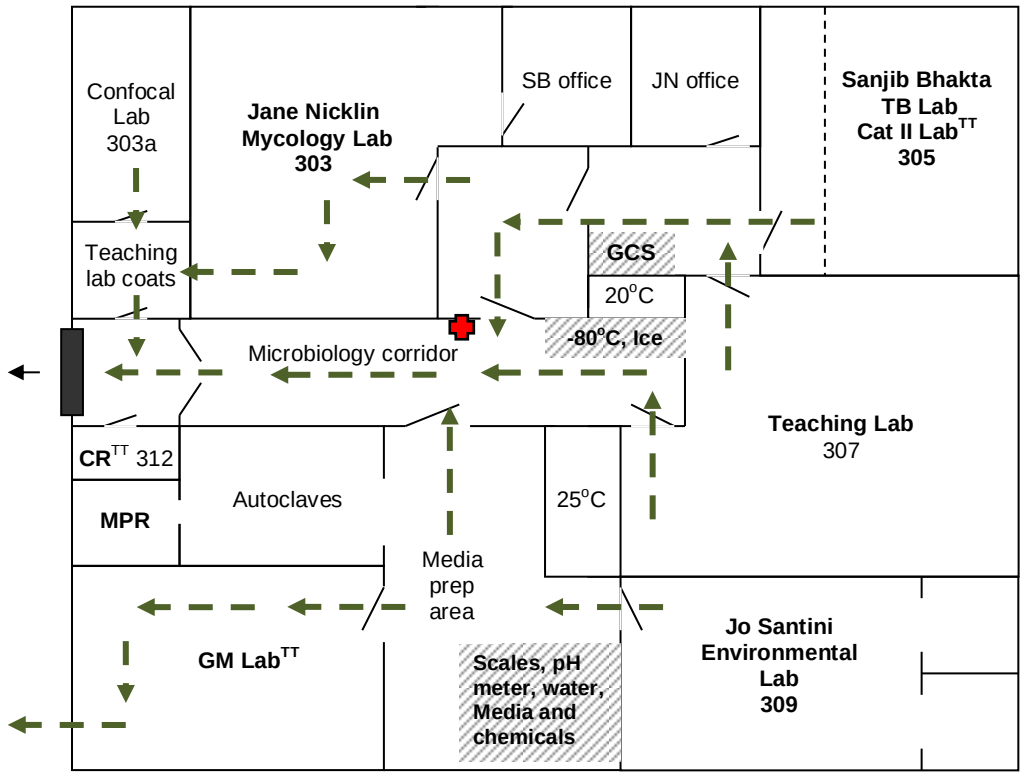
Department of Biological Sciences
Microbiology Research Unit: Induction, Safety and Equipment Training Notes for iGEM 2015

1. Map of the Unit



Main First Aid box
MPR - Media pouring room
CR - Centrifuge room
GCS - General chemical shelves
TT - Laboratories requiring authorisation for entry
- Secure doors, card entry only
- Fire Exit Routes

2. Safety Procedures for the Microbiology Unit

The College Safety Code of Practice for the Department of Biological Sciences can be found at:
http://www.bbk.ac.uk/so/policies/MAINLABS

The department's safety policies can be found at:
http://www.bbk.ac.uk/biology/our-research/safety

3. Microbiology Research Unit Code of Conduct and Good Laboratory Practices

Prior to commencing any experimental work within the area:

- ✓ Please ensure that you have read, understood and signed all the relevant COSHH forms.
- ✓ Every individual that enters the laboratory has a duty of care to all the other members within the unit, and is expected to behave in a way that does not compromise the safety of others.

