510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION MEMORANDUM
ASSAY ONLY TEMPLATE

A. 510(k) Number:

k141689

B. Purpose for Submission:

New assay

C. Measurand:

C-reactive protein (CRP)

D. Type of Test:

Quantitative

E. Applicant:

Qualigen, Inc.

F. Proprietary and Established Names:

FastPack High Sensitivity C-Reactive Protein Immunoassay
FastPack High Sensitivity C-Reactive Protein Calibrator Kit
FastPack High Sensitivity C-Reactive Protein Controls
FastPack High Sensitivity C-Reactive Protein Method Verification Kit

G. Regulatory Information:

1. Regulation section:

   21 CFR 866.5270 – C-reactive protein immunological test system
   21 CFR 862.1150 – Calibrator
   21 CFR 862.1660 – Quality Control Material (Assayed and Unassayed)
2. Classification:

   Class II
   Class II
   Class I, reserved

3. Product code:

   DCK – C-reactive, protein, Antigen, Antiserum
   JIT – Calibrator, Secondary
   JJX – Quality Control Material (Assayed and Unassayed)

4. Panel:

   Clinical Chemistry

H. Intended Use:

1. Intended use(s):

   See indication for use.

2. Indication(s) for use:

   FastPack High Sensitivity C-Reactive Protein Immunoassay
   FastPack High Sensitivity C-Reactive Protein Immunoassay is to be used for evaluation of conditions thought to be associated with inflammation, in otherwise healthy individuals. The FastPack High Sensitivity C-Reactive Protein Immunoassay is intended for use with the FastPack Analyzer. Not intended for Point-of-Care use.

   FastPack High Sensitivity C-Reactive Protein Calibrator Kit
   FastPack High Sensitivity C-Reactive Protein Calibrators are used for calibrating the quantitative FastPack High Sensitivity C-Reactive Protein Immunoassay on the FastPack Analyzer.

   FastPack High Sensitivity C-Reactive Protein Controls
   FastPack High Sensitivity C-Reactive Protein Controls are used for quality control of the FastPack High Sensitivity C-Reactive Protein Immunoassay on the FastPack Analyzer.

   FastPack High Sensitivity C-Reactive Protein Method Verification Kit
   FastPack High Sensitivity C-Reactive Protein Verifiers are used in the quantitative verification of calibration and assay range of the quantitative FastPack High Sensitivity C-Reactive Protein Immunoassay on the FastPack Analyzer.

3. Special conditions for use statement(s):

   For prescription use only.
   For in vitro diagnostic use only.
   Not intended for Point-of-Care use.

4. Special instrument requirements:

   FastPack Analyzer

I. Device Description:

Each FastPack High Sensitivity C-Reactive Protein Immunoassay Kit contains:

- 30 FastPack High Sensitivity C-Reactive Protein Reagent Packs
- Sample diluent A, 32 vials, 2.475 ml each

Each FastPack High Sensitivity C-Reactive Protein Reagent Pack contains:

- Paramagnetic Particles coated with streptavidin, 150 1-JL
- Murine monoclonal anti-CRP antibody covalently linked to alkaline phosphatase and Murine monoclonal anti-CRP antibody covalently linked to biotin, 100 1-JL
- Wash Buffer, 2.0 ml
  Tris buffer containing surfactants
- Substrate, 145 1-JL lmmuGlow: lndoxyl-3-phosphate and lucigenin in buffer containing preservatives

The test reagents include:

- Conjugate solution –
  - a murine monoclonal anti-C-reactive protein (CRP) monoclonal antibody conjugated to alkaline phosphatase
  - a biotinylated murine monoclonal anti-CRP monoclonal antibody
- Solid support streptavidin coated paramagnetic particles
- Substrate solution – ImmuGlow
- Wash solution: Tris buffer containing detergents
- Sample diluent A – the defined protein (bovine serum albumin) matrix provided within the reagent kit for dilution of samples above the assay range

- FastPack High Sensitivity C-Reactive Protein Calibrator Kit
  One level of calibrator material is provided ready to use in 1.0 mL/vial. Contains known quantities of human C-reactive protein. Contains 0.09% Sodium azide as preservative.

- FastPack High Sensitivity C-Reactive Protein Control Kit
  Two levels of hsCRP controls are provided ready to use in 1.0 mL/vial. hsCRP controls are prepared from human plasma and human proteins. Preservatives (0.09% sodium azide) and stabilizers have been added to maintain product integrity.

