FISH 'n' Tips

What's wrong with my haematology FISH?

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PROBLEM	INSUFFICIENT FIXATION ON BLOOD SMEARS	HIGH LEVELS OF GREEN BACKGROUND	BRIGHT, GREEN SIGNALS ON CELL SEPARATED SAMPLES	SMALL, WEAK SIGNALS	BACTERIAL CONTAMINATION
LIKELY CAUSES	• High levels of red background caused by presence of erythrocytes and cellular debris on the slide • The fixation time is critical and depends on the size and erythrocyte density of the smear	• Positively-charged slides are designed for tissue sections to ensure good adhesions, however these can produce higher levels of green background on other cell samples	• CD138+ separated samples can produce a high volume of cytoplasm and debris, which can be seen as bright green fluorescence	• Caused by baking or ageing slides • Could also be a result of high stringency in the post-hybridisation wash	• Bacteria may fluoresce very brightly under both the FITC (Fluorescein isothiocyanate) and Texas Red® filters, depending on the type of bacteria • The level of contamination can vary depending on the length of culture i.e 24 or 72 hours
SOLUTION	✓ 10-30 minutes fixation time with 3:1 methanol/ glacial acetic acid is recommended ✓ Replace with fresh fixative (3:1 methanol/ glacial acetic acid fix) after 10 minutes ✓ Hypotonic solutions, such as potassium chloride can be used with smear slides to improve FISH results	✓ Review the type of slides used, try using non-adhesive slides	✓ Use <3µl cell suspension and treat with a hypotonic solution, such as potassium chloride, followed by an enzymatic digestion step using a protease	✓ Avoid baking (>45°C) or ageing slides in heated SSC solutions. This reduces signal intensity and can be time consuming ✓ Re-make 0.4xSSC ensuring that the pH=7 and the wash temperature is 72+/-1°C	✓ Always use fresh cell samples and reset the culture if required ✓ Ensure aseptic techniques are correctly followed when culturing cells to minimise risk of contamination ✓ Regularly refresh your solutions, including reagent bottles and coplin jars

INTERPRETATION OF SIGNALS

Breakapart:

Normal Signal Pattern (2F)

Abnormal Signal Pattern (1R, 1G, 1F)

In some breakapart probes, the red and green signals may not be tightly co-localised in all cells, especially when chromatin is extended in interphase. It is therefore important to determine the cut-off levels for a normal result[1].

Translocation:

Normal Signal Pattern (2R, 2G)

Abnormal Signal Pattern (1R, 1G, 2F)

The dual-fusion translocation probes span the breakpoints of interest. The abnormal pattern is rarely seen as an artefact of chromosome overlap in interphase[1]. In an abnormal cell, this produces two fusion signals (co-localised red and green signals).

Scoring Recommendations[1]:

- Analyse only intact nuclei, not overlapped or crowded nuclei or nuclei covered by cytoplasmic debris or high degree of auto-fluorescence
- Signal intensity may vary, even within a single nucleus. In such cases, use single filters and/or adjust the focal plane.

1. Analysis and scoring guidelines recommended by The AGT Cytogenetics Laboratory Manual (fourth edition).

For more troubleshooting tips, visit our FISH 'n' Tips page:
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