CODE OF PRACTICE FOR WORK WITH

VIRUS INFECTED MATERIAL

(HIV HCV)

IN THE CONTAINMENT LEVEL 3 (CL3)

LABORATORY (Rooms 664.20.27, 664.20.27a

and 664.20.27b) LOCATED

IN THE NDM RESEARCH BUILDING, NUFFIELD

DEPARTMENT OF MEDICINE, UNIVERSITY OF

OXFORD, OLD ROAD CAMPUS, OXFORD

Updated October 2016
Version 5

TO BE DISPLAYED ON THE DOOR OF CL3 ROOMS

This facility is a Containment Level 3 (CL3) laboratory. It contains six Class II microbiological safety cabinets, and is a fully self-contained facility. Four of these cabinets lie within the larger laboratory (Room 664.20.27) and two within the smaller laboratory (Room 664.20.27a).

Access to the laboratory is restricted to authorised users and visitors. An authorised user must accompany visitors at all times. Access to the CL3 laboratory is made only to authorised cardholders.

The type of work carried out in this laboratory includes the separation of blood from HIV or HCV infected people and the culture of HIV infected lymphocytes.

Further segregation of the work into “high” titre & “low” titre work is made according to the guidance that “high” titre constitutes the *in vitro* culture and propagation of active HIV virus and “low” titre constitutes the handling of samples that may or may not have been infected with HIV virus. Both CL3 laboratory suites have been designated for “high” and “low” titre work

Big lab. (Big CL3 lab, Room 664.20.27) is designated for “High” and “Low” titre work.

Small lab. (Small CL3 lab, Room 664.20.27a) is designated for “High” and “Low” titre work.

Overall, the location of this laboratory suite is:

Room 664.20.27
The NDM Research Building,
Nuffield Department of Medicine,
University of Oxford
Old Road Campus
Headington
Oxford.

See Appendix 1 for telephone numbers & emergency contacts.

GENERAL ARRANGEMENTS AND LABORATORY RULES

The room is suitable for work with pathogens in Hazard Group 3 of the ACDP categorisation of pathogens, and GM work requiring CL3 containment.

The doors of the laboratory are labelled 'Containment Level 3'. When work is in progress, the laboratory door must be kept closed. Entrance to the laboratory is by means of a magnetic "swipe" card into the lobby and both CL3 suites, and issue of access cards will be strictly restricted to authorised users. Authorised users must under go a safety induction by then a period of supervised training under the supervision of a dedicated trainer in each group, before card access will be granted. The personnel department, Mrs Ling Jinks (Building and Facility Manager) and Dr Hongbing Yang (CL3 lab supervisor) hold a record of current cardholders authorised by Professor McMichael, which is reviewed annually.

Laboratory work with HIV must be restricted to the CL3 laboratory. Paper work must not be transferred out of the CL3 laboratories. Equipment should not be moved out of the laboratory without prior agreement and thorough appropriate decontamination.

The use of sharps (needles, blades) is not permitted in this laboratory. Glassware must be avoided where possible. Liquids normally contained in glass bottles should be decanted into plastic containers before being brought into the lab. The use of glass will only be permitted after full discussion has taken place with Dr Hongbing Yang and Mrs Ling Jinks and they are satisfied that its use is unavoidable and that the protocols to be used will not lead to increased risk.

1. Authorised Personnel

All personnel working in this laboratory must demonstrate competence in safety procedures, and must receive instruction and training on the procedures they wish to undertake by their line manager or other recognised user. The line manager or other recognised user will supervise the initial training of new users and authorisation to use the room will not be given until Dr Hongbing Yang is satisfied of their competence. This will involve the reading of the Code of Practice and users must sign their training record to state that they will comply with the Code at all times. Also, supervisors must sign the training record to testify that the proposed user is competent in carrying out the scientific technique(s) on their training record. Dr Hongbing Yang assigns new personnel to a probationary period for this training and supervision of their work, until the new user has reached an acceptable level of competence to work safely unsupervised.

When an individual has satisfactorily completed the training they will become an authorised user and their name added to the Users e-mail list. **Anyone who repeatedly fails to comply with the Code of Practice or conduct themselves in a safe and satisfactory way in the laboratory will have access withdrawn completely.** Access will only be granted subsequently after satisfactory retraining.

Users of the laboratory must conduct themselves in such a way that they do not put themselves or others at risk. Personnel must be adequately trained in the handling and the safe disposal of Hazard Group 3 microorganisms and be aware of

the risks they present. A high standard of supervision of the work must be maintained.

Eating, drinking and smoking are absolutely forbidden in the laboratory. While in the laboratory the face should not be touched with the hands. (Wearers of contact lenses should be particularly aware of this fact).

Only authorised personnel may work in this HIV room and a list of these people will be displayed at the entrance. The current authorised users are listed in the Appendix 9. There is also a CL3 users e-mail list, accessed only via Dr Hongbing Yang & this is to be used to facilitate information between users.

Visitors to the laboratory may only work under the supervision of an authorised user, with the agreement of Hongbing Yang and Andrea Keepence-Keyte.

When access to the laboratory is required for maintenance purposes, a temporary shutdown will be implemented and the room and equipment disinfected or sterilised as appropriate. Authorised maintenance personnel will be provided with information on the nature of the hazards in the area, and should be accompanied at all times by an authorised member of the group to ensure that they work safely.

A maximum of 8 - 9 people may work in the big CL3 laboratory and 4-5 people in small CL3 lab at any one time. On the occasions when out-of-hours work is deemed necessary and there is no-one else in the Laboratory, the line manager, another member of the team or another contact (such as a close friend or family) should be told of the expected completion time for the work and what action to take if the worker fails to meet or contact them at the expected time.

Undergraduate students are not permitted to work in this laboratory.

2. Protective Clothing

No outdoor clothes or lab coats will be brought into the laboratory area. On entry to the laboratory, all personnel must wear one pair of gloves followed by a protective gown.

Wearing of eye protection **MUST** take place. (University of Oxford Safety Memo: M14 / 06).

Gloves should be changed whilst in the CL3 laboratory after working with particularly infectious material such as high titre virus cultures. “Double gloving” (nitrile + latex outer) gives additional protection and **MUST** be adopted for work in both CL3 labs. The outer gloves being removed prior to touching door & incubator handles / switches / microscopes, etc. in order to reduce the chances of contamination of communal surfaces with infectious material. Gloves may be changed, or sprayed with 70% IMS before exiting, i.e. before touching door handles and / or switches to ensure as clean a pair of gloves as practicable before lab coat removal.

All protective clothing will be removed before leaving the lobby. Gowns when removed to be hung up on the hooks. Gloves to be removed last, on the assurance

that these are clean “inner” gloves. Used gowns must be changed every week, or when known or suspected to be contaminated, and discarded. The wearing of open-toed sandals is **forbidden** in the CL3 laboratories.

