SUMMARY OF SAFETY AND PROBABLE BENEFIT (SSPB)

I. GENERAL INFORMATION

Device Generic Name: KIT D816V Assay

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

Device Procode: OWD

Applicant's Name and Address: ARUP Laboratories
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Date(s) of Panel Recommendation: None

Humanitarian Device Exemption (HDE) Number: H140006

Humanitarian Use Device (HUD) Designation Number: HUD # 10-0247

Date of HUD Designation: May 14, 2012

Date of Notice of Approval to Applicant: December 18, 2015

II. INDICATIONS FOR USE

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.

The indication for use statement has been modified from that granted for the HUD designation. The HUD designation was for use in skin or bone marrow biopsies. It was modified for the HDE approval to limit the assay use to fresh bone marrow biopsies.

III. CONTRAINDICATIONS

There are no known contraindications.

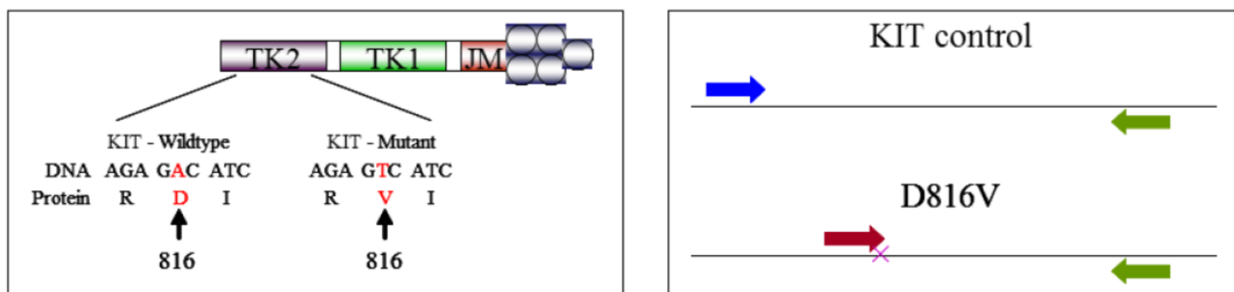
IV. WARNINGS AND PRECAUTIONS

Warnings and precautions relating to procedure and interpretation can be found in the KIT D816V Mutation Detection by PCR for Gleevec Eligibility in Aggressive Systemic Mastocytosis (ASM) labeling.

V. DEVICE DESCRIPTION

Aggressive systemic mastocytosis (ASM) is a rare subset of systemic mastocytosis characterized by the progressive growth of neoplastic mast cells in multiple organs. In >80% of ASM, a somatic A to T missense mutation at position 2447 of the coding sequence of KIT occurs that results in the substitution of aspartic acid (D) to valine (V) at position 816. This mutation is located in the kinase domain of the protein and leads to auto-activation of the KIT receptor tyrosine kinase. The KIT D816V mutation is mainly found in mastocytosis, however it is also sometimes found in gastrointestinal stromal tumors, acute myeloid leukemia, and germ cell tumors. ASM patients **without** the KIT D816V mutation may benefit from treatment with kinase inhibitors such as Gleevec.

Figure 1: The KIT D816V Mutation and the Allele-specific PCR Strategy



The analysis for KIT D816V mutation status is performed on fresh bone marrow specimens by allele-specific PCR using 2 sets of primers in separate PCR reactions. The first set of primers (blue and green in figure above) amplifies a region of exon 17 which serves as a control for both WT and D816V KIT. The second set of primers (red and green in figure above) targets the D816V mutation, forming a different sized amplicon only when the D816V mutation is present.

The device also includes a positive control made up of normal human bone marrow spiked with the HMC-1.2 cell line that contains the KIT D816V mutation. Normal human bone marrow is used as the assay negative control. For each specimen, 2 mutation specific PCR replicates are run along with a single WT PCR reaction. The presence of mutant amplicons at or above the cutoff of 400 relative fluorescence units (RFU) along with WT amplicons at or above the cutoff of 6000 RFU indicates a mutation is present. If the 2 mutant PCR reactions are discordant, the entire assay will be repeated. If the 2 reactions are discordant again, then the assay is reported as “mutant not detected”. If the WT and MUT PCR reactions are below the cut-offs, the reactions are repeated. If they both remain below the cutoff after the repeat, the assay is reported as being “invalid” and no result is returned.

The assay procedure is summarized briefly below:

- Genomic DNA is isolated from white blood cells and quantitated by spectrophotometry.

- A fixed concentration of DNA is utilized for PCR amplification in 2 separate PCR reactions in2 tubes.
    - Tube 1 uses primers that amplify a control sequence to ensure the presence of amplifiable DNA.
    - Tube 2 uses a mutant allele-specific forward primer and the same reverse primer as the control reaction to only amplify D816V mutant *KIT*.

- For each specimen, 2 mutation-specific PCR replicates (tube 2) and 1 wild-type replicate (tube 1) are performed.

- Detection of the PCR products is performed by capillary electrophoresis for high-sensitivity amplicon detection and accurate fragment size determination.

All instruments required to perform this assay are qualified for their use by the laboratory.

VI. ALTERNATIVE PRACTICES AND PROCEDURES

There are no cleared or approved alternatives to the *KIT* D816V Mutation Detection by PCR for Gleevec Eligibility in Aggressive Systemic Mastocytosis (ASM) assay.

VII. MARKETING HISTORY

The *KIT* D816V Mutation Detection by PCR for Gleevec Eligibility in Aggressive Systemic Mastocytosis (ASM) assay has not been marketed in the United States or any foreign country.

VIII. POTENTIAL ADVERSE EFFECTS OF THE DEVICE ON HEALTH

Failure of the assay to perform as expected or failure to correctly interpret test results may lead to incorrect test reporting, and subsequently improper patient management decisions. Patients receiving a false-positive result may be excluded from treatment with Gleevec when they could potentially benefit from treatment. Patients receiving a false-negative result may be treated with Gleevec and be associated with the risks of treatment without the potential for benefit.

IX. SUMMARY OF PRECLINICAL STUDIES

A. Laboratory Studies

1. Accuracy

    a. Objective: In order to demonstrate that the KIT D816V assay could accurately detect the variant of interest, the sponsor compared results of the KIT D816V assay to a Next Generation Sequencing Assay as the comparator method. In order to justify the choice of comparator method, the sponsor additionally provided the independent validation information for the next generation sequencing (NGS) assay (data not shown).

b. Testing: Forty-three blinded, residual genomic DNAs from fresh bone marrow aspirate with known (based on NGS results) KIT D816V status were used. The sample set included 5 KIT D816V positive samples. The acceptance criteria for the study were 95% PPA, 85% NPA, and 90% OPA between the 2 assays. The results of this study are shown below in Table 1.

Table 1: Accuracy Study using Residual DNAs from Clinical Specimens

	Kit D816V Positives (NGS)	Kit D816V Negatives (NGS)	Total
Kit D816V Positives (HDE Assay)	5 (100% PPA)	1	6
Kit D816V Negatives (HDE Assay)	0	37 (97.4% NPA)	37
Total	5	38	43

The study met the pre-determined acceptance criteria with 100% PPA, 97.4% NPA, and 97.7% OPA comparing the 2 assays.

By NGS the 5 positive clinical samples had variant allele frequencies ranging from 3.1% to 41.1%. The sample that was found to be negative by NGS, but positive by the HDE KIT D816V assay underwent manual review of the NGS. It was determined that the sample contained KIT D816 sequence at an allelic frequency below the limit of detection of the NGS assay but within the limit of detection of the HDE KIT D816V assay.

c. Conclusions: These data demonstrate that the HDE KIT D816V assay can accurately detect the variant of interest.

2. Assay Cut-off

a. Objective: In order to determine when an assay can be called mutation positive, a cut-off in terms of relative fluorescence units (RFU) should be determined. Likewise, a cut-off should also be specified for the wild-type (WT) sequence to determine if the assay has performed adequately.

b. Testing: The mutation and WT RFU thresholds were originally established using 173 bone marrow surrogate specimens covering a range of D816V concentrations around the postulated assay limit of detection. The threshold of 400 RFU was determined based on the fact that >95% of the individual PCR replicates and >95% of the surrogate specimens containing D816V were above the threshold, and 0% of the WT specimens would have a determination of mutant detected. Using these same 173 bone marrow specimens, the >6000 RFU threshold for the WT sequence was determined because 100% of the PCR replicates and specimens were >6000 RFU. During design verification a separate set of samples was used

to confirm these thresholds (400 RFU for mutant and 6000 RFU for WT). In these studies 98.9% of wild-type PCR replicates were below the 400 RFU cut-off for mutant detected and 100% were above 6000 RFU cut-off for WT.

c. Conclusions: For each specimen, 2 mutation specific PCR replicates are run along with a single WT PCR reaction. The presence of mutant amplicons at or above the cutoff of 400 RFU along with WT amplicons at or above the cutoff of 6000 RFU indicates a mutation is present.

3. Limit of Detection (LoD) and Limit of Blank (LoB),

a. Objective: The objective of this testing was to establish the detection limits and blank of the KIT D816V assay.

b. Testing:

i. LoD using Surrogate Specimens: Freshly grown HMC-1.2 cells (heterozygous for KIT D816V) were diluted into freshly procured normal bone marrow aspirate. DNA from the cell lines served as a positive control, and DNA from the normal bone marrow served as the negative control. Mutant cell concentrations ranged from 0-15% by dilution.

Twenty samples for each of the following concentrations were examined (0.05%, 0.1%, 0.15%, 0.3%, 1.0%, 2.5%, 5%, 7.5%, and 15%. The definition of ASM is <20% mast cells, therefore it was estimated that 15% would the highest percentage of mutant cells that would be expected to be observed in a specimen. Determination of LoD was based on a point estimate of 95% accurate results defined as 19 out of 20 samples having detectable KIT D816V amplicon in both replicates above the cut-off of 400 RFU. For allelic fractions 0.05%, and 0.15% one sample had discrepant results between the 2 MUT PCR reactions and were repeated in accordance with the pre-specified protocol. Additionally, for allelic fractions 0.05%, 0.15%, and 15%, the capillary electrophoresis reaction failed on one replicate, and so in accordance with the pre-specified protocol the PCR reactions were repeated. Using cell line blends, the results demonstrated 100% correct results after repeat testing supporting the LoD claim of 0.1%. Results are shown in Table 2 below:

