510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I Background Information:

A 510(k) Number

K250408

B Applicant

ZEUS Scientific

C Proprietary and Established Names

Alegria Flash ENA Screen

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
LLL	Class II	21 CFR 866.5100 - Antinuclear Antibody Immunological Test System	IM - Immunology

II Submission/Device Overview:

A Purpose for Submission:

New assay

B Measurand:

Human IgG autoantibodies to Jo-1, SSA-52, SSA-60, SS-B, Scl-70, Sm, and SmRNP

C Type of Test:

Automated qualitative chemiluminescence immunoassay

III Intended Use/Indications for Use:

A Intended Use(s):

See Indications for Use below

B Indication(s) for Use:

The Alegria Flash ENA Screen kit is a chemiluminescent immunoassay (CLIA) for the qualitative screening of IgG autoantibodies to Jo-1, SSA-52, SSA-60, SS-B, Scl-70, Sm, or SmRNP in human serum. The presence of these autoantibodies is intended for use as an aid in the diagnosis of the connective tissue diseases (CTD) Sjögren's syndrome (SS), systemic lupus erythematosus (SLE), systemic sclerosis (SSc), mixed connective tissue disease (MCTD), Limited Cutaneous Systemic Sclerosis (CREST Syndrome), polymyositis, and dermatomyositis along with other laboratory and clinical findings. The test must be performed on the Alegria Flash instrument.

C Special Conditions for Use Statement(s):

Rx - For Prescription Use Only

D Special Instrument Requirements:

For use with the Alegria Flash instrument (Zeus Solinas α instrument, K230863).

IV Device/System Characteristics:

A Device Description:

Each Alegria Flash ENA Screen kit contains the following materials:

- One (1) Assay Cartridge containing bead suspension, sample diluent, and conjugate reagents.
  - Bead suspension: magnetic particles that are coated with a cocktail of ENA antigens in 5 mL of storage buffer. The suspension contains Tween-20, bovine serum albumin, phosphate-buffered-saline, and <0.1% sodium azide. Ready to use.
  - Sample diluent: 10 mL of phosphate-buffered-saline solution containing detergent, proteins, <0.1% sodium azide, and ProClin 300. Ready to use.
  - Conjugate: 10 mL of anti-human IgG that is conjugated with Acridinium Ester in a buffered solution containing, ProClin 300. Ready to use.

- One (1) Calibrator 1 (blue-capped vial): 0.5 mL of human serum that contains anti-ENA autoantibodies at levels above the cutoff value and <0.1% sodium azide. Ready to use. Assay and lot specific.

- One (1) Calibrator 2 (white-capped vial): 0.5 mL of human serum containing anti-ENA autoantibodies at levels near the cutoff value and <0.1% sodium azide. Ready to use. Assay and lot specific.

A separate Alegria Flash ENA Control kit is sold separately. The Alegria Flash Control kit contains:

- One (1) positive control ("CTRL +"): a 1.0 mL vial of human serum containing anti-ENA antibodies at levels above the cutoff value and <0.1% sodium azide. Ready to use and assay specific.

- One (1) negative control ("CTRL –"): a 1.0 mL vial of human serum containing anti-ENA antibodies at levels below the cutoff value and <0.1% sodium azide. Ready to use and assay specific.

B Principle of Operation:

The Alegria Flash ENA Screen kit is designed to detect human anti-Jo-1, SSA-52, SSA-60, SS-B, Scl-70, Sm, or SmRNP IgG autoantibodies, in human serum. The Alegria Flash ENA Screen Kit test procedure involves three main steps. Sample diluent, test sera, and antigen-coated magnetic particles are added to a reaction cuvette. During the initial incubation, autoantibodies specific to Jo-1, SSA-52, SSA-60, SS-B, Scl-70, Sm, or SmRNP present in the serum will bind to the immobilized antigens. The beads are then washed to remove unbound antibodies and other serum components. An acridinium ester-conjugated anti-human IgG solution is then added to the reaction cuvette. During the second incubation, the conjugate reacts with IgG autoantibodies that are immobilized on the magnetic particles in step 1. The beads are then washed to remove unbound conjugate. Two trigger solutions are added to the cuvette containing immobilized acridinium ester conjugate, causing a flash chemiluminescence reaction to occur. The light signal released by the chemiluminescence reaction is measured by a photomultiplier within the Alegria Flash analyzer and the signal is reported as relative light units (RLU). RLU values are converted to CLIA Units using the instrument's Alegria Flash software. CLIA Unit values that are above or equal to the cut-off value of 100 CLIA Units are indicative of the presence of selected anti-ENA antibodies within the original serum sample.

