SPARQ-ed Risk Assessment Sheet : Bacterial Work

Description of Risk	Hazard				Analyse / Evaluate Risk			Overall Risk Category (see explanation on last page)
	Source	Current Controls	Event	Category	Consequences	Exposure	Probability	
Use of S4 Drug : Antibiotics are used to select for the transformed bacteria, carrying a plasmid of interest. Selection of the antibiotic will depend upon the nature of the antibiotic resistance imparted by the vector. We most commonly use ampicillin, but other antibiotics, including tetracycline, kanamycin and chloramphenicol, are also used. Many selective antibiotics used in bacterial culture are on the Poisons Schedule and are rated S4. The concentrated form (usually powder) must be securely stored and logged out when made into a working concentration. Even at working concentrations, long term exposure can result in damage. The working stock is usually added to media at a 1:1000 dilution.	Chemical	S4 drugs securely stored in cabinet (room temperature) or fridge/freezer (4C or -20C). there is a log sheet for each chemical which is to be filled out each time that the concentrate is removed to make up a working stock. SPARQ-ed participants do make up stock solutions of these drugs.	Spill of concentrated power generating dust -exposure to inhalation and contact with skin and eyes	Other contact with chemical or substance	**Serious :** acute hypersensitivity reaction can result in death within minutes	**Very rare :** SPARQ-ed Participants do not make up stock solutions and will only be coming into contact with diluted working solutions	**Conceivable :** Solutions used are dilute and in very small volumes. Participants are supervised by well-trained staff.	Prob \ Exposure: VR / R / U / O / F / C AC: Mod / Subs / High / VH / VH / VH QP: Mod / Mod / Subs / High / VH / VH UP: Low / Mod / Mod / Subs / High / High RP: Low / Low / Low / Mod / Mod / Subs **C**: **Low** / Low / Low / Low / Mod / Mod PI: Low / Low / Low / Low / Low / Low
General Sharps (exposure) Risk - Use of Biological Agents : Risk associated with pipetting of common biological agents within the laboratory. Pipetting is a common technique used in a laboratory. Specific hazards relate to the nature of the substance in the tip and if exposure occurs. General hazards relating to the event of a sharps injury (i.e. piercing of skin).	Mechanical	PPE worn (gloves - some biologicals may require double gloving, lab gown, closed footwear). Tip disposal into sharps containers which are destroyed by incineration.	Exposure to microbiological agents (recombinant or non recombinant) when gloves break, pipette ejector fails (manually removing tips) or tips bounce out of sharps container	Contact with, or exposure to, biological factors.	**Substantial :** general biological agents used are not specifically hazardous	**Frequent** : pipetting is a common practice	**Remotely Possible :** conceivable that tips leak or aerosols may occur (exposure risks increased when no hood is used)	Prob \ Exposure: VR / R / U / O / **F** / C AC: Low / Mod / Subs / Subs / High / VH QP: Low / Low / Mod / Mod / Subs / High UP: Low / Low / Low / Mod / Mod / Subs **RP**: Low / Low / Low / Low / **Low** / Mod C: Low / Low / Low / Low / Low / Low PI: Low / Low / Low / Low / Low / Low
Plating Cells on Agar : This task can create aerosols for the person doing the task and anyone whose "breathing zone" is nearby. If an experienced person is flaming the loop/spreader, this should not occur. Hence it is important for students and inexperienced people to be taught the correct technique of flaming using cultures that do not have a route of infection via aerosol production.	Microbiological	Training before doing task using aseptic techniques to prevent contact with bacteria, ensure loop/spreader has extinguished before dipping in ethanol, cooling down of the loop/spreader before contacting cultures.	Loop/spreader has not cooled down and aerosol is created. Loop/spreader is flamed and then placed in ethanol and catches on fire.	Contact with, or exposure to, biological factors.	**Minor :** many factors would need to come into play for a severe infection to be caused by aerosol from flaming. Given that very low risk organisms are to be used by inexperienced people, the risk is minimal with little chance of substantial consequence.	**Occasional :** other factors such as necessary dosage for that particular organism for the aerosol to cause infection would need to be in place.	**Conceivable :** this will also vary dependent on several factors eg. individual persons susceptibility.	Prob \ Exposure: VR / R / U / **O** / F / C AC: Low / Low / Low / Low / Mod / Subs QP: Low / Low / Low / Low / Low / Mod UP: Low / Low / Low / Low / Low / Low RP: Low / Low / Low / Low / Low / Low **C**: Low / Low / Low / **Low** / Low / Low PI: Low / Low / Low / Low / Low / Low

SPARQ-ed Risk Assessment Sheet : Bacterial Work (continued)

