510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
DEVICE ONLY

A. 510(k) Number:
k032796

B. Analyte:
Complement component C3d – human IgG circulating immune complexes

C. Type of Test:
Semi-quantitative enzyme immunoassay

D. Applicant:
Lehnus and Associates for Immuno Biological Laboratories – Hamburg

E. Proprietary and Established Names:
IBL C3d-CIC Test Kit; complement C3 antigen, antiserum, control

F. Regulatory Information:
1. Regulation section:
   21 CFR 866.5240 Complement components immunological test system
2. Classification:
   Class II
3. Product Code:
   CZW
4. Panel:
   Immunology 82

G. Intended Use:
The IBL C3d-CIC test is a semi-quantitative enzyme immunoassay for the in vitro diagnostic detection of circulating immune complexes that bind C3d in human serum. The measurement is performed as an aid in the diagnosis of various autoimmune and other CIC related diseases. Levels of these complexes are one indicator in a multi-factorial diagnostic regime
1. Indication(s) for use:
   Aid in the diagnosis of various autoimmune and other CIC related diseases
2. Special condition for use statement(s):
   None
3. Special instrument Requirements:
   None

H. Device Description:
The assay is a sandwich solid phase enzyme immunoassay (ELISA) utilizing coated microplates. Microplates coated with antibody recognizing the C3d portion of C3d-

circulating immune complexes capture complexes from patient serum samples, calibrators, or controls. Patient serum samples and controls are diluted 1:5 with sample diluent. Horseradish peroxidase labeled anti-human IgG is added after initial incubation and washing of the specimen in the coated microplate wells. After a second incubation and washing, enzyme substrate (tetramethylbenzidine; TMB) is added to develop color when enzyme is captured onto wells of the plate. The color reaction is stopped after 15 minutes at room temperature (18-24ºC) by addition of stop solution. The amount of color produced in each well is measured in a microtiter plate reader capable of reading optical density at 450 nm. The amount of color developed by standards of previously determined concentration is proportional to the concentration of calibrators. The concentration of samples or controls is calculated from the linear relationship of absorbance with concentration of the calibrators. Calibrators of 2 different arbitrary concentrations are utilized to derive sample concentration, expressed as µg/ml. Positive and negative controls are also included to monitor the satisfactory performance of the assay.

I. **Substantial Equivalence Information:**
1. Predicate device name(s):
   Scimedx Corp. C3d ScanLisa
2. Predicate K number(s):
   K861362
3. Comparison with predicate:

Similarities		
Item	**Device**	**Predicate**
Assay technology	Enzyme immunoassay	Enzyme immunoassay
Analyte capture mechanism	Monoclonal anti-C3d antibody coated microplates	Monoclonal anti-C3d coated microplates
Calibration material	Heat aggregated human IgG	Heat aggregated human IgG
Differences		
Item	**Device**	**Predicate**
Calibrators	2 of different concentration	3 of different concentration
Equivocal zone	16-24 µg/ml	No equivocal zone
Intended Use	A semi-quantitative enzyme immunoassay for the in vitro diagnostic detection of circulating immune complexes that bind C3d in human serum. The measurement is performed as an aid in the diagnosis of various autoimmune and other CIC related diseases. Levels of these complexes are one indicator in a multi-factorial diagnostic regime	Not stated in labeling

J. Standard/Guidance Document Referenced (if applicable):
None

K. Test Principle:
Enzyme immunoassay utilizing antibody to complement component C3d coated to microwells to capture complexes of human IgG containing complement component C3d. Enzyme-labeled antibody to human IgG recognizes captured complexes absorbed on microwells.

L. Performance Characteristics (if/when applicable):
　1. Analytical performance:
　　　a. *Precision/Reproducibility:*
　　　　To assess inter-day reproducibility, 5 samples of varying concentration were tested on 8 days. The samples included 3 with positive concentrations, 1 with an equivocal concentration, and 1 with a negative concentration. To assess intra-assay imprecision, 5 samples similar to the inter-day description of imprecision were tested in 12 replicates. For each sample on each day the mean, standard deviation, and %coefficient of variation (CV) were calculated.

Precision	Range (μg/ml)	CV(%)
Intra-Assay	4.0 – 83.6	8.1 – 3.2
Inter-Assay	4.0 – 83.0	12.3 – 2.5

　　　b. *Linearity/assay reportable range:*

　　　　A high positive sample was serially diluted from 1:0.625 to 1:20 with diluent buffer. The observed concentration was determined from a standard curve developed by linear regression analysis of the optical density and the concentration of the blank, standard, positive control and negative control. From this analysis, an expected concentration for the serial dilutions of the sample was determined. The observed concentration and expected concentration were compared by regression analysis to determine if the slope was equivalent with 1.0. The slope of the regression line of observed vs. expected C3d CIC concentration was 1.14 (95% confidence interval 0.87 to 1.41). The correlation coefficient was 0.986. The slope is equivalent with 1.0 (i.e. the confidence interval contains 1.0). Therefore, the dilution analysis indicates linearity of observed C3d concentration with dilution of the sample up to 1:20.

