Original Research

Evaluation of the Vysis IntelliFISH Hybridization Buffer and Vysis IntelliFISH Universal FFPE Pretreatment and Wash Kit in FISH Assays

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Standard fluorescence in-situ hybridization (FISH) technique requires a hybridization time of 12 hours or greater, thus amounting to a turn-around time (TAT) of 24 hours or greater for result reporting. To improve the TAT for the FISH tests, a new Vysis IntelliFISH Hybridization Buffer has been recently developed by Abbott Molecular. It was evaluated in 20 pairs of matched bone marrow samples and 10 pairs of matched formalin-fixed lymphoma tissue samples against the standard Vysis LSI/WCP Hybridization Buffer protocol. Compared to the standard Vysis LSI/WCP Hybridization Buffer protocol, the new fast working Vysis IntelliFISH Hybridization Buffer protocol reduced FISH hybridization time and simplified the workflow of the standard overnight hybridization protocol. In addition, signal intensity, slide background and signal specificity of FISH probes were comparable to those generated with the standard hybridization protocol.
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INTRODUCTION

FISH, as a specific assay, is widely used in hematological malignancies. It uses fluorescently labeled probes for detection of specific chromosome aberrations, and offers high sensitivity and specificity.[1] The advantage of this technique is its application in both dividing cells (metaphase chromosome preparation) and non-dividing cells (interphase nuclei). It has been proven to be very reliable and can also be performed on formalin-fixed, paraffin-embedded tissue (FFPE) samples.[2,3] In contrast to conventional cytogenetics, it rapidly identifies specific genomic abnormalities needed for clinical diagnosis.

In addition, FISH can detect cryptic aberrations.[4,5] It plays a central role in bridging conventional cytogenetics techniques (5-10 Mb) with molecular biology techniques (base pairs). Clinical application of FISH technology has upgraded classical cytogenetics to molecular cytogenetics. Furthermore, FISH is currently applied to monitor the response to treatment, especially when complete cytogenetics response (CCyR) is the therapeutic goal. With good quality commercial probes, FISH assays can achieve sensitivities of 1%-6%.[6]

In the past decades, although there have been great innovative technical advances in the field of cytogenetics which have enhanced the detection of chromosomal alterations, a typical FISH assay has changed minimally. Standard FISH techniques still require more than 12-hour hybridization times, thus amounting to turn around times (TAT) of 24 hours or greater for results reporting. To improve the TAT for the FISH tests, a new fast working Vysis IntelliFISH Hybridization Buffer has been recently developed by Abbott Molecular. With the improvement of hybridization buffer, the hybridization time was significantly reduced to less than 3 hours, allowing the FISH assay to be a rapid assay.

METHODS

Specimen Preparation

Bone marrow specimens were collected according to institutional guidelines. The specimens were prepared according to the laboratory developed protocol and fixed in Carnoy's fixative.

FFPE lymphoma tissue specimens were sectioned at 4μm and were mounted onto positively charged slides. The specimens were pretreated using Vysis IntelliFISH Universal FFPE Tissue Pretreatment and Wash Reagents (Abbott Molecular Inc., Des Plaines, IL) as previously deescriped,[7] with SSC pretreatment at 80ºC for 25 min and protease digestion at 37ºC for 20 min.

Probe Preparation and FISH Denaturation and Hybridization Conditions

Vysis IntelliFISH Hybridization Buffer was evaluated in bone marrow and formalin-fixed, paraffin-embedded (FFPE) lymphoma tissue samples. The sample slides from 20 bone

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marrow samples and 10 FFPE lymphoma tissue specimens were probed with Vysis TP53/CEP17 FISH Probe Kit (Abbott Molecular Inc., Des Plaines, IL) and Vysis IGH/MYC/CEP8 Tri-color DF FISH Probe Kit (Abbott Molecular Inc., Des Plaines, IL), respectively, in duplicate by using: (1) the standard Vysis LSI/WCP Hybridization Buffer protocol and (2) Vysis IntelliFISH Hybridization Buffer (Abbott Molecular Inc., Des Plaines, IL) as previously described[8]. The preparation of probe mixture using Vysis LSI/WCP Hybridization Buffer and Vysis IntelliFISH Hybridization Buffer is presented in **Table 1**. **Table 2** showed the denaturation and hybridization conditions using Vysis LSI/WCP Hybridization Buffer and Vysis IntelliFISH Hybridization Buffer protocols.