- ✓ Eating or drinking is not permitted anywhere within the microbiology research unit. Avoid placing any object in your mouth - (pencils, pens, fingers etc.).
- ✓ Laboratory coats and gloves must be worn whilst performing experiments within the Unit. Safety goggles are provided (to be used whenever there is even a slight risk to the eyes) along with face masks (to be used where there is a risk of inhaling contaminated dust, e.g., spores, silica). Please use protective equipment as required.
- ✓ Regard all organisms and biological materials used within the microbiology research unit as potentially infectious and pathogenic to humans.
- ✓ Always wash and dry your hands thoroughly, with the anti-bacterial wash provided, before leaving the laboratory.
- ✓ Tie long hair back neatly away from the shoulders prior to any experimental work being undertaken. Also wear enclosed footwear (i.e. no open toe sandals) within the Microbiology Research Unit.
- ✓ Keep your work area/bench clean and tidy. Wipe with IMS prior and after any experimental work. Remove waste material from your area as soon as possible and dispose of in an appropriate manner (see waste disposal table in the Appendix). Do not leave your waste for somebody else to clear away.
- ✓ Label all samples, reagents, tubes, bottles etc. with the contents, your name and the so that you can recognise what belongs to you. Do not leave unlabelled used/filled containers in the lab.
- ✓ It is also good practise to write down the molarity of a solution, the name of the agar in a poured plate, the step you are performing if you have many tubes etc., name of isolate if storing microorganisms, and the date prepared all this. etc.
- ✓ Wash your hands with detergent before you leave the lab.
- ✓ If you have an open cut on your hands or other exposed skin surfaces please cover with a plaster and/or wear gloves.
- ✓ When working with GM or cat II pathogens please ensure that all work is carried out within the designated working area / microbiological class II cabinet. Do not place any books, writing materials and other personal belongings within the designated area.
- ✓ Ensure you carry out procedures which will minimise the risks of spills, splashes and the production of aerosols. If you have an accident of any kind, call your supervisor or laboratory manager immediately and follow all instructions for clearing up the waste. For minor microbiological spills, put on gloves, spray the area with IMS. Leave for a minimum of 30 minutes and then wipe up with paper towel. Give a final wipe with 100% IMS spray.
- ✓ Report all accidents or incidents to the laboratory manager or your supervisor who will help you to fill in an incident form.
- ✓ Do not open any doors with gloved hands. Ensure you remove your gloves (or one glove if transporting biological materials) prior to leaving the laboratory within which you are working.
- ✓ Do not pour solvents down the sink. Discard in the appropriate solvent waste containers provided. If you are unsure of which category your solvent falls under please ask.
- ✓ Please make sure you know the location of the following:
    - First aid kit (main corridor)
    - Fire extinguisher (one situated either in or just outside each laboratory)
    - Fire Blanket (Lab 303 and 307)
    - Eyewash station (Lab 307 and main corridor)
    - Spillage Kit (Spillage granules and sodium bicarbonate - one set located in each laboratory)

4. Microbiology Waste Disposal and Cleaning

Appropriate waste disposal and cleaning is designed to separate different types of waste and minimise risks when handling hazardous products or by-products.
Please follow disposal instructions carefully and if in doubt ask your supervisor or the laboratory manager.

- ✓ Biological waste - Microbiological material in plastic containers (e.g., cultures on agar or liquid cultures in centrifuge tubes) and used gloves should be disposed of in the autoclave bags provided. Once autoclaved the bags are placed in the general waste.
- ✓ Bleach Pots – For contaminated tips, used slides, used cuvettes (containing microbiological cultures) and used glass / plastic Pasteur pipettes.
- ✓ Contaminated glass waste – Glass equipment (e.g., conical flasks, beakers) should be placed in a autoclave bucket and labelled with autoclaved tape.
- ✓ Glass waste - When any glassware is broken, please notify the laboratory manager or your supervisor immediately for assistance with disposal. Non-contaminated broken glass can be directly disposed of in the glass bin (one located in each lab). Contaminated broken glass will need to be treated.
- ✓ Sharps bins – These are for the disposal of sharps including scalpel or razor blades, disposable scalpels and needles. Always carry the sharps bin to the sharps and not the sharps to the sharps bin.

General Waste
There are general waste bins located in each laboratory for the disposal of non-contaminated waste such as paper towels, empty agar bottles etc.

Paper waste
There are a couple of paper waste bins for the disposal of paper, catalogues etc. for recycling.

Cleaning of General Work Areas
Prior to any experimental work and after the completion of experimental wipe down the surface of the work bench by spraying 100% IMS and then wiping away the excess using paper towels.

Spillages
Each lab should have a 'Spillage Kit' consisting of spillage granules (for alkaline liquids and solvents), sodium bicarbonate (for acid spillages). Personal protective clothing is also available including laboratory coats, face masks, goggles and gloves. Each spillage kit is checked and maintained by the laboratory manager. Please ensure that access to the spillage kit is free from obstruction at all times. If the kit is used, please notify the laboratory manager so that stocks can be replenished. In the event of a non-biohazard spillage cover the liquid with the appropriate spillage granule and leave to absorb. Sweep up and place contents in a sealable container and place in a fume hood.

