Bio & GM Safety Working to the Code

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https://workspace.nottingham.ac.uk/display/safety/Biosafety+&+GM

Regulations

- **C**ontrol **o**f **S**ubstances **H**azardous to **H**ealth [HSE]
- **G**enetic **M**odification **C**ontained **U**se Regs [HSE]
  - Specific approval required
- **S**pecified **A**nimal **P**athogens **O**rder [DEFRA]

- **A**nti **T**errorism **C**rime & **S**ecurity Act
  - Schedule 5 list – pathogens & toxins

Biological Agents

- COSHH Definition
- Any of the the following if they can cause infection, allergy, toxicity or other human harm.
  - micro-organism
  - cell culture
  - human endoparasite

Classification & Notification & Containment

- ACDP Approved List of Biological Agents 2013
  - List of Bacteria, Viruses, Parasites & Fungi
  - Hazard grouping 1 - 4 (low to high)

- Schedule 3 of COSHH Regulations
  - Requires notification of HG 3 /4 agents and certain HG 2
  - Prescribes Containment facilities/lab standards & control measures to be applied

Control measures = containment level

- Increasing levels of stringency CL 1 – 4
- Prescribes lab facilities
- Access controls
- Use of Microbiological Safety cabinets
- Provision of S O Ps
- Disinfection & Decontamination regimes
- Information instruction & training
- PPE requirements

Biological Agents

- Hazard Group 1
  - Unlikely to cause human disease
    - Animal tissues and cell lines (not known to contain human pathogens)
    - Well established human cell lines - history of safe use e.g. MRC 5
    - Plant cells/materials

Assign to CL 1

Hazard Group 2

- Can cause disease
- May be a hazard to employees
- Unlikely to spread to community
- Prevention or treatment available
  - Bacillus cereus, Clostridium spp, campylobacter
  - Most wild type E. coli,
  - Pseudomonas aeruginosa,
  - Proteus vulgaris, Staph aureus.
  - Fungi – Aspergillus spp, fusarium spp
  - Human tissues & primary cell cultures

Assign to CL 2

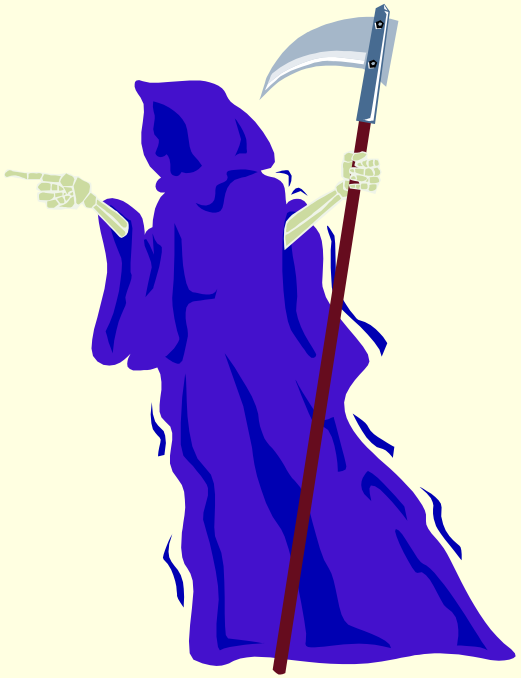
Hazard Group 3

- Can cause severe human disease
- Serious risk to employees
- May spread to community
- Prevention or treatment available
  - Anthrax; Brucella abortus/canis/suis; E.coli O157 Mycobacterium bovis/leprae/tuberculosis; Salmonella typhi/paratyphi; Yersinia pestis.
  - HIV; SIV; Hepatitis; Hantaan
  - Plasmodium faliciparum, Trypanosoma cruzi
  - Human/Bovine TSE (prions)

Assign to CL 3

Hazard Group 4

- Severe human disease – likely to spread – no treatment
- Virusus such as
  - Lassa fever
  - Rabies
  - Congo heamorrahagic fever
  - Ebola
  - Marburg
  - Variola
- **CANNOT BE USED HERE**



Containment – increasing levels of control

Level 1

Level 2

Level 3

Level 4

Containment level 2 - facilities.

- Bench - impervious, washable, chemical resistant.
- Floor - coved, continuous, sealed.
- Wash-hand basin by the door.
- Negative pressure to corridor - [mechanical ventilation].
- Restricted access, door kept closed.
- Autoclave - in building
- Lab coat storage - enough for occupants

Containment level 2 - practices.