Table 1. Probe mixture composition using Vysis IntelliFISH Hybridization Buffer and the standard Vysis LSI/WCP Hybridization Buffer.

Buffer	**Vysis IntelliFISH Hybridization Buffer Probe Mixture Composition**			**Standard Vysis LSI/WCP Hybridization Buffer Probe Mixture Composition**		
Component	Hybridization buffer	Water	Probe	Hybridization buffer	Water	Probe
Volume added	12 µL	2 µL	1 µL	7 µL	2 µL	1 µL
Total Volume	15 µL			10 µL		

Table 2. Denaturation and hybridization conditions for bone marrow and FFPE lymphoma tissue specimens.

	Bone Marrow Specimens				**FFPE Specimens**			
	Denat Temp	Denat Time	Hyb Temp	Hyb Time	Denat Temp	Denat Time	Hyb Temp	Hyb Time
Vysis IntelliFISH Hybridization Buffer	80°C	5 min	37°C	3 hrs	73°C	5 min	37°C	3 hrs
Vysis LSI/WCP Hybridization Buffer	78°C	5 min	37°C	18 hrs	73°C	5 min	37°C	18 hrs

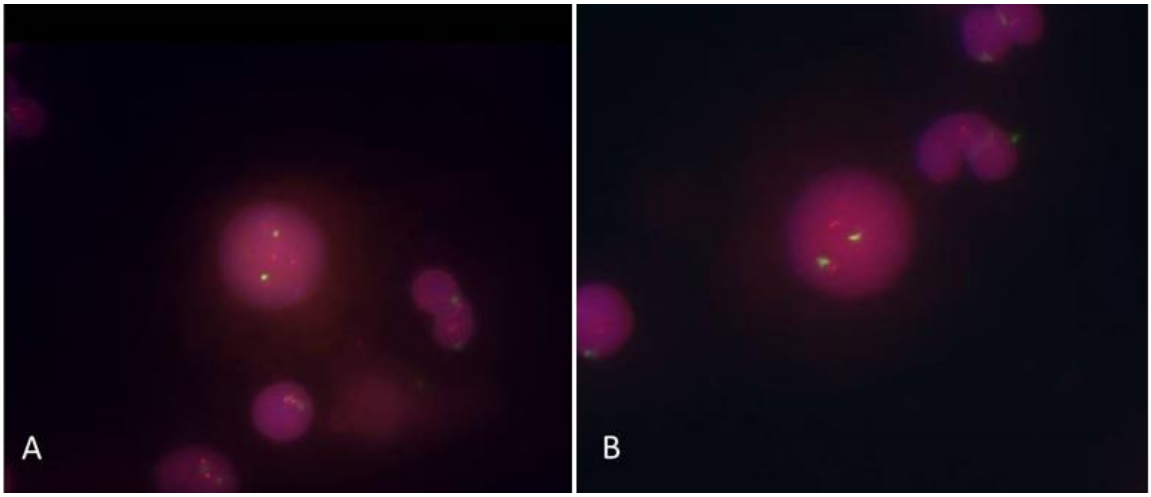

Figure 1. The representative hybridization results of (**A**) standard Vysis LSI/WCP Hybridization Buffer and (**B**) Vysis IntelliFISH Hybridization Buffer in Bone Marrow samples.

Performance Comparison

To compare the hybridization performance using Vysis LSI/WCP Hybridization Buffer and Vysis IntelliFISH Hybridization Buffer, each hybridized sample were given a score of 1 to 5 for two elements of performance: (1) signal intensity and (2) slide background; and a score of 1 to 4 for hybridization specificity. Incremental score for each element indicated improvement in the signal intensity, slide background and hybridization specificity.

For signal intensity, ideal signals should be bright and distinct so that the user can easily evaluate the interphase cells in the target hybridization area using the prescribed filter set. Score of 1 referred to no detectable signals in any of the cells analyzed in within the hybridization area, and score of 2 to 5 referred to incremental signal detected in the hybridization area, from approximately 25% to more than 90%.

