INSTRUCTIONS FOR USE

EN

dsDNA (Crithidia l.)

REF FA1001 IVD Rx Only Σ 100 CE

INTENDED USE

The dsDNA (Crithidia l.) kit is an indirect immunofluorescence assay utilizing Crithidia luciliae for the qualitative and semi-quantitative determination of double-stranded DNA (dsDNA) IgG antibodies to DNA in human serum by manual fluorescence microscopy or with dIFine® automated microscope. The presence of dsDNA antibodies in conjunction with other serological and clinical findings can be used to aid in the diagnosis of systemic lupus erythematosus (SLE).

SUMMARY AND EXPLANATION

dsDNA antibodies, are frequently found in sera from patients with active spontaneous systemic lupus erythematosus (SLE) and drug-induced lupus diseases (1 - 9). The presence of dsDNA antibodies is indicative of active SLE and correlates closely with the onset of lupus nephritis (5, 10 - 13). The specificity of dsDNA antibodies for SLE is much greater than antinuclear antibodies (5, 12). Therefore, detection of dsDNA antibodies provides valuable diagnostic, as well as prognostic information for the differential diagnosis of SLE (5, 10 - 13). DNA antibodies were discovered in sera of patients with SLE several decades ago (1 - 4). Since then, DNA antibodies have been studied by a number of techniques, including gel diffusion (1, 14 - 15), complement fixation (2, 14, and 16), agglutination (17, 18), DNA spot tests (13, 19), radioimmuno-electrophoresis (20), counter-immunoelectrophoresis (21, 22), ammonium sulfate precipitation (10, 23, and 24) and ELISA (38). Considerable effort has been made to determine the specificity of DNA antibodies. It is now apparent that antibodies have been found which react with either dsDNA or denatured single stranded (sDNA) or both (8, 12, 14, and 20). dsDNA antibodies are thought to correlate with the clinical activity of the disease (2, 5, 10, 25, 39, 40). In addition, antibodies to dsDNA have been eluted from the kidneys of patients with SLE and one report demonstrated the presence of DNA-anti-dsDNA complexes in sera from patients with active SLE (26). However, these antibodies have been found in patients with and without active lupus nepharitis (27, 28).

The dsDNA (Crithidia l.) indirect fluorescent assay (IFA) assay is based on the use of the Crithidia l. kinetoplast substrate first described by Aarden, et al (29). This method is a useful laboratory test to detect dsDNA antibodies in patients with systemic lupus erythematosus (30 - 33).

PRINCIPLE OF THE ASSAY

The dsDNA (Crithidia l.) kit is an indirect fluorescent antibody assay (IFA) for the qualitative and semi-quantitative determination of anti-dsDNA IgG antibody in human sera. The reaction occurs in two steps:

1. Step one; If dsDNA antibodies are present, a reaction between dsDNA antibodies and the kinetoplast of the Crithidia l. substrate takes place in the first step.
2. Step two; goat anti-human IgG conjugate labeled with fluorescein isothiocyanate (FITC) is added to the substrate. If the patient's sera contain anti-dsDNA IgG antibody, a positive apple-green fluorescent antigen-antibody reaction will be observed when the slides are examined with the fluorescence microscope. A positive reaction is recognized as an intense staining reaction in the small kinetoplasts of the *Crithidia l.* organism.

REAGENTS

Materials Provided:

Each Test System contains the following components in sufficient quantities to perform the number of tests indicated on the packaging label. **NOTE: Conjugate and controls contain a combination of proclin (0.05% v/v) and Sodium azide (<0.1% w/v) as preservatives.**

SLD	1	Crithidia l. Substrate Slides: Ten, 10-well Slides with blotter.
CONJ	2	Conjugate: Goat anti-human IgG labeled with FITC. Contains phosphate buffer with BSA and counterstain. Two 3.5ml amber-capped bottles. Ready to use.
CTRL +	3	Positive Control (Human Serum): Will produce positive apple-green staining of the kinetoplast in the Crithidia l. organisms. One, 0.5mL, red-capped, vial. Ready to use.
CTRL -	4	Negative Control (Human Serum): Will produce no detectable dsDNA staining. One, 0.5mL, green-capped, vial. Ready to use

BUF PBS	5	Phosphate-buffered-saline (PBS): pH 7.2 ± 0.2. Empty contents of each buffer packet into one liter of distilled or deionized water. Mix until all salts are thoroughly dissolved. Two packets, sufficient to prepare 2 liters.
MNTMED	6	Mounting Media (Buffered Glycerol): One, 3.0 ml white capped, dripper tripper vials
COVGLS	7	Cover Glass. Package of twelve, 24 x 60 mm, Thickness #1**.**

NOTES:

The following components are not kit lot number dependent and may be used interchangeably with the Sebia IFA products, as long as the product numbers are identical: Mounting media (Product #: FA0009S), Negative control (FA2005-IUNC), Cover glass (Product S8007), and PBS (Product #: 0008S).

MATERIALS REQUIRED BUT NOT PROVIDED

1. dIFine® automated microscope or a properly equipped fluorescence microscope.
2. Pipettor(s) capable of pipetting volumes between 10 and 200 uL.
3. Disposable pipette tips.
4. Small test tubes, dilution plate or similar for preparing sample dilutions.
5. Slide Washer, or a large staining dish with a magnetic stir plate for washing slides between incubation steps.
6. Distilled or deionized water.
7. 1 Liter Graduated Cylinder.
8. Laboratory timer to monitor incubation steps.
9. Disposal basin, disposable gloves, and disinfectant (i.e.: 10% household bleach – 0.5% Sodium Hypochlorite).

STORAGE CONDITIONS

2°C – 8°C	Unopened Kit.
	Mounting Media, Conjugate, Slides, Positive and Negative controls.
	Rehydrated PBS (Stable for 30 days).
2°C – 25°C	Phosphate-buffered-saline (PBS) Packets.

PRECAUTIONS

1. For *In vitro* diagnostic use.
2. Follow normal precautions exercised in handling laboratory reagents. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing, gloves, and eye/face protection. Do not breathe vapor. Dispose of waste observing all local, state, and federal laws.
3. The wells of the slide do not contain viable organisms. However, consider the slide **potentially bio-hazardous materials** and handle accordingly.
4. The controls are **potentially bio-hazardous materials**. Source materials from which these products were derived were found negative for HIV-1 antigen, HBsAg and for antibodies against HCV and HIV by approved test methods. However, since no test method can offer complete assurance that infectious agents are absent, these products should be handled at the Bio-safety Level 2 as recommended for any potentially infectious human serum or blood specimen in the Centers for Disease control/National Institutes of Health manual "Biosafety in Microbiological and Biomedical Laboratories": current edition; and OSHA's Standard for Bloodborne Pathogens (20).
5. Adherence to the specified time and temperature of incubations is essential for accurate results. **All reagents must be allowed to reach room temperature (20 - 25°C) before starting the assay**. Return unused reagents to their original containers immediately and follow storage requirements.
6. Improper washing could cause false positive or false negative results. Be sure to minimize the amount of any residual PBS, by blotting, before adding conjugate. Do not allow the wells to dry out between incubations.
7. Conjugate, and controls contain Sodium azide at a concentration of <0.1% (w/v). Sodium azide has been reported to form lead or copper azides in laboratory plumbing which may cause explosions on hammering. To prevent, rinse sink thoroughly with water after disposing of solution containing Sodium Azide. This preservative may by toxic if ingested.
8. Dilution or adulteration of these reagents may generate erroneous results.
9. Never pipette by mouth. Avoid contact of reagents and patient specimens with skin and mucous membranes.
10. Avoid microbial contamination of reagents. Incorrect results may occur.
11. Cross contamination of reagents and/or samples could cause erroneous results.
12. Reusable glassware must be washed and thoroughly rinsed free of all detergents.
13. Avoid splashing or generation of aerosols.
14. Do not expose reagents to strong light during storage or incubation.

15. Allowing the slide packet to equilibrate to room temperature prior to opening the protective envelope will protect the wells and blotter from condensation.
16. Collect the wash solution in a disposal basin. Treat the waste solution with disinfectant (i.e.:10% household bleach - 0.5% Sodium Hypochlorite). Avoid exposure of reagents to bleach fumes.
17. Do not expose any of the reactive reagents to bleach-containing solutions or to any strong odors from bleach-containing solutions. Trace amounts of bleach (Sodium Hypochlorite) may destroy the biological activity of many of the reactive reagents within this Test System.
18. Do not apply pressure to slide envelope. This may damage the substrate.
19. The components of this Test System are matched for optimum sensitivity and reproducibility. Reagents from other manufacturers should not be interchanged. Follow Package Insert carefully.
20. Unopened/opened components are stable until the expiration date printed on the label, provided the recommended storage conditions are strictly followed. Do not use beyond the expiration date. Do not freeze.
21. Evans Blue Counterstain is a potential carcinogen. If skin contact occurs, flush with water. Dispose of according to local regulations.
22. Do not allow slides to dry during the procedure. Depending upon lab conditions, it may be necessary to place slides in a moist chamber during incubations.

SPECIMEN COLLECTION

1. Carry out specimen collection in accordance with CLSI document M29: Protection of Laboratory Workers from Occupationally Acquired Infectious Diseases. No known test method can offer complete assurance that human blood samples will not transmit infection. Therefore, all blood derivatives should be considered potentially infectious.
2. Only freshly drawn and properly refrigerated sera obtained by approved aseptic venipuncture procedures with this assay (34, 35). No anticoagulants or preservatives should be added. Avoid using hemolyzed, lipemic, or bacterially contaminated sera.
3. Store sample at room temperature for no longer than 8 hours. If testing is not performed within 8 hours, sera may be stored between 2 - 8°C, for no longer than 48 hours. If delay in testing is anticipated, store test sera at –20°C or lower. Avoid multiple freeze/thaw cycles which may cause loss of antibody activity and give erroneous results. It is the responsibility of the individual laboratory to use all available references and/or its own studies to determine stability criteria for its laboratory (37).

ASSAY PROCEDURE

1. Remove slides and other kit components from refrigerated storage and allow them to warm to room temperature (20 - 25°C). Tear open the protective envelope and remove slides. **Do not apply pressure to flat sides of protective envelope.**
2. Identify each well with the appropriate patient sera and controls. **NOTE: The controls are intended to be used undiluted**. Prepare a 1:10 dilution (e.g.: 10µL of serum + 90µL of PBS Buffer) of each patient serum.

Semi-Quantitative Options:

a. Users may titrate the Positive control to endpoint to serve as a semi-quantitative (1+ Minimally Reactive) control. In such cases, the control should be diluted two-fold in PBS. An endpoint dilution is established and printed on the Positive control vial (± one dilution). It should be noted that due to variations within the laboratory (equipment, etc.), each laboratory should establish its own expected end-point titer for each lot of Positive control.

b. When titrating patient specimens, initial 1:10 dilutions should be in PBS and all subsequent dilutions should also be prepared in PBS.

3. With suitable dispenser, dispense 20µL of each control and each diluted patient sera in the appropriate wells.
4. Incubate slides at room temperature (20 - 25°C) for 35 ± 5 minutes.
5. Gently rinse slides with PBS. If washing manually **do not direct a stream of PBS into the test wells.**
6. Wash slides for two, 5-minute intervals, changing PBS between washes.
7. Remove slides from PBS one at a time. Invert slide and key wells to holes in blotters provided. Blot slide by wiping the reverse side with an absorbent wipe. CAUTION: Position the blotter and slide on a hard, flat surface. Blotting on paper towels may destroy the slide matrix. **Do not allow the slides to dry during the test procedure**.
8. Add 20-40 µL of conjugate to each well.
9. Repeat steps 4 through 7.
10. Apply 3-5 drops of mounting media to each slide between wells and apply the cover glass. Alternatively, one may apply a small number of mounting media to each well and apply cover glass. Examine the slides immediately with an appropriate fluorescence microscope.

NOTE: If delay in examining slides is anticipated, seal coverslip with clear nail polish and store in refrigerator. It is recommended that slides be examined on the same day as testing.

QUALITY CONTROL

1. Every time the assay is run, a Positive control and a Negative control must be included.
2. It is recommended that one read the Positive and Negative controls before evaluating test results. This will assist in establishing the references required to interpret the test sample. If controls do not appear as described, results are invalid.

a. Negative control - characterized by the absence of fluorescent staining of the kinetoplast. Staining of the nucleus only and/or staining of the basal body should be interpreted as a negative test.

b. Positive control - characterized by any apple-green fluorescent staining of the kinetoplast. Staining of the basal body **in conjunction with** the kinetoplast should be considered a positive result.

3. Additional controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.

NOTES:

a. The intensity of the observed fluorescence may vary with the microscope and filter system used.

b. The kinetoplast is generally located closer to the basal body than the nucleus; however, because of the fluid nature of the endoplasm, the location of the kinetoplast may vary from cell to cell (36).

c. Read only single, well-defined organisms in each field. Not all organisms will appear optimal; morphology may vary between organisms because of fixation, their stages of growth, and/or their orientation on the slide as they dried (36).

INTERPRETATION OF RESULTS

1. Titers less than 1:10 are considered negative.
2. Positive Test: Any observed apple-green staining of the small kinetoplast of the *Crithidia l.* substrate organism, at a 1:10 dilution based on a 1+ to 4+ scale. 1+ is considered a weak reaction, and 4+ a strong reaction.
3. For semi-quantitative results, all sera positive at 1:10 should be titrated to endpoint dilution. This is accomplished by making a 1:10, 1:20, 1:40, etc., serial dilution of all positive patient samples. The endpoint is the highest dilution that produces a positive reaction.
4. Staining of both the small kinetoplast and the adjacent larger *Crithidia l.* nucleus simultaneously should be interpreted as a positive test.
5. Polar staining at the base of the flagella is not significant.
6. Staining of the nucleus only should not be interpreted as a positive test.

LIMITATIONS OF THE ASSAY

1. The dsDNA (Crithidia l.) kit is a diagnostic aid. It is therefore imperative that the dsDNA antibody results be interpreted in light of the patient's clinical condition by a medical authority.
2. SLE patients undergoing steroid therapy may have negative test results (5, 8, and 9).
3. Some drugs, particularly hydralazine, may induce dsDNA antibody production (5, 6, and 8).

EXPECTED RESULTS

Expected values in a normal population are negative at a 1:10 starting dilution. However, certain drugs may induce a positive dsDNA antibody test (5, 6).

PERFORMANCE CHARACTERISTICS

NOTE: When establishing Performance Characteristics of the dsDNA (Crithidia l.), slides were interpreted using three different methods as outlined below:

Interpretation Method:
Method A. Method A was a completely manual interpretation method. It was accomplished using a traditional fluorescent microscope equipped with objective and ocular lenses. Determining the qualitative outcome was accomplished using trained laboratory technicians.
Method B. Method B was accomplished by scanning the slides using dIFine® and subsequently having a trained laboratory technician interpret the qualitative results using the digital image appearing on the computer monitor.
Method C. Method C is the ***suggested outcome*** predicted by dIFine®; Method C predicts the qualitative result. If Method C is "UNC" (uncertain), the level of fluorescence measured by dIFine is borderline between positive and negative, or other features within the slide well that prevented a definitive suggestion. Method C must be "validated" or accepted by the laboratory technician or modified or invalidated completely. For purposes of this study and the data presented below, **Method C** is logged "AS IS" without any modification by the laboratory technician(s). It is therefore presented for *informational purposes only*.

1. Analytical Performance Studies:

a. Linearity:

Two low positive serum samples (~1:10-1:20 endpoint), two medium positive serum samples (~1:40-1:80 endpoint), and two strong positive serum samples (≥ 1:320 endpoint) were identified. The six samples were assayed at a 1:10 screening dilution, as well as at serial dilutions ranging from 1:20 through 1:5120, then interpreted by all three methods noted above. This study was conducted internally at the manufacturer. The endpoints for each sample and each method are presented below:

Sample	Method A	Method B	Method C
Low Positive-1	1:10	1:20	1:20
Low Positive-2	1:20	1:40	1:40*
Medium Positive-1	1:40	1:80	1:80
Medium Positive-2	1:40	1:80	1:80
High Positive-1	1:640	1:640	1:640
High Positive-2	1:640	1:640	1:640

* 1 UNC result at the 1:80 dilution for Low Positive-2 sample counted as Negative

Sample	Method A	Method B	Method C
Low Positive-1	1:10	1:20	1:20
Low Positive-2	1:20	1:40	1:80**
Medium Positive-1	1:40	1:80	1:80
Medium Positive-2	1:40	1:80	1:80
High Positive-1	1:640	1:640	1:640
High Positive-2	1:640	1:640	1:640

** 1 UNC result at the 1:80 dilution for Low Positive-2 sample counted as Positive

For Methods A and B, the fluorescence intensity was recorded at each dilution using a scale of 4 being very intense and 0 indicating no fluorescence. The sample dilutions and the associated fluorescence intensities are summarized in the tables appearing below:

		Fluorescence Intensity (4+ to 0); Method A									
Sample ID	Description	1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1280	1:2560	1 5120
1	Low Positive	1	0	0	0	0	NT	NT	NT	NT	NT
2	Low Positive	2	1	0	0	0	NT	NT	NT	NT	NT
3	Medium Positive	2	1	1	0	0	0	0	0	0	0
4	Medium Positive	2	1	1	0	0	0	0	0	0	0
5	High Positive	4	3	3	2	2	1	1	0	0	0
6	High Positive	4	3	3	2	2	1	1	0	0	0
NT; Not Tested											

		Fluorescence Intensity (4+ to 0); Method B									
Sample ID	Description	1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1280	1:2560	1 5120
1	Low Positive	1	1	0	0	0	NT	NT	NT	NT	NT
2	Low Positive	2	1	1	0	0	NT	NT	NT	NT	NT
3	Medium Positive	2	2	1	1	0	0	0	0	0	0
4	Medium Positive	2	2	1	1	0	0	0	0	0	0
5	High Positive	4	3	3	2	2	1	1	0	0	0
6	High Positive	4	3	3	2	2	1	1	0	0	0
NT; Not Tested											

b. Lot-to-Lot Reproducibility:

Nine negative serum samples, two low positive serum samples (~1:10-1:20 endpoint), two medium positive serum samples (~1:40-1:80 endpoint), and two strong positive serum samples (≥ 1:320 endpoint) were identified. This group of 15 specimens was assayed at a 1:10 screening dilution. For the 6 positive samples, additional serial dilutions ranging from 1:20 through 1:5120, were also assayed and interpreted by all three methods noted above, towards determining an endpoint titer.

Results:

i. **Qualitative Agreement:** There was 100% agreement in the qualitative results at the screening dilution of all 15 specimens across all 3 kit lots, for interpretation methods A and B. For lot 3, 1 UNC result was obtained via interpretation method C at the 1:10 screening dilution, for 1 of the low positive samples.

ii. **Endpoint Titer Agreement:** All 6 positive specimens resulted in the same endpoint titers ± one dilution regardless of reagent kit lot or method interpretation.

c. Reference Range Study:

One hundred and eighty random serum samples were acquired from healthy donors in the Northeastern US. The samples were assayed at the screening dilution of 1:10 and interpreted via all three methods. The results of the screening test are summarized below:

Interpretation Method	Number of Positives	% Positives	Number of Negatives	% Negatives	Number of Uncertain	% Uncertain
A	2	1.11%	178	98.89%	N/A	N/A
B	2	1.11%	178	98.89%	N/A	N/A
C	1	0.56%	176	97.78%	3	1.67%

d. Twenty-day Repeatability Study:

Two negative serum samples, two low positive serum samples (~1:10-1:20 endpoint), two medium positive serum samples (~1:40-1:80 endpoint), and two strong positive serum samples (≥ 1:320 endpoint) were identified. These eight specimens were assayed at a 1:10 screening dilution in triplicate, on twenty different days. Qualitative results were interpreted by two technicians for Methods A and B, and by a single dIFine instrument for Method C. Sample identities were blinded and randomized independently prior to each day of testing.

The qualitative result agreement values for the within-method repeatability evaluation are depicted within the tables below and also summarized as follows: There was 100% within-method qualitative result agreement for all eight samples when interpreted via Methods A and B, for both technicians. For Method C, the medium positive-1 sample, high positive-2 sample, and both negative samples yielded 100% within-method qualitative result agreement. The low positive-1 sample, low positive-2 sample, medium positive-2 sample, and high positive-1 sample yielded within-method qualitative result agreement values of 96.7%, 98.3%, 98.3%, and 95.0% respectively. For samples that yielded less than 100% within-method agreement, all results were labeled as "UNC" (uncertain) for Method C interpretations.

Within-Method Qualitative Result Agreement (Technician 1)

Sample	Method A Agreement (95% CI)	Method B Agreement (95% CI)
Low Positive-1	100% (88.7 - 100%)	100% (88.7 - 100%)
Low Positive-2	100% (88.7 - 100%)	100% (88.7 - 100%)
Medium Positive-1	100% (88.7 - 100%)	100% (88.7 - 100%)
Medium Positive-2	100% (88.7 - 100%)	100% (88.7 - 100%)
High Positive-1	100% (88.7 - 100%)	100% (88.7 - 100%)
High Positive-2	100% (88.7 - 100%)	100% (88.7 - 100%)
Negative-1	100% (88.7 - 100%)	100% (88.7 - 100%)
Negative-2	100% (88.7 - 100%)	100% (88.7 - 100%)

Within-Method Qualitative Result Agreement (Technician 2)

Sample	Method A Agreement (95% CI)	Method B Agreement (95% CI)
Low Positive-1	100% (88.7 - 100%)	100% (88.7 - 100%)
Low Positive-2	100% (88.7 - 100%)	100% (88.7 - 100%)
Medium Positive-1	100% (88.7 - 100%)	100% (88.7 - 100%)
Medium Positive-2	100% (88.7 - 100%)	100% (88.7 - 100%)
High Positive-1	100% (88.7 - 100%)	100% (88.7 - 100%)
High Positive-2	100% (88.7 - 100%)	100% (88.7 - 100%)
Negative-1	100% (88.7 - 100%)	100% (88.7 - 100%)
Negative-2	100% (88.7 - 100%)	100% (88.7 - 100%)

Within-Method Qualitative Result Agreement (n = 2 Technicians Com

Sample	Endpoint Titer	Method A Agreement (95% CI)	Method B Agreement (95% CI)
Negative-1	N/A	120/120 = 100% (96.9 - 100%)	120/120 = 100% (96.9 - 100%)
Negative-2	N/A	120/120 = 100% (96.9 - 100%)	120/120 = 100% (96.9 - 100%)
Low Positive-1	1:10 - 1:20	120/120 = 100% (96.9 - 100%)	120/120 = 100% (96.9 - 100%)
Low Positive-2	1:20 - 1:40	120/120 = 100% (96.9 - 100%)	120/120 = 100% (96.9 - 100%)
Medium Positive-1	1:40 - 1:80	120/120 = 100% (96.9 - 100%)	120/120 = 100% (96.9 - 100%)
Medium Positive-2	1:40 - 1:80	120/120 = 100% (96.9 - 100%)	120/120 = 100% (96.9 - 100%)
High Positive-1	1:320 - 1:640	120/120 = 100% (96.9 - 100%)	120/120 = 100% (96.9 - 100%)
High Positive-2	1:160 - 1:320	120/120 = 100% (96.9 - 100%)	120/120 = 100% (96.9 - 100%)

Sample	Method C Agreement (95% CI)
Low Positive-1	96.7% (88.6 - 99.1%)
Low Positive-2	98.3% (91.1 - 99.7%)
Medium Positive-1	100% (88.7 - 100%)
Medium Positive-2	98.3% (91.1 - 99.7%)
High Positive-1	95.0% (86.3 - 98.3%)
High Positive-2	100% (88.7 - 100%)
Negative-1	100% (88.7 - 100%)
Negative-2	100% (88.7 - 100%)

The qualitative result agreement values for the between-method repeatability evaluation are depicted within the tables below and also summarized as follows: There was 100% within-method qualitative result agreement for all eight samples when interpreted via Method A versus Method B, for both technicians. When Method A and Method B were compared to Method C, the medium positive-1 sample, high positive-2 sample, and both negative samples yielded 100% between-method qualitative result agreement. The low positive-1 sample, low positive-2 sample, medium positive-2 sample, and high positive-1 sample yielded between-method qualitative result agreement values of 96.7%, 98.3%, 98.3%, and 95.0% respectively. For samples that yielded less than 100% between-method agreement, all results were labeled as "UNC" (uncertain) for Method C interpretations.

Between-Method Qualitative Result Agreement (Technician 1)

Sample	Method A vs Method B Agreement (95% CI)	Method A vs Method C Agreement (95% CI)	Method B vs Method C Agreement (95% CI)
Low Positive-1	100% (88.7 - 100%)	96.7% (88.6 - 99.1%)	96.7% (88.6 - 99.1%)
Low Positive-2	100% (88.7 - 100%)	98.3% (91.1 - 99.7%)	98.3% (91.1 - 99.7%)
Medium Positive-1	100% (88.7 - 100%)	100% (88.7 - 100%)	100% (88.7 - 100%)
Medium Positive-2	100% (88.7 - 100%)	98.3% (91.1 - 99.7%)	98.3% (91.1 - 99.7%)
High Positive-1	100% (88.7 - 100%)	95.0% (86.3 - 98.3%)	95.0% (86.3 - 98.3%)
High Positive-2	100% (88.7 - 100%)	100% (88.7 - 100%)	100% (88.7 - 100%)
Negative-1	100% (88.7 - 100%)	100% (88.7 - 100%)	100% (88.7 - 100%)
Negative-2	100% (88.7 - 100%)	100% (88.7 - 100%)	100% (88.7 - 100%)

Between-Method Qualitative Result Agreement (Technician 2)

Sample	Method A vs Method B Agreement (95% CI)	Method A vs Method C Agreement (95% CI)	Method B vs Method C Agreement (95% CI)
Low Positive-1	100% (88.7 - 100%)	96.7% (88.6 - 99.1%)	96.7% (88.6 - 99.1%)
Low Positive-2	100% (88.7 - 100%)	98.3% (91.1 - 99.7%)	98.3% (91.1 - 99.7%)
Medium Positive-1	100% (88.7 - 100%)	100% (88.7 - 100%)	100% (88.7 - 100%)
Medium Positive-2	100% (88.7 - 100%)	98.3% (91.1 - 99.7%)	98.3% (91.1 - 99.7%)
High Positive-1	100% (88.7 - 100%)	95.0% (86.3 - 98.3%)	95.0% (86.3 - 98.3%)
High Positive-2	100% (88.7 - 100%)	100% (88.7 - 100%)	100% (88.7 - 100%)
Negative-1	100% (88.7 - 100%)	100% (88.7 - 100%)	100% (88.7 - 100%)
Negative-2	100% (88.7 - 100%)	100% (88.7 - 100%)	100% (88.7 - 100%)

Between-Method Qualitative Result Agreement (n = 2 Technicians Combined)

Sample	Endpoint Titer	Method A vs Method B Agreement (95% CI)	Method A vs Method C Agreement (95% CI)	Method B vs Method C Agreement (95% CI)
Negative-1	N/A	120/120 = 100% (96.9 - 100%)	120/120 = 100% (96.9 - 100%)	120/120 = 100% (96.9 - 100%)
Negative-2	N/A	120/120 = 100% (96.9 - 100%)	120/120 = 100% (96.9 - 100%)	120/120 = 100% (96.9 - 100%)
Low Positive-1	1:10 - 1:20	120/120 = 100% (96.9 - 100%)	116/120 = 96.7% (91.7 - 98.7%)	116/120 = 96.7% (91.7 - 98.7%)
Low Positive-2	1:20 - 1:40	120/120 = 100% (96.9 - 100%)	118/120 = 98.3% (94.1 - 99.5%)	118/120 = 98.3% (94.1 - 99.5%)
Medium Positive-1	1:40 - 1:80	120/120 = 100% (96.9 - 100%)	120/120 = 100% (96.9 - 100%)	120/120 = 100% (96.9 - 100%)
Medium Positive-2	1:40 - 1:80	120/120 = 100% (96.9 - 100%)	118/120 = 98.3% (94.1 - 99.5%)	118/120 = 98.3% (94.1 - 99.5%)
High Positive-1	1:320 - 1:640	120/120 = 100% (96.9 - 100%)	114/120 = 95% (89.5 - 97.7%)	114/120 = 95% (89.5 - 97.7%)
High Positive-2	1:160 - 1:320	120/120 = 100% (96.9 - 100%)	120/120 = 100% (96.9 - 100%)	120/120 = 100% (96.9 - 100%)

e. Five-day, Multi-Site Reproducibility Study:

Two negative serum samples, two low positive serum samples (~1:10-1:20 endpoint), two medium positive serum samples (~1:40-1:80 endpoint), and two strong positive serum samples (≥ 1:320 endpoint) were identified. These eight specimens were assayed at a 1:10 screening dilution in triplicate, twice per day, on five different days, at three different laboratories. Qualitative results were interpreted by two technicians at each laboratory for Methods A and B, and by a single dIFine instrument at each laboratory for Method C. Sample identities were blinded and randomized independently prior to each day of testing.

The results of the qualitative agreement are depicted below:

i. Qualitative Result Agreement

a. Within Method

Site 1 - Within-Method Qualitative Result Agreement (Technician 1)

Sample	Method A (95% CI)	Method B (95% CI)
Low Positive-1	30/30 - 100% (88.65 - 100.00%)	30/30 - 100% (88.65 - 100.00%)
Low Positive-2	30/30 - 100% (88.65 - 100.00%)	30/30 - 100% (88.65 - 100.00%)
Medium Positive-1	30/30 - 100% (88.65 - 100.00%)	30/30 - 100% (88.65 - 100.00%)
Medium Positive-2	30/30 - 100% (88.65 - 100.00%)	30/30 - 100% (88.65 - 100.00%)
High Positive-1	30/30 - 100% (88.65 - 100.00%)	30/30 - 100% (88.65 - 100.00%)
High Positive-2	30/30 - 100% (88.65 - 100.00%)	30/30 - 100% (88.65 - 100.00%)
Negative-1	30/30 - 100% (88.65 - 100.00%)	30/30 - 100% (88.65 - 100.00%)
Negative-2	30/30 - 100% (88.65 - 100.00%)	30/30 - 100% (88.65 - 100.00%)

Site 1 - Within-Method Qualitative Result Agreement (Technician 2)

Sample	Method A (95% CI)	Method B (95% CI)
Low Positive-1	30/30 - 100% (88.65 - 100.00%)	30/30 - 100% (88.65 - 100.00%)
Low Positive-2	30/30 - 100% (88.65 - 100.00%)	30/30 - 100% (88.65 - 100.00%)
Medium Positive-1	30/30 - 100% (88.65 - 100.00%)	30/30 - 100% (88.65 - 100.00%)
Medium Positive-2	30/30 - 100% (88.65 - 100.00%)	30/30 - 100% (88.65 - 100.00%)
High Positive-1	30/30 - 100% (88.65 - 100.00%)	30/30 - 100% (88.65 - 100.00%)
High Positive-2	30/30 - 100% (88.65 - 100.00%)	30/30 - 100% (88.65 - 100.00%)
Negative-1	30/30 - 100% (88.65 - 100.00%)	30/30 - 100% (88.65 - 100.00%)
Negative-2	30/30 - 100% (88.65 - 100.00%)	30/30 - 100% (88.65 - 100.00%)

Site 1 - Within-Method Qualitative Result Agreement

Sample	Method C (95% CI)
Low Positive-1	28/30 - 93.33% (78.68 - 98.15%)
Low Positive-2	29/30 - 96.67% (83.33 - 99.41%)
Medium Positive-1	30/30 - 100% (88.65 - 100.00%)
Medium Positive-2	28/30 - 93.33% (78.68 - 98.15%)
High Positive-1	30/30 - 100% (88.65 - 100.00%)
High Positive-2	28/30 - 93.33% (78.68 - 98.15%)
Negative-1	29/30 - 96.67% (83.33 - 99.41%)
Negative-2	29/30 - 96.67% (83.33 - 99.41%)

Site 2 - Within-Method Qualitative Result Agreement (Technician 1)

Sample	Method A (95% CI)	Method B (95% CI)
Low Positive-1	30/30 - 100% (88.65 - 100.00%)	30/30 - 100% (88.65 - 100.00%)
Low Positive-2	30/30 - 100% (88.65 - 100.00%)	30/30 - 100% (88.65 - 100.00%)
Medium Positive-1	30/30 - 100% (88.65 - 100.00%)	30/30 - 100% (88.65 - 100.00%)
Medium Positive-2	30/30 - 100% (88.65 - 100.00%)	30/30 - 100% (88.65 - 100.00%)
High Positive-1	30/30 - 100% (88.65 - 100.00%)	30/30 - 100% (88.65 - 100.00%)
High Positive-2	30/30 - 100% (88.65 - 100.00%)	30/30 - 100% (88.65 - 100.00%)
Negative-1	30/30 - 100% (88.65 - 100.00%)	30/30 - 100% (88.65 - 100.00%)
Negative-2	30/30 - 100% (88.65 - 100.00%)	30/30 - 100% (88.65 - 100.00%)

Site 2 - Within-Method Qualitative Result Agreement (Technician 2)

Sample	Method A (95% CI)	Method B (95% CI)
Low Positive-1	30/30 - 100% (88.65 - 100.00%)	30/30 - 100% (88.65 - 100.00%)
Low Positive-2	30/30 - 100% (88.65 - 100.00%)	30/30 - 100% (88.65 - 100.00%)
Medium Positive-1	30/30 - 100% (88.65 - 100.00%)	30/30 - 100% (88.65 - 100.00%)
Medium Positive-2	30/30 - 100% (88.65 - 100.00%)	30/30 - 100% (88.65 - 100.00%)
High Positive-1	30/30 - 100% (88.65 - 100.00%)	30/30 - 100% (88.65 - 100.00%)
High Positive-2	30/30 - 100% (88.65 - 100.00%)	30/30 - 100% (88.65 - 100.00%)
Negative-1	30/30 - 100% (88.65 - 100.00%)	30/30 - 100% (88.65 - 100.00%)
Negative-2	30/30 - 100% (88.65 - 100.00%)	30/30 - 100% (88.65 - 100.00%)

Site 2 - Within-Method Qualitative Result Agreement

Sample	Method C (95% CI)
Low Positive-1	28/30 - 93.33% (78.68 - 98.15%)
Low Positive-2	27/30 - 90.00% (74.38 - 96.54%)
Medium Positive-1	30/30 - 100% (88.65 - 100.00%)
Medium Positive-2	28/30 - 93.33% (78.68 - 98.15%)
High Positive-1	30/30 - 100% (88.65 - 100.00%)
High Positive-2	30/30 - 100% (88.65 - 100.00%)
Negative-1	30/30 - 100% (88.65 - 100.00%)
Negative-2	30/30 - 100% (88.65 - 100.00%)

Site 3 - Within-Method Qualitative Result Agreement (Technician 1)

Sample	Method A (95% CI)	Method B (95% CI)
Low Positive-1	30/30 - 100% (88.65 - 100.00%)	29/30 - 96.67% (83.33 - 99.41%)
Low Positive-2	30/30 - 100% (88.65 - 100.00%)	30/30 - 100% (88.65 - 100.00%)
Medium Positive-1	30/30 - 100% (88.65 - 100.00%)	30/30 - 100% (88.65 - 100.00%)
Medium Positive-2	30/30 - 100% (88.65 - 100.00%)	30/30 - 100% (88.65 - 100.00%)
High Positive-1	30/30 - 100% (88.65 - 100.00%)	30/30 - 100% (88.65 - 100.00%)
High Positive-2	30/30 - 100% (88.65 - 100.00%)	30/30 - 100% (88.65 - 100.00%)
Negative-1	30/30 - 100% (88.65 - 100.00%)	29/30 - 96.67% (83.33 - 99.41%)
Negative-2	30/30 - 100% (88.65 - 100.00%)	30/30 - 100% (88.65 - 100.00%)

Site 3 - Within-Method Qualitative Result Agreement (Technician 2)

Sample	Method A (95% CI)	Method B (95% CI)
Low Positive-1	30/30 - 100% (88.65 - 100.00%)	29/30 - 96.67% (83.33 - 99.41%)
Low Positive-2	30/30 - 100% (88.65 - 100.00%)	30/30 - 100% (88.65 - 100.00%)
Medium Positive-1	30/30 - 100% (88.65 - 100.00%)	30/30 - 100% (88.65 - 100.00%)
Medium Positive-2	30/30 - 100% (88.65 - 100.00%)	30/30 - 100% (88.65 - 100.00%)
High Positive-1	30/30 - 100% (88.65 - 100.00%)	30/30 - 100% (88.65 - 100.00%)
High Positive-2	30/30 - 100% (88.65 - 100.00%)	30/30 - 100% (88.65 - 100.00%)
Negative-1	30/30 - 100% (88.65 - 100.00%)	29/30 - 96.67% (83.33 - 99.41%)
Negative-2	30/30 - 100% (88.65 - 100.00%)	30/30 - 100% (88.65 - 100.00%)

Site 3 - Within-Method Qualitative Result Agreement

Sample	Method C (95% CI)
Low Positive-1	27/30 - 90.00% (74.38 - 96.54%)
Low Positive-2	29/30 - 96.67% (83.33 - 99.41%)
Medium Positive-1	30/30 - 100% (88.65 - 100.00%)
Medium Positive-2	27/30 - 90.00% (74.38 - 96.54%)
High Positive-1	29/30 - 96.67% (83.33 - 99.41%)
High Positive-2	30/30 - 100% (88.65 - 100.00%)
Negative-1	30/30 - 100% (88.65 - 100.00%)
Negative-2	30/30 - 100% (88.65 - 100.00%)

b. Between Method:

Site 1 - Between-Method Qualitative Result Agreement (Technician 1)

Sample	Method A vs Method B (95% CI)	Method A vs Method C (95% CI)	Method B vs Method C (95% CI)
Low Positive-1	30/30 - 100% (88.65 - 100.00%)	28/30 - 93.33% (78.68 - 98.15%)	28/30 - 93.33% (78.68 - 98.15%)
Low Positive-2	30/30 - 100% (88.65 - 100.00%)	29/30 - 96.67% (83.33 - 99.41%)	29/30 - 96.67% (83.33 - 99.41%)
Medium Positive-1	30/30 - 100% (88.65 - 100.00%)	30/30 - 100% (88.65 - 100.00%)	30/30 - 100% (88.65 - 100.00%)
Medium Positive-2	30/30 - 100% (88.65 - 100.00%)	28/30 - 93.33% (78.68 - 98.15%)	28/30 - 93.33% (78.68 - 98.15%)
High Positive-1	30/30 - 100% (88.65 - 100.00%)	30/30 - 100% (88.65 - 100.00%)	30/30 - 100% (88.65 - 100.00%)
High Positive-2	30/30 - 100% (88.65 - 100.00%)	28/30 - 93.33% (78.68 - 98.15%)	28/30 - 93.33% (78.68 - 98.15%)
Negative-1	30/30 - 100% (88.65 - 100.00%)	29/30 - 96.67% (83.33 - 99.41%)	29/30 - 96.67% (83.33 - 99.41%)
Negative-2	30/30 - 100% (88.65 - 100.00%)	29/30 - 96.67% (83.33 - 99.41%)	29/30 - 96.67% (83.33 - 99.41%)

Site 1 - Between-Method Qualitative Result Agreement (Technician 2)

Sample	Method A vs Method B (95% CI)	Method A vs Method C (95% CI)	Method B vs Method C (95% CI)
Low Positive-1	30/30 - 100% (88.65 - 100.00%)	28/30 - 93.33% (78.68 - 98.15%)	28/30 - 93.33% (78.68 - 98.15%)
Low Positive-2	30/30 - 100% (88.65 - 100.00%)	29/30 - 96.67% (83.33 - 99.41%)	29/30 - 96.67% (83.33 - 99.41%)
Medium Positive-1	30/30 - 100% (88.65 - 100.00%)	30/30 - 100% (88.65 - 100.00%)	30/30 - 100% (88.65 - 100.00%)
Medium Positive-2	30/30 - 100% (88.65 - 100.00%)	28/30 - 93.33% (78.68 - 98.15%)	28/30 - 93.33% (78.68 - 98.15%)
High Positive-1	30/30 - 100% (88.65 - 100.00%)	30/30 - 100% (88.65 - 100.00%)	30/30 - 100% (88.65 - 100.00%)
High Positive-2	30/30 - 100% (88.65 - 100.00%)	28/30 - 93.33% (78.68 - 98.15%)	28/30 - 93.33% (78.68 - 98.15%)
Negative-1	30/30 - 100% (88.65 - 100.00%)	29/30 - 96.67% (83.33 - 99.41%)	29/30 - 96.67% (83.33 - 99.41%)
Negative-2	30/30 - 100% (88.65 - 100.00%)	29/30 - 96.67% (83.33 - 99.41%)	29/30 - 96.67% (83.33 - 99.41%)

Site 2 - Between-Method Qualitative Result Agreement (Technician 1)

Sample	Method A vs Method B (95% CI)	Method A vs Method C (95% CI)	Method B vs Method C (95% CI)
Low Positive-1	30/30 - 100% (88.65 - 100.00%)	28/30 - 93.33% (78.68 - 98.15%)	28/30 - 93.33% (78.68 - 98.15%)
Low Positive-2	30/30 - 100% (88.65 - 100.00%)	27/30 - 90.00% (74.38 - 96.54%)	27/30 - 90.00% (74.38 - 96.54%)
Medium Positive-1	30/30 - 100% (88.65 - 100.00%)	30/30 - 100% (88.65 - 100.00%)	30/30 - 100% (88.65 - 100.00%)
Medium Positive-2	30/30 - 100% (88.65 - 100.00%)	28/30 - 93.33% (78.68 - 98.15%)	28/30 - 93.33% (78.68 - 98.15%)
High Positive-1	30/30 - 100% (88.65 - 100.00%)	30/30 - 100% (88.65 - 100.00%)	30/30 - 100% (88.65 - 100.00%)
High Positive-2	30/30 - 100% (88.65 - 100.00%)	30/30 - 100% (88.65 - 100.00%)	30/30 - 100% (88.65 - 100.00%)
Negative-1	30/30 - 100% (88.65 - 100.00%)	30/30 - 100% (88.65 - 100.00%)	30/30 - 100% (88.65 - 100.00%)
Negative-2	30/30 - 100% (88.65 - 100.00%)	30/30 - 100% (88.65 - 100.00%)	30/30 - 100% (88.65 - 100.00%)

Site 2 - Between-Method Qualitative Result Agreement (Technician 2)

