Centre for Biological Engineering

RISK ASSESSMENT REVIEW/REVISION RECORD

Risk Assessment Ref No:	BRA/CBE/13	Version Number
		1.0

This risk assessment should be reviewed **annually** or more frequently if there is any change in the work, or if new information becomes available that indicates the assessment may no longer be valid. **This form should be attached to the front of the current version of the risk assessment or to the new version of the risk assessment if one is issued**

The following review/revision has been carried out on the dates indicated and either the assessment remains valid or it has been amended as indicated.

Name(s) of reviewer: Danny Bayliss	Date: 19/02/10
Signature:	

Amendments:

Due to delays in getting the plasma system ready and safe to use in the CBE labs plasma treatment work will be postponed until all safety aspects and SOPs have been fully satisfied.

The work I will be carrying out in the CBE lab is with the EPICS Altra Flow Cytometer and cell sorter; refer to risk assessment and SOP 081 "Use and maintenance of EPICS Altra Flow Cytometer" for correct use and safety advice. Bacterial samples will be analysed to assess the different sub populations produced from treatments and these cells will be sorted according to their different sub populations for further experiments to be conducted if required.

Specific training for the calibration, maintenance and use of the EPICS Altra Flow Cytometer will be undertaken before any work can commence and all training will be recorded in my training file available for review in the CBE office.

Samples to be transferred into and out of the CBE Laboratory Unit are constrained within the University site. Samples that have received treatments will be brought into the CBE lab as small volumes of a suspension (10ml max) in universal glass bottles. These bottles will be transported in bottle racks to ensure secure transportation of bottles with minimal damage. The bottles in the racks will be placed inside a suitable leak proof container which is sealed with autoclave tape and decontaminated with 1% Virkon and 70% IMS then placed inside a cooler box that can hold at least double the total volume of liquid inside all the bottles should any breakages occur during

transport. Material will be transferred into the Autoclave Room (H31) where the containment package will be removed from the cooler box and decontaminated with 70% IMS before transporting to H23. All transport will be subject to controlled procedures according to the local CoP and SOP005 (see below). For example, transfers will use double containment procedures as described above. Transport of research material between laboratories is done using sealed containers which are put into tube racks and trays and transported using trolleys according to the SOPs listed below. Any waste generated from analysis potentially containing viable agents is not removed from the laboratories until it has been autoclaved. Samples in the analytical room will be processed for analysis with EPICS Altra flow Cytometer inside the biological safety cabinet until ready for use. Any cell sorting will be collect on agar dishes or as small liquid suspensions and transported back out of the CBE lab to Chemical Engineering lab S128 for further analysis or culturing according to the following SOPs.

1) SOP003, "Disposal of Biological Waste"
2) SOP005, "Storage and Transport of Biological Material"
3) SOP038, "Biological Spill Response

This review or revision must be approved by the person supervising the work and the CBE Quality Manager. Significant changes may require a revised version of the risk assessment to be issued for re-approval by the local BGMSA and/ or the BSO and/or GM Safety Committee, as appropriate.

Name of Approver: PAUL HOURD	Date: 19/02/10.
Position: CBE QUALITY MANAGER	
Signature: P Hourd	
Name of Approver:	Date:
Position:	
Signature:	
Name of Approver:	Date:
Position:	
Signature:	

Name of Approver:	Date:
Position:	
Signature:	

Loughborough University

For Health and Safety Unit Use only ASSESSMENT NO. BRA/CBE/13

RISK ASSESSMENT OF WORK WITH BIOLOGICAL MATERIALS

Please note the following before completing this form:

1. University Health and Safety Policy requires that risk assessment of all work with biological materials must be carried out in advance of work commencing. A key requirement of The Control of Substances Hazardous to Health Regulations (COSHH) is to assess the risks associated with any work activity involving the use of biological materials that may contain biological agents.
2. YOU SHOULD COMPLETE ALL OF PART A, THE APPROPRIATE SECTION(S) OF PART B, AND ALL OF PART C. THIS FORM SHOULD BE SUBMITTED TO THE HEALTH AND SAFETY UNIT FOR REVIEW (VIA YOUR DEPARTMENTAL SAFETY OFFICER)
3. It is the responsibility of the Principal Investigator to ensure compliance to these requirements and that this risk assessment remains valid.
4. This risk assessment form **IS NOT** for assessing the risks associated with **Genetically Modified Organism activities.**

Date Submitted:	28/9/09	Date Approved:	28/9/09

PART A: Please provide the following general information:

School/Department			
Centre for Biological Engineering			
The Project			
Title of Project: Understanding the mode of action of atmospheric cold plasmas in decontaminating of food borne pathogens.			
Project Reference Number: BB/E528144/1			
Person responsible for this work (*Principle Investigator*):			
Name: Professor Michael Kong		Position: Supervisor	
Department: Electrical Engineering		University School: Electrical Engineering/CBE	
Person conducting this assessment			
Name: Danny Bayliss		Position: Post Graduate Researcher	
Department:	Electrical Engineering	Date Risk Assessment Undertaken:	21.09.09
Proposed Project Start Date:	01/10/2009	Proposed Project End Date:	01/10/2011

