Friday, August 25, 2017

The Notifications Officer
Health and Safety Executive
Hazardous Installations Directorate
Biological Agents Unit
5S2 Redgrave Court
Merton Road
Bootle
Merseyside L20 7HS

Dear Sir/Madam,

Subject: Significant change to GM Notification GM6/09.1

We wish to expand the notified activity GM 6/09.1, entitled "Investigation of in vitro and vivo cellular processes of Hazard Group 2 bacterial pathogens clinically relevant in the UK", to include Hazard Group 2 bacterial pathogens clinically relevant in the UK that are primarily transmitted via the aerosol/airborne route. Such pathogens were specifically excluded from the original notification and we were advised that extending the connected program of work to include them would constitute a significant change – see attached letter dated 19 May 2009.

The aerosol/airborne Hazard Group 2 pathogen we are proposing to work with initially is *Streptococcus pyogenes*, but there are proposals to work with other aerosol/airborne Hazard Group 2 pathogens in the near future, e.g. *Streptococcus pneumoniae* and *Pseudomonas aeruginosa*.

Two risk assessments relating to the *S. pyogenes* work are attached, one covering in vitro work (GMM1403) and one covering in vivo work (GMM1411). Comments from the University Biological Agents Committee on the risk assessments have been appended to the risk assessment forms. I have also enclosed copies of the Containment Level 2 SOPs referenced in the risk assessments. SOP48, referenced in assessment GMM1403, is our generic SOP for working in CL2 Tissue Culture facilities. SOP222, referenced in assessment GMM1411, is specific to the Containment Level 2 facility in our animal unit.

Please note that we are now the School of Life Sciences, as opposed to the College of Life Sciences, and Prof. Doreen Cantrell, former Head of College, has been succeeded by Prof. Julian Blow FRSE FMedSci, Dean of School.

If you require further information please do not hesitate to contact me.

Sincerely,

Lisa Grayson

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Enclosures:

1. Letter from HSE dated 19/5/09
2. GMM1403
3. GMM1411
4. SOP48
5. SOP222

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Head of Unit
Dr Joanne Nettleton

Dr Ian G Scragg
University of Dundee
3 Cross Row
Dundee
Scotland
DD1 4HN

Date: 19 May 2009

Reference: GM6

Dear Dr Scragg

GENETICALLY MODIFIED ORGANISMS (CONTAINED USE) REGULATIONS 2000, AS AMENDED

NOTIFICATION OF CLASS 2 ACTIVITY

Thank you for your notification under Regulation 10 of the above Regulations, of the intention to undertake activities involving Class 2 genetically modified micro-organisms (GMMs).

The Competent Authority has no objection to your performing the notified activity, GM 6/09.1 originally entitled "Life Sciences research involving Class 2 genetically modified micro-organisms". But to more accurately reflect the proposed connected programme the Competent Authority has changed the title to "Investigation of in vitro and vivo cellular processes of hazard group 2 bacterial pathogens clinically relevant in the UK".

The Competent Authority are content to clear this connected programme of work based on the genetic modification activities being restricted to hazard group 2 bacterial pathogens, as specified in the submitted documentation. Should the work extend to others HG2 bacterial pathogens, transmitted via an airborne route, then this would in all likelihood constitute a significant change to the notification and require subsequent notification to the Competent Authority.

This letter does not imply endorsement by the Competent Authority of any physical containment and associated safety precautions at your centre, or that the measures you have taken are sufficient to comply with the Regulations and other relevant legislation. HSE inspectors may have additional observations and requirements when conducting site inspections under the Health & Safety at Work etc Act.

Regulation 15(2) and (3) requires that the Competent Authority be kept informed of any changes to the notified details.

Yours sincerely

Dr Michael Paton, Health & Safety Executive
On behalf of the Competent Authority

David Barnes
Scottish Government

Full GMM Risk Assessment 1403

FullSerialNo	GMM1403
Version	3
Title	Working with genetically modified Group A Streptococcus (Streptococcus pyogenes)
Final_class	Class 2
PI responsible	Dorfmueller, Helge
Division	Molecular Microbiology
Building	Medical Sciences Institute
Lab_No	2
Name of assessor	Dorfmueller, Helge
Approval_date	12/04/2017
Review date	12/04/2018

1. Brief description of project

The aims of our studies are to understand the biosynthesis (in parts and in whole) of the Group A Carbohydrate from Group A Streptococcus (GAS), also known as Streptococcus pyogenes. We are studying the biosynthesis pathway in a combination of approaches, using synthetic biology, enzymology and related techniques. Recent work by van Sorge et al. (2015) (https://www.ncbi.nlm.nih.gov/pubmed/24922575) has shown that genetic manipulation of the Group A Carbohydrate cluster (genes gacA-L) manipulates the biosynthesis pathway of the Group A Carbohydrate. The Group A Carbohydrate is a surface polysaccharide, linked to cell wall peptidoglycan, as identified by Munoz et al., 1967 (https://orbi.ulg.ac.be/bitstream/2268/93329/1/Ghuysen%20-%2013.pdf). The genetic deletion strains of gacI, gacJ and gacK lack an immuno-dominant sidechain (GlcNAc) on the cell wall. The gacI deletion strain was shown to have reduced virulence in a mouse and rabbit model (https://www.ncbi.nlm.nih.gov/pubmed/24922575).

Van Sorge et al. have created a mutant library of this cluster, and we want to use this existing library of genetic deletion mutants (https://www.ncbi.nlm.nih.gov/pubmed/24922575) to complement several of our studies.