Table 2: LoD using Surrogate Specimens

Spiked HMC-1.2 Mutant Cells	Samples	PCR Replicates D816V detected (≥ 400 RFU)	Samples D816V detected (both PCR reps (≥ 400 RFU)	Samples D816V detected (both PCR reps ≥ 400 RFU)
0%	25	1/54[#]	0/25	0%
0.05%	20	42/43*[$]	20/20	100%
0.10%	20	40/40	20/20	100%
0.15%	20	41/42[$]	20/20	100%
0.30%	20	40/40	20/20	100%
1.0%	20	40/40	20/20	100%
2.5%	20	40/40	20/20	100%
7.5%	20	40/40	20/20	100%
15.0%	20	42/43[$]	20/20	100%

[#] Original peak heights discrepant for one sample. Upon repeat PCR per protocol both peaks < 400 RFU.

* Original peak heights discrepant for one sample. Upon repeat PCR per protocol both peaks ≥ 400 RFU.

[$] Sample failed at capillary electrophoresis. PCRs repeated.

The average MUT peak height for the 25 WT samples was 104 RFU with only a single replicate above 40. Linear regression indicated that the assay is not linear with regard to peak height however there is a trend towards higher peak height with higher percent mutant cells (data not shown). There was no evidence of a hook effect (data not shown).

ii. LoD using Mixed Genomic DNA (gDNA) from Cell lines and Bone Marrow: A second study testing LoD was conducted on 20 samples consisting of gDNA from HMC-1.2 cells mixed with gDNA from healthy bone marrow. The following mutant gDNA concentrations were examined, 0%, 0.05%, 0.1%, 0.15%, 0.3%, 1.0%, 2.5%, 5%, 7.5%, and 15%. DNA from the mutant cell line was the positive control and DNA from healthy donors was the negative control. The acceptance criterion was based on 95% of the replicates having detectable mutant above the 400 RFU cut-off. Results confirmed the LoD at 0.1% (highlighted in yellow in Table 3 below). The average RFU for the WT samples was 91 with no replicates above the cut-off. Once again analysis showed that the assay is not linear in regards to RFU peak height and percent mutant DNA. The results are shown in Table 3 below:

Table 3: Limit of Detection using Genomic DNA

Spiked mutant HMC-1.2 gDNA	"Samples" (1 WT, 2 MUT PCR replicates each)	PCR replicates D816V detected (≥400 RFU)	PCR replicates D816V detected
0%	20	0/40	0%
0.05%	20	29/40	72.5%
0.10%	20	39/40	97.5%
0.15%	20	40/40	100%
0.30%	20	40/40	100%
1.0%	20	40/40	100%
2.5%	20	40/40	100%
5.0%	20	40/40	100%
7.5%	20	40/40	100%
15.0%	20	39/39*	100%
100% (POS control)	10	10/10	100%

* No template DNA added to one MUT PCR replicate of one "sample" due to operator error.

One replicate for the 15% samples failed as a result of operator error (no template DNA added). This replicate was not used in the LoD calculation.

iii. Limit of Blank: To demonstrate whether there is any background signal for the mutant DNA in the presence of wild-type DNA only, a LoB study was conducting using 26 normal bone marrow specimens over multiple days. For each specimen, 2 PCR replicates and 1 WT PCR control was tested. These 52 replicates gave a LoB of 277.5 RFU. No RFU above 400 was detected. A LoB study was also conducted on 20 purified gDNAs from normal bone marrow specimens. For each specimen, 2 mutant allele-specific PCR replicates and 1 WT PCR control were performed. These 40 replicates gave a LoB of 55.2 RFU.

c. Conclusions: The LoD for the KIT D816V assay is established at 0.10%.

4. <u>Reproducibility and Precision</u>

a. Objective: This study was designed to assess operator-to-operator, day-to-day, and within-run assay precision.

b. Testing: Three operators ran the assay using 3 batches of 4 surrogate samples, 3 replicates per sample on non-consecutive days across 21 days. The samples were DNA extracted from surrogate samples (healthy bone marrow plus HMC-1.2 cells). Each batch of samples included 3 WT, 0.3% MUT cells, 1% MUT cells, and 15% MUT cells. This design yielded 18 total MUT PCR reactions per operator per sample type. Acceptance criteria were 90% concordance for inter-and intra-operator comparisons. Results met acceptance criteria.

Table 4: Reproducibility and Precision

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%)	

c. Conclusions: The assay is reproducible within- and between-operators at the single site where testing will take place.

5. Reagent Lot-to-lot and Instrument-to-instrument Reproducibility

a. Objective: This study was designed to demonstrate lot-to-lot concordance for the extraction, PCR primer and enzyme, and capillary electrophoresis reagents in addition to the instrument-to-instrument concordance for the thermal cycler and capillary electrophoresis primary and back-up instruments.

b. Testing: Surrogate specimens were used that consisted of HMC-1.2 cells spiked into fresh normal bone marrow. Two samples were evaluated at 0% mutant, 0.3% mutant (3x LoD), and 1.0% mutant (10x LoD). Three lots of reagent were tested. Four thermal cyclers were tested and three capillary electrophoresis instruments were tested. Acceptance criteria were 100% PPA, NPA and OPA for each experimental condition.

Results:

- For the extraction reagent testing there was 100% PPA, NPA, and OPA.
- For PCR primer testing there was 100% PPA, NPA, and OPA, however 1 repeat was performed for discrepant results in the replicates.
- For the PCR enzyme testing there was 100% PPA, NPA, and OPA.
- For the capillary electrophoresis polymer reagent testing there was 100% PPA, NPA, and OPA.
- For PCR thermal cycler testing there was 100% PPA, NPA, and OPA.
- For the capillary electrophoresis instrument testing there was 100% PPA, NPA, and OPA.

c. Conclusions: The assay demonstrated suitable lot-to-lot and instrument-to-instrument reproducibility.

6. Control Validation

a. Objective: The KIT D816V assay requires samples to be run alongside 1 positive control and 1 negative control. Control validation is performed to make sure each new lot of controls demonstrates adequate results.

b. Testing: The negative control is made of gDNA purified from normal bone marrow. The positive control is DNA purified from normal bone marrow spiked with HMC-1.2 cells to a concentration of 0.3%. A lot of controls is

validated by taking 6 representative aliquots of each control type. The samples are tested in duplicate in the mutant PCR and in singlet for the WT PCR reaction. The current control lots are also run alongside the new control lots. Acceptance criteria are 100% PPA, NPA and OPA for the qualitative results. For the negative control the WT peak must be > 6000 RFU and the MUT peak height must be < 400 RFU. For the positive control the MUT peak height must be > 400 RFU.

c. Conclusions: Assay controls serve to ensure that the assay performs adequately.

7. Cross-reactivity of the KIT D816V Assay with other Codon D816 Mutations

a. Objective: The KIT D816V assay is allele-specific PCR specifically designed to amplify the D816V mutation and not amplify the WT sequence. While the mutation is present in >80% of ASM patients, there are other rare substitutions found in the 816 codon. D816H and D816Y are the 2 most common other mutations that may be found at that codon. Therefore, testing was performed to ensure that the assay would not amplify D816H or D816Y.

b. Testing: Residual genomic DNA samples from clinical specimens containing D816H (2 samples) and D816Y (2 samples), as determined by sequencing, were tested in addition to plasmids containing KIT D816H and D816Y.

In regards to D816Y, 1 clinical sample did not contain enough DNA, but neither the other clinical sample nor the plasmid returned a result indicating that D816Y is not amplified by the assay.

In regards to D816H, 1 clinical sample returned a result but the second clinical sample and the plasmid did not. The sponsor then performed NGS on this clinical sample and found the sample to be heterogeneous for D816H, and D816V (data not shown). It was concluded that the D816V cross-contamination allowed the D816H sample to amplify.

c. Conclusions: The KIT D816V assay is specific for a DNA mutation that results in the amino acid valine at position 816.

8. Carry-over/Cross-contamination

a. Objective: This testing was performed to ensure that signal cross-contamination is not observed in the assay.

b. Testing:
   i. Injection-to-Injection Cross-Contamination: In the first experiment, 24 identical replicates containing mutant DNA from HMC-1.2 cells at 10x LoD (represents 1% mutant allele fraction) were run followed by 24 replicates containing NTC. In the second

experiment 24 replicates were analyzed using alternating wells between 10xLoD mutant DNA and NTC. The plate layout along with the injection format is displayed below.

Table 5: Carry-over and Cross-Contamination Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	10X LOD	10X LOD	10X LOD	NTC	NTC	NTC	EMPTY	EMPTY	EMPTY	10X LOD	NTC	10X LOD
B	10X LOD	10X LOD	10X LOD	NTC	NTC	NTC	EMPTY	EMPTY	EMPTY	NTC	10X LOD	NTC
C	10X LOD	10X LOD	10X LOD	NTC	NTC	NTC	EMPTY	EMPTY	EMPTY	10X LOD	NTC	10X LOD
D	10X LOD	10X LOD	10X LOD	NTC	NTC	NTC	EMPTY	EMPTY	EMPTY	NTC	10X LOD	NTC
E	10X LOD	10X LOD	10X LOD	NTC	NTC	NTC	EMPTY	EMPTY	EMPTY	10X LOD	NTC	10X LOD
F	10X LOD	10X LOD	10X LOD	NTC	NTC	NTC	EMPTY	EMPTY	EMPTY	NTC	10X LOD	NTC
G	10X LOD	10X LOD	10X LOD	NTC	NTC	NTC	EMPTY	EMPTY	EMPTY	10X LOD	NTC	10X LOD
H	10X LOD	10X LOD	10X LOD	NTC	NTC	NTC	EMPTY	EMPTY	EMPTY	NTC	10X LOD	NTC
	Injection #1			Injection #2			Injection #3			Injection #4		

Acceptance criteria were 100% PPA or NPA as applicable (expected results). All samples met the acceptance criteria.

ii. Well-to-Well Cross-contamination: A second study to evaluate the possibility of carry over contamination was conducted using samples in a checkerboard pattern with a higher concentration of mutant DNA next to no template controls. A total of 24 gDNA replicates derived from a single bone marrow specimen spiked with gDNA from HMC-1.2 cells to represent an allelic fraction of 15% were run followed by 24 samples containing only PCR master mix (NTC). In the second experiment 48 replicates were analyzed with alternating wells containing 15% mutant DNA cells versus NTC. Acceptance criteria were 100% PPA or NPA as applicable (expected results). All samples met the acceptance criteria.

c. Conclusions: There is no apparent signal carry-over or cross-contamination in the assay format.

