

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

A. 510(k) Number:

k072166

B. Purpose for Submission:

New device

C. Measurand:

Cystatin C

D. Type of Test:

Quantitative

E. Applicant:

The Binding Site Ltd.

F. Proprietary and Established Names:

Human Cystatin C Kit

G. Regulatory Information:

1. Regulation section:

21 CFR 866.1225

2. Classification:

II

3. Product code:

NDY

4. Panel:

Clinical Chemistry

H. Intended Use:

1. Intended use(s):

See indications for use below.

2. Indication(s) for use:

This Cystatin C Kit is intended for the quantitative determination of Cystatin C in human serum, lithium heparin plasma and EDTA plasma by turbidimetry using the SPAPLUS analyzer. Cystatin C measurements in serum and plasma are used as an aid in the diagnosis and treatment of renal diseases in conjunction with other laboratory and clinical findings.

3. Special conditions for use statement(s):

For prescription use only.

4. Special instrument requirements:

SPAPLus analyzer

I. Device Description:

The Human Cystatin C Kit is comprised of a latex reagent, calibrators, controls and a supplementary reagent. The latex reagent is comprised of monospecific sheep antibody coated onto polystyrene latex along with preservatives. The calibrator and controls consist of pooled human serum and are supplied in stabilized form.

The sponsor states that all donors of human serum supplied with the kit have been tested and found negative for hepatitis B surface antigen (HBsAg) and antibodies to human immunodeficiency virus (HIV1 and HIV2) and hepatitis C virus.

J. Substantial Equivalence Information:

1. Predicate device name(s):

Dade Behring N Latex Cystatin C reagents

2. Predicate K number(s):

k041878 and k003503

3. Comparison with predicate:

| Similarities | | |
|--------------------|---|---|
| Item | Binding Site Cystatin C | Dade Behring N LatexCystatin C (predicate device) |
| Intended use | An <i>in vitro</i> diagnostic assay for the quantitative determination of cystatin C in human serum, heparinized and EDTA plasma. Cystatin C measurements are used in the diagnosis and treatment of renal diseases | An <i>in vitro</i> diagnostic assay for the quantitative determination of cystatin C in human serum and heparinized plasma. Cystatin C measurements are used in the diagnosis and treatment of renal diseases |
| Differences | | |
| Item | Binding Site Cystatin C | Dade Behring N LatexCystatin C (predicate device) |
| Method | Particle enhanced turbidimetric immunoassay | Particle enhanced immunonephelometry |
| Instruments | SPA _{PLUS} analyser | Dade Behring BN systems |
| Calibrators | Binding Site cystatin C calibrator set | N protein standard UV |
| Controls | Binding Site cystatin C control set (2 levels) | N latex cystatin C control set (2 levels) |
| Measuring range | 0.4mg/L-7.35mg/L | Approx 0.23-8mg/L |
| Reference Interval | 0.58-1.03mg/L | 0.53 – 0.95mg/L |
| Antibodies | Sheep | Rabbit |

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

None referenced.

L. Test Principle:

The Cystatin C uses turbidimetric methods involves reaction with specific antiserum to form insoluble complexes. When light is passed through the suspension formed, a portion of the light is transmitted and focused onto a photodiode by an optical lens system. The amount of transmitted light is indirectly proportional to the specific protein concentration in the test sample. Concentrations are automatically calculated by reference to a calibration curve stored within the instrument.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. *Precision/Reproducibility:*

A precision study was conducted with the Human Cystatin C Kit assay according to the CLSI Evaluation of Precision Performance of Clinical Chemistry Approved Guideline (EP5-A). Three different samples using three different reagent lots using different analyzers were run twice a day for 21 days. Total, within-run, between-run and between-day precision was calculated for the three concentrations: high (75-95% of the upper limit of the measuring range), medium (medical decision) and low level (140-180% of the lower limit of the measuring range). The results are shown in the chart below.

| Precision | | | | | | |
|--------------|--------------------------------|-----|-----------------------------------|-----|---------------------------------|-----|
| | Low control mean 0.533 mg/L | | Median control mean 0.963 mg/L | | High control mean 6.029 mg/L | |
| | SD | CV% | SD | CV% | SD | CV% |
| Within run | 0.02 | 2.8 | 0.02 | 1.9 | 0.11 | 1.8 |
| Between run | 0.01 | 1.9 | 0.02 | 2.4 | 0.11 | 1.8 |
| Between -day | 0.05 | 8.8 | 0.05 | 5.4 | 0.29 | 4.7 |
| Total | 0.05 | 9.4 | 0.06 | 6.2 | 0.32 | 5.4 |

b. *Linearity/assay reportable range:*

The sponsor conducted linearity studies on clinical serum samples containing high levels of Cystatin c. The samples were serially diluted in 8 concentrations (samples 1-3) and 14 concentrations (sample 4) and each dilution was assayed twice on the SPAplus. The reportable range for the assay is 0.4 to the value of the high calibrator (target range 7.0-7.7mg/L) at the standard 1/10 dilution. The high calibrator value tested was 7.35 mg/L. The linearity was validated up to 7.24 mg/L (shown in sample 4 below). The four study results are shown in the table below.

| | N | Range mg/L | Slope | Intercept |
|----------|----|------------|--------|-----------|
| Sample 1 | 16 | 0.05-6.77 | 1.0017 | 0.01 |
| Sample 2 | 16 | 0.05-6.25 | 1.0059 | 0.03 |
| Sample 3 | 16 | 0.05-6.33 | >0.99 | 0.02 |
| Sample 4 | 28 | 0.05-7.24 | 1.0015 | 0.01 |

An antigen excess study was conducted to assess the linearity of the of high

Cystatin C concentrations. The sponsor tested calibrator material spiked to 2x and 3x the concentration of the high calibrator in order to look for a hook effect. The results were compared to a normal curve produced with the same calibrators. The sponsor observed no hook effect up to approximately 22 mg/L for Cystatin C.

