Biosciences

Risk Assessment for the growth and manipulation of *Acinetobacter baumanii*

Date reviewed:	11/12/18 11/05/21	**Reviewer:**	Rick Titball Julie Fletcher	**Revision required?**	
Nature of revision:	Addition of section 4.8 – Isolation of bacteriophage with activity against *A. baumanii* (JF)				

1. Responsibilities

1.1 Academic supervisors are responsible for authorising this procedure and ensuring that this procedure is implemented and complied with.

1.2 Individuals working with *A. baumanii* strains are responsible for ensuring that they read and understand the risk assessment and for complying with any duties or control measures.

2. Related documentation and other procedures

2.1 School of Biosciences "Code of Safety Practice"

2.2 Other relevant risk assessments

3. The Risk Assessment

3.1 Agent Specific Information

Acinetobacter baumanii is an ACDP category 2, Gram-negative bacterium. It is an opportunistic bacterial pathogen primarily associated with hospital-acquired infections. The recent increase in incidence, largely associated with infected combat troops returning from conflict zones, coupled with a dramatic increase in the incidence of multidrug-resistant (MDR) strains, has significantly raised the profile of this emerging opportunistic pathogen

3.2 Identification

Acinetobacter baumannii is a Gram-negative bacillus that is aerobic, pleomorphic and non-motile. Acinetobacters may be identified presumptively to the genus level as Gram-negative, catalase-positive, oxidase-negative, non-motile, non-fermenting coccobacilli. However, the organisms are often difficult to de-stain and, as such, are often incorrectly identified as Gram-positive. There is no definitive metabolic test that can distinguish Acinetobacters from other non-fermenting Gram-negative bacteria but strains can be confirmed as members of the *A. baumannii* complex using an API 20NE Kit. More advanced molecular diagnostic methods have been developed for identification of *Acinetobacters* to the species level. For example, using blaOXA-51-like gene tracking and a multiplex polymerase chain reaction (PCR) method using *gyrB*-directed primers (Higgins et al., 2010).

3.3 Pathogenicity & Virulence

A. baumannii is found only rarely as part of the normal skin microflora, with one study estimating that only 3% (at most) of the population are colonized by the bacterium. Acinetobacter may "colonize" or live in a patient without causing infection or symptoms, especially in tracheostomy sites or open wounds. Acinetobacter can be spread to susceptible persons by person-to-person contact or contact with contaminated surfaces. There is no evidence of airborne infection. Acinetobacter may survive in the environment for several days. Careful attention to infection control procedures, such as hand hygiene and environmental cleaning, can reduce the risk of transmission.

As a pathogen, *A. baumannii* specifically targets moist tissues such as mucous membranes or areas of the skin that are exposed, either through accident or injury. Acinetobacter causes a variety of diseases, ranging from pneumonia to serious blood or wound infections, and the symptoms vary depending on the disease. Acinetobacter poses very little risk to healthy people. However, people who have weakened immune systems, chronic lung disease, or diabetes may be more susceptible to infections with Acinetobacter. An opportunistic pathogen, *A. baumannii* has a high incidence among immunocompromised individuals, particularly those who have experienced a prolonged (> 90 d) hospital stay. Very ill patients on a ventilator, those with a prolonged hospital stay, those who have open wounds, or any person with invasive devices like urinary catheters are at greater risk for Acinetobacter infection.

Decisions on treatment of infections with Acinetobacter should be made on a case-by-case. Acinetobacter infection typically occurs in ill patients and can either cause or contribute to death in these patients. First line agents for susceptible organisms include a broad-spectrum cephalosporin (ceftazidime or cefepime), a combination beta-lactam/beta-lactamase inhibitor (i.e. one that includes sulbactam), or a carbapenem (eg, imipenem, meropenem, or doripenem). Carbapenems are highly bactericidal against susceptible strains of Acinetobacter.

Acinetobacter is often resistant to many commonly prescribed antibiotics. The following definitions were established based on the extent of resistance to antibiotics that would otherwise serve as treatments for Acinetobacter (ie, cephalosporins, fluoroquinolones, and carbapenems):
●Multidrug-resistant: isolate is non-susceptible to at least one agent in three or more antibiotic classes
●Extensively drug-resistant: isolate is non-susceptible to at least one agent in all but two or fewer antibiotic classes
●Pandrug-resistant: isolate is non-susceptible to all agents

Higgins, P. G., Lehmann, M., Wisplinghof, H., and Seifert, F. H. (2010). gyrB multiplex PCR to differentiate between *Acinetobacter calcoaceticus* and *Acinetobacter* genomic species 3. J. Clin. Microbiol. 48, 4592–4594.

