KIT D816V PRODUCT DATASHEET

Proprietary Name: *KIT* D816V Mutation Detection by PCR for Gleevec Eligibility in Aggressive Systemic Mastocytosis (ASM)

Established Name: *KIT* D816V for Gleevec Eligibility in ASM

INTENDED USE

Humanitarian Device. Authorized by Federal law for use in qualitative polymerase chain reaction (PCR) detection of *KIT* D816V mutational status in patients with aggressive systemic mastocytosis (ASM). 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.

KIT D816V Mutation Detection by PCR for Gleevec Eligibility in Aggressive Systemic Mastocytosis (ASM) (referred to as the "*KIT* D816V assay") is an in vitro diagnostic test intended for qualitative polymerase chain reaction (PCR) detection of *KIT* D816V mutational status from fresh bone marrow samples of patients with aggressive systemic mastocytosis. The *KIT* D816V mutational assay is indicated as an aid in the selection of ASM 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

Aggressive systemic mastocytosis (ASM) is a rare subtype of systemic mastocytosis (SM) characterized by the progressive growth of neoplastic mast cells in multiple organs. In the majority of ASM cases (estimated >80%), the clonal nature of the disease can be established through demonstration of a somatic A to T missense mutation at position 2447 of the coding sequence in the *KIT* gene.[1] The resulting substitution of aspartic acid (D) to valine (V) at amino acid 816 is referred to as the D816V mutation. The D816V mutation, which is located in the kinase domain, leads to auto-activation of the KIT receptor tyrosine kinase.

The *KIT* D816V mutation is mainly found in mastocytosis; however it is uncommonly identified in gastrointestinal stromal tumors, acute myeloid leukemia, and germ cell tumors. The tyrosine kinase inhibitor Gleevec® (Novartis Pharmaceuticals) has been used to treat patients with a variety of *KIT* mutations. However, *in vitro* studies have demonstrated that the *KIT* D816V mutation confers resistance to Gleevec®.[2-4] Therefore, patients harboring the *KIT* D816V mutation will not benefit from Gleevec® therapy. Approximately 90% of ASM patients with a mutation at *KIT* codon 816 have the D816V substitution. The *KIT* D816V assay is a companion diagnostic test for the use of Gleevec® in the treatment of ASM. ASM patients <u>without</u> the *KIT* D816V mutation may benefit from treatment with kinase inhibitors such as Gleevec®.

BIOLOGICAL PRINCIPLES OF THE PROCEDURE

The *KIT* D816V assay is only performed on fresh bone marrow aspirate. Genomic DNA is isolated from white blood cells and quantitated by spectrophotometry. DNA concentrations are adjusted, if necessary, and a fixed concentration of DNA is utilized for PCR amplification. A two-tube PCR format is employed to maximize PCR sensitivity for detection of the *KIT* D816V mutation.[5] Tube one uses primers that amplify a 184 base pair sequence of *KIT* exon 17, which is used as a control to ensure the presence of amplifiable DNA. Tube one amplifies regardless of the mutation status of the specimen. Tube two uses a mutant allele-specific forward primer and the same reverse primer as the control reaction to only amplify D816V mutant *KIT*. The presence of a D816V mutant allele will be indicated by a 90 base pair product. Wild-type *KIT* sequence will not be amplified by tube two. For each specimen, two mutation-specific PCR replicates (tube two) and one wild-type replicate (tube one) are performed. A preparation of genomic DNA containing 0.3% of *KIT* D816V mutant is used as a positive PCR control and a preparation of genomic DNA from normal human bone marrow (0% mutant) is used as a negative PCR control. Detection of the PCR products is performed by capillary electrophoresis for high-sensitivity amplicon detection and accurate fragment size determination.

SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS

Collect fresh bone marrow aspirate. Transfer 3 mL (minimum 1 mL) of bone marrow to an EDTA tube. Submit specimen in an ARUP Standard Transport Tube and ship refrigerated. Specimen must be received in the laboratory within 72 hours of collection. Frozen, clotted, or grossly hemolyzed specimens are all unacceptable specimen types.

Because the *KIT* D816V assay 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 Internal Review Board (IRB) for *KIT* D816V for Gleevec testing. Go to http://www.aruplab.com/KITD816V 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 specimen. Specimens submitted with incomplete information may delay specimen testing.
3. Physicians are instructed as follows: ARUP does not accept specimens directly from physician offices. ARUP only accepts specimens from established clients. To send a specimen to ARUP, contact your local hospital/reference laboratory to determine if they are an ARUP client and can send the specimen. If they cannot send the specimen to ARUP, contact ARUP Client Services at (800) 522-2787 to be directed to an alternative ordering mechanism.