3. Cuts etc.

Exposed cuts and scratches **must** be adequately covered before entry into the room. **DO NOT** work with open cuts or sores. If any potential worker has a dermatological condition, which might increase the risk of exposure, this must be reported to and discussed with the University occupational Health Physician (282679) before commencing work in this facility.

4. Clean Surfaces

It CANNOT be assumed that any surface in the CL3 lab is automatically clean and free of contamination. Extra care should be taken however when touching the following:

Equipment such as centrifuges & microscopes
Door handles
Hand washbasin tap levers
Light switches
Telephone

5. Servicing and repair of equipment

The biological safety cabinets should be VHP fumigated before routine servicing. Any equipment released from the laboratory for maintenance or repair must be disinfected or sterilised by an authorised user of the room, and a release certificate signed by Hongbing Yang, before any equipment leaves the room.

If it is not possible to disinfect or sterilise completely potentially contaminated equipment, the release certificate must give details of the parts that remain contaminated and the precautions that have been taken to prevent infection. The equipment must not be released until either Hongbing Yang or Andrea Keepence-Keyte has ensured that the recipient fully understands the measures that have to be taken to avoid infection and the matter has been discussed with the University Biological Safety Officer (Mr Andrew Thompson 270819).

6. Tidying Up, Weekly maintenance and cleaning

At the end of each work session, the areas used must be left in a clean, tidy and safe condition, by thoroughly disinfecting all work surfaces and disposing of all contaminated material. If the safety cabinets have been used, then the surfaces should be wiped down with 1% Virkon disinfectant and rinsed with distilled water. The floor will be cleaned and disinfected using 1% Virkon, at least once a month. Other CL3 duties are to be completed by paired users as shown on a rota system posted on the outside of the entrance lobby door. This is a monthly assignment. The signed initials of the participants indicating which tasks have been completed.

7. Disposal

All materials that have been used in the laboratory and are to be discarded must be decontaminated either by autoclaving or by soaking in fresh Virkon solution. Material not soaked by Virkon from the CL3 room bins will be autoclaved in CL3. Autoclaved waste can then go into yellow clinical waste bags and taken to the clinical waste bin in the glass wash room. Autoclave duties will carried out by CL3 users on a rota system posted on the outside of the entrance lobby door. Virkon soaked material will be put into double sealed autoclave bags and autoclaved in CL3. Radioactive waste from the use of 51 - Cr will be disposed of down the lab sinks and the amount of radioactivity accounted for in MBqs and recorded on the appropriate drain sheet. Remaining plastic ware will be monitored and if at background count level, removed to the autoclavable waste bags for autoclaving & disposal. Users of 3H – Thymidine to remove plastic waste after disinfection into plastic radioactive bags, ensuring **ALL** liquid has been drained and then immediately to the red 3H waste bins outside the CL3 laboratory.
Glass blood bottles will be disposed of into the sharps bins in the CL3 labs. These will be removed & autoclaved in the CL3 autoclave. If the blood tubes are **FULL / UNUSED,** Hongbing Yang to be notified & autoclaving to be carried out at once to ensure destruction of infectious material as rapidly as possible.

8. Safety Cabinet Maintenance

The Class II microbiological safety cabinets in the CL3 laboratory have been fully commissioned prior to use. The cabinets should be subjected to regular 'in use' protection factor testing, which must take account of any variations placed upon the system by mechanisms incorporated into the laboratory air conditioning. The cabinets should be capable of achieving a protection factor of 10^5 or greater when in normal use. The air conditioning settings should not be altered without discussion with the laboratory head, and cabinets should not be moved. The cabinets will be tested every six months for operator protection (KI test).

9. Liquid Nitrogen Storage and -80^{o}C Storage

No high-risk samples should be stored in liquid Nitrogen. Two -80^{o}C freezers are reserved for high-risk storage.

10. Incubators

All sera, cell lines, and culture supernatants within the incubators derived from HIV+ donors must be regarded as potentially infectious. Any flasks or cell culture plates / dishes / flasks containing viral cultures **MUST** be placed on a tray that will collect spillage. If any spillage occurs onto the tray, the tray must be removed to the safety cabinet and cleaned Virkon. If spillage occurs onto the incubator surface, "NO ENTRY" signs MUST be placed over the incubator door and a risk assessment of the spill must be made via the NDMRB Biological Safety Officer (Mrs Ling Jinks) before cleaning is carried out. If seemed necessary, the incubator may be sterilised by heating, and / or fumigation.

Water levels in the bottom tray of the incubators will be checked weekly by those on the weekly "monitor" rota. For intensive cleaning see "Deep Clean" protocol.

11. Centrifugation

ALL blood samples must be centrifuged in sealed buckets. The opening of the sealed buckets **MUST** take place inside the safety cabinet. Inspecting for any blood spill inside the centrifuge inserts / adapters is highly recommended, together with inspecting the integrity of the spun tubes as well. **ANY SPILLAGE OF BLOOD WITHIN THE CENTRIFUGE ADAPTERS MUST BE CLEANED UP AT ONCE AND DISINFECTED WITH 1% VIRKON AFTER CLEANING.**
Also, if there is a major malfunction of the centrifuge the room should be evacuated, the door closed and the centrifuge should not be opened until expert advice has been sought.

12. Removal of samples from the laboratory

Samples (e.g. cell-lines, frozen ampoules of cells) can only be removed from the laboratory in an approved container using the approved method (and must be transferred to a similar containment facility without delay. In general, these cells will be human EBV-transformed B-cell-lines from HIV-infected individuals, which are at low risk of HIV infection, or peripheral blood mononuclear cells (PBMC) from uninfected donors to be used as feeder cells for T-cell-lines and clones. Cells to be irradiated must be transported to the irradiator in the ORCRB in an approved container (See Appendix 15 for Approved Method). Similarly cells frozen in the CL3 room should be transferred to the -80ºC freezer in a safe container. The container should be robust, have a secure lid and be made of a smooth impervious material (e.g. plastic or metal), which can be easily cleaned and disinfected, such as a cool box. This MUST be clearly labelled with the information on the enclosed sheet for Transport of Infectious Substances.

Samples being transported from clinics etc to the laboratory must be carried in boxes clearly marked ‘Biohazard’ and ‘Danger of Infection’.

Samples carried from one suite to the other must be in a primary sealed container (use lids id using coulter cuvettes) and secured within a secondary sealed container (Tupperware box for example)

13. Radioactive work

All experiments in the CL3 laboratory involving radio-isotopes must follow accepted protocols and guidelines. Guidance is to be found within University of Oxford Policy Statement S8 / 05: **“General Local Rules for Work with Sealed & Unsealed Radioactive Substances”.** All radionuclide users will have received and hold a personal copy of this documentation and must familiarise themselves with the information.

