THE REPUBLIC OF UGANDA
MINISTRY OF HEALTH

UGANDA Supranational® Reference Laboratory
Timely Accurate Diagnostics for a TB-Free Africa

Training on New and rapid Tuberculosis diagnostics (first and second line Probe Assay)

Module 10: Detection-automated systems

Uganda Supranational Reference Laboratory

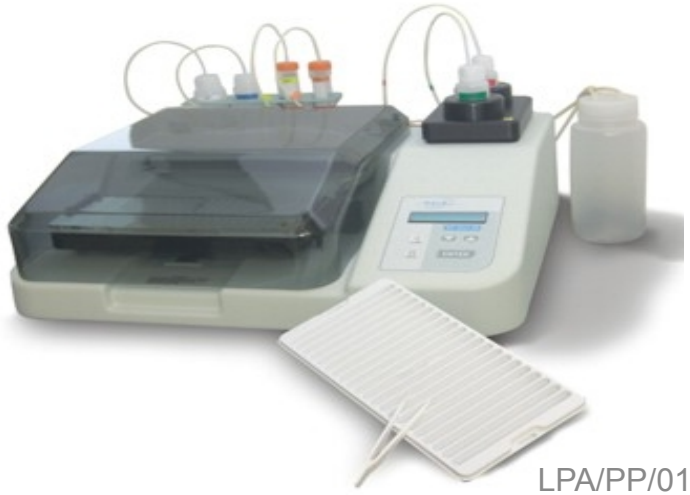
Content outline

- Introduction
- Laboratory layout
- Equipment and materials
- Safety precautions
- Procedure for use
- Cleaning procedures

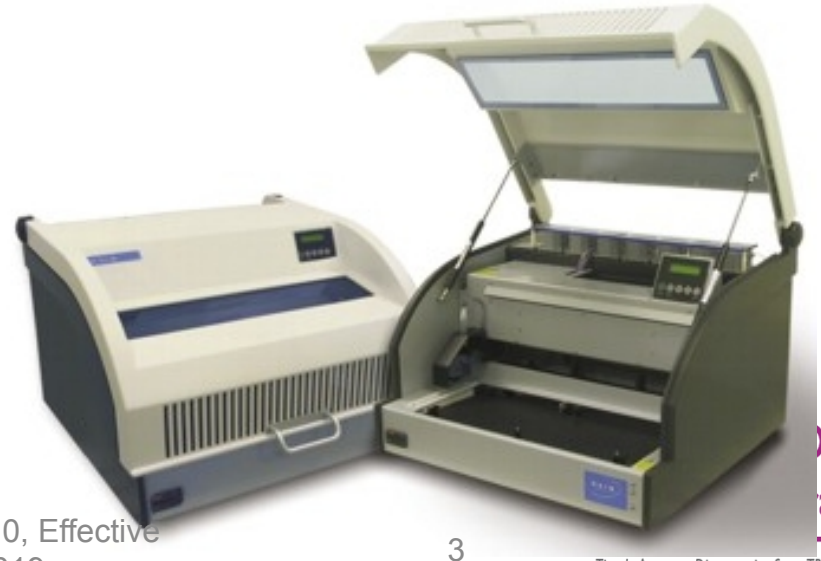
Introduction

- The GT Blot 48 or GT Blot 20 instruments are automated hybridization washers that incubate line probe assay (LPA) strips at the appropriate temperature and perform the various incubation and wash steps automatically.

