Bordetella Reagent and Process Control Preparation

PURPOSE

- This procedure provides instructions for preparation of reagents and procedural controls

ABBREVIATIONS

- BORD: Bordetella
- BORDP: *Bordetella* PCR
- Bp: *Bordetella pertussis*
- Bpp: *Bordetella parapertussis*
- BSC: biosafety cabinet
- Ct: crossing threshold
- F/T: freeze/thaw
- IC: internal control
- MM: master mix
- NEGC: negative control
- NFW: nuclease free water
- 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
- Area/Room 1: Clean room
- Area/Room 2: Processing room
- Area/Room 3: Amplification room

SAFETY CONSIDERATIONS

- 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

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 ▪ Dedicated set of pipettes: 2 µl, 10 µl, 20 µl, 100 µl, 200 µl, and 1000 µl pipettes ▪ Pipet-Aid Room 2: Processing ▪ BSC, Process rm 2 ▪ Refrigerator, 2 to 8° C ▪ Freezer, ≥ - 70°C ▪ Nalgene cooling block ▪ Vortex ▪ Microcentrifuge ▪ Dedicated set of pipettes: 2 µl, 10 µl, 20 µl, 100 µl, 200 µl, and 1000 µl pipettes Room 3: Amplification and detection ▪ Liaison MDX Location: Microbiology ▪ McFarland densitometer	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 Control (POSC)	Sharps disposal container
	Bordetella process control (PCTL)	Gripper rack, rm 2
	TA MasterMix	Orange barrier wipes
	Sani-Cloth Bleach wipes	12X75 sterile plastic test tubes
	70% alcohol	Sterile Q – Tipped applicator swabs
	5% Extran	50 ml sterile conical tube
	Bordetella pertussis ATCC 8467	Eppendorf 5 ml tips
		Serological pipettes, 5 and 10 ml

PROCEDURE A: Follow the activities in the table below for Process Control preparation

Preparing Process Control Suspension

Activity	Step	Action	Related Doc
Prepare **Matrix** Room 2	1	Pool approximately 10 ml of nasal wash matrix in a 50 ml sterile conical tube	
	2	Dilute matrix in NFW to achieve a 30 – 35 ml suspension	
	3	Vortex well	
	4	Test suspension in duplicate according to the BORDP assay procedure to ensure that it is target free	MB 6.05 Bordetella PCR Assay
0.5 McFarland Micro	5	Prepare a 0.5 McFarland suspension of *Bordetella pertussis* ATCC 8467 a: Pick isolated colonies (3 – 4 d growth) with sterile CultureSwab b: Suspend in saline; vortex c: Adjust suspension to 0.5 McFarland (~1.5 X 10^8 CFU/mL) using densitometer d: **Dilution 1**: make a 1:100 dilution of this suspension in NFW (~1.5 X 10^6 CFU/mL) ▪ Pipette 10 µl into 990 µl NFW ▪ Vortex well e: **Dilution 2:** make a 1:100 dilution from dilution 1 in NFW (~1.5 X 10^4 CFU/mL) ▪ Pipette 40 µl into 4.0 mL NFW ▪ Vortex well	0.5 McFarland Standard turbidity range = 0.5 – 0.63
Working suspension Room 2	6	**Dilution 3** (working suspension): Make a 1:10 dilution from dilution 2 (final concentration ~1.5 X 10^3 CFU/mL) a: Pipette 3 mL from BORDP dilution 2 into 27 ml of matrix b: Mix well by inversion/vortexing c: Test suspension prior to freezing (3 µl Bp suspension into 7 µl BORDP mm) d: Target control range: Ct values 30 – 32 e: I f necessary, adjust suspension to obtain specified range with NFW; retest	1 log = ~ 3 Ct
Aliquot and Freeze	7	Label 1.5 ml micro-centrifuge tubes with contents and date of preparation (approx. 60 tubes)	
	8	Dispense 0.5 ml of working suspension into tubes	
	9	Freeze aliquots at –70° C	
Decontaminate Hood **Room 2**	10	Wipe down BSC with Bleach Sani – Cloth followed by water and 70% alcohol	
	11	UV hood for 15 min	
Test aliquots before use	12	Before use: ▪ Thaw one BORDP PCTL aliquot ▪ Vortex ▪ Perform direct testing 5 times to determine average Ct value	
	13	▪ Document Ct values on BORDP PCTL New Reagent Worksheet MB 6.09.F3	
	14	Place worksheet and BORDP Segment report including graphs in *New Lot Inventory and QC* manual	

