EVALUATION OF AUTOMATIC CLASS III DESIGNATION FOR *ipsogen*® JAK2 RGQ PCR KIT DECISION SUMMARY

A. DEN Number:

DEN160028

B. Purpose for Submission:

De novo request for evaluation of automatic class III designation of the *ipsogen*® JAK2 RGQ PCR Kit

C. Measurand:

Janus Tyrosine Kinase 2 (JAK2) gene mutation G1849T (V617F)

D. Type of Test:

Allele-specific, quantitative, polymerase chain reaction (PCR) using an amplification refractory mutation system (ARMS)

E. Applicant:

QIAGEN

F. Proprietary and Established Names:

Trade Name: QIAGEN *ipsogen* JAK2 RGQ PCR Kit

G. Regulatory Information:

1. Regulation section:

   21 CFR 866.6070

2. Classification:

   Class II (Special Controls)

3. Product code:

   PSU

4. Panel:

   88 − Pathology

H. Indications For Use:

1. Indications for Use:

   The *ipsogen* JAK2 RGQ PCR Kit is a qualitative in vitro diagnostic test for the detection of the JAK2 V617F/G1849T allele in genomic DNA extracted from EDTA whole blood. The *ipsogen* JAK2 RGQ PCR Kit is a real time PCR test performed on the QIAGEN Rotor-Gene Q MDx instrument. The test is intended for use as an adjunct to evaluation of suspected Polycythemia Vera, in conjunction with other clinicopathological factors.

   This test does not detect less common mutations associated with Polycythemia Vera including mutations in exon 12 and is not intended for stand-alone diagnosis of Polycythemia Vera.

   Special conditions for use statement(s):

   For prescription use only.

   For *in vitro* diagnostic use only.

2. Special instrument requirements:

   QIAGEN Rotor-Gene Q MDx platform using Rotor-Gene AssayManager software version 1.0.4 or higher

I. Device Description:

The *ipsogen*® JAK2 RGQ PCR Kit (hereafter also referred to as the JAK2 Kit or JAK2 assay) employs allele-specific, quantitative, polymerase chain reaction (PCR) using an amplification refractory mutation system (ARMS). DNA is extracted from K2-EDTA anti-coagulated whole blood using the QIAsymphony instrument (QSSP) and QIAsymphony® DSP DNA Mini Kit. Purified DNA must be diluted to 10 ng/µl using the TE buffer provided in the JAK2 Kit. Each PCR reaction of the Rotor-Gene Q MDx is optimized for 50 ng of purified gDNA diluted in a final volume of 5 µl. A total of 100 ng per tested sample (50 ng for each reaction) is needed. The Kit contains sufficient reagents to test 24 reactions. Table 1 describes the components of the *ipsogen* JAK2 RGQ PCR Kit.

Table 1. Components of the *ipsogen* JAK2 RGQ Assay

Item	Description	Use
JAK2 Mutant Control	100% V617F allele	Assay Positive Control
JAK2 WT Control	100% WT allele	Assay Negative

		Control
JAK2 MT Quant Standard 1	$5x10^1$ V617F copies in 5 µL	Mutation Standard Curve
JAK2 MT Quant Standard 2	$5x10^2$ V617F copies in 5 µL	Mutation Standard Curve
JAK2 MT Quant Standard 3	$5x10^3$ V617F copies in 5 µL	Mutation Standard Curve
JAK2 MT Quant Standard 4	$5x10^4$ V617F copies in 5 µL	Mutation Standard Curve
JAK2 WT Quant Standard 1	$5x10^1$ wild-type copies in 5 µL	Wild-type Standard Curve
JAK2 WT Quant Standard 2	$5x10^2$ wild-type copies in 5 µL	Wild-type Standard Curve
JAK2 WT Quant Standard 3	$5x10^3$ wild-type copies in 5 µL	Wild-type Standard Curve
JAK2 WT Quant Standard 4	$5x10^4$ wild-type copies in 5 µL	Wild-type Standard Curve
JAK2 MT Reaction Mix	Primers, probes, and necessary components for the mutation-specific and internal control PCR reaction	Mutation Specific PCR Reaction
JAK2 WT Reaction Mix	Primers, probes, and necessary components for the wild-type and internal control PCR reaction	Wild-type Specific PCR Reaction
Taq DNA Polymerase	PCR reaction enzyme	Mutation and Wild-type PCR
TE buffer	Tris-EDTA	Sample Dilution
Nuclease-Free Water	Water	No Template Control
Rotor-Gene AssayManager JAK2 plug-in	JAK2 Assay-specific software	Results Acquisition and Analysis
JAK2 Assay Profile	JAK2 Assay-specific software parameters	Results Acquisition and Analysis

Additional materials required but not provided with the JAK2 RGQ PCR Kit:

- QIAsymphony® DSP DNA Mini Kit
- QIAsymphony Sample Preparation instrument and accessories
- QIAsymphony software version 4.0 that operates the QIAsymphony instrument
- QIAGEN Rotor-Gene Q MDx Platform
- Rotor-Gene AssayManager® software version 1.0.4 or higher that operates the Rotor-Gene Q MDx

J. Substantial Equivalence Information:

1. Predicate device name(s) and DEN number(s):
   Not applicable.

2. Comparison with predicate:
   Not applicable.

K. Standards/Guidance Documents Referenced:

- CLSI EP05-A2: Evaluation of Precision Performance of Quantitative Measurement Methods
- CLSI EP06-A: Evaluation of the Linearity of Quantitative Measurement Procedures. A Statistical Approach
- CLSI EP7-A2: Interference Testing in Clinical Chemistry
- CLSI EP12-A2: User Protocol for Evaluation of Qualitative Test Performance
- CLSI EP15-A2: User Verification of Performance for Precision and Trueness
- CLSI EP17-A2: Protocols for Determination of Limits of Detection and Limits of Quantitation
- CLSI EP25-A: Evaluation of stability of in vitro diagnostic reagents
- CLSI MM13-A: Guidance for the Collection, Transport, Preparation and Storage of Specimens for Molecular Methods

L. Test Principle:

The *ipsogen* JAK2 RGQ PCR Kit uses the qPCR oligonucleotide hydrolysis principle coupled with an Amplification Refractory Mutation System (ARMS) technique for detecting the single base change G1849T in the JAK2 gene on exon 14. This single-nucleotide mutation results in a unique valine (V) to phenylalanine (F) substitution at position 617 of the protein (JH2 domain). DNA is extracted from cells in the K2-EDTA blood sample using an automated instrument and silica-membrane-based nucleic acid purification reagents. A specific concentration of DNA is then added to the JAK2 qPCR reagents.

The JAK2 assay qPCR reaction uses sequence specific forward and reverse primers to hybridize to the specific DNA sequence (target sequence) to amplify it. A DNA allele specific probe, which consists of an oligonucleotide labeled with a 5' reporter dye (FAM) and a downstream, 3' dye-free quencher (BHQ1), also hybridizes to the target sequence between the primers. The hydrolysis probe method exploits the 5'- 3' exonuclease activity of the Taq DNA polymerase. When the probe is intact, the proximity of the reporter dye to the quencher results in suppression of the reporter fluorescence. During PCR, if the target sequence is present, both forward and reverse primers specifically anneal and flank the probe. The 5'-3' exonuclease activity of the DNA polymerase cleaves the probe between the reporter and the quencher displacing the probe fragments from the target, leading to detectable reporter fluorescence. The process is repeated through multiple cycles and the increase in fluorescence signal is directly proportional to the target amplification during PCR. The number of PCR cycles necessary to detect a signal above the threshold is called the Cycle threshold (CT) and is directly proportional to the amount of target present at the beginning of the reaction.

The JAK2 Kit contains standards for the quantification of the JAK2 allele. Mutant (MT) and wild-type (WT) Quant standards are amplified, and a standard curve is generated by plotting the log of the initial template copy number against the CT generated for each standard. Using the equation of this standard curve allows the determination of percent mutant JAK2 copy number in the sample. Results above 1% are “positive” for JAK2 G1849T mutation and results below 1% are “negative” for the mutation.