Ideal slide background in the target areas should appear dark or black without any fluorescent particles or haze. Score of 1 indicated numerous background fluorescent particles on the slide and the particle covers the entire target area. Score of 2 to 5 indicated progressive improvement of the background to absence of background particle on the slide.

Hybridization specificity rated the presence of non-specific hybridization, from less than 25% of the cells showing weak non-specific hybridization (score of 4) to obvious presence of in all the cells (score of 1).

The performance of Vysis IntelliFISH Universal FFPE Tissue Pretreatment and Wash Reagents was evaluated based on the first time success rate and the specimens were scored using the rating system for signal intensity and slide background.

The results from the two cohorts were analyzed using t-test: paired two samples for means, with p value of < 0.05 considered as significant.

RESULTS

Bone Marrow Specimens

Hybridization results of Vysis TP53/ CEP17 FISH Probe Kit using Vysis IntelliFISH Hybridization Buffer for 2 to 3 hours demonstrated equivalent performance in bone marrow samples in terms of signal intensity when compared to overnight hybridization using Vysis LSI/WCP Hybridization Buffer ($p = 0.5$). Slide background and signal specificity improved using Vysis IntelliFISH Hybridization Buffer when compared to Vysis LSI/WCP Hybridization Buffer ($p = 0.04$ and $p = 0.02$ for slide background and signal specificity respectively). The representative hybridization results using the two protocols were presented in **Figure 1**).

FFPE Lymphoma Tissue Specimens

The first time success rate when Vysis Universal FFPE Tissue Pretreatment and Wash Kit was 100%. The slides processed with Vysis Universal FFPE Tissue Pretreatment and Wash Kit and Vysis IntelliFISH Hybridization Buffer tend to have greenish background, as shown in **Figure 2**. However, the greenish background did not interfere with the signal interpretation as the signal-to-noise ratio on the slides was still comparable to Vysis LSI/WCP Hybridization Buffer.

The hybridization results from FFPE lymphoma tissue specimens from 3-hour hybridization using IntelliFISH Hybridization Buffer were equivalent to the results from overnight hybridization using Vysis LSI/WCP Hybridization Buffer. The p value for signal intensity, slide background and signal specificity between the two cohorts was 0.2, 0.3 and 0.2 respectively, showing no significant difference between 3-hour hybridization and overnight hybridization (**Figure 2**).

