1. **TITLE**

Preparation of Dried Tube Specimen (DTS) Quality Controls (QC), Training Panels (TP) and Proficiency Testing (PT) panels

1. **PURPOSE**

The purpose of this procedure is to provide guidance on how to prepare dried tube specimens (DTS) quality control (QC), training panels (TP), and proficiency testing (PT) panels to ensure the quality of HIV Rapid Tests.

1. **SCOPE**

This procedure applies to quality assurance activities for the HIV Rapid Test.

1. **RESPONSIBILITIES**

National Reference Laboratory Staff who have been trained on HIV Rapid Testing

1. **EQUIPMENT**
   1. Biosafety cabinet (optional)
   2. Vacuum pump unit for PBS-Tween-20 buffer filtration (optional)
   3. Serological pipet aide
   4. Multi-dispensing pipettes capable of 20µL and up to 2mL (Rainin E4 Electronic Pipette, LTS E4-300XLS+ - catalog # 17014488; Thermo Scientific™ Finnpipette Stepper Pipette, Fisher #21-377-147, Globe Scientific Diamond RV-Pette PRO™ Repeat Volume Pipette - Fisher 22-010-1162, or equivalent)
   5. Centrifuge for plasma separation (accommodates 50mL tubes)
   6. -20°C Freezer for long-term storage of samples
2. **SUPPLIES**
   1. 2.0mL conical bottom Sarstedt cryo-tubes (catalog # 72-694-007; www.sarstedt.com)
   2. 1L deionized water
   3. PBS with 0.05% Tween 20, pH 7.4 powder (cat P3563, www.sigmaaldrich.com)
   4. Green food coloring dye
   5. Pipette or syringe tips for multi-dispensing pipettes (example, 5mL Fisher #21-377-154, 0.5mL #21-377-148, 50mL #21-377-160)
   6. Disposable transfer pipet (Samco #282 transfer pipet, Fisher, catalog # 13-711-43) or 200µL precision pipette with tips for DTS rehydration
   7. Freezer boxes (catalog #05-541-38, Fisher)
   8. Tube racks to accommodate 2.0mL tubes for DTS aliquoting (Fisher, Catalog #05-541-38)
   9. Freezer boxes to accommodate 2.0mL tubes (Fisher, catalog # 05-541-38)
   10. Cryo labels for tubes (Diversified Biotech, catalog #LCRY-2380) and freezer boxes (Diversified Biotech, catalog #LCRY-1258)
   11. 50mL conical tubes (Fisher, catalog # 14-432-22)
   12. 15 mL conical tubes (Fisher, catalog # 14-959-49D)
   13. 50mL and 15mL conical tube stands
   14. Serological pipet, 10mL (Fisher, catalog # 170374N)
   15. 250mL storage bottles (Fisher, catalog # 02-893B)
   16. Zip Lock bags, small, for distributing DTS QC and PT panels
   17. Zip Lock bags, large, for storing plasma units in the freezer
   18. 1L Disposable 0.2 µM filter flask for filtering DTS buffer (Fisher #09-761-104)
   19. Absorbent surface protectors or bench pad/linen saver
   20. Paper towels
   21. Personal protective equipment (PPE) : lab gloves, lab coats, face shields/safety goggles, hand soap. In line with COVID-19 mitigation requirement, disposable face mask is also needed.
   22. Benchtop biohazard bag and bag holder
   23. Biohazard autoclave bags
   24. Bleach-based surface disinfectant (Decon Labs, IRADECON lab surface disinfectant)
   25. Regular fine tip permanent markers
   26. Ink pens
   27. Timers
   28. HIV rapid tests kits used in national algorithm
3. **SPECIAL SAFETY PRECAUTIONS**
   1. Wear PPE while handling plasma, DTS, and other biological materials.
   2. Handle DTS as potentially infectious material.
   3. Ensure that equipment, supplies and, linen contaminated with infectious material is either disinfected or discarded properly as biohazardous waste.
   4. Follow universal safety precautions for handling biological materials and manufacturer’s safety precautions for handling chemical materials.
   5. Do not interchange vial caps; this will lead to cross contamination of specimens.
   6. If available, leave the DTS in the biosafety cabinet (BSC) for overnight drying of the specimens.
   7. If BSC is not available, ensure that the DTS QC/PT panels are left in a secure location on the lab bench and covered with Kimwipes or paper towels to avoid contamination
4. **PROCEDURE** 
   1. **Preparation of cryo labels for tubes and storage boxes**
      1. Decide how many sets of panels need to be prepared.
         1. Number of QC panels is dependent on the QC testing frequency and the number of HIV Rapid Testing sites.
         2. Number of training panels (TP) is dependent on the number of testers to be trained.
         3. Number of PT panels is dependent on number of sites and/or testers enrolled and existing PT program guidelines for PT IDs.
      2. Print labels for each QC, TP, and PT sample with the following information:
         1. Sample ID (QC HIV Negative, QC HIV Positive, TP Sample 1 to 10, PT Sample 1 to 5) – see Tables 1 and 2
