Biosciences

Risk Assessment for General Bacteriological Methods at CL2

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Nature of revision:	Addition of individual metal chlorides within Section 4, and review of associated hazard information.				

RISK ASSESSMENT FOR GENERAL BACTERIOLOGICAL METHODS AT CL2

1. RESPONSIBILITIES

- Academic supervisors are responsible for authorising this procedure and ensuring that this procedure is implemented and complied with.
- Staff working with hazardous chemicals are responsible for ensuring that they read and understand the risk assessment and for complying with any duties or control measures.

2. RELATED DOCUMENTATION & OTHER PROCEDURES

a) CLES Microbiological Safety Policy

b) Operating Procedure and Workplace Risk Assessment for Containment Level 3 Laboratory, Geoffrey Pope Building, University of Exeter

c) Other risk assessments which detail the specific hazards associated with handling specific microorganisms.

3. PROCESS DESCRIPTIONS

3.1 BACTERIAL GROWTH & BACTERIAL INACTIVATION.

3.1.1 Autoclaving of contaminated waste

All waste containing biological material will be autoclaved using a destruct cycle at 130°C for at least 25 min, except where it contains formaldehyde or organic solvents. Records of the load will be kept, detailing the nature of the load and chart records of the temperature profile. The autoclave will be serviced and validated annually using thermocouples

3.1.2 Inactivation of bacteria with 10% Chloros (max volume 100ml).

10% Chloros is used in waste (discard) pots. Inactivation of microorganisms requires exposure to Chloros solution for at least 1 hour.

Hazards

- Handling of bacteria – see microorganism-specific risk assessments
- Chloros - See part 4

Safe Disposal

- Please refer to section 8 “Safe Disposal”.

3.1.3 Inactivation of bacteria with formaldehyde (max volume 1ml of formaldehyde).

Small volumes of formaldehyde might be added to microorganisms or proteins to inactivate them. Formaldehyde is also used in silver staining kits and for the fumigation purposes.

Hazards

- Handling of bacteria – see microorganism-specific risk assessments
- Formaldehyde - see part 4

Safe Disposal

- Please refer to section 8 “Safe Disposal”.

3.1.4 Preparation of bacterial culture media.

This method is a general guide to making bacterial plates and agarose gels. Selective plates may include the addition of other reagents not considered here.

1. Agar can be prepared freshly using an autoclave. Before using the autoclave, be sure you know how to operate it. If not, please consult an appropriate member of staff who is trained to use the autoclave.
2. Place bottles containing solid agar in a microwave, making sure that the caps are loose. To prevent any risk of the contents overflowing, the volume of materials should not exceed 80% the volume of the bottle (i.e. 400 mL in 500 mL Duran bottle).
3. Use the microwave on a low power setting.
4. Approximately halfway through, pause microwave, remove the bottles of agar and mix the contents by gently swirling bottle. Replace bottles and continue microwaving. To prevent injury, the user should always wear thick gloves when handling any hot contents from the microwave.

5. If the agar is not completely melted, continue heating and mixing for short periods. A cooling period of at least 5 min should be allowed before removing from microwave.
6. Allow melted agar to cool to approximately 50°C prior to the addition of any supplements (e.g. antibiotics, IPTG, X-Gal). To prepare the plates, aseptically pipette 12-20ml aliquots of the agar into petri dishes located on a flat surface. To minimise contamination risks, agar plates should be prepared in a class1 fumehood or near a Bunsen flame.
7. Once set (typically after 30 min) the agar plates can be inverted and stored at 4°C.

Hazards

- Agar
- Microwaving of agar and injury from hot contents
- Electrical hazard
- Supplements (e.g. antibiotics, IPTG and X-gal; See part 4)

Safe Disposal

- Refer to section 3.1.1, "Autoclaving of contaminated waste".

3.2 METHODS FOR ANALYSIS OF DNA & RNA.

3.2.1 Isolation and precipitation of genomic DNA

Phenol should not be used for any DNA isolation procedures as it cannot be autoclaved and is difficult to treat with an approved and compatible biocide.

DNA can be isolated using a commercially available kit which does not use phenol, for example, the Promega Wizard Genomic DNA Purification kit, which contains no hazardous agents in its pre-made solutions. Refer to the manufacturer's instructions before carrying out the procedure, which is outlined here:

1. Centrifuge 1 ml of overnight culture at 13,000 rpm for 2 mins, discard supernatant
2. Add 600 µl of Nuclei Lysis solution and pipette gently to mix. Incubate at 80ºC for 5 minutes, then cool to room temperature. Add 3 µl of RNase A solution, mix by inversion, then incubate at 37ºC for 15-60 mins. Cool to room temperature.
3. Add 200 µl of Protein Precipitation solution. Vortex vigorously for 20 s. Incubate on ice for 5 mins before centrifuging at 13,000 rpm for 3 mins.
4. Remove supernatant to a fresh tube containing 600 µl of room temperature isopropanol and mix by inversion until DNA strands are visible.
5. Centrifuge at 13,000 rpm for 3 mins and discard supernatant.
6. Wash pellet with 600 µl room temperature 70% ethanol. Re-centrifuge for 2 mins before draining off ethanol thoroughly. Air-dry pellet for 10-15 mins
7. Rehydrate in DNA rehydration solution, either at 65ºC for 1 hr or by leaving at 4ºC overnight.

If necessary, genomic DNA can be precipitated by adding 1/10 volume of 3M sodium acetate and 2 volumes of ice-cold absolute ethanol to the DNA solution, mixing and placing at -20° for at least 15 mins. DNA can then be pelleted, washed and rehydrated by following steps 5-7 above.

Hazards

- Handling of bacteria – see microorganism-specific risk assessments
- Centrifugation – see part 4
- Chemicals: Ethanol, isopropanol, sodium acetate. See part 4

3.2.2 Isolation of plasmid DNA

Kits are available for the small scale purification of plasmids and should be used according to the manufacturer's instructions. Brief details are given below for the Thermo Scientific GeneJet kit.

1. Centrifuge overnight culture of bacteria at 8,000 rpm for 2 mins, discard supernatant
2. Resuspend cells in 250 µl Resuspension Solution and mix thoroughly
3. Add 250 µl of Lysis Solution and mix by inversion
4. Add 350 µl of Neutralization Solution and mix immediately by inversion
5. Centrifuge at 13,000 rpm for 5 mins
6. Transfer supernatant to GeneJet spin column and centrifuge at 13,000 rpm for 1 min. Discard flow-through and replace column in collection tube
7. Wash column with 500 µl of Wash Solution. Centrifuge for 30-60 s and discard flow-through. Repeat wash step.
8. Transfer spin column to fresh microcentrifuge tube and add 50 µl Elution Buffer to the centre of the membrane. Incubate at room temp. for 2 mins, then centrifuge for 2 mins

If necessary, plasmid DNA can be precipitated by adding 1/10 volume of 3M sodium acetate and 2 volumes of ice-cold absolute ethanol to the DNA solution, mixing and placing at -20° for at least 15 mins. DNA can then be pelleted, washed and rehydrated by following steps 5-7 of the procedure outlined in section 3.2.1

Hazards

- Handling of bacteria – see microorganism-specific risk assessments
- Centrifugation – see part 4
- Chemicals; Ethanol, sodium dodecyl sulfate, sodium hydroxide, guanidine hydrochloride, sodium acetate. See part 4

3.2.3 Electrophoresis of DNA

Prepare agarose (usually 0.8-1.2 %) in Tris-acetate, EDTA (TAE buffer) in a bottle which will withstand microwaving (e.g. a Duran bottle) or in a conical flask. Heat in the microwave, mix regularly by gentle swirling and repeat until all the agarose has dissolved (see local rules covering the use of the microwave).

Allow agarose to cool to 50 °C before adding Midori Green nucleic acid stain to a final concentration of 0.5 µg/ml. Pour gel, place in gel tank, flood tank with TAE buffer. Add DNA in loading dye (typically ThermoScientific 6x LD; bromophenol blue & xylene cyanol FF) and electrophorese at 10-100V. Length of time will depend on voltage selected, but typically 30-40 minutes. Visualise gel using a UV transilluminator.

Electrophoresis of gels is carried out by attaching a gel tank to a suitable power pack. All connections should be made and checked before the powerpack is switched on. Only well maintained connectors of the correct type should be used. The connections and cables should be kept dry.

Hazards

- Chemicals; TAE buffer, Midori Green, bromophenol blue & xylene cyanol FF - see part 4
- UV light, - see part 4
- Electrical hazards- see part 4
- Microwaving of agarose – see part 4

3.2.4 PCR amplification of DNA

The polymerase chain reaction (PCR) is a method for amplifying DNA and RNA using an enzymatic process driven by thermal cycling of a reaction cocktail. PCR reactions are optimised from a set of core reagents. This set of core reagents includes buffers, nucleic acids, enzymes, adjuncts, intercalating dyes, and fluorescent dyes. The buffers are Tris based and vary in pH (7.5-9.0), $MgCl_2$ concentration, and other salts such as KCl.

A mastermix is made up using these reagents and dispensed into PCR tubes, into which the template DNA is added. The amplification reaction is then carried out in a thermal cycler on a program specific to the primers being used for amplification.

PCR may be carried out in parallel, in which case the products can be pooled by ethanol precipitation according to protocols outlined above (Section 3.2.1)

Hazards

- Tris buffer - see part 4
- Electrical hazards - see part 4
- PCR cyclers- see part 4

3.3 METHODS FOR ANALYSIS OF PROTEINS & POLYSACCHARIDES.

3.3.1 SDS-PAGE of proteins and polysaccharides

Protein extracts for analysis are often generated by incubating bacterial cells or purified proteins in the presence of SDS-PAGE buffer at 100°C (in a water bath or heating block). A maximum of 100µl of sample should be contained within an eppendorf tube. Heating should be carried out with lids closed, in a fume cupboard. Samples must be cooled on ice before analysis by electrophoresis

Analytical separation of proteins and polysaccharides is carried out by polyacrylamide gel electrophoresis (PAGE) with or without the strongly anionic detergent sodium dodecyl sulphate (SDS). PAGE uses a stacking gel to group the sample into a thin band which is then applied to the separating gel. PAGE separates proteins on the basis of their size by sieving through the polyacrylamide gel pores. The size of the pores is dependent on the concentration of acrylamide and amount of cross-linking (formed by bisacrylamide) in the gel.

Samples will be mixed with sample buffer (2X Sample buffer Tris-HCl pH 6.8, 0.0625M, 10% Glycerol, 0.01% bromophenol blue, 3% SDS (if required), 5% 2-mercaptoethanol) and boiled for 1-5 minutes in a boiling water bath.

Polyacrylamide gels can be purchased pre-cast or made in the laboratory (for the latter, refer to Section C-2). The use of pre-cast gels reduces the hazard of contact with acrylamide. For ready-made gels read the manufacturer's instructions for setting up and running.

Electrophoresis of gels is carried out by attaching a gel tank to a suitable power pack and running until the dye front has reached the bottom of the gels. The Phast system is self-contained and will only apply a current to the gel when the lid is closed. As long as the apparatus is well maintained and used in accordance with the manufacturer's directions, it should not pose a hazard. With the Bio-Rad gel system, all connections should be made and checked before the powerpack is switched on. Only well maintained connectors of the correct type should be used. The connections and cables should be kept dry.

Hazards

- Handling of bacteria – see microorganism-specific risk assessments

- Chemicals: Tris buffer, sodium dodecyl sulfate, bromophenol blue, acrylamide, bisacrylamide, 2-mercaptoethanol, ammonium persulphate, TEMED, formaldehyde, citric acid, sodium hydroxide, silver nitrate, ammonium hydroxide, methanol, acetic acid - See part 4
- Electrical hazards - See part 4

3.3.2 Preparation of laboratory-made PAGE Gels

Use electrophoresis grade acrylamide which should not contain yellow crystals.

Make a 29% acrylamide, 1% N,N-bis-methylene acrylamide solution in warm water. pH should be less than 7.0. Filter under gravity and store in foil wrapped bottles at 4°C. TOXIC !! Alternatively, this may be made by diluting a purchased 40% solution to 30 % with water.

Buffers used are Tris-HCl, 1.5M, pH 8.8 with 0.4% SDS added before pH adjustment and Tris-HCl, 0.5M, pH 6.8 with 0.4% SDS added before pH adjustment (if required). Ammonium persulphate - 10% solution in dH_2O and TEMED are used to catalyse gel polymerisation but typically <50 µl volumes of these materials are used and can be added to the acrylamide and bis-methylene acrylamide mixture on the bench.

For details on electrophoresis, refer to Section 3.3.1.

3.3.3 Staining and destaining (Coomassie Blue or Silver Stain)

After electrophoresis, gels can be fixed and stained with Coomassie Brilliant Blue or silver salts.

Coomassie Brilliant Blue:
Gels can be stained in a solution of 0.1% Coomassie Blue R-250 in 50% (v/v) methanol and 10% acetic acid. Gels are destained in 40% (v/v) methanol and 10% acetic acid and can be stored in 5% acetic acid + 10% glycerol. Small gels (e.g. Phast gels) can be stained on the bench but staining and destaining of larger gels where more than 10ml of staining and destaining solution are used should take place in a fume hood or in a microbiological safety cabinet vented to atmosphere.

Silver staining, Method 1:
This method requires gels to be fixed in 40% (v/v) methanol, 5% (v/v) acetic acid followed by oxidation with 0.7% (w/v) periodic acid. Gels are stained with silver staining solution (2 ml of ammonium hydroxide [specific gravity 0.88] added to 28ml of 0.1M sodium hydroxide with stirring and 1g of silver nitrate dissolved in 5ml of distilled water added dropwise while stirring) washed and developed with 0.05% (v/v) formalin and 0.005% (w/v) citric acid in distilled water. Development is stopped with 5 % (w/v) acetic acid.

Silver staining, Method 2:
This method employs the BioRad kit, which uses silver nitrate rather than ammoniacal silver solutions (the latter can form potentially explosive by-products). Gels are fixed in 40% (v/v) methanol, 5% (v/v) acetic acid and oxidised with potassium dichromate and nitric acid. After incubation with Silver Reagent (contains silver nitrate) the gels are developed with a solution that contains sodium carbonate and paraformaldehyde and development is stopped with 5 % (w/v) acetic acid.

Hazards

- Chemicals; Coomassie Brilliant Blue, methanol, acetic acid, periodic acid, sodium hydroxide, silver nitrate, ammonium hydroxide, citric acid, formalin, potassium dichromate, nitric acid, Bio Rad kit silver staining kit - See part 4

3.3.4 In-Gel Digestion of Peptides Prior to Mass Spectrometry Analysis

Low amounts of proteins will be digested using commercially available enzymes after having been separated using polyacrylamide gel electrophoresis (PAGE).

The enzymes that will be used to carry out the digestion are trypsin, pepsin endoproteinase Lys-C, endoproteinase Arg-C, and endoproteinase Glu-C. These enzymes are commercially available as lyophilised powders.

The digestion will be carried out in ammonium bicarbonate at concentrations between 1 and 100 mM. Calcium chloride may or may not be added to these buffers to prevent autolysis. Amounts of formic or acetic or trifluoroacetic acid between 0.1 to 10% may also be used in the digestion and extraction stages.

There are many procedures for in gel digestion. A generalised method is shown below but other protocols exist. These changes would involve different amounts and/or concentrations of the reagents listed, and different incubation times. The order of events may also be changed and an increased number of steps may be added, e.g. extra washing stages. The hazards remain the same, and all volumes of liquid remain less than 100 μl.

1. Protein spots can be excised from gels with pipette tips cut to the desired diameter (e.g. 2mm). After transfer of the gel pieces to a 1.5 ml siliconised micro-centrifuge tubes, or 96-well plates (for multiple samples) a small quantity of acetonitrile will be added to cover the gel pieces. After approximately 20 min the acetonitrile will be removed and the tube transferred to a vacuum concentrator where the gel pieces will be dried. If no vacuum concentrator is available, gel pieces may be dried by aspiration of liquid using pipette tips. Pipette aspiration is also the case for samples in 96-well plates.
2. Dried gel pieces will be swollen in a buffer containing enzyme (~12ng/μl) and 50 mM ammonium bicarbonate (pH ~8.0) at 4 °C for 45 min.
3. The sealed tube will incubated at 37 °C for up to 24 hours in duration. 96-well plates are placed in an incubator at 37°C overnight. The tube will be centrifuged and supernatant collected. For 96-well plates, extraction of liquids is via aspiration of the liquid from the well. Further extraction will be carried out by one change of 20 mM ammonium bicarbonate and three changes of 5% formic acid in 50% acetonitrile, allowing 30 min between changes.
4. Extracts will then be combined and the volume reduced using a vacuum concentrator, where available. The sample will then be ready for storage at –20 °C until analysis by ESI-MS or MALDI-MS. Alternatively, extracted peptides can be mixed immediately with MALDI-TOF MS matrix solution (refer to MALDI SWOP). MALDI-TOF matrix is a solution containing Alpha-Cyano-4-hydroxycinnamic acid.
5. If reduction and alkylation of the substrate is required the following will be carried out after step 1: Following drying the gel pieces will be covered with a 10 mM solution of dithiothreitol (DTT) in 100 mM ammonium bicarbonate and incubated at 56 °C for 1h.
6. After cooling to room temperature the DTT solution will be removed and replaced by an equal volume of 55 mM iodoacetamide in 100 mM ammonium bicarbonate. Incubation will be carried out for 45 min in the dark at room temperature.
7. Gel pieces will be washed with 100 μl aliquots of 100 mM ammonium bicarbonate for 10 min. Acetonitrile will be used to dehydrate the gel pieces before swelling them once more in 100 mM ammonium bicarbonate and dehydrating them again with acetonitrile.
8. Any liquid phase will be removed and the gel pieces dried completely in a vacuum concentrator.
9. Steps 3-6 will then be carried out to digest the now reduced and alkylated substrate.

Additional steps may be required to destain protein bands or spots prior to performing the rest of the procedure. Coomassie stained gels can be destained with several changes of ammonium bicarbonate solution or ammonium bicarbonate:acetonitrile solution. Silver stained gels can be destained with potassium ferricyanide:sodium thiosulphate solution (30 mM: 100 mM).

Hazards.

- Chemicals: Ammonium bicarbonate, Acetonitrile, Dithiothreitol, Iodoacetamide, Potassium ferricyanide, Sodium thiosulphate; See part 4.

3.3.5 Protein assays

Assays typically used are detailed below, Bovine serum albumin is usually used as a protein standard (2mg/ml and less).

The Bradford protein assay. Commercially available from BioRad. Measures binding of Coomassie blue dye to proteins.

UV absorption. Can be measured against a control sample. An approximate correction when nucleic acid is present is as follows:
Protein Concentration (mg/ml) = $1.5 \times A_{280} - 0.75 \times A_{260}$
This assay assumes average proportions of hydrophobic (tyr, phe & tryp) amino acids.

Bicinchoninic acid protein quantitation. Commercially available from Perbio. Contains:
Reagent A (1% w/v BCA-N_2, 2% w/v sodium carbonate, 0.16% w/v sodium tartrate, 0.4% w/v sodium hydroxide and 0.95 % sodium bicarbonate) & Reagent B (4% w/v copper sulphate).

Hazards
Chemicals: Coomassie blue, Bicinchoninic acid, sodium carbonate, sodium tartrate, sodium hydroxide, sodium bicarbonate, copper sulphate, bovine serum albumin. – Part 4

3.3.6 TLC analysis of glycolipids

1. Grow seed cultures in brain heart infusion broth for 14 hours, dilute 1:10 in saline and use 10ml to seed Roux bottles containing brain heart infusion agar. Incubate at 37°C for 24 hours.
2. Harvest cells with glass beads and pellet (5,000 x g, 10 minutes) and wash twice with saline. All glass beads will be decontaminated in 10% Chloros.
3. Extract glycolipids from the cell pellet with chloroform / methanol (1:2, 2:1 and 1:4 v/v respectively and pool the extracts.
4. Remove solvent with rotary evaporator and dissolve the extractable lipids in chloroform/methanol (1:1, v/v) at a concentration of 5mg/ml. Separate lipids present using thin-layer chromatography (TLC) on silica gel G-plates using chloroform / methanol / acetic acid (65:25:10, v/v) as solvent.
5. For analytical purposes separated lipids can be visualised by spraying with p-anisaldehyde / sulfuric acid / ethanol, (5:5:90, v/v/v) in a chemical fume hood and subsequently heating at 110°C for 10 minutes. For preparative purposes, lipids can be temporarily visualised and then marked using iodine vapour in a sealable container (again in fume hood).
6. Identified lipids can be scraped off, purified through a small column of C-200 silica gel and eluted with chloroform / methanol (1:4, v/v). This latter process is repeated until a single spot is apparent by TLC.

Hazards

- Handling of bacteria – see microorganism-specific risk assessments
- Chemicals: chloroform, methanol, acetic acid, p-anisaldehyde, sulfuric acid - see part 4

3.3.7 Tricholoroacetic acid (TCA) precipitation of proteins

1. Prepare 100% (w/v) Trichloroacetic acid (TCA) solution. recipe: dissolve 500g TCA (as shipped) into 350 ml dH2O, store at RT.
2. Grow bacteria in a suitable liquid growth medium for as long as required
3. Harvest the cell free supernatant by centrifugation
4. **In a chemical fume cupboard** add 1 volume of TCA stock to 4 volumes of protein sample (i.e. in 1.5ml tube with maximum vol., add 250µl TCA to 1.0ml sample.)
5. Incubate 10 min on ice / at 4°C.
6. Centrifuge at 14,000 x g, 5 min.
7. **In a chemical fume cupboard** remove supernatant to a suitable acid waste bottle, leaving protein pellet intact. Pellet should be formed from whitish, fluffy ppt.
8. Wash pellet with 200µl cold acetone (for 1ml of starting culture supernate).
9. Centrifuge at 14,000 x g, 5 min.
10. **In a chemical fume cupboard** remove the acetone to a suitable solvent waste bottle
11. Repeat steps for a total of 2 acetone washes.
12. **In a chemical fume cupboard** dry the pellet by placing tube in 95°C heat block for 5-10 min to drive off acetone.

Hazards

- Handling of bacteria – see microorganism-specific risk assessments
- Chemicals: TCA, acetone - see part 4

4. HAZARDS, RISKS AND CONTROL MEASURES

Risk Rating	Action Required
Minimal	Controls Adequate (Unlikely harm would ever result from the activity)
Low	Review Controls, take action as necessary. (Harm would seldom result)
Medium	Action to be taken to reduce risk. (It is reasonably possible that harm could result)
High	Urgent action required. Consider halting activity/process. (It is certain or almost certain that could result)

	Acetic acid
Hazard	Concentrated (glacial) acetic acid may be toxic by inhalation or ingestion. May cause skin burns and sever damage to eyes. Individuals with pre-existing skin disorders or impaired respiratory function may be more susceptible to the effects of concentrated acetic acid
Control measures	Lab coat and gloves will be worn when handling. Handle Concentrated (glacial) acetic acid in a fume cupboard. Dilute (e.g. <10%) solutions can be handled on the open bench but with care.
Risk	**Low for concentrated acid. Minimal for dilute solutions**

	Acetone
Hazard	Highly flammable. Irritating to eyes. Repeated exposure may cause skin dryness or cracking. Vapours may cause drowsiness and dizziness.
Control measures	Lab coat and gloves will be worn when handling. Must handle in a fume cupboard. Avoid proximity to naked flames
Risk	**Low when handled in a fume cupboard dilute solutions**

	Acetonitrile
Hazard	Highly flammable. Harmful if swallowed, in contact with skin or by inhalation. Irritating to eyes.
Control measures	Lab coat and gloves will be worn when handling. Avoid sources of ignition. In case of contact with eyes or skin, flush with copious amounts of water and seek medical advice.
Risk	**Low. Used in small dilute quantities.**

	Acrylamide
Hazard	Toxic. It is a possible human carcinogen, teratogen and may cause heritable damage. It is readily absorbed through the skin and inhalation maybe fatal. It is a skin, eye and respiratory irritant. It may cause CNS damage, the effects of which become apparent only after a delay of several months or years. MEL 0.3mg/m3
Control measures	Must be handled in a fume hood whilst wearing gloves and a lab coat. In case of contact flush eyes or skin with copious amounts of water and obtain medical attention. Polymerised acrylamide does not present the

	same hazard, but traces of unpolymerised acrylamide may be present in gels.
Risk	**Low if handled as indicated**

	N,N-bis-methylene acrylamide
Hazard	Irritant
Control measures	Should be handled whilst wearing gloves and a lab coat. In case of contact flush eyes or skin with copious amounts of water
Risk	**Minimal**

	Agar and agarose
Hazard	An irritant to mucous membranes
Control measures	Weighed out carefully to avoid dust generation.
Risk	**Minimal**

	Ammonium bicarbonate
Hazard	Harmful if swallowed.
Control measures	If swallowed, rinse mouth and seek medical advice.
Risk	**Low**

	Ammonium Hydroxide
Hazard	Corrosive - may cause burns. Harmful by ingestion, inhalation or absorption through the skin
Control measures	Should be handled wearing lab coat and gloves. In case of contact wash with copious amounts of water. Wear face mask if handling significant quantities.
Risk	**Minimal. Only small quantities are handled**

	Ammonium persulfate
Hazard	Destructive to tissue of the mucous membranes and the upper respiratory tract, eyes and skin. Inhalation may be fatal.
Control measures	Must be handled in a fume hood whilst wearing lab coat and gloves. In case of contact wash with copious amounts of water. It must be stored away from combustible material and is a strong oxidiser. Will be disposed via incineration.
Risk	**Minimal because of the small quantities handled**

	Antibiotics and other media supplements
Hazard	Ampicillin – may cause sensitisation by inhalation and skin contact. Do not breathe dust.Kanamycin – may cause harm to the unborn child. In the case of an accident or if feeling unwell, seek medical advice. Chloramphenicol – may cause cancer. Harmful if swallowed, in contact with skin and by inhalation. In the case of an accident or if feeling unwell seek medical advice. Do not breathe dust. Wear suitable protective clothing and gloves. Keep container in a cool, well ventilated place. Streptomycin – may cause harm to the unborn child. Harmful if swallowed. Do not breathe dust. Tetracycline – Harmful if swallowed, inhaled or absorbed through the skin. May cause irritation Antimicrobials derived from plants such as GOD-24, a trisulphide from *Allium* sp., WS-1 an acylphloroglucinol from *Hypericum* sp. and PJ-141-1 a

	quaternary pyridine alkaloid from *Prosopis* sp are not known to be hazardous. However, they should be handled as any other antibiotic i.e. wear gloves, do not breathe dust, avoid skin contact and do not swallow. IPTG – may cause cancer and heritable genetic damage. Harmful by inhalation, in contact with skin and if swallowed. Irritating to eyes, respiratory system and skin. In case of contact with eyes wash with copious amounts of water. Do not breathe dust X-GAL – harmful by inhalation and if swallowed. Irritating to the eyes. In case of contact with eyes, wash with copious amounts of water and seek medical advice.
Control measures	Chemicals should be carefully weighed to avoid the generation of airborne dusts. Gown and latex gloves are worn at all times. When weighing dusts wear eye protection to: BSEN 166.4. Where possible prepare concentrated stock solutions of antibiotics which can be stored frozen in 0.5ml aliquots
Risk	**Minimal. Only small quantities are handled**

	Bicinchoninic acid (BCA) protein quantitation reagent A
Hazard	The product contains no substances which at their given concentration, are considered to be hazardous to health.
Control measures	Skin contact. Rinse with plenty of water. Immediate medical attention is not required. Eye contact. Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Ingestion. Not expected to present a significant ingestion hazard under anticipated conditions of normal use. If you feel unwell, seek medical advice. Inhalation. Not expected to be an inhalation hazard under anticipated conditions of normal use of this material. Consult a physician if necessary. Notes to Physician Treat symptomatically.
Risk	**minimal**

	Bicinchoninic acid (BCA) protein quantitation reagent B
Hazard	The product contains no substances which at their given concentration, are considered to be hazardous to health.
Control measures	Skin contact. Rinse with plenty of water. Immediate medical attention is not required. Eye contact. Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Ingestion. Not expected to present a significant ingestion hazard under anticipated conditions of normal use. If you feel unwell, seek medical advice. Inhalation. Not expected to be an inhalation hazard under anticipated conditions of normal use of this material. Consult a physician if necessary. Notes to Physician Treat symptomatically.
Risk	**minimal**

	Bisphenol A (BPA)
Hazard	May cause sensitisation by skin contact. Possible risk of impaired fertility, Irritating to respiratory system. Risk of serious eye damage.
Control measures	Wear protective gloves/eye protection. In case of eye contact rinse immediately with water and seek medical advice. If swallowed seek medical advice.
Risk	**Low**

	Blood
Hazard	May contain pathogens. particular care should be taken with human blood, which may contain HIV and hepatitis viruses
Control measures	Handle wearing gown and gloves. Only work with human blood from a known source, which has been tested for HIV and hepatitis and is free of other pathogens.
Risk	**Minimal because of the small quantities handled**

	BioRad ReadyPrep Reagent 1, BioRad ReadyPrep Reagent 2, BioRad ReadyPrep Reagent 3
Hazard	Reagents 2 and 3 contain the following: Urea: Used in re-suspension buffers for extracted proteins. May be irritating to eyes, skin and may be harmful if ingested in quantity (LD50 oral 8.5g/kg, rat). Must not be autoclaved due to release of ammonia Thiourea: Used in re-suspension buffers for extracted proteins. Toxic. Possible risk of irreversible effects. Irritating to skin, may cause sensitisation by skin contact. Toxic to aquatic organisms. (LD_{50} 8.5g/kg oral, rat). Must not be autoclaved or added to chloros due to release of ammonia. CHAPS. Harmful if inhaled, swallowed or absorbed through the skin. May cause irritation to mucous membranes. May cause congenital malformation of the foetus. 3-(decyldimethylammonio)-propanesulfonate (SB) 3-10. Irritant to skin and mucous membranes on prolonged exposure. Toxic to aquatic species.
Control measures	Lab coat and nitrile gloves will be worn when handling
Risk	**Minimal. Only small quantities are handled**

	BioRad protein Assay kit
Hazard	This kit uses a range of hazardous chemicals including silver nitrate 40% (v/v) methanol, 5% (v/v) acetic acid, potassium dichromate, nitric acid, sodium carbonate and paraformaldehyde. For specific details of the hazards posed by individual chemicals see the chemical-specific risk assessment
Control measures	These materials are sufficiently dilute to be handled on the open bench if wearing Lab coat and gloves
Risk	**Minimal because of the dilute nature of materials**

	Boric Acid
Hazard	Readily absorbed through skin and is irritating to the eyes.
Control measures	Should be handled wearing lab coat, chemical resistant gloves and eye protection. In case of contact flush with copious amounts of water.
Risk	**Minimal.**

	Bovine serum albumin
Hazard	May cause skin and eye irritation
Control measures	In case of skin contact rinse with plenty of water. Immediate medical attention is not required. In case of eye contact rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do.
Risk	**Minimal**

	Bromophenol blue
Hazard	This may be harmful by inhalation, ingestion or skin absorption.

Control measures	Should be handled whilst wearing gloves and a lab coat. It is incompatible with strong oxidising agents. In case of contact wash out eyes with copious amounts of water and wash skin with soap and water
Risk	**Minimal. Only small quantities are handled**

	Butanol
Hazard	May be harmful by inhalation, ingestion or skin absorption. Causes eye, skin and mucous membrane irritation. Flammable.
Control measures	Should be handled whilst wearing gloves and a lab coat. In case of contact wash with copious amounts of water. It should be stored in a flammables cabinet. Anything other than trace amounts of waste butanol should be destroyed by incineration. Do not handle near a naked flame.
Risk	**Minimal, because of the small volumes used**

	Cadmium chloride
Hazard	Toxic if swallowed, fatal if inhaled. Very toxic to aquatic life.
Control measures	In case of contact remove any contaminated clothing and wash with copious amounts of water. Should be handled wearing lab coat and gloves. Take care when weighing out dry powders to avoid dust generation. If swallowed or inhaled, immediately contact doctor/physician. If swallowed, rinse mouth. Do not let product enter drains. Discharge to the environment must be avoided.
Risk	**Minimal (due to low volumes being handled)**

	Calcium chloride
Hazard	May be harmful by inhalation, ingestion or skin absorption. Incompatible with strong acids.
Control measures	Should be handled wearing lab coat and gloves. In case of contact wash with copious amounts of water.
Risk	**Minimal**

	Centrifugation and microcentrifugation
Hazard	Generation of aerosols of bacteria or toxic chemicals
Control measures	For centrifuging in a microcentrifuge, the microcentrifuge is kept inside the cabinet, and only sealable tubes are used. For larger scale centrifugations, use only tubes rotors which are sealable with O-ring seals and open rotors in safety cabinets
Risk	**Low**

	CHAPS
Hazard	Irritant. Harmful if swallowed or inhaled.
Control measures	Wear resistant gloves. The chemical is weighed out at ACDP level II and only a small amount, dissolved in a sealed container, is brought into the Cat Ill suite. Lab coat and nitrile gloves will be worn when handling
Risk	**The risk is assessed as low as small dilute samples are handled.**

	Chloroform
Hazard	Harmful by inhalation, skin absorption or ingestion. Possibility of serious damage from chronic exposure and should be substituted where possible. Animal carcinogen and suspect human carcinogen following long term exposure. Flammable. Solvent vapour may travel considerable distance to source of ignition and flash back.
Control measures	Keep container tightly closed Avoid contact with skin. Anything more than small quantities should be used in a fume cupboard. Non sparking equipment should be used. Compatible chemical resistant gloves should be worn. A face shield to BSEN 166 3 must be worn whenever there is a risk of splashing. All large volume toxic solvent work should all take place in a fume cupboard. Waste must be disposed of via the incinerator facility. Lab coat and nitrile gloves will be worn when handling. Chloroform must not be autoclaved and should be stored in a suitable waste container in a solvents cupboard for incineration. Keep away from sources of ignition.and do not handle near a naked flame.
Risk	**Minimal. The main risk arises from spills during decanting**

	Chloros
Hazard	500-1000mls at a concentration of 10% (v/v) will be used as a general disinfectant. Oxidising, corrosive, causes burns, contact with acids liberates toxic gases. Possible sensitiser. The risk is damage to skin or eyes caused by splashes of solutions.
Control measures	The solution will be handled by staff wearing marigold gloves, gown and a face shield. Because of the volumes involved dispense with extreme care. Chloros is diluted to a final volume of 10% before use. Materials inactivated with chloros, may be disposed of with copious tap water down the drain.
Risk	**Minimal. The main risk arises from spills during decanting**

	CIDEX® OPA
Hazard	50mls neat will be used as a high level disinfectant for the needle holder of the Zebrafish embryo microinjection equipment. Irritant to eyes and skin, may produce vapours. The risk is damage to skin or eyes caused by splashes of solutions and vapour production in an enclosed environment.
Control measures	The solution will be handled by staff wearing gloves and gown. Ensure adequate ventilation at all times. Materials inactivated with CIDEX® OPA, may be disposed of with copious tap water down the drain.
Risk	**Minimal. The main risk arises from spills during decanting**

	Citric Acid
Hazard	Harmful by ingestion, inhalation or absorption through the skin
Control measures	Lab coat and nitrile gloves will be worn when handling. Take care when weighing out dry powders to avoid dust generation. Wear face mask if handling significant quantities
Risk	**Minimal**

	Cobalt (II) chloride hexahydrate
Hazard	Harmful if swallowed. May cause allergic skin reaction, or breathing difficulties if inhaled. Very toxic to aquatic life.
Control measures	In case of contact remove any contaminated clothing and wash with copious amounts of water. Take care when weighing out dry powders to avoid dust generation. Should be handled wearing lab coat and gloves. If swallowed or inhaled, consult a doctor/physician. If swallowed, rinse mouth. Do not let product enter drains. Discharge to the environment must be avoided.
Risk	**Minimal (due to low volumes being handled)**

	Coomassie Blue
Hazard	Causes respiratory tract eye and skin irritation.
Control measures	Lab coat and nitrile gloves will be worn when handling. Take care when weighing out dry powders to avoid dust generation. Wear face mask if handling significant quantities.
Risk	**Minimal.**

	Copper (II) chloride
Hazard	Harmful if swallowed or in contact with skin. Causes skin irritation and eye damage. Very toxic to aquatic life.
Control measures	In case of contact remove any contaminated clothing and wash with copious amounts of water. Take care when weighing out dry powders to avoid dust generation. Should be handled wearing gloves and eye protection. In case of contact with eyes, rinse thoroughly with water for at least 15 minutes. Consult a physician. Discharge to the environment must be avoided.
Risk	**Minimal (due to low volumes being handled)**

	Dexamethasone
Hazard	Skin and eye irritant. May cause sensitisation by inhalation and skin contact.
Control measures	Wear protective gloves. In case of eye contact rinse eyes with plenty of water and seek medical advice.
Risk	**Low**

	Dimethyglutaric acid
Hazard	May cause irritation and is harmful if inhaled or swallowed
Control measures	In case of contamination wash eyes with copious amounts of water and skin with soap and water. Should be handled whilst wearing gloves and lab coat.
Risk	**Minimal.**

	Dithiothreitol (DTT)
Hazard	Toxic. Skin and eye irritant.
Control measures	Lab coat and gloves will be worn when handling. In case of contact with eyes or skin, flush with copious amounts of water.
Risk	**Low. Used in small quantities, and in dilution.**

	Dry ice
Hazard	Very cold and can cause burns.
Control measures	Only the minimum amount needed is used each time, and when handling dry ice, heat-resistant gloves MUST be worn. When the dry ice is in the cabinet, it is handled through gloves and gauntlets so there is never direct contact with skin.
Risk	**Minimal if gloves are worn**

	Egg yolk emulsion
Hazard	May contain *Salmonella* serotypes
Control measures	Handle with care wearing lab coat and gloves.
Risk	**Minimal.**

	Electricity
Hazard	Risk of electrocution
Control measures	Use only equipment with factory-fitted plugs. Inspect cables periodically for fraying. Do not lay cables across the floor. Periodically have equipment checked.
Risk	**Minimal. All equipment is checked for electrical safety periodically**

	Electrophoresis power packs
Hazard	electrocution
Control measures	The Phast system is self-contained and will only apply a current to the gel when the lid is closed. As long as the apparatus is well maintained and used in accordance with the manufacturer's directions, it should not pose a hazard. With the Bio-Rad gel system, all connections should be made and checked before the powerpack is switched on. Only well maintained connectors of the correct type should be used. The connections and cables should be kept dry.
Risk	**Minimal if used as described above**

	Ethanol
Hazard	Flammable and toxic by inhalation or if swallowed. Irritating to eyes and skin. Keep container tightly closed. Keep away from sources of ignition. Solvent vapour may travel considerable distance to source of ignition and flash back.
Control measures	Avoid contact with skin. Anything more than small quantities should be used in a fume cupboard. Non sparking equipment should be used. Compatible chemical resistant gloves should be worn. A face shield to BSEN 166 3 must be worn whenever there is a risk of splashing. All large volume toxic solvent work should all take place in a fume cupboard. Waste solvents must be disposed of via the incinerator facilty. Lab coat and nitrile gloves will be worn when handling. Organic solvents must not be autoclaved and should be stored in a suitable waste container in a solvents cupboard for incineration. Do not handle near a naked flame.
Risk	**Low.**

	Ethylene diamine tetraacetic acid (EDTA)
Hazard	may irritate the skin
Control measures	wear latex gloves and gown
Risk	**Minimal, only small quantities are handled**

	Ethylene glycol tetraacetic acid (EGTA)
Hazard	may irritate the skin
Control measures	wear latex gloves and gown
Risk	**Minimal, only small quantities are handled**

	Formaldehyde (including formalin)
Hazard	Formaldehyde (1ml) will be used to inactivate bacteria. Do not breathe vapour, get in eyes, on skin or on clothes. Change gloves after handling. Formaldehyde is toxic, may cause cancer and inheritable genetic defects. Toxic by inhalation and in contact with the skin. Causes burns. May cause sensitisation by inhalation and skin contact. Readily absorbed through the skin. Lachrymator. Formaldehyde is a listed chemical under EH40 (WEL 2ppm). Formalin 0.05% (v/v) is also used to develop silver stained gels
Control measures	Dispense with care. A face visor should be used during decanting and whenever there is a risk of splashing. Wear chemical resistant gloves for anything other than brief contact with formaldehyde containers. Fumigant will be vented to atmosphere at high level after fumigation is completed.
Risk	**Low. Only relatively small quantities (<1ml are handled). Formalin 0.05% (v/v) used to develop silver stained gels poses minimal risk because of the low concentration**

	Formic acid
Hazard	Formic acid at a final concentration of 0.1 % will be used is corrosive.
Control measures	Lab coat and nitrile gloves will be worn when handling
Risk	**Minimal. Only small volumes are handled**

	Guanidine hydrochloride
Hazard	May be toxic by ingestion, inhalation or skin absorption. They causes eye and skin irritation and are irritating to mucous membranes and upper respiratory tract.
Control measures	Lab coat and nitrile gloves will be worn when handling
Risk	**Low. The volumes used are small and pose minimal risk**

	Hydrochloric acid (HCl)
Hazard	May irritate the skin and mucosal surfaces,
Control measures	Dispense concentrated solutions of HCl in a fume cupboard. Dilute to working strength (1M) by adding acid to water with mixing. Wear latex gloves and gown but due to the small quantities used, minimal risks arise.
Risk	**Minimal. Working solutions will be 1M solutions**

	Iodoacetamide
Hazard	Toxic. May cause allergic reactions through contact or inhalation.
Control measures	Lab coat and gloves will be worn whilst handling. If swallowed, seek medical advice immediately.
Risk	**Low. Used in diluted form.**

	Isopropanol & isoamyl alchohol;
Hazard	Flammable and toxic by inhalation or if swallowed. Irritating to eyes and skin. Keep container tightly closed. Keep away from sources of ignition. Solvent vapour may travel considerable distance to source of ignition and flash back.
Control measures	Avoid contact with skin. Anything more than small quantities should be used in a fume cupboard. Non sparking equipment should be used. Compatible chemical resistant gloves should be worn. A face shield to BSEN 166 3 must be worn whenever there is a risk of splashing. All large volume toxic solvent work should all take place in a fume cupboard. Waste

	solvents must be disposed of via the incinerator facilty. Lab coat and nitrile gloves will be worn when handling. Organic solvents must not be autoclaved and should be stored in a suitable waste container in a solvents cupboard for incineration. Do not handle near a naked flame.
Risk	**Low.**

	Lysozyme
Hazard	Lysosyme: (5mls) May be harmful if inhaled, swallowed or absorbed through skin
Control measures	Wear resistant gloves. This chemical is weighed out at ACDP level II and only a small amount, dissolved in a sealed container, is brought into the Cat Ill suite. Lab coat and nitrile gloves will be worn when handling.
Risk	**Minimal. Only small quantities are handled**

	2-Mercaptoethanol
Hazard	Highly toxic and may be fatal if inhaled or absorbed through the skin. It is also a possible mutagen and a severe irritant.
Control measures	It must be handled in fume hood whilst wearing gloves and a lab coat. In case of contact immediately flush eyes or skin with copious amounts of water and seek medical advice.
Risk	**Low if handled in a fume hood**

	Methanol
Hazard	Flammable and toxic by inhalation or if swallowed. Irritating to eyes and skin. Keep container tightly closed. Keep away from sources of ignition. Solvent vapour may travel considerable distance to source of ignition and flash back.
Control measures	Avoid contact with skin. Anything more than small quantities should be used in a fume cupboard. Non sparking equipment should be used. Compatible chemical resistant gloves should be worn. A face shield to BSEN 166 3 must be worn whenever there is a risk of splashing. All large volume toxic solvent work should all take place in a fume cupboard. Waste solvents must be disposed of via the incinerator facilty. Lab coat and nitrile gloves will be worn when handling. Organic solvents must not be autoclaved and should be stored in a suitable waste container in a solvents cupboard for incineration. Do not handle near a naked flame.
Risk	**Low.**

	Microwaving of agar or agarose
Hazard	The main risk is for boiling during mixing of microwaved agar or agarose which may escape from the bottle potentially causing burns.
Control measures	Protective clothing, consisting of heat resistant gloves (BSEN 407) and a face shield (BSEN 166.3), should be worn. The setting used on the microwave should be as low as possible. Agar or agarose which has been microwaved should be allowed to “rest” for at least 1 minute before removal and mixing
Risk	**Low**

	Midori Green stain
Hazard	May be irritating to mucous membranes and upper respiratory tract. May be harmful if inhaled. May be harmful if swallowed.
Control measures	Lab coat and nitrile gloves will be worn when handling.
Risk	**Low. Only small volumes are handled.**

	Nitric acid
Hazard	Oxidising agent, keep away from reducing agents. Corrosive. Keep away from sources of ignition. Harmful by inhalation or skin contact.
Control measures	Lab coat, gloves and eye/face protection will be worn when handling. In case of contact immediately flush eyes or skin with copious amounts of water and seek medical advice.
Risk	**Low. Only small volumes are handled, and diluted for the procedure.**

	Nonylphenol (4-tert)
Hazard	Harmful if swallowed, skin corrosion. Very toxic to aquatic organisms, may cause long term adverse effects in the aquatic environment.
Control measures	Wear protective gloves/clothing/eye protection. If in eyes rinse cautiously with water for several minutes. Remove contact lenses if present and easy to do. Continue rinsing. Immediately call doctor. Dispose of contents/container to an approved waste disposal plant. Avoid release into the environment.
Risk	**Low**

	PCR cyclers
Hazard	PCR thermal cyclers reach temperatures in excess of 95°C.
Control measures	Machines must be clearly labelled to show this hazard.
Risk	**Low**

	Periodic acid
Hazard	Corrosive. May cause burns. Harmful by ingestion, inhalation or absorption through the skin. Possible irreversible effects. Listed as calif. Prop. 65 carcinogen. Possible sensitiser
Control measures	Concentrated periodic acid must be handled in fume hood whilst wearing gloves and a lab coat. In case of contact immediately flush eyes or skin with copious amounts of water and seek medical advice. Dilute (e.g. 1% periodic acid can be handled on the open bench whilst wearing gloves and a lab coat.
Risk	**Low if concentrated acid is handled in a fume hood. Low for dilute acid**

	Phenol
Hazard	Toxic by inhalation, in contact with skin or if swallowed. Absorbed through the skin.
Control measures	Chemical resistant gloves and eye protection should be worn when handling quantities of phenol compounds which could splash. Phenol must not be autoclaved and must be disposed of safely.
Risk	**Minimal. Only small volumes are handled**

	Phenylmethylsulphonylfluoride (PMSF)
Hazard	Toxic by inhalation, skin contact and ingestion and causes burns.
Control measures	Chemical resistant gloves, splash eye protection and lab coat should be worn when handling solid PMSF or solutions containing PMSF. The maximum amount of PMSF stock solution (0.1M) handled will be 1ml. This would contain 17.4μg of PMSF. Solid PMSF should be handled, weighed out and dissolved in n-propanol in a chemical fume hood. The working concentration will be 1:1000 dilution. Solutions containing 1mM PMSF can be handled safely on the laboratory bench. In case of contact wash with

	copious amounts of water. Stock solutions will be disposed of via the onsite destructor facility. Contact between PMSF and water liberates highly flammable gases.
Risk	**Minimal. Only small quantities are handled**

	p-Nitrophenolphosphorylcholine (pNPPC):
Hazard	P-nitrophenol is released on hydrolysis of this compound, and is toxic.
Control measures	Gloves should be worn when handling solid pNPPC or solutions containing pNPPC. pNPPC will be handled in quantities of less than 25mg. The powdered chemical should be handled in a chemical fume hood or safety cabinet. Solutions of pNPPC (40mM, <2ml; 12mg/ml) can be handled on the laboratory bench.
Risk	**Minimal. Only small quantities are handled**

	Potassium borohydride
Hazard	Highly flammable - When exposed to water or moist air it may react violently and start a fire. Produces flammable gases on contact with water. Strong reducing agent - Reacts rapidly and dangerously with oxygen and with other oxidizing agents, even weak ones. Thus, they are likely to ignite on contact with alcohols. Hydrides are incompatible with acids, alcohols, amines, and aldehydes. Irritant - Inhalation or contact with vapours, substance or decomposition products may cause severe injury or death.
Control measures	Lab coat and gloves will be worn when handling; face mask if handling significant quantities. Powdered chemical should be handled in a fume hood. In case of fire, dry powders such as soda ash or powdered sodium chloride should be used to extinguish. In case of contact flush eyes or skin with copious amounts of water. Solution can be decomposed with a drop wise addition of ethanol
Risk	**Low. Only small quantities are handled and dilute solutions are used in procedures**

	Potassium dichromate
Hazard	Harmful to skin, eyes and by inhalation. Confirmed carcinogen. Oxidizing material, should be kept away from reducing agents.
Control measures	Lab coat and gloves will be worn when handling. In case of skin or eye contact, flush with copious amounts of water and seek medical advice.
Risk	**Low. Only small quantities are handled and dilute solutions are used in procedure.**

	Potassium ferricyanide
Hazard	Contact with acid liberates very toxic gas.
Control measures	Lab coat and gloves will be worn when handling. Keep away from acids. In case of contact with eyes or skin, flush with copious amounts of water.
Risk	**Low.**

	Proteases; Trypsin & proteinase K, pepsin, endoproteinase Lys-C, endoproteinase Arg-C and endoproteinase Glu-C
Hazard	All of these proteases may be harmful as dry powders but the solution used presents a minimal hazard because of the small volumes involved.
Control measures	Lab coat and gloves will be worn when handling. Take care when weighing out dry powders to avoid dust generation. Wear face mask if handling significant quantities

Risk	**Minimal. Only small quantities are handled and dilute solutions are used in procedures**

	Sodium acetate
Hazard	The dust is combustible, but presents a minimal hazard as volumes involved are small. Do not breathe dust. Avoid contact with skin and eyes.
Control measures	Lab coat and nitrile gloves will be worn when handling. Avoid dust formation.
Risk	**Low. Only small quantities are handled**

	Sodium dodecyl sulphate (SDS)
Hazard	Sodium dodecyl sulphate may be harmful if swallowed, inhaled or absorbed through the skin. Wear gloves and handle with care.
Control measures	Should be handled whilst wearing gloves and a lab coat. In case of skin or eye contact wash with copious amounts of water for 15mins and seek medical advice. Wear face mask if handling significant quantities.
Risk	**Minimal. Only small quantities are handled**

	Sodium hydroxide
Hazard	Harmful if swallowed, inhaled or absorbed through the skin or eyes. Wear gloves and handle with care.
Control measures	Should be handled whilst wearing gloves and a lab coat. In case of skin or eye contact wash with copious amounts of water for 15mins and seek medical advice. Wear face mask if handling significant quantities.
Risk	**Low. Only small quantities are handled**

	SDS-PAGE buffer.
Hazard	Contains sodium dodecyl sulphate and dithiothreitol. DTT at 5mM is irritant.
Control measures	Lab coat and nitrile gloves will be worn when handling
Risk	**Low. Only small volumes are handled**

	Silver nitrate
Hazard	Corrosive. Possible risk of irreversible effects. Harmful by ingestion, inhalation or absorption through the skin.
Control measures	Silver nitrate powder and concentrated silver nitrate solutions should be handled wearing lab coat and gloves in a fume cupboard. Dilute solutions can be handled wearing lab coat and gloves with care on the open bench.
Risk	**Minimal. if handled as above**

	Sodium perchlorate
Hazard	Harmful if inhaled or swallowed. May acts as a skin, eye or respiratory irritant. LD_{50} dose 550 – 2100 mg/kg. Shock-sensitive and potentially explosive. Incompatible with organics, other combustibles, powdered metals, acids, reducing agents.
Control measures	Lab coat and gloves will be worn when handling. Take care when weighing out dry powders to avoid dust generation. Wear face mask if handling significant quantities. Store refrigerated.
Risk	**Minimal. Only small quantities are handled**

	TEMED
Hazard	Harmful if swallowed, inhaled or absorbed through the skin. It is destructive to the mucous membranes, eyes and skin.
Control measures	Must be handled in the fume hood whilst wearing gloves and a lab coat. In case of contact wash with copious amounts of water. Must be stored in the flammables cabinet. Anything other than trace amounts of TEMED should be incinerated.
Risk	**Minimal. Only small volumes are handled**

	TAE buffer
Hazard	Contains tris, acetic acid and EDTA. May be harmful by ingestion or skin absorption. Causes eye and skin irritation. If preparing from individual components rather than purchased as a pre-prepared solution then see individual assessments for handling tris, acetic acid and EDTA
Control measures	Lab coat and gloves will be worn when handling solution.
Risk	**Minimal as a solution**

	TE buffer
Hazard	TE buffer may be harmful if swallowed, inhaled or absorbed through the skin.
Control measures	Wear gloves and handle with care.
Risk	**Minimal**

	Tributyl phosphine (TBP)
Hazard	Harmful if ingested, inhaled or absorbed through skin. Destructive to mucous membranes. Inhalation in large amounts may be fatal.
Control measures	As TBP may cause damage to mucous membranes it must only be handled within a microbiological safety cabinet when in concentrated form. Continuous exposure may allow penetration of skin. Suitable latex gloves must be also be worn at all times while handling in powdered form and liquid solutions. If in doubt contact the safety advisor for information regarding a suitable choice of glove for use.
Risk	**Minimal. Only small quantities are handled**

	Trichloroacetic acid (TCA)
Hazard	Very hazardous in case of skin contact (irritant), of eye contact (irritant), of ingestion, of inhalation. Hazardous in case of skin contact (corrosive), eye contact (corrosive). Slightly hazardous in case of skin contact (permeator). The amount of tissue damage depends on length of contact. Eye contact can result in corneal damage or blindness. Skin contact can produce inflammation and blistering. Inhalation of dust will produce irritation to gastro-intestinal or respiratory tract, characterized by burning, sneezing and coughing. Severe over-exposure can produce lung damage, choking, unconsciousness or death. Inflammation of the eye is characterized by redness, watering, and itching. Skin inflammation is characterized by itching, scaling, reddening, or, occasionally, blistering. Harmful if ingested, inhaled or absorbed through skin. Destructive to mucous membranes. Inhalation in large amounts may be fatal.
Control measures	It must be handled in fume hood whilst wearing gloves and a lab coat. In case of contact immediately flush eyes or skin with copious amounts of water

	and seek medical advice. In the case of inhalation, remove to a well ventilated area and seek medical advice
Risk	**Low if handled in a fume hood**

	3,3',5-Triiodo-L-thyronine sodium salt (Thyroid hormone T3)
Hazard	Not thoroughly investigated.
Control measures	Wear gloves. In event of contact with eyes, wash with plenty of water. In event of skin contact, wash with soap and water.
Risk	**Low**

	Tris
Hazard	May be harmful by inhalation, ingestion or skin absorption. Causes eye and skin irritation.
Control measures	Should be handled wearing lab coat and gloves. In case of contact wash with copious amounts of water. Wear face mask if handling significant quantities.
Risk	**Minimal**

	UV transilluminator
Hazard	UV light can cause retinal damage and promote the development of skin tumours
Control measures	Always wear a UV face shield (reference number EN166 with UV filter, gloves and a laboratory coat
Risk	**Minimal with protective equipment**

	Xylene cyanol FF
Hazard	This may be harmful by inhalation, ingestion or skin absorption.
Control measures	Should be handled whilst wearing gloves and a lab coat. It is incompatible with strong oxidising agents. In case of contact wash out eyes with copious amounts of water and wash skin with soap and water
Risk	**Minimal. Only small quantities are handled**

	Zinc chloride
Hazard	Causes severe skin burns and eye damage. Harmful if swallowed or inhaled. Very toxic to aquatic life.
Control measures	In case of contact remove any contaminated clothing and wash with copious amounts of water. Should be handled wearing lab coat and gloves. Take care when weighing out dry powders to avoid dust generation. If swallowed or inhaled, immediately contact doctor/physician. If swallowed, rinse mouth. If in eyes, rinse cautiously with water for several minutes. Do not let product enter drains. Discharge to the environment must be avoided.
Risk	**Minimal (due to low volumes being handled)**

	Zinc sulphate
Hazard	Irritant to the eyes, respiratory system and skin
Control measures	In case of contact wash with copious amounts of water. Should be handled whilst wearing lab coat and gloves.
Risk	**Minimal**

4.1 Where and under what circumstances will the work be done?

All procedures detailed above will be carried out within a designated ACDP containment level 2 laboratory.

All chemicals must be transported in quantities required for use and in labelled containers. Risk assessments for the decanting of liquids must be made, where appropriate, in the sourcing laboratories.

5. WHO MIGHT BE AFFECTED?

1. Staff working using the above procedures
2. Individuals not directly involved with the process/procedure/equipment etc but who may be affected; for example co-workers, students, contractors, cleaners etc.
3. Any individual who enters the laboratory whilst work is ongoing. Visitors to a CL 3 laboratory are only permitted after decontamination of the entire laboratory by fumigation. The HSE and Head of Department may enter the workplace by arrangement
4. Individuals working in close proximity.
5. Individuals working in the workplace who are likely to be at increased risk include
    - Pregnant workers or those who are breast feeding
    - Young persons (ie < 18 years of age)
    - Persons on work experience or training schemes
    - Temporary employees
    - Contractors
    - Lone workers
    - Persons with known ailments e.g. asthmatics and diabetics.

6. HEALTH SURVEILLANCE

No additional health checks are needed as a result of these procedures.

7. EMERGENCY PROCEDURES

7.1 Spillages

Any spillage of biological materials should be contained and covered with absorbent paper/cloth, which should subsequently be placed in an appropriate bin for autoclaving. The contaminated area should be washed exhaustively with 70% ethanol.

In normal use the risk to the environment is zero because working practices prevent the organisms from escaping.

7.2 Personal contamination

In the event of personal contamination with biological materials, remove any contaminated clothing as quickly as possible.

Eye contact: Wash exhaustively with the emergency eye bath.
Skin contact: Wash with soap and cold water.
Mouth contact: Flush mouth exhaustively with water. Avoid ingestion.
Puncture wounds: Encourage to bleed. Wash minor cuts and similar lesions with soap and water before applying a dressing as required.

Medical advice must be sought if there is a risk of infection.

8. SAFE DISPOSAL

8.1 Autoclaving of contaminated waste

All waste containing biological material will be autoclaved using a destruct cycle at 130^{o}C for at least 25 min, except where it contains formaldehyde or organic solvents. Records of the load will be kept, detailing the nature of the load and chart records of the temperature profile. The autoclave will be serviced and validated annually using thermocouples.

8.2 Contaminated waste which cannot be autoclaved

Procedures for disposal of biological waste containing organic solvents or formaldehyde are outlined below. These must be read in conjunction with procedure- and chemical-specific information contained within the relevant sections of the “Risk Assessment for general bacteriological methods at CL2”.

8.2.1 Anything other than trace amounts of organic solvents must not be autoclaved and should be stored in a suitable waste container in a solvents cupboard for treatment with a biocide and subsequent incineration. Solvent waste which cannot be autoclaved should be treated with 10% v/v Teknon Biocleanse for at least 24 hr, and then removed from the laboratory for incineration.

8.2.2 Solutions containing formaldehyde should not be autoclaved. Waste formaldehyde should be stored in a suitable waste container in a solvents cupboard and sterility checked before removal from the laboratory for incineration.