Activity	Step	Action	Related Doc
Stability	15	Once thawed, process control is stable for 7 days at refrigerated temperature	
	16	Do not refreeze (only 1 F/T cycle)	

PROCEDURE B: Follow the activities in the table below for aliquoting TE buffer (sample buffer tubes) and Nuclease Free Water (NFW) used for NEGC and MM

Preparing TE buffer and NFW

Activity	Step	Action	Related Doc
PPE	1	Wear lab coat and gloves dedicated to the Clean room 1	
TE buffer and NFW	2	Label cryo-storage box with contents ▪ BORDP TE buffer: reagent lot, expiration date and date of preparation ▪ NEGC NFW: lot number (L/N), expiration date (1 year), and date of preparation	
Room 1	3	Aliquot the following amounts into 1.5 micro-centrifuge tubes ▪ BORDP Elution buffer: 200 µl of TE buffer into 1.5 ml micro-centrifuge tube ▪ NEGC: 500 µl of NFW into 1.5 ml micro-centrifuge tube	
Storage	5	Refrigerate aliquots in room 1	
	6	Keep working supply in room 2	

PROCEDURE C: Follow the activity below for preparing master mix (MM)

Preparing RT-PCR Master Mix (MM)

Activity	Step	Action
	1	***MM must be used within 30 min of preparation***.
	2	Wear lab coat and gloves dedicated to the Clean room 1
Warm reagents to RmTemp Room 1	3	Thaw Primer Probe mix, IC and the Master Mix at room temperature ▪ Protect from light ▪ Use within 1 hour
	4	Gently mix each component Component / Mixing action TA mm — Vortex 2 – 3 sec, setting 8 Bp PP — Gently flick Bpp PP — Gently flick IC DNA — Vortex 2 – 3 sec, setting 8 IC PP — Gently flick
	5	Quick spin reagents
	6	Prepare MM in a 1.5 mL micro-centrifuge tube by combining the reagents according to **Table 1**
	7	Gently vortex MM 2 – 3 sec to mix; vortex setting 8 ***Note:*** Adjust mixing time according to volume.
	8	Quick spin MM

Activity	Step	Action
Refrigerate reagents	9	Do not refreeze reagents; store in refrigerator up to 30 days ***Note:*** Refer to procedure MB 6.03 for storage conditions and expiry dates
Transport Room 2	10	Transport to room 2
	11	Keep the MM in refrigerator or cooling block protected from light until PCR reaction set-up.

Table 1: BORDP Master Mix Table

No. of samples	1	2	3	4	5	6	7	8	9	10	11	12
TA Master Mix (µl)	6	10	14	18	24	28	32	36	40	44	48	52
Bp Primer Mix (µl)	0.6	1	1.4	1.8	2.4	2.8	3.2	3.6	4	4.4	4.8	5.2
Bpp Primer Mix (µl)	0.6	1	1.4	1.8	2.4	2.8	3.2	3.6	4	4.4	4.8	5.2
IC DNA (µl)	0.3	0.5	0.7	0.9	1.2	1.4	1.6	1.8	2	2.2	2.4	2.6
IC Primer Mix (µl)	0.3	.05	0.7	0.9	1.2	1.4	1.6	1.8	2	2.2	2.4	2.6
NFW (µl)	2.7	4.5	6.3	8.1	10.8	12.6	14.4	16.2	18	19.8	21.6	23.4
Total volume (µl)	10.5	17.5	24.5	31.5	42	49	56	63	70	77	84	91