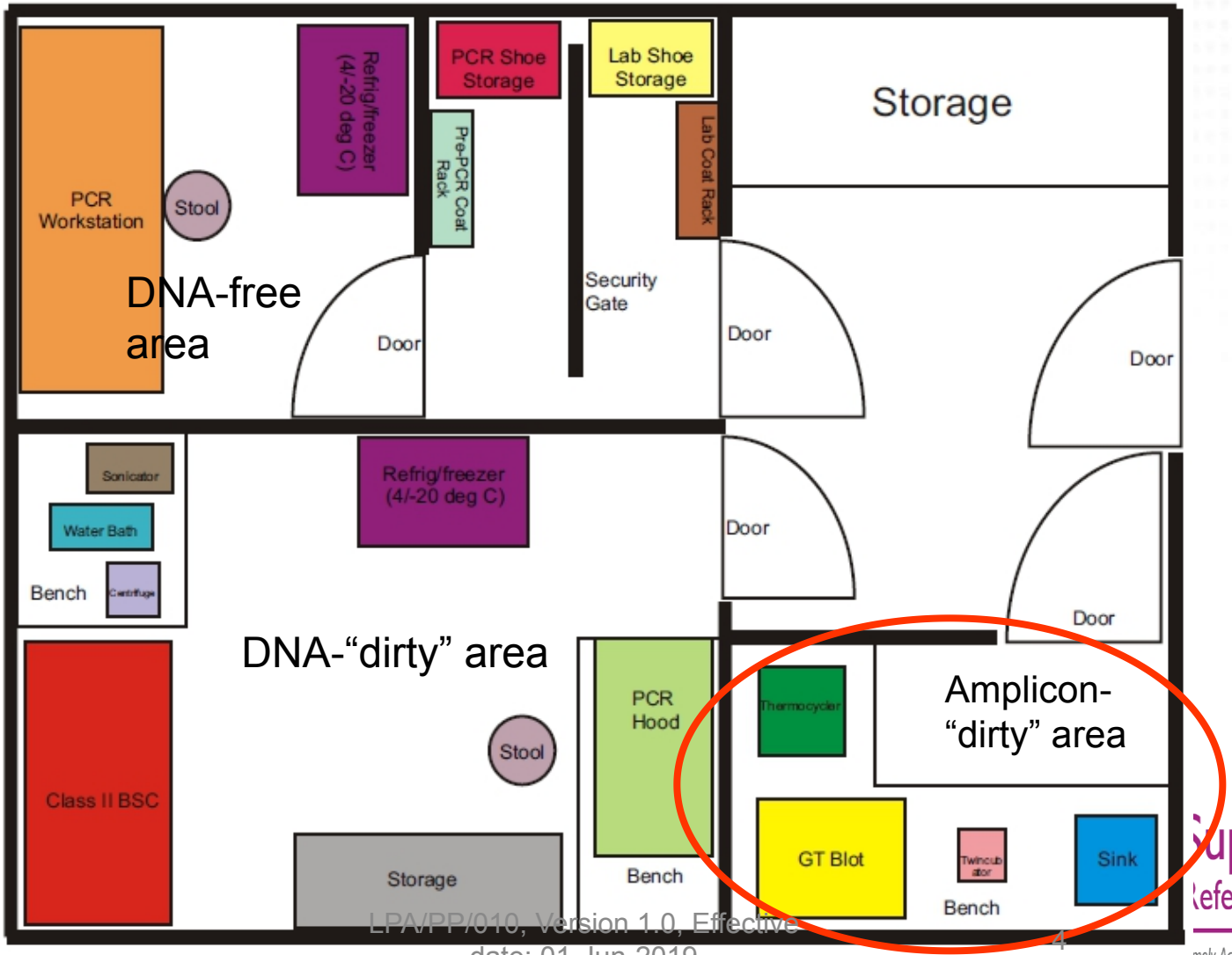
GT-blot 20-perfoms 20 tests



GT-Blot 48-perfoms 48 tests



Optimal PCR laboratory lay out



Equipment and materials

- equipment/ materials are similar to those used when using the manual procedure

-Gloves

-Tweezers

-p200 pipette

-1st or 2nd line LPA kit

-50 ml graduated cornical tubes for dilutions

-sterile filter tips

-Discard container

-Adsorbent paper

Safety precautions

- Do not touch moving parts of the instrument while in operation
- Do not attempt to open the electrical supply or interfere with the electrical components of the instrument at any time.

- It is important not to attempt to run the instrument without liquids. Serious damage to the instrument may occur.
- The operator must position the sensor in the tray prior to starting the assay.

Instructions for use-1

Before using the instrument check the following:

i. Ensure the power is switched OFF.

ii. Ensure the waste tubes are routed into a suitable empty container.

iii. One of the waste tubes is gravity fed. Ensure the waste container is below the level of the machine and the waste tubes are not trapped under feet.

iv. Switch power ON (back left of the machine) and ensure that the instrument initializes properly.

Instructions for use-2

The initialization sequence of events is as follows:

- The display will indicate “BeeBlot Ready Press Start”
- The aspirating needles move upwards to their home position
- The arm will move left to its home position
- The tray mechanism will move into its home position
- The green LED at the front of the instrument will come ON
- The rear fan should be rotating

Instructions for use-3

After initialization/washing step:

- Place all tubings in a container filled with distilled/deionised water.

- Follow the keypad commands, and ensure the water flows though the appropriate dispensing needles under the arm when washing is in progress.
  - “BeeBlot press start”

Instructions for use-4

Washing step

- Using either the left or right arrow select “Washing – Assay 11” then press START three times:
  - “Cleaning cycle A” Press START
  - After cleaning cycle A is complete press START
  - “Cleaning cycle B” Press START
- While the cleaning cycle is in progress, prepare dilutions of the conjugate and substrate solutions.

