



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

I Background Information:

A 510(k) Number

K250666

B Applicant

ZEUS Scientific

C Proprietary and Established Names

Alegria Flash CTD 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 SSA-60, SSA-52, SS-B, Jo-1, Scl-70, SmRNP, Sm, dsDNA, Ribosomal P, Nucleosome, and Centromere-B

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 CTD Screen kit is a chemiluminescent immunoassay (CLIA) for the qualitative screening of IgG autoantibodies to SSA-60, SSA-52, SS-B, Jo-1, Scl-70, SmRNP, Sm, dsDNA, Ribosomal P, Nucleosome, and Centromere-B 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 CTD Screen kit contains the following materials:

- One (1) Assay Cartridge containing bead suspension, sample diluent, and conjugate, described below:
 - Bead suspension: Magnetic particles coated with a cocktail of ANA antigens in 5 mL of storage buffer. 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 conjugated to Acridinium Ester. A protein, buffered solution containing, ProClin 300. Ready to use.
- One (1) Calibrator 1: Blue-capped vial. 0.5mL human serum containing anti-CTD positive antibodies at levels above the cutoff value. <0.1% sodium azide. Ready to use. Assay and Lot Specific.
- One (1) Calibrator 2: White-capped vial. 0.5mL human serum containing anti-CTD positive antibodies at levels near the cutoff value. <0.1% sodium azide. Ready to use. Assay and Lot Specific.

An Alegria Flash CTD 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 autoantibodies at levels above the cutoff value and <0.1% sodium azide. Ready to use.
- One (1) negative control (“CTRL –”): a 1.0 mL vial of human serum containing no autoantibodies or antibody at levels below the cutoff value and <0.1% sodium azide. Ready to use.

B Principle of Operation:

The Alegria Flash CTD Screen kit is designed to detect IgG class antibodies against a variety of autoantigens in human sera. The test procedure involves three main steps: (1) Sample diluent, test sera, and antigen-coated magnetic particles are added to a reaction cuvette. Antigens used in the manufacture of this assay are: SSA-60, SSA-52, SS-B, Sm, Sm/RNP, Scl-70, Jo-1, dsDNA, Ribosomal P, Nucleosome, and Centromere-B. During the initial incubation, autoantibodies specific for the listed antigens present in the serum will bind to the immobilized antigen(s). The beads are then washed to remove unbound antibodies and other serum components. (2) An acridinium ester-conjugated anti-human IgG solution is then added to the reaction cuvette. During the second incubation, the conjugate reacts with IgG antibodies immobilized on the magnetic particles in step 1. The beads are then washed to remove unbound conjugate. (3) 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 reported as relative light units (RLU). RLU values are converted to CLIA Units within the software. CLIA Unit values above a predetermined cutoff are indicative of the presence of selected anti-ANA antibodies within the original serum sample.

V Substantial Equivalence Information:

A Predicate Device Name(s):

ANA Screen Elisa Test System

B Predicate 510(k) Number(s):

K940362

C Comparison with Predicate(s):

Device & Predicate Device(s):	<u>K250666</u> (Candidate Device)	<u>K940362</u> (Predicate Device)
Device Trade Name	Alegria Flash CTD Screen	ANA Screen ELISA Test System
General Device Characteristic Similarities		
Intended Use/ Indications For Use	The Alegria Flash CTD Screen kit is a chemiluminescent immunoassay (CLIA) for the qualitative screening of IgG autoantibodies to SSA-60, SSA-52, SS-B, Jo-1, Scl-70, SmRNP, Sm, dsDNA, Ribosomal P, Nucleosome, and Centromere-B 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 Scientific, Inc. ANA Screen ELISA Test System is a qualitative screening assay designed to detect anti-nuclear antibodies (ANA) in human sera. When performed according to the enclosed instructions, this test system is capable of detecting all ANAs commonly tested for, such as those against double stranded DNA (dsDNA), Jo-1, Sm, Sm/RNP, SSA, SSB, and Scl-70. The test is also capable of detecting ANA demonstrating centromere, nucleolar, peripheral, and spindle indirect immunofluorescence antibody (IFA) patterns. This device is for in vitro diagnostic use.
Sample matrix	Serum	Same
Detection	Qualitative	Same
General Device Characteristic Differences		
Antigens	SSA-60, SSA-52, SS-B, Jo-1, Scl-70, SmRNP, Sm, dsDNA, Ribosomal P, Nucleosome, and Centromere-B	dsDNA, Jo-1, Sm, Sm/RNP, SSA, SSB, Scl-70, centromere, nucleolar, peripheral, and spindle
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: 0.90–1.09 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
- CLSI GP44-A4 – Procedures for the Handling and Processing of Blood Specimens for Common Laboratory Tests

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:

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

Within-laboratory precision:

To evaluate the within-laboratory precision of the Alegria Flash CTD Screen, 12 samples were prepared by diluting 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.