Assessment Review:
required at least once a year or immediately following any significant change to the project

	Review 1	Review 2	Review 3	Review 4	Review 5
Due Date	01/10/2010	19/02/10			

Date Conducted					

A1 PROJECT SUMMARY

A1.1 Scientific Goals of the Project *Brief yet clear outline only*

Cold Atmospheric plasmas have emerged over recent years as a potential sterilisation tool due to its advantages over current sterilisation methods. Plasmas have the ability to treat heat sensitive materials without leaving toxic chemicals behind after treatment and potentially operate in continuous processes rather than in batch. Although plasmas have been shown to inactivate a wide number of pathogens it is still unclear the mechanisms in which this is achieved. The aim of this project is to gain an understanding of how cold atmospheric plasmas inactivate food borne pathogens. Focusing on the main targets of the bacteria that may cause increases susceptibility to plasmas such as membrane lipids or proteins and also looking at the main plasma gas species that cause inactivation.

Understanding of the main bacterial targets will allow better understanding for the uses of plasma within other areas such as treatment of fresh foods, healing wounds or cancer treatment. Greater understanding can also result in the development of more efficient plasma sources that produce greater amounts of inactivating plasma gas species for improved efficacy

A1.2 Description of the Experimental Procedures

Describe laboratory procedures to be used and highlight any non-standard laboratory operations

Treatment of bacteria (*Listeria innocua* ATCC 33090 and *E.coli* Type 1 W87001 Bacillus Subtilis ATCC 6633) with cold atmospheric plasma to look at decontamination kinetics and differences in resistances by varying conditions under which bacteria are cultivated (pH, nutrient starvation, temperature) and also varying plasma treatment conditions. This will involve minimal manipulation of bacterial samples according to the attached protocol and following Standard Operating Procedures. All procedures will be conducted in accordance with the laboratory Quality Management System (QMS) requirements, Good Aseptic Technique, the local Code of Practice (COP) and the Loughborough University Biological Safety Policy.

PART B: Please provide information in one or more of the following sections, as appropriate. Only sections which you complete should be submitted:

Section 1: *micro-organisms (prions, viruses, bacteria, fungi, parasites in ACDP category 2 and pathogens controlled by the Department for the Environment, Food and Rural Affairs).* ***[Work with ACDP category 3 and 4 pathogens is not currently permitted in the University.]***

Section 2: *cell cultures, tissues, blood, body fluids or excreta*

Section 3: *plants and plant material*

Section 4: *animals and animal tissues*

SECTION 1: MICRO-ORGANISMS

B1.1 HAZARD AND RISK IDENTIFICATION: NATURE OF MICRO-ORGANISMS

*This information gives an indication of the **potential** harm that the biological material may cause*

B1.1.1 List all micro-organisms to be used

Name	Strain	ADCP cat*	Source
Listeria innocua	**ATCC 33090**	1	National culture collection
E.coli	**Type 1 W87001**	1	National culture collection
Bacillus subtilis	**ATCC 6633**	1	National culture collection

see The Approved List of Biological Agents – available on the Health & Safety website

B1.1.2 Has any strain been genetically modified in any way?

Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	NO
If Yes, complete Genetically Modified Organisms (GMO) Risk Assessment Form	

B1.2 DESCRIPTION OF RISK TO HUMANS

B1.2.1 The disease(s) caused to humans

Describe the type and severity of effects or disease(s) on human health (including colonisation, infection, allergy, toxin-mediated disease) by each of the agents or strains to be used

Indicate in the adjacent box if Not Relevant (N/R)		N/R
Name	Type	Severity
Listeria innocua ATCC 33090	Non pathogenic	N/R
E.coli Type 1	Non pathogenic	N/R
Bacillus subtilis ATCC 6633	Non pathogenic	N/R

B1.2.2 What is the likelihood of infection of this material? Indicate as None, Low Risk, Medium Risk, High Risk, Known Infected

Name of agent	Risk Category	Justification for Selection
Listeria innocua ATCC 33090	None	Non pathogenic
E.coli Type 1 W87001	None	Non pathogenic
Bacillus subtilis ATCC 3366	None	Non pathogenic
		If none proceed to section B1.3

B1.2.3 Infectivity to humans

Describe ALL the route(s)of infection (relevant to the laboratory setting) and the minimum infectious dose(s) if known (eg percutaneous, mucocutaneous, inhalation, ingestion)

Name of agent(s)	**Route(s) of infection**	**Minimum infectious dose**

B1.2.4 Drug resistance
Is there any known or suspected drug resistance amongst the strains to be used? Identify & describe.

B1.2.5 Attenuation or increased virulence
Are the strains attenuated or do they have an increased virulence in any way?

Identify and describe:

B1.2.6 Ability to survive
In what form is the agent present eg spores or vegetative bacteria, and are there any issues about the agents robustness, including any resistance to chemical disinfectants?

Identify and describe:

B1.2.7 Most hazardous procedure?
Identify and describe the most hazardous procedure(s) to be used.

