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| Simplexa Bordetella Direct Assay | | | | | |
| **Purpose** | This procedure provides instructions for preparing samples, setting up the PCR reaction and running the *Simplexa™* Bordetella direct assay on Nasopharyngeal (NP) swabs in UTM. | | | | |
| **Policy Statements** | This procedure applies to all technical staff performing testing on the Liaison MDX instruments. | | | | |
| **Principle and Clinical Significance** | *Bordetella pertussis* (Bp) and *Bordetella parapertussis* (Bpp) are small gram negative bacteria that cause the highly contagious acute respiratory disease, whooping cough (pertussis). The World Health Organization estimates that there are 50 million whooping cough cases worldwide each year, resulting in 350,000 fatalities. The disease presents with a prolonged cough and patients often have episodes of violent coughing that may be followed by an inspiratory whoop and vomiting. The illness occurs in all age groups but primarily in young children, finding the most serious in unvaccinated infants. In severe cases, seen most commonly in young infants, these symptoms can lead to hypoxia, permanent brain damage, or death. In older children and adults, particularly those who have been vaccinated or previously had disease, the illness can be milder and present as a prolonged cough.  *B. parapertussis, B. holmseii* and *B. bronchiseptica* are often attributed to causing less severe forms of the respiratory disease.1  Individuals with pertussis disease are most infectious during the catarrhal period and the first 2 weeks after the onset of cough, often before a diagnosis has been made. This often has drastic consequences because pertussis is highly infectious; 90–100% of susceptible contacts develop pertussis when exposed to a symptomatic household member, and the infection rate of household contacts older than 15 years of age has been reported to be as high as 83%. After infection with *B. pertussis*, immunity wanes over time.2  Pertussis can be detected among all age groups (e.g., neonates, children, adolescents and adults). A great majority of these cases are caused by *Bordetella pertussis*; however, 2-20% of cases are caused by *Bordetella parapertussis*, which can present clinically as a milder pertussis-like disease. It is widely believed that vaccine-conferred immunity wanes after 7 to 10 years.1  **Principle**  The Simplexa™ Bordetella Direct assay system is a real-time PCR assay that enables the direct amplification, detection and differentiation of *Bordetella pertussis* and *Bordetella parapertussis* DNA from unprocessed nasopharyngeal (NP) swabs without nucleic acid extraction. The system consists of the Simplexa™ Bordetella Direct assay, the LIAISON® MDX (with LIAISON® MDX Studio Software), the Direct Amplification Disc and associated accessories.1  In the Simplexa™ Bordetella Direct assay, primers and fluorescent probes are used together to amplify and detect *Bordetella pertussis*, *Bordetella parapertussis* and internal control targets. Insertion sequences IS481 and IS1001 are targeted to identify *Bordetella pertussis* and *Bordetella parapertussis* DNA, respectively. An internal control is used to detect PCR failure and/or inhibition.1  In the past, laboratory diagnosis was traditionally based on culture which is considered the gold standard. Although culture is highly specific, the sensitivity of culture is generally low (around 36%). With the continuing resurgence of pertussis, PCR is being used more frequently for the detection of *Bordetella pertussis* and *Bordetella parapertussis* with a noticeable improvement in diagnostic accuracy and turn-around-time. Two target sequences, IS*481* and IS*1001,* are used for *B. pertussis* and *B. parapertussis*. The *B. pertussis* genome contains 50 – 200 copies of the IS*481* sequence making the IS*481* target very sensitive but not species specific. This same sequence is present in *B. holmseii* (8 – 10 copies) and occasionally *B. bronchiseptica* causing cross reactivity to occur. The *B. parapertussis* genome contains 20 – 22 copies of the IS*1001*target sequence that is used for the detection of *B. parapertussis.