Instructions for use-5

- After cleaning cycle B is complete switch the power to the instrument OFF.
- Dry tubings with a dry cloth.
- Place all solutions in their respective places (colour matched(and insert the conduction tubes (e.g. the HYB solution is green, and the slot is marked with a green dot).

Instructions for use-6

- Switch the power to the instrument ON.
  - "BeeBlot start" Press START.
- By pressing the right arrow key select "Genotype 45" Press START three times.
- Lift the temperature sensor up carefully and put into the tray. Make sure that the sensor is properly positioned down before starting the assay.
- Reagent preheat of HYB and STR solutions for 15 minutes will start.

Instructions for use-7

- While the pre-heat is in progress, dispense 20µl of denaturation “DEN” solution in a corner of each of the wells in the tray.

- Add 20µl of amplified sample to the DEN solution, pipette up and down to mix and incubate at room temperature for 5 minutes.

- Take the LPA strips out of the tube using tweezers and label with a pencil/pencil. Always use gloves when handling strips.

Instructions for use-8

- After the pre-heat is complete, press START and select the number of wells- must be an even number between 2 and 48.
- Position sensor and press START.
- Begin assay – press START
- Close lid – press START

Instructions for use-8

- After the priming sequence is completed the instrument dispenses pre-warmed hybridization reagent (HYB) into the tray and it will dispense water into the wells that are not used, in order to gain optimal temperature

Instructions for use-9

According to the assay program, the instrument will aspirate and add the necessary reagents at the correct time interval and incubate the reagents appropriately as per the table below

HYB (hybridization)	green	30 minutes at 45ºC
STR (stringent wash)	red	15 minutes at 45 ºC
RIN (rinse solution)		1 minute
CON (diluted conjugate)	Orange	30 minutes at 25 ºC
RIN		1 minute at 25 ºC
Distilled water wash		1 minute at 25 ºC
SUB (diluted substrate)	yellow	3 minutes at 25 ºC
Distilled water wash (twice)		1 minute each

Instructions for use-10

After the reaction has stopped:

- ASPIRATE tray? Will be displayed. Press START.
- After aspiration is complete open the door and remove the tray and put it on the bench
- Take out the tubings from the reagent containers and place them in the container filled with 1% bleach solution and run the washing assay twice and repeat the washing cycle twice with de-ionised water.
- Dry tubings and place them in an empty container.

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Instructions for use-11

Cleaning of GT-Blot trays

i. Soak the trays thoroughly using diluted bleach solution in the sink.

ii. Remove excess liquid and then spray with 70% ethanol and wipe wells thoroughly to remove residues (a tooth brush could be used at this point)

iii. After cleaning, wash well using distilled water to ensure all bleach is removed. Bleach residues not removed may affect the colour development of the strips.

iv. Discard the water and wash a second time with distilled water.

v. Pat remaining liquid on a fresh paper towel and leave to air dry.

vi. Trays can be reused for quite some time, but check during cleaning that

Instructions for use-12

Cleaning the GT Blot 48

- Switch off the instrument and close the lid prior to cleaning.
- Clean the outside of the instrument on a monthly basis using a moist lint free cloth.
- On a weekly basis clean the insert for the tray inside the instrument carefully using 70% ethanol and a cotton tipped applicator stick to remove any residues from between the wells.
- Do not use bleach inside the instrument.

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Assessment

1. Identify any 2 safety precautions taken when using the GT-blot system of detection.

2. What is the risk of having precipitates in the reagents/ poor reagent pre-heat stage.

3. Why does the GT Blot instrument also dispense water in the wells that are not used during the run.

4. After how long should the GT blot trays be used?
5. Briefly describe the maintenance procedure for the trays and GT blot instrument.

Summary

- The GT-Blot system is an automated detection system.
- Important to always follow manufactures' instructions on operation of the GT blot.
- A good service and maintenence program is important for the GT blot.

References

- GenoType MTBDR*plus* product insert. Hain Lifescience. Version 2.0.

- Barnard M, Albert H, Coetzee G, O'Brien R, Bosman ME. Rapid molecular screening for drug-resistant tuberculosis in a high-volume public health laboratory in South Africa. Am J Respir Crit Care Med. 2008 Apr 1;177(7):787-92. Epub 2008 Jan 17.

Acknowledgments