# MicroFISH: Increased Efficiency and Cost Savings Utilizing a Multi-well Based System for FISH Hybridization

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## INTRODUCTION

FISH assays are among the most widely used diagnostic tests for human cancers. However, the combination of the high cost of DNA probes used in a labor intensive, complex workflow and shrinking cost reimbursements is causing mounting economic strain on cytogenetics laboratories. We describe a simple, slide-based method with streamlined workflow that reduces up to 10-fold the cost per test for routine FISH assays. The methodology is based upon an eight-well slide, transfer racks and slide hybridization and incubation equipment (MicroFISH Assay System; SciGene) for performing up to eight independent microvolume FISH assays on a single slide (Figure 1).

Slides have eight preformed wells with a novel hydrophobic coating with three unique properties for this application: 1) solutions containing cell samples or probe are retained within the boundary of the wells; 2) coverslips bond to the hydrophobic coating during incubation eliminating use of sealants such as rubber cement, and; 3) coverslips are easily removed after incubation by mechanical agitation.

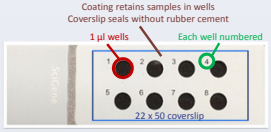


Figure 1. Features of the MicroFISH Slide

These unique features provide several important advantages over existing methods. Assays can now be performed using as little as 1 µl of sample and probe per well preserving precious samples and dramatically reducing probe cost per test. Since up to eight assays can be performed on a single slide, work is reduced by eliminating handling and processing of multiple slides when running a panel of probes. Eliminating the requirement for coverslip sealant removes a time consuming and tedious step in the workflow and reduces repetitive motion. Lastly, the ability to remove coverslips by mechanical agitation eliminates the time consuming step of removing each coverslip by hand and allows batch processing of slides using a slide rack. Further efficiencies are gained by utilization of a single slide rack for transfers between equipment for performing the denaturation and hybridization steps allowing denaturation of multiple batches of slides on a single heating unit with bulk overnight incubation of slides in an oven.

In this first report, we present the results of a series of technical studies that compare the reliability and extensibility of the MicroFISH method to existing methods using a variety of different probes from major suppliers. We will also report the results of a clinical study that examines the quality of clinical results obtained using this new method and the impact of its simplified workflow on labor costs and retest rates.

## MATERIALS AND METHODS

### Probes and Reagents

Probes used in these studies were from CytoCell (OGT) and Abbott . Abbott probes were prepared according to manufacturer's recommendations. CytoCell probes were prepared to manufacturer's recommendations but, were prehydrated 10% with $H_2O$. Wash buffer consisted of 0.4X SSC; 0.3% NP40 (VWR) at pre-established temperatures and counterstained with DAPI (Vector).

### Equipment

CytoBrite Slide Incubation System and CytoBrite Slide Oven (SciGene) were used for performing the denaturation and overnight incubation steps respectively. Slides were imaged using standard methods (Cytovision) after manual examination.

### Slide Preparation

1-2 µl of Carnoy's fixed bone marrow pellet was dropped within each well to be used on the MicroFISH slide(s) and air dried. Slides were placed in CytoBrite trays then transferred to a 90˚C oven for 10 minutes. 1 µl of each probe (Cytocell or Abbott) was placed in the center of the sample wells and a single 22 x 50 mm coverslip placed over all wells. No coverslip sealant was used.

### Hybridization

CytoBrite slide trays with coverslipped MicroFISH slides were transferred to a CytoBrite Slide Incubation System and heated from 37 to 80˚C, held for 5 minutes for co-denaturation, then cooled to 37˚C. Trays were transferred to a sealed chamber with a moist absorbent pad and placed in a CytoBrite Slide Oven at 37˚C overnight.

## MATERIALS AND METHODS (cont.)

### Post-Hybridization Processing

After overnight incubation, the sealed humidity chamber was taken from the oven and CytoBrite slide trays removed. MicroFISH slides were immediately placed in Little Dipper® Processor slide rack (SciGene) without removing coverslips.

The slides were then processed using the following protocol: Bath #1 - 600 RPM - 5 seconds - wash buffer @ room temperature (shakes off coverslip); Bath # 2 - No agitation - 60 seconds - Wash Buffer @ 72˚C; Bath # 3 - No agitation - 30 seconds - Wash Buffer @ room temperature; Centrifuge 30 seconds to dry; MicroFISH slides were removed from the rack; 5 µl of DAPI placed on each sample well and a 22x50 mm coverslip placed over the wells.

### Imaging

Images were obtained under 100X oil objectives using standard methods after manual evaluation of signals.