Sample	(CLIA Units)		Expected Result	Positive		
	Mean	Range		n(+)/N	% pos.	(95% CI)
1	397.8	310.7–463.0	Positive	80/80	100.0	(95.42–100.00)
2	328.8	259.2–409.5	Positive	80/80	100.0	(95.42–100.00)
3	325.6	215.7–367.0	Positive	80/80	100.0	(95.42–100.00)
4	163.6	137.8–191.5	Positive	80/80	100.0	(95.42–100.00)
5	133.0	97.8–176.7	Positive	79/80	98.8	(93.25–99.78)
6	120.7	104.3–138.1	Positive	80/80	100.0	(95.42–100.00)
7	105.1	85.5–121.9	Positive	59/80	73.8	(63.18–82.14)
8	91.9	72.9–115.5	Negative	12/80	15.0	(75.59–91.21)
9	82.6	67.0–92.7	Negative	0/80	0.0	(95.42–100.00)
10	58.1	46.1–76.0	Negative	0/80	0.0	(95.42–100.00)
11	24.6	14.9–41.3	Negative	0/80	0.0	(95.42–100.00)
12	14.9	12.2–18.7	Negative	0/80	0.0	(95.42–100.00)

Lot-to-lot imprecision:

To evaluate the between-lot imprecision of the Alegria Flash CTD Screen, eight samples were prepared by diluting 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, 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	777.2	675.4–893.1	75/75	100.0	25/25	100.0	25/25	100.0	25/25	100.0
2	Positive	220.7	181.6–253.4	75/75	100.0	25/25	100.0	25/25	100.0	25/25	100.0
3	Positive	175.0	146.4–198.9	75/75	100.0	25/25	100.0	25/25	100.0	25/25	100.0
4	Positive	117.8	100.0–137.0	0/75	100.0	25/25	100.0	25/25	100.0	25/25	100.0
5	Negative	91.6	79.4–112.6	11/75	14.7	0/25	0.0	2/25	8.0	9/25	36.0
6	Negative	82.0	67.5–93.8	0/75	0.0	0/25	0.0	0/25	0.0	0/25	0.0
7	Negative	70.7	61.7–94.1	0/75	0.0	0/25	0.0	0/25	0.0	0/25	0.0
8	Negative	23.2	16.0–35.0	0/75	0.0	0/25	0.0	0/25	0.0	0/25	0.0

Site-to-site reproducibility:

The reproducibility of the Alegria Flash CTD Screen was evaluated at three sites using 11 samples that were prepared by diluting 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	455.8	388.3–555.5	75/75	100.0	25/25	100.0	25/25	100.0	25/25	100.0
2	Positive	372.1	329.8–439.2	75/75	100.0	25/25	100.0	25/25	100.0	25/25	100.0
3	Positive	369.2	326.7–436.5	75/75	100.0	25/25	100.0	25/25	100.0	25/25	100.0
4	Positive	184.6	159.3–217.4	75/75	100.0	25/25	100.0	25/25	100.0	25/25	100.0
5	Positive	144.5	122.3–212.4	75/75	100.0	25/25	100.0	25/25	100.0	25/25	100.0
6	Positive	118.8	107.5–139.5	75/75	100.0	25/25	100.0	25/25	100.0	25/25	100.0
7	Positive	100.5	84.9–121.1	34/75	45.3	9/25	36.0	5/25	20.0	5/25	20.0
8	Negative	79.8	66.0–96.9	0/75	0.0	0/25	0.0	0/25	0.0	0/25	0.0
9	Negative	65.5	55.7–89.9	0/75	0.0	0/25	0.0	0/25	0.0	0/25	0.0
10	Negative	28.5	17.6–41.6	0/75	0.0	0/25	0.0	0/25	0.0	0/25	0.0
11	Negative	18.5	11.8–27.2	0/75	0.0	0/25	0.0	0/25	0.0	0/25	0.0

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 as shown in the table below.

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 CTD Screen to illustrate the analytical specificity of the assay. The results are outlined below.

Sample ID	CDC Description	Alegria Flash CTD Screen Result
ANA 01	ANA homogeneous positive/ anti-native DNA	Positive

Sample ID	CDC Description	Alegria Flash CTD Screen Result
ANA 02	ANA speckled positive/ anti-SSB/La	Positive
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	Positive
ANA 09	ANA anti-Scl-70 positive	Positive
ANA 10	Anti-Jo-1 positive	Positive
ANA 11	Anti-PM/Scl positive	Negative
ANA 12	Anti-Ribosomal P positive	Positive
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 SSA-60, SSA-52, SS-B, Jo-1, Scl-70, SmRNP, Sm, dsDNA, Ribosomal P, Nucleosome, and Centromere-B. 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 CTD Screen kits, six serum samples and two controls were assessed in singlicate using Alegria Flash CTD Screen kits that were stored for one day, or for up to six months at 2-8°C. The results support that the Alegria Flash CTD 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 CTD Screen kit was determined by testing three serum samples and two controls using Alegria Flash CTD Screen reagents stored opened within the Alegria Flash's refrigerated reagent bay (8-12°C) for five weeks or within the refrigerator at 2-8°C for nine weeks. The results of the study support that Alegria Flash CTD Screen reagents are stable when stored onboard for four weeks or at 2-8°C for eight weeks.

Sample stability:

The sample stability studies were conducted by testing three serum samples stored up to 17 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 16 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 five 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 four times.

6. Detection Limit:

Not applicable

7. Assay Cut-Off:

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

To validate this cut-off, 28 ANA positive clinically characterized serum samples and 25 apparently healthy serum donor samples that were not used in any other studies were tested using the Alegria Flash CTD Screen. All known ANA positive samples were found to be positive for the Alegria Flash CTD Screen.

B Comparison Studies:

1. Method Comparison with Predicate Device:

The 943 samples used in the Alegria Flash CTD 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			Total
		Positive	Indeterminate	Negative	
Alegria Flash CTD Screen	Positive	394	7	51	452
	Negative	74	6	411	491
	Total	468	13	462	943

Indeterminate as positive		
PPA	83.4%	95% CI: 79.8 – 86.4%
NPA	89.0%	95% CI: 85.8 – 91.5%
Total	86.1%	95% CI: 83.6 – 88.2%
Indeterminate as negative		
PPA	84.2%	95% CI: 80.6 – 87.2%
NPA	87.8%	95% CI: 84.5 – 90.4%
Total	86.0%	95% CI: 83.6 – 88.1%

2. Matrix Comparison:

Not applicable

C Clinical Studies:

1. Clinical Sensitivity and Specificity:

A cohort of 943 characterized samples were used to validate the clinical performance of the Alegria Flash CTD Screen. Among 943 samples, 589 samples were from patients diagnosed with ANA-associated connective tissue diseases (CTDs) and 354 samples were from patients with 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 CTD Screen		
			No. of positives	% of positives	95% CI
Sensitivity Cohort	Sjögren's syndrome	135	113	83.7	76.6 – 89.0
	Systemic Sclerosis	55	39	70.9	57.9 – 81.2
	Systemic Sclerosis (CREST Syndrome)	55	41	74.6	61.7 – 84.2
	Polymyositis	51	24	47.1	34.1 – 60.5
	Dermatomyositis	53	22	41.5	29.3 – 55.0
	Mixed Connective Tissue Disease	60	39	65.0	52.4 – 75.9
	Systemic Lupus Erythematosus	180	139	77.2	70.6 – 82.8
Specificity Cohort	Autoimmune Hepatitis	30	6	20.0	9.5 – 37.3
	Antiphospholipid Syndrome	20	3	15.0	5.2 – 36.0
	Cancer	20	0	0.0	0.0 – 16.1
	Celiac Disease	24	0	0.0	0.0 – 13.8
	Drug Induced Lupus	5	2	40.0	11.8 – 77.0
	Fibromyalgia	20	1	5.0	0.90 – 23.6
	Crohn's Disease	31	0	0.0	0.0 – 11.0
	Ulcerative Colitis	29	1	3.5	0.6 – 17.2
	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	2	16.6	4.7 – 44.8
	Herpes Simplex Virus infection	10	0	0.0	0.0 – 27.8
	Primary Biliary Cholangitis	30	4	13.3	5.3 – 29.7
	Rheumatoid Arthritis	20	3	15.0	5.2 – 36.0
	Vasculitis*	16	0	0.0	0.0 – 19.4
	Atrophic Gastritis	20	4	20.0	8.1 – 41.61
	Graves' Disease	20	1	5.0	0.90 – 23.6
	Hashimoto's Thyroiditis	19	4	21.1	8.5 – 43.3
Total		943			

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

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

		Diagnosis		
		ANA-Associated CTDs	Non ANA-Associated CTD	Totals
Alegria Flash CTD Screen	Positive ≥ 100.0	417	35	452
	Negative < 100.0	172	319	491
	Total	589	354	943

Sensitivity	70.8% (417/589)	95% CI: 67.0 – 74.3%
Specificity	90.1% (319/354)	95% CI: 86.6 – 92.8%

D Clinical Cut-Off:

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

E Expected Values/Reference Range:

The frequency of CTD 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 CTD Screen on the Alegria Flash instrument. Ten (10) out of 200 samples (5.0%) 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.