Loughborough University **Biological Risk Assessment**	Safety Department use only		Material(s) Classification	
	Reference Number:		Hazard Group 1	☐
			Hazard Group 2	☑
	CBE Use only		GMO	☐
	Reference Number:	CBE/BRA/194	HTA Licensable	☐

FORM CBE-RA-Form/002 Version 1.0

RISK ASSESSMENT AND PROJECT REGISTRATION FOR WORK INVOLVING BIOLOGICAL MATERIAL

PLEASE READ CAREFULLY

This form acts to register projects involving the use of Biological Agents and / or Genetically Modified Micro-Organisms, or of materials that may be contaminated with these agents. It assesses the hazards and risks associated with the project as well as identifying those at risk and the measures necessary for preventing, or controlling these risks. Please ensure that sufficient detail is provided when completing this form and that the relevant written SOPs are referenced where required. Once completed and approved, all risk assessments must be supplied to all those working within this project. The work described within this form must not commence until this risk assessment has been completed and approved and that all necessary control measures are in place.

Any changes to the work, or the persons involved, must be notified to the authorised person. All changes requested must be recorded within the risk assessment change control form and may also need to be incorporated within an amended version of this form.

A separate risk assessment will be required for assessing risks associated with GMO activities.

The following declaration must be completed and undersigned by the Principal Investigator or Person Responsible for the project

- All information contained in this form is accurate and comprehensive.
- All workers involved will be instructed that their work must remain within the boundaries of this project registration & assessment.
- All workers have been given, or will be given before they become involved, adequate training and where necessary their competency assessed.
- All workers have, or will be before their involvement begins, enrolled with Occupational Health for health clearance where necessary.
- It is understood that this risk assessment shall not be transferred to a third party without the PI/Supervisor/Line Manager named in this form either taking responsibility for the new activities, or ensuring that a new proposal is submitted.
- All changes to the work covered by this form will be reassessed & the changes submitted to the authorised person before those changes are made to the work.

Principal Investigator		Person conducting this risk assessment	
Name	Sourav Ghosh	Name	Jakub Nasterski
Position	Senior Lecturer	Position	PhD Reseacher
Department	Centre of Biological Engineering	Department	Centre of Biological Engineering
School	Wolfson of MEME	School	Wolfson of MEME

The Project Activity			
Title	Detection of spiked concentration of bacteria in buffer and artificial urine using Anharmonic Detection Technique (ADT) method.		
Reference Number			
Start Date	2 Dec 2020	End Date	31 Dec 2021

Others involved in the work	
Names	Praveenkumar Kaveri

Name	Jakub Nasterski	Signature	Jakub Nasterski	Digitally signed by Jakub Nasterski Date: 2020.12.02 15:56:44 Z	Date	2 Dec 2020

1. INTRODUCTION

1.1 Background & aim of project	Aims: To culture and store hazard Group 1 and 2 bacteria in liquid growth medium. To perform quantitative and qualitative detection of bacteria in spiked buffer, using Anharmonic Detection Technique (ADT) instrument.
1.2 Description of experimental procedures	1. Bacterial strains from beads will be cultured in either tryptone soy broth or nutrient broth. The bacterial culture will then be washed in PBS Buffer using centrifugation. (Location : Wolfson school T208.b). 2. The desired concentration of bacteria Eg. 1X10^1 to 1 X 10^8 will be spiked into PBS buffer or Artificial urine medium (COSHH approved). 3. The quartz crystal will be prepared by washing with 50% acetone and isopropanol. 4. The quartz crystal will be dried in a stream of nitrogen gas. 5. The quartz crystal resonator (QCR) will be cleaned using a plasma cleaner and functionalised overnight with a mixed biotin and hydroxyl thiol. 6. The next day, the crystal will be cleaned of unbound thiol and placed inside a microfluidic cell connected to SensAND (custom network analyser). SOP for SensAND - SOP186. 7. Streptavidin and 0.5% BSA will be passed through the microfluidic cell using a syringe pump. 8. A biotinylated aptamer designed for bacterial detection will then be passed through the cell and captured on streptavidin functionalised QCR. 9. The bacterial suspension will be passed over the quartz crystal and then analysed using the anharmonic detection technique to detect binding of bacteria to the aptamer. The ADT technique involves a closed system. The bacteria sample starts in a sealed eppendorf tube. It is pumped through the flow cell via silicone tubing and finishes in a syringe. The elements in contact with the bacteria are: eppendorf tube, silicone tubes, needle, syringe, flow cell and quartz crystal. At the end of the experiment the remaining bacteria sample liquid will be submerged in 1% virkon solution for 24 hours and disposed down the sink. 1% virkon solution will then be slowly flowed through the system for 24 hours, disinfecting all the elements that were in contact with the bacteria prior to disposal/reuse.
1.3 Where will this work be carried out?	Rooms/areas: T208b
	Building(s): Wolfson

