UKCRF NETWORK

Guidance for Delivering Laboratory Skills Training to Clinical Research Staff

Version 3 – July 2023



UKCRF NETWORK

Guidance for Delivering Laboratory Skills Training to Clinical Research Staff

Version 3.0 – July 2023

© Copyright December 2023 – The *UKCRF Laboratory Training Delivery Team* - part of the UKCRF Network Education & Training Theme Group, hosted by Manchester University Hospitals NHS FT

Permission is granted to reproduce for personal and education use only on the condition that authorship of the *UKCRF Network Education & Training Group* is being acknowledged.

Commercial copying is prohibited.

Contact details:
ukcrf.network@nihr.ac.uk
www.ukcrfnetwork.co.uk

UKCRF NETWORK

Contents

Introduction		4
How to use the Documents		5
Using the Teaching Resources to Facilitate a Workshop		6
Laboratory Skills Training Delivery		10
1.	Legislation and Regulations	11
2.	Risk Assessment	15
3.	Safety in the Laboratory	21
4.	Pre-analytics	31
5.	Centrifuge Training: Principles and Practice	36
6.	Principles and Practice of Pipetting	50
7.	Tracking and Storage of Research Samples	55
8.	Dangerous Goods Regulations	64
9.	Practical Session	80
Abbreviations and Glossary		81
Further Reading		85
Appendix 1: Laboratory Training Delivery Team		87
Appendix 2: List of Training Resources		88

Introduction

The **Guidance for Delivering Laboratory Skills Training to Clinical Research Staff** has been designed to help Clinical Research Facilities (CRFs) and other research organisations set up and deliver comprehensive laboratory skills training. The guidance document is accompanied by a slide set and various teaching resources (appendix 2) available on the UKCRF Network Portal. The training has been developed by the UKCRF Network Education & Training Group and is based on regulatory requirements, guidance documents and on best practice from a number of established CRFs.

The Good Clinical Practice Directive (2005) stipulates that staff undertaking clinical research have to be trained in the interventions and skills they are providing for a clinical research study. In addition, correct sample collection, handling and subsequent laboratory analysis has a significant contribution to research data. This includes both primary and secondary outcomes but importantly in clinical trials, essential safety information. It is therefore imperative that research samples are acquired, processed and analysed by appropriately trained staff (UKCRC Network of Clinical Trial Units, no date).

An employer also has a statutory responsibility to protect staff from any occupational hazards that they may be exposed to in the course of their duties (ECMC, 2011). Sample handling and processing may expose the handler to a multitude of different risks. Most commonly these will include biohazards when handling blood, serum or plasma. It is important to check that risk assessments are undertaken, the required safety measures are honoured and that any training required has been undertaken by any persons involved in the sample management process (ECMC, 2011).

This guidance document recommends a framework for training research staff in the essential laboratory skills required to perform safe and accurate handling and processing of research samples. It is envisaged that it will be used to train new research staff but could also be delivered as a refresher to experienced research staff to update skills and practice. Due to the variety of sample processing facilities, the level and style of training required will vary (UKCRC Network of CTUs, no date). To ensure that the training delivered is appropriate, the guidance document has been designed so that it can be adapted to meet local needs. The document has been independently reviewed and the training sessions have been piloted by the UKCRF Network Education & Training Group.

How to use the Documents

Workshop Topics

In order to achieve an appropriate level of laboratory skills training for research staff, this guidance document and the teaching resources provided (appendix 2), cover the following topics:

1. Legislation and Regulations
2. Risk Assessment
3. Safety in the Laboratory
4. Pre-analytics
5. Centrifuge Training: Principles and Practice
6. Principles and Practice of Pipetting
7. Tracking and Storage of Research Samples
8. Dangerous Goods Regulations
9. Practical session

Teaching Resources

Each topic provides the trainer with sufficient information and tools to deliver a laboratory skills workshop to research staff. This document includes guidance for delivering each topic including:

- Learning objectives for each topic
- Background information about the topic
- A slide set with additional information for describing the slide content
- Workshop activities if applicable
- Suggested handouts if applicable
- Trainer Tips and Answers booklet

Accessing Teaching Resources

A full list of available resources (agenda, slide sets, workshop activities and handouts) can be found in appendix 2. All resources are available for free download from the UKCRF Network Portal. If you do not already have access to the Network's portal you will need to complete a request form on the UKCRF Network website by clicking here.

Using the Teaching Resources to Facilitate a Workshop

Delivery

It is important to consider the research staff and their learning needs. Most research nurses and clinical staff will require the full set of workshops but a trained lab technician coming to work in a research environment may need a smaller selection. The trainer can tailor the workshop selection to suit the audience. The workshops can be delivered as a full day training event or as individual sessions depending on the availability of the trainer, staff and the resources required. Approximate times for each topic session are found on the Agenda, in the Trainer Tips and Answer Booklet, located on the UKCRF Network Portal (see page 5 for more details).

Trainers

If delivering the workshops as a full day, it might be appropriate to use 2 or more trainers. The UKCRF Network Education & Training Group piloted the full training day with 2 trainers who interchanged after each workshop, and one other additional laboratory staff member who helped with the practical workshop. Evaluation found that this worked well.

Audience

It may be necessary to limit the audience size depending on the number of trainers and size of laboratory/practical space. The pilot session revealed that for a practical exercise, 1 trainer for approximately 4 or 5 staff was appropriate, although the class-room sessions could be delivered to a larger audience.

Environment

Most of the workshops contain elements of group work so it is important to consider the training environment to facilitate this. It is also necessary to demonstrate and practice laboratory procedures so access to a laboratory setting is essential for some of the workshops. Consider the proximity of the training room to the laboratory area and ensure timings of the training schedule reflect transfer time. A practical session requires a large space for attendees to practise. It may be necessary to use a clinical space in addition to a Laboratory area.

Teaching Resources

This document contains guidance for each topic, giving advice about delivering each workshop to facilitate appropriate learning. It will advise on practised methods of delivering the workshop and how to incorporate activities, demonstrations and practical elements to the course. The teaching resources include:

- **Learning Objectives**

  These indicate what the attendee is expected to achieve from each session.

- **Background Information**

  The information booklet for each topic provides background information for the trainer. It could also be used as pre-course reading for the research staff if appropriate. An example of this might be a small audience of 1 or 2 staff. It might not be resourceful to deliver a lecture but providing the information booklet, followed by a discussion of the main points and issues might suffice.

- **Slide Sets**

  The slide sets contain appropriate information that has been quality checked and assured by the UKCRF Network Education & Training Group. The content and design of the slides has been kept simple to ensure readability and effective teaching. Further information for the trainer is given in the notes section of each slide set to aid understanding of the slide content and delivery of the workshop.

  * The slides have been designed to 'build' and the next bullet point will appear on 'clicking'. To help the presenter know when the slide will change, the last bullet point always ends with a full stop.
  * The slides are available for free download from the UKCRF Network Portal (see page 5 for more details).

- **Workshop Activities**

  The individual members of the audience will naturally learn in different ways so it is important to add some activities into the workshops. This helps to engage the research staff and maintain interest and focus. A full list of workshop activities is provided in appendix 2. These can be altered to reflect the needs of the facility or laboratory setting where the staff will be working. The activities are available for free download from the UKCRF Network Portal (see page 5 for more details).

- **Trainer Tips and Answer Booklet**

  A booklet containing trainer tips has been provided containing tips and suggestions on how to run practical sessions. Answers to the workshop questions have also been provided to assist trainers in giving feedback at the workshop and practical sessions. This is available for free download from the UKCRF Network Portal (see page 5 for more details).

- **Suggested Handouts**

  It might be applicable to deliver some workshops as a practical demonstration in the laboratory setting and not use the slide set at all, e.g. Safety in the Laboratory. In addition, as the information on the slide set is minimal, some workshops require detailed instructions for the sample processing techniques involved. A selection of handouts has therefore been included in the guidance document. Again these can be altered to reflect the needs of the facility or laboratory setting where the staff will be working. The handouts are available for free download from the UKCRF Network Portal (see page 5 for more details).

- **Workshop Booklet**

  The handouts and workshop activities have been amalgamated into one booklet for ease of reference and access, but these may be used individually if presenting sessions independently. This is available for free download from the UKCRF Network Portal (see page 5 for more details).

- **Competency**

  Competency assessment is a method of ensuring a fully trained and competent workforce. It verifies that a member of staff has full knowledge and comprehension of a procedure, shows that they can discuss the principles in detail, can perform the procedure competently without supervision or assistance, but recognises their own limitations and seeks appropriate advice when necessary.

  The UKCRF Laboratory Competencies for Clinical Research Staff can be accessed in the UKCRF Network Portal. This can be used in full, but it is recognised the type of laboratory work carried out by Clinical Research Facilities varies depending on the studies undertaken. This document has therefore been amended to meet the

requirements of this course and can be found as the Sample Processing Competency Record, also available from the UKCRF Network Portal (see page 5 for more details).

- **Evaluation**

  Evaluation of the course will provide you with detailed feedback about the delivery of the course and an example evaluation has been provided. The *UKCRF Laboratory Skills Training Delivery Team* also analyse the quality and content of this course which, in turn, helps to define ways in which we can continually improve it. We ask CRFs to provide feedback on the training document and we amend the course as appropriate.

Laboratory Skills Training Delivery

The information in each section below provides background information for the trainer. Reading and understanding this information will enable the trainer to have sufficient knowledge to deliver the presentation and any accompanying workshops.

Introduction

Correct sample collection, handling and subsequent laboratory analysis has a significant contribution to research data. This includes both primary and secondary outcomes, and importantly in clinical trials, essential safety information. It is therefore essential that research samples are acquired, processed and analysed by appropriately trained staff.

Correct processing will guarantee that the quality, integrity and viability of a sample remain unaffected at any point during the study. Different samples require different processes and poor or unsafe handling techniques can impact on research study results.

In clinical trials, blood samples are sent as whole blood to hospital laboratories for analysis, or as separated components to central, study specific laboratories. Separation of the blood components preserves the sample integrity, allowing for accurate results.

1: Legislation and Regulations

Learning objectives

- To understand the regulatory and legal framework relating to laboratory practice.
- To understand the responsibilities of employers, employees and visitors in relation to the legislation.

Background Information

Health and safety are of paramount importance when working in a laboratory. There is also legislation and regulation that we must follow to safely process biological samples in a laboratory setting.

Overview of Relevant UK legislation and regulations

1. Health and Safety at Work etc. Act (HSWA) (1974)
2. Management of Health and Safety at Work Regulation (1999)
3. Control of Substances Hazardous to Health (COSHH) Regulations (2002)
4. Personal Protective Equipment at Work Regulation (1992)
5. IATA Dangerous Goods Regulations (2023)
6. Research Requirements
7. Local Policy

1. **Health and Safety at Work etc. Act (HSWA) (1974)**

   The Act sets out the general duties which employers have towards employees and members of the public, and employees have to themselves and to each other.

   - It is the duty of every employer, so far as is reasonably practicable, to ensure the health, safety and welfare at work of all employees (this includes students).
   - It is the duty of every employee while at work to take reasonable care of himself (herself) and of other persons who may be affected by his (her) acts or omissions at work.
   - The employees must cooperate with the employer with regard to health and safety procedures.

Here the law requires what good management and common sense would lead employers to do anyway: that is to look at what the risks are and take sensible measures to tackle them.

2. **Management of Health and Safety at Work Regulation (1999)**

This regulation places a duty on employers to assess and manage risks to their employees and others arising from work activities.

Every employer shall make a suitable and sufficient assessment of:

- the risks to the health and safety of his employees to which they are exposed whilst they are at work; and
- the risks to the health and safety of persons not in his employment arising out of or in connection with the conduct by him of his undertaking.

3. **Control of Substances Hazardous to Health (COSHH) Regulations (2002)**

This regulation requires every employer to ensure that the exposure of his employees to substances hazardous to health is either prevented or, where this is not reasonably practicable, adequately controlled.

The employer has a responsibility to:

- assess risks
- provide adequate control measures
- provide information and training
- provide health surveillance if appropriate

Further information is provided in **Section 2 - Risk Assessment** (page 14).

4. **Personal Protective Equipment at Work Regulation (1992)**

The main requirement of this regulation is that personal protective equipment is to be supplied and used at work wherever there are risks to health and safety that cannot be adequately controlled in other ways.

The Regulations also require that PPE:

- is properly assessed before use to ensure it is suitable
- is maintained and stored properly

- is provided with instructions on how to use it safely
- is used correctly by employees

5. IATA Dangerous Goods Regulations – 64th edition 2023

The **I**nternational **A**ir **T**ransport **A**ssociation (IATA) is a trade association of the world's airlines. The IATA Dangerous Goods Regulation is a global reference for shipping dangerous goods by air. More information on this will be covered in **Section 8 - Dangerous Goods Transportation and Regulations** (page 62).

Note: While COSHH talks about "substances hazardous to health", IATA refers to "dangerous goods". These terms are used as equivalent terms.

6. Research Requirements

Regulatory factors that need to be considered come from varying perspectives. Any factor which may influence the way a sample is collected and subsequently handled is important and adherence to regulations is mandatory.

- Good Clinical Practice (GCP) - All research staff involved in the collection of any sample type for the purpose of research should have adequate training. This includes training and compliance with the principles of GCP as well as study specific training. Details of this should be documented in both the study delegation log and the individual's training record.
- Good Laboratory Practice (GLP) - this is the UK government legislation governing any test facility which conducts, or intends to conduct regulatory studies and safety tests on Chemicals – including pharmaceuticals. These provide guidelines to the standards that should be observed when processing samples for storage and/or shipment for Clinical Trials or Studies.
- Good Clinical laboratory practice (GCLP) – this document has been created by the Research Quality Association (RQA) (UK) as an interpretation of the GLP legislation – to provide guidance on the quality systems required in laboratories that undertake the analysis of samples from clinical trials. GCLP is applying those standards established under GLP which are relevant to the analyses of samples from a clinical trial, whilst at the same time ensuring that the purpose and objectives of the GCP regulations are satisfied. In so

doing, the reliability and integrity of data generated by analytical laboratories can be assured.

- A useful source of information is the 'Reflection paper for laboratories that perform the analysis or evaluation of clinical trial samples' (European Medicines Agency, 2012). This document provides laboratories that perform analysis or evaluation of human samples for clinical trials with information to help and maintain quality systems.

7. **Local and internal policies & procedures**

   It is also important to consider your local policies including:

   - Infection Control
   - Health and Safety
   - Standard Operating Procedures.

Slide Set

A slide set is available for this topic that can be downloaded from the UKCRF Network Portal (see page 5 for more details).

Workshop Activities

This session works best given as a lecture as an introduction to why it is necessary to consider all of the following sessions.

Suggested Handouts

There are no suggested handouts for this session.

2: Risk Assessment

Learning objectives

- Demonstrate how to identify and evaluate the hazards associated with sample handling and processing.
- Understand how to complete a risk assessment.

Background Information

The legal responsibility for health and safety in the laboratory cannot be delegated and rests primarily with the employer. This involves assessment of the risks, development of policies, and implementation and monitoring of those policies. Effective management can only be achieved through the involvement of staff and ultimately each employee has a duty to take care of their own health and safety and that of others who may be affected by actions at work. Employees must work in accordance with training, policies and guidance given by their employer and report any situations they believe to be unsafe.

The extent to which clinical research staff will be exposed to risk during the course of their work will vary. The exposure to risk will be dependent upon the type of study or processes required. Some will be exposed to biohazards when handling biological samples or exposed to substances that are hazardous to health due to the processing technique.

What is the risk?

Risk in relation to occupational safety and health is *the likelihood that a person may be harmed or suffers adverse health effects if exposed to a hazard.*

What is a hazard?

A Hazard is a potential source of harm or adverse health effect on a person or persons.

What is the difference between a 'hazard' and a 'risk'?

A hazard is something that can cause harm, a risk is the chance, high or low, that any hazard will actually cause somebody harm. The terms hazard and risk are often used interchangeably. The following example demonstrates how the terms are used correctly:

A spill of water in a room would present a slipping **hazard** to persons passing through it. If access to that area was prevented by a physical barrier then the hazard would remain though the **risk** would be minimised.

What is Risk Assessment?

A risk assessment is not about creating huge amounts of paperwork, but about identifying sensible measures to control the risks. A laboratory risk assessment requires careful examination of what may cause harm. We need to think about how accidents and ill health could happen and concentrate on real risks – those that are most likely and which will cause the most harm. For some risks, other regulations require particular control measures, for example, Control of Substances Hazardous to Health (COSHH) Regulations. Risk assessment can help identify where you need to look at certain risks and these particular control measures in more detail. Sample handling and processing may expose those involved to a multitude of different risks, for example, risk of infection, handling of dry ice or liquid nitrogen, manual handling of loads, ionising radiation, fire hazards, lone working and slips, trips or falls.

Prior to starting a study, the procedure for obtaining and processing biological samples needs to be clear. This will be dictated by the protocol and the trial laboratory manual. The research staff should assess the practicalities involved in obtaining and processing the samples and identify potential risks and any training requirements which would minimise the risk.