Sample	Method A vs Method B (95% CI)	Method A vs Method C (95% CI)	Method B vs Method C (95% CI)
Low Positive-1	30/30 - 100% (88.65 - 100.00%)	28/30 - 93.33% (78.68 - 98.15%)	28/30 - 93.33% (78.68 - 98.15%)
Low Positive-2	30/30 - 100% (88.65 - 100.00%)	27/30 - 90.00% (74.38 - 96.54%)	27/30 - 90.00% (74.38 - 96.54%)
Medium Positive-1	30/30 - 100% (88.65 - 100.00%)	30/30 - 100% (88.65 - 100.00%)	30/30 - 100% (88.65 - 100.00%)
Medium Positive-2	30/30 - 100% (88.65 - 100.00%)	28/30 - 93.33% (78.68 - 98.15%)	28/30 - 93.33% (78.68 - 98.15%)
High Positive-1	30/30 - 100% (88.65 - 100.00%)	30/30 - 100% (88.65 - 100.00%)	30/30 - 100% (88.65 - 100.00%)
High Positive-2	30/30 - 100% (88.65 - 100.00%)	30/30 - 100% (88.65 - 100.00%)	30/30 - 100% (88.65 - 100.00%)
Negative-1	30/30 - 100% (88.65 - 100.00%)	30/30 - 100% (88.65 - 100.00%)	30/30 - 100% (88.65 - 100.00%)
Negative-2	30/30 - 100% (88.65 - 100.00%)	30/30 - 100% (88.65 - 100.00%)	30/30 - 100% (88.65 - 100.00%)

Site 3 - Between-Method Qualitative Result Agreement (Technician 1)

Sample	Method A vs Method B (95% CI)	Method A vs Method C (95% CI)	Method B vs Method C (95% CI)
Low Positive-1	29/30 - 96.67% (83.33 - 99.41%)	27/30 - 90.00% (74.38 - 96.54%)	26/30 - 86.67% (70.32 - 94.69%)
Low Positive-2	30/30 - 100% (88.65 - 100.00%)	29/30 - 96.67% (83.33 - 99.41%)	29/30 - 96.67% (83.33 - 99.41%)
Medium Positive-1	30/30 - 100% (88.65 - 100.00%)	30/30 - 100% (88.65 - 100.00%)	30/30 - 100% (88.65 - 100.00%)
Medium Positive-2	30/30 - 100% (88.65 - 100.00%)	27/30 - 90.00% (74.38 - 96.54%)	27/30 - 90.00% (74.38 - 96.54%)
High Positive-1	30/30 - 100% (88.65 - 100.00%)	29/30 - 96.67% (83.33 - 99.41%)	29/30 - 96.67% (83.33 - 99.41%)
High Positive-2	30/30 - 100% (88.65 - 100.00%)	30/30 - 100% (88.65 - 100.00%)	30/30 - 100% (88.65 - 100.00%)
Negative-1	29/30 - 96.67% (83.33 - 99.41%)	30/30 - 100% (88.65 - 100.00%)	29/30 - 96.67% (83.33 - 99.41%)
Negative-2	30/30 - 100% (88.65 - 100.00%)	30/30 - 100% (88.65 - 100.00%)	30/30 - 100% (88.65 - 100.00%)

Site 3 - Between-Method Qualitative Result Agreement (Technician 2)

Sample	Method A vs Method B (95% CI)	Method A vs Method C (95% CI)	Method B vs Method C (95% CI)
Low Positive-1	29/30 - 96.67% (83.33 - 99.41%)	27/30 - 90.00% (74.38 - 96.54%)	26/30 - 86.67% (70.32 - 94.69%)
Low Positive-2	30/30 - 100% (88.65 - 100.00%)	29/30 - 96.67% (83.33 - 99.41%)	29/30 - 96.67% (83.33 - 99.41%)
Medium Positive-1	30/30 - 100% (88.65 - 100.00%)	30/30 - 100% (88.65 - 100.00%)	30/30 - 100% (88.65 - 100.00%)
Medium Positive-2	30/30 - 100% (88.65 - 100.00%)	27/30 - 90.00% (74.38 - 96.54%)	27/30 - 90.00% (74.38 - 96.54%)
High Positive-1	30/30 - 100% (88.65 - 100.00%)	29/30 - 96.67% (83.33 - 99.41%)	29/30 - 96.67% (83.33 - 99.41%)
High Positive-2	30/30 - 100% (88.65 - 100.00%)	30/30 - 100% (88.65 - 100.00%)	30/30 - 100% (88.65 - 100.00%)
Negative-1	29/30 - 96.67% (83.33 - 99.41%)	30/30 - 100% (88.65 - 100.00%)	29/30 - 96.67% (83.33 - 99.41%)
Negative-2	30/30 - 100% (88.65 - 100.00%)	30/30 - 100% (88.65 - 100.00%)	30/30 - 100% (88.65 - 100.00%)

If one combines all eight samples resulting in 240 results, the qualitative results can also be summarized as follows:

Method A Multisite Reproducibility:

Qualitative results from three sites and two technicians per site comparing site to site and tech to tech.

			Site 1		**Site 2**		**Site 3**	
			Technician 1	Technician 2	Technician 1	Technician 2	Technician 1	Technician 2
			Method A		Method A		Method A	
Site 1	Technician 1	Method A		240/240 - 100% (98.42 - 100.00)	240/240 - 100% (98.42 - 100.00)	240/240 - 100% (98.42 - 100.00)	240/240 - 100% (98.42 - 100.00)	240/240 - 100% (98.42 - 100.00)
	Technician 2				240/240 - 100% (98.42 - 100.00)	240/240 - 100% (98.42 - 100.00)	240/240 - 100% (98.42 - 100.00)	240/240 - 100% (98.42 - 100.00)
Site 2	Technician 1	Method A				240/240 - 100% (98.42 - 100.00)	240/240 - 100% (98.42 - 100.00)	240/240 - 100% (98.42 - 100.00)
	Technician 2						240/240 - 100% (98.42 - 100.00)	240/240 - 100% (98.42 - 100.00)
Site 3	Technician 1	Method A						240/240 - 100% (98.42 - 100.00)
	Technician 2							

Method B Multisite Reproducibility:

Qualitative results from three sites and two technicians per site comparing site to site and tech to tech.

			Site 1		Site 2		Site 3	
			Technician 1	Technician 2	Technician 1	Technician 2	Technician 1	Technician 2
			Method B		Method B		Method B	
Site 1	Technician 1	Method B		240/240 - 100% (98.42 - 100.00)	240/240 - 100% (98.42 - 100.00)	240/240 - 100% (98.42 - 100.00)	238/240 - 99.17% (97.01 - 99.77)	238/240 - 99.17% (97.01 - 99.77)
	Technician 2				240/240 - 100% (98.42 - 100.00)	240/240 - 100% (98.42 - 100.00)	238/240 - 99.17% (97.01 - 99.77)	238/240 - 99.17% (97.01 - 99.77)
Site 2	Technician 1	Method B				240/240 - 100% (98.42 - 100.00)	238/240 - 99.17% (97.01 - 99.77)	238/240 - 99.17% (97.01 - 99.77)
	Technician 2						238/240 - 99.17% (97.01 - 99.77)	238/240 - 99.17% (97.01 - 99.77)
Site 3	Technician 1	Method B						240/240 - 100% (98.42 - 100.00)
	Technician 2							

Method C Multisite Reproducibility Comparing Site to Site

		Site 1	Site 2	Site 3
		Method C		
Site 1	Method C		227/240 - 94.58% (90.95 - 96.81)	230/240 - 95.83% (92.50 - 97.72)
Site 2				231/240 - 96.25% (93.03 - 98.01)
Site 3				

f. Interference Study:

Two negative serum samples, two low positive serum samples (~1:10-1:20 endpoint), two medium positive serum samples (~1:40-1:80 endpoint), and two strong positive serum samples (≥ 1:320 endpoint) were identified. These 8 specimens were spiked with two different concentrations (low spike and high spike) of nineteen different interferents as outlined in the table below. All specimens were assayed in triplicate by the dsDNA (Crithidia l.) kit and interpreted by all three methods noted above. Qualitative results were interpreted by two technicians for Methods A and B, and by a single dIFine instrument for Method C.