Please pay special attention to the following sections:

a) **Part 4.8** (p.35 - 40): “Routine work with aqueous radioactive liquids”.
Briefly, this addresses the need for segregation of work, containment and surface contamination monitoring.

b) "**Appendix 16:** Contingency Plan for Incidents Involving Unsealed Radioactive Substances."
Briefly this pays special emphasis on the need for evacuation, restriction, containment, clean-up & monitoring of the affected area, in the event of a spillage of an unsealed radionuclide.
(Appendix 16 is posted outside on the front door to the CL3 suite).

14. Spillage of Radionuclide Material (51Cr & 3H)

A spillage kit is available in the CL3 lobby, comprising of the following items:

A small bottle of Decon 90 (to be diluted to a working concentration of 20%)
A bottle of "Clearoff" solution
Wipal roll
Large, medium & small radiation bags
Nitrile gloves (small, medium & large)

These items to be used only in the event of a spillage. All items wiped up to be inserted into the appropriate radiation bin outside the CL3 suite and counts / second used to give an approximation to the amount of radiation spilled in MBqs. Incident to be reported to TDI RPS as soon as possible.

15. Working & Monitoring for Tritium (3H)

Tritium is a Beta emitter – 18.6 keV
Range in air – 6mm; Water – 6 x 10-3
Proton exchange
Monitor by scintillation counting
ALI 476 MBq per year
Poses an internal hazard from ingestion or inhalation
Half - life of 12.3 years.

As 3H contamination cannot be detected by portable monitors, wipe tests should be performed regularly by individual users on areas & equipment after use. All users of 3H must know how to do this, see Dr Suzanne Campion and Dr Benedikt Kessler for training before they use 3H - Thymidine in the QuanTRT kits.

16. To swab for Tritium (3H)

Take a 2 cm square of white filter paper and soak with 70% alcohol and holding the swab in clean forceps, wipe the swab over the area or item and then place into a Maxi plastic counting vial.

Rinse forceps in 10% Decon solution or CountOff between each sample taken. For swabs taken in the CL3 labs. Use 70% IMS on the swabs as well. Include 2 blank controls, which contain the swab, alcohol, decontaminant and 5mls of scintillant.

Label vials on the lid, add 5mls of scintillant to each vial, mix well by inverting several times (with the lid on) and leave to rest for at least 30 mins. The scintillant is kept in Room 472 and must be used in the fume hood. The outside of the vials to be

removed from the CL3 labs. must be decontaminated first by wiping with 70% IMS. Count for 1 min on Prog. User # 1 on the scintillation counter.

Determine the results and re-swab and count until areas / items are cleaned to background. If this is not possible, label accordingly with the print out results.

See Suzanne or Benedikt if you have any problems.

17. Accidents – see notice on CL3 room wall (see Appendix 10, as posted on laboratory wall).

Accidental exposure to HIV may occur from splashes to the skin and eyes, or through inoculation injury. Aerosols of high-titre virus could potentially be a hazard.

In the event of suspected exposure to HIV, the following first aid measures should be used:
Splashes to eyes or mucous membranes should be irrigated thoroughly with water (eye wash may be used, there is a eye wash station in CL3 lobby), and any skin contamination be washed off with soap and water (but do not rub the injury site). Inoculation injuries of the skin should be washed thoroughly with soap and water and made to bleed freely if possible. It should be noted that the CL3 laboratory DOES NOT contain a First – aid kit and therefore a user requiring treatment needs to evacuate the facility and use the First-aid kit at the right hand side lab. Hand-wash sink in CL2 lab.

The next step is to consider the need and desirability of administering anti-retroviral treatment, by firstly contacting the John Warin Ward Infectious Diseases Unit; tel. 225214 (within 2 hours). With appropriate counselling, the worker will be advised of the need for anti-retroviral therapy and appropriate testing for HIV and any other suspected blood-borne viruses, following a risk assessment by contacting the University Occupational Health service. Further testing will be carried out at intervals over the following year if the person agrees.

The accident must also be reported to the Biological Safety Officer (Mrs Ling Jinks). The supervisor should also be informed.

Finally, all incidents, accidents and near misses **MUST** be reported to the NDMRB reception, where the Administrator will complete an accident report. Also, the NDMRB BSO (Andrea Keepence-Keyte) must be notified as well

18. Spillage of Infected Material (see Appendix 9, as posted on laboratory wall).

In the Cabinet
In the case of spillage, Virkon powder should be added immediately. The cabinet front should be closed and the cabinet fumigated as soon as possible. Contaminated gloves and gowns **MUST** be immediately removed and autoclaved. Hands should be thoroughly washed before leaving the CL3 lobby.

Outside the Cabinet
After a significant spillage of infected material such as virus cultures, causing considerable splashing and / or aerosol production, **ALL** personnel to exit the laboratory as soon as possible. A red & white "NO ENTRY – SPILLAGE" sign **MUST** be placed on the entrance door into the CL3 laboratory. Also, use plentiful amounts of the black & orange warning tape on the door and frame in order to hamper any possible attempt at entry. (A "lock-out" mechanism to be installed at a future date). Contaminated gowns or other clothing should immediately be removed & discarded. A risk assessment should be carried out to determine the extent of the exposure risk, and the University safety office should be contacted to discuss the potential need for fumigation. After a spillage such as this, the biological safety cabinet should be left on to help ventilate the area and capture any aerosol raised. If the room has to be fumigated the protocol below should be followed. (See "Appendix 11" for printable version and criteria that constitute "high" and "low" risk spills).

19. Experimental Protocols

All experimental protocols for use in the CL3 room must be approved by the project supervisor and either Professor Andrew McMichael and / or Hongbing Yang.

20. Equipment available

Room 664.20.27:

Four CAS class II biological safety cabinets
Four CO2 incubators
Two + 4ºC Refrigerator
One - 20ºC Freezers
One - 80ºC Freezer
Three bench centrifuges + 1 microfuge
Microscope
Luminex
ELISA reader
Cell counter
Computer
Water bath

Room 664.20.27a:

Two CAS class II biological safety cabinets
Two CO2 incubators
One + 4ºC Refrigerators
One - 20ºC Freezers
One - 80ºC Freezers
Two bench centrifuges + 1 microfuge
Microscope
One Ultracentrifuge
One autoclave

Room 664.20.27b:

One autoclave

21. Disinfection policy

Virkon is the only disinfectant approved for use in this CL3 laboratory. No other should be used without seeking approval from Hongbing Yang or Ling Sung. The concentrations and contact times that should be used are given below:

Item	Concentration	Contact Time
Plastic ware	1% final solution or 2% if heavy contamination	At least 30 mins fully immersed. All pipettes (5, 10 & 25 ml) types MUST be aspirated up and down within the Virkon solution in order that the contents of the pipette are FULLY disinfected.
Liquids e.g. Samples, culture supernatant etc.	Final concentration of 2% Virkon when liquid is added. Increase to 5% where FCS is used.	at least 30 mins.
Surfaces including benches and floors	1% solution	Wipe over
Minor surface contamination	1% solution	10 mins
Larger spillage including body fluids	Virkon powder directly onto spill + 1% solution for any post – sprinkling clean up.	10 mins

After disinfection waste fluid may be poured down the laboratory sink with copious amounts of water.
All plastic ware etc. must be autoclaved before final disposal.

