**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION**
**DECISION SUMMARY**
**ASSAY ONLY TEMPLATE**

**A. 510(k) Number:**

k072264

**B. Purpose for Submission:**

new device

**C. Measurand:**

human chorionic gonadotropin (hCG)

**D. Type of Test:**

quantitative chemiluminescent assay

**E. Applicant:**

Qualigen Inc.

**F. Proprietary and Established Names:**

Qualigen Fastpack® hCG Immunoassay

**G. Regulatory Information:**

1. Regulation section:

   21CFR862.1155, Human Chorionic Gonadotropin

   21CFR862.1150, Calibrator

2. Classification:

   Class II

3. Product code:

   JHI and JIT, respectively

4. Panel:

   Chemistry (75)

## H. Intended Use:
1. Intended use(s):
   The Fastpack® hCG Immunoassay is a paramagnetic particle immunoassay for the in vitro quantitative determination of hCG in human serum or EDTA or lithium-heparin plasma.  The assay is designed for use with the Fastpack® System and is indicated for the early detection of pregnancy.

2. Indication(s) for use:
   See intended use above.

3. Special conditions for use statement(s):
   For prescription use.

4. Special instrument requirements:
   The assay is designed for use with the Fastpack® System.

## I. Device Description:

Each Fastpack® contains biotin mouse monoclonal anti-hCG antibody bound to streptavidin-coated paramagnetic particles; hCG antibody solution; wash buffer and substrate (indoxyl-3-phosphate and lucigenin) in buffer with preservatives.

## J. Substantial Equivalence Information:

1. Predicate device name(s):

   Beckman Coulter Access hCG

2. Predicate K number(s):

   k023480, k980173

3. Comparison with predicate:

   This assay has a similar intended use to the predicate and uses a similar methodology (chemiluminescence).  Specific reagents and antibodies differ.  The predicate device is for use on the Access® System; this device is for use on the Fastpack® System.

## K. Standard/Guidance Document Referenced:
- *Evaluation of Precision Performance of Quantitative Measurement Methods, EP5-A2*

- *Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach,* EP6-A
- *Method Comparison and Bias Estimation Using Patient Samples,* EP9-A; *Protocols for Determination of Limits of Detection and Limits of Quantitation,* EP17-A

**L. Test Principle:**

The test is a chemiluminescence "sandwich" assay. A monoclonal anti-hCG antibody labeled with alkaline phosphatase reacts with hCG in a sample. A second monoclonal anti-hCG antibody, covalently couple to biotin and pre-bound to streptavidin-coated paramagnetic particles is added. Particles are washed to remove unbound materials. Chemiluminogenic substrate is added, resulting in chemiluminescence measured by the Fastpack® System. The amount of labeled antibody is directly proportional to the concentration of hCG in the measured sample.

**M. Performance Characteristics (if/when applicable):**

1. Analytical performance:

   a. Precision/Reproducibility:

   Imprecision evaluations were performed using serum-based control materials. Each control was tested in duplicate for 10 days. Two reagent lots (40 replicates each) and 3 instruments (1 instrument used for each lot, plus one in common to both lots) were used in testing. There were 80 replicate measurements for each control. Data was analyzed using ANOVA. Results are shown below.

   |  | **Control 1** | **Control 2** | **Control 3** | **Control 4** |
   | --- | --- | --- | --- | --- |
   | **Mean hCG (mIU/mL)** | 3.4 | 19.0 | 347.3 | 722.3 |
   | **SD within-assay** | 0.37 | 1.8 | 21.5 | 73.7 |
   | **CV within-assay** | 10.8 | 9.4 | 6.2 | 10.2 |
   | **SD within-assay** | 0.5 | 2.2 | 27.1 | 98.3 |
   | **CV within-assay** | 14.5 | 11.4 | 7.8 | 13.6 |

   b. *Linearity/assay reportable range:*

   The reportable range is 1.8-1000 mIU/mL. The evaluations described in this section, together with the sensitivity evaluations (see Sensitivity Section, below) support this range.

A linearity evaluation was performed according to CLSI document EP-6A to assess the range of linearity. A single pool with an endogenous concentration of 940.6 mIU/mL was diluted to the concentrations shown below. Differences between expected and measured values were all within 10%. Evaluation based on the EP-6A guideline resulted in less than 5 mIU/mL deviation between a linear fit and a higher order fit in the concentration range 0-50 mIU/mL, and less than 25 mIU/mL deviation in the range 50-1000 mIU/mL. A table of expected and measured values from this evaluation is shown below.

| Percent of original concentration | Expected value (mIU/mL), hCG | Measured value (mIU/mL), hCG | |
|---|---|---|---|
| 0 | 0.0 | 0.2 | |
| 1.56 | 14.7 | 13.4 | 91% |
| 3.13 | 29.4 | 31.1 | 106% |
| 6.25 | 58.8 | 64.5 | 110% |
| 12.5 | 117.6 | 124.1 | 106% |
| 25 | 235.1 | 230.9 | 98% |
| 50 | 470.3 | 454.8 | 97% |
| 75 | 705.4 | 664.1 | 94% |
| 90 | 846.5 | 814.7 | 96.2% |
| Neat | N/A | 940.6 | N/A |

A dilution evaluation was also performed. Two serum pools with high endogenous concentrations of hCG were diluted with either PBS or Fastpack® hCG diluent, and measured in triplicate using the Fastpack® System. Percent recoveries (based on observed/expected concentrations) are shown below for the 2 diluents tested. Results in the table are results for one of the serum pools. Results of the evaluation with the second serum pool were comparable. Expected concentrations are based on the measured value of the neat sample and the dilution factor.

| | Dilution with Fastpack® diluent | | Dilution with PBS | |
|---|---|---|---|---|
| **Expected concentration (mIU/mL)** | **Measured concentration (mIU/mL)** | **% recovery** | **Measured concentration (mIU/mL)** | **% recovery** |
| N/A | 699.2 | N/A | 699.2 | N/A |
| 349.6 | 366.6 | 104.9 | 361.9 | 103.5 |
| 174.8 | 174.7 | 99.9 | 187.6 | 107.3 |
| 87.4 | 90.1 | 103.1 | 94.3 | 107.9 |
| 43.7 | 46.5 | 106.4 | 46.0 | 105.4 |

Additional dilution studies with high samples (up to near 200,000 mIU/mL), show no significant bias in recovery (< 5%).

High dose hook effect was observed at high values outside the reportable range. These values are reported by the instrument as >1000 mIU/mL, and should be diluted and retested to obtain quantitative results.

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*

A master calibration curve is generated for each lot of FastPack® reagents. Since FastPack® System analyzers vary in response levels, the initial starting point needs to be determined on each analyzer and normalized to fit the manufacturer's generated calibration curve. The FastPack® hCG Calibrator Kit is used for this purpose.

The nominal lot concentrations for the calibrator in the Fastpack calibration kit is 250 mIU/mL. The allowed variability between lots is +/- 10%. The allowed uncertainty in measurement of the calibrator is +/- 10%. The traceability process is based on ISO 17511, Primary standards are prepared gravimetrically from a reconstituted vial of WHO 75/589. Values are transferred from primary to secondary materials using the Fastpack hCG assay. The secondary standards are used to assign values to individual test lots.

Opened and closed vial calibrator stability is performed at 2-8 degrees C. Measurements are made on at least 2 replicates, and 2 instruments. The acceptance criterion for recovery is 90-110% of time 0.

(Control materials were not included within this 510(k)).

d. *Detection limit:*

The limit of blank (LOB), limit of detection (LOD), and limit of quantitation (LOQ) were determined according to CLSI EP17-A. The limit of blank was determined from 10 replicate determinations of the FastPack® hCG sample diluent on each of three different FastPack® instruments using two different lots of reagent sets. Raw RLUs from the assays were converted to apparent mIU/mL based on the calibration curve for each assay. The LOB was determined as the maximum observed value. This value was 0.8 mIU/mL hCG.

