

FLUORESCENCE IN SITU HYBRIDIZATION (FISH) TEMPERATURE-DEPENDENT EQUIPMENT COMPARISONS: TEMPERATURE GRADIENT EFFECTS AND ANALYTICAL OUTCOME



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PROBLEM/QUESTION

Differences in hybridization platforms used in fluorescence in situ hybridization (FISH) analysis experiments can lead to significant differences in hybridization results. To address some of the challenges involved in genomic hybridization technology as a clinical tool, variations in performance characteristics of hybridization platforms were evaluated (HyBrite and ThermoBrite (Abbott Molecular), CytoBrite (SciGene)). We proposed that based on the thermoelectric technology (i.e. Peltier technology) for rapid heating and cooling, the performance of the CytoBrite would allow for improved and more accurate probe denaturation and hybridization.

BACKGROUND

Historically, our laboratory has experienced several FISH "failures" due to inefficient probe hybridization. The primary reason for the failure of a patient assay is the uncertainty of analytical results as a function of diffuse signal patterns (i.e. "spatter") or lack of hybridization. This has been especially evident in the evaluation of peripheral blood and bone marrow smears for straight-forward assays such as BCR/ABL (t(9;22) fusions). In a single month, a total of eight FISH failures (requiring repeat preparation and processing) all involving hematology FISH targets (BCR/ABL, PDGFRA, PDGFRB, PML/RARA). While FFPE tissues can be problematic, fresh specimens should be highly accurate, analyzable, and reproducible. Technologist notes in the analysis log trace back to diffuse, unable to analyze signals. In the first 6 months of 2013, a total of 60 FISH failures (requiring repeats) were logged. As with any other DNA hybridization assay, the main factors influencing failures and/or background are: (1) the amount of repetitive sequences of the probe, and the extent to which they are blocked; (2) hybridization temperature (lowering it increases non-specific binding of the repetitive sequences); (3) the balance between hybridization time and amount of DNA probe; and (4) the stringency of the post-hybridization washes.

VARIABLES/RESEARCH

Controlled variables

- Commercially available FISH probes

Independent variables

- Hybridization platforms
 - HyBrite
 - ThermoBrite
 - CytoBrite

Dependent variables

- Hybridization efficiency
 - Good, Okay, Poor
- Probe signal intensity
 - Good, Okay, Poor
- Overall assay Results
 - Acceptable, Unacceptable

METHOD

- Using expired probes from commercial vendors, comparative studies were performed using the CytoBrite, HyBrite, and ThermoBrite. In a preliminary study to confirm denaturation conditions that lead to probe splatter, parallel sets of blood/bone marrow samples were analyzed using BCR/ABL and PML/RARA probes (CytoCell) and run on the three instruments with the first set of samples following recommended denaturation times and the second set denatured for twice the recommended denaturation times.
- Subsequently, slide preparation, denaturation and hybridization conditions were performed according to the probe manufacturer's specifications and DeltaMDx standard laboratory protocol. Post-hybridization wash solutions were performed using the ready-to-use FISH Wash Buffer 1 (0.4%SSC/0.3% IGEAL, pH7) and FISH Wash Buffer 2 (2xSSC/0.1% IGEAL, pH7) (SciGene). All samples were sealed with CytoBond prior to denaturation and processing. Specimen types included both FFPE tissues, cytogenetic preparations (pellets fixed in Carnoy's), and peripheral blood/bone marrow smears. Triplicate slides were prepared on all specimens. Positive controls (if available) were incorporated into the study as appropriate.
- Analytical parameters were recorded for all probe sets according to laboratory protocol.

RESULTS - SUMMARY

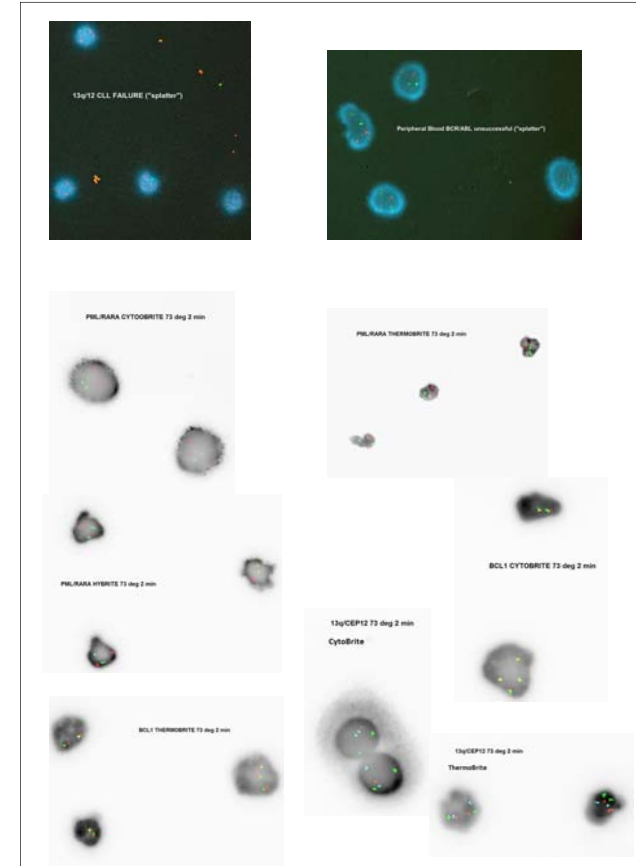
A total of 50 prepared slides from various specimen types and various probes were compared. FISH slides were compared for hybridization efficiency, probe signal intensity, and overall assay results. Based on the initial visual interrogation, the CytoBrite hybridization platform appeared to produce less "spatter" and more analyzable signals (regardless of the probe). Out of 25 fresh specimens (bone marrow/peripheral blood smears) and 25 FFPE specimens, 20 (80%) and 25 (100%) fresh and FFPE, respectively showed improved analyzable signals using the CytoBrite. Improved signal data included subjective and objective parameters such as signal intensity/strength, background, and tissue architecture (FFPE).

CONCLUSIONS

Peltier technology is a vast improvement over existing FISH hybridization platforms. Used for many years in traditional polymerase chain reaction (PCR) assay procedures, the rapid heating and cooling parameters allows for efficient denaturation and hybridization in FISH assays. This efficiency results in reproducible and successful assays for many distinctly different tissues and FISH probe types.



RESULTS- COMPOSITE IMAGES



ACKNOWLEDGEMENTS

- SciGene – for technical and scientific support
- CytoCell/Rainbow Scientific – for providing FISH probes
- CymoGen Dx- for providing FISH probes