- FastPack High Sensitivity C-Reactive Protein Method Verification Kit
  Three levels (Low, Mid and High) are provided ready to use in 0.5 mL/vial. hsCRP Verifiers contain components of human origin prepared with protein stabilizers and preservatives to yield predetermined concentrations. Contains 0.09% sodium azide as preservative.

The Fast Pack High Sensitivity C-Reactive Protein Calibrator Kit, Controls and Verification

Kit contain human source material. Each serum/plasma donor unit used in the manufacture of these products has been tested by FDA accepted methods and found non-reactive for the presence of HBsAg and antibody to HIV-1/2, HCV and HIV-1 Ag.

J. Substantial Equivalence Information:

1. Predicate device name(s):
   - Olympus CRP Latex Reagent
   - Bio-Rad Laboratories Liquichek™ Cardiac Markers Plus Control
   - Ortho-Clinical Diagnostics, Inc. VITRO Chemistry Products hsCRP Performance Verifier, I, II, and III

2. Predicate 510(k) number(s):
   - k051564
   - k050537
   - k041799

3. Comparison with predicate:

Similarities and Differences between FastPack and Olympus hsCRP Assays		
Item	Qualigen FastPack High Sensitivity C-Reactive Protein Immunoassay	Olympus America, Inc. CRP Latex reagent k051564
Intended Use/ Indications for Use	High Sensitivity C-Reactive Protein Immunoassay is to be used for evaluation of conditions thought to be associated with inflammation, in otherwise healthy individuals.	Same
Sample Type	Serum or plasma (EDTA or lithium heparin)	Same
Reagent Storage Temperature	2-8 ºC	Same
Testing Environment	Professional use	Same
Precision (% CV)	Within-run: ≤ 1.0% Between-run: ≤ 5.2% Total: ≤9.0%	Within-run: ≤ 3.2% Total: ≤ 3.8%
Linearity	Assay linear from 0.2 mg/L to 15 mg/L in high sensitivity application	Assay linear from 0.2 - 160 mg/L

Interfering Substances/Specificity	No interference found at the below tested concentrations: Bilirubin (conjugated) up to 40 mg/dL Bilirubin (unconjugated) up to 40 mg/dL Hemoglobin up to 750 mg/dL Lipids up to 1000 mg/dL Human serum albumin up to 7.7 g/dL Transferrin up to 567 mg/dL Human IgG up to 2961 µg/mL	No interference found at the below tested concentrations: Bilirubin up to 40 mg/dL Hemoglobin up to 500 mg/dL Intralipid up to 1000 mg/dL
Expected Values/Reference Intervals	0.2 – 11.4 mg/L	Cardiac risk assessment categories: Low < 1 mg/L Average 1.0 to 3.0 mg/L High > 3.0 mg/L
Methodology	The FastPack High Sensitivity C-Reactive Protein Immunoassay is a paramagnetic particle, chemiluminescent immunoassay employing specific murine monoclonal antibodies.	The Olympus CRP Latex reagent is a turbidimetric assay employing rabbit antibodies coated on latex particles.
Assay principle	Chemiluminescence	Turbidimetry
Assay procedure	Automated	Automated
Assay range	0.2 - 15 mg/L for High Sensitivity Application	0.2 - 160 mg/L (provides measurements both for “Normal Application” and “Highly Sensitive Application”)
Traceability	Traceable to the ERM-DA474/IFCC reference which serves as the Primary Reference Material	Traceable to an external standard

Similarities and Differences between FastPack and Olympus CRP Calibrators		
Item	Qualigen FastPack High-Sensitivity C- Reactive Protein Immunoassay	Olympus America, Inc. CRP Latex reagent k051564

Intended Use/Indication for Use	For in-vitro diagnostic use in calibrating C-Reactive Protein Immunoassay	Same
Antigen used in	Human CRP	Same
Matrix	Liquid human serum matrix containing a predetermined level of human CRP	Same
Storage temperature	2-8 ºC	Same
Number of calibrators	1	5 (additional calibrators provided at higher concentrations to enable CRP measurements in the "Normal Application")

Similarities and Differences between FastPack and Predicate CRP Controls		
Item	Qualigen FastPack High-Sensitivity C-Reactive Protein Immunoassay	Bio-Rad Laboratories Liquichek™ Cardiac Markers Plus Control k050537
Intended Use/Indication for Use	For in-vitro diagnostic use to monitor the precision and accuracy of the High-Sensitivity C-Reactive Protein	Same
Antigen used in controls	Human CRP	B-type Natriuretic Peptide, Creatine Kinase (Total), C-Reactive Protein, Homocysteine, Digitoxin, N-terminal pro-B-type Natriuretic Peptide, CK-MB, Myoglobin, Troponin I, Troponin T
Matrix	Liquid human serum matrix containing a predetermined level of human CRP	Prepared from human serum with added constituents of human and animal origin, preservatives, and stabilizers. The controls are in liquid form.
Storage temperature	2-8 ºC	2-8 ºC (Opened), or -20 to -70 ºC (Unopened)
Number of levels	2	3

