887, 9,085,800, 9,169,256 and EP patent numbers 1068358, 1144429, 1232157, 1261616, 1430147, 1781675, 1789587, 1975256, 2714939 as well as applications that are currently pending.

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