V Substantial Equivalence Information:

A Predicate Device Name(s):

Zeus ENA Screen ELISA Test System

B Predicate 510(k) Number(s):

K941014

C Comparison with Predicate(s):

Device & Predicate Device(s):	K250408 (Candidate Device)	K941014 (Predicate Device)
Device Trade Name	Alegria Flash ENA Screen	ENA Screen ELISA Test System
General Device Characteristic Similarities		
Intended Use/ Indications For Use	The Alegria Flash ENA Screen kit is a chemiluminescent immunoassay (CLIA) for the qualitative screening of IgG autoantibodies to Jo-1, SSA-52, SSA-60, SS-B, Scl-70, Sm, or SmRNP in human serum. The presence of these autoantibodies is intended for use as an aid in the diagnosis of the connective tissue diseases (CTD) Sjögren's syndrome (SS), systemic lupus erythematosus (SLE), systemic sclerosis (SSc), mixed connective tissue disease (MCTD), Limited Cutaneous Systemic Sclerosis (CREST Syndrome), polymyositis, and dermatomyositis along with other laboratory and clinical findings. The test must be performed on the Alegria Flash instrument.	The ZEUS ELISA ENA Screen Test System is a qualitative screening assay designed to detect antibodies to extractable nuclear antigens (anti-ENA) in human sera. When performed according to the enclosed instructions, this test is capable of detecting all anti-ENAs commonly tested for, such as those against Jo-1, Sm, Sm/RNP, SSA, SSB, and Scl-70. This device is for In Vitro diagnostic use
Sample matrix	Serum	Same
Detection	Qualitative	Same
General Device Characteristic Differences		
Antigens	Jo-1, SSA-52, SSA-60, SS-B, Scl-70, Sm, SmRNP	SSA 60, SS-B, Jo-1, Scl70, SmRNP, Sm
Technology	Chemiluminescent Immunoassay (CLIA)	Enzyme Linked Immunosorbent Assay (ELISA)
Solid Phase	Magnetic microscopic beads	96 well polystyrene ELISA
Cut-off	100 CLIA Units	0.90 and 1.10 Units
Interpretation	Negative: <100 CLIA Units Positive: ≥100 CLIA Units	Negative: ≤0.90 Units Equivocal: 0.91–1.09 Units Positive: ≥1.10 Units

VI Standards/Guidance Documents Referenced:

The following Clinical and Laboratory Standards Institute (CLSI) guidelines were used:

- CLSI EP07-Ed3 – Interference Testing in Clinical Chemistry
- CLSI EP12-Ed3 – Evaluation of Qualitative, Binary Output Examination Performance

- CLSI EP17-A2 – Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures
- CLSI EP25-Ed2 – Evaluation of Stability of In Vitro Medical Laboratory Test Reagents
- CLSI EP28-A3c – Defining, Establishing and Verifying Reference Intervals in the Clinical Laboratory

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:

The precision of the Alegria Flash ENA Screen was evaluated based on the CLSI EP12-Ed3.

Within-laboratory precision:

To evaluate the within-laboratory precision of the Alegria Flash ENA Screen, 11 samples were prepared by mixing positive native serum samples with negative serum samples. The prepared samples, containing various concentrations of autoantibodies, were assayed in duplicate, twice a day, for 20 days, using one reagent lot, one instrument, and one operator, for a total of 80 measurements per sample. The percent of positive (% Pos), with 95% confidence interval (CI), for each sample was calculated. The results are summarized in the following table.

Sample	Expected Result	(CLIA Units)		Positive		
		Mean	Range	n(+)/N	% Pos.	(95% CI)
1	Positive	457.4	398.6–594.5	80/80	100	(95.4–100)
2	Positive	303.3	254.6–386.6	80/80	100	(95.4–100)
3	Positive	262.3	213.9–342.6	80/80	100	(95.4–100)
4	Positive	259.5	227.7–337.6	80/80	100	(95.4–100)
5	Positive	167.4	124.7–204.8	80/80	100	(95.4–100)
6	Positive	113.7	102.3–131.9	80/80	100	(95.4–100)
7	Negative	97.2	76.0–120.3	28/80	35.0	(25.4–45.9)
8	Negative	78.4	64.7–91.3	0/80	0.0	(0.0–4.6)
9	Negative	47.2	35.9–65.0	0/80	0.0	(0.0–4.6)
10	Negative	17.2	9.8–29.4	0/80	0.0	(0.0–4.6)
11	Negative	11.5	7.4–21.5	0/80	0.0	(0.0–4.6)

Lot-to-lot imprecision:

To evaluate the between-lot imprecision of the Alegria Flash ENA Screen, eight samples were prepared by diluting positive native serum samples with negative serum samples. The samples, containing various concentrations of autoantibodies, were assayed were assayed in quintuplicate, once a day, for 5 days, using three reagent lots, using one instrument, for a total of 75 replicates per sample. The results are summarized in the following table.