9. Assay Robustness

a. Objective: This study was designed to assess performance robustness to variation in critical assay parameters.

b. Testing: The PCR enzyme amount (+/- 0.1 units), PCR annealing temperature (+/- 0.5˚C), electrophoresis injection voltage (+0.2 kV) and electrophoresis injection time (+/- 1 sec) were examined for robustness.

Samples included 2 of each of the following conditions:

- WT genomic DNA (gDNA) derived from normal bone marrow
- gDNA derived from normal bone marrow spiked with 0.3% D816V gDNA from HMC-1.2 cells (3x LoD)
- gDNA derived from normal bone marrow spiked with 1% D816V gDNA from HMC-1.2 cells (10x LoD)

Acceptance criteria are 100% PPA, NPA, and OPA for each variable as compared to the standard assay condition. All of the variables met the acceptance criteria.

c. Conclusions: The KIT D816V assay is robust to minor variability in PCR enzyme amount, annealing temperature, electrophoresis injection voltage, and electrophoresis injection time.

10. Assay Interference

a. Objective: This testing was performed to assess whether the presence of expected interferents (hemoglobin, intralipid, bilirubin, heparin, and excess EDTA) would impact the performance of the assay.

b. Testing: Samples containing 0% or 0.3% HMC-1.2 cells were utilized, and 2 concentrations of each substance were tested first, but then a lower concentration of bilirubin was tested subsequently due to specimen clotting at higher concentrations.

Acceptance criteria were 100% PPA, NPA, and OPA for each interferent compared to no interfering substance included. Results are shown below. All testing met acceptance criteria except for the high and medium concentrations of bilirubin. Bilirubin caused clotting of the sample.

Table 6: Interference Studies

Interfering Substance	Conc	Sample Type	Total Samples	WT Peak ≥6000 RFU	MUT Peak ≥400 RFU	Samples D816V Detected	Comments
None	-	0% MUT cells added	4	4/4	0/8	0/4	no repeats necessary
		0.3% MUT cells added	4	4/4	8/8	4/4	no repeats necessary
Hemoglobin	2 g/L	0% MUT cells added	2	2/2	0/4	0/2	no repeats necessary
		0.3% MUT cells added	2	2/2	4/4	2/2	no repeats necessary
	1 g/L	0% MUT cells added	2	2/2	0/4	0/2	no repeats necessary
		0.3% MUT cells added	2	2/2	4/4	2/2	no repeats necessary
Unconjugated bilirubin	342 µmol/L	0% MUT cells added	2	1/1	0/2	0/1	1 sample lost due to clotting (not evaluable)
			2	2/2	0/4	0/2	repeated samples
		0.3% MUT cells added	2	2/2	4/4	2/2	no repeats necessary
	171 µmol/L	0% MUT cells added	2	2/2	0/4	0/2	no repeats necessary
			2	1/1	0/2	0/1	1 sample lost due to clotting (not evaluable)
		0.3% MUT cells added	2	2/2	4/4	2/2	no repeats necessary

	50 µmol/L	0% MUT cells added	2	2/2	0/4	0/2	no repeats necessary
		0.3% MUT cells added	2	2/2	4/4	2/2	no repeats necessary
	25 µmol/L	0% MUT cells added	2	2/2	0/4	0/2	no repeats necessary
		0.3% MUT cells added	2	2/2	4/4	2/2	no repeats necessary
Intralipid	37 mmol/L	0% MUT cells added	2	2/2	0/4	0/2	no repeats necessary
		0.3% MUT cells added	2	2/2	4/4	2/2	no repeats necessary
	18.5 mmol/L	0% MUT cells added	2	2/2	0/4	0/2	no repeats necessary
		0.3% MUT cells added	2	2/2	4/4	2/2	no repeats necessary
EDTA	3.6 mg/mL	0% MUT cells added	2	2/2	0/4	0/2	no repeats necessary
		0.3% MUT cells added	2	2/2	4/4	2/2	no repeats necessary
	1.8 mg/mL	0% MUT cells added	2	2/2	0/4	0/2	no repeats necessary
		0.3% MUT cells added	2	2/2	4/4	2/2	no repeats necessary

Heparin	30 USP units /mL	0% MUT cells added	2	2/2	0/4	0/2	no repeats necessary
		0.3% MUT cells added	2	2/2	4/4	2/2	no repeats necessary
	15 USP unite s/mL	0% MUT cells added	2	2/2	0/4	0/2	no repeats necessary
		0.3% MUT cells added	2	2/2	4/4	2/2	no repeats necessary

c. Conclusions: Hemoglobin, intralipid, heparin, and excess EDTA do not interfere with assay performance. Excess bilirubin causes the sample to clot which would trigger rejection of the sample at the sample acceptance stage of the assay.

11. Assay Concordance

a. Objective: In order to demonstrate the equivalency between the final assay version and an earlier version of the assay, an assay concordance study was performed between the first assay version to enter validation and the current assay.

b. Testing: In the first concordance study, 43 purified DNA samples from residual clinical specimens were used. The sample set included 6 known mutations positive samples (as determined with the sponsor's validated NGS assay).Acceptance criteria were 95% PPA, NPA, and OPA between the two test versions. The results are shown below.