1) We intend to use GM GAS to further validate in Streptococcus our knowledge with regards to catalytic residues, which show either reduced activity or no activity. Our research in a recombinant E. coli system sets the groundwork to establish that no mutants should be more active than the WT GAS. Therefore, the risk to human health is low, compared to the WT GAS strains. These insertion mutants are predicted to be comparable to the gacA-L deletion mutants in terms of reduced virulence. Insertion mutants, such as gacI inactive, we will test in a mouse model and compare to the gacI deletion mutant strain.

2) We intend to grow the GM GAS mutant strains with and without complementation with point mutations for cell-based assays, agglutination tests and carbohydrate extractions. Complementation mutants are defined as GM GAS that contain the native gene in a modified version, where a key residue has been mutated on the genetic level.

3) In the future, we aim to insert into the library of GM GAS selected point mutants of the native genes, that are in the binding site of identified inhibitors. These so called inhibitor-resistant mutants will help to validate our inhibitors, that have been identified from E.coli expression systems (plate based assay, micro-titre plate assay).

4) These studies can be complemented with cell-based assays and where required carbohydrate analysis and microscopy of cells.

Strains we intend to use and further modify:
*M5448 WT deletion mutant strains, GAS B42, C121, C265, C132, B403; Lancefield collection strains, M2, M3, M6, M11, M12, M28, M75, M89 strains.

2a. Hazards to human health associated with the recipient microorganism

Streptococcus pyogenes is an aerobic, gram-positive extracellular bacterium (1, 2). It is made up of non-motile, non-sporing cocci that are less then 2 µm in length and that form chains and large colonies greater then 0.5 mm in size (3, 4). It has a β-hemolytic growth pattern on blood agar and there are over 60 different strains of the bacterium (5, 6)
PATHOGENICITY/TOXICITY: This bacterium is responsible for a wide array of infections (7, 8). It can cause streptococcal sore throat, which is characterized by fever, enlarged tonsils, tonsillar exudate, sensitive cervical lymph nodes and malaise (6, 9). If untreated, strep throat can last 7-10 days (9). Scarlet fever (pink-red rash and fever) as well as impetigo (infection of the superficial layers of skin) and pneumonia are also caused by this bacterium (3, 6, 7, 10). Septicaemia, otitis media, mastitis, sepsis, cellulitis, erysipelas, myositis, osteomyelitis, septic arthritis, meningitis, endocarditis, pericarditis, and neonatal infections are all less common infections due to S. pyogenes (3, 6, 7). Streptococcal toxic shock syndrome, acute rheumatic fever (joint inflammation, carditis and CNS complications), post-streptococcal glomerulonephritis (inflammation, hematuriia, fever, edema, hypertension, urinary sediment abnormalties and severe kidney pain) and necrotizing fasciitis (rapid and progressive infection of subcutaneous tissue, massive systematic inflammation, hemorrhagic bullae, crepitus and tissue destruction) are some of the more serious complications involving S. pyogenes infections (1, 6-8). There are at least 517,000 deaths globally each year due to severe S. pyogenes infections and rheumatic fever disease alone causes 233,000 deaths (8). 1,800 invasive S. pyogenes disease-related deaths are reported in the USA yearly, necrotizing fasciitis kills about 30% of patients and streptococcal toxic shock syndrome has a mortality rate of 30-70% (3, 11, 12).
MODE OF TRANSMISSION: Transmission via respiratory droplets, hand contact with nasal discharge and skin contact with impetigo lesions are the most important modes of transmission (5, 9, 13). The pathogen can be found in its carrier state in the anus, vagina, skin and pharynx and contact with these surfaces can spread the infection (5, 14, 15) The bacterium can be spread to cattle and then back to humans through raw milk as well as through contaminated food sources (salads, milk, eggs); however, cattle do not contract the disease (16-18). Necrotizing fasciitis is usually because of contamination of skin lesions or wounds with the infectious agent (12).
INCUBATION PERIOD: The incubation period is usually 1-3 days (9).
COMMUNICABILITY: If untreated, patients with streptococcal pharyngitis are infective during the acute phase of the illness, usually 7-10 days, and for one week afterwards; however, if antibiotics are used, the infective period is reduced to 24 hours (9). The bacterium can remain in the body in its carrier state without causing illness in the host for weeks or months and is transmissible in this state (5).

2b. Hazards to human health arising directly from the inserted genetic material

1) As described above, we intend to replace deleted genes from the GM GAS collection that are required for the biosynthesis of the GAC virulence factor. These deletion constructs are predicted to have no direct negative effect on human health. For example, deletion of the gene gacI leads to a strongly reduced virulent GM GAS strain (van Sorge et al., 2015, Cell Host & Microbe). The insertion of replacement genes, that have been shown to be inactive in the E.coli system, will result into a GM GAS that has a similar phenotype to the existing GM GAS (deletion mutants), since we anticipate to have validated the 'inactivity' of the gene product.

Gene deletion introduces one antibiotic resistance cassette and the insertion plasmid introduces another. Therefore, GM bacteria will acquire antibiotic resistance to two of the following antibiotics: Erythromycin, Ampicillin, Kanamycin, Chloramphenicol.

2) S. pyogenes infections are susceptible to a variety of drugs: β-lactams such as penicillin, as well as erythromycin, clindamycin, imipenem, rifampin, vanomycin, macrolides and lincomycin; however, certain strains of the bacterium have been found to resistant to macrolides, lincomycin, chloramphenicol, tetracyclines and cotrimoxazole (5, 7, 19, 20). The student/staff has to inform the GP of any antibiotic used in the study to get proper treatment.

2c. Hazards to human health arising indirectly from the inserted genetic material

No additional hazards to human health are anticipated.