         2. Date of Preparation (month/year)
      3. Ensure there is a link of the original specimen bank ID and the new ID.
      4. Print labels for DTS rehydration buffer with the following information:
         1. DTS Rehydration Buffer
         2. Expiration date of 1 year from the date of preparation
         3. Volume: 1.5 mL
   2. **Preparation of DTS Rehydration Buffer (PBS-Tween-20)** 
      1. Powder of phosphate buffered saline (PBS) with Tween-20 can be commercially purchased from Sigma (catalog # 3563).
      2. Dissolve one foil pouch of PBS with Tween-20, pH 7.4 in 1L of deionized water. It will yield 0.01 M PBS; NaCl 0.138 M; KCl 0.0027 M; 0.05% Tween 20; pH 7.4.
      3. Optional: if vacuum pump unit is available, filter solution through a 0.2µM filter flask.
      4. Prepare 50mL aliquots in pre-labeled 50mL conical tubes as master stock for NRL lab.
      5. Aliquot 1.5 mL to each labeled 2.0mL Sarstedt conical bottom tubes for testers to rehydrate DTS QC, TP or PT panel prior to HIV RT testing.
   3. **Preparation and HIV Serostatus Verification of Specimen Bank Units (Prior to Panel Preps)**
      1. Obtain rejected whole blood or plasma units from local blood bank of different HIV status including both HIV positive and negative samples. Initially acquire >50 units to build specimen inventory. Continue to acquire more units to build a large specimen inventory.
      2. When using whole blood units, transfer whole blood from bags to clean 50 mL conical tubes, and centrifuge at 1000-1300 RCF for 10 minutes to separate plasma. Using a serological pipet, carefully transfer plasma from the conical tubes to clean and labeled 250mL storage bottles.
      3. When using plasma unit, transfer plasma from bags directly to a clean and labeled 250mL storage bottle.
         1. If plasma is not freshly prepared, likely it needs to be clarified with centrifugation. Transfer to clean 50mL conical tubes, centrifuge at 1000-1300 RCF for 10 minutes. Carefully remove any large particles floating on top with pipette and make sure the samples are clear of any clots or fibroids.
         2. Transfer supernatant to 250mL storage bottle.
      4. Store plasma at 2-8°C up to 7 days until further testing has been conducted. **If further testing cannot be conducted within 7 days, transfer the plasma to -20°C.** Minimize freeze-thaw cycles (up to 5 freeze-thaw cycles is acceptable).
      5. Regardless of HIV status provided by the blood bank, the specimen reactivity must be verified by the laboratory that is responsible for preparing the panel by using either of the following methods per country guideline.
         1. National Rapid Test Algorithm: Test plasma specimens based on the country’s HIV rapid test diagnostic algorithm. Make sure the rapid test algorithm has the capability of identifying and distinguishing HIV-2 positive samples if appropriate.
         2. ELISA and Confirmatory Test Algorithm (if applicable): Test plasma specimens using ELISA and confirm positive results with either Western Blot or Geenius confirmatory tests.
      6. Ensure that you have at least 50 confirmed HIV-1 positive plasma specimens prior to preparation.
      7. Record unit information and HIV serostatus verification results on **Blood Bank Sample Inventory**.
   4. **Preparation of DTS QC, TP and PT Panel**
      1. Identify samples that are clearly HIV negative and HIV positive from the specimen bank and select as candidates for QC, TP, CP and PT.
         1. Negative samples should have the presence of a control line and clear absence of test line
         2. Positive samples should have the presence of a control line and a strong and clear test line.
      2. If your standard operating procedure includes heat inactivation, inactivate the confirmed HIV positive units:
         1. Set up water bath at 56°C
         2. Incubate samples in 250mL bottles or 50mL tubes for 30 minutes
         3. Ensure sample is completely immersed in the water bath
      3. **QC panel composition:** There should be one HIV negative, one HIV positive sample.
      4. **Training Panel Composition**: There should be no less than 10 samples for training panel (TP) and competency panel (CP). Both TP and CP panel should include samples representing different status of HIV infection: HIV negative and HIV positive as indicated in Table 1 below.
         1. TP-1 through TP-5 will be used as “practice” training samples.
         2. CP-1 through CP-5 will be used as “competency” samples.

**Table 1. An Example of Composition of Training Panels for HIV Rapid Testing**

|  |  |
| --- | --- |
| **Specimen IDs** | **HIV Status** |
| TP-1 | HIV Positive |
| TP-2 | HIV Positive |
| TP-3 | HIV Negative |
| TP-4 | HIV Positive |
| TP-5 | HIV Negative |
| CP-1 | HIV Positive |
| CP-2 | HIV Negative |
| CP-3 | HIV Negative |
| CP-4 | HIV Positive |
| CP-5 | HIV Positive |

* + - 1. Although not optimal, you may need to alter the TP composition based upon availability of HIV Positive plasma units(must include at least two Positive for practice and competency).
    1. **PT Panel Composition**: There should be no less than 5 samples for PT panel. Each panel should include samples representing different status of HIV infection: HIV negative and HIV positive as indicated in Table 2 below.

**Table 2. An Example of Composition of QC and PT Panels for HIV Rapid Testing**

|  |  |
| --- | --- |
| **Specimen IDs** | **HIV Status** |
| DTS QC Positive | HIV Positive |
| DTS QC Negative | HIV Negative |
| PT-1 | HIV Positive |
| PT-2 | HIV Positive |
| PT-3 | HIV Negative |
| PT-4 | HIV Positive |
| PT-5 | HIV Negative |