Howard A, O'Donoghue M, Feeney A, Sleator RD. (2012) *Acinetobacter baumannii*: an emerging opportunistic pathogen. Virulence. 3:243-50

3.4 Infectious Dose
Not known.

3.5 Host range
Opportunistic pathogen in immunocompromised/hospitalised humans.

3.6 Laboratory Acquired Infection
To our knowledge, there have been no reports of laboratory-acquired *A. baumanii* infection.

3.7 Environmental Impact
A. baumanii is a cosmopolitan, but rare microorganism in the environment.

4 Process Descriptions

4.1 Growth of *A. baumanii* strains
Stocks of the agent will be stored at -80°C in cryotubes containing media and glycerol.

1. Suitable growth media include nutrient agar/broth, Luria-Bertani medium and tryptone-soya broth (TSB).
2. Streak bacteria on to an agar plate from a -80°C stock. Grow overnight aerobically at 37°C or if required, store streak plate at 4°C for short periods of time (approximately 1 week).
3. Pick a single colony from the streak plate into broth or re-suspend bacteria into PBS and inoculate plastic sterilins. Grow overnight at 37°C aerobically.
4. Inactivation can be performed using hypochlorite-based solutions. Decontamination of surfaces can be performed with alcohol-based products.
5. To sterility check materials, Inoculate 10% or 5 ml (whichever is the lesser) of the processed bacterial suspension into liquid broth (e.g. L-broth), incubate for at least 3

days. Plate the incubated broth onto agar and incubate for a further three days. The limit of detection is 10-100 viable organisms.

4.5 Isolation, PCR and electrophoresis of DNA.
See "Risk Assessment for general bacteriological methods at CL2"

4.6 PCR of DNA
See "Risk Assessment for general bacteriological methods at CL2"

4.7 Infection of *G. mellonella* larvae
See "Safe handling of insect larvae *Galleria mellonella*, *Manduca sexta*, *Chilecomadia moorei* (Butterworms), *Hermetia illucens* (Phoenix worms), *Tenebrio molitor* (Mealworms) and *Pachnoda marginata* and challenge with ACDP category I/II microorganisms"

4.8 Isolation of bacteriophage with activity against *A. baumanii*

4.8.1 Phage enrichment
Samples such as river water, animal faeces and raw sewage will be collected from the environment and filtered through a 0.22 µm filter. Separate risk assessments are available for these processes (Working with animal faeces, Working with raw sewage). The filtrates will be incubated with *A. baumanii* in a 96 deep well plate. The plate will be covered with a plate seal and incubated at 30°C/37°C with shaking. Following this initial enrichment step, the deep well plate will be transferred to the biological safety cabinet (Class 2). This is to prevent any potential release of phage aerosols into the lab environment which could result in potential contamination of lab cultures. 200 µL from each well will be transferred into a 96 well filter plate on top of a regular 96 well plate. The plate stack will be placed in a safety centrifuge cup or sealed with parafilm and centrifuged (900 g, 4 minutes). A second enrichment step with *A. baumanii* will be carried out and the resulting filtrates used in spot assays. Filtrates containing potentially active phage will be handled in the safety cabinet.

4.8.2 Spot plaque assays
A lawn of *A. baumanii* will be created by mixing an overnight culture with molten top agar and pouring over the surface of bottom agar plates. Once the top agar has set, the plates will be transferred to the biological safety cabinet (Class 2) and the (phage) filtrates will be spotted onto the surface. The lids will be left off of the plates to allow the drops to dry. Once dry, the lids will be replaced and the stack of plates taped together. The plates will be placed in a plastic storage box with holes in the lid and incubated at 37°C. Following incubation any resulting plaques will be picked using a pasteur pipette and placed into buffer. This will be carried out in the safety cabinet. Any plates containing phage that need to be transferred to the fridge will be sealed with parafilm.

Any spillages of *A. baumanii* will be decontaminated with 70% ethanol and the contaminated blue roll placed directly in the autoclave waste bin. Spent cultures will be autoclaved as described in section 10.1. Any spillages of (phage) filtrates will be decontaminated with 70% ethanol and the contaminated blue roll placed in a plastic bag prior to placing in the autoclave waste bin. Any plasticware containing (phage) filtrates which are no longer required will be placed in a plastic bag prior to placing in the autoclave waste bin.