NOTE:
NEW SOLUTION MUST BE MADE UP AFTER 1 WEEK OR WHENEVER THE PINK COLOUR HAS FADED.

General rules for the disposal of waste from the CL3 room:

All solid material from the CL3 lab will be autoclaved and disinfected liquids disposed of down the nearest sink or autoclaved in an autoclavable plastic container.

All waste containers used within the Class 2 cabinets and on the benches MUST be labelled with the users **NAME & DATE & TIME.** This identifies users and can speed up identification of who has recently worked in the facility. Containers can then be sieved in the lab sink and used disinfected plastics can then be tipped into the autoclave bags supported within the leak proof autoclave containers. Waste is kept within the CL3 lab until it is taken to the autoclave. The un-disinfected waste is then loaded directly into the autoclave for immediate treatment; it is not stored in the autoclave room. Liquid waste after disinfection should be disposed down the sinks in the CL3, all GM containing liquid waste should be autoclaved before disposal.

There are three types of bin in CL3 lab. Waste disinfected by Virkon will be placed in double autoclave bags in metal bin and transferred to the yellow bin outside CL3 lab in the morning by members of CL3 users. This involves tie the plastic bags with black cable tie loosely and transferring the bags to the yellow bin. Uncontaminated Paper waste, from the larger grey bins is similarly treated. Black bin in CL3 is only for contaminated gloves, paper towel, gowns, which are not disinfected with Virkon. Waste in black bin must be autoclaved in CL3.

It is important that AT ALL TIMES when placing items into the yellow bin outside CL3 that **clean** gloves are used to handle the bags and that the lid or any other surface of the wheeled yellow bin are touched by clean INNER nitrile gloves. This will ensure the lack of any contaminants being transferred OUT of the CL3 facility. The lid of the yellow bin is then closed. James Scott will transfer waste from yellow bin to the CL2 autoclaves.

Waste is taken to the CL2 autoclave room in the afternoon such that it can be transferred directly into the autoclaves for immediate treatment. Transport of waste, transfer to the autoclave trolley and removal of lids should only be undertaken by fully trained CL2 laboratory workers. The CL2 autoclave is loaded (trolley pushed in) and operated only by autoclave room staff who have been trained in autoclave operation.

In the event of a spillage outside the containment level 3 laboratory, a risk assessment would be undertaken by management staff to determine the most appropriate means of dealing with the spillage. Virkon disinfectant would be applied to the spill and the affected area would be vacated and secured to prevent access, until the area had been decontaminated and made safe.

Radioactive waste

To dispose of items that contain radioactivity and may be contaminated with HIV, the item should first be soaked for at least 30 mins in freshly-prepared 1% Virkon. Liquid waste should be disposed of in the radio-active sink in the CL3 room (making sure to record the amount of radio-activity put into the drains) and solids should go into an appropriately sized plastic radiation waste bag and then into the appropriate

radioactive waste bin. Radioactive waste has to be disposed of immediately and not stored for decay.

WASTE MANAGEMENT MEASURES

Consumables (mainly Virkon treated plasticware eg pipettes, flasks, tubes) - autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993 ,134^{o}C (+/-2) for 10 minutes (+/-2), discharge any excess liquids to drains, dispose of solids via clinical waste stream for incineration.
Liquids (eg residue samples, culture supernatants, tissue culture media) – autoclave using a make safe cycle as specified in BS 2646, Part 3, 1993, 121^{o}C (+/-2) for at least 10 minutes (+/-2), discharge to drains.

Degree of kill

Autoclaving: effectively 100% kill (annual validation).

Incineration: effectively 100% kill (licensed incinerator).

Appendix 1

Emergency Contact Telephone Numbers:

The phone numbers of the Cat.3 laboratories are:
Large CL3 lab. 612870 Small CL3 lab. 612903

FUNCTION	NAME	GUIDANCE	TEL. No.
CL3. lab Safety Officer	Dr Hongbing Yang	Overall responsibility for management of the CL3 suite	Work: 01865 - 612914 Mobile: 07534563503
NDMRB Building and Facilities Manager	Mr Darren Blase	Overall responsibility for management of the CL3 suite	Work: 01865 – 612872 Mobile: 07788443286
Lab Manager, Biological Safety Officer	Andrea Keepence-Keyte	Management of equipment and general guidance	Work: 01865 – 612927 Mobile: 07960086093
McMichael group	Dr Suzanne Campion	Consultation, advice & guidance	Work: 01865 – 612913 Mobile: 07712887298
Borrow group	Dr Angharad Fenton-May	Consultation, advice & guidance	Work: 01865 – 612887 Mobile: 07974792364
Dorrell group	Dr Hongbing Yang	Consultation, advice & guidance	Work: 01865 – 612914 Mobile: 07534563503
Rowland-Jones group	Dr Louise Marie Yindom	Consultation, advice & guidance	Work: 01865 – 612912 Mobile: 07405446362
NDMRB Radiation Protection Supervisor	Dr Benedikt Kessler Dr Suzanne Campion	Safe use of radionuclides & radioactive spillage	Work: 01865 – 612921 Work: 01865 – 612913 Mobile: 07712887298
University Biological Safety Officer	Dr. Andrew Thompson	Consultation, advice & guidance	Work: 01865 – (2)70819
John Warin Ward Churchill Hospital	On-call microbiologist	Advice on accidental exposure to infectious agents	Work: 01865 – (2)25214
University of Oxford Occupation Health Department	None	Advice on accidental exposure to infectious agents. 8.30 – 5.00 pm “out of hours “	01865 – 282676 01865 - 741166

Appendix 2

Disposal of Waste from the CL3 room

Autoclave metal bin only (lined with clear autoclave bag):
Uncontaminated:
Gloves
Paper
Wrappers
Gowns

Any items in contact with blood, media or other liquids should be disinfected by 1% Virkon for at least 30min and be placed in the autoclave bag bin after being drained of Virkon solution:
Universal tubes
Flasks
Pipettes
Plates
Tips
NO SYRINGES to be placed into this bin.
These bags will be taken out every morning and autoclaved in the CL3 lab.

Sharps bin only:
Syringes
Glass tubes (contaminated or partially full with blood).
This bin will be autoclaved in the CL3.

Appendix 3

LABORATORY EQUIPMENT

CAS class II biological safety cabinets

The biological safety cabinets must be kept tidy and swabbed out with Virkon solution after use.

Fumigation (see later detailed protocol)

20 ml of formalin and 20ml of water is placed in the box. The front of the biological safety cabinet is shut securely. The formalin unit is switched on for 15 minutes, and then the biological safety cabinet is left overnight. The hood is opened and run for 30 minutes before use.

Filter integrity and operator protection factor should be checked six monthly by service engineer.

Centrifuge

The centrifuge should only be operated with the tubes placed in safety buckets with clear tops.

In case of a major malfunction in the centrifuge (spillage in the centrifuge, jammed centrifuge, unbalanced centrifuge), the machine should immediately be turned off, the room evacuated and the door closed (to allow all aerosols to settle) and expert advice sought before the machine is opened.

Inverted Phase Microscope

This is used both for inspection of cultures and for counting cells. The stage may be cleaned with Virkon solution or alcohol.

Protocols for dealing with other apparatus, clothing, cuts, maintenance personnel, liquid nitrogen storage, and -80 º C storage, removal of samples from the laboratory, radioactive work and accidents are dealt with in the code of practice.

Vortex

Any sample (blood, plasma, cell culture medium, cell lysis, etc.) need to be vortexed in a tightly secured tube or flask, and make sure the outside of tube or flask is dried.

Coulter Counter

This is used for counting cell suspension. Infected samples must be added to the cuvettes must be filled in the Biological Safety Cabinet, cuvettes must have a secure lid whilst being transported to the counter. A beaker of Virkon solution must be kept next to the counter so that solution is disinfected immediately after use and to avoid a risk of spill.

Appendix 4

PERMIT TO WORK – Cat3 facility First floor, NDMRB

Overview			
Individual(s) involved:			
Company/organisation:		Telephone no:	
Date of work:		Duration of permit:	
Location of work:	664.20.27		
Description of work:			

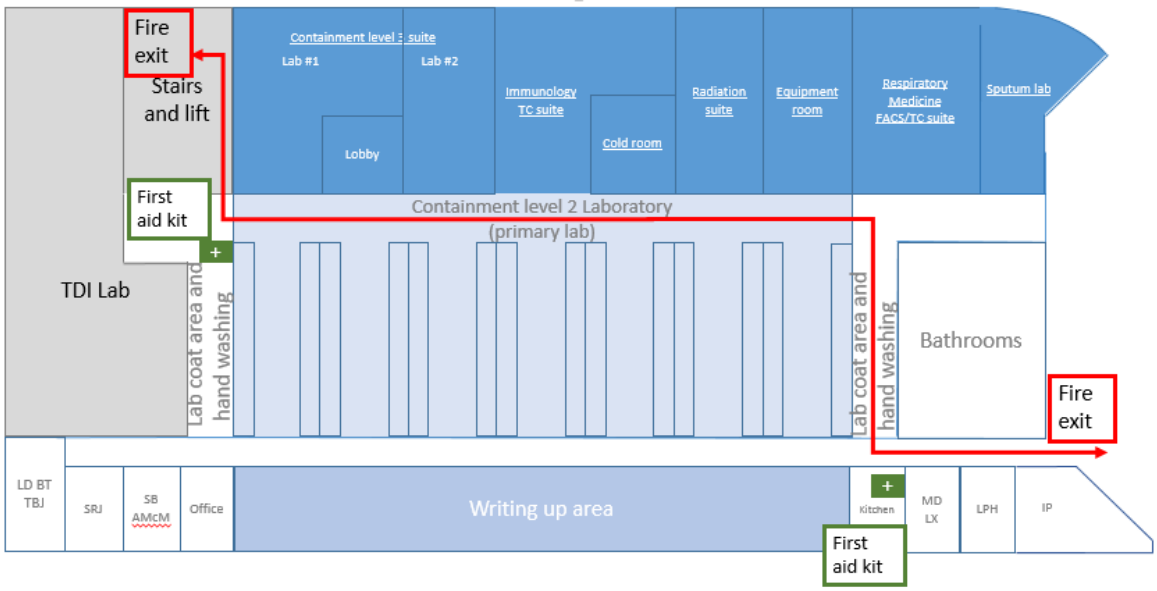
Hazard identification - as identified by a risk assessment

Infection from exposure to pathogens _ HIV work

Safety precautions - as identified by a risk assessment and including access arrangements, personal protective equipment and/or equipment, alarm or guard isolation

1. The suite must be shut down and not experimental activities can take place whilst an engineer is servicing an equipment.
2. The engineer will be given an induction to the Cat3 facility, highlighting procedures in case of common alarm and fire alarm, how to gown up upon entry and safely de-gown upon exit.
3. The area of work will be cleared and the equipment suitably decontaminated.
4. The engineer must not under any circumstances open any fridge, freezer or incubator.

Authorisation – by department		**Acceptance – by contractor**	
I authorise the work to be carried out as detailed within this permit to work and confirm that all prior precautions are in place:		I understand the hazards outlined and will ensure the work is conducted under the conditions of this permit to work:	
Name:		Name:	
Signature:		Signature:	
Date/time:		Date/time:	



Fire Emergency Procedure

- Fire alarm is tested on Thursdays 11am, not expected to leave the building.
- On hearing fire alarm, vacate building calmly, do not use the lift, and assemble on the grassy area behind the NDMRB.
- On finding a fire, raise the alarm, vacate the building safely, do not use the lift, assemble on the grassy area behind the triangular building.

First aid

- First aid kits are available in the kitchenette and at the far end of the laboratory.
- Names of first aiders are displayed on the door of the cupboards where the first aid kit is stored.
- Eye wash basin are present in each entrance to the primary laboratory and in each secondary lab space.

CL2 laboratory

- Strictly no eating, drinking, chewing or cosmetic application within the laboratory.
- Tools are allowed to be brought in the lab, areas of active work must be clearly marked to prevent contamination of tools.
- Personal Protective Equipment:
  - Labcoat
  - Gloves
  - Safety spectacles
- Hands must be washed upon exit from the laboratory.

CL3 laboratory

- Equipment will have been adequately decontaminated prior to the arrival of the engineer.

- Strictly no eating, drinking, chewing or cosmetic application within the suite.
- Engineer can enter under the supervision of the lab manager or an experienced cat3 users (permit of work is necessary).
- Tools are allowed to be brought in if no work is being carried out at the time of the servicing/repair. Tools which are suspected to have become contaminated must be sprayed with 1% Virkon, rinsed with water and then sprayed with 70% IMS.
- Upon entry in the lobby, contractor must put on:
  - Nitrile gloves
  - Disposable labcoat
  - Secondary pair of gloves
  - Safety spectacles
- Once in the cat3 suite, the engineer must not open any incubator/fridge/freezer without supervision of the users.
- Contractor may remain unattended in the suite if he/she feels comfortable with it and if no user input is required, he/she must have received necessary information as to the expected conduct in the suite.
- Outer pair of gloves must be removed before existing the inner suite. Exit is by depressing the round circle "Exit" – care must be taken the door are heavy and the door handle must be pushed all the way down to open correctly. There is an interlock and inner doors can only be opened if all other doors are shut within the facility.
- Before leaving the lobby, the engineer must de-gown by first removing the disposable labcoat, safety glasses, then the nitrile gloves, hands must be thoroughly washed and any waste must be disposed of in the autoclave bin provided in the lobby.
- If the AHU alarm sounds, the contractor can still work in the suite safely, however, given the volume of the alarm, it is recommended that the contractor exited the suite until the alarm is resolved.

Radiation laboratory

- Equipment will have been monitored and adequately decontaminated prior to the arrival of the engineer.
- Strictly no eating, drinking, chewing or cosmetic application within the suite.
- Engineer can enter under the supervision of the lab manager or an experienced radiation user.
- Tools are allowed to be brought in if no work is being carried out at the time of the servicing/repair.
- Upon entry in the suite, contractor must put on:
  - Nitrile gloves
  - Disposable labcoat
  - Secondary pair of gloves
  - Safety spectacles
- Once in the radiation suite, the engineer must not open any incubator/drawer/cupboard without supervision of the users.
- Contractor may remain unattended in the suite if he/she feels comfortable with it and if no user input is required, he/she must have received necessary information as to the expected conduct in the suite.
- All waste must be contained in a radiation waste bag, placed in a bin and logged in the appropriate paperwork under the supervision of a radiation user.

- Gloves, labcoat and safety spectacles must be removed before existing the laboratory. Before leaving the suite, hands must be thoroughly washed. Exit is by depressing the green push button “Exit”.

NDM RB First Floor Contractor induction

- Fire emergency procedure
- First Aid
- Protective clothing
- Cat2 lab
- Cat3 suite
- Radiation suite

Name of contractor:
Date:
Signature:

Name of inductor:
Date:
Signature:

Appendix 5

CL3 LABORATORY TRAINING RECORD

Name of trainee………………………………………………………………………………

Technique / Assay	Induction on the technique	End of probation on the technique	**Training completed for radionuclide use: Yes / No** *(see Radiation folder for training records)*
Name of technique:	*Name of trainer:*	*Name of trainer:*	
Date + Signature of trainee	*Date + Signature of trainer*	*Date + Signature of trainer*	
Name of technique:	*Name of trainer:*	*Name of trainer:*	
Date + Signature of trainee	*Date + Signature of trainer*	*Date + Signature of trainer*	
Name of technique:	*Name of trainer:*	*Name of trainer:*	
Date + Signature of trainee	*Date + Signature of trainer*	*Date + Signature of trainer*	
Name of technique:	*Name of trainer:*	*Name of trainer:*	
Date + Signature of trainee	*Date + Signature of trainer*	*Date + Signature of trainer*	
Name of technique:	*Name of trainer:*	*Name of trainer:*	
Date + Signature of trainee	*Date + Signature of trainer*	*Date + Signature of trainer*	

Appendix 5….cont.

Declaration

I have read and understood the CL 3 laboratory Code of Practice and will comply with the code at all times.

Signed:

Print Name:

Date:

Authorisation by nominated trainer of specified technique (s)

Signed:

Print name:

Date:

Authorisation by Group leader

Training has been satisfactorily completed and ……
Is authorized to work unsupervised in the CL3 laboratory.

Signed:

Print name:

Date:

Appendix 6

List of techniques to be carried out in CL3 labs.

All new procedures should only be carried out after a risk assessment has been performed and the protocol agreed by either Tao Dong or Alastair Waugh.

1. **E.B.V. transformation of cells**
2. **Separation of lymphocytes from seropositive blood**
3. **Separation of serum and plasma from seropositive blood**
4. **Preparation of CTL**
5. **CTL Assays**
6. **Freezing and thawing cells**
7. **Preparation of samples for FACS analysis, tetramer staining etc.**
8. **Mycoplasma testing**
9. **Preparation of DNA**
10. **Viral load/HIV-1 RNA quantitation**
11. **Dynabeads**
12. **ELISpot assays**
13. **Assays of NK function**
14. **T cell proliferation assays**
15. **Preparation of dendritic cells**
16. **Preparation of HIV virus stocks and assay for RT activity**
17. **Protocol for producing HIV virus and infecting CD4+T lymphocytes**
18. **Measuring ^{3}H uptake in HIV-infected macrophages**

Appendix 7

List of authorised NDMRB CL3 Room users: *March ~ 2015*

McMichael Group
suzanne.campion@imm.ox.ac.uk
elena.brenna@ndm.ox.ac.uk
Geraldine.gillespie@ndm.ox.ac.uk
Lucy.walters@ndm.ox.ac.uk
Simon.Brackendridge@ndm.ox.ac.uk

Borrow Group
Angharad.fenton-may@ndm.ox.ac.uk
thomas.partridge@ndm.ox.ac.uk
isabela.pedroza-pacheco@ndm.ox.ac.uk
Katherine.roberts@sjc.ox.ac.uk
Anna.Kliszczak@ndm.ox.ac.uk
Dimitra.peppa@ndm.ox.ac.uk
Ane.ogbe@ndm.ox.ac.uk

Dorrell Group
gemma.hancock@ndm.ox.ac.uk
hongbing.yang@imm.ox.ac.uk
zoe.wallace@keble.ox.ac.uk
emma.ghaffari@ndm.ox.ac.uk

Rowland-Jones Group
glenn.wong@wolfson.ox.ac.uk
Sengeziwe.sibeko@ndm.ox.ac.uk
sophie.andrews@msdtc.ox.ac.uk
louis-marie.yindom@ndm.ox.ac.uk
Shmona.simpson@ndm.ox.ac.uk

Tao Group

NDMRB Facilities
darren.blase@ndm.ox.ac.uk
ross.macrae@ndm.ox.ac.uk
andrea.keepence-keyte@ndm.ox.ac.uk

Appendix 8

What to do in the event of an accidental exposure to potentially HIV-infected material

Accidental exposure to HIV may occur from splashes to the skin and eyes, or through inoculation injury: aerosols of high-titre virus could potentially be a hazard.

In the event of suspected exposure to HIV, the following first aid measures should be used immediately:

- Splashes to eyes or mucous membranes should be irrigated thoroughly with water (eye wash may be used).
- Skin contamination should be washed off with soap and water (but do not rub the injury site).
- Inoculation injuries of the skin should be washed thoroughly with soap and water and made to bleed freely if possible.

The next step is to consider the need and desirability of administering anti-retroviral treatment, following a risk assessment and in the light of expert advice.

This should be sought immediately (within 2 hours) from the consultants on the John Warin Ward Infectious Diseases Unit; tel. 225214. After contacting the on-call Infectious Diseases consultant, the worker should arrange to take a taxi to attend the John Warin ward as soon as possible.

The consultants will offer counselling and testing of the worker and advise on post-exposure prophylaxis with anti-retroviral drugs if appropriate. Testing for HIV and other blood-borne viruses may be arranged at intervals over the following year if the person agrees.

The accident must also be reported to the **NDMRB Biological Safety Officer** and the **University Occupational Health Service** as soon as possible:

Tel: -

01865 – (2)82676: MON. – FRI: 8:30am to 5:00pm.

01865 – 741166: Out of hours: (on-call Microbiologist via JR Hospital switchboard)

The supervisor and University Safety Officer should also be informed.

Appendix 9

Spillage of Infected Material

In the event of a spillage causing considerable splashing and / or aerosol production, place a "NO ENTRY – SPILLAGE" sign on the entrance door into the CL3 laboratories. (Sign located in hold-all in lobby).
Report incident to CL3 Safety Officer (Hongbing Yang: 07534563503) or Biological Safety Officer (Andrea Keepence-Keyte: 07960086093).

In the Cabinet

In the case of spillage, Virkon powder should be added immediately. For minor spills (<50ml), the powder can be scraped into the autoclave waste 10 minutes after all liquid has been absorbed and the surface of the cabinet wiped with 1% Virkon solution. For major spills (>50ml), the granules should be left in situ for a minimum of 30 mins. The cabinet front should be closed. If major spills are high titre virus, Tiph and Hongbing will be informed immediately and the cabinet need to be fumigated as soon as possible. Contaminated gloves and gowns **MUST** be immediately removed and autoclaved. Hands should be thoroughly washed before leaving the CL3 facility.

Outside the Cabinet

After a significant spillage of infected material such as virus cultures, causing considerable splashing and / or aerosol production, **ALL** personnel to exit the laboratory as soon as possible. A red & white "NO ENTRY – SPILLAGE" sign **MUST** be placed on the entrance door into the respective CL3 laboratory. Also, use plentiful amounts of the black & orange hazard warning tape on the door and frame in order to hamper any possible attempt at entry. Contaminated gowns MUST be immediately removed in the lobby area & autoclaved and the facility vacated. The outer door should be locked and warning notices ("NO ENTRY – SPILLAGE) plus black & orange hazard warning tape put up to prevent access A risk assessment should be carried out to determine the extent of the exposure risk, and the University Safety Office should be contacted to discuss the potential need for fumigation. After a spillage such as this, the biological safety cabinet should be left on to help ventilate the area and capture any aerosol raised. If the room has to be fumigated, contact the NDMRB Biological Safety Officer and CL3 laboratory manager.

Criteria for spillages outside of the cabinet:

High risk:
High titre HIV – 1 culture (culture medium or contents of vials with infected cells);
Whole blood, PBMCs, plasma where status for HIV / HBV / HCV either known to be positive AND patient is viraemic or status is unknown.

Medium risk:
Whole blood, plasma and / or PBMCs from HIV / HBV / HCV + donors where virus RNA (HIV / HCV) or DNA (HBV) undetectable.

Low risk:
Whole blood, plasma and / or PBMCs from donors where status of HIV / HBV / HCV confirmed negative.

Appendix 10

Appendix 11

CL3 DEEP CLEAN ROTAs (please sign initials + date against those duties completed):

Task / duties:

Sink area cleaning & clearing away

Clearing away unnamed media from fridge

Clean inside / outside of centrifuge with 1% Virkon

Surface area clean of work surfaces with 70% IMS + areas where dust collects.

Cleaning of under surface of Class 2 cabinets (removal of steel work surface)

(These duties to be tabled on a chart at the entrance to the CL3 facility).

Please sign after completing the deep clean:

Name	Date	Signature

Appendix 12

Cat. 3 LABORATORY INDUCTION TRAINING RECORD

Name……**Sign** :……..……

Practice	**Comment**	**Date**
Labcoat		
Double gloving		
Disinfection		
Accidental exposure		
Incubators		
Disposal		
Cleaning rota		
Cabinets		
Radioactive monitoring		
Centrifuge		
Spillage / Cleaning up		
Spillage within incubators		
Cuts		

I have read and understood the Containment Level 3 laboratory Code of Practice and will comply with the code at all times.

Signed:

Print Name:

Appendix 13

Virkon assay

To test the ability of 1% Virkon to kill high titre HIV virus in 30 and 10 minutes.

Protocol:

1% Virkon solution: dissolve 1g Virkon in 100ml water
5% Virkon solution: dissolve 5g Virkon in 100ml water

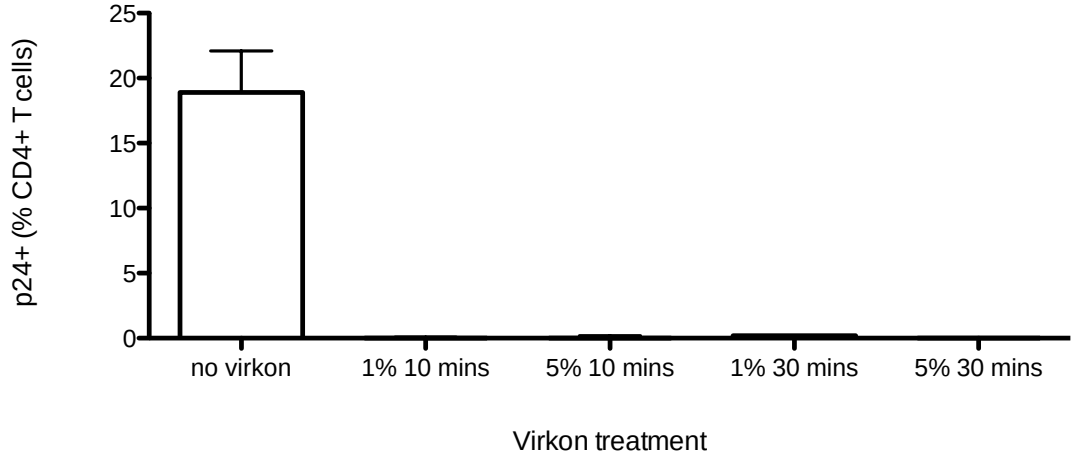
High titre HIV-1 virus: HIV_{IIIB}.

Infect PHA activated healthy donor's PBMC with HIV_{IIIB} at M.O.I of 0.01 by spin-occupation at 2000rpm 27^{o}C for 2 hours. Infected cells are then divided into 5 tubes:
1) No Virkon treatment,
2) 1% Virkon for 10 minutes,
3) 5% Virkon for 10 minutes,
4) 1% Virkon for 30 minutes,
5) 5% Virkon for 30 minutes

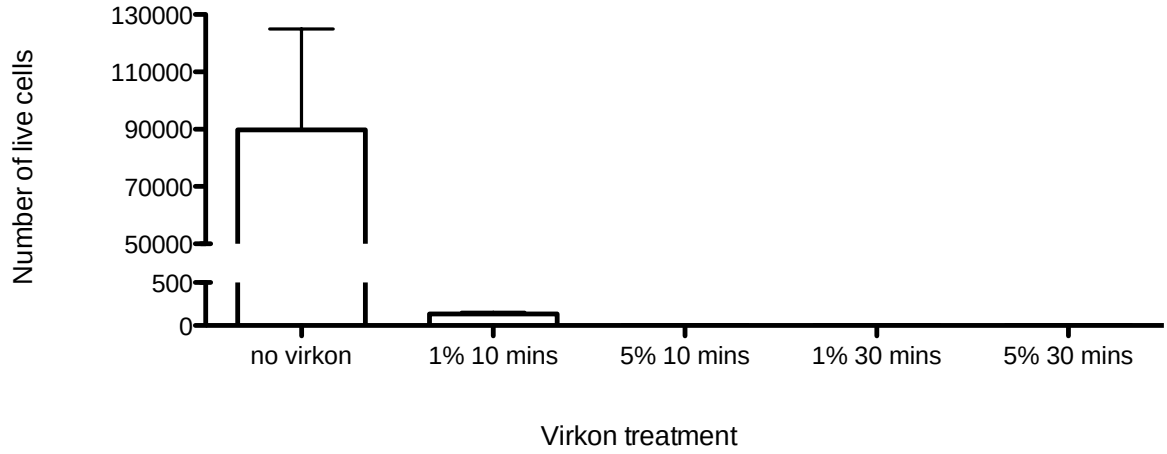
After Virkon treatment, cells are washed 3x times with RPMI-1640 medium with 10% FCS (R10) and then cultured in R10 at concentration of 1 million cells /ml in 96-well plates in triplicates for each condition for 7 days. At day 7, infected cells are harvested and stained for intracellular HIV protein p24 and analysed by Flow cytometry.

Results

Result 1: No HIV p24+ cells are detected after Virkon treatment (graph below)



Result 2: no live cells detected after treatment with 5% Virkon for 10 and 30 minutes or 1% Virkon for 30 minutes, only 0.1% cells are alive after 1% Virkon for 10 minutes.



Appendix 14

University of Oxford- NDM Research Building

Decontamination Release Certificate

Dr. Hongbing Yang
(CL3 Lab Supervisor)
***Telephone :* 01865 612914**

E-mail :
Hongbing.yang@ndm.ox.ac.uk

Room 664.20.27
NDM Research Building,
Nuffield Department of Medicine,
University of Oxford,
Old Road Campus,
Oxford, OX3 7FZ
United Kingdom.

Containment Level 3
Item / equipment…….

Parts
decontaminated……..……
……..……
……
……

Non decontaminated
parts……..……
……
……
……

Date: ……

Signed: ……Lab Manager

Signed: ……Researcher

Appendix 15

INSPECTION CHECKS – CONTAINMENT LEVEL 3 LABORATORIES		
DATE:	LAB NO.	664.20.27, 27A and 27B
CHECKED BY:	NAME:	SIGNATURE:
TYPE OF CHECK	VISUAL INSPECTION	MONTHLY
Dust trails (check around pipework, cracks in walls, sensors)		
Cracks on the walls		
Cardboard (should be covered in plastic)		
Paper (should be in sleeve and folders)		
Chairs (check for break in the furnishing)		
Incubators (water level, spill marks)		
Fridge/freezer (tidy? Door closing properly?)		
Centrifuges (O-ring centrifuge lids all present, spill marks?)		
Other not listed above:		
ACTIONS REQUIRED:		
ACTIONS COMPLETED:		

Appendix 16

Sealability Test Record Sheet
NDMRB Containment Level 3 suite

Inspection carried out by: _____

Date: _____

Room inspected: 20.27 (Large suite)

Note on previous findings:

Area inspected	Results	Further action needed
Door frame		
Light fittings		
Ventilation fittings		
Outer Window frames		
Electrical trunking		
Wall corner at the far end of the suite_right handside		
Wall corner at the far end of the suite_left handside		
Around pipework by the safety cabinets		
Wall corner by the sink area _right handside		
Wall corner by the sink area _left handside		
Inner window frame		
Pipework by the sink		
Other area not mentioned above:		
Other area not mentioned above:		

Room inspected: 20.27A (Small suite)
Note on previous findings:

Area inspected	Results	Further action needed
Door frame		
Light fittings		
Ventilation fittings		
Outer Window frames		
Electrical trunking		
Wall corner at the far end of the suite_right handside		
Wall corner at the far end of the suite_left handside		
Around pipework by the safety cabinets		
Wall corner by the sink area		
Wall corner by the door		
Inner window frame		
Pipework by the sink		
Other area not mentioned above:		
Other area not mentioned above:		

Approved Method for packaging

The following sets out the approved requirements for packaging.

The total packaging must include:

a) An inner packaging comprising:

i) Watertight primary receptacle(s);

ii) A watertight secondary packaging; and

iii) Absorbent material in sufficient quantity to absorb the entire contents placed between the primary receptacle(s) and the secondary packaging; if several primary receptacles are placed in a single secondary packaging then they shall be individually wrapped so as to prevent contact between them.

b) An outer packaging of adequate strength for its capacity, mass and intended use, and with minimum external dimensions of 100 mm.

Inner packaging containing infectious substances may not be packed together with inner packaging containing unrelated types of goods.

Items must also be packed in accordance with the following:

a) Lyophilized substances

Among other suitable receptacles, flame-sealed glass ampoules or rubber-stoppered glass vials fitted with metal seals, may be used for the primary receptacle.

b) Liquid or solid substances

i) For substances consigned at ambient or higher temperatures, primary receptacles shall be of glass, metal or plastics. Positive means of ensuring a leak-proof seal shall be provided, e.g. a heat seal, a skirted stopper or metal crimp seal. If screw caps are used, they shall be reinforced with adhesive tape.

ii) For substances consigned refrigerated or frozen, ice or dry ice shall be placed around the secondary packaging(s). Interior supports shall be provided to secure the secondary packaging(s) in position after the ice or dry ice has dissipated. If ice is used, the outer packaging needs to be leak-proof. If dry ice is used, the outer packaging shall permit the release of carbon dioxide gas. The primary receptacle and the secondary packaging need to be able to maintain their integrity at the temperature of the refrigerant used.

iii) For substances consigned in liquid nitrogen, plastic primary receptacles capable of withstanding very low temperatures need to be used. The secondary packaging shall also be capable of withstanding very low temperatures, and in most cases will need to be fitted over the primary receptacle individually. The requirements for the consignment of liquid nitrogen shall also be fulfilled. The primary receptacle and the secondary packaging need to be able to maintain their integrity at the temperature of the liquid nitrogen. Note: - University departments should consign substances in liquid nitrogen only if there is no suitable alternative means -please contact the University Safety Office for further advice on the particular consignment requirements.

Whatever the intended temperature of the consignment, the primary receptacle or the secondary packaging shall be capable of withstanding without leakage an internal pressure producing a differential pressure of not less than 95kPa and temperatures in the range -40°C to +55°C.

Version	Amendments/changes
1.2	List of user updated Page X of Y instead of Page X Addition of the Engineer induction page
1.3	Addition of Lab manager contact details Modification of the training records Addition of Inspection checks and sealability checks Addition of transport of sample in between suites
1.4	Update of HY contact details Update of CL3 user list Update of waste stream Addition of coulter counter in the equipment list
5	Update of contacts – new buildings manager and lab manager Change of version format