Description of Risk	Hazard				Analyse / Evaluate Risk			Overall Risk Category (see explanation on last page)
	Source	Current Controls	Event	Category	Consequences	Exposure	Probability	
Centrifugation of Bacterial Cultures : Bacterial broth cultures are centrifuged to wash, concentrate or extract plasmids.	Microbiological	Unbreakable plastic centrifuge tubes, appropriate tube for the speed that is going to be used. Sealed lid to prevent aerosol escape.	Tube collapse during spin, liquid spills or aerosol escape due to lid not securely tightened.	Contact with, or exposure to, biological factors.	**Minor :** risk groups 1 and 2 are low risk and therefore can be treated.	**Unusual :** bacterial work performed irregularly.	**Remotely Possible :** possible with improper technique	Prob \ Exposure: VR R **U** O F C AC: Low Low Low Low Mod Subs QP: Low Low Low Low Low Mod UP: Low Low Low Low Low Low **RP**: Low Low **Low** Low Low Low C: Low Low Low Low Low Low PI: Low Low Low Low Low Low
Growing Bacteria in Shaking Broth Cultures : Many bacteria require aeration to grow and are shaken on a platform shaker to incubate overnight. Smaller mini-preparations are often performed in plastic tubes with caps loosened and gently held down by tape. Care must be taken to ensure lids/caps are still secure and will not fall off during rotation.	Microbiological	Training in performing the procedures. Caps should be loosened and taped down to tube to prevent cross-contamination and aerosol production.	Aerosol could contaminate the incubator room or splash on user if caps come off during incubation. Incident category exposure to biological factors	Contact with, or exposure to, biological factors.	**Minor :** exposure to GMO's - exempt and NLRDs. Risk Groups 1 and 2 are low risk and therefore can be treated.	**Unusual :** Has occurred, but not in SPARQ-ed laboratory.	**Conceivable** : No infections known to have occurred from this type of exposure.	Prob \ Exposure: VR R **U** O F C AC: Low Low Low Low Mod Subs QP: Low Low Low Low Low Mod UP: Low Low Low Low Low Low RP: Low Low Low Low Low Low **C**: Low Low **Low** Low Low Low PI: Low Low Low Low Low Low
Vortexing of Bacterial Suspension : Vortex mixers used to create bacterial suspensions in growth media or with other solutions. This is primarily done in capped plastic culture tubes or eppendorfs.	Microbiological	There should be a delay (5 minutes) before opening the bottle or eppendorf tube to minimize risk of exposure to aerosol or the opening could be done in biohazard cabinet. Also special care should be taken when opening the lid to eppendorf tubes - they can be tight and give way suddenly and when opening the lid a drop of the suspension can flick out.	Tube lids flick open or break and exposure to bacterial culture as a liquid or aerosol occurs - skin contact, inhalation.	Contact with, or exposure to, biological factors.	**Minor :** exposure to GMO's - exempt and NLRDs. Risk Groups 1 and 2 are low risk and therefore can be treated.	**Unusual :** sometimes occurs - such as dislodging of lid and exposure.	**Remotely Possible :** possible for this to occur, however participants are supervised by well-trained staff and PPE worn.	Prob \ Exposure: VR R **U** O F C AC: Low Low Low Low Mod Subs QP: Low Low Low Low Low Mod UP: Low Low Low Low Low Low **RP**: Low Low **Low** Low Low Low C: Low Low Low Low Low Low PI: Low Low Low Low Low Low
Innoculating Bacteria from Agar Plates, Cultures, Frozen Glycerol Stocks : Transformed bacteria (from ligations) or streaked out cultures grow as colonies. The bacteria are harvested using a pipette tip which is then dipped into LB growth medium contained within a plastic eppendorf or tube. This task is associated with the use of pipette tips (sharps) risks - see separate risk assessments	Microbiological	PPE worn (labcoat, gloves and closed footwear). Supervision & training from experienced personnel in the use of risk 1 & 2 organisms using aseptic techniques to prevent contact with bacteria. Minimizing aerosol formation when spreading bacteria onto plates. Immediate antiseptic cleaning of contaminated area if contact occurs with bacteria.	Contact with bacteria can result in infection	Contact with, or exposure to, biological factors.	**Minor :** pipette tips used so consequence of exposure to recombinant organisms through skin contact is minor. during recombinant DNA/RNA projects.	**Occasional :** bacterial inoculation is occasional	**Practically Impossible :** not likely - a puncture or skin exposure may take place with scraping frozen, liquid or colony stocks.	Prob \ Exposure: VR R U **O** F C AC: Low Low Low Low Mod Subs QP: Low Low Low Low Low Mod UP: Low Low Low Low Low Low RP: Low Low Low Low Low Low C: Low Low Low Low Low Low **PI**: Low Low Low **Low** Low Low

SPARQ-ed Risk Assessment Sheet : Bacterial Work (continued)

Description of Risk	Hazard				Analyse / Evaluate Risk			Overall Risk Category (see explanation on last page)
	Source	Current Controls	Event	Category	Consequences	Exposure	Probability	
Open Flame / Use of Bunsen Burner : A portable bunsen burner is used to flame sterilise metal loops or glass spreader with 70% ethanol before plating agar plate. staff are trained in the technique open flame could result in burns to the user or surrounding environment. the burns can range from redness and swelling to more serious blistering. Contact or exposure to heat and cold	Thermal	Participants are trained in the technique.	Open flame could result in burns to the user or surrounding environment. The burns can range from redness and swelling to more serious blistering.	Contact or exposure to heat and cold.	**Substantial :** serious burns - a fire could destroy equipment and laboratory.	**Occasional :** several times a month or less.	**Remotely Possible :** could happen if improper technique. Working around open flame with caution should minimise risk.	

