

QUANTA Lite® HA dsDNA ELISA

704615

For *In Vitro* Diagnostic Use
CLIA Complexity: High

Intended Use

This 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. Sufficient materials are supplied to allow a maximum of 40 samples to be tested in duplicate or 88 in single, with a calibration curve and positive, negative and single stranded DNA controls.

Summary and Explanation of the test

The use of anti-dsDNA antibodies as a diagnostic marker for SLE and in the subsequent monitoring of disease activity has been of great benefit to clinicians¹ since first being recognised by Ceppellini *et al* in 1957² and confirmed by its inclusion in the 1982 revised American College of Rheumatology criteria for the classification of SLE³.

There are three commonly used assay methods employed today that can be differentiated on the basis of the avidity of the dsDNA antibodies detected⁴: Enzyme linked immunosorbant assay (ELISA), of which there are many variants, classically detect a wide spectrum of antibody avidities. The *Crithidia luciliae* immunofluorescent assay (IFA) detects antibodies biased to those with lower avidities and Farr radioimmunoassays (RIA)⁵ classically measure only the high avidity antibodies by virtue of the ammonium sulphate precipitation step which precludes low avidity antibodies.

The HA dsDNA assay incorporates a more stringent set of assay conditions⁸, which are intended to remove the low avidity weakly bound antibodies.

Some studies^{6,7} have shown a relationship between antibody avidity and the progression of the disease, where the selection of the assay facilitates the differentiation between patients with a benign form of SLE and those with skin and renal involvement, particularly in cases of lupus nephritis^{8,9}.

Generally there is good agreement among all the assays using sera from patients with 'definite' systemic lupus erythematosus (SLE), however there is significant discordance in patients with inactive disease, or those that do not meet the standard classification for SLE.

Principles of the Procedure

Microwells are pre-coated with calf thymus dsDNA antigen. The 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 labelled goat anti-human IgG conjugate is added. The conjugate binds to the captured human autoantibody and the excess unbound conjugate is removed by a further wash step. The bound conjugate is visualised 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. Sulfuric acid is added to each well to stop the reaction. This produces a yellow end point color, which is read at 450nm.

Reagents

1. Polystyrene microwell ELISA plate coated with calf thymus dsDNA antigen (12-1 x 8 wells), with holder in foil package containing desiccants
2. ELISA Negative Control, 1 vial of buffer containing preservative and human serum with no human antibodies to dsDNA, prediluted, 1.2mL
3. HA dsDNA ELISA Calibrator A, 1 vial of buffer containing preservative and human serum antibodies to dsDNA, prediluted, 1.2mL
4. HA dsDNA ELISA Calibrator B, 1 vial of buffer containing preservative and human serum antibodies to dsDNA, prediluted, 1.2mL
5. HA dsDNA ELISA Calibrator C, 1 vial of buffer containing preservative and human serum antibodies to dsDNA, prediluted, 1.2mL
6. HA dsDNA ELISA Calibrator D, 1 vial of buffer containing preservative and human serum antibodies to dsDNA, prediluted, 1.2mL
7. HA dsDNA ELISA Calibrator E, 1 vial of buffer containing preservative and human serum antibodies to dsDNA, prediluted, 1.2mL
8. HA dsDNA Positive Control, 1 vial of buffer containing preservative and human serum antibodies to dsDNA prediluted, 1.2mL
9. Type III Sample Diluent, 2 vials – colored yellow containing Tween 20, protein stabilizers and preservative, 50mL
10. HA dsDNA Wash Concentrate (10x), 2 vials of 10x concentrate - colorless containing Tween 20 and a preservative, 50mL. Refer to the Methods Section for dilution instructions.
11. HA dsDNA Single Stranded Control, 1 vial of buffer containing preservative and human serum antibodies to ssDNA, pre-diluted, 1.2mL
12. HRP HA dsDNA IgG Conjugate, anti-human IgG, 1 vial – colored blue containing buffer, protein stabilizers and preservative, 10mL
13. TMB Chromogen, 1 vial containing stabilizers, 10mL
14. HRP Stop Solution, 0.344M Sulfuric Acid, 1 vial – colorless, 10mL

Warnings

1. **WARNING:** This product contains a chemical (0.02% chloramphenicol) in the controls, diluent and conjugate known to the State of California to cause cancer. The wash and sample diluent contain Proclin 150. To prevent injury, avoid inhalation, ingestion or contact with skin and eyes.

