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)

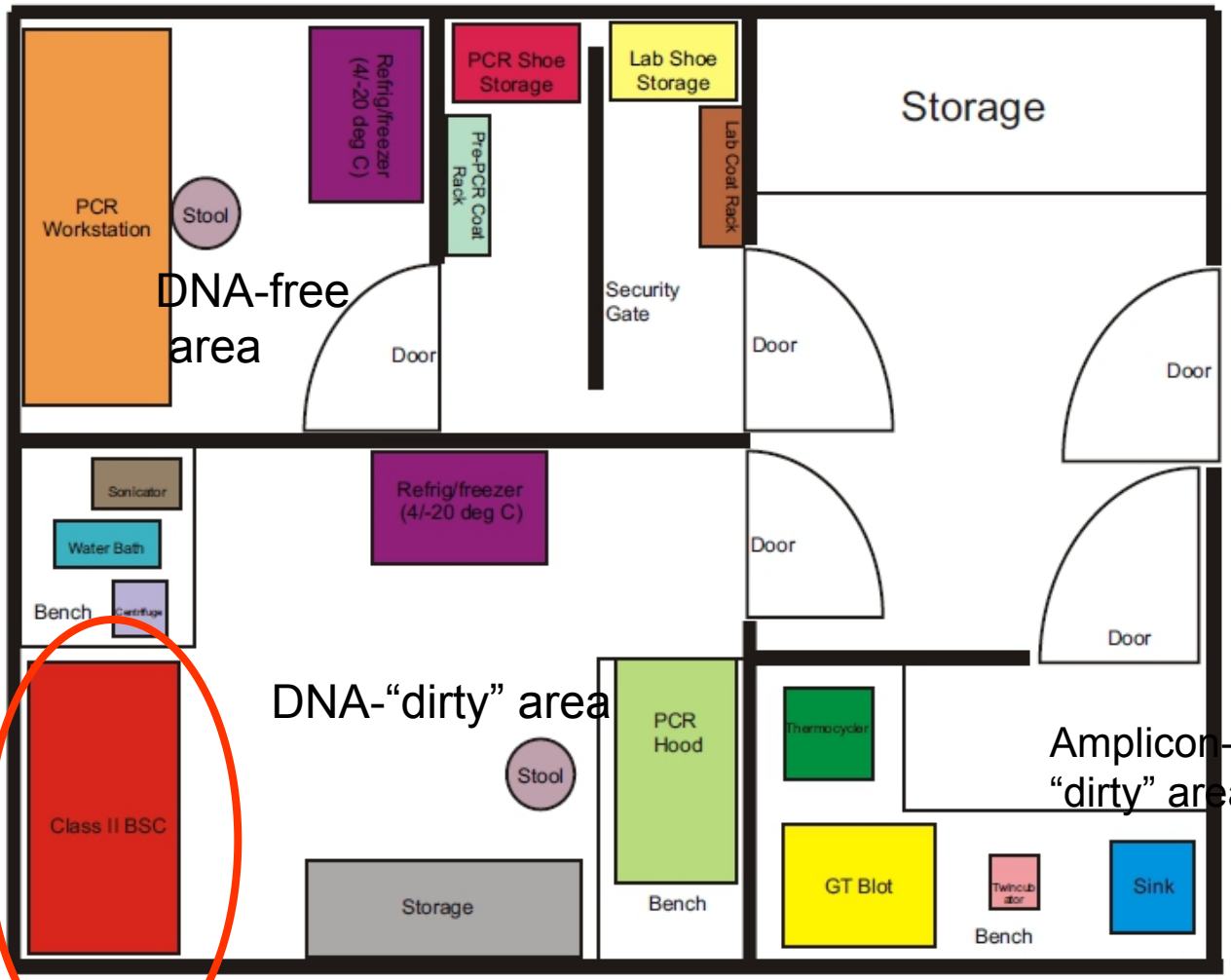
Module 8: Specimen preparation-DNA extraction

Uganda Supranational Reference Laboratory

Content outline

- PCR laboratory layout
- Checklist
- Personal protective equipment (PPE)
- Working in a Biological Safety Cabinet (BSC)
- Specimen preparation for PCR
- Contamination control

Optimal PCR laboratory layout



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Group exercise-5 minutes

1) Prepare a written checklist for all materials and tools required during specimen processing or DNA extraction for LPA.

