



UGANDA

Supranational[®]
Reference Laboratory

Timely Accurate Diagnosis for a Better Future

Training on Proficiency Testing Scheme GeneXpert DTS

Module 10: Panel Aliquoting

Venue

Facilitator's name

Date

Introduction

- Based on the pretest results obtained, suitable stock isolates are selected to be composed in panel by aliquoting according to the desired dilution factor and dyeing the panels.

Objectives

By the end of this module participants should be able to:

- To understand the principles of panel aliquoting
- To understand the quality control procedures taken during the process of panel aliquoting

Module Outline

- Select 5 of inactivated stocks for panel preparation
- Determine the order of aliquoting
- Calculate amount of saline for diluent
- Label racks and dilution tubes
- Clean and set up BSC

Next Steps

- Out of the 8 inactivated stock select 5 for panel preparation
- determine order of aliquoting
- Calculate amount of saline for diluent
- Label racks and dilution tubes
- Clean and set up BSC
- Vortex with beads, settle 15 minutes
- Dilute with saline
- Aliquot
- Dry 7-10 days
- Cap and store at consistent 2-25 °C in the dark

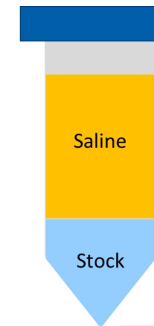


Determining Dilutions to Prepare

How much do you need to make?

How low are your Ct results from pre-test?

- **1:10 Dilution of stock**
 - Use if mean Ct of Probe A on pretest is below 17
- **Entire stock plus enough saline to make needed aliquots**
 - Use if Ct on pretest is higher
- **Neat stock**
 - Use if higher Ct and need less than 110 panels
 - No dilution prep, add food color to stock



1:10 Dilution of stock

- Examples

A. Aliquots Per Stock	B. Total Volume 1:10 Diluted Stock Required [(A x 0.1 ml) + 5 ml]	C. Volume Stock Solution Vortexed with Beads [(B/10) + 0.5 ml]	D. Volume Vortexed Stock Added to Saline for 1:10 Dilution (B/10)	E. Volume of saline for 1:10 Dilution (B - D)	Volume Blue Food Grade Dye for 1:1000 Dilution (B/1000)
250	25 ml + 5 ml = 30 ml	3.0 + 0.5 = 3.5 ml	3 ml	27 ml	30 µl
400	40 ml + 5 ml = 45 ml	4.5 + 0.5 = 5.0 ml	4.5 ml	40.5 ml	45 µl

Entire stock plus enough saline to make needed aliquots

- Examples

A. Aliquots Per Stock	B. Total Volume Diluted Stock Required [(A x 0.1 ml) + 5 ml]	C. Volume Stock Solution	D. Volume of Stock Added to Saline	E. Volume of Saline for Dilution (B - D)	Volume Blue Food Grade Dye for 1:1000 Dilution (B/1000)
250	(250 x 0.1 ml) + 5 ml = 30 ml	Entire volume of stock _____ml	Entire volume of stock above beads (9 ml)	30 ml - 9 ml = 21 ml	30 ml / 1000 = 30µl
500	(500 x 0.1 ml) + 5 ml = 55 ml	Entire volume of stock _____ml	Entire volume of stock above beads (9 ml)	55 ml - 9 ml = 46 ml	55 ml / 1000 = 55µl

Labeling and Preparation

- Label:
 - Racks and dilution tubes with:
 - isolate name
 - stock number
 - Initials
- Rack 4 ml cryovials in racks
- Document stock use
 - Fill in the “date stock used/purpose” column on Stock Log

Aliquoting Samples

Sample should always be aliquoted in this order to reduce chance of cross-contamination

- Clean BSC intensively with disinfectant to destroy DNA
 - Such as 0.5% sodium hypochlorite or DNA Away
 - Ensure BSC walls and pan under work surface are disinfectant
 - Rinse with 70% Ethanol

Order of Aliquoting Samples

To minimize chances of cross contamination

- Order of samples
 - Aliquot TB Negative sample(s)
 - Aliquot TB Detected/RIF Susceptible sample(s)
 - Aliquot TB Detected/Rif Resistant sample(s)

Aliquoting: Tips for Success

- Never mix sample types in the same cabinet at the same time
- Never carry out routine work while samples are open and drying

GXPT/PP/010, Version 2.0, Effective date: 01-Apr-2022



THE REPUBLIC OF UGANDA

Prepare BSC for Use

- -Remove selected stocks from storage at 2-8 degrees Celsius
- Prepare working solution of disinfectant
 - Add ~200 ml to a beaker for pipette discard
- Place absorbent bench liner on work surface and soak with disinfectant
- Place needed items in the BSC:
 - Stock solutions, sterile saline, food coloring, racks of 4 ml cryos, repeat pipette and syringe tip

Dilution Preparation

- Transfer 10 glass beads to stock conical tube
- Cap tightly and vortex for 5 minutes
 - Ensure a full vortex is obtained
- Allow each tube to settle for 15 minutes undisturbed
- Aliquot saline and food color to the dilution tubes in calculated volumes
- Transfer the required/entire volume of stock from above the beads to dilution tube
 - Do not push pipette into or disturb beads



Aliquot Dilution

- Remove caps on cryovials and place in plastic zipper bag inside BSC
- Vortex each dilution for 30 seconds
- Use a repeat pipette with 5-10 ml tip to aliquot 100 µl of the dilution into open cryovials
 - When aspirating dilution into syringe always keep tip in the upper 1/3 of the dilution to avoid clumps that have settled to the bottom
 - Always prime the pipette 2-3 times by expressing back into the dilution tube before beginning to aliquot
- QC-always hold rack of cryovials up and at an angle to visualize the liquid in the bottom of each and every tube



Final Steps

- Return any remaining dilution to storage at 2-8C
- Place sign on BSC notifying others not to use cabinet
- Turn off light in BSC
- Allow tubes to sit open in the BSC in BSL3 7-10 days
 - Ensuring tubes are visually dry before capping
- Cap snug and store samples:
 - in large plastic, sealable specimen transport bags
 - at a consistent 2-28C
 - in the dark

Assessment

- What measures are employed to avoid mix-ups of stock in DTS preparation?
- What is the volume aliquoted in each tube for a DTS preparation?
- What are the storage activities performed during DTS preparation?

Summary

- Out of the 8 inactivated stock select 5 for panel preparation and determine order of aliquoting
- Calculate amount of saline for diluent
- Label racks and dilution tubes to avoid mix-ups
- Vortex with beads, settle 15 minutes
- Dilute with saline
- Aliquot 100 µl
- Dry 7-10 days
- Cap and store at consistent 2-25°C in the dark



References

- ISO 13528:2005, Statistical methods for use in proficiency testing by interlaboratory comparisons
- ISO Guide 34, General requirements for the competence of reference material producers
- ISO Guide 35, Reference materials – General and statistical principles for certification
- Guide 34, ISO Guide 35 and ISO 13528 (homogeneity and stability)
- ISO/IEC Guide 98-3, Uncertainty of measurement – Part 3: Guide to the expression of uncertainty in measurement (GUM:1995)
- ISO/IEC 17011:2004, Conformity assessment – General requirements for accreditation bodies accrediting conformity assessment bodies
- ISO/IEC 17025, General requirements for the competence of testing and calibration laboratories

Acknowledgments