Endogenous Substances		
Substance	**Low Concentration**	**High Concentration**
Bilirubin (unconjugated)	0.02 mg/mL	0.15 mg/mL
Cholesterol (total)	1.5 mg/mL	2.2 mg/mL
Triglycerides (total)	1 mg/mL	2.5 mg/mL
Albumin	35 mg/mL	52 mg/mL
Hemoglobin	100 mg/mL	200 mg/mL
RF	200 U/mL	400 U/mL
Exogenous Substances		
Substance	**Low Concentration**	**High Concentration**
Intralipids	2.0 mg/mL	20 mg/mL
Cyclophosphamide	0.183 mg/mL	0.549 mg/mL
Ibuprofen	0.073 mg/ml	0.219 mg/ml
Hydroxychloroquine	0.006 mg/mL	0.024 mg/mL
Simvastatin	0.0000277 mg/mL	0.000083 mg/ml
Prednisone	0.000033 mg/mL	0.000099 mg/mL
Azathioprine	0.00086 mg/mL	0.00258 mg/mL
Diltiazem	0.0003 mg/mL	0.0009 mg/mL
Mycophenolate mofetil	0.012 mg/mL	0.048 mg/mL
Rituximab	0.5 mg/mL	2 mg/mL
Belimumab	2 mg/mL	8 mg/mL
Methotrexate	0.454 mg/mL	1.36 mg/mL
Naproxen	0.12 mg/mL	0.36 mg/mL
Enalapril	Not tested	819 ng/mL
Voclosporin	Not tested	210 ng/mL

None of the interferents affected the expected results of any samples when read by Methods A and B. When the interferent/samples combinations were tested, Method C yielded uncertain results in several samples: 'low negative-2' sample spiked with a high concentration of cyclophosphamide, 'low positive-2' sample spiked with low and high concentration of hydroxychloroquine, 'medium positive-2' sample spiked with a low concentration of azathioprine and a high concentration of Bilirubin, 'high positive-1' sample spiked with low concentration of triglycerides a high concentration of albumin. Overall, it can be concluded that the dsDNA (Crithidia l.) kit is not at risk of generating erroneous results due to the presence of the interferents tested.

2. Clinical Performance Study Design:

The 660 clinically characterized specimens that were utilized are outlined in the table below. These 660 specimens were aliquoted, blinded, randomized, and evaluated at a 1:10 screening dilution via the dsDNA (Crithidia l.), in conjunction with the dIFine® automated microscope system, at three independent laboratories. Qualitative results were interpreted by two technicians at each laboratory for Methods A and B, and by a single dIFine® instrument at each laboratory for Method C. Samples positive via interpretation Methods A and B at the 1:10 screening dilution were subsequently serially diluted towards determining an endpoint dilution via all three interpretation methods.

For each laboratory, the results were used to assess clinical specificity (potential cross reactivity), clinical sensitivity, qualitative agreement between interpretation methods, and endpoint titer agreement between interpretation methods.

Target Disease			n
Systemic Lupus Erythematosus			300
Control Diseases	**ANA-Associated Diseases**		***n***
	Connective Tissue Diseases	Sjögren's Syndrome	30
		Scleroderma	20
		Autoimmune Myositis	30
		Mixed Connective Tissue Disease	20
		CREST	20
	Other ANA-Associated Autoimmune Diseases	Autoimmune Hepatitis	20
		Primary Biliary Cholangitis	10
		Drug-Induced Lupus	20
	Non-ANA-Associated Diseases		***n***
	Other Autoimmune Diseases	Celiac	20
		Vasculitis (ANCA)	30
		Crohn's Disease	10
		Rheumatoid Arthritis	30
		Autoimmune Thyroiditis	30
		Inflammatory Bowel Disease	10
		Ulcerative Colitis	10
	Other Diseases	Fibromyalgia	10
		Infectious Disease	20
		Malignancy/Cancer	20
		Total:	660

3. Clinical Sensitivity and Clinical Specificity:

The clinical sensitivity was calculated at each site using the qualitative results derived from the Systemic Lupus Erythematosus samples (n = 300). Specificity was calculated the combined set of qualitative results derived from the control disease samples (n = 360).

a. Clinical Performance at Site 1

Diagnostic Sensitivity and Specificity			SLE (n = 300)	Control Diseases (n = 360)
			% Sensitivity (95% CI)	% Specificity (95% CI)
Site 1	Method A	Technician A	26.67 (21.98 - 31.94)	99.17 (97.58 - 99.72)
	Method A	Technician B	27.00 (22.29 - 32.29)	99.72 (98.44 - 99.95)
	Method B	Technician A	26.67 (21.98 - 31.94)	99.17 (97.58 - 99.72)
	Method B	Technician B	27.00 (22.29 - 32.29)	99.44 (98.00 - 99.85)
	Method C	dIFine	27.00 (22.29 - 32.29)	99.17 (97.58 - 99.72)

b. Clinical Performance at Site 2

Diagnostic Sensitivity and Specificity			SLE (n = 300)	Control Diseases (n = 360)
			% Sensitivity (95% CI)	% Specificity (95% CI)
Site 2	Method A	Technician A	24.33 (19.82 - 29.49)	99.72 (98.44 - 99.95)
	Method A	Technician B	25.00 (20.44 - 30.20)	99.17 (97.58 - 99.72)
	Method B	Technician A	25.00 (20.44 - 30.20)	99.72 (98.44 - 99.95)
	Method B	Technician B	24.33 (19.82 - 29.49)	99.17 (97.58 - 99.72)
	Method C	dIFine	22.33 (17.99 - 27.38)	99.17 (97.58 - 99.72)

c. Clinical Performance at Site 3

Diagnostic Sensitivity and Specificity			SLE (n = 300)	Control Diseases (n = 360)
			% Sensitivity (95% CI)	% Specificity (95% CI)
Site 3	Method A	Technician A	25.33 (20.74 - 30.55)	98.89 (97.18 - 99.57)
	Method A	Technician B	25.67 (21.05 - 30.90)	99.17 (97.58 - 99.72)
	Method B	Technician A	25.33 (20.74 - 30.55)	97.78 (95.68 - 98.87)
	Method B	Technician B	25.67 (21.05 - 30.90)	97.50 (95.32 - 98.68)
	Method C	dIFine	24.33 (19.82 - 29.49)	97.50 (95.32 - 98.68)

Sensitivity values for the SLE cohort ranged from 22.33% to 27.0% across all three methods and all three sites. The percent positivity observed in the SLE cohort seemed lower than expected; however, it was in line with FDA 510k summaries from similar devices. The lower percent positivity may be due to a variety of patient-dependent factors at the time of serum collection, such as: presence of

strong immunosuppressive treatments, low disease activity, or disease remission. The percent positivity within this SLE cohort was further confirmed using another FDA-cleared Crithidia l. anti-dsDNA IFA product. Clinical specificity among the control Diseases cohort ranged from 97.5% to 99.72% across all three methods, across all three sites. If one averages all methods of interpretation across all three sites, the clinical sensitivity in the SLE group averaged 25.44% and the clinical specificity in the control Disease group averaged 99.06%.

4. Interpretation Method Comparisons:

There were 660 clinical samples that were tested at all three clinical sites. Considering the interpretations recorded from all three sites for these 660 specimens, there were a total of 3,960 instances where one could compare the results of Method A versus Method B, Method A versus Method C, and Method B versus Method C. A summary of those qualitative comparisons appears in the tables below:

a. Method A vs Method B Qualitative Comparison

Method A vs Method B		Positive Sample Agreement (95% CI)	Negative Sample Agreement (95% CI)	Total Sample Agreement (95% CI)
Site 1	Technician A	83/83, 100.00% (95.58 - 100.00)	577/577, 100.00% (99.34 - 100.00)	660/660, 100.00% (99.42 - 100.00)
	Technician B	81/82, 98.78% (93.41 - 99.78)	576/578, 99.65% (98.75 - 99.91)	657/660, 99.55% (98.67 - 99.85)
Site 2	Technician A	74/74, 100.00% (95.07 - 100.00)	584/586, 99.66% (98.76 - 99.91)	658/660, 99.70% (98.90 - 99.92)
	Technician B	76/78, 97.44% (91.12 - 99.29)	582/582, 100.00% (99.34 - 100.00)	658/660, 99.70% (98.90 - 99.92)
Site 3	Technician A	80/80, 100.00% (95.42 - 100.00)	576/580, 99.31% (98.24 - 99.73)	656/660, 99.39% (98.45 - 99.76)
	Technician B	80/80, 100.00% (95.42 - 100.00)	574/580, 98.97% (97.76 - 99.53)	654/660, 99.09% (98.03 - 99.58)

b. Combined Qualitative Agreement for Method A vs Method B All Sites/All Technicians

		Method A	
		Positive	Negative
Method B	Positive	474	14
	Negative	3	3469
	Positive Percent Agreement =	99.37% (474/477)	95% Confidence Interval = 98.17 - 99.79%
	Negative Percent Agreement =	99.60% (3469/3483)	95% Confidence Interval = 99.33 - 99.76%
	Total Percent Agreement =	99.57% (3943/3960)	95% Confidence Interval = 99.31 - 99.73%

c. Method A vs Method C Qualitative Comparison

For comparisons between Method A and Method C, these were calculated twice; once assuming that all Method C results that were UNC were considered as "negative" and once assuming that all Method C results that were UNC were considered as positive.

