510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I Background Information:

A 510(k) Number

K250814

B Applicant

ZEUS Scientific

C Proprietary and Established Names

Alegria Flash SSA-60
Alegria Flash SSA-52
Alegria Flash SSB

D Regulatory Information

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

II Submission/Device Overview:

A Purpose for Submission:

New devices

B Measurand:

Human anti-SSA-52 (anti-Ro52) IgG autoantibodies
Human anti-SSA-60 (anti-Ro60) IgG autoantibodies
Human anti-SSB IgG autoantibodies

C Type of Test:

Alegria Flash SSA-52: automated qualitative, chemiluminescent immunoassay (CLIA)
Alegria Flash SSA-60: automated qualitative, chemiluminescent immunoassay (CLIA)
Alegria Flash SSB: automated semi-quantitative, chemiluminescent immunoassay (CLIA)

III Intended Use/Indications for Use:

A Intended Use(s):

See Indications for Use below.

B Indication(s) for Use:

Alegria Flash SSA-52
The Alegria Flash SSA-52 kit uses chemiluminescent immunoassay (CLIA) technology for the qualitative detection of IgG antibodies to SSA-52 in human serum. It is intended for use as an aid in the diagnosis of Sjögren's syndrome (SJS), Systemic Lupus Erythematosus (SLE), Systemic Sclerosis (SS), Polymyositis (PM), and Dermatomyositis (DM) in conjunction with other laboratory and clinical findings. The test must be performed on the Alegria Flash instrument.

Alegria Flash SSA-60
The Alegria Flash SSA-60 kit uses chemiluminescent immunoassay (CLIA) technology for the qualitative detection of IgG antibodies to SSA-60 in human serum. It is intended for use as an aid in the diagnosis of Sjögren's syndrome (SS) and Systemic Lupus Erythematosus (SLE) in conjunction with other laboratory and clinical findings. The test must be performed on the Alegria Flash instrument.

Alegria Flash SSB
The Alegria Flash SSB kit uses chemiluminescent immunoassay (CLIA) technology for the semi-quantitative measurement of IgG antibodies to SSB (La) in human serum. It is intended for use as an aid in the diagnosis of Sjögren's syndrome (SS) and Systemic Lupus Erythematosus (SLE) in conjunction 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:

Zeus Alegria Flash instrument (K230863)

IV Device/System Characteristics:

A Device Description:

Alegria Flash SSA-52

The Alegria Flash SSA-52 kit contains the following materials:

- One (1) Assay Cartridge containing bead suspension, sample diluent, and conjugate reagents.
  - Bead suspension (5 mL): Magnetic particles coated with recombinant SSA-52 antigen in storage buffer containing Tween-20, bovine serum albumin, phosphate-buffered-saline, and <0.1% sodium azide. Ready to use.
  - Sample diluent (10 mL): Phosphate-buffered-saline solution containing detergent, proteins, <0.1% sodium azide, and ProClin 300. Ready to use.
  - Conjugate (10 mL): Anti-human IgG conjugated with Acridinium Ester in a buffered solution containing ProClin 300. Ready to use.
- One (1) Calibrator 1 (blue-capped vial) (0.5 mL): Human serum that contains anti-SSA-52 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): Human serum containing anti-SSA-52 autoantibodies at levels near the cutoff value and <0.1% sodium azide. Ready to use. Assay and lot specific.

A separate Alegria Flash SSA-52 Control kit is sold separately. It contains:

- One (1) positive control ("CTRL +") (1.0 mL/vial): Human serum containing anti-SSA-52 antibodies at levels above the cutoff value and <0.1% sodium azide. Ready to use and assay specific.
- One (1) negative control ("CTRL –") (1.0 mL/vial): Human serum containing anti-SSA-52 antibodies at levels below the cutoff value and <0.1% sodium azide. Ready to use and assay specific.

Alegria Flash SSA-60

The Alegria Flash SSA-60 kit contains the following materials:

- One (1) Assay Cartridge containing bead suspension, sample diluent, and conjugate reagents.
  - Bead suspension (0.5 mL): Magnetic particles coated with recombinant SSA-60 antigen in storage buffer containing Tween-20, bovine serum albumin, phosphate-buffered-saline, and <0.1% sodium azide. Ready to use.
  - Sample diluent (10 mL): Phosphate-buffered-saline solution containing detergent, proteins, <0.1% sodium azide, and ProClin 300. Ready to use.
  - Conjugate (10 mL): Anti-human IgG conjugated with Acridinium Ester in a buffered solution containing ProClin 300. Ready to use.
- One (1) Calibrator 1 (blue-capped vial) (0.5 mL): Human serum that contains anti-SSA-60 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): Human serum containing anti-SSA-60 autoantibodies at levels near the cutoff value and <0.1% sodium azide. Ready to use. Assay and lot specific.