There are certain prerequisites required before conducting a risk assessment:

- Knowledge of the biological, chemical and physical hazards that will be encountered
- Understanding of the principles of biological, chemical and physical safety
- Knowledge regarding modes of transmission of potential infectious agents
- Knowledge regarding legislation, organisation policies and procedures

How to Conduct Risk Assessment

There are 5 steps to risk assessment:

1. **Identify the hazards** - use of a Hazard Identification/Inspection Checklist provides a structured approach achieving the identification of potential sources of harm. Every aspect of a study protocol and laboratory manual must be thought out in advance so

that all sample handling and processing activities are conducted safely. Consider both the workplace and activities, identify hazards which might reasonably result in significant harm and gather information from staff and accident reports. A risk assessment should include consideration of what might cause harm, for example, biological samples, chemicals, processes, equipment, waste management, hazards in the working environment, such as walkways, floor surfaces, lighting, storage, stairs, and electricity. Identify hazards by conducting regular health and safety inspections using your checklist. The compiled list of tasks and identified hazards should then undergo a detailed risk assessment.

2. **Identify who might be harmed and how** – all groups who may be harmed should be considered, for example, nursing, laboratory staff and support workers. It is important to identify workers who may have particular needs, for example those with disabilities, pregnant women, and workers who may not be in the workplace all of the time, including external contractors, visiting researchers and members of the public.

3. **Evaluate the risk**

   The level of risk should be balanced against the measures needed to control the real risk in terms of money, time or effort. However, it is not necessary to take action if it would be grossly disproportionate to the level of risk. A Risk Assessment Matrix can be used as a tool to aid the evaluation of risk.

Severity **Likelihood**	**Negligible** **1**	**Minor** **2**	**Moderate** **3**	**Major** **4**	**Catastrophic** **5**
Certain **5**	Medium 5	Medium 10	High 15	High 20	High 25
Probable **4**	Low 4	Medium 8	High 12	High 16	High 20
Possible **3**	Low 3	Medium 6	Medium 9	High 12	High 15
Unusual **2**	Low 2	Low 4	Medium 6	Medium 8	Medium 10
Remote **1**	Low 1	Low 2	Low 3	Low 4	Medium 5

Figure 1: Example of a Risk Assessment Matrix

When deciding on appropriate controls, list what is already in place to reduce the likelihood of harm or make the harm less serious and consider what is reasonably practicable. The evaluation should be guided by good practice and how current workplace practice compares with this.

Pose the question:

Can the hazard be removed altogether?

If not, how can the risks be controlled?

- can the hazard be replaced?
- prevent access to the hazards
- involve and consult with workers
- provide facilities such as first aid and washing facilities
- use protective equipment

The hierarchy of controls below can be used to guide appropriate control measures.

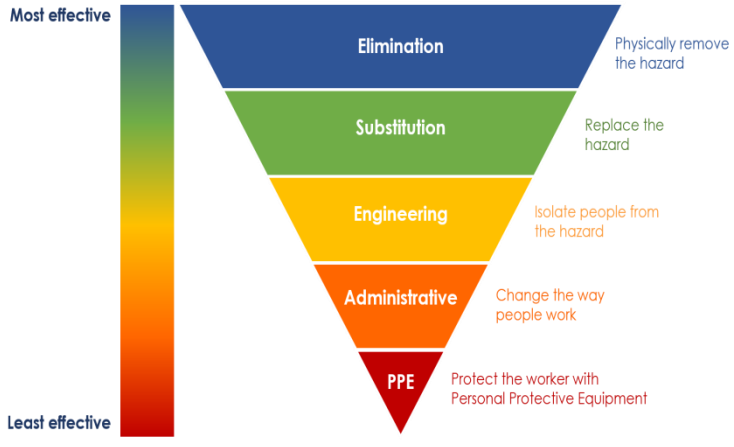


Figure 2: Hierarchy of Controls

4. **Record your assessment and implement findings** – it is important that the assessment is recorded and shared with the rest of the team. Risk assessment should be accessible by the workforce and written in a way that is clear and easily

understood. Each organisation may have Risk Assessment and Action Plan templates. Prioritise hazards identified, and deal with those hazards that have high risks and serious consequences first. Examples of risk assessment documents are available for free download from the UKCRF Network Portal (see page 5 for more details).

5. **Continually review** - Changes to the trial protocol, the working environment, or workforce may occur and therefore it is important to review what you are doing on an ongoing basis. An appropriate review date is usually required when documenting a risk assessment. This should be adhered to and the risk assessment should be reviewed in a timely fashion.

Control of Substances Hazardous to Health

Occasionally processing biological samples may require the use of additional hazardous agents. Using chemicals or other hazardous substances at work can put people's health at risk. The law requires employers to control exposure to hazardous substances to prevent ill health by complying with the Control of Substances Hazardous to Health Regulations (COSHH) (2002).

Substances can take many forms and include:

- chemicals
- products containing chemicals
- fumes
- dusts
- vapours
- mists
- nanotechnology
- gases and asphyxiating gases and
- biological agents

COSHH Risk Assessment

The COSHH risk assessment consists of several additional steps to a standard risk assessment.

A risk assessment must be completed for each task associated with exposure to a hazardous substance. Risk assessing the procedures required to process biological

samples is an integral first step in ensuring the safety of the workforce involved. A standard set of risk assessments for processing biological samples may be sufficient to incorporate the majority of research studies conducted in a particular department. However, each research study protocol should be checked to ensure that all procedures are included in the standard risk assessment. A study specific risk assessment is required for any additional procedures and processes.

The safety of the people involved in sample processing is at the forefront of laboratory work. Risk assessment will minimise harm and ensure that all sample handling and processing activities are conducted safely.

Slide Set

A slide set is available for this topic that can be downloaded from the UKCRF Network Portal (see page 5 for more details).

Workshop Activities

A risk assessment activity conducted in small groups is useful in this section to engage the attendees. This could be asking the group to carry out a risk assessment on a simple sample processing activity using the blank risk assessment provided, or by completing the Risk Assessment Workshop questions, listed in appendix 2. A full list of teaching resources can be found in appendix 2 and all resources can be downloaded for free from the UKCRF Network Portal (see page 5 for more details).

Suggested Handouts

It is helpful in this session to hand out a blank copy of your local risk assessment documentation. You may also like to demonstrate a simple risk assessment that has previously been completed in your area. An example of a blank risk assessment can be downloaded for free from the UKCRF Network Portal (see page 5 for more details).

3: Safety in the Laboratory

Learning Objectives

- Describe the safety precautions to be observed in a laboratory setting.

Background Information

The laboratory setting (or equivalent) can be considered a “high-risk” area due to the nature of the work and of the biological samples being handled. This may pose a health risk to staff; therefore, it is important that staff are trained and observe precautions to protect themselves and others as well as the environment and the quality of the research samples whilst minimising any associated risks.

Every piece of equipment should have an SOP, copies of which are kept in the lab and electronically. The SOP covers details such as the operation of the equipment and how to deal with spillages. A working document covers the maintenance and what to do in the event of breakdown. There should also be SOPs covering sample management, use of hazardous substances (for example, liquid nitrogen and reagents), sharps, and disposal procedures. All staff must be aware of these documents, and they should be read and signed off before carrying out laboratory procedures or using any equipment.

Laboratory Hazards

There are both biological and chemical risks to staff in a laboratory area. Biological risk arises from the samples which are being processed as they are opened to the air when pipetting. Chemical risk can arise from disinfectant used in the laboratory or solutions used for specific sample processing. Laboratories are considered as Hazard group 2 (please see below) and should have “Class 2 containment” which includes restricting access to the area by means of a keypad or swipe access. Visitors and children should not be allowed to enter laboratory areas. Appropriate warning and safety signage including “no smoking”, “no eating” and “no drinking” signs and the International Biohazard warning symbol should be on laboratory doors where Hazard group 2 samples are handled.



A hazard is anything with the potential to cause harm e.g., electricity, chemicals, infectious materials, fire, equipment etc. There are a number of potential hazards involved in working

in a laboratory setting, hence it is important that risk assessments are carried out and staff are appropriately trained. All equipment should be in good working condition at all times with periodic inspection, cleaning and maintenance carried out, alongside annual calibration. Even if you are not dealing with a hazardous substance/procedure, it is important to remember that the people around you may be.

Think about the following areas:

Activity	**Hazard**
Sample processing	Bodily fluid contamination
Equipment use	Imbalanced centrifuge
Sample storage	-80ºC freezer burns
Sample Transportation	Inhalation of CO2 from dry ice
Waste removal	Hazardous material contamination

Electrical safety

Electrical safety helps prevent the misuse of electronic instruments, electric shocks and other injuries and ensure that any damaged equipment, cords or plugs are reported appropriately so they can be repaired or replaced.

Centrifuge

Centrifuges are potentially dangerous equipment unless used correctly and maintained. Common centrifuge hazards can be seen in the table below:

Problem	**Hazard**
Compartment load exceeds maximum specified	Mechanical failure of rotating parts (often violent)
Lids not secured fully	Contact with rotating parts
Sample leak	Aerosols, stress corrosion, contamination
Sample imbalance	Machine movement/walking or stress failure of component parts

Using inappropriate chemicals which are highly explosive or flammable	Fire or explosion
Rotor speed/temperature combination exceeds the solubility of the gradient material and causes it to precipitate	Health (contact with contaminated components/ vapours

Centrifuges should be placed at a level that workers can see into the bowl and to place buckets correctly. The centrifuge needs to be properly balanced to prevent damage to the rotor. An imbalanced centrifuge can damage the rotor as well as causing serious harm to the operator. If the weight is uneven then balance tubes filled with water can be used (see **Section 5 - Centrifuge training: Principles and Practice**; page 34). Once operational the centrifuge should not be left unattended.

Safety caps must be used when centrifuging samples as this will prevent harmful aerosols escaping if a blood tube should break in the centrifuge. In the event of this occurring the lid must be kept closed for 30 minutes to allow the aerosols to settle. Cleaning and decontamination can then be carried out.

Regular calibration and servicing as per local policy is essential to ensure their safety and correct operation. In addition routine maintenance should be performed weekly including cleaning and inspection of buckets, adaptors and lids for hairline cracks or damage.

Staff are required to have appropriate training to operate a centrifuge and risk assessments should be in place. When working with a centrifuge appropriate personal protective equipment should be worn. Hair should be tied back to prevent the risk of entrapment in machinery and to reduce the risk of infection.

There are also hazards associated with multiple users sharing a centrifuge to spin multiple samples. These include:

- Assuming all users can use the centrifuge correctly
- Samples can be mixed up if not correctly labelled
- Risk of contamination if tubes not fully closed or bucket lids not used
- Spinning parameter may differ, so samples may not be spun for long enough or separate fully

Liquid Nitrogen and Dry Ice

Liquid nitrogen is used for many cooling and cryogenic applications. It is liquefied under high pressure conditions and can expand to a very large volume of gas. Liquid nitrogen temperature is -196ºC and the vapour can rapidly freeze skin tissue and eye fluid, causing cold burns, severe frostbite, and eye damage. When working with liquid nitrogen, lab coats, face masks and cryogenic gloves must be worn at all times to prevent injury. Gloves should be loose fitting so they can be easily removed if cryogenic liquid is spilt on them. Open pockets should be avoided, and open toed shoes should not be worn under any circumstances. Liquid nitrogen is odourless and colourless. The greatest risk posed is asphyxiation as increased nitrogen in the air displaces the oxygen. Processing must only be done in large, well-ventilated spaces. Appropriate cryovials and insulated, impact resistant containers must be used and tongs to withdraw objects immersed in liquid nitrogen. In the event of a large spillage or accidental release, the following procedures should be followed:

- Evacuate the area. Deploy warning signs if necessary
- Ventilate the area. Open doors and windows or activate forced ventilation to allow spilt liquid to evaporate and gas to disperse.
- Try to stop the release, if possible, e.g., turn off valves, but only if safe to do so – always wear protective clothing.
- Do not re-enter the area unless it is proved safe to do so.
- Prevent liquid nitrogen from entering drains, basements, pits or any confined spaces where accumulation may be dangerous.

If moving liquid nitrogen between locations, an assessment of the route and potential hazards must be completed.

Dry ice is the solid form of carbon dioxide (CO_2) and has a temperature of -78.5ºC. Contact with dry ice can cause severe cold burns and frostbite within a short period of time. Appropriate insulated gloves must be worn when handling dry ice. Dry ice must only be used in well-ventilated areas to avoid risk of asphyxiation. Placing dry ice into tightly sealed containers can produce sufficient gas build up to cause an explosion so suitable insulated and unsealed containers must be used; however, lids must be kept on containers as much as possible to avoid evaporation. Dry ice must never be stored in a freezer as CO_2 gas can build up leading to explosions, asphyxiation when the freezer door is opened, and damage to the freezer's internal temperature control systems. (Please see **Section 8 - Dangerous Goods Regulations** for further information; page 62).

First aid measures for both liquid nitrogen and dry ice incidents must be outlined in COSHH assessments. Where inhaled, the victim (who may be unconscious) should be removed to a well-ventilated area and kept warm and rested. If breathing has stopped, resuscitation should be commenced by a trained first aider. Where contact has occurred, the aim should be to slowly raise the temperature of the affected area back to normal. Clothing should not be pulled away from burned or frozen skin. The affected area should be doused with copious amounts of tepid water (40ºC) for at least 15 mins and a sterile burn dressing applied to protect the injury

Infection

Laboratory Acquired Infections (LAIs) are defined as:
"All infections acquired through laboratory or laboratory-related activities regardless of whether they are symptomatic or asymptomatic in nature".

Many hazards can pose a risk of infection, including handling blood and other bodily fluid samples. Infection can be acquired by a number of routes;
Inhalation for example from aerosols.
Ingestion from hand–mouth contact, thus it is important to avoid such contact and to carry out correct hand washing.
Absorption through non-intact skin, it's therefore important to cover cuts and grazes and wear appropriate PPE as protection.
Inoculation sustained from sharps injuries. Staff must be aware of local procedures in the event of sustaining a sharps injury.

All clinical samples should be deemed as infectious and universal precautions must be observed. However, if there is a known risk from a blood borne virus, precautions pertaining to Hazard Group 2 infections, or use of Class 2 containment, particular care should be taken when pipetting and staff should attempt to keep their distance to avoid inhalation from the sample.

COSHH and Reagents

The Health and Safety Executive (HSE) publishes "The Approved List of biological agents" (current version: 4th edition 2021) which provides classification of biological agents referred to by the COSHH Regulations (2002). This is relevant to risk assessment for work with biological agents and the application of appropriate control measures. The list classifies substances into Hazard groups 1-4

- Hazard Group 1: unlikely to cause human disease;
- Hazard Group 2: can cause human disease and may be hazard to employees; It is unlikely to spread to the community and there is usually effective prophylaxis or treatment available;
- Hazard Group 3: can cause severe human disease and may be a serious hazard to employees; it may spread to the community, but there is usually effective prophylaxis or treatment available;
- Hazard Group 4: causes severe human disease and is a serious hazard to employees; it is likely to spread to the community and there is usually no effective prophylaxis or treatment available.

COSHH specifies four containment levels for activities which involve working with biological agents. These correspond to the classification of biological agents into Hazard Groups 1 to 4. COSHH then detail containment measures appropriate to the containment level. However, as no laboratory has complete control over the specimens it receives, laboratory workers may be exposed to organisms in higher risk groups than anticipated.

Some studies involve the use of reagents. All reagents require a COSHH and a Safety Data Sheet (SDS) highlighting risks of use to all users. Depending on the storage conditions, these must be stored in locked reagent/flammables cabinets, or securely in cold storage appliances. A reagent log must be in place with a list of all reagents and their location and expiry dates. All reagents must be labelled with date of receipt, date made up (if a combination) and date of expiry to avoid incorrect reagent use. All unused reagents, kits and materials are to be disposed of as per the national and international guidelines.

Care must also be given to allow enough time for ordering, defrosting or making up of reagent mixes before any scheduling or processing so patient samples are not compromised, or have to be repeated due to delays.

Prevention

Laboratory Layout, Hygiene, and Sanitation

Laboratory hygiene and sanitation deal with the basic upkeep, tidiness, and maintenance of a safe laboratory. Laboratory personnel should follow safe hygienic practices including hand washing and wearing PPE. Workbench cleaning should be done before starting and after finishing work daily with industrial methylated spirit (IMS), Chemgene or equivalent. Equipment should be cleaned every time before starting and finishing work. First Aid and Eyewash kits must be available in the laboratory area, and if possible, these should be located as close as possible to a sink. In the event of a chemical splashing into eyes or on skin, immediately flush the affected area with running water for at least 20 mins/eye wash facility.

Ample space must be provided for the safe conduct of laboratory work and for cleaning and maintenance. To avoid cross-contamination there should be basic designated areas for storage of supplies, sample and reagents, sample processing, administrative work, waste disposal and packaging of samples. The skill mix of staff employed should be appropriate to the level of facility and workload. The organisational structure of the laboratory must support an optimal path of workflow from collection to shipping by allowing processes that yield efficient sample handling whilst minimising error. Care must be taken to ensure eye washes, exits or hallways are not blocked by boxes or other items.

Personal Protective Equipment (PPE)

Laboratory staff must comply with Personal Protective Equipment (PPE) regulations and wear PPE appropriate to the task to be undertaken. This will be outlined in the relevant risk assessment for the task being carried out. It is important that staff working in a laboratory setting consider their personal safety.

- Laboratory coveralls, gowns or coats must be worn at all times for work in the laboratory. These should preferably be buttoned. However, long sleeved gowns are acceptable. Aprons should be worn over laboratory coats or gowns where necessary to give further protection against spillage of chemicals or biological materials.
- Appropriate gloves must be worn for all procedures that may involve direct or accidental contact with blood, bodily fluids and other potentially infectious materials.
- Goggles and face masks should be available and must be worn when appropriate, for example where there is a risk of splashing.

