CytoCell

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A Sysmex Group Company

Interpretation Guide

CDA-LPH013 KMT2A Breakapart FISH Probe Kit PDx FISH Analysis

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1. Introduction & Purpose

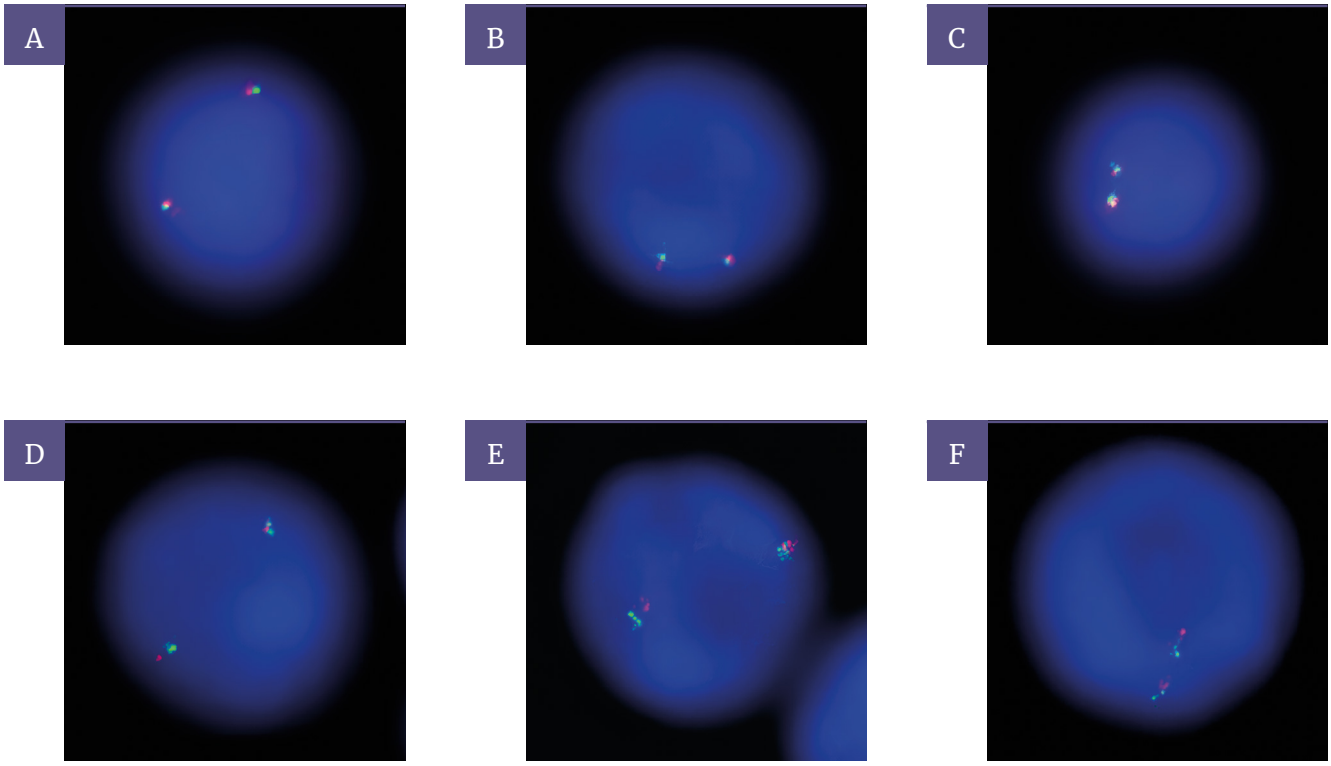
This interpretation guide has been developed to assist users in accurately interpreting fluorescence in situ hybridization (FISH) signals generated using the CDA-LPH013 KMT2A Breakapart FISH Probe Kit PDx.

