Appendix H: Environmental Monitoring for Nucleic Acid Contamination

Purpose: To ensure that testing areas are not contaminated with extraneous nucleic acids.

Principle: Nucleic acid amplification is based on a simple principle: an enzymatic reaction that increases (amplifies) the amount of nucleic acids initially present in a sample. Contamination can occur when extraneous nucleic acids are inadvertently introduced into a PCR reaction or surrounding work areas. Routine environmental monitoring of work areas for nucleic acid contamination ensures that any potential contamination is detected and remediated as soon as possible, before testing results can be affected.

1. Safety Precautions

1.1. Wear designated personal protective equipment (PPE) for each area- Pre-Amplification, Extraction/ Template Addition, Amplification and Post- Amplification.

1.2. Refer to individual assay standard operating procedures (SOP) for additional safety information related to the testing method.

2. Materials

2.1. Supplies

- 2.1.1. Gloves
- 2.1.2. Disposable or dedicated lab coats as applicable
- 2.1.3. Sterile Nuclease-free Water
- 2.1.4. Sterile 2 ml Microcentrifuge Tubes
- 2.1.5. Sterile 15 ml Centrifuge Tube
- 2.1.6. Sterile swabs

3. Frequency

3.1. Each area will be swabbed monthly. Refer to **Appendix C: Molecular Work Areas by Room Number** for the sites need to be tested per PCR assay.

3.2 All trained staffs performing monitored PCR assays are responsible for their section's environmental monitoring for nucleic acid contamination data collection and records. **(Appendix I.1-4)**

4. Procedure

4.1. Obtain sterile swabs, four/ five sterile 2 ml microcentrifuge tubes, a 15 ml centrifuge tube and sterile nuclease-free water.

4.2. Prepare 5 collection vials of 500 µl sterile nuclease-free water in sterile 2 ml microcentrifuge tubes. Label four tubes with each testing area (pre-amplification, extraction/template addition, amplification and post-amplification). Label the fifth tube "blank." Only 4 collection vials are needed if there is no post-amplification step.

4.3. Fill a 15 ml centrifuge tube with 5 ml of sterile nuclease-free water.

4.4. Swab sites, following unidirectional workflow at all times. Refer to signs posted on the entrance of the rooms or the end of the work bench **(Appendix D- G of Best Practices for Nucleic Acid Amplification Assays)** and **Appendix B: Molecular Areas Map**.

4.4.1. Don designated PPE for the working area.
4.4.2. Open a sterile swab and moisten with sterile nuclease-free water from the 15 ml centrifuge tube.
4.4.3. Using one swab for each site, rub the swab over the appropriate surface(s). Refer to Section 5 for examples.
4.4.4. Swirl the swab in the appropriate collection vial for 5 seconds. Dispose the swab in a biohazard container.
4.4.5. Repeat steps 4.4.2. to 4.4.4. for each site in the area.
4.4.5.1. Change gloves between each swab.
4.4.5.2. Use a new swab for each site.
4.4.5.3. Swirl swabs from the same area (pre-amplification, extraction/template addition, amplification or post-amplification) in the same collection vial.

4.5. Vortex and quick-spin the collection vials after all samples have been collected. Store samples according to the monitored assays procedures before use.

4.6. Samples will be drawn from the final collection vials and will be processed in the same manner as extracted patient sample templates for all targets that were utilized and recorded on the **BSC and PCR Enclosure Decontamination Records (Appendix A)** during the monitoring period.

4.7. Mark sites tested and detail description of locations swabbed on the Section specific **Environmental Monitoring Quality Control Record (Appendix I.1-4)**. If no target was utilized at a site during the monitoring period, the site need not be tested.

5. Monitoring Sites

5.1. Refer to the **Environmental Monitoring Quality Control Record (Appendix I.1-4)** for a list of monitoring sites.

5.2. Swab any surfaces that could potentially become contaminated.

5.2.1. For PCR enclosures, dead air boxes, BSCs and bench work sites: pipets, vortex mixers switches, mini-centrifuges switches/ lid, box lid of pipet tips, orange biohazard waste rack etc. Refer to **Figure 1** as an example.

