Overview of Cdiff PCR Procedure

Specimen Processing in BSC

1 Specimen Preparation

- A positive and negative external control must be included with each run.
  → Thaw frozen control suspensions and process as a specimen.



Clean sample preparation hood and bench tops before and after use with 10% bleach followed by 70% alcohol.
Don a new pair of gloves for handling specimens.

- Vortex each specimen at high speed for 15 s.
- In biosafety cabinet, use a sterile swab to sample stool.
- Place swab in sample buffer tube (blue cap).
- Use a 4x4 to lift and break swab shaft.
- Vortex swab in sample buffer for 1 min.

2 Lysis - DNA Extraction


Don a new pair of gloves for handling and pipetting from sample buffer tubes. Wipe down pipette from top to bottom before use with a 70% alcohol prep. Do not saturate the pipette.

- Add 40 uL of sample buffer from additional sample buffer tube (blue cap) to the lysis tube (yelllow cap).
- In a biosafety cabinet, transfer 10 uL of the cell suspension (blue cap) to a lysis tube (yellow cap).
- Vortex at high speed for 5 min.

Pre-Amplification Room

- Centrifuge briefly.
- Verify heat block temperature and place tubes at 95° C for 5-7 min to inactivate potential inhibitors.
- Place lysis tube on a cooling block for a minimum of 10 min.

3 Reconstitution of Molecular Reagents

While specimen lysis tubes are cooling, retrieve the mastermix from the refrigerator. Wear gloves while getting all reagents from the refrigerator. Use 1 master mix for up to 6 specimens and 2 controls. Keep master mix cold at all times!


Don a new pair of gloves before handling SmartCycler reaction tubes. Place enough tubes on the SmartCycler cooling block for each specimen and each of the controls.

Wipe down pipettes from top to bottom before use with a 70% alcohol prep. Do not saturate the pipette.

- Add 225 uL of diluent to lyophilized master mix and vortex. Use a 4x4 to remove the cap from the tube.
- Transfer 25 uL of master mix to the reservoir of each reaction tube on the cooling block and partially close the lids.
- Label the lids of the reaction tubes to correspond with the batch log.
- With <u>one tube open at a time</u>, transfer 3 uL of each lysate to a separate reaction tube. Pipette and mix 3-4 times and dispense against the inside wall of the SmartCycler tube. Fully close the lid to each tube.
- Add 225 uL of sample buffer to control DNA tube and vortex. Use a 4x4 to remove the cap from the tube.
- Transfer 3 uL control DNA to positive control tube in the same manner as the specimen lysates.
- Transfer 3 uL of sample buffer (blue cap) to negative control tube in the same manner as the specimen lysates.
- Centrifuge all tubes briefly.
- Transport tubes on cooling block to the amplification area.

Amplification/Detection Area

4 Real-Time PCR Analysis

- Program run: choose Cdiff assay, enter lot/exp., enter accession #'s
- Insert each reaction tube into the assigned I-CORE on the Smart Cycler
- Start run and obtain results in approximately 1 h

5 Verify Results

- Verify that both controls passed. Document <u>all</u> QC results and corrective action in LIS.
- Look for any unresolved specimens. The lysate for an unresolved specimen may be retested after a freez/thaw cycle. Refer to the test procedure for details.
- Print results
- Enter results in LIS using the computer in the amplification area. <u>Verify</u> all entries before filing results.



Don a new pair of gloves to unload reaction tubes.
Decontaminate and clean area with 10% bleach followed by 70% alcohol. Remove trash. Discard gloves.

Overview of MRSA ACP PCR Procedure

Specimen Processing in BSC

1 Specimen Preparation



Clean sample preparation hood and bench tops before and after use with 10% bleach followed by 70% alcohol. Don a new pair of gloves for handling specimens.

- Place swab in sample buffer tube (blue cap).
- Use a 4x4 to lift and break swab shaft.
- Vortex at high speed for 1 min.

2 Prepare Samples & Processing Controls


Don a new pair of gloves for handling and pipetting from sample buffer tubes. Wipe down pipette from top to bottom before use with a 70% alcohol prep. Do not saturate the pipette.

- Transfer 90 uL of the cell suspension from the sample buffer to lysis tube
- Add 225 uL of sample buffer to control DNA tube and vortex 5-10 s
- Use a 4x4 to open the POS control tube. Add 90 uL of POS control DNA to a lysis tube.


Don a new pair of gloves after handling the control DNA tube.

- Add 90 uL of NEG control (sample buffer) to a lysis tube

Pre-Amplification Room

3 Lysis - DNA Extraction

- Verify heat block temperature and place all lysis tubes at 37° C for 20 min
- Verify heat block temperature and transfer tubes to a second heating block at 99° C for 5 min
- Place tubes on cooling block for at least 10 min.

4 Preparation of Molecular Reagents

While specimen lysis tubes are cooling, retrieve the mastermix from the refrigerator. Wear gloves while getting all reagents from the refrigerator. Use 1 master mix for up to 6 specimens and 2 controls. Keep master mix cold at all times!