Data analysis for the JAK2 Kit is done by the Rotor-Gene Assay Manager (RGAM) software associated to the JAK2 Plug-in, no manual analysis is required.

M. Interpretation of Results

The run characteristics on the Rotor-Gene Q MDx Instrument are enclosed in the JAK2 Plug-in and in the AssayProfile. No run settings need to be entered by the user. Based on the Ct values obtained for controls, standards and samples, the RGQ software determines if runs are valid and if individual samples give valid results and identifies which samples contain the V617F mutation. The information from all runs is saved as a result file on the RGQ.

On the RGAM report each sample is assigned with one of the following results:

- Invalid:
  - if one of the curve checks fails
  - if one of the run controls criteria failed
  - or if one of the sample release criteria failed
- Mutation detected:
  - if all curve checks passed
  - if all run controls criteria as well as sample release criteria were met
  - and the Mutant percentage of the sample is above the positivity cut-off of 1%
- No Mutation Detected:
  - if all curve checks passed
  - if all run controls criteria as well as sample release criteria were met
  - and if the Mutant percentage of the sample is below the positivity cut-off of 1%.

N. Performance Characteristics:

* References to the detection of the V617F mutation or V617F positive should be inferred as the detection of the mutant G1849T allele.

1. <u>Analytical Performance:</u>

   a. Precision:

      i. *Within-laboratory Precision:*
      The purpose of this study was to establish the precision of the JAK2 assay under normal conditions of use over a range of mutation levels. The study was designed in accordance with CLSI EP5-A3. Mutation positive PV (MUT) specimens and healthy whole blood (WT) samples were used in this study. Genomic DNA (gDNA) from specimens were pooled to generate sufficient quantity of samples for the various mutation levels. A panel of 11 samples representing a range of percent mutations (%MUT; 0% to 70%) including samples near the clinical cut-off (i.e., 1%) were tested in the study. Two runs per day were performed using 3 JAK2 Kit lots, 3 RGQ instruments by 3 different operators over the course of 27 non-consecutive days. The two runs on each day used the same lot, instrument, and operator. Two replicates were tested per PCR run and per %MUT level. A total of 108 measurements were obtained per sample (3 lots * 3 instruments * 3 operators * 2 runs/day * 2 replicates = 54 runs * 2 replicates = 108 measurements). Each run

included the WT and MUT controls as well as the internal control and NTC control in order to assess the validity of the run controls. Precision was calculated for between-operator, within-run, between-run, between-day, and total imprecision. The min, max, mean, standard deviation, and %CV were calculated for each sample. The results are summarized for each panel member with the mean percent mutation in Table 2 below.

Imprecision increased as the percent mutation decreased. Imprecision at the clinical decision point (1%) ranged from 0 to 27%. The assay has acceptable precision for its intended use.

Table 2. Within Laboratory Precision

% JAK2 MUT Mean (Min-Max)	Between Operator SD, %CV	Between Day SD, %CV	Between Run within Day SD, %CV	Within-run SD, %CV	#Total SD, %CV
72.68 (61.69-88.60)	0.12, 0.16	1.42, 1.96	2.99, 4.11	2.0, 2.75	5.40, 7.43
53.69 (40.32-67.53)	0.43, 0.81	0.00, 0.00	3.24, 6.04	4.12, 7.67	7.06, 13.15
23.13 (14.15-33.19)	0.00, 0.00	0.40, 1.72	1.95, 8.44	1.59, 6.86	4.47, 19.31
11.97 (6.81-17.27)	0.47, 3.95	0.67, 5.62	1.17, 9.75	1.10, 9.18	2.76, 23.03
6.01 (3.38-9.63)	0.16, 2.61	0.24, 4.08	0.63, 10.52	0.70, 11.72	1.56, 25.90
2.39 (1.14-4.23)	0.09, 3.74	0.24, 9.85	0.36, 15.22	0.31, 12.79	0.69, 28.97
1.23 (0.60-2.08)	0.00, 0.00	0.10, 8.21	0.16, 12.70	0.17, 13.74	0.34, 27.18
0.63 (0.22-1.44)	0.04, 6.88	0.07, 10.66	0.12, 19.61	0.13, 20.84	0.24, 37.62
0.13 (0.02-0.42)	0.00, 0.00	0.02, 11.86	0.03, 24.87	0.05, 40.67	0.07, 51.75
0.07 (0.00-0.21)	0.01,17.84	0.00, 0.00	0.02, 30.95	0.03, 48.82	0.04, 65.13
0.01 (0.00-0.048)	0.00, 0.00	0.00, 44.42	0.00, 0.00	0.01, 146.91	0.01, 156.72

ii. Between-instrument and Between Lot

Data to determine the precision between three instruments and between 3 reagent kit lots was assessed in the precision study. The results are shown in Table 3 and demonstrate that the between instrument and between lot reproducibility are acceptable.

Table 3. Between-Instrument and Between-Lot Precision

% JAK2 MUT Mean (Min-Max)	Between Instrument	Between Kit Lot
	SD, %CV	SD, %CV
72.68 (61.69-88.60)	0.00, 0.00	4.62, 6.35
53.69 (40.32-67.53)	0.00, 0.00	5.76, 10.73
23.13 (14.15-33.19)	0.00, 0.00	4.48, 19.37
11.97 (6.81-17.27)	0.00, 0.00	2.57, 21.49
6.01 (3.38-9.63)	0.00, 0.00	1.47, 24.48
2.39 (1.14-4.23)	0.00, 0.00	0.54, 22.57
1.23 (0.60-2.08)	0.00, 0.00	0.27, 21.96
0.63 (0.22-1.44)	0.00, 0.00	0.16, 26.08
0.13 (0.02-0.42)	0.00, 0.00	0.03, 20.20
0.07 (0.00-0.21)	0.01, 11.63	0.02, 30.11
0.01 (0.00-0.048)	0.00, 38.69	0.00, 6.94

iii. Site-to-Site Reproducibility:

The object of this study was to establish the between-site reproducibility of the assay by testing samples at 3 independent test sites. Two studies assessing between site reproducibility were performed. The first study was conducted using cell lines and the second study was conducted using patient specimens.

Study 1: Samples were prepared by mixing healthy whole blood with MUTZ-9 cell lines to create 8 samples with %MUT fraction ranging from 50% to 0%. For each of the 8 MUT levels, 16 aliquots were prepared and 4 were sent to each of the 3 external sties (3 to be tested and 1 for repeat testing as needed). Four test sites (2 in the US, 2 in Europe) participated in this study. Each testing site performed a minimum of 3 gDNA extractions per sample and tested each sample in 8 PCR runs over 4 days for a total of 96 data points per mutation level (4 sites x 3 extractions X 8 runs). Testing used a single lot of DNA extraction kits and a single lot of JAK2 assay. The acceptance criteria for the study were as follows:

- The A260/280 OD should be within 1.7-2.0 and the gDNA concentrations should be $\geq$ 10 ng/µL with a 90% pass rate.
- The variability between sites must be less than that of within-laboratory study.

The study met pre-specified acceptance criteria and the site-to-site reproducibility of the assay is determined to be acceptable. The mean mutation value and the minimum and maximum values observed across the replicates are shown with the SD and %CV results in Table 4 below. One invalid run resulted in the loss of 3 data points for each specimen (n = 96-3). For other samples, additional run or sample processing failures returned invalid results accounting for the missing data. Table 5 displays the qualitative results as a function of the 1% clinical cut-off for each of the data points.

Table 4. Summary of Variant Component Analysis for Reproducibility Study 1

%JAK2 MUT Mean (Min, Max)	n/N Positive/Total Tests	Within Run SD, %CV	Between Run Within Day SD, %CV	Between Day SD, %CV	Between Site SD, %CV	#Total SD, %CV
67.6 (54, 76)	93/93	2.62, 3.87	2.06, 3.05	2.0, 2.96	1.53, 2.26	4.07, 6.02
40.0 (31, 56)	86/86	3.48, 8.70	1.01, 2.53	2.39, 5.97	0.99, 2.46	4.39, 10.96
22. (4, 41)	93/93	3.32, 14.90	1.26, 5.64	1.26, 5.64	0.80, 3.61	3.81, 17.10
8.0 (2, 18)	90/91	1.77, 22.06	0.52, 6.44	0.00, 0.00	0.00, 0.00	1.84, 22.95
4.4 (2, 7)	93/93	0.71, 16.23	0.55, 12.57	0.00, 0.00	0.2, 4.53	0.91, 20.82
2.0 (1, 3)	93/93	0.25, 12.15	0.36, 18.00	0.06, 3.11	0.00, 0.00	0.44, 21.76
1.02% (NA, 2)	25/92	0.10, 8.62	0.06, 4.72	0.21,17.43	0.00, 0.00	0.19,15.64
0% (NA, NA)	0/92	NA	NA	NA	NA	NA

NA = not applicable

Table 5. Qualitative Analysis for Site to Site Reproducibility

	Tested Sample (Mean % mutation)							
JAK2 Status	L1 (67.63%)	L2 (40.03%)	L3 (22.26%)	L4 (8.02%)	L5 (4.35%)	L6 (2.03%)	L7 (1.02%) *	L8 (0%) **
JAK2 positive	93/93	86/86	93/93	90/91	93/93	93/93	25/92	-
JAK2 negative	-	-	-	1/91	-	-	67/92	92/92
Invalid	3	10	3	5	3	3	4	4
****no mean available as all samples are negative for the JAK2 V617F mutation,***								

Study 2: A second 3 site reproducibility study was performed with 6 clinical samples which included 4 JAK2 V617F mutation positive PV clinical samples (the low positive (4%) sample was created by blending two clinical samples) and 2 mutation negative samples. The gDNA extracts were prepared at a single site for the 6 specimens and shipped to each of the 3 testing sites. For each clinical sample, 36 measurements were obtained using 2 operators per site, 2 runs per day per operator over 3 non-consecutive days (n = 3sites X 2operators X 3days X 2 runs). Testing involved one batch of JAK2 Kit and one RGQ instrument per site. Each study sample was assessed with the expected JAK2 V617 status in all three sites. For all 6 specimens, 100% of calls were correctly positive or negative (95% CI, 90.3%, 100%). The data from the study is summarized in the tables below

Results from the variant component analysis for the 3 site reproducibility studies is summarized below in Table 6. The observed variability was lower than that in precision study when comparable mutation levels were measured. The study met pre-specified acceptance criteria and supports the sponsor conclusion that the JAK2 Kit provides reliable and reproducible results on clinical samples spanning the % claimed mutation range.

Table 6. Summary of Variant Component Analysis for Reproducibility Study 2

JAK2 % MUT Mean*	Within Run SD, %CV	Between Run within Day SD, %CV	Between Day SD, %CV	Between Site SD, %CV	#Total SD, %CV
95.19	1.0, 1.04	0.00, 0.00	0.541, 0.57	0.00, 0.00	1.13, 1.19
22.83	3.99,17.47	0.00, 0.00	1.707, 7.48	1.55, 6.80	4.50, 19.72
14.44	2.26,15.63	1.4, 9.68	0.00, 0.00	1.42, 9.84	2.89, 20.01
4.03	0.19, 4.63	0.84, 20.74	0.00, 0.00	0.61, 15.09	0.92, 22.91

*For the 2 negative samples, no % MUT data is reported. These samples are therefore excluded from the variant component analysis presented above. Qualitatively these samples were reported as negative in all testing replicates.

iv. Precision of Controls

Repeatability and reproducibility of controls was assessed in the single site precision studies conducted above. Precision was calculated considering the variation between operators, kits, instruments and days and the results are summarized in Table 7 and 8 below.

Table 7. Precision of the MT and WT controls

	Mutation Percentage							
Sample	**N**	**No of Missing Obs**	**%JAK2 MT Mean**	**Median**	**Standard Deviation**	**Percent CV**	**Minimum**	**Maximum**

Mutant Control	54	0	99.98	99.98	0.02	0.02	99.91	100.00
Wild Type Control	54	0	0.00	0.00	0.00	0.00	0.00	0.00
Legend: JAK2%MT: JAK2 mutation %; N: Number of sample; CV: coefficient of variation.								

Table 8. Precision of the internal controls (HEX channel)

Analysis	Ct Mean	Between Operator SD, %CV	Between Instrument SD, %CV	Between Kit Lot SD, %CV	Between Day SD, %CV	Between Run within Day SD, %CV	Residual SD, %CV	#Total SD, %CV
HEX Mutant Ct	33.09	0.11, 0.32	0.36, 1.08	0.21, 0.63	0.10, 0.31	0.23, 0.69	0.54, 1.63	0.70, 2.11
HEX Wild Type Ct	32.90	0.08, 0.25	0.62, 1.89	0.24, 0.74	0.20, 0.62	0.30 0.91	0.49, 1.48	0.83, 2.53

v. *DNA Extraction Method Reproducibility*

A study was conducted to examine assay variation attributed to the extraction process using different instruments and different extraction kit lots. Ten whole blood samples (8 healthy donor and 2 PV patient) samples were used. The 2 PV samples were pooled and mixed with healthy donor blood to create 8 samples with different mutation allelic frequencies (0.25-39%). This sample set was then distributed into 12 tubes (96 aliquots total). The healthy whole blood was distributed into 196 aliquots. These samples were then extracted on multiple days using 3 different batches of QIAsymphony DSP DNA Mini Kit on 3 QS instruments with 2 replicates per extraction run. A total of 18 extraction runs and 24 PCR runs were performed.

One sample in the control extraction run produced a result of 0.012% MUT, and 1 sample in the test run returned a result of 0.001% MUT which was lower than that observed in the control run; however both observations were much lower than the clinical cut-off of 1%.

b. Linearity/Reportable Range and DNA Input:

The linearity of the JAK2 assay measuring interval (from high mutation percentage to the LoD) was assessed for 5 different DNA input levels. The study was performed using a set of 5 samples with different DNA input levels (30, 20, 10, 5, and 2 ng/µL; assay instructions are to use an input level of 10 ng/µL). For each input level, the sample was diluted to generate 11 different mutation percentages (range 0 to 70% mutation including a sample near the clinical cut-off of 1%). One PV whole blood and one human whole blood were used to generate the test sample sets. The 11 sample dilution series is described in Table 9 below.

Table 9. Dilution series for Linearity study

Sample ID	JAK2 MUT%
S01	**70%**
S02	**46%**
S03	**23%**
S04	**12%**
S05	**6%**
S06	**3%**
S07	**1.5%**
S08	**0.7**
S09	**0.4**
S10	**0.2**
S11	**0**

For each DNA input level 2 runs were performed with 2 replicates per sample (4 data points per %MUT per DNA input). In accordance with CLSI EP6-A, linearity was assessed at each input level using polynomial least-squared regression testing. The results were then assessed against assay precision to conclude whether the test was linear. In addition, the impact of DNA input on mutation status was assessed at the 1% clinical cut-off using both ANOVA and linear regression analysis. At the 1% cut-off the acceptance criterion was that the degree of linearity must not be statistically different from 0.

Regression analysis demonstrated that the assay is linear at DNA inputs of 5, 10, and 20 ng/µL. he assay input amount of 10 ng/µL. The assay is not linear at 2 or 30 ng/ µL. The impact of DNA input amount on mutation status determination was also examined. There was a significant effect when DNA input was 2 ng/µL and %MUT was < 25% and when DNA input was 5 ng/µL and %MUT was <6%. T. The results support the conclusion that JAK2 assay is linear for DNA inputs 5-20 ng/µL over 0-70% MUT. Table 10 indicates the slope, intercept and 95% CI on the data obtained from the linearity assessment.

Table 10. Slope and Intercept of linearity demonstrated with 3 DNA inputs.

DNA concentration (ng/µl)	Slope [95% confidence intervals]	Intercept [95% confidence intervals]
5	1.024 [1.008 ; 1.041]	-0.461 [-0.906 ; -0.017]
10	1.007 [0.991 ; 1.023]	-0.010 [-0.427 ; 0.407]
20	0.988 [0.971 ; 1.006]	0.156 [-0.307 ; 0.619]

Figure 1: 1st order Regression output With All Observations for Mutation Percentage against Sample Percentage by DNA Input (DNA Input = 5)

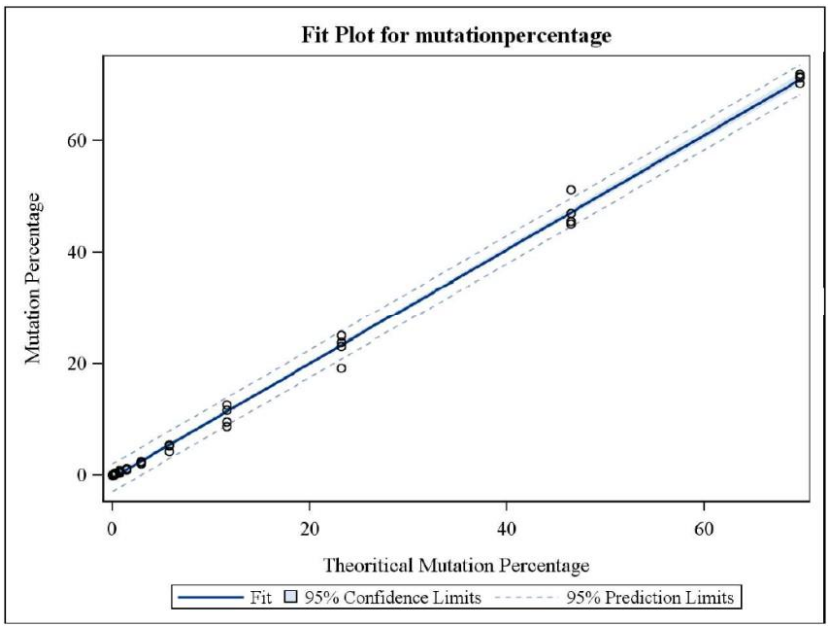


Figure 2: 1st order Regression output With All Observations for Mutation Percentage against Sample Percentage by DNA Input (DNA Input = 10)

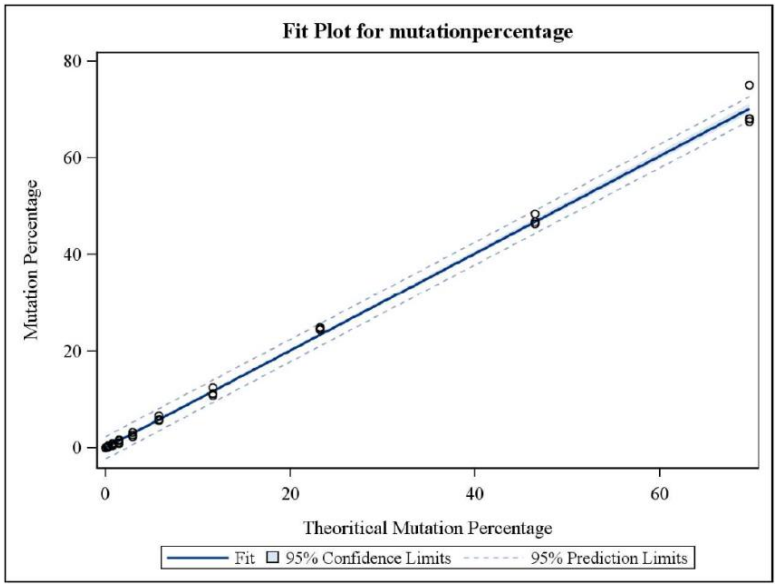
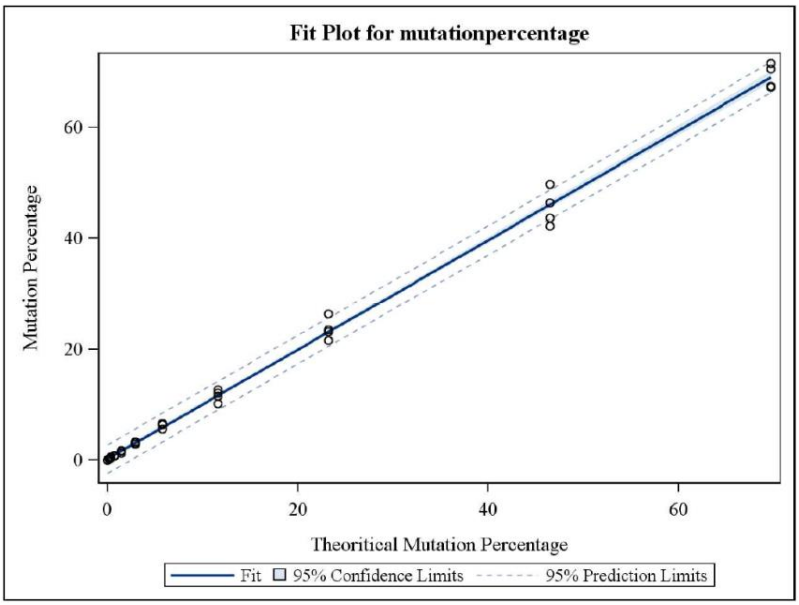


Figure 3: 1st order Regression output With All Observations for Mutation Percentage against Sample Percentage by DNA Input (DNA Input = 20)



c. *Traceability/Calibration:*

The *ipsogen* JAK2 RGQ PCR Kit is not traceable to any known standard. The quality control process for the standards is set to internal primary standards for which an independent orthogonal method is used to confirm the values. The JAK2 Kit uses 4 standards for the generation of a WT standard curve and 4 standards for the generation of the MT curve. The slope and intercept for the wild type and mutant standard curves are determined. The mutant or wild type standard curve equations are used to determine the wild type and mutant copy numbers present in a test sample using the equations below.

$$\log_{10} CN_{WT} = \frac{(C_{TWT} - \text{standard curve intercept}_{WT})}{\text{standard curve slope}_{WT}} \qquad \log_{10} CN_{Mutant} = \frac{(C_{TMutant} - \text{standard curve intercept}_{Mutant})}{\text{standard curve slope}_{Mutant}}$$

From the total wild type and mutant copy numbers a percentage mutation (%MT) is calculated for the specimen.

$$\text{JAK2 V617F (\%)} = \frac{CN_{Mutant}}{(CN_{Mutant} + CN_{WT})} \times 100$$

Linearity of Standards
Linearity for mutant and wild type standards was assessed with data obtained from 192 runs performed in assay validation using which slope and intercept were calculated. Table 11 shows the slope, intercept and correlation.

Table 11. Slope and Intercept for Mutant and Wild Type Standards

Standard	N. of runs	Slope [min-max]	Intercept [min-max]	R^2 [min-max]

Mutant	192	-3.371 [-3.733 ; -3.091]	41.346 [39.632 ; 42.992]	0.998 [0.983 ; 1.000]
WildType	192	-3.367 [-3.703 ; -3.119]	40.114 [38.778 ; 41.805]	0.998 [0.986 ; 1.000]

d. Controls

Positive control: The HEL cell line has been characterized by RT-PCR and sequencing and both methods confirmed the presence of JAK2 mutated allele. With the use of a HEL 0.625 ng/µl gDNA solution as positive control, the collected verification data (Refer to Section 4.0. Pre-clinical studies) showed that the mutant percentage result was close to the target 100%.

Negative control: The K-562 cell line has been characterized as a Wild-Type profile. With the use of a K-562 5 ng/µl gDNA solution as negative control, the collected verification data (refer to Section 3.0 of this direct *de novo* Application) showed that the mutant percentage result was close to the target 0%.

No template control: PCR amplification with the JAK2 Kit is conducted for 45 cycles. Therefore, the acceptance criterion for the No Template Control is that no Ct value should be generated at or below 45. This criterion is applied to both the Mutant and Control Reaction Mixes.

Internal control reaction: The internal control reaction must give a positive result with the No Template Control in each of the Mutation, and the Wild-Type Reaction Mixes for a test run to be considered valid. In test samples, the lack of internal control amplification may indicate the presence of inhibitors or competition for the FAM labeled reactions. A mutation negative result with a failed internal control assay indicates an invalid result and that the test (for that sample) should be repeated. The Ct specification for the internal control reaction is 25 to 37.79 for both reaction mixes.

e. Assay Cut-off:

The *ipsogen* JAK2 RGQ PCR Kit using a clinical cut-off of 1% . Specimens < 1% are considered negative and no value is generated. Specimens ≥1% are considered positive. The assay is not intended to be quantitative. There is currently no consensus on the clinical value of extremely low JAK2 V617F loads, however a 1% mutation load is considered by experts and literature (Tefferi, 2011 and Martinaud, 2010) as a meaningful cut-off for JAK2 V617F positivity.

f. Detection Limit:

i. Limit of Blank (LoB):

Testing was conducted to determine the LoB of the JAK2 assay in accordance with CLSI EP17-A2 (the data point corresponding to the upper 95th percentile of the results obtained with blank (100% WT) samples.

A total of 30 blood samples selected for absence of mutations were tested with the JAK2 assay. Each sample was tested using 3 kit lots, and 4 replicates were included for each sample and lot (120 data points for each lot over 3 days with multiple instruments and operators = 360 data points total). The Shapiro-Wilk test showed that the testing results were not normally distributed. Therefore, LoB was

calculated as specified in CLSI EP17-A2 for non-normally distributed data. All results were below the claimed limit of detection and not reported by the assay. The results support a claim that LoB is below the LoD and therefore not detectable for the JAK2 assay.

ii. *Limit of Detection (LoD):*

The limit of detection of the JAK2 assay for the JAK2 mutation when 10 ng/µL DNA input is used was established in 2 separate studies. In the first study, the LoD was determined by testing 3 positive clinical samples and 3 negative clinical samples using DNA input of 10ng/µL. The samples were used to create a 6 point dilution series in 2-fold increments spanning the estimated LoD (determined to be 0.068% from an initial study assessing serial dilutions of the assay). Three (3) sets of a 6 point dilution series (0.192 to 0.006%) were tested in 20 replicates each in 3 assay kit lots (20 replicates x 3 lots x 3 donor sets = 180 total measurements). Probit analysis of the data as described in CLSI EP17-A2 indicated that the LoD for the JAK2 assay is 0.042% MUT.

In the second study, 2 samples were normalized to the LoD determined in study 1 above. Testing used 30 replicates for each of the 2 samples in order to generate 60 data points for LoD verification. The acceptance criteria of the testing were that the claimed LoD must be less than the clinical cut-off (< 0.1% MUT). The 10^{th} percentile was calculated and determined to be greater than the LoB >90% of the time. Therefore, the JAK2 assay LoD was verified to be 0.042%.

g. *Analytical Specificity:*

i. *Interfering Substances:*

Substances present in both the blood samples of PV patients (table below) as well as substances present in the DNA extraction kit were tested in samples at the LoD for their potential to interfere with the assay performance. The interfering substances used in the study and the concentrations are shown in Table 13 below. The results of the preliminary study demonstrated no interference with the assay with the exception of Proteinase K, which was shown to inhibit the PCR reaction. Therefore Proteinase K was examined in more detail in design verification.

A worst case concentration of Proteinase K was tested through the entire JAK2 assay system following CLSI EP7-A2.

A pool of healthy whole blood and PV whole blood were generated and spiked with Proteinase K or TE prior to extraction (in this instance the TE buffer was serving as a volume control). Two levels of Proteinase K, representing 1.5x and 3x the normal use volume were tested. Testing used 1 lot of assay kit and 4 replicates per sample. The acceptance criteria for this testing were as follows:

- The control pool and control pool + TE (volume control) should not be different.
- The internal control Ct values should be > 38.51.
- The A260/280 should be within 1.7-2.0
- The gDNA concentration for each sample should be ≥ 10 ng/µL

- The % MUT compared between the test and control samples by a 2-sided t-test should not have a p-value < 5%.
- The mutation call for all test samples should be 100% concordant with controls.

The testing met all acceptance criteria. The assay is robust to the effect of potential interfering substances encountered under normal use conditions.

Table 12. Interfering substances tested

Interfering Substance	Tested Concentrations
Busulfan	38.4µg/mL
Citalopram hydrobromide	0.75µg/mL
Paroxetine hydrochloride hemihydrate	1.14µg/mL
Sertraline hydrochloride	0.67µg/mL
Fluoxetine hydrochloride	3.87µg/mL
Acetaminophen	200.7µg/mL
Bilirubin unconjugated	150.3µg/mL
K2 EDTA	3600µg/mL
Hemoglobin (human)	2000µg/mL
Triglycerides	30000µg/mL
Lisinopril dihydrate	0.33µg/mL
Hydroxyurea	28.2µg/mL
Acetylsalicylic acid	651.6µg/mL
Salicylic Acid	0.6µg/mL
Thio-Tepa	48µg/mL
Anagrelide	6µg/mL
Interferon alpha 2b [§]	1.8MU

ii. *Primer Specificity:*

An in silica BLAST alignment of the JAK2 assay kit primers and probes was performed to ensure that no unexpected binding would lead to non-specific amplification. All alignments gave a "no significant similarity found".

a.Specimen Carryover Contamination (Sample Handling):

A study was conducted to demonstrate the absence of within-run cross-contamination during DNA extraction and subsequent PCR testing procedures. Cross-contamination was evaluated by arranging alternate WT samples (0% mutation) with samples MUT samples during extraction and with PCR reaction layout in a checkerboard pattern alternating WT and MUT samples. Specimens from the extraction study were used for the cross-contamination study. The acceptance criteria for cross-contamination analysis were that there is no evidence of cross-contamination greater than the clinical cut-off (1%) by ANOVA. The study established the lack of cross-contamination.

h. *Reagent and Specimen Stability:*

i. *Reagent Stability:*

The purpose of this study was to establish the shelf-life and in-use stability of the JAK2 Kit. Samples were created by mixing WT and PV gDNA to encompass 3 mutation levels (0.3-0.5%, 3-5%, and > 30%). Reference testing for kit stability at time zero was established in 3 runs performed per kit lot for 3 kit lots (9 total) by 3 operators and on 3 instruments. Once the time zero was established kits were stored as required by the study. The following studies were performed:

a. Closed Bottle (Shelf-life) Stability: In this testing, kits were stored at either 12.5±2.5˚C (high temp) or -35±5˚C (low temp) storage conditions and assessed for stability.

b. Open Bottle (In-Use) and Transport Stability (including freeze-thaw): A simulations study to assess the stability of in-use reagents, elevated temperatures and freeze thaw conditions was conducted using the baseline data from the closed-bottle stability study. One (1) lot of assay kit reagents were tested over 2 time periods (12 months and 24 months). Each kit was re-used and tested 3 times per time period (6 kits per time period). Of the 6 kits that are tested per time period 2 are exposed transport simulation 1, 2 to transport simulation 2, and 2 are controls. Transport conditions 1 and 2 are described in Tables 13, and 14 below:

Table 13. Transport Condition 1

Duration	Place	Temperature
1 day (overnight)	Freezer	-15 to -25˚C
3 days	Shipping box	Dry Ice (<70°C)
1 day (8 hours)	Lab	-15 to -25˚C
1 day (overnight)	Freezer	-15 to -25˚C
3 days	Shipping box	Dry Ice
1 day (8 hours)	Lab	-15 to -25˚C
Storage	Freezer	-15 to -25˚C

Table 14. Transport Condition 2

Duration	**Place**	**Temperature**
1 day (overnight)	Freezer	-15 to -25˚C
3 days	Shipping box	Dry Ice (<70°C)
1 day (8 hours)	Lab	-15 to -25˚C
1 day (overnight)	Freezer	-15 to -25˚C
3 days	Shipping box	Dry Ice (<70°C)
1 day (8 hours)	Lab	-15 to -25˚C

1 day (overnight)	Freezer	-15 to -25˚C
3 days	Shipping box	Dry Ice (<70°C)
1 day (8 hours)	Lab	-15 to -25˚C
Storage	Freezer	-15 to -25˚C

The testing included freeze-thaw cycling to ensure the absence of kit degradation and performance in the case of multiple freeze-thaw cycles. Kits were subjected to 0, 3, 4, and 5 freeze-thaw cycles.

The acceptance criteria for these studies were as follows for PV samples –

- 0.5 to 3% sample must be quantified at less than 1%, i.e. mutation not detected.
- 3 to 5% sample must be quantified at greater than 1%, i.e. mutation detected.

The JAK2 Kit is stored at -15 to -30˚C. The JAK2 Kit is shipped on dry ice following warehouse storage at -20±5˚C. All testing met the acceptance criteria and supports a shelf life of (b) (4) months and (b) (4) freeze thaw cycles for the JAK2 Assay kit with the study ongoing.

ii. *Specimen Stability (Whole Blood Stability):*

The objective of this study is to assess the stability of whole blood after being collected in K2 EDTA tubes. The study is designed to establish stability for up to 4 days after collection under 2 different storage conditions (2-8˚C and room temperature) prior to DNA extraction. Ten whole blood samples are collected on Day 1 and shipped for receipt on Day2. Upon receipt, 14 aliquots of the blood are prepared. Two (2) of these are tested for a baseline measurement, and the remaining 12 aliquots are stored for testing at +2/+8°C and room temperature (+25°C). On each successive day till day 5 (96 hrs.) 2 tubes are tested. The study is designed to support a specimen stability claim of 96 hours for whole blood samples in collected in K EDTA tubes which is consistent with stability of this specimen type in literature.

iii. *Specimen Stability (Genomic DNA Stability):*

The objective of this study was to verify the stability of DNA extracted from PV whole blood samples. During storage at -15 to -30˚C and after 4 freeze/thaw cycles: Ten PV whole blood samples collected in EDTA were used for this study. After receipt the samples were stored at room temperature for 4 days and then gDNA was extracted. For each sample, 2 tubes were extracted in 1 run. The 2 extracts were then pooled and divided into 4 tubes and stored at -15 to -30˚C until testing. At each time point the gDNA samples were taken out of the freezer, quantified, normalized and assessed with the JAK2 assay. Testing parameters are within specifications to 18 months and testing will continue to 25 months. Thus the extracted gDNA can be stored up to 15 months with studies ongoing to extend this claim. The study is ongoing to support extracted DNA stability claim of 24 months.

2. Comparison Studies

a. Accuracy Method comparison

To determine the accuracy of the *ipsogen* JAK2 RGQ PCR Assay, specimens were obtained from patients enrolled into the clinical trial (see Section 2 Clinical Performance). A total of 276 specimens were available and evaluated with the JAK2 assay and compared to results obtained with bidirectional Sanger sequencing. Seven (7) specimens were not available for testing because the patients did not complete the study. The range of percent mutation in these specimens based on the JAK2 Assay was 5% to 93%. Only 1 discordant case was observed between the JAK2 Kit and bidirectional sequencing for determination of JAK2V617F mutational status. This subject, who was negative by Sanger, but tested mutation (MUT) positive for JAK2V617F with the *ipsogen* JAK2 RGQ PCR Kit, had a percent mutation (5.6%) below the bidirectional sequencing limit of detection The observed percent positive agreement (PPA) was 100% (71/71 subjects; 95% CI: 94.4%, 100%) and the observed negative percent agreement (NPA) was 99. 5% (204/205 subjects; 95% CI: 97.3%, 100%). The results are shown in Table 15 below.

Table 15. Agreement Between the *ipsogen* JAK2 RGQ PCR Kit and Bi-Directional Sanger Sequencing

***ipsogen* JAK2 RGQ PCR Kit**	**JAK2 V617F Bidirectional Sanger Sequencing**		
	Positive	**Negative**	**Total**
Positive	71	1	72
Negative	0	204	204
Total	71	205	276

b. Matrix comparison:

Not applicable.

3. Clinical Performance:

A study was conducted to establish the clinical performance of the *ipsogen* JAK2 RGQ PCR Kit for detecting JAK2 G1869T/V617F mutation as an aid in the evaluation of patients with suspicion of polycythemia vera (PV) which is a myeloproliferative neoplasm (MPN). Detection of the JAK2 V617F mutation is part of the reference World Health Organization (WHO 2008) and newly updated 2016 criteria for the diagnosis of BCR-ABL-negative MPN, and presence of this mutation is a major criterion for diagnostic confirmation of PV (refer to Table 16 below). The V617F mutation in exon 14 of the JAK2 oncogene is present in greater than 95% of PV patients. Other PV JAK2 mutations are also encountered in exon 12 and represent 3% of the patient population but are not detected by this assay. The remaining 2% of the patient population are exon 14 and exon 12 mutation negative (Wild Type).

Study Design: The clinical performance of the JAK2 Kit in the diagnosis of PV was evaluated during a multicenter, international, prospective, interventional study. The primary objective was to evaluate the specificity and sensitivity of the *ipsogen* JAK2

RGQ PCR Kit with a 1% cutoff for positivity. The reference for JAK2 status determination was the independent assessment of patient status at the clinical site based on the 2008 WHO diagnostic criteria shown in Table 16 below. The secondary objectives were to compare JAK2V617F status determination between the *ipsogen* JAK2 RGQ PCR Kit (1% positivity cutoff) and BDS in a PV population (for positive percentage agreement; PPA) and a non-PV population (for negative percentage agreement; NPA).

Table 16. 2008 WHO criteria for the diagnosis of MPN

Major	1. Hemoglobin (Hgb): • >18.5 g/dl^{-1} (men) or >16.5 g/dl^{-1} (women) or, • Hgb or hematocrit (Hct) >99th percentile of reference range for age, sex, or altitude of residence or, • Hgb >17 g/dl^{-1} (men) or >15 g/dl^{-1} (women) if associated with sustained increase of ≥2 g/dl^{-1} from baseline that cannot be attributed to correction of iron deficiency or Elevated red cell mass >25% above mean normal predicted value. 2. Presence of JAK2 V617F or similar mutation.
Minor	1. Bone marrow trilineage myeloproliferation. 2. Subnormal serum erythropoietin level. 3. Endogenous erythroid colony (EEC) growth.

The study was conducted at 9 study sites in the United States (US; 7 enrolled subjects), 12 study sites in France (all 12 enrolled subjects), and 9 study sites in Italy (5 enrolled subjects). Subjects were screened and selected based on inclusion and exclusion criteria that suggested a diagnosis of PV. All enrolled subjects received blood tests for both the investigational device and the reference test (bidirectional sequencing determination of the JAK2V617F and JAK2 exon 12 status). In order to assure ascertainment of clinical truth, bone marrow from patients was tested by bidirectional sequencing if the blood tests by bi-directional assessment was negative. The inclusion and exclusion criteria for patient enrollment were as follows:

Inclusion Criteria

1. Aged ≥18 years.
2. Suspected of having PV based on at least 1 of the following confirmed biological abnormalities:
    a. Hemoglobin ≥18.5 g/dL for males or ≥16.5 g/dL for females
    b. Hemoglobin or hematocrit in the ≥99th percentile based on a reference range for age, sex, or altitude of residence
    c. Hemoglobin ≥17 g/dL for males or ≥15 g/dL for females if the value was associated with a sustained increase of ≥2 g/dL from baseline and the increase could not be attributed to a correction of iron deficiency
    d. An elevated red cell mass ≥25% above the mean normal predicted value
    e. Confirmation of the biological abnormality should have been obtained within

3 months

3. Willing, able, and committed to participating in baseline and follow-up evaluations for the full duration of the study.
4. Provided written informed consent to participate in the study provided by the subject.

Exclusion Criteria:

1. The subject was previously diagnosed with MPN (e.g., PV, essential thrombocythemia, or primary myelofibrosis). Including secondary polycythemia of any cause
2. The subject had a known JAK2V617F status.

Subject/Specimen Accountability: A total of 286 subjects were consented to the trial. A diagnosis of PV following the WHO criteria was necessary for inclusion. Three (3) were excluded due to screen failure. An additional 67 subjects were not evaluable for the reasons described in Table 18 (some subjects were not evaluable for more than one reason). A total of 216 subjects were evaluable for the study.

Table 17. Reasons for Exclusions in the All Enrolled Population

Reasons	Number of subjects
Failure of inclusion or exclusion criteria	9
Missing EPO results	22
Missing BM biopsy if required for PV diagnosis	26
• Positive JAK2V617F by BDS and Serum EPO within normal limits	15
• Positive JAK2 Exon 12 by BDS and Serum EPO within normal limits	1
• Negative JAK2V617F by BDS and Serum EPO abnormal low	10
Patient did not complete study and/or Missing final diagnosis of PV	15

Analyses: Subjects with clinical features compatible with the diagnosis of PV (including increased hemoglobin and decreased erythropoietin [EPO] levels), but with negative JAK2 V617F and exon 12 determination by BDS, and subjects with positive JAK2 V617F and exon 12 determination by BDS and normal or high EPO levels, were to undergo a bone marrow biopsy with histologic and cytogenetic analysis, as required by the 2008 WHO diagnostic algorithm for myeloproliferative diseases. The final diagnosis (PV or non-PV) was established based on the results of the non-investigational study procedures (i.e., the 2008 WHO algorithm with JAK2 mutation determination using the reference BDS assay). Results of the *ipsogen* JAK2 RGQ PCR Kit using a clinical cut-off of 1% were compared to clinical diagnoses (JAK2 results based on sequencing) and WHO criteria. The results demonstrated that the majority of subjects (94.6%; 53/56 subjects) diagnosed with PV by the investigator were identified as JAK2 V617F positive by the *ipsogen* JAK2 RGQ PCR Kit and the WHO diagnostic algorithm. Similarly, almost all subjects (98.1%; 157/160 subjects) diagnosed as non-PV by the investigator were also V617F negative using the *ipsogen* JAK2 RGQ PCR Kit. The JAK2 Kit gave a diagnosis which disagreed with the

investigator's diagnosis in 3 subjects. One subject had serum EPO levels within the normal range (at 16.5 IU/l) and no JAK2 or exon 12 mutation; however, the subject was diagnosed as having PV according to the investigator's opinion. A second subject had serum EPO levels below the normal range and a JAK2V617F mutation by BDS, but was given a diagnosis of non-PV according to the investigator's opinion. A third subject was JAK2 wild type for BDS and positive using the *ipsogen* JAK2 RGQ PCR. This subject's serum EPO concentration was at the lower limit of normal and no bone marrow biopsy examination was performed. The results are summarized in Table 18 below.

Table 18. Final PV diagnosis based on the Investigator's opinion informed by bi-directional testing and the 2008 World Health Organization algorithm using the *ipsogen* JAK2 RGQ PCR Kit

	Final diagnosis by investigator based on WHO algorithm And JAK2 Asessment by BDS		
Diagnosis based on WHO algorithm combined with JAK2 assessment done by on *ipsogen* JAK2 RGQ PCR Kit	PV N (% of Total)	Non-PV N (% of total)	Total N (% of total)
PV	53 (94.6%)	1 (0.6%)	54 (25.0%)
Non-PV	1 (1.8%)	157 (98.1%)	158 (73.1%)
Inconclusive	2 (3.6%)*	2 (1.3%)†	4 (1.9%)
Total	56	160	216

The mutation status of exon 12 as well as JAK2 V617F was determined by BDS to identify those PV cases missed by the the *ipsogen* JAK2 RGQ PCR Kit. There were 3 exon 12 cases identified in the study. In regard to the missing specimens, comparison of sequencing results to the JAK2 Kit demonstrates agreement for 60 of the missing subjects. Seven (7) subjects did not have sequencing or JAK2 assay results. In the absence of these results the sensitivity and/or specificity described below may be mildly inflated.

Overall, as summarized in Table 19, the sensitivity of the assay was 94.6% (53/56 subjects; CI: 85.1%, 98.8%), indicating that this assay is expected to detect PV in the vast majority of subjects with the disease.
Similarly, the specificity of PV diagnosis using this assay was 98.1% (157/160 subjects; 95% CI: 94.6%, 99.6%), indicating that it is also expected to rule out PV in the vast majority of subjects without PV.

Table 19. Sensitivity and specificity analysis (evaluable population)

Variable	**Estimate**	**Lower 95% Confidence Limit**	**Upper 95% Confidence Limit**
Sensitivity	94.6%	85.1%	98.9%
Specificity	98.1%	94.6%	99.6%

O. Instrument Name:

QIAGEN Rotor-Gene Q MDx platform using Rotor-Gene AssayManager software version 1.0.4 or higher. The instrument was cleared by FDA under K113319 on February 06, 2012.

P. System Descriptions:

1. Modes of Operation:
   QIAGEN Rotor-Gene Q MDx platform using Rotor-Gene AssayManager software version 1.0.4 or higher instrument outputs qualitative results based on the measured fluorescence signals.

2. Software:
   FDA has reviewed the applicant's Hazard Analysis and software development processes for this line of product types:

   Yes __X__ or No _____

3. Calibration and Quality Controls:
   Installation and calibration are performed by the manufacturer. The assay uses standards for generation of a curve by which the % mutation is assessed. The Instrument and assay employ both in-process QC checks and array QC metrics to assist in identifying problems in the assay and instances in which the assay has failed.

Q. Other Supportive Performance Characteristics Data Not Covered in the "Performance Characteristics" Section above:

1. Correlation of PV Clinical Samples with the MUTZ-8 Cell Line:
   The purpose of this development study was to compare the amplification efficiencies of DNA extracted from the MUTZ-8 cell line (containing the JAK2 1849 G>T mutation) and DNA extracted from healthy donor blood (wild type with respect to the JAK2 1849 G>T mutation) with DNA extracted from clinical samples from the Myeloproliferative Neoplasms (MPN) patient population (with Polycythemia Vera (PV) disorder). This comparison was used to establish that the cell line and healthy donor blood are suitable substitutes for clinical samples for the purpose of analytical validation studies.

   A total of 12 clinical PV samples were used (6 positive and 6 negative for the mutation). Each sample was extracted twice for a total of 24 samples. Additionally 6 healthy donor blood samples were collected and extracted twice for a total of 12 samples. Finally the MUTZ-8 cell line was used. MUTZ-8 is 100% mutation positive, so a set of 6 dilutions were prepared.

   Each DNA was diluted to give input copy numbers over the input range of the assay (10-50,000 copies). It was found that it requires twice the concentration of input DNA from MUTZ-8 cells to get the same copy number as MPN sample DNA.

   Each dilution was tested in a single run in triplicate. The results for each dilution series was analyzed using summary statistics (mean, sd, %CV) and linear regression

modeling. Amplification efficiency was evaluated to determine if the MUTZ-8 samples fell within the 2-sided 95% predication interval for a PV JAK2 positive clinical sample. To assess healthy donor blood, amplification efficiency was evaluated to determine if the results fell within the 2-sided 95% predication interval for PV (mutation negative) blood.

Based on the results generated from the statistical analysis of the data produced in this study, limits of the 2-sided 95% prediction intervals for the mean of the amplification efficiencies of the PV positive samples with the MT Assay are 0.824 and 1.051. For the PV negative samples using the WT assay, the 2 sided 95% prediction intervals are 0.559 and 1.306. Based on the calculated mean amplification efficiencies of MUTZ-8 and healthy donor blood DNA is 0.846 and 0.914 respectively. These values fall within the stated 2 sided 95% prediction interval of the PV samples of the same mutation status. The mean amplification efficiency for the MUTZ-8 cell line DNA is at the low end of the 2 sided 95% prediction intervals range, however this was considered acceptable. DNA extracted from normal healthy donors may supplement analytical validation studies

2. Guard-band/Robustness Studies:
The objective of the guard-band studies was to determine the robustness of the JAK2 assay to common variables introduced to the system by equipment or users.

a) Temperature Fluctuations: The objective of this study was to determine the effect on results caused by temperature fluctuations. This was achieved by changing the activation, denaturation, and annealing/elongation temperatures by ±2˚C in 1˚C increments.

