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
- POSC: positive 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 room
- Area/Room 2: Processing room
- Area/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 -30° C ▪ Refrigerator, 2 to 8° C ▪ Microcentrifuge ▪ Nalgene cooling block ▪ Vortex ▪ Eppendorf Repeater pipette	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)

Equipment	Reagents	Supplies
▪ 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 8° C ▪ Freezer, ≥ - 70°C ▪ 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)	*Bordetella* Molecular Control (POSC) *Bordetella* process control (PCTL) TA MasterMix Sani-Cloth Bleach wipes 70% alcohol 5% Extran *Bordetella pertussis* ATCC 8467	Sharps disposal container Gripper rack, rm 2 Orange barrier wipes BBL™CultureSwab™ 12X75 sterile plastic test tubes Sterile Q – Tipped applicator swabs 50 ml sterile conical tube Eppendorf 5 ml tips Serological pipettes, 5 and 10 ml Sterile scissors

QUALITY CONTROL

A. Assay Controls
   1. A PCTL, POSC and NEGC must be included in each assay run.
   2. An IC is incorporated into each reaction mixture.

B. QC Monitors:

Control	Control Monitor
Positive Control (POSC)	Reagent failure and primer-probe integrity
Negative Control (NEGC)	Reagent and/or environmental contamination, cumulative effect
Process Control (PCTL)	Elution and/or lysis failure; reagent failure
Internal Control (IC)	PCR inhibition in specimen, reagent failure or process error

C. 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
Sample Order **Room 2**	1	Call worksheet **BORDP**; use this worksheet for sample identification throughout testing.	MB 1.01 Specimen Management
	2	Position samples and controls in disc as follows: Sample: Patient samples — Position: 1 – nn PCTL — 3rd to last position POSC — 2nd to last position NEGC — Last position	MB 3.01 Engineering Controls MB 2.01 Safe Work Practices

<table>
<tr><th>Activity</th><th>Step</th><th>Action</th><th>Related Doc</th></tr>
<tr><td>Organizing run

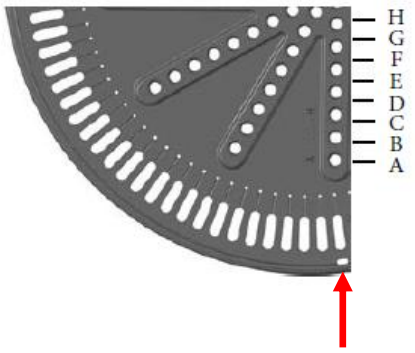
Room 2</td><td>3</td><td>Using the BORDP worksheet as a layout, organize patient specimens and labels<table><tr><th>Step</th><th>Action</th></tr><tr><td>a</td><td>Color code worksheets and labels per run</td></tr><tr><td>b</td><td>Number patients on worksheet in consecutive order</td></tr><tr><td>c</td><td>Number corresponding patient labels according to assigned numbers on worksheet, color coded by run</td></tr><tr><td>d</td><td>Number each primary patient specimen according to worksheet</td></tr></table></td><td></td></tr>
<tr><td>Process NP swabs</td><td>4</td><td>Elute NP swabs in 200 µl TE buffer<table><tr><th>Step</th><th>Action</th></tr><tr><td>a</td><td>Number cap of each 200 µl TE tube according to assigned number on worksheet</td></tr><tr><td>b</td><td>Properly label TE tube with patient aliquot label matching the number on the cap to the number on the label</td></tr><tr><td>c</td><td>Verify number on primary and secondary container before transfer</td></tr><tr><td>d</td><td>Cut the wire mini-tip swab into the TE buffer tube with corresponding number on cap</td></tr><tr><td>e</td><td>Vortex 5 min, vortex setting 8</td></tr></table></td><td></td></tr>
<tr><td>Process Bronchs, nasal washes/aspirates</td><td>5</td><td>Number and label a 2.0 ml cryovial for each nasal wash/aspirate and bronch specimen to be tested<table><tr><th>Step</th><th>Action</th></tr><tr><td>a</td><td>Number cap of each cryovial according to assigned number on worksheet</td></tr><tr><td>b</td><td>Properly label the tube with patient aliquot label matching the number on the cap to the number on the label</td></tr><tr><td>c</td><td>Vortex specimen in original container until well mixed</td></tr><tr><td>d</td><td>Verify number on primary and secondary container before transfer</td></tr><tr><td>e</td><td>Transfer specimen into tube with corresponding number on cap
Only one tube can be open at a time</td></tr></table></td><td></td></tr>
<tr><td rowspan="4">Change gloves</td><td>6</td><td>Change gloves when possible contamination is suspected or every 8 samples</td><td rowspan="4"></td></tr>
<tr><td>7</td><td>Place numbered tubes (washes and NP swabs) in consecutive order in rack</td></tr>
<tr><td>8</td><td>Decontaminate hood and scissors; bleach wipe followed by alcohol and water</td></tr>
<tr><td>9</td><td>Change gloves</td></tr>
</table>

