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| GeneXpert Xpert Enterovirus (EV) Assay Quality Control |
| **Purpose** | This procedure provides instructions for Quality Control procedures required for the Xpert EV Assay.  |
| **Policy Statements** | This procedure applies to all employees that work in microbiology. |
| **Sample** | **New Lot/Shipment and Monthly Quality control:*** Enterovirus positive control
* Made in-house with ZeptoMetrix Coxsackievirus A9 or Echovirus Type 9 culture fluid
* Enterovirus negative control
* SeraCare synthetic CSF

**Wipe test control (monthly):*** Culturette swab collection and placed into tris-EDTA (TE) buffer

**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 | -Every 30 days-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 |
| * ZeptoMetrix Coxsackievirus A9 culture fluid (cat no. 0810017CF)
* ZeptoMetrix Echovirus Type 9 culture fluid (cat no. 0810077CF)
* Tris-EDTA (TE) buffer
* SeraCare synthetic CSF (cat no. 0175-0007)
* 10% bleach
* 70% ethanol
 | * Xpert Enterovirus cartridges
* Transfer pipettes
* Pipette tips
* Sample racks
* Cartridge transfer tray
* Absorbent biohazard squares
* Culturette swabs
* 200 uL extended pipette tips

Store kits at 2-28°C. Kits are stable until the expiration date printed on the outer box. Store controls at 2-25°C. Swabs are stable until the expiration date printed on the package. | * Biosafety Hood
* Cepheid GeneXpert Instrument and computer
* Printer
* Pipettes
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| **QC Prep Procedure****Procedure** | **Positive Control QC Prep: Coxsackievirus A9 (CXA9)** (TCID50: 1.38 x 107 U/mL)1. Allow stock solution to thaw.
2. Label three 1.5 mL cryovials and aliquot TE buffer:
	1. **CXA9 dil A**
		1. 990 uL TE buffer
	2. **CXA9 dil B**
		1. 900 uL TE buffer
	3. **CXA9 dil C**
		1. 900 uL TE buffer
3. Label a 15 mL conical:
	1. **CXA9 QC stock**
		1. 4,017 uL TE buffer
4. Make **CXA9 dil A** (1:100):
	1. Add 10 uL stock solution to the tube.
5. Make **CXA9 dil B** (1:10):
	1. Add 100 uL **CXA9 dil A** to the tube.
6. Make **CXA9 dil C**  (1:10):
	1. Add 100 uL **CXA9 dil B** to the tube.
7. Make **CXA9 QC stock** (TCID50: ~120.00 U/mL):
	1. Add 383 uL **CXA9 dil C** to the tube.
	2. Test in triplicate as you would a patient sample.
	3. If sample tests positive (~27-33 Ct) proceed to the next step.
8. Label 20 – 22 1.5 mL cryovials with **CXA9 pos QC**, the prep date, and expiration date (1 year).
9. Aliquot 200 uL **CXA9 pos QC** into each cryovial and cap tightly.

**NOTE:** vortex 5 – 10 second between every 5 samples 1. Store at -70 °C for 1 year.
2. Record prep and expiration dates in the Xpert EV QC binder.

**NOTE:** vortex well (8 – 10 seconds) before pipetting the stock solution and before all subsequent dilution steps **Positive Control QC Prep: Echovirus Type 9 (EC9)** (TCID50: 1.17 x 105 U/mL)1. Allow stock solution to thaw.
2. Label three 1.5 mL cryovials and aliquot TE buffer:
	1. **EC9 dil A**
		1. 990 uL TE buffer
	2. **EC9 dil B**
		1. 900 uL TE buffer
3. Label a 15 mL conical:
	1. **EC9 QC stock**
		1. 3,949 uL TE buffer
4. Make **EC9 dil A** (1:100):
	1. Add 10 uL stock solution to the tube.
5. Make **EC9 dil B** (1:10):
	1. Add 100 uL **EC9 dil A** to the tube.
6. Make **EC9 QC stock** (TCID50: ~120.00 U/mL):
	1. Add 451 uL **EC9 dil B** to the tube.
	2. Test in triplicate as you would a patient sample.
	3. If sample tests positive (~27-33 Ct) proceed to the next step.
7. Label 20 – 22 1.5 mL cryovials with **EC9 pos QC**, the prep date, and expiration date (1 year).
8. Aliquot 200 uL **EC9 pos QC** into each cryovial and cap tightly.