5. Hazard, risk and control measures

Risk Rating	Action Required
Minimal	Controls Adequate

	(Unlikely harm would ever result from the activity)
Low	Review Controls, take action as necessary. (Harm would seldom result)
Medium	Action to be taken to reduce risk. (It is reasonably possible that harm could result)
High	Urgent action required. Consider halting activity/process. (It is certain or almost certain that could result)

Hazard	Exposure to *A. baumanii* strains. Any procedures which could result in contact with broken skin poses the greatest risk. Low Risk in healthy workers.
Control measures	Handle under ACDP 2 conditions, by individuals who have been trained in microbiological practices and work according to the School of Biosciences "Code of Safety Practice". In the event of a spill, swab with 70% ethanol. Wear gloves as required, particularly if worker has broken skin on hands.
Risk	**Low in healthy workers** **Medium or high in immunocompromised or severely injured individuals**

Hazard	Exposure to hazardous chemicals
Control measures	See "Risk Assessment for general bacteriological methods at CL2"
Risk	**Low**

5.1 Where and under what circumstances will the work be done.

All procedures detailed above will be carried out within a designated ACDP containment level (CL) 2 laboratory.

6. Who might be affected?

1. Staff competent to work at CL2 or those under their direct supervision.
2. Individuals not directly involved with the process/procedure/equipment etc but who may be affected; for example co-workers, students, contractors, cleaners etc.
3. Any individual who enters the laboratory whilst work is ongoing. Individuals working in close proximity.
4. Individuals working in the workplace who are likely to be at increased risk include
   - Pregnant workers or those who are breast feeding
   - Young persons (ie < 18 years of age)
   - Persons on work experience or training schemes
   - Temporary employees
   - Contractors
   - Lone workers
   - Individuals with known hypersensitivity to antibiotics added to media
   - Individuals receiving antibiotic therapy
   - Immunocompromised individuals (specifically cystic fibrosis patients)

SEE HEALTH SURVEILLANCE BELOW

7 Information, instruction and training

Individuals should be trained in the safe use of this microorganism.

8 Health surveillance

Certain groups of individuals will be excluded from working with *A. baumanii* . Essentially, this includes any staff member with systemic immune suppression (either as a result of immunosuppressive agents or as a consequence of disease/infection). *A. baumanii* may also cause infection if there is local disruption of the immune system (e.g. broken skin). Consequently, staff who have breaks in their skin will be required to have barrier protection where possible (e.g. gloves), or will be excluded from working with the organism until break in skin has healed.

9. Emergency Procedures

9.1 Spillages

Any spillage of biological materials should be absorbed onto tissue and placed in an autoclave bag for autoclaving (see "10.1 Autoclaving of waste"). Decontaminate the affected area with 70% ethanol. In normal use the risk to the environment is zero because working practices prevent the organisms from escaping.

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9.2 Personal contamination

In the event of personal contamination with biological materials, remove any contaminated clothing as quickly as possible.

Eye contact: Wash exhaustively with the emergency eye bath.
Skin contact: Wash with soap and cold water.
Mouth contact: Flush mouth exhaustively with water. Avoid ingestion
Puncture wounds: Encourage to bleed. Wash minor cuts and similar lesions with soap and water before applying a dressing as required.

Medical advice must be sought if there is a risk of infection.
Also see School of Biosciences "Code of Safety Practice"

10 Safe disposal

10.1 Autoclaving of waste

All waste containing contaminated material will be autoclaved using a destruct cycle, at 130^{o}C for at least 25min, except where it contains formaldehyde or organic solvents. Records of the load (details of load and chart records of the temperature profile will be kept). The autoclave will be serviced and validated annually using thermocouples. Waste containing anything other than trace amounts of formaldehyde or organic solvents should not be autoclaved (see below for disposal method).

10.2 Contaminated waste which cannot be autoclaved

Procedures for disposal of biological waste containing organic solvents or formaldehyde are outlined below. These must be read in conjunction with procedure- and chemical-specific information contained within the relevant sections of the "Risk Assessment for general bacteriological methods at CL2".

10.2.1 Anything other than trace amounts of organic solvents must not be autoclaved and should be stored in a suitable waste container in a solvents cupboard for treatment with a biocide and subsequent incineration. Solvent waste which cannot be autoclaved

should be treated with 10% v/v Teknon Biocleanse for at least 24 hr, and then removed from the laboratory for incineration.

10.2.2. Solutions containing formaldehyde should not be autoclaved. Waste formaldehyde should be stored in a suitable waste container in a solvents cupboard and sterility checked, before removal from the laboratory for incineration.