- Appropriate footwear covering the entire foot should be worn to protect from injury or damage from hazardous substances.
- When working in the laboratory all cuts and grazes should be adequately covered to prevent contamination.
- Contact lenses must not be handled in laboratory areas.
- Hair should be tied back to prevent the risk of entrapment, for example in moving parts of a centrifuge.
- Hand-mouth contact should also be limited. Correct hand washing technique should be carried out, both before and after sample processing and after removing gloves or leaving the laboratory.
- Gloves must be removed before touching door handles, telephones, and computers. All PPE must be removed prior to leaving the laboratory.
- Materials must not be placed in the mouth. Labels must not be licked.

Waste

Waste is separated into clinical (orange bag) and non- clinical waste (black bag). It is important to dispose of waste appropriately to reduce the risk of infection. A local e-learning module may be available on “Segregation of waste” or refer to your local hospital policy.
Sharps bins should be used for the correct disposal of sharps, including micro-pipette tips and re-capped blood tubes. Sharps bins should not be over-filled or filled with non-sharps materials such as gauze swabs or cotton wool. Full sharps bins should be disposed of in line with hospital policy.
Laboratories should ensure there are proper procedures in place for **disposal of equipment** that is unserviceable or no longer required. These should be as per the SOP and/or relevant existing policies or local national guidelines for disposal as applicable.

Biological Safety Cabinets

Biological safety cabinets are advised for Biosafety Level 2 laboratories for potential aerosols. These should be used whenever:

- There is an increased risk of airborne infection.
- Procedures with a high potential for producing aerosols are used. These may include centrifugation, grinding, blending, vigorous shaking or mixing, sonic disruption, or opening of containers of infectious materials whose internal pressure may be different from the ambient pressure.

Spillages

It is essential to keep all bench areas clean and tidy and deal with any spillages promptly. A surface disinfectant such as 1% Distel or less harmful disinfectant such as 2% Chemgene can be used for general cleaning and for small spillages.

For larger spillages a gelling agent such as presept granules are used as described in Standard Infection Control Precautions. All spills, accidents and overt or potential exposures to infectious materials must be reported to the laboratory manager/supervisor. A written procedure for the clean-up of all spills should be developed and followed.

General Rules for Laboratory Safety

Only use equipment that you have received training in.

Use the appropriate personal protective equipment (PPE).

Always remove any PPE and wash your hands before leaving the lab area.

Smoking, eating, drinking, chewing gum, and applying cosmetics is prohibited.

Laboratory glassware should never be used as food or beverage containers.

All sharps should be handled carefully and disposed of in an appropriate sharps container.

Do not dispose of chemicals down the drain.

Report any misuse/fault to the laboratory manager.

Be aware of any laboratory safety and Trust/Health Board policies, risk assessments or Control of Substances Hazardous to Health (COSHH) assessments before you carry out your procedure.

Exclude highly susceptible individuals, e.g. pregnant women, from highly hazardous laboratory work.

A list of hazardous materials used in the laboratory should be prepared and all hazardous materials should be accounted for on a periodic basis.

Biohazard symbols should be used on all containers/equipment containing biohazardous material.

COSHH forms and Safety Data Sheets for all reagents are kept in the lab and electronically.

Accident/incident/injuries record of laboratory personnel should be maintained and reported as per local Policy. The report should include a description of the event, factors contributing to the event and information on first aid or other health care provided. This information can be analysed periodically towards effectively controlling and preventing future events.

Slide Set

A slide set is available for this topic that can be downloaded from the UKCRF Network Portal (see page 5 for more details).

Workshop Activities

This session could be taught as a practical session in a laboratory, not following the slides, but using the safety elements around the lab to describe potential hazards and precautions. Some of the attendees may not have been in a research laboratory so this session allows them to visualise the safety precautions, as well as the processing equipment and environment.

Suggested Handouts

If conducting this session as a practical laboratory workshop, the slide set could still be given to the attendees as reference material. An additional safety handout is available and could be given to the attendees to provide more detailed information.

A full list of teaching resources can be found in appendix 2 and all resources can be downloaded for free from the UKCRF Network Portal (see page 5 for more details).

4. Pre Analytics

Learning objectives

- Explain the term pre-analytics.
- Demonstrate an understanding of how to obtain, handle and process samples.

Background Information

Laboratory testing plays an important part in the delivery of healthcare. There are many steps in the total testing process and errors can have serious consequences potentially leading to misdiagnosis, incorrect treatment or the need for re-sampling.

There are three recognised phases in the total testing process –

1. Pre analytical – this includes the test request, patient & specimen identification, specimen collection, transport and processing
2. Analytical – the specimen testing
3. Post analytical – reporting of test results, interpretation, follow up, storage and retesting if required.

The pre analytical phase of sample processing includes all those steps that occur in practice before a sample is analysed in the laboratory.
Advances in technology and automation of laboratories have improved the quality of laboratory tests. However it is estimated that a high percentage of errors (50 – 75%) occur in the pre analytical phase with an obvious effect on the reliability of patient samples.

Good specimen collection technique and handling are of the utmost importance because significant errors occur in the pre analytical phase of laboratory testing. Standard operating procedures (SOPs) and protocols are intended to prevent these problems and protect the quality of patient results.

There are three areas to consider in the pre analytical phase of the trial

1. Patient preparation
2. Sample collection
3. Sample processing.

1. Patient Preparation

Patient variables

There are patient variables which we must consider which may affect blood samples. Fasting is one such variable as some biochemistry analytes, for example glucose and cholesterol require the patient to be fasted. It is therefore important to explain this to the patient to gain their compliance. The length of fast may vary from a minimum 4 hour fast up to 12 hours for glucose for example which requires a longer fast. It is also important to point out to patients that they can still drink water when fasting; this helps to prevent dehydration which may make venepuncture more difficult.

Other tests, such as cortisol, have diurnal variations which could lead to variability of results depending on when the test is done. There may be a requirement for exercise restriction as moderate to strenuous exercise can influence results particularly in CK, LDH, ACTH and creatinine as it leads to muscular and protein breakdown.

Dietary restrictions may be outlined in the protocol, for example, limiting caffeine intake as it has the potential to increase the blood pressure which may be significant for renal tests. Smoking can have a similar effect. In addition alcohol consumption may affect glucose measurement.

2. Sample Collection

There are a number of considerations which are important when selecting the site for venepuncture and preparing it.

- **Selecting a suitable vein** is an important first step. Normally a vein in the ante-cubital fossa and one which is free from bruising or signs of infection would be selected.
- The **tourniquet** should be applied 7-10cm above the venepuncture site and prolonged application of the tourniquet should be avoided. Ideally the tourniquet should be released on successful venepuncture or as soon as is reasonably possible. Prolonged use of a tourniquet can lead to haemoconcentration which can result in test errors, for example an increase in serum calcium and potassium.
- If the venepuncture site has been **cleansed with alcohol** it should be allowed to air dry before the needle is inserted. This helps to ensure that the specimen isn't

contaminated with alcohol which can lead to erythrocyte membrane rupture also known as **haemolysis**.

- **Fist clenching** by the patient should also be avoided as it can cause an increase in serum CK and potassium.
- **Good venepuncture technique** is also important and excessive probing should be avoided as it may lead to a poor quality sample.
- **Blood tubes** should be stored at the **correct temperature** (4-25 degrees centigrade) and the **expiry date** checked prior to use. If an expired tube is used the vacuum, barrier or additive performance may be affected.
- It is important to follow the correct "**order of draw**", this means that samples are collected in a specific order. This minimises the risk of transferring additives from one tube to another which can lead to erroneous test results. The order of draw will be outlined in the sample processing protocol or laboratory manual.
- Similarly it is essential that blood collection tubes are filled to their **fill tolerance,** often a black line on the draw tube. This ensures the correct blood to additive ratio. An incorrect blood to additive ratio may also lead to incorrect results.

Haemolysis occurs when the membrane of the red blood cell bursts allowing the release of haemoglobin into the surrounding fluid. This results in a pink or red tinge to the serum or plasma. It can result from certain conditions such as haemolytic anaemia or a transfusion reaction. However it also results from improper specimen collection or vigorous handling and can lead to specimen rejection or an inaccurate result. Haemolysis mainly affects potassium but may also affect sodium, calcium or haemoglobin.

3. Sample Processing

It is essential that staff responsible for sample processing follow the relevant sample processing protocol and any relevant SOPs. The sample processing protocol will outline the speed and time of centrifugation and the temperature of the centrifuge. It is essential that these instructions are followed. Incorrect centrifugation may lead to incorrect separation and contribute to erroneous results. Centrifugation is covered in more detail in section 5 of the guidance document (page 30).

It is important to note the **time to processing**, for example a whole blood sample should be spun within 2 hours of venepuncture. Serum Separating Tube (SST) samples must be allowed to clot in an upright position ideally in a rack for 30 minutes. This allows adequate time for clot formation. Insufficient clotting time results in fibrin formation which interferes

with barrier formation. **Mixing** is also critical to achieve appropriate clotting times and clot formation.

Some samples may have quite specific instructions which are essential to follow. For example there are some chemistry analytes which require the **tube to be chilled** after collection to maintain the stability of the sample. Chilling inhibits metabolism of blood cells and stabilises constituents which may otherwise be destroyed or change in response to heat. Tubes can be chilled by placing them on ice or ice and water.

There are also **analytes which are photo-sensitive** and need to be protected from light, both sunlight and fluorescent light. In this instance the tubes can be wrapped in foil to protect them. It is considered best practice not to leave any samples in direct sunlight.

How can we minimise pre-analytical errors?

- Phlebotomy education
  - Good phlebotomy technique is important to prevent trauma to the patient and minimise the need for re-bleed of the patient.
  - Good technique can also reduce the incidence of haemolysis
  - As well as initial assessment an annual competency assessment is also recommended to ensure good technique is ongoing.
- Use of appropriate products
  - closed blood collection systems are safer and less traumatic than using a syringe and needle
  - Plastic tubes have replaced glass as there is less risk of shattering and they offer increased centrifugation speed tolerance.
- Order of draw – blood collection should be done in a specific order. This is necessary to minimise the risk of transferring tube additives which may affect test results.
- It is important to invert blood tubes gently and for the specified number of times depending on the type of tube.
- Identification – technologies such as barcodes can help to reduce mistakes in patient identification but clear labelling and identification are essential.
- Adhering to protocols/SOPs – Following specific processing protocols and SOPs in the laboratory helps to reduce errors. Written procedures should be in place for patient identification, collection and labelling as well as transportation and preparation for analysis.

- Specific criteria for sample rejection should be established by laboratories and staff informed appropriately so that corrective action can be taken, for example collection of a fresh sample.

Slide Set

A slide set is available for this topic that can be downloaded from the UKCRF Network Portal (see page 5 for more details).

Workshop Activities and Handouts

After the introduction to pre analytics (slide 5 of this section), the groups can be asked to consider factors that can affect the blood sample. Pre Analytics workshop question sheet is available to aid this discussion. In this case it might be appropriate to withhold the slides and give to the attendees as handouts at the end of the session. A full list of teaching resources can be found in appendix 2 and all resources can be downloaded for free from the UKCRF Network Portal (see page 5 for more details).

5. Centrifuge Training: Principles and Practice

Learning Objectives

- To understand the principles of centrifugation.
- To demonstrate an awareness of the maintenance and calibration of centrifuges.
- To operate a centrifuge safely.

Background Information

It is important that samples are processed correctly to protect patient sample integrity. If samples are not centrifuged correctly then serum/plasma may not separate, and the results generated will be incorrect due to contamination from blood cells. Poorly spun and separated samples can lead to samples being rejected by central laboratories. Appropriate use of the centrifuge can prevent misdiagnosis, improper treatment/therapy, and additional pain or discomfort due to multiple blood collections and delay in appropriate treatment/therapy. It also decreases the chances of prolonged or unnecessary hospital stays due to inappropriate treatment, increased cost of staff, resources for improper investigations and lab testing and possible legal action due to serious or fatal medical errors.

Whether in the pre analytical or analytical phase of a clinical trial, centrifugation is a vital step in the testing of patient samples. It allows researchers to separate samples increasing the purity of the sample. Centrifuges move at high speed so can potentially be dangerous. Therefore, it is vital they are properly maintained and used correctly. Centrifuge maintenance and calibration is an everyday tool to maintain optimal condition and safety. Incorrect loading can also affect the lifetime of the rotor and uncontrolled, heavy vibration can lead to permanently damaging the centrifuge, injury to users, or in worst case, a rotor crash (large amounts of aerosol escaping with the centrifuge exhaust). To prevent injury personnel should be trained before using a centrifuge and the centrifuge service and maintained correctly.

For simplicity this document and the slides will only focus on common equipment used in the pre analytical phase of a clinical trial. Extended information found in this document can be sourced from equipment manuals.

Attendees must present a solid understanding of these modules before processing samples. The benefits of having trained and competent centrifuge users can greatly reduce pre-analytical and analytical errors, prolong centrifuge shelf-life, and prevent the risk of injury.

Principle of Centrifugation

A centrifuge is used to separate particles according to their size, shape, density, viscosity of the medium and rotor speed.

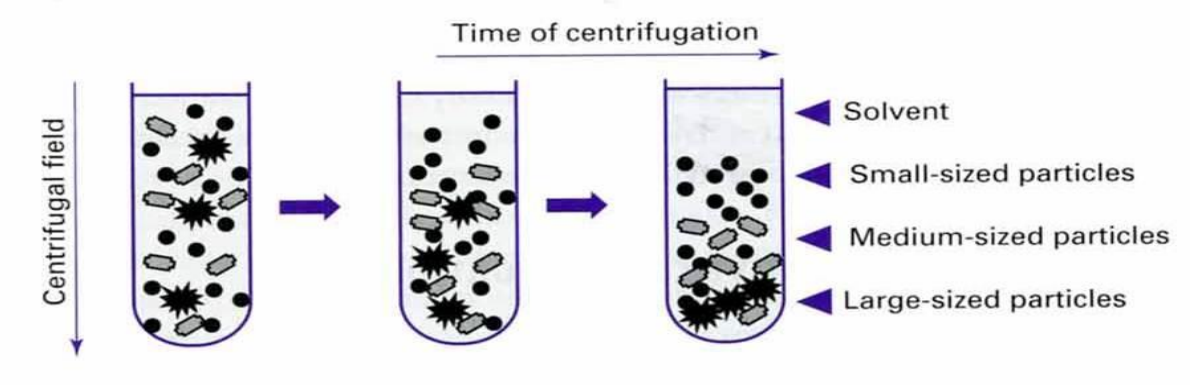


Figure 3: Principles of Centrifugation: Particles of different densities or size will sediment at different rates with the largest and most dense particles sedimenting the fastest followed by less dense and smaller particles

The most common application in the clinical laboratory is the separation of blood into cells and either serum (from blood collected without an anticoagulant) or plasma (from blood collected with an anticoagulant such as EDTA).

Blood is separated in bags and blood tubes into its various components:

- **Red Blood Cells (RBC),** erythrocytes, carry oxygen from the lungs to the rest of the body.
- **White blood cells (WBC), or leucocytes**, aid the immune system in fighting infections. There are five types of WBC: lymphocytes, monocytes, eosinophils, basophils, and neutrophils (granulocytes).
- Both serum and plasma appear as a straw-coloured layer in a normal sample after centrifugation, however, there are differences:
    - **Plasma** contains proteins, glucose, clotting factors, hormones, and carbon dioxide. The hazy colour of the plasma comes from the fibrinogen inside.

- **Serum** is blood plasma without fibrinogen or the clotting factors (i.e. Whole blood minus both the cells and the clotting factors). There is a lower risk of haemolysis in plasma compared to serum.

Separation of cellular constituents within blood can be achieved by a process known as differential centrifugation. In differential centrifugation, acceleration force is adjusted to sediment certain cellular constituents and leave others in suspension.

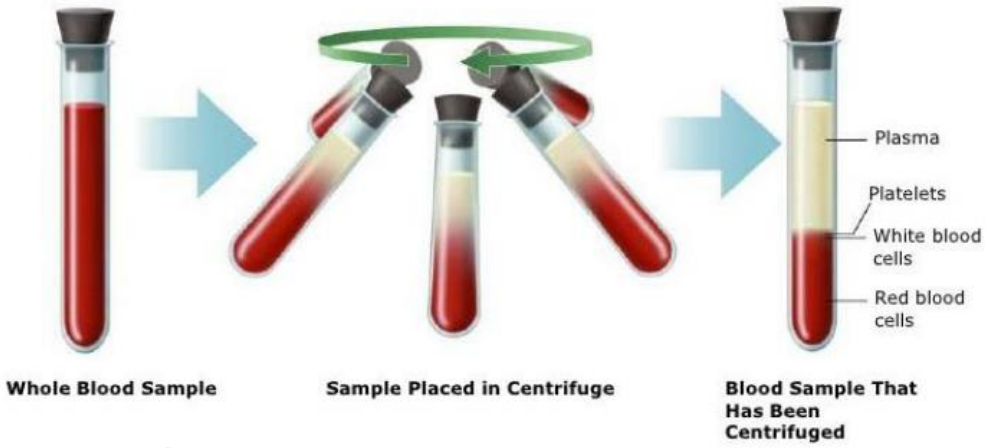


Figure 4: stages of centrifugation

During the process of differential centrifugation of blood, the sample is separated into two phases: a pellet consisting of cellular sediment and a supernatant that may be either cellular or cell-free. The supernatant can be found above the pellet at the bottom of the tube. Patient samples are spun and separated according to the lab manual guidelines provided by the Sponsors.

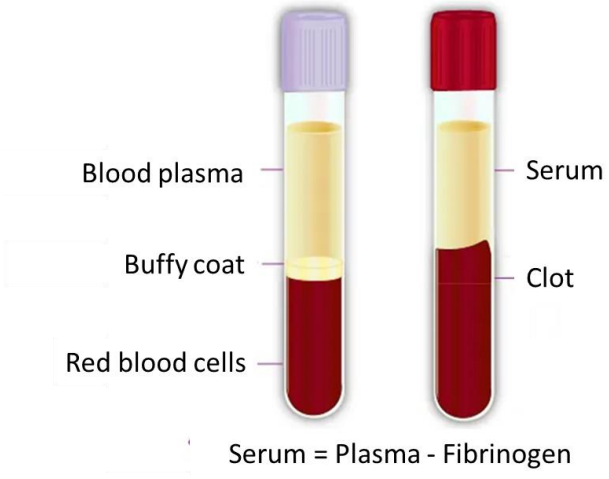


Figure 5: PLASMA (from blood collected with an anticoagulant such as EDTA) and **SERUM** (from blood collected without an anticoagulant such as SST)

In Figure 5, the cloudy coloured thin layer between the plasma and RBC is named as “buffy coat” which consists of a mixture of WBCs and platelets.

Samples should be spun as soon as possible to separate the plasma from the red cells. This prevents a leakage of analytes e.g. potassium from the red cells into the serum and also minimises the risk of haemolysis.

Serum is the watery portion of blood that remains after coagulation and is free from fibrin. Serum can be obtained by centrifugation of the blood collected into the tube without an anticoagulant. In tests that require serum, it is important to collect and centrifuge the sample properly. If a tube is spun too soon after drawing the blood, you will send plasma to the laboratory and not serum. However, once a serum sample has completed its 30 minute clotting time, it should be spun as soon as possible. Like plasma, this also prevents a leakage of analytes e.g. potassium from the red cells into the serum and also minimises the risk of haemolysis. If samples are spun incorrectly the results will not be accurate and redraw may be required. Examples of incorrect centrifugation are shown in figure 6 below.

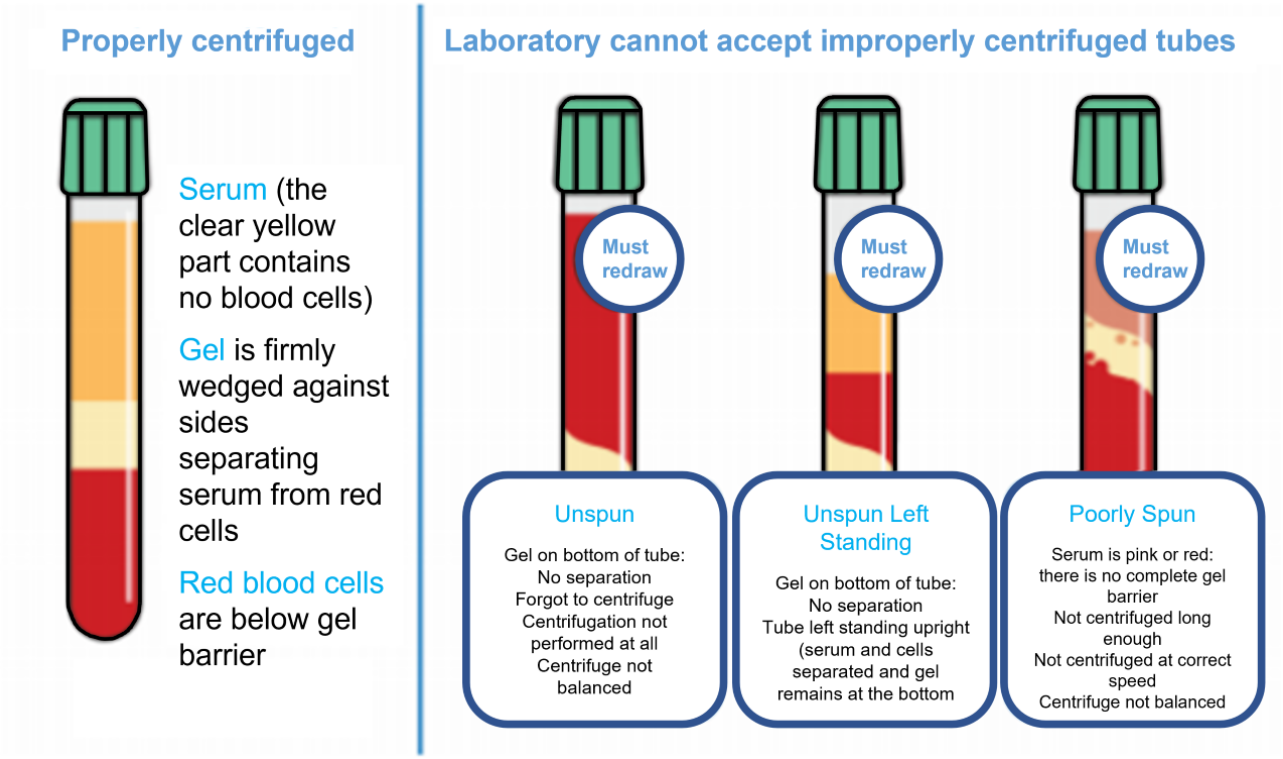


Figure 6: Examples of incorrectly spun samples

Common Rotors

There are different types of centrifuges used to process patient samples but the most common two used in clinical research are swinging bucket centrifuges and smaller table top fixed angle centrifuges. It is important to clarify which rotor the lab manual requires as settings and sedimentation results can vary depending on which one is used.

Swinging bucket rotors allow longer distance of travel which may allow better separation, such as in density gradient centrifugation. This makes it easier to withdraw supernatant without disturbing the pellet. Fixed angle rotor results in sedimenting particles having only a short distance to travel before pelleting. This usually results in a shorter running time. - The radius of the centrifuge determines the relative centrifugal force (RCF) (measured in g), along with the speed of the centrifuge. A rotor with swing out buckets will therefore allow higher RCF (g) at a slower speed and makes them ideal for separating cell layers such as white blood cells e.g. PBMCs and neutrophils.

Balancing a centrifuge

Rotors must be balanced for safe and correct separation during spinning. In order to balance a centrifuge the following steps should be carried out along with the specific equipment instructions:

- All buckets must be in the rotor during spin otherwise the rotor can be distorted.
- Loads must equal the weight of the opposing bucket.
- Opposing samples must be arranged diametrically (i.e., if you twist the centrifuge around 180^{o} the layout should be the same on each side.)
- A balance of an identical tube of equal weight by filling with an appropriate amount of water should be used if an odd number of tubes are to be centrifuged.

It is important that a centrifuge is not left alone until the centrifuge has reached its required settings. This ensures that there is someone present to stop it if violent vibrations occur. An imbalanced rotor can destroy a centrifuge and also cause serious harm to the user.

The following figures demonstrate examples of balanced and unbalanced loads.

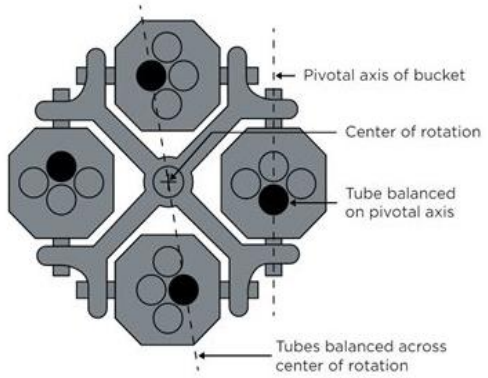


Figure 7: Example of a balanced centrifuge. Assuming all tubes have been filled with an equal amount of liquid, this rotor is balanced. The opposing buckets are loaded with equal number of tubes and are balanced across the centre of rotation.

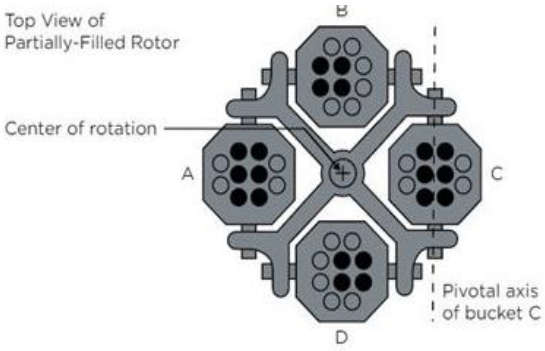
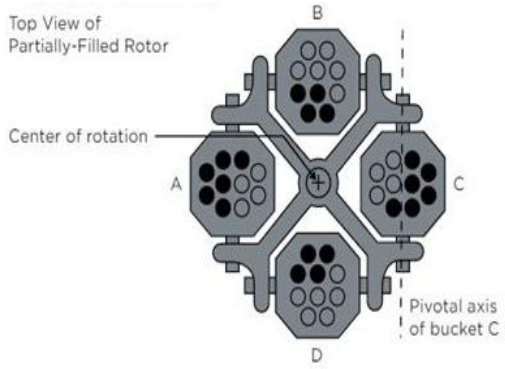


Figure 8: Top and Side-view of a swinging bucket during a correctly balanced load.



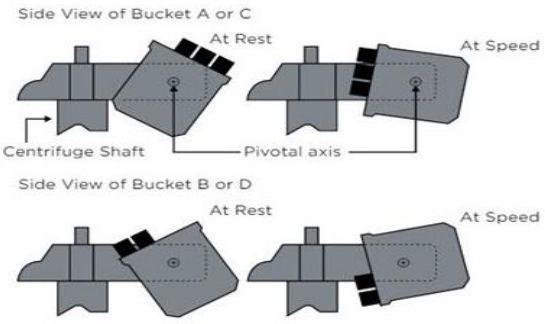


Figure 9: Top view of an unbalanced load. Even if all the tubes are filled equally, this rotor is improperly loaded. None of the bucket loads are balanced with respect to their pivotal axes. At operating speed, buckets A and C will not reach the horizontal position. Buckets B and D will pivot past the horizontal. Also note that buckets B and D are not diametrically opposed.

Settings

Once the centrifuge is correctly loaded, the settings must be accurately entered. Variable settings used while centrifuging a sample can have different outcomes in terms of quality and types of sedimentation. In study lab manuals, the optimum centrifuge settings are to be

followed to ensure that correct separation takes place. These along with a correctly calibrated and routinely maintained centrifuge ensure accurate results.

There are four standard types of settings found on centrifuges.

- **Temperature**
  - Centrifuges are either refrigerated or not refrigerated. Refrigerated centrifuges have a built-in refrigeration unit surrounding the rotor, with a temperature sensor and thermostat permitting selection of a particular temperature or a permissible temperature range that is maintained during centrifugation.
  - Sample collection tubes are normally spun at 4° C or ambient (21°C)
  - Many biological samples are temperature sensitive, and centrifugation at 4°C is frequently required.
  - Whichever temperature is required, the centrifuge must be at the appropriate temperature before spinning commences to ensure sample integrity remains intact.
  - The sample viability may be affected if spun at the incorrect temperature

- **Time (Total duration of spin)**
  - This is the amount of time required for accurate separation of particles.

- **Rotor Speed (RCF/RPM)**
  - Laboratory Manuals give the centrifuge speed in relative centrifugal force (RCF) measured in g (gravity), or in revolutions per minute (RPM).
  - **RPM does not equal RCF and must not be used interchangeably!** They differ, as a setting in RPM will exert a different pressure on the sample depending on the size of the centrifuge, whereas a setting in RCF will ensure the same force is exerted whatever centrifuge is used. This might be important in multi centre studies where the machines are different.
  - A conversion calculation can be done:

$$RCF = 11.18 \times r \times \left(\frac{Q}{1000}\right)^2$$

Where **Q** is the centrifuge speed in **RPM**

r is the radius of the centrifuge rotor (and swing out buckets if being used)

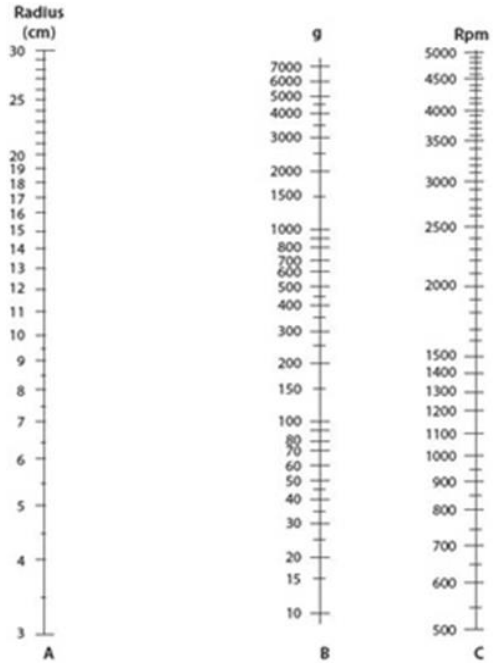


Figure 10: A conversion chart is often included in commercial lab manuals.

- Although newer centrifuges usually allow you to set the speed in RCF, for those that only control speed through RPM suppliers have conversion tools on line for specific centrifuges and rotors.
- It is important that the correct speed is set when spinning samples. There can be a big difference in the outcome of the separated samples if spun too slow/too fast. Using high centrifugal forces is not recommended as this may induce platelet activation and lysis of RBCs. Samples should be spun according to the lab manual requirements. These requirements will be based on the type of tube and tests required. Through centrifugation, one obtains a separation of two particles but any particle in the mixture may end up in the supernatant or in the pellet or it may be distributed in both fractions, depending upon its size, shape, density, and conditions of centrifugation.

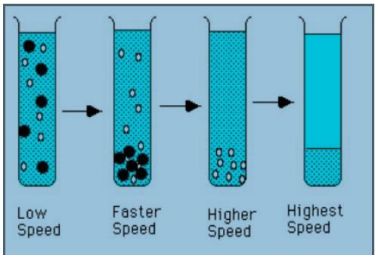


Figure 11: The effects of speed on sample separation

- **Brake settings**
    - The impact of deceleration and acceleration on sedimentation is often perceived to be negligible. However high deceleration can have negative effects on the rotor life.
    - When centrifuging to create gradients such as for the isolation of peripheral blood mononuclear cells (PBMCs) slow deceleration is often essential, so the layers are not disturbed. Lab Manuals will usually state soft brake or no brake.

Sample Quality

Different research studies require samples to be spun in specific ways to ensure accurate results. Failure to follow the given settings causes insufficient separation of the samples leading to can lead to:

- Poor gel formation
- Breakages
- Poor sample quality causing inaccurate results.

Incorrect Spin

Incorrect spinning will not produce the correct separation. In some cases, it might be possible to re-spin the sample (e.g. if it has been remixed by mistake). With SST tubes, serum from the original SST tube must be transferred to a new SST tube and then re-spun as this prevents elevated potassium levels.

Safety when centrifuging:

Modern centrifuges will stop if they are unbalanced however there are extenuating situations, such as a bucket not being secured correctly falling off the rotor or a tube shattering, where they will become unbalanced and start to shake.

Turn off mains supply immediately and follow the sample spillage guide below.

Aerosols

Working in research laboratories usually means working with potentially infectious blood or bodily fluid samples. Statistics show that 80% of all laboratory acquired infections (LAIs) are unsuspected (aerosols). Due to their mode of operation, centrifuges can present a high risk

if not operated correctly. To maintain a desired temperature, air-cooled and refrigerated centrifuges use an air ventilation system. This system discharges warm air from inside the centrifuge into the environment. Infectious agents present in the exhaust air will be dispersed quickly and broadly throughout the laboratory. Tube breakage poses an even greater risk since this can produce a large amount of aerosol. The use of aerosol tight lids or caps will reduce this risk significantly and is essential.

Manual lid opening procedure

In the event of a mains power cut, opening of the lid is prevented by the lid locking safety device. It is recommended to wait for the mains to be switched back on so that this safety device enables the lid to be unlocked. During rotation, the slightest leak from a sample is enough to create an aerosol. Rotation of the rotor can only be detected when the machine is switched on. Opening the lid manually if the rotor is still rotating would disperse the aerosol into the environment. Rotating parts are also a risk as these could come into contact with the user or be ejected. Manual lid unlocking must only be done by someone informed of the danger and of the necessary precautions to be undertaken. Wait 30 mins before opening the lid. Despite the absence of noise, the rotor could still be rotating when you need to open the lid manually. Always set the mains switch to the OFF position, even in the case of a mains power cut.

Further cautions

Never try to bypass the lid safety lock whilst the rotor is spinning. By opening the lid when the centrifuge is still spinning you may be exposed to dangerous pathogens and the risk of injury due to the high speed at which the rota is moving. High voltage is present behind the panels of the centrifuge. DO NOT remove these panels unless the centrifuge has first been disconnected from the power outlet.

