EVALUATION OF AUTOMATIC CLASS III DESIGNATION FOR
Cytocell FISH Probe Kits for AML and MDS
DECISION SUMMARY

A. DEN Number:

DEN170070

B. Purpose for Submission:

De novo request for evaluation of automatic class III designation for the following Cytocell FISH Probe Kits for AML (Acute Myeloid Leukemia) and/or MDS (Myelodysplastic Syndromes) (refer to the list of devices in Section C below).

C. Measurands:

Chromosomal rearrangements as listed below:

Device	**Chromosomal Rearrangement**
MLL (KMT2A) Breakapart FISH Probe Kit	Rearrangement of MLL (KMT2A) region on chromosome 11 at location 11q23.3
P53 (TP53) Deletion FISH Probe Kit	Deletions of the P53 (TP53) region on chromosome 17, at location 17p13
Del(20q) Deletion FISH Probe Kit	Deletions within the long arm of chromosome 20 at locations 20q12 and 20q13.1
CBFβ (CBFB) /MYH11 Translocation, Dual Fusion FISH Probe Kit	Rearrangements of chromosome 16 causing the CBFβ-MYH11 (CBFB-MYH11) fusion
Del(5q) Deletion FISH Probe Kit	Deletions within the long arm of chromosome 5 at location 5q31.2
Del(7q) Deletion FISH Probe Kit	Deletions within the long arm of chromosome 7 at locations 7q22 and 7q31.2
AML1/ETO (RUNX1/RUNX1T1)) Translocation, Dual Fusion FISH Probe Kit	Rearrangements involving the AML1 (RUNX1) region on chromosome 21 at location 21q22.1 and the ETO (RUNX1T1) region on chromosome 8 at location 8q21.3
EVI1 (MECOM) Breakapart FISH Probe Kit	Rearrangements involving the EVI1 (MECOM) region on chromosome 3 at location 3q26.2

D. Type of Test:

Fluorescent *in situ* hybridization (FISH).

E. Applicant:

Cytocell, Ltd

F. Proprietary and Established Names:

- MLL (KMT2A) Breakapart FISH Probe Kit
- P53 (TP53) Deletion FISH Probe Kit
- Del(20q) Deletion FISH Probe Kit
- CBFβ (CBFB) /MYH11 Translocation, Dual Fusion FISH Probe Kit
- Del(5q) Deletion FISH Probe Kit
- Del(7q) Deletion FISH Probe Kit
- AML1/ETO (RUNX1/RUNX1T1) Translocation, Dual Fusion FISH Probe Kit
- EVI1 (MECOM) Breakapart FISH Probe Kit

G. Regulatory Information:

1. Regulation section:

   21 CFR 864.1880

2. Classification:

   Class II

3. Product code(s):

   QDI

4. Panel:

   Pathology

H. Indications for use:

1. Indications for use:

   The MLL (KMT2A) Breakapart FISH Probe Kit is a fluorescence in situ hybridization (FISH) Test used to detect rearrangement of the MLL (KMT2A) region on chromosome 11 at location 11q23.3 in fixed bone marrow specimens from patients with acute myeloid leukemia (AML) or myelodysplastic syndrome (MDS). The test is indicated for characterization of patient specimens consistent with World Health

Organization (WHO) guidelines for Classification of Tumours of Haematopoietic and Lymphoid Tissues (Revised 4th Edition) and in conjunction with other clinicopathological criteria. The assay results are intended to be interpreted by a qualified pathologist or cytogeneticist. The test is not intended for use as a stand-alone diagnostic, disease screening, or as a companion diagnostic.

The P53 (TP53) Deletion FISH Probe Kit is a fluorescence in situ hybridization (FISH) Test used to detect deletion of the P53 (TP53) region on chromosome 7 at location 17p13 in fixed bone marrow specimens from patients with acute myeloid leukemia (AML) or myelodysplastic syndrome (MDS). The test is indicated for characterization of patient specimens consistent with World Health Organization (WHO) guidelines for Classification of Tumours of Haematopoietic and Lymphoid Tissues (Revised 4th Edition) and in conjunction with other clinicopathological criteria. The assay results are intended to be interpreted by a qualified pathologist or cytogeneticist. The test is not intended for use as a stand-alone diagnostic, disease screening, or as a companion diagnostic.

The Del(20q) Deletion FISH Probe Kit is a fluorescence in situ hybridization (FISH) Test used to detect deletion within the long arm of chromosome 20 at locations 20q12 and 20q13.1, in fixed bone marrow specimens from patients with myelodysplastic syndrome (MDS). The test is indicated for characterization of patient specimens consistent with World Health Organization (WHO) guidelines for Classification of Tumours of Haematopoietic and Lymphoid Tissues (Revised 4th Edition) and in conjunction with other clinicopathological criteria. The assay results are intended to be interpreted by a qualified pathologist or cytogeneticist. The test is not intended for use as a stand-alone diagnostic, disease screening, or as a companion diagnostic.

The CBFβ (CBFB)/MYH11 Translocation, Dual Fusion FISH Probe Kit is a fluorescence in situ hybridization (FISH) Test used to detect rearrangement of the chromosome 16 causing the *CBFβ-MYH11* (*CBFB-MYH11*) fusion in fixed bone marrow specimens from patients with acute myeloid leukemia (AML). The test is indicated for characterization of patient specimens consistent with World Health Organization (WHO) guidelines for Classification of Tumours of Haematopoietic and Lymphoid Tissues (Revised 4th Edition) and in conjunction with other clinicopathological criteria. The assay results are intended to be interpreted by a qualified pathologist or cytogeneticist. The test is not intended for use as a stand-alone diagnostic, disease screening, or as a companion diagnostic.

The Del(5q) Deletion FISH Probe Kit is a fluorescence in situ hybridization (FISH) Test used to detect deletions within the long arm of chromosome 5 at location 5q31.2 in fixed bone marrow specimens from patients with acute myeloid leukemia (AML) or myelodysplastic syndrome (MDS). The test is indicated for characterization of patient specimens consistent with World Health Organization (WHO) guidelines for Classification of Tumours of Haematopoietic and Lymphoid Tissues (Revised 4th Edition) and in conjunction with other clinicopathological criteria. The assay results are intended to be interpreted by a qualified pathologist or cytogeneticist. The test is not

intended for use as a stand-alone diagnostic, disease screening, or as a companion diagnostic.

The Del(7q) Deletion FISH Probe Kit is a fluorescence in situ hybridization (FISH) Test used to detect deletions within the long arm of chromosome 7 at locations 7q22 and 7q31.2 in fixed bone marrow specimens from patients with acute myeloid leukemia (AML) or myelodysplastic syndrome (MDS). The test is indicated for characterization of patient specimens consistent with World Health Organization (WHO) guidelines for Classification of Tumours of Haematopoietic and Lymphoid Tissues (Revised 4th Edition) and in conjunction with other clinicopathological criteria. The assay results are intended to be interpreted by a qualified pathologist or cytogeneticist. The test is not intended for use as a stand-alone diagnostic, disease screening, or as a companion diagnostic.

The AML1/ETO (RUNX1/RUNX1T1) Translocation, Dual Fusion FISH Probe Kit is a fluorescence in situ hybridization (FISH) Test used to detect rearrangement involving the *AML1* (*RUNX1*) region on chromosome 21 at location 21q22.1 and the *ETO* (*RUNX1T1*) region on chromosome 8 at location 8q21.3 in fixed bone marrow specimens from patients with acute myeloid leukemia (AML). The test is indicated for characterization of patient specimens consistent with World Health Organization (WHO) guidelines for Classification of Tumours of Haematopoietic and Lymphoid Tissues (Revised 4th Edition) and in conjunction with other clinicopathological criteria. The assay results are intended to be interpreted by a qualified pathologist or cytogeneticist. The test is not intended for use as a stand-alone diagnostic, disease screening, or as a companion diagnostic.

The EVI1 (MECOM) Breakapart FISH Probe Kit is a fluorescence in situ hybridization (FISH) Test used to detect rearrangement involving the *EVI1* (*MECOM*) region on chromosome 3 at location 3q26.2, in fixed bone marrow specimens from patients with acute myeloid leukemia (AML) or myelodysplastic syndrome (MDS). The test is indicated for characterization of patient specimens consistent with World Health Organization (WHO) guidelines for Classification of Tumours of Haematopoietic and Lymphoid Tissues (Revised 4th Edition) and in conjunction with other clinicopathological criteria. The assay results are intended to be interpreted by a qualified pathologist or cytogeneticist. The test is not intended for use as a stand-alone diagnostic, disease screening, or as a companion diagnostic.

2. Special conditions for use statement(s)
   For prescription use.
   For *in vitro* diagnostic use

I. Device Description:

Each Cytocell FISH Probe Kit device consists of one vial containing specific probes as described below. The probes are provided premixed in hybridization solution (formamide; dextran sulfate; saline-sodium citrate (SSC)) and are ready to use. The Kit also includes one

vial of 4,6-diamidino-2-phenylindole (DAPI) counterstain. The kits are available in a 10-test format.

The MLL (KMT2A) Breakapart FISH Probe Kit consists of an 87kb probe, labeled in Texas red, covering a region telemetric to the MLL (KMT2A) gene including the marker SHGC-111513 and a FITC green probe covering a 170kb region centromeric to the MLL (KMT2A) gene spanning the CD3G and UBE4A genes.

The P53 (TP53) Deletion FISH Probe Kit consists of a 161kb probe, labeled in Texas red, covering the whole P53 (TP53) gene, extending 74kb telomeric to the gene and covering a region centromeric to the gene, to just beyond the marker D17S655; and a probe, labelled in FITC green, covering the chromosome 17 centromere (D17Z1) region.

The Del(20q) Deletion FISH Probe Kit consists of a 331kb probe, labeled in Texas red, covering a region within the *PTPRT* gene and including the D20S108 marker; and two (141kb and 174kb) probes labeled in FITC green covering the *MYBL2* gene and including the D20S150 marker.

The CBFβ (CBFB) /MYH11 Translocation, Dual Fusion FISH Probe Kit consists of a 617kb probe, labeled in Texas red, covering a region, within 16q22 including the *CBFB* gene; and a 621kb probe, labeled in FITC green, covering a region within 16p13.1 including the *MYH11* gene.

The Del(5q) Deletion FISH Probe Kit consists of a 186kb probe, labeled in Texas red, covering a region within 5q31.2, including the D5S500 marker; and a 376kb probe, labeled in FITC green, within 5p15.3, including the D5S630 marker.

The Del(7q) Deletion FISH Probe Kit consists of a 396kb probe, labeled in Texas red, covering a region within 7q22 including the telomeric end of the *RELN* gene and extending beyond the D7S658 marker; and a 203kb probe, labeled in FITC green, covering a region within 7q31.2 including the *TES* gene.

The AML1/ETO (RUNX1/RUNX1T1) Translocation, Dual Fusion FISH Probe Kit consists of a 156kb probe labeled in Texas red, centromeric to the *AML1* (*RUNX1*) gene, including the *CLIC6* gene; a 169kb probe labelled in Texas red, telomeric to *AML1* (*RUNX1*) gene, extending beyond the marker D21S1921; and two (151kb and 194kb) probes, labeled inn FITC green, on either side of the *ETO* (*RUNX1T1*) gene.

The EVI1 (MECOM) Breakapart FISH Probe Kit consists of a 158kb probe, labeled in Texas red, telomeric to the D3S4415 marker and including the *LRRC34* gene, a FITC green probe covering a 181kb region, including the entire *EVI1* (*MECOM*) gene and flanking regions and a PF-415 blue probe, which covers a 563kb region centromeric to the *EVI1* (*MECOM*) gene, including the D3S1614 marker.

K. Standard/Guidance Document Referenced:

Guidance for Industry and FDA Staff – Content and Format for Abbreviated 510(k)s for Early Growth Response 1 (EGR1) Gene Fluorescence In-Situ Hybridization (FISH) Test

System for Specimen Characterization Devices

L. Test Principle:

Fluorescence in situ hybridization (FISH) is a technique that allows chromosomal aberrations to be detected on metaphase chromosomes or in interphase nuclei from fixed cytogenetic samples.

Bone marrow cells from patients are attached to microscope slides using standard cytogenetic procedures. After fixation and denaturation of cellular DNA to single-stranded form, target DNA is available for annealing to a similarly denatured, fluorescently labelled DNA probe, which has a complementary sequence. Following hybridization, unbound and non-specifically bound DNA probe is removed by a series of washes, and the DNA is counterstained for visualization with DAPI, a DNA-specific stain that fluoresces blue. Fluorescence microscopy equipped with appropriate excitation and emission filters then allows the visualization of the hybridized probe on the target material. Two analysts should analyze each sample. Each analyst scores independently 100 nuclei for each sample. In some cases, depending on the number of abnormal nuclei each analyst has seen, a third reader may be required.

The expected signal pattern is dependent on the number of copies of each probe. Enumeration of the signals provide a mechanism for determining absolute copy number of the probe targets and the presence of chromosomal aberrations of interest. Refer to Table 1 - Supported Signal Patterns & Cut-Offs for the expected normal signal pattern and expected abnormal signal pattern for each probe kit (R = red, G = Green, B = blue).

Table 1 - Supported Signal Patterns & Cut-Offs

Catalogue	Name	Negative Signal	Positive Signal
USA-LPH013	MLL (KMT2A) Breakapart	(b)(4)	
USA-LPH017	P53 (TP53) Deletion		
USA-LPH020	Del (20q) Deletion		
USA-LPH022	CBFβ (CBFB) /MYH11 Translocation, Dual Fusion		
USA-LPH024	Del (5q) Deletion		
USA-LPH025	Del (7q) Deletion		
USA-LPH026	AML1/ETO (RUNX1/RUNX1T1) Translocation, Dual Fusion		
USA-LPH036	EVI1 (MECOM) Breakapart Inversion Signal		
	EVI1 (MECOM) Breakapart Translocation Signal		

M. Performance Characteristics:

Analytical validation was provided in support of each probe kit:

1. Analytical performance:

a. Precision/Reproducibility

Repeatability and reproducibility of each probe kit was assessed using (b)(4) bone marrow specimens to represent the range of results [two normal (negative), two near cut-off or low positive, and two high positive]. Specimens were analyzed according to protocol and tested over 5 non-consecutive days in duplicates at (b)(4 sites to assess precision (n =30 per specimen).

In some cases, to ensure there was ample volume to allow for consistent testing amongst all sites one or more normal samples were combined (pooled) and distributed to testing sites. The majority of the near cut-off and low positive samples were contrived by spiking known normal samples with known positive specimens. All samples were confirmed to be normal by an established alternative cytogenetic method (competitor probe or G-banding) prior to distribution.

Data was analyzed for intra-day, between-day, between-site and total SD.

Two probe kits [CBFβ (CBFB) /MYH11 Translocation, Dual Fusion FISH Probe Kit (LPH 022) and EVI1 (MECOM) Breakapart FISH Probe Kit (LPH 036)]) had specimens too close the cut-off to assess precision and therefore additional specimens were re-assessed at (b)(4) and (b)(4) the cut-off at one site with 2 operators and 5 days in duplicate to confirm acceptable precision at the low end of the enumeration range (n (b)(4)). The data with the clinical thresholds used in the study are shown in Tables 2 through 10 demonstrate that all probe kits had acceptable precision.

Table 2. Precision of the MLL (KMT2A) Breakapart (cut-off: 3.8%)

Specimen	Mean of abnormal percentage	Range	Agreement replicates	Agreement (%)	Intra-Day SD	Inter-Day SD	Between-site SD	Total SD
Negative 1	(b)(4)			100	0.00	0.00	0.00	0.00
Negative 1				100	0.00	0.00	0.00	0.09
Near cut-off 1				63	0.00	0.00	1.17	1.98
Near cut-off 2				90	0.00	1.69	0.87	3.12
Positive 1				100	0.00	0.77	4.57	6.60
Positive 2				100	0.81	0.35	3.06	4.14

N/A = not applicable

Table 3. Precision of the P53 (TP53) Deletion (cut-off: 6.8%)

Specimen	Mean of abnormal percentage	Range	Agreement replicates	Agreement (%)	Intra-Day SD	Inter-Day SD	Between-site SD	Total SD
Negative 1	(b)(4)			97	0.00	0.00	1.11	2.09
Negative 1				93	0.00	0.00	1.65	2.50
Near cut-off 1				83	0.00	0.00	1.71	3.62
Near cut-off 2				100	0.00	2.18	1.34	4.46
Positive 1				100	0.00	0.00	0.00	5.84
Positive 2				100	0.00	0.92	4.18	6.04

Table 4. Precision of the Del (20q) Deletion (cut-off: 5.7%)

Specimen	Mean of abnormal percentage	Range	Agreement replicates	Agreement (%)	Intra-Day SD	Inter-Day SD	Between-site SD	Total SD
Negative 1	(b)(4)			100	0.00	0.48	0.00	1.41
Negative 1				100	0.00	0.33	0.67	1.01
Near cut-off 1				80	0.00	0.00	0.00	4.45
Near cut-off 2				100	0.00	2.01	2.79	5.31
Positive 1				100	0.00	0.00	0.00	6.93
Positive 2				100	0.00	0.00	0.48	4.96

Table 5. Precision of the CBFβ (CBFB) /MYH11 Translocation, Dual Fusion (cut-off: 2.3%)

Specimen	Mean of abnormal percentage	Range	Agreement replicates	Agreement (%)	Intra-Day SD	Inter-Day SD	Between-site SD	Total SD
Negative 1	(b)(4)			100	0.00	0.00	0.00	0.00
Negative 1				100	0.00	0.00	0.00	0.09
Near cut-off 1				27	0.00	0.51	0.00	1.30
Near cut-off 2				43	0.75	0.00	0.10	2.47
Positive 1				100	0.00	0.84	0.64	4.55
Positive 2				100	0.00	1.76	3.20	7.50

N/A = not applicable

Table 5.a. Additional supporting date for precision of the CBFβ (CBFB) /MYH11 Translocation, Dual Fusion

Specimen	Mean of abnormal percentage	Range	Agreement replicates	Agreement (%)	Intra-Day SD	Inter-Day SD	Total SD
Near cut-off 1	(b)(4)			100	0.56	0.25	1.34
Near cut-off 2				100	0.00	0.00	2.42

Table 6. Precision of the Del (5q) Deletion (cut-off: 6.3%)

Specimen	Mean of abnormal	Range	Agreement replicates	Agreement (%)	Intra-Day SD	Inter-Day SD	Between-site SD	Total SD
Negative 1	(b)(4)			100	0.32	0.58	0.73	1.25
Negative 1				100	0.00	0.06	1.30	1.59
Near cut-off 1				80	0.00	2.47	3.67	5.85
Near cut-off 2				97	2.06	0.00	1.08	4.49
Positive 1				100	1.93	3.27	0.89	7.04
Positive 2				100	0.00	1.21	3.18	5.09

Table 7. Precision of the Del (7q) Deletion (cut-off: 7.4%)

Specimen	Mean of abnormal percentage	Range	Agreement replicates	Agreement (%)	Intra-Day SD	Inter-Day SD	Between-site SD	Total SD
Negative 1	(b)(4)			100	0.00	0.11	0.87	1.12
Negative 1				100	0.00	0.00	0.98	1.67
Near cut-off 1				100	0.00	0.30	2.54	4.86
Near cut-off 2				100	0.39	0.00	2.28	4.21
Positive 1				100	0.00	0.00	3.00	5.09
Positive 2				100	0.00	0.31	2.16	3.79

Table 8. Precision of the AML1/ETO (RUNX1/RUNX1T1) Translocation, Dual Fusion (cut-off: 2.3%)

Specimen	Mean of abnormal percentage	Range	Agreement replicates	Agreement (%)	Intra-Day SD	Inter-Day SD	Between-site SD	Total SD
Negative 1	(b)(4)			100	0.00	0.00	0.00	0.00
Negative 1				100	0.00	0.00	0.00	0.00
Near cut-off 1				80	0.23	0.00	0.12	1.72
Near cut-off 2				97	1.57	0.00	0.82	2.40
Positive 1				100	0.00	1.06	0.00	8.30
Positive 2				100	0.25	0.00	0.00	6.25

N/A = not applicable

Table 9. Precision of the EVI1 (MECOM) Breakapart_Inversion (cut-off: 4.0%)

Specimen	Mean of abnormal percentage	Range	Agreement replicates	Agreement (%)	Intra-Day SD	Inter-Day SD	Between-site SD	Total SD
Negative 1	(b)(4)			100	0.24	0.40	0.54	1.02
Negative 1				100	0.52	0.00	0.26	1.24
Near cut-off 1				53	0.00	1.20	0.00	3.49
Near cut-off 2				73	0.00	1.25	1.06	4.13
Positive 1				100	0.68	3.01	7.05	11.93
Positive 2				100	2.42	0.00	5.56	13.26

** these two specimens are too close to cut-of to allow assessment of precision An additional one-site precision study with low positive specimens is provided below.*

Table 9.a. Additional supporting date for precision of the EVI1 (MECOM) Breakapart Inversion

Specimen	Mean of abnormal percentage	Range	Agreement replicates	Agreement (%)	Intra-Day SD	Inter-Day SD	Total SD
Near cut-off 1	(b)(4)			100	0.26	1.78	2.75
Near cut-off 2				100	0.00	0.00	2.11

Table 10. Precision of the EVI1 (MECOM) Breakapart Translocation (cut-off: 4.0%)

Specimen	Mean of abnormal percentage	Range	Agreement replicates	Agreement (%)	Intra-Day SD	Inter-Day SD	Between-site SD	Total SD
Negative 1	(b)(4)			100	0.00	0.00	0.95	1.02
Negative 1				100	0.00	0.32	0.84	1.09
Near cut-off 1				97	0.00	1.47	2.87	4.27
Near cut-off 2				100	0.00	0.00	1.27	3.38
Positive 1				100	0.00	0.00	3.34	6.79
Positive 2				100	1.11	0.37	0.00	6.78

Between- Lot reproducibility:
Three reagent lots were evaluated using one normal, one low positive and one high positive specimens were tested with (b)(4) replicates per lot to assess lot to lot variation (total of (b)(4) replicates evaluated). Intra-lot, inter-lot and total SD for each lot are shown in Tables 11-19 below.

Table 11. Lot to lot reproducibility of the MLL (KMT2A) Breakapart (cut-off: 3.8%)

Specimen	Mean of abnormal percentage	Range	Agreement replicates	Agreement (%)	Intra-lot SD	Inter-lot SD	Total SD
Negative	(b)(4)			100	0.0	0.00	0.00
Near cut-off				100	0.9	1.43	2.09
Positive				100	0.0	1.46	3.97

N/A = not app

Table 12. Lot to lot reproducibility of the P53 (TP53) Deletion (cut-off: 6.8%)

Specimen	Mean of abnormal percentage	Range	Agreement replicates	Agreement (%)	Intra-lot SD	Inter-lot SD	Total SD
Negative	(b)(4)			100	0.00	0	1.20
Near cut-off				100	0.00	0	4.85
Positive				100	2.25	0	2.76

Table 13. Lot to lot reproducibility of the Del (20q) Deletion (cut-off: 5.7%)

Specimen	Mean of abnormal percentage	Range	Agreement replicates	Agreement (%)	Intra-lot SD	Inter-lot SD	Total SD
Negative	(b)(4)			92	0.00	0.00	2.17
Near cut-off				67	1.41	0.00	3.25
Positive				100	0.00	2.62	4.95

Table 14. Lot to lot reproducibility of the CBFβ (CBFB) /MYH11 Translocation, Dual Fusion (cut-off: 2.3%)

Specimen	Mean of abnormal percentage	Range	Agreement replicates	Agreement (%)	Intra-lot SD	Inter-lot SD	Total SD
Negative	(b)(4)			100	0.00	0	0.00
Near cut-off				33	1.12	0	2.06
Positive				100	0.00	0	7.69

Table 15. Lot to lot reproducibility of the Del (5q) Deletion (cut-off: 6.3%)

Specimen	Mean of abnormal percentage	Range	Agreement replicates	Agreement (%)	Intra-lot SD	Inter-lot SD	Total SD
Negative	(b)(4)			83	0.98	0.49	2.09
Near cut-off				92	0.00	0.00	4.06
Positive				100	0.00	1.40	3.66

Table 16. Lot to lot reproducibility of the Del (7q) Deletion (cut-off: 7.4%)

Specimen	Mean of abnormal percentage	Range	Agreement replicates	Agreement (%)	Intra-lot SD	Inter-lot SD	Total SD
Negative	(b)(4)			100	0.00	0.08	0.94
Near cut-off				100	0.00	0.00	5.78
Positive				100	1.82	0.00	4.03

Table 17. Lot to lot reproducibility of the AML1/ETO (RUNX1/RUNX1T1) Translocation, Dual Fusion (cut-off: 2.3%)

Specimen	Mean of abnormal percentage	Range	Agreement replicates	Agreement (%)	Intra-lot SD	Inter-lot SD	Total SD
Negative	(b)(4)			100	0.00	0.00	0.14
Near cut-off				100	0.87	0.58	1.84
Positive				100	0.80	1.18	4.85

Table 18. Lot to lot reproducibility of the EVI1 (MECOM) Breakapart Inversion (1RG/1B/1RGB) (cut-off: 4.0%)

Specimen	Mean of abnormal percentage	Range	Agreement replicates	Agreement (%)	Intra-lot SD	Inter-lot SD	Total SD
Negative	(b)(4)			92	0.00	0.00	2.52
Near cut-off				67	1.63	2.82	6.29
Positive				100	1.80	3.01	6.99

Table 19. Lot to lot reproducibility of the EVI1 (MECOM) Breakapart Translocation (1R/1GB/1RGB) (cut-off: 4.0%)

Specimen	Mean of abnormal percentage	Range	Agreement replicates	Agreement (%)	Intra-lot SD	Inter-lot SD	Total SD
Negative	(b)(4)			100	0.25	0.00	0.70
Near cut-off				100	0.96	1.80	3.55
Positive				100	4.67	3.74	7.62

b. Linearity/assay Reportable Range:

Not applicable

c. Traceability, Stability, Expected Values (controls, calibrators, or methods):

Several stability studies were conducted in support of the probe kits. The specifics of each study are described below and summarized in Table 20 below.

Real-time Kit stability study:

The real-time stability of the probes was assessed using an isochronal study whereby probe lots of differing ages were assessed at the same time on identical samples. One normal, one low positive, and one high positive specimens were each tested in triplicate on each lot. Three lots of probes with minimum age of 25 months were tested in support of a 24-month shelf-life claim. Probe lots of intermediate ages between 0 and 25 months were also included to provide interim data. The result supported the claimed shelf life of 24 months when stored at -20 C.

Transport Stability
The transport study was conducted by stressing probes under potential extremes of transport conditions. One normal, one low positive, and one high positive specimens were each tested in triplicate using one lot of probe. There was no change in device performance under the stress conditions.