No. of samples	13	14	15	16	17	18	19	20	21	22	23	24
TA Master Mix (µl)	56	60	66	70	74	78	82	86	90	94	98	102
Bp Primer Mix (µl)	5.6	6	6.6	7	7.4	7.8	8.2	8.6	9	9.4	9.8	10.2
Bpp Primer Mix (µl)	5.6	6	6.6	7	7.4	7.8	8.2	8.6	9	9.4	9.8	10.2
IC DNA (µl)	2.8	3	3.3	3.5	3.7	3.9	4.1	4.3	4.5	4.7	4.9	5.1
IC Primer Mix (µl)	2.8	3	3.3	3.5	3.7	3.9	4.1	4.3	4.5	4.7	4.9	5.1
NFW (µl)	25.2	27	29.7	31.5	33.3	35.1	36.9	38.7	40.5	42.3	44.1	45.9
Total volume (µl)	98	105	115.5	122.5	129.5	136.5	143.5	150.5	157.5	164.5	171.5	178.5

PROCEDURE D: Follow the activity below for preparing miscellaneous reagents

Preparing miscellaneous reagents

<table>
<tr><th>Reagent</th><th>Step</th><th>Action</th></tr>
<tr><td rowspan="2">5% Extran Working solution

Room 3</td><td>1</td><td>Prepare in room 3/amplification room
Caution: Protective eyewear must be worn when working with concentrated Extran</td></tr>
<tr><td>2</td><td>Make working solution as follows:
<table>
<tr><th>Working Volume</th><th>Conc. Extran</th><th>Water</th></tr>
<tr><td>2000 ml</td><td>100 ml</td><td>1900 ml</td></tr>
<tr><td>3000 ml</td><td>150 ml</td><td>2850 ml</td></tr>
<tr><td>4000 ml</td><td>200 ml</td><td>3800 ml</td></tr>
</table></td></tr>
<tr><td rowspan="2">70% alcohol

Room 3 or Recycling room</td><td>1</td><td>Prepare from 100% Dehydrant alcohol located in the Flammable cabinet in the Recycling room.</td></tr>
<tr><td>2</td><td>Make working solution as follows:
<table>
<tr><th>Working Volume</th><th>100% Dehydrant</th><th>Water</th></tr>
<tr><td>1000 ml</td><td>700 ml</td><td>300 ml</td></tr>
</table></td></tr>
</table>

REFERENCES

1. *Bordetella* PCR Clinical Verification and Validation Study performed at Children's Hospitals and Clinics of MN, 2015
2. Simplexa™ *Bordetella* Universal Direct Circular PI.MOL2700.IVD, Rev. F, 18-July-2012, Focus Diagnostics, Cypress, CA 90630
3. *Bordetella pertussis* Primer Pair (50 µl) ASR, Circular PI.MOL9006 Rev. B, 20-January-2011, Focus Diagnostics, Cypress, CA 90630
4. *Bordetella parapertussis* Primer Pair (50 µl) ASR, Circular PI.MOL9007 Rev. B, 07-February-2011, Focus Diagnostics, Cypress, CA 90630
5. Simplexa™ *Bordetella* Molecular Control, Circular PI.MOL8006 Rev. A, 06-Feb-2013, Focus Diagnostics, Cypress, CA 90630
6. Simplexa™ Extracton & Amplification Control Set, Circular PI.MOL9000, Rev. D, CE, 7 Mar 2013, Focus Diagnostics, Cypress, CA 90630

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 004
3	P. Ackerman	03.29.17	Instrument name change from Focus Integrated Cycler to DiaSorin Liaison MDX; fixed hyperlinks for SharePoint upload