☐ **2.1 Human or animal tissues, cells, body fluids or excreta will be used in this project**

☑ **2.11 Biological agents will be used in this project**

2. BIOLOGICAL AGENTS (i.e. micro-organisms such as bacteria, fungi, microscopic endoparasites)

2.12 List the biological agents to be used	Name of Agent	Strain(s)	ACDP / Defra Classification
	Escherichia coli	ATCC 33780	Hazard Group 2
	Escherichia coli	ATCC 12014	Hazard Group 2
	Staphyloccocus epidermidis	ATCC 14990	Hazard Group 1
	Escherchia coli	ATCC 11775	Hazard Group 2
	Salmonella enterica	ATCC 19585	Hazard Group 2
	Escherchia coli	ATCC 25922	Hazard Group 1
2.13 Describe the type and severity of the disease that can be caused to humans, animals or plants by each of the agents and if relevant, the particular strains in use	The selected HG2 bacteria strains can cause disease. However, treatment is readily available, and the likelihood of an infection is low as the experimental procedure will be controlled as described.		
2.14 Has any strain listed in Section 2.12 been genetically modified in any way?	○ Yes ☑ No		

3. CLASSIFICATION OF HAZARD GROUP

3.1. Are you confident that any non-GM organism, tissue, cell, body fluid, excreta or any component thereof covered by this assessment cannot potentially pose a threat to humans or cause human diseases?	○ Yes - Classify as HG1
3.1.1. Can any non-GM organism, tissue, cell, body fluid, excreta or any component thereof cause human disease and potentially be a hazard to humans but is unlikely to spread to the community and for which there is usually effective prophylaxis or treatment available?	☑ Yes - Classify as HG2
3.1.2. Can any non-GM organism, tissue, cell, body fluid, excreta or any component thereof cause severe human disease and potentially be a serious hazard to humans and that may spread to the community, where effective prophylaxis or treatment may or may not be available?	○ Yes
3.2. Do any of the materials contain pathogens or toxins covered by the Anti-Terrorism Crime and Security Act?	○ Yes **ATCSA Schedule 5**
ASSIGNMENT OF CONTAINMENT LEVEL	HG2

4. TISSUES, CELLS, BODY FLUIDS OR EXCRETA

4.2. Will any culturing of the material described in section 2 take place? *If Yes, describe which cell(s) will be cultured and under what conditions.*	☑ Yes ○ No	Bacterial cells will initially be cultured overnight in 100 ml flasks at 37 degrees with shaking. This culture will be used to inoculate a fresh culture and grown to approximately 1x10^7 in 100 ml flasks at 37 degrees for approximately 3 hours with shaking. The number of cells will be determined via a spectrophotometer at OD 600.
4.3. Could HIV permissive cells be present*? *If Yes, describe the cells and for how long these cultures will be allowed to grow. If unsure seek advice. Refer to CBE Code of Practice for details on additional precautions.*	○ Yes ☑ No	
4.4. What is the maximum volume of culture grown?	Per Vessel	100
	Number of vessels	2
4.5. Will the tissues, cells, body fluids or excreta be manipulated in any way that could result in the concentration of adventitious biological agent present? *If Yes, explain.*	○ Yes ☑ No	
4.6. Will any of the tissues, cells or fluids be donated by you or your colleagues working in or with access to the labs?	○ Yes ☑ No	

4. BIOLOGICAL AGENTS (ie micro-organisms such as bacteria, viruses, fungi, microscopic endoparasites)

4.8. Describe ALL route(s) of infection (relevant to the laboratory setting) and the minimum infectious dose(s), if known	Name of agent	Route(s)	Minimum infectious dose
	Escherichia coli	Inhalation / ingestion	
	Staphylococcus epidermidi	Inhalation / ingestion	
	Salmonella enterica	Inhalation / ingestion	