Similarities And Differences between FastPack CRP Verifiers and Predicate Verifiers		
Item	Qualigen FastPack High-Sensitivity C-Reactive Protein Immunoassay	Ortho-Clinical Diagnostics, Inc. VITRO Chemistry Products hsCRP Performance Verifier, I, II, and III k041799
Intended Use/Indication for Use	For use in the quantitative verification of calibration and assay range of the High-Sensitivity C-Reactive Protein.	Same
Antigen used	Human CRP	Same
Storage temperature	2-8 ºC	Same
Matrix	Low Verifier: HEPES buffer with Bovine Serum Albumin (BSA) and Detergent Mid and High Verifiers: Liquid human serum matrix containing a predetermined level of human CRP	A base matrix of human plasma proteins to which stabilizers and preservative have been added.
Number of levels	3	Same

K. Standard/Guidance Document Referenced (if applicable):

- CLSI EP5-A2: Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline, Second Edition, 2005
- CLSI EP6-A: Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline 2014
- CLSI EP7-A2: Interference Testing in Clinical Chemistry, Approved Guideline, Second Edition, 2014
- CLSI EP9-A3: Method Procedure Comparison and Bias Estimation Using Patient Samples; Approved Guideline, Third Edition 2014
- CLSI EP14-A2: Evaluation of Matrix Effects; Approved Guideline, Second Edition 2014
- CSLI EP17-A2: Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline, Second Edition 2013
- CLSI EP28-A3c: Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline, Third Edition 2014
- CLSI-I/LA21-A2: Clinical Evaluation of Immunoassays; Approves Guideline, Second Edition 2008

- CLSI-I/LA30-A: Immunoassay Interference by Endogenous Antibodies; Approved Guideline 2009

L. Test Principle:

The FastPack High Sensitivity C-Reactive Protein Immunoassay employs a Sandwich immunoassay principle. Endogenous CRP in a patient sample, calibrator, control, or verifier is dispensed into a FastPack reagent pack. In the reagent pack, the sample binds with a murine monoclonal anti-CRP antibody covalently linked to alkaline phosphatase (ALP) and a different murine monoclonal anti-CRP antibody linked to biotin. After incubation, immunoreacted complex (Monoclonal anti-CRP antibody-ALP conjugate and anti-CRP antibody linked to biotin reacted with CRP in the sample) is mixed with streptavidin coated paramagnetic particles. After washing steps (using a Tris buffer containing detergents) to separate bound from unbound anti-CRP monoclonal antibody-ALP, a chemiluminogenic substrate mixture is added to the system. This mixture contains indoxyl-3-phosphate, a substrate for ALP, and lucigenin (N,N'- dimethyl-9,9'-biacridinium dinitrate). ALP dephosphorylates indoxyl-3-phosphate to indol-3-ol, which subsequently undergoes oxidation. As a result, lucigenin is reduced to form a dioxetane structure that is cleaved to yield N-methylacridone. This compound produces a sustained luminescent glow following excitation. The raw relative luminescence units (RLUs) generated are measured by a photomultiplier tube in the FastPack Analyzer and are directly proportional to the concentration of CRP in the sample.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Precision was evaluated following the CLSI EP5-A2 guidance. Six serum patient samples with concentrations of 0.5, 1.0, 2.5, 5.0, 7.5 and 12.5 mg/L CRP were tested in duplicate determinations in each of two runs per day on each of two FastPack Reagent lots, two FastPack Analyzers per reagent lot, over a period of 20 days to yield 320 replicate determinations of each sample. A single FastPack calibrator lot was utilized for all runs. Within-run, between-run, and between-day components of variation were calculated and total imprecision using general linear model (GLM). The table below presents the results:

Sample	Average	Within-run		Between-run		Between-day		Total	
		SD	CV%	SD	CV%	SD	CV%	SD	CV%
1	0.38	0.002	0.53	0.014	3.68	0.005	1.32	0.033	8.68
2	1.00	0.004	0.43	0.038	3.80	0.013	1.30	0.087	8.70
3	2.06	0.008	0.39	0.071	3.45	0.030	1.46	0.180	8.74
4	5.01	0.023	0.46	0.206	4.11	0.073	1.46	0.362	7.23
5	7.67	0.035	0.46	0.314	4.09	0.111	1.45	0.684	8.92
6	12.54	0.073	0.58	0.649	5.18	0.230	1.83	1.091	8.70

b. Linearity/assay reportable range:

Linearity:
This linearity study followed CLSI EP6-A. A high patient sample was intermixed with a low sample to generate 8 concentration levels each tested in triplicate determinations using one FastPack reagent lot on one FastPack analyzer using one FastPack calibrator lot. Linear results were compared to 2nd and 3rd order polynomial fits against a pre-specified allowable error. The linearity range was found to extend from the LOQ (0.063 mg/L) to 15.0 mg/L.