Sample	Expected Result	Total (across lots)				Lot 1		Lot 2		Lot 3	
		Mean (U)	Range (U)	n(+)/N	% Pos	n(+)/N	% Pos	n(+)/N	% Pos	n(+)/N	% Pos
1	Positive	663.0	422.2 – 864.7	75/75	100	25/25	100	25/25	100	25/25	100
2	Positive	243.9	188.8 – 379.7	75/75	100	25/25	100	25/25	100	25/25	100
3	Positive	144.5	124.9 – 174.8	75/75	100	25/25	100	25/25	100	25/25	100
4	Positive	115.5	96.6 – 129.7	74/75	98.7	25/25	100	25/25	100	24/25	96.0
5	Positive	103.8	87.1 – 121.1	48/75	64.0	24/25	96.0	16/25	64.0	8/25	32.0
6	Negative	89.2	75.9 – 125.6	3/75	4.0	0/25	0.0	3/25	12.0	0/25	0.0
7	Negative	79.2	59.8 – 126.2	2/75	2.7	0/25	0.0	2/25	8.0	0/25	0.0
8	Negative	16.7	5.1 – 60.3	0/75	0.0	0/25	0.0	0/25	0.0	0/25	0.0

Site-to-site imprecision:

The reproducibility of the Alegria Flash ENA Screen was evaluated at three sites using 11 samples that were prepared by mixing positive native serum samples with negative serum samples. The samples, containing various concentrations of autoantibodies, were assayed in quintuplicate, once a day, for five days to generate 25 data points per sample per site for a total of 75 replicates per sample. One reagent lot was used in the study. The instrument and operator variables were nested within the multiple site component. The resulting data are summarized in the following table:

Sample	Expected Result	Total (across sites)				Site 1		Site 2		Site 3	
		Mean (U)	Range (U)	n(+)/N	% Pos	n(+)/N	% Pos	n(+)/N	% Pos	n(+)/N	% Pos
1	Positive	512.7	417.8 – 644.2	75/75	100	25/25	100	25/25	100	25/25	100
2	Positive	352.9	284.9 – 426.3	75/75	100	25/25	100	25/25	100	25/25	100
3	Positive	288.3	228.1 – 374.3	75/75	100	25/25	100	25/25	100	25/25	100
4	Positive	287.0	233.3 – 420.6	75/75	100	25/25	100	25/25	100	25/25	100
5	Positive	197.0	144.2 – 242.4	75/75	100	25/25	100	25/25	100	25/25	100
6	Positive	115.6	84.6 – 154.3	66/75	88.0	24/25	96.0	18/25	72.0	24/25	96.0
7	Negative	97.0	80.3 – 123.5	22/75	29.3	0/25	0	1/25	4.0	21/25	84.0
8	Negative	85.1	70.3 – 99.5	0/75	0	0/25	0	0/25	0	0/25	0
9	Negative	62.5	45.3 – 81.8	0/75	0	0/25	0	0/25	0	0/25	0
10	Negative	30.4	17.0 – 49.4	0/75	0	0/25	0	0/25	0	0/25	0
11	Negative	24.8	14.6 – 41.3	0/75	0	0/25	0	0/25	0	0/25	0

2. Linearity:

Not applicable

3. Analytical Specificity/Interference:

Endogenous and Exogenous Interference:

Two serum samples (a negative and a positive serum samples), were spiked (<10% volume/volume) with the test endogenous or exogenous test substance at the indicated concentrations or with an appropriate blank vehicle solvent. The samples were tested in triplicate using an Alegria Flash instrument. The percent recovery of the spiked samples was

determined relative to the blank vehicle solvent spike in sample. None of the interferents changed the expected result at the indicated testing concentrations.