4.9. What is the highest concentration and volume of agent(s) to be worked with?	Per experiment: 200 ml	Total stored: 200 ml
4.10. Are there any known drug resistances amongst the strains to be used? If Yes, explain what these are and the consequences	○ Yes ☑ No	
4.11. What forms of agent will be used e.g. spores, vegetative forms and are there any issues over the robustness of these particular forms e.g. resistance to disinfectants or increased stability on dry surfaces?	Vegetative forms with no known resistance to disinfectant.	

4. BIOLOGICAL AGENTS (ie micro-organisms such as bacteria, viruses, fungi, microscopic endoparasites)

4.12. What will be the most hazardous procedure involving the use of this material?	The most hazardous procedure will be handling of the bacteria (inoculation and testing with AD

5. RISKS AND CONTROL MEASURES

Risk		How will this be controlled?	Reference to SOP's / Other documentation
5.1. Might infectious droplets, aerosols or splashes be created, either deliberately or by accident?	☑ Yes ○ No	A class II BSC will be used for all bacterial culture work to protect against potential aerosols. All work will be carried out using aseptic technique. Any spillages inside the BSC will be dealt with according to SOP038, depending on the volume of the spill.	Biological spill response: SOP038 and SOP009 Use and maintenance of Class II BSC.
5.2. Will this material be transported within the laboratory e.g. between BSC & incubator?	☑ Yes ○ No	The material will be transfered in a sealed flask from BSC to incubator. In the event of a small spill, the spill area and adjacent area will be cleaned by covering with paper towels soaked with 1% Virkon solution. In the event of a large spill, SOP038 will be followed and a spill kit will be used.	Storage and transport of biological agents: SOP005 Biological spill response: SOP038 and SOP009
5.3. Will this material (including waste) be transported locally between sites on campus but outside the laboratory?	○ Yes ☑ No		
5.4. Will material(s) listed in section 2.2 or section 2.3 be shipped to organisations elsewhere in the UK or abroad?	○ Yes ☑ No		
5.5. Will this material be received from organisations elsewhere in the UK or abroad?	○ Yes ☑ No		
5.6. Will this material be stored?	☑ Yes ○ No	A portion of bacterial cells will be frozen in order to maintain a bank of comparable cells to work with. Storage of bacteria is via beads, no liquid is frozen.	Storage and transport of biological agents: SOP005
5.7. Will infectious material be centrifuged?	☑ Yes ○ No	Centrifuging takes place during the washing of bacterial cultures, in order to be resuspended into buffer/ AUM samples. Bacterial cultures are centrifuged within closed centrifuge tubes with a maximum of 50 ml per tube. Sealed buckets will be used and opened within the BSC. In the case of a small spill (less than 10 ml), the spill area and adjacent area will be cleaned by covering with paper towels soaked with 1% Virkon solution. Virkon soaked paper towels will be disposed as hazardous waste. In the case of a large spill, a spill kit will be used.	Biological Spill response: SOP038
5.8. Are biological samples to be cultured in an incubator?	☑ Yes ○ No	Bacterial strains will be cultured in a shaking incubator. Sealed culture flasks will be used in the shaking incubator. In case of a small spill (less than 10 ml), the shaking incubator will be stopped and cleaned by covering with paper towels soaked in virkon. If the spill is large, a biological spill kit will be used.	Biological Spill response: SOP038
5.9. Are sharps to be used at any stage during this activity?	☑ Yes ○ No	Sharps include pipette tips and a needle. A needle and syringe are used to draw liquid through the microfluidic device using a syringe pump during the ADT experiment. This will be set up without the presence of microbiological material to reduce risk. Once testing has finished, users must not attempt to re-sheath the needle. Any accidents or near misses must be reported immediately. The sharps will be placed inside a sharps bin and autoclaved as solid waste on cycle 4. Indicator strips are used on every load. Once the sterilisation cycle is complete, the sharps container will be allowed to cool and it will be verified that the sterilisation cycle was successful according to the indicator strip.	Use and maintenance of Systec VX Autoclave | H&S document reference: CBE SOP 24 SOP024, SOP025, SOP054