* The IS1001 target sequence can occasionally be found in *B. bronchiseptica* 1  **Table 1: Gene target**   |  |  |  |  |  | | --- | --- | --- | --- | --- | | **Analyte** | **Gene Targeted** | **Probe Fluorophore** | **Excitation** | **Emission** | | *Bordetella pertussis* | IS481 | FAM | 495 nm | 520 nm | | *Bordetella parapertussis* | IS1001 | CFR610 | 590 nm | 610 nm | | DNA Internal control | DNA IC | Q670 | 644 nm | 670 nm |   **Figure 1: Scorpion Primer Function**   |  |  | | --- | --- | | http://research.chem.ox.ac.uk/Data/Sites/4/media/brown-t/t-brown-fig2.png | 1. The Scorpion primer acts as a primer and a probe. The probe forms a hairpin loop with a self-complimentary stem sequence so that the quenched reporter does not fluoresce. The primer is linked to the probe at the start of the hairpin loop. 2. During the annealing, the primer binds to the template and is extended. 3. The probe part of the Scorpion is complementary to the extension product of the attached primer. When the complementary strands are separated in the denaturation step of the next PCR step, the reporter separates from the quencher and opens the loop. When cooled to annealing temperature, the probe sequence binds to the internal target sequence. The reporter and the quencher are now far enough apart to generate detectable fluorescence.3,4 | | | | | |
| **Test Code** | **BORDP** | | | | |
| **Sample** | 1. **Acceptable specimens:**  |  |  |  | | --- | --- | --- | | **Specimen type** | **Volume** | **Transport Containers** | | Flocked Nasopharyngeal (NP) swab in UTM | 3 mL UTM (100uL minimum) | * 3 mL UTM * CHC# 32788 |  1. **Unacceptable specimens:** Improperly labeled or unlabeled samples. Calcium alginate swabs, other body fluids, other swabs, other respiratory samples. 2. Transport and Storage: For additional information refer to [Lab Test Directory](http://intranet.childrensmn.org/departments-and-committees/lab-test-directory/)  |  |  | | --- | --- | | Temperature  Refrigerated , 2 – 8 °C | Sample Stability | | * NP swab | * 7 days at 2-8 °C\* |   \*If there is >7 day delay before testing, store at -70°C | | | | |
| **Special Safety Precautions** | * Standard precautions. Refer to MB 2.02 Biohazard Containment * Use of engineering controls: Refer to MB 3.01 Engineering Controls to Prevent Nucleic Acid Contamination   Microbiologists are subject to occupational risks associated with specimen handling. Refer to the safety policies located in the safety section of the *Microbiology*, *Virology, and Molecular Policy Manual*:   1. [*Safety in the Microbiology/Virology Laboratory*](file:///G:\Lab%20Procedures\Microbiology\1NEW%20Micro%20Procedure%20Manual.%20(same%20as%20in%20Starnet)\MCVI%203%20Safety\MCVI%203.2%20Safety%20in%20the%20Microbiology%20Lab.docx) 2. [*Safe Work Practices in Molecular*](https://starnet.childrenshc.org/References/labsop/molbio/safety/mb-2.01-safe-work-practices-in-molecular.pdf)  * [*Biohazardous Spills*](file:///G:\Lab%20Procedures\Microbiology\1NEW%20Micro%20Procedure%20Manual.%20(same%20as%20in%20Starnet)\MCVI%203%20Safety\MCVI%203.4%20Biohazardous%20Spills.docx) * [*Biohazardous Spill in Molecular*](https://starnet.childrenshc.org/References/labsop/molbio/safety/mb-2.03-biohazardous-spills-in-molecular.pdf) * [*Biohazard Containment*](https://starnet.childrenshc.org/References/labsop/index.php?view=folder&folder=molbio)  1. Wear appropriate personal protective equipment (PPE) including disposable gloves and lab coats. 2. Handle all samples and waste materials as if they were capable of transmitting infectious agents. 3. Change gloves often when handling reagents or samples. 4. Dispose of materials used in this assay, including reagents, used buffer vials in biohazardous waste. | | | | |
| **Materials** | |  |  |  | | --- | --- | --- | | **Reagents** | **Supplies** | **Equipment** | | Simplexa Bordetella Direct Kits (MOL 2750)   * Reaction Mix (24) 50 µl * Store at -10 to – 30 °C   Simplexa Bordetella Positive Control Pack (MOL 2760)   * 10 tubes, 100 µl * Direct Amplification Disc (DAD) Kit (MOL 1455) * Negative control – UTM * Sani-Cloth Bleach wipes * 70% alcohol * 5% Extran | * Gloves (powder-free) * Filtered pipette tips, 100 and 200 uL, extended tips * Sharps disposal container * Replacement foil wedge | Room 1: Clean room   * -10 to -30° C freezer * Laminar flow Hood   Room 2: Processing   * Refrigerator 2 – 8° C * BSC BSL-2 * -70⁰ C freezer * 100 or 200 µl pipette   Room 3: Amplification  Liaison MDX | | | | | |
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| **Calibration** | Spectral calibrations performed on instruments by a DiaSorin Molecular Technical Field Specialist. | | | | |
| **Quality Control** | **Daily Quality Control:**  Internal quality control is included in all reactions. The internal control must be valid in order to obtain valid negative patient results. A valid internal control result is not required for valid positive results.  **External Quality Control:**   * Perform QC using external manufactured positive and negative controls every 30 days AND/OR with new lots/shipments. Record and file results in the appropriate binder. * Run a previous positive and negative sample with new lot/new shipment QC * Either a BP or BPP positive sample * See IQCP document * POSC – Simplexa Bordetella Positive Control Pack, stored at -70°C * NEGC – UTM, stored at 2-25°C * An IC is incorporated into each reaction mixture   QC Monitors:   |  |  | | --- | --- | | **Control** | **Control Monitor** | | Positive Control (POSC) | Reagent failure and primer-probe integrity | | Negative Control (NEGC) | Reagent and/or environmental contamination, cumulative effect | | Internal Control (IC) | PCR inhibition in specimen, reagent failure or process error |  * Before reporting patient results, all controls must yield valid results   + NOTE: a valid positive result does not require an IC result * If results are invalid, obtain new reagents and controls; repeat testing   **Preparing Negative Control (NEGC)**   1. Wear lab coat and gloves dedicated to the Clean room 1 2. Label cryo-storage box with contents 3. Lot number (L/N), expiration date and date of preparation 4. Aliquot 300 µl of UTM into 1.5 microcentrifuge tubes 5. Refrigerate aliquots in room 2 6. Record lot information in appropriate binder   **Preparing Manufactured Positive Control (POSC)**   1. Remove POSC from – 70⁰ C, thaw POSC at room temperature    1. Do not refreeze 2. Label with open date and expiration date (24 hours) 3. Gently flick tube to mix    1. *Do not vortex* 4. Quick spin POSC before use   Test controls as you would patient samples.  **Record and file results in QC binder**  **NOTE:** QC testing on each instrument is to be performed on a rotating basis.  **Expected Control Results5**   |  |  |  |  | | --- | --- | --- | --- | | **Control Type** | ***Bordetella pertussis* (481)** | ***Bordetella parapertussis* (IS1001)** | **DNA Internal Control (DNA IC)** | | **POSC: Simplexa™ Bordetella Positive Control1** | Detected | Detected | Not applicable2 | | **NEGC: UTM** | Not Detected | Not Detected | Valid |   1. Typical Ct values for the Positive Control range between 21-26.  2. Detection of the Simplexa™ DNA Internal Control (DNA IC) is not required for a valid result when Bp and/or Bpp are detected.  **Wipe testing:**   * Perform wipe testing every 30 days to monitor for contamination. * See MB 3.02 Wipe Testing for Amplicon Contamination   **NOTE:** External quality control may be performed on an as needed basis if certain circumstances arise. Examples include:   * Drift in results (e.g., increasing/decreasing positivity rates) * Potential contamination (negative control) * After dramatic instrument maintenance or movement | | | | |
| **Assay Procedure** | **NOTE:** Always clean hood before sample handling.  **All testing supplies must be cleaned with 10% bleach followed by water and 70% alcohol.**  **Testing Preparation: Room 2**   1. Call worksheet **BORDP**; use this worksheet for sample identification throughout testing. 2. Position samples and controls (when applicable) in disc as follows:  |  |  | | --- | --- | | Sample | Position | | Patient samples | Position 1-nn | | POSC | After last patient sample | | NEGC | After POSC |  1. Using the BORDP worksheet as a layout, organize patient specimens and labels    1. Color code worksheets and labels per run    2. Number patients on worksheet in consecutive order    3. Number corresponding patient labels according to assigned numbers on worksheet, color coded by run    4. Number each primary patient specimen according to worksheet   **PCR set-up (Room 2) and amplification (Room 3):**   1. Remove one MM for each sample to be tested from - 20⁰ C freezer (Room 1) and thaw at room temperature (approximate range 18 to 25⁰C).   **NOTE:** Use MM within 30 minutes.   1. When thawed, gently flick MM tubes to mix; briefly centrifuge. Do not vortex or refreeze. 2. Vortex specimen tubes prior to set-up 3. Remove DAD from package and set on disc cold block 4. Number wedges according to worksheet layout 5. Peel back the foil cover, one at a time, to expose the SAMPLE and Reaction (R) wells.   **!