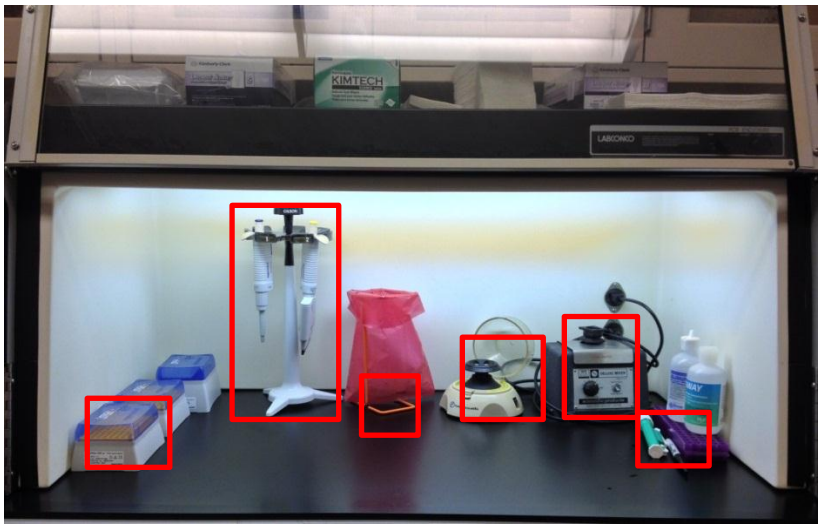

Figure 1

5.2.2. For instrumentation sites: points of contact with sample, user interfaces, handles, switches etc. Refer to **Figure 2, Figure 3** and **Figure 4** as examples.

Figure 2

Figure 3

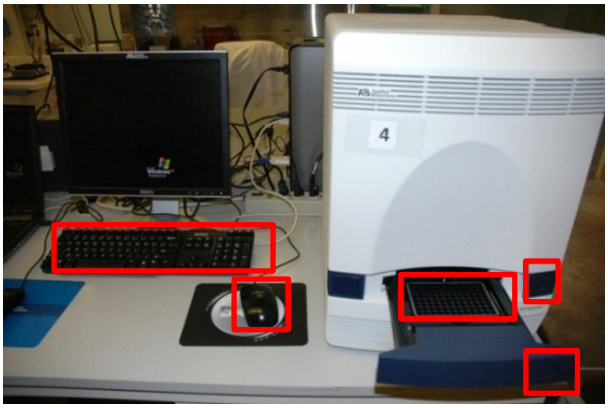

Figure 4

6. Decontamination

6.1. In the event that a sample comes back positive for contamination, decontaminate the affected site(s) following the procedure in Section 2 of **Best Practices for Amplification Assays**.

6.1.1. Wipe all surfaces with a freshly prepared 10% hypochlorite solution followed by isopropyl alcohol to prevent corrosion of metal surfaces. Other validated chemical alternatives (DNA Away, Eliminase, etc.) may also be used following manufacturer guidelines for use or as described in section standard operating procedures (SOPs).

6.1.2. Follow manufacturer recommendations for decontamination of instruments.

6.2. Re-swab the affected site(s) and retest for the presence of contamination. Start a RFRA to determine the cause of the contamination and preventive action(s).

7. Documentation

7.1. Attach a copy of PCR worksheet and test results to the completed **Environmental Monitoring Quality Control Record (Appendix I.1-4)**.

7.2. Records are maintained by the Quality Assurance section.

7.3. Records are retained on site for a minimum of 2 years.

8. Related documents

8.1. Best Practices for Nucleic Acid Amplification Assays

8.2. Appendix A: BSC and PCR Enclosure Decontamination Record

8.3. Appendix B: Molecular Areas Map

8.4. Appendix C: Molecular Work Areas by Room Number

8.5. Appendix D- G: Pre-Amplification, Extraction/ Template Addition, Amplification and Post Amplification Door Signs

8.6. Appendix I.1-4: Environmental Monitoring Quality Control Record

Signature Approvals:

_____ /_____
Originator Date

_____ / _____
QA Officer Date

_____ / _____
Laboratory Director Date