Biosciences

Risk Assessment for the growth and manipulation of non pathogenic *Escherichia coli* (*E. coli*) species and their related bacteriophages

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Nature of revision:	Sections 9 & 10 (Safety procedures & Disposal of Waste)				

Risk Assessment for the growth and manipulation of *Escherichia coli* strains and related bacteriophages

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 *E. coli* and *E. coli* bacteriophages (coliphages) 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

Strains of *E. coli* that cause disease or have the potential to cause disease in humans are not covered in this risk assessment. These include O157:H7, O121 and O104:H21 and EPEC, EHEC, EAggEC etc. strains which have been implicated in severe diarrhea disease. This also includes any *E. coli* strains that have been isolated from clinical specimens. Strains that are covered in this risk assessment are those that are "lab adapted" i.e. they have lost their ability to colonise the intestine and survive outside laboratory conditions. It does include those that have been engineered to temperature sensitive and carry certain plasmids e.g. pLys S, a lysozyme producing plasmid. It also covers handling of *E. coli* host strains that are used for general cloning and protein expression (details of which are covered in a separate Class I GMMO risk assessment entitled “Routine cloning and expression of genes from ACDP Hazard group 2 pathogens using attenuated *Escherichia coli*”).

Serotypes of *E. coli* belong to the family Enterobacteriaceae and are found as part of the normal commensal gut flora. They are Gram-negative, facultative anaerobic and non-sporulating. Growth can be by aerobic and anerobic respiration. In anaerobic conditions *E. coli* produces lactate, succinate, ethanol, acetate and carbon dioxide through the process of mixed-acid fermentation. Typically, *E. coli* are 2 µm in length and 0.5 µm in diameter with a cell volume of 0.6 - 0.7 μm^3. Strains that are motile have peritrichous arrangement of flagella. Optimal growth for most strains occurs at 37 °C. Laboratory adapted *E. coli* strains possess the ability to transfer DNA via the methods of transduction, transformation or bacterial conjugation.

Due to its normal commensal role in the gut and the fact that laboratory cultivated strains of *E. coli* have not retained the ability to colonise humans they are classified as an ACDP category 1 organism.

Coliphages are ubiquitous viruses found in the same environments as their host, including waste water, rivers, and the mammalian gut. Some commonly studied coliphages include T-even phages, phage λ, and MS2. They appear in a variety of structures and sizes; most range between 25-110 nm. All carry some form of nucleic

acid and protein. Coliphages can be classified as either virulent (displaying solely the lytic cycle) or temperate (exhibiting both lytic and lysogenic cycles). Optimal growth occurs at 37 °C.

Coliphages may also be classified as an ACDP category 1 organism, as they only infect *E. coli* and are therefore harmless to humans.

3.2 Identification

Growth of *E. coli* and coliphages occurs optimally at 37 °C though growth at other temperatures may occur. *E. coli* are Gram negative rods and have no particular cell arrangement. It is lactose positive and forms deep red colonies on MacConkey agar. It produces black colonies with a greenish-black metallic sheen when grown on Levine EMB agar. *E. coli* is Lysine positive, indole positive, methyl red positive, VP negative, citrate negative and oxidase negative. When grown on LB agar *E. coli* forms opaque shiny domed cream colonies. Combination of biochemistry (commercial kits available) and serotyping required for full identification.

The presence of virulent coliphages may be determined using a plaque assay of *E. coli*, although full identification requires electron microscopy.

3.3 Pathogenicity & Virulence

Laboratory-adapted strains of *E. coli* are by their nature unable to colonise humans due to mutations in various genes e.g. rfb-50 mutation is an IS5 insertion that results in the absence of O-antigen synthesis in *E. coli* strain K12 and its derivatives. Other strains have mutations in metabolic genes e.g. ilcG mutation is a frameshift mutation that knocks out acetohydroxyl acid synthase II. Thus laboratory strains are non-pathogenic.

Coliphages are unable to infect eukaryotic cells, and are thus non-pathogenic. Numerous studies have been conducted on the therapeutic use of phages to treat bacterial infection. Doses as high as 10^{11} pfu were orally administered to patients with no serious side effects reported. Furthermore, phages are highly specific to their host; therefore, disruption of the normal gut flora should not occur, if ingested.

3.4 Infectious Dose

Due to their non-pathogenic properties it is impossible to determine an infectious dose.

3.5 Host range

E. coli is found as a commensal in the human gut and found in the intestinal lumen of many animals. It has a broad host range.

Coliphages infect numerous strains of *E. coli* and are found inhabiting similar environments as their hosts.

3.6 Laboratory Acquired Infection

No cases of laboratory acquired infection for non-pathogenic *E. coli* nor its bacteriophages have been described.

3.7 Environmental Impact

Non-pathogenic strains are part of the normal flora of the gut and produce vitamin K2 which benefits their hosts. *E. coli* are not always confined to the intestine and have the ability to survive in the environment for brief periods. However, many laboratory-adapted strains are unable to survive in the environment.

Coliphages are found in a wide range of environments and are deemed harmless to all eukaryotic organisms.

4 Process Descriptions

This risk assessment covers the growth of *E. coli* on agar plates and in broth in volumes up to 1 litre. Cells harvested by centrifugation may be processed to isolate proteins or DNA for subsequent analysis.

This risk assessment also covers the growth of coliphages on agar plates seeded with *E. coli* and in broth in volumes up to 1 litre. A soft-agar/host overlay method may be used to propagate and enumerate coliphages. Broth propagation methods may also be used.

The risk assessment covers the growth of *E. coli* and its related bacteriophages. It does not cover the isolation and purification of toxins (which is detailed in a separate GMMO risk assessment entitled, “Cloning bacterial toxins”).

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 *E. coli* and/or coliphages. Any procedures which could result in ingestion or accidental inoculation through the skin pose the greatest risk. Low Risk
Control measures	Whilst all strains covered by this risk assessment are ACDP category 1, they will be routinely handled within our ACDP containment level (CL) 2 laboratory, 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, surfaces will be wiped with 70% ethanol. Gloves will be worn at all times in the laboratory to reduce the likelihood of transmission to a minimum.
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?

- Staff competent to work at CL2 or those under their direct supervision.
- Individuals not directly involved with the process/procedure/equipment etc but who may be affected; for example co-workers, students, contractors, cleaners etc.
- Any individual who enters the laboratory whilst work is ongoing. Individuals working in close proximity.
- 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
    - Immunosuppressed individuals (individuals should seek professional medical advice before working with *E. coli*).

7 Information, instruction and training

Individuals should be trained in the safe use of this procedure

8 Health surveillance

Immunosuppressed individuals should seek professional medical advice before working with *E. coli*.

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.

11. References

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