510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

A. 510(k) Number:

k131330

B. Purpose for Submission:

New device

C. Measurand:

Anti-Nuclear Antibodies (ANA)

D. Type of Test:

Manual, qualitative enzyme linked immunosorbent assay (ELISA)

E. Applicant:

Gold Standard Diagnostics Corporation

F. Proprietary and Established Names:

Proprietary Name: Gold Standard Diagnostics Anti-nuclear Antibody (ANA) Screen ELISA Test Kit

Established Name: Antinuclear Antibody (Enzyme-Labeled), Antigen, Controls

G. Regulatory Information:

1. Regulation section:

   21 CFR §866.5100 Antinuclear antibody immunological test system

2. Classification:

   Class II

3. Product code:

   LJM, antinuclear antibody (enzyme-labeled), antigen, controls

4. Panel:

   82 (Immunology)

H. Intended Use:

1. Intended use(s):

   The Gold Standard Diagnostics Antinuclear Antibody (ANA) Screen ELISA Test Kit is a qualitative assay for the detection of ANAs in human serum. The assay collectively detects in one well ANAs against double stranded DNA (dsDNA), SSA (Ro60 and Ro52), SSB (La), Sm, Sm/RNP, Scl-70, Jo-1, Ribosomal P, and Centromeric antibodies along with sera positive for immunofluorescent HEp-2 ANAs.

   The assay is used as an aid in the diagnosis of Systemic Lupus Erythematosus, Mixed Connective Tissue Disease, Sjögren's Syndrome, Progressive Systemic Sclerosis, and Polymyositis/Dermatomyositis, and should be used in conjunction with other laboratory tests and clinical findings.

2. Indication(s) for use:

   Same as Intended Use

3. Special conditions for use statement(s):

   Prescription use only

4. Special instrument requirements:

   Microwell plate reader capable of measuring OD at 450 nm and at 620 nm for dual wavelength readings.

I. Device Description:

The device, as described in the labeling, is a kit composed of the following reagents:

1. One microtiterplate consisting of 96 wells with coated antigen (lyophilized) from which single wells can be broken.
2. Sample Buffer (orange cap) 100 mL ready to use solution, with preservative and Tween 20.
3. Wash Buffer (blue cap), 100 mL 10x concentrate solution, with preservative and Tween 20. Add to 900 mL deionized water.
4. Negative Control, 2.0 mL, human serum with protein-stabilizer and preservative, ready to use.
5. Positive Control, 2.0 mL, human serum containing antibodies positive for ANA with

protein-stabilizer and preservative, ready to use.

6. Cutoff Control, 2.0 mL, human serum containing antibodies positive for ANA with protein-stabilizer and preservative, ready to use.
7. Conjugate (red cap), 14 mL ready to use solution, peroxidase-labeled goat antihuman IgG conjugate with protein-stabilizer and preservative.
8. Substrate (3, 3', 5, 5'-TetraMethylBenzidine), 14 mL ready to use solution.
9. Stop Solution, 14 mL ready to use solution, contains acid.

J. Substantial Equivalence Information:

1. Predicate device name(s) and 510(k) number(s):

   Aeskulisa ANA Hep2, k040953

2. Comparison with predicate:

Similarities		
Item	Device	Predicate
Intended Use	A qualitative assay for the detection of ANAs in human serum	Same
Measured analytes	Antibodies to double stranded DNA (dsDNA), SS-B (La), Sm, RNP/Sm, Scl-70, Jo-1 and centromeric antigens, and antibodies detected against Hep2 cells by immunofluorescence test	Same
Assay technology	Colorimetric enzyme immunoassay	Same
Immunoglobulin detected	Human IgG autoantibodies	Same
Captured antigens	Lysed Hep-2 extract Purified native: Ro60, Sm Recombinant: SSB, RNP, Scl-70, Jo-1, CENP-B	Same
Detection antibody	Horseradish peroxidase (HRP) labeled goat anti-human IgG	Same
Substrate	3, 3', 5, 5'-tetramethylbenzidine (TMB)	Same
Controls	Positive, negative and cut-off controls	Same
Sample matrix	Serum	Same

Differences		
Item	Device	Predicate
Measured analytes and captured antigens	Antibodies to SSA [Ro60 (purified native) and Ro52 (recombinant)] Ribosomal P (recombinant)	Antibodies to SS-A (Ro) only Ribosomal P not detected
Interpretation	Convert to units: Negative <0.83 units; Equivocal units 0.83-1.2 units Positive >1.2 units	Convert to units: Negative <1.0 units; no equivocal zone Positive >1.0 units

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

1. CLSI EP07-A2 Interference Testing in clinical chemistry
2. FDA Guidance for Industry and Staff. Recommendations for anti-nuclear antibody (ANA) test systems premarket submissions

L. Test Principle:

The Antinuclear Antibody (ANA) Screen ELISA Test kit is an enzyme linked immunosorbent assay where natural and recombinant Extractable Nuclear Antigens (ENAs) are collectively bound to microwells in polystyrene microtiter plates. Human serum containing ANA (IgG) antibodies is added to the wells and any ANAs bind to their cognate antigen(s). After incubation, the wells of the plate are washed to remove unbound serum components and non-ANA IgGs. A detection antibody conjugated with HRP is added to detect human IgG antibodies bound to antigens on the microtiter well. After an incubation period, the wells of the plate are washed to remove unbound enzyme-labeled anti-human IgG. Upon its addition to the washed wells the substrate TMB is converted by bound enzyme-conjugate antibody producing a blue end product. The Stop Solution is an acid that stops the reaction and turns the substrate yellow. The amount of color produced is proportional to the amount of antibody is present in the patient serum.

The antigens used in the Screen ELISA Test are a lysed HEp-2 cell extract with added purified antigens. The assay collectively detects in one well antibodies against double stranded DNA (dsDNA), SSA (Ro60 and Ro52), SSB (La), Sm, Sm/RNP, Scl-70, Jo-1, Ribosomal P, and Centromeric antibodies along with antibodies with reactivity against HEp-2 cells.

Optical density values read by the spectrophotometer are converted into 3 categories based upon a ratio of optical density of the samples (or controls) to the optical density of a cutoff control reagent present in the assay kit. Three result categories are determined by optical density ratio values as follows:

Optical density ratio	Interpretation
< 0.83 units	negative
0.83 – 1.2 units	equivocal
> 1.2 units	positive

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Assessment of the repeatability of the assay was performed on seven samples; three positive samples, one equivocal sample, and three negative samples. One positive sample was diluted with normal human serum to give a unit value 20% above the positive ratio cutoff (1.36 U). The other two positive samples gave assay units approximately 2.56 and 5.60. One negative sample was diluted to 20% below the negative cutoff ratio (approximately 0.70 units). The other two negative samples gave assay units approximately 0.3 and 0.15. Each sample was tested twice a day for ten days for a total of 20 replicates. All the observed results matched the expected results. The results, presented as ratio Units, are summarized in the following table:

Repeatability:

Sample	**Mean (U)**	**Range (U)**	**Expected qualitative result**	**% Observed matching expected result**
1	5.60	5.18–5.82	Positive	100%
2	2.56	2.13–2.96	Positive	100%
3	1.36	1.24–1.67	Positive	100%
4	1.06	0.93–1.30	Equivocal	90%
5	0.73	0.62–0.90	Negative	90%
6	0.34	0.27–0.38	Negative	100%
7	0.16	0.12–0.20	Negative	100%

The reproducibility of the assay (between-lab imprecision) was done by testing three samples in duplicate for five days, twice a day, at three sites with two technicians per site for a total of 60 replicates. The mean results, reported as ratio Units, are summarized in the table below:

Reproducibility:

Sample	Mean (U)	Site	Range (U)	Expected qualitative result	% Observed result
1	0.20	1	0.18-0.24	Negative	100%
1		2	0.18-0.26	Negative	100%
1		3	0.20-0.27	Negative	100%
2	1.45	1	1.05-1.57	Equivocal	95%
2		2	1.06-1.45	Equivocal	95%
2		3	1.4-1.55	Equivocal	100%
3	3.08	1	2.94-3.14	Positive	100%
3		2	2.51-3.18	Positive	100%
3		3	3.04-3.37	Positive	100%

Lot to Lot:

Three samples were tested five times each on three different lots. The mean results, reported as ratio Units, are summarized in the table below:

Sample	Mean (U)	Lot Number	Range (U)	Expected qualitative result	% Observed result matching expected result
1	1.474	1	1.42-1.51	Positive	100%
1		2	1.48-1.60	Positive	100%
1		3	1.39-1.47	Positive	100%
2	0.960	1	0.92-0.96	Equivocal	100%
2		2	0.92-1.01	Equivocal	100%
2		3	0.96-1.00	Equivocal	100%
3	0.519	1	0.51-0.58	Negative	100%
3		2	0.50-0.52	Negative	100%
3		3	0.50-0.51	Negative	100%

b. Linearity/assay reportable range:

Linearity: Not applicable.

Assay reportable range: Not applicable.

Hook effect:

To evaluate the hook effect on the assay, five sera with high antibody concentrations were diluted 1:100 to 1:12,000 and the units were calculated after completion of the testing. No hook effect was seen at the maximum measurement of 12 U.

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

Traceability:

There is no recognized standard or reference material, so the assay is calibrated in arbitrary units. Each new lot of the cutoff control is traceable to an internal master control.

Positive and Negative Control:

Positive control was prepared from a pool of eight pathological sera obtained from various plasma brokers determined to be positive for ANAs by HEp-2 immunofluorescence. Negative control was prepared by diluting the positive control pool 100-fold with sample diluent and protein stabilizer. The package insert states that the negative control must have an OD of <0.15; the positive control must have an OD of 0.6 to 1.6.

Cut-off Control:

Cutoff controls are derived from a pool of eight pathological sera obtained from various plasma brokers determined to be positive by HEp-2 immunofluorescence. The cutoff control is prepared by diluting the serum pool with analyte-free serum to the appropriate concentration. The OD value of the cutoff control is compared with the master cutoff control. The unit ratio (OD cutoff control / OD master cutoff control) during the in-house QC procedure has to be 0.87 – 1.15. Each new lot of the cutoff controls, which are prepared by dilution of the pool stock, is compared with the master control and must meet the pre-specified acceptance criteria. Further, a set of 12 sera representing the antigen specificities is measured for each new lot of the controls and have to fall within their pre-specified ranges (which was obtained from several tests with different lots).

Calibrators: Not applicable

Stability: The sponsor provided data demonstrating real-time stability of an opened kit for 6 months when stored at 2-8°C.

d. Detection limit:

Not applicable

e. Analytical specificity:

The effect of endogenous substances in serum specimens that could cause interference with the ANA ELISA Screen Test was evaluated. Five samples ranging from 1.4 to 2.5 U were spiked with the concentrations of hemoglobin, bilirubin, rheumatoid factor and triglycerides indicated below. The recovery in relation to the un-spiked sample without interferent was calculated. In addition, to assess the interference in the assay from heterophilic antibodies, two samples were spiked with three concentrations of HAMA type 1 antibody. The recommended concentrations from the guideline "Interference Testing in Clinical Chemistry" from the Clinical and Laboratory Standards Institute were used (CLSI EP7-A2). The tested substances as listed below did not affect the performance of the ELISA Screen Test.

Substance	Concentration	Mean Percent Inhibition
Hemoglobin	2 g/L	-2.1%
Bilirubin	20 mg/dL	-3.0%
Rheumatoid Factor	100 IU/mL	8.8%
Triglycerides	3000 mg/dL	-1.4%
Heterophile	65 µg/mL	5.4%
Heterophile	32.5 µg/mL	2.2%
Heterophile	16.25 µg/mL	-0.6%

Ten CDC and AMLI samples were tested in duplicate. The following table summarizes the specific reactivity of each sample and the result of each replicate.

Sample	Specificity	Result 1	Result 2
CDC #1	DNA	Positive	Positive
CDC #2	SS-A/SS-B	Positive	Positive
CDC #3	RNP, SS-A, SS-B	Positive	Positive
CDC #4	RNP	Positive	Positive
CDC #5	Sm	Positive	Positive
CDC #7	SS-A/Ro	Positive	Positive
CDC #8	CENP-B	Positive	Positive
CDC #9	Scl-70	Positive	Positive
CDC #10	Jo-1	Positive	Positive
CDC #12	Ribosomal-P	Positive	Positive
AMLI #1	Negative	Negative	Negative
AMLI #2	SS-A/SS-B	Positive	Positive
AMLI #3	SmRNP	Positive	Positive
AMLI #4	SS-A/Ro	Positive	Positive
AMLI #5	SS-A/SS-B	Positive	Positive
AMLI #6	Scl-70	Positive	Positive
AMLI #7	Jo-1	Positive	Positive
AMLI #8	CENP-B	Positive	Positive
AMLI #9	dsDNA	Positive	Positive
AMLI #10	Negative	Negative	Negative

ƒ *Assay cut-off*

The cutoff was determined by testing 99 normal blood donors and three known negative samples. The mean value plus three standard deviations of the negative samples was established as the cutoff. To encourage a repeat testing of samples close to the cutoff, an equivocal range of ± 20% of the cutoff was established.

2. Comparison studies:

a. Method comparison with predicate device:

A comparison of the performance of the test device and the predicate was performed at three different sites using a total of 848 samples. The sera used for this study were prospectively obtained from the reference labs that were from samples that were

submitted for ANA serology testing. The results are summarized in the following table with the percent positive (PPA) and percent negative (NPA) agreements:

	Predicate device		
Gold Standard Diagnostic ANA ELISA	Positive	Negative	**Total**
Positive	251	22	**273**
Equivocal	7	23	**30**
Negative	14	531	**545**
Total	**272**	**576**	**848**

Equivocals considered as Positive: PPA = 94.9% (95% C.I. 91.5% - 97.2%),
NPA = 92.2 % (95% C.I. 89.7% - 94.2%),
Overall = 93.0 % (95% C.I. 91.1% - 95.7%),

Equivocals considered as Negative: PPA = 92.3% % (95% C.I. 88.4% - 95.2%)
NPA = 96.2 % (95% C.I. 94.3% - 97.6%)
Overall = 94.9 % (95% C.I. 93.2% - 96.3%)

To demonstrate the proposed test has comparable performance to the individual analyte assays, five samples known to be positive for each analyte (dsDNA, SS-A/Ro 60, SS-A/Ro 52, SS-B, Sm, Sm/RNP, Scl-70, Jo-1, Ribosomal P, Centromere, and HEp-2 IFA, total of 55 samples) were obtained and were tested on the proposed ANA Screening test, on an FDA-cleared test that measures each analyte individually, and by ANA HEp-2 IFA test. The percentage of the five samples from each analyte that were positive in the different tests is shown in the following table:

Sample reactivity	**GSD test**	**Individual analyte assay- % positive**										
		DNA	SSA (Ro60)	SSA (Ro52)	SSB (Ro52)	Sm	SM/RNP	SCL-70	Jo-1	Ribosomal P	Centromere	Hep-2
dsDNA	**100%**	**100%**	60%	20%	20%	40%	40%	0%	0%	20%	0%	100%
SSA (Ro60)	**100%**	60%	**100%**	40%	20%	0%	0%	0%	0%	20%	0%	100%
SSA (Ro52)	**80%** (1 equivocal)	20%	80%	**100%**	20%	0%	0%	0%	0%	0%	0%	80%
SSB	**100%**	20% (1 equivocal)	100%	60%	**100%**	0%	0%	0%	0%	0%	0%	100%
Sm	**100%**	60%	0%	0%	0%	**100%**	100%	0%	0%	0%	0%	100%
Sm/RNP	**100%**	80%	20%	0%	0%	60%	**100%**	0%	0%	40%	0%	100%
SCL-70	**100%**	20%	0%	0%	0%	0%	0%	**100%**	0%	0%	0%	100%
Jo-1	**100%**	0%	20%	40%	0% (1 equivocal	0%	0%	0%	**100%**	0%	0%	20%
Ribosomal	**100%**	80%	40%	0%	0%	0%	40%	0%	0%	**100%**	0%	80%
Centromere	**100%**	0%	0%	0%	0%	0%	0%	0%	0%	0%	**100%**	100%
Hep-2	**100%**	40%	40%	0%	20%	20%	40%	20%	0%	40%	0%	**100%**

b. Matrix comparison: Not applicable.

3. Clinical studies:

a. Clinical Sensitivity and Specificity:

A comparison of the assay results to clinical diagnosis was performed using 753 retrospective samples with associated clinical diagnosis and demographic information obtained from serum brokers. No NHS or proficiency samples were used in the Clinical Sensitivity and Specificity study.

Clinical Diagnosis	Number Tested	Positive (%)	Equivocal (%)	Negative (%)
Systemic Lupus Erythematosus (SLE)	322	269 (82.9)	11 (3.4)	42 (13)
Systemic Sclerosis (SSc)	40	29 (72.5)	1 (2.5)	10 (25)
Polymyositis (PM)	12	4 (33.3)	2 (16.7)	6 (50)
Dermatomyositis (DM)	15	4 (26.7)	2 (13.3)	9 (60)
PM or DM Overlap	5	1 (20)	1 (20)	3 (60)
Myositis	10	4 (40)	1 (10)	5 (50)
Mixed Connective Tissue Disease (MCTD)	28	28 (100)	0	0
Undifferentiated CTD (UTCD)	3	3 (100)	0	0
Sjögren's Syndrome (SjS)	75	63 (84)	4 (5.3)	8 (10.7)
Total (CTD)	**510**	405 (79.4)	22 (4.3)	83 (16.3)

Clinical Diagnosis	Number Tested	Positive (%)	Equivocal (%)	Negative (%)
Rheumatoid Arthritis (RA)	100	2 (2)	0	98 (98)
Osteoarthritis	20	5 (25)	0	15 (75)
Primary Biliary Cirrhosis (PBC)	8	1 (12.5)	0	7 (87.5)
Autoimmune Hepatitis (AIH)	2	0	0	2 (100)
Hashimoto's Thyroiditis	17	0	0	17 (100)
Grave's Disease	17	0	0	17 (100)
Ulcerative Colitis	5	0	0	5 (100)
Celiac Disease	5	0	0	5 (100)
Primary Anti-phospholipid Syndrome (PAPS)	22	0	0	22 (100)
Granulomatosis with polyangitis (Wegener's)	5	0	0	5 (100)
Total (non-CTD)	**201**	8 (4)	**0**	193 (96)

Clinical Diagnosis	Number Tested	Positive (%)	Equivocal (%)	Negative (%)
Herpes Simplex Virus (HSV)	7	0	0	7 (100)
Epstein Barr Virus (EBV)	7	0	0	7 (100)
Syphilis	7	0	0	7 (100)
Varicella Zoster Virus (VZV)	7	0	0	7 (100)
Mumps	7	0	0	7 (100)
Rheumatoid Factor	7	0	0	7 (100)
Total (other)	**42**	0	0	42 (100)

Equivocals considered as Positive: Sensitivity = 83.5% (95% C.I. = 79.1% - 86.0%)
Specificity = 96.7% (95% C.I. = 93.6% - 98.6%)
Overall = 87.8% (95% C.I. = 85.2% - 90.0%)

Equivocals considered as Negative: Sensitivity = 79.2% (95% C.I. = 75.4% - 82.7%)
Specificity = 96.7% (95% C.I. = 93.6% - 98.6%)
Overall = 84.9% (95% C.I. = 82.1% - 87.4%)

b. Other clinical supportive data (when a. is not applicable):

Not applicable

4. Clinical cut-off**:**

Not applicable.

1) Expected values/Reference range:
The expected value is negative; however, it is known that a certain percentage of NHS will be positive. In the sponsor's cutoff study, of the 99 samples from normal healthy donors, two were positive, for a prevalence of ANAs in the healthy population detected by this assay of 7%. The results are presented below.

Total	
N	99
Positives	2
Equivocals	7
Negatives	92
Prevalence	7.0 %
Ratio Mean (SD) (U)	0.59 (0.2)
Ratio Range (U)	0.1 – 1.58

By Gender	Number Tested	Mean Units	SD	95th Percentile
Male	51	0.62	0.24	1.09
Female	48	0.55	0.13	0.82

N. Proposed Labeling:

The labeling meets 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.