- Practice Good Occupational Hygiene
  - No eating, drinking, chewing, pen sucking
  - Handwashing
  - Cover cuts
  - Avoid hand to mouth contact
- Minimise aerosols
- Contain harmful/infectious aerosols. [MSC]
- Avoidance of sharps/glass
- Prevention/containment of spills
- PPE
- Disinfection and waste disposal procedures.
- Waste to autoclave in robust spill/leak proof containers.
- Safe, secure storage of organisms.
- Immunisation where available
- TRAINING & COMPETENCE

ROUTES OF EXPOSURE & TRANSMISSION

Inhalation - aerosols. [airborne e.g. TB/adeno]

- **Pouring**
- **Resuspending/mixing**
- **Sonication**

Skin penetration [blood born pathogens]

- **Sharps injury**
- **Defective skin barrier [cuts/skin lesions/eczema]**
- **Mucous membrane contact**

Ingestion [enteric pathogens]

- **Hand to mouth contact**
- **Pens/papers**
- **Ingestion of aerosol**

Prepare your workstation

Avoiding/minimising aerosols

Pipetting

- Use “to deliver” pipettes/ reverse pipetting techniques to avoid blowing out the last drop
- Drain pipettes gently with the tip against the inner wall of the receiving vessel
- Use pipettes with plugs to reduce contamination of the pipetting device
- Work over an absorbent, plastic-backed pad to avoid aerosol dispersion from drops falling on hard surfaces
- Do not resuspend/mix materials using a pipette – this creates bubbles - use vortex mixer

Opening tubes:

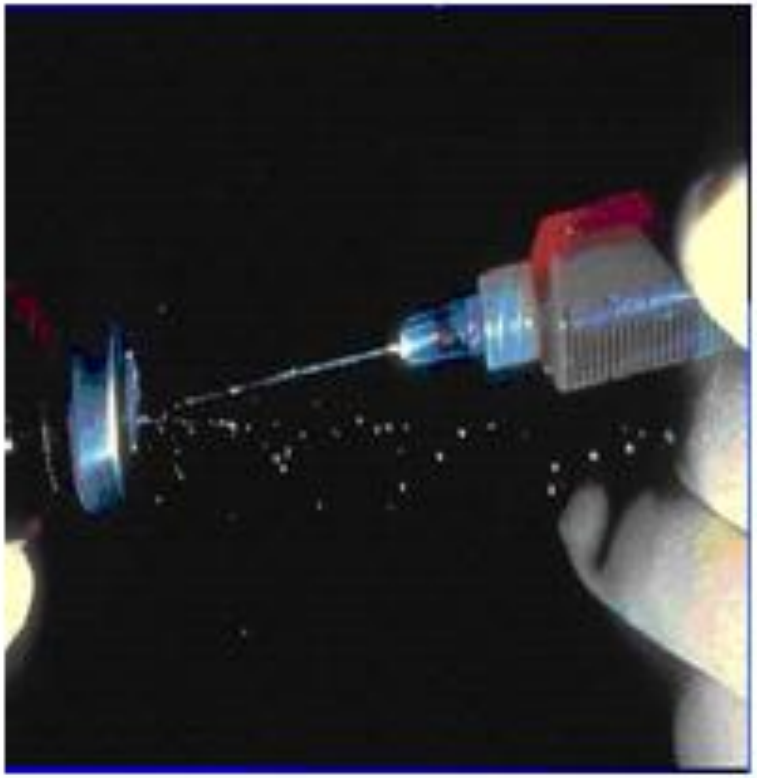
- Avoid push-in/flip top closures (when opened, the film of liquid trapped between tube and closure breaks and releases aerosols)
- Use a vortex mixer instead of inverting tubes
- Wait 30 seconds after shaking a tube before opening/briefly centrifuge

Pouring infectious liquids:

- Avoid pouring off supernatant – use pipettes or vacuum line instead
- Always pour down side of cylinder
- Pour infectious liquid waste through a funnel where the end is below the surface of the disinfectant in the discard container;
- Pour disinfectant through the funnel after use

