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

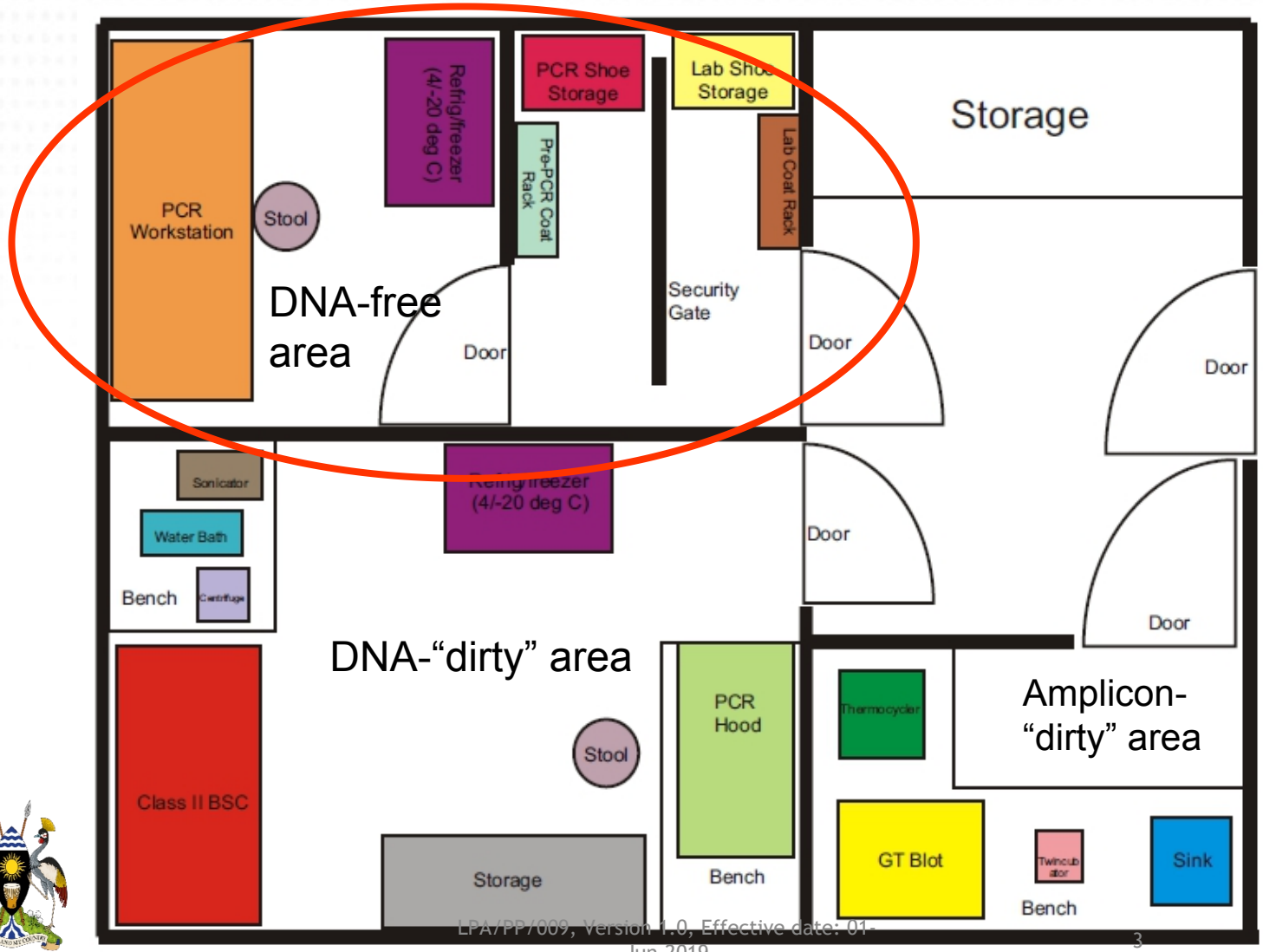
Module 9: Reagent preparation

Uganda Supranational Reference Laboratory

Content outline

- PCR laboratory layout
- Calculation of volume of master mix components
- Personal protective equipment (PPE)
- Reagent preparation for PCR
- Contamination control

Optimal PCR laboratory layout



Group exercise-10 minutes

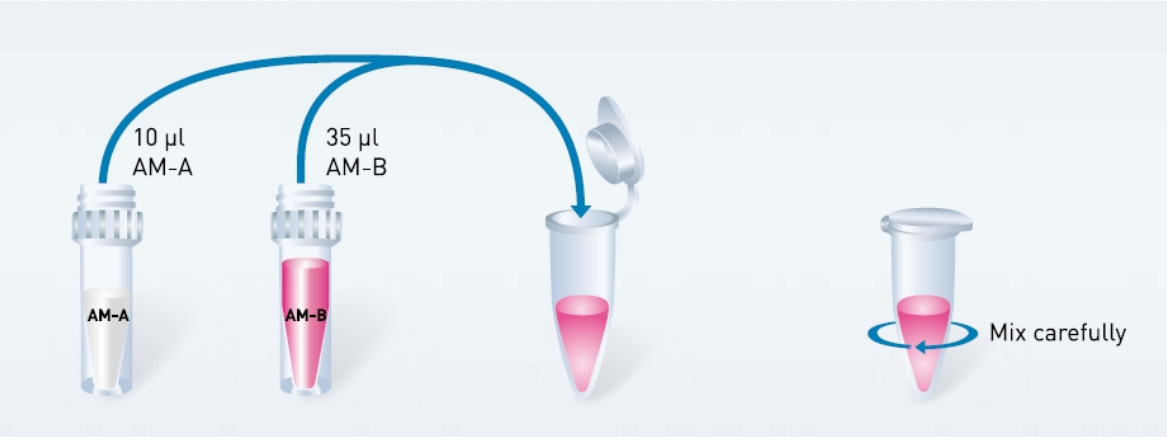
1. What precautions should be taken when preparing the LPA reagents (master mix)?

2. Identify the materials or tools that are required in the reagent preparation room.

3. If you have 18 samples plus 3 controls: what is the final volume of master mix to be prepared?

Reagent preparation 1
Calculation of components

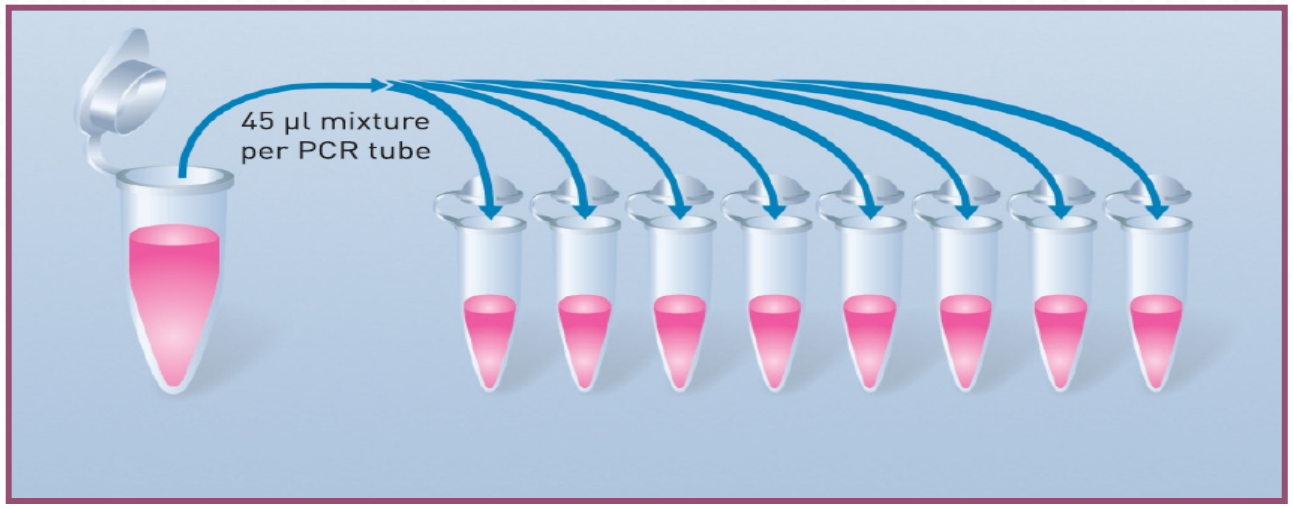
DNA free area



- Determine the number of specimens and controls
- Use this number plus 1 to calculate the volume of the different components of the master mix
- The volume of master mix is 45 ml per specimen
- Prepare a worksheet to calculate the volume of the master mix ingredients (put on wall near work area)

Reagent preparation 2
Dispensing master mix area

DNA free



- Label PCR tubes in line with numbers of specimens and controls on your worksheet
- Place PCR tubes onto the PCR tube rack
- Dispense 45 ml master mix into each PCR tube using filtered tip (same tip may be used but should be changed if it touches any outside part of the tubes or PCR hood contents)
- Add 5 ml of same sterile, molecular grade water used to prepare master mix into **negative control PCR** tube
- If no sterile molecular grade water is available then dispense 50 µl of master mix into the negative control PCR tube

Reagent preparation 3
Clean-up of work place DNA free area

- After each use, clean pipettes, racks, instruments and the BSC with freshly diluted 1% bleach , followed by 70% alcohol and UV light

- Use area-dedicated spray flasks or beakers (separate beakers for surface cleaning and instruments)

- NB: The use of UV light does not undermine the need for effective disinfection with bleach and alcohol

Contamination control
Reagent Preparation Area

- Tools:
  - Tools and instruments that are used here have to be labeled accordingly and cannot be used elsewhere
  - Do not move the beaker to Reagent Preparation Area from one room to another.
  - Use 0.5 ml individual PCR tubes instead of 0.2 ml strips
  - Use a PCR hood with UV
- Good Laboratory Practice:
  - Change shoes or use shoe cover before entering Specimen Prep. Area. Decontaminate and calibrate pipettes regularly
  - After you are finished working in the Specimen Prep. Area, do not come back and clean the Reagent Prep Area workplace

Contamination control
Reagent Preparation Area

- Reagents:
  - Always prepare fresh bleach (sodium hypochlorite) solution
  - Check undiluted bleach concentration
  - Final concentration of bleach should not fall below 0.5%
  - Containers containing bleach CANNOT be autoclaved!
  - Use 70% alcohol instead of distilled water for cleaning to avoid contamination of distilled water stocks
  - Aliquot sterile molecular grade water into 1.5 ml tubes
- Laboratory cleaning:
  - Always properly clean the work area after completing the work
  - Establish a regular (*e.g.* weekly) and thorough laboratory cleaning protocol (floors, doors, walls)

Assessment

1. What steps are taken to minimize the introduction of DNA into the reagent preparation room?
2. At what temperature should the new stocks of master mix components (AM-A & AM-B) be stored?
3. What is the final concentration of the bleach to be used in the reagent preparation room?

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

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