Overview of Containment Level 3 (CL3) Laboratories

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- MSc in Medical Microbiology from The London School of Hygiene and Tropical Medicine

- Healthcare Scientist for the National Mycobacterium Reference Laboratory – Public Health England

- Currently a Research Scientist at University College London working on the TB Alliance Clinical Trials
Linzy Elton

- MSc in Medical Parasitology from The London School of Hygiene and Tropical Medicine
- PhD in host/pathogen/vector biology for *Yersinia pestis* (plague) at The University of Nottingham, where I was also BSL3 laboratory manager
- Currently a postdoctoral scientist working for PANDORA and CANTAM (my main roles include BSL3 laboratory assessment and accreditation, TB and AMR)
### Hazard Groups

- Biological agents are classified into hazard groups

<table>
<thead>
<tr>
<th>Hazard Groups</th>
<th>Group 1</th>
<th>Group 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unlikely to cause human disease</td>
<td>Can cause human disease</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Unlikely to spread to the community</td>
</tr>
</tbody>
</table>
We work with *Mycobacterium tuberculosis* (HG3) in our Containment Level 3 laboratories

<table>
<thead>
<tr>
<th>Hazard Group 3</th>
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</thead>
<tbody>
<tr>
<td>• Can cause <strong>severe</strong> human disease</td>
</tr>
<tr>
<td>• May be a <strong>serious</strong> hazard to employees</td>
</tr>
<tr>
<td>• May spread to the community</td>
</tr>
<tr>
<td>• Effective prophylaxis or treatment available</td>
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</tbody>
</table>
1. Negative Pressure
2. Extracted air is HEPA filtered
3. All work is carried out inside a Microbiological Safety Cabinet: Class 1 recommended
4. Personal Protective Equipment (PPE)
5. Sealable
6. Restricted access
7. A means of viewing occupants
8. Biological agents stored safely
9. Autoclaved waste
• Air pressure on the inside of the laboratory is lower than the outside

• Prevents release of HG3 pathogens to surrounding areas
Small CL3 pressure gauges. **Always check the pressures before entering the laboratory.**
Extracted air is HEPA filtered

- **High Efficiency Particulate Air Filter**

- Installed in the Microbiological Safety Cabinets & CL3 extracts

- Retain 99.97% of particles of ≥0.3μm in diameter

- Must be tested at least every 14 months (external contractor)
Extracted air is HEPA filtered

Small CL3 ceiling extract with HEPA filter

Small CL3 Microbiological Safety Cabinet with HEPA filter
Class 1 Microbiological Safety Cabinet

- Protect the individual from infectious aerosol release
- Does **NOT** protect the work *i.e.* not sterile
- HEPA filter present to prevent infectious aerosols releasing to environment
- Airflow readings are taken daily and recorded
- Airflow values should be between 0.7 and 1.0 ms⁻¹
Class 1 Microbiological Safety Cabinet

Exhaust to outdoors

HEPA filter

Pre-filter

Potentially contaminated air

Work generating aerosols

Room air (intake 0.7 – 1.0 ms⁻¹)

Schematic diagram of a Class 1 MSC
Class 2 Microbiological Safety Cabinet

- Protects the individual and the work being carried out
- Process involves recirculated filtered air
- Safety of the cabinet can be disturbed more easily by sudden movements by operator or around cabinet
• Rear fastening impermeable gowns are worn in CL3

• These cannot be removed from CL3 except for disposal in autoclave bags

• Gloves must be worn when handling specimens, cultures, and waste

• Gloves must be changed regularly
PPE

- Rear fastening impermeable gown
- Gloves
  - Date written on gown so that it can be changed weekly
The laboratory **must** be sealable to permit disinfection.

Fumigation (decontamination process involving formaldehyde) of the laboratory is necessary if there is a uncontrolled release of *Mycobacterium tuberculosis* e.g. dropping a culture outside of the Microbiological Safety Cabinet.

- Walls/ceilings should **not** have cracks.
- Pipes entering the room must be sealed around entry/exit points.
- Sealability must be regularly checked: monthly integrity checks/annual room integrity checks by external contractors.
# Monthly Integrity/Laboratory Check and Alarm Test

This form must be completed by different individuals. It is everyone’s responsibility to check the integrity of the CL3 laboratories. If you open the laboratory on the 1st working day of a month then it is your responsibility (unless you did it the previous month, in which case nominate someone else).

<table>
<thead>
<tr>
<th>Component</th>
<th>Action</th>
<th>Initials</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cracks, Dust Trails, Loose Mastic or Beading?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Walls</td>
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<tr>
<td>Windows</td>
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<td></td>
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<tr>
<td>Door frames</td>
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<tr>
<td>Door view-panel</td>
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<tr>
<td>Floor-level pipes</td>
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<tr>
<td>Sink waste pipes</td>
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<tr>
<td>MSC Ducting</td>
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<tr>
<td>Cable ports</td>
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• Swipe card entry for those who have completed CL3 inductions and passed competency

• All visitors must have read and signed the relevant documents

• List on the of doors of CL3 showing who has access, this must be regularly updated
We are able to view our colleagues working.

Windows on both ante room and laboratory doors.
Biological agents stored safely

- Storage in cupboards, incubators, fridges and freezers
- All cultures must be in suitable racking
- Tabletop storage is avoided as much as reasonably possible
- Stacking is prohibited
Biological agents stored safely

Suitable racks

Suitable box

Stacking
Autoclaved Waste

- All cabinet waste is placed into disposable jars e.g. tips and loops
- The dispo jars are placed into autoclave bags and then placed inside a tin
- The tin is then taken out in order to autoclave: 134°C for 5 min
• All other waste is placed into autoclave bags and taken out to be autoclaved in the media room

• The autoclaved waste is then placed in a yellow bag and sent for incineration
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