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

　　　　Values for standards and controls were determined from standards in the predicate assay. Two approximate values were chosen, 100

µg/ml and 10 µg/ml. Values for controls are assigned an acceptable range based on the mean ± 2 standard deviations.

d. *Detection limit:*

Sample diluent buffer was tested in 26 replicates on a single plate. The concentration was calculated using the standard curve. The mean value was 1.9 µg/ml ± 1 standard deviation (SD) 0.13 µg/ml. The limit of detection was defined as the mean concentration plus 3 SDs. The limit of detection is 2.2 µg/ml.

e. *Analytical specificity:*

Four samples of different concentrations were obtained and supplemented with 4 concentrations of 3 different interfering substances. The 4 samples ranged in concentration from 10 to 37 µg/ml. Bilirubin at concentrations from 1 mg/dL to 250 mg/dL caused less than ±25% interference at the concentrations tested (10 – 37 µg/ml). Hemoglobin at concentrations from 100 mg/dL to 1000 mg/dL caused less than ± 25% interference at the concentrations tested. Finally, EDTA at concentrations ranging from 1 to 50 mM caused less than ±15% interference at the concentrations tested.

Monoclonal anti-C3d utilized to capture complement component C3d in the assay does not cross-react with human IgG but due to the structure of C3d will cross-react with C3 and C4 and not C3b.

f. *Assay cut-off:*

A gray zone was initially suggested by utilizing a normal population (using 3 and 5 standard deviations above the mean of normal subjects) to yield equivalent results with the predicate device. Thirty eight normal subjects provided the samples utilized. The equivocal zone was initially determined as 16 to 24 µg/ml. Analysis of 128 separate samples from normal donors indicated that the 90th percentile value was 14 µg/ml. The upper 95% confidence interval of the 90th percentile was 24 µg /ml. The 90th percentile and the upper 95% confidence limit of the 90th percentile values are similar to the chosen values of 16 to 24 µg/ml for the equivocal zone.

2. Comparison studies:
    a. *Method comparison with predicate device:*

        To evaluate the performance of the assay compared with the predicate device, 81 samples were tested in both assays. The samples were identified as provided by normal asymptomatic subjects and autoimmune patients. Of the 81 samples, 38 samples were identified as normal subjects. The remaining 43 samples were identified as autoimmune patients (28 CIC, 4 SLE, 2 ANA, 1 RA, 2 TPO, 3 ENA, 1 ANCA, 1 gliadin, and 1 cardiolipin). The following table summarizes the agreement of the proposed device

with 3 result categories (positive, equivocal, and negative) vs. the predicate device with 2 result categories (positive, negative):

SciMedx result

IBL	>9 µg/ml	≤ 9 µg/ml	Total
>24 µg/ml	19	2	21
16≤ x ≤ 24 µg/ml	1	0	1
<16 µg/ml	2	57	59
total	22	59	81

The amount of agreement between assay results was 93.8%. The amount of chance agreement between assay results was 60.1%. The kappa statistic for agreement compared with random chance agreement was 0.845 ± 1 standard error of 0.109. The probability of perfect agreement between assays was 0.15. Therefore, the agreement of assay results is not significantly different from perfect agreement (kappa = 1). This would support the hypothesis that the assay results are equivalent. The relative agreement of positive result was 0.864 (exact binomial 95% confidence interval 0.651 to 0.971). The relative agreement of negative result was 0.966 (exact binomial 95% confidence interval (0.883 to 0.996).

   b. *Matrix comparison:*
   Not performed. Only serum is used in the assay.
3. Clinical studies:
   a. *Clinical sensitivity:*
   Not performed
   b. *Clinical specificity:*
   Not performed
   c. *Other clinical supportive data (when a and b are not applicable):*

   Fifty-two samples from rheumatoid arthritis subjects and 45 samples from SLE subjects were purchased from a commercial supplier having an identified disease state. The mean rheumatoid factor result for the 52 subjects was 170 IU/ml. For the 45 SLE subjects, the mean double stranded DNA (dsDNA) titer was 1:16. Among rheumatoid arthritis subjects, 51.9% had assay results greater than 24 µg/ml while 44.2% had assay results less than 16 µg/ml. Of the SLE subjects, 33.3% had assay results greater than 24 µg/ml while 64.4% had assay results less than 16 µg/ml. Samples from 129 normal blood donors were also tested in the proposed assay. Among normal blood donors, 4.7% had assay results greater than 24 µg/ml while 93.8% had assay results less than 16 µg/ml.

4. Clinical cut-off:
   Not performed
5. Expected values/Reference range:

Positive assay result > 24 μg/ml circulating immune complexes
Equivocal assay result 16 to 24 μg/ml circulating immune complexes
Negative assay result < 16 μg/ml circulating immune complexes

M. Conclusion:

Based on data shown in the submission, the IBL C3d–CIC Test Kit is substantially equivalent to other devices regulated under 21 CFR §866.5240, product code CZW, Class II.