2d. Hazards to human health arising from transfer of genetic material to a related

pDCErm and similar non-mobilisable vectors will be used.
No hazard anticipated, since only single genes will be inserted using plasmids and these will be validated as being inactive. A whole cluster of 12 genes is required to make the genetic material functional in a related organism.

3. Assign a provisional containment level

Containment Level 2

4a. Hazards to the environment associated with the recipient microorganism

The bacterium (WT) can survive on a dry surface for 3 days to 6.5 months (22). Similar survival rate is expected for GM GAS.
S. pyogenes is an exclusively human pathogen (5, 7), although cattle can also act as a reservoir (16 -18). Cows infected by humans are intermediate hosts and can pass the bacterium in their milk, which, if consumed unpasteurized, can infect other humans (16).

4b. Hazards to the environment arising from the genetic material

The engineered genetic material is located on small narrow spectrum non-mobilisable plasmids. Some genetic materials consist of synthetic gene sequences that do not exist in nature. A whole

cluster of 12 genes is required to make the genetic material functional in a related organism, therefore, the hazard arising from the genetic material to the environment is regarded as very low.

5a. Brief description of nature of work (include maximum culture volumes)

GAS cells are grown for several purposes (maximal volume listed).

1) isolate genomic DNA for cloning purposes (20 ml cultures)
2) grown in agar plates for plate based assays
3) growth curves (5 ml cultures)
4) microtitre plate for inhibitor testing (500 µl per well)
5) Carbohydrate extraction (500 mL cultures, if required 1000 mL)

5b. Is a microbiological safety cabinet or isolator required to protect the worker from aerosol transmission?

Yes

5c. Waste disposal

As per SOP 48: Containment Level 2: Tissue Culture Facilities.

Our standard autoclaving conditions will be used: 134 degrees C for 5 min for solid waste; 121 degrees C for 20 min for liquid waste.

This bacteria is susceptible to 70% ethanol which will be used for the day-to-day cleaning of work surfaces and equipment. 1% Virkon has been shown to be effective against S. pyogenes [see Virkon efficacy data at http://www.euromar.co.il/files/Virkonefficacydata.32373086.pdf] and will be used for neutralising spills and bio-decontaminating non-autoclavable items.

5d. Are sharps required? Yes or no. If yes, justify use.

no

5e. If the work involves experimental infection of animals is it known if the animal will shed the GM microorganisms?
If yes, give details and measures to prevent exposure.

A separate risk assessment will be prepared to cover animal work as and when the project progresses to this stage.

5f. If the work involves experimental infection of plants what is known about the likely route of transmission of the GM microorganisms?

n/a

5g. Where will the GM microorgansim be stored?

Transformed GAS strains will be stored on agar plates at 4 degree Celsius during the experimentation period and then disposed of by autoclaving. Transformed GM microbes will be stored at -80 degree

Celsius in a freezer labelled with a Containment Level 2 sign. Plates will be stored in the fridge for temporary storage only. All fridges/freezers are located within the CL2 facility.

5h. How will the GM microorganism be transported within/between buildings to minimise risk of spillage/escape?

We do not anticipate transporting GM organisms between different departments. Within the department, cells will be transported in sealed and identified container as per SOP 48: Containment Level 2: Tissue Culture Facilities.

5i. Will staff/students receive any vaccination or health surveillance? If yes, give details.

No vaccine available. Staff/students will be informed to raise any symptoms (tonsillitis, skin infection) and to inform GP of strains used, i.e. which antibiotic marker is present in the strains used.

5j. Emergency plan, if required.

Small scale CL2 activity, therefore, no emergency plan is required.

5k. Monitoring

As detailed on the SLS Safety web site at https://www.lifesci.dundee.ac.uk/services/healthandsafety/other-topics/microorganisms/cl_1_and_2.html#validation

6. Final classification

○ Class 1 ● Class 2 ○ Class 3

7. Additional information

Most information has been taken from (very useful read):
http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/strep-pyogenes-eng.php

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Comments from University Biological Agents Committee

LG, 8/3/2017: Made a couple of straight forward amendments. Antibiotic resistance cassettes should be detailed for cross checking against drugs used in treatment of infection.

NRH, 10/3/17:
In the brief description:
1. Discuss the strains referred to at the bottom of that section in more detail. Where will these come from, what are their characteristics? Are any already GM or are they WT and natural mutants?
2. Which of those strains will then be used to make "GM GAS" and how. I would like to see more detail on the Group A Carbohydrate virulence factor too and why it is so important not to produce it. Presumably various of the strains to be used already produce it, so why is it important not to do so in the GM GAS?
Section 2a should be enough to ensure anyone working with these bugs understands how nasty they are.
Section 2b helps a bit with the issues I was talking about above, but again I don't understand some comments. For example: "Deletion constructs are predicted to have no direct effect on human health". Does it actually mean that introduction of deletion constructs into a host bacteria is not predicted to affect the pathogenicity of that bacteria because it will remove the target genes? How about the antibiotic that will be introduced as part of this process? That could have an effect on pathogenicity. I also think there needs more clarity on which antibiotic resistance will be conferred. Currently, the wording allows for all 4 of those antibiotic resistances to be conferred. Is that the case? If so, how could that affect pathogenicity? I do note that later there is comment about clinically used antibiotics, so maybe the ones used for the GM work are not clinically important? Needs to be discussed.
4b states "Their ability to be recognized and processed by natural competence systems in the environment is expected to be very low". This needs explanation as to why this is expected and references to back it up.
5g. Will plates be stored in a sealed condition?
5j. An emergency plan is normally not required at CL2 unless there are very unusual activities.