Don a new pair of gloves before handling SmartCycler reaction tubes. Place enough tubes on the SmartCycler cooling block for each specimen and 2 controls.

Wipe down pipettes from top to bottom before use with a 70% alcohol prep. Do not saturate the pipette.

- Add 225 uL of diluent to lyophilized master mix and vortex. Use a 4x4 to remove the cap from the tube.
- Transfer 25 uL of master mix to the reservoir of each reaction tube on the cooling block and partially close the lids.
- Label the lids of the reaction tubes to correspond with the batch log.
- With <u>one tube open at a time</u>, transfer 3 uL of each lysate to a separate reaction tube. Pipette and mix 3-4 times and dispense against the inside wall of the SmartCycler tube. Fully close the lid to each tube.
- Add 3 uL POS control lysate to the positive control reaction tube in the same manner as the specimen lysates.
- Add 3 uL NEG control lysate to the negative control reaction tube in the same manner as the specimen lysates.
- Centrifuge all SmartCycler reaction tubes briefly.
- Place tubes on cooling block until ready to load. Transport tubes in cooling block to the amplification area.

Amplification/Detection Area

5 Real-Time PCR Analysis

- Program run: choose MRSA ACP assay, enter lot/exp. for the Lysis Kit, enter accession #'s.
- Insert each reaction tube into the assigned I-CORE on the SmartCycler.
- Start run and obtain results in approximately 1 h.

6 Verify Results

- Verify that both controls passed. If there is a control failure, the assay must be started from the beginning (sample buffer tubes). Document <u>all</u> QC results and corrective action in LIS.
- Look for any unresolved specimens. The lysate for an unresolved specimen may be retested after a freez/thaw cycle. Refer to the test procedure for details.
- Print results
- Enter results in LIS using the computer in the amplification area. <u>Verify</u> all entries before filing results.



Don a new pair of gloves to unload reaction tubes.
Decontaminate and clean area with 10% bleach followed by 70% alcohol. Remove trash. Discard gloves.

- Perform culture verification on all positives. Refer to the test procedure for details.

Overview of Group B Strep PCR Procedure

Specimen Processing in BSC

1 Specimen Preparation

- Use patient swab specimen to inoculate LIM broth and incubate 12 - 24 h.
- A positive and negative external control must be included with each run.
  → Prepare a 0.5 McFarland suspension of each test strain in LIM broth.



Clean sample preparation hood and bench tops before and after use with 10% bleach followed by 70% alcohol.
Don a new pair of gloves for handling LIM broth tubes.

- Number each LIM broth tube to correspond with the Group B Strep PCR log.
- Vortex each LIM broth tube.
- Using a graduated pipette, transfer 0.2 mL of the LIM broth to a sample buffer (blue cap) and let stand for 5 min.
- Vortex specimen/sample buffer for 15 s.

2 Lysis – DNA Extraction


Don a new pair of gloves for handling and pipetting from sample buffer tubes. Wipe down pipette from top to bottom before use with a 70% alcohol prep. Do not saturate the pipette.

- Transfer 50 uL of sample to the lysis tube (yellow cap).
- Vortex at high speed for 5 min.

Pre-Amplification Room

- Centrifuge briefly.
- Verify heat block temperature and place lysis tubes at 95° C for 2 min to inactivate potential inhibitors.
- Place lysis tube on a cooling block for a minimum of 10 min.

3 Reconstituion of Master Mix


Don a new pair of gloves before handling SmartCycler reaction tubes. Place enough tubes on the SmartCycler cooling block for each specimen and 2 external controls. Also include 1 POS run control and 1 NEG run control.

- Use tool to open all of the SmartCycler tubes.

Wipe down pipettes from top to bottom before use with a 70% alcohol prep. Do not saturate the pipette.

- Add 25 uL diluent to each master mix and control tube on the cooling block and partially close the lids.
- Label the lids of the reaction tubes to correspond with the batch log.
- With <u>one tube open at a time</u>, transfer 1.5 uL of each lysate to a separate reaction tube. Pipette and mix 3-4 times and dispense against the inside wall of the SmartCycler tube. Fully close the lid to each tube.
- Centrifuge reaction tubes for 5-10 s
- Vortex reaction tubes in cooling block upside down for 5-10 s
- Leave tubes on cooling block until ready to load. Transport tubes in cooling block to the amplification area.

Amplification/Detection Area

4 Real-Time PCR Analysis

- Program run: choose StrepB assay, enter lot/exp., enter accession #'s
- Insert each reaction tube into the assigned I-CORE on the Smart Cycler
- Start run and obtain results in less than 45 minutes

5 Verify Results

- Verify that both controls passed. Document <u>all</u> QC results and corrective action in LIS.
- Look for any unresolved specimens. The lysate for an unresolved specimen may be retested after a freez/thaw cycle. Refer to the test procedure for details.
- Print results
- Enter results in LIS using the computer in the amplification area. <u>Verify</u> all entries before filing results.
- For positive samples, check to see if the patient is PEN allergic and subculture if indicated.



Don a new pair of gloves to unload reaction tubes.
Decontaminate and clean area with 10% bleach followed by 70% alcohol. Remove trash. Discard gloves.