2. All human source material used in the preparation of controls for this product has been tested and found negative for antibody to HIV, HBsAg, and HCV by FDA cleared methods. No test method however can offer complete assurance that HIV, HBV, HCV or other infectious agents are absent. Therefore, the HA dsDNA Positive Control, HA dsDNA Single Stranded Control, HA dsDNA Calibrators A through E and ELISA Negative Control should be handled in the same manner as potentially infectious material.¹²
3. Sodium Azide is used as a preservative. Sodium Azide is a poison and may be toxic if ingested or absorbed through the skin or eyes. Sodium azide may react with lead or copper plumbing to form potentially explosive metal azides. Flush sinks, if used for reagent disposal, with large volumes of water to prevent azide build-up.
4. The HRP conjugate contains a dilute poisonous/corrosive chemical, which may be toxic if ingested in large amounts. To prevent possible chemical burns, avoid contact with skin and eyes.
5. TMB Chromogen contains an irritant, which may be harmful if inhaled, ingested or absorbed through the skin. To prevent injury, avoid inhalation, ingestion or contact with skin and eyes.
6. The HRP Stop Solution consists of a dilute sulfuric acid solution. Avoid exposure to bases, metals, or other compounds, which may react with acids. Sulfuric acid is a poison and corrosive, which may be toxic if ingested. To prevent chemical burns, avoid contact with skin and eyes.
7. Use appropriate personal protective equipment while working with the reagents provided.
8. Spilled reagents should be cleaned up immediately. Observe all federal, state and local environmental regulations when disposing of wastes.

Precautions

1. This product is for *In Vitro Diagnostic Use*.
2. This product should only be used by appropriately trained personnel.
3. Strict adherence to the protocol is recommended. Any deviation may affect assay performance, and the results obtained. Pay attention to specific '**Notes**' and warnings throughout these Instructions for Use.
4. Calibrator, controls, conjugate and plate batch numbers are **not** interchangeable. Substitution of such components with batch numbers that differ from those that are provided in the kit could lead to inconsistent and inaccurate results. All strips must be taken from the same foil pouch.
5. To avoid reagent contamination, only use new or clean plastic / glassware. **Never** return unused reagents to the bottles.
6. Do **not** leave reagent bottles uncapped; any resulting evaporation or contamination will lead to inconsistent results.
7. TMB substrate must not be exposed to light or water.
8. Microbially contaminated, haemolysed or lipaemic serum and specimens containing particulate matter should not be used.
9. Inaccurate sample dilution cannot be checked, as kit controls are ready to use. The use of calibrated pipettes and appropriate internal QC samples is recommended.
10. The use of automated assay systems, sample dilutors and other automated equipment may lead to differences in results when compared to the manual procedure. It is the responsibility of any laboratory to fully validate the system, and ensure the results fall within the limits as defined in this insert and associated QC certificate.
11. All equipment used must be calibrated and maintained according to the manufacturer's instruction.

Storage Conditions

1. The kit should be stored at 2-8°C and should **not** be frozen. Inappropriate storage temperatures will affect the results.
2. Diluted wash buffer is stable for 1 week at 2-8°C.
3. The expiry date of the kit is shown on the outer label.

Specimen Collection

1. Blood samples should be collected by venepuncture allowed to clot naturally and the serum separated.
2. The serum may be stored at 2-8°C for up to 7 days prior to assay¹⁰, or for prolonged storage, aliquoted and stored at -20°C or below.
3. Repeated thawing and freezing should be avoided.
4. Serum samples should **not** be heat-inactivated, as this may give false positive results.

Procedure

Materials provided

- **Instruction leaflet:** Giving full assay details.
- **QC Certificate:** Indicating the expected performance of the batch.
- **HA dsDNA Coated Wells:** 12 breakapart 8 well strips coated with calf thymus dsDNA antigen. Each plate is packaged in a re-sealable foil bag containing two desiccant pouches.
- **Type III Sample Diluent:** 2 bottles containing 50mL of buffer for sample dilution. Colored yellow, ready to use.
- **HA dsDNA Wash Concentrate (10X):** 2 bottles containing 50mL of a 10-fold concentrated buffer for washing the wells.
- **HA dsDNA Calibrators:** 5 bottles, each containing 1.2mL of diluted human serum, with the following concentrations of anti-dsDNA autoantibody: 1000, 333, 111, 37, 12.3 IU/mL. Ready to use.
- **HA dsDNA Positive Control:** 1 bottle containing 1.2mL of diluted human serum. The expected value is given on the QC certificate. Ready to use.
- **ELISA Negative Control:** 1 bottle containing 1.2mL of diluted human serum. The expected value is given on the QC certificate. Ready to use.
- **HA dsDNA Single Stranded Control:** 1 bottle containing 1.2mL of diluted human serum. The expected value is given on the QC certificate. Ready to use.
- **HRP HA dsDNA IgG Conjugate:** 1 bottle containing 10mL of purified peroxidase labelled antibody to human IgG. Colored blue. Ready to use.

