

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

A. 510(k) Number:

k062183

B. Purpose for Submission:

New device

C. Measurand:

Double-stranded DNA (dsDNA) autoantibodies (high avidity)

D. Type of Test:

Enzyme-linked immunosorbent assay (ELISA)

E. Applicant:

The Binding Site

F. Proprietary and Established Names:

FARRZYME Human High Avidity anti-dsDNA Enzyme Immunoassay Kit

G. Regulatory Information:

1. Regulation section:

CFR §866.5100 Antinuclear antibody immunological test system

2. Classification:

Class II

3. Product code:

LRM: Anti-DNA antibody (enzyme-labeled), antigen, control

4. Panel:

Immunology (82)

H. Intended Use:

1. Intended use:

The FARRZYME assay is intended for the in-vitro measurement of specific, high avidity IgG autoantibodies against double stranded deoxyribonucleic acid (dsDNA) present in human serum, as an aid to the diagnosis of systemic lupus erythematosus (SLE), in conjunction with other serological test results and clinical findings.

2. Indication for use:

Same as Intended Use

3. Special conditions for use statement(s):

Prescription use only

4. Special instrument requirements:

Microplate reader capable of measuring OD at 450nm referenced on air

I. Device Description:

The device consists of dsDNA (calf thymus) coated microwell strips; Sample Diluent; Wash Buffer; Calibrators containing anti-dsDNA autoantibodies at levels of 1000, 333, 111, 37, and 12.3 IU/mL; Positive Control containing diluted human serum and an assayed amount of anti-dsDNA autoantibodies; Negative Control containing diluted human serum; Single-Stranded DNA antibody Control; Conjugate with purified peroxidase labeled antibody to rabbit anti-human IgG; TMB Substrate; and 3M phosphoric acid Stop Solution.

J. Substantial Equivalence Information:

1. Predicate device name(s):
TBS BINDAZYME Human Anti-dsDNA Enzyme Immunoassay Kit
TBS *CRITHIDIA LUCILIAE* dsDNA Kit/Substrate Slides
Farr Radioimmunoassay (RIA) (pre-amendment, laboratory method)
2. Predicate 510(k) number(s):
K993727
K930987
3. Comparison with predicate devices:

Similarities		
Item	Device	Predicate
	FARRZYME	BINDAZYME
Indication for Use	Aid in the diagnosis of SLE	Same
DNA antigen source	Calf thymus	Same
Method	ELISA	Same
Calibrators	5 levels: 1000, 333, 111, 37, 12.3 IU/mL	Same
Controls	Positive and negative	Same
Second negative control	ssDNA antibody control	Same
Labeled conjugate	Peroxidase labeled rabbit anti-human IgG	Same
Substrate	TMB	Same
Stop solution	3M phosphoric acid	Same

Differences		
Item	Device	Predicate
	FARRZYME	BINDAZYME
dsDNA antibodies detected	High avidity	High and low avidity
Wash buffer	More stringent	Conventional

Similarities		
Item	Device	Predicate
	FARRZYME	<i>Crithidia luciliae</i>
Indication for Use	Aid in the diagnosis of SLE	Same

Differences		
Item	Device	Predicate
	FARRZYME	<i>Crithidia luciliae</i>
DNA antigen source	Calf thymus	<i>C. luciliae</i>
Method	ELISA	Indirect immunofluorescence

Differences		
Item	Device	Predicate
Calibrators	5 levels: 1000, 333, 111, 37, 12.3 IU/mL	None
Controls	Positive, negative and ssDNA control	Positive (apple-green kinetoplast staining) and negative
Labeled conjugate	Peroxidase labeled rabbit anti-human IgG	Fluorescein isothiocyanate (FITC) labeled sheep anti-human IgG
Counterstain	Not applicable	Evans Blue
Substrate	TMB	Not applicable
Stop solution	3M phosphoric acid	Not applicable
Mounting medium	Not applicable	Necessary for reading results

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

None referenced

L. Test Principle:

Calibrators, controls, and diluted patient samples are added to the wells and autoantibodies recognizing the dsDNA antigen bind during the first incubation. After washing the wells to remove all unbound proteins, purified peroxidase labeled rabbit anti-human IgG (γ chain specific) conjugate is added. The conjugate binds to the captured human autoantibody and the excess unbound conjugate is removed by a further wash step. The unbound conjugate is removed by a further wash step. The bound conjugate is visualized with 3,3',5,5' tetramethylbenzidine (TMB) substrate which gives a blue reaction product, the intensity of which is proportional to the concentration of autoantibody in the sample. Phosphoric acid is added to each well to stop the reaction. This produces a yellow end point color, which is read at 450nm. The controls and patient results are compared to a 5 point calibration curve for interpretation.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. *Precision/Reproducibility:*