Examples of operations that generate aerosols

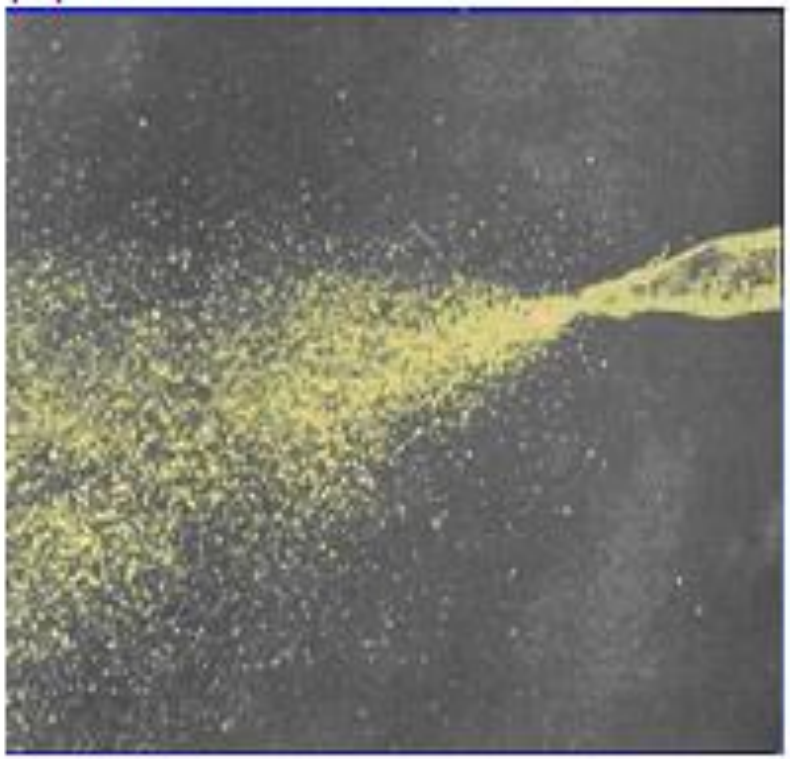
Withdrawing needle from a vial



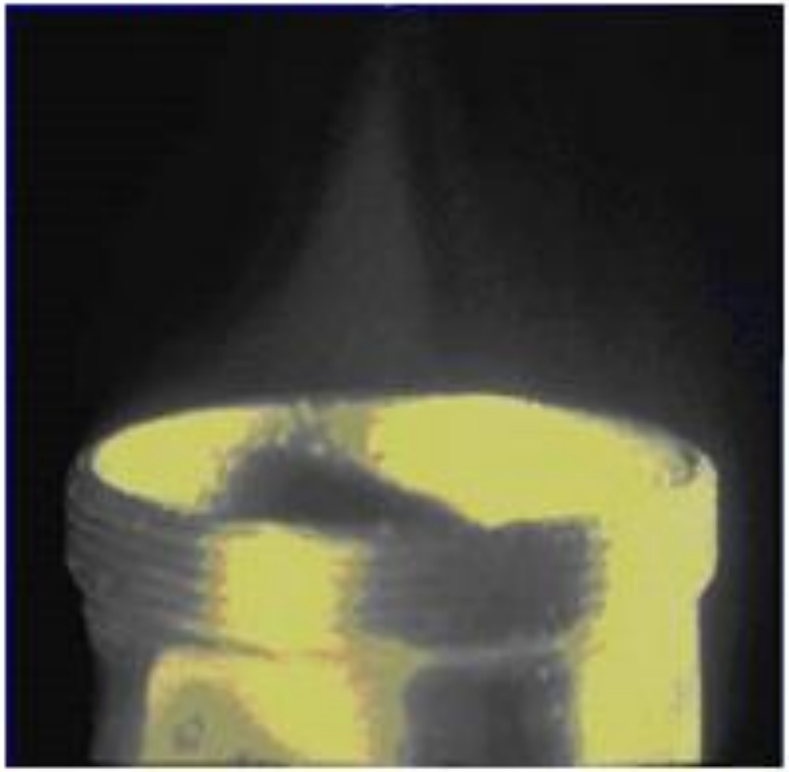
Vortexing

Examples of operations that generate aerosols

Pushing out the last drop from a pipette



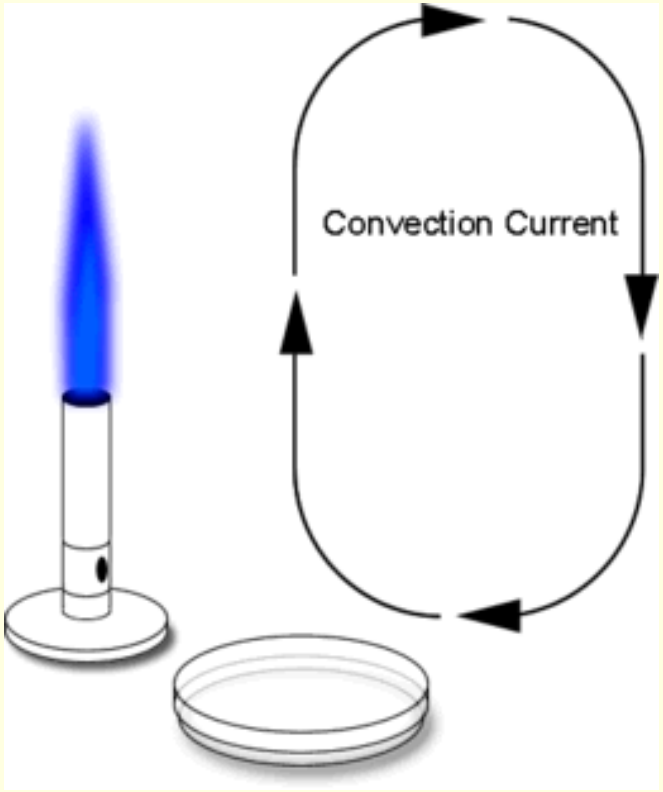
Opening up a tube with contents recently centrifuged