A separate Alegria Flash SSA-52 Control kit is sold separately. It contains:

- One (1) positive control ("CTRL +") (0.1 mL/vial): Human serum containing anti-SSA-60 antibodies at levels above the cutoff value and <0.1% sodium azide. Ready to use and assay specific.
- One (1) negative control ("CTRL –") (1.0 mL/vial): Human serum containing anti-SSA-60 antibodies at levels below the cutoff value and <0.1% sodium azide. Ready to use and assay specific.

Alegria Flash SSB

The Alegria Flash SSB kit contains the following materials:

- One (1) Assay Cartridge containing bead suspension, sample diluent, and conjugate reagents.
  - Bead suspension (5 mL): Magnetic particles that are coated with SSB (La) antigen in storage buffer containing Tween-20, bovine serum albumin, phosphate-buffered-saline, and <0.1% sodium azide. Ready to use.
  - Sample diluent (10 mL): Phosphate-buffered-saline solution containing detergent, proteins, <0.1% sodium azide, and ProClin 300. Ready to use.
  - Conjugate (10 mL): 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): Human serum containing anti-SSB 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): Human serum containing anti-SSB autoantibodies at levels near the cutoff value and <0.1% sodium azide. Ready to use. Assay and lot specific.

A separate Alegria Flash SSB Control kit is sold separately. It contains:

- One (1) positive control ("CTRL +") (1.0 mL/vial): Human serum containing anti-SSB antibodies at levels above the cutoff value and <0.1% sodium azide. Ready to use and assay specific.
- One (1) negative control ("CTRL –") (1.0 mL/vial): Human serum containing anti-SSB 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 SSA-52, SSA-60, SSB assay kits are designed to detect human anti- SSA-52, SSA-60, and SSB IgG autoantibodies, in human serum. The Alegria Flash SSA-52, SSA-60, SSB test procedures involve 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 SSA-52, SSA-60, or SSB 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 (RU). RU values above or equal to the cut-off value of 100 RU are indicative of the presence of anti-SSA-52, anti-SSA-60, or anti-SSB antibodies within the original serum sample.

V Substantial Equivalence Information:

A Predicate Device Name(s):

QUANTA Flash Ro52
QUANTA Flash Ro60
AtheNA Multi-Lyte ANA-II Plus Test System (SS-B)

B Predicate 510(k) Number(s):

K141655
K141328
K021103

C Comparison with Predicate(s):

Alegria Flash SS-A 52

Device & Predicate Device(s):	**K250814 (Candidate Device)**	**K141655 (Predicate Device)**
Device Trade Name	Alegria Flash SSA-52	QUANTA Flash SSA-52
General Device Characteristic Similarities		
Detection Principle	Chemiluminescent immunoassay	Same
Sample Matrix	Serum	Same
Antigen	Recombinant Ro52	Same
General Device Characteristic Differences		
Intended Use/ Indications For Use	The Alegria Flash SSA-52 kit uses chemiluminescent immunoassay (CLIA) technology for the qualitative detection of IgG antibodies to SSA-52 in human serum. It is intended for use as an aid in the diagnosis of Sjögren's syndrome (SJS), Systemic Lupus Erythematosus (SLE), Systemic	QUANTA Flash Ro52 is a chemiluminescent immunoassay for the semi-quantitative determination of IgG anti-Ro52 autoantibodies in human serum. The presence of anti-Ro52 autoantibodies, in conjunction with clinical findings and other

	Sclerosis (SS), Polymyositis (PM), and Dermatomyositis (DM) in conjunction with other laboratory and clinical findings. The test must be performed on the Alegria Flash instrument.	laboratory tests, is an aid in the diagnosis of Systemic Lupus Erythematosus, Sjögren's Syndrome, Systemic Sclerosis, Idiopathic Inflammatory Myopathies.
Type of Test	Qualitative	Semi-quantitative
Instrumentation	Alegria Flash	QUANTA Flash
Assay Units	Relative light units (RU)	Chemiluminescent units (CU)
Analytical Measuring Interval	Not applicable	2.3 CU – 1685.3 CU
Cut-off	100 RU	20 CU

Alegria Flash SS-A 60

Device & Predicate Device(s):	**K250814 (Candidate Device)**	**K141328 (Predicate Device)**
Device Trade Name	Alegria Flash SSA-60	QUANTA Flash SSA-60
General Device Characteristic Similarities		
Detection Principle	Chemiluminescent immunoassay	Same
Sample Matrix	Serum	Same
General Device Characteristic Differences		
Intended Use/ Indications For Use	The Alegria Flash SSA-60 kit uses chemiluminescent immunoassay (CLIA) technology for the qualitative detection of IgG antibodies to SSA-60 in human serum. It is intended for use as an aid in the diagnosis of Sjögren's syndrome (SS) and Systemic Lupus Erythematosus (SLE) in conjunction with other laboratory and clinical findings. The test must be performed on the Alegria Flash instrument.	QUANTA Flash Ro60 is a chemiluminescent immunoassay for the semi-quantitative determination of IgG anti-Ro60 autoantibodies in human serum. The presence of anti-Ro60 autoantibodies, in conjunction with clinical findings and other laboratory tests, is an aid in the diagnosis of Systemic Lupus Erythematosus and Sjögren's Syndrome.
Instrumentation	Alegria Flash	QUANTA Flash
Assay Units	Relative light units (RU)	Chemiluminescent units (CU)
Antigen	Purified Native SSA-60	Recombinant SSA-60
Type of Test	Qualitative	Semi-quantitative
Analytical Measuring Interval	Not applicable	4.9 CU – 1374.8 CU
Cut-off	100 RU	20 CU