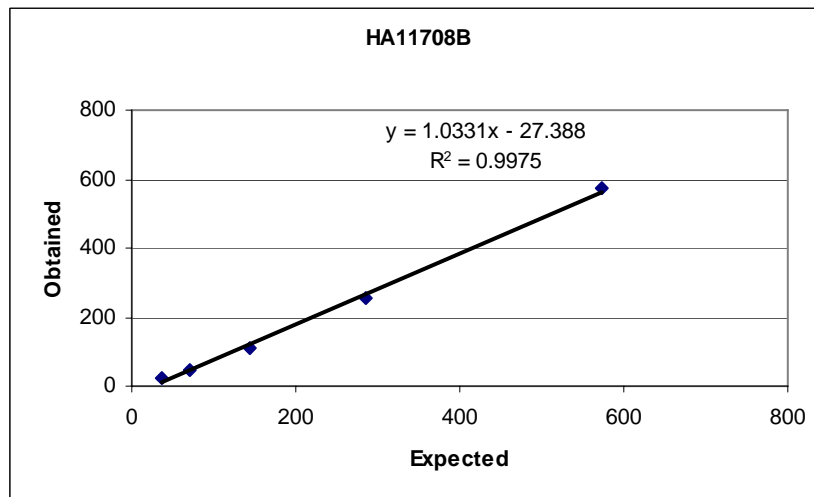
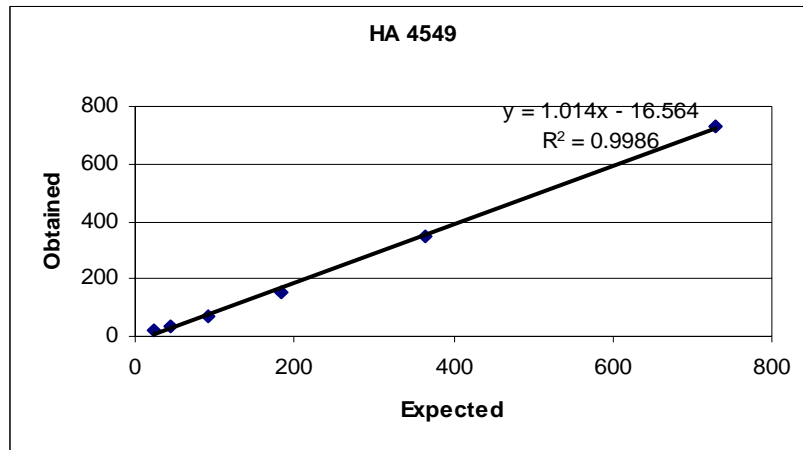
The intra- and inter-assay precisions were measured using 6 samples covering the range of the calibration curve. Intra-assay precision was measured using 20 replicates in one assay. Inter-assay precision was measured by testing samples in duplicate in 6 assays performed over 3 days.

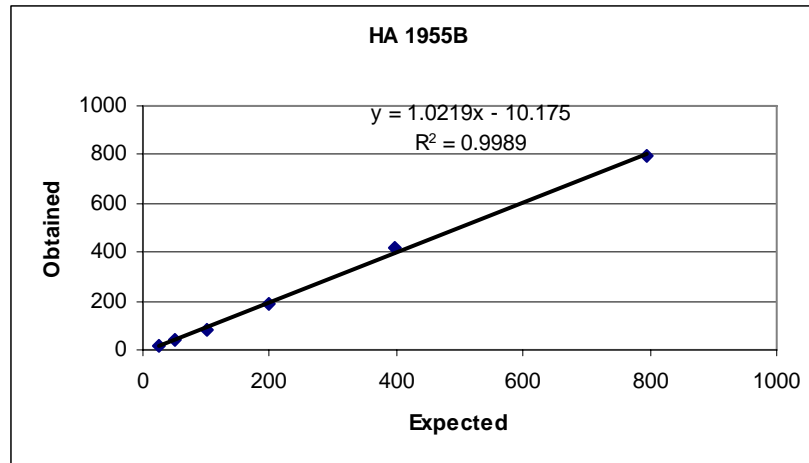
Intra-Assay Precision				Inter-Assay Precision			
N=20	IU/mL	SD	% CV	N=6	IU/mL	SD	% CV
Sample 1	25.1	1.35	5.4	Sample 1	24.6	3.32	13.5
Sample 2	39.0	1.86	4.8	Sample 2	42.3	1.66	3.9
Sample 3	74.5	1.64	2.2	Sample 3	61.3	7.13	11.6
Sample 4	205.5	6.74	3.3	Sample 4	123.2	6.02	4.9

Intra-Assay Precision				Inter-Assay Precision			
N=20	IU/mL	SD	% CV	N=6	IU/mL	SD	% CV
Sample 5	361.2	15.60	4.3	Sample 5	272.4	18.96	7.0
Sample 6	528.6	27.19	5.1	Sample 6	474.1	32.46	6.9

b. *Linearity/assay reportable range:*

The reportable range for the assay of 12.3-1000 IU/mL is based on the calibrators. In the linearity studies, high samples were first diluted at the original assay dilution of 1:100 then doubling dilutions were made.





c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*
The calibrators are standardized against the WHO reference material Wo-80.

d. *Detection limit:*

Confirmation that the FARRZYME assay can distinguish between two samples with values close to the bottom of the measuring range (120 and 165% of the lowest calibrator (12.3 IU/mL)) was obtained by statistical analysis (Student's t-test) of the results obtained from testing 20 replicates of each sample.

