510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:

K172287

B. Purpose for Submission:

Expansion of the Indications for Use.

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

3. Product code:

   PSU

4. Panel:
   88 – Pathology

H. Intended Use:

1. Intended use(s):
   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 myeloproliferative neoplasms, in conjunction with other clinicopathological factors.

   This test does not detect less common JAK2 mutations associated with Myeloproliferative Neoplasms including mutations in exon 12 and is not intended for stand-alone diagnosis of Myeloproliferative Neoplasm.

2. Indication(s) for use:
   Same as Intended use

3. Special conditions for use statement(s):

   For prescription use only.
   For *in vitro* diagnostic use only.

4. Special instrument requirements:

   QIAGEN Rotor-Gene Q MDx platform using Rotor-Gene AssayManager software version 2.1.

I. Device Description:

The *ipsogen* JAK2 RGQ PCR Kit 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 2.1 that operates the Rotor Gene Q MDx

J. Substantial Equivalence Information:

1. Predicate device name(s):
   ipsogen® JAK2 RGQ PCR Kit

2. Predicate 510(k) number(s):
   DEN160028

3. Comparison with predicate:

Characteristic	Device	Predicate
Similarities		
Intended 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.	Same
Specimen Type	Genomic DNA extracted from EDTA whole blood	Same
Assay Targets	JAK2 V617F/G1849T allele	Same
Genomic DNA Extraction	DNA should be extracted using the QIAsymphony SP instrument in combination with the QIAsymphony DSP DNA Mini Kit	Same
Amplification and Detection Technology	Real-time PCR DNA amplification	Same
Amplification and Detection Instrument System	Assay uses the Rotor-Gene Q MDx	Same
Assay Controls	Positive Control, Negative Control and Internal Control included in the kit	Same

Characteristic	Device	Predicate
Differences		
Indications for Use	The test is intended for use as an adjunct to evaluation of suspected Myeloproliferative	The test is intended for use as an adjunct to evaluation of suspected Polycythemia Vera,

	Neoplasms, in conjunction with other clinicopathological factors. This test does not detect less common JAK2 mutations associated with Myeloproliferative Neoplasms including mutations in exon 12 and is not intended for stand-alone diagnosis of Myeloproliferative Neoplasms.	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.
Amplification and Detection Instrument System Software	Rotor-Gene AssayManager® software version 2.1	Rotor-Gene AssayManager® software version 1.04

K. Standard/Guidance Document Referenced (if applicable):
CLSI EP25-A: Evaluation of stability of in vitro diagnostic reagents

L. Test Principle:
Refer to DEN160028.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

Overall analytical performance of the QIAGEN *ipsogen*® JAK2 RGQ PCR Kit was previously demonstrated using specimens from patients with suspected polycythemia vera (PV). Refer to decision summary for DEN160028 available at https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpmn/denovo.cfm?ID=DEN160028 for a description of the following analytical performance studies:

- *Repeatability/Reproducibility/Lot-to-Lot*
- *Linearity/assay reportable range and DNA input*
- *Traceability/Calibration*
- *Detection limit (Limit of Blank and Limit o Detection)*
- *Analytical specificity (including Interfering Substances)*
- *Reagent Stability*
- *Extracted DNA stability*

Analytical performance studies provided to support expansion of the indications for use claim to include additional JAK2 positive myeloproliferative neoplasms included accuracy study using specimens from essential thrombocytopenia (ET) and primary

myelofibrosis (PMF) patients, and specimen stability study. The studies are described below.

a. Accuracy study – Comparison to a Reference Method

A study was conducted to demonstrate the accuracy for detecting JAK2 V617F/G1849T allele in clinical specimens from patients representing the target population. Because the original study included suspected MPNs, specimens in this study were selected based on diagnosis which included both JAK2 positive and negative specimens. A total of 197 specimens (98 ET and 99 PMF) was tested with the *ipsogen* JAK2 RGQ PCR Kit and compared with validated bi-directional Sanger Sequencing (BDS).

All specimens were concordant except for 9 ET and 5 PMF samples, all of which were JAK2 V617F positive with the *ipsogen* JAK2 RGQ PCR Kit and negative with BDS. Concordance between the two methods is shown in Table 2 for ET specimens and Table 3 for PMF specimens.

Table 2. Concordance table between *ipsogen* JAK2 RGQ PCR Kit and Sanger Bidirectional Sequencing in ET population

		Sanger bi-directional sequencing		
		JAK2 V617F positive	JAK2 V617F negative	Total
ipsogen JAK2 RGQ PCR Kit	JAK2 V617F positive	43	9	52
	JAK2 V617F negative	0	46	46
	Total	43	55	98

The overall agreement is 90.8% (89/98 subjects; 95% CI: [83.3% – 95.7%]), the positive agreement is 100% (43/43 subjects; 95% CI: [91.8% – 100%]), and the negative agreement is 83.6% (46/55 subjects; 95% CI: [71.2% – 92.2%]).

Table 3. Concordance table between *ipsogen* JAK2 RGQ PCR Kit and Sanger Bidirectional Sequencing in PMF population

		Sanger Bi-directional sequencing		
		JAK2 V617F positive	JAK2 V617F negative	Total
ipsogen JAK2 RGQ PCR Kit	JAK2 V617F positive	51	5	56
	JAK2 V617F negative	0	43	43
	Total	51	48	99

The overall agreement is 94.9% (94/99 subjects; 95% CI: [88.6% – 98.3%]), the positive agreement is 100% (51/51 subjects; 95% CI: [93.0% – 100%]), and the negative agreement is 89.6% (43/48 subjects; 95% CI: [77.3% – 96.5%]).

Previously in DEN160028, a clinical study was conducted in which 276 PV samples were evaluated with the *ipsogen* JAK2 RGQ PCR Kit and the results were compared to results obtained with BDS. One PV sample was found JAK2 V617F positive with the *ipsogen* JAK2 RGQ PCR Kit and negative with BDS. Concordance between the two methods is shown in Table 4 for PV specimens. The overall agreement is 99.6% (275/276 subjects; 95% CI: [98.0% – 100%]), the positive agreement is 100% (71/71 subjects; 95% CI: [94.9% – 100%]), and the negative agreement is 99.5% (204/205 subjects; 95% CI: [97.3% – 100%]).

Table 4. Concordance table between *ipsogen* JAK2 RGQ PCR Kit and Sanger Bidirectional Sequencing in PV population

		Sanger Bi-directional sequencing		
		JAK2 V617F positive	JAK2 V617F negative	Total
ipsogen JAK2 RGQ PCR Kit	JAK2 V617F positive	71	1	72
	JAK2 V617F negative	0	204	204
	Total	71	205	276

Accuracy with all MPN specimens evaluated combined

The overall accuracy of the test in MPN specimens was assessed by combining the

data obtained from each specimen cohort. A total of 473 MPN specimens were evaluated and a total of 15 samples were discordant. The overall agreement is 96.8% (458/473 subjects; 95% CI: [94.8%; 98.2%]). The positive agreement was 100% (165/165 subjects; 95%CI: [97.8%; 100%] and the negative agreement was 95.1 % (293/308 subjects; 95% CI: [92.1%; 97.2 %]). The results are shown in Table 5.

Table 5. Concordance table between *ipsogen* JAK2 RGQ PCR Kit and Sanger Bidirectional Sequencing in MPN population (combined ET, PMF and PV populations)

		Sanger bi-directional sequencing		
		JAK2 V617F positive	JAK2 V617F negative	Total
ipsogen JAK2 RGQ PCR Kit	JAK2 V617F positive	165	15	180
	JAK2 V617F negative	0	293	293
	Total	165	308	473

The specimens yielding discordant results appeared to have mutation levels below the BDS detection capability (around 10%). Because Sanger sequencing is not as sensitive as the JAK2 assay, and the JAK2 assay sensitivity is 1%, a separate study was conducted using a validated next generation sequencing (NGS) method to detect JAK2 V617F allele in the 15 discordant samples (9 ET, 5 PMF, and 1 PV), as well as a randomly selected set of 22 JAK2 V617F concordant specimens (11 positive and 11 negative specimens). All 15 discordants tested positive by NGS, agreeing with the *ipsogen* JAK2 RGQ PCR Kit. All concordant samples tested the same with NGS.

b. Whole blood stability

A specimen stability was conducted with 6 PV specimens (3 JAK2 V617F positive and 3 JAK2 V617F negative samples in K2-EDTA tubes) collected and stored at 4°C or room temperature (RT) before proceeding to gDNA extraction. Specimen stability was tested by assessing the agreement between qualitative results, and separately, for trends in changes in the percent mutation measured with the JAK2 Kit at each time point (i.e., baseline, day 2, 3, 4 and day 4 + 2h) and storage condition (RT or 2-8°C). At each extraction, 3 aliquots per sample were extracted using QIAsymphony. The results passed acceptance criteria and support the claim that whole blood can be stored at RT and 2-8°C for 96 hours before being processed for gDNA extraction to assess the JAK2 V617F status with the JAK2 Kit.

2. Comparison studies:

   a. Method comparison with predicate device:

   Not applicable.

   b. Matrix comparison:

   Not applicable.

3. Clinical studies:

   Not applicable.

4. Clinical cut-off**:**

   Specimens < 1% are considered negative and no value is generated. Specimens ≥1% are considered positive. The assay reports the JAK2 percentage because the additional information on potential mutant allele burden enhances diagnostic evaluation, however the assay is not intended for quantitative use. 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 reporting JAK2 V617F positivity.

5. Expected values/Reference range:

   The assay is not intended for quantitative use. Low JAK2 allele has been detected in subjects without MPNs. .

N. Instrument Name:

QIAGEN Rotor-Gene Q MDx platform using Rotor-Gene AssayManager software version 2.1. The instrument was cleared by FDA under K113319 on February 06, 2012.

O. System Descriptions:

1. Modes of Operation:

   Does the applicant's device contain the ability to transmit data to a computer, webserver, or mobile device?

   Yes ____X____ or No _____

   Does the applicant's device transmit data to a computer, webserver, or mobile device using wireless transmission?

   Yes _____ or No ___X_____

2. Software:

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

   Yes ___X____ or No _____

3. Specimen Identification:
   Whole blood.

4. Specimen Sampling and Handling:
   4C or RT for 96 hours.

5. Calibration and Quality Control:
   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.

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

None.

Q. Proposed 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.

R. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.