A two factor (activation/denaturation and annealing/elongation), 5 level design was used to assess the impact of temperature variations on Ct values. Experiments were conducted across 3 days using MUTZ-8 cell line samples diluted into WT DNA extracted from healthy donor blood samples. Samples were created at 0%, 0.5%, 5%, and 50% allelic frequency. The acceptance are shown in Table 20:

Table 20. Percentage Mutation Acceptance Criteria Cycling Tolerance Experiments

Sample Used	Accepted %Mutation Range
0%	≤ 0.001%
0.5%	0.25 to 1%
5 %	2.62 to 6.33%
50%	36.94 to 55.38%

Results demonstrated an overall trend of decreasing percentage mutation found with increasing denaturation/activation temperatures, however the results within ±

1˚C were acceptable. The effect of annealing temperature on percent mutation values was insignificant across the variables tested. Therefore the JAK2 assay is robust to temperature fluctuations within the expected range for the RGQ platform (± 1˚C) from standard conditions across allelic frequencies.

b) Whole blood extraction volume limits (recommended volume 300 µL): The objective of this study was to assess the input volume limits from 190 to 300 µL. Whole blood from 2 donors was added to extraction tubes and 3 extraction experiments were conducted for each. The limit of input volume to avoid a QIAsymphony pipetting error is > 265 µL. No failure was observed for volumes > 265 µL in the studies. Therefore the sponsors conclude that the JAK2 assay is validated at 300 µL and performs adequately at volumes > 265 µL.

c) Robustness: Robustness studies tested the performance of the assay over multiple conditions. The 4 components analyzed were the Taq DNA Polymerase, PCR buffer, $MgCl_2$, and dNTPs. Ct values, total copy number and % mutant detected were the 3 outputs analyzed in these studies. Each component was tested at high, medium, and low concentrations. The samples tested contained 0.5%, 2%, and 20% mutant DNA.

R. Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Parts 801 and 809 and the special controls for this device type.

S. Patient Perspectives:

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

T. Identified Risks and Required Mitigations:

Identified Risks to Health	Required Mitigations (See Section V below for Special Controls)
False negative results	Special Controls (1) and (2)
False positive results	Special Controls (1) and (2)

U. Benefit/Risk Analysis:

Summary	
Summary of the Benefit(s)	This kit has significant benefit as an adjunct to the physician's evaluation of suspected Polycythemia Vera as demonstrated in the analytical and clinical performance studies.

Summary of the Risk(s)	There are risks associated with false negative and false positive results. However, the risks associated with these misclassification have to be taken into the context that the test is intended to be used as an adjunct to diagnosis in patients suspected of having PV along with additional clinicopathological factors and is not intended to be used as a standalone diagnostic. False negative: There is a possibility that an individual with a very low but positive JAK2 V617F mutation would be assigned a negative test result. In this scenario, it would still be possible that the patient be correctly diagnosed with PV, because the presence of the JAK2 V617F mutation is not required for diagnosis. The risk of this type of false negative result is mitigated by the analytical accuracy of the device; furthermore, this test is not the sole determinant of diagnosis. The risk of a false negative is mitigated also because the device is intended to be used as an adjunct to diagnosis along with other clinicopathological factors and not as a standalone diagnostic. There is also a risk that the test fails to detect another JAK2 mutation. However, this possibility has been addressed in the labeling which states that this test does not detect less common mutations associated with Polycythemia Vera including mutations in exon 12. False positive: There is a possibility that the test spuriously reports a sample negative for a JAK2 V617F mutation as positive. In the clinical study, 157 patients had a final WHO diagnosis as non-PV based on the *ipsogen* JAK2 RGQ PCR test out of 158 that had a non-PV WHO diagnosis based on bi-directional Sanger. The 1 discordant patient was actually also positive by bi-directional Sanger. Thus, the risk of a false positive in the case of the *ipsogen* assay is mitigated by the high degree of specificity of the assay.
Summary of Other Factors	The ordering physician should be aware that this test does not pick up less common mutations associated with Polycythemia Vera including mutations in exon 12. This information is included in the intended use statement.
Conclusions **Do the probable benefits outweigh the probable risks?**	The probable clinical benefits of this device, which allow for qualitative ascertainment of JAK2 V617F mutational status, outweigh the potential risks in light in light of the listed special controls and applicable general controls, including design controls.

V. Conclusion:

The information provided in this *de novo* submission is sufficient to classify this device into class II under regulation 21 CFR 866.6070. FDA believes that the stated special controls, and applicable general controls, including design controls, provide reasonable assurance of the safety and effectiveness of the device type. The device is classified under the following:

Product Code:	PSU
Device Type:	Mutation detection test for myeloproliferative neoplasms
Class:	II (special controls)
Regulation:	21 CFR 866.6070

(a) *Identification*. A mutation detection test for myeloproliferative neoplasms is an in vitro diagnostic device intended for the detection of the JAK2 V617F/G1849T allele in genomic DNA extracted from whole blood. The test is intended for use as an adjunct to evaluation of suspected Polycythemia Vera, in conjunction with other clinicopathological factors.

(b) *Classification.* Class II (special controls). A mutation detection test for myeloproliferative neoplasms must comply with the following special controls:

1) Premarket notification submissions must include the following:
    i. The indication for use must indicate the variant(s) for which the assay was designed and validated, for example JAK2 G1849T.
    ii. A detailed description of all components in the test, including the following:
        (A) A detailed description of the test components, all required reagents, instrumentation and equipment, including illustrations or photographs of non-standard equipment or methods.
        (B) Detailed documentation of the device software including, but not limited to, standalone software applications and hardware-based devices that incorporate software.
        (C) A detailed description of methodology and assay procedures including appropriate internal and external quality controls that are recommended or provided. The description must identify those control elements that are incorporated into the testing procedure.
        (D) A detailed specification for sample collection, processing, and storage.

(E) A description of the criteria for test result interpretation and reporting including result outputs, analytical sensitivity of the assay, and the values that will be reported.

iii. Information that demonstrates the performance characteristics of the test, including:

(A) For indications for use based on a threshold established in a predicate device of this generic type, device performance data from either a method comparison study to the predicate device or through a clinical study demonstrating clinical validity using well-characterized prospectively or retrospectively obtained clinical specimens, as appropriate, representative of the intended use population.

(B)For indications for use based on a threshold not established in a predicate device of this generic type, device performance data from a clinical study demonstrating clinical validity using well characterized prospectively or retrospectively obtained clinical specimens, as appropriate, representative of the intended use population.

(C) Device reproducibility data generated, using a minimum of three sites, of which at least two sites must be external sites, with two operators at each site. Each site must conduct study that includes at least 2 operators per site, 2 runs per operator per day over a minimum of 3 non-consecutive days evaluating a sample panel that contains allelic frequencies that span the claimed measuring range, and include the clinical threshold allelic frequency. Pre-specified acceptance criteria must be provided and followed.

(D) Information on device traceability and a description of the value assignment process for calibrators and controls.

(E) Device precision data using clinical samples and controls to evaluate the within-lot, between-lot, within-run, between run, and total variation.

(F) Device linearity data generated from samples covering the device measuring range and for any standards used in the quantitation of allelic frequencies.

(G) Device analytic sensitivity data including limit of blank and limit of

detection.

(H) Device specificity data, including interference and cross-contamination.

(I) Device and clinical specimen stability data, including real-time stability (long term storage and in-use stability) and stability evaluating various storage times, temperatures, and freeze-thaw conditions, as appropriate.

iv. Identification of risk mitigation elements used by the device, including a detailed description of all additional procedures, methods, and practices incorporated into the instructions for use that mitigate risks associated with testing using the device.

2) The 21 CFR 809.10(b) compliant labeling must include a detailed description of the performance studies conducted to comply with section b.1.iii and a summary of the results.