Importance of Maintenance

Centrifuge Calibration

To ensure samples are centrifuged accurately and consistently the SPEED, TIME and TEMPERATURE of the centrifuge should be calibrated. Although it is recommended that

centrifuges are calibrated yearly, end users should be vigilant about any change in the appearance of routine samples and changes in the sound of the centrifuge whilst spinning. Calibration certificates confirm that these measurements are reliable, accurate and repeatable when spinning samples according to study lab manuals and are usually required by the Sponsors of clinical research trials. Calibration should be carried out by trained personnel e.g. external supplier

Maintenance

It is important that before learning how to use a centrifuge, users must understand how to maintain a centrifuge. If a centrifuge breaks down due to poor maintenance, there can be a delay in processing samples, preventable accidents may occur and also costly repairs may be required. Staff should follow the manufacturer's instructions for decontamination and cleaning to prevent the risk of infection and to ensure that equipment is not damaged. Only non-corrosive disinfectants and cleaners may be used on metal parts. Do not use strong acids or alkalis for cleaning as this can cause further damage to the centrifuge. Always keep buckets clean and dry to prevent further corrosion. It is advised that the centrifuge should be checked and cleaned on a routine basis.

Example Routine Daily Maintenance tasks:

- Check the rotor and buckets for damage
- Seals are greased and not worn
- Check for any broken glass/tubes.
- Clean in and around the centrifuge according to centrifuge manual instructions.
- Lid kept open to prevent build-up of condensation after refrigerated spins e.g. 4°C.

It is important that maintenance is carried out regularly and recorded on a maintenance log. Centrifuge maintenance varies on the type of centrifuge so specific maintenance instructions can be found in the centrifuge instruction manuals and must be adhered to. It is advisable that an **annual service** is arranged by an external engineer. A calibration of the centrifuge can be arranged during this visit also.

Routine Weekly Maintenance tasks

- Centrifuges should be routinely cleaned once a week with 10% Chemgene and 70% IMS, or equivalent.
- Make detailed inspection of the centrifuge rotor, rotor lid and buckets for cracks and corrosion. For a fixed angle rotor, check underneath the rotor as well as the tube bores. For a swing bucket rotor, pay special attention to the pivots and grooves.
- The bucket pins should be cleaned and lubricated monthly using the supplied centrifuge grease to allow free swing.
- Do not scratch the protective anodised surface of the buckets. Light use of a plastic scouring pad is acceptable in extreme circumstances.

Cleaning of the centrifuge rotor

The cleaning of the rotor should be carried out every 200 spins. Some centrifuges have a message that appears when it is due, for others the centrifuge logbook can be used to see when this cleaning needs to be done. Cleaning of the rotor will only be carried out by those trained to do so. External users should inform the laboratory manager/supervisor if this message appears – they are not permitted to remove the rotor from the centrifuge themselves.

Cleaning of small sample spillages

- Always wear a laboratory coat, gloves and goggles. This will minimise the risk of dermal or mucous contact with the material (e.g., splatter, droplets)
- Switch off the centrifuge and disconnect it from the power supply before starting any cleaning or disinfection.
- Remove all tube adaptors from buckets.
- Use Chemgene and tissue to clean up any small spillages before spraying with IMS
- Ensure all parts are completely dry before reassembling as trapped moisture will cause corrosion.
- Disinfect hands before washing them thoroughly.

Cleaning of large sample spillages/tube breakage

- Always wear a laboratory coat, gloves and goggles. This will minimise the risk of dermal or mucous contact with the material (e.g., splatter, droplets)

- Switch off the centrifuge and disconnect it from the power supply before starting any cleaning or disinfection.
- Do not open the sealed bucket lid for at least 30 mins after the run to allow the aerosols to settle.
- NB: if bucket lids were not used to contain spillages there is an extremely high risk of aerosol contamination on opening the centrifuge. Once identified, close the centrifuge and leave for 30 mins to allow aerosols to settle.
- If there is broken glass: retrieve bigger pieces with forceps, remove small shards and glass powder with damp lint-free cloth.
- Absorb blood with gauze or paper towel and seal the material in a biohazard bag for subsequent safe disposal.
- Wipe contaminated parts with 10% Chemgene.
- If necessary, remove contaminated rotors, rotor lids, buckets and bucket caps and soak in 10% Chemgene for 15 mins before rinsing in warm water then spraying with IMS.
- Leave the centrifuge out of action for 24 hrs then respray with IMS.
- Ensure all parts are completely dry before reassembling as trapped moisture will cause corrosion.
- Disinfect hands before washing them thoroughly.

Annual cleaning and Maintenance

It is important that maintenance is carried out regularly and recorded on a maintenance log. Centrifuge maintenance varies on the type of centrifuge so specific maintenance instructions can be found in the centrifuge instruction manuals and must be adhered to. When using refrigerated centrifuges, clean the refrigeration mesh of the heat exchanger with a brush at least twice a year. It is advisable that an **annual service** is arranged by an external engineer. A calibration of the centrifuge can be arranged during this visit also.

Check List

- The rotor is free of damage and spillage and any ‘o’ring seals are greased
- The seals of the buckets or lids aren’t damaged
- The adaptors are correct for your tubes – different sizes of bucket inserts are available for different size tubes.
- The samples are balanced correctly

- Lids are attached securely - Ensure the rotor and all lids are tightened correctly before use (but not over tightened as centrifugation can tighten even further and make it difficult to open the buckets).
- The swinging buckets swing freely
- The speed intended for centrifuging samples does not exceed the maximum for rota.
- Operator stands at a safe distance (at least 1m) whilst centrifuge reaches maximum speed.

Slide Set

A slide set is available for this topic that can be downloaded from the UKCRF Network Portal (see page 5 for more details).

Workshop Activities

Slide 20 of this session is a media clip of centrifugation. This is a useful tool to deliver teaching through a new medium to keep the attention of the attendees. It also allows them to visualise the process. If delivering the workshop as a full day, the media clip could be used here; otherwise, if this is an individual session, it might be more applicable to demonstrate the techniques immediately. The media clip can be found on the UKCRF Network Portal (see page 5 for more details) as well as on the Network's YouTube page.

A practical activity is essential to demonstrate safe centrifuge loading, balancing and setting, and allow the attendees to practise these skills. This can be done at the end of this session or, if delivering a full day workshop, the practical elements can be carried out all together in one practical session.

Suggested Handouts

A Good Centrifuge Practice Handout is available. This may be useful for the attendee to use whilst completing practical tasks. Competency documents can be used for the attendees to demonstrate safe centrifuge skills.

A full list of teaching resources can be found in appendix 2 and all resources can be downloaded for free from the UKCRF Network Portal (see page 5 for more details).

6. Principles and Practice of Pipetting

Learning Objectives

- To demonstrate knowledge of pipetting theory and practice

Background Information

A pipette is a piece of lab equipment which is used to dispense a measured volume of liquid. A pipette works by creating a vacuum above the liquid-holding chamber and selectively releasing this vacuum to draw up and dispense liquid. There are several different types of pipette. The most common types are transfer pipettes and air displacement pipettes.

Disposable/Transfer Pipette

A transfer (Pasteur) pipette is the most common type of pipette which is provided in pre analytical laboratory kits. There are several designs, and some have graduated markings for volumes on the side (normally 0.5mL, 1mL etc.). When only an approximate amount of serum/plasma is required, transfer pipettes are sufficient. This type of pipette is not calibrated and relies on a visual inspection by the user to confirm the correct amount has been aspirated.

- **Transfer pipette technique**
    1. Hold the pipette at 90°.
    2. Apply pressure to Bulb.
    3. Insert pipette into sample just below the top of the serum or plasma layer.
    4. Release pressure until the required amount of sample has been aspirated into the pipette, moving down with the plasma/serum level as it is removed to stay below the surface whilst avoiding the red blood cells.
    5. Leave 0.5mL plasma/serum in the tube to avoid contamination with red blood cells.
    6. Do not apply/release any pressure until the pipette has been removed from the sample and placed into the transport container.
    7. Slowly reapply pressure to the bulb and dispense liquid at a 45°C angle against the inside of the wall.

8. Once all has been dispensed, touch the tip of the pipette against the inside of the transport container wall to allow for any liquid on the outside of the tip to be dispensed.
9. Dispose of pipette once finished with collection tube

- **Key Points:**
    - Different blood collection tubes may/may not contain different additives which if mixed may affect test results. Therefore it is important to use a separate pipette for each type of tube per patient. This ensures that the serum/plasma is not cross-contaminated.
    - It is also important not to use the same pipette when dealing with two different patients. This can cause a mixture of patient samples which will cause incorrect and unidentifiable results.
    - Once a sample has been centrifuged and sedimented, it is important that the correct pipetting technique is used. The bulb of the pipette must be "squeezed" before placing it in the serum/plasma. If squeezed while in the spun sample, this can cause the sample to remix which can lead to re-spinning, increased turnaround time and possible inaccurate results due to haemolysis.
    - If a sample has been remixed even slightly, the serum/plasma should not be transferred until re-spun.
    - Only the top section i.e. Plasma/serum should be transferred unless the lab manual includes other requirements. If the "middle section" i.e. Buffy coat or "bottom section" i.e. Red blood cells are interfered with by accident, the sample should be re-spun.
    - Once the sample has been taken up it is not good practice to return the sample back into the collection tube unless re-spinning is required. This will cause the sample to remix.

Air-Displacement Pipette

Air-Displacement pipettes are the recommended pipette for accurate laboratory work. These are used when smaller volumes are to be dispensed. The same principles of transfer pipettes apply to air-displacement pipettes. When the push-button is pressed, the piston inside the instrument moves down to let air out.

Air is displaced by the piston. The volume of air displaced is equivalent to the volume of liquid aspirated. Pipette volume ranges are usually presented on the side of the pipette. Do not turn the pipette past its minimum or maximum setting as this may damage the mechanism and cause the pipette to go out of calibration.

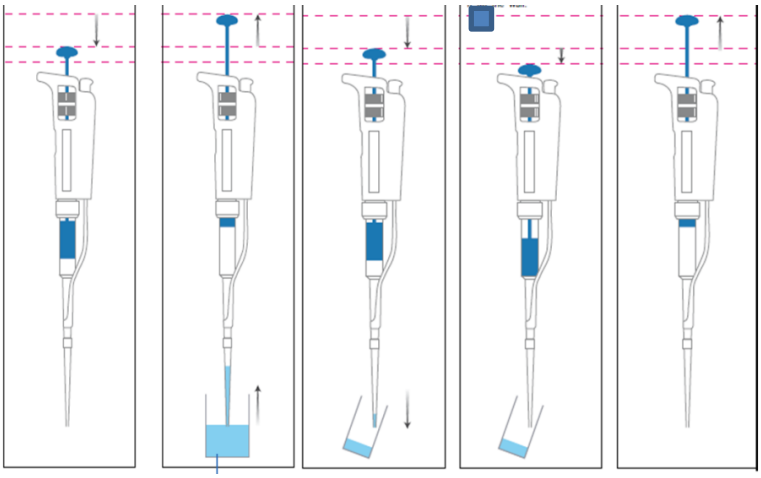
If pipettes are not supplied with the laboratory kits, there are a number available on the market designed to be ergonomic. When choosing the pipette it should be as close to the volume you wish to pipette, for example:

- 200-1000µl for volumes >200µl
- 200µl for volumes between 20-200µl
- 20µl for volumes between 5-20µl
- 5µl for volumes between 1-5µl
- 2µl 0.5-2µl –consider if this is accurate enough- is there an alternative such as diluting the samples or reagents.

Basic rules for selection of a pipette are that they become less accurate when used at the lower end of their range so always use the highest volume pipette possible.

Pipetting Technique

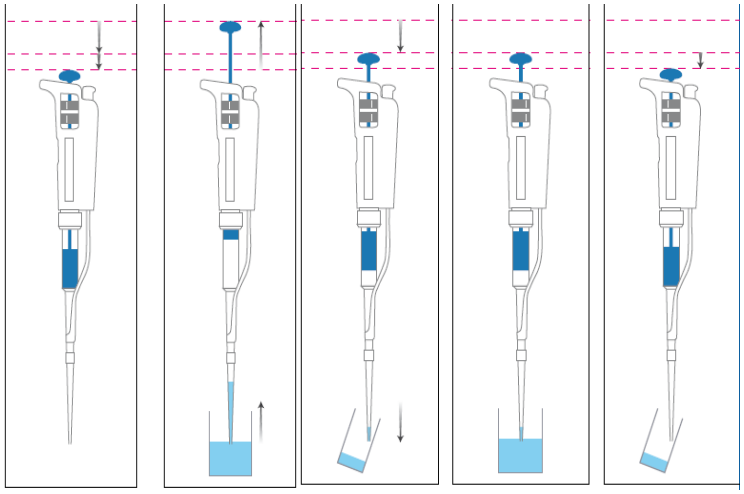
Check that the pipette is not blocked or contaminated, and that the tips to be used are the appropriate size and recommended by the manufacturer. A new pipette tip is recommended for each sample (tube). For biological samples consider using filtered tips to prevent contaminating the pipette. Pipette tips should be discarded into a sharps bin as they have the potential to cause puncture wounds if discarded into domestic or clinical waste and are potentially biologically contaminated.



Depress Plunger smoothly to the **first** stop position.

Immerse pipette into liquid and allow

Figure 12: Forward pipetting technique



Depress Plunger smoothly to the **second** stop position.

Immerse pipette into liquid and allow plunger to release up.

Depress

Figure 13: Reverse pipetting technique

Repetitive Strain Injury (RSI)

To avoid RSI the workstation should be set up so it is ergonomic, with the researcher sitting comfortably and does not have to stretch to reach a sample and apply or discard pipette tips. There are a number of ergonomic pipettes that are available on the market which are as accurate as conventional ones. If excessive numbers of aliquots are required from the same sample then use of an electronic or repetitive pipette should be considered.

Calibration

The calibration of a pipette should be checked regularly, depending on the frequency of use, (but at least once a month) by weighing the weight of water displaced at minimum recommended volume, a midrange volume and maximum recommended volume of the pipette. The accuracy of the pipette should be determined by taking three readings at each

setting. The balance used should be calibrated so changes in the weight of the water dispensed by the pipette can be attributed to pipette inaccuracies rather than the balance. Pipettes should be calibrated at least every 12 months; reliable external suppliers are available for this.

- **Key points**
  - Check the barrel of the pipette is not blocked or contaminated.
  - Always use the appropriate pipette for the volume
  - Do not immerse the pipette in the sample just the tip
  - Use a different tip for each sample and discard safely
  - Regularly check the calibration of the pipette and have pipette calibrated

Slide Set

A slide set is available for this topic that can be downloaded from the UKCRF Network Portal (see page 5 for more details).

Workshop Activities

Slide 7 of this session is a media clip of pipetting. This is a useful tool to deliver teaching through a new medium to keep the attention of the attendees. It also allows them to visualise the techniques. If delivering the workshop as a full day, the media clip could be used here; otherwise, if this is an individual session, it might be more applicable to demonstrate the techniques immediately. The media clip can be found on the UKCRF Network Portal (see page 5 for more details) as well as on the Network's YouTube page.

A practical activity is essential to demonstrate accurate pipetting, and allow the attendees to practise the skills. This can be done at the end of this session or, if delivering a full day workshop, the practical elements can be carried out all together in one practical session.

Suggested Handouts

A handout of the key points noted in this section could be given to the attendees to remind them of the correct process during a practical session. Competency documents can be used for the attendees to demonstrate accurate pipetting skills.

A full list of teaching resources can be found in appendix 2 and all resources can be downloaded for free from the UKCRF Network Portal (see page 5 for more details).

7. Tracking and Storage of Research Samples

Learning Objectives

- To understand that incorrect storage and tracking of samples can affect sample integrity and patient safety.
- To describe correct sample labelling and storage methods.
- To describe correct documentation for tracking and storage of samples.

Background Information

In clinical trials, blood samples are sent as whole blood to hospital laboratories for analysis, or as separated components to central, study specific laboratories. Separation of the blood components preserves the sample integrity whilst being transported to a laboratory. Either route requires accurate labelling and tracking to ensure patient safety and accurate trial results.

In order to guarantee high quality, robust data collection and integrity of samples whilst maintaining the confidentiality of study participants, it should be made certain that any study sample can be traced from its point of origin to its final storage place or destruction.

To ensure the quality of this process, laboratories should have Standard Operating Procedures (SOPs) or documented study specific procedures, detailing labelling, storage and tracking processes. SOPs or documented policies should cover all key activities; examples for storage and tracking include, but are not limited to:

- Procedures for the receipt, storage, processing and transfer of samples and reference materials.
- Procedures for the analysis and reporting of clinical trial samples.
- Procedures for dealing with the receipt of unexpected, unscheduled or poorly labelled samples.
- Policies that control the installation, validation, calibration, maintenance and servicing of apparatus, equipment and computerised systems.

Sample labelling

Correct labelling enables logging and tracking of samples from start to finish. It also prevents harm to others that unintentionally come into contact with that sample. Individual samples should be clearly, accurately and concisely labelled to allow unambiguous identification. Confidentiality should be maintained at all times so that the safety and rights of any clinical trial subject, who has provided the study samples, are not compromised. Details to go on the tube include:

- Study sample type e.g. plasma, serum,
- Time and date collected
- Unique identifier, number or barcode
- Study number or name

Use of fridge/-80^{O}C freezer suitable pens and labels is recommended. The study reference number, ID of participant, visit number and date /time of sample acquisition should be written on the label. To add the name of the Principal Investigator is recommended, though this may be referenced on the accompanying sample requisition form.

Labels may need to be affixed in a certain way so as not to occlude expiry dates on collection tubes or to avoid covering up information on the study label e.g. labels should be placed lengthways and not around the tube.

Study SOPs or laboratory specific protocols, however, may dictate how individual study samples should be labelled and how those labels should be applied. Some labels will be supplied pre-printed for the study with only the date and time to be added. Other labels will be completely pre-printed with bar code identification where an electronic scanning system is used for tracking and storage of samples.