** Do not touch underside of foil to prevent cross contamination    Pipette 50 µl of MM into the Reaction (R) well first before sample  **NOTE:**   * To prevent aerosols and possible contamination, hold the pipette at a 30-degree angle inserting the tip under the roof of the well     *Caution:* Avoid placing pipette tip at the bottom of the well to prevent possible punctures in the foil that may lead to instrument contamination   1. Vortex sample 5-10 seconds. With a 200uL extended pipette tip, pipette 50 µl of sample/control into the SAMPLE well   *Caution:* Pipette leakage outside of well may lead to external disc contamination when resealing wedge   1. Seal the foil wedge before opening the next foil cover 2. After all wedges are filled, carefully remove the perforated foil tab    1. If foil is torn, it must be replaced with a replacement foil wedge to prevent carryover contamination 3. Use the disc applicator to seal the foil firmly on all wedges 4. Remove lab coat and change gloves   **Computer Set-up: Room 3**   1. Set up Liaison; take run specific patient labels and DAD into room 3 2. Turn on the Liaison MDX (ABC) by flipping the switch in the back and the Liaison computer 3. Log on computer    1. User: Administrator    2. Password: focusIC#1 4. Double-click on Integrated Cycler DX icon to open program 5. Enter personal user and password code    1. To switch users: Select **File: Switch Users** 6. From the main screen, scan the reagent lot barcode, small data matrix located on the lower left corner of the REF card 7. Scan the disc barcode on the DAD to show disc layout  * Used wedges are shown in black and unavailable for use * Available wedges are shown in gray Fig. 1   **Figure 1**     1. Enter sample IDs: scan barcode ID from each label consecutively    1. **Type** drop down box: **:** select **Unknown** (default) 2. When applicable**,** enter controls according to layout  * POSC – scan the barcode provided on the positive control vial label * NOTE: the positive QC vial label is to be placed on the back of the Bordetella reagent lot barcode card after use of the first vial. If the QC barcode is unavailable type in the lot number. * NEGC – select **NTC** from the Type drop down box  1. Load the DAD into instrument 2. Select the instrument from the drop down box (lower right) 3. Click **Run** to begin processing the disc; Approximate run time: 65 min. The progress bar on screen indicates time to completion.   **!** Once run is started, it cannot be cancelled; canceling will require reloading new samples into unused wedges.  **!** Users cannot be changed while running   1. Recycle labels when run is complete; do not take back to room 2 2. Remove lab coat and change gloves before leaving area 3. When run is complete, remove disc from instrument; *check well volumes.* Place disk in bio-bag and discard if completely used. If there are unused wedges, retain disc in a sealed bio-bag in room 2. Upon completion of the run, the software automatically calculates and displays results.   **Note:** in room 2 - soak applicator and disc cold block in 5% extran followed by a water rinse. | | | | |
| **Interpretation/ Results and Reporting** | **Reviewing and Printing Completed Runs** When the run is complete, the results are interpreted by the software and will display on the screen; positive results appear red **Figure 2:** Analysis Complete   Click the Print button to print a full report of the results, Fig. 2  * 1. √ Include Ct values   2. √ Include graphs   3. Scroll through the report , reviewing comments, failures and amplification curves      + A valid curve shows a smooth, exponential increase, Fig. 3      + Invalid curve may be linear or a curve with data “spikes” where the curve crosses the threshold      + Review “QC statement/Note” on the Segment Report for failures and error messages   4. Click **Print**   5. Export results to LIS; refer to procedure   **Figure 3:** Valid and invalid amplification curves  **Valid Valid Invalid**    **For a detailed analysis of the completed run, click the** Details **button to open the Analysis Window** Click on the run Details tab to display a summary of the run, fluid checks, Ct values and any sample failures that are highlighted in yellow **Figure 4**: Details Screen     1. For each CID (Sample ID) entered, the software displays a result (“Detected”, “Not Detected”, “Invalid” or “EC500”) for the IS481 (Bp) and IS1001 (Bpp) targets.  |  |  |  |  | | --- | --- | --- | --- | | **Result** | **Interpretation** | **Notes** | **Action** | | **Detected** | Indicates the presence of IS481 (Bp) and/or IS1001 (Bpp) DNA in the patient sample. |  | Export results to LIS | | **Not Detected** | Indicates the absence of IS481 (Bp) and/or IS1001 (Bpp) DNA in the patient sample. |  | Export results to LIS | | **Invalid** | Indicates the inability to determine the presence or absence of IS481 (Bp) and/or IS1001 (Bpp) DNA in the patient sample. | Results may be due to:   1. DNA internal Control failure 2. Failure to detect sufficient specimen. | Repeat testing (see procedure below). Document result in problem log. | | **EC500** | Indicates an error for the particular analyte(s). | Data processing error due to noise, weak or late amplification in the signal. | Repeat testing. (see procedure below)  NEAT and 1:4 dilution  Document result in problem log. | | **EC505** | Indicates an error for the particular viral analyte(s). | Insufficient information to determine whether amplification was present. | Repeat testing. (see procedure below)  NEAT and 1:4 dilution  Document result in problem log. | | **EC515** | Indicates an error for the particular viral analyte(s). | Internal control amplification is not within specification. Result is invalid, repeat the sample. | Repeat testing. (see procedure below)  NEAT and 1:4 dilution  Document result in problem log. | | **System Error** |  | Read error dialog box containing software messages regarding the cause of the problem and possible solutions. | Follow directions given by software, repeat testing if necessary. Contact DiaSorin technical support  **1-800-838-4548, option 3.**  See “Exporting a Service Packet” procedure below if necessary |  Click Data tab to *Select* or *Deselect* samples to be exported to LISSelect or deselect samples to view graphs (optional)Select or deselect samples to export to LISExport results to LIS (see procedure below) **Figure 5: Data Screen**  Export drop down    To view graphs by dye, click on the dye checkbox  Data / Detail tabs  Select and Deselect buttons  **Exporting Data to LIS**   1. When applicable, confirm POSC and NEGC are valid before reporting patient results 2. Positive patient results: Confirm name, CID number and disc location of primary sample before releasing results 3. If all test results were valid upon review, select **√** results to be exported onthe **Data** tab, refer to Fig.5    1. *Do not* send invalid patient results or POSC and NEGC. Deselect by clicking on individual box(es) 4. From the Export drop down box, select **LIS** and then **LIS folder;** click **OK**   **Figure 6:** Export to LIS     1. A message that the run exported successfully will appear. Click **OK** 2. Patient results will be translated in LIS as *Positive* or *Negative* for *Bordetella pertussis* and *Bordetella parapertussis*. 3. If the sample is interpreted as “Invalid” by Simplexa, results will need to entered manually as *Equivocal* or *Unresolved* after review.   Do not report patient results until problem is resolved  Record problem and corrective action in the ***QC and Equipment Failure* *Log*** | | | | |
| **Result Reporting: Sunquest** | 1. After results have been exported to LIS: log into Sunquest:    1. Click on the Sunquest icon to log on    2. Enter user, password and location [R] 2. Click on **Result Entry** from the menu options 3. Select **SIM** from drop down box   **Figure 1:** Interface Configuration:     1. Click on the  button located in the lower left corner    1. If the page says “Waiting for cups….”, the results were not successfully transmitted or the results page was accessed too quickly before the transmission was completed  |  |  | | --- | --- | | If | Then | | * Specimen box reads *Preprocessing passed* with no further messages * Test box has no messages * Sample results are acceptable | Click Save and then Accept (Fig. 5) |  1. Staple worksheet containing specimen identifiers used during testing and RIP Segment Report together 2. Place report in the Bordetella result log book   **Duplicate results**   1. If a run is exported more than once, uncheck the duplicate results OR valid result and release the checked results      1. Click  button located on the lower left corner 2. **Click** Release All and accept | | | | |
| **Retesting Procedure: Invalids and EC500, EC505, or EC515 errors** | 1. Perform repeat testing from original specimen aliquot 2. Repeat within 7 days if stored at 2 – 8⁰ C 3. Vortex the specimen tubes prior to retesting  |  |  | | --- | --- | | **If** | **Then** | | Result repeats – valid result | Export results to LIS | | Result repeats – invalid results or error | Send results across to LIS and report as UNR, call result\*\* and request new sample for testing |   **\*\***See “Critical and Phoned Results” Procedure below | | | | |