Clean up procedure for small biohazard spills
Stop work and make safe the immediate work area within which the spillage has occurred
Wash hands if they have been contaminated with antimicrobial soap and water or remove and replace contaminated protective gloves and/or clothing, prior to clean up.
Put on a fresh pair of gloves.
Place absorbent material (e.g., blue paper towel) pre-wetted with Virkon, bleach or 100% IMS and allow to sit for a minimum of 10 minutes.
Mop up the spill and place the absorbent material in an autoclave bag.
Wipe down the area again with Virkon, bleach or 100% IMS, discard, remove gloves and wash hands.

In the event of a major biohazard or chemical spill, notify the laboratory manager and leave the area immediately and do not re-enter until clearance is given by the College Safety Officer.

AUTOCLAVE BAGS	BLEACH/VIRKON POTS	BUCKETS	GLASS BOXES	SHARPS BIN
These bags located around the lab are autoclaved to sterilise the contents prior to disposal	Bleach pots are located on work benches and contain a 10% Bleach solution	Contents are autoclaved before washing. Buckets are located in the autoclave room	Cardboard box for containment on broken glassware. One located in each lab.	Contents are sent for incineration. One or two located in each lab.
USED FOR: Paper Any disposable plastics: Petri dishes Agar plates Loops Microfuge tubes Gloves and facemasks Swabs	**USED FOR:** Microscope slides and coverslips Glass and plastic Pasteur pipettes Pipette tips Cuvettes	**USED FOR:** General glassware Test-tubes Boiling tubes Conical flasks Medical flats Bottles (Schott/Duran/etc.) Universals Re-useable plastics	**USED FOR:** General broken glassware For contaminated broken glass, spray with 100% IMS prior to disposal in glass waste box.	**USED FOR:** Razor blades Scalpels Scalpel blades Syringes and needles
NOT FOR: Sharps Glassware	**NOT FOR:** Sharps	**NOT FOR:** Sharps Microscope slides and coverslips Pasteur pipettes Non-re-useable plastics	**NOT FOR:** Unbroken glass items Sharps Any plastic-ware Paper Gloves	**NOT FOR:** Paper General plastics Pasteur pipettes Microscope slides and coverslips

5. Equipment Available for use within the Microbiology Unit

In this section, you will find some notes to remember on the various techniques and equipment you may use while you are working in this laboratory.

Lab coats – located in the room to the left of the main entrance.
Available in small, medium large and extra-large.
A sticker will be given to you to write you name on and stick to the coat you will be using.

Balance - Located in the Media prep area of the Microbiology unit.
Weighing is one of the most common tasks in the laboratory.
Check that the balance displays the correct SI unit that is required.
Check that the balance displays exactly zero at the start of each weighing.
Tare, if needed, to avoid zero errors.
Always place the weighing sample in the middle of the weighing pan. This will prevent corner load errors.
Keep the weighing chamber and weighing pan clean.
NOTE: Wash the spatula and weighing boat after use and place on the shelf above sink to dry.

pH meter – Located in the Media prep area of the Microbiology unit.
Remove the cap from the probe; place it in an upright position so that the storage solution doesn't spill out.
Next, rinse the probe in clean, running water to remove the storage solution (about 15 seconds).
Finally, wipe-off the excess water from the meter with a clean, dry paper towel, taking care not to touch the glass probe itself.
To begin, you have to calibrate the probe against a known set of values before it will yield accurate readings.
This is done by going through the probe's calibration protocols and using a set of calibrating solutions that have a known pH of 7.01 and 4.01, respectively.
In general, once you have entered the calibration mode, the meter will first ask you to place it in one of these solutions (usually the 7.01). Then, once it has locked this value in, it will ask you to place it in the other solution (the 4.01). When it has completed the cycle, it will then signal that it has been calibrated and it is now ready to be used.
Once you have successfully calibrated your meter, it should give you accurate results providing you follow some basic guidelines.
pH meters are very sensitive and you need to take care not to contaminate your samples, or you will get inaccurate results.
NOTE: When you are done, rinse and dry the probe, and replace the protective cap on the meter (making sure it is full of the storage solution).

