

NDMRB-RA-002

**Risk Assessment:** Protein Expression in Bacteria

**Scope**

Large scale protein expression in prokaryotic expression systems including BL21, Rosetta and HMS174, leads to the formation of inclusion bodies that comprise aggregates of misfolded recombinant proteins. To recover the proteins for downstream studies, the bacterial lysates require sonication, homogenisation in a Triton-X100 based wash buffer and finally, solubilisation in Urea-MES buffer. The risks associated with this technique include

1. Working with bacterial culture and GMOs (RA-Protein expression in Bacteria and NDMRB-GMO-RA-021),
2. The use of bunsen burners (TDI-RA-008)
3. The use of laboratory centrifuges (TDI-RA-003) and the manual handling of rotors for floor standing centrifuges (TDI-RA-012)
4. The use of chemical with known health risks (Ampicillin-RA, Carbenicillin\_RA, Chloramphenicol\_RA and TDI-RA-002).
5. Use of orbital shaker incubators.

It is the users responsibility to ensure what controls are needed to ensure that the health of themselves and others around them. It is imperative that you **DO NOT** start any work until you are absolutely sure of the appropriate precautions that need to be employed. If you are unsure seek advice from your line/laboratory manager or your departmental safety officer (DSO).

<b>Name of assessor:</b>	G Gillespie	<b>Date of Assessment:</b>	Feb 2016	<b>Review Date:</b>	Every three years
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**Risk Matrix:**

Risk Matrix		Likelihood			
		High	Medium	Low	Negligible
Consequence	Severe	High	High	Medium	Effectively Zero
	Moderate	High	Medium	Medium/low	Effectively Zero
	Insignificant	Medium/Low	Low	Low	Effectively Zero
	Negligible	Effectively Zero	Effectively Zero	Effectively Zero	Effectively Zero

**Risk Assessment:**

Hazard (Cause and consequence)	Affected Groups	Existing controls	Risk	Further Action
<b>1. Working with bacteria/GMOs and the waste generated.</b>	Staff/students/visitors	<p>All staff to be trained in accordance with microbial safety practices before commencing biological work. All staff should familiarise themselves with the related RA documents.</p> <p>Cultures should be inoculated in a dedicated microbial workspace, using aseptic technique (workplace swabbing with 70% Isopropanol before and after set-up, with inoculation in the presence of a flame).</p> <p>Soak liquid waste, including cellular debris, culture supernatants, and contaminated culture flasks/centrifugation containers in a final concentration of 2% Virkon (w/v) overnight.</p> <p>Consumables will be autoclaved at 136°C for 12 minutes resulting in 100% kill. Waste will subsequently discharged to landfill.</p>	Low	All users must read SOPs and RAs associated with handling bacterial cultures (NDMRB-GMO-RA 021).
<b>2. Use of Bunsen burners</b> Burns from flames, fire risk, explosions	Staff, students and visitors	<p>All users receive appropriate training from experienced personnel.</p> <p>Use the Bunsen in an uncluttered and dedicated area, with no paperwork, plastic or flammable objects/liquids/chemicals stored overhead in the immediate vicinity. Never lean over a lit bunsen. Never leave unattended and shut off flame once work is completed.</p>	Medium	<p>Ensure that staff are adequately trained.</p> <p>Ensure that the canister connectors are in good repair and are replaced regularly</p>

		<p>Allow the bunsen to cool down before handling after use.</p> <p>Unused gas canisters should be stored in flammable cabinet. Check the integrity of new cannisters. Dispose of damaged canisters through the appropriate channels.</p>		<p>Associated RA – <a href="#">TDI-RA-008</a> Working with open flames and Bunsen burners</p>
<p><b>3. Use of centrifuges</b></p> <p>Mechanical failure, contact with rotating parts, electrocution.</p>	<p>Staff, students and visitors</p>	<p>All users receive appropriate training from experienced personnel and/or attend the NDMRB induction for the use of centrifuges. Users should comply with RA TDI-RA-012 if they are required to remove the JLA-8.100 rotor. Ensure that the centrifuge and rotors are thoroughly cleaned and in good repair. Ensure that the lid lock is in good working order. Ensure that the spin load is properly balanced. NEVER attempt to open a centrifuge whilst the spin programme is running.</p> <p>Regular PAT test should be performed.</p>	<p>Medium</p>	<p>Associated RA – <a href="#">TDI-RA-003</a> Safe use of laboratory centrifuges</p> <p>And</p> <p><a href="#">TDI-RA-012</a> Manual handling of rotors for the floor standing centrifuge</p>
<p><b>4. Chemicals with known health risks</b></p> <p>a. antibiotics</p> <ul style="list-style-type: none"> <li>• Amplicillin ( H317, H334)</li> <li>• Carbenicillin (H317, H334)</li> <li>• Chloramphenicol (H350)</li> </ul> <p>IPTG (isopropyl B-D-1-thiogalactopyranoside) is also used but does not have a hazard assignment.</p>	<p>Staff, students and visitors</p>	<p>Ensure all users are adequately trained to work with chemicals that pose health hazards. All associated SOPs and RAs Concentrated master stock should be dissolved in a chemical fume hood to avoid contact with dust and aerosols.</p> <p>Handle all other chemicals according to GLP and relevant RAs.</p> <p>All spend buffer should be disposed of in accordance with the measures that comply with the disposal of bacterial/GMO-derived waste outlined in Section 1 above.</p>	<p>Low</p>	<p><a href="#">COSHH</a></p>

<p><b>5. Use of orbital shaker incubators</b></p> <p>Injury via the breakage of glass, electrocution.</p>	<p>Staff, students and visitors</p>	<p>Confirm that the appropriate clamps are securely in place. Ensure that flasks fit snugly in the clamp attachments. Take extra care when removing or placing flasks in the clamps.</p> <p>In the event of glass breakage, immediately disconnect electrics. Isolate the spillage and disinfect with Virkon powder. Dispose of broken glassware in a dispo jar and autoclave before disposal.</p> <p>Ensure regular PAT testing.</p>	<p>Low</p>	<p>Check the integrity of clamp attachments and accessories. Replace as required.</p>
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Signed By Author:

Approved by (sign and print):

Reviewed by:

Review date: