#  *Simplexa™ Bordetella* PCR Assay Procedure

**PURPOSE**

* This procedure provides instructions for preparing samples, setting up the PCR reaction and running the *Simplexa™ Bordetella* PCR assay for the detection of *B. pertussis and B. parapertussis* from nasal and bronchial specimens

#### POLICY STATEMENT

* PCR testing is performed daily, 0700 –1530
* Alert value: Call patient’s caregiver with positive results for *B. pertussis* and *B. parapertussis*. Document name of person called, date and time.

**ABBREVIATIONS**

|  |  |
| --- | --- |
| * ABC: Analyzer Before Computer
* BOR: *Bordetella*
* BORDP: *Bordetella* PCR
* Bp: *Bordetella pertussis*
* Bpp: *Bordetella parapertussis*
* BSC: BioSafety Cabinet
* BSL: BioSafety level
* CBA: Computer Before Analyzer
* CFU: colony forming unit
* Ct: crossing threshold
* F/T: freeze/thaw
* IC: internal control
* MM: master mix
* NA: Nucleic Acid
* NEGC: negative control
 | * NFW: nuclease free water
* NP: nasopharyngeal swab
* NW: nasal wash specimen
* PCR: polymerase chain reaction
* PCTL: process control
* PP: primer – pair
* PPE: personal protective equipment
* SEAC: Simplexa extraction and amplification control
* TE buffer: Tris – EDTA buffer
* UNAC: Specimen unacceptable, please recollect

Area/Room 1: Clean roomArea/Room 2: Processing roomArea/Room 3: Amplification room |

## DOCUMENTATION/RECORDS

* Simplexa BORD run-specific Segment Report
* LIS Incomplete and Completed worksheets
* Daily Maintenance Log

## SAFETY CONSIDERATIONS

* Standard precautions for infectious agents. Refer to MB 2.02, Biohazard containment
* Use of engineering controls: Refer to MB 3.01 Engineering Controls to Prevent Nucleic Acid Contamination
* General Safety: MB 2.01 Safe Work Practices
* *Caution:* PPE including protective eyewear must be worn when working with concentrated Extran

#### MATERIALS REQUIRED

| **Equipment** | **Reagents** | **Supplies** |
| --- | --- | --- |
| Room 1: Clean room* Laminar-flow hood, Clean rm 1
* Freezer, -10 to -30⁰ C
* Refrigerator, 2 to 8⁰ C
* Microcentrifuge
* Nalgene cooling block
* Vortex
* Eppendorf Repeater pipette
* Dedicated set of pipettes: 2 µl, 10 µl, 20 μl, 100 μl, 200 μl, and 1000 μl pipettes

Room 2: Processing* BSC, Process rm 2
* Refrigerator, 2 to 8⁰ C
* Freezer, ≥ - 70⁰C
* Nalgene cooling block
* Vortex
* Micro-centrifuge
* Dedicated set of pipettes: 2 µl, 10 µl, 20 μl, 100 μl, 200 μl, and 1000 μl pipettes
* Gilson Concept pipette, 100 µl

Room 3: Amplification and detection* Liaison MDX

Room: Microbiology* McFarland densitometer (micro)
 | TE buffer | Micro tube racks |
| Nuclease Free Water (NFW) | 2 ml cryovials |
| SEAC* Internal control PP
* Internal control DNA
 | Sterile filtered pipette tips for 10 µl, 20 µl, 100 μl, 200 µl, 1000 µl pipettes |
| Bp PP | Micro tubes 1.5 ml, RNase / DNase free |
| Bpp PP | Nitrile gloves (powder-free) |
| Bordetella Molecular Controls ( Bp and Bpp PCTL, NEGC) | Sharps disposal container  |
| TA MasterMix | Gripper rack, rm 2 |
| Sani-Cloth Bleach wipes | Orange barrier wipes |
| 70% alcohol | BBL™CultureSwab™ |
| 5% Extran | 12X75 sterile plastic test tubes |
| *Bordetella pertussis* ATCC 8467 | Sterile Q – Tipped applicator swabs |
| *Bordetella parapertussis* ATCC 9305 | 50 ml sterile conical tube |
|  | Eppendorf 5 ml tips |
|  | Serological pipettes, 5 and 10 ml |
|  | Sterile scissors |
|  |  |

## QUALITY CONTROL

1. Assay Controls
	1. A PCTL and NEGC must be included in each assay run.
	2. Bp and Bpp PCTL use is to be rotated daily. See form MB 6.09.F10.
	3. An IC is incorporated into each reaction mixture.
2. QC Monitors:

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| --- | --- |
| **Control** | **Control Monitor** |
| Negative Control (NEGC) | Reagent and/or environmental contamination, cumulative effect  |
| Process Control (Bp or Bpp PCTL) | Reagent failure and primer-probe integrityElution and/or lysis failure; reagent failure |
| Internal Control (IC) | PCR inhibition in specimen, reagent failure or process error |

1. Before reporting patient results, all controls must yield valid results. Refer to MB 6.05, Procedures F and G, Evaluating and Interpreting Results.

**PROCEDURE A:** Follow the steps in the table below to prepare specimens for testing

Testing Preparation

| **Activity** | Step | **Action** | **Related Doc** |
| --- | --- | --- | --- |
|  | 1 | Call worksheet **BORDP**; use this worksheet for sample identification throughout testing. | MB 1.01Specimen Management |
| **Sample Order****Room 2** | 2 | Position samples and controls in disc as follows:

|  |  |
| --- | --- |
| Sample | Position |
| Patient samples | 1 – nn |
| PCTL | 2nd to last position |
| NEGC | Last position |

 | MB 3.01 Engineering ControlsMB 2.01Safe Work Practices |
| **Organizing run****Room 2** | 3 | Using the BORDP worksheet as a layout, organize patient specimens and labels

|  |  |
| --- | --- |
| Step | Action |
| a | Color code worksheets and labels per run |
| b | Number patients on worksheet in consecutive order |
| c | Number corresponding patient labels according to assigned numbers on worksheet, color coded by run |
| d | Number each primary patient specimen according to worksheet |

 |  |
| **Process NP swabs** | 4 | Elute NP swabs in 200 µl TE buffer

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| Step | Action |
| a | Number cap of each 200 µl TE tube according to assigned number on worksheet |
| b | Properly label TE tube with patient aliquot label matching the number on the cap to the number on the label |
| c | Verify number on primary and secondary container before transfer |
| d | Cut the wire mini-tip swab into the TE buffer tube with corresponding number on cap |
| e | Vortex 5 min, vortex setting 8 |

 |  |
| **Process Bronchs, nasal washes/aspirates** | 5 | Number and label a 2.0 ml cryovial for each nasal wash/aspirate and bronch specimen to be tested

|  |  |
| --- | --- |
| Step | Action |
| a | Number cap of each cryovial according to assigned number on worksheet |
| b | Properly label the tube with patient aliquot label matching the number on the cap to the number on the label |
| c | Vortex specimen in original container until well mixed  |
| d | Verify number on primary and secondary container before transfer  |
| e | Transfer specimen into tube with corresponding number on cap* Only one tube can be open at a time
 |

 |  |
| **Change gloves** | 6 | Change gloves when possible contamination is suspected or every 8 samples |  |
|  | 7 | Place numbered tubes (washes and NP swabs) in consecutive order in rack |  |
|  | 8 | Decontaminate hood and scissors; bleach wipe followed by alcohol and water |  |
|  | 9 | Change gloves |  |

**PROCEDURE B:** Follow the steps in the table below for setting up the computer

Computer set-up

|  |  |  |  |
| --- | --- | --- | --- |
| **Activity** | Step | **Action** | **Related Doc** |
| **Computer Set-up****Room 3** | 1 | Set up Liaison MDX; take run specific patient labels into room 3

|  |  |  |
| --- | --- | --- |
| Step | Prompt | Action/Entry |
| a | ------ | Turn on the Liaison MDX (ABC) |
| b | ------ | Turn on the Liaison computer |
| c | ------ | Log on computer |
| d | User name | administrator |
| e | Password | focusIC#1 |
| f | ------ | Double-click on program icon to open  |
| g | User name | Enter personal user code  |
| h | Password | Enter personal password code  |
| i | ----- | Select **Setup Run** from Quick pick list |
| j | Assay definition | Select **BORD** from drop down box |
| k | Run Name Prefix | Type in **BORD** |
| l | Lot information | PP lot: Add/deactivate reagent lot numbers as needed |
| m | Add Samples | Scan barcode ID from each label consecutively |
| n | Controls | Assign controls according to layout |
| o | ----- | Click **Move to Disc** button |
| p | ----- | Click **Save** to save the run for later use *or*  |
| q | ----- | Click **Run** to save the run and open the **Start Run** window |
| r | ----- | (Optional) Click the **Print Preview** button to generate a layout report, refer to Fig.1 |
| s | ----- | Recycle labels when run is complete; do not take back to room 2 |

 |  |
| **New user** | 2 | To switch users: Select **File: Switch Users***Note*: Users cannot be changed while instrument is running |  |
| **Delete or Edit Segment** | 3 | To delete or edit segments, right click one of the wells in the segment

|  |  |
| --- | --- |
| Step | Action |
| a | Select action: Edit Segment or Delete Segment* Delete Segment will remove all test samples from run
* Edit Segment will move samples from the disc back to the sample list where changes can be made
 |
| b | To move samples back to disc, click starting well location in Disc View |
| c | Click **Move to Disc** button |

 |  |
| **Change PPE** | 4 | Remove lab coat |  |
|  | 5 | Change gloves; move to room 1 |  |

**Figure 1:** Spoke 1 isidentified by theopen slot on the outer ring of the disc. The wells are

 identified from the outer–edge inward A – H. Numerical assignment of the wells is in vertical order.

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**PROCEDURE C:** Follow the steps in the table below for preparing the MM

Master Mix preparation

| **Activity** | Step | **Action** | **Related Doc** |
| --- | --- | --- | --- |
| **Thaw/warm reagents****Room 1** | 1 | Remove MM components from –20° C freezer/refrigerator; warm to room temperature (approx 15 min) protected from light; use within 1 h |  |
|  | 2 | Gently mix each MM component prior to each use; briefly centrifuge* Larger volumes: Vortex 2 – 3 sec, setting 8 (IC DNA and TA MM)
* Lower volumes: flick tube 4 – 5 times (IC, Bp and Bpp PP)
* Centrifuge: 1 – 2 sec
 | MB 6.04Refer to MM chart  |
| **MasterMix** | 3 | Prepare MM in 1.5 micro-centrifuge tube according to chart volumes |  |
| **Room 1** | 4 | Gently vortex MM; briefly centrifuge* Vortex setting: 8
* Time: 2 sec
* Centrifuge: 1 – 2 sec
 |  |
|  | 5 | Return reagents to refrigerator, do not refreeze | MB 6.03 Storage and Stability |
|  | 6 | Proceed to PCR set-up |
|  | 7 | Remove lab coat; move to room 2 |  |
| **Room 2** | 8 | Place MM in cooling block until use |  |
|  | 9 | *Keep MM protected from light. Use MM within 30 min of preparation* |  |

**PROCEDURE D:** Follow the steps in the table below for PCR set-up and amplification

**PCR set-up and amplification**

| **Activity** | Step | **Action** | **Related Doc** |
| --- | --- | --- | --- |
| **Vortex****Room 2** | 1 | Vortex specimen tubes prior to set-up if they have been sitting for more than 30 min after initial processing |  |
|  | 2 | Remove Universal disc from package and set on disc cold block |  |
| **Load MM**  | 3 | Position spoke 1 over silver plate groove (refer to Fig. 1) |  |
| **Room 2** | 4 | Pipette 7 µl of MM into each well to be used

|  |  |
| --- | --- |
| ***Tip*** | * Automatic pipettor: hold at slight angle to maintain accuracy
 |
| * Manual pipetting: hold the pipette at a 30-degree angle inserting the tip under the roof of the well to reduce possible contamination

  |

 | [Simplexa Operator Manual](http://khan.childrensmn.org/Manuals/Lab/SOP/MolBio/UserMan/212340.pdf) |
| **Load samples** | 5 | Slowly pipette 3 µl of each patient sample and each control into appropriate well* NP swabs: swab elution in TE buffer
* Bronch, nasal wash: undiluted
* PCTL: undiluted
* NEGC: undiluted

*Caution*: Do not go to second stop to avoid introduction of bubbles and producing aerosols |  |
|  | 6 | Apply the cover tape on the disc in horizontal position |  |
| **Seal disc** | 7 | Use the disc applicator to seal the cover tape  |  |
|  | 8 | Remove cover tape tabs by gently pulling outwards |  |
| **Change gloves** | 9 | Remove lab coat  |  |
|  | 10 | Change gloves; move to room 3 |  |
| **Room 3** | 11 | Place disc into the instrument; close lid |  |
| **Start Run** | 12 | Click **Run** button to move to status screen |  |
|  | 13 | Select test instrument from drop down box |  |
|  | 14 | Click **Start***Note:* Once the run is started, it cannot be canceled and then restarted using the same disc. Canceling will require a new disc. |  |
|  | 15 | Remove lab coat  |  |
| **Change gloves** | 16 | Change gloves before leaving room 3 |  |
|  | 17 | Approximate run time: 1 h |  |
| **Run** | 18 | Run progress can be viewed in the **Run Status Window**: refer to Fig. 2 |  |
|  | 19 | Remove disc from instrument; *check well volumes for pipetting accuracy* |  |
| **Run completion** | 20 | Place in bio-bag |  |
|  | 21 | Discard in red biohazard container |  |

**Figure 2**: The graph plots detection progress in Real-Time

Amplification curve

 (Data acquisition)

Dye drop down box for Bp (FAM), Bpp (CFR610) and IC (Q670)

Instrument drop down box

Progress bar shows estimated end time

**PROCEDURE E:** Follow the steps in the table below for analysis of data

Analyzing Completed Runs

| **Activity** | **Step** | **Action** | **Related doc** |
| --- | --- | --- | --- |
| **Analyze Results** | 1 | Click the Analyze button at the bottom of the screen to open the Analysis Window |  |
| **Summary** | 2 | Click on the run Details tab to display a summary of the run, target Ct and IC Ct values |  |
|  |  |  |  |
| Room 3**Review amplification curves** | 3 | Review IC Ct results and amplification curves for exponential growth and possible inhibition or low target amplification, refer to Figures 3 and 4

|  |  |
| --- | --- |
| Step | Action |
| a |  Select **Data** tab |
| b | Click on **Print Preview** |
| c | Check **Include Graphs** |
| d | Scroll through the report , reviewing comments, failures and amplification curves |
| e | A valid curve shows a smooth, exponential increase |
| f | Invalid curve may be linear or a curve with data “spikes” where the curve crosses the threshold |
| g | If curve is valid, the Ct values may be used to interpret the results |
| h | Confirm results by a second reviewer before releasing  |
| I | Positive results: Confirm name and accession number on primary sample/TE buffer before releasing |
| j | Select or deselect results to be released |

 | Refer to procedures F, G and H for interpretation of QC and patient results and Exporting results to LIS |
| **Print Report** | 4 | Print report after review (include graphs) Fig. 3

|  |  |
| --- | --- |
| Step | Action |
| a | Click **Print Preview** button for multi-page analysis report |
| b | Checkbox: **Include Graphs**  |
| c | **Print** |

 |  |
| **Analysis Window**Data / Detail tabsReview channels by clicking dye box(es) to be reviewed |  5 | **Figure 3:** Analysis WindowExport drop downSelect and Deselect buttonsPrint Preview |  |
| **Curve examples** | **6** | **Figure 4:** Valid and invalid amplification curves **Valid Valid Invalid** |  |

**PROCEDURE F:** Follow the activities below for evaluating QC acceptability

**Evaluating and Interpreting QC Results**

| **Activity** | **Step** | **Action** | **Related doc** |
| --- | --- | --- | --- |
|  | 1 | Check QC to determine if the run is valid before reporting patient results |  |
|  | 2 | Failure indications will be highlighted in yellow

|  |  |
| --- | --- |
| Step | Action |
| a | Click the Print Preview button to review the “Data Quality message” on the Segment report under QC Notes |
| b | Review associated amplification curves and Ct values |
| c | Click the **Print** button to generate a report for the ***QC and Equipment Failure* *Log*** documentation |
| d | Record corrective action on QC log |
| e | Record number of failed samples on **Failed Run** log |

 | [Simplexa Operator Manual](http://khan.childrensmn.org/Manuals/Lab/SOP/MolBio/UserMan/212340.pdf)Appendix B: Troubleshooting |
| QC / Valid assay | 3 | For a valid run, the following QC conditions must be met: *ranges are subject to change based on periodic re-calculated values*

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Control | Bp Ct | Bpp Ct | IC Ct | Assay Result |
| PCTL (Bp or Bpp) | 28 – 34  | 28-34 | NA | Positive |
| NEGC  | 0 | 0 | 25 – 31  | Negative |

 | 3SD ranges periodically determined in EP Evaluator and programmed into the Simplexa |
| **QC conditions not met****Invalid assay**  | 4 | If | Then | Refer to MB 6.05, Proc. I for repeat testing |
| **Valid assay:** Controls as expected | * Report patient results
 |
| **Invalid assay conditions:**PCTL/NEGC failure | * Do not report patient results
* Failure caused by inhibition, reagent or system failure
* Repeat patient testing
 |
| PCTL negative or out of range | * Review the specimen handling/ preparation technique
* Repeat patient testing
 |
| NEGC positive | * Possible contamination of samples
* Review the specimen handling/ preparation technique
* Repeat patient testing
 |
| IC not detected in the NEGC | * Failure caused by reagent or system failure
* Repeat patient testing
 |
| IC fails in negative patient sample but negative control acceptable | * Failure caused by inhibition, reagent or system failure
* Review disc well for proper volume
* Check sample for blood/mucus
* Repeat patient testing
 |
|  |  | Problem unresolved | * Call DiaSorin technical service, **1-800-838-4548, option 3**
* Notify section technical director or designee
 |  |
|  |  | Do not report patient results until problem is resolved |  |  |
| **Problem Log** | 5 | Record problem/operator action in the ***QC and Equipment Failure* *Log*** |  |

**PROCEDURE G:** Follow the activities below for evaluating the acceptability of patient results

**Evaluating and Interpreting Patient Results**

|  |  |  |  |
| --- | --- | --- | --- |
| **Activity** | **Step** | **Action** | **Related doc** |
| Patient Results | 1 | Review amplification curves for each result for exponential growth and data spikesReview “QC statement/Note” on the Segment Report for failures* Document operator action for failures on QC log and Segment report
 | Refer to Fig. 3, 4 |
|  | 2 | If the amplification curve is valid, the patient Ct values will be interpreted by LIS when the results are exported |  |
|  | 3 | Patient results will be reported as *Positive* or *Negative* for Bp and Bpp |  |
| **Internal Control** | 4 | **If** | **Then** | MB 6.07Reporting and Archiving BORDP Results |
| IC is detected | * Negative results are valid
* Positive results are valid
 |
| IC is not detected | * Negative results are invalid
* If the Bp or Bpp amplification curves are positive, the IC is not required to be detected ; positive result valid
 |
| Invalid result | * Failure caused by inhibition: Extract 200 µl sample on the EasyMag (RVP protocol); repeat testing from eluate
* Reagent or system failure: Repeat testing from original sample
* If repeat testing remains unresolved, report UNAC
 |
|  | 5 | Refer to **Table 1** for interpretation of results. |  |

###### Table 1: Interpretation of Patient Results: Refer to MB 6.07 *Reporting and Archiving Results*

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Scenario | Bp Ct value | Bpp Ct value | IC Ct value | Interpretation |
| 1 | 0 | 0 | 20 – 38 | Bp and Bpp negative |
| 2 | 13 – 39  | 0 | 0 – 40  | Bp positive, Bpp negative |
| 3 | 0 | 13 – 39  | 0 – 40  | Bpp positive, Bp negative |
| 4 | 13 – 39  | 13 – 39  | 0 – 40  | Bp and Bpp positive |
| 5 | 0 | 0 | 0 | Invalid: repeat |

**PROCEDURE H:** Follow the steps in the table below for exporting data to LIS from the analysis screen

Exporting Data to LIS

| **Activity** | Step | **Action** | **Related Doc** |
| --- | --- | --- | --- |
| **Select data** | 1 | If all test results were valid upon review, select **√** results to be exported onthe **Data** tab, refer to Fig.3  | MB 6.07Reporting and Archiving BORDP Results |
|  | 2 | *Do not* send failed patient results or PCTL/NEGC. Deselect by clicking on individual box(es) |  |
| **Export** | 3 | From the Export drop down box, select **LIS** and then **LIS folder;** click **OK**  |  |
|  | 4 | A message that the run exported successfully will appear. Click **OK** |  |

**PROCEDURE I:** Follow the activities below for repeat testing

**Repeat Testing**

| **Activity** | **Step** | **Action** | **Related doc** |
| --- | --- | --- | --- |
|  | 1 | Perform repeat testing from original specimen aliquot or TE buffer tube  | Refer toMB 6.05, Proc. D |
| **Timeframe** | 2 | Repeat within 5 day if stored at 2 – 8⁰ C |  |
|  | 3 | Repeat samples may be retested in the same run as new samples |  |
| **Vortex** | 4 | Vortex the specimen tubes prior to retesting for 1 min; vortex setting 9 |  |
| Type of Failure | 5 | Review type of failure (not all inclusive)

|  |  |
| --- | --- |
| Failure | Action |
| Inhibition | * Specimen
1. Extract 100 - 200 µl on EasyMag (RVP protocol)
2. Test eluate
* Include extraction Controls
1. Extract 100 µl PCTL
2. Extract 200 µl NEGC (NFW)
3. Test eluates
* If sample remains unresolved, call caregiver for new collection
 |
| PCTL  | * Vortex PCTL and specimen tubes; repeat testing
* Include PCTL and NEGC
* If PCTL fails on repeat, thaw new PCTL
 |
| NEGC | * Repeat run from patient NW/bronch aliquot or NP TE buffer tubes
* Replace NEGC if contamination is indicated; review patient results
* Pipette carefully to avoid possible aerosol contamination
 |
| System error | * Repeat run from patient NW/bronch aliquot or NP TE buffer tubes
* Include PCTL/NEGC
 |
| Failure unresolved | * Call DiaSorintechnical service, **1-800-838-4548, option 3**
* Notify section technical director or designee
 |

 | [Simplexa Operator Manual](http://khan.childrensmn.org/Manuals/Lab/SOP/MolBio/UserMan/212340.pdf)Appendix B: TroubleshootingMB 6.06 Troubleshooting Guide |

**PROCEDURE J:** Follow the steps in the table below for Liaison MDX instrument shutdown in room 3

Computer and Instrument Shutdown

| **Activity** | **Step** | **Action** |
| --- | --- | --- |
| **CBA** | 1 | **CBA**: Shut down computer and then the analyzers when all runs are completed (Computer before analyzer) |
|  | 2 | Click on the **Close** button or “X” out of the program |
| **Shutdown menu** | 3 | Click on the **Start** button (Windows icon) |
|  | 4 | Next to **Restart**, click on  |
|  | 5 | Select **Shutdown** from the drop down menu |
| **CBA** | 6 | After the computer has shutdown, turn off the analyzers |

**PROCEDURE K:** Follow the steps in the table below for storing test specimens

Storage and Retention of test specimens

| **Activity** | **Step** | **Action** |
| --- | --- | --- |
| **Positive samples** | 1 | For storage of positive samples, label 2 boxes, one *Positive Bp* and one *Positive Bpp* including date range  |
|  | 2 | Store positive test samples in -70⁰ C freezer, shelf 3, for at least 1 year |
| **Negative samples** | 3 | Number freezer boxes 1 – 4 for negative samples |
|  | 4 | Rotate boxes once filled; discard box after rotation is complete starting with box 1 |

####  METHOD PERFORMANCE

* Clinical Sensitivity/Specificity 2: 96% / 100%
* Analytical Sensitivity 2: *B. pertussis*:1 CFU/3 µl reaction and *B. parapertussis*: 6 CFU/3 µl reaction

**PROFICIENCY TESTING**

* CAP *B. pertussis/B.parapertussis* (BOR), 2 shipments per year, 3 challenges each

#### ALTERNATE METHOD

1. *Bordetella pertussis* and *Bordetella parapertussis*, Molecular detection by PCR
2. Reference Lab: Mayo Medical Laboratories (Test ID: BPRP)
3. Sunquest Order code: BPPCR
4. Logistics:
* NP Swab in Liquid Stuart’s or Amies Charcoal transport medium
* Nasal wash/aspirate (0.5 ml) in sterile screw top container, no transport media
* Transport at RT or refrigerated : Stable up to 7 days
* Analytic time: 1 day
* Testing Monday – Friday, Sunday

## LIMITATIONS

1. Negative results do not rule out Bp and Bpp.
2. PCR detection of *B. pertussis* and *B. parapertussis* does not distinguish between viable and non-viable organism. Results should be used in conjunction with an evaluation of signs and symptoms of pertussis and available exposure information.
3. This test should not be used as a test for cure for *B. pertussis* and *B. parapertussis*.
4. This test does not distinguish between *B. pertussis* and *B. holmseii*. Some strains of *B. bronchiseptica* also contain the IS*481* gene and will cross-react at a lower level.
5. The IS1001target sequence can occasionally be found in *B. bronchiseptica* 4, 5, 6,
6. False-positive PCR results and pseudo-outbreaks have been associated specimen contamination at the point of collection from some vaccines containing *B. pertussis* DNA 6, 7, 8.
7. False-negative results can occur when low numbers of organism are present. PCR has optimal sensitivity during the first 3 weeks of cough9.
8. False negative results may occur if Bp or Bpp has genomic mutations, insertions, deletions or rearrangements.
9. Consider culture back-up during outbreak situations to rule out possible contamination9.

**REFERENCES**

1. Simplexa™ 3M™ Integrated Cycler Studio 5.0 , 3M™ Integrated Cycler Operator Manual Reference 34-8710-8382-9, PI.MOL1101.UD\_REV. F for use with user defined assays, Focus Diagnostics 2009-2012, Focus Diagnostics, Inc. Cypress, CA
2. *Bordetella* PCR Clinical Verification and Validation Study performed at Children’s Hospitals and Clinics of MN, 2015
3. Simplexa™ *Bordetella* Universal Direct Circular PI.MOL2700.IVD, Rev. F, 18-July-2012, Focus Diagnostics, Cypress, CA 90630
4. Tilley PA, Kanchana MV, Knight I, Blondeau J, Antonishyn N, Deneer H, Detection of *Bordetella pertussis* in a clinical laboratory by culture, polymerase chain reaction, direct fluorescent antibody staining; accuracy and cost, Diagn Microbiology Infect Dis. 2000 May; 37(1): 17-23.
5. Pittet LF, Emonet S, Francois P, et al, Diagnosis of Whooping cough in Switzerland: Differentiating *Bordetella pertussis* from *Bordetella holmseii* by Polymerase Chain Reaction, PLOS Feb 2014, vol 9, issue 2, e88936 pg 1-5.
6. Michael Loeffelholz, Towards Improved Accuracy of *Bordetella pertussis* Nucleic Acid Amplification Tests, Journ of Clin Micro, Volume 50, Number 7: 2186-2190
7. Mandal, Sema, Tatti KM, Woods-Stout D, Cassiday A, Faulkner E, et al, Pertussis Pseudo-outbreak linked to Specimens Contaminated by *Bordetella pertussis* DNA from Clinic Surfaces, Pediatrics; Volume 129, Number 2, Feb 2012.
8. California Department of Health – February 2011 newsletter: Pertussis: Laboratory Testing.
9. MMWR Weekly August 24, 2007/56(33); 837-842. Outbreaks of Respiratory Illness Mistakenly Attributed to Pertussis---New Hampshire, Massachusetts, and Tennessee, 2004-2006

|  |  |
| --- | --- |
| Historical Record |  |
|  | **Version** | **Written/Revised by:** | **Effective Date:** | **Summary of Revisions** |
|  | 1 | P. Ackerman | 1.23.16 | Initial Version |
|  | 2 | P. Ackerman | 07.20.16 | Reformatted for CMS upload; prev BOR 005 |
|  | 3 | P. Ackerman | 03.29.17 | Instrument name change from Focus Integrated Cycler to DiaSorin Liaison MDX; fixed hyperlinks for SharePoint upload |
|  | 4 | J. Laramie | 02.26.18 | -Added  *Bordetella parapertussis* ATCC 9305 to reagent list-Eliminated manufactured positive control (POSC) notes -Added Bp and Bpp PCTL rotation to quality control section |