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| Agena MassArray SARS-CoV-2 Assay Quality Control |
| **Purpose** | This procedure provides instructions for Quality Control procedures required for the Agena MassArray SARS-CoV-2 Assay.  |
| **Policy Statements** | This procedure applies to all employees that work in molecular biology. |
| **Sample** | **New Lot/Shipment Quality control:*** SARS-CoV-2 positive amplification control
* Twist Bioscience Synthetic SARS-CoV-2 RNA Control 1 (#102019)
* SARS-CoV-2 LOW positive control
* Made in-house with ZeptoMetrix SARS-CoV-2 Culture Fluid (Heat Inactivated)
* Previously tested positive and negative patient samples
* SARS-CoV-2 negative control
* VTM

**Wipe test control (monthly):*** Culturette swab collection and placed into VTM – See MB 3.01 Wipe testing for Amplicon or Nucleic Acid Contamination

**Instrument Performance Verification after repairs:*** One known positive and one known negative patient sample OR Positive and Negative External Controls
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| Frequency | -With every patient run-Receipt of new shipments-Receipt of new lots-Drift in results (e.g., unexpected significantly increasing/decreasing positivity rates)-Potential contamination (negative control)-After drastic system maintenance-Wipe testing: Monthly  |
| **Special Safety Precautions** | Microbiologists/virologists are subject to occupational risks associated with specimen handling. Refer to the safety policies located in the safety section of the *Microbiology*and *Virology Policy Manual***:**1. *Biohazard Containment*
2. *Safety in the Microbiology/Virology Laboratory*
* *Biohazardous Spills*
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| **Materials** |

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| Reagents | Supplies | Equipment |
| -Sani-Cloth Bleach wipes or 1:10 diluted bleach solution-70% alcohol-5% Extran-DNA Away-Deionized water-Nuclease Free Water (NFW)-Clean Resin-HPLC-grade water-Negative control – VTM – store at 2 – 8 °C-Positive Amplification Control material: Twist Bioscience Synthetic SARS-CoV-2 RNA Control 1 (#102019) - store at – 70 °C-Low Positive control material: ZeptoMetrix SARS-CoV-2 Culture Fluid (Heat Inactivated) (0810587CFHI) – store at – 70 °C-EasyMag Extraction reagents: Buffers 1-4 and Silica (buffer 3 and silica stored in fridge, other reagents at room temp)-KingFisher Extraction reagents: MagMax Viral Pathogen Kit II (stored at room temp)-80% ethanolRoom 1:-Agena SARS-CoV-2 Panel (REF 12379F) – store at -25 °C to -10 °C:* SC2 PCR Primers
* SC2 Panel Extend Primers
* UNG (heat labile)
* MMLV Enzyme
* RNase Inhibitor

-PCR Reagent Set with dUTP - store at -25 °C to -10 °C:* MgCl2, 25mM
* 10x PCR Buffer
* dUTP/dNTP Mix
* PCR Enzyme

-iPLEX Pro Reagent Set - store at -25 °C to -10 °C:* 3 Point Calibrant
* iPLEX Termination Mix
* iPLEX Buffer Plus, GPR
* iPLEX Pro Enzyme
* SAP Buffer
* Shrimp Alkaline Phosphatase (SAP)

Room 2:* MS2 phage internal control

**NOTE:** The MassArray SARS-CoV-2 Panel Kit can be used up to ten times (freeze/thaw), as long as components are maintained on ice or a cold block during access and returned to storage conditions after use. **NOTE:** Keep all reagents in cold blocks once thawed and only take enzymes out of freezer immediately prior to use. Mark white board for each freeze-thaw cycle. | -Gloves (powder-free)-Filtered pipette tips, various sizes including 200 uL, extended tips-Sharps disposal container-Greiner strip plates -Microtubes -Clear adhesive plate seals-96 well semi-skirted, color microtiter plates-0.2 mL PCR strip tubes (8)-Pipette reservoirs ­-EasyMag consumables-Kingfisher consumables  | -Plate centrifuge-Vortex-Mini tube centrifuge -Tube centrifuge -Thermocycler-PCR work station with UV irradiation-Multichannel pipettes-Single channel Pipettes -Tube racks -Sealing paddle -Pipeting reservoirs-SpectroCHIP arrays-10 to -30° C freezers-Laminar flow Hood-Refrigerators 2 – 8° C-BSC BSL-2-70⁰ C freezer-Agena MassArray with Chip Prep Module (CPM) 96, including:* Typer software v5.01 or greater
* RT-Workstation v4.1 or greater
* Chip Prep Controller v2.2 or greater
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| **QC Prep Procedure****Procedure** | **Positive Amplification Control Prep**Upon receipt of control stock, label 8 vials with stock lot and expiration date. Aliquot 14 uL into vials for QC prep. To avoid freeze-thaw cycles of the stock solution. Prepare the positive control by diluting the Twist Bioscience Synthetic SARS-CoV-2 RNA Control 1 (1x106 copies/uL) to a working stock of 300 copies/uL. **NOTE:** Exercise caution when performing the serial dilutions. Risk of lab contamination is high due to the highly concentrated RNA control being used. **NOTE: do not vortex synthetic QC material or aliquots.**  1. Allow the control stock solution to thaw.
2. Label 5 1.5 mL cryovials with the dilution name AND copies/uL, and aliquot NFW into each.
	1. **Twist Dilution A (100,000 copies/uL)**
		1. 90 uL NFW
	2. **Twist Dilution B (10,000 copies/uL)**
		1. 450 uL NFW
	3. **Twist Dilution C (1,000 copies/uL)**
		1. 450 uL NFW
	4. **Twist Working Dilution (300 copies/uL)**
		1. 350 uL NFW

**NOTE:** Save dilutions in -70 °C freezer for future Pos QC prep.1. Make **Twist Dilution A** (1:10)
	1. Add 10 uL of SARS-CoV-2 RNA Control stock. Mix well by pipetting up and down, then quick spin.
2. Make **Twist Dilution B** (1:10)
	1. Add 50 uL **Dilution A**. Mix well by pipetting up and down, then quick spin.
3. Make **Twist Dilution C** (1:10)
	1. Add 50 uL **Dilution B**. Mix well by pipetting up and down, then quick spin.
4. Make **Working Pos QC**
	1. Add 150 uL of **Dilution D**. Mix well by pipetting up and down, then quick spin.
5. Label ~50 1.5 mL cryovials with **SC2 Pos QC**, the prep date, and expiration date (1 year from prep date)…Lot # = Prep Date
6. Aliquot 10 uL of the **SC2 Pos QC Stock** into each cryovial and cap tightly.
7. Freeze Samples
	1. Test new lot on 3 separate runs.
	2. Record results on the Positive Control Prep Worksheet
8. Store at -70 °C for 1 year.
9. Record prep and expiration dates in the Agena QC binder.

**NOTE:** If a QA failure occurs, document observation, record corrective action and notify technical specialist **Low Positive Control QC Prep (LoD x10): ZeptoMetrix SARS-CoV-2 Culture Fluid (Heat Inactivated)** 1. Allow stock solution to thaw.