Alegria Flash SSB

Device & Predicate Device(s):	K250814 (Candidate Device)	K021103 (Predicate Device)
Device Trade Name	Alegria Flash SSB	Zeus AtheNA Multi-Lyte SSB
General Device Characteristic Similarities		
Intended Use/ Indications For Use	The Alegria Flash SSB kit uses chemiluminescent immunoassay (CLIA) technology for the semi-quantitative measurement of IgG antibodies to SSB (La) in human serum. It is intended for use as an aid in the diagnosis of Sjögren's syndrome (SS) and Systemic Lupus Erythematosus (SLE) in conjunction with other laboratory and clinical findings. The test must be performed on the Alegria Flash instrument.	The ZEUS AtheNA Multi-Lyte ANA-II Plus Test System is intended for the semi-quantitative detection of IgG class antibody to eight separate analytes (SSA, SSB, Sm,RNP, Scl-70, Jo-1, Centromere B, and Histone) in human serum, the quantitative detection of IgG class antibody to dsDNA in human serum, and the qualitative detection of IgG class antibody to ANA. The test system is intended to be used as an aid in the diagnosis of various systemic autoimmune disorders. This test is for In Vitro Diagnostic use.
Type of Test	Semi-quantitative	Same
Sample Matrix	Serum	Same
General Device Characteristic Differences		
Detection Principle	Chemiluminescent immunoassay	Luminex-based, multiplex Immunoassay
Instrumentation	Alegria Flash	AtheNA Multi-Lyte platform
Antigen	Recombinant SSB	Purified SSB
Analytical Measuring Interval	20.2 – 526.2 RU	Not specified
Cut-off	100 RU	< 100 Units Negative 100 – 120 Units Equivocal > 120 Units Positive

VI Standards/Guidance Documents Referenced:

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

- CLSI EP05-A3, Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline – Third Edition

- CLSI EP06-Ed2, Evaluation of the Linearity of Quantitative Measurement Procedures – Second Edition
- CLSI EP07-Ed3 – Interference Testing in Clinical Chemistry
- CLSI EP09c 3rd Edition, Measurement Procedure Comparison and Bias Estimation Using Patient Samples
- 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:

Precision and reproducibility of the Alegria Flash SSA-52, Alegria Flash SSA-60, and Alegria Flash SSB were evaluated in accordance with the CLSI guideline EP05-A3. The samples panels were prepared by mixing positive native serum samples with negative serum samples.

Within-Lab Precision:

To evaluate within-laboratory precision of the assays, a variable number of samples containing various concentrations of antibodies were assayed in duplicate, twice a day, for 20 days, for a total of 80 measurements per sample, using one reagent lot at one laboratory site, by one operator.

- For the qualitative Alegria Flash SSA-52 and Alegria Flash SSA-60 assays, the percent of positive (% Pos), with 95% confidence interval (CI), for each sample was calculated.
- For the semi-quantitative Alegria Flash SSB assay, the data was analyzed using Analyze-it for Excel software by analysis of variance (ANOVA) methods and the within-run (repeatability), between-run, between-day, and within-laboratory precision were determined.

The results are summarized in the following tables:

Alegria Flash SSA-52:

Sample	Expected Result	Relative Units (RU)		Positive		
		Mean	Range	n(+)/N	% Pos.	(95% CI)
1	Positive	403.1	361.8–441.6	80/80	100	(95.4–100)
2	Positive	333.7	286.0–462.1	80/80	100	(95.4–100)
3	Positive	250.6	198.4–370.3	80/80	100	(95.4–100)

Sample	Expected Result	Relative Units (RU)		Positive		
		Mean	Range	n(+)/N	% Pos.	(95% CI)
4	Positive	162.8	123.2–209.5	80/80	100	(95.4–100)
5	Positive	101.2	74.4–132.5	80/80	100	(95.4–100)
6	Negative	85.2	73.4–99.4	47/80	58.8	(47.8–68.9)
7	Negative	66.8	49.0–93.4	0/80	0.0	(0.0–4.6)
8	Negative	35.5	28.4–42.9	0/80	0.0	(0.0–4.6)

Alegria Flash SSA-60:

Sample	Expected Result	Relative Units (RU)		Positive		
		Mean	Range	n(+)/N	% Pos.	(95% CI)
1	Positive	365.6	271.8–425.7	80/80	100	(95.4–100)
2	Positive	269.2	213.4–317.5	80/80	100	(95.4–100)
3	Positive	168.4	125.6–202.3	80/80	100	(95.4–100)
4	Negative	98.0	77.6–111.2	36/80	45.0	(34.6–55.9)
5	Negative	88.4	64.2–107.3	6/80	7.5	(3.5–15.4)
6	Negative	65.8	47.1–79.2	0/80	0.0	(0.0–4.6)
7	Negative	27.8	20.0–35.5	0/80	0.0	(0.0–4.6)

Alegria Flash SSB

		Within-Run		Between-Run		Between-Day		Within-Laboratory	
Sample	Mean (RU)	SD (RU)	%CV	SD (RU)	%CV	SD (RU)	%CV	SD (RU)	%CV
1	399.3	16.3	4.1	0.0	0.0	21.8	5.5	27.2	6.8
2	238.6	7.5	3.2	4.6	1.9	10.4	4.4	13.7	5.7
3	118.7	6.1	5.1	0.9	0.7	5.2	4.4	8.1	6.8
4	92.3	3.2	3.5	2.8	3.0	6.4	7.0	7.7	8.3
5	71.1	2.8	3.9	0.7	1.0	3.3	4.7	4.4	6.2
6	41.9	2.3	5.4	0.5	1.3	1.5	3.6	2.8	6.6

Site-to-Site Reproducibility:

The reproducibility of the Alegria Flash assays was conducted at three sites using a variable number of samples containing various concentrations of antibodies. Samples were assayed in quintuplicate, once a day, for five days to generate 25 data points per sample per site using one reagent lot and a total of 75 replicates per sample. Instrument variables were nested within the multiple site component – i.e., a different instrument was used at each of the three sites.

- For the qualitative Alegria Flash SSA-52 and Alegria Flash SSA-60 assays, the percent of positive (% Pos), with 95% confidence interval (CI) for all sites combined, for each sample was calculated.
- For the semi-quantitative Alegria Flash SSB assay, the data was analyzed using Analyze-it for Excel software by analysis of variance (ANOVA) methods and the within-run

(repeatability), between-run, between-day, and within-laboratory precision were determined.

The results are summarized in the following tables:

Alegria Flash SSA-52

Sample	Expected Result	Total (across sites)				Site 1		Site 2		Site 3	
		Mean (RU)	Range (RU)	n(+)/N	% Pos	n(+)/N	% Pos	n(+)/N	% Pos	n(+)/N	% Pos
1	Positive	403.9	361.2–436.4	75/75	100	25/25	100	25/25	100	25/25	100
2	Positive	239.9	208.2–273.9	75/75	100	25/25	100	25/25	100	25/25	100
3	Positive	129.2	116.6–150.0	75/75	100	25/25	100	25/25	100	15/25	60
4	Positive	102.9	91.2–121.0	47/75	62.7	8/25	32	24/25	86	25/25	100
5	Negative	72.7	61.2–84.8	0/75	0	0/25	0	0/25	0	0/25	0
6	Negative	34.1	27.0–44.0	0/75	0	0/25	0	0/25	0	0/25	0

Alegria Flash SSA-60

Sample	Expected Result	Total (across sites)				Site 1		Site 2		Site 3	
		Mean (RU)	Range (RU)	n(+)/N	% Pos	n(+)/N	% Pos	n(+)/N	% Pos	n(+)/N	% Pos
1	Positive	399.3	243.1–464.8	75/75	100	25/25	100	25/25	100	25/25	100
2	Positive	241.9	190.2–275.6	75/75	100	25/25	100	25/25	100	25/25	100
3	Positive	130.5	103.6–149.9	75/75	100	25/25	100	25/25	100	15/25	60
4	Negative	93.6	60.0–109.7	16/75	21.3	7/25	28	2/25	8	7/25	28
5	Negative	75.8	55.5–86.9	0/75	0	0/25	0	0/25	0	0/25	0
6	Negative	41.5	30.2–50.1	0/75	0	0/25	0	0/25	0	0/25	0

Alegria Flash SSB

		Within-Run		Between-Day		Between-Site/Instrument		Reproducibility	
Sample	Mean (RU)	SD (RU)	%CV	SD (RU)	%CV	SD (RU)	%CV	SD (RU)	%CV
1	406.7	17.7	4.3	21.7	5.3	2.0	0.5	28.0	6.9
2	245.3	11.3	4.6	8.7	3.6	8.2	3.3	16.4	6.7
3	122.7	5.5	4.5	3.9	3.2	5.1	4.2	8.5	6.9
4	93.3	3.3	3.6	3.7	4.0	5.2	5.6	7.2	7.7
5	74.7	3.6	4.9	2.4	3.2	6.5	8.8	7.9	10.5
6	48.1	2.8	5.8	1.7	3.5	4.6	9.6	5.7	11.7

Lot-to-Lot Imprecision

To evaluate the between-lot imprecision of the Alegria Flash, a variable number of samples containing various concentrations of antibodies 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.