**NOTE:** vortex 5 – 10 second between every 5 samples 1. Store at -70 °C for 1 year.
2. Record prep and expiration dates in the Xpert EV QC binder.

**NOTE:** vortex well (8 – 10 seconds) before pipetting the stock solution and before all subsequent dilution steps **Negative Control QC Prep: Synthetic CSF** 1. Allow stock solution to thaw.
2. Label 50 1.5 mL cryovials with **EV Neg QC**, the prep date, and expiration date (listed by manufacturer).
3. Aliquot 200 uL synthetic CSF into each cryovial and cap tightly.
4. Record the prep and expiration dates in the Xpert EV QC binder.
5. Store at -70 °C until the expiration date.

**New Lot/Shipment and Monthly Quality control:**1. Obtain a positive and negative control from the feezer: let come to room temp.
2. Clean hood and supplies: 10% bleach followed by 70% ethanol.
3. Change gloves.
4. Obtain two test cartridges.
5. Label cartridges for the positive and negative controls.

**NOTE:** Set up the positive control first.1. Vortex the control vial for 10 seconds.
2. Run cartridges as patient samples. (see GeneXpert Xpert EV procedure)

**NOTE:** Under the “Test Type” field select “Positive Control 1” or “Negative Control 1”.1. Change gloves and clean hood in-between processing of controls AND before moving to the instrument.
2. Clean hood with 10% bleach followed by 70% ethanol.
3. Document QC in the GeneXpert Assay binder.

**NOTE:** Before reporting patient results, all controls must yield valid results.**NOTE:** Rotate modules for QC testing.**Wipe test:**1. Aliquot 500 uL TE buffer into a cryovial.
2. Dip a culturette swab in the TE buffer and swab the processing hood surface, counter around the GeneXpert instrument (including the keyboard, mouse, and scanner), and door handles on the instrument.
3. Break swab off into the cryovial using an absorbent biohazard pad (orange) as a barrier on the top of the tube.
4. Process and run as a patient sample.
5. Document testing in the GeneXpert EV QC binder.

**NOTE:** In the event of positive result notify the tech specialist, decontaminate, and re-test.  |
|  | **Reviewing results:**1. Ensure that the printer is turned on.
	1. Reports will print automatically.
2. Review reports for valid QC results or any reason to retest

**Reasons to retest:**1. An **INVALID** result. This may indicate:
	1. The sample was not properly processed.
	2. PCR was inhibited.
2. An **ERROR** result. This may indicate:
	1. The reaction tube was filled improperly.
	2. A reagent probe integrity problem was detected.
	3. The maximum pressure limit was exceeded.
	4. A valve positioning error was detected.
3. **NO RESULT**:
	1. This result indicated that insufficient data were collected. (e.g. test stopped while in progress or power failure occurred.)

**NOTE:** if retesting is required, obtain a new vial of QC material**Valid Results:*** Coxsackievirus A9 (CXA9) or Echovirus Type 9 (EC9) control: Enterovirus Positive
* Synthetic spinal fluid negative control: Enterovirus Negative

**Desirable Results:*** Wipe test control: Enterovirus Negative

**NOTE:** Record any failures, errors, and repeat testing in the “GeneXpert Maintenance and Problem Log” **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. Xpert EV Package Insert, 300-5052, Rev. H, October 2012. Sunnyvale, CA: Cepheid.
2. CAP Microbiology Checklist, College of American Pathologists, 325 Wakegan Road, Northfield, IL 60093-2750, 08/17/2016.
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| **Historical Record** |  |  |  |  |
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| 1 | Julie Laramie / Matthew Meyer | 5/25/2020 | Initial Version |
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