Table 7: Results of Assay Concordance Study with Clinical Samples

	First Version		
Current Version	**Mutation Detected**	**Mutation Not Detected**	**Total**
Mutation Detected	6	0	6
Mutation Not Detected	0	36	37[1]
No Result	0	0	0
Total	6	36	43

[1] One specimen was invalid by the first test but mutation not detected by the current test.

One sample tested with revision A was invalid and as revision A has no provisions for repeat testing, the sample was deemed "no result".

In the second concordance study a set of 6 surrogate samples consisting of 0%, 0.3%, and 1% KIT D816V DNA were tested with both revisions A and 9 of the assay technical procedure. No acceptance criteria were pre-specified. There was 100% NPA, PPA, and OPA between the 2 assay versions.

c. Conclusions: Modifications made to the first assay did not impact assay performance.

12. Reagent Stability

a. Objective: The objective of these studies was to validate the expiry dating of the assay reagents comprising the KIT D816V assay.

b. Testing:

i. PCR Enzyme Stability (open container): These studies were performed on 2 lots of 500 U tubes of enzyme. One tube for each lot underwent 39 openings and closings with removal of enzyme. The tube was returned to the freezer for 30 min between openings. Tubes were stored at -20˚C (liquid state). The enzyme aliquots removed at the 1, 10, 20, 30, and 39th opening were used to perform the assay. Upon testing, however, 1 of the enzyme lots failed to meet pre-specified cut-offs for the WT peak height at the 39th opening. Therefore, it was decided that enzymes should be aliquoted to 100 U tubes. The study was repeated with a 3rd lot of enzyme in 100 U aliquots. Acceptance criteria were 100% PPA, NPA and OPA for the last opening as compared to the unopened container of enzyme.

ii. PCR Enzyme Stability (shelf-life): These studies are being conducted to confirm the enzyme manufacturer's expiry dating. For each 3 month time point PCR enzyme stability will be assessed using residual DNAs extracted from 0% and 0.3% HMC-1.2 spiked bone marrow specimens (3x LoD). Testing will continue until 1 month past the manufacturer's stated expiry dating and will incorporate 3 lots of enzyme. Acceptance criteria are 100% PPA, NPA, and OPA as compared to the first time point in the study. Testing will continue until November of 2017.

iii. PCR Primer Stability (freeze-thaw): PCR primers are received from the vendor as lyophilized powders that reconstituted into 200 µM stocks, and then diluted to create 2 µM working stocks. The working stocks were used to conduct freeze-thaw studies. For each cycle, the tube of primer was removed from the freezer, thawed at room temperature until liquid (~15 min) and returned to the freezer for a minimum of 1 hour prior to the next cycle. No volume was removed from the tubes. Two lots of primers were included in the testing. Testing used 2 samples from each of the following 3 conditions:

- Bone marrow gDNA (WT)
- Bone marrow gDNA spiked with HMC-1.2 gDNA (to represent 3x LoD; 0.3% mutant fraction)
- Bone marrow gDNA spiked with HMC-1.2 gDNA (to represent 10x LoD; 1% mutant fraction)

Acceptance criteria were 100% PPA, NPA, and OPA as compared to the tubes that had not undergone freeze/thaw. Testing was performed on 0, 5, and 10 freeze-thaw cycles, and all samples met acceptance criteria.

iv. PCR Primer Stability (shelf-life): This study will demonstrate PCR primer stability of diluted primers to 13 months (1 month past desired shelf-life) in 3 month increments. Stability is being assessed in the working primer stock (2 µM). Testing will be performed on a single tube per primer from 2 different primer set lots. Testing will use 2 samples from each of the following 3 conditions:
   - Bone marrow gDNA (WT)
   - Bone marrow gDNA spiked with HMC-1.2 gDNA (to represent 3x LoD; 0.3% mutant fraction)
   - Bone marrow gDNA spiked with HMC-1.2 gDNA (to represent 10x LoD; 1% mutant fraction)

   Acceptance criteria are 100% PPA, NPA, and OPA as compared to time zero. Results from the first 2 time-points are available in the submission. Both time points met acceptance criteria.

v. Control Stability (shelf-life): This testing was designed to assess the PCR control shelf-life stability to 4 months when stored at 2-8˚C. Testing will be conducted at 1-month intervals for 4 months which is 1 month longer than the desired shelf life of the refrigerated controls. Three aliquots of each control type will be tested with 1 WT and 2 MUT PCR reactions per aliquot tested. Acceptance criteria are 100% PPA, NPA, and OPA as compared to time zero. At the time of submission, 2 results are available (time zero and 1 month). Both have passed acceptance criteria.

vi. Capillary Electrophoresis Array Injection Capacity: Ongoing array injection capacity studies are being conducted with identical specimens run at pre-specified injections. Three capillary array lots will be assessed. Samples will be residual DNAs extracted from wild-type (0% mutant) and HMC-1.2-spiked bone marrow specimens at 0.3% and 1.0% allelic frequency. The baseline time point will be injection 160 (the manufacturer's recommended expiry). The array will then be tested at the first available injection after 150, 200, and 300. Where possible, studies will also include the first available injection after 400, 450 and 500 injections. The sponsor will collect these data in the post-market setting. Until

such time as the claim of 500 injections has been demonstrated for 3 lots, the assay will be run with the manufacturer's recommended use of 150 injections. Pre-determined acceptance criteria for this testing are 95% PPA, NPA, and OPA for the assay compared to the baseline.