- For the qualitative Alegria Flash SSA-52 and Alegria Flash SSA-60 assays, the percent of positive (% Pos), with 95% confidence interval (CI) for all lots combined, for each sample was calculated.
- For the semi-quantitative Alegria Flash SSB assay, the data was analyzed using Analyze-it for Excel software by analysis of variance (ANOVA) methods and the within-run (repeatability), between-run, between-day, and within-laboratory precision were determined.:

Alegria Flash SSA-52:

Sample	Expected Result	Total (across lots)				Lot 1		Lot 2		Lot 3	
		Mean (RU)	Range (RU)	n(+)/N	% Pos	n(+)/N	% Pos	n(+)/N	% Pos	n(+)/N	% Pos
1	Positive	414.6	361.2–483.1	75/75	100	25/25	100	25/25	100	25/25	100
2	Positive	243.8	208.2–279.1	75/75	100	25/25	100	25/25	100	25/25	100
3	Positive	135.6	121.0–150.9	75/75	100	25/25	100	25/25	100	25/25	100
4	Positive	110.0	97.7–121.0	73/75	97.3	24/25	96	25/25	100	24/25	96.0
5	Negative	79.9	67.8–87.4	0/75	0.0	0/25	0.0	0/25	0.0	0/25	0.0
6	Negative	38.1	30.7–44.4	0/75	0.0	0/25	0.0	0/25	0.0	0/25	0.0

Alegria Flash SSA-60:

Sample	Expected Result	Total (across lots)				Lot 1		Lot 2		Lot 3	
		Mean (RU)	Range (RU)	n(+)/N	% Pos	n(+)/N	% Pos	n(+)/N	% Pos	n(+)/N	% Pos
1	Positive	409.3	308.1–463.3	75/75	100	25/25	100	25/25	100	25/25	100
2	Positive	250.3	218.3–281.7	75/75	100	25/25	100	25/25	100	25/25	100
3	Positive	141.4	110.4–158.9	75/75	100	25/25	100	25/25	100	25/25	100
4	Positive	105.7	86.5–122.7	56/75	74.7	7/25	28	24/25	96	25/25	100
5	Negative	85.7	74.4–99.3	0/75	0.0	0/25	0.0	0/25	0.0	0/25	0.0
6	Negative	42.9	32.2–51.1	0/75	0.0	0/25	0.0	0/25	0.0	0/25	0.0

Alegria Flash SSB:

		Within-Run		Between-Day		Between-Lot		Total	
Sample	Mean (RU)	SD (RU)	%CV	SD (RU)	%CV	SD (RU)	%CV	SD (RU)	%CV
1	389.9	19.4	5.0	18.2	4.7	0	0	26.6	6.8
2	230.6	10.0	4.3	9.0	3.9	1.4	0.6	13.5	5.9
3	117.1	5.6	4.8	4.9	4.1	2.2	1.9	7.8	6.6
4	87.7	2.8	3.2	4.0	4.6	2.5	2.9	5.5	6.3
5	68.9	2.4	3.5	3.1	4.5	2.7	4.0	4.8	6.9
6	43.3	2.5	5.9	2.2	5.2	2.3	5.2	4.1	9.4

2. Linearity:

The linearity of the semi-quantitative Alegria Flash SSB analytical measuring range was evaluated by following CLSI EP06-Ed2. Various samples were prepared as overlapping dilution series by mixing human serum samples with high antibody concentrations and

samples with low antibody concentrations to cover portions of the analytical measuring interval (AMI) of each analyte. Each sample was tested in five replicates and the results from each sample were analyzed separately using a weighted least squares regression analysis. All dilutions included in the sample regressions were within ±10% deviation from the linearity. The results are summarized below.

Alegria Flash SSB:

Sample	Test Range (RU)	Slope (95% CI)	R^2	Range of Linearity Deviations
1	172.8 – 526.2	0.96 (0.90 – 1.02)	1.00	-3.7% – 3.2%
2	97.5 – 245.3	0.99 (0.85 – 1.14)	0.98	-4.4% – 6.2%
3	20.2 – 99.9	1.01 (0.96 – 1.05)	1.00	-5.4% – 4.1

The data supports a linearity of the claimed analytical measuring interval (AMI) of 20.2–526.2 RU for the Alegria Flash SSA-60 assay.

The Alegria Flash SSB assay does not have an extended measuring range for samples above the AMI. Samples exceeding the AMI are reported as "above assay" in the result report. The package insert instructs users to report the result as >526.2 RU.

3. Analytical Specificity/Interference:

An interference study was performed based on the recommendations contained in CLSI EP07-A2. Two human serum specimens − one positive specimen at ~125 RU and one negative specimen at ~75 RU, were assessed as vehicle-control or interferent-spiked contrived specimens in five replicates using the Alegria Flash SSA-52, Alegria Flash SSA-60, and Alegria Flash SSB assays. The percent recovery for each sample that was spiked with the potential interfering substance was calculated by comparing its result to that of the corresponding control sample without the interfering substance. The following endogenous and exogenous substances showed <±15% recovery than the control:

Endogenous Substances		Exogenous Substances	
Interferent	**Testing Concentration**	**Interferent**	**Testing Concentration**
Bilirubin	0.15 mg/mL	Ibuprofen	0.219 mg/ml
Cholesterol	2.2 mg/mL	Hydroxychloroquine	0.024 mg/mL
Triglycerides	2.5 mg/mL	Prednisone	0.000099 mg/mL
Albumin	52 mg/mL	Azathioprine	0.00258 mg/mL
Hemoglobin	200 mg/dL	Diltiazem	0.0009 mg/mL
RF	400 U/mL	Rituximab	2 mg/mL
		Methotrexate	1.36 mg/mL
		Enalapril	0.000819 mg/mL
		Omeprazole	0.0084 mg/mL
		Losartan	0.000306 mg/ml
		Atenolol	0.009 mg/mL

Endogenous Substances		Exogenous Substances	
Interferent	**Testing Concentration**	**Interferent**	**Testing Concentration**
		Erythromycin	0.138 mg/mL
		Amoxicillin	0.054 mg/mL
		Ranitidine	0.0105 mg/mL
		Furosemide	0.0159 mg/mL
		Alendronate	0.000034 mg/mL
		Atorvastatin	0.00075 mg/mL

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 three Alegria Flash assays to illustrate the analytical specificity of the assay. The results are outlined below.

Panel ID	CDC Description	Alegria Flash Assay Result		
		SSA-52	**SSA-60**	**SSB**
ANA 01	ANA Homogenous Positive/ anti-native DNA	Negative	Negative	Negative
ANA 02	ANA Speckled Positive/anti-SSB/La	Positive	Positive	Positive
ANA 03	ANA Speckled Positive/ anti-U1 RNP, SSB/La, SSA/Ro	Negative	Positive	Positive
ANA 04	anti-U1 RNP Positive	Negative	Negative	Negative
ANA 06	ANA Nucleolar (U3RNP) Positive	Negative	Negative	Negative
ANA 07	anti-SSA Ro Positive	Positive	Positive	Negative
ANA 08	ANA Centromere Positive	Negative	Negative	Negative
ANA 09	ANA anti-Scl70 Positive	Negative	Negative	Negative
ANA 10	anti Jo-1 Positive	Positive	Negative	Negative
ANA 11	anti-PM/Scl Positive	Negative	Negative	Negative
ANA 12	anti-Ribosomal P Positive	Negative	Negative	Negative
ANA 15	anti-MPO Positive	Negative	Negative	Negative
ANA 16	anti-PR3 Positive	Negative	Negative	Negative

4. Assay Reportable Range:

Not applicable for the qualitative Alegria Flash SSA-52 and Alegria Flash SSA-60.

The assay reportable range is the same as the analytical measuring intervals (AMI) for the Alegria Flash SSB assay: 20.2 – 526.2 RU

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

Traceability

There are no international reference standards for anti-SSA-52, anti-SSA-60, and anti-SSB antibodies that allow metrological standardization of the assays. Calibrator and control values are directly traceable to in-house reference materials that are used to create the master curves for each Alegria Flash assay lot.

Stability:

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, serum sample panels were assessed in triplicate on three lots of Alegria Flash SSA-52, Alegria Flash SSA-60, and Alegria Flash SSB kits at various time points. The results support that the assay kits are stable when stored unopened at 2-8°C for up to six months.

On-board stability: The onboard storage stability of the three Alegria Flash kits was determined by testing three serum samples and two assay-specific Alegria Flash controls using the Alegria Flash reagents stored opened within the Alegria Flash's refrigerated reagent bay. The results of the study support that Alegria Flash SSA-52, Alegria Flash SSA-60, and Alegria Flash SSB ENA 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, two serum samples – a low positive and a high negative – were stored up to 15 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 14 days at 2-8°C or at room temperature.

To determine the stability of patient samples subjected to multiple freeze/thaw cycles, two serum samples – a low positive and a high negative – were subjected to six 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 five times.

6. Detection Limit:

Limit of Blank (LoB), Limit of Detection (LoD), and Limit of Quantitation (LoQ) for the Alegria Flash SSB assay were determined by following CLSI EP17-A2.

LoB: To determine the LoB, four analyte-free blank samples were run in 20 replicates, once a day for three days using two reagent lots, for a total of 60 data points per sample and lot (480 total replicates). The LoB was determined for each analyte, on each reagent lot separately, at the 95th percentile using the non-parametric method for all analyses. The higher LoB result between the two lots was selected for the final LoB value. The LoB of the assay is 17.4 RU.

LoD: To determine the LoD for each analyte, four low level samples were assayed in 20 replicates, for three days using two reagent lots, to generate a total of 60 data points for each sample on each reagent lot. The LoD was determined separately for each sample on each reagent lot and the highest LoD result was determined for the final LoD value. The LoD of the assay is 20.1 RU.