Freeze/Thaw Stability

The freeze/thaw stability was assessed by testing the probes at 0, 5, and 11 freeze/thaw cycles to support the (b)(4) cycles claim. One normal, one low positive, and one high positive specimens were each tested in triplicate using one lot of probe. Ten freeze-thaw cycles were found to be acceptable for this kit.

Post Hybridization Stability
The post hybridization stability was assessed by re-analyzing hybridized slides at (b)(4) and (b)(4) weeks after hybridization to support the 1month claim. One normal, one low positive, and one high positive specimens were each tested in triplicate using one lot of probe. The post-hybridization signal was determined to be stable to one month.

Photostability
The photostability of the probes was assessed by exposing probes to light for 0, 24, and (b)(4) hours to support the limited exposure to light claim. One normal, one low positive, and one high positive specimens were each tested in triplicate using one lot of probe. The photo-stability of slides was supported to (b)(4) hours. Prolonged exposure of slides to light should be avoided.

Table 20. Summary of stability studies

Test	Samples	Lots	Storage	Intervals	Baseline
Real Time Stability	(b)(4)		-20 °C	(b)(4)	
Transport Stability			2 weeks at +40 °C, then -20 °C		
Freeze/Thaw Stability			-20 °C		
Post Hybridizatio Stability			Refrigerator temperature (in the dark)		
Photostabilit			Room Temperature (fluorescent light)		

BM – bone marrowN = negative; LP = low positive; HP = high positive; F/T = freeze thaw; t = time point

d. Detection Limit:

The analytical sensitivity of the probes was established by analyzing interphase nuclei from 25 karyotypically normal bone marrow samples. Each sample was analyzed by 2 independent analysts and the signal pattern of each interphase was recorded. Each analyst analyzed 100 nuclei per sample, for a total of 200 nuclei per sample, resulting in 5000 scorable nuclei per probe evaluated. All nine probes evaluated in this study have an analytical sensitivity of greater than 95%, meeting the required acceptance criteria for analytical sensitivity (Table 21).

Table 21. Analytical sensitivity of the probes

Probe name	Number of interphase nuclei with the expected normal signal pattern	Total number of interphase nuclei analyzed	Analytical sensitivity (%)	95% Confidence interval (%)
MLL (KMT2A) Breakapart probe	(b)(4)			
P53 (TP53) Deletion Probe				

Del(20q) Deletion Probe	4924	5000	98.48	98.1-98.78
CBFβ (CBFB)/MYH11 Translocation, Dual Fusion Probe	(b)(4)			
Del(5q) Deletion Probe				
Del(7q) Deletion Probe				
AML1/ETO (RUNX1/RUNX1T1) Translocation, Dual Fusion Probe				
EVI1 (MECOM) Breakapart Probe				

e. *Analytical specificity:*

Analytical specificity is defined as the percentage of signals that hybridize to the correct locus and no other location. To establish the probe analytical specificity, each probe was assessed to verify that it hybridizes to the specific target chromosome location. Five (b)(4) normal male peripheral blood samples ((b)(4) metaphase spreads in each sample, or (b)(4) metaphase spreads if the probe is located on a sex chromosome – giving (b)(4) unique loci evaluated for each analysis). The analytical specificity of each product was calculated as the number of metaphase chromosome FISH signals hybridized to the correct locus divided by the total number of metaphase chromosome FISH signals hybridized. All probes met the required acceptance criteria for analytical specificity with all components having a specificity of 100%. (Table 22).

Table 22. Analytical specificity of the probes

Probe	Target	Number of metaphase chromosomes hybridized	Number of correct hybridized loci	Analytical Specificity	95% Confidence Interval
MLL (KMT2A) Breakapart FISH Probe Kit					
(b)(4)					

Probe	Target	Number of metaphase chromosomes hybridized	Number of correct hybridized loci	Analytical Specificity	95% Confidence Interval
(b)(4)					
EVI1 (MECOM) Breakapart FISH Probe Kit					
EVI1, Red	3q26.2	200	200	100%	98.12% - 100%
EVI1, Green	3q26.2	200	200	100%	98.12% - 100%
EVI1, Blue	3q26.2	200	200	100%	98.12% - 100%

f. Assay Cut-off (Upper Reference Limit):

The assay cut off was assigned as follows: For seven of the nine probes, a central clinical laboratory generated the clinical cut-off using a large patient dataset exceeding the ACMG minimum sample size guidelines. In addition, analytical sensitivity data from 25 karyotypically normal bone marrow samples was used to confirm the normal cut-offs. None of the 25 normal samples showed an abnormal signal pattern at or above the normal cut-offs confirming the clinical cut-off established.

For two of the nine probes included in this study, AML1 (RUNX1) Breakapart FISH Probe Kit and EVI1 (MECOM) Breakapart FISH Probe Kit, the results generated by the analytical sensitivity study were utilized (25 karyotypically normal bone marrow samples). In this case: ≥ 25 karyotypically normal bone marrow samples were enumerated and the normal cut-offs were calculated using the β inverse function (BETAINV) (Table 23).

An analytical result above the normal cut-off (upper reference limit) is deemed to be positive. Conversely, an analytical result below the cut-off is deemed to be negative.

Table 23: Supported Signal Patterns & Cut-Offs

Name	Negative Signal	Positive Signal	Cut-off
MLL (KMT2A) Breakapart	(b)(4)	1F/1R/1G	3.8%
P53 (TP53) Deletion		1R/2G	6.8%
Del (20q) Deletion		1R/1G	5.7%
CBFβ (CBFB) /MYH11 Translocation, Dual Fusion		2F/1R/1G	2.3%
Del (5q) Deletion		1R/2G	6.3%
Del (7q) Deletion		1R/1G	7.4%
AML1/ETO (RUNX1/RUNX1T1) Translocation, Dual Fusion		2F/1R/1G	2.3%
EVI1 (MECOM) Breakapart Inversion Signal		1RG/1B/1RGB	4.0%
EVI1 (MECOM) Breakapart Translocation Signal		1R/1GB/1RGB	4.0%

g. Probe limit
Not applicable. The probes are intended to be used only at the concentration provided and are not intended to be diluted.

2. Comparison Studies:

a. Method comparison with predicated device:

Not applicable

b. Matrix Comparison

Not applicable

3. Clinical Studies:

a. Clinical sensitivity
Not applicable

b. Clinical specificity
Not applicable

c. Other clinical supportive data

Acute myeloid leukemia (AML) and myelodysplastic syndromes (MDS)

AML and MDS are neoplastic hematological disorders that arise from myeloid progenitor cells in the bone marrow. AML is characterized by the clonal expansion of myeloid blasts in the peripheral blood, bone marrow or other tissues, while MDS is characterized by the simultaneous proliferation and apoptosis of hematopoietic cells.

Refer to the WHO Guidelines for the most up-to-date use of the probes.

Incident rate

Reference to the clinical significance in WHO guidelines and peer-reviewed published papers (3 for AML and 3 for MDS) were provided to support the clinical validity of the device in characterizing bone marrow specimens from patients with AML and/or MDS. Prevalence rates based on clinical thresholds defined in the literature were reviewed and summarized. Clinical specimens were tested in using the Cytocell AML FISH probe sets and assigned cut-offs and the incidence rates were compared to literature. Laboratory 1 (GOP) analyzed 100 known and suspected AML and MDS specimens in total. Laboratory 2 (YAL) re-analyzed specimen data (266-742 AML or MDS specimens depending on the probe) tested in their laboratory based on the assigned cut-off. See Table 24 for the summary of results and Tables 25-33 for individual probe results.

Table 24. Summary Table of Clinical Validation of Cytocell Probes

Probes/ Rearrangement	Expected Prevalence Rate from Literature	GOP data set Prevalence (95% CI)	YAL data set Prevalence (95% CI)
MLL (KMT2A) Breakapart	(b)(4)		
P53 (TP53) deletion			
Del(20q) deletion			
CBFB/MYH11 translocation, dual fusion			
Del(5q) deletion			

Del(7q) deletion	(b)(4)
AML/ETO (RUNX1/RUNX1T1) dual fusion, translocation	
EVI1 (MECOM) Breakapart	

N/A = not applicable as data was not obtained from this site for this probe.

Table 25. MLL (KMT2A) breakapart probe incidence rate

Condition	Literature Source 1 Schanz et al.	Literature Source 2 Zhao et al.	Literature Source 3 Wang et al.	Literature Source 4 Papaemmanuil et al.	Literature Source 5 Grimwade et al.	Literature Source 6 Bacher et al.	Data Source 1 GOP	Data Source 2 YAL
Was the specific device under review in the submission used in the study?	No	No	No	No	No	No	Yes	Yes
Was the specimen type in the study representative of the claimed specimen type(s)	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Target population (disease status)	Confirmed MDS	Confirmed MDS	Confirmed MDS	Confirmed AML	Confirmed AML	Confirmed AML	Known or suspected MDS or AML	Known or suspected MDS or AML
Upper reference limit - 'Cut-off value' (percentage and per 200 nuclei)	N/A	N/A	N/A	N/A	N/A	N/A	(b)(4) 1R1G1F patterns per 200 scoreable interphase nuclei	1R1G1F patterns per 200 scoreable interphase nuclei

Total Number of specimens tested for each claimed type	2902	2404	435	1540	5876	2235	100	413
Number of specimens with a positive probe result	N/A	N/A	N/A	N/A	N/A	N/A	2	6
Range of positive probe results	N/A	N/A	N/A	N/A	N/A	N/A	(b)(4)	
Source incidence rate for rearrangement (95% CI)	0.2%	1.24%	1.9%	2.86%	4.5%	2.72%		
			*'11q abnormalities'		Expected range from literature:			

Table 26. P53(TP53) deletion probe incidence rate

Condition	Literature Source 1 Schanz et al.	Literature Source 2 Bernasconi et al.	Literature Source 3 Wang et al.	Literature Source 4 Papaemmanuil et al.	Literature Source 5 Grimwade et al.	Literature Source 6 Lazarevic et al.	Data Source 1 GOP
Was the specific device under review in the submission used in the study?	No	No	No	No	No	No	Yes
Was the specimen type in the study representative of the claimed specimen type(s)	Yes	Yes	Yes	Yes	Yes	Yes	Yes

Target population (disease status)	Confirmed MDS	Confirmed MDS	Confirmed MDS	Confirmed AML	Confirmed AML	Confirmed AML	Known or suspected MDS or AML
Upper reference limit - 'Cut-off value' (percentage and per 200 nuclei)	N/A	N/A	N/A	N/A	N/A	N/A	(b)(4) r 14 1R2G patterns per 200 scoreable interphase nuclei
Total Number of specimens tested for each claimed type	2902	331	435	1540	5876	3251	100
Number of specimens with a positive probe result	N/A	N/A	N/A	N/A	N/A	N/A	5
Range of positive probe results	N/A	N/A	N/A	N/A	N/A	N/A	(b)(4)
Source incidence rate for rearrangement (95% CI)	0.6%	1.8%	1.9%	4%	4%	8.8%	
					Expected range from literature:		

Table 27. Del(20q) deletion probe incidence rate

Condition	Literature Source 1 Schanz et al.	Literature Source 2 Haas et al.	Literature Source 3 Wang et al.	Data Source 1 GOP	Data Source 2 YAL
Was the specific device under review in the submission used in the study?	No	No	No	Yes	Yes

Was the specimen type in the study representative of the claimed specimen type(s)	Yes	Yes	Yes	Yes	Yes
Target population (disease status)	Confirmed MDS	Confirmed MDS	Confirmed MDS	Known or suspected	Known or suspected
Upper reference limit - 'Cut-off value' (percentage and per 200 nuclei)	N/A	N/A	N/A	(b)(4)	
Total Number of specimens tested for each claimed type	2902	2072	435	100	742
Number of specimens with a positive probe result	N/A	N/A	N/A	5	9
Range of positive probe results	N/A	N/A	N/A	(b)(4)	
Source incidence rate for rearrangement (95% CI)	1.7%	3.6%	6.6%		

Table 28. CBFB/MYH11 translocation, dual fusion probe incidence rate

Condition	Literature Source 1 Papaemmanuil et al.	Literature Source 2 Grimwade et al.	Literature Source 3 Dores et al.	Data Source 1 GOP	Data Source 2 YAL
Was the specific device under review in the submission used in the study?	No	No	No	Yes	Yes
Was the specimen type in the study representative of the claimed specimen type(s)	Yes	Yes	Yes	Yes	Yes
Target population (disease status)	Confirmed AML	Confirmed AML	Confirmed AML	Known or suspected MDS or AML	Known or suspected MDS or AML
Upper reference limit - 'Cut-off value' (percentage and per 200 nuclei)	N/A	N/A	N/A	2.3% or 5 1R1G2F patterns per 200 scoreable interphase nuclei	2.3% or 5 1R1G2F patterns per 200 scoreable interphase nuclei
Total Number of specimens tested for each claimed type	(b)(4)				
Number of specimens with a positive probe result	N/A	N/A	N/A	2	7
Range of positive probe results	N/A	N/A	N/A	83.0%-93.0%	14%-99.5%

Source incidence rate for rearrangement % (95% CI)	5.26%	5%	1.04%	2% (0.24% to 7.04%)	2.63% (1.06% to 5.35%)
		Expected range from literature:		1.04% - 5.26%	

Table 29. Del(5q) deletion probe incidence rate

Condition	Literature Source 1 Schanz et al.	Literature Source 2 Haase et al.	Literature Source 3 Bernasconi et al.	Literature Source 4 Papaemmanuil et al.	Literature Source 5 Grimwade et al.	Literature Source 6 Sanderson et al.	Data Source 1 GOP	Data Source 2 YAL
Was the specific device under review in the submission used in the study?	No	No	No	No	No	No	Yes	Yes
Was the specimen type in the study representative of the claimed specimen type(s)	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Target population (disease status)	Confirmed MDS	Confirmed MDS	Confirmed MDS	Confirmed AML	Confirmed AML	Confirmed AML	Known or suspected MDS or AML	Known or suspected MDS or AML
Upper reference limit - 'Cut-off value' (percentage and per 200 nuclei)	N/A	N/A	N/A	N/A	N/A	N/A	6.3% or 13 1R2G patterns per 200 scoreable interphase nuclei	6.3% or 13 1R2G patterns per 200 scoreable interphase nuclei
Total Number of specimens tested for each claimed	2902	2072	331	1540	5876	1709	100	723

type								
Number of specimens with a positive probe result	N/A	N/A	N/A	N/A	N/A	N/A	9	73
Range of positive probe results	N/A	N/A	N/A	N/A	N/A	N/A	8%-98%	8%-99.5%
Source incidence rate for rearrangement (95% CI)	8%	15.1%	16%	6%	5%	11%	9% (4.20% to 6.40%)	10.9% (8% to 12.53%)
					Expected range from literature:		5% - 16%	

Table 30. Del(7q) deletion probe incidence rate

Condition	Literature Source 1 Schanz et al.	Literature Source 2 Haase et al.	Literature Source 3 Bernasconi et al.	Literature Source 4 Papaemmanuil et al.	Literature Source 5 Grimwade et al.	Literature Source 6 Sanderson et al.	Data Source 1 GOP	Data Source 2 YAL
Was the specific device under review in the submission used in the study?	No	No	No	No	No	No	Yes	Yes
Was the specimen type in the study representative of the claimed specimen type(s)	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Target population (disease status)	Confirmed MDS	Confirmed MDS	Confirmed MDS	Confirmed AML	Confirmed AML	Confirmed AML	Known or suspected MDS or AML	Known or suspected MDS or AML

Upper reference limit - 'Cut-off value' (percentage and per 200 nuclei)	N/A	N/A	N/A	N/A	N/A	N/A	7.4% or 15 1R1G patterns per 200 scoreable interphase nuclei	7.4% or 15 1R1G patterns per 200 scoreable interphase nuclei
Total Number of specimens tested for each claimed type	2902	2072	331	1540	5876	1709	100	746
Number of specimens with a positive probe result	N/A	N/A	N/A	N/A	N/A	N/A	4	48
Range of positive probe results	N/A	N/A	N/A	N/A	N/A	N/A	21.5%-96%	9%-98%
Source incidence rate for rearrangement (95% CI)	3.6%	11.1%	7.8%	5.7%	8%	9%	4% (1.10% to 9.93%)	6.43% (4.78% to 8.44%)
					Expected range from literature		3.6% - 11.1%	

Table 31. AML1/ETO (RUNX1/RUNX1T1) dual fusion translocation probe incidence rate

Condition	Literature Source 1 Papaemmanuil et al.	Literature Source 2 Grimwade et al.	Literature Source 3 Dores et al.	Data Source 1 GOP	Data Source 2 YAL
Was the specific device under review in the submission used in the study?	No	No	No	Yes	Yes

Was the specimen type in the study representative of the claimed specimen type(s)	Yes	Yes	Yes	Yes	Yes
Target population (disease status)	Confirmed AML	Confirmed AML	Confirmed AML	Known or suspected MDS or AML	Known or suspected MDS or AML
Upper reference limit - 'Cut-off value' (percentage and per 200 nuclei)	N/A	N/A	N/A	2.3% or 5 1R1G2F patterns per 200 scoreable interphase nuclei	2.3% or 5 1R1G2F patterns per 200 scoreable interphase nuclei
Total Number of specimens tested for each claimed type	1540	5876	19497	100	414
Number of specimens with a positive probe result	N/A	N/A	N/A	0	6
Range of positive probe results	N/A	N/A	N/A	N/A	69.5%-98.5%
Source incidence rate for rearrangement % (95% CI)	3.8%	7%	1.6%	0% (0.00% to 3.62%)	1.45% (0.53% to 3.13%)
		Expected range from literature		1.6% - 7.0%	

Table 32. EVI1 Breakapart Probe incidence rate

Condition	Literature Source 1 Schanz et al	Literature Source 2 Haase	Literature Source 3 Pozdnyakova et al	Literature Source 4 Papaemmanuil et al	Literature Source 5 Grimwade et al	Literature Source 6 Byrd et al	Data Source 1 GOP
Was the specific device under review in the submission used in the study?	No	No	No	No	No	No	Yes
Was the specimen type in the study representative of the claimed specimen type(s)	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Target population (disease status)	Confirmed MDS	Confirmed MDS	Confirmed MDS	Confirmed AML	Confirmed AML	Confirmed AML	Known or suspected MDS or AML
Upper reference limit - 'Cut-off value' (percentage and per 200 nuclei)	N/A	N/A	N/A	N/A	N/A	N/A	4% or 8 1RG/1B/1RGB or 1R/1GB/1RGB patterns per 200 scoreable interphase nuclei
Total Number of specimens tested for each claimed type	2902	2072	1029	1540	5876	1213	100
Number of specimens with a positive probe result	N/A	N/A	N/A	N/A	N/A	N/A	4

Range of positive probe results	N/A	N/A	N/A	N/A	N/A	N/A	11%-90.5%
Source incidence rate for rearrangement (95% CI)	0.4%	2%	0.3%	1.3%	1.5%	1%	4% (1.10% to 9.93%)
					Expected range from literature		0.3% - 2.0%

Additional supporting data

To further support the performance of AML1/ETO (RUNX1/RUNX1T1) and CBFβ (CBFB) /MYH11 probes, additional clinical data sets covering the full range of the signal distribution were provided to demonstrate concordance between FISH and G-band. 100% agreement were observed for both data sets expect for one specimen that falls into re-test zone. Tables 33 and 34 demonstrate acceptable clinical concordance.

Table 33. Concordance between the AML1/ETO (RUNX1/RUNX1T1) Probe kit and Karyotyping

	AML1/ETO (RUNX1/RUNX1T1) Translocation, Dual Fusion FISH probe kit % agreement (n)	
G-band result	**Normal**	**Abnormal**
No t(8;21) rearrangement	(b)(4)	
Confirmed t(8;21) rearrangement		

Table 34. Concordance between the CBFβ (CBFB) /MYH11 Probe kit and Karyotyping

	CBFβ (CBFB) /MYH11 Translocation, Dual Fusion FISH Probe Kit % agreement (n)	
G-band result	**Normal**	**Abnormal**
No inv(16)/t(16;16) rearrangement	(b)(4)	
Confirmed inv(16)/t(16;16) rearrangement		

* The discordance was determined to be a sample with results very near the cut-off.

