Best Practices for Nucleic Acid Amplification Assays

Purpose: This document provides guidelines for best practices involved with performing nucleic acid amplification assays. This document addresses the general concepts of unidirectional workflow, use of personal protective equipment (PPE), dedicated equipment and supplies, decontamination, and quality assurance activities designed to minimize the presence or introduction of contaminants at critical points in the amplification process.

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 an amplification reaction or surrounding work areas. Extraneous nucleic acids may originate from clinical material or isolates as well as from amplicons produced in previous amplification reactions. "Open" amplification assays, where the amplified product is further manipulated, are more prone to contamination than "closed" amplification assays, where the amplified product remains sealed throughout the procedure. Contamination impacts assay results by potentially producing false positives and once present, is difficult to remediate. Standard work practices and strict adherence to a unidirectional workflow are required in the laboratory to minimize the opportunities for contamination of materials and experiments.

1. Unidirectional Workflow

1.1. Nucleic acid sample preparation, reaction mixture assemblage, the amplification process, and any subsequent reaction product analysis should be performed in separate areas moving from cleanest to dirtiest.

1.2. Practice good housekeeping at all times.

1.2.1. Only store necessary equipment within biological safety cabinets (BSCs) and dead-air boxes (or PCR enclosures). Do not store excess supplies (e.g. tips, paper towels, plates, etc.). See **Figure 1** for example.

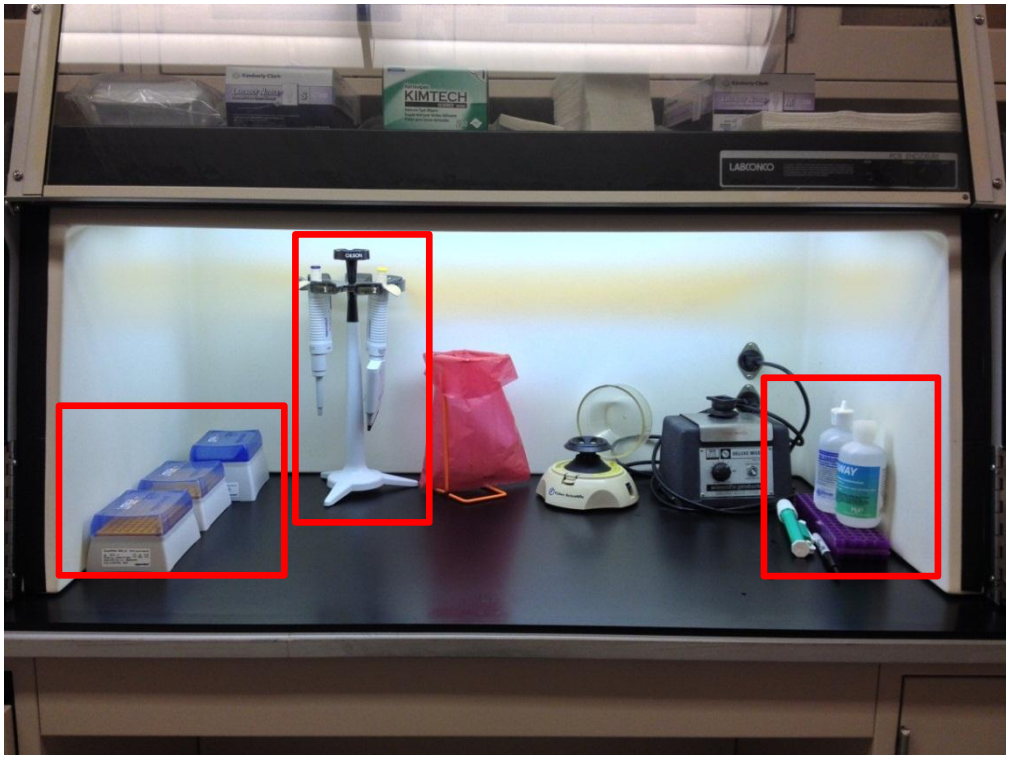

Figure 1

1.2.2. All reagents, reaction tubes etc. should be clearly labelled.

1.2.3. Record batch numbers of all reagent batches used in individual assays.

1.3. Ensure that all equipment (including pens, PPE, pipettes and other consumables) are dedicated for use only in that particular room or laboratory area.

1.4. Color-coded signs are located on the entranceways to molecular testing rooms/areas so that the status of the room/area can be easily identified. Signs are located at the end of the bench as applicable. (**Appendices D-G**)

1.4.1. Green – "Pre-amplification/Assay Set-up"
1.4.2. Yellow –"Extraction/Template Addition Area"
1.4.3. Orange – "Amplification Area"
1.4.4. Red – "Post-amplification Area"

1.5. If it is necessary to enter a cleaner room after working in a dirty room, ensure that proper PPE is donned/ removed and hands are washed prior to entering the cleaner room/area.

1.6. If any reagents or consumables must be relocated from a dirty area to a cleaner area, ensure that they are decontaminated as outlined in Section 2.

1.7. Discrete areas are established as described below. Refer to **Appendix C: Molecular Work Areas by Room Number** for a table listing the designated areas at ODH and **Appendix B: Molecular Areas Map** for a color coded layout.

1.7.1. Pre-amplification/Assay Set-up – Green area

1.7.1.1. It is very important to keep this room free of any biological material (i.e. DNA/RNA extracts, samples, cloned materials and amplification products).

1.7.1.2. This area is used for the preparation and aliquoting of reagent stocks and preparation of reaction mixes prior to the addition of clinical nucleic acid.

1.7.1.2.1. Reagents should be aliquotted to avoid excessive freeze-thawing and to protect stock reagents from contamination.