LoQ: To determine the LoQ for each analyte, the four LoD samples and an additional four low-level samples greater than the LoD were run in 20 replicates, for three days using two reagent lots, to generate 60 data points for each sample on each reagent lot. The LoQ was determined separately for each sample on each reagent lot by calculating the total imprecision of each sample. The LoQ was defined as the lowest concentration level that meets the within-laboratory imprecision of ≤15% for each lot. The LoQ of the assay is 21.1 RU.

7. Assay Cut-Off:

The assays' cut-offs are 100 RU.

B Comparison Studies:

1. Method Comparison with Predicate Device:

Clinically characterized samples were tested on the Alegria Flash assays and their predicate devices. Positive percent agreement (PPA) and negative percent agreement (NPA), with 95% confidence intervals were calculated for each analyte comparison. For the qualitative Alegria Flash SSA-52 and Alegria Flash SSA-60 assays, all values were included in the PPA and NPA determination. For the semi-quantitative SS-B assay, values that were outside of the measuring ranges of either assay were excluded from the calculations. The following tables summarize the assays' method comparison study results:

Alegria Flash SSA-52:

		Predicate		
		Positive	Negative	Total
Alegria Flash SSA-52	Positive	57	6	163
	Negative	0	54	54
	Total	57	60	117

PPA 100% (95%CI 93.7 – 100.0)
NPA 90% (95%CI 79.9 – 95.3)

Alegria Flash SSA-60:

		Predicate		
		Positive	Negative	Total
Alegria Flash SSA-52	Positive	57	6	163
	Negative	0	54	54
	Total	57	60	117

PPA 100% (95%CI 93.7 – 100.0)
NPA 90% (95%CI 79.9 – 95.3)

Alegria Flash SSB:

		Predicate SSB			
		Positive	**Equivocal**	**Negative**	**Total**
Alegria Flash SSB	**Positive**	59	1	7	67
	Negative	6	4	313	323
	Total	65	5	320	390

Equiv as Neg **PPA** 90.8% (95%CI 81.3 – 95.7)
NPA 97.5% (95%CI 95.2 – 98.8)

Equiv as Pos **PPA** 85.7% (95%CI 75.7 – 92.0)
NPA 97.8% (95%CI 95.6 – 98.9)

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 SSA-52, Alegria Flash SSA-60, and Alegria Flash SSB assays. The clinical validation study included 913 total samples including samples from patients with Sjögren's syndrome (SjS, *N*=134), systemic lupus erythematosus (SLE, *N* =178), systemic sclerosis (SSc, *N*=124)[1], polymyositis (PM, *N* =50), dermatomyositis (DM, *N* =50) and control samples (*N* =376) from patients with various types of autoimmune and infectious diseases. The performance summarized below is organized by assay:

[1] The SSc cohort consisted of 70 samples diagnosed with SSc using the 2013 EULAR/ACR Classification Criteria but were not further categorized and 54 samples diagnosed with limited cutaneous systemic sclerosis (lcSSc, formerly known as CREST syndrome).

Alegria Flash SSA-52:

		Diagnosis						
		SLE	**SjS**	**SSc**	**PM**	**DM**	**Controls**	**Total**
Alegria Flash SSA-52	**Positive**	74	99	25	11	13	16	238
	Negative	104	35	89	39	37	360	674
	Total	178	134	124	50	50	376	912

SLE	**Sensitivity**	41.6%	(95%CI 34.6 – 48.9%)
SjS	**Sensitivity**	73.9%	(95%CI 65.8 – 80.6%)
SSc	**Sensitivity**	20.2%	(95%CI 14.0–28.1%)
PM	**Sensitivity**	22.0%	(95%CI 12.8 – 35.2%)
DM	**Sensitivity**	26.0%	(95%CI 15.9 – 39.6%)
	Specificity	95.7%	(95%CI 93.2 – 97.4%)

SSA-52
Distribution of clinical study samples and antibody positivity rates:

Diagnostic Group	***N***	**SSA-52 Positive**	
		n	**(%)**
Target conditions			
Systemic lupus erythematosus (SLE)	178	74	41.6
Sjögren's Syndrome (SjS)	134	99	73.9
Systemic sclerosis (SSc)	124	25	20.2
Polymyositis (PM)	50	11	22.0
Dermatomyositis (DM)	50	13	26.0
Differential diagnosis controls			
Mixed Connective Tissue Disease	20	4	20.0
Autoimmune Hepatitis	30	2	6.7
Antiphospholipid Syndrome	20	0	0
Cancer	19	0	0
Celiac Disease	24	0	0
Drug Induced Lupus	5	2	40.0
Fibromyalgia	20	0	0
Crohn's Disease	30	0	0
Ulcerative Colitis	29	0	0
HBV	17	2	11.8
HIV	12	0	0
HCV	10	1	10.0
HSV	10	0	0
Primary Biliary Cholangitis	30	1	3.3
Rheumatoid Arthritis	20	2	10.0
Vasculitis (Undifferentiated)	6	0	0
Vasculitis (ANCA Associated)	5	0	0
Vasculitis (Large/Med Vessel)	10	0	0
Atrophic Gastritis	20	0	0
Graves' Disease	20	0	0
Hashimoto's Thyroiditis	19	2	10.5
Total Controls	**376**	**16**	**4.3%**