Prob	Exposure					
	VR	R	U	**O**	F	C
AC	Low	Mod	Subs	Subs	High	VH
QP	Low	Low	Mod	Mod	Subs	High
UP	Low	Low	Low	Mod	Mod	Subs
RP	Low	Low	Low	**Low**	Low	Mod
C	Low	Low	Low	Low	Low	Low
PI	Low	Low	Low	Low	Low	Low

Description of Risk	Hazard				Analyse / Evaluate Risk			Overall Risk Category (see explanation on last page)
	Source	Current Controls	Event	Category	Consequences	Exposure	Probability	
Use of Genetically Modified Organisms : Genetically modified bacteria contain a plasmid(s) that are independently replicable and encode genes including for antibiotic resistance. The release of such an organism may mean that it spreads to the environment. The use of PC 2 facilities and procedures are to prevent the unintentional release of such an organism.	Microbiological	Training / supervision includes UQ PC2 training and induction at DI. PPE worn (gloves, lab coat, safety glasses) written procedures and signs. No gloves or lab coats outside of PC2 area. Triple packaging of GMO outside PC2 area.	Unintentional release of GMO, spread of antibiotic resistance into the environment (including ourselves), spread of genes not natural to those organisms.	Contact with, or exposure to, biological factors.	**Minor :** general microbiological agents used are not specifically hazardous.	**Unusual :** no more than once a month.	**Conceivable :** Unlikely - participants are supervised by well-trained staff.	

Prob	Exposure					
	VR	R	**U**	O	F	C
AC	Low	Low	Low	Low	Mod	Subs
QP	Low	Low	Low	Low	Low	Mod
UP	Low	Low	Low	Low	Low	Low
RP	Low	Low	Low	Low	Low	Low
C	Low	Low	**Low**	Low	Low	Low
PI	Low	Low	Low	Low	Low	Low

Description of Risk	Hazard				Analyse / Evaluate Risk			Overall Risk Category (see explanation on last page)
	Source	Current Controls	Event	Category	Consequences	Exposure	Probability	
Transport of Bacterial Culture : It is possible that bacterial cultures (especially with larger preparations) are spilled during transport from incubator to working area.	Mechanical	Transport in a lockable container (esky with wheels).	Liquid spills from large bacterial culture exposure to infectious organisms either by direct contact or aerosol can result in infection.	Contact with, or exposure to, biological factors.	**Minor :** exposure to risk group 1 and 2 is minor.	**Unusual :** this procedure does not occur very often.	**Conceivable :** unlikely to happen if using lockable containers to transport bacterial cultures around	

Prob	Exposure					
	VR	R	**U**	O	F	C
AC	Low	Low	Low	Low	Mod	Subs
QP	Low	Low	Low	Low	Low	Mod
UP	Low	Low	Low	Low	Low	Low
RP	Low	Low	Low	Low	Low	Low
C	Low	Low	**Low**	Low	Low	Low
PI	Low	Low	Low	Low	Low	Low

Explanation of Overall Risk Categories

Exposure					
Very Rare (VR) – extremely rare (has not yet occurred	**Rare (R)** – hardly every occurs (but has been known to occur)	**Unusual (U)** – does not occur often (from once per month to once per year)	**Occasional (O)** – sometimes occurs (from once per week to once per month)	**Frequent (F)** – occurs often (approximately once daily)	**Continuous (C)** – occurs repeatedly (or many times daily)

Probability (Prob)					
Almost Certain (AC) – is the most likely and expected result if the hazard event takes place	**Quite Possible (QP)** – is quite possible, not unusual, has an even 50/50 chance	**Unlikely but Possible (UP)** – Would be an unusual sequence or coincidence	**Remotely Possible (RP)** – would be a remotely possible coincidence	**Conceivable (C)** – has never happened after many years of exposure, but is conceivably possible	**Practically Impossible (P)** – has never happened after many years of exposure and is virtually impossible

Overall Risk Category				
Low – risk is normally acceptable	**Moderate (Mod)** – should be dealt with as soon as possible but situation is not an emergency	**Substantial (Subs)** – should receive attention as soon as possible	**High** – immediate correction required	**Very High (VH)** – immediate correction required

The Overall Risk Category for each element is highlighted in **green**.