## RESULTS

### Cell Quantity per Well

Routine FISH analysis for hematologic diseases typically ranges from 100-500 nuclei. A sufficient number of scorable cells must be present in each well for anticipated performance gains. Analysis of 200 nuclei was routinely attainable on MicroFISH slides. Census of total number of nuclei was taken in a representative number of wells. With medium to high density, 1000-1500 DAPI-positive nuclei were available for analysis, giving ample number of cells for most standard interphase FISH analysis.

### Well-to-Well Cross-contamination

Cross-contamination of probe solutions into hybridization areas is an area of concern with a multi-well format. To determine if this would occur, 1.0 µl of cell sample was applied to each of four adjoining wells. Alternate wells had 1.0 µl of CEP4 or CEP10 probe applied while remaining wells received 1ul of hybridization solution without probe. Slides were processed per protocol and counterstained with DAPI. Areas with probe showed appropriate signal patterns. Areas with no probe sequence were then analyzed for evidence of cross contamination. No probe cross contamination was observed (Figure 2).

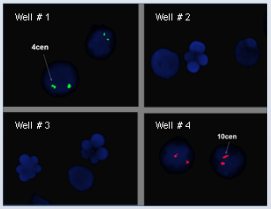


Figure 2. Examination of wells for cross-contamination.

### Rubber Cement/Probe Prehydration

Sealing of the hybridization areas with rubber cement or similar sealant maintains moisture content within the hybridization area and prevents contamination by exogenous humidity sources. The hydrophobic slide coating used on MicroFISH slides was formulated to eliminate coverslip sealant. To test this, parallel runs using TP53 and 4-14 probes were performed with or without rubber cement sealant. Results were indistinguishable between the rubber cement sealed slides and those without coverslip sealant (Figure 3). In this same study, we also examined the effects of serial prehydration of the Cytocell (OGT) probes on signal quality and found that 10% prehydration generally increased signal intensity. Additional prehydration beyond 10% decreased signal quality.

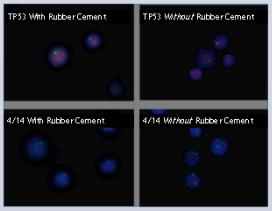


Figure 3. MicroFISH slides processed with and without rubber cement.

## RESULTS (cont.)

### Evaluation of Standard Probe Libraries

To determine if MicroFISH slides and associated workflow was amenable to a variety of probe sizes and designs, 16 common probes from CytoCell (OGT) and Abbott were studied. All probes including, spot counting, dual fusion, and break-apart probe designs performed satisfactorily. (Figure 4)

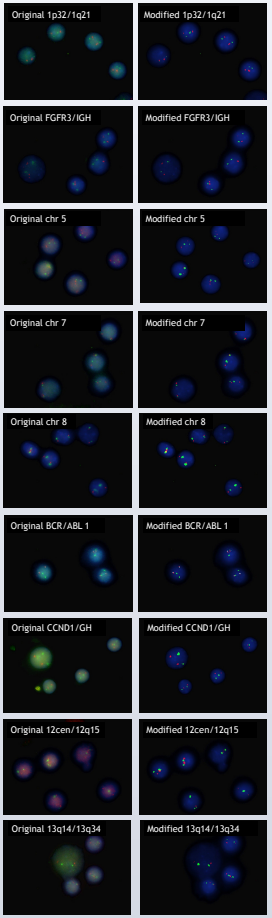


Figure 4. Original and modified images of common probes processed with the MicroFISH method.

### Work Analysis

We performed stop-watched time and motion studies of the steps used for performing a FISH assay on 12 patients using a four probe MDS panel following traditional and MicroFISH assay methods. The traditional method used standard slides with two cell spots per slide (two slides per patient), automated pretreatment using the VP2000, rubber cement to seal coverslips and manual post-hybridization processing. The MicroFISH method used 10 min heating of slides at 90˚C for pretreatment, no rubber cement and automated removal of coverslips and post-hyb processing on the Little Dipper Processor (Figure 5). We found a single technician using the MicroFISH system can prepare 12 patients slides ready for imaging in under one hour. The traditional method used in this example required three times the hands-on time and four times the elapsed time (Table 2).

### MicroFISH Workflow

Pipette 1-2µl fixed cells/well.

**CytoBrite® Duo System**
Heat @ 90˚C for 10 min.

Pipette 1µl probe per well.
Place coverslip.

**CytoBrite® System**
Denature @ 80˚C for 5 min.

**CytoBrite® Slide Oven**
Incubate @ 37˚C overnight.

**Little Dipper® Processor**
Removes coverslip.
Post-hyb wash and dry.

Apply DAPI.
Place coverslip.