SA, 10/3/17: I agree with NRH's comments about antibiotic resistance. I think this need to be better explained. I also think more detail in 5i/j would be helpful.

AHF, 10/3/17: Having read the cited article on the gacI deletion mutant, I have some issues with the definition of "avirulent" used in the application. My reading is that the gacI mutant shows "reduced virulence" in mice and avirulence in rabbits. These conclusions assume that the authors have not missed "delayed virulence" due to early termination of their animal experiments.
I am not an expert in this area, but I would like reassurance that adding back a catalytically inactive gacI point mutant is as safe as claimed in the risk assessment. First, what is the level of "inactivity" in your mutants (likely to be greater than zero) and second, what is the likely rate of reversion of a single point mutation in gacI to wild-type? What is the minimum inoculum required to initiate an infection?

HD, 10/3/17: You are absolutely right, we have to tune down the avirulence statement. Your suggested experiment - insertion of point mutation - is our intention, however, we can only conduct them once we are allowed to have the GM strains and the risk assessment approved to conduct the experiments.
We cannot predict the reversion rate to WT. However, since the growth of the deletion mutant is not effected, we can only speculate that there is no selective pressure to revert a potentially inactive

mutant. Kinetic experiments conducted with recombinant enzymes will most likely reveal that the enzyme is almost inactive (very low kcat and / or very high Km values are expected for these active site mutants) and we cannot guarantee 100% inactivity of the inserted point mutant. However, the result of a partially active insertion mutant would be the enzymatic function equivalent to the wild type GAS strains we use.
Regards minimum inoculum: Unfortunately, this has never been investigated or at least described. I have asked Prof Sriskandan, a worldwide recognised expert in GAS research, and she responded to this very same question that it is not known, which dose / cell number is required to infect a healthy human being.

NRH, 13/3/17: Thank you for your revised texts, comments and explanations, which assist greatly in understanding what is to be done. I agree completely with the comments about avirulence. This needs to be revised for clarity, but the key point is that no complementation mutant should have the ability to produce GAC more effectively than the original WT organism. The prediction is most will have zero or next to zero ability. Since the WT organism is a HG2 organism, CL2 is appropriate as a containment measure and so the GM organisms will also be appropriate to handle under CL2 even though the prediction is that they will be significantly less virulent than the WT (noting that the GAC mutants being used as the starting point for this study appear to be much less virulent than the WT organism – AHF's comments noted). HD, correct me if I am wrong please, but I am thinking that you don't intend to handle the WT organism at all as part of this study?
A key element of risk assessment is always to reduce the risk so far as is reasonably practicable and this proposed study is a continuation of a previous study undertaking work in GM E. coli, so the groundwork to establish that no mutants should be more active than the WT has been done in a model organism of low hazard to human health. I am confident, therefore, that the appropriate thought has been put into this work and that steps have been taken to control the risk suitably.
With the amendments discussed, I support the assessment being classified as Class 2.

HD, 13/3/17: many thanks for your reply and comments! Yes, that is correct, WT GAS is not required for this study. I will revise the RA as attached and highlighted in turquoise and submit it for approval.

LG, 12/4/17: Further amendments suggested by LG and NRH. Modified/approved by PI. Now version 3. Ready for submission to HSE.

Full GMM Risk Assessment 1411

FullSerialNo	GMM1411
Version	3
Title	Screening GM strains in an in vivo model of Group A Streptococcus (Streptococcus pyrogenes)
Final_class	Class 2
PI responsible	Dorfmueller, Dr Helge
Division	Molecular Microbiology
Building	Medical Sciences Institute
Lab_No	2
Name of assessor	Sarah Thomson
Approval_date	24/08/2017
Review date	24/08/2018

1. Brief description of project

We are studying the biosynthesis pathway of Group A Carbohydrate, a Group A Streptococcus (GAS) virulence factor as described in previously approved assessment GMM1403.

The aim aims of this part of the project are to establish an in vivo model of GAS infection in mice, to screen GM GAS strains to identify the functional role and to test potential vaccine candidates for efficacy in the model.

2a. Hazards to human health associated with the recipient microorganism

See GMM1403.

2b. Hazards to human health arising directly from the inserted genetic material

See GMM1403.

2c. Hazards to human health arising indirectly from the inserted genetic material

No additional hazards to human health are anticipated.

2d. Hazards to human health arising from transfer of genetic material to a related

See GMM1403.

3. Assign a provisional containment level

Containment Level 2

4a. Hazards to the environment associated with the recipient microorganism

See GMM1403.

4b. Hazards to the environment arising from the genetic material

See GMM1403.

5a. Brief description of nature of work (include maximum culture volumes)

GAS cells for in vivo infection are grown outwith the WBRU in the Molecular Biology CL2 facility in culture volumes of up to 200ml. Within the WBRU mice will be infected with a small volume (<200ul) of bacterial suspension. Tissue samples will be harvested, homogenised and small volumes (<200ul) plated onto agar and incubated to monitor GAS infection.
Live animals will be housed and manipulated in accordance with SOP222: Infection studies in the Wellcome Trust Biocentre Resource Unit (WBRU) Containment Level 2 Facility.

5b. Is a microbiological safety cabinet or isolator required to protect the worker from aerosol transmission?

Yes

5c. Waste disposal

Waste disposal procedures to be followed within the WBRU are detailed in SOP222 (which includes parameters for autoclave cycles). When working with S. pyogenes outwith the WBRU follow the waste disposal procedures in SOP48, Containment Level 2, TC Facilities, and apply the standard autoclaving conditions of 134 degrees C for 5 min for solid waste and 121 degrees C for 20 min for liquid waste.