vii. Capillary Electrophoresis Cathode and Anode Buffer Stability: The manufacturer limits for cathode and anode buffers is 7 days. In order to demonstrate reagent stability at 7 days, a retrospective analysis of buffer dates where surrogate specimens were analyzed was undertaken. A total of 80 samples consisting of the following sample types were included.
    - Bone marrow gDNA (WT)
    - Bone marrow gDNA spiked with HMC-1.2 gDNA (to represent 3x LoD; 0.3% mutant fraction)
    - Bone marrow gDNA spiked with HMC-1.2 gDNA (to represent 10x LoD; 1% mutant fraction)

    Acceptance criteria are 100% PPA, NPA, and OPA between data collected using 7 day old buffer and data collected on the first day of buffer use. The study met its acceptance criteria.

viii. Capillary Electrophoresis Polymer Stability: The manufacturer limits for capillary electrophoresis polymer stability is 7 days. In order to demonstrate reagent stability at 7 days, a retrospective analysis of buffer dates where surrogate specimens were analyzed was undertaken. A total of 80 samples consisting of the following sample types were included.
    - Bone marrow gDNA (WT)
    - Bone marrow gDNA spiked with HMC-1.2 gDNA (to represent 3x LoD; 0.3% mutant fraction)
    - Bone marrow gDNA spiked with HMC-1.2 gDNA (to represent 10x LoD; 1% mutant fraction)

    Acceptance criteria are 100% PPA, NPA, and OPA between data collected using 7 day old capillary electrophoresis polymer and data collected on the first day of polymer use. The study met its acceptance criteria.

ix. Capillary Electrophoresis Injection Plate Stability: This testing was to determine the stability of injection plates containing PCR amplicon, HiDi formamide and size standards when stored in the dark at -20˚C. Eight PCR plates were prepared, and 1 was run immediately (day 0) while the other 7 were frozen and one plate was taken out daily and run. Samples included 2 each of the following.
    - Bone marrow gDNA (WT)
    - Bone marrow gDNA spiked with HMC-1.2 gDNA (to represent 3x LoD; 0.3% mutant fraction)

- Bone marrow gDNA spiked with HMC-1.2 gDNA (to represent 10x LoD; 1% mutant fraction)

Acceptance criteria are 100% PPA, NPA, and OPA between the plate run on day 7and the plate run as soon as it was prepared (day 0). Each of the plates from days 0-6 returned expected results, with 1 mutant sample on day 2 showing discrepant mutant peaks requiring a repeat. However the day 7 plate did not perform as expected, with no WT peaks evident. The sponsor suspects operator error. However, despite the suspicion of operator error, the sponsor states that they have set their limit of plate storage to 6 days at -20˚C. This represents the assay procedure back-up plan if the instrument is inoperable or unavailable.

13. Specimen Stability
    a. Objective: The purpose of this testing was to demonstrate the stability of fresh bone marrow collected in EDTA tubes for this assay.

    b. Testing: ARUP's protocol requires DNA extraction within 5 days of collection if bone marrow is refrigerated. Normal bone marrow, and normal bone marrow spiked with HMC-1.2 cells at 3x LoD were used for this testing. Three aliquots per sample type were prepared and stored at 4˚C. Tubes were prepared out to 7 days following receipt and 8 days following collection. Acceptance criteria were 100% PPA, NPA, and OPA between the latest time point as compared to the samples that were run on day 1. Two samples had to be repeated (1 WT sample from day 7, and 1 MUT sample from day 2) and 1 sample failed due to a failure in DNA extraction.

    c. Conclusions: Fresh bone marrow specimens are stable for 7 days if stored at 4˚C in regards to KIT D816V assay performance.

B. Animal Studies
None

C. Additional Studies
None

X. SUMMARY OF CLINICAL INFORMATION

The testing for the KIT D816V mutation in ASM to determine Gleevec eligibility was supported by 1 open-label, multicenter, phase 2 study testing Gleevec in diverse populations of patients suffering from life-threatening diseases associated with Abl, Kit or PDGFR protein tyrosine kinases (Novartis NDA 21-588, supplement approved October 2006). This study included 5 patients with ASM. The ASM patients were treated with 100 mg to 400 mg of Gleevec daily. These 5 patients ranged from 49 to 74 years of age. In addition to these 5 patients, 10 published case reports and case series were provided that described the use of Gleevec in 23 additional patients with ASM aged 26 to

85 years. These 23 patients also received 100 mg to 400 mg of Gleevec daily.

Two patients had a KIT mutation in the juxtamembrane region (one Phe522Cys and one K509I) and four patients had a D816V c-Kit mutation (not considered sensitive to Gleevec), one with concomitant CML. Of the total population of 28 patients treated for ASM, 8 (29%) achieved a complete hematologic response and 9 (32%) a partial hematologic response (61% overall response rate). A summary of the response rates to Gleevec in ASM is provided below. Response durations of literature patients ranged from 1+ to 30+ months.