* + 1. **Mini DTS Panel:** Before preparing the bulk panels,verify the expected HIV RT result of each selected plasma unit by preparing a “mini DTS Panel”:
       1. Prepare at least three tubes of DTS for each plasma unit, following the same procedure as bulk panel preparation detailed in steps 8.5.8.
       2. Rehydrate each DTS tube with 200 µL DTS buffer (see section 8.6). For the “mini DTS Panel”, overnight rehydration period is required. **For bulk production, quality checks and routine DTS QC/PT testing at sites, overnight rehydration should be performed.**
       3. Using the rapid tests from the national algorithm, test each DTS sample and document the DTS expected result of each plasma unit to guide the large-scale panel preparation.
       4. Record results on the **RT Data Capture Form** while working in the lab. Use the **Blood Bank Sample Inventory** as an electronic notebook to record final results.
    2. Once reactivity is confirmed, identify the final candidates for QC, TP, CP and PT samples based on step 8.4.1 criteria and proceed with the bulk panel preparation.
    3. Bulk Panels Preparation: Prepare a 1:1001 dilution of green food coloring dye to specimen. For example, add 20µL of dye to 20mL of QC specimen. Vortex the specimen to mix the dye.
       1. Prepare DTS by pipetting 20µL of colored plasma specimen to the bottom of a Sarstedt 2.0mL conical bottom cryo-tube.
       2. Aliquot each specimen into properly labeled tubes.
       3. **Aliquot only one specimen type at a time to avoid cross-contamination.**
       4. **Carefully ensure the 20µL drop is on the bottom of the tube, especially if using a repeat pipette.**
       5. Leave tubes uncapped in a Biosafety Cabinet (if available) or in a clean, secure lab space (cover tubes with clean paper towel) overnight at room temperature to facilitate drying. To avoid cross-contamination, keep tubes made from plasma units with different reactivity separate in the BSC or clean lab space.
       6. The following day ensure the specimens have dried completely; a visible colored pellet should be present at the bottom of each tube. Once completely dry, cap each tube.
       7. Store capped DTS at room temperature (18-30°C) for one month or 2-8°C for up to 12 weeks until ready for shipment to the participating laboratories. For long term storage, store the DTS at -20oC or colder for up to 2 years and 6 months.
       8. **For PT panels**, create a panel of 5 samples from the characterized specimens with a composition suggested in section 8.5.5. Carefully blind the panel before distribution. Ensure there is a link of the original ID and the new ID.
  1. **Establish DTS Panel Reference Data**
     1. Randomly select at least 3 sets of QC, TP and PT panels from the beginning, middle and end of production.
     2. At least three different laboratorians should perform testing independently on the panels.
        1. Rehydrate each DTS tube with 200µL DTS buffer and verify the expected RT result. For this quality check procedure overnight rehydration should be used.
        2. Test all DTS samples using the national HIV RT algorithm as well and record the results as the Confirmed HIV Status of the **Reference Results Form**. Enter the final interpretation, which is the best of 3 results.
        3. Among testers, DTS samples results should be clear and unambiguous.
  2. **DTS Rehydration (see DTS Rehydration Job Aid)**
     1. Tap the tube gently to ensure that the colored pellet falls to the bottom of the tube before uncapping.
     2. Rehydrate DTS sample overnight at room temperature
     3. Use either of the following methods to rehydrate:
        1. Use a precision pipette to add 200uL of DTS buffer to each tube.
        2. Use Samco transfer pipet (Fisher, catalog # 13-711-43) to carefully add 7 drops of DTS buffer to each tube.
        3. **Use of any other transfer pipet must be validated by the national reference lab to ensure the specific number of drops is equal to the required volume of 200µL.**
     4. **Once rehydrated, DTS samples must be tested the next day within 24 hours.**
     5. Mix the specimen by gently tapping the tube before testing.
  3. **Arrange the Panels into Sets**
     1. Arrange the panels into sets in labelled cryo boxes and store at -20°C or colder until use:
        1. For sample testing quality assurance, store QC, TP, CP and PT sets in labeled cryo boxes until ready to distribute to sites and/or testers in small Ziploc bags.
        2. Store DTS rehydration buffer tubes separately at 2-8oC just before distribution.
  4. **Storage of DTS QC, TP and PT panels**
     1. DTS QC and PT panels can be stored at:
        1. For short-term storage, room temperature (18-30°C) for one month or 2-8°C for up to 12 weeks prior to shipping to testing sites
        2. For long-term storage, -20°C or colder for up to 2 years and 6 months.
     2. The panel should be rehydrated and tested immediately upon receipt at the testing site.
  5. **Usage of QC, TP, CP and PT Panels**
     1. **Training:** Use the QC and TP during HIV RT training to ensure the competency and readiness of the trainees to provide this testing service
     2. **Quality Assurance during Sample Testing:**
        1. All testing sites that provide HIV RT testing should use the QC panel to verify performance of HIV RT kits:
        2. Recommended weekly or at least once a month per site - Only one tester per entry point/site, but try to rotate the testers each month
        3. When storage temperature exceeds the manufacturer’s recommended storage conditions
        4. Upon receipt of each new lot or shipment of HIV RT kit (NRL only)
     3. **Proficiency Testing:** PT panel should be provided to all sites and/or testers that provide HIV rapid testing at least 2 times per year. Only competent testers should perform HIV RT.
  6. **Packaging and Transport of DTS QC, TP and PT panels** 
     1. Prepare DTS QC and TP or PT panels for shipment to include one tube of each QC and/or TP/CP/PT panel member and one to two DTS Buffer tubes.
        1. **Note:** Number of DTS buffer tubes provided in DTS PT panel package is dependent on number of samples in the panel and if QC is also included. One tube of 1.5 mL buffer is enough to rehydrate 5 samples. If more than 5 then recommend sending at least two DTS buffer tubes with the panel.
     2. Provide two disposable transfer pipets specified for DTS rehydration along with **DTS Rehydration Job Aid**, and results recording form.
     3. Pack DTS QC and TP or PT panel, DTS buffer, and transfer pipet, job aid and result form in labeled Ziploc bags (or boxes for training).
     4. The collated DTS panels can be stored at room temperature (18-30°C) for one month or 2-8°C for up to 12 weeks prior to shipping to testing sites.
     5. A sample of the DTS QC and TP or PT panel should be tested prior to shipping as an additional quality check to verify sample integrity.
     6. The DTS panels can be transported at room temperature (18-25℃) to the testing sites.
     7. Once received at the testing sites, the DTS panels must be rehydrated and tested immediately.
  7. **Reporting and Result Analysis**
     1. Collect report from all participating sites and/or testers.
     2. Enter data in the appropriate data management tool.
     3. Analyze the data using the data management tool.
     4. Send the final report to all the participating sites and/or testers.
     5. Follow up with a supervisor for those sites and/or testers who do not receive a 100% passing grade**.**

1. **REFERENCES** 
   1. Dried tube specimens: a simple and cost-effective method for preparation of HIV proficiency testing panels and quality control materials for use in resource-limited settings. Parekh BS, Anyanwu J, Patel H, Downer M, Kalou M, Gichimu C, Keipkerich BS, Clement N, Omondi M, Mayer O, Ou CY, Nkengasong JN. Journal of Virological Methods, v163, issue 2, Feb 2010, pp 295-300.
2. **RELATED DOCUMENTS** 
   1. **HIV Rapid Test National Algorithm and SOPs**
3. **APPENDICES**
   1. **DTS Rehydration Job Aid**
   2. **Blood Bank Sample Inventory**
   3. **RT Data Capture Form**
   4. **Panel Prep Reference Results Form**