4. Forms and information about the *KIT* D816V for Gleevec testing, and IRB registration, may be accessed at http://www.aruplab.com/KITD816V.

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.

This test will only detect the *KIT* codon D816V mutation. Other mutations at codon 816 will not be detected.

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

PERFORMANCE CHARACTERISTICS

Expected Values

Population Characteristics

The *KIT* D816V mutation is expected in approximately 93% of ASM patients.

Analytical Sensitivity - Limit of Detection

The limit of detection was defined as the minimum amount of input that produces the correct result 95% of the time. The limit of detection was assessed using two types of specimens:

- Cell blends: cells from a *KIT* D816V mutant cell line were diluted into healthy bone marrow aspirate containing known concentrations of nucleated cells as determined by quantitative flow cytometry.
- Genomic DNA blends: genomic DNA extracted from a *KIT* D816V mutant cell line was mixed with genomic DNA extracted from healthy bone marrow.

Analytical Sensitivity Using Cell Blends

Freshly grown cells from a *KIT* D816V mutant cell line were diluted into known concentrations of nucleated cells from healthy bone marrow to generate nine (9) known mutant cell concentrations (0.05%, 0.10%, 0.15%, 0.3%, 1.0%, 2.5%, 5%, 7.5% and 15%). Twenty (20) replicates of each mutant cell concentration were tested. Twenty-five (25) replicates of the negative control, DNA extracted from healthy bone marrow (0% mutant cell concentration), were tested. DNA extracted from 100% D816V mutant cells was used as the positive control. The D816V mutation was detected in all twenty (20) replicates of the lowest mutant cell concentration (0.05%) tested. While the precise limit of detection could not be determined from this study using cell blends, this study demonstrated that the assay can accurately detect mutant cell concentrations of 0.05%.

Analytical Sensitivity Using Genomic DNA

Genomic DNA extracted from a *KIT* D816V mutant cell line was mixed with genomic DNA extracted from healthy bone marrow.to generate nine (9) known mutant genomic DNA concentrations (0.05%, 0.10%, 0.15%, 0.30%, 1.0%, 2.5%, 5.0%, 7.5%, 15%). DNA extracted from 100% D816V mutant cells was used as the positive control (10 replicates) and DNA extracted from healthy bone marrow was used as the negative control (20 replicates). Twenty (20) replicates of each mutant concentration were tested. Sensitivity was determined by the lowest percentage of *KIT* D816V mutant DNA that was detectable at least 95% of the time. The results of the sensitivity study using genomic DNA are shown in Table 1 below. The assay demonstrated a 97.5% mutation detection rate at the 0.10% mutant DNA concentration.

Table 1. Analytical Sensitivity of the *KIT* D816V Assay using Genomic DNA

KIT D816V Mutant DNA (%)	Replicates	Rate of Mutation Detection
0%	20	0%
0.05%	20	72.5%
0.10%	20	97.5%
0.15%	20	100%
0.30%	20	100%
1.0%	20	100%
2.5%	20	100%
5.0%	20	100%
7.5%	20	100%
15.0%	20	100%
100%	10	100%

The 0.10% limit of detection established by the DNA-based dilution study was utilized as the limit of detection for the KIT D816V assay.

Analytical Sensitivity - Limit of Blank

Normal bone marrow samples were evaluated to ensure that a blank bone marrow sample does not generate an analytical signal above the minimum threshold, indicating a mutation at a low concentration. Twenty-six (26) normal bone marrow samples were extracted and evaluated using the *KIT* D816V assay to ensure that any detectable analytical signal for the mutation was below the minimum threshold. No signals were above the mutation threshold for the 100% wild-type samples. Likewise, twenty (20) genomic DNA samples purified from normal bone marrow samples were evaluated using the *KIT* D816V assay. No signals were above the mutation threshold for the genomic DNA samples purified from 100% wild-type samples.

Accuracy

Accuracy of the *KIT* D816V assay was evaluated by testing blinded, genomic DNA samples purified from clinical bone marrow specimens that had previously been tested for the *KIT* D816V mutation using Next Generation Sequencing (NGS). Forty-three (43) DNA samples were tested, including five positive samples containing the *KIT* D816V mutation. The results of the accuracy study are shown in Table 2 below. The *KIT* D816V assay demonstrated an overall accuracy of 97.7%.

Table 2. Accuracy study using residual DNAs from clinical specimens previously tested by NGS

	***KIT* D816V Positives (NGS)**	***KIT* D816V Negatives (NGS)**	**Total**
***KIT* D816V Positives (*KIT* D816V assay)**	5 (100%)	1	6
***KIT* D816V Negatives (*KIT* D816V assay)**	0	37 (97.4%)	37
Total	5	38	43

One of the samples originally found to be negative by the NGS assay returned a positive result when tested using the *KIT* D816V assay. Upon manual review of the NGS results, it was determined that the sample contained a low level of the *KIT* D816V mutation near the *KIT* D816V assay limit of detection.

Cross-Reactivity

Cross-reactivity of the *KIT* D816V assay was evaluated by testing other *KIT* codon 816 mutations. Samples included:

- *KIT* D816H: two (2) bone marrow specimens and one (1) plasmid
- *KIT* D816Y: two (2) bone marrow specimens and one (1) plasmid

The *KIT* D816V assay showed no cross-reactivity for the D816Y mutant in either of the two bone marrow specimens or plasmid. For the D816H mutant, no cross-reactivity was observed for one of the bone marrow specimens and the plasmid. The cross-reactivity observed for the second D816H bone marrow specimen was determined to be from a minor amount (1%) of D816V allele, as demonstrated by Next Generation sequencing. The *KIT* D816V assay does not detect the non-D816V mutations tested (D816H and D816Y).

Interference

Warning: High concentrations of unconjugated bilirubin (≥171 µmol/L) have been shown to cause clotting of bone marrow collected in EDTA. Specimens that are clotted are an unacceptable type and will be rejected. Specimens with high concentrations of unconjugated bilirubin that are not clotted may be processed normally.

The addition of hemoglobin (2 g/L), unconjugated bilirubin (50 µmol/L), intralipid (37 mmol/L), EDTA (3.6 mg/mL) or heparin (30 USP units/mL) to bone marrow prior to testing did not adversely affect the assay.

Precision and Reproducibility

The precision and reproducibility of the *KIT* D816V PCR assay was established using three batches of twelve bone marrow specimens. Each batch of samples included the following: three samples with 0% mutant cells (healthy bone marrow), three samples with 0.3% mutant cells, three samples with 1% mutant cells, and three samples with 15% mutant cells. The three batches were tested by three operators on non-consecutive days to determine intra-operator precision and inter-operator reproducibility. Intra-operator precision results (Tables 3 - 5) show 100% agreement. PPA – positive percent agreement; NPA – negative percent agreement

Table 3. Operator "A" Precision

Sample Type	Day	Total Samples	Samples D816V Detected	Concordance
0% MUT cells	1	3	0/3 (0%)	100% NPA
	2	3	0/3 (0%)	
	3	3	0/3 (0%)	
0.3% MUT cells	1	3	3/3 (100%)	100% PPA
	2	3	3/3 (100%)	
	3	3	3/3 (100%)	
1% MUT cells	1	3	3/3 (100%)	100% PPA
	2	3	3/3 (100%)	
	3	3	3/3 (100%)	
15% MUT cells	1	3	3/3 (100%)	100% PPA
	2	3	3/3 (100%)	
	3	3	3/3 (100%)	

Table 4. Operator "B" Precision

Sample Type	Day	Total Samples	Samples D816V Detected	Concordance
0% MUT cells	1	3	0/3 (0%)	100% NPA
	2	3	0/3 (0%)	
	3	3	0/3 (0%)	
0.3% MUT cells	1	3	3/3 (100%)	100% PPA
	2	3	3/3 (100%)	
	3	3	3/3 (100%)	
1% MUT cells	1	3	3/3 (100%)	100% PPA
	2	3	3/3 (100%)	
	3	3	3/3 (100%)	
15% MUT cells	1	3	3/3 (100%)	100% PPA
	2	3	3/3 (100%)	
	3	3	3/3 (100%)	

Table 5. Operator "C" Precision

Sample Type	Day	Total Samples	Samples D816V Detected	Concordance
0% MUT cells	1	3	0/3 (0%)	100% NPA
	2	3	0/3 (0%)	
	3	3	0/3 (0%)	
0.3% MUT cells	1	3	3/3 (100%)	100% PPA
	2	3	3/3 (100%)	
	3	3	3/3 (100%)	
1% MUT cells	1	3	3/3 (100%)	100% PPA
	2	3	3/3 (100%)	
	3	3	3/3 (100%)	
15% MUT cells	1	3	3/3 (100%)	100% PPA
	2	3	3/3 (100%)	
	3	3	3/3 (100%)	

Inter-operator reproducibility results (Table 6) show 100% agreement. PPA – positive percent agreement; NPA – negative percent agreement

Table 6. Inter-Operator Reproducibility

Sample Type	Day	Operator	Total Samples	Samples D816V Detected	Concordance
0% MUT cells	1	A	3	0/3 (0%)	100% NPA
		B	3	0/3 (0%)	
		C	3	0/3 (0%)	
	2	A	3	0/3 (0%)	100% NPA
		B	3	0/3 (0%)	
		C	3	0/3 (0%)	
	3	A	3	0/3 (0%)	100% NPA
		B	3	0/3 (0%)	
		C	3	0/3 (0%)	
0.3% MUT cells	1	A	3	3/3 (100%)	100% PPA
		B	3	3/3 (100%)	
		C	3	3/3 (100%)	
	2	A	3	3/3 (100%)	100% PPA
		B	3	3/3 (100%)	
		C	3	3/3 (100%)	
	3	A	3	3/3 (100%)	100% PPA
		B	3	3/3 (100%)	
		C	3	3/3 (100%)	
1% MUT cells	1	A	3	3/3 (100%)	100% PPA
		B	3	3/3 (100%)	
		C	3	3/3 (100%)	
	2	A	3	3/3 (100%)	100% PPA
		B	3	3/3 (100%)	
		C	3	3/3 (100%)	
	3	A	3	3/3 (100%)	100% PPA
		B	3	3/3 (100%)	
		C	3	3/3 (100%)	
15% MUT cells	1	A	3	3/3 (100%)	100% PPA
		B	3	3/3 (100%)	
		C	3	3/3 (100%)	
	2	A	3	3/3 (100%)	100% PPA
		B	3	3/3 (100%)	
		C	3	3/3 (100%)	
	3	A	3	3/3 (100%)	100% PPA
		B	3	3/3 (100%)	
		C	3	3/3 (100%)	

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Manufacturer's Address:

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You are receiving this information because you have been diagnosed with aggressive systemic mastocytosis or ASM. This is a rare disorder in which certain blood cells grow in multiple organs.

Certain patterns in the genes of these cells, called biomarkers, may be present that can help your physician in deciding what treatments might be best for you. A sample of your bone marrow can be tested for a specific biomarker called the *KIT* D816V gene mutation. Measuring the presence or absence of this *KIT* D816V gene mutation in bone marrow, along with all other available clinical information, may aid in the assessment of patients diagnosed with ASM for whom Gleevec (imatinib) treatment is being considered.

The *KIT* D816V Mutation Detection by PCR for Gleevec Eligibility in Aggressive Systemic Mastocytosis (ASM) test (referred to as the *KIT* D816V assay) is a “humanitarian use” device test developed by ARUP Laboratories. This test is approved by the U.S. Food and Drug Administration (FDA) as a "humanitarian use" device, which means the effectiveness of this device for this test has not been demonstrated.

What is involved in the testing?

If you choose to take part in this testing, a sample of your bone marrow will be tested with the *KIT* D816V assay at ARUP Laboratories. The test results, along with all other available clinical and laboratory data, will be used to make decisions about your care.

Risks of testing

The assay tests for the presence of a gene mutation (positive result). If the test yields a false-positive result, a patient who is a candidate for Gleevec would be excluded from treatment. If the test yields a false-negative result, a patient who would not be a candidate for Gleevec treatment may be administered Gleevec and therefore be subjected to the associated risks of treatment without the potential for benefit. Because the gene mutation may not be present in every cell, a false-negative test result is possible.

Benefits of testing

The effectiveness of this device has not been demonstrated, however, the detection of *KIT* D816V gene mutation may allow your doctor to have additional information on your eligibility for treatment with Gleevec (imatinib), which is currently approved for treatment of adult patients with ASM and without the *KIT* D816V gene mutation or mutation status unknown.

What are the costs?

The cost of the test may not be reimbursable by your health insurance; therefore, you may be required to pay part or all of the costs associated with this testing. You may want to check with your insurance company to see if this test is covered.

Your alternatives

You may choose not to have this testing. Your decision to have this testing or not will not influence the availability of future medical care and will involve no penalty or loss of benefits to which you are otherwise entitled.

Who to call with questions

If you have any questions or problems during this testing, or if you think that you may have experienced an injury related to this testing, you should contact the doctor who ordered this test for you.

IRB protocol contact name and phone number to be provided (TBD).