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

Computer set-up

<table>
<tr><th>Activity</th><th>Step</th><th>Action</th><th>Related Doc</th></tr>
<tr><td>Computer Set-up Room 3</td><td>1</td><td>Set up Liaison MDX; take run specific patient labels into room 3
<table>
<tr><th>Step</th><th>Prompt</th><th>Action/Entry</th></tr>
<tr><td>a</td><td>------</td><td>Turn on the Liaison MDX (ABC)</td></tr>
<tr><td>b</td><td>------</td><td>Turn on the Liaison computer</td></tr>
<tr><td>c</td><td>------</td><td>Log on computer</td></tr>
<tr><td>d</td><td>User name</td><td>administrator</td></tr>
<tr><td>e</td><td>Password</td><td>focusIC#1</td></tr>
<tr><td>f</td><td>------</td><td>Double-click on program icon to open</td></tr>
<tr><td>g</td><td>User name</td><td>Enter personal user code</td></tr>
<tr><td>h</td><td>Password</td><td>Enter personal password code</td></tr>
<tr><td>i</td><td>-----</td><td>Select Setup Run from Quick pick list</td></tr>
<tr><td>j</td><td>Assay definition</td><td>Select BORD from drop down box</td></tr>
<tr><td>k</td><td>Run Name Prefix</td><td>Type in BORD</td></tr>
<tr><td>l</td><td>Lot information</td><td>PP lot: Add/deactivate reagent lot numbers as needed</td></tr>
<tr><td>m</td><td>Add Samples</td><td>Scan barcode ID from each label consecutively</td></tr>
<tr><td>n</td><td>Controls</td><td>Assign controls according to layout</td></tr>
<tr><td>o</td><td>-----</td><td>Click Move to Disc button</td></tr>
<tr><td>p</td><td>-----</td><td>Click Save to save the run for later use or</td></tr>
<tr><td>q</td><td>-----</td><td>Click Run to save the run and open the Start Run window</td></tr>
<tr><td>r</td><td>-----</td><td>(Optional) Click the Print Preview button to generate a layout report, refer to Fig.1</td></tr>
<tr><td>s</td><td>-----</td><td>Recycle labels when run is complete; do not take back to room 2</td></tr>
</table></td><td></td></tr>
<tr><td>New user</td><td>2</td><td>To switch users: Select File: Switch Users
Note: Users cannot be changed while instrument is running</td><td></td></tr>
<tr><td>Delete or Edit Segment</td><td>3</td><td>To delete or edit segments, right click one of the wells in the segment
<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>a</td><td>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</td></tr>
<tr><td>b</td><td>To move samples back to disc, click starting well location in Disc View</td></tr>
<tr><td>c</td><td>Click Move to Disc button</td></tr>
</table></td><td></td></tr>
<tr><td rowspan="2">Change PPE</td><td>4</td><td>Remove lab coat</td><td rowspan="2"></td></tr>
<tr><td>5</td><td>Change gloves; move to room 1</td></tr>
</table>

Figure 1: Spoke 1 is identified by the open 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.