S. pyogenes is susceptible to 70% ethanol which will be used for the day-to-day cleaning of work surfaces and equipment. 1% Virkon has been shown to be effective against S. pyogenes [see Virkon efficacy data at http://www.euromar.co.il/files/Virkonefficacydata.32373086.pdf] and will be used for neutralising spills and bio-decontaminating non-autoclavable items.

5d. Are sharps required? Yes or no. If yes, justify use.

Yes. Animals will be infected by injection and vaccine therapies need to be given by injection. Sharps will be disposed of directly into a sharpsafe after use. Once the experiment is complete, animals will be dissected with sharp scissors and forceps for tissue analysis. No alternatives at present.

5e. If the work involves experimental infection of animals is it known if the animal will shed the GM microorganisms?
If yes, give details and measures to prevent exposure.

Yes, during nasalpharynx infection, nasal shedding will peak around day 2-4 post infection. During systemic infection it may be shed via faeces. Shedding from either route of administration to the bedding of animals may spread infection to animals within the same cage. The risk of infection to humans is minimal as and is further reduced by adhering to SOP 222. Cage waste is removed within a Class II cage-change cabinet, double bagged then autoclaved before disposal. Carcasses are double bagged and autoclaved prior to disposal. Used cages are treated with Sanosil Super 25 (2%

solution for 3 min) prior to regular cage washing procedure.

5f. If the work involves experimental infection of plants what is known about the likely route of transmission of the GM microorganisms?

Not applicable.

5g. Where will the GM microorgansim be stored?

Transformed GAS strains will be stored on agar plates at 4 degree Celsius during the experimentation period and then disposed of by autoclaving. Transformed GM microbes will be stored at -80 degree Celsius in a freezer labelled with a Containment Level 2 sign. Plates will be stored in the fridge for temporary storage only. All fridges/freezers are located within the CL2 facility.

5h. How will the GM microorganism be transported within/between buildings to minimise risk of spillage/escape?

Cells will be transported in sealed and identified container as per SOP 48.

5i. Will staff/students receive any vaccination or health surveillance? If yes, give details.

No vaccine available. Staff/students will be informed to raise any symptoms (tonsillitis, skin infection) and to inform GP of strains used, i.e. which antibiotic marker is present in the strains used.

5j. Emergency plan, if required.

Small scale CL2 activity, therefore, emergency plan not strictly required. However, SOP 222 includes emergency procedures to follow in the event of the air supply to the IVCs failing and if a worker receives an animal bite/needlestick injury.

5k. Monitoring

As detailed on the SLS Safety web site at https://www.lifesci.dundee.ac.uk/services/healthandsafety/othertopics/microorganisms/cl_1_and_2.html#validation

6. Final classification

○ Class 1 ● Class 2 ○ Class 3

7. Additional information

Please refer to GMM1403 for in vitro work.

Comments from University Biological Agents Committee

LG, 8/6/17: This covers the animal work associated with Helge's S. pyogenes work. Will go to HSE as part of the significant change notification. I'll compare it to the S. pyogenes GM risk assessment 1403 to make sure both assessments concur.

NRH, 22/6/17: I have concerns about section 5e. The wording is somewhat clumsy in places and also vague. For example "wearing appropriate PPE". What does that mean? They should refer to the SOP for animal work (which hopefully states what PPE is needed?). Once that is tightened up, I am happy with this one.

LG, 23/8/17: Sections 5a/c/e/j have been reworded to refer to SOP222. Section 5a has been reworded for clarity. Rather than copying and pasting blocks of text, GMM1403 is now referenced in sections 1/2a/2b/2d/4a/4b/7.

Safe Operating Procedure 48

FullSerialNo	SOP48
Version	13
Title	Containment Level 2: Tissue Culture Facilities
Author	Lisa Grayson
Division	Life Sciences
Building	All
Lab No	
Approval date	21/08/2017
Review date	21/08/2018

Procedure

Note: This is a summary of the main points. For full details see "Procedures for Safe Working With Micro-organisms" at www.lifesci.dundee.ac.uk/services/healthandsafety/other-topics/microorganisms/microorganisms_home.html.

Procedure

1. Read and understand the relevant risk assessment and receive adequate training from your supervisor.
2. The CL2 TC facility must be clearly signed as such and access restricted to authorised personnel only.
3. Wear appropriate personal protective equipment (PPE). Note: dark blue TC lab coat is mandatory. Requirement for additional PPE must be determined by risk assessment.
4. Use centrifuges in strict accordance with the manufacturer's instructions. Aerosol containment tubes, canisters or rotors must be used during centrifugation and must only be opened within the microbiological safety cabinet (MSC). Disinfect with 1% Virkon after use.
5. Follow correct working procedures when using a MSC in order to ensure operator protection. Incorrect working practice can cause operator protection to fail. Complete the on-line TC Safety training package and undergo practical training before first use. (Your Group Leader will appoint a competent person to deliver practical training.).
6. Ensure incubators are labelled with a large biohazard sign and all culture vessels are labelled with your name and the nature of the sample. Use culture vessels that will prevent leaks/spills if at all possible, e.g. filter cap flasks. Otherwise, place culture vessels in a tray that will contain any leaks/spills.
7. Aerosol resistant pipette tips & plugged pipettes are recommended to prevent cross contamination. Use pipettes slowly & carefully. Eject pipette tips carefully & directly into the waste container. Keep the waste container within the MSC.
8. Take steps to minimise the risk of contaminating personal items that you will take with you when you leave the CL2 facility, e.g. do not touch mobile phones/tablets/laptops without first removing gloves and washing hands and do not place such items on surfaces where work with live cultures is undertaken. If you suspect that an item may be contaminated, wipe it down with a disinfectant wipe before removing it from the CL2 facility. Extra care should be taken to avoid accidental contamination of paperwork/notebooks that cannot be readily disinfected.

9. All waste containers must be clearly labelled and must not be overfilled.
   - o The use of sharps must be avoided unless essential. True sharps waste must be disposed of into an approved sharpsafe container kept within the MSC. Once the sharpsafe is full to the max fill line, engage the permanent closure and place in the designated waste collection area. Do not place sharpsafes into the red boxes used for solid waste.
   - o Plastic pipette tips must be disposed of as true sharps if a sharpsafe container is already in use. This is to minimise the number of items within the MSC and, thereby, turbulence within the cabinet. Otherwise, dispose of into a robust, plastic screw-cap container (available from Central Technical Services) kept within the MSC. Once full, cap container and dispose of as solid waste.
   - o Make sure plastic pipettes are fully discharged of liquid before disposal as solid waste.
   - o Large, glass pipettes are not used in TC/for CL2 work.
   - o Solid waste must be collected in a red, lined, biohazard labelled, autoclavable box (available from Central Technical Services). There must be no liquid or sharps in the solid waste bag. At the end of the work session: loosely tape the liner around the neck; place the lid on the box; secure with a strip of autoclave tape; label as “SOLID WASTE” and place the box in the designated waste collection area.
   - o Avoid using the aspirator setups if at all possible. If there is no alternative a safe operating procedure must be agreed with SLS Safety.
   - o Other liquid waste must be collected in a robust, autoclavable container. Once three quarters full, cap and place in the red, biohazard labelled, autoclavable box. At the end of the work session: place the lid on the box; secure with a strip of autoclave tape; label as “LIQUID WASTE” and place the box in the designated waste collection area.
   - o Blood and tissue waste (recognisable as such) must be disposed of as clinical waste (see the SLS Safety web site Waste Disposal section for further details).
   - o Radioactive or chemically toxic waste must not be autoclaved. Consult your BSO for advice.
10. Surfaces must be decontaminated after work. Use fresh 1% Virkon solution. Thoroughly spray area, leave for at least ten minutes then wipe down thoroughly with 70% ethanol to remove residue. Do not spray sensitive equipment with Virkon.
11. Contaminated reusable glass/plastic ware, if autoclavable, must be placed with liquid waste containers in the designated collection area. If reusable items are not autoclavable, consult your BSO for advice on disinfection.
12. Tip boxes should be sprayed down with 70% ethanol prior to sending for refilling. This is to prevent exposing staff who refill the tip boxes, prior to sterilisation, to any contamination picked up within the CL2 facility.
13. In the event of a spill, follow SOP number 62.
14. Samples must be doubly contained during transport outwith the containment facility & clearly labelled with a contact name & number, the nature of the sample & the biohazard symbol. Inner container/tube must be robust & leak-proof. Outer container must be robust, leak-proof & contain enough absorbent material to absorb the total volume of sample should the inner container leak.
15. Samples in liquid nitrogen cryo-stores must be contained in proper cryo-tubes & stored in the liquid nitrogen vapour phase to eliminate risk of tube explosion upon initial warming. Samples in fridges/freezers must be doubly contained. Fridges, freezers & cryogenic storage vessels must be secure, biohazard labelled & subject to a well maintained inventory system.
16. Before leaving the TC suite, remove PPE then wash hands.
17. Any accident or incident - including a major spill or any spill resulting in personal contamination - must be formally reported to your Lab Manager.

Safe Operating Procedure 222

FullSerialNo	SOP222
Version	3
Title	Infection studies in the Wellcome Trust Biocentre Resource Unit (WBRU) Containment Level 2 Facility
Author	S Thomson
Division	Life Sciences
Building	Wellcome Trust Biocentre
Lab No	4L6-46/47/48
Approval date	21/08/2017
Review date	21/08/2018

Procedure

Only authorised, trained personnel may enter the WBRU Containment Level 2 (CL2) Facility. Entry is via proximity card and only the WBRU Manager can assign card access privileges.

Using the Facility

> All personnel must be trained in the use of the CL2 Facility and training records kept by the facility manager.
> Personnel must also have read and understood the specific risk assessments for the work they will undertake.
> Access to the room must be kept to a minimum.
> Work with different pathogens must be kept separate and all equipment must be disinfected prior to continuing with a different pathogen and at the end of the work session.
> Several different Hazard Group 2 pathogens are used in this facility. See the specific risk assessments for details on hazards to human health and the environment, surface disinfection method, waste treatment and any other pathogen specific control measures.
> DETAILS of each ongoing experiment will be written on the wipeboard with reference to the study plan in case of animal bite/escaped animal.

Entering the room

> Where possible, please timetable work in the CL2 Facility for the end of the working day to avoid going back into the Main Unit.
> In the ante-room, put on a dedicated red lab coat and an extra pair of overshoes.
> Hats will be provided for workers with long hair.
> Enter the main room, wash your hands and put on a pair of disposable gloves.

Using the Class II Microbiological Safety Cabinet (MSC)

> This must be prepared and in operation before any cages are removed from the individually ventilated cage (IVC) rack.
> Switch it on in the normal way and spray with either 1% Virkon (leave for at least two full minutes then use 70% ethanol to remove Virkon residue) or the specified disinfectant for the pathogen last used in the hood.
> If you find equipment in the MSC which you do not intend to use, do not remove it without

ascertaining that it has been properly decontaminated.
> The cage to be used should be placed in the MSC and unsealed immediately to maintain a supply of air to the animals.
> Try not to disturb dirty bedding when handling the mice.
> Do not have more than one cage in the MSC at any one time unless absolutely necessary.
> ALWAYS clean/weigh/treat UNINFECTED CONTROLS first to prevent any potential cross contamination!

