FISH Slide Preparation

Notes

- Ideal environmental conditions for slide making are between 45-55% humidity and 24°C (72-75°F) with minimum drafts. A hygrometer/thermometer should be positioned near the slide-making bench to monitor environmental conditions. If conditions are not ideal, adjustments should be attempted prior to slide making (see Step 2 - Drying Time Indicators for details). Use of a thermotron is ideal.
- Specimen quality is important and difficult to adjust once fixative is added. It is important to follow standard Cytogenetics protocols using reagents that have been parallel tested prior to use.
- Optional (if excessive debris is seen on slides): Clean glass microscope slides by placing in a coplin jar with 70% alcohol for five (5) minutes, wiping vigorously in one direction several times with a tissue to remove debris, then placing in a coplin jar with fresh 3:1 methanol : glacial acetic acid fixative. Slides may be used directly or dried and stored in the freezer (-20°C).
- Make slides on only one patient sample at a time to avoid cross-contamination between specimens.
- Further optimization of the protocol may be required.

Required Reagents & Equipment (Not Supplied)

Fixative – 3:1 Methanol : Glacial Acetic Acid
Hygrometer/Thermometer
Thermotron (ideal, but slides can be dropped at bench)
Phase Contrast Microscope

Step 1: Slide Making

1. Harvest sample using standard Cytogenetics protocols (see References or Cell Fixation Protocol).
2. Change fixative (3:1 methanol : glacial acetic acid) in sample tube until supernatant is colorless. Re-fix sample in fresh fixative just prior to slide making.
3. Aspirate supernatant ~0.5 ml above cell pellet and re-suspend cells by using slight agitation of conical tube.
4. Add enough fresh cold fixative to produce a slightly milky suspension.
5. Remove one slide from fixative coplin jar (optional step- if metaphases are not spreading well, dropping on a wet fixative slide may help spread the chromosomes), drain on paper towel. If a wet slide is unnecessary, remove dry slide from package.
6. Hold the slide diagonally by the frosted labeling area and drop three to five (3-5) drops of suspension along the top of the slide just below the frosted area, allowing the drops to run along the length of the slide. (Many technicians use different methods of dropping- for instance, hold the slide horizontally and drop 3 drops on the slide at the top, middle, and bottom. Choose what works best for you in your lab).
7. Gently rotate and tap the slide slightly after ~15 seconds to drain excess suspension on a paper towel.
8. Place slide vertically until a grainy appearance is observed and the slide completely dries. Time will vary according to atmospheric humidity (increase drying time if low humidity, decrease time if high humidity).
9. Wipe back of slide with a tissue.
10. Label the slide with the appropriate patient slide label. Never leave an unlabeled slide on slide warmer! To label, use an HB pencil or permanent alcohol resistant marker. Label slide with at least two unique patient identifiers, date and initial. Some labs prefer a sticker labeling system.
11. Assess slide under phase contrast microscope (10X and 40x objective). Check cells and metaphases. You should see about 50 cells per field of view. No cells should be touching/ clumping or overlapping. No cytoplasm should be surrounding metaphases. Common Protocols & Procedures FISH Slide Preparation Protocol Rev Date: 4/8/2021 Rev: 5 1 of 2 Chromosomes should be well spread with minimal overlapping. If cytoplasm is present and metaphases are not well spread, slow drying time but increasing humidity.

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Step 2: Assessing Slide Quality

Ideally the concentration should have ~50 interphase cells/field; interphase cells should be large, grey and flat. Metaphases should be well spread with minimum crossovers, intact (not overspread), no cytoplasm and dark grey in color. If not, consider the following variables:

1. Concentration

Too thick - Cells will be under spread and probably be in cytoplasm (metaphases will appear in 3D with a distinct halo surrounding them). Add additional fixative accordingly.

Too thin - Metaphases may not be intact, more probe is required for larger surface area. Re-centrifuge tube (1200 rpm for 10 minutes) and remove excess fixative accordingly.

2. Drying time indicators

Dried too fast - Interphase cells appear refractile, small and black; metaphase cells are under-spread, in cytoplasm (appear 3D with a halo around the metaphase) and black.

If humidity level is too low (below 40-45%) create a more humid environment by one of the following methods:

- Add a room humidifier,
- Make slides over a beaker of steam,
- Make slides over a sink with hot running water, place wet tissues on slide warmer and place slide briefly (~5 seconds) before placing directly on slide warmer (Step 1-11 above).

Dried too slowly - Metaphases are light grey and may be over spread or not intact. Both scenarios may produce poor quality hybridization. *Lower* humidity level.

If humidity level is too high (above 55%) a room dehumidifier may assist in lowering humidity level.

Correct drying - Interphase cells are flat, plump and pale; metaphase cells are dark grey, well spread with few crossovers, intact, with no cytoplasm.

3. Cytoplasm around metaphases

If cytoplasm around metaphases continues to be an issue after making adjustments in Concentration and Drying Time, add 1-2 drops of fresh fixative to slide after Step 1-8 above to try and wash away cytoplasm.

See references below for additional slide-making troubleshooting methods.

Step 3: Slide Storage

1. If slide will not be hybridized within 24-48 hours they may be stored in a sealed container in the fridge at 4°C for up to two (2) weeks.
2. Short term storage (2 weeks) of fixed cell suspensions- store in conical tubes at 4°C in the fridge. Long term storage- transfer pellet to cryovials and place in the freezer at -20°C.

References

Barch MJ, Knutsen T, Spurbeck JL. The AGT Cytogenetics Laboratory Manual, Third Edition, Chapter 3 – Peripheral Blood Cytogenetics Methods (Brown MG, Lawce HJ). Lippincott-Raven Philadelphia. 1991. Dunn B, Mouchrani P, Keagle M. The Cytogenetics Symposia – AGT, Second Edition.

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