Glasswasher – Located in the Media prep area of the Microbiology unit.
NOTE: Place **dirty, uncontaminated** glassware in the cleaning dishes provided. These will be washed and returned to you.

Water – Located in the Media prep area of the Microbiology unit.
We have two storage towers of 5-10 Ωm water, a 15 Ωm dispenser and an 18 Ωm (molecular grade water/HPLC) dispenser.

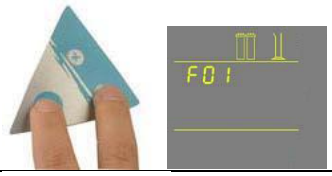
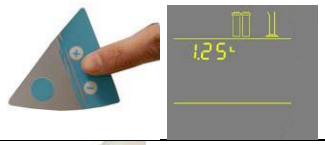
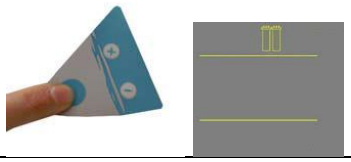
HOW TO GET WATER FROM THE SYNERGY

1. USING THE DISPENSING BUTTON

There are two ways to get water using the Dispensing Button:

Press once and release.  To stop dispensing water, press the Dispensing Button once again.	**OR**	Press and hold down.  To stop dispensing water, release the Dispensing Button.

2. HOW TO DISPENSE AN EXACT AMOUNT OF PRODUCT WATER (F01)

Press the Main and "-" buttons together to enter the menu. The Display will show F01.	
Press the "+" or "-" button to adjust the exact amount of product water (in Litres) needed. Pre-set volumes of water can be adjusted from 0.25 L to 9.75 L in 0.25 increments.	
Press the Dispensing Button once. The system will dispense water. The Display will show the amount of water dispensed and the Product resistivity. NOTE: To stop dispensing water, press the Dispensing Button once again.	
To exit the menu, press and hold the Main Button for 2 seconds. To display the next menu option, press the Main Button once.	

HOW TO GET WATER FROM THE ELIX

Manual water dispense

The mast and the arm supporting the E-POD® dispensers are designed to accommodate all commonly used glassware and the height can be adjusted by pressing the button at the back.
Water can be obtained by pressing the plunger of the E-POD® unit.

Automatic volumetric water dispense

Volumetric water dispensing is set on the base of the E-POD® unit. The user can adjust the volume to be delivered with the (+) and (-) keys, and then press the volumetric dispensing button to start delivery of the selected volume

Autoclaves - Located in the autoclave room within the Media prep area.
Instructions are stuck on the wall, but you will not operate these machines without supervision from the lab manager.
NOTE: Autoclaving of media, tips, glassware etc. can take up to four hours, so you need to plan in advance when you require these, so they can be autoclaved the day before.

Water baths - Located in the autoclave room.
Use distilled water from the storage tanks to fill the water bath.
For temperatures higher than 60^{o}C, please use the gloves provided when placing or removing items from the water.
Switch off after use.

Constant temperature rooms – There are two temperature control rooms, 20^{o}C and 25^{o}C.

Incubators – There are 3 Innova Incubators located in the 25^{o}C room.
These will be used only with the lab manager present.

Fume hood – Located in Lab 307.
Perform all work involving hazardous or volatile materials in a fume hood.
Check that the fume-hood is operating correctly before you start work. To check the air flow a strip of paper, tissue, or ribbon can be taped to fume-hood sash.
Do NOT use a fume hood as a storage area, they should contain only working volumes of chemicals.

Cat II hood – Located in Lab 303.
Microbiological Safety Cabinets are designed to protect users and the environment (which includes other people in the laboratory) from aerosol risks arising from the handling of hazardous biological material. Some types of cabinet are also designed to protect the materials being handled within them from environmental contamination. Air discharged from the exhaust of the cabinet is filtered to remove microbial contamination and is either ducted to outside or recirculated into the laboratory.

There are three types or "Class" of Microbiological Safety Cabinet which differ significantly in design and mode of operation. These are referred to as Class I, Class II and Class III cabinets. This lab has a class II cabinet.
CLASS II - Safety cabinet with a front aperture through which the operator can carry out manipulations inside the cabinet and which is constructed so that the worker is protected, the risk of product and cross contamination is low and the escape of airborne particulate contamination generated within the cabinet is controlled by means of an appropriate filtered internal airflow and filtration of the exhaust air.