B1.3 HUMANS AT INCREASED RISK OF INFECTION

B1.3.1 Are there any pre-existing medical conditions that increase the risk associated with this agents listed in section 1.1 (including immunocompromised workers, pregnant workers, breast feeding mothers, diabetic workers)?

Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	NO
If yes, Occupational Health must be consulted:	

B1.4. PROPAGATION OR CONCENTRATION OF ADVENTITIOUS AGENTS

B1.4.1 Give details of the volumes and concentrations of organisms to be used

Name & Strain	Volume	Concentration
Listeria innocua ATCC 33090	1 ml filtered onto membranes 30 membranes maximum	1 E+9 CFU/ml
E.coli Type 1	1 ml filtered onto membranes 30 membranes maximum	1 E+9 CFU/ml
Bacillus subtilis ATCC 6633	1 ml filtered onto membranes 30 membranes maximum	1 E+9 CFU/ml

B1.5 ENVIRONMENTAL CONSIDERATIONS:

B1.5.1 Are any of the agents capable of causing disease or other harm in animals, fish or plants?

Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	NO

If yes, describe briefly here (A separate risk assessment may be required if the agent to be used poses a significant risk to the environment):

B1.5.2 Will there be any other environmental risks?

Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	NO
If yes, describe briefly here (NOTE: A separate risk assessment may be required if the agent to be used poses a significant risk to the environment):	

B1.6 OTHER HAZARDS

B1.6.1 Are there any other hazards associated with this work? For example, hazardous chemicals, cryogenic gases ionising radiation.

Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	YES
If yes, identify these: Plasma produces an ionised gas, UV radiation, radicals. Procedures described in SOP062-071.	
If yes, have these been risk assessed and any necessary approval obtained? Yes. This has been reviewed by the CBE Area Safety Advisor (Bob Temple). Reference to be assigned.	

PART C: CONTROL MEASURES

C1. CONTROL MEASURES

The risk of exposure must be prevented or adequately controlled to minimise the chance of harm arising. COSHH Regulations require minimum containment measures for laboratories handling organisms from the different ACDP hazard groups (http://www.hse.gov.uk/pubns/misc208.pdf)
The hazard group number typically indicates the level of containment (includes physical measures & working practices) that must be used for its handling).

C1.1 Preventing Exposure

C1.1.1 Substitution with a Safer Alternative

Is substitution with a safer alternative practical, by for example, replacement of a clinical strain or pathogen with one that is lab adapted? Provide reasons for your answer:

The use of Listeria innocua is itself a non-pathogenic surrogate for Listeria monocytogenes. E.coli Type 1 is a non pathogenic laboratory strain to act as a surrogate E.coli 0157:H7. B.subtilis is also a non-pathogenic class 1 strain. These represent the safer alternatives of the organisms.

C1.1.2 Isolation/Segregation

(i) Is/Are the laboratory(s) to be used for this work to be shared with other workers not directly involved in this activity?

Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	YES

If yes, provide details:

Access to the Containment Level 2 CBE Laboratory Unit is restricted to authorised laboratory workers with appropriate training in accordance with documented local Code of Practice (COP) and Quality Management System (QMS) requirements for Containment Level 2 work activities involving biological materials.

The laboratories are locked at all times outside of normal working hours to ensure safe storage of biological agents and unauthorised entry. Keys to the laboratories are only issued to authorised users. Access is also restricted to the building (swipe card) and CBE (key entrance) during normal working hours. Out of Hours/Lone working is logged and permitted subject to risk assessment.

No cleaning personnel are permitted in the CBE Laboratory Unit. Access by other Non-Laboratory or maintenance personnel is subject to risk assessment and Permit-to-Work system documented in the local COP.

(ii) Is access to the laboratory(s) to be used for this work restricted?

Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	YES

If yes, provide details:

Access is restricted to people with documented training (authorised access documented in each individual's training record) in accordance with the COP and QMS (see C1.1.2).

C1.2 Controlling Exposure

C1.2.1 Are sharps (needles, blades, scissors, forceps, glass or capillary tubes) to be used at any stage during this activity?

Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	YES

If yes, list the sharps: scissors, glass, forceps

Forceps, Glass universal bottles.

If yes, justify there use – is there an alternative?:

It is local practice in the CBE laboratory unit that the use of sharps is avoided wherever possible. Glass items are replaced with plastic alternatives where possible, however the use of glass universal bottles is necessary for this protocol.

If yes, describe there use and disposal:

Forceps will be used for transferring treated membranes into sterile universal bottles containing 10ml buffer (PBS). Universals are glass bottles used for the suspension of bacteria from the membranes after plasma treatment. Forceps are reusable and are decontaminated using 1% Virkon after use. All bottles will be transferred in secure containers out of the CBE (see C1.2.4). If any bottles are broken (see below) these are placed directly into a sharps containers conforming to BS 7320. Sharps bins are removed when three quarters full and contents rendered safe by autoclaving prior to their removal from site.

If yes, describe any additional precautions employed to reduce risk:

In order to minimise the likelihood of accidents arising as a result of poor working practices and to take the correct action in the event of any accident that may occur, all individuals using sharps are aware of, and must use, guidance detailed in the local CBE CoP.