Method A vs Method C (UNC = neg)		Positive Sample Agreement (95% CI)	Negative Sample Agreement (95% CI)	Total Sample Agreement (95% CI)
Site 1	Technician A	79/83, 95.18% (88.25 - 98.11)	572/577, 99.13% (97.99 - 99.63)	651/660, 98.64% (97.43 - 99.28)
	Technician B	77/82, 93.90% (86.51 - 97.37)	571/578, 98.79% (97.52 - 99.41)	648/660, 98.18% (96.85 - 98.96)
Site 2	Technician A	68/74, 91.89% (83.42 - 96.23)	585/586, 99.83% (99.04 - 99.97)	653/660, 98.94% (97.83 - 99.49)
	Technician B	69/78, 88.46% (79.50 - 93.81)	582/582, 100.00% (99.34 - 100.00)	651/660, 98.64% (97.43 - 99.28)
Site 3	Technician A	77/80, 96.25% (89.55 - 98.72)	580/580, 100.00% (99.34 - 100.00)	657/660, 99.55% (98.67 - 99.86)
	Technician B	76/80, 95.00% (87.84 - 98.04)	579/580, 99.83% (99.03 - 99.97)	655/660, 99.24% (98.24 - 99.68)

Method A vs Method C (UNC = POS)		Positive Sample Agreement (95% CI)	Negative Sample Agreement (95% CI)	Total Sample Agreement (95% CI)
Site 1	Technician A	81/83, 97.59% (91.63 - 99.34)	568/577, 98.44% (97.06 - 99.18)	649/660, 98.33% (97.04 - 99.07)
	Technician B	79/83, 95.18% (88.25 - 98.11)	567/578, 98.10% (96.63 - 98.93)	646/660, 97.88% (96.47 - 98.73)
Site 2	Technician A	73/74, 98.65% (92.73 - 99.76)	577/586, 98.46% (97.11 - 99.19)	650/660, 98.48% (97.23 - 99.17)
	Technician B	76/78, 97.44% (91.13 - 99.29)	576/582, 98.97% (97.77 - 99.53)	652/660, 98.79% (97.63 - 99.38)
Site 3	Technician A	78/80, 97.50% (91.34 - 99.31)	571/580, 98.45% (97.08 - 99.18)	649/660, 98.33% (97.04 - 99.07)
	Technician B	77/80, 96.25% (89.55 - 98.72)	570/580, 98.28% (96.86 - 99.06)	647/660, 98.03% (96.66 - 98.85)

d. Combined Qualitative Agreement for Method A vs Method C All Sites/All Technicians

		Method A	
		Positive	Negative
Method C (If UNC = Neg)	Positive	446	14
	Negative	31	3469

Positive Percent Agreement =	93.50% (446/477)	95% Confidence Interval = 90.92 - 95.38%
Negative Percent Agreement =	99.60% (3469/3483)	95% Confidence Interval = 99.33 - 99.76%
Total Percent Agreement =	98.31% (3893/3960)	95% Confidence Interval = 97.86 - 98.66%

		Method A	
		Positive	Negative
Method C (If UNC = POS)	Positive	464	54
	Negative	13	3429

Positive Percent Agreement =	97.27% (464/477)	95% Confidence Interval = 95.39 - 98.40%
Negative Percent Agreement =	98.45% (3429/3483)	95% Confidence Interval = 97.98 - 98.81%
Total Percent Agreement =	98.31% (3893/3960)	95% Confidence Interval = 97.86 - 98.66%

e. Method B vs Method C Qualitative Comparison

For comparisons between Method B and Method C, these were calculated twice; once assuming that all Method C results that were UNC were considered as "negative" and once assuming that all Method C results that were UNC were considered as positive.

Method B vs Method C (UNC = neg)		Positive Sample Agreement (95% CI)	Negative Sample Agreement (95% CI)	Total Sample Agreement (95% CI)
Site 1	Technician A	79/83, 95.18% (88.25 - 98.11)	572/577, 99.13% (97.99 - 99.63)	651/660, 98.64% (97.43 - 99.28)
	Technician B	78/83, 93.98% (86.66 - 97.40)	571/577, 98.96% (97.75 - 99.52)	649/660, 98.33% (97.04 - 99.07)
Site 2	Technician A	68/76, 89.47% (80.58 - 94.57)	583/594, 99.83% (99.04 - 99.97)	651/660, 98.64% (97.43 - 99.28)
	Technician B	69/76, 90.79% (82.19 - 95.47)	584/584, 100.00% (99.35 - 100.00)	653/660, 98.94% (97.83 - 99.49)
Site 3	Technician A	77/84, 91.67% (83.78 - 95.90)	576/576, 100.00% (99.34 - 100.00)	653/660, 98.94% (97.83 - 99.49)
	Technician B	77/86, 89.53% (81.29 - 94.40)	574/574, 100.00% (99.34 - 100.00)	651/660, 98.64% (97.43 - 99.28)

Method B vs Method C (UNC = POS)		Positive Sample Agreement (95% CI)	Negative Sample Agreement (95% CI)	Total Sample Agreement (95% CI)
Site 1	Technician A	81/83, 97.59% (91.63 - 99.34)	568/577, 98.44% (97.06 - 99.18)	649/660, 98.33% (97.04 - 99.07)
	Technician B	80/83, 96.39% (89.90 - 98.76)	567/577, 98.27% (96.84 - 99.06)	647/660, 98.03% (96.66 - 98.85)
Site 2	Technician A	74/76, 97.37% (90.90 - 99.28)	576/584, 98.63% (97.32 - 99.30)	650/660, 98.48% (97.23 - 99.17)
	Technician B	75/76, 98.68% (92.92 - 99.77)	577/584, 98.80% (97.55 - 99.42)	652/660, 98.79% (97.63 - 99.38)
Site 3	Technician A	81/84, 96.43% (90.02 - 98.78)	570/576, 98.96% (97.75 - 99.52)	651/660, 98.64% (97.43 - 99.28)
	Technician B	82/86, 95.35% (88.64 - 98.18)	569/574, 99.13% (97.98 - 99.63)	651/660, 98.64% (97.43 - 99.28)

f. Combined Qualitative Agreement for Method B vs Method C All Sites/All Technicians

		Method B	
		Positive	Negative
Method C (If UNC = Neg)	Positive	448	12
	Negative	40	3460

Positive Percent Agreement =	91.80% (448/488)	95% Confidence Interval = 89.03 - 93.92%
Negative Percent Agreement =	99.65% (3460/3472)	95% Confidence Interval = 99.40 - 99.80%
Total Percent Agreement =	98.69% (3908/3960)	95% Confidence Interval = 98.28 - 99.00%

		Method B	
		Positive	Negative
Method C (If UNC = POS)	Positive	473	45
	Negative	15	3427

Positive Percent Agreement =	96.93% (473/488)	95% Confidence Interval = 94.99 - 98.13%
Negative Percent Agreement =	98.70% (3427/3472)	95% Confidence Interval = 98.27 - 99.03%
Total Percent Agreement =	98.48% (3900/3960)	95% Confidence Interval = 98.06 - 98.82%

In all cases, the qualitative agreement between interpretation methods is quite high indicating that all three methods (manual microscope, digital read of the dIFine® and automated call from the dIFine®) correlate well with each other and exhibited few discrepancies.

Taken altogether, these data demonstrate that the auto-call identified by dIFine® (Method C) agrees with Method A and/or Method B (non-automated identification methods) for the vast majority of the samples. However, it is still the responsibility of the trained operator to make the final decision.

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GLOSSARY OF SYMBOLS

The following symbols **may** have been used in the labelling of this product.

Symbol	Description	Symbol	Description
	Manufacturer		Keep away from sunlight
IVD	*In vitro* diagnostic medical device	CE	Conformity with Directive 98/79
REF	Catalogue number	COVGLS	Cover Glass
Σ n	Sufficient for *n* tests	SLD	Substrate Slide
LOT	Batch code	BUF PBS	PBS Buffer
	Use by	MNTMED	Mounting Media
	Storage Temperature limitations	CONJ	Conjugate
RX Only	For Prescription Use Only	CONTROL +	Positive Control
	Consult electronic instructions for use	CONTROL -	Negative Control
	Store in the upright position	Made in the USA	Made in the USA

ZEUS Scientific.
200 Evans Way, Branchburg, New Jersey, 08876, USA
Toll Free (U.S.): 1-800-286-2111, Option 2
International: +1 908-526-3744
Fax: +1 908-526-2058
Website: www.zeusscientific.com

For US Customer Service contact your local distributor.
For US Technical Support contact ZEUS Scientific, call toll free or e-mail support@zeusscientific.com.
For Non-US Customer Service and Technical Support inquiries, please contact your local distributor.

EC REP
EMERGO EUROPE
Westervoortsedijk 60
6827 AT Arnhem
The Netherlands