This version includes:

- Visual examples of common signal types, including near cut-off cases
- Rules for which cells should and should not be counted
- Access information via QR code

2. Image Gallery: Cell Interpretations

2.1. Normal cells (2F)

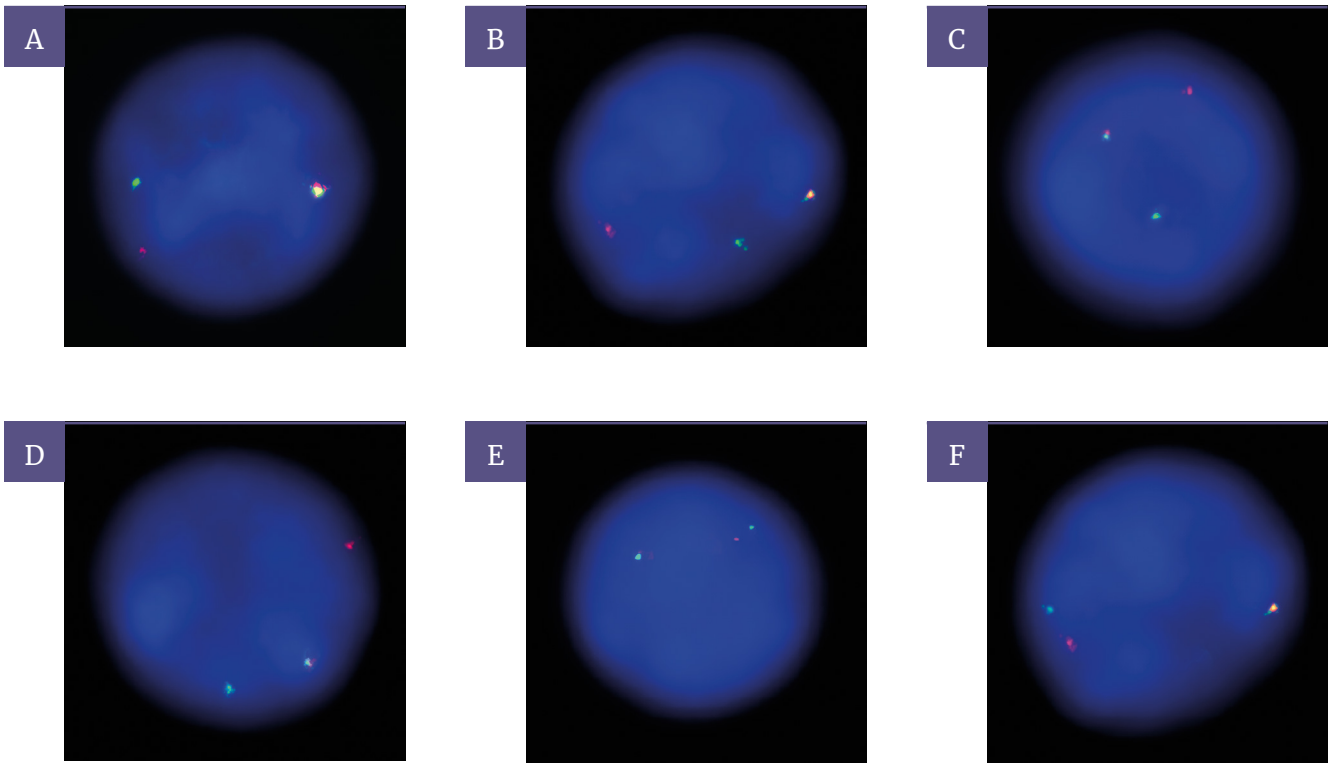


Cells A to C display the expected normal **2F signal pattern**, with two distinct red/green fusion signals, consistent with a negative result.

Cells D and E each show a red and green signal with a small gap; however, the distance between these signals is **less than two signal widths**. Per the Package Insert, "if there is a gap between the red and green signal no greater than two signal widths apart, count as not rearranged/fused signal." Therefore, these cells are also interpreted as **normal 2F signal pattern** consistent with a negative result.

Cell F shows diffuse green signals, each with a faint strand connecting to the red signals. From the Package Insert, "If two signals of the same color touch each other, or the distance between them is no greater than two signal widths, or when there is a faint strand connecting the two signals, count as one signal". Therefore, this cell is also interpreted as **normal 2F signal pattern** consistent with a negative result.

2.2. Abnormal cells (1F1R1G)



Images A to D show representative nuclei with **1F1R1G *KMT2A*** abnormal signal patterns. This signal pattern is consistent with a *KMT2A* rearrangement and should be counted as abnormal during analysis. The example images demonstrate clearly defined signals in well-spread, intact nuclei and serve as a reference for identifying abnormal cells.

Images E and F also display **1F1R1G *KMT2A*** abnormal signal patterns, in which the separation between the red and green signals is just greater than two signal widths. As defined in the Package Insert, these signals meet the criteria for a separated (i.e., not fused) signal and should therefore be counted as *KMT2A* abnormal, alongside the more clearly separated examples shown in A to D.

Note: While these individual cells display *KMT2A* abnormal patterns, a sample is only interpreted as positive when the overall percentage of abnormal nuclei exceeds the established cut-off (3.8% for AML, 3.1% for ALL). However, if the abnormal signal count result falls between 1x and 2x the cut-off, interpret with caution.

2.3. Other *KMT2A*-Abnormal Signal Patterns

In addition to the expected abnormal signal pattern 1F1R1G other signal patterns are possible in samples with aneuploid/unbalanced rearrangements. A signal pattern is considered *KMT2A* abnormal (indicative of *KMT2A* rearrangement) when it includes:

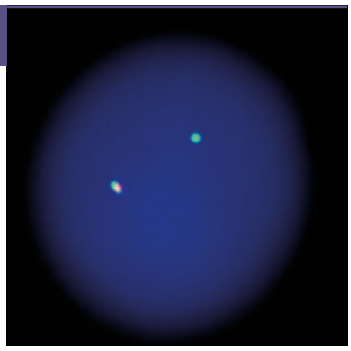
nFnRnG, where:

- nF (number of Fusion signals) ≥ 0
- nR (number of Red signals) ≥ 0
- nG (number of Green signals) ≥ 1

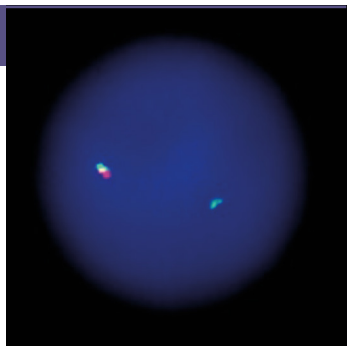
Any signal pattern fitting this description may be interpreted as consistent with *KMT2A* rearrangement and revumenib eligibility (see *Limitations*).

The images below (A–H) show representative examples of such nuclei observed during the clinical study:

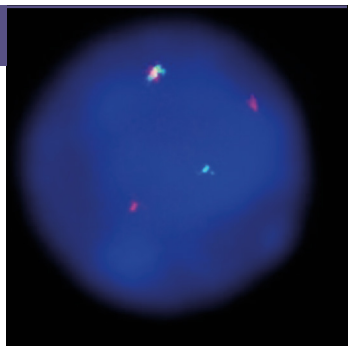
A



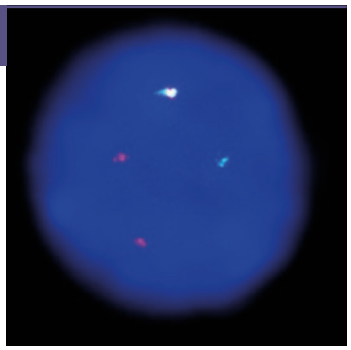
B

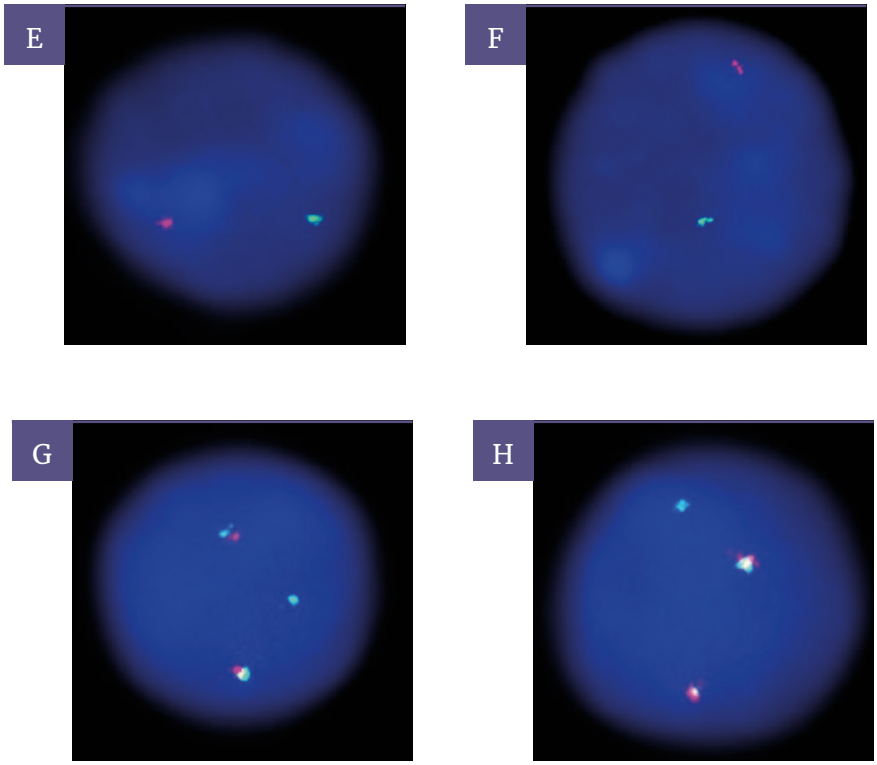


C



D





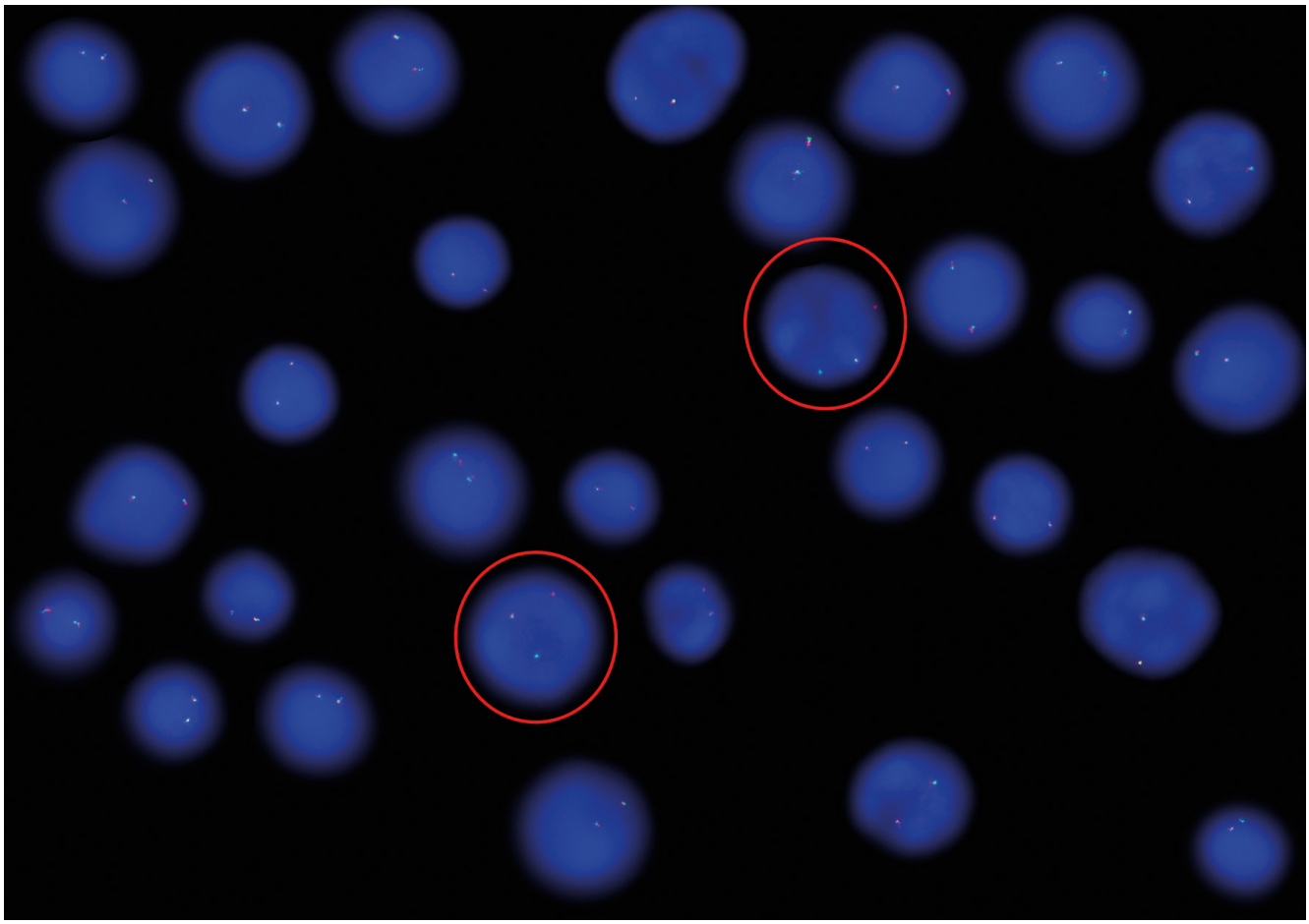
Images A and B (1F1G): One fusion and one green signal. This signal pattern satisfies the package insert rule (nF ≥ 0, nR ≥ 0, nG ≥ 1) and should be treated as an abnormal signal pattern.

Images C and D (1F2R1G): One fusion, two red and one green signals. This signal pattern satisfies the package insert rule (nF ≥ 0, nR ≥ 0, nG ≥ 1) and should be considered an abnormal signal pattern.

Images E and F (1R1G): One red and one green signal, with no fusion present. As the red and green signals are clearly separated and nG ≥ 1, this pattern should be interpreted as an abnormal signal pattern.

Images G and H (2F1G): Two fusion signals and one green signal. The additional green signal may represent a rearrangement and fulfils the package insert definition of an abnormal signal pattern.

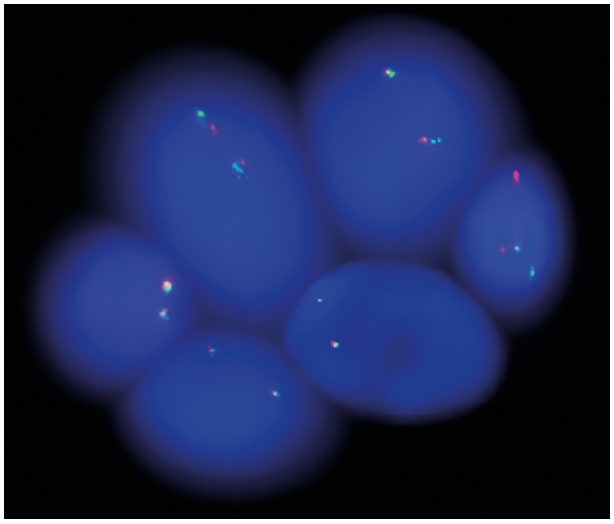
2.4. Near Cut-off Positive



The above field of view from a near cut-off sample shows predominantly negative cells displaying the expected normal 2F signal pattern. Two cells (circled in red) demonstrate an abnormal 1F1R1G signal pattern consistent with a *KMT2A* rearrangement. In this representative example, the proportion of abnormal cells is 2/30=6.7% (although a full 200 cell analysis per the guidelines in the Package Insert is required to fully categorize the sample). This positivity level is close to the cut-off threshold for AML (3.8%).

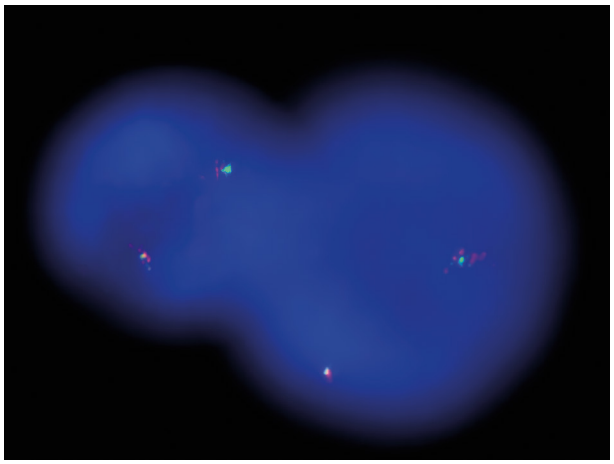
3. Do Not Count

Clumped nuclei



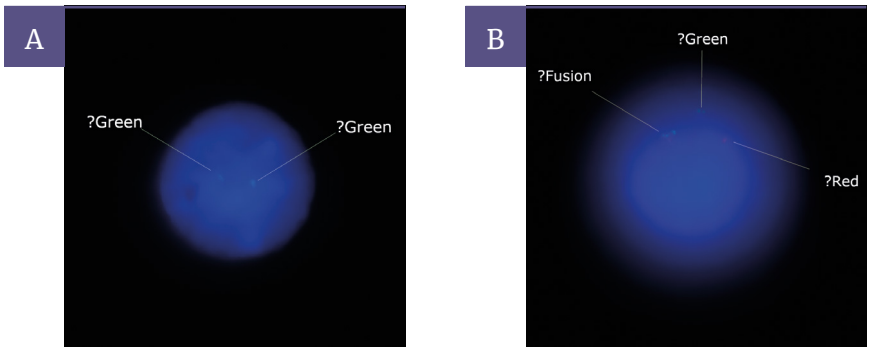
This image shows a cluster of six nuclei in close proximity, each displaying technically analyzable FISH signals. However, due to the degree of clumping, the boundaries between nuclei are not clearly defined. According to the Package Insert “analyze only intact nuclei, not overlapped or crowded nuclei…” Cells in clumped arrangements should be excluded from analysis, even if signals appear interpretable, to avoid misidentification.

Overlapping nuclei



This image shows two overlapping nuclei, each with clearly visible FISH signals. However, due to the physical overlap, the full extent and boundaries of each nucleus cannot be confidently distinguished. Per the Package Insert: “analyze only intact nuclei, not overlapped or crowded nuclei…”. Overlapping nuclei should be excluded from analysis, regardless of signal clarity, ensure accurate cell identification and signal allocation.

Weak signals



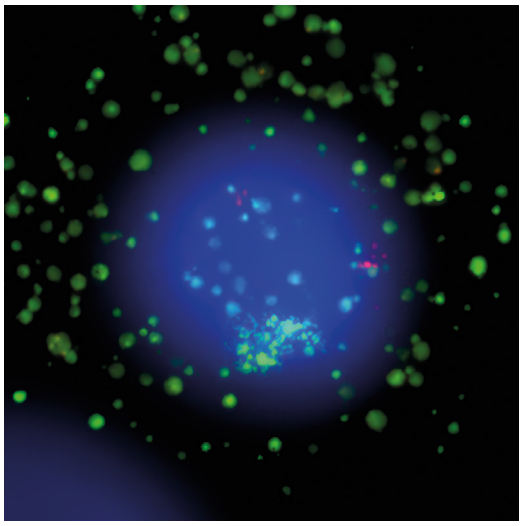
These images demonstrate nuclei where signals are present but too weak or poorly defined to support reliable interpretation. According to the Package Insert: "*Signals should appear bright, distinct and easily evaluable... If in doubt about whether a cell is analyzable or not, then do not analyze it.*"

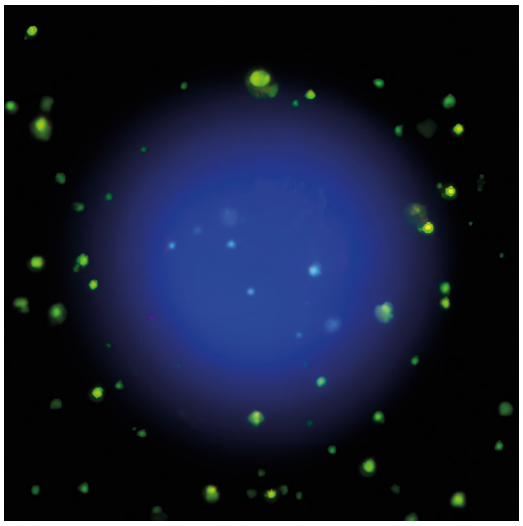
Cell A gives the impression of two weak green signals, but the corresponding red signal(s) are either absent or too faint to distinguish. The signal intensity and clarity are insufficient to confidently determine the signal configuration, making this cell unsuitable for analysis.

Cell B appears to show a potential breakapart pattern; however, both the red and green signals are too dim and diffuse to be interpreted reliably. The inability to reliably confirm whether the signals are fused or split makes this cell not analyzable under the Package Insert guidelines.

In such instances, cells should be excluded from scoring to avoid introducing uncertainty into the analysis.

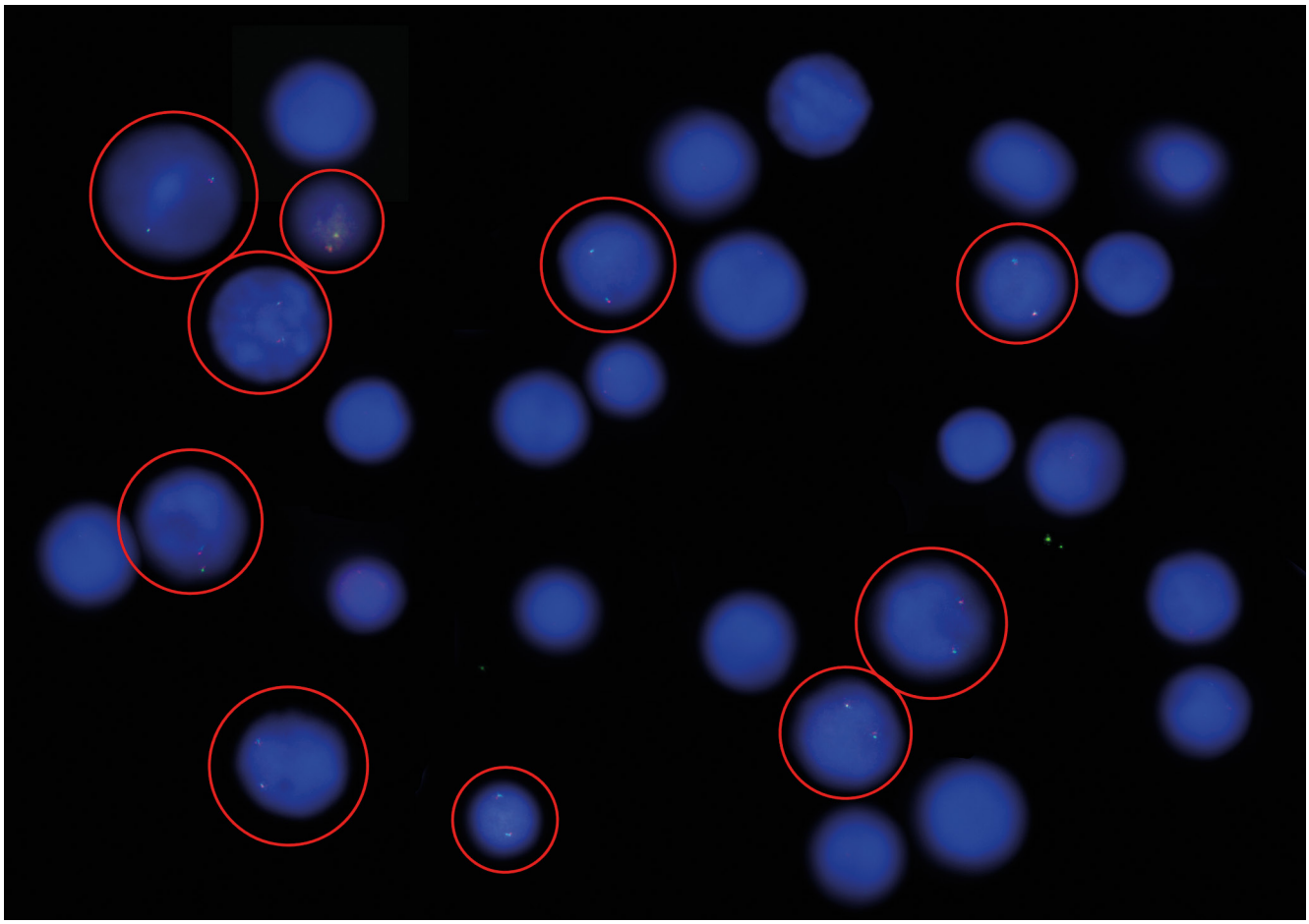
Autofluorescence





These nuclei are partially obscured by autofluorescence extending beyond the nuclear boundary. Autofluorescence will usually be visible across multiple filter channels. According to the Package Insert, cells "*should not be analyzed if… there is excess fluorescent particles between cells and/or a fluorescent haze that interferes with the signals.*" Cells affected by such artifacts must be excluded from scoring.

Unhybridized nuclei



This field of view includes 30 nuclei. While 10 nuclei (circled in red) display clear normal 2F signal patterns consistent with a negative result, the remaining 20 nuclei are either completely unhybridized or display signal intensity too weak to support reliable analysis. According to the Package Insert: "*The slide should not be analyzed if… >50% of the cells are not hybridized.*"

In this example, ~67% (10/30) of the cells are suitable for interpretation. Should a similar distribution of unhybridized cells be observed across the slide, the criteria for analysis are not met and the slide should be excluded. In such cases, make a new slide and then get new samples if this fails again.

4. Counting Rules (Summary)

Count only intact, well-separated nuclei

Less than 2 signal widths between red/green = fusion signal

If in doubt, exclude the cell

Do not analyze slides where greater than 50% of cells are unhybridized

Make new slide on 1st fail attempt. Get new samples if it fails again

5. Access and Updates

The product packaging contains a QR code that links to a landing page with the latest Package Insert and this interpretation guide

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What binds us, makes us.

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Indications for Use:

The CDA-LPH013 *KMT2A* Breakapart FISH Probe Kit PDx is a fluorescence in situ hybridization (FISH) test used to detect rearrangement of the *KMT2A* region on chromosome 11 at location 11q23.3 in 3:1 methanol/glacial acetic acid fixed bone marrow specimens from patients with acute leukemia with *KMT2A* rearrangement.

The assay is indicated for detecting the presence of rearrangements involving the *KMT2A* region as a companion diagnostic to aid in identifying those patients for whom treatment with REVUFORJ® (revumenib) is indicated in accordance with the approved therapeutic product labeling. The CDA-LPH013 *KMT2A* Breakapart FISH Probe Kit PDx is not intended for monitoring of residual disease.

Limitations:

This device is designed to detect rearrangements with breakpoints in the region bounded by the red and green clones in this probe set, which includes the *KMT2A* gene. Breakpoints outside of this region, or variant rearrangements wholly contained within this region, may not be detected with this device.

This device is not intended for monitoring of residual disease or for use as prenatal test, or population-based screening test.

This device has not been validated for sample types, disease types, or purposes outside of those stated in the indications for use.

Reporting and interpretation of FISH results should be performed by a qualified pathologist or cytogeneticist.

Reporting and interpretation of FISH results should be consistent with professional standards of practice and should take into consideration other clinical and diagnostic information. Failure to adhere to the protocol may affect the performance and lead to false results.

The reproducibility of the test in samples with positivity ranging between 3.1-6.2% for ALL and 3.8-7.6% for AML are 65.3% and 75%, respectively. Therefore, use caution when interpreting results within this range.

Analytical validation demonstrating performance is limited to 1F1R1G signal pattern. Other signal patterns that are considered positive for *KMT2A* rearrangement have not been validated.

This device is intended for in vitro diagnostic use only.

For prescription use only.

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