# GAS Reagent and Process Control Preparation

**PURPOSE**

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

**ABBREVIATIONS**

|  |  |
| --- | --- |
| * BSC: BioSafety Cabinet
* Ct: crossing threshold
* F/T: freeze/thaw
* GAS: Group A Strep
* GASD: Group A Strep Detection
* IC: internal control
* MM: master mix
* NEGC: negative control
* NFW: nuclease free water
* PCTL: process control
 | * PP: primer – pair
* PPE: personal protective equipment
* RT: room temperature
* SEAC: Simplexa extraction and amplification control
* TE buffer: Tris – EDTA buffer

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

#### 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 in each working area: 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 8⁰ C
* Freezer, ≥ - 70⁰C
* Nalgene cooling block
* Vortex
* Microcentrifuge Dedicated set of pipettes in each working area: 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 |
| GAS pp | Micro tubes 1.5 ml, RNase /DNase free |
| GAS process control (PCTL) | Nitrile gloves (powder-free) |
| TA MasterMix | Sharps disposal container  |
| Sani-Cloth Bleach wipes | Gripper rack, rm 2 |
| 70% alcohol | Orange barrier wipes |
| 5% Extran | BBL™CultureSwab™ |
| *Streptococcus pyogenes* ATCC 19615 | 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 |
|  |  |

## SAFETY CONSIDERATIONS

* Standard precautions. Refer to [MB 2.02](http://khan.childrensmn.org/Manuals/Lab/SOP/MolBio/Safety/212201.pdf) Biohazard Containment
* Use of engineering controls: Refer to [MB 3.01](http://khan.childrensmn.org/Manuals/Lab/SOP/MolBio/EngCtl/212209.pdf) Engineering Controls to Prevent Nucleic Acid Contamination

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

Preparing Process Control Suspension

| **Activity** | **Step** | **Action** | **Related Doc** |
| --- | --- | --- | --- |
| **Prepare** | 1 | Pour 7 ml of TE Buffer in a 50 ml sterile conical tube  |  |
| **Matrix**Room 2 | 2 | Add NFW to achieve a 30 ml suspension |  |
|  | 3 | Test suspension in duplicate according to the GAS assay procedure to ensure that it is target free. If preparing the Positive Process Control proceed to step 4. If preparing the Negative Process Control proceed to step 6. | MB 8.05 GAS Assay |
| **GAS 0.5 McFarland****Micro** | 4 | Prepare a 0.5 McFarland suspension of *Streptococcus pyogenes* ATCC 19615

|  |  |
| --- | --- |
| Step | Action |
| a | Pick isolated colonies (18 – 24 h growth) with sterile CultureSwab |
| b | Suspend in saline; vortex |
| c | Adjust suspension to 0.5 McFarland(~1.5 X 108 CFU/mL) using densitometer  |
| d | **Dilution 1**: Make a 1:10 dilution of this suspension in NFW (~107 CFU/mL)* Pipette 0.5 mL of suspension into 4.5 mL NFW
* Vortex well
 |

 | 0.5 McFarland Standard turbidity range = 0.5 – 0.63 |
| **Working suspension****Room 2** | 5 | **Dilution 2:** working suspension ~ 106 CFU/mL

|  |  |
| --- | --- |
| Step | Action |
| 1 | Pipette 3.0 mL from dilution 1 into 30 ml of the TE Buffer and NFW suspension |
| 2 | Mix well by inversion/vortexing  |
| 3 | Absorb ~100 µl unto 2 swabs; test prior to freezing suspension |
| 4 | Target control range: Ct values 29-31 |
| 5 | I f necessary, adjust suspension to obtain specified range; retest |

 | 1 log = ~ 3 Ct |
| **Aliquot and Freeze** | 6 | Label 2 ml cryovials with contents and date of preparation (approx. 29 tubes) |  |
| 7 | Dispense 1 ml of working suspension into tubes |  |
|  | 8 | Freeze aliquots at –70° C |  |
| **Decontaminate Hood** | 9 | Wipe down BSC with Bleach Sani – Cloth followed by water and 70% alcohol  |  |
| **Room 2** | 10 | UV hood for 15 min  |  |
| **Test aliquots before use** | 11 | Before use:* Thaw one aliquot
* Prepare 5 swabs
* Test each swab
 | Refer to MB 8.04Proc. B |
|  | 12  | Document Ct values on GAS PCTL or GAS NEGC New Reagent Worksheet | MB 8.09.F8MB 8.09 F10 |
|  | 13 | Place worksheet and GAS Segment report including graphs in *New Lot Inventory and QC* manual |  |
| **Stability** | 14 | Once thawed, process controls are stable for 7 days at refrigerated temperature |  |
|  | 15 | Do not refreeze (only 1 F/T cycle) |  |