	Replicates	Mean (IU/mL)	Std. Dev.	% CV
Sample 1	20	20.30	1.06	5.25
Sample 2	20	14.96	0.97	6.49

e. *Analytical specificity:*

Various elevated serum components (free and conjugated bilirubin, hemoglobin, chyle and rheumatoid factor) were added to low (normal) and high (positive) sera which were then assayed to test the possible effect of interfering substances provided in an Interference Check A plus kit (Kokusai, Japan). No interference was observed at the following concentrations.

Substance	Concentration
Bilirubin F (free)	20.3 mg/dL
Bilirubin C (conjugated)	20.2 mg/dL
Hemoglobin	486 mg/dL
Chyle	1460 Units
Rheumatoid factor	45 IU/mL

Interference due to high IgG was evaluated in a separate study by testing 6 IgG myeloma sera on the FARRZYME assay and all gave negative results.

f. *Assay cut-off:*

The assay cut-off was established based on the results obtained from testing samples from healthy blood donors (n=150). At the established cut-off of ≤ 30

IU/mL as negative and >30 IU/mL as positive, the 150 normal sera all gave results below 17.0 IU/mL, with 146 (97%) of the results below 12.3 IU/mL. Of the 224 SLE patients also tested, 36% of the patient sera gave a positive result which is similar to the range found in the furnished literature of 28-63% for an unselected (active versus inactive) population.

2. Comparison studies:

a. *Method comparison with predicate device:*

Two-hundred fifty-two samples including 189 from patients with SLE, 28 samples from healthy normals, and 35 samples positive for dsDNA antibodies by *C. luciliae* or ELISA were tested by FARRZYME, by the TBS conventional dsDNA ELISA and by the TBS *CRITHIDIA LUCILIAE* immunofluorescent assay. The low positive agreements with both predicate devices are due to the fact that both the conventional dsDNA ELISA and the *C. luciliae* detect low, moderate and high avidity antibodies whereas the FARRZYME only detects high avidity antibodies.

		BINDAZYME dsDNA ELISA		
		+	-	Total
FARRZYME	+	47	4*	51
	-	33	168*	201
	Total	80	172	252
Positive Percent Agreement		58.8% (47/80)		
Negative Percent Agreement		97.7% (168/172)		
Overall Agreement		85.3% (215/252)		

* Includes (3) Borderline BINDAZYME results as negative

** Includes (51) Borderline BINDAZYME results as negative

		<i>Crithidia luciliae</i> assay		
		+	-	Total
FARRZYME	+	37	14	51
	-	18	183	201
	Total	55	197	252
Positive Percent Agreement		67.3% (37/55)		
Negative Percent Agreement		92.9% (183/197)		
Overall Agreement		87.3% (220/252)		

b. *Comparison to the Farr RIA*

A comparison was performed between the FARRZYME ELISA and the Farr RIA assay which detects high avidity antibodies. One hundred sixty-six samples were included.

		Farr RIA		
		+	-	Total
FARRZYME	+	44	7	51
	-	5	110	115
	Total	49	117	166

Positive Percent Agreement	89.8% (44/49)
Negative Percent Agreement	94.0% (110/117)
Overall Agreement	92.8% (154/166)

c. Matrix comparison:

The FARRZYME assay and all other assays in the comparison studies use serum as the matrix.

3. Clinical studies:

To check that the type of samples tested in a clinical laboratory did not give a higher incidence of positive samples than seen within the normal range, samples from patients with other diseases and samples known to contain other autoimmune antibodies were run with the FARRZYME assay. The study results were:

Disease controls	N	Result
Ulcerative colitis	13	Negative
Crohn's disease	16	Negative
Samples tested for Diphtheria toxoid	9	Negative
Known autoantibodies		
Scl-70	3	Negative
Jo-1	4	Negative
PR3	3	Negative
MPO	4	Negative
GBM	3	Negative
Gliadin IgG	3	Negative
TPO	3	Negative
ASCA	3	Negative

4. Clinical cut-off:

See Assay cut-off

5. Expected values/Reference range:

See Assay cut-off

N. Proposed Labeling:

The labeling is sufficient and it satisfies 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.