Injuries from broken glass are avoided by ensuring (i) glassware is in good condition (without chips or cracks) before use, (ii) glass items are carried in a box or similar to minimise the risk of dropping them (iii) have a sharps bin available at the point of use to enable immediate disposal (iv) a procedure is in place for reporting, recording and follow up of all accidents and incidents involving sharps. Accident procedures for sharps and glass injuries are displayed in posters in all laboratories within the Unit (v) Specific procedures for handling contaminated broken glass are detailed in SOPs (SOP038 – Biological spill response).

C1.2.2 Containment and Ventilation

(i) Is the use of BSC required for the protection of the worker ie do the work procedures generate aerosols or splashes that pose a risk to workers?

Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	YES

If yes, specify the type(s) and when they will be used:

A Class II Biological Safety Cabinet (located in Laboratory H29) will be used for all manipulations that may produce aerosols or splashes of Hazard Group (HG) 1 and 2 Biological Agents (BAs). Procedures to be carried according to SOP009, "Use and Maintenance of HERASAFE KS Class II BSC". This engineering control measure is specifically to protect the worker (when handling HG2 BAs) and ensure protection of research materials as part of a quality assurance discipline (when handling both HG1 and 2 BAs).

All manipulations of samples containing HG1 microbiological cultures will be carried out in the HERASAFE KS safety cabinet located in laboratory H29.

Preparation of microbiological samples for plasma treatment will be prepared as described by the protocol in the attached document. Plasma treatment of bacteria will occur in a specially designated Class II BSC in room H29 according to the following SOPs:

1) SOP062, "Use and Maintenance of Plasma Source System"
2) SOP063, "Use and Maintenance of Functional Generator"
3) SOP064, "Use and Maintenance of Amplifier"
4) SOP065, "Use and Maintenance of Oscilloscope"
5) SOP066, "Use and Maintenance of Pulse Generator"
6) SOP067, "Use and Maintenance of Power Meter"
7) SOP068, "Use and Maintenance of Voltage Probe"
8) SOP069, "Use and Maintenance of MFC Flow Controller"
9) SOP070, "Use and Maintenance of MFC Controller Readout"
10) SOP071, "Use and Maintenance of Plasma Rig

(ii) Are there any requirements for room ventilation e.g. negative pressure, temperature control?

Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	NO

If yes, specify:

C1.2.3 Transport and Storage within the laboratory

How and where are materials to be stored?

No hazardous or potentially hazardous materials will be stored in the CBE for the purposes of this experiment?

How will this material be transported within the laboratory e.g. between BSC and incubator? Detail the containment measures which will be used to prevent or contain accidental splashes or spills.

Bottles and Petri dishes that contain a bacterial culture will be secured in racks or sealed bags which will be placed inside secondary containers with a lid to contain spills from leaking or broken vessels (see attached protocol for details). These will be transported and handled according to the following SOPs:

1) *SOP005, "Storage and transport of Biological Materials"*
2) *SOP038, "Biological spill response"*

C1.2.4 Local transport out of the laboratory

How will this material be transported on-site (e.g. research material between labs on campus or movement of waste containing viable agents e.g. to a remote autoclave? Detail the containment measures which will be used to prevent or contain accidental splashes or spills

Transfer into and out of the CBE Laboratory Unit is constrained within the University site (see attached protocol). Material will be transferred into the Autoclave Room (H31) where the containment package will be decontaminated before transporting to H29. All transport will be subject to controlled procedures according to the local CoP and SOP005 (see below). For example, transfers will use double containment procedures as described in the attached protocol. Transport of research material between laboratories is done using sealed containers which are put into tube racks and trays and transported using trolleys according to the following SOPs. Waste potentially containing viable agents is not removed from the laboratories until it has been autoclaved.

1) SOP003, "Disposal of Biological Waste"
2) SOP005, "Storage and Transport of Biological Material"
3) SOP038, "Biological Spill Response"

C1.2.5 Shipment of Biological Material

Will this material be shipped elsewhere in the UK or abroad?	
Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	NO
If yes, give details to support compliance to the relevant regulation (e.g. category of material, correct packaging instruction):	

C1.2.6 Receipt of material

If material will be received from other sites or organisations, what precautions are being taken to ensure that the material is shipped correctly?

N/R

C1.2.7 Centrifugation

(i) If material is to be centrifuged will sealed buckets and rotors be used?	
Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	N/R
(ii) Where will these rotors/buckets be opened?	
(iii)Describe the procedures in place to deal with leaks and spillages in the centrifuge	

C1.2.8 Incubators

If incubators are to be used, what type of incubator (e.g. shaking, static) is used and describe procedures to prevent and contain spillages.

N/R. All samples pre-prepared and brought over to the CBE lab for treatment.