Risk		How will this be controlled?	Reference to SOP's / Other documentation
5.10. Are animals to be used in this project?	○ Yes ☑ No		
5.11. Will a fermenter / bioreactor be used to culture a biological agent or material?	○ Yes ☑ No		
5.12. Is there any stage within the experimental procedures when an infectious material is inactivated (other than for disposal)?	○ Yes ☑ No		
5.13 Are any of the following to be used in conjunction with the project?	☐ Carcinogens or Mutagens		
	☐ Toxins		
	☐ Liquid Nitrogen		
	☐ Ionising radiation		
You must complete a lone working risk assessment before work begins and add the reference here.	☑ Lone working	Attached with this risk assessment. Lone working will only be for ADT experiments in case they extend beyond office hours.	
5.14. Are there any conditions associated with the hazards described in section 5.13 that require additional control measures?	○ Yes ☑ No		

6. PPE AND HYGENE

Control Measure	Details		Reference to SOPs / other documentation
6.1 When will gloves be worn?	Autoclave gloves stored near the autoclave will be worn at all times when operating the autoclave. Disposable latex powder free gloves will be worn at all times when inside the laboratory.		Use of personal protective equipment: SOP037
6.2 What type and where will they be stored?	Nitrile	In Lab	Use of personal protective equipment: SOP037
6.3 When will laboratory coats be worn and what type are these?	At all times	Coloured Howie	Use of personal protective equipment: SOP037
6.4 Where will lab coats be stored and what are the arrangements for cleaning or disposal?	They are stored outside the laboratory in a dedicated change area. The lab coats will be autoclaved and sent for cleaning every month. If a lab coat is severely contaminated, it should be immediately autoclaved and a new one should be used.		Use of personal protective equipment: SOP037
6.5 Provide details of any other types of PPE to be used?	Safety glasses. Whilst using the autoclave, a face shield and heat proof apron will also be worn.		
6.6 Describe the lab hygiene facilities available and where they are located	Hand washing facilities and eye wash stations are available in the change room of each laboratory.		Use of personal protective equipment: SOP037 SOP004
6.7 Where are the first aid boxes and emergency spill kits located?	A first aid kit is located outsi		

7. WASTE

7.1 How will waste be treated prior to disposal

(Note that all differently treated wastes must be included e.g. if some liquid is autoclaved, but others not, then describe both)	Treatment prior to disposal	Is the treatment validated?	Reference to SOPs / other documentation
☑ Liquid waste	Contaminated material is treated with Vikron disinfectant for 24 hours prior to disposal down the sink with copious amounts of water. Acetone is disposed of by collecting in a designated labeled glass winchester bottle and placed in the waste disposal area (downstairs next to stores). The non hazardous liquids (isopropanol, ethanol, PBS buffer) can be disposed down the sink with copious amounts of water.	○ Yes ☑ No	
☑ Solid waste	Solid waste that has been in contact with biological material is placed in an autoclave bag next to the BSC and loosely tied once half full. This bag is then autoclaved on cycle 4 and then placed in a secondary orange bio-hazard bag. Once this secondary bag is half full, it is zip tied and placed in the waste disposal area (downstairs, next to stores). Indicator strips are used on every load. Solid waste that has not been in contact with biological material (for example packaging) or has been in contact with chemicals that mean it cannot be autoclaved e.g Virkon will be placed in a yellow bag and zip tied once half full and placed in the waste area. Sharps waste will be placed in an autoclavable sharps bin. Once the sharps bin is filled to the indicator line, it is closed and wrapped in indicator tape and autoclaved on cycle 4. Once autoclaved, sharps bins are placed in the waste area.	○ Yes ☑ No	
☐ Other (Specify)			

7.2 Is any waste being autoclaved?	☑ Yes ○ No	Decontamination and disposal of healthcare waste: SOP003
All cycles have been validated for the actual load types used? *(If Yes, documentary evidence of the validation must be available)*	☑ Yes ○ No	Validation documents available in CBE office.
The successful completion of every load is checked prior to disposal?	☑ Yes ○ No	Decontamination and disposal of healthcare waste: SOP003