Moving Animals
> Uninfected animals should be moved into the isolation room to acclimatise 10 days before the procedure starts.
> Uninfected animals should be brought to the isolation area in their standard cages and transferred to clean isolation IVCs outwith the MSC.
> The standard cages may then be removed for washing as normal.
> Live animals may be taken OUT of the isolation area ONLY for in-vivo imaging (see details below), therefore changes in plan after they have been brought in may not be possible.

Carrying Out Procedures
> Gloves should be sprayed with the specified disinfectant between handling (potentially) infected animals/equipment within the MSC and touching anything outwith the MSC.
> All spills, body fluids and faeces must be cleaned up immediately with the specified disinfectant.
> If using plastic pipette tips, ensure minimal aerosol formation by using aerosol resistant plugged tips and ejecting tips carefully and directly into an autoclavable sharp-safe.
> Other small contaminated plastics, e.g. vials, should also be disposed of in this way. True sharps waste must also be disposed of in an autoclavable sharp-safe.
> Liquid waste must be collected in a sealable, robust, autoclavable container.
> Carcasses and tissue waste must be double-bagged in clear plastic bags, dated and stored in the fridge awaiting disposal by technical staff Closing the Cage
> Before removing a cage from the MSC, check that the large cage changing station in the main room is unoccupied and that its dunk tank is operational.
> Seal the cage and spray thoroughly with the specified disinfectant.
> Disinfect your gloves with the specified disinfectant before picking up the cage to pass it through the dunk tank and replace it on the cage rack. Note: the yellow indicator flag will show if the cage has not been fitted correctly.
> If there is an unavoidable delay, return the cage to the hood and unseal it. Re-seal, decontaminate and replace it on the rack as soon as possible thereafter.
> If you empty a cage, i.e. all occupants are killed during your procedure, then seal and decontaminate it as above, replace it on the rack and label as ‘dirty’. MAKE SURE you tell a member of the resource unit staff that the cage is ready for autoclaving.

Sedating and/or Anaesthetising Animals
Animals may have to be sedated and/or anaesthetised for non-invasive in-vivo imaging. Sedative will be injected ip and anaesthetic will be gaseous. Ensure that you are assessed and signed off as competent to perform these techniques by the relevant managers and training records are updated accordingly. Sharps must be placed in the sharp-safe provided.

In-vivo Imaging
This procedure will require 2 people to co-ordinate the transfer between the different areas and monitor recovering animals. Infected mice will undergo imaging on the Xenogen IVIS-Lumina

instrument in the Main Unit. A containment box (XIC-3 Animal Isolation Imaging Chamber: Caliper LifeSciences) will be used to house the animals during transport from the CL2 Facility to the IVIS, and throughout the imaging procedure. The containment box is made of cleanable materials and is sealed on all sides apart from the ports for the supply and exhaust air lines. The two air lines are used for connection to the anaesthesia machine and are both protected by in-line Iso-Gard® HEPA filters. The following precautions will be taken to minimise the risk of contamination of personnel, the environment and other animals.

In the IVIS Room

> Activate all anaesthesia systems.

> Prepare the floor of the IVIS instrument by wiping with 70% ethanol.

> Place an appropriate liner on the stage of the instrument. Reusable liners, e.g. plastic sheet protectors, must be easy to clean/disinfect. Disposable liners may also be used.

> Detach carefully the tubing from gas in and gas out for “the standard” setup and place on bench.

> Initialise system.

> Position the anaesthetic trolley immediately outside the CL2 Facility doors.

In the CL2 Facility

> Weigh charcoal filter and note weight on side.

> Sedate/anaesthetise mice - set a flow rate of 4 for both the O2 and the isofluorane.

> Reduce the flow rate to 1 for O2 and 3 for isofluorane. Transfer the mice from the anaesthetic chamber to the containment box and attach it to the anaesthetic to fill with gas and to ensure animals are fully anaesthetised and in position (may need to use tape to ensure animals do not slide within the box).

> Spray the outside of the containment box with ethanol (be careful not to scratch or smudge the imaging window).

> Detach the containment box from the anaesthetic system, transfer the containment box out of the CL2 Facility and attach it to the anaesthetic system outwith the facility door. Same setting: flow rate of O2 at 1 and isofluorane at 3.

> Check the animals have not moved then wheel the anaesthetic trolley holding the containment box to the IVIS room.

Imaging

> Place the containment box on the lined imaging surface of the instrument and connect the anaesthesia outlets to the IVIS anaesthetic station (flow rate of 1 for O2 and 3 for isofluorane) and image. Note: When using the containment chamber for imaging animals there should be no vacuum system connected to the IVIS as this would cause negative pressure to build up inside the chamber and cause harm to the animals and damage the containment chamber. Instead, connect the gas out to a charcoal filter housed within the IVIS.

> After completion of imaging, disconnect the containment chamber from the anaesthesia outlets and transport the animals back to the CL2 Facility under O2 only. The sedative will be reversed and animals will be monitored for recovery then transferred back into their cage.

> Weigh charcoal filter – if there is a gain in 50g, replace with a new one.

Cleaning the IVIS

Dispose of liner or, if reusable, disinfect in the IVIS with the specified disinfectant ready for reuse.

