510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:

k120169

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, Inc

F. Proprietary and Established Names:

Immunofixation Electrophoresis Test using Interlab G26 v2.0 Instrument

G. Regulatory Information:

1. Regulation section:

   21 CFR §866.5510 Immunoglobulins A, G, M, D and E Immunological Test System
   21 CFR §866.5550 Immunoglobulin (light chain specific) Immunological Test System
   21 CFR §862.1630 Protein (fractionation) Test System

2. Classification:

   Class II

3. Product code:

   CFF – Immunoelectrophoretic, 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(s):

   The Immunofixation Electrophoresis (IFE) Test using the Interlab G26 v2.0 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 v1.0 and v2.0 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 only.

4. Special instrument requirements:

   Automated Interlab G26 v2.0 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 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.

Test components required for the test but not supplied in 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 antisera are from Grifols which were cleared in k103757.

The Automated Interlab G26 v2.0 Electrophoresis Analyzer includes automated pipetting of samples from barcode sample tubes in a rack and dilutes the samples into a sample tray for dispensing onto an agarose gel; protein fraction separation (using the principle of gel electrophoresis); followed by assay specific staining, destaining, washing and drying. The Interlab G26 v2.0 instrument is pre-programmed with firmware to conduct and manage all phases of assay analytical procedures, including instrument control, selection of analytical

methods, and data evaluation. The Interlab Easy Mask Antisera Applicator Device is a standalone electronic instrument designed to work in conjunction with the Interlab G26 v2. This device allows for processing of electrophoretic agarose gel assays using reagent or antisera overlays.

J. Substantial Equivalence Information:

1. Predicate device name(s) and 510(k) number(s):
   Immunofixation Electrophoresis Test using Interlab G26 v1.0 Instrument, k103757.

2. Comparison with predicate:

Similarities		
Item	Device	Predicate
Intended Use	For detection and the characterization of monoclonal proteins	Same
Antisera Specificity	Antibody specificity to heavy chains (IgG, IgA, IgM) and to kappa (bound) and lambda (bound) light chains.	Same
Antisera Storage	2 – 8ºC	Same
Methodology	Gel electrophoresis	Same
Technology	Agarose gel Electrophoretic migration with immunofixation	Same
Sample Type	Serum and urine	Same
Sample Size	30 µL	Same
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	Same
Results Interpretation	Qualitative	Same

Differences		
Item	Device	Predicate
Instrument	Interlab G26 version 2.0	Interlab G26 version 1.0
Application of Samples on the Agarose Gel	Automated	Manual
Patient ID from Tube Barcode	Automated	Manual
Primary Tube Sampling	Automated	Manual
Sample Dilutions	Automated	Manual

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

CLSI EP-7A: Interference Testing in Clinical Chemistry; Approved Guideline.

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 un-precipitated 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.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Within-Run Reproducibility: Eight serum samples were run in two series in six replicates within a run. Each series had one normal serum and seven sera with confirmed monoclonal bands representing specific subtypes were run. For series one, IgGκ, IgGλ, IgAκ, IgAλ, IgMκ, IgMλ, κ free were tested and for series two IgGκ, IgGλ, IgAκ, IgAλ, IgMκ, IgMλ, λ free were tested. 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	Series 1 Sample Number	Series 2 Sample Number	Sample Range
IgGκ/ IgGλ	620 – 1400 mg/dL	2	2	957 – 201,890 mg/dL
IgAκ/ IgAλ	80 – 350 mg/dL	2	2	142 – 1,840 mg/dL
IgMκ/ IgMλ	45 – 250 mg/dL	2	2	118 – 1,280 mg/dL
κ free	<20 mg/L	1	0	36 – 707 mg/L
λ free	<15 mg/L	0	1	31 – 717 mg/L

Between-Run Reproducibility: Eighteen serum samples were run 3 times and repeated in 3 runs using the same batch of reagents. The 18 samples comprised of 3 normal samples and 1-3 samples each of the 8 subtypes, including 3 IgGκ, 3 IgGλ, 2 IgAκ, 2 IgAλ, 2 IgMκ, 1 IgMλ, 1 free λ, and 1 free κ. The total Ig levels were between 1 g/L and 5 g/L. According to the identified monoclonal component characterization, concordant and reproducible between-run results were obtained.

Lot-to-Lot Reproducibility: Nine samples were run with three different antisera lot numbers nine times. The 9 samples 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, concordant and reproducible between-run results were obtained.

b. Linearity/assay reportable range:

Not applicable.

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

Stability: Real time stability studies were performed using four packs of IFE Kit and four packs of antisera kit on IgGk, 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 IFE kit stability of 24 months. Open kit studies were performed using four packs of IFE Kit and four packs of antisera kits. Open IFE kits were stored at room temperature (15 – 30ºC) and open antisera kits were stored at 2 – 8ºC. Testings were performed at day 1 and 6 months. Results support the open kit stability for 6 months.

d. Detection limit:

IFE Test detection limit was performed on three pathological samples with twofold serial dilutions from 1:2 through 1:256. Dilutions were made from the dilutor on the G26v2.0 (with auto sampler on board). 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	IgGλ	20.5	0.05
2	IgAκ	22.5	0.03
3	IgMλ	12.8	0.06

(ii) Urine:

Sample No.	Type	Concentration (g/L) in original sample	Detection limit (g/L)
1	IgAλ*	0.46	0.028
2	IgGκ*	1.83	0.050
3	IgMκ*	0.50	0.062
4	κ Free	4.70	0.140
5	λ Free	4.00	0.060

*Spiked with serum samples.

e. Analytical specificity:

Interference:

Twelve serum samples comprised of one normal and eleven pathological sera (1 IgGκ, 2 IgGλ, 1 IgAκ, 1 IgGλ/IgAλ, 1 IgMκ, 1 IgMλ, 2 IgGκ/free κ, 1 free λ, 1 IgGλ/free λ, with concentration levels of 7 IgG from 779 – 2,040 mg/dL, 2 IgA from 551 – 2,012 mg/dL and 2 IgM from 225 – 450 mg/dL). These samples were spiked with endogenous substances namely bilirubin, hemoglobin, and triglycerides (lipids) and tested on the new device using Interlab G26 v.2 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 IgGκ, 3 free κ, 2 free λ, 1 IgGλ/free λ) with concentration ranges from 39 – 4,730 mg/dL. These samples were spiked with prepared hemolysate prepared from whole blood with 8.8 g/L hemoglobin. No effects were observed on the monoclonal bands in spite of greater background shown due to residual serum proteins from hemolysate.

Applicator and sample probe 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 102 serum samples (92 pathological and 10 normal) were performed on Grifols Immunofixation Electrophoresis (IFE) Test using both Interlab G26 v1.and G26 v2.0 Instruments. The study demonstrated 100% agreement between the two methods. The number of samples and immunoglobulin concentrations for different subtypes are shown in the following tables:

Sample Types	Quantity
Normal/Absent	10
IgGκ	26
IgGλ	21
IgAκ	6
IgAλ	10
IgMκ	12
IgMλ	8

Sample Types	Quantity
IgGλ & IgAλ	1
IgGλ & IgMλ	1
IgGκ & IgMλ	1
IgGλ & λ free	1
IgMκ & IgMλ	1
IgGκIgGλ	2
λ free	2
Total	102

Number of samples	Ig Concentration	Subtype	Total Serum Protein (g/dL)
35	< 0.8 g/dL	IgMκ/λ, IgAκ/λ, IgGκ/λ	8 – 9.5
1	< 0.8 g/dL	Bi-clonal: IgGλ & IgAλ	8 – 9.5
1	< 0.8 g/dL	Bi-clonal: IgGλ & IgMλ	8 – 9.5
1	< 0.8 g/dL	Bi-clonal: IgGκ & IgMλ	8 – 9.5
30	0.8 – 2 g/dL	IgMκ/λ, IgAκ/λ, IgGκ/λ	10 – 10.9
1	0.8 – 2 g/dL	Biclonal: IgMκ & IgMλ	10 – 10.9
1	0.8 – 2 g/dL	Bi-clonal: IgGλ & λ free	10 – 10.9
17	>2 g/dL	IgMκ/λ, IgAκ/λ, IgGκ/λ	11 – 13.0
2	>2 g/dL	Bi-clonal: IgGκ & IgGλ	11 – 13.0
3	>20 mg/L	λ free	11.0
10	Not applicable	Negative	9.0

Urine Samples Study:

A total of 64 concentrated urine samples were performed on Grifols IFE Test using Interlab G26 v1 and G26 v2Instruments. They comprised of 8 normal and 56 pathological samples, which included 5 polyclonal, 29 kappa free, 8 lambda free, 1 IgGκ/kappa free, 1 IgG λ/lambda free, 1 IgG λ and10 IgGκ. Of the 64 samples, 6 samples had no detectable total protein, 39 had <2,000 mg/24 hr urine total protein, and 19 samples had >2,000 mg/24hr urine total protein. The study demonstrated 100% agreement between the two methods.

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

Comparison between the three kit configurations were performed using 38 serum samples including 32 pathological samples containing monoclonal components (12 IgGκ, 4 IgGλ, 4 IgAκ, 1 IgAλ, 7 IgMκ, 3 IgMλ, 1 Biclonal IgGλ) and 6 normal samples. The results from testing with different kit configurations were found to be comparable. The immunoglobulin concentrations for different sub-types were as follows:

Number of Samples	Concentration	Subtype
21	< 0.8 g/dL	IgMκ/λ, IgAκ/λ, IgGκ/λ
5	0.8 – 2 g/dL	IgMκ, IgAκ, IgGκ
1	0.8 – 2 g/dL	Bi-clonal: IgGκ & IgGλ
5	>2 g/dL	IgGκ/λ, IgMκ/λ
6	n.a.	Negative
Total = 38		

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 Immunoelectrophoresis with interpretation of Pathologic Human Serum Patterns', S. Karger, pp 6-29. Arcquembourg, P.C.; Salvaggio J.E.; Bicker J.N. (1970).

N. Instrument Name:

Interlab G26 v.2.0 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 v. 2.0 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.

   Yes ____X____ or No _____

3. Specimen Identification:

   Barcode sample identification available on version 2 with on-board sample pipette.

4. Specimen Sampling and Handling:

   Samples should be collected using standard techniques following good laboratory practice. Fresh serum and urine samples are recommended for the tests. If necessary, serum and urine samples can be stored tightly capped up to one week at 2 - 8°C and up to one month if stored at 20°C.

   Serum
   Dilute serum sample as follows:
   IgG Track: 1:8 (10 µL of sample + 70 µL of Immunofixation Diluent SRE 153M)
   Other tracks: 1:5 (50 µL of sample + 200 µL of Immunofixation Diluent SRE 153M)

For an abnormal band concentration of more than 15 g/L, dilute the sample with the Immunofixation Diluent, to a final concentration between 2.5-5 g/L. For the IgG track, dilute the sample with the Immunofixation Diluent, to a final concentration between 1-3 g/L.
If the total immunoglobulin concentration is less than 5 g/L, it is recommended to use lower dilutions of the samples. Distribute diluted serum in all the wells. Analyze within 10 minutes after samples are added to the sample tray.

Concentrated Urine
Most urine samples must be concentrated. Concentrate the urine to a final total protein value of about 5 g/L or to a total immunoglobulins concentration of about 1 g/L. If the total protein or immunoglobulin concentration value is not available, concentrate the sample in the range of 25X – 80X. Centrifuge the samples before use. If salt concentration is high, dialyze the urine.
Distribute concentrated urine in all the wells. Analyze within 10 minutes after samples are added to the sample tray.

The immunofixation analysis on the Interlab G26, with and without the integrated sampler, is a semi-automated procedure.

Interlab G26 Without On-Board Sampler - application of the samples on the agarose gel plate, electrophoretic migration, washing, drying, staining, destaining and final gel drying.

Interlab G26 With On-Board Sampler – Sample Tray preparation from sample primary tubes including dilutions, application of the samples on the agarose gel plate, electrophoretic migration, washing, drying, staining, destaining and final gel drying. Some steps require external treatment of the agarose gel after the electrophoretic migration: application of the fixative solution and the antisera, gel incubation at 20°C, gel blotting at 40°C and gel denaturation at 60°C.

5. Calibration:

   Not applicable

6. Quality Control:

   With Elfolab, it is possible to calculate quality control for both precision and accuracy of results. The steps to perform the quality control are the following:

   - Choose the traces that will be considered useful for the C.V. (coefficient of variation) calculation (Precision);
   - Create a database where the data will be inserted;
   - Save the data into the database that was just created;
   - Control the values: Average, Variance, Standard Deviation, CV and Levey-Jennings curves.

   The user is guided through an automated, menu-driven QC process.

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.