**PROCEDURE B:** Follow the activities in the table below for preparation of process control swabs

Preparing Process Control Swabs

| **Activity** | **Step** | **Action** | **Related Doc** |
| --- | --- | --- | --- |
| **Thaw** | 1 | Thaw the working suspension; vortex |  |
| Room 2 | 2 | Each working suspension is enough for approximately 10 control swabs |  |
| **Swabs Prep** | 3 | Label CultureSwab transport container with contents and date of preparation |  |
|  | 4 | Dip CultureSwab into suspension for 10 sec. to absorb approx. 100 µl (final concentration per swab ~ 1.0 x 105 CFU/mL) |  |
|  | 5 | Rotate swab against the wall of the tube above the liquid to remove excess inoculum. |  |
|  | 6 | Place swab into transport container allowing contact with medium at the bottom of the tube. |  |
|  | 7 | Let swabs stand at room temp for 5 minutes before use. |  |
|  | 8 | Wipe down BSC with Bleach Sani – Cloth followed by water and 70% alcohol  |  |
| **Stability** | 9 | Test controls the same as a clinical specimen. |  |
|  | 10 | Process control swabs are stable for 5 days at 2 – 8⁰ C. |  |

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

Preparing RT-PCR Master Mix (MM)

| Activity | Step | **Action** |
| --- | --- | --- |
| **PPE** | 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** | 3 | Thaw Primer Probe mix, IC and the Master Mix at room temperature* Protect from light
* Use within 1 hour
 |
| Room 1 | 4 | Gently mix each component

|  |  |
| --- | --- |
| Component | Mixing action |
| TA mm | Vortex 2 – 3 sec, setting 8 |
| GAS pp | Gently flick  |
| IC DNA | Vortex 2 – 3 sec, setting 8 |
| IC pp | Gently flick  |

 |
|  | 5 | Quick spin regents |
|  | 6 | Prepare MM in a 1.5 micro-centrifuge tube by combining the reagents according to **Table 1** |
| **Prepare MM** | 7 | Gently vortex MM 2 – 3 sec to mix; vortex setting 8  ***Note:***Adjust mixing time according to volume.  |
|  | 8 | Quick spin MM |
| **Refrigerate reagents** | 9 | Do not refreeze reagents; store in refrigerator up to 30 days ***Note:*** Refer to procedure [MB 8.03](http://khan.childrensmn.org/Manuals/Lab/SOP/MolBio/GAS/212286.pdf) for storage conditions and expiry dates |
| **Transport** | 10 | Transport to room 2 |
| Room 2 | 11 | Keep the MM in refrigerator or cooling block protected from light until PCR reaction set-up. |

**Table 1: GAS 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 |
| **GAS 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)** | 0.6 | 1 | 1.4 | 1.8 | 2.4 | 2.8 | 3.2 | 3.6 | 4 | 4.4 | 4.8 | 5.2 |
| **IC Primer Mix (µl)** | 0.23 | 0.38 | 0.53 | 0.68 | 0.9 | 1.05 | 1.2 | 1.35 | 1.5 | 1.65 | 1.8 | 1.95 |
| **NFW (µl)** | 4.58 | 7.63 | 10.68 | 13.73 | 18.3 | 21.35 | 24.4 | 27.45 | 30.5 | 33.55 | 36.6 | 39.65 |
| **Total volume (µl)** | 12 | 20 | 28 | 36 | 48 | 56 | 64 | 72 | 80 | 88 | 96 | 104 |

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **No. of samples** | **13** | **14** | **15** | **16** | **17** | **18** | **19** | **20** | **21** | **22** | **23** | **24** |
| **TA Master Mix (µl)** | 58 | 62 | 66 | 70 | 76 | 80 | 84 | 88 | 92 | 96 | 100 | 104 |
| **GAS Primer Mix (µl)** | 5.8 | 6.2 | 6.6 | 7.0 | 7.6 | 8 | 8.4 | 8.8 | 9.2 | 9.6 | 10 | 10.4 |
| **IC DNA (µl)** | 5.8 | 6.2 | 6.6 | 7.0 | 7.6 | 8 | 8.4 | 8.8 | 9.2 | 9.6 | 10 | 10.4 |
| **IC Primer Mix (µl)** | 2.18 | 2.33 | 2.48 | 2.63 | 2.85 | 3 | 3.15 | 3.3 | 3.45 | 3.6 | 3.75 | 3.9 |
| **NFW (µl)** | 44.23 | 47.28 | 50.33 | 53.38 | 57.95 | 61 | 64.05 | 67.1 | 70.15 | 73.2 | 76.25 | 79.3 |
| **Total volume (µl)** | 116 | 124 | 132 | 140 | 152 | 160 | 168 | 176 | 184 | 192 | 200 | 208 |

