Risk assessment made under the
Control of Substances Hazardous to Health Regulations

WORK WITH BIOLOGICAL MATERIAL

This form is to be used to identify the handling precautions to be adopted for biological material e.g. blood or blood products, tissues and other clinical samples, protein or RNA fractions, cell lysates, etc. Use the specific risk assessment form for naked DNA work. If the material is going to be used for genetic modification (e.g. cDNA library) an additional risk assessment is required under the Genetically Modified Organisms (Contained Use) Regulations. Guidance on risk assessment of biological agents is provided in University Policy Statement S5/09.

If the material is to be obtained from outside the UK or its use is otherwise controlled by DEFRA then advice should be sought and licences should be obtained as appropriate.

If the material is to be administered to laboratory animals an Animal Care Workers Risk Assessment must be undertaken (see UPS S5/09). Any persons handling the material who might have compromised resistance to disease for any reason should seek further advice regarding the need for additional precautions from the University Occupational Health Physician.

TITLE OF PROJECT:	Sexually Transmitted infections diagnostic Evaluation using Point of care isothermal amplification (STEP)
PURPOSE OF EXPERIMENT:	To determine performance of a point of care LAMP test for *Neisseria gonorrhoeae* (NG), *Chlamydia trachomatis* (CT), *Trichomonas vaginalis* (TV), Herpes Simplex Virus (HSV) in vaginal swab, throat swab, rectal swab and first void urine (FVU) or ulcer/vesicle swab via fluorescence and lateral flow readout
LOCATION OF WORK:	NDCLS Laboratory 5501, John Radcliffe Hospital

Supervisor (PRINT): Dr Monique Andersson **Signature:** **Date:** 2023-01-20	**Assessed by (if not Supervisor) (PRINT):** Professsor Stephen Hyde **Signature:** **Date:** 2023-01-25
Work approved by **(Biological) Safety Committee:** **YES~~/NO~~** **Biological Safety Officer:** Prof Stephen Hyde **Signature:** **Date:** 2023-01-25 Chairs action after meeting 2022-12-06	**Permission granted by HoD** **for work to commence (if required):** **YES/NO** **Head of Department:** Professor Deborah Gill **Signature:** **Date: 2023 01 31**

Persons involved: Yasaman Ahmadi, Yejiong Yu, Monique Andersson	

NATURE OF MATERIAL:	
FULL NAME OF SOURCE ORGANISM: Including species, subspecies, strain	Vaginal swab, throat swab, rectal swab and first void urine (FVU) or ulcer/vesicle swab suspected to contain pathogens such as *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, *Trichomonas vaginalis*, Herpes Simplex Virus (HSV)
HAZARD GROUP OF SOURCE ORGANISM (For micro-organisms):	2
Is source organism pathogenic or is material a potential/actual host to pathogenic organisms?	YES
HAZARD GROUP OF CO-PATHOGENS (e.g. For micro-organisms or zoonotic agents in animal or human tissue):	1 **2** 3 4 Samples are suspected to contain HG 2 organisms, but have the potential to contain HG 3
If pathogenic can material be biologically inactivated before use? (e.g. Pasteurization for Mycobacterium)	No, it is not possible to optimise the assay if sample is heated before opening the vial. The process will be conducted in a Biosafety cabinet at CL2. If yes give details:
If material is pathogenic and cannot be inactivated does it pose an infection risk?	YES
If infection risk is posed, specify consequences of infection(s) (severity and type or illness caused):	The risk of infection is related to direct exposure to the organism. Specifically, infection is only possible where there is direct contact of infected sample with the throat, eye or broken skin. In order to minimise any risk, potentially infectious samples will be managed in CL2 wearing PPE with GLP compliance. Likely risk is for conjunctivitis – red, painful eye (NG, CT and TV, HSV), pharyngitis (NG) – sore throat or skin ulceration with vesicle formation where skin already broken or damaged (HSV). Disease is generally mild and is treatable with antibiotics/antivirals. HSV establishes latent infection.
IS THE MATERIAL OR THE CO-PATHOGENS PATHOGENIC TO ANIMALS?	NO
IS THE MATERIAL OR CO-PATHOGENS ON THE LIST OF SPECIFIED ANIMAL PATHOGENS ORDER?	NO
ASSIGN DEFRA HAZARD GROUP	1 **2** 3 4
If pathogenic to animals please contact University Biological Safety Officer for further advice as different licences may be required.	
Is the material or co-pathogen listed on Schedule 5 of ATCSA?	NO
Route(s) of infection:	**Ingestion (NG)** / Inhalation / **Percutaneous (HSV)** / **Ocular (NG, TV, CT and HSV)**
Can material from a less hazardous source be used?	NO

If yes, why not use it?	
Are there any other hazards of the material or its preparation?	NO
If yes identify type:	Toxic / Allergenic / Oncogenic / Carcinogenic / Other (specify)

CONTROL MEASURES:	
Containment Level:	2
Additional precautions:	
- Wear gloves at all times	YES
- Avoid use of sharps	YES
- Use microbiological safety cabinet for aerosols	YES
- All material to be handled in microbiological safety cabinet	YES
- Centrifugation	YES
- Vortexing	YES Human samples will be centrifuged in sealed rotors and vortexing will be done in sealed tubes
-	NO
FACS	YES
Other (specify) Eye Protection **Goggles/visor**	
- **Good microbiological practice:**	YES
Registration with Occupational Health Service	YES (NB Monique Andersson is registered with NHS equivalent; Yasaman Ahmadi, Yejiong Yu are/will be registered with University's Occupational Health Service)