Examples of operations that generate aerosols

Flipping open an Eppendorf tube

Bunsen Burners in the Micro Lab



Why use.

convection current created by heat prevents potentially contaminating particles from falling onto the agar plate.

BUT

It will also carry infectious aerosol created in/above flame into the wider environment.

Correct technique for flaming loops.

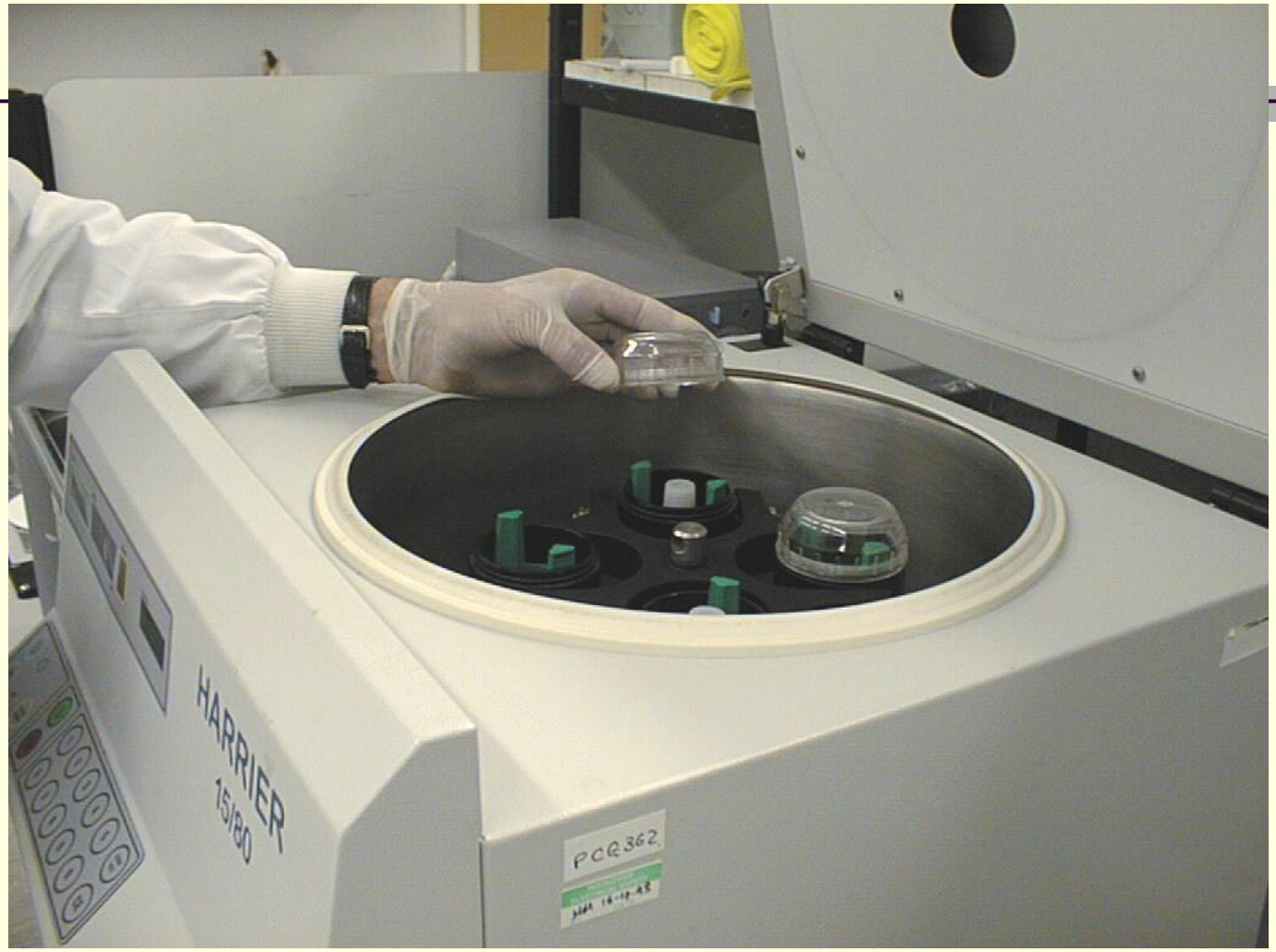
Must not but used in MSC

Fire risk – turn off after use!