Use appropriate BSL3 PPE

- Gowns
  - Must have solid front and can be tied in the back
  - Long-sleeved with elastic cuffs
  - Must be either autoclavable/washable (soak in bleach) or disposable

- Respirators
  - N95
- Gloves
  - Single-use

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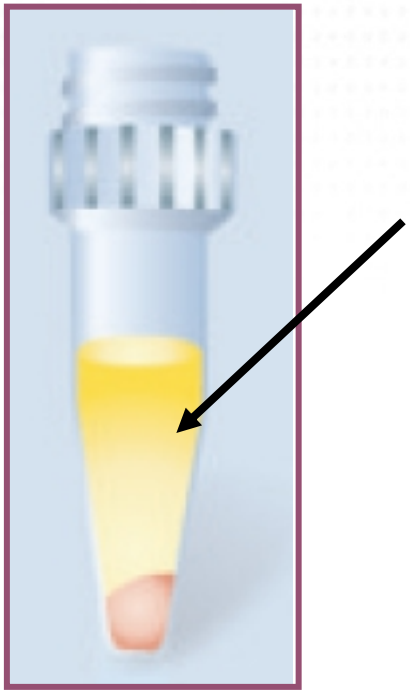
Working in BSC II

- Decontaminate the BSC with freshly diluted 1% bleach or 5% lysol (phenol), followed by 70% alcohol
- Cover the working area with paper towels and spray it with suitable disinfectant
- Use a discard container with a plastic bag and freshly diluted 1% bleach or 5% lysol (phenol).
- DO NOT block the grill
- Perform all operations at least 12 cm away from the front grill on the work surface
- Place all materials and aerosol-generating equipment away from the front grill

Preparation of clinical specimens

Clinical specimens have to be processed by N-acetyl-L-cystein/NaOH method before specimen preparation for LPA



- Label 1.5 ml screw-cap conical tubes (both tubes and caps) for each specimen
- Completely thaw frozen and processed clinical sediments
- Vortex processed clinical specimen for 1 min
- Process only one specimen at a time; do not leave open containers or centrifuge tubes in the BSC
- Transfer 500 ml processed clinical specimen into 1.5 ml conical screw-capped vial. Change tips!

Infectious material: all steps have to be performed in a BSC at least in an appropriate BSL2 Laboratory

Preparation of isolates on solid culture

- Re-suspend a half loopful but representative no. of colonies of *M. tuberculosis* culture on solid medium in either sterile PBS or sterile distilled water. Change loops!
- Do not scrape the surface of the medium since components of egg-based medium can be PCR inhibitor
- Vortex suspension for 1 min to completely break up colonies and clumps
- Label 1.5 mL screw cap conical tubes (both tubes and caps) for each sample
- Transfer 500 ml processed clinical specimen into 1.5 ml conical screw-capped vial. Change tips!

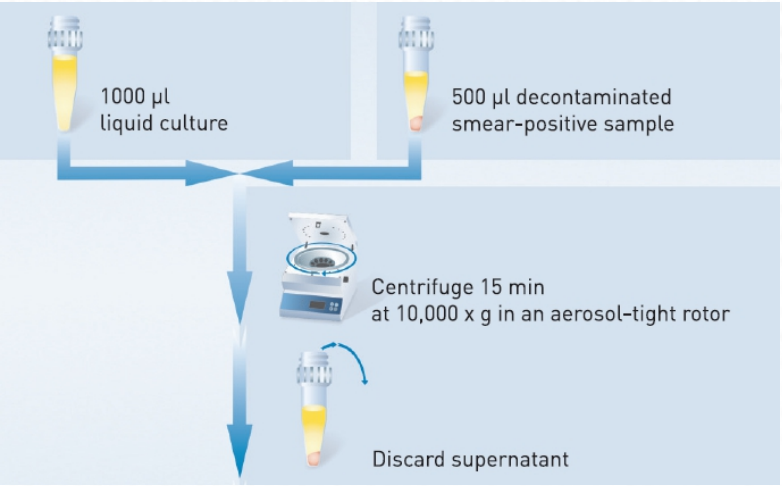
Infectious material: all steps have to be performed in a BSC at BSL3 Laboratory