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

There is not an internationally recognized reference standard for Cystatin C. Calibrator value assignment is controlled during kit production using set target values for the initial fluid of pooled human serum. An internal reference standard with a value of 1.7mg/L, assigned using the DAKO Cystatin C reagents on the Modular P, is used to validate curves and standardize kit calibration.

The assayed controls have assigned values with a +/-10% in-house tolerance and target values of 1mg/L for the (low) control and 4mg/L for a high control. The controls are assigned on calibration curves validated with the internal reference standard. The controls supplied with each lot are assayed on the kit, and information on the values obtained are supplied in a quality control certificate that accompanies the product that includes lot specific calibrator and control values.

Closed vial stability testing was conducted on three kit lots of Cystatin C. The kits were stored at 22 C for 1 week to mimic shipping conditions. The results support the sponsor's closed vial stability claim of 3 months.

Open vial stability testing was conducted with three reagent kits, controls and calibrators. The results support the sponsor's open vial stability of 3 months at 2-8 °C.

On-board stability was conducted with 100 test vials of Cystatin C reagent on the SPAPLUS. The results show that the control values were within +/-10% of the assigned value and show that the reagents are stable on board the SPAPLUS analyzer for up to 46 days.

d. Detection limit:

The sponsor conducted a lower limit of detection (LoD) study. LoD was estimated at the lower end of the calibration curve (~0.037 ng/mL) by measuring two samples with concentrations close to the lower limit ten times each. The sponsor states an LoD of 0.0518 mg/L.

The sponsor also conducted a limit of the blank (LoB) study. A blank sample was assayed 21 times against a calibration curve for Cystatin C on the SPAPLUS analyzer. The LoB was calculated (mean + 3SD) to be 0.0013 mg/L.

e. Analytical specificity:

The sponsor conducted an interference study by adding high concentrations of triglycerides (intralipid), hemoglobin, and bilirubin to test serum samples (low control diluted with diluent) that contained known concentrations of Cystatin C. Rheumatoid factor interference was also assessed. A purified sample with high concentration of RF (943 IU/mL) was added to a low control at a dilution of 2.95 to give an RF concentration of 320 IM/mL. The triplicate interference results were compared to saline blanks. Percentage interference was calculated as an interferent comparison with the saline blank. Deviations greater than 10% of the blank value were considered to interfere.

| | Mean | CV | % deviation from blank |
|---------------------------------|-------|------|------------------------|
| Saline blank | 0.45 | 0 | ----- |
| Bilirubin (200 mg/L) | 0.43 | 0 | -4.44 |
| Triglycerides (Intralipid 0.5%) | 0.44 | 0 | -2.22 |
| Hemoglobin (5 g/L) | 0.49 | 1.19 | +8.15 |
| Saline blank | 0.606 | 0.42 | ----- |
| Rheumatoid Factor | 0.608 | 0.40 | +0.33 |

The sponsor has stated in their labeling the following “Minimal assay interference by 200 mg/L bilirubin (-4.4%), 5 g/L Hemoglobin (+8.2%) and 0.5% intralipid (-2.2%) has been demonstrated using a sample containing 0.45 mg/L Cystatin C. Rheumatoid factor (320 IU/mL) showed no significant interference (+0.33%) when added to a serum sample containing 0.61 mg/L of Cystatin C.”

f. Assay cut-off:

Not applicable.

2. Comparison studies:

a. Method comparison with predicate device:

The sponsor conducted a method comparison study with the Dade Behring N Latex Cystatin C reagents. 63 serum samples ranging from 0.58 to 7.10 mg/L were tested with both assays in singlet on the SPAPLUS analyzer and the Dade Behring BNII analyzer. The passing-Bablok regression statistics for the 63 samples was $y=1.066x+0.037$ with a correlation coefficient of 0.996.

b. Matrix comparison:

A matrix comparison study was conducted with 53 plasma samples- 33

Lithium heparin that ranged from 0.57- 4.87 mg/L and 20 EDTA samples (from the method comparison above) that ranged from 0.45-0.98 mg/L) . The passing-Bablok regression statistics for the 53 plasma samples was $y=0.939x+ 0.144$ with a correlation coefficient with the predicate device/instrument of 0.991.

The sponsor conducted a paired matrix study to compare thirty-eight lithium heparin plasma and serum paired samples obtained from known/suspected renal patients. The sponsor also conducted a matrix study to compare twenty paired serum and EDTA plasma samples from normal subjects. The resulting linear equations are shown below.

| | Range (mg/L) | Equation | Correlation coefficient |
|---|--------------|--------------------|-------------------------|
| 38 paired lithium heparin plasma and serum samples | 0.57-4.81 | $y=1.0264x +0.002$ | 0.998 |
| 20 paired normal range serum and lithium heparin plasma samples | 0.48-0.98 | $y=1.03x +0$ | 0.999 |
| 20 paired normal range serum and EDTA plasma samples | 0.48-0.98 | $y=1.01x - 0.04$ | 0.979 |

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:

The sponsor conducted a normal range study using 127 normal sera (63 females and 64 males) obtained from healthy adult blood donors. The reference range interval was calculated using non-parametric statistics and represents the central 95% of the population. The following table is in the package insert.

| | Number (n) | Mean (mg/L) | Median (mg/L) | 95 Percentile Range (mg/L) |
|------------|------------|-------------|---------------|----------------------------|
| Cystatin C | 127 | 0.75 | 0.73 | 0.58-1.03 |

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.