Cleaning Containment Box (Read Instruction Manual before starting)

At end of each experiment, after all runs have been completed over several days, remove the HEPA filters from the supply and exhaust lines of the containment box, and store in a plastic container with lid. Wipe exterior of the plastic container and label with PI name. The filters may be reused throughout the duration of the experiment, then finally disposed of in the biohazard waste and autoclaved. Soak the containment box in the specified disinfectant making sure that it is fully submerged for the required length of time.

Sick Animals

Report all signs of ill-health to the relevant personal and/or project licence-holder, the facility manager, and/or the NVS. Normally the personal licence holder will be expected to attend to deal with the issue. Deliberately infected mice may also develop signs of adverse welfare and these, if mild, may reverse by themselves or may progress to more serious illness. It is again vital that you report any ill health as promptly as you can. In all cases, the project licence should describe the end-points to be applied.

Killing Animals

Animals may have to be killed because an experiment has come to an end, because it has reached the required welfare end-point or because they are experiencing unanticipated serious effects. Mice for lung infection models will be killed by overdose of anaesthetic given ip. Sharps must be placed in the sharp-safe provided. When ready for disposal, this should be sealed and then wiped down with the specified disinfectant. Cadavers should be double-bagged in clear plastic bags, dated and the outer bags sprayed with the specified disinfectant before they are placed in the fridge in the CL2 Facility. Make sure that the facility staff know that they are there and whether they are to be autoclaved as soon as possible, or whether the NVS has asked for them to be retained until he/she can examine them.

Waste Disposal and Shutdown/Change of Pathogen

> ALL items must be sprayed thoroughly with the specified disinfectant prior to removal from the MSC.

> Bagged carcasses/tissues can be stored in the refrigerator within the facility, but ENSURE that you tell the technical staff so that the waste can be autoclaved promptly.

> General waste, such as overshoes and hand-towels, should be placed in the bin provided.

> Sharps must be placed in the sharp-safe provided. When ready for disposal, this should be sealed and then wiped down with the specified disinfectant.

> Spray and wipe down all accessible internal surfaces of the MSC and any equipment used within the MSC with the specified disinfectant. Remove the decontaminated equipment before shutting down the MSC in the normal way.

> Spray your gloves with the specified disinfectant before exiting. If you are switching to work with another pathogen, remove and discard your gloves, wash your hands and then re-glove. If you are leaving the CL2 Facility, remove and discard your gloves, wash your hands, then exit to the ante-room.

> In the ante-room, remove and discard the outer pair of overshoes as you cross the threshold into the ante-room, then remove your lab coat. If this is still clean, hang it back on the coat hook. If it has been visibly contaminated, bag it and identify it for autoclaving. ALL lab coats will be collected once a week by resource unit staff for autoclaving and laundering. RED LAB COATS will be changed weekly by technical staff.

> Spray hands with alcohol spray (on wall) and exit.

Emergency Procedures

Continued forced ventilation of the cages on the rack is vital if the animals are not to suffocate. There are two possible ways in which this might fail.

Failure of the Mains Electrical Supply

The racks are on maintained supply, i.e. if the mains power fails the back-up generator should start up and immediately provide power to the racks. If the back-up generator does not activate as expected the back-up battery on the racking system should provide 6 hours of ventilation to the

cages until either the mains or the back-up generator supplies become available. The NVS should be notified as soon as the power failure is detected, so that he/she can advise on what to do if neither supply can be restored in time. In the event of a complete power failure, empty racks and the back-up air-handling unit should be switched off to conserve their battery power.

Mechanical Failure of a Fan Unit

The racks will emit an alarm via the BEMS in this eventuality. If it is purely a mechanical fan unit failure, where possible, place in use cages onto the other cage storing rack in the room. There is also a back-up fan unit in the store room, in the corridor opposite the cleaner's cupboard in the unit, for use in the event of the primary fan failing. It should be wheeled into the CL2 Facility to replace the broken fan unit, plugged into the socket, attached to the rack and activated. If the back-up fan unit does not work, start up the cage changing station and the MSC and stack the cages in these cabinets, with covers unsealed. Attempt to couple the back-up air handling unit to the affected rack. When/if this has been achieved, cages should be surface disinfected then replaced in the rack. If storage in the cabinets is likely to be required for more than two hours, the licence-holders, facility manager and NVS must be notified. If possible, the bone marrow chimera cages should be segregated from those known to contain microbiological agents. The animals should be inspected regularly to ensure that they are getting enough air, without the air gaps being big enough for escape.

First-aid Procedures for Bites or Needle-stick Injuries

1. Encourage wound to bleed while washing thoroughly with soap and warm water.
2. Dry thoroughly then cover with a waterproof plaster.
3. Complete an on-line accident report form. This is automatically sent to Safety Services and SLS Safety.
4. Steps 5 to 7 should be followed if one or more of the following applies:
   a. animal has been deliberately infected with a human pathogen (details on current studies will be written on the wipeboard);
   b. animal is infection free but bite is deep/dirty and you are concerned that it may become infected;
   c. animal is infection free and bite appears minor but later becomes infected (indicated by redness);
   d. there is swelling, pain and/or tracking up hand/arm.
5. Make urgent appointment with your GP or, if unable to be seen on the day, go to A&E and explain that you have been bitten by a laboratory animal.
6. If animal has been deliberately infected with a pathogen give details of pathogen.
7. Inform Occupational Health by telephone, on (3)86948 or (3)85410, at the earliest opportunity.

Autoclave Cycle Parameters

Mice/Rats 20: 121 degrees C; 15 min
Plastic Discard: 126 degrees C; 15 min
Fluid Discard: 121 degrees C; 15 min
Fabrics: 134 degrees C; 3 min
Cages Bedding: 121 degrees C; 15 min
Drum with Cages: 121 degrees C; 15 min