The linear fit yields an equation of y= 0.00 + 0.97x with percent deviation from linearity of ≤ ± 10%.

Samples recovering above the range may be diluted using Sample Diluent A. Dilutional linearity has been validated by the sponsor in their Hook effect study.

Hook effect:
Four patient serum samples with endogenous values of approximately 450, 300, 300, and 400 mg/L, respectively were run neat and diluted 1:100 in Sample Diluent A in triplicate determinations using one FastPack Reagent lot, one calibrator lot on two FastPack Analyzers. The objective of the study was to demonstrate that the Neat values exceeded the upper limit of the assay (15 mg/L).
The results demonstrate that all high level samples recover > 15 mg/L when run neat (range: 21.51 - 35.03 mg/L) and that back calculated actual values range from 309.5 - 442.6 mg/L CRP.

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

The sponsor bases its traceability process for hsCRP on the International Standard prEN ISO 17511:2003, Metrological Traceability of Values Assigned to Calibrator and Control Material. It is traceable to the ERMDA474/IFCC reference which serves as the Primary Reference Material. The value assignment process for calibrators, controls and verifiers was reviewed and found to be adequate.

Shelf Life and Open Vial Stability for Calibrators:
The FastPack calibrator materials have a shelf life stability of 12 months stored at 2-8°C, and have open-vial stability of 30 days when stored at 2-8°C.

Shelf Life and Open Vial Stability for Controls and Verifiers:
The FastPack controls and verifiers have a shelf life stability of 24 months stored at 2-8°C, and have open-vial stability of 30 days when stored at 2-8°C.

The stability study protocols and acceptance criteria were reviewed and found to be adequate.

d. Detection limit:

The limit of blank (LOB), limit of detection (LOD), and limit of quantitation (LOQ) were determined according to CLSI EP17.

LoB:
In this study, the limit of blank was determined from 80 replicate determinations of a blank sample tested on four different FastPack instruments using two reagent lots. The LOB was determined as the upper 95th percentile of the distribution. This value was 0.005 mg/L CRP.

LoD:
The LOD was estimated from 80 replicate determinations of three low samples. Per the CLSI EP17-A guideline, LOD was determined by the following equation:
LOD = LOB + (cß * SDS),
where cß = 1.645/(1-(1/(4 *f)), where f is the degrees of freedom, and SDS is the pooled standard deviation of the observations. In this study, the LOD was found to be 0.032 mg/L CRP.

LoQ:
The LoQ was determined as the lowest sample which provided <20% CV. The LOQ was set to 0.063 mg/L CRP.

e. Analytical specificity:

Endogenous substance interference was evaluated by spiking two serum samples (1.0 and 6.0 mg/L CRP, respectively) with hemoglobin, lipid, bilirubin, albumin, rheumatoid factor (RF), transferrin, human anti-mouse IgG HAMA and Heterophile/HAMA. The samples testing interference with hemoglobin, lipid, bilirubin, albumin, rheumatoid factor (RF), transferrin were analyzed in 5 replicate determinations using one FastPack analyzers and one lot of Fast Pack reagents. The samples testing interference with human anti-mouse IgG HAMA were tested in duplicate and samples testing interference with Heterophile/HAMA in triplicate determinations, respectively. There was no significant interference for the following endogenous substances in the ranges tested below:

Bilirubin up to 40 mg/dL
Hemoglobin up to 750 mg/dL
Lipids up to 1000 mg/dL
Albumin up to 7.7 g/dL
Rheumatoid factor (RF) up to 1000 IU/mL
Transferrin up to 567 mg/dL
Human IgG up to 2961 µg/mL
Human anti-mouse IgG HAMA up to 4 mg/dL
Heterophile/HAMA up to 3641 ng/mlL.