| **Retesting Procedure: EC500, EC505, or EC515 errors** | 1. Dilute 50 uL of specimen in 150uL UTM to obtain a 1:4 dilution    1. Label a cryovial with a patient foot label with 1:4 written on it    2. Pipette 150 uL UTM into the cryovial    3. Vortex the patient sample 5-10 seconds and pipette 50 uL sample into the cryovial    4. Vortex the cryovial 5-10 seconds 2. Retest the sample NEAT (undiluted) and test the 1:4 dilution  |  |  | | --- | --- | | **If** | **Then** | | Error resolves with undiluted sample | Report the valid result | | Error resolves with diluted sample | Report the result along with the comment code DILUT to indicate "Sample diluted due to inhibition.  Please consider submission of a new sample if clinical suspicion is high.” | | Error does not resolve | Report as UNR and request new sample for testing. | | | | | |
| **Critical and Phoned Results** | **Alert Value:** Positive *Bordetella pertussis* results must be called to the patient’s caregiver.  **Phoned Results, Sunquest GUI Interface**   1. Enter phoned results in **Result Entry** 2. Click on the interpretation box to expand the result 3. At the blinking cursor, add the code **CAL**, press tab, enter semi-colon, record who the result was relayed to and the time/date. 4. Type the first name and last initial of the person called and the date/time 5. Close the interpretation box 6. Click **Save** and then **Accept** on the Verify Release screen to file results in LIS | | | | |
| **Manual Entry of Results** | 1. Open Result Entry, select the Manual resulting mode (top left corner), from the configuration drop down select the appropriate test code. Click  in the lower right corner. 2. Enter the Specimen ID or scroll to the correct patient if necessary (lower left corner). 3. Type in results and applicable comments when necessary. 4. Check results against instrument print out and click .  | **Result** | **Sunquest code** | **Interpretation** | | --- | --- | --- | | **Positive** | **POS** | 1. Positive | | **Negative** | **NEG** | 1. Negative | | **Unresolved Results** | **UNR**  **CAL** | 1. Unresolved: This sample is inhibitory to amplification and the results are inconclusive. Consider repeat collection if clinically indicated. | | **Diluted Results** | **POS/NEG**  **DILUT** | 1. DILUT: "Sample diluted due to inhibition.  Please consider submission of a new sample if clinical suspicion is high.” | | | | | |
| **Correcting Results** | 1. Open Result Entry, select the Manual resulting mode (top left corner), from the configuration drop down select the appropriate test code. Click  in the lower right corner. 2. Enter the Specimen ID, enter Tab and click Yes to modify the result. 3. Change the incorrect result. The corrected result comment will automatically append. Add the CAL comment, press tab, enter a semi-colon and record who was called and the time/date. 4. Click . Click  when the “Verify Release Destination” window opens. | | | | |
| **Sample Storage** | **Storage and Retention of Test Specimens**   1. Mark all positive samples on cap.    1. Write positive results on the side of the tube 2. Store in rack in the -70 °C freezer for a minimum of 1 month. 3. Discard samples after elapsed time in red biohazard container | | | | |
| **Equipment and Room Decontamination** | **Refer to:**  [MB 3.03 Cleaning and Decontamination of Equipment and Work Areas](https://starnet.childrenshc.org/References/labsop/molbio/engctl/mb-3.03-cleaning-and-decontamination-of-equipment-and-work-areas.pdf)  [MB 4.02 DiaSorain Liaison MDX Instrument Maintenance and Troubleshooting](file:///G:\Lab%20Procedures\Molecular%20Procedure%20Manual\MB%204.0%20Equipment\MB%204.02%20DiaSorain%20Liaison%20MDX%20Instrument%20Maintenance%20and%20Troubleshooting.docx) | | | | |
| **Customer and Technical Support** | Call DiaSorin Technical Service at 1-800-838-4548 option #3. Technical service may ask you to generate and send a Service Packet file; see Troubleshooting above for downloading a \*.icz file. If it is determined that the instrument must be returned for service, decontaminate the Liason MDX before shipping, refer to procedure MB 4.02. Document all problems and actions in the QC and Equipment Failure Log. | | | | |