Figure 5. MicroFISH Assay Workflow

Table 2. Time Study of Traditional vs. MicroFISH Methods.

| | Traditional | | MicroFISH | |
|---|---|---|---|---|
| # Slides | 24 | | 12 | |
| # Coverslips | 48 | | 12 | |
| | Time (min) | | | |
| | Hands-On | Elapsed | Hands-On | Elapsed |
| Cell Dropping | 30 | 30 | 15 | 15 |
| Pretreatment | 5 | 60 | 0 | 10 |
| Probe + Coverslipping | 60 | 60 | 18 | 18 |
| Coverslip Removal | 15 | 15 | 0 | 0 |
| Post-hyb Wash/Dry | 12 | 36 | 5 | 5 |
| DAPI + Coverslip | 24 | 24 | 12 | 12 |
| **Total** | **146** | **225** | **50** | **60** |

| | Time Savings | | | |
|---|---|---|---|---|
| | Minutes | | Percentage | |
| | Hands-On | Elapsed | Hands-On | Elapsed |
| Cell Dropping | 15 | 15 | 50 | 50 |
| Pretreatment | 5 | 50 | 100 | 83 |
| Probe + Coverslipping | 42 | 42 | 70 | 70 |
| Coverslip Removal | 15 | 15 | 100 | 100 |
| Post-hyb Wash/Dry | 7 | 31 | 58 | 86 |
| DAPI + Coverslip | 12 | 12 | 50 | 50 |
| **Total** | **96** | **165** | **66%** | **73%** |

### Clinical Validation

Finally, this method must show approximate levels of detection to current methods. Twenty consecutive myelodysplasia panels (5q, 7q, +8, 20q) were run in parallel. Concordant negative results were obtained. Clinical cut-off values were calculated for the 20 MicroFISH slides and compared to current, extensive continuous validation data utilizing standard protocol at GAI. Both 8-inverse normal values and 3SD abnormal cut-off values were comparable in the limited study (Table 3). More limited data, (not shown) is suggestive the trend will hold against all probe panels.

Table 3. Clinical cut-off values for 20 MicroFISH slides

| Probe | 8-inverse normal cut-off | | Abnormal patterns 3 Std Deviation | |
|---|---|---|---|---|
| del(5q) Cytocell | 2r2g | 1r2g | 1r1g | 2r1g |
| Continuous validation | 94% | >2% | >1% | >1% |
| MicroFISH 20 normals | 94% | >2% | >2% | >2% |
| | | | | |
| del(7q) Cytocell | 2r2g | 1r2g | 1r1g | 2r1g |
| Continuous validation | 96% | >1% | >2% | >1% |
| MicroFISH 20 normals | 93% | >3% | >3% | >1% |
| | | | | |
| CHARGE, DB22 Cytocell | 2r2g | 3r3g | | |
| Continuous validation | 96% | >1% | | |
| MicroFISH 20 normals | 95% | >2% | | |
| | | | | |
| del(20q) Cytocell | 2r2g | 1r2g | 1r1g | 2r1g |
| Continuous validation | 96% | >2% | >2% | >1% |
| MicroFISH 20 normals | 92% | >2% | >3% | >1% |

Example: 12 Patients / MDS Panel/ Four Probes

Table 4. Annual Probe Cost Savings Using MicroFISH

| Probe Cost | # Cases / Week | | | |
|---|---|---|---|---|
| $/ul | 25 | 50 | 100 | 250 |
| $2 | $41,600 | $83,200 | $166,400 | $416,000 |
| $4 | $83,200 | $166,400 | $332,800 | $832,000 |
| $6 | $124,800 | $249,600 | $499,200 | $1,248,000 |
| $8 | $156,000 | $312,000 | $624,000 | $1,560,000 |
| $10 | $208,000 | $416,000 | $832,000 | $2,080,000 |
| $12 | $249,600 | $499,200 | $998,400 | $2,496,000 |
| $14 | $291,200 | $582,400 | $1,164,800 | $2,912,000 |

Annual Probe Savings with a Four Probe Panel

## CONCLUSIONS

We describe here a new microvolume, multiwell slide (MicroFISH Assay Slide, SciGene) and associated simplified workflow for performing FISH. This new system uses 1 µl of FISH probe per sample with up to 8 probes run on a single slide. The specially engineered slide coating keeps the sample and probe within the well area and eliminates the need for rubber cement to seal coverslips. The simplified MicroFISH workflow demonstrates that one technician can set up and process 12 patient samples (12 slides) ready for imaging in 60 minutes or less hands-on time.

We have shown that the MicroFISH system has been successfully used with probes across major vendors and probe designs. In a clinical study, results were comparable to standard methods with similar cut-off values.

The combination of lower probe volume and reduced labor when performing FISH using the MicroFISH system will provide a much needed reduction in cost per test in reimbursement constrained cytogenetics laboratories.

## CONTACT INFORMATION

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