Alegria Flash SSA-60:

		Diagnosis			
		SLE	**SjS**	**Controls**	**Totals**
Alegria Flash SSA-60	**Positive**	92	104	30	226
	Negative	86	30	570	686
	Total	178	134	600	912

SLE	**Sensitivity**	51.7%	(95%CI 44.4 – 58.9)
SjS	**Sensitivity**	77.6%	(95%CI 69.8 – 83.8)
	Specificity	95.0%	(95%CI 93.0 – 96.5)

SSA-60
Distribution of clinical study samples and antibody positivity rates:

Diagnostic Group	***N***	**SSA-60 Positive**	
		n	**(%)**
Target conditions			
Systemic lupus erythematosus (SLE)	178	92	51.7
Sjögren's Syndrome (SjS)	134	104	77.6
Differential diagnosis controls			
Systemic sclerosis (SSc)	124	9	7.3
Polymyositis (PM)	50	3	6.0
Dermatomyositis (DM)	50	4	8.0
Mixed Connective Tissue Disease	20	2	10.0
Autoimmune Hepatitis	30	2	6.7
Antiphospholipid Syndrome	20	0	0
Cancer	19	0	0
Celiac Disease	24	0	0
Drug Induced Lupus	5	2	40.0
Fibromyalgia	20	1	5.0
Crohn's Disease	30	0	0
Ulcerative Colitis	29	0	0
HBV	17	1	5.9
HIV	12	0	0
HCV	10	0	0
HSV	10	0	0
Primary Biliary Cholangitis	30	1	3.3
Rheumatoid Arthritis	20	1	5.0
Vasculitis (Undifferentiated)	6	1	16.7
Vasculitis (ANCA Associated)	5	0	0
Vasculitis (Large/Med Vessel)	10	0	0
Atrophic Gastritis	20	0	0
Graves' Disease	20	1	5.0
Hashimoto's Thyroiditis	19	2	10.5
Total Controls	**600**	**30**	**5.0%**

Alegria Flash SSB:

		Diagnosis			
		SLE	**SjS**	**Controls**	**Totals**
Alegria Flash SSB	**Positive**	36	69	10	115
	Negative	142	65	590	797
	Total	178	134	600	912

SLE	**Sensitivity**	20.2%	(95%CI 14.0 – 25.5)
SjS	**Sensitivity**	51.5%	(95%CI 43.1 – 59.8)
	Specificity	98.3	(95%CI 97.0 – 99.1)

SSB
Distribution of clinical study samples and antibody positivity rates:

Diagnostic Group	***N***	**SSA-60 Positive**	
		n	**(%)**
Target conditions			
Systemic lupus erythematosus (SLE)	178	36	20.2
Sjögren's Syndrome (SjS)	134	69	51.5
Differential diagnosis controls			
Systemic sclerosis (SSc)	124	3	2.4
Polymyositis (PM)	50	1	2.0
Dermatomyositis (DM)	50	2	4.0
Mixed Connective Tissue Disease	20	0	0
Autoimmune Hepatitis	30	1	3.33
Antiphospholipid Syndrome	20	0	0
Cancer	19	0	0
Celiac Disease	24	0	0
Drug Induced Lupus	5	0	0
Fibromyalgia	20	0	0
Crohn's Disease	30	0	0
Ulcerative Colitis	29	0	0
HBV	17	0	0
HIV	12	0	0
HCV	10	0	0
HSV	10	0	0
Primary Biliary Cholangitis	30	0	0
Rheumatoid Arthritis	20	1	5.0
Vasculitis (Undifferentiated)	6	0	0
Vasculitis (ANCA Associated)	5	0	0
Vasculitis (Large/Med Vessel)	10	0	0
Atrophic Gastritis	20	0	0
Graves' Disease	20	0	0
Hashimoto's Thyroiditis	19	2	10.5
Total Controls	**600**	**10**	**1.7%**

D Clinical Cut-Off:

The assays' cut-offs are 100 Units.

E Expected Values/Reference Range:

A negative Alegria Flash SSA-52, Alegria Flash SSA-60, and Alegria Flash SSB test result is expected for apparently healthy individuals. To determine the expected values for the three assays, a panel of 200 apparently healthy blood donors (100 females/100 males, ages 16 to 80 years, mean 51 years of age, median 54 years of age) were tested. The results are as follows:

Assay	Number of samples positive	Mean concentration (RU)	Range (RU)
Alegria Flash SSA-52	3 (1.5%)	N/A	N/A
Alegria Flash SSA-60	4 (2.0%)	N/A	N/A
Alegria Flash SSB	1 (0.5%)	6.0	0.0 – 1042.5

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.