LABORATORY / EXPERIMENTAL PROCEDURES (typical volumes, frequency of use, etc.):

The sample vials will contain ~1.5ml of patient sample (urine, VTM with swab). The reaction mixture (typically: 12.5 ul LAMP master mix + 2.5 ul 10X primers + 0.5 ul SYTO9 (25 uM) + 9 ul RNase/DNase Free water + 5 ul sample of interest including NG, CT, TV or HSV) will be mixed by pipetting each of these substances from their original container (a tube of maximum 1 mL volume) into PCR-tubes (0.2 mL volume). The PCR tubes will be placed in a heat block/qPCR machine (temperature: 65 °C) for 30 min for a fluorescent readout. The sample will also be incubated at 95°C for 5 min to denature the sample. After cooling back to room temperature, the PCR tubes will be then discarded as clinical waste to be further incinerated. All the surfaces will be cleaned by wiping out with disinfectant (eg. 1% Virkon) and then 70% ethanol. Any residual clinical sample will be placed directly in an autoclave bag then into the orange clinical waste bags for disposal via the OUH clinical waste stream.

MOST LIKELY & WORST CASE SCENARIO. INDICATE MITIGATING CONTROLS: (What could happen in extreme circumstances or is the most likely negative occurrence to happen)

The worst case scenario is to spill the vial of 1.5ml including live bacteria or virus. As risk mitigating controls, all the bacteria/virus samples will be handled under the designated microbiological safety cabinet as a HG2 organism.

DISINFECTION PROCEDURES:

The safety cabinet will be cleaned with disinfectant (0.5-1% Sodium hypochlorite (Chloros) or 1% Virkon with a final wipe of 70% ethanol and subjected to 20 min of UV irradiation before and after using the safety cabinet. Other working surfaces and equipment will be regularly cleaned with 1%Virkon and 70% ethanol to minimise any surface contamination. All laboratory procedures such as handling reagents or specimens will be performed while wearing protective equipment (laboratory coat and gloves and safety specs (if required visor).

EMERGENCY PROCEDURES:

In the event of spillages, cover the spill with absorbent material soaked in 1% Virkon. Allow 10-15minutes depending on the volume of the spill. Clean the surface with a fresh cloth (eg blue roll) with disinfectant then 70% Ethanol. The contaminated absorbent material will be disposed in biohazardous waste and incinerated.

WASTE DISPOSAL PROCEDURES: (Please consult Departmental Policy on Waste Disposal)

- Patient samples will be denatured by heating at 95^{o}C for 5 min/disinfected according to manufacturer's protocol, and then disposed of via the OUH clinical waste streams (incineration). 96-well plates will be immediately disposed of into discard bins (limb bins) for disposal via the OUH clinical waste streams (incineration). There is no requirement to unseal plates prior to disposal. Posters are on display in the lab as a visual reminder of the OUH waste segregation policy.
- Disposables (tips, pipettes, gloves, eppendorf tubes, etc): Tips and pipettes will initially be discarded into a disposal jar. Disposal jars and other items (gloves, Eppendorf tubes etc) must be disposed in appropriate waste containers (limb bins or orange clinical waste bags) for disposal via the OUH clinical waste stream (incineration). Any contaminated absorbent material used to wipe up spillages/decontaminate surfaces will be placed directly in an autoclave bag then into the orange clinical waste bags for disposal via the OUH clinical waste stream. The rate of waste removal is governed by OUH clinical waste stream practises.
- Surface decontamination: will be achieved by allowing 70% Ethanol to evaporate from relevant surfaces. All tips, pipettes will be discarded into disposal jar.
- Sample identification: All samples are labelled only with a study number only, no information that could reveal patient identification will be used.

STORAGE: (State where material and its products will be stored if containment levels 2 and 3 apply)

There is no plan to store material in the NDCLS Laboratory. If patient samples need to be stored for a short period of time, they will be transported back to the Oxford University Hospitals NHS Foundation Trust Microbiology Laboratory in accordance with standard procedures for the safe transport of hazardous biological materials outlined in the University's Biorisk Management policy statement S5/09 (briefly, within a sealed secondary vessel that contains sufficient absorbent material to absorb any spillage/leakage).

ADDITIONAL INFORMATION: (Please consult University Policy Statement S5/09 if handling human blood, blood products or tissues)

Samples known to be positive for HIV and/or HepB will not be utilised. However, as is standard practice, not all samples will have previously been tested for these pathogens, thus all samples will be managed with universal precautions.

HEALTH SURVEILLANCE All workers must register with the University Occupational Health Service when working with human samples. All workers will be hepatitis B vaccinated.

VACCINATION(S): **Is an appropriate vaccination available?** NO **Will it be offered? YES / NO / <u>NOT APPLICABLE</u>** **If "no" why not?**

Additional controls may be required if the following techniques are being used as part of this project (highlight):

Generation of aerosols: **<u>Centrifugation</u>** Sonication **<u>Mixing</u>** Vigorous pipetting Any other action generating splashes or aerosols	**<u>Wear appropriate eye protection (spectacles, goggles, visor)</u>** **<u>Use microbiological safety cabinet.</u>** Implement any other necessary controls (list): Tubes/plates will be sealed for vortexing and vortexer will be located within the class II safety cabinet. Sealed rotors will be used for any centrifugation steps.
Dissection **Use of syringe needles** **Use of other sharps or glass** **N/A**	Use cut and puncture resistant gloves if unable to avoid these procedures. Implement any other necessary controls (list):
Consider all other potential routes of infection: **Skin**	**Implement appropriate controls:** **Wear gloves, appropriate hand washing remove gloves when touching door handles, phones etc to avoid accidental contamination).**