Endogenous Substances	
Substance	**Maximum Testing Concentration**
Bilirubin (unconjugated)	0.15 mg/mL
Cholesterol	2.2 mg/mL
Triglycerides	2.5 mg/mL
Albumin	52 mg/mL
Hemoglobin	2 mg/mL
Rheumatoid Factor (RF)	400 U/mL

Exogenous Substances	
Substance	**Maximum Testing Concentration**
Ibuprofen	0.219 mg/mL
Hydroxychloroquine	0.024 mg/mL*
Prednisone	9.9×10^{-5} mg/mL
Azathioprine	2.58×10^{-3} mg/mL
Diltiazem	9×10^{-4} mg/mL
Rituximab	2 mg/mL
Methotrexate	1.36 mg/mL
Enalapril	8.19×10^{-4} mg/mL
Omeprazole	8.4×10^{-3} mg/mL
Losartan	3.06×10^{-4} mg/mL
Atenolol	9×10^{-3} mg/mL
Erythromycin	0.138 mg/mL
Amoxicillin	5.4×10^{-2} mg/mL
Ranitidine	1.05×10^{-2} mg/mL
Furosemide	1.59×10^{-2} mg/mL
Alendronate	3.4×10^{-5} mg/mL
Atorvastatin	7.5×10^{-4} mg/mL

* The concentration tested is lower than the recommended testing concentration of three times the maximum concentration observed in human serum.

Reference Sera:

Selected Antinuclear Antibodies(ANA) IUIS Reference Standards samples, previously known as the CDC (Center for Disease Controls and Prevention) ANA Reference Panel, were tested in singlicate using one reagent lot of the Alegria Flash ENA Screen to illustrate the analytical specificity of the assay. The results are outlined below.

Sample ID	CDC Description	Alegria Flash ENA Screen Result
ANA 01	ANA homogeneous positive/ anti-native DNA	Negative
ANA 02	ANA speckled positive/ anti-SSB/La	Positive

Sample ID	CDC Description	Alegria Flash ENA Screen Result
ANA 03	ANA speckled positive/ anti-U1 RNP, SSB/La, SSA/Ro	Positive
ANA 04	Anti-U1 RNP positive	Positive
ANA 06	ANA nucleolar (U3RNP) positive	Negative
ANA 07	Anti-SSA Ro positive	Positive
ANA 08	ANA centromere positive	Negative
ANA 09	ANA anti-Scl70 positive	Positive
ANA 10	Anti-Jo-1 positive	Positive
ANA 11	Anti-PM/Scl positive	Negative
ANA 12	Anti-Ribosomal P positive	Negative
ANA 15	Anti-MPO positive	Negative
ANA 16	Anti-PR3 positive	Negative

4. Assay Reportable Range:

   Not applicable

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

   Traceability:

   Currently there are no recognized international standards for the measurement of Jo-1, SSA-52, SSA-60, SS-B, Scl-70, Sm, and SmRNP. The calibrators and controls are directly traceable to in house standards.

   Reagent stability:

   Shelf-life: The reagent stability studies were designed and conducted following CLSI EP25-Ed2. To assess the shelf-life stability of the Alegria Flash ENA Screen kits, six serum samples and two controls were assessed in singlicate using Alegria Flash ENA Screen kits that were stored for 1 day, or for up to 6 months at 2-8°C. The results support that the Alegria Flash ENA Screen kits are stable when stored unopened at 2-8°C for up to five months.

   On-board stability: The onboard storage stability of the Alegria Flash ENA Screen kit was determined by testing three serum samples and two controls using Alegria Flash ENA Screen reagents stored opened within the Alegria Flash's refrigerated reagent bay or within the refrigerator at 2-8°C. The opened Alegria Flash ENA Screen kits were assessed after up to 5 weeks of storage. The results of the study support that Alegria Flash ENA Screen reagents are stable when stored onboard or at 2-8°C for four weeks.

   Sample stability:

   The sample stability studies were designed and conducted following CLSI EP25-Ed2. To assess the stability of patient samples, three serum samples were stored up to 20 days at 2-8°C or at room temperature. The samples were then assessed using three replicates and the

percent recovery in comparison to the unmanipulated, baseline sample was determined for each storage condition. The results support that patient serum samples are stable when stored for up to 19 days at 2-8°C or at room temperature.

To determine the stability of patient samples subjected to multiple freeze/thaw cycles, three serum samples were subjected up to 5 rounds of freeze/thaw cycles, consisting of 12 hours of storage at -20°C and a subsequent thaw. The samples were assessed for each condition using three replicates and the percent recovery in comparison to the unmanipulated, baseline sample was determined for each freeze/thaw cycle. The results support that patient serum samples are stable when frozen at -20°C and thawed for a maximum of 4 times.

6. Detection Limit:

Not applicable

7. Assay Cut-Off:

The cut-off of the Alegria Flash ENA Screen is 100 Units.