C1.2.9 Disinfection

Specify the type and concentration of disinfectants to be used:

The disinfectants were carefully chosen for effectiveness in use. The number of disinfectants in use is strictly limited to avoid errors and ambiguities in use and accidental mixing of compounds that may give

rise to hazardous reactions or the formation of toxic products. Unless there are compelling reasons to do otherwise, Virkon (1% w/v) is the sole disinfectant used in the CBE Laboratory Unit other than 70% IMS which is used for general disinfection cleaning (SOP004) where Virkon cannot be used; for example stainless steel surfaces.

Virkon has a wide range of bactericidal, virucidal, fungicidal and sporocidal activities. Representative viruses from all the major virus families are inactivated by Virkon. Working solutions of 1% w/v have low toxicity and no irritancy. Selection and procedures detailed in the following SOPs:

1) SOP004, "General Laboratory Housekeeping"
2) SOP006, "Selection and Use of Virkon Disinfectant"
3) SOP039, "Storage, Handling and Disposal of Chemicals"

COSHH Risk Assessment reference for Virkon SAF/MM/1745

Have these disinfectants been validated for use with the recipient biological material?	
Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	YES

If yes, describe the procedure:

For Hazard Group 1 and 2 Biological Agents it is normally sufficient to rely on the manufacturer's data, providing the recommended concentrations and contact times are used. Hence Virkon (1%) is used as per manufacturers instruction and according to standard procedures detailed in the following SOP:

1) SOP006, "Selection and Use of Virkon Disinfectant"

Further supporting evidence is provided in the following reference: Walker, A.J. et al. Letters in applied microbiology 15 (2): pg 80 (1992)

C1.2.10 Personal Protective Equipment (PPE)

(i) What type of lab coats will be worn and where will they be stored?

Side fastening Howie type lab coats are worn at all times within the CBE Laboratory Unit. They are stored outside the laboratory where the work will take place. Proper use of PPE is described in the following SOP:

1) SOP037, "Use of Personal Protective Equipment (PPE)"

(ii) What type of gloves will be worn and where will they be stored?

1. Autoclave gloves - stored in close proximity to the autoclave equipment in the Autoclave Room (H31)
2. Latex powder free gloves for general use - stored in the change rooms and/or point of entry to each laboratory within the CBE Laboratory Unit.

Correct use of PPE is described in SOP037, "Use of Personal Protective Equipment (PPE)"

(iii) Describe any other PPE to be used:

1. Laboratory safety glasses (including those for spectacle wearers)
2. Face masks, in case of aerosol generation during plasma treatment.
3. Shoe protection for moving heavy equipment (if necessary

Correct use of the above PPE is described in SOP037, "Use of Personal Protective Equipment (PPE)"

C1.2.11 Hygiene Measures

Describe the hygiene facilities available and where they are located
1. Designated hand washing facilities are located in each laboratory change room and in the Analytical Laboratory (H23). The nearest hand washing facilities to H29 are located in H26. This is signposted on the wall in in H29. 2. Eye Wash stations are located next to each 'hand washing only' sink in each laboratory change room and in the Analytical Laboratory (H23). The nearest eye wash stations to H29 are located in H26. This is signposted on the wall in H29.

C1.2.12 Vaccination

Are effective vaccines available against any of the agents listed in Section1, 2, 3, or 4 of Part B?	
Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	NO
If yes, describe:	

C1.2.13 Waste Treatment before Disposal

How must waste to be treated before disposal and how has it been validated as being effective?		
	Treatment before disposal	Validation
Liquid waste	Virkon sterilise (SOP003 – Disposal of biological waste)	According to manufacturers instructions; see section C2.1.9
Solid waste	Autoclave sterilise (SOP003 – disposal and disinfection of biological waste)	Treatment Cycle validated according to SOP024 & 025, " Use and maintenance of the Systec VX95 Autoclave No CBE44 and CBE 45"

C1.2.14 Autoclave sterilisation

If waste is treated by autoclave sterilisation then this section must be completed. If this section is not relevant then hatch the box			
	Type of waste	***Autoclave cycle (temp, cycle time)***	***Treatment monitor***
Liquid waste	*None*	*N/R*	*N/R*
Solid waste	Petri dishes with thin agar slabs	*121°C for 20 min sterilisation time*	*Internal temperature probe to measure inside the container holding the solid waste being decontaminated.* Designated autoclave tape monitors
Location of autoclave	**Servicing details**	**Location of back-up autoclave**	**Designated area for storage of unsterilised waste**
Autoclave Room H31	Annual	H31 and H22	In secure cage within the Autoclave Room (H31)

C1.2.15 Liquid Waste Disposal

How will liquid waste be disposed of?
No liquid waste will be generated within the CBE Laboratory Unit.