Well Identity Matrix - Universal Disc

Well-Spoke 1	2	3	4	5	6
1 (1A)	9 (2A)	17 (3A)	25 (4A)	33 (5A)	41 (6A)
2 (1B)	10 (2B)	18 (3B)	26 (4B)	34 (5B)	42 (6B)
3 (1C)	11 (2C)	19 (3C)	27 (4C)	35 (5C)	43 (6C)
4 (1D)	12 (2D)	20 (3D)	28 (4D)	36 (5D)	44 (6D)
5 (1E)	13 (2E)	21 (3E)	29 (4E)	37 (5E)	45 (6E)
6 (1F)	14 (2F)	22 (3F)	30 (4F)	38 (5F)	46 (6F)
7 (1G)	15 (2G)	23 (3G)	31 (4G)	39 (5G)	47 (6G)
8 (1H)	16 (2H)	24 (3H)	32 (4H)	40 (5H)	48 (6H)

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	MB 6.04 Refer to MM chart
	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	
MasterMix Room 1	3	Prepare MM in 1.5 micro-centrifuge tube according to chart volumes	MB 6.03 Storage and Stability
	4	Gently vortex MM; briefly centrifuge ▪ Vortex setting: 8 ▪ Time: 2 sec ▪ Centrifuge: 1 – 2 sec	
	5	Return reagents to refrigerator, do not refreeze	
	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*	

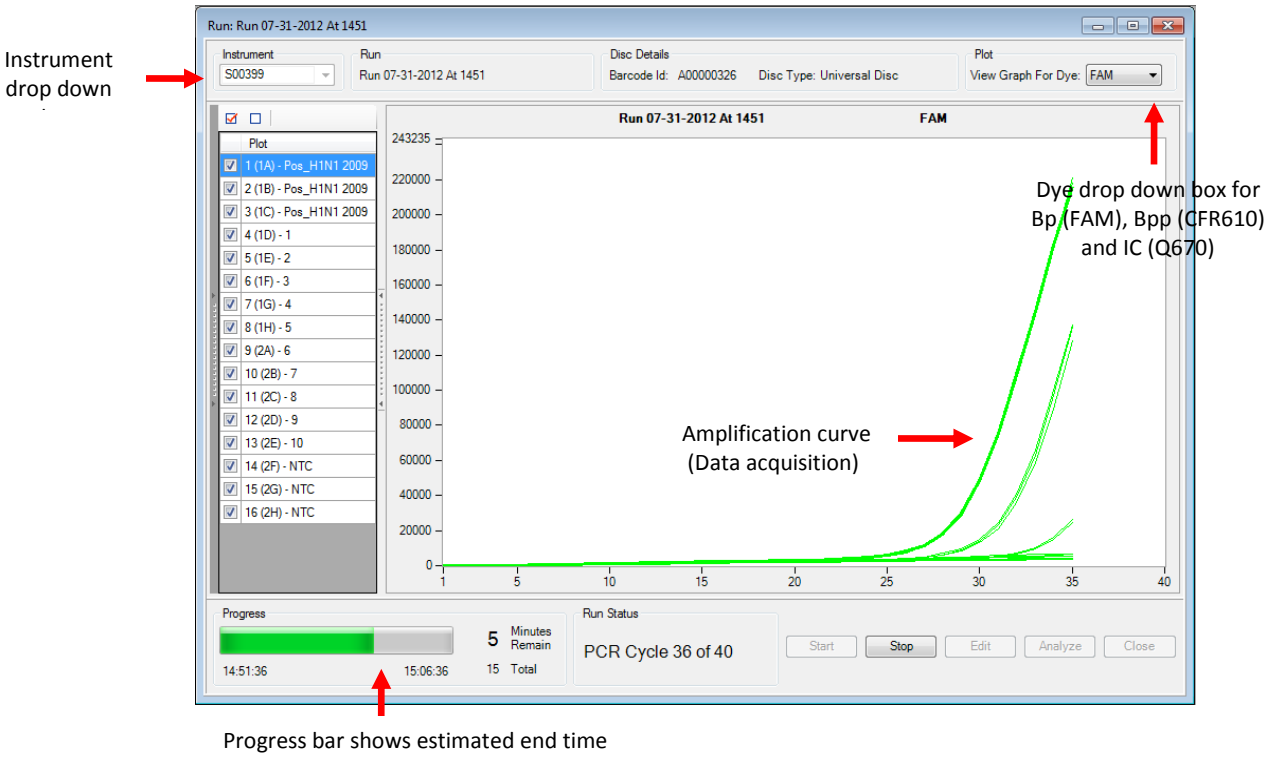
PROCEDURE D: Follow the steps in the table below for PCR set-up and amplification