**NOTE:** current stock solution: 1.51 x 106 TCID50/mL. SOP subject to change upon next shipment of material. 1. Label three 1.5 mL cryovials and aliquot VTM:
	1. **Zepto Dilution A**
		1. 990 uL VTM
	2. **Zepto Dilution B**
		1. 990 uL VTM
	3. **Zepto Dilution C**
		1. 1,980 uL VTM
2. Label one Large conical tube **Working Pos QC Stock**
3. Make **Zepto Dilution A** (1:100):
	1. Add 10 uL stock solution to the tube.
4. Make **Zepto Dilution B** (1:10):
	1. Add 10 uL of **Dilution A** to the tube.
5. Make **Zepto Dilution C**  (1:10):
	1. Add 20 uL of **Dilution B** to the tube.
6. Make **Working (final) SC2 Low Pos QC stock** (TCID50/mL: ~0.2):
	1. Add 1,193 uL of **Dilution C** to 7,807 uL of VTM in a large conical tube.
	2. Test in triplicate before aliquoting
	3. If 3 or more targets are detected from each extraction, proceed to the next step.
7. Label 70 1.5 mL cryovials with **SC2 Low Pos QC**, the prep date, and expiration date (1 year)…Lot#=Prep Date
8. Aliquot 250 uL of the **Working Low Pos QC Stock** into each cryovial and cap tightly.

**NOTE:** vortex 5 – 10 second between every 5 samples 1. Freeze Samples
	1. Test three different samples on three different runs.
	2. Record results on the Positive Control Prep Worksheet.
2. Store at -70 °C for 1 year.
3. Record prep and expiration dates in the Agena QC binder.

**NOTE:** vortex well (8 – 10 seconds) before pipetting the stock solution and before all subsequent dilution steps **NOTE:** If a QA failure occurs, document observation, record corrective action and notify technical specialist**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 VTM into 1.5 microcentrifuge tubes
5. Refrigerate aliquots (2 – 8 °C) in room 2
6. Record lot information in appropriate binder
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| **re** | **Expected Control Results**

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| **Control Type** | **Expected Results** | **SC2-N1 target** | **SC2-N2 target** | **SC2-N3 target** | **SC2-ORF1 target** | **SC2-ORF1ab** | **MS2 Phage Internal Control1** |
| **Historical Positive and Negative Patient Samples** | Match to historical qualitative results |
| **POSC: Positive Amplification Control\*** | SARS-CoV-2 Detected | Detected | Detected | Detected | Detected | Detected | Not applicable1 |
| **Low POSC: Low Positive Control\*\*** | SARS-CoV-2 Detected\* | Detected | Detected | Detected | Detected | Detected | Not applicable1 |
| **NEGC:** **VTM\*** | SARS-CoV-2 Not Detected | Not Detected | Not Detected | Not Detected | Not Detected | Not Detected | Detected |

1. Detection of the MS2 Phage Internal Control (RNA IC) is not required for a valid result when ≥2 SARS-CoV-2 targets are detected, POSC does not contain MS2. \*Run report will indicate “QC Status Passed” if results match according to the table\*\*Required qualitative result, all genes detected, not required for control to pass**NOTE:** record all new QC prep results on MB 15.0.F5 Agena MassArray SARS-CoV-2 Positive Amplification Control Prep Worksheet and/or MB 15.0.F6 Agena MassArray SARS-CoV-2 Low Positive Control Prep Worksheet**NOTE:** record new lot/ship QC on MB 15.0.F2 Agena SARS-CoV-2 New reagent QC worksheet**NOTE:** If there is a QC failure, document observation and correction action. Report QC problems that cannot be resolved to the tech specialist. For repeated failures contact Cepheid Technical Support. Do not report patient results until problem is resolved. |
| **References** | 1. Multiplex RT-PCR/MALDI-TOF test intended for the qualitative detection of nucleic acid from SARS-CoV-2, IFU-CUS-001 R02. In. San Diego, CA: Agena Bioscience; 2020. |
| **Historical Record** |  |  |  |  |
|  | **Version** | **Written/Revised by:** | **Effective Date:** | **Summary of Revisions** |
| 1 | Julie Laramie / Matthew Meyer | 10/19/2020 | Initial Version |
| 2 | Julie Laramie | 12/14/2020 | Added Twist Bioscience amplification control and removed Zeptometrix Pos control |
| 3 | Julie Laramie | 02/22/2021 | Changed amplification control prep from 50 copies/uL to 300 copies/uL |
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| **Archived by:** |  | **Archived Date:** |  |