Preparation of liquid cultures

- Gently invert the liquid culture 4-5 times for homogenization of the positive broth.
- Label 1.5 ml screw-cap conical tubes (both tubes and caps) for each sample
- Transfer 1 ml of liquid *M. tuberculosis* culture into 1.5 ml conical tubes. Change transfer pipettes or filtered tips!

Infectious material: all steps have to be performed in a BSC at BSL3 Laboratory

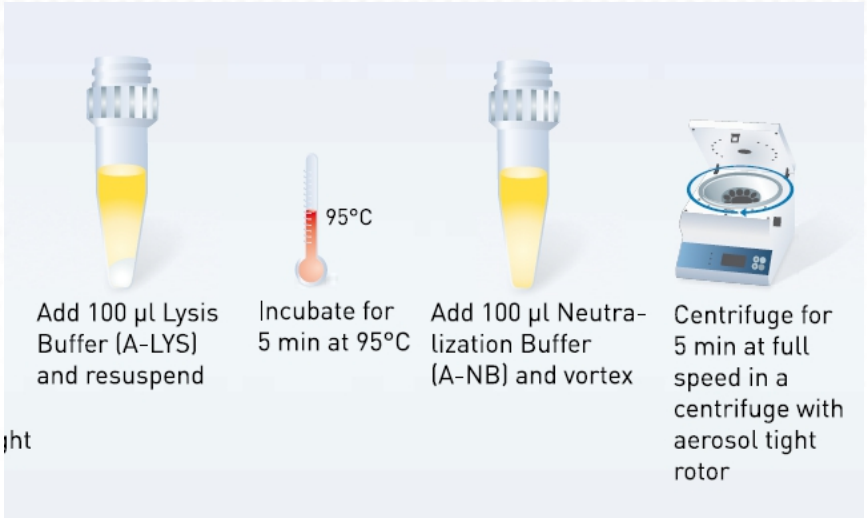
Specimen pr



- Pellet bacteria by centrifuging specimen for 15 min at 10,000 x g
- Use table top centrifuge with an aerosol tight rotor (open rotor in the BSC)
- Let the vials stand in the rack for 1-2 min to avoid aerosol generation when opening the tubes
- Gently discard off the supernatant taking care not to pour off the pellet.
- If there is no visible pellet then a 1 ml pipette with filtered tips should be used to carefully aspirate the supernatant leaving behind about 100ml. Change tips!

Infectious material: all manipulations have to be performed in a BSC at BSL 3 Laboratory

Specimen preparation 3: DNA extraction with GenoLyse



- Add 100µl Lysis buffer (A-LYS) to the sediment and suspend using vortex mixer
- Incubate for 5 minutes at 95 C, preferably in a heating block or hot air oven (monitor temperature using calibrated thermometer)
- Add 100µl Neutralisation buffer (A-NB) and vortex
- If the procedure was properly carried out, sample can be considered non-infectious after this point

Infectious material: all manipulations have to be performed in a BSC at BSL2 Laboratory

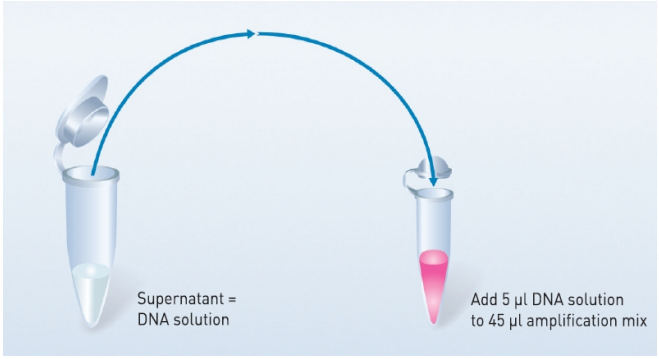
preparation 4

- Centrifuge specimens for 5 min at 13,000 x g (maximum speed)
- Use table top centrifuge with an aerosol tight rotor (open rotor in the BSC)