7.3 How will liquid waste be disposed of?

☑ To drain?	After 1% Virkon decontamination for 24 hours, waste is po	○ Yes ☑ No	
☐ As solid waste?			
☐ Other (Specify)			

7.4 How will solid waste be disposed of?

Categorisation	Waste stream colour code	Disposal method (Edit as required)
☑ Sharps	**Orange**	Yellow/Orange lidded sharps bin > autoclave sterilisation if known or potentially infected > clinical waste disposal (incineration)
☐ Sharps contaminated with cytotoxic or cytostatic material		
☐ Human body parts, organs, including blood bags and blood preserves and excreta that have been pretreated before leaving the site		

Categorisation	Waste stream colour code	Disposal method (Edit as required)
☐ Animal body carcasses or recognisable parts that have been pretreated before leaving the site		
☐ Potentially or known infected lab wastes contaminated or potentially contaminated with cytotoxic or cytostatic material that have **NOT** been pretreated before leaving the site		
☐ Potentially or known infected lab wastes that have **NOT** been pretreated before leaving the site		
☑ Infected or potentially infected lab wastes that **HAVE** been pretreated before leaving site	**Orange**	Disinfection or sterilisation in the lab site > orange clinical waste bags > clinical waste disposal (incineration)

8. MAINTENANCE

8.1 Are preventative maintenance and monitoring regimes in place for the following laboratory equipment?

	Inspection / Servicing Frequency	Cleaning / Disinfection Frequency	Monitoring / Alarms Frequency	Reference to SOPs
☑ Centrifuges	Inspected before use and during weekly clean. Centrifuges will be serviced every 2 years. PAT testing will be done every two years.	During the weekly clean the inside of the chamber, all parts of rotation assembly and any head accessories are cleaned and dried.	Centrifuge will be monitored throughout use.	General laboratory housekeeping: SOP004 SOP122 Use and Maintenance of Eppendorf minispin centrifuge: SOP088 in Wolfson school T208b.
☑ BSCs	Inspected before every use and during weekly clean. Inspected and tested by a contractor annually. PAT testing will be done every two years.	BSCs are cleaned before and after every use with virkon and 70% IMS and undergo deep clean once a week. After each use, BSC also undergo a round of UV disinfection.	Record is kept of downflow velocity (m/s) and performance factor before each use.	SOP009- use and maintenance of Class II BSC SOP004- General laboratory housekeeping.
☐ Fume Hoods				
☑ Autoclaves	Inspected before every use and serviced twice a year. Pressure vessel inspection annually.	Autoclave cleaned weekly. Inside not cleaned as its routinely sterilised during use.	Record kept of cycle number, cycle type and whether or not the cycle passed via indicator strip. Integrated temperature, pressure and water monitor.	Use and maintenance of Systec VX Autoclave | H&S document reference: CBE SOP 24
☑ Incubators	Inspected once a week and regularly by operator prior to use.	Cleaned weekly	Constant monitoring for the shaker speed and temperature. Alarm is raised if there is an issue with temperature or shaker speed.	Use and maintenance of Sartorius Certomat BS 1 incubator: SOP 124 at Wolfson school T208b
☐ Liquid N_2 Stores				
☑ Freezers	Weekly inspection, PAT tested yearly	Cleaned and defrosted as needed.	Alarm raises if temperature falls below -70 degrees.	Use and Maintenance of Fridges & Freezers: SOP 016
Failure contingency plan				
☑ Fridges	Weekly inspection, PAT testing will be done every two years.	Cleaned every month	Visual inspection frequently during lab hours to check for any errors.	Use and maintenance of fridges and freezers: SOP016
Failure contingency plan				
☐ Others				

9. TRAINING

☐ 9.2. This work involves HTA 'Relevant Material', confirm that all project research workers have undertaken HTA training

Name of researcher	Had Training	Date training completed (or will be completed)	If no, state why

9.1. Have all project research workers undertaken safety training for working with hazardous or potentially hazardous biological materials and agents at CL2?

