510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I Background Information:

A 510(k) Number

K203184

B Applicant

Sebia

C Proprietary and Established Names

HYDRASHIFT 2/4 isatuximab

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
CFF	Class II	21 CFR 866.5510 - Immunoglobulins A, G, M, D, and E Immunological Test System	IM - Immunology

II Submission/Device Overview:

A Purpose for Submission:

New device

B Measurand:

Monoclonal immunoglobulins (IgG, IgA, IgM) and light chains (kappa, lambda) in serum

C Type of Test:

Qualitative

III Intended Use/Indications for Use:

A Intended Use(s):

The HYDRASHIFT 2/4 isatuximab kit is intended for the qualitative detection of monoclonal proteins in human serum by immunofixation electrophoresis. The HYDRASHIFT 2/4 isatuximab kit is to be used in conjunction with the HYDRAGEL IF kit and the semi-automated HYDRASYS 2 electrophoresis apparatus. The electropherograms are evaluated visually for the presence of specific reactions with the suspect monoclonal proteins. The HYDRASHIFT 2/4 isatuximab kit removes the isatuximab IgG kappa interference and enables the visual evaluation of the presence or absence of monoclonal proteins on the HYDRAGEL IF kits in patients who have received isatuximab therapy.

For In Vitro Diagnostic use. For Prescription Use Only.

B Indication(s) for Use:

Same as Intended Use(s) above.

C Special Conditions for Use Statement(s):

Rx – For Prescription Use Only

D Special Instrument Requirements:

HYDRASYS 2 electrophoresis apparatus

IV Device/System Characteristics:

A Device Description:

Reagents and materials supplied in the HYDRASHIFT 2/4 isatuximab kits include the following:

Items	(40 tests)
Anti-isatuximab antiserum (ready to use)	1 vial, 0.8 mL
Sample diluent (ready to use)	1 vial, 2.2 mL
Green applicators (ready to use)	2 packs of 10 (15 teeth)

Reagents required but not supplied include the following:

Items
HYDRAGEL 2 or 4 IF Acid Violet – Dynamic Mask
Antisera and fixative for immunofixation IF – Dynamic Mask
Or
HYDRAGEL 2 or 4 IF Acid Violet – Standard mask
Antisera and fixative for immunofixation IF – Standard Mask
And
Isatuximab CONTROL
Destaining solution
HYDRASYS wash solution
HYDRAGEL IF sample diluent
FLUIDIL

Dithiothreitol (DTT) diluent (IF/IT)
Dithiothreitol (DTT)
Beta-Mercaptoethanol

Equipment and accessories required but not supplied:

1. HYDRASYS 2 System SEBIA: HYDRASYS 2 SCAN, HYDRASYS 2, HYDRASYS 2 SCAN FOCUSING or HYDRASYS 2 FOCUSING.
2. HYDRASHIFT 2/4 Accessories which contain: one applicator carrier specific for the HYDRASHIFT isatuximab procedure and 2 guides for anti-isatuximab antiserum application on 15 teeth green applicator.
3. Wet Storage Chamber, supplied with HYDRASYS 2.
4. Container Kit supplied with HYDRASYS 2.
5. Template guide Bar SEBIA supplied with HYDRASYS 2.
6. Accessory Kit for HYDRASYS IF, or Dynamic mask.
7. Pipettes: 10 µL, 20 µL, 100 µL and 200 µL.

B Principle of Operation:

Abnormal bands in serum protein electropherograms (SPEP), primarily those in the beta globulin and gamma globulin zones, are suspected to be monoclonal proteins (M-proteins, paraproteins, monoclonal immunoglobulins). The presence of monoclonal protein as indicated by SPEP leads to testing of sample with an immunofixation (IF) technique to detect and identify the monoclonal protein components, using the HYDRAGEL IF assay.

Isatuximab is a human therapeutic IgG kappa monoclonal antibody and as such, during the clinical monitoring of patients treated with isatuximab, this antibody is visualized as a band detected by serum protein electrophoresis and immunofixation in the gamma region. The presence of isatuximab can be interpreted as an endogenous IgG kappa monoclonal protein, and thus confound the clinical interpretation of the assay results.

The HYDRASHIFT isatuximab immunofixation procedure performed on the HYDRAGEL IF 2/4 gel is based on the creation of an isatuximab/anti-isatuximab antibody complex that is shifted outside the gammaglobulin zone. With the HYDRASHIFT isatuximab procedure, the isatuximab/anti-isatuximab antibody complex is visualized in alpha-1 zone on the IgG and Kappa immunofixation tracks and the isatuximab interference is removed from the gamma zone. This can allow for the visualization of any endogenous IgG kappa monoclonal proteins by IF that may have been masked by the endogenous isatuximab.

V Substantial Equivalence Information:

A Predicate Device Name(s):

HYDRAGEL IF

B Predicate 510(k) Number(s):

K960669

C Comparison with Predicate(s):

Device & Predicate Device(s):	K203184	K960669
Device Trade Name	HYDRASHIFT 2/4 isatuximab	HYDRAGEL IF
General Device Characteristic Similarities		
Intended Use / Indications For Use	The HYDRASHIFT 2/4 isatuximab kit is intended for the qualitative detection of monoclonal proteins in human serum by immunofixation electrophoresis. The HYDRASHIFT 2/4 isatuximab kit is to be used in conjunction with the HYDRAGEL IF kit and the semi-automated HYDRASYS 2 electrophoresis apparatus. The electropherograms are evaluated visually for the presence of specific reactions with the suspect monoclonal proteins. The HYDRASHIFT 2/4 isatuximab kit removes the isatuximab IgG kappa interference and enables the visual evaluation of the presence or absence of monoclonal proteins on the HYDRAGEL IF kits in patients who have received isatuximab therapy. For In Vitro Diagnostic use. For Prescription Use Only.	The HYDRAGEL 1 IF, 2 IF, 4 IF and 9 IF kits are designed for detection of monoclonal proteins in human serum and urine by immunofixation electrophoresis. The kits are used in conjunction with the semi-automated HYDRASYS electrophoresis apparatus. The proteins, separated by electrophoresis on alkaline buffered agarose gels, are incubated with individual antisera that are specific against gamma (Ig G), alpha (Ig A) and mu (Ig M) heavy chains, and kappa (free and bound) and lambda (free and bound) light chains, respectively. After removing the non-reacted proteins, the immunoprecipitates are stained either with acid violet or amidoblack. The electrophoregrams are evaluated visually for the presence of specific reactions with the suspect monoclonal proteins.
Assay Principle	Agarose Gel Electrophoresis	Same
Software Program	IF Program	Same
Reagents • Gel Kit • Antisera Kit	 • Hydragel IF • Antisera and fixative for IF	 • Same • Same
Visualization of Target Protein	Acid Violet Gel Staining	Same
Antisera Specificity	Gamma (IgG), alpha (IgA), and mu (IgM) heavy chains; kappa (free and bound) and lambda (free and bound) light chains.	Same
Results	Qualitative	Same

Instrument	HYDRASYS electrophoresis apparatus.	Same
General Device Characteristic Differences		
Specimen Type	Human Serum	Human Serum, Human Urine
Device-Specific Reagents	Anti-isatuximab antibody	No anti-isatuximab antibody
Isatuximab Band	With the Hydrashift 2/4 procedure, the isatuximab band is moved from the gamma zone to the alpha zone.	Remains in the gamma zone.

VI Standards/Guidance Documents Referenced:

CLSI EP07 3rd Ed. *Interference testing in clinical chemistry; Approved Guideline – Third Edition*. Clinical and Laboratory Standards Institute.

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

Unless stated otherwise, all of the results described below met the manufacturer's pre-determined acceptance criteria.

1. Precision:

To evaluate precision, a panel of 10 serum samples were tested using the HYDRASHIFT 2/4 isatuximab procedure using either the HYDRAGEL 4 IF Acid Violet Standard Mask, or the HYDRAGEL 4 IF Acid Violet Dynamic Mask. The panel of 10 samples contained a normal isatuximab control, a normal serum control, and eight human serum samples with different monoclonal protein components. These eight serum samples were analyzed as native samples or with the addition of an isatuximab spike-in to verify the concordance between native and spiked samples. The samples tested for precision are described in the table below:

Sample #	Sample Type	Samples examined
1	Normal isatuximab control	Native serum
2	Normal serum control	Native serum
3	IgG kappa	Native serum and 1.0 g/L isatuximab-spiked serum
4	IgG lambda	Native serum and 1.0 g/L isatuximab-spiked serum
5	IgA kappa	Native serum and 1.0 g/L isatuximab-spiked serum
6	IgA lambda	Native serum and 1.0 g/L isatuximab-spiked serum
7	IgM kappa	Native serum and 1.0 g/L isatuximab-spiked serum
8	IgM lambda + IgA kappa	Native serum and 1.0 g/L isatuximab-spiked serum
9	Free Kappa component	Native serum and 1.0 g/L isatuximab-spiked serum
10	Free Lambda component	Native serum and 1.0 g/L isatuximab-spiked serum

