

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY**

A. 510(k) Number:

k103757

B. Purpose for Submission:

New device

C. Measurand:

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

D. Type of Test:

Immunofixation Electrophoresis, Qualitative

E. Applicant:

Grifols USA, LLC

F. Proprietary and Established Names:

Immunofixation Electrophoresis Test using Interlab G26 Instrument

G. Regulatory Information:

1. Regulation section:

21 CFR §866.5510 Immunoglobulins (A, G, M, D, E) Immunological Test Systems

21 CFR §866.5550 Immunoglobulin (light chain specific) Immunological Test Systems

21 CFR §862.1630 Electrophoretic, Protein Fractionation

2. Classification:

Class II

3. Product code:

CFF – Immuno-electrophoretic, Immunoglobulins (G, A, M)

DFH – Kappa, Antigen, Antiserum, Control

DEH – Lambda, Antigen, Antiserum, Control

CEF – Electrophoretic, Protein Fractionation

4. Panel:

Immunology (82)

Clinical Chemistry (75)

H. Intended Use:

1. Intended use:

The Immunofixation Electrophoresis (IFE) Test using the Interlab G26 instrument is for the qualitative *in vitro* diagnostic separation and identification of abnormal immunoglobulins (IgG, IgA and IgM), and kappa and lambda light chains in human serum and concentrated urine using agarose gel supported on Mylar®. The test is useful as an aid in identifying suspected monoclonal proteins. The test result will be used in conjunction with clinical and other laboratory findings.

The Interlab IFE kits, (2, 4, 6 samples per gel) are intended to be used with the automated Interlab G26 electrophoresis analyzer in conjunction with the Easy Mask antisera application device.

2. Indication(s) for use:

Same as Intended Use.

3. Special conditions for use statement(s):
For prescription use only.
4. Special instrument requirements:
Automated Interlab G26 electrophoresis analyzer in conjunction with the Easy Mask antisera application device and Elfolab software system version 16.1.0. (This Elfolab software system version 16.1.0 is an upgrade of version 7.3.0 previously cleared with Interlab Microgel Electrophoresis system cleared under 510(k) number k053571).

I. Device Description:

The Immunofixation Electrophoresis (IFE) Test kit is packaged as a 20 (2 samples/ gel), 40 (4 samples/ gel) or 60 (6 samples/ gel) test kits. The kit contains ready-to-use components: 10 gel plates, 2 buffered sponges, acid violet stain (500 mL), washing solution for applicators (80 mL), washing solution 1 for IFE (80 mL), washing solution 2 for IFE (80 mL), IFE diluent (6 or 12 mL), disposable sample trays 26 (10 pcs) or 39 (10 pcs), blotters A (10 pcs), blotters L (10 pcs), blotters G (10 pcs), and 1 CD Package Insert.

The following components are required for the test but are not supplied in the test kit: de-stain solution pack (6x100 mL), fixative solution (1.5 mL) and specific antisera Anti-Human-IgG (1 mL), Anti-Human-IgA (1 mL), Anti-Human-IgM (1 mL), Anti-Human-Kappa (1 mL) and Anti-Human-Lambda (1 mL).

The Automated Interlab G 26 Electrophoresis Analyzer provides protein fraction separation using the principle of electrophoresis; separation involving electrically charged molecules that orient and migrate at different rates when subjected to an electric field. The migration is performed at a constant temperature, obtained through the use of a Peltier device, on assay specific buffered agarose gel plates. The agarose gel medium provides a support and molecular sieve allowing the different fractions to migrate to points based on individual net charges.

After electrophoresis, the gel is heated to “fix” the focalized proteins, followed by assay specific staining, destaining, washing and drying. All methods utilizing a quantitative assessment are immediately processed using the on-board densitometer. The signal obtained for each specimen result is sent to the personal computer and presented using the Elfolab interpretive software. The Interlab G26 instrument is pre-programmed with all necessary firmware to conduct and manage all phases of the analytical procedures used in Interlab manufactured assays. The instrument works in conjunction with a personal computer using Windows® based software featuring pull down menus and intuitive icons for easy instrument control, selection of analytical methods, and data evaluation.

Instrument design includes: automated application of the samples on the agarose gel; electrophoretic migration; “heat fixing” proteins to the gel; gel staining/ destaining/ drying; densitometric reading of the gel; and data transmission and processing.

The Interlab Easy Mask Antisera Applicator Device is a standalone electronic instrument identical to the peltier contained within the Interlab G26 and is designed to work in conjunction with the Interlab G26. This device allows for accurate and

simplified processing of various electrophoretic agarose gel assays that require reagent or antisera overlays. This device allows for easier user processing of the manual steps necessary in antisera type assays (IFE, BJ, Penta) by allowing the user to work unencumbered from mechanical arms and instrument covers. The Easy Mask provides functions identically to the processing steps used in other agarose gel systems that require the user to perform the manual antisera steps directly on the instrument. Through the use of a Peltier and vacuum pump, the temperature across the surface of the gel remains at a precise and controlled temperature, thus improving assay quality and decreasing processing time. Instrument is designed to receive the Gel Holder from the Interlab G26 Instrument. The Gel Holder is inserted into a template which places the gel in direct contact with the peltier plate assuring uniform and controlled temperature over the entire surface of the gel. Perfect adhesion of the gel to the peltier plate is accomplished using a vacuum pump. Assay specific application masks are placed in the frame, which provide precise application of the antisera or reagents during the incubation phase. After the incubation phase is complete, the frame locks over the gel providing a calibrated heated press to blot away un-bound antisera and reagents. When processing is completed on the Easy Mask, the operator places the Gel Holder back in the parking location on the Interlab G26 for the final steps of the analysis.

J. Substantial Equivalence Information:

1. Predicate device name(s) and 510(k) number(s):
Sebia Hydragel Kit (k960669)
2. Comparison with predicate:

Similarities		
Item	Device	Predicate
Methodology/ Technology	Agarose Gel Immunofixation Electrophoresis	Same
Sample Type	Serum and Urine	Same
Antisera Specificity	Antibody specificity to heavy chains (IgG, IgA, IgM) and to light chains (kappa, lambda).	Same
Results Interpretation	Qualitative	Same

Differences		
Item	Device	Predicate
Intended Use	Qualitative <i>in vitro</i> diagnostic separation and identification of abnormal immunoglobulins (IgG, IgA and IgM), and kappa and lambda chains in	Detection of monoclonal proteins in human serum and urine.

Differences		
Item	Device	Predicate
	human serum and concentrated urine as an aid in identifying suspected monoclonal proteins.	
Instrument	Automated Interlab G26 electrophoresis analyzer. Instrumentation includes the Easy Mask antisera application device and the Elfolab software system.	Semi-automated HYDRASYS electrophoresis apparatus
Serum sample volume	30 µL	10 µL
IFE Antisera Storage	2 – 8°C	2 - 8°C or room temperature (15 - 30°C)
Lowest Detectable Limit	Serum: IgGλ: 0.05 g/L IgAκ: 0.03 g/L IgMλ: 0.06 g/L Urine: IgGκ: 0.028 g/L IgAλ: 0.050 g/L IgMκ: 0.062 g/L	Serum: 12 - 25 mg/dL Urine: <5 mg/dL

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

CLSI EP-7A: Interference Testing in Clinical Chemistry

L. Test Principle:

The principle of Immunofixation electrophoresis (IFE) is based on the protein separation at alkaline pH. After protein migration, one of the gel lanes is treated with fixative to fix all proteins to provide a reference pattern and the other gel lanes are treated with specific antisera. Reaction with patient samples results in the formation of insoluble antigen-antibody complex that produces a band of precipitate when the proportion of antibodies and antigen is appropriate. The precipitation rate depends on temperature, pH, and ionic strength of the solution. The gels are washed to remove excess unprecipitated proteins, then blotters are applied to remove excess buffer twice. Gels are then stained with acid violet, de-stained and dried.

The comparison of the positions of immunofixed bands and that of the suspected monoclonal band in the reference pattern allows assessment of the biochemical identity of the protein. IFE usually displays discrete and sharply focused bands with monoclonal proteins in monoclonal gammopathies. In polyclonal gammopathies a diffuse zone is shown in the corresponding antiserum in contrast to the sharp band observed in monoclonal gammopathies.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. *Precision/Reproducibility:*

Within-Run Reproducibility: Nine serum samples were run two times in four replicates within a run. The nine samples were comprised of one normal sample and one each of the 8 subtypes including IgGκ, IgGλ, IgAκ, IgAλ, IgMκ, IgMλ, free λ, and free κ. According to the identified monoclonal protein, the concordant and reproducible within-run results were obtained.

Tabulated below are the immunoglobulin concentrations levels studied:

Subtype	Normal Range	Number of samples		Sample Range
		Series 1	Series 2	
IgG	620 – 1400 mg/dL	2	2	779 – 2040 mg/dL
IgA	80 – 350 mg/dL	2	2	551 – 2012 mg/dL
IgM	45 – 250 mg/dL	2	2	225 – 450 mg/dL
κ free	<20 mg/L	1	1	54 – 169 mg/L
λ free	<15 mg/L	1	1	24 – 48 mg/L

Between-Run Reproducibility: Ten serum samples were run 4 times and repeated in 3 runs on same batch of reagents. The ten samples were comprised of 2 normal samples and one each of the 8 subtypes including IgGκ, IgGλ, IgAκ, IgAλ, IgMκ, IgMλ, free λ, and free κ. The total Ig levels were between 1 g/L to 5 g/L. According to the identified monoclonal component characterization, the concordant and reproducible between-run results were obtained.

Inter-Lot Reproducibility: Nine samples were run on three different antisera lot numbers nine times. The nine samples were comprised of one normal and one each of the 8 subtypes including IgGκ, IgGλ, IgAκ, IgAλ, IgMκ, IgMλ, free λ, and free κ. The total Ig levels were between 1 g/L to 5 g/L. According to the identified monoclonal component characterization, the concordant and reproducible between-run results were obtained.

b. *Linearity/assay reportable range:*

Not applicable.

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

No reference standards and method available.

Stability: Real time stability studies were performed on four packs of IFE Kit and four packs of antisera kit on IgGκ, IgGλ, IgAκ, IgAλ, IgMκ, and IgMλ samples. Kits were stored at room temperature and tested every 6, 12, 18, and 24 months. The studies support the antisera stability for 24 months. Open kit and open antisera vial studies were performed on four packs of IFE Kit and four packs of antisera kit. IFE kits were stored at room temperature (15-30°C) and antisera kits were stored at 2 – 8°C. Both open IFE kits and antisera kits were tested at 6 months. The studies support the open kit and open vial stability for 6 months.

d. *Detection limit:*

IFE Test detection limit was performed with twofold serial dilutions from 1:2 through 1:256. The detection limit of the Immunofixation Electrophoresis Kit was determined by the lowest concentration of the monoclonal component examined by visual inspection.

Results are listed below:

(i) Serum:

Sample No.	Type	Concentration (g/L) (in original sample)	Detection limit (g/L)
1	IgG λ	3.0	0.050
2	IgA κ	62.0	0.030
3	IgM λ	7.2	0.060

(ii) Urine

Sample No.	Type	Concentration (g/L) in original sample	Detection limit (g/L)
1	Ig A λ *	6.4	0.028
2	Ig G κ *	14.7	0.050
3	Ig M κ *	2.0	0.062
4.	κ Free	4.7	0.140
5.	λ Free	4.0	0.060

*Spiked with serum samples

e. *Analytical specificity:*

Interference: Twelve serum samples comprised of one normal and eleven pathological sera (1 IgG κ , 2 IgG λ , 1 IgA κ , 1 IgG λ /IgA λ , 1 IgM κ , 1 IgM λ , 2 IgG κ / free κ , 1 free λ , 1 IgG λ / free λ , with concentration levels of 7 IgG from 779-2040, 2 IgA from 551-2012 and 2 IgM from 225-450mg/dL). These samples were spiked with endogenous substances namely bilirubin, hemoglobin, and triglycerides (lipids) and tested on the new device using Interlab G26 Instrument. No effects were observed with bilirubin (up to 20 mg/dL), hemoglobin (up to 500 mg/dL) and triglyceride (up to 220 mg/dL).

Eight urine samples comprised of one normal and seven pathological urine samples (1 IgG κ , 3 free κ , 2 free λ , 1 IgG λ / free λ) with concentration ranges from 39-4730 mg/dL. These samples were spiked with prepared hemolysate with 8.8 g/L hemoglobin (hemolysate was from whole blood). No effects were observed on the monoclonal bands in spite of greater background shown due to residual serum proteins from hemolysate.

Applicator Carryover: Both pathological and normal samples were processed to simulate a standard day of laboratory analysis on the Interlab G26 instrument. Normal samples were processed immediately following pathological samples. The final gel run was processed using saline. Gels were examined visually to check for the appearance of bands as expected in known samples. Successive gels were examined visually to check for the presence or absence of the previous sample appearing as carryover. The final gel was examined visually to check for the absence of all protein bands. No sample carryover by the applicator was observed in the study after washing procedure protocol.

- f. *Assay cut-off:*
Not applicable
2. Comparison studies:
a. *Method comparison with predicate device:*

Serum Sample Study: A total of 122 serum samples (75 pathological and 47 normal) were performed on Grifols Immunofixation Electrophoresis (IFE) Test using Interlab G26 Instrument and on the Sebia Hydragel Immunofixation (IFE) violet Kit. The study demonstrated 100% agreement between the two methods. The numbers of samples for different subtypes in the study are listed below.

Sample Types	Quantity	Sample Types	Quantity
Normal/Absent	47	IgGκ & IgMλ	2
IgGκ	27	IgGκ & IgMκ	1
IgGλ	12	IgAλ & λ free	1
IgAκ	6	IgGκ & λ free	3
IgAλ	2	IgGλ & λ free	2
IgMκ	9	IgGκ & κ free	2
IgMλ	5	λ free	1
IgGκ & IgAλ	1	κ free	1
		Total	122

Concentration levels of the serum samples according to the subtypes were as follows:

Number of Samples	Concentration	Subtype	Total Serum Protein (g/dL)
32	< 0.8 g/dL	IgMκ/λ, IgAκ/λ, IgGκ/λ	8 – 9,5
26	0.8 – 2 g/dL	IgMκ/λ, IgAκ/λ, IgGκ/λ	10 – 10,9
15	> 2 g/dL	IgMκ/λ, IgAκ/λ, IgGκ/λ	11 – 13
1	>20 mg/L	κ free	11
1	>20 mg/L	λ free	9
47	Not applicable	Negative	6 – 8
Total 122			

Urine Samples Study: A total of 45 concentrated urine samples (6 normal and 39 pathological: 7 polyclonal, 11 free Kappa, 15 free Lambda, 1 Lambda/ free Kappa, 1 Kappa/ free Lambda, 4 IgGκ) were performed on Grifols IFE Test using Interlab G26 Instrument and on the Sebia Hydragel Immunofixation (IFE) violet Kit. The study demonstrated 100% agreement between the two methods. The numbers of samples for different subtypes according to immunoglobulin concentration levels in this study are listed below.

Number of Samples	Concentration	Subtype	Total Urine Protein
28	>20 mg/L	κ free and λ free	200 – 1000 mg/24 hr
7	>100 mg/L	Polyclonal	1000 – 2000 mg/24 hr
4*	< 0.8 g/dl	IgG κ	8 – 9.5 g/ dL
6	Not applicable	Negative	0-200 mg/ 24 hr
Total 45			

*24 hr normal urine sample spiked with serum samples

Kit Configuration Comparison: The IFE test is available with three configurations for 2 (SRE627K), 4 (SRE628K), and 6 (SRE639K) samples.

Comparison between kit configuration 2 samples (SRE627K) and 4 samples (SRE628K) were performed on forty six serum samples (19 normal and 27 pathological containing monoclonal components: 10 IgG κ , 5 IgG λ , 3 IgA κ , 2 IgA λ , 5 IgM κ , 2 IgM λ) were tested and were found to be comparable. The immunoglobulin concentrations for different sub-types were as follows:

Number of Samples	Concentration	Subtype
14	< 0.8 g/dL	IgM κ/λ , IgA κ/λ , IgG κ/λ
8	0.8 – 2 g/dL	IgM κ/λ , IgA κ/λ , IgG κ/λ
5	>2 g/dL	IgM κ/λ , IgA κ/λ , IgG κ/λ
19	Not applicable	Negative
Total 46		

Comparison between kit configuration 4 samples (SRE628K) and 6 samples (SRE639K) were performed on thirty six serum samples (10 normal, 6 polyclonal and 20 pathological containing monoclonal components: 5 IgG κ , 4 IgG λ , 3 IgA κ , 3 IgA λ , 3 IgM κ , 2 IgM λ) were tested and were found to be comparable. The immunoglobulin concentrations for different sub-types were as follows:

Number of samples	Concentration	Subtype
10	<0.8 g/dL	IgM κ/λ , IgA κ/λ , IgG κ/λ
7	0.8 – 2 g/dL	IgM κ/λ , IgA κ/λ , IgG κ/λ
3	>2 g/dL	IgM κ/λ , IgA κ/λ , IgG κ/λ
16	Not applicable	No monoclonal proteins
Total 36		

- b. *Matrix comparison:*
Not applicable.
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:

Same as Expected values/Reference range.

5. Expected values/Reference range:

Absence of monoclonal immunoglobulins.

References cited: ‘*Tietz Fundamentals of Clinical Chemistry*’, Carl A. Burtis, Edward R. Ashwood, MD, page 346, Fifth Edition,(1996) and ‘*Primer of Immuno-electrophoresis with interpretation of Phathologic Human Serum Patterns*’, S. Karger, pp 6-29. Arcquembourg, P.C.; Salvaggio J.E.; Bicker J.N. (1970).

N. Instrument Name:

Automated Interlab G26 electrophoresis analyzer in conjunction with the Easy Mask antisera application device

O. System Descriptions:

1. Modes of Operation:

Protein separation and detection of the separated proteins on Immunofixation gels (2, 4, or 6 samples per gel).

2. Software:

The Interlab operating system Elfolab software version 16.1.0 is designed to work with the Interlab G26 instrumentation. FDA has reviewed and found the following software documents acceptable: Device Hazard Analysis, SRS, Architecture Design Chart, SDS, Traceability Analysis, Software development Environment Description, Summary of Verification and Validation Results, Revision Level of history, Unresolved anomalies (bugs and defect) and Operation Manual for the Serum and Urine IFE line of product types.

3. Specimen Identification:

Not applicable

4. Specimen Sampling and Handling:

Not applicable

5. Calibration:

Not applicable.

6. Quality Control:

Not Applicable

P. ~~Other Supportive Instrument Performance Characteristics Data Not Covered In The “Performance Characteristics” Section above:~~

None.

Q. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

R. Conclusion:

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