As solid waste? N/R
Other? None

C1.2.16 Solid Waste Disposal

Describe the waste category and disposal route. *(For guidance refer* to http://www.environment-agency.gov.uk)

European Waste Catalogue Code	Categorisation		Disposal Method
		Hatch relevant box(es)	
18 01 01	Sharps		Sharps bin > autoclave sterilisation if known or potentially infected > clinical waste disposal (incineration)
18 01 02 [human]	Human body parts, organs, including blood bags and blood preserves and excreta (unless identified as medium or high risk or known infected in Section 2.16 of this RA in which case they must be pre-treated before disposal and classified 18 01 04 [sealed bins])		Rigid one way sealed tissue bins > incineration only
18 01 02 [animal]	Animal body carcases or recognisable parts ((unless identified as medium or high risk or known infected in Section 2.16 of this RA in which case they must be pre-treated before disposal and classified 18 01 04 [sealed bins])		Rigid one way sealed tissue bins > incineration only
18 01 03	Potentially or known infected lab wastes (including sharps) of HG2, GM Class 2, DEFRA Cat 2 or higher, that have not been pre-treated before leaving the site.		This is not a route of preference and is subject to special requirements
18 01 04 [bags]	Infected or potentially infected lab wastes that have been pre treated before leaving the site		Disinfection or sterilisation (as identified in C1.2.13) in the lab suite > placement in yellow clinical waste bags > clinical waste disposal (incineration)
18 01 04 [sealed bins]	Infected or potentially infected animal or human body parts, organs or excreta that have been pre treated before leaving site		Disinfection or sterilisation (as identified in C1.2.13) in the lab suite > placement in yellow one way sealed tissue bins > incineration)

C1.2.17 Work with Animals or Vectors (if none proceed to Section C1.2.18)

(i) Are animals or vectors to be infected with any of these biological agents?	
Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	N/R
If yes, describe the procedure and describe where this aspect of the work will be conducted:	
(ii) Is shedding of infectious materials by the infected animals possible or expected?	

Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	N/R
If yes, describe the routes of shedding, risk periods for such shedding and the additional precautions required to control exposure:	
(iii) Who will perform the inoculations of animals/vectors? What training have they received?	
Indicate in the adjacent box if Not Relevant (N/R)	N/R
Provide details of the training required:	

C1.2.18 Bioreactor/Fermenters (if none proceed to Section C1.2.19)

Will a fermenter be used to culture a pathogen?	
Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	N/R
If yes, describe the size, and type of the fermenter.	
(ii) Are any supplementary containment measures required, for example, the use of a BSC or spill tray.	
Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	N/R
If yes, describe:	

C1.2.19 Other Control Measures Required?

None

C1.3 Emergency Procedures

C1.3.1 Describe the procedures in place for dealing with spillages *(specify disinfectants and any special containment for large volumes)*

Within the BSC: Procedures for dealing with small and large spillages are detailed in the following SOPs: 1) SOP006, "Selection and use of Virkon Disinfectant" 2) SOP009, "Use and Maintenance of HERASAFE KS Class II BSC" 3) SOP038, "Biological Spill Response" Labelled Biological Spill kits are located in each laboratory change room within the CBE Laboratory Unit. The nearest biological spill kit for use in H29 is located in H26. Signs are posted throughout the Laboratory Unit to enable workers to locate the nearest biological (and chemical) spill kits. Posters are also displayed in each laboratory within the Unit where a BSC is located to advise on spill response (inside the BSC) and reporting procedures.
Within the laboratory but outside the control measure e.g. BSC, spill tray Procedures for dealing with small and large spillages are detailed in the following SOPs: 1) SOP006, "Selection and use of Virkon Disinfectant" 2) SOP038, "Biological Spill Response" Labelled Biological Spill kits are located in each laboratory change room within the CBE Laboratory Unit. Signs are posted throughout the Laboratory Unit to enable workers to locate the nearest biological (and chemical) spill kits. Posters are also displayed in each laboratory within the Unit to advise on spill response

(outside the BSC) and reporting procedures.

Outside the laboratory e.g. during transport

Procedures for dealing with small and large spillages are detailed in the COP and the following SOPs:

1) SOP005, "Storage and Transport of Biological Material"
2) SOP006, "Selection and use of Virkon Disinfectant"
3) SOP038, "Biological Spill Response

Describe the procedures in place for an accidental exposure (if necessary describe different procedures for different types of exposure e.g. eye splash or percutaneous inoculation)

1. Procedures to respond to accidental exposure are detailed in SOP038, "Biological Spill Response" and the local COP. These are detailed in spill response posters located in each laboratory within the Unit. Accident procedures in the case of glass or sharps injury are described in the local COP and displayed in posters located in each laboratory within the Unit
2. Designated hand washing facilities are located in each laboratory change room and in the Analytical Laboratory (H23). Signs are posted in H29 and H30 to enable workers to locate the nearest hand wash facility.
3. Eye Wash stations are located next to each 'hand washing only' sink in each laboratory change room and in the Analytical Laboratory (H23). Signs are posted in H29 and H30 to enable workers to locate the nearest eye wash station.
4. A First Aid Kit is located outside the Laboratory Unit. Signs are posted throughout the Laboratory Unit to enable workers to locate the nearest Medical Kit. Contact details for First Aiders are posted in each laboratory within the Unit

Essential and Emergency Contact details are posted in each laboratory within the Unit.