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	

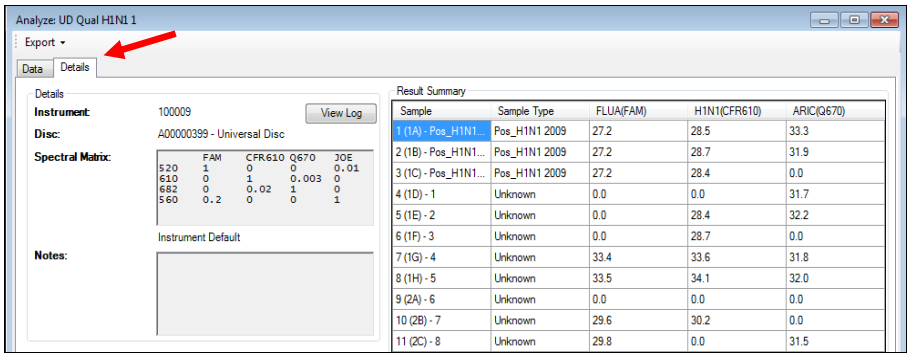
Activity	Step	Action	Related Doc
Load MM Room 2	3	Position spoke 1 over silver plate groove (refer to Fig. 1)	Simplexa Operator Manual
	4	Pipette 7 µl of MM into each well to be used Tip ▪ Automatic pipettor: hold at slight angle to maintain accuracy ▪ Manual pipetting: hold the pipette at a 30-degree angle inserting the tip under the roof of the well to reduce possible contamination 	
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 ▪ POSC: undiluted ▪ NEGC: NFW *Caution*: Do not go to second stop to avoid introduction of bubbles and producing aerosols	
Seal disc	6	Apply the cover tape on the disc in horizontal position	
	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	
Run	17	Approximate run time: 1 h	
	18	Run progress can be viewed in the **Run Status Window**: refer to Fig. 2	
Run completion	19	Remove disc from instrument; *check well volumes for pipetting accuracy*	
	20	Place in bio-bag	
	21	Discard in red biohazard container	

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



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

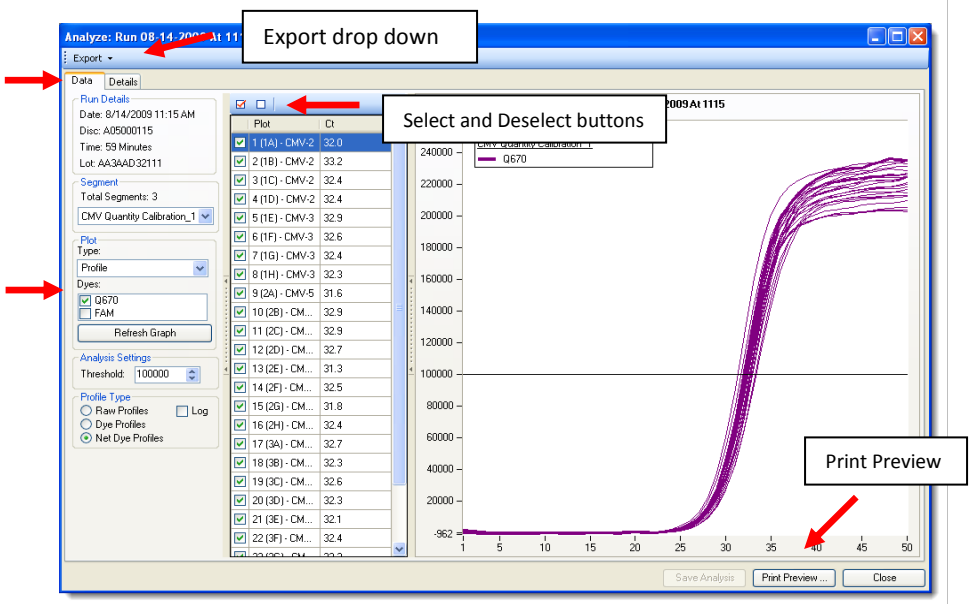
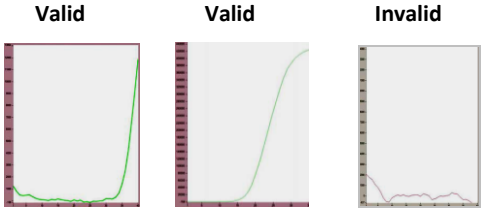
Analyzing Completed Runs