To validate this cut-off, 30 ANA positive clinically characterized serum samples and 90 apparently healthy serum donor samples that were not used in any other studies were tested using the Alegria Flash ENA Screen. All known ANA positive samples were found to be positive for the Alegria Flash ENA Screen.

B Comparison Studies:

1. Method Comparison with Predicate Device:

The 943 samples used in the Alegria Flash ENA Screen clinical validation study (Section C.1 below) were compared to the predicate in a method comparison analysis. All samples were tested in singlicate. Positive percent agreement (PPA) and negative percent agreement (NPA), with their associated 95% confidence intervals (CIs), were calculated. The results are summarized in the following tables.

		Predicate			
		Positive	Indeterminate	Negative	Total
Alegria Flash ENA Screen	Positive	352	14	55	421
	Negative	12	19	491	522
	Total	364	33	546	943

Indeterminate as positive		
PPA	92.2%	95% CI: 89.1 − 94.2%
NPA	89.9%	95% CI: 87.1 − 92.2%
Indeterminate as negative		
PPA	96.7%	95% CI: 94.3 – 98.1%
NPA	88.1%	95% CI: 85.2 – 90.5%

2. Matrix Comparison:

Not applicable

C Clinical Studies:

1. Clinical Sensitivity and Specificity:

A cohort of characterized samples were used to validate the clinical performance of the Alegria Flash ENA Screen. The clinical validation study included 589 from ENA-associated connective tissue diseases (CTDs) and 354 samples from diseases that might be considered in the differential diagnosis of CTDs. The results stratified by the disease conditions are summarized in the table below:

Diagnosis		n	Alegria Flash ENA Screen		
			No. of Positives	% of Positives	95% CI
Sensitivity Cohort	Sjögren's syndrome	135	114	84.4	77.4 - 89.6
	Systemic Sclerosis	55	33	60.0	46.8 - 71.9
	Systemic Sclerosis (CREST Syndrome)	55	25	45.4	33.0 - 58.5
	Polymyositis	51	25	49.0	35.9 - 62.3
	Dermatomyositis	53	26	49.1	36.1 - 62.1
	Mixed Connective Tissue Disease	60	41	68.3	55.7 - 78.7
	Systemic Lupus Erythematosus	180	129	71.7	64.7 - 77.7
Specificity Cohort	Autoimmune Hepatitis	30	4	13.3	5.3 - 29.7
	Antiphospholipid Syndrome	20	3	15.0	5.2 - 36.0
	Cancer	20	0	0	0 - 16.1
	Celiac Disease	24	0	0	0 - 13.8
	Drug Induced Lupus	5	2	40.0	11.7 - 76.9
	Fibromyalgia	20	2	10.0	2.8 - 30.1
	Ulcerative Colitis	29	0	0	0 - 11.0
	Crohn's Disease	31	0	0	0 - 11.7
	Hepatitis B Virus infection	18	3	16.7	5.8 - 39.2
	Hepatitis C Virus infection	10	1	10.0	1.8 - 40.4
	HIV infection	12	1	8.3	1.5 - 35.4
	Herpes Simplex Virus infection	10	0	0	0 - 27.8
	Primary Biliary Cholangitis	30	3	10.0	3.5 - 25.6
	Rheumatoid Arthritis	20	3	15.0	5.2 - 36.0
	Vasculitis*	16	2	12.5	3.5 – 36.0
	Atrophic Gastritis	20	1	5.0	0.9 - 23.6
	Graves' Disease	20	1	5.0	0.9 - 23.6
	Hashimoto's Thyroiditis	19	3	15.8	5.5 - 37.6
Total		**943**			

* The vasculitis sample panel consisted of five ANCA-associated vasculitis samples, five large/medium vessel vasculitis samples, and six undefined vasculitis sample.

Clinical sensitivity and specificity for the Alegria Flash ENA Screen are summarized in the table below:

		Diagnosis		
		ENA-Associated CTDs	Non ENA-Associated CTD	Totals
Alegria Flash ENA Screen	Positive ≥100.0	393	29	422
	Negative <100.0	196	325	521
	Total	589	354	943

Sensitivity	66.7% (393/589)	95% CI: 62.8; 70.4%
Specificity	91.8% (325/354)	95% CI: 88.5; 94.2%

D Clinical Cut-Off:

Refer to the assay cut-off section (VII.A.7) above.

E Expected Values/Reference Range:

The frequency of ENA autoantibody positivity was investigated in a cohort of 200 serum samples from apparently healthy donors. The samples were equally distributed across age and sex. The samples were tested using the Alegria Flash ENA Screen on the Alegria Flash instrument. Eight samples (4.0%) were positive.

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.