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4. Clinical cut-off

   Not applicable

5. Expected values/Reference range:

   Same as Assay Cut-off (Upper Reference Limit) (section 1.f) above

N. Other Supportive Instrument Performance Characteristics Data Not Covered In The "Performance Characteristics" Section above:

The labeling supports the decision to grant the De Novo request for this device

O. Patient Perspective

This submission did not include specific information on patient perspectives for this device.

P. Identified Risks to Health and Mitigation Measures:

Identified Risks to Health	Mitigation Measures
Incorrect test results	Special controls (1), (2), (3), and (4)
Incorrect interpretation of test results	Special controls (1), (2), (3), and (4)

Q. Benefit/Risk Summary:
Clinical reviewer memo will contain details for benefit risk determination.

Summary of the Benefits	Summary of the Risks	Summary of Other Factors	Conclusions
AML and MDS patients may benefit by use of the device on bone marrow specimens to obtain results that can be used in accordance with the World Health Organization criteria for assessment of these patient specimens when assay results are interpreted by a qualified pathologist or cytogeneticist.	Erroneous device performance can yield false negative or false positive results or incorrect interpretation of test results by the user which may adversely influence management of AML or MDS patients.	Risks are mitigated by analytical and clinical validation studies using the device probes, along with labeling and supports the intended use. The device use requires a qualified pathologist or cytogeneticist in the context of histopathological evaluation (e.g., immunohistochemistry).	Yes. Based on the supporting clinical studies for the diagnostic device along with review of the analytical performance and labeling, the probable benefits outweigh the probable risks in light of the mitigations provided by the special controls, in addition to the general controls.

S. Conclusion:

The information provided in this de novo submission is sufficient to classify this device into class II under regulation 21 CFR 864.1880. FDA believes that the stated special controls, in combination with the general controls, provide a reasonable assurance of the safety and effectiveness of the device type. The device is classified under the following:

Product Code: QDI

Device Type: Fluorescence in-situ hybridization based detection of chromosomal abnormalities from patients with hematologic malignancies
Class: II (Special Controls)
Regulation: 21 CFR 864.1880

(a) IDENTIFICATION: Fluorescence in-situ hybridization based detection of chromosomal abnormalities from patients with hematologic malignancies is used to detect chromosomal abnormalities in human specimens from patients with hematologic malignancies. The test is indicated for the clinical management of patients consistent with internationally accepted guidelines (e.g. World Health Organization guidelines for Classification of Tumours of Haematopoietic and Lymphoid Tissues) and in conjunction with other clinical and clinicopathological criteria. The results are to be interpreted by a pathologist or equivalent professional.

(b) CLASSIFICATION: Class II (special controls). The special controls for this device are:

(1) Design verification and validation (b)(4) must include:

(i) A detailed description of all probes included in the kit;

(ii) Purpose of each probe;

(iii) Probe molecular specificity;

(iv) Probe specificity;

(v) Probe limits;

(vi) Probe sensitivity;

(vii) Specification of required ancillary reagents, instrumentation, and equipment;

(viii) Specification of the specimen collection, processing, storage and slide preparation methods;

(ix) Specification of the assay procedure;

(x) Specification of control elements that are incorporated into the recommended testing procedures;

(xi) Specification of the criteria for test result interpretation and reporting;

(xii) Documentation demonstrating analytical validation that includes:

(A) Device analytical sensitivity data with a minimum of 25 specimens from karyotypically normal males.

(B) Device analytical specificity data with a minimum of 5 specimens from karyotypically normal males.

(C) Description of how the clinical threshold was assigned and verification of the assigned clinical threshold.

(D) Device precision/reproducibility data with a minimum of 6 clinical specimens including 2 negative specimens, 2 positive specimens near the clinical decision threshold (cut-off) and 2 positive specimens. The data must include results obtained from 3 sites (as applicable), with 2 operators at each site, with the assay run for a minimum of 3-5 non-consecutive days and each specimen run in duplicate for a minimum of 30 replicates.

(E) Between-reagent lot reproducibility using 3 reagent lots and 3 clinical specimens representing negative, near cut-off /low positive, and positive.

(F) Device stability data to include:

(1) Real-time Stability,

(2) Freeze-Thaw Stability,

(3) Transport and Temperature Stability, as applicable,

(4) Post-Hybridization Signal Stability, and

(5) Photostability of Probe.

(xiii) Documentation demonstrating the clinical validity of the device that includes:

(A) A summary of the prevalence and clinical thresholds reported in 3 peer-reviewed published literature references for the intended use population of the device and device performance data demonstrating conformance with the published prevalence as reported in peer-reviewed published literature references based on testing clinical specimens, selected without bias (e.g., consecutively selected) from the intended use population using the specific device seeking marketing clearance. A minimum number of clinical specimens must be tested to ensure sufficient positives are evaluated by the device, or alternatively, in the absence of a sufficient number of positives, an additional comparison of results obtained with the device to clinical truth (e.g., confirmed clinical diagnosis and/or G-banded karyotyping) with an independent specimen set must be conducted.

(B) Documentation for peer–reviewed published literature references must include the following elements:

(1) Whether the specific device was used in the literature reference;

(2) Number and type of specimens;

(3) Target population studied;

(4) Upper reference limit; and

(5) Prevalence range estimated based on the number of positive probe results.

(C) In the absence of clinical data obtained from paragraphs (b)(1)(xiii)(A) and (b)(1)(xiii)(B) of this section, clinical data obtained from a method comparison to the predicate with positives and negative clinical specimens.

(2) The intended use required on the label under 21 CFR 809.10(a)(4) and on the labeling under 21 CFR 809.10(b)(5)(ii), must include a statement that

"The test is not intended for use as a stand-alone diagnostic, disease screening, or as a companion diagnostic."

(3) The 21 CFR 809.10(b) labeling must include information that demonstrates the performance characteristics of the test, including a detailed summary of the performance studies conducted and their results, as described in paragraphs (b)(1)(iv) through (b)(1)(xiii) of this section. The 21 CFR 809.10(b) labeling must include the pre-specified acceptance criteria for these performance studies, justification for the pre-specified acceptance criteria, and whether the pre-specified acceptance criteria were met.

(4) The 21 CFR 809.10(b) labeling must include the following limiting statements:

(i) "Reporting and interpretation of FISH results should be consistent with professional standards of practice and should take into consideration other clinical and diagnostic information. This kit is intended as an adjunct to other diagnostic laboratory tests and therapeutic action should not be initiated on the basis of the FISH result alone. Failure to adhere to the protocol may affect the performance and lead to false results."

(ii) "Each lab is responsible for establishing their own cut-off values. Each laboratory should test sufficiently large number of samples to establish normal population distribution of the signal levels and to assign a cut-off value. The product is for professional use only and is intended to be interpreted by a qualified Pathologist or Cytogeneticist."