ALWAYS follow the study protocol, lab manual and SOPs for the trial you are working on.

Storage of samples

Many samples are stored in freezers after separation and prior to shipment to central labs. Both tubes and labels need to withstand temperature conditions to avoid them becoming unidentifiable. Where duplicate sets of samples are produced (primary and backup) there should be separation of these sets for storage as a contingency method. Temperature of

the storage area should be monitored to demonstrate correct storage conditions and there should be contingency plans in place should the storage fail so sample integrity is not affected. There should also be a disaster recovery plan in place with a clear plan of action in case of storage and/or equipment breakdown. Routine maintenance should be carried out to limit ice build-up and damage to doors and seals. Care must be taken to avoid freezer burns so appropriate PPE must be worn when storing or removing samples or scraping ice build-up.

Sample Requisition Form

A sample requisition form will accompany a blood sample to the laboratory for analysis. This may be a local Laboratory Request Form if the sample is being analysed at the local hospital laboratory or a study specific form if the sample is being sent to a central laboratory. In addition to the information on the tube, the following should be included on the sample requisition form:

- Type of sample taken
- Study ID
- Date and time collected.
- Details of any pre-processing at a local laboratory before transporting for further processing and analysis e.g. centrifugation/incubation/freezing

Samples and accompanying requisition forms should have no identifiable information other than that required by the study. If it is found that confidentiality has been compromised then identifiable information should be covered or masked. This should be done in a way that does not obliterate any study required labelling. Anonymised study identifiable labelling must remain visible on the sample in order for analysis and evaluation to be performed. In hospital laboratory settings confidentiality is not normally an issue when local procedures are being followed. It must be highlighted though that the study protocol must be followed to ensure blinding is not compromised.

Tracking of samples

There should be an audit trail of a sample from acquisition to final analysis/storage or destruction. The custodian of that sample is the PI. However, there should be a chain of custody following the sample from the beginning to the end of the study.

If a computerised system is used to track samples, computer software validation should be performed on a regular basis for QA purposes. This should be documented and held in the site file. When a sample has been acquired and labelled a tracking log should be completed in preparation of transport/transfer to the laboratory.

All data should be recorded directly, promptly, accurately and legibly. It should be possible to determine the date the sample was taken, transported, analysed or an evaluation performed. The identity of the person responsible for each part of the chain should be recognisable from the documentation. Any change to the data has to be auditable. Who has changed it, why and when? The change should be justified and should be documented.

A process should be put in place to track the movement of each sample from arrival to analysis or evaluation. This practice should be maintained throughout the study and for each sample collected.

A sample log should be used to document that the sample has been collected and a copy may accompany the sample to the lab. Maintain any original log at site. This should include details included on the specimen collection vessel as described above but also the following:

- Date/time taken/sent to Laboratory
- Date/time of receipt at lab
- Storage location; e.g. freezer, rack/tray/position
- Where appropriate, log the date that the sample is to be destroyed by - For example as per protocol, or the consenting agreement with participant

Sample Transit

Samples need to be transported so that their integrity and viability remains unaffected regardless of length of transport time or distance. Whether the samples are being transported from one laboratory to another within the same organisation or transported further afield, things to consider include:

- The time samples remain in transit
- Temperature monitoring during transit

- If not refrigerated or frozen, the types of conditions they may encounter during transit time

All specimens must be delivered to the correct laboratory in a timely manner to avoid any delay in processing. Local policies for NHS laboratories highlight transport arrangements where specimens are under certain conditions - specimens sent in ice, specimens marked emergency or those which require special transport instructions must be delivered to the correct laboratory in a timely manner to ensure the integrity of the sample. These local procedures will usually specify how any samples are to be transported.

- Ensure all sample bags are sealed and appropriately labelled if “High Risk” if required by hospital policy
- Do not carry samples by hand – samples can be dropped or lost. Use an appropriate secondary transport bag.
- Do not put samples in your pockets.
- Do not drop or manhandle specimens in a rough manner.
- Do not remove specimens and or Laboratory Request Forms from the plastic bag.
- In the event of a sample spillage or broken container DO NOT leave unattended but ask a member of staff to contact the appropriate department in your locality e.g. Porter Control, stating where the spill or broken container has occurred. Porter Control will instruct a member of their staff to clean and dispose of the spill/breakage safely.
- Inform your clinic area/ ward area that the sample(s) which have been spilt or leaked are unsuitable for analysis and repeat specimens are required (subject to study protocol).

Communication and collaboration with local staff is important where samples are acquired and taken to clinical laboratories for testing. Use of designated staff experienced in transportation of specimens should be considered. Otherwise, research staff compliance with local rules is essential to maintain safety.

Study protocols may indicate differing procedures for study samples and these instructions should be followed. Where samples are to be transported at a particular temperature then it may be required to use temperature logs to monitor the temperature during transit. Where samples are being transported ‘off-site’ this may be possible from some couriers with ‘data

loggers'. The MHRA do require confirmation that samples were transported at the intended temperature.

Storage

In the feasibility stage it should be considered whether the facilities for storage are appropriate. Are storage facilities within the same building or on another site and what issues are there for the transit of the samples between venues?

Freezer logs should be kept and updated when samples are received into the storage facility/laboratory. These should detail the name/number of the freezer the samples have been placed into. A storage grid can be used to include the number of the shelf and place in the storage box (see example grid in Fig: 14) Where possible, best practice is to separate duplicate samples so that not all are kept in the same freezer. This is an advantage in case of equipment failure.

Name of Trial **Trial Number** **Type of Samples** **Box no** **Location**

	1	2	3	4	5	6	7	8	9	10
A										
B										
C										
D										
E										
F										
G										
H										
I										
J										

Figure 14: Example of a sample grid for freezer storage box

The study may have access to an electronic storage system whereby the bar code labels are used on both the sample storage vessels and freezer shelves/boxes. These are then scanned to automatically record where they are kept. A spare bar code label is often assigned to a hard copy storage log as a further back up.

Monitoring of refrigerators and freezers should be undertaken to ensure they are operating within acceptable parameters and evidence of the monitoring should be documented and retained in the study file. If temperature ranges are given within a protocol/laboratory manual then these must be adhered to. Written procedures should be in place with instructions to follow if there is a breach of these acceptable limits. There should be provision of a backup refrigerator and freezer with capacity for samples to be stored in the event of breakdown of equipment. All staff involved in the handling and storage of samples should be aware of these procedures and there should be clear details of who to contact 'out of hours' in the event of an emergency.
The action taken in the event of temperature excursions should also be documented and retained. Equipment used for the purpose of monitoring temperatures should be subject to periodic calibration. Checks on what the local or study specific requirements are, should be investigated and established prior to study start.

Researchers need to consider what to do if a participant wishes to withdraw from a study. When a researcher applies to the Research Ethics Committee (REC) they need to specify how and if data will be used after withdrawal from the study. This should be clear in the protocol and all staff should be aware if samples need to be destroyed at this point. Documentation of discussions and actions taken in the event of participant withdrawal is required.

Sample Receipt

- When study samples arrive in the laboratory, the receiving personnel should check them for physical integrity. The study staff (including PI and Sponsor) should be notified if this has been compromised in any way.
- The date and time of receipt should be logged. A check should be made that all expected samples are accounted for. These activities should all be documented as evidence of compliance with the study protocol.
- If samples have been received that are unexpected, poorly labelled or samples are missing from the consignment then the study team (including PI and Sponsor) should be notified so the issue can be investigated and resolved.
- If the identity of the sample is not known it should not be analysed until the identity of the sample is confirmed. However, if the integrity of the sample would be compromised by a delay in analysis then processing should go ahead. The result should then be quarantined until the identity of that sample has been established.

The sample should only be destroyed after concerted efforts to find the identity have failed. In this instance the results should not be reported.

- Version controlled documented policies which detail the process of receiving and dealing with missing, unexpected or poorly labelled samples should be available. Laboratory Staff should be aware of these and comply with them by keeping documentary evidence of their activities.
- Auditing of the sample receipt procedure should be performed via regular monitoring to monitor the receipt and accurate 'logging in' of samples. This should be part of regular Quality Control (QC) checks which helps to ensure compliance with laboratory policies is being upheld. Where these checks are not routinely carried out, the integrity of the data produced by those laboratories may be in question.

Quality Assurance (QA)

QA processes should be in place to ensure the following when dealing with study:

- Patient safety and confidentiality are not compromised.
- Analysis and/or evaluation of study samples are conducted in compliance with the principles of Good Clinical Practice.
- Analysis and/or evaluation of study samples are conducted in accordance with the protocol and in line with any contract agreement/work instruction and associated methods.
- Laboratory policies and SOPs are adhered to.
- Trial data is recorded and reported accurately, legibly, completely and in a timely fashion.
- At the end of the study, the data is archived.
- It should be noted that any staff involved in the QA processes should not be directly involved in generating any of the study data.

Documents used in tracking the sample, validation of testing methods, analysis and results need to be retained in accordance with the requirements of GCP and any relevant national legislation.

Slide Set

A slide set is available for this topic that can be downloaded from the UKCRF Network Portal (see page 5 for more details).

Workshop Activities

A group work session can be done here to question how they would label and track certain samples, and a worksheet is available on the UKCRF network Portal. An alternative method would be to ask the group to label and log samples in a practical laboratory based exercise.

Suggested Handouts

Competency documents can be used for the attendees to demonstrate accurate tracking and labelling. A full list of teaching resources can be found in appendix 2 and all resources can be downloaded for free from the UKCRF Network Portal (see page 5 for more details).

8. Dangerous Goods Regulations

Learning Objectives

- Understand the legal requirements for safe packaging of Dangerous Goods
- Describe the safety measures required when handling dry ice

Background Information

All persons involved in the transport of dangerous goods are required to have appropriate training. Research trial sponsors regularly ask for a certificate of proof for this training for all research staff involved in shipping samples. The International Air Transport Association (IATA) have detailed a minimum training criteria for Dangerous Goods Training and the UK Civil Aviation Authority (CAA) have compiled a list of CAA approved Dangerous Goods Training organisations. This course is **not** approved by the CAA but it does include all the training elements required by IATA. The CAA has advised that a member of staff can disseminate the Dangerous Goods Training within their department if they have completed an approved course themselves. **The *UKCRF Lab Workshop Project Group* therefore advises that the trainer for this section undertakes an approved course if a separate certificate for Dangerous Goods (IATA) training is to be issued to the participants.** Details of approved courses can be found by clicking here.

Trainers should also deliver at least 1 course annually to maintain competence.

What is Dangerous Goods training?

The IATA *Dangerous Goods Regulations* is an easy-to-use manual based on the International Civil Aviation Organization (ICAO) *Technical Instructions for the Safe Transport of Dangerous Goods by Air*. It incorporates additional operational requirements, which provide a harmonised system for operators to accept and transport dangerous goods safely and efficiently on all commercial air transport. There are also similar regulations for transport of goods safely by road, rail and sea and the differences for air transport are highlighted later in this booklet.

What is IATA?

IATA is an international trade body, created over 60 years ago by a group of airlines. Today, IATA represents 290 airlines comprising 82% of total air traffic. The organisation also represents, leads and serves the airline industry in general.

Dangerous Goods

Dangerous goods are defined as those goods which meet the criteria of one or more of the nine UN hazard classes and, where applicable, to one of the three UN packing groups. They are capable of posing a risk to health, safety, property or the environment.

The IATA *Dangerous Goods Regulations*

Dangerous goods can be transported safely by air transport provided certain principles are strictly followed. Packaging is the essential component in the safe transport of dangerous goods by air. The IATA *Dangerous Goods Regulations* classify dangerous goods and provide packing instructions acceptable for air transport, with a wide range of options for inner, outer, and single packaging. The packaging instructions require the use of UN performance-tested specification packaging.

Training requirements

By law, any person who is involved with shipping dangerous goods by air, road, sea, rail, or inland waterway, must receive training appropriate to their job. This includes those who:

- Load, unload, or handle dangerous goods
- Label, mark or package dangerous good shipments
- Prepare documentation for dangerous good shipments
- Operate a vehicle transporting dangerous goods

An untrained (un-certified) individual may handle dangerous goods provided the goods are handled in the presence and under the direct supervision of an individual who holds a training certificate. A transport of dangerous goods certificate must be held whenever preparing or receiving dangerous goods for shipment. The certificate must be provided to an inspector upon request and must include:

- the individual's name
- the most recent training and assessment completion month

- a description, copy or reference to training and assessment materials used to meet the training and assessment requirements
- the name and address of the organisation providing the training and assessment
- evidence which shows that personnel have been assessed as competent to perform their responsibilities.

The employer must maintain a record of training and assessment for personnel.

Current Legal Documents

Air

- Technical Instructions for the Safe Transport of Dangerous Goods by Air (ICAO) - These are the legally binding international regulations. The International Air Transport Association (IATA) publishes Dangerous Goods Regulations (DGR) that incorporate the ICAO provisions. The ICAO rules apply on all international flights.
- The Air Navigation Order 2009 SI 3015 provides the legal basis for the Air Navigation (Dangerous Goods) Regulations 2002 (SI 2786) (As amended)

Road and Rail

- European Agreement Concerning the International Carriage of Dangerous Goods by Road (ADR)
- Regulations concerning the International Carriage of Dangerous Goods by Rail (RID)
- The Carriage of Dangerous Goods and Use of Transportable Pressure Equipment Regulations 2009 (2009 No 1348) (As Amended)
- The Carriage of Dangerous Goods and Use of Transportable Pressure Equipment Regulations (Northern Ireland) 2010

Maritime

- International Maritime Dangerous Goods Code (IMDG)
- The Merchant Shipping (Dangerous Goods and Marine Pollutant) Regulations 1997 (SI 2367)

Classification of Dangerous Goods

Class	Type of material
1	Explosive substances and articles
2.1	Flammable gas (e.g. butane)
2.2	Non-flammable and non-toxic gases which could cause asphyxiation (e.g. nitrogen, helium, carbon dioxide) or oxidisers (e.g. oxygen)
2.3	Toxic gases (e.g. chlorine, phosgene)
3	Flammable liquids (e.g. lighter fluid, petrol)
4.1	Flammable solids, self-reactive substances and solid desensitised explosives
4.2	Substances liable to spontaneous combustion
4.3	Substances which, in contact with water, emit flammable gases
5.1	Oxidising substances
5.2	Organic peroxides
6.1	Toxic substances
6.2	Infectious substances
7	Radioactive material
8	Corrosive substances
9	Miscellaneous dangerous substances and articles

Class 6.2 - Infectious Substances

Infectious substances are substances which are known, or are reasonably expected to contain pathogens. Pathogens are defined as microorganisms (including bacteria, viruses, rickettsiae, parasites, fungi) and other agents such as prions, which can cause disease in humans or animals. They are subdivided into Category A and B substances.

Category A is an infectious substance which is transported by any mode in a form that, when exposure to it occurs, is capable of causing permanent disability, life-threatening or fatal disease to humans or animals.

Materials containing Category A must be consigned/shipped as either UN2814 INFECTIOUS SUBSTANCE AFFECTING HUMANS or UN2900 INFECTIOUS SUBSTANCE AFFECTING ANIMALS only, as appropriate. A pathogen that affects both humans and other animals is assigned to UN2814.

In addition, if there is any doubt as to whether or not a pathogen falls within this category, it must be transported as a Category A infectious substance. Clinical wastes containing Category A infectious substances shall be assigned to UN2814 or UN2900 as appropriate.

Category B is an infectious substance, referred to as a biological substance, which does not meet the criteria for inclusion in Category A and must be consigned / shipped as UN3373 BIOLOGICAL SUBSTANCE.

For the purpose of guidance, UN3373 could be deemed to include patient specimens of human or animal material including, but not limited to, excreta, secreta, blood and its components, tissue and tissue fluid swabs, and body parts being transported for purposes such as research, diagnosis, investigational activities, disease treatment or prevention.

Examples of Infectious Substances Assigned to Category A

These entries are for infectious substances carried in any form, unless otherwise indicated. Note: The following list is not exhaustive. Infectious substances, including those containing new or emerging pathogens, which do not appear in the following list but which meet the same criteria, must be transported as a Category A infectious substance. In addition, if there is doubt as to whether or not a pathogen falls within this category it must be transported as a Category A infectious substance.

UN2814 Infectious Substances Affecting Humans

*For land modes only: when cultures are intended for diagnostic or clinical purposes, they may nevertheless be classified as Category B

Bacillus anthracis (cultures only)

Brucella abortus (cultures only)

Brucella melitensis (cultures only)

Brucella suis (cultures only)

Burkholderia mallei–Pseudomonas mallei–Glanders (cultures only)

Burkholderia pseudomallei–Pseudomonas pseudomallei (cultures only)

Chlamydia psittaci–avian strains (cultures only)

Clostridium botulinum (cultures only)

Coccidioides immitis (cultures only)

Coxiella burnetii (cultures only)

Crimean-Congo haemorrhagic fever virus

Dengue virus (cultures only)

Eastern equine encephalitis virus (cultures only)

Escherichia coli, verotoxigenic (cultures only)

Ebola virus

Flexal virus

Francisella tularensis (cultures only)

Guanarito virus

Hantaan virus

Hantavirus causing hemorrhagic fever with renal syndrome

Hendra virus

Hepatitis B virus (cultures only)

Herpes B virus (cultures only)

Human immunodeficiency virus (cultures only)

Highly pathogenic avian influenza virus (cultures only)

Japanese Encephalitis virus (cultures only)

Junin virus

Kyasanur Forest disease virus

Lassa virus

Machupo virus

Marburg virus

Monkeypox virus

Mycobacterium tuberculosis (cultures only)

Nipah virus

Omsk haemorrhagic fever virus

Poliovirus (cultures only)

Rabies virus (cultures only)

Rickettsia prowazekii (cultures only)

Rickettsia rickettsii (cultures only)

Rift Valley fever virus (cultures only)

Russian spring-summer encephalitis virus (cultures only)

Sabia virus

Shigella dysenteriae type 1 (cultures only)

Tick-borne encephalitis virus (cultures only)

Variola virus

Venezuelan equine encephalitis virus (cultures only)

West Nile virus (cultures only)

Yellow fever virus (cultures only)

Yersinia pestis (cultures only

UN 2900 Infectious Substances Affecting Animals Only

African swine fever virus (cultures only)

Avian paramyxovirus Type 1 -Velogenic Newcastle disease virus (cultures only)

Classical swine fever virus (cultures only)

Foot and mouth disease virus (cultures only)

Lumpy skin disease virus (cultures only)

Mycoplasma mycoides -Contagious bovine pleuropneumonia (cultures only)

Peste des petits ruminants virus (cultures only)

Rinderpest virus (cultures only)

Sheep-pox virus (cultures only)

Goatpox virus (cultures only)

Swine vesicular disease virus (cultures only)

Vesicular stomatitis virus (cultures only)

Exemptions from the Regulatory Provisions

1. Substances which do not contain infectious substances or substances which are unlikely to cause disease in humans or animals are not subject to the specific modal requirements unless they meet the criteria for inclusion in another class.
2. Substances containing microorganisms which are non-pathogenic to humans or animals are not subject to the specific modal requirements unless they meet the criteria for inclusion in another class.
3. Substances in a form that any present pathogens have been neutralized or inactivated such that they no longer pose a health risk are not subject to the specific modal requirements unless they meet the criteria for inclusion in another class.
4. Environmental samples (including food and water samples) which are not considered to pose a significant risk of infection are not subject to the specific modal requirements unless they meet the criteria for inclusion in another class.
5. Dried blood spots, collected by applying a drop of blood onto absorbent material, or faecal occult blood screening tests.
6. Blood or blood components which have been collected for the purposes of transfusion or for the preparation of blood products to be used for transfusion or transplantation and any tissues or organs intended for use in transplantation are not subject to the specific modal requirements.
7. Human or animal specimens for which there is minimal likelihood that pathogens are present are not subject to the specific modal requirements if the specimen is transported in a packaging which will prevent any leakage and which is marked with the words “Exempt human specimen” or “Exempt animal specimen”, as appropriate.

PLEASE NOTE THE FOLLOWING INFORMATION BELOW FOR FURTHER GUIDANCE ON EXEMPTION 7 ABOVE.

It is the opinion of UK authorities that exemption 7 can only apply to substances which are known not to contain pathogens (for example following testing or action taken to neutralise or inactivate any pathogens present). Expert medical advice in the United Kingdom is that it is not always possible to determine which human or animal samples contain pathogens to some degree and consequently it cannot be assumed that, for example, a blood sample taken for the purposes of cholesterol level testing, will necessarily be exempt from the requirements.

It is therefore strongly recommended that unless it is known or reasonably believed to contain infectious substances of Category A, all human or animal material should be regarded as UN 3373; failure to do so may result in the inadvertent consignment of Category B infectious substances not in compliance with modal regulations, for which legal penalties may result.

Current Packaging, Marking, Labelling and Documentation Requirements

- Category A must follow Packing Instruction 620
- Category B must follow Packing Instruction 650

Packing Instruction 650

This course will concentrate on Packaging Instruction 650

Differences for AIR carriage are underlined

Selecting the proper packaging is one of the most important functions of the shipper. With proper packaging you ensure the safety of those who may handle or unpack the contents of the package. Proper packaging ensures that the material is not released during transportation. The shipper is responsible for all aspects of the packing of dangerous goods.

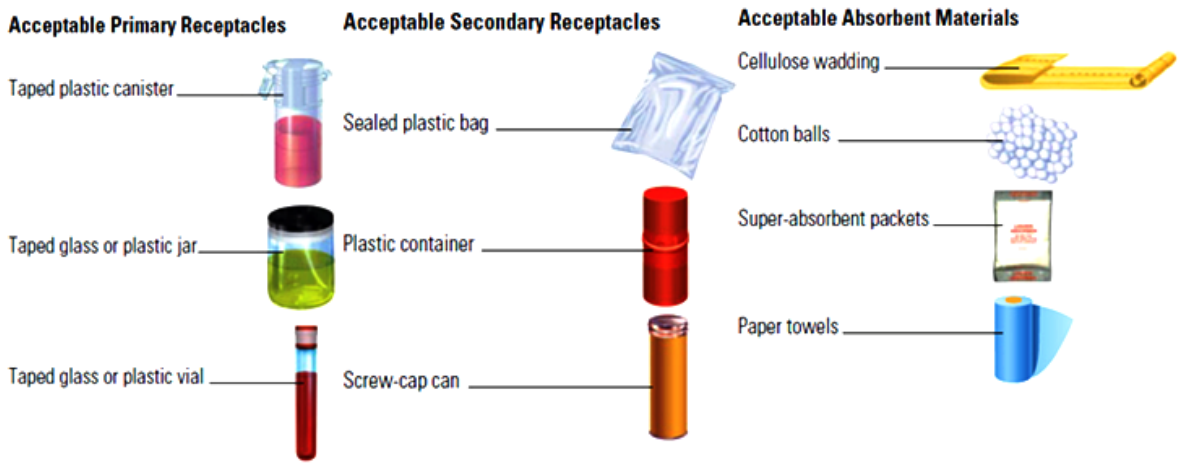
	Packaging Instruction	**Useful Tips**
1	The packaging shall be of good quality, strong enough to withstand the shocks and loadings normally encountered during transport. Packaging shall be constructed and closed to prevent any loss of contents that might be caused under normal conditions of transport by vibration or by changes in temperature, humidity or pressure.	Individual primary receptacles and the secondary packages should be tightly closed and sealed with tape or other means to prevent leakage (“Ziplock”® bags would meet this requirement). Outer packaging should be securely closed by

		means such as clasps, catches or tape.
2	The packaging shall consist of at least three components: (a) a primary receptacle; (b) a secondary packaging; (c) an outer packaging of which either the secondary or the outer packaging shall be rigid. The **outer packaging** must be rigid	Note: It is quite common that the samples when completely packaged may have more than three components; there is no definition of which "layer" of packaging is the secondary.
3	Primary receptacles shall be packed in secondary packaging in such a way that, under normal conditions of transport, they cannot break, be punctured or leak their contents into the secondary packaging. Secondary packaging shall be secured in outer packaging with suitable cushioning material. Any leakage of the contents shall not compromise the integrity of the cushioning material or of the outer packaging.	If a specimen is contained in another dangerous substance (see 13 below), it must be ensured that the cushioning material is compatible with the dangerous substance.
4	UN 3373 must be marked on the outer packaging adjacent to the words 'Biological Substance Category B'. The mark shall be displayed on the external surface of the outer packaging on a background of a contrasting colour and shall be clearly visible and legible. The mark shall be a square diamond-shaped with each side having a length of at least 50 mm; the letters and numbers shall be at least 6 mm high.	The mark must be placed on the outer so that it is readily visible. Where road vehicles collect from clinics, surgeries etc., it is recommended that this mark is at least 100mm square to ensure visibility. No colour is specified for this marking.
5	At least one surface of the outer packaging shall have a minimum dimension of 100 mm x 100 mm.	
6	The completed package shall be capable of successfully passing the drop test referred to in the specific modal requirements at a height of 1.2 m. Following the appropriate drop sequence, there shall be no leakage from the primary receptacle(s) which shall remain protected by absorbent material, when required, in the secondary packaging.	The drop tests are determined according to the type of packaging material. The test must be of a complete package as prepared for transport: i.e. the primary receptacles must be filled with a substance that represents the infectious substance (e.g. water) and absorbent/cushioning material must be in place. The test may be carried out by consignors/shippers and a record must be kept. The use of accredited testing facilities is not required.
7	For liquid substances (a) The primary receptacle(s) shall be leak proof;	There is no defined test method for determining an internal pressure. For

	(b) The secondary packaging shall be leak proof; (c) If multiple fragile primary receptacles are placed in a single secondary packaging, they shall be either individually wrapped or separated to prevent contact between them; (d) Absorbent material shall be placed between the primary receptacle(s) and the secondary packaging. The absorbent material shall be in quantity sufficient to absorb the entire contents of the primary receptacle(s) so that any release of the liquid substance will not compromise the integrity of the cushioning material or of the outer packaging; (e) The primary receptacle or the secondary packaging shall be capable of withstanding, without leakage, an internal pressure of 95 kPa (0.95 bar). The primary receptacle must not contain more than 1 litre (f) The outer package must not contain more than 4 litres; this quantity excludes ice, dry ice for liquid nitrogen used to keep the specimen cold.	surface journeys (land and sea) a record of continuous use of a particular packaging e.g. plastic bag, without known leakage, would meet this requirement.
8	8) For solid substances (a) The primary receptacle(s) shall be sift proof; (b) The secondary packaging shall be sift proof; (c) If multiple fragile primary receptacles are placed in a single secondary packaging, they shall be either individually wrapped or separated to prevent contact between them; (d) If there is any doubt as to whether or not residual liquid may be present in the primary receptacle during transport then a packaging suitable for liquids, including absorbent materials, shall be used.	"Sift proof" means impermeable to dry content. Except for packages containing body parts, organs or whole bodies, the outer package must not contain more than 4kg. This quantity excludes ice, dry ice or liquid nitrogen when used to keep specimens cold. *Whole bodies are subject to separate rules and advice from airlines should be sought.*
9	Refrigerated or frozen specimens: Ice, dry ice and liquid nitrogen (a) When dry ice or liquid nitrogen is used to keep specimens cold, all applicable requirements shall be met. When used, ice or dry ice shall be placed outside the secondary. Interior supports shall be provided to secure the secondary packaging in the original position after the ice or dry ice has dissipated. If ice is used, the outside packaging or over pack shall be leak proof. If solid carbon dioxide (dry ice) is used, the packaging shall be designed and constructed to permit the release of carbon dioxide gas to prevent a build-up of pressure that could rupture the packaging and the package shall be marked “Carbon dioxide, solid” or “Dry ice”; (b) The primary receptacle and the secondary packaging shall maintain their integrity at the temperature of the refrigerant used as well as the temperatures and the pressures which could result if refrigeration were lost.	It is very important to follow these procedures carefully. Placing refrigerants in the wrong place can lead to serious accidents. Note: certain packaging materials become brittle at very low temperatures, particularly plastics.
10	When packages are placed in an over pack, the package markings	

	required by this packing instruction shall either be clearly visible or be reproduced on the outside of the over pack.	
11	Infectious substances assigned to UN 3373 which are packed and marked in accordance with this packing instruction are not subject to any other requirements except for the following: a) The name and address of the shipper and of the consignee must be provided on the outer packaging. b) The name, address and telephone number of a person responsible must be provided on a written document (such as an air waybill, attached to the outer packaging) or on the package; c) The package must be classified in accordance with the dangerous goods regulations. *d)* The dangerous goods regulations incident reporting requirements must be met. *e)* The dangerous goods regulations inspection for damage or leakage requirements must be met. f) Passengers and crew members are prohibited from transporting infectious substances either as, or in, carry-on baggage or checked baggage or on their person.	It is recommended that substances should not be carried on any form of passenger transport other than an individual sample carried by a patient. Note: When the shipper or consignee is also the "person responsible" as referred to in b) above, the name and address need be marked only once, on or attached to the outer packaging, in order to satisfy the name and address marking provisions in both a) and b), above.
12	Clear instructions on filling and closing such packages shall be provided by packaging manufacturers and subsequent distributors to the consignor or to the person who prepares the package (e.g. patient) to enable the package to be correctly prepared for transport.	This information can be printed on the packaging or provided in a leaflet. It is particularly important that this is provided to patients who are responsible for sending their own samples.
13	Other dangerous goods shall not be packed in the same packaging as Division 6.2 infectious substances unless they are necessary for maintaining the viability of, stabilizing or preventing degradation or neutralizing the hazards of the infectious substances. A quantity of 30 ml or less of dangerous goods of Class 3, 8 or 9 may be packed in each primary receptacle containing infectious substances (provided these substances meet the requirements of 1;2.4.2 and 1;2.4.3 of the Technical Instructions). When these small quantities of dangerous goods are packed with infectious substances in accordance with this Packing Instruction, no other requirements in need be met.	

Figure 15: Examples of acceptable packaging



Working with Dry Ice

Dry ice is solid carbon dioxide (CO_2). It is made by compressing and cooling gaseous CO_2 until it liquefies. Expansion converts the liquid into the snow form of the solid state. The snow is then compressed by a hydraulic press into dry ice blocks, slices or pellets.
Solid Carbon Dioxide is -78°C. It sublimes (turns from a solid to gas) to an asphyxiant gas that is heavier than air. It is important to note that a little bit of dry ice will sublime to a large volume of gas.

Hazards

- Asphyxiation - In high concentrations sublimed vapour may cause asphyxiation. 10kg of dry ice sublimes into about 5.4 m^3 of carbon dioxide gas
- Extreme cold - Contact with dry ice can cause cold burns and frostbite

Storage

ALWAYS store dry ice in an area which is:

- Well ventilated.
- Preferably not below ground.
- Out of direct sunlight and sources of heat.
- Secure – to prevent unauthorised access.

Suitable dry ice containers are available from your supplier. Generally, the principle is – the better the insulation, the slower the sublimation rate and the longer the quality of the

product will be maintained. DO NOT store or use dry ice in any gas tight container. Within large containers, gas rich atmospheres will have built up.

Handling

Many applications of dry ice result in the sublimation of the dry ice volume into the working area. REMEMBER – a little bit of dry ice will sublime to a large volume of CO_2 gas.

- Do not handle dry ice with bare hands as it can cause severe cold burns and frostbite. Dry ice should always be handled with protective gloves.
- DO NOT play with dry ice. Playing games with dry ice is dangerous.
- ALWAYS keep dry ice away from children.
- USE AN INSULATED CONTAINER TO STORE DRY ICE
- DO NOT PLACE IN REFRIGERATOR OR FREEZER

Safe Disposal

Dry ice sublimates leaving no residue. Surplus dry ice should be allowed to sublime naturally:

- In a well-ventilated area
- Secured against access to passers-by
- DO NOT dispose of dry ice in an area where CO_2 gas can collect in low-lying areas – drains, confined spaces, etc.

Shipping with Dry Ice

Dry ice should never be placed in a sealed container. The package must be able to release vapour at all times. The lid of a Styrofoam cooler should not be taped in place, although the outer cardboard box should be securely fastened. Basic safety precautions should be followed even when transporting dry ice by ground; ventilation of the cooler and the vehicle should be allowed as CO_2 can build up in a vehicle with closed windows.

Labelling summarised

In addition to this dry ice labelling, there are specific requirements for labelling dangerous goods packages that have been covered in this session. The following is a list to summarise the requirements.

1. The name and address of the shipper and of the

Name:……

Address: ……..

consignee must be provided on the outer packaging. The name, address and telephone number of a person responsible must be provided on a written document (such as an air waybill) or on the package

2. 

UN 3373 must be marked on the outer packaging adjacent to the words Biological Substance Category B in letters greater than 6mm high.

3. 

Biological Substance Category B hazard label is often on inner packaging. **The biohazard symbol is not required on the outer box.**

4. 

Orientation labels are required on combination packages and over packs containing liquid dangerous goods. The labels must be visible on at least two opposite sides to show the proper package orientation so that the closures will be in an upright position. The orientation arrows are not required on single packages or on packages or over packs containing solid dangerous goods. The orientation label is not necessary for flammable liquids in inner packaging of 120 millilitres or less, infectious substances in primary receptacles of 50 millilitres or less, or radioactive materials.

Arrows must not be marked on dangerous goods packages containing liquids for any purpose other than indicating proper orientation.

Air Passenger Provisions

Infectious substances in Category A or B are not permitted for transport in carry-on or checked baggage and must not be carried on the person.

Emergency Response Procedures

The following guidance is primarily of use by operators in the event that a package containing infectious substances (of either Category A or B) is involved in an incident resulting in spillage.

Containing Spillage

- Isolate spill or leak area immediately in all directions.
- Keep unauthorised personnel away.
- Obtain the identity of the substance involved if possible and report the spill to the appropriate authorities.
- Do not touch or walk through spilled material.
- Do not touch damaged containers or spilled material unless wearing appropriate protective clothing.
- Be particularly careful to avoid contact with broken glass or sharp objects that may cause cuts or abrasions that could significantly increase the risk of exposure.
- Damaged packages containing solid CO2 as a refrigerant may produce water or frost from condensation of air. Do not touch this liquid as it could be contaminated by the contents of the package.
- Liquid nitrogen may be present and can cause severe burns.
- Absorb spilled materials with earth, sand or other non-combustible material while avoiding direct contact.
- Cover damaged package or spilled material with damp towel or rag and keep wet with liquid bleach or other disinfectant. Liquid bleach will generally effectively inactivate the released substance.

PLEASE NOTE: Clean-up or disposal should only be carried out or supervised by a person who has undertaken appropriate training.