A better alternative

- Sterile – no flaming / no aerosol risk
- Can be used in MSC
- Calibrated to deliver set amount of culture – reproducability

If you can't avoid it CONTAIN IT!



CONTAINING AEROSOL

Centrifuges

- Use sealed buckets or rotors
- Balancing
- Check seals before use
- CL2 & 3 - open in safety cabinet
- Clean and disinfect centrifuge and rotor after use.
- Some disinfectants attack metal rotors!
- Spillage/breakage procedure


CONTAINING AEROSOL

Microbiological Safety Cabinets

- Class I to III
- Classes DO NOT relate to containment level!!
  - Class III - highest protection
  - Class I - good general operator protection
  - Class II - combines protection of work and worker against contamination.

Video Training

- Safe use of Microbiological safety cabinets

- Access at

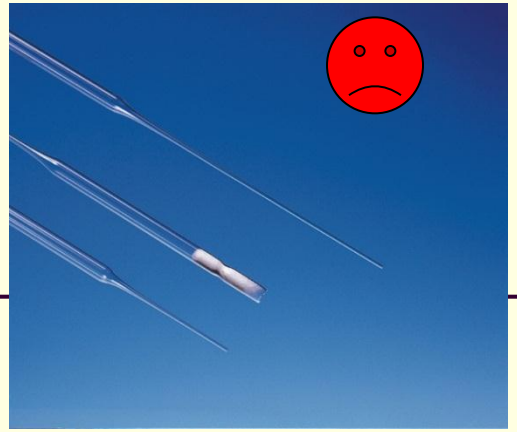
http://moodle.nottingham.ac.uk/course/view.php?id=7616

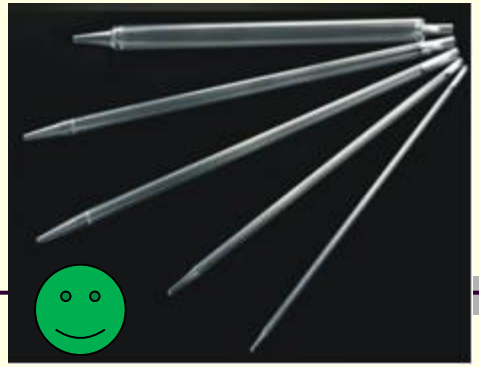
Log in, view video & supplementary info

Complete on-line assessment

Laminar Flow Hoods

- DO NOT confuse laminar flow hoods with Microbiological Safety Cabinets.
- LFHs draw clean filtered air vertically or horizontally across the work to protect the work from external contamination.
- There is no worker protection as there is no inward air flow - horizontal units direct air towards the operator!!!
- Use only for non hazardous organisms

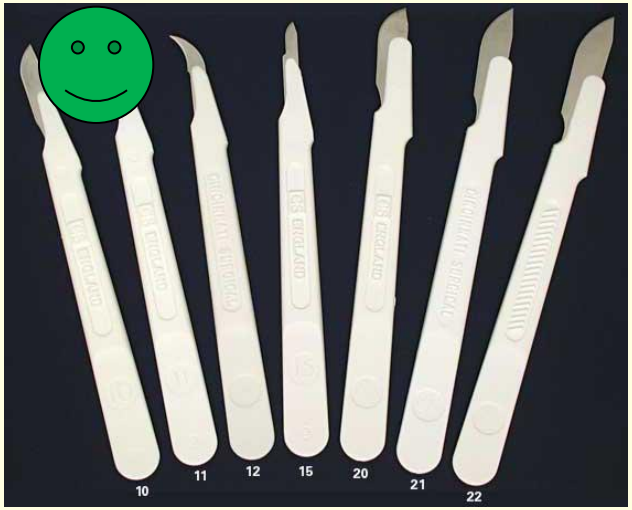





NEVER
RE-SHEATH


CONTAMINATED WASTE
SHARPS ONLY


UNSTERILE
SINGLE USE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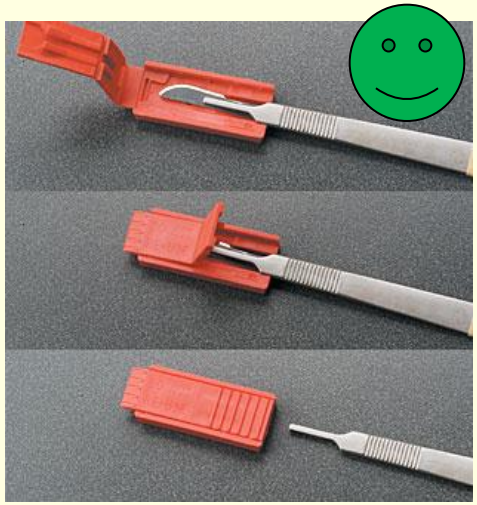

Personal Protective Equipment

Lab Coat

- Correct type
- Fastened to neck
- Hang up – no double hanging
- Change regularly

Nitrile Gloves
Cuffs worn over lab coat

ASTM F1671
Viruses

EN373 2

Level 2

Safety Specs

- Working on bench with harmful organisms/substances
- Where there is risk of splashing

After you have finished your work.

- Deal with your waste
- Disinfect you work area and any associated equipment
- Store your cultures safely
- Remove lab coat – hand it up!
- Wash your hands thoroughly
- Use hand care products – **outside of lab**

Social Hand Washing:
For routine hand washing, liquid soap and water is adequate, using the technique described

Aseptic Hand Washing:
(Invasive Therapy)
After hand washing, apply sufficient skin sanitiser to completely cover the hands. Rub in until the alcohol has evaporated using the technique described

Remember:
1. Keep nails short and clean
2. Wet hands first under running water
3. Hand wash for 10-15 seconds
4. Pay particular attention to thumbs, fingertips and between fingers
5. Rinse thoroughly under running water
6. Dry thoroughly
7. Apply conditioning cream regularly

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Hand Hygiene Product Application Guide

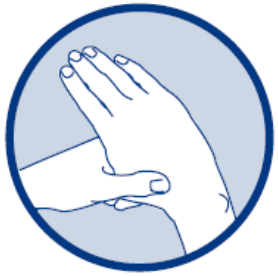
Follow these simple steps when washing, sanitising or applying moisturising cream.

❶ Rub palm to palm

❷ Palm to palm, fingers interlocked and around wrists

❸ Palm to back of hand & round wrist

❹ Finger tips and back of fingers into palm



❺ Thumbs clasped in palm

❻ Clasped fingers into palms

NB. When using hand cleansers, wet hands before applying washing agent.
Don't forget to wash wrists as well before rinsing and drying thoroughly.

deb Be the world's leading away from home skin care system company

UK LIT0152Z

Safe storage of cultures/organisms

Labelling	**meaningful, clear, ownership**
	Biohazard signs on fridges/freezers
Secure	**racks, trays, away from bench edge**
	plates sealed, secure stacks.
	Designated facilities,
	Within lab areas
	Separate from non viable material.
	Cold rooms
	Regular housekeeping

Inventory

Archive material **Segregate and label ‘non active’**


How not to do it!

Unscreened human tissue and fluids

- Risk of “hidden” pathogens.
- CL2 if unknown, CL3 if known HG3 present.
- Very low aerosol risk - cuts, scratches, injection
- Hep B vacc. before start - contact Occ Health.
- Use screened/low risk group donors where possible
- Designate area, written protocols followed,
- Strict adherence to CL practices
- MSC if aerosols produced - mixing, shaking, sonication
- Avoid sharps
- Cover cuts with waterproof plasters, wear gloves,
- Follow Sharps Injury procedure
- Rigorous decontamination procedures,

SHARPS INJURY & EYE /MOUTH SPLASH PROCEDURE

- **Immediate action**
  - **Sharps Injury**

    Encourage wound to bleed – **DO NOT** suck
    Wash wound with soap and water, dry and apply dressing
  - **Body fluid contact with eyes/mouth** use large amounts of water to wash away.
  - **Report incident** immediately to your line manager or supervisor and in conjunction with your manager assess the risk and take appropriate action as identified in the table below:

SUBSEQUENT ACTIONS

Nature of hazard	Action to be taken
Unused clean sharp which is definitely uncontaminated	Complete University accident report If concerned seek further advice as below.
Used sharp not known to be contaminated with any harmful biological agent or toxic substance.	Contact Occupational Health Ext 14329 at earliest opportunity to discuss whether attendance at A&E is required
• Used or dirty sharp, contaminated with human material/harmful biological agent • Human bite or scratch • Human body fluid splash SOURCE KNOWN OR UNKNOWN	Immediately attend Accident & Emergency department at Nottingham University Hospital Queen Medical Centre : 0115-9249924 Additionally during normal hours contact Occupational Health Mon-Thursday 0830 -1630 Friday 0900 – 1630 Tel: 0115 951 4329 Outside normal hours contact: Contact OH at first available time above. OH MUST be provided with an incident risk assessment After A and E intervention the injured person should attend OH as soon as possible for potential HEP B vaccination and blood storage services

Disinfectants - selection

- Type
  - Spectrum of activity
  - Specific activity for different micro-organisms
  - Check validation data
- Circumstances
  - Dirty or clean - organic load
  - intra or extracellular viruses
  - Chemical incompatability
  - Temperature, pH, hardness of water.

Disinfectants - selection

- Consider material/surfaces to be disinfected
  - Metal equipment surfaces – avoid acids, alkalis, hypochlorites
  - Plastics could be damaged by phenolics
  - Spills – consider powder form, or gel absorbents
- Consider Hazardous properties
  - toxic/corrosive [phenolics /hypochlorite]
  - irritant [Virkon , formaldehyde]
  - Sensitising [gutaraldehyde]
  - Reaction products [formaldehyde + Chlorine]

Virkon [peroxygen compound]

- Broad spectrum of effectiveness
- Activity reduced by protein/salts
- WC = 1% discard jars/bug cultures
  2% buffered systems
- Colour indication
- Stable for 7 days
- Does not bleach clothing
- Prolonged exposure can cause corrosion [10m]
- 1% working solutions are relatively safe
- Powder irritant

Distel (formerly Trigene)

- Halogenated tertiary amine+ surfactants
- Small efficacy spectrum
- WC = 2% discard jars, 10% body fluids/spills
- non toxic, less irritant than Virkon

Clorine - based hypochlorite

- Chloros/Presept/Chlor-clean
- Rapid action - Protein denaturation
- WC
  - General use 1,000-2,500 ppm
  - Discard containers 5,000-10,000 ppm
  - Spills 20,000 ppm
- Chlorine produced if mixed with acid
- Carcinogens if mixed with formaldehyde
- Corrosive, damages metal .
- Limited shelf life - chemical reaction

- Not commonly used but essential for Prion work & Clostridia spp

Alcohols

- 70% ethanol; 60% iso- propanol
  - Relatively poor efficacy.
  - Susceptible to interference.
  - Flammability risk - do not use sprays in MSCs
  - Only suitable for use on physically clean surfaces.

Autoclaves

- Mandatory for HG 3 waste & certain HG 2/GM waste
- Portable/benchtop models not suitable for waste inactivation
- Transport - robust leakproof containers
- ID source of waste
- Written operating procedure
- Training and authorisation needed
- Visual check of seals and steam leaks pre-use
- Protective clothing - lab coat; impervious apron; heavy duty gauntlets; face visor, robust shoes
- NO RADIOACTIVE MATERIALS/ toxic chemicals
- Maintenance, validation & calibration

Autoclaves

Process	Sterilising temperature (range, °C)	Sterilisation time (minutes)
Liquids sterilisation*	121 - 124	15
Equipment/glassware sterilisation	121 - 124 134	15 3
Make-safe cycles (decontamination of materials for discard or for re-use)	121 - 124 134	15 3'

Biohazard (Clinical) Waste

- Requirements
  - Segregation
  - Identification
  - Packaging
    - Yellow bags
    - Label source - dept name tape
    - 3/4 full max.
    - Seal - tie, knot, proprietary clip.
  - Remove to secure collection point
  - Final Disposal - Incineration
  - Infective wastes - autoclave first

Transport of Biological Material

- Regulations for road, rail and air.
- Classification, labelling and packaging by competent person.
- Infectious Substances Cat A & Cat B
- Package - UN approved to prevent release for Cat A
  - Special package if dry-ice used.
- Shipper's declaration for air transport.
- Advise use of Courier for HG2 and above
- School/departmental safety officer must be consulted first.
- University Code of Practice
- Further Advice – DGSA Road Safe Europe

Transport of Biological Material

Mini Tube

Biofreeze - 1ltr

University Code of Practice on Transport of Potentially Dangerous Goods [2004]

GENETIC MODIFICATION CONTAINED USE REGULATIONS 2000

GM - What is it?

