PDGFRB FISH PRODUCT DATASHEET

Proprietary Name: *PDGFRB* FISH for Gleevec Eligibility in Myelodysplastic Syndrome/Myeloproliferative Disease (MDS/MPD)

Established Name: *PDGFRB* FISH for Gleevec in MDS/MPD

INTENDED USE

Humanitarian Device. Authorized by Federal law for use in the qualitative detection of *PDGFRB* gene rearrangement in patients with MDS/MPD. The effectiveness of this device for this use has not been demonstrated.

Caution: Federal Law restricts this device to sale by or on the order of a licensed practitioner.

PDGFRB FISH for Gleevec Eligibility in Myelodysplastic Syndrome/Myeloproliferative Disease (MDS/MPD) is an in vitro diagnostic test intended for the qualitative detection of PDGFRB gene rearrangement from fresh bone marrow samples of patients with MDS/MPD with a high index of suspicion based on karyotyping showing a 5q31~33 anomaly. The *PDGFRB* FISH assay is indicated as an aid in the selection of MDS/MPD patients for whom Gleevec® (imatinib mesylate) treatment is being considered. This assay is for professional use only and is to be performed at a single laboratory site.

SUMMARY AND EXPLANATION OF THE TEST

The *PDGFRB* FISH assay detects rearrangement of the *PDGFRB* locus at chromosome 5q31~33 in adult patients with myelodysplastic syndrome/myeloproliferative disease (MDS/MPD). The *PDGFRB* gene encodes a cell surface receptor tyrosine kinase that upon activation stimulates mesenchymal cell division. Rearrangement of *PDGFRB* has been demonstrated by classical cytogenetic analysis in an exceedingly small fraction of MDS/MPD patients (~1%), usually cases with a clinical phenotype of chronic myelomonocytic leukemia (CMML). In particular, these patients harbor a t(5:12)(q31;p12) translocation involving the *PDGFRB* and ETV6 genes. Fusion of *PDGFRB* to the ETV6 gene on chromosome 12 generates a constitutively active tyrosine kinase fusion protein, ETV6-*PDGFRB*, which is sensitive to the tyrosine kinase inhibitor Gleevec.[1,2]

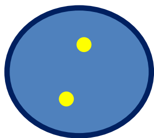
Given the therapeutic implications of Gleevec, all patients with a presumptive diagnosis of MDS/MPD and suspicion of a 5q31~33 anomaly based on karyotyping should be confirmed by *PDGFRB* FISH analysis, especially, but not only, if there is concomitant eosinophilia. The *PDGFRB* FISH assay aims to aid in the identification of MDS/MPD patients that may benefit from Gleevec treatment, as only patients harboring a *PDGFRB* rearrangement are likely to benefit from treatment.

BIOLOGICAL PRINCIPLES OF THE PROCEDURE

The FISH (fluorescence in situ hybridization) technique utilizes fluorescently labeled DNA probes to target specific genes or particular chromosome regions within a sample of cells. The *PDGFRB* FISH assay utilizes live cells from patient bone marrow, which are grown in culture and subsequently fixed. The *PDGFRB* FISH assay may also be utilized with live cells from patient bone marrow that are directly fixed without prior cell culture. The fixed cells are dropped onto slides and pre-treated chemically to remove proteins that block DNA access. The DNA is denatured to its single-stranded form and subsequently allowed to hybridize with the DNA probes of interest. Following hybridization, the unbound probe is removed by a series of washes, and the cell nuclei are counterstained with DAPI (4, 6 diamidino-2-phenylindole), a DNA specific stain that fluoresces blue. Hybridization of the *PDGFRB* probe is viewed using a fluorescence microscope equipped with appropriate filters allowing visualization of the red and green fluorescent signals. Detection of signals is conducted by manual microscopic examination of the nucleus.

