

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

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

k031563

**B. Analyte:**

Complement C1q CIC (circulating immune complexes)

**C. Type of Test:**

Semi-quantitative enzyme immunoassay

**D. Applicant:**

The Binding Site, Ltd.

**E. Proprietary and Established Names:**

BINDAZYME™ C1q Binding Circulating Immune Complex Enzyme Immunoassay Kit

**F. Regulatory Information:**

1. Regulation section:

21 CFR 866.5240

Complement Components Immunological Test System

2. Classification:

Class II

3. Product Code:

DAK

Complement C1q, Antigen, Antiserum, Control

4. Panel:

Immunology (82)

**G. Intended Use:**

1. Intended use(s):

The BINDAZYME™ C1q Binding Circulating Immune Complex Enzyme Immunoassay Kit is designed for the *in vitro* measurement of circulating immune complexes (CIC) that bind C1q, present in human serum. It is intended for the determination of CIC in serum of patients with various autoimmune and other CIC-related diseases, and is used in conjunction with other clinical findings.

2. Indication(s) for use:

The BINDAZYME™ C1q Binding Circulating Immune Complex Enzyme Immunoassay Kit is designed for the *in vitro* measurement of circulating immune complexes (CIC) that bind C1q, present in human serum. It is intended for the determination of CIC in serum of patients with various autoimmune and other CIC-related diseases, and is used in conjunction with other clinical findings.

3. Special condition for use statement(s):

For prescription use only

4. Special instrument Requirements:

None given

**H. Device Description:**

The device is an enzyme immunoassay using microtiter plates as the solid phase. The plate wells are coated with human C1q which captures C1q binding immune complexes present in the patient sample. The conjugate is peroxidase labeled antibody to human IgG which uses 3,3',5,5' tetramethylbenzidine (TMB) as substrate. The kit contains 5 levels of calibrators (aggregated human IgG) with concentrations of 100, 33.3, 11.1, 3.7, and 1.23 µg Eq/mL of immune complex. A positive and a negative control are included with the kit. The kit also contains sample diluent, wash buffer concentrate and stop solution.

**I. Substantial Equivalence Information:**

1. Predicate device name(s):  
ALPCO CIC-C1q Circulating Immune Complexes EIA
2. Predicate K number(s):  
k012576
3. Comparison with predicate:

<b>Similarities</b>		
<b>Item</b>	<b>Device</b>	<b>Predicate</b>
Indication for Use	Measurement of circulating immune complexes (CIC) that bind C1q present in human serum. It is intended for the determination of CIC in serum of patients with various autoimmune and other CIC-related disease and is used in conjunction with other clinical findings.	Quantitative determination of circulating immune complexes (CIC) in serum and plasma of patients with various autoimmune and other CIC-related diseases.
Methodology	Enzyme immunoassay	Enzyme immunoassay
Solid Phase	Microtiter wells coated with human C1q	Microtiter wells coated with human C1q
<b>Differences</b>		
<b>Item</b>	<b>Device</b>	<b>Predicate</b>
Sample Matrix	Serum (fasting 12 hours)	Serum/plasma (fasting 12 hours)
Cut-offs	Neg. < 4.4 µg Eq/mL Equiv. 4.4-10.8 Pos. > 10.8	Neg. <3.2 µg Eq/mL Equiv. 3.2-5.0 Pos. > 5.0
Interfering substances	Rheumatoid Factor	None listed
Calibrators	Aggregated human IgG 100, 33.3, 11.1, 3.7 and 1.23 µg Eq/mL	Aggregated human IgG 50, 20, 10, 4, and 1 µg Eq/mL
Controls	Positive - aggregated human IgG	High and Low levels containing lot specific

	Negative - diluted human sera	amounts of aggregated human IgG
Conjugate	Peroxidase labeled antibody to human IgG	Protein A conjugated to alkaline phosphatase
Substrate	TMB	pNPP
Stop Solution	3M phosphoric acid	1N sodium hydroxide
Total Incubation Time	90 minutes	125 minutes

**J. Standard/Guidance Document Referenced (if applicable):**

None

**K. Test Principle:**

Microwells are pre-coated with purified human C1q. The calibrators, controls and diluted patient samples are added to the wells and C1q binding immune complexes are bound by the C1q during the first incubation. After washing the wells to remove all unbound proteins, purified peroxidase labeled rabbit antihuman IgG ( $\gamma$  chain specific) conjugate is added. The conjugate binds to the captured immune complex and the excess unbound conjugate is removed by a further wash step. The bound conjugate is visualized with TMB substrate which gives a blue reaction product, the intensity of which is proportional to the concentration of C1q binding CIC in the sample. Phosphoric acid is added to each well to stop the reaction. This produces a yellow end point color, which is measured at 450 nm. The concentration of CIC in the sample is read from the calibration curve and results are expressed as heat aggregated human IgG equivalents per mL ( $\mu\text{g Eq/mL}$ ).