C2 ASSIGNMENT OF CONTAINMENT LEVEL

The laboratory Containment Level is directly related to each of the 4 Hazard Groups; organisms categorised as HG1 (lowest hazard rating) should normally be handled in CL1 facilities (minimum level of containment), and likewise up to HG4 (highest hazard rating) in CL4 facilities (maximum level of containment). Where the identity or presence of a biological agent is not known the following rules apply: a) where uncertainty exists over the presence of pathogenic biological agent – minimum of CL2; b) where the presence of a pathogenic biological agent is known or suspected – minimum of Containment Level appropriate to the agent, where the assessment is inconclusive but where the activity might involve serious risk – minimum CL3

C2.1. What containment level is required for this work?

All procedures will be carried out under Containment level 2 (CL2) within the CL2 CBE Laboratory Unit. The work activities within this project involve biological agents (BAs) assessed as Hazard Group 1. Work with the lower Hazard Group will be carried out under the management standards imposed by the higher level (Containment level 2). This project, involving the use of Hazard Group 1 BAs that require Containment Level 1 are carried out at Containment Level 2 for reasons other than worker protection; this includes the need to ensure research material protection (e.g. the use of a class II safety cabinet) and to impose a quality assurance discipline

C2.2. Describe extra controls or derogation from certain controls

:

None

C3 FACILITIES

C3.1 Where will this work take place?

Room(s)	Building	Campus	Person in Control of area
CBE Laboratory Unit; Rooms H29	Centre for Biological Engineering	Holywell Park campus	Professor Chris Hewitt Carolyn Kavanagh Bob Temple

C4 PERSONNEL

C4.1 Names of Personnel involved in the Project

Surname	Initials	ID	Position
Bayliss	DLB	A738821	Research Student

C4.2 Information, Instruction and Training

Describe the training that will be given to all those affected (directly or indirectly) by the work activity. Instruction should include the 'Local Rules' or 'Local Codes of Practice' which focus on the working instructions to be followed by all persons involved in the work activity to control or prevent exposure to hazardous biological agent(s). These should be written and readily available to all workers working at Containment Level 2. A formal record of training should be kept for all individuals working at Containment Level 2.

Formal records of training are kept for all workers authorised to work at Containment Level 2 (CL2) within the CBE CL2 Laboratory Unit. Instruction against local Code of Practice and QMS i.e. SOPs is provided to all authorised personnel. Include specific documented training where necessary ie use and maintenance of the Plasma Source (SOP071).

C4.3 Relevant Experience/Training:

Surname	Experience/Training
Danny Bayliss	Documented in Personal Training File available for review in CBE Office

C4.4 Other people who may be at risk from the activity e.g. cleaners, maintenance workers or other workers in shared laboratory

Details: Workers sharing the laboratory

NONE: Cleaners and Maintenance workers are not authorised to enter the laboratory. All laboratory cleaning is undertaken by authorised personnel (i.e. CBE staff). Access for non-laboratory workers is subject to local permit-to-work procedures. If access is needed for essential maintenance of equipment for example a clean down and decontamination of the laboratories will be performed. This will be documented with decontamination certificates and the maintenance worker fully supervised according to SOP004; "General Laboratory Housekeeping" and the local Code of Practice. Two laboratory shut downs occur every year for a week for maintenance work to be done in the CBE Laboratory Unit. Prior to these shut down weeks a full deep clean decontamination will be performed in the all laboratory areas.

C5 OCCUPATIONAL HEALTH

C5.1 Vaccination

Is an effective vaccination available for any of the pathogens associated with this work? Advice can be obtained from the Occupational Health Adviser if required. All workers involved with handling unscreened blood, blood products and other tissues are recommended to have Hepatitis B immunization

Not required for HG1 non-human microbiological work

C5.2 Health Surveillance

Is health surveillance required? (Health surveillance is typically applied if working with a hazardous substance that: a) produces an identifiable disease or adverse health effect that can be related to exposure; b) there is a reasonable likelihood that the disease or effect may occur under the conditions of work, and c) there are valid techniques for detecting indications of the disease or effect).

University Forms submitted.

C6. NOTIFICATIONS: Human Tissue Act

C6.1.1 Relevant material covered by the Human Tissue Act

Are any of the cell, tissues or fluids to be used covered by the Human Tissue Act?	
Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	N/R

C6.1.2 Does This Work Have Ethical Approval? If Yes, Provide Details

Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	N/R
Approval number:	
Date obtained:	Ethics committee name:

C6.1.3 Are other registrations/notifications required for this work? For example HSE notification under COSHH, Home Office notification under anti-terrorism, crime and security act etc

Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	N/R
If Yes, give details:	

7. LICENSING REQUIREMENTS FOR ANIMAL PRODUCTS

C7.1.1 Are there any licensing requirements for this work?

Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	N/R

NOTE: The regulations covering the import of animal products (including tissue cultures, tissues, body fluids or fractions thereof) are in a state of flux. See the DEFRA website for details.