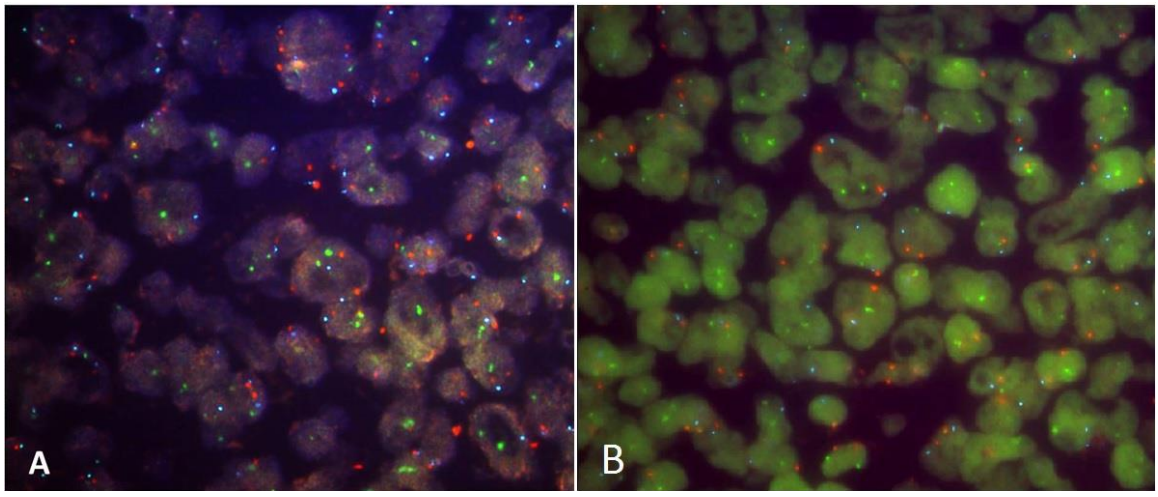

Figure 2. The hybridization results of (**A**) standard Vysis LSI/WCP Hybridization Buffer and (**B**) Vysis IntelliFISH Hybridization Buffer in FFPE lymphoma tissue samples. The slides were processed using Vysis Universal FFPE Tissue Pretreatment and Wash Kit and probed with Vysis IGH/MYC/CEP8 Tri-Color DF FISH Probe Kit.

DISSCUSION

FISH is a widely used laboratory method. It has a broad spectrum of clinical and research applications, such as diagnostics in hematologic and solid tumors. FISH has the advantage that it can be used in metaphase chromosomes and interphase nuclei, and to identify chromosomal abnormalities through fluorescent labeled DNA probes.[9] Recurrent chromosomal abnormalities including translocations, deletions, duplications, and gene amplifications have been characterized. Specifically designed FISH panels have also been widely performed in the diagnosis and monitoring of acquired chromosomal abnormalities in hematologic and solid tumors.[9-11]

FISH results often offer a quick evaluation of targeted abnormal patterns and their percentage within the cells from bone marrow or solid tumors. For example, acute

promyelocytic leukemia (APL) patients with underlying *PML/RARa* fusions require immediate treatment. Rapid FISH result of *PML/RARa* test is mandated for the administration of all-trans retinoic acid (ATRA).[12] Currently, most laboratories perform overnight hybridization, resulting in a minimum turnaround time of 20 - 24 hours. In this study, a newly developed, commercially available hybridization buffer, Vysis IntelliFISH Hybridization Buffer was evaluated. The evaluation compared the signal intensity, slide background and signal specificity in paired bone marrow and FFPE lymphoma tissue specimens using the current overnight hybridization and 3 hours hybridization with Vysis IntelliFISH Hybridization Buffer.

The result of the evaluation showed that Vysis IntelliFISH Hybridization Buffer offered a faster turnaround time for both bone marrow and FFPE lymphoma tissue specimens. In addition, the shorter turnaround time was not at the expense of the quality of the hybridization result. The hybridization results from Vysis IntelliFISH Hybridization Buffer were equivalent to the hybridization results using Vysis LSI/WCP Hybridization Buffer in both bone marrow and FFPE lymphoma tissue specimens. This will offer an attractive option to FISH laboratories to generate a faster turnaround time for FISH tests.

The first time success rate when using Vysis IntelliFISH Universal FFPE Pretreatment and Wash Kit was 100%, which eliminates the need to repeat failed hybridizations. This can help to increase the efficiency of processing and ultimately save the reagent costs. It also offers a simplified, standardized pretreatment protocol for processing FFPE tissue specimens. In combination with Vysis IntelliFISH Hybridization Buffer, the workflow can reduce the turnaround time significantly, at the same time retaining equivalent signal quality compared to overnight hybridization.

In the near future, the fast working hybridization buffer and the availability of more disease-specific probes will further accelerate and expand the clinical and research application of FISH.

CONCLUSIONS

To ensure safe and effective diagnostic application, the analytical validity of the Vysis IntelliFISH Hybridization Buffer has been evaluated by its signal intensity, specificity and slide background using both the standard overnight protocol and Vysis IntelliFISH fast working protocol. In the present study, the Vysis IntelliFISH Hybridization Buffer has significantly reduced FISH hybridization time and simplified the workflow of the standard overnight Vysis hybridization protocols. Signal intensity, specificity and slide background were comparable to standard hybridization protocols. Vysis IntelliFISH Universal FFPE Pretreatment and Wash kit provides a standardized pretreatment protocol for processing FFPE specimens. In combination with IntelliFISH Hybridization Buffer, it provides an efficient workflow for FFPE in increasing the success rate and reducing the turnaround time.

CONFLICT OF INTEREST

The authors have no conflict of interest to disclose. We would like to thank Abbott Molecular Inc. for the providing Vysis IntelliFISH reagents and Vysis FISH probes for the study.

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