First Aid:

- Move the exposed person to a safe isolated area. CAUTION: exposed person may be a source of contamination.
- Remove and isolate contaminated clothing.
- In case of contact with substance, immediately flush skin or eyes with running water for at least 20 minutes.
- Effects of exposure (inhalation, ingestion or skin contact) to substance may be delayed.
- For further assistance, contact the appropriate public health authority.
- Ensure that medical personnel are aware of the substances involved and take precautions to protect themselves.
- If appropriate, report the accident or incident to the appropriate authorities of the State of the operator and the State in which the accident or incident occurred in accordance with the reporting requirements of those appropriate authorities.

Slide Set

A slide set is available for this topic that can be downloaded from the UKCRF Network Portal (see page 5 for more details).

Workshop Activities

Using examples of different types of packaging is useful during this session so that the attendees can visualise the different materials.

If a separate IATA Dangerous Goods Regulations Certificate is required for this session it is necessary to test the attendees on their knowledge of dangerous goods. An example test is available on the UKCRF Network Portal. This can be completed as an open book test using the Dangerous Goods Regulations Training Notebook. A sample certificate is also available. The content of this certificate meets the minimum criteria requested by IATA.

Suggested Handouts

It is advisable that the Dangerous Goods Regulations Training Notebook is sent to the attendee as pre-course reading. A full list of teaching resources can be found in appendix 2 and all resources can be downloaded for free from the UKCRF Network Portal (see page 5 for more details).

9. Practical Session

Learning Objectives

- To demonstrate safe research laboratory skills for research samples

Background Information

This session is useful if you are running a full day laboratory workshop. It is fully practical and amalgamates all of the theoretical information given to the attendees allowing them to practise all lab skills in one session. There is therefore no additional slide set for this section.

Workshop Activities

This practical session requires demonstration of sample processing techniques followed by the attendees practising the techniques. There are several points for consideration when organising the practical session. These are highlighted in the 'Practical Skills – Trainer Tips' document, available on the UKCRF Portal. This document also contains information about a case study that can be used to guide the practical session.

Depending on the number of attendees and available trainers, this session may need to be organised as a 'round robin', with the attendees rotating through various practical stations. A small group, however, could work through the case study in chronological order.

It may be appropriate to introduce the UKCRF Network competency documents during this session.

Suggested Handouts

A case study with questions and tasks for the practical session is available, with an example Freezer Sample Log, and a Sample Requisition Log that can be completed during the practical exercise. A full list of teaching resources can be found in appendix 2 and all resources can be downloaded for free from the UKCRF Network Portal (see page 5 for more details).

Abbreviations and Glossary

Ambient – relating to the immediate surroundings of something e.g. the sample is kept at room temperature

Analytic - this phase includes the laboratory testing or diagnostic procedures, processes, and products that ultimately provide the required results.

Biological agent - a microorganism, cell culture, or human endoparasite, whether or not genetically modified, which may cause infection, allergy, toxicity or otherwise create a hazard to human health

Buffy Coat - the buffy coat is the fraction of an anticoagulated blood sample that contains most of the white blood cells and platelets following density gradient centrifugation of the blood

Clotting - a sequence of complex chemical and physical reactions that results in conversion of blood into a coagulated mass, making the specimen unacceptable for coagulation or Haematology testing.

Fibrin Clotting - an elastic, insoluble, whitish protein produced by the action of thrombin on fibrinogen and forming an interlacing fibrous network in the coagulation of blood.

Containment level - the standard of controls required based on the hazard group of the biological agent being processed.

COSHH - Control of Substances Hazardous to Health Regulations. These Regulations require employers to control exposure to hazardous substances to prevent ill health.

CRFs – **Clinical Research Facilities**

CTA – **Clinical Trial Authorisation. A** CTA has to be given prior to the start of a study when the study is a CTIMP. The authorisation is given by the MHRA

CTIMP – **Clinical Trial of an Investigational Medicinal Product.**

CTU – **Clinical Trial Unit**

Dry Ice – dry ice is the solid form of carbon dioxide. It is used primarily as a cooling agent. Its advantages include lower temperature than that of water ice and not leaving any residue (other than incidental frost from moisture in the atmosphere).

EDTA – **Ethylenediamine tetra-acetic acid.** This is a strong anticoagulant used in blood tubes where a plasma sample is required. The lavender top tube contains EDTA as an anticoagulant -- used for most haematological procedures. **Note:** After the tube has been filled with blood, immediately invert the tube several times to prevent coagulation.

GCLP – **Good Clinical Lab Practice.** Applies those principles established under Good Lab Practice for data generation used in regulatory submissions relevant to the analysis of samples from a clinical trial. At the same time it ensures that the objectives of the GCP principles are carried out. This ensures the reliability and integrity of data generated by analytical laboratories.

GCP – **Good Clinical Practice.** Good Clinical Practice is an international ethical and scientific quality standard for designing, conducting, recording and reporting trials that involve the participation of human subjects.

g- Force – This is the force exerted on the contents of the rotor when centrifuging samples. It results from the revolutions of the rotor.

Hazard Group 1 - unlikely to cause human disease;

Hazard Group 2 - can cause human disease and may be hazard to employees; it is unlikely to spread to the community and there is usually effective prophylaxis or treatment available;

Hazard Group 3 - can cause severe human disease and may be a serious hazard to employees; it may spread to the community, but there is usually effective prophylaxis or treatment available;

Hazard Group 4 - causes severe human disease and is a serious hazard

Haemolysis - haemolysis results when RBCs are lysed (the cell breaks apart and is destroyed); haemoglobin is released; and serum or plasma, which is normally straw coloured, becomes tinged with pink or red. If a specimen is grossly haemolysed, the serum or plasma appears very dark red.

HTA – **Human Tissue Authority.** The HTA is an executive non-departmental public body of the Department of Health. It regulates organisations that remove, store and use human tissue for research, medical treatment, post-mortem examination, education and training, and display in public. They also give approval for organ and bone marrow donations from living people.

IATA Dangerous Goods Regulations – International Air Transport Association. It is a Global reference for shipping dangerous goods by air.

IB – **Investigator Brochure**. In drug development, the Investigator's Brochure (IB) is a comprehensive document summarising the body of information about an investigational product ("IP" or "study drug") obtained during a drug trial. The IB is a document of critical importance throughout the drug development process and is updated with new information as it becomes available. The purpose of the IB is to compile data relevant to studies of the IP in human subjects gathered during preclinical and other clinical trials

Inversion (Invert) - The turning of a sample collection tube by 180°C.

Liquid Nitrogen - in its liquid form nitrogen is used as a cryogen for many applications where very low temperatures or rapid temperature reduction is required. Medical liquid nitrogen can be used for the storage of biological material, such as cells, tissues, blood, gametes at cryogenic temperatures, for subsequent medical procedures and analysis

Lysis – the destruction of cells through damage or rupture of the plasma membrane, allowing escape of the cell contents.

MHRA – **Medicines and Healthcare products Regulatory Agency.** The MHRA is the competent authority for the UK in relation to the Directive 2001/20/EC and the Clinical Trials Regulations, and for Medical Devices, the competent authority in relation to the Medical Devices Regulations 2002. They are responsible for ensuring that medicines and medical devices work, and are acceptably safe.

Microbiological safety cabinet - is an enclosed, ventilated laboratory workspace for safely working with materials contaminated with (or potentially contaminated with) pathogens requiring a defined biosafety level.

Mislabel - the placement of incorrect label/information on a sample collection or transport tube. This can lead to samples being rejected by labs.

MTA - **Material Transfer Agreement**. This is a contract that governs the transfer of tangible research materials between two organisations, when the recipient intends to use it for his or her own research purposes. The MTA defines the rights of the provider and the recipient with respect to the materials and any derivatives.

NHS R&D - **National Health Service Research and Development departments.** NHS R&D departments support service for clinical research projects ensuring research is conducted to a high scientific, ethical & financial standard.

NRES - **National Research Ethics Service (NRES).** The National Research Ethics Service (NRES) is the core function of the Health Research Authority (HRA) and provides help and leadership for NHS Research Ethics Committees (RECs) by coordinating the development of operational and infrastructure arrangements in support of their work.

Pellet – the precipitate at the bottom of a test tube post centrifugation**.**

Pipette - a pipette is a lab instrument which is used to dispense a measured volume of liquid. A pipette works by creating a vacuum above the liquid-holding chamber and selectively releasing this vacuum to draw up and dispense liquid.

PI - **Principal Investigator**. For CTIMPs, the authorised health professional responsible for the conduct of that trial at a trial site, and if the trial is conducted by a team of authorised health professionals at a trial site, the Investigator is the leader responsible for that team. For research other than CTIMPs: The person responsible, individually or a leader of the researchers at a site, for the conduct of a study at that site.

Plasma - this is the fluid part of the blood in which the blood cells are suspended. It makes up about 55% of the total blood volume. It is a clear, straw-coloured liquid made up of 92 % water. It contains plasma proteins, inorganic salts, nutrients including glucose, clotting factors, gases such as carbon dioxide, waste materials from the cells and various hormones, secretions and enzymes.

Post Analytic - the final phase of the laboratory process. This culminates in the production of a final value, result, or a diagnostic report.

PPE – **Personal Protective Equipment.** Various additional uniform or equipment to be used for specific tasks as instructed by the employer and/or study protocol

Pre Analytic – the pre-analytic phase includes specimen acquisition and handling issues that occur prior to the time the specimen is received in the laboratory for processing. Important errors can occur during the pre-analytic phase with specimen handling and identification. Therefore, the pre-analytical phase must have rigorous control measures.

QA - **Quality Assurance**. QA programmes should always be designed to assure compliance with the relevant European Union Directives, associated guidance and the facility's internal policies and SOPs. All those planned and systematic actions that are established to ensure that the trial is

performed and that data are generated, documented (recorded), and reported in compliance with good clinical practice (GCP) and the applicable regulatory requirement(s).

RCF – **Relative Centrifugal Force.** This is measured in force x gravity or g-force**.** The force is exerted by spinning. This force is constant for different size machines. **NB**. RCF does not equal RPM (revolutions per minute) and must not be used interchangeably

REC – **Research Ethics Committee.** A research ethics committee is a group of people appointed to review research proposals to assess formally if the research is ethical. This means the research must conform to recognised ethical standards, which includes respecting the dignity, rights, safety and well-being of the people who take part

RBCs – **Red Blood Cells**. Erythrocytes

RIDDOR 2013–**Reporting of Injuries, Diseases and Dangerous Occurrences Regulations 2013.** RIDDOR is the law that requires employers, and other people in charge of work premises, to report and keep records of work-related accidents which cause deaths**.**

RPM – **Revolutions per Minute.** How fast a centrifuge spins. **NB**. RPM does not equal RCF (Relative Centrifugal force) and must not be used interchangeably

Sample Integrity – consistent, sound, uncorrupted or reliable

Serum (Blood) – The liquid part of the blood that remains after the solid elements have been separated out. It is the clear, straw coloured liquid portion of the plasma that does not contain any fibrinogen or blood cells.

Shipper - the person packing the samples to be sent to labs.

SOPs – **Standard Operating Procedures**. Detailed, written instructions to achieve uniformity of the performance of a specific function. They may be study specific or local.

SST – Serum Separator Tube. This tube contains a clot activator and serum gel separator -- used for various laboratory tests. **Note:** Invert the tube to activate clotting; let stand for 20 to 30 minutes before centrifuging.

Supernatant – the clear fluid that after the settling out of an insoluble liquid or solid by the action of normal gravity or of centrifugal force, takes up the upper portion of the contents of a vessel.

TAT – Turn Around Time. The time it takes for a sample to be tested and results reported.

UKCRC – UK Clinical Research Collaboration

UKCRF Network – UK Clinical Research Facility Network

Viscosity - the viscosity of a fluid is a measure of its resistance to gradual deformation by shear stress or tensile stress. For liquids, it corresponds to the informal concept of "thickness"; for example, honey has a much higher viscosity than water.

WBCs – White Blood Cells **or** Leucocytes. There are five types; lymphocytes, monocytes, eosinophils, basophils and neutrophils (granulocytes).

Further Reading

Department of Transport, Transport of Infectious Substances UN2814, UN2900 & UN3373 Guidance Note Number: 17/2012[REV.7]

European Medicines Agency 'Reflection paper for laboratories that perform the analysis or evaluation of clinical trial samples' 23rd Feb 2012 EMA/INS/GCP/532137/2010 GCP Inspectors Working Group

Experimental Cancer Medicine Centre (ECMC) Early Phase and transitional Research Nurse Network Group: 'Sample Handing Guidance Document for Research Nurses and Junior Clinical Laboratory Staff' version 1 March 2011

European Directive 2005/28/EC. Available at: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2005:091:0013:0019:en:PDF (Last accessed May 2023)

Health and Safety at Work Act (1974). Available at: http://www.hse.gov.uk/legislation/hswa.htm (Last accessed May 2023)

Health and Safety Executive (1992). Personal Protective Equipment at Work Regulation Available at: http://www.hse.gov.uk/pUbns/priced/l25.pdf (Last accessed May 2023)

Health and Safety Executive (1991) Safe working and the prevention of infection in clinical laboratories and similar facilities Available At: http://www.hse.gov.uk/pubns/clinical-laboratories.pdf (Last accessed May 2023)

Health and Safety Executive (2021). The approved list of biological agents. Available at: http://www.hse.gov.uk/pubns/misc208.pdf (Last accessed May 2023)

Health and Safety Executive (2002) The Control of Substances Hazardous to Health Regulations as amended. Available at: http://www.hse.gov.uk/pUbns/priced/l5.pdf (Last accessed May 2023)

International Air Transport Association (IATA) (2017). Dangerous Goods Regulations 64th Edition. IATA Publications

MHRA. Good Clinical Practice Guide, 2012. Chap 13 Clinical Trial Samples – analysis and evaluation

UKCRC Guidance for CTUs on Assessing the Suitability of Laboratories Processing Research Samples. Available at: https://ukcrc-ctu.org.uk/wp-content/uploads/2021/04/ukcrc_guidance_for_ctus_on_a.pdf (Last accessed May 2023)

UKCRF Network 'Laboratory Competencies for Clinical Research Staff' Version 1 March 2015

UKCRF NETWORK

Appendix 1: UKCRF Network Laboratory Training Delivery Team

The members of the Laboratory Training Delivery Team responsible for developing and reviewing this document are listed below.

Originally developed by

- Janet Johnstone - Research Nurse Manager/Education and Training Officer, Glasgow
- Naomi Hickey - Education and Training Officer, Glasgow
- Karen Secombes -Training Facilitator, Grampian
- Sean Gaffney – Laboratory Technician, London
- Kay Walker - Clinical Research Nurse Manager, Tayside

Many thanks to Melissa Wardale, Laboratory Manager, and team at NIHR Clinical Research Facility, University Hospitals Birmingham NHS Foundation Trust, for independently reviewing the guidance document and slide set for accuracy and quality assurance.

Many thanks also to Kornelia Hathaway, NIHR Cambridge Clinical Research Facility, for her input and advice at the beginning of the project.

Version 2, December 2019, updated by

- Janet Johnstone - Research Nurse Manager/Education and Training Officer, Glasgow
- Naomi Hickey - Education and Training Officer, Glasgow
- Karen Secombes -Training Facilitator, Grampian
- Kay Walker - Clinical Research Nurse Manager, Tayside
- Helen Smith - Laboratory Manager, Birmingham

Version 3, June 2023, updated by

- Naomi Hickey, Education and Quality Lead, Glasgow
- Helen Smith - Laboratory Manager, Birmingham
- Kimberley Driver, Laboratory Manager, Sheffield
- Deborah McGlynn, Education and Training Facilitator, Glasgow
- Barbara McLaren, Research Nurse Manager, Glasgow
- Kanom Bibi, Research Photographer and CRF Training Lead, London

Appendix 2: List of Training Resources

All resources are available for free download from the UKCRF Network Portal. If you do not already have access to the Network's portal you will need to complete a request form on the UKCRF Network website by clicking here. The answer booklet is available to trainers by contacting the UKCFRN at ukcrf.network@nihr.ac.uk.

	Supporting Documents
Powerpoint slide set	Trainer information in notes section of powerpoint
Trainer Tips and Answer Booklet	Agenda with suggested timings Risk Assessment Workshop Answers Pre-analytics Workshop Answers Centrifuge Tasks – Trainer Tips Pipetting Tasks – Trainer Tips Tracking and Storage Workshop Answers Dangerous Goods Regulations Test Answers Practical Skills – Trainer Tips
Administration Documents	Sample Processing Competency Record (abbreviated) Certificate of Attendance Evaluation Form
Workshop Booklet ● Legislation and Regulations ● Risk Assessment ● Safety in the Laboratory ● Pre-analytics ● ● Centrifuge Training: Principles and Practice ● ● Principles and Practice of Pipetting ● Tracking and Storage of Research Samples ● Dangerous Goods Regulations ● Practical Session	None Required Risk Assessment Workshop - Pipetting and Centrifugation Risk Assessment Form Blank Safety in the Laboratory Handout Pre-analytics Workshop Centrifuge Tasks Centrifuge Tips Pipetting Tasks Pipetting Tips Tracking and Storage Workshop Dangerous Goods Regulations Notebook (separate to workshop booklet) Dangerous Goods Regulations Test Dangerous Goods Record of Training (separate to workshop booklet) Practical Skills Case Study Freezer Sample Log Sample Requisition Form