The sponsor also tested potential interference to the exogenous substances in 5 replicate determinations using one FastPack analyzer and one lot of Fast Pack reagents. No significant interference was found when these substances were tested in the concentration ranges indicated below:

L-Ascorbic Acid up to 200 mg/L
Oxaloacetic Acid up to 300 μM
Glutathione up to 300 μM
Isoniazid up to 300 μM
L-DOPA up to 300 μM

The sponsor's definition of non-significant interference is <10% difference between the spiked and unspiked samples.

The labeling includes a limitation that heterophilic antibodies may interfere with this method.

f. Assay cut-off:

Not applicable

2. Comparison studies:

a. Method comparison with predicate device:

In this method comparison study, one hundred and thirty three human serum samples with CRP values across the measuring range were analyzed on the candidate device and the results were compared to those determined with the predicate device. Deming regression results from the method comparison study are summarized in the table below:

No. Samples	Range Tested (mgl/L)	Slope (95% CI)	y-intercept (95% CI)	R (95% CI)
143	0.21 – 15.00	0.98 (0.95-1.01)	-0.12 (-0.21 to -0.02)	0.99 (0.99 - 0.99)

b. Matrix comparison:

Serum and lithium-heparin plasma

The sponsor performed a matrix comparison study to assess the performance of the assay when different sample types/tubes (serum vs. EDTA plasma and. Lithium Heparin plasma) were tested. Forty one human blood samples were processed to serum, lithium-heparin plasma, or EDTA plasma in parallel. The samples were then

tested in duplicate determinations each in the FastPack High Sensitivity C-Reactive Protein Immunoassay using two lots of FastPack Reagents and one lot of calibrators. The data analysis was performed using singlicate results. Deming regression results for comparisons of EDTA and lithium-heparin plasma to serum are summarized in tables below:

Comparison of serum and EDTA plasma:

Parameter	Result
N compared	41
Range of observations, mg/L	Serum: 0.33 – 14.72 EDTA Plasma: 0.29 – 14.76
Absolute bias, mg/L	-0.225
% Bias	-6.1
Deming regression results	
Slope	0.94
y-intercept	0.0
R	0.984
R^2	0.967

Comparison of serum and Lithium-Heparin plasma:

Parameter	Result
N compared	41
Range of observations, mg/L	Serum: 0.33 – 14.72 Lithium-Heparin Plasma: 0.31 – 14.86
Absolute bias, mg/L	0.002
% Bias	0.5
Deming regression results	
Slope	1.00
y-intercept	0.00
R	0.993
R^2	0.986

The studies support the sponsor's claims that EDTA and Lithium-Heparin are acceptable anticoagulants to be used with The FastPack hsCRP Assay.

3. Clinical studies:
   a. Clinical Sensitivity:

      Not applicable

   b. Clinical specificity:

      Not applicable

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

      Not applicable

4. Clinical cut-off:

   Not applicable

5. Expected values/Reference range:

   A reference interval study was carried out with serum samples from 211 subjects representing 4 different geographic regions of the United States yielded the results in the table below. The non-parametric 2.5th - 97.5th percentile of 0.2 - 11.4 mg/L provides the reference interval determined from this study, which is in accord with literature[1-4].

Observed values	
Mean (SD)	3.2 (3.1) mg/L
Median (Min - Max)	1.9 (0.2 - 13.1) mg/L
2.5th - 97.5th percentile	0.2 - 11.4 mg/L

   Newborns with no evidence of infection have CRP concentrations of< 2 mg/L.[5]

   References:

[1] Aziz N, Fahey JL, Detels R, Butch AW. Analytical performance of a highly sensitive C-reactive protein-based immunoassay and the effects of laboratory variables on levels of protein in blood. Clinical and Diagnostic Laboratory Immunology 2003;10:652-7.

[2] Imhof A, Frohlich M, Loewel H, et al. Distributions of C-reactive protein measured by high-sensitivity assays in apparently healthy men and women from different populations in Europe. Clin Chem 2003;49:669-72.

[3] Sennels HP, Jacobsen S, Jensen T, et al. Biological variation and reference intervals for circulating osteopontin, osteoprotegerin, total soluble receptor activator of nuclear factor kappa B ligand and high-sensitivity C-reactive protein. Scand J Clin Lab Invest 2007;67:821-35.

[4] Charuruks N, Laohajinda B, Rujiwanitgun S, Chaiworaporn M. Reference interval for C-reactive protein and its distribution pattern in thai adults. Circ J 2005;69:339-44.

[5] Soldin OP, Bierbower LH, Choi JJ, et al. Serum iron, ferritin, transferrin, total iron

binding capacity, hs-CRP, LDL cholesterol and magnesium in children; new reference intervals using the Dade Dimension Clinical Chemistry System; Clin Chim Acta 2004;342:211-7.

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.