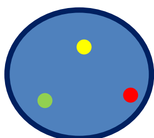
The *PDGFRB* FISH assay utilizes a break-apart probe to detect rearrangement of *PDGFRB* at chromosome locus 5q31~33. If the *PDGFRB* locus is intact, the probe will appear as adjacent (touching) red and green signals or as a fused (overlapping) yellow signal. Each normal cell will display two fusion "2F" (yellow) signals. If the *PDGFRB* locus is rearranged, the probe will most often appear as one red and one green signal separated by at least two signal distances. In the most common form of *PDGFRB* rearrangement, the abnormal cell will display an "RGF" signal, with one fusion (yellow), one red, and one green signal. Rarely, both *PDGFRB* loci are rearranged to generate the "2G2R" signal, resulting in two red and two green signals separated by at least two signal distances. Another rare signal pattern that can result from a PDGFRB rearrangement is called "FR", which is generated when one red signal is rearranged and one green signal is lost. The diagram shown below illustrates the normal 2F signal pattern and the three abnormal signal patterns indicative of PDGFRB rearrangement.

NORMAL SIGNAL PATTERNS INDICATIVE OF NO *PDGFRB* REARRANGEMENT

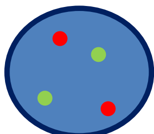


NORMAL 2F: 2 Fused (adjacent red and green appear yellow)

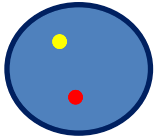
ABNORMAL SIGNAL PATTERNS INDICATIVE OF A *PDGFRB* REARRANGEMENT



COMMON RGF: 1 Red, 1 Green, 1 Fused



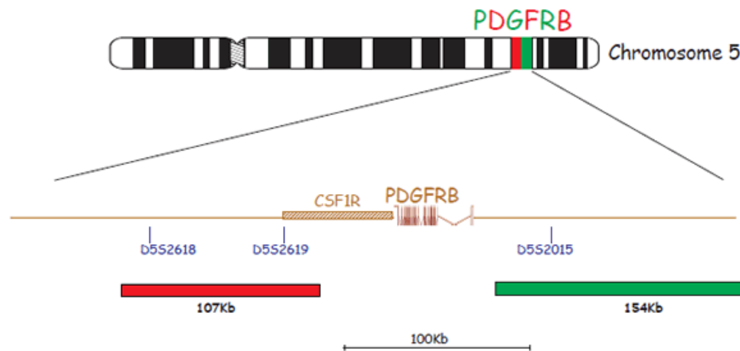
RARE 2G2R: 2 Green, 2 Red



RARE FR: 1 Red, 1 Fused (green signal lost due to rearrangement)

PROBE DESCRIPTION

The *PDGFRB* FISH probe is a mixture of a 107Kb red-labeled probe, located centromeric to the *PDGFRB* gene, and a 154Kb green-labeled probe located telomeric to the *PDGFRB* gene. The probes are pre-mixed in hybridization buffer.



SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS

Collect fresh bone marrow aspirate. Transfer 3 mL (minimum 1 mL) of bone marrow to a green, sodium heparin tube. Submit specimen in an ARUP Standard Transport Tube and ship at room temperature. Specimen must be received in the laboratory within 72 hours of draw. Specimens received >4 days (96 hours) from the time of collection are unacceptable and will not be tested. Frozen, paraffin-embedded, and clotted specimens are all unacceptable specimen types.

Because *PDGFRB* FISH for Gleevec Eligibility in Myelodysplastic Syndrome/ Myeloproliferative Disease (MDS/MPD) is a test approved by the FDA under a Humanitarian Device Exemption, testing must be ordered using the following procedures:

1. The ordering physician must register with the Institutional Review Board (IRB) for the *PDGFRB* FISH testing for Gleevec eligibility in MDS/MPD. Go to www.aruplab.com/*PDGFRB* to obtain IRB registration online.
2. The test should be ordered using the ARUP test request form or via ARUP's web-based ordering interface (available only to existing ARUP clients). The full name of the ordering physician must be included on the ARUP form to ensure timely testing of the sample. Samples submitted with incomplete information may delay specimen testing.
3. To send a specimen to ARUP, contact your local hospital/reference lab to determine if they are an ARUP client and can send the specimen.

If they cannot send the specimens to ARUP, contact ARUP Client Services at (800) 522-2787 to be directed to an alternative ordering mechanism.

4. Forms and information about the *PDGFRB* FISH Assay, and IRB registration, may be accessed at www.aruplab.com/*PDGFRB*.

LIMITATIONS OF THE PROCEDURE

FOR IN VITRO DIAGNOSTIC USE ONLY

Optimal performance of this test requires proper specimen preparation, handling, storage, and transport as described in these instructions for use.

Results of this test should be interpreted within the context of clinical findings.

EXPECTED VALUES

Population Characteristics

An abnormal result (rearranged *PDGFRB* locus) is expected in approximately 1% of MDS/MPD patients.

Normal Cutoff

The normal cutoff value for FISH is the maximum percentage of scoreable interphase nuclei with a specific abnormal signal pattern at which the specimen is considered normal for that signal pattern. The cutoff values for the *PDGFRB* FISH assay were established using interphase nuclei from twenty normal bone marrow specimens, as determined by flow cytometry and anemia results. For each specimen, two hundred cells, which is the standard number of scored cells, were evaluated by counting the number of fused (F), red (R), or green (G) signals. The expected normal signal pattern in interphase cells for the *PDGFRB* probe is two fused (2F) signals. The normal cutoff values were determined using the beta inverse function and are shown in Table 1 below.

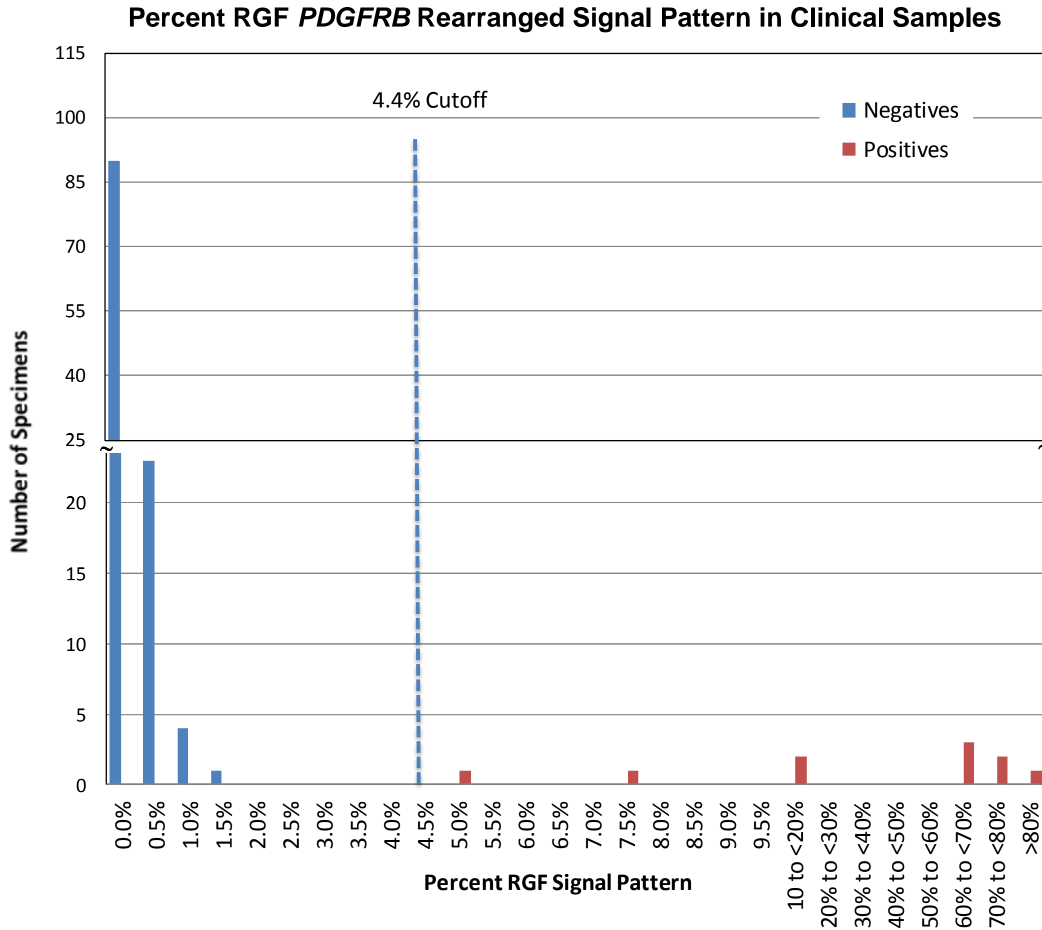
Table 1. Normal Cutoff Values (%) for Determination of Normal/Abnormal for the *PDGFRB* FISH assay

# of Cells	RGF	1F	3F	4F	FG	FR	2FR	2FG	2G2R	2FRG
200	4.4	5.1	4.4	4.4	2.3	1.5	2.3	2.3	2.3	2.3

Patient Distribution

The *PDGFRB* FISH assay has been used to test clinical specimens in the intended use population. Shown in the figure below is the distribution of 10 positive specimens and 117 sequentially tested negative specimens with regard to percent of cells exhibiting the RGF signal

pattern indicative of a *PDGFRB* rearrangement. The RGF cutoff value of 4.4% is shown for reference.



