



CytoCell



A Sysmex Group Company

Application Note

**Validation of signal pattern cut-off levels in
CytoCell FDA-cleared AML/MDS probe kits**

Background

In December 2018, Oxford Gene Technology received FDA clearance for eight CytoCell® fluorescence *in situ* hybridization (FISH) probes:

Probe Name	Cat. No.
AML1/ETO (RUNX1/RUNX1T1) Translocation, Dual Fusion FISH Probe Kit	USA-LPH 026
CBFB (CBFB)/MYH11 Translocation, Dual Fusion FISH Probe Kit	USA-LPH 022
Del(5q) Deletion FISH Probe Kit	USA-LPH 024
Del(7q) Deletion FISH Probe Kit	USA-LPH 025
Del(20q) Deletion FISH Probe Kit	USA-LPH 020
EVI1 (MECOM) Breakapart FISH Probe Kit	USA-LPH 036
MLL (KMT2A) Breakapart FISH Probe Kit	USA-LPH 013
P53 (TP53) Deletion FISH Probe Kit	USA-LPH 017

These CytoCell FDA-cleared FISH probe kits are FISH tests used to detect common chromosomal rearrangements found in 3:1 methanol/acetic acid fixed bone marrow specimens from patients with AML and MDS.

In order to fulfil the high standards required for an FDA clearance, all eight CytoCell FISH probes in this submission underwent stringent performance assessments to underpin the probe performance and ensure that the products were safe and effective for their intended use. A summary of these performance assessments can be found in each of the pack inserts of these probe sets.

Practical Advice

The pack inserts for these probe kits contain information including guideline cut-off values for the expected abnormal signal pattern(s) for each probe set, but recognize that other signal patterns may be possible in the presence of aneuploid or unbalanced rearrangements.

The purpose of this support document is to aid customers wishing to introduce these tests into their laboratory, by providing calculation guidance and giving example cut-off values for non-standard signal patterns calculated from the analysis data included in the CytoCell probe FDA submission. This data is intended to be used by laboratories solely as a benchmark when determining their own internally-generated cut-off values. These cut-off values will be required when assessing and reporting samples with non-standard signal patterns in accordance with standard laboratory accreditation guidelines.

When introducing new FISH tests into the laboratory, cut-off values for each probe set should be generated for use specifically in the validating laboratory. There are a number of published methodologies available and it is up to the laboratory to select the appropriate one in accordance with their standard practices^{1,2,3}. This assessment will result in a data set of analyses which detail the frequency of all signal patterns seen on these specimens. This data set can then be used to generate cut-off values for any signal pattern required. If a signal pattern has not been seen in the dataset, then the frequency is counted as zero; if a particular signal pattern was seen in the dataset, record the highest number of cells seen in one individual specimen. One available method to calculate the cut-off from the data obtained is to use the BETAINV Microsoft Excel formula:

BETAINV (probability, alpha, beta).

The BETAINV function syntax has the following arguments:

Probability: Probability associated with the beta distribution, for 95% confidence level enter 0.95

Alpha: The number of abnormal nuclei seen (in the sample with the highest level of this abnormality) +1

Beta: The total number of nuclei analyzed in the sample

For example, to calculate a cut-off with a 95% confidence level in which six false-positive cells for a given signal pattern were seen in a specimen with 200 nuclei examined, using Microsoft Excel enter: (BETA INV(0.95,6+1,200))*100; the result is 5.67% cut-off.

If sample results are tabulated in Microsoft Excel with the signal patterns seen, the BETAINV function can be embedded at the bottom of the spreadsheet to provide a comprehensive set of cut-off values for all signal patterns.

Tables 1-8 show illustrative data generated during the FDA submission process for these probes.

Further Guidance

It is important to note that reporting the presence of non-standard signal patterns in a specimen will be at the discretion of the testing laboratory and should be provided on the laboratory report for informational purposes only. Similarly, the presence of abnormal signal patterns should not be extrapolated to indicate the presence of particular karyotype or rearrangement, for example, when using the USA-LPH 026 AML1/ETO (RUNX1/RUNX1T1) Translocation, Dual Fusion FISH Probe Kit, the presence of cells with a 2 red, 3 green signal pattern higher than the cut-off level for this pattern will be consistent with, but not indicative of, the presence of trisomy 8.

Regulatory Disclaimers

This technical supplement is not intended to replace the information in the pack insert. Cut-offs for all FISH probe kits should always be validated by the user. These FISH probe kits are not validated for use outside of the stated intended use.

Example Data

Other than standard normal signal pattern	1R1G1F	1F	3F	1R2F	1G1F	4F	1G2F	1R1G2F	6F	1R1F	8F	All other signal patterns (i.e zero incidence in dataset)	No. cells assessed	No. samples assessed
USA-LPH 013 Cut-off	% 3.8	% 5.7	% 6.3	% 2.3	% 5.1	% 6.3	% 3.8	% 2.3	% 3.1	% 2.3	% 2.3	% 1.5	200	1600

Table 1. Cut-off values for signal patterns seen in USA-LPH 013: MLL (KMT2A) Breakapart FISH Probe Kit.

Other than standard normal signal pattern	1R2G	2R1G	3R2G	2R4G	3R1G	2R3G	1R1G	4R4G	1R3G	3R3G	4R2G	4R1G	3R4G	4R3G	6R6G	All other signal patterns (i.e zero incidence in dataset)	No. cells assessed	No. samples assessed
USA-LPH 017 Cut-off	% 6.8	% 7.9	% 3.8	% 5.1	% 4.4	% 5.1	% 5.7	% 5.7	% 3.8	% 3.8	% 3.1	% 2.3	% 2.3	% 2.3	% 2.3	% 1.5	200	1600

Table 2. Cut-off values for signal patterns seen in USA-LPH 017: P53 (TP53) Deletion FISH Probe Kit.

Other than standard normal signal pattern	1R1G	3R2G	2R1G	4R4G	2R3G	3R3G	1R2G	8R8G	All other signal patterns (i.e zero incidence in dataset)	No. cells assessed	No. samples assessed
USA-LPH 020 Cut-off	% 5.7	% 3.1	% 4.4	% 5.1	% 6.3	% 3.8	% 4.4	% 3.8	% 1.5	200	1300

Table 3. Cut-off values for signal patterns seen in USA-LPH 020: Del(20q) Deletion FISH Probe Kit.

Other than standard normal signal pattern	1R1G2F	1R2G	2R1G	5R3G	1R1G	2R3G	3R2G	3R3G	4R4G	1R1G1F	1G2F	2F	2R2G2F	All other signal patterns (i.e zero incidence in dataset)	No. cells assessed	No. samples assessed
USA-LPH 022 Cut-off	% 2.3	% 6.8	% 3.8	% 2.3	% 3.8	% 6.3	% 3.1	% 3.8	% 5.7	% 7.9	% 3.1	% 3.1	% 3.1	% 1.5	200	1300

Table 4. Cut-off values for signal patterns seen in USA-LPH 022: CBFβ (CBFB)/MYH11 Translocation, Dual Fusion FISH Probe Kit.

Example Data

Other than standard normal signal pattern	1R2G	2R1G	2R3G	3R3G	1R1G	2R2G1F	4R4G	2R4G	3R2G	8R8G	3R4G	6R6G	All other signal patterns (i.e zero incidence in dataset)	No. cells assessed	No. samples assessed
USA-LPH 024 Cut-off	% 6.3	% 5.1	% 5.7	% 4.4	% 6.3	% 2.3	% 4.4	% 3.1	% 5.7	% 3.1	% 2.3	% 3.1	% 1.5	200	1290

Table 5. Cut-off values for signal patterns seen in USA-LPH 024: Del(5q) Deletion FISH Probe Kit.

Other than standard normal signal pattern	1R1G	1R2G	4R4G	2R1G	2R3G	2R4G	3R3G	1R1G1F	3R2G	3R4G	6R6G	All other signal patterns (i.e zero incidence in dataset)	No. cells assessed	No. samples assessed
USA-LPH 025 Cut-off	% 7.4	% 3.1	% 6.8	% 6.8	% 3.1	% 2.3	% 3.8	% 2.3	% 3.1	% 2.3	% 2.3	% 1.5	200	1300

Table 6. Cut-off values for signal patterns seen in USA-LPH 025: Del(7q) Deletion FISH Probe Kit.

Other than standard normal signal pattern	1R1G2F	1R2G	3R2G	2R1G	2R3G	4R4G	1R1G	1R4G	1R1G1F	2R1G1F	2R5G	3R3G	4R3G	2R4G	2R2G1F	5R4G	4R2G	3R4G	All other signal patterns (i.e zero incidence in dataset)	No. cells assessed	No. samples assessed
USA-LPH 026 Cut-off	% 2.3	% 6.3	% 7.4	% 5.1	% 6.3	% 5.1	% 3.1	% 3.1	% 5.1	% 2.3	% 2.3	% 5.1	% 3.8	% 3.8	% 2.3	% 2.3	% 2.8	% 8.5	% 1.5	200	1290

Table 7. Cut-off values for signal patterns seen in USA-LPH 026: AML1/ETO (RUNX1/RUNX1T1) Translocation, Dual Fusion FISH Probe Kit.

Other than standard normal signal pattern	1R1GB1RGB	1RG1B1RGB	1R1G1B1RGB	1RGB	2RG2B	All other signal patterns (i.e zero incidence in dataset)	No. cells assessed	No. samples assessed
USA-LPH 036 Cut-off	% 4*	% 4*	% 2.3	% 2.3	% 3.1	% 1.5	200	25

Table 8. Cut-off values for signal patterns seen in USA-LPH 036: EVI1 (MECOM) Breakapart FISH Probe Kit.

*Cut-off rounded up to the next whole decimal.

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References

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What binds us, makes us.

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