Genomics Training Academy (GTAC)

NHS England

Virtual reality resource supporting material: Preparation of samples for cytogenetic analysis

Model answers document

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About this document

This document has been designed to support the ‘Preparation of samples for cytogenetic analysis’ virtual reality resource in training sessions. The questions and model answers below provide a reference point for discussions that will have happened during the session, and can be kept by learners for future reference, or used by trainers to aid in session planning.

Model answers

Bookmark 1: Cell culture (05:27)

1. **Why do we check the patient details carefully and only process one sample at a time?**

Checks are in place to ensure that the correct patient sample is being processed. We process one patient sample at a time so that they can’t be mixed up.

2. **Some laboratories may have the labelling of tubes witnessed by another technical member of staff. Why would this be in place?**

By witnessing the process, it provides an additional check to ensure that there is a minimal risk of the samples being mixed. The check would need to be done by a member of the team who understands the process.

3. **Why would you undertake a cell count? How is it done, and when would you not do it?**

Cell counts in bone marrow samples vary from patient to patient. By doing a cell count, it ensures that the optimal number of cells for culturing are present. If there are too many cells, they will use all of the nutrients in the media before harvesting and will die. Conversely, there must be enough cells at the start to result in sufficient cells at harvesting.

Some laboratories have automated cell counters due to the number of samples they receive (as shown in the VR film), however, not all laboratories have an automated cell counter. Those that don’t instead use a haemocytometer or counting chamber.

There is no requirement to do a cell count for routine blood cultures. Blood cultures are more standardised as we are not stimulating different cell lines like we do in the leukaemia cultures.

4. **Why is it important to record the batch number of reagents which have been used?**

Recording the batch numbers of reagents is an important part of quality control and quality assurance, and is a requirement of UKAS ISO15189 accreditation.

By recording the batch numbers, it is possible to track when a reagent isn't performing to an accepted standard, and manufacturers can then be contacted as part of troubleshooting. Specifically for culture media, if a poor batch is noted, it is possible to contact the manufacturers and request a different batch.

5. What are the main components of blood culture media?

Blood culture media contains:

- Base media such as RPMI, which contain vitamins, minerals and amino acids essential for the growth of cells.
- Fetal calf serum, which provides the hormones for cell growth and essential nutrients.
- Penicillin and streptomycin, antibiotics that prevent bacterial infection in the culture.
- Glutamine, an amino acid which supports growth of cells.
- Heparin, which prevents clotting of samples.
- PHA, which stimulates cell growth of T lymphocytes.

6. Is anything else required for culturing cells from bone marrow?

Cultures from bone marrow aspirates require the addition of specific growth factors to stimulate the growth of specific cell lines.

Malignant cells in the bone marrow are generally dividing, therefore additional stimulants are not always added. However, for T-cell malignancies, PHA can be added to stimulate the T-cells.

Different chemicals are added at different times during cell culture depending on the culturing regime, but they all ultimately synchronise the cell cycle.

7. What temperature should the incubator be at and why? What would the impact be if it was too low or too high?

The incubator should be at 37ºC, which is the temperature of the human body.

An incorrect temperature will result in the cells potentially dying. Therefore, there will be insufficient metaphases, ultimately failing the sample for cytogenetic analysis. Incubator temperatures are monitored closely in accordance with UKAS ISO15189 accreditation.

8. What are the typical culturing times for blood samples, PHA-stimulated bone marrow samples and standard bone marrow samples?

- Cultures from blood samples require 72 hours in the incubator.
- PHA stimulated bone marrow sample cultures also require 72 hours in the incubator.
- Most other bone marrow samples are cultured overnight or for 24 hours to ensure that the malignant cells are being examined.

Bookmark 2: Harvesting (09:52)

9. Why do we centrifuge the samples?

Centrifuging samples ensures that the cell pellet is at the bottom of the culture vessel and the waste material is above and can either be pipetted or poured off.

10. What is the role of the fixative?

Fixative fixes the cells in metaphase and preserves the chromosome morphology. It also removes nuclear proteins.

11. Why do we add the first fix slowly? What would be the result if the fix was added quickly?

The first fix is added slowly otherwise the cells are shocked, which will damage them and make them difficult to analyse due to cell debris and cytoplasm.

Please note that the volume of fix added will depend on your local laboratory procedure.

12. Why is it important to clean the cabinet after completing processing?

Work areas should always be kept clean and tidy in accordance with aseptic techniques. This protects samples from contamination and is good laboratory practice.

13. Why do we need to remove unfixed blood from the sides of the tubes?

Blood is a biological agent and may contain infectious organisms, therefore there is a risk of infection if it has not been fixed. Although gloves will be worn for the majority of the downstream processes, it is still advisable to clean the culture vessels to minimise the risk of infection.

14. Why do we store the newly harvested samples in the cold room/fridge/freezer?

Leaving the culture vessels out in the laboratory areas may result in some of the final fix evaporating off. In addition, there is a risk that the vessels could be knocked off the bench. If this happened when the technician came to prepare slides, there is a risk they would pour off the cell pellet.

15. **Are there any differences seen in the video that are performed differently in your local laboratory? For example, pipetting off supernatants, centrifugation time and speeds, and hypotonic times.**

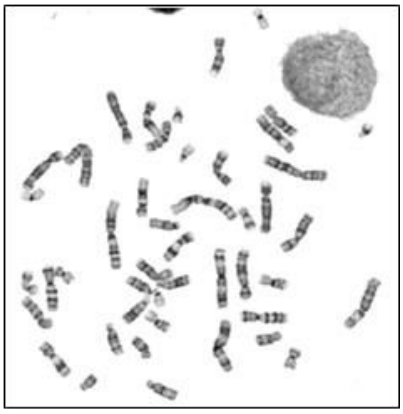
Bookmark 3: Slide preparation (14:18)

16. **Why are temperature and humidity controlled for slide preparation? If they are not, what would be the impact on the preparations?**

Temperature and humidity are controlled to get the best possible quality of chromosome spreading and banding on the slides.

Ideally there will be few crossovers of chromosomes – they will be spread out but not so spread out that chromosomes are lost from cells. When analysing chromosomes, you need to be able to check each region of each one, therefore slides with cells with lots of chromosomes on top of each other will result in more cells having to be checked to ensure all chromosome regions have been examined.

If the temperature is too high, then the chromosomes are more likely to be tight and crossed over. If the slides dry too slowly, the chromosomes will spread out too much and chromosomes from one cell will spread into those of neighbouring cells. An ideal example is shown in the image.



In addition, controlling temperature and humidity often makes the G-banding simpler and more consistent.

17. **Why is an additional drop of fixative added to the slide?**

Adding an additional drop of fixative helps slow the drying process down, which means the chromosomes will spread out better. This improves the quality of the preparations and makes downstream processes easier.

18. **How would you dilute the cell suspension – what would you use?**

To dilute a cell suspension, you would use a freshly made fixative 3:1 methanol to acetic acid.

19. **Why do the slides go into a drying oven for one hour at 90ºC? Is this the same for all laboratories?**

This ages the slides which improves the G-banding downstream. An oven at a lower temperature for longer, a hotplate or UV light may be used depending on works best for the laboratory. Aging the slides means that the trypsin does not over-digest the chromosomes.

20. **What is the role of hydrogen peroxide? Is this used in all laboratories?**

Not all laboratories use hydrogen peroxide, but it is a reagent that can be used to age the slides and harden the chromosomes prior to digesting with trypsin.

21. **What is the role of the trypsin?**

The trypsin removes excess proteins and some of the histones. The removal of the proteins relaxes the structure of the chromosome.

The stains then bind to the AT-rich regions of the chromosomes. The unbound regions are GC-rich regions.

22. **Are there any other stains which could be used instead of Leishman's?**

Some laboratories use Giemsa stain, which is where the 'G' in G-banding comes from.

23. **What is the role of the hot plate. Are there any other options? What does your laboratory do?**

The role of the hot plate is to dry the slides prior to putting the glue on the coverslip. Any remaining water on the slides will interact with the glue and prevent the coverslip from attaching; ultimately, this would result in damage to the preparations. Leaving the slides to air dry will have the same result, it will just take longer.

24. **Why is the glue added to the coverslips in a safety cabinet? Where would you find the necessary information about the risks of the glue?**

The glue can be irritating to the respiratory tract, eyes and skin. Hazards will be included in the risk assessment for the slide making procedure and also in the COSHH database.

25. **Does your laboratory culture and harvest cells and prepare slides in the same way as in the film? If not, how does it differ?**

Bookmark 4: FISH processing (16:41)

26. **What does pre-treatment do?**

It allows the probes to penetrate the cell membrane. The chemicals used in the VIP2000 are:

- Pepsin
- 2xSSC
- Formaldehyde
- 75%, 80%, 100% ethanol

Note: not all laboratories will have an automated system for FISH processing and will do pre-treatments via various methods – such as pepsin or UV.

27. What are the different types of FISH probe and what are they used for?

Unique sequence probes

- Detect gains, loss and amplification.
- Bind to a specific region of the chromosome.
- Can also be used to look for the location of a region.
- Generally have a control probe on the same chromosome.

Centromeric probes

- Hybridise to the centromeric region of the chromosome.
- Support the determination of copy number.
- Cross hybridisation is known between various chromosomes.

Dual fusion probes

- Dual colour – each probe hybridises to a different region. When the rearrangement is present, the probes produce a fusion signal.

Break-apart probes

- Used generally in leukaemias/cancer to identify rearrangements of a particular gene.

28. Why is it necessary to keep the probes protected from the light as much as possible?

The probes are fluorescently labelled and exposure to the light will bleach out the label making it challenging to detect.

29. What quality control is required when adding the probes to the slides?

It is important to check that the correct probe is added for each patient sample. When analysing metaphases we can check the correct probe was used by confirming the chromosome position. However, in leukaemia FISH analysis, we often use interphase cells where the chromosomes are not visible and are therefore unable to confirm probe position. Incorrect probes being added will impact on the result being reported to the patient and incur a cost for a wasted probe and processing time.

30. Why do the slides go in a water bath overnight?

Before putting the slides into the water bath, they are heated to denature the DNA and the probes. Incubating the slides in the water bath overnight holds them at a set temperature which allows the probes to bind to the target DNA.

End of film

31. Why is it important that the reagents are made up correctly? If the washes (SSC) were not made correctly, what would you expect to see when analysing the FISH?

The concentration of the wash buffers (stringency) is vital - too stringent (wash is too strong) and the probe may be washed off the chromosomes; not stringent enough (too weak) would mean that unbound probe is left on the chromosomes and in the cells. This could either result in a false positive, failed results where there are no signals at all or too much background to accurately determine the probe signal.

32. Why are the coverslips removed in the hood? What hazards are there?

The FISH probes contain formaldehyde, a known carcinogen, to denature the DNA. Therefore, caution should be used with appropriate personal protective equipment (PPE). Some labs use a safety cabinet.

The coverslips should be disposed of in a sharps bin – they will easily break and contain residual unbound probe.

33. What are the basic principles of the second day of FISH processing?

The second day of FISH processing washes excess probe off the preparations and applies the DAPI stain which visualises the cells and chromosomes whilst not masking the fluorescent FISH probes. As discussed, washes need to be the correct stringency so that excess probe is removed.

34. Does your laboratory process FISH slides in the same way as in the film? What differences are there, or does everyone follow the same principles?

35. What personal protective equipment (PPE) and other health and safety aspects should we be aware of throughout the cytogenetics procedure?

PPE

- Gloves should be worn and changed as required.
- Lab coats should be worn in designated areas. Some labs use different coloured lab coats in different areas. It is important to make sure you know when you should be wearing a lab coat and what colour it should be.

Other health and safety aspects

- No eating or drinking in the lab.
- Long hair is always tied up.
- No mobile phones in the lab.
- All cabinets and equipment should be checked to be working correctly with no alarms sounding.
- All reagents should be handled and stored as set out in the local risk assessments and COSHH assessments are available for all members of staff.
- There is a risk that blood-borne viruses may be present in any sample, therefore we treat all samples with care and work in a Class II+ environment to protect ourselves from infection.
- Known high-risk samples may be handled with extra care. Clinical teams that are aware that a patient is infected with a blood-borne pathogen will indicate this on the referral form. Some laboratories will process known high-risk samples at the end of the day when there are less people in the lab and all other samples have been processed. They may also use stickers to alert staff, a separate incubator and extra PPE, and increase the containment levels and class of cabinets.

36. What does each class of cabinet do?

Class I

Class II

Class III

Class I

Protects the operator but not the sample. Air flows into the cabinet through the front.

Class II

Protects both sample and the operator. Open fronted, but prevents the samples being contaminated.

Class III

Designed to deal with class 4 pathogens, protecting the operator and sample. Samples are handled using a gloved entrance. Samples such as those infected with airborne viruses can only be processed in a class III cabinet.

37. Where do we dispose of waste/sharps?

Appropriate bins will be available in the laboratory area, including:

- biobins for non-sharp contaminated waste – including reagents;
- large bins for contaminated waste; and
- large bins for domestic waste such as non-contaminated gloves and paper towels.