Table 8: ASM Response by Cytogenetic Abnormality

Cytogenetic Abnormality	Number of Patients	Complete Hematologic Response N (%)	Partial Hematologic Response N (%)
FIP1L1-PDGFRα Fusion Kinase (or CHIC2 Deletion)	7	7 (100%)	0 (0%)
Juxtamembrane Mutation	2	0 (0%)	2 (100%)
Unknown or No Cytogenetic Abnormality Detected	15	0 (0%)	7 (44%)
D816V Mutation	4	1* (25%)	0 (0%)
Total	28	8 (29%)	9 (32%)

*Patient had concomitant CML and ASM

The Gleevec labeling includes the following warning:
Gleevec has not been shown to be effective in patients with less aggressive forms of systemic mastocytosis (SM). Gleevec is therefore not recommended for use in patients with cutaneous mastocytosis, indolent systemic mastocytosis (smoldering SM or isolated bone marrow mastocytosis), SM with an associated clonal hematological non-mast cell lineage disease, mast cell leukemia, mast cell sarcoma or extracutaneous mastocytoma. Patients that harbor the D816V mutation of c-Kit are not sensitive to Gleevec and should not receive Gleevec.

These data are supportive of the selection of patients without the c-Kit mutation to determine Gleevec eligibility. To date, no clinical studies have been conducted using this device.

XI. FINANCIAL DISCLOSURE

The Financial Disclosure by Clinical Investigators regulation (21 CFR 54) requires applicants who submit a marketing application to include certain information concerning the compensation to, and financial interests and arrangement of any clinical investigator conducting clinical studies covered by the regulation. No new clinical studies were conducted in support of this application, and so no clinical investigator financial information was reviewed.

XII. RISK PROBABLE BENEFIT ANALYSIS

The ARUP *KIT* D816V assay is designed to qualitatively detect the D816V mutation the *KIT* gene and is indicated as an aid in the assessment of ASM patients for whom Gleevec treatment is being considered. Results from the *KIT* D816V assay are intended for use as an adjunct to existing clinical and pathologic information currently used for estimating

prognosis in patients with ASM. Some adverse drug reactions that occurred in >10% of Gleevec treated patients included nausea, vomiting, musculoskeletal and joint pains, rash, diarrhea, headache, and fluid retention. Severe adverse reactions which occurred in <10% of the Gleevec treated patients included elevations in liver function tests, hemorrhage, and severe fluid retention, congestive heart failure and left ventricular dysfunction. In the MDS/MPD patient trial, the adverse reactions regardless of relationship to study drug in > 10% of the patients included nausea, diarrhea, anemia, fatigue, muscle cramp, arthralgia and periorbital edema.

The potential risk to the patient lies in the test yielding either a false positive, a false negative or a delay in treatment (e.g., due to an invalid test result and the need to repeat testing). The assay tests for the presence of the gene mutation – a positive result. If the test yielded a false negative, a patient to whom Gleevec should not be recommended may be administered Gleevec and therefore be subjected to the associated risks of treatment without the potential for benefit. If the test result is invalid and needs to be repeated, a patient who would be eligible for treatment with Gleevec might experience a delay in receiving treatment benefit. However, if the patient was eventually found to be ineligible for Gleevec treatment due to detection of the *KIT* D816V mutation, there would be little or no risk of a delay in receiving treatment due to an invalid test result. Based on the risk assessment provided by the sponsor, the impact to a patient of a false positive result is higher than the impact of a false negative or delayed result, because the patient might be incorrectly denied a potentially effective treatment. There is therefore a potential benefit in knowing *KIT* D816V mutation status in ASM patients so that clinicians can make more informed decisions to improve the overall management of their ASM patients.

The sponsor stated that the incidence rate of all types of systemic mastocytosis is < 300 cases annually, and aggressive systemic mastocytosis represents 10-12% of these cases. Using this incidence rate, one would expect 10-30 cases of ASM annually, making it virtually impossible to generate the clinical data needed to support a PMA in a reasonable amount of time.

Therefore, it is reasonable to conclude that the probable benefit to health from using the device for the target population outweighs the risk of illness or injury, taking into account the probable risks and benefits of currently available devices or alternative forms of treatment when used as indicated in accordance with the directions for use.

XIII. PANEL RECOMMENDATION

This HDE was not taken to a meeting of the Clinical and Molecular Genetics Devices Panel because other marketing applications for PCR assays for similar indications with similar design have been reviewed by the Panel. It was determined, therefore, that the clinical issues raised by this HDE are similar to those previously reviewed.

XIV. CDRH DECISION

CDRH has determined that, based on the data submitted in the HDE, the *KIT* D816V Mutation Detection by PCR for Gleevec Eligibility in Aggressive Systemic Mastocytosis (ASM) assay will not expose patients to an unreasonable or significant risk of illness or injury and the probable benefit to health from using the device outweighs the risks of illness

or injury. CDRH issued an approval order on December 18, 2015.

The requirement for inspection of the applicant's manufacturing facilities prior to approval has been waived.

XV. APPROVAL SPECIFICATIONS

Directions for use: See the device labeling.

Hazards to Health from Use of the Device: See indications, limitations and patient information in the labeling.

Post-approval Requirements and Restrictions: See approval order.