Name of researcher	Had Training	Date training completed (or will be completed)	If no, state why
Jakub Nasterski	(•) Yes ( ) No	10 Oct 2019	
Praveenkumar Kaveri	(•) Yes ( ) No	18 Nov 2018	

☐ 9.2. This work involves HTA 'Relevant Material', confirm that all project research workers have undertaken HTA training

10. EMERGENCY PROCEDURES

10.1 Are procedures in place for dealing with spillage of infectious or potentially infectious material

Equipment	Reference to SOPs
☑ Within the BSC	SOP038 – Biological Spill Response, SOP004 - general laboratory housekee
☑ Within the centrifuge	SOP122 Use and Maintenance of Eppendorf minispin centrifuge: SOP088 in
☑ Within the laboratory, but outside any primary control measures (e.g. BSC)	SOP 038 biological spill response
☐ Outside the laboratory	

Are procedures in place for the security of these HTA Relevant samples?

☐ Loss or theft of samples (including whilst in transit)	
☐ Loss of traceability of samples	
☐ Incorrect disposal of samples	

10.2 Describe the procedures in place for an accidental exposure

Immediate action	For a large spill, leave the lab and alert anyone else inside to do so. Consult MSDS for any chemicals involved in the spill. Remove any contaminated PPE and leave inside lab. Wait for at least 30 minutes for any aerosols to settle. Make sure other lab users are aware and do not enter the lab until it has been deemed safe to do so. Assemble clean up team of 3 people, one to observe and direct, other two to carry out the procedure. Spill kit can be found on the left hand side as you enter the lab. Put on PPE including mask. Use forceps to remove sharps and place in sharps container. Remove non-sharp items and place in yellow bag. Cover spill area with virkon powder working from outside in and slowly push inwards. Scrape into yellow bag. Wipe area with towel soaked in 1% virkon. Remove all PPE and autoclave/dispose. Wash hands and inform lab users when complete. Sharps injury-encourage bleeding, then wash with soap and water and seek medical attention. Skin exposure-flush with running water and wash with soap. Seek medical attention. Eyes-flush with eyewash for 15 minutes whilst holding eyes open. Ingestion/inhalation - seek medical attention.	Ref to SOP's	Biological Spill response: SOP038, HTA-MI-SOP008 repo
When and whom to report the incident	Report to lab manager once everyone has evacuated. For spills above	Ref to SOPs	Biological Spill response: SOP038, SOP 050 corrective ap

11. ACCESS

11. ACCESS

		Explanation	References
11. Is/are the lab(s) adequately separated from other areas (e.g. offices)?	☑ Yes ○ No		
11.2. Is/are the lab(s) or other work areas shared with other users not involved in the project?	☑ Yes ○ No	This work will be conduction in Wolfson T208b, which is a shared laboratory. Laboratory coats are segregated into microbiology (green) and non-microbiology (blue). Benches are not shared. As much work as possible will be completed inside the BSC.	SOP009- use and maintenance of Class II BSC. SOP003- Disposal of biological waste. SOP004-General lab housekeeping.
11.3. Describe the measures in place to ensure that hazardous biological agents or **HTA relevant** material is secure	○ Yes ☑ No		

12. OCCUPATIONAL

12.1. All workers involved with handling unscreened blood, blood products and other tissues are recommended to have Hepatitis B immunisation. Have all workers involved in this project been immunized?	☑ Yes ○ No
12.2. Is health surveillance required?	☑ Yes ○ No

13. NOTIFICATIONS

☐ 13.1. Are any of the cells, tissues or fluids covered by the Human Tissue Act (HTA) under the University HTA Licence?	
☐ 13.2. Are any of the cells, tissues or fluids obtained from a HTA licensed biobank with REC approval for generic research use?	
☐ 13.3. Does this work have ethical approval from a recognised NHS Research Ethics Committee?	
☐ 13.4. Does any of the work require approval from the University Ethical Committee?	
☐ 13.5. Do any of the materials require approval for use from the UK Stem Cell Bank Steering Committee (MRC)?	
☐ 13.6. Do any of the materials or biological agents listed require any other licenses?	

14. APPROVALS

Authorised Person	Sourav Ghosh Digitally signed by Sourav Ghosh Date: 2020.12.10 11:38:42 Z
Departmental Biological Safety Advisor	Kulvindar Sikand Digitally signed by Kulvindar Sikand Date: 2020.12.11 10:44:00 Z
University Biological Safety Officer (or Deputy)	C Kavanagh (C. Kavanagh on behalf of Julie Turner)

Approved by Julie Turner by email 15/12/20