Within-in run precision (repeatability):
Each of the above 10 samples was tested four times within the same gel. As noted above, sample #1 and #2 were run without the addition of isatuximab as controls. Samples #3─#10 were run as native samples without isatuximab or with an isatuximab spike-in (1.0 g/L). All samples were run with both the Standard Mask (SM) and Dynamic Mask (DM) for a total of eight assessments per sample. The gel results were read and evaluated by two readers for the presence or absence of monoclonal proteins and the presence of the isatuximab/anti-isatuximab antibody complex visualized in the alpha-1 zone on the IgG and kappa immunofixation tracks. The repeatability was evaluated visually for the concordance of monoclonal bands for each sample. The results showed 100% concordance for each sample, and between the same sample tested with the SM and DM, and between readers. The results are summarized below:

- Native samples were 100% concordant when visualized using the SM and DM.
- Replicates of isatuximab spiked samples were 100% concordant between SM and DM. Samples with monoclonal proteins other than the IgG kappa (isatuximab monoclonal component) were 100% concordant when compared with native samples.
- An isatuximab/anti-isatuximab band was observed in the alpha-1 zone on the IgG and kappa immunofixation tracks in isatuximab-spiked samples.

Between-day, between-lot/instrument precision:
To evaluate the total imprecision of between the gel, lot, and instrument components, the same 10 samples in the format described above were assessed on three HYDRASYS 2 instruments using one HYDRASYS 2/4 isatuxmab lot per instrument, over three working days. Samples were run with both the HYDRAGEL 4 IF Acid Violet Standard Mask (SM) and HYDRAGEL 4 IF Acid Violet Dynamic Mask (DM) for a total of nine assessments per sample for each method. The normal isatuximab control was assessed on nine gels over three working days, yielding a total of 27 assessments each using the SM and the DM. In addition, samples #3─#10 were tested with or without an isatuximab spike-in (1.0 g/L), as described in the repeatability study. All results were read by two readers. The results were evaluated visually for the concordance of monoclonal proteins and the presence of isatuximab/anti-isatuximab antibody complex in the alpha-1 zone for each sample. The results showed 100% concordance for all of the samples between days, instruments and lots, between SM and DM visualization methods, and between the two readers.

2. Linearity:

   Not applicable

3. Analytical Specificity/Interference:

   Endogenous interference:
   The performance of the HYDRASHIFT 2/4 Isatuximab procedure was evaluated in the presence of common interfering factors. Testing was performed using four samples: one control (isatuximab control) and three samples containing monoclonal components (IgG kappa; IgA kappa; and IgM kappa). The samples were assessed as native samples or isatuximab spiked samples (1.0 g/L), in the presence or absence of each of potential interference compounds with concentrations recommended based on CLSI EP07 3rd Ed. No

interference with the HYDRASHIFT 2/4 isatuximab procedure was detected for the following endogenous compounds up to the concentrations indicated below:

Endogenous Interfering Factor	Concentration Tested
Unconjugated Bilirubin	20 mg/dL (342µM)
Conjugated Bilirubin	20 mg/dL (342µM)
Triglycerides	3000 mg/dL (33.96 mM)
Hemoglobin	0.2 g/dL
Rheumatoid Factor	2000 IU/mL
Human anti-mouse Antibody (HAMA)	1:640 Titer

Exogenous interference:
The performance of the HYDRASHIFT 2/4 isatuximab procedure was evaluated in the presence of potential exogenous interfering factors. Testing was performed using four samples: one control (isatuximab control), and three samples with monoclonal components (IgG kappa; IgA kappa; and IgM kappa), native and spiked with isatuximab (1.0 g/L), and in the presence or absence of the below compounds based on CLSI EP07 3rd Ed Guideline. No interference was detected for the following exogenous compounds at or below the concentration indicated:

Exogenous Interfering Factor	Maximum Concentration Tested
Pomalidomide	1.0 mg/L
Carfilzomib	1.0 mg/L
Dexamethasone	1.0 mg/L
Ixazomib	1.0 mg/L
Cyclophosphamide	1.0 mg/L
Melphalan	1.0 mg/L
Prednisone	1.0 mg/L
Lenalidomide	4.0 mg/L
Bortezomib	2.0 mg/L

4. Assay Reportable Range:

   Not applicable

5. Traceability, Stability, Expected Values (Controls, Calibrators, or Methods):

   Traceability:
   The isatuximab control and the anti-isatuximab antibody are traceable to internal reference controls.

   Isatuximab CONTROL:
   The isatuximab control is manufactured from a pool of human sera complemented with isatuximab, which is a therapeutic human IgG kappa monoclonal antibody. The isatuximab control is in a stabilized lyophilized form and sold separately.

The freeze-dried isatuximab control vial must be stored refrigerated (at 2–8°C). It is recommended to run the isatuximab Control after each change of lot of a reagent (agarose gel, diluent or antisera).

Stability:
Except for the istuximab control and the anti-isatuximab antibody, the test reagents of HYDRASHIFT 2/4 isatuximab kit were cleared in K960669. All the components are stable until the expiration date indicated on the kit box or on the component labels.

Kit Stability: To determine the expiration date for the HYDRASHIFT 2/4 isatuximab kit components, including IgG anti-isatuximab antibody and HYDRAGEL IF diluent, an accelerated and real time stability studies were, or are currently being, performed. Briefly, IgG anti-isatuixmab reagent was stored for 10 weeks at 15–30°C in an accelerated shelf-life stability study, or stored for 6 months at 2–8°C in a real time stability study. Similarly, the stability of HYDRAGEL IF diluent reagent was stored for 3 months at 37°C in an accelerated stability study and for 6 months at 2–8°C or 30°C in a real time stability. The results from the accelerated stability studies support the following stability claims:

- IgG anti-isatuximab reagent is stable for up to 2 years at 2–8°C.
- HYDRAGEL IF diluent is stable for up to 3 years at 2–8°C or 3 years at 30°C.

Sample stability: To assess the stability of serum samples before their analysis using the HYDRASHIFT 2/4 isatuximab kit in conjunction with the HYDRAGEL 4 IF Acid Violet Dynamic Mask, six serum specimens with different monoclonal components (IgG kappa, IgA lambda, IgM kappa, IgG lambda, IgA kappa, and IgM lambda) were spiked with isatuximab antibody, and stored under various conditions (fresh, 10 days at 2–8°C, 3 days at 15–30°C, 3 months at −18 to −30°C) before being assessed for their monoclonal components via immunofixation electrophoresis (IFE). The tests were performed by one operator on three separate days and evaluated for 100% concordance between the reference and test condition. The results demonstrate that samples are stable under the following conditions:

- Samples are stable for 10 days when stored at 2–8°C
- Samples are stable for 3 days when stored at 15–30°C
- Samples are stable for 3 months when stored at −18°C to −30°C

6. Detection Limit:

Sensitivity:
A sensitivity study was performed in order to determine the threshold detection value for visualization of the isatuximab/anti-isatuximab antibody complex. Five serum samples containing different monoclonal gammaglobulinemia components were used in this study and shown as follows:

Sample	Sample Type	Total Band Protein Concentration
Sample A	High gamma globulin serum pool	
Sample B	Pooled normal serum	
Sample C	High IgG kappa	9.3 g/L
Sample D	High IgA kappa	38.2 g/L
Sample E	High IgM kappa	12.4 g/L

Each of above samples was spiked with different concentrations of isatuximab for six final concentration points (0.0 g/L, 0.1 g/L, 0.2 g/L, 0.3 g/L, 0.5 g/L, 1.0 g/L, and 3.0 g/L isatuximab). Each native and isatuximab-spiked serum sample was assessed by one operator in one run on three instruments utilizing both the SM and the DM methods. The results showed that the limit of detection for the isatuximab/anti-isatuximab antibody complex in the alpha-1 zone of the IgG and kappa tracks is 0.3 g/L.

7. Assay Cut-Off:

Not applicable. The detection value for visualization of the isatuximab / anti-isatuximab antibody complex in the alpha-1 zone of the IgG kappa tracks is 0.3 g/L.

B Comparison Studies:

1. Method Comparison with Predicate Device:

Three method comparison studies were conducted to evaluate the performance of the HYDRASHIFT 2/4 isatuximab kit in comparison to the predicate HYDRASYS IF device. One internal study and two complementary external studies were conducted.

Internal Study:
An internal study was conducted using 53 serum specimens, including 26 normal specimens and 27 specimens with monoclonal components, and analyzed using the HYDRASHIFT 2/4 isatuximab procedure in conjunction with either HYDRAGEL 2 or 4 IF Acid Violet Standard Mask or Dynamic Mask for a total of four different configurations in the study. The samples were evaluated by one operator using two separate HYDRASYS instruments over seven working days as native specimens or as isatuximab-spiked (1.0 g/L) specimens. The results demonstrated 100% concordance between the various samples and masking procedures.

The results were summarized as follows:

Initial Characterization	Isatuximab Spike-in Characterization	Number of serum samples
Normal	Normal	26
IgG kappa	IgG kappa	9
IgG lambda	IgG lambda	4
IgA kappa	IgA kappa	3
IgA lambda	IgA lambda	3
IgM kappa	IgM kappa	3
IgM lambda	IgM lambda	2
Free Kappa	Free Kappa	3
Total		53

External studies 1 and 2:
The external method comparison studies were performed at two U.S. sites. A collection of 203 native serum samples from healthy, untreated multiple myeloma, and isatuximab-treated multiple myeloma donors in complete remission (CR), very good partial response (VGPR),

partial response (PR), minimal response (MR), stable disease (SD), and progressive disease (PD) were evaluated sites 1 and 2. An additional sample was evaluated at site 2 for a total of 204 (203+1) samples. The studies were conducted using the HYDRAGEL 4 IF Acid Violet Standard Mask and Dynamic Mask kits.

The results were summarized as follows:

Initial Characterization	Isatuximab HYDRASHIFT 2/4 Characterization	Number of serum samples
2* IgA kappa	2* IgA kappa	1
2* IgG kappa	2* IgG kappa	2
2* IgM kappa + IgG lambda	2* IgM kappa + IgG lambda	1
2* Lambda	2* Lambda	1
IgA kappa	IgA kappa	17
IgA kappa + IgG kappa	IgA kappa + IgG kappa	1
IgA lambda	IgA lambda	10
IgA lambda + IgG lambda	IgA lambda + IgG lambda	1
IgA lambda + Lambda	IgA lambda + Lambda	4
IgG kappa	IgG kappa	45
IgG kappa + IgG lambda	IgG kappa + IgG lambda	5
IgG kappa + Kappa	IgG kappa + Kappa	1
IgG kappa + Lambda	IgG kappa + Lambda	2
IgG lambda	IgG lambda	29
IgG lambda + Lambda	IgG lambda + Lambda	5
Kappa	Kappa	2
Lambda	Lambda	8
Normal	Normal	68
Total		203

* An asterix (*) indicates multiple distinct molecular weight monoclonal gammaglobulin proteins. For example, 2* IgA kappa indicates that the sample contained two separate IgA kappa monoclonal gammaglobulin bands.

Each specimen was assessed for concordance between the disease reference and HYDRASHIFT 2/4 Isatuximab test analysis. If differences were observed between the two, the result was considered equivalent if the difference was attributable to the presence of isatuximab in the serum sample. For example, an IgA kappa sample would be considered concordant if it contained an isatuxumab monoclonal band when assayed using the HYDASYS 2/4 IF device, but the isatuximab interference was eliminated when assayed using the HYDRASHIFT 2/4 isatuximab test result, yielding an IgA kappa result. For the purpose of the study, the disease reference condition for the samples was predetermined by a clinician based on 2016 International Myeloma Working Group (IMWG) diagnostic criteria.

The results demonstrated that the samples were 100% concordant between the reference result and the HYDRASHIFT 2/4 isatuximab test result in both external studies.

Please note, while the purpose of the HYDRASHIFT 2/4 isatuximab assay is to detect endogenous serum monoclonal proteins in the presence of isatuximab by removing isatuximab interference, if isatuximab is present in the sample at a concentration below the

limit of detection, an isatuximab/anti-isatuximab band may not be observed. In addition, an isatuximab/anti-isatuximab band may not be reliably seen in sera obtained from isatuximab treated multiple myeloma patients that are not in complete (CR) or very good partial response (VGPR), such as partial response (PR), minimal response (MR), stable disease (SD) or progressive disease (PD) patients. This information may also be found in the package insert of the HYDRASHIFT 2/4 isatuximab kit.

2. Matrix Comparison:

Not applicable

C Clinical Studies:

1. Clinical Sensitivity:

Not applicable

2. Clinical Specificity:

Not applicable

3. Other Clinical Supportive Data (When 1. and 2. Are Not Applicable):

Not applicable

D Clinical Cut-Off:

Not applicable

E Expected Values/Reference Range:

Not applicable

VIII Proposed Labeling:

The labeling supports the finding of substantial equivalence for this device.

IX Conclusion:

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