SPECIFIC PERFORMANCE CHARACTERISTICS

Assay Analytical Sensitivity and Specificity

Assay analytical sensitivity is defined as the percentage of scoreable cells with the expected normal signal pattern (true positives). The assay sensitivity of the *PDGFRB* FISH assay was evaluated using twenty (20) individual karyotypically normal male bone marrow specimens plus a pool of fixed cell pellets prepared from peripheral blood of five (5) karyotypically normal males. For each of the twenty (20) individual bone marrow specimens and the pool, 100 cells were scored by two (2) different operators. In total, 4200 cells were scored. For the sensitivity calculation, the *PDGFRB* probe signal patterns were enumerated for each cell. Two normal signals (fused red and green) were expected per cell for a total of 8400 signals. The assay sensitivity was calculated to be 97.2% (Table 2).

Table 2. Probe Sensitivity

Signal Pattern	No. of Signal Patterns: Total True Positives Observed	No. of Signal Patterns: Total Expected Signal Patterns	Sensitivity: Point Estimate (%)	Sensitivity: 95% Confidence Interval (%)*
PDGFRB 2 Fused Red-Green	4081	4200	97.2	(96.7, 97.7)

**Confidence intervals calculated using the Exact method (Clopper and Pearson, Biometrika 26:404-413, 1934).*

Assay analytical specificity is defined as the percentage of probe signals that hybridize to the correct chromosomal location and to no other location (total signals minus false positives). The probe specificity of the *PDGFRB* probe were evaluated using a pool of fixed cell pellets prepared from peripheral blood of five karyotypically normal males. The mitotic index of the pooled sample was sufficient to evaluate the probe signal patterns and hybridization localization in 200 metaphase cells (400 loci). Two normal signals (fused red and green) were expected per metaphase for a total of 400 signals. For the specificity calculation, the number of metaphase chromosome probe signals hybridized to the correct locus and the number of metaphase chromosome probe signals hybridized to the incorrect locus were enumerated.

Table 3. Probe Specificity

Probe	No. of Metaphase Chromosome Signals: Total False Positives Observed	No. of Metaphase Chromosome Signals: Total True Positives Observed	No. of Metaphase Chromosome Signals: Total Observed	Specificity: Point Estimate (%)	Specificity: 95% Confidence Interval (%)*
PDGFRB Centromeric-Red	0	399	399	100	(99.08, 100)
PDGFRB Telomeric-Green	0	399	399	100	(99.08, 100)

**Confidence intervals calculated using the Exact method (Clopper and Pearson, Biometrika 26:404-413, 1934).*

Precision

The precision of the *PDGFRB* probe was established using three separate pools of healthy bone marrow (two individual samples per pool). The three separate specimen pools were tested in triplicate by three operators on non-consecutive days to determine intra-operator precision. All 81 slides produced results that fell below the cutoff for positive signal patterns and were therefore interpreted as “normal”. Intra-operator precision results (Tables 4 – 6) show 100% agreement and coefficient of variation less than 1%.

Table 4. Operator "A" Precision

Normal Bone Marrow Sample Pool	Day	Mean # of Normal Signals	Standard Deviation	Concordance and % CV
Pool A	1	198.33	1.53	100% PPA, NPA, OPA 0.70% CV
	2	199.00	1.73	
	3	196.33	1.53	
Pool B	1	199.00	1.00	100% PPA, NPA, OPA 0.34% CV
	2	198.33	2.08	
	3	197.67	1.53	
Pool C	1	199.67	0.58	100% PPA, NPA, OPA 0.77% CV
	2	198.67	1.15	
	3	196.67	1.53	