To estimate LOD, a low control sample (3.5 mIU/mL) was measured over 10 days, using 2 instruments and 2 lots, and 2 assays each day, for a total of 80 measurements. The standard deviation for the low sample was 0.57mIU/mL. The standard deviation for the "0" sample was 0.2. Using the equation: LOD=LOB + ($c_B$ * $SD_s$), LOD was calculated as 1.4 mIU/mL.

(In the equation, $c_\beta = 1.645/(1-(1/(4*f)))$, where f is the degrees of freedom, and $SD_S$ is the standard deviation of the observations.) In this study, the LOD was 1.8 mIU/mL hCG.

For the LOQ analyses, the prospectively defined goals for accuracy were 80-120% recovery and <20% CV. The concentration of a serum control sample was determined by multiple measurements on the predicate device to be 3.34 mIU/mL. The sample was measured on the hCG Fastrack®; twenty replicates were measured using 2 lots and 2 instruments, for a total of 80 measurements, over 3 day. Results were as follows:
Mean: 3.13 mIU/mL; SD: 0.59 mIU/mL; %CV: 18.7; recovery relative to the predicate device: 94%. This supports the claimed LOQ of 3.1 mIU/mL hCG.

   e. *Analytical specificity:*
   The potential cross-reactants LH, FSH, and TSH were tested for cross-reactivity with the assay in the absence of hCG. Each substance was tested at a concentration of 1000 mIU/mL. No significant cross-reactivity (<0.5 mIU/mL) was observed.

   Bilirubin (30 mg/dL), hemoglobin (500 mg/dL), lipemia (1800 mg/dL), and human albumin (3 g/dL) were tested for interference at hCG concentrations of 3 mIU/mL and 500 mIU/mL. No significant cross-reactivity (<0.5 mIU/mL) was observed.

2. Comparison studies:

   a. *Method comparison with predicate device:*

   Serum samples from 106 apparently healthy women, containing varying hCG concentrations were evaluated using the FastPack® hCG assay and the predicate device. Samples ranged from 0 to 865 mIU/mL using the FastPack Assay. Results were evaluated using Deming regression. The following regression equation was determined:

   (Predicate device)=1.11(FastPack) + 8.04, $R^2$=0.99, standard error of the estimate = 19.88.

   b. *Matrix Comparison*

   To support the indications for use of this device with EDTA and Lithium heparin plasma, blood collections were obtained from over 106 healthy volunteers. Samples from 80 of the volunteers were spiked with hCG to obtain concentrations ranging from 0 to 645 mIU/mL. All samples were processed to either matched serum and EDTA plasma, or serum and Lithium heparin plasma. Results are shown below:

6

| Parameter | EDTA plasma vs. serum | Lithium heparin plasma vs. serum |
|---|---|---|
| N | 61 | 45 |
| Absolute bias (mIU/mL) | 8.7 | -4.9 |
| % Bias | 8% | -3% |
| Deming regression | | |
| Slope | 1.07 | 0.96 |
| Y-intercept (mIU/mL) | 1.16 | 0.41 |
| $R^2$ | 0.99 | 0.99 |

3. Clinical studies:

   a. *Clinical Sensitivity:*

      Not typically provided for this device type.

   b. *Clinical specificity:*

      Not typically provided for this device type.

   c. Other clinical supportive data (when a. and b. are not applicable):

4. Clinical cut-off:

   Not applicable.  The device is for quantitative measurements.


5. Expected values/Reference range:
   Samples were obtained from 126 non-pregnant apparently healthy female blood donors without any clinically abnormal indications. The hCG levels were determined using the FastPack® hCG Immunoassay in conjunction with the FastPack® System in order to establish the hCG concentrations in the reference populations. The reference interval (5th to 95th percentiles) for the FastPack® hCG Immunoassay for males is <0.8 – 1.7 mIU/mL and <0.8 – 3.0 mIU/mL for non-pregnant females. These reference intervals reflect the donor population of this study group. Each laboratory should determine their own reference intervals appropriate for their population.  The distribution of values for non-pregnant females is presented in the histogram below.

**N. Proposed Labeling:**

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

**O. Conclusion:**

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