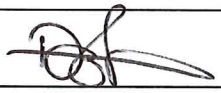
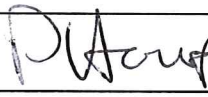
UNLESS THIS SECTION IS NOT RELEVANT (N/R) ie THE INTENDED WORK DOES NOT USE ANIMAL PRODUCTS - CONSULT THE LU H&S OFFICE TO REVIEW APPLICATION REQUIREMENTS BEFORE ANY SUBMISSIONS

8. DECLARATION

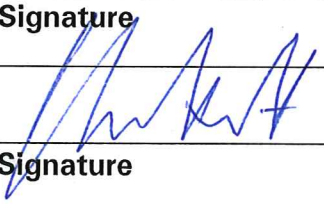
*The declaration must be signed **before** submitting this assessment to the Departmental Safety Officer and University Biological Safety Officer*

I, the undersigned:

- confirm that all information contained in this assessment is correct and up to date
- will ensure that **suitable and sufficient instruction, information and supervision** is provided for all individuals working on the activity
- will ensure that no work will be carried out until this **assessment has been completed and approved** and that all necessary control measures are in place
- accept that for some Containment Level 2 and all CL3 activities **a statutory notification period of 20 days** may be required before work can commence
- that all information contained in this assessment must remain correct and up to date (the assessment should be **reviewed once a year** and whenever any **significant changes** to the work activity occur)
- will re-submit the assessment for approval if any significant changes occur

Name: Person conducting assessment	**Signature**	**Date**
Danny Bayliss		**23/09/09**
Name: Other signature (s) (if required – please state position)		
P.Hourd (CBE QM)		25/09/09
Name: Principal Investigator		
Micheal Kong	* 	25/09/09

9.APPROVAL

Name: Departmental Safety Officer	**Signature**	**Date**
C.J. Hewett		28/9/09.
Name: University Biological Safety Officer	**Signature**	**Date**

Bacterial Treatment Protocol

1. ***L. innocua/ E.coli / B.subtilus*** are the micro organisms that will be used. These micro organisms are classed as a hazard group 1, which is a biological agent unlikely to cause human disease.
2. **In Chemical Engineering (CE) Department:** They will be vacuum filtered on to pre-sterilised membranes producing a total population of $1x10^9$ CFUs maximum on each membrane.
3. Each membrane containing the bacteria or spores is placed onto thin slabs of agar (technical agar no. 3) in small Petri dishes (~55m diameter) to fix down the membrane and left to dry for 60mins.
4. Each Petri dish containing bacterial or spore deposit are covered with the Petri dish lid then sealed with parafilm.
5. All sealed Petri dishes are placed inside Petri dish bags which are taped shut with autoclave tape. The Petri dish bags are then sprayed down with 70%IMS and placed inside a leak proof secondary sealable container that has been previously disinfected with 1% virkon and 70% IMS.
6. The secondary container is placed inside a leak proof cooler box for transportation from the CE department across to the CBE lab.
7. Universal bottles containing 10ml PBS which are used to resuspend the bacteria after plasma treatment are also transported across to the CBE lab inside the leak proof cooler box. The bottles will be placed in racks to keep them upright and stop the bottles banging together.
8. **In the CBE Laboratory Unit:** The cooler box containing the Petri dish bags is taken to H31 (by hand) where the secondary sealed container is removed in and sprayed down with 70% IMS. This is then transferred (by hand or trolley?) to the Biophotonics lab (H29). The cooler box containing the universal bottles is also taken to H31 and sprayed down with 70% IMS. This is then transferred (by hand or trolley?) to the Biophotonics lab (H29).
9. The Plasma rig is set up (SOP071) inside the Hera Safe class II safety cabinet. Refer to SOP009 for using the safety cabinet correctly.
10. All plasma rig parts that will go inside the safety cabinet are to be sprayed down with 70% IMS and left for the solvent to completely dry off before placing inside the BSC.

11. The Petri dish bags are removed from the secondary container (outside the BSC). The sealed Petri dish samples to be treated are removed from the Petri dish bags and placed inside the safety cabinet.

12. Parafilm seals and Petri dish lids are removed inside the BSC just before treatment in order to minimise exposure of the membranes to any outside cross contamination.

13. NOTE: Placing the plasma rig inside the safety cabinet for the plasma treatment may disrupt air flow inside the safety cabinet and may therefore compromise worker protection. However, samples containing HG1 biological agents are unlikely to cause human disease and minimal manipulation of the samples inside the BSC will minimise the likelihood of aerosols or splashes. The BSC will still offer some protection to both the worker and the work.

14. After completion of the plasma treatment the membranes are aseptically removed from the agar dish and transferred using forceps into the corresponding universal bottle containing 10ml PBS.

15. The agar dish is then placed inside an autoclave bag. Once all the samples have been treated the autoclave bag with all the waste agar plates are to be transported to the autoclave room for sterilisation. Refer to SOP005 and SOP003 for transport and disposal of biological waste.

16. Once the experiment is complete, all plasma equipment is to be removed from the class II safety cabinet. The plasma equipment is sprayed down with 70% IMS. The BSC is cleaned down and left switched on in accordance with SOP009.

17. Universal bottles containing the resuspended bacteria or spores are placed in racks which are then placed inside a suitable secondary container which has been cleaned with 70% IMS.

18. The container is then transported by hand to H31 and placed into the cooler box for transportation back to the Chemical Engineering Department for plating out.