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

1. Analytical performance:

a. Precision/Reproducibility:

Inter-assay

The inter-assay precision was measured using three samples within the measuring range of the calibration curve. The studies compare the mean duplicate result of each of the three samples across three different kit batches. CV's ranged from 3.9-13.0%.

Intra-assay

The intra-assay precision was measured using three samples within the measuring range of the calibration curve. The studies show the CV's within a single plate based on 16 individual results for each of the three samples. CV's ranged from 5.5-7.5%.

b. Linearity/assay reportable range:

Linearity (dilution recovery)

Studies were performed to demonstrate that all samples accurately dilute across the standard curve range and correlate to their predicted values based on dilution. Samples were diluted to 1:100 and double dilutions were made from this. The values were then plotted and a correlation coefficient produced. One high sample (69.7  $\mu\text{g Eq/mL}$ ) and 2 moderate level samples (28.5 and 28.7  $\mu\text{g Eq/mL}$ ) were serially diluted. For the high sample regression analysis yielded  $y =$

$1.0047x - 1.6536, R^2 = 0.9977$ . The two moderate samples showed  $y = 1.0091x - 0.4153, R^2 = 0.996$  and  $y = 1.0083x - 0.3005, R^2 = 0.999$ , respectively.

c. *Traceability (controls, calibrators, or method):*

N/A

d. *Detection limit:*

The limit of detection was determined as the mean concentration + 2SD given by 20 determinations of the sample diluent. A value of 0.1  $\mu\text{g Eq/mL}$  was obtained.

e. *Analytical specificity:*

The studies compared a normal and high positive sera spiked with known amounts of specific serum factors. The interfering substances were tested at elevated levels to mimic high contamination. No interference was observed with free or conjugated bilirubin, hemoglobin, or chyle. Rheumatoid Factor (RF) did cause interference at a level of 500 IU/mL and is reported in the package insert.

f. *Assay cut-off:*

See Clinical cut-off

2. Comparison studies:

a. *Method comparison with predicate device:*

Forty eight samples including 29 normal serum and 19 clinical (12 SLE and 7 other disease positive) were tested using the new and the predicate device. The overall agreement between the two assays was 83.3%. Linear regression analysis yielded  $y = 0.862x - 3.14, R^2 = 0.8082$ .

b. *Matrix comparison:*

N/A - for use with serum only.

3. Clinical studies:

a. *Clinical sensitivity:*

Not provided

b. *Clinical specificity:*

Not provided

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

4. Clinical cut-off:

The normal range (cut-off for negative) was assessed using 200 normal adult blood donor samples (112 males and 88 females). C1q-CIC are found sporadically in the normal population as a result of infection, and can also be elevated after eating. To arrive at a logical cut-off for the 'true normal', the elevated samples have been eliminated by three iterative cycles. Each cycle involves calculating the mean and SD and removing any samples with values greater than the mean +3SD. After two further cycles, a total of 19 samples were removed and the resulting mean +2SD gave a value for the cut-off of 4.4  $\mu\text{g Eq/mL}$ . The same iterative procedure was used in the predicate device cut-off determination. An equivocal range has been set between 4.4 and 10.8  $\mu\text{g}$

Eq/mL with positive being  $>10.8 \mu\text{g Eq/mL}$ . Using this cut-off, 95% of the normal samples were negative.

5. Expected values/Reference range:

The expected value in the normal population should be negative. However, up to 5-6% of normal donors can have levels of C1q-CIC above the cut-off.

**M. Conclusion:**

The Binding Site BINDAZYME™ C1q Binding Circulating Immune Complex Enzyme Immunoassay Kit is substantially equivalent to other devices regulated under 21 CFR §866.5240, product code DAK.