1.7.1.2.2. Pulse-centrifuge tubes before opening the reagents.

1.7.1.2.3. Uncap and close tubes carefully to prevent aerosols.

1.7.1.2.4. Maintain reagents on ice or cold racks as necessary.

1.7.1.2.5. Reduce reagent exposure to UV light as recommended by the manufacturer.

1.7.1.3. Dead air boxes are used to prevent potential contamination within the working area.

1.7.1.4. Working supplies are stored in the room and remain closed and sealed until needed for use.

1.7.1.5. Single use disposable gowns and gloves are required PPE during master mix preparations.

1.7.2. Extraction/Template Addition – Yellow area

1.7.2.1. While this area is not as clean as reagent preparation rooms, this area is still designated as pre-amplification. Amplicons and stocks of cloned material are not to be brought into this area.

1.7.2.2. This area is used for sample processing, extraction of nucleic acid from clinical samples, and addition of sample template to master mix.

1.7.2.3. The samples should never enter rooms where amplified products and cloned DNA are present.

1.7.2.4. Class II Type B2 Biological Safety Cabinets (BSCs) or PCR enclosures are used for containment purposes.

1.7.2.5. Dedicated disposable gowns and gloves are required PPE during extraction or template addition.

1.7.2.6. Change gloves before and after template addition.

1.7.2.7. Date and dispose of gowns weekly or maximum biweekly based on usage for Room 143 Extraction Area.

1.7.2.8. For Newborn Screening and General Microbiology Lab Extraction Areas, dedicated cloth lab coats should be used. Change lab coat at least monthly.

1.7.2.9. Single use gown from Pre-amplification should be disposed after template addition.

1.7.2.10. Take off PPE and wash hands before exiting the areas.

1.7.3. Amplification Area – Orange area

1.7.3.1. This area is used for the amplification of nucleic acids. Location of thermal cyclers and real-time PCR instrumentation.

1.7.3.2. This is a “contaminated” area and therefore no reagents, equipment, laboratory coats etc. from this room should be used in any of the other cleaner areas.

1.7.3.3. Gloves are required PPE.

1.7.3.4. Take off gloves and wash hands before exiting the areas.

1.7.4. Post-amplification Area – Red area

1.7.4.1. This area is designated for the use or further manipulation of amplified products.

1.7.4.2. This is a "contaminated" area and therefore no reagents, equipment, laboratory coats etc. from this room should be used in any of the other cleaner areas.

1.7.4.3. Dedicated disposable gowns and gloves are required PPE during post-amplification.

1.7.4.4. Take off PPE and wash hands before exiting the areas.

2. Decontamination

2.1. Decontaminate all benches and containment area (BSCs, PCR Enclosure, etc.) surfaces both prior to and following use.

2.1.1. 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).

2.1.2. Follow manufacturer recommendations for decontamination of instruments.

2.1.3. Document completion on the **BSC and PCR Enclosure Decontamination Record (Appendix A)**.

3. Quality Control

3.1. Refer to individual assay SOPs for specific instructions on including in-assay controls and section-specific quality assurance activities not listed here.

3.2. New batches or kit lots of reagents (primers, probes, PCR mix etc.) are assessed for performance against previous kit lots or quality control material with a pre-established result. Records are maintained as per the individual assay SOP.

3.3. Decontamination records are maintained by the Quality Assurance section. Records are stored on-site for a minimum of two years.

3.4. ODH participates in quality assurance programs, such as proficiency testing, as required by CLIA. Any deviation from expected results is investigated to determine the cause of the deficiency, as per the ODH **Proficiency Testing Policy**.

3.5. Environmental monitoring is conducted on a routine basis to ensure work areas have not become contaminated. **Environmental Monitoring Quality Control Records (Appendix I.1-4)** are maintained by the Quality Assurance section. Refer to **Appendix H** for details on frequency and scope.

4. Appendices

4.1. Appendix A: BSC and PCR Enclosure Decontamination Record
4.2. Appendix B: Molecular Areas Map
4.3. Appendix C: Molecular Work Areas by Room Number
4.4. Appendix D: Pre-amplification/Assay Setup Door Sign
4.5. Appendix E: Extraction/Template Addition Door Sign
4.6. Appendix F: Amplification Door Sign
4.7. Appendix G: Post-Amplification Door Sign
4.8. Appendix H: Environmental Monitoring for Nucleic Acid Contamination
4.9. Appendix I.1-4: Environmental Monitoring Quality Control Record- BT, Gen Micro, Newborn Screening and Special Micro

5. References

5.1. Health Protection Agency. (2012). Good Laboratory Practice when Performing Molecular Amplification Assays. UK Standards for Microbiology Investigations. Q 4 Issue 4.2. http://www.hpa.org.uk/SMI/pdf.
5.2. Raquel V. Viana and Carole L. Wallis (2011). Good Clinical Laboratory Practice (GCLP) for Molecular Based Tests Used in Diagnostic Laboratories, Wide Spectra of Quality Control, Dr. Isin Akyar (Ed.), ISBN: 978-953-307-683-6, InTech, Available from: http://www.intechopen.com/books/wide-spectra-of-quality-control/goodclinical-laboratory-practice-gclp-for-molecular-based-tests-used-in-diagnostic-laboratories
5.3. US Department of Health and Human Services. Biosafety in Microbiological and Biomedical Laboratories, 5th Edition. HHS Publication No. (CDC) 21-1112. Revised December 2009.
5.4. Ohio Department of Health Laboratory. Proficiency Testing Policy. Revision 3-24-2014.

Signature Approvals:

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