FISH SLIDE PREPARATION PROTOCOL

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 2b for details).
- 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.
- Clean glass microscope slides by placing in a coplin jar with 70% alcohol for 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 g acetic acid:methanol 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.

Step 1 – Slide Making

1. Harvest sample using standard Cytogenetics protocols.*
2. Change fixative (Carnoy's 3:1 methanol:g acetic acid) in sample tube until supernatant is colorless. Refix sample in fresh fixative just prior to slide making.
3. Set slide warmer to ~45ºC.
4. Aspirate supernatant ~0.5ml above cell pellet and resuspend cells using a glass Pasteur pipette.
5. Add enough fresh cold fixative to produce a slightly milky suspension.
6. Remove one slide from fixative coplin jar, drain on paper towel. Alternatively remove one cold slide from the freezer.
7. Hold slide slightly vertically and drop 3 drops of suspension at top, middle and bottom of slide.
8. Gently rotate, tipping slightly after ~15 seconds to drain excess suspension on a tissue.
9. Keep slide horizontal until a grainy appearance is observed and the edges of the slide begin to dry. Time will vary according to atmospheric humidity (increase drying time if low humidity, decrease time if high humidity).
10. Wipe back of slide with a tissue, blow gently over slide and wave 2-3 times slowly.
11. Place slide directly on slide warmer until completely dry.
12. Label the slide with the appropriate patient slide label. Never leave an unlabelled 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.
13. Assess slide under phase contrast microscope (10X objective).

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:

a. **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 fix accordingly. **Too thin-** metaphases may not be intact, more probe is required for larger surface area. Recentrifuge tube (1200rpm for 10 minutes) and remove excess fix accordingly.

b. **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: 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. Decrease time in steps 9 and 10 prior to placing slide on slide warmer. 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 cross-over's, intact, with no cytoplasm.

c. **Cytoplasm around metaphases:**
If cytoplasm around metaphases continues to be an issue after making adjustments in step 2-a and 2-b above, add 1-2 drops of fresh fixative to slide after step 1- #9 above.

***See references below for additional slide making troubleshooting methods**

Step 3 – Slide Aging/Storage

1. Store dried slides in a desiccator at least 24 hours at room temperature to age sufficiently before the FISH step. If results are required quickly, leave the slide on the slide warmer for at least 15 minutes prior to FISH.
2. If slide will not be hybridized within 24-48 hours they may be stored in a sealed container in the freezer @ -20ºC for up to 2 weeks.
3. Fixed cell suspensions should be stored in cryovials in the freezer (-20°C).

*References:

1. Barch MJ, Knutsen T, Spurbeck JL. The AGT Cytogenetics Laboratory Manual Third Edition.
Chapter 3 - Peripheral Blood Cytogenetic Methods (M.G. Brown, H.J. Lawce). Lippincott-Raven Philadelphia1991.

2. Dunn B, Mouchrani P, Keagle M. The Cytogenetic Symposia - AGT. Second Edition.