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 	

<table>
<tr><th>Activity</th><th>Step</th><th>Action</th><th>Related doc</th></tr>
<tr><td>Room 3

Review amplification curves</td><td>3</td><td>Review IC Ct results and amplification curves for exponential growth and possible inhibition or low target amplification, refer to Figures 3 and 4
<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>a</td><td>Select Data tab</td></tr>
<tr><td>b</td><td>Click on Print Preview</td></tr>
<tr><td>c</td><td>Check Include Graphs</td></tr>
<tr><td>d</td><td>Scroll through the report , reviewing comments, failures and amplification curves</td></tr>
<tr><td>e</td><td>A valid curve shows a smooth, exponential increase</td></tr>
<tr><td>f</td><td>Invalid curve may be linear or a curve with data “spikes” where the curve crosses the threshold</td></tr>
<tr><td>g</td><td>If curve is valid, the Ct values may be used to interpret the results</td></tr>
<tr><td>h</td><td>Confirm results by a second reviewer before releasing</td></tr>
<tr><td>I</td><td>Positive results: Confirm name and accession number on primary sample/TE buffer before releasing</td></tr>
<tr><td>j</td><td>Select or deselect results to be released</td></tr>
</table></td><td>Refer to procedures F, G and H for interpretation of QC and patient results and Exporting results to LIS</td></tr>
<tr><td>Print Report</td><td>4</td><td>Print report after review (include graphs) Fig. 3
<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>a</td><td>Click Print Preview button for multi-page analysis report</td></tr>
<tr><td>b</td><td>Checkbox: Include Graphs</td></tr>
<tr><td>c</td><td>Print</td></tr>
</table></td><td></td></tr>
<tr><td>Analysis Window

Data / Detail tabs

Review channels by clicking dye box(es) to be reviewed</td><td>5</td><td>Figure 3: Analysis Window
 
</td><td></td></tr>
<tr><td>Curve examples</td><td>6</td><td>Figure 4: Valid and invalid amplification curves
 
</td><td></td></tr>
</table>

PROCEDURE F: Follow the activities below for evaluating QC acceptability

Evaluating and Interpreting QC Results

<table>
<tr><th>Activity</th><th>Step</th><th>Action</th><th>Related doc</th></tr>
<tr><td></td><td>1</td><td>Check QC to determine if the run is valid before reporting patient results</td><td></td></tr>
<tr><td></td><td>2</td><td>Failure indications will be highlighted in yellow
<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>a</td><td>Click the Print Preview button to review the “Data Quality message” on the Segment report under QC Notes</td></tr>
<tr><td>b</td><td>Review associated amplification curves and Ct values</td></tr>
<tr><td>c</td><td>Click the Print button to generate a report for the QC and Equipment Failure Log documentation</td></tr>
<tr><td>d</td><td>Record corrective action on QC log</td></tr>
<tr><td>e</td><td>Record number of failed samples on Failed Run log</td></tr>
</table></td><td>Simplexa Operator Manual Appendix B: Troubleshooting</td></tr>
<tr><td>QC / Valid assay</td><td>3</td><td>For a valid run, the following QC conditions must be met: <u>ranges are subject to change based on periodic re-calculated values</u>
<table>
<tr><th>Control</th><th>Bp Ct</th><th>Bpp Ct</th><th>IC Ct</th><th>Assay Result</th></tr>
<tr><td>POSC</td><td>23 – 34</td><td>20 - 30</td><td>NA</td><td>Positive</td></tr>
<tr><td>PCTL</td><td>28 – 34</td><td>0</td><td>NA</td><td>Positive</td></tr>
<tr><td>NEGC</td><td>0</td><td>0</td><td>25 – 31</td><td>Negative</td></tr>
</table></td><td>3SD ranges periodically determined in EP Evaluator and programmed into the Simplexa</td></tr>
<tr><td>QC conditions not met

Invalid assay</td><td>4</td><td>
<table>
<tr><th>If</th><th>Then</th></tr>
<tr><td>Valid assay: Controls as expected</td><td>▪ Report patient results</td></tr>
<tr><td>Invalid assay conditions:
PCTL/POSC/ NEGC failure</td><td>▪ Do not report patient results
▪ Failure caused by inhibition, reagent or system failure
▪ Repeat patient testing</td></tr>
<tr><td>PCTL negative or out of range</td><td>▪ Review the specimen handling/ preparation technique
▪ Repeat patient testing</td></tr>
<tr><td>POSC negative or out of range</td><td>▪ Review the specimen handling/ preparation technique
▪ Repeat patient testing</td></tr>
<tr><td>NEGC positive</td><td>▪ Possible contamination of samples
▪ Review the specimen handling/ preparation technique
▪ Repeat patient testing</td></tr>
<tr><td>IC not detected in the NEGC</td><td>▪ Failure caused by reagent or system failure
▪ Repeat patient testing</td></tr>
<tr><td>IC fails in negative patient sample but negative control acceptable</td><td>▪ Failure caused by inhibition, reagent or system failure
▪ Review disc well for proper volume
▪ Check sample for blood/mucus
▪ Repeat patient testing</td></tr>
<tr><td>Problem unresolved</td><td>▪ Call DiaSorin technical service, 1-800-838-4548, option 3
▪ Notify section technical director or designee</td></tr>
</table></td><td>Refer to MB 6.05, Proc. I for repeat testing</td></tr>
<tr><td>Problem Log</td><td>5</td><td colspan="2">Do not report patient results until problem is resolved</td></tr>
<tr><td></td><td>6</td><td colspan="2">Record problem/operator action in the QC and Equipment Failure Log</td></tr>
</table>

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 spikes ▪ Review "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 6.07 Reporting 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.		

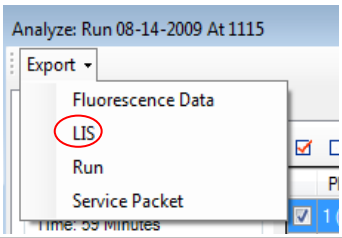
Table 1: Interpretation of Patient Results: Refer to MB 6.07 *Reporting and Archiving Results*

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 on the **Data** tab, refer to Fig.3	MB 6.07 Reporting and Archiving BORDP Results
	2	*Do not* send failed patient results or PCTL, POSC and NEGC. Deselect by clicking on individual box(es)	

Activity	Step	Action	Related Doc
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
Timeframe	1	Perform repeat testing from original specimen aliquot or TE buffer tube	Refer to MB 6.05, Proc. D
	2	Repeat within 5 day if stored at 2 – 8° 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)	Simplexa Operator Manual Appendix B: Troubleshooting MB 6.06 Troubleshooting Guide

Failure	Action
Inhibition	▪ Specimen a. Extract 100 - 200 µl on EasyMag (RVP protocol) b. Test eluate ▪ Include extraction Controls a. Extract 100 µl PCTL b. Extract 200 µl NEGC (NFW) c. Test eluates ▪ If sample remains unresolved, call caregiver for new collection
PCTL	▪ Vortex PCTL and specimen tubes; repeat testing ▪ Include POSC and NEGC ▪ If PCTL fails on repeat, thaw new PCTL
POSC	▪ Repeat run from patient NW/bronch aliquot or NP TE buffer tubes ▪ Vortex POSC and specimen tubes before repeat testing ▪ If POSC fails on repeat, thaw new POSC
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/POSC/NEGC
Failure unresolved	▪ Call DiaSorintechnical service, **1-800-838-4548, option 3** ▪ Notify section technical director or designee

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 -70° 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 cough[9].
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 contamination[9.]

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