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **No. of samples** | **25** | **26** | **27** | **28** | **29** | **30** | **31** | **32** | **33** | **34** | **35** | **36** |
| **TA Master Mix (µl)** | 110 | 114 | 118 | 122 | 128 | 132 | 136 | 140 | 144 | 148 | 152 | 156 |
| **GAS Primer Mix (µl)** | 11 | 11.4 | 11.8 | 12.2 | 12.8 | 13.2 | 13.6 | 14 | 14.4 | 14.8 | 15.2 | 15.6 |
| **IC DNA (µl)** | 11 | 11.4 | 11.8 | 12.2 | 12.8 | 13.2 | 13.6 | 14 | 14.4 | 14.8 | 15.2 | 15.6 |
| **IC Primer Mix (µl)** | 4.13 | 4.28 | 4.43 | 4.58 | 4.8 | 4.95 | 5.1 | 5.3 | 5.4 | 5.55 | 5.7 | 5.85 |
| **NFW (µl)** | 84 | 87 | 90 | 93 | 97.6 | 100.7 | 103.7 | 106.8 | 109.8 | 112.9 | 115.9 | 119 |
| **Total volume (µl)** | 220 | 228 | 236 | 244 | 256 | 264 | 272 | 280 | 288 | 296 | 304 | 312 |

**PROCEDURE D:** Follow the activities in the table below for aliquoting TE buffer (sample buffer tubes) and Nuclease Free Water (NFW) used for 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 * GASDN 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* 250 µl of TE buffer into each tube
* 500 µl of NFW into each tube
 |  |
| **Storage** | 4 | Refrigerate aliquots in room 1 |  |
|  | 5 | Keep working supply in room 2 |  |

**PROCEDURE E:** Follow the activity below for preparing miscellaneous reagents

Preparing miscellaneous reagents

| Reagent | Step | **Action** |
| --- | --- | --- |
| 5% Extran Working solution | 1 | Prepare in room 3/amplification room *Caution: Protective eyewear must be worn when working with concentrated Extran* |
| Room 3 | 2 | Make working solution as follows:

|  |  |  |
| --- | --- | --- |
| Working Volume | Conc. Extran | Water |
| 2000 ml | 100 ml | 1900 ml |
| 3000 ml | 150 ml | 2850 ml |
| 4000 ml | 200 ml | 3800 ml |

 |
| **70% alcohol** | 1 | Prepare from 100% Dehydrant alcohol located in the Flammable cabinet in the Recycling room. |
| Room 3 or Recycling room | 2 | Make working solution as follows:

|  |  |  |
| --- | --- | --- |
| Working Volume | 100% Dehydrant | Water |
| 1000 ml | 700 ml | 300 ml |

 |

**REFERENCES**

1. Simplexa™ Bordetella Universal Direct, PI.MOL2700.IVD EN CE Marked, REV F, July 18, 2012, DiaSorin Molecular, Cypress, CA 90630
2. Clinical Verification and Validation Study performed at Children’s Hospitals and Clinics of MN August 2014

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| --- | --- |
| Historical Record |  |
|  | **Version** | **Written/Revised by:** | **Effective Date:** | **Summary of Revisions** |
|  | 1 | P. Ackerman | 9.5.14 | Initial Version |
|  | 2 | P. Ackerman | 08.16.2016 | Reformatted for CMS upload; prev. designation GAS 004 |
|  | 3 | P. Ackerman | 03.29.17 | Instrument name change from Focus Integrated Cycler to DiaSorin Liaison MDX |
|  | 4 | P. Ackerman | 06.23.17 | Changed dilution of working suspension for GAS PCTL  |
|  | 5 | J. Laramie | 02.12.18 | Eliminated Positive Control (manufactured) from abbreviations and reagent list  |
|  | 6 | J. Laramie | 02.12.18 | Added Negative control preparation and testing  |
|  | 7 | J. Laramie | 05.16.18 | Eliminated the use of nasal matrix for control prep |