Spectrophotometer - Located in Lab 303.
UV-visible spectroscopy is the measurement of the absorbance of light at a specific wavelength in a sample. This is used to identify the presence and concentration of molecular entities within the sample.
There are four main components of a spectrophotometer. These are a light source to emit a high and constant amount of energy over the full wavelength range; a method for separating the light into discreet wavelengths; a sample holder and a light detector.
NOTE: Machine should be blanked first before sample is read.

Centrifuges – There are several centrifuges located around the Microbiology Unit.

- ✓ **Beckman Avanti 26XP** - Located in room 312 which is kept locked. Samples can be centrifuged up to speeds of 25000 rpm (dependent on sample size and rotor used). You will not use this machine.
- ✓ **Rotina 38R Mettich Centrifuge** – Located in lab 303. Samples can be centrifuged up to speeds of 24000 rcf (dependent on sample size and rotor used).
- ✓ **Chilled microfuge** – Located in 303. Training is required before you are allowed to use this centrifuge.

PCR machine - Located in lab 303 and 310.
WARNING: UV light can be harmful to unprotected eyes and skin. Make sure of the following before using the UV light:

- ✓ Glass screen is down in resting position (glass blocks UV transmission).
- ✓ Door closure(s) are in place

Gel electrophoresis - Located in lab 303.

1. Dissolve the agar, cool the solution, and pour the gel.
✓ When the agar has solidified, carefully remove the comb.
2. Load samples in the wells in the gel.
✓ Make a written record of which sample you will load in each well of the gel.
✓ Place the gel form on a black or dark surface to help you see the wells in the agar.
✓ You may find it helpful to only load samples in every other well.
✓ Be careful to not puncture the bottoms of the wells
3. Place the gel in the electrophoresis chamber with the wells closest to the negative (black) electrode.
4. Prepare the salt solution and add it to the chamber.
✓ Add salt to tap water and swirl it to dissolve.
5. Place the lid on the chamber and connect the electrode leads to the power supply.
6. Connect the black lead to the negative terminal and the red lead to the positive terminal.
7. Turn on the power supply and adjust the voltage to 50-100 volts.
8. Run the gel for set time.
9. Turn off the power supply, disconnect the electrode leads, and remove the chamber lid.
10. Remove the gel from the electrophoresis chamber.
11. Read the gel – see gel doc system
12. Discard the gel in the bin provided.
13. Rinse the electrophoresis chamber and gel form with tap water; turn them upside down to dry.

Gel doc system - Located in lab 303.
Using gloves, spray a bit of IMS and wipe down the surface.
Place the gel inside and close the door.
Use the PC to view the gel.
Save changes
Discard the gel in the appropriate bin.
Clean the surface with IMS.
Connected to PC and uses GeneSnap to capture images.

Other Equipment
Microtitre plate reader - Located in lab 303. Uses Mantra to save results.
Freeze-dryer – Located in lab 303.
Ice machine – Located in the Microbiology corridor opposite the entrance to lab 307.
Microwave – Located in lab 303
-80^{o}C freezers - Located in the Microbiology corridor. There are two -80^{o}C freezers available for use
Gas –available in lab 307. Turn key to activate, wait for system to initialise, then gas will be available.

Correct pipetting techniques

Micropipettes are instruments used to accurately transfer small volumes (1 µl to 1 ml) of solution. Because of their accuracy, ease of use, and convenience in sterile techniques they are a practically universal lab tool. If you look at the dot on the plunger of each micropipette you will see a number that represents the maximum volume, in microliters (µl) that can be transferred by that micropipette.
The minimum volume appropriate for each micropipette is typically ten percent of the maximum.
The dot on the plunger is also color-coded and generally matches the colour of the disposable tips used with that micropipette.