- alteration of genetic material of an organism -
  - **bacteria**
  - **fungi**
  - **yeast**
  - **mammalian cells including human cells/cell lines**
  - **viruses**
  - **plants**
  - **animals**

⌘ in a way which does not occur naturally – a variety of methods involving:

- **removal of genetic material from one organism**
- **cutting or copying sequences from that material and**
- **re-inserting them into another organism of the same or a different species**

Some Examples

- Bacterial cloning using plasmid vector containing ‘foreign’ DNA insert
- Use of viral vectors containing DNA for use in mammalian expression systems
- Production of GM animals or plants [transgenics, knock outs, chimeras]

Contained use - what is it?

- **Production**
- **Culture**
- **Storage**
- **Transport**
- **Disposal ..... of a GMO**

Main provisions of Regs

- Set up a GM Safety Committee to approve RAs
- Risk Assessment – humans and environment
- RA to be approved & recorded
- Assign containment level to protect H & E
- CL determines activity class.
- Prescribes standards for containment facilities
- Notify HSE [class 2 or above – Fee!!]
- Emergency plan
- Report certain incedents /accidents

Risk assessment - Matters to be considered

Consider properties of:

- **Recipient [host] organism**
- **The inserted genetic material**
- **The vector**
- **The donor organism [where it is used during the GM activity]**
- **The resulting GMO**

What harmful properties?

- Disease to humans
- Disease to animals/plants
- Adverse effects on treatment of disease /prophylaxis
- allergenic/toxic effects/ adverse biological effects- humans
- ability to transfer to other organisms in environment
- adverse effects of transfer to other organisms/ dissemination to environment
- adverse effects of interaction with other GMOs in the lab area

HOST [the organism that will be modified]

- can it cause disease in humans,
- is it a disabled strain/wild type
- Check ACDP group/animal pathogens list

- Assign to one of 4 Hazard groups
  - Disabled K12 strains of e coli = HG 1
  - Wild type candida = HG 2
  - Wild type c botulinum = HG 2
- If parental/host organism is HG 2 or above the activity is notifiable - Complete PART 2

VECTOR

Plasmid/yeast vectors

mobility status - ability to transfer to other organisms

Mobilisation defective, non-mobilisable, *self mobilisable*

Viral

is it an attenuated strain

e.g. Ad5 with E1/E3 deletion = HG 1

wild type Adenovirus = HG 2 -

Check ACDP group

Consider ability to infect human cells in culture

ecotropic v amphotropic

INSERT DNA

does it code for a harmful protein, is it an oncogene

Will it be produced in an active form

Will it be expressed at high level

FINAL GMM –

is it more harmful to humans than parental organism

- **Assign provisional containment level [1, 2 or 3]**
- **Consider potential harm to environment - is a higher CL required ?**

ANIMALS

Additional RA forms must be completed for:

- Infection of an animal with a GMM [CL 1-4]
- Production of a transgenic animal [AC A or B]
- Submit to GMSC - and send copy to Manager of Animal facility
- Must have project license number

Process

- Discuss with supervisor
- Complete risk assessment form with supervisor/PI
- Seek advice of Local BSO
- Get approval from HOD/HOS
- Submit to GMSC for approval
- Commence work on receipt of approval
- Review work regularly - if change submit to GMSC

University GM Safety Committees

Review and Approval of RAs

C	**[amalgamated A B & C] UP/Med School/QMC/Derby GEMS**
	All groups in CBS
	Life Sciences
	Pharmaceutical Sciences
	Medicine
D	**Sutton Bonington Campus**
	Biosciences
	Vets School
E	**City Hospital Site**
	Clinical Sciences Building
	Academic Unit of Oncology [hospital]

If your project involves GM work …..

Discuss with academic supervisor/Principal Investigator *

Contact your local BSO

UP/Med School/QMC

Life Sciences	Simon D awson
Medicine	TBA
Pharmacy [outside CBS]	Martin Garnett
CBS	Alan Cockayne /Louise Cupitt
City Hospital	Dan Duthie [CSB] Ian Spendlove [AUO]

Complete relevant risk assessment form with your supervisor

Submit form to Dan Duthie [City Hospital]
UBSA – UP/Med Sch/QMC

- Note – PI must be head of research group /similar.

If your project involves GM work …..

Discuss with academic supervisor/PI *

Contact your local BSO

Biosciences

Plant & Crop Sciences	Rupert Fray – also School BSO
Food Sciences	Cath Rees
BBS	Chris Powell
Nutritional Sciences	Simon Welham
Animal Sciences	Pat Fisher

Vet School Mike Jones – School BSO & Chair of GMSC

Complete relevant form
Submit form to Mike Jones for GMSC review and approval

* Note – PI must be head of research group /similar.