| **Limitations** | 1. For *in vitro* diagnostic use. 2. For professional and prescription use only. 3. In the United States, this product is intended for use in healthcare facilities with a minimum CLIA certification of moderate complexity. 4. Results from this test must be considered in conjunction with the clinical history, epidemiological data, and other laboratory information available to the clinician evaluating the patient. 5. The detection of bacterial nucleic acid is dependent upon proper sample collection, transport, handling, storage, and preparation. Failure to observe proper procedures in any one of these steps can lead to incorrect results. 6. IS481 is also present in *Bordetella holmesii.* Additional testing should be performed if necessary to differentiate between *Bordetella holmesii* and *Bordetella pertussis*. 7. The prevalence of bacterial infections may affect the test’s predictive value. 8. Negative results do not rule out Bordetella infections and should not be used as the sole basis for treatment or other patient management decisions. 9. False-negative results may occur if the bacteria have genomic mutations, insertions, deletions, or rearrangements or if performed very early in the course of illness. 10. False-negative results may occur if inadequate numbers of bacteria are present in the specimen due to improper collection, transport or handling. False-negative results may also occur if the bacteria are present at a level that is below the analytical sensitivity of the assay. 11. As with other tests, false-positive results may occur. Repeat testing may be required in some in some settings. 12. This test cannot rule out diseases caused by other bacterial or viral pathogens. 13. This test is a qualitative test and does not provide the quantitative value of detected organisms present. 14. The performance of this test has not been established for patients without symptoms of Bordetella infection. 15. The performance of this test has not been established for monitoring treatment of Bordetella infection. 16. The performance of this test has not been established for use in donor screening tests.5 17. Vaccines contain high copy numbers of B. pertussis DNA, which can be aerosolized, causing false-positive B. pertussis PCR results if samples are collected in the same vicinity.6,7 | | | | |
| **Method Performance Specifications** | **According to the manufacturer (per the package insert):**  Simplexa Bordetella Direct *Bordetella* pertussis Results Versus FDA Cleared NAAT Prospectively Collected Fresh Samples  PPA: 100%, 95% CI: 90.4% to 100%  NPA: 97.9%, 95% CI: 95.7 to 99.0%  Simplexa Bordetella Direct *Bordetella parapertussis* Results Versus PCR/Bi-Directional Sequencing Method Prospectively Collected Fresh Samples  PPA: 100%, 95% CI: 34.2% to 100%  NPA: 95.0%, 95% CI: 97.8 to 100%  For additional performance characteristics refer to the [Simplexa Bordetella Direct Package Insert](file:///G:\LAB\Molecular%20Biology\Simplexa,%20BORD%20direct\PIs\BORDETELLA%20DIRECT%20PI.pdf) | | | | |
| **References** | 1. Simplexa Bordetella Direct Package Insert, REF MOL2750, Rev. 02. (December, 2018). In: DiaSorin Molecular  2. Tan TJTPidj. Summary: epidemiology of pertussis. 2005;24(5):S35-S38.  3. Persing DH, Tenover FC, Tang Y-W, Nolte FS, Hayden RT, Belkum Av. *Molecular microbiology: diagnostic principles and practice.* ASM press; 2011.  4. Buckingham L. *Molecular diagnostics: fundamentals, methods and clinical applications.* FA Davis; 2019.  5. Simplexa Bordetella Positive Control Pack Package Insert, REF MOL2760, Rev.01. (August, 2018). In: DiaSorin Molecular.  6. Salimnia H, Lephart PR, Asmar BI, Prebelich D, Paulson E, Fairfax MRJJocm. Aerosolized vaccine as an unexpected source of false-positive Bordetella pertussis PCR results. 2012;50(2):472-474.  7. Mandal S, Tatti KM, Woods-Stout D, et al. Pertussis pseudo-outbreak linked to specimens contaminated by Bordetella pertussis DNA from clinic surfaces. 2012;129(2):e424-e430. | | | | |
| **Alternate Methods** | 1. Send out test to Mayo: Bordetella pertussis and Bordetella parapertussis, Molecular Detection, PCR, Varies (Mayo lab test code: BPRP) | | | | |
| **Proficiency Testing** | CAP (BOR): 2 shipments a year with 3 samples | | | | |
| **Training Plan/ Competency Assessment** | **Training Plan** | | | **Initial Competency Assessment** | |
| 1. Employee must read the procedure. 2. Employee will demonstrate the ability to perform procedure, record results, and document corrective action after instruction by the trainer. | | | 1. Direct observation | |
| **Historical Record** |  |  |  | |  |
|  | **Version** | **Written/Revised by:** | **Effective Date:** | | **Summary of Revisions** |
| 1 | Julie Laramie | 1/21/2020 | | Initial Version |
| 2 | Julie Laramie | 6/8/2020 | | Added sample dilution with EC500 errors |
|  |  |  | |  |
| **Archived by:** |  | **Archived date:** | |  |
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