The table below shows the volume range, expected accuracy, and the appropriate tips for the micropipettes that you will be using

Micropipette	Volume Range	Accuracy	Tips
P1000	100-1000 µl	+/- 10 µl	Blue
P200	20-200 µl	+/- 1 µl	Yellow (white)
P20	1-20 µl	+/- 0.5 µl	White

To transfer solution using a micropipette:

1. Set the volume setting to the desired volume by rotating the volume adjustment knob. Note: the digital volume setting should never be adjusted above the maximum volume specified for a particular micropipette. Remember that the maximum volume is the largest volume shown on the plunger.
2. Seat a disposable tip on the micropipette by firmly placing the end of the barrel into a tip.
3. Depress the plunger to the **FIRST STOP** and immerse the end of the tip into the solution to be transferred. **SLOWLY** release the pressure of your thumb on the plunger to **SMOOTHLY** draw the solution up into the tip.
4. To expel the solution, put the tip into the next tube (or just above a sheet of paper). Then depress the plunger **ALL THE WAY TO THE SECOND STOP**.
5. If expelling into a liquid solution, **REMOVE** the end of the tip from the solution **BEFORE** releasing the pressure on the plunger with your thumb.
6. You should generally use a new tip for each transfer
7. When you wish to eject the tip you are using, place the tip over the appropriate waste container (small plastic beaker) and press the tip ejector button. If the tip is difficult to eject it is likely that you are jamming the tips onto the micropipette harder than necessary.

Your training session is now over and hopefully you will put into practise what you have learnt today.

6. Microbiology Unit Staff and Useful Contacts

Dr Jane Nicklin
Head of Microbiology Unit, College Biological Safety Officer
Room 304
Tel: 02076316232
Email: j.nicklin@bbk.ac.uk

Bilkis Kazi
Microbiology Laboratory Manager
Room 350
Tel: 02076316237 (office) or 02076316350 (lab)
Email – b.kazi@bbk.ac.uk

Department of Biological Sciences
Microbiology Research Unit: Fire instructions for Students and Visitors

Our fire alarms are tested between 08.00 and 08.40 on week-days.
Alarm tests involve intermittent bursts of sound of only a few seconds duration.

The main fire alarm is a *continuous* ringing bell or *continuous* siren in all Birkbeck buildings.
When a *continuous* alarm sounds you must leave the building immediately

There will be no other warning messages.

If you hear a continuous fire alarm:

1. Leave the building immediately by the nearest exit. Do not delay to collect your belongings.
2. Do not use the lifts or the phone.
3. Follow the instructions of your tutor, course leader and/or fire marshals.
4. Report the location of anyone unable to evacuate due to a disability to the Duty Attendant or the Fire Brigade.
5. Move well away (100 metres) from the exits once outside.
6. Do not stand in the road/street.
7. Do not re-enter the building unless told it is safe to do so

If you discover a fire:

1. Operate the nearest fire alarm (red "break-glass" boxes on walls)
2. The Duty Attendant at Malet Street will be automatically be contacted in every case and will immediately call the Fire Brigade.
3. Do not try to fight a fire unless you have been trained to use fire extinguishers.
4. Leave the building by the nearest exit

Explore the College. Get to know all the fire exit routes available to you.
In the event of a fire you may need to use more than one.

Printed from: http://www.bbk.ac.uk/so/guidance/fireinfo/STUDENTFIRE
Date printed: 16/06/2015

Once you have completed your induction tour/ equipment training and have read the fire safety instructions, please fill in the visitor registration form and hand it in to your supervisor who will sign it, and then once completed, hand it to the laboratory manager.

Visitor Registration Form

Please hand in completed forms to the Laboratory Manager

About you:		
Title:	First (given) name:	Surname (family name):
Gender:	Date of birth:	Nationality:
Mobile number:		E-mail address
Home address: Postcode:		
London address (if different): Postcode:		
Home Institution:		

You and the Department:	
Status: Birkbeck Student: Undergraduate ☐ MSc ☐ DPhil ☐ / Academic visitor/sponsored researcher ☐ / Employee ☐ / Visiting student ☐ / Other ☐ Specify:.....	
Supervisor:	
Start date:	End date:
Lab phone no:	Visitor card number:

Emergency contact:	
Next of kin surname (family name):	Next of kin first (given) name:
Relationship to you:	Next of kin phone number:
Next of kin address:	

Health & Safety*: - to be completed by supervisor	
COSHH form signed: ☐	Fire instructions and all relevant safety information issued: ☐
Signature of visitor:	Signature of supervisor:
Date:	Date: