Biosciences

Risk Assessment for the growth and manipulation of *Pseudomonas aeruginosa*

Date reviewed:	16/02/18 14/11/19 11/05/21	**Reviewer:**	Josh Dyer Julie Fletcher Julie Fletcher	**Revision required?**	Yes Yes Yes
Nature of revision:	Addition of section 4.4.1 and 4.4.2 – Microscopy of *P. aeruginosa*-infected J774 & HL-60 (mammalian) cells (JD) Addition of section 4.4.3 – Microscopy of live biofilms of *P. aeruginosa* (JF) Addition of section 4.5 – Isolation of bacteriophage with activity against *P. aeruginosa* (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 *P. aeruginosa* 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

Pseudomonas aeruginosa is an ACDP category 2, Gram-negative bacterium. It is widespread in the environment, in both land and water, and is metabolically versatile. Healthy adults with a competent immune system are considered to be at negligible risk for infection with *P. aeruginosa*. However, the organism is an important opportunistic pathogen for humans, animals and plants, producing severe infection in immunocompromised hosts (particularly HIV patients and burns patients). In the human host, *P. aeruginosa* is implicated in respiratory infections, urinary tract infections, gastrointestinal infections, keratitis, otitis media and bacteremia. *P. aeruginosa* is the major factor for morbidity and mortality in individuals with cystic fibrosis (CF). Certain strains of *P. aeruginosa* have a propensity for patient-to-patient transmission, most extensively observed within CF patients.

3.2 Identification

Typically, *P. aeruginosa* can be identified using commercially-available bacterial identification systems such as the API20NE system. Positive identification can also be presumed by growth on *Pseudomonas* selective agar – *Pseudomonas* Agar Base (Oxoid; CM0559) supplemented with *Pseudomonas* CN Selective Supplement (Oxoid; SR0102). Typical *P. aeruginosa* strains will exhibit distinctive pigmented (green-blue) growth on this agar. *P. aeruginosa* species-specific and *Pseudomonas* genus-specific PCR assays are also available.

3.3 Pathogenicity & Virulence

Whilst *P. aeruginosa* almost never infects uncompromised tissues, there is hardly any tissue that it cannot infect if the tissue defences are compromised in some manner. Most commonly, *P. aeruginosa* causes urinary tract infections, respiratory infections and soft tissue infections. Systemic infections are a particular problem in patients with severe burns and those who are immunosuppressed (e.g. cancer and AIDS patients). Cystic fibrosis patients are particularly susceptible to *P. aeruginosa* infection – it is the most frequent and important pathogen responsible for chronic infection of the CF lung, and is associated with increased morbidity and mortality. Initial isolates of *P. aeruginosa* from the CF lung are nonmucoid and generally sensitive to antimicrobials. As a result, these early infecting strains can be eradicated by aggressive antibiotic treatment. However, over time, a mucoid antibiotic-resistant phenotype develops which is associated with accelerated pulmonary deterioration and mortality. Chronic mucoid *P. aeruginosa*, characterized by the production of the exopolysaccharide alginate and growth in biofilms within the lung, is usually impossible to eradicate.

In addition to alginate, which is observed exclusively in the course of CF lung infection, *P. aeruginosa* possesses numerous other virulence factors, including secreted factors such as elastase, proteases, phospholipase C, hydrogen cyanide, exotoxin A and exoenzyme A.

Many of these factors are quorum-sensing regulated. Biofilm formation is also a significant part of the *P. aeruginosa* infection process.

A variety of effective antibiotics are available treatment of *P. aeruginosa* infections, including quinolones (e.g. ciprofloxacin), aminoglycosides (e.g. gentamicin), anti-pseudomonal cephalosporins (e.g. ceftazidime), anti-pseudomonal carbapenems (e.g. meropenem), and polymyxins. Usually, two antimicrobials with different modes of action are used together. Effective vaccines are not currently available.

3.4 Infectious Dose

Not known.

3.5 Host range

Opportunistic pathogen in immunocompromised humans.

3.6 Laboratory Acquired Infection

To our knowledge, there have been no reports of laboratory-acquired *P. aeruginosa* infection.

3.7 Environmental Impact

P. aeruginosa is a ubiquitous microorganism in the environment.

4 Process Descriptions

4.1 Growth of *P. aeruginosa* 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.

4.2 Isolation, PCR and electrophoresis of DNA.

See “Risk Assessment for general bacteriological methods at CL2”

4.3 Isolation and Electrophoresis of proteins

See “Risk Assessment for general bacteriological methods at CL2”

4.4 Microscopy

4.4.1 Microscopy of fixed *P. aeruginosa*-infected J774 & HL-60 (mammalian) cells

P. aeruginosa infected-cell imaging should be carried out to ensure there is low risk to other microscope users. Cells infected with *P. aeruginosa* mounted onto microscope slides should be sealed with a coverslip and a suitable fixing agent such as nail polish or VECTASHIELD®. Slides should be maintained in a sealed box during transportation to Bioimaging facility. Alternatively, cells may be grown on tissue culture plates then infected with *P. aeruginosa*. Prior to transport to Bioimaging facility, the infected cells should be fixed using a suitable fixing agent such as VECTASHIELD® and the lid of the tissue plate sealed with parafilm. Tissue culture plates should be maintained in a sealed box during transportation to Bioimaging facility. After microscope analysis is completed the area should be decontaminated with 70% ethanol if necessary. All samples should be removed from the Bioimaging facility after microscopical observation. Glass slides should be discarded in suitable sharps bin and tissue culture plates disposed of according to protocols described in section 10.

4.4.2 Microscopy of live *P. aeruginosa*-infected J774 & HL-60 (mammalian) cells

Cells will be grown on tissue culture plates then infected with *P. aeruginosa*. Prior to transport to Bioimaging facility, the tissue culture's lid should be sealed with parafilm. Tissue culture plates should be maintained in a sealed box during transportation to Bioimaging facility. After microscope analysis is completed the area should be decontaminated with 70% ethanol if necessary. All live samples should be removed from the Bioimaging facility after microscopical observation and disposed of according to protocols described in section 10.

4.4.3 Microscopy of live biofilms of *P. aeruginosa* on the surface of titanium and stainless steel coupons

Bacterial biofilms of *P. aeruginosa* will be grown on the surface of stainless steel and titanium coupons. Prior to transport to the Bioimaging facility, plates will be sealed with parafilm and placed in a sealed box. After microscope analysis is completed the area should be decontaminated with 70% ethanol if necessary. All live samples must be removed from the Bioimaging facility after microscopical observation and disposed of according to protocols described in section 10.

4.5 Isolation of bacteriophage with activity against *P. aeruginosa*

4.5.1 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 *P. aeruginosa* 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 *P. aeruginosa* will be carried out and the resulting filtrates used in spot assays. Filtrates containing potentially active phage will be handled in the safety cabinet.

4.5.2 Spot plaque assays

A lawn of *P. aeruginosa* 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 *P. aeruginosa* 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 *P. aeruginosa* strains. Any procedures which could result in ingestion, inhalation and contact with broken skin poses the greatest risk. Medium Risk
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 after measures described above are taken**

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 *P. aeruginosa*. Essentially, this includes any staff member with systemic immune suppression (either as a result of immunosuppressive agents or as a consequence of disease/infection), and individuals with cystic fibrosis. *P. aeruginosa* 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 25 min, 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.