Table 5. Operator "B" Precision

Normal Bone Marrow Sample Pool	Day	Mean # of Normal Signals	Standard Deviation	Concordance and % CV
Pool A	1	199.33	0.58	100% PPA, NPA, OPA 0.54% CV
	2	199.00	1.00	
	3	197.33	1.53	
Pool B	1	198.33	2.89	100% PPA, NPA, OPA 0.17% CV
	2	198.67	1.53	
	3	198.00	1.00	
Pool C	1	199.00	0.00	100% NPA, 0.35% CV
	2	198.00	1.00	
	3	197.67	1.53	

Table 6. Operator "C" Precision

Normal Bone Marrow Sample Pool	Day	Mean # of Normal Signals	Standard Deviation	Concordance and % CV
Pool A	1	198.00	1.73	100% PPA, NPA, OPA 0.54% CV
	2	197.67	1.53	
	3	196.00	1.73	
Pool B	1	198.67	1.15	100% PPA, NPA, OPA 0.39% CV
	2	198.67	1.53	
	3	197.33	0.58	
Pool C	1	199.33	0.58	100% PPA, NPA, OPA 0.17% CV
	2	199.00	0.00	
	3	198.67	1.15	

Reproducibility

The reproducibility of the *PDGFRB* probe was established using three separate pools of normal bone marrow (two individual samples per pool). The three separate specimen pools were tested in triplicate by three operators on non-consecutive days to determine inter-operator reproducibility. All 81 slides produced results that fell below the cutoff for positive signal patterns and were therefore interpreted as "normal". Inter-operator reproducibility results (Tables 7) show 100% agreement and coefficient of variation less than 1%.

Table 7. Reproducibility

Normal Bone Marrow Sample Pool	Day	Operator	Replicate Mean # of Normal Signals	Inter-Operator Mean	Inter-Operator Standard Deviation	Concordance
Pool A	1	A	198.33	198.56	0.69	100% PPA, NPA, OPA 0.35% CV
		B	199.33			
		C	198.00			
	2	A	199.00	198.56	0.77	100% PPA, NPA, OPA 0.39% CV
		B	199.00			
		C	197.67			
	3	A	196.33	196.56	0.69	100% PPA, NPA, OPA 0.35% CV
		B	197.33			
		C	196.00			
Pool B	1	A	199.00	198.67	0.33	100% PPA, NPA, OPA 0.17% CV
		B	198.33			
		C	198.67			
	2	A	198.33	198.55	0.19	100% PPA, NPA, OPA 0.10% CV
		B	198.67			
		C	198.67			
	3	A	197.67	197.67	0.33	100% PPA, NPA, OPA 0.17% CV
		B	198.00			
		C	197.33			
Pool C	1	A	199.67	199.33	0.33	100% PPA, NPA, OPA 0.17% CV
		B	199.00			
		C	199.33			
	2	A	198.67	198.56	0.51	100% PPA, NPA, OPA 0.26% CV
		B	198.00			
		C	199.00			
	3	A	196.67	197.67	1.00	100% PPA, NPA, OPA 0.51% CV
		B	197.67			
		C	198.67			

Potentially Interfering Substances

The performance of the *PDGFRB* FISH Assay was evaluated in the presence of elevated levels of potentially interfering substances. Two normal bone marrow samples were pooled and aliquots were incubated with hemoglobin, bilirubin, intralipid, EDTA, or heparin prior to cell harvest. FISH results were compared to aliquots that were not treated with an interferent. All samples produced normal results. No interference in the performance of the *PDGFRB* FISH Assay was observed in the presence of the following substances:

- Hemoglobin (2g/L)
- Bilirubin (342µmol/L)
- Intralipid (37 mmol/L)
- EDTA (3.6 mg/mL)
- Heparin (30 USP units/mL)

BIBLIOGRAPHY

1. Golub TR, et al. Fusion of PDGFR receptor beta to a novel ets-like gene, tel, in chronic myelomonocytic leukemia with t(5;12) chromosomal translocation. Cell 1994; 77:307-316.
2. Apperley JF, et al. Chronic myeloproliferative diseases involving rearrangements of the platelet-derived growth factor beta receptor (PDGFRB) showing rapid responses to the tyrosine kinase inhibitor STI571. N Engl J Med 2002; 347:481-487.
3. Clopper C, Pearson ES. (1934). The use of confidence or fiducial limits illustrated in the case of the binomial. Biometrika 1934: 26:404–413.

Manufacturer's Address:

ARUP Laboratories, Inc.
500 Chipeta Way
Salt Lake City, UT 84108