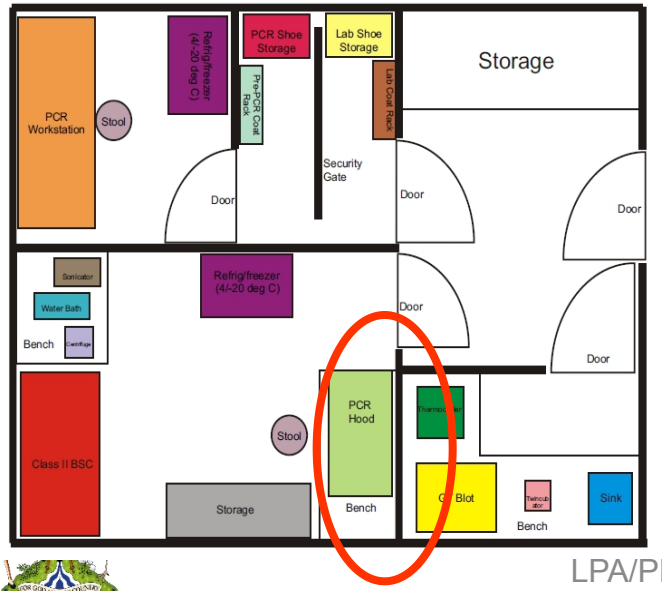
! Always use screw-cap, not flip-cap, vials in order to avoid contamination

- Transfer 50-70 µl supernatant containing DNA to new 1.5 ml screw-cap vials using a 100-200 ml pipette with filtered tips. Change tips!
- Do not stir up pellet
- Specimens may be kept at 4°C for not more than 7 days

Specimen preparation 5



- Work in a PCR hood (optional) in DNA-"dirty area"
- Completely thaw supernatant
- Pipette up and down



- Add 5 l of each DNA sample to the corresponding tube containing the 45µl master mix
- Change filtered pipette tips between each addition and dispose of them in 1% bleach solution
- Close PCR tubes and place them on appropriate rack for transfer to the amplification room.

Cleaning up- decontamination

- Decontaminate any materials (vials, tubes etc.) before removal from the BSC and PCR hood
- Clean pipettes, racks, instruments and the BSC with freshly diluted 1% bleach (20 min), followed by 70% alcohol
- Use area-dedicated spray flasks or beakers (separate beakers for surface cleaning and instruments)
- Do not take anything from this area to Reagent Preparation Area

Contamination control

Specimen Preparation Area

Tools and instruments used here have to be labeled accordingly and *should not* be used elsewhere

Never take anything from this site to the Reagent Preparation Area

You may only take the PCR tubes in there rack that has been disinfected appropriately.

Always use filtered tips

Do not use flip-cap tubes, always use screw-cap tubes

If applicable, dedicate a BSC only for molecular work

Change gloves if they get contaminated by specimens

Assessment

1. What type/ forms of samples can DNA be extracted for LPA procedures
2. Mention 4 ways to minimize contamination during sample preparation.
3. Why should the disinfectant e.g. 1% bleach or 5% lysol be freshly prepared prior to use.
4. Identify one source of inhibitor that may affect the PCR reaction due to the sample preparation procedure.

Summary

- Organize your work (use checklist)
- Work uni-directionally (laboratory layout)
- Develop and follow SOP for procedure and instrument maintenance
- Keep tools and work place clean!

References

- GLI TB training package http://www.stoptb.org/wg/gli/trainingpackages.asp
- www.hain-lifesciences.com

Acknowledgments