- **TMB Chromogen:** 1 bottle containing 10mL TMB chromogen. Ready to use.
- **HRP Stop Solution:** 1 bottle containing 10mL of 0.344M Sulfuric Acid. Ready to use.

Additional materials required but not provided

- **Automatic microplate plate washer:** This is recommended, however, plate washing can be performed manually.
- **Plate reader:** Capable of measuring optical densities at 450nm referenced on air.
- **Distilled or deionised water:** This should be of the highest quality available
- **Calibrated micropipettes:** For dispensing 1000, 100 & 10 μ L
- **Multichannel pipette:** Recommended for dispensing 100 μ L volumes of conjugate, substrate and stop solution.
- **Glass/plastic tubes:** For sample dilution

Method

Before you start

1. **Bring the kit to room temperature**
 - The kit is designed for room temperature operation (20-24°C).
 - Remove the kit from storage and stand at room temperature for approximately 60 minutes. Wells must **not** be removed from the foil bag until they have reached room temperature. **Note:** The kit may be maintained at room temperature for up to 1 week.
2. **Kit components**
Gently mix each kit component before use.
3. **Wash buffer (10 x concentrate) dilution**
Add 100mL of the wash buffer concentrate to 900mL of distilled water (1 in 10 dilution) into a clean container and mix. Smaller volumes can be diluted as appropriate. **Note:** Diluted wash buffer is stable for 1 week at 2-8°C, therefore only dilute the appropriate amount.
4. **Sample dilution**
Dilute 10 μ L of each sample with 1000 μ L of sample diluent (1:100) and mix well. **Note:** Diluted sample **must** be used within 8 hours.
5. **Strip and frame handling**
Place the required number of wells in the strip holder. Position from well A1, filling columns from left to right across the plate. When handling the plate, squeeze the long edges of the frame to prevent the wells falling out. **Note:** Return unused wells to the foil bag immediately with the two desiccant pouches and re-seal tightly to minimise exposure to moisture. Take care not to puncture or tear the foil bag, see below. **WARNING: Exposure of wells to moisture or contamination by dust or other particulate matter will result in antigen degradation, leading to poor assay precision and potentially false results.**

Assay procedure

1. **Sample addition**
Dispense 100 μ L of each calibrator, controls and diluted (1:100) sample into the appropriate wells of the plate provided. **Note:** Samples should be added as quickly as possible to the plate to minimise assay drift, and the timer started after the addition of the **last** sample. **Incubate at room temperature for 30 minutes.**
2. **Washing**
The washing procedure is critical and requires special attention. An improperly washed plate will give inaccurate results, with poor precision and high backgrounds. After incubation remove the plate and wash wells 3 times with 200-300 μ L wash buffer per well. Wash the plate either by using an automatic plate washer or manually as indicated below. After the final automated wash, invert the plate and tap the wells dry on absorbent paper.
Automated plate washers should not be programmed to include a soak step.
Plates can be washed manually as follows:
 - a. Flick out the contents of the plate into a sink
 - b. Tap the wells dry on absorbent paper.
 - c. Fill each well with 200-300 μ L wash buffer using a multichannel pipette.
 - d. Gently shake the plate on a flat surface.
 - e. Repeat a-d twice
 - f. Repeat a and b.**Do not leave the wash in the wells for longer than it takes to fill the whole plate.**
3. **Conjugate addition**
Dispense 100 μ L of conjugate into each well, blot the top of the wells with a tissue to remove any splashes. **Note:** To avoid contamination, never return excess conjugate to the reagent bottle. **Incubate at room temperature for 30 minutes.**
4. **Washing**
Repeat step 2.
5. **Chromogen (TMB) addition**
Dispense 100 μ L of TMB chromogen into each well, blot the top of the wells with a tissue to remove any splashes. **Note:** To avoid contamination never return excess TMB to the reagent bottle. **Incubate at room temperature in the dark for 30 minutes.**
6. **Stopping**
Dispense 100 μ L of stop solution into each well. This causes a change in colour from blue to yellow.
7. **Optical density measurement**
Read the optical density (OD) of each well at 450nm on a microplate reader, within 30 minutes of stopping the reaction.

Quality Control

1. Quality control

In order for an assay to be valid, all the following criteria must be met:

- Calibrators, positive, negative and single stranded controls must be included in each run.
- The values obtained for all the controls should be in the ranges specified on the QC Certificate.
- The curve shape should be similar to the calibration curve, shown on the QC Certificate.

If the above criteria are not met, the assay is invalid and the test should be repeated.

2. Calculate mean optical densities (For assays run in duplicate only).

For each calibrator, control and sample calculate the mean OD of the duplicate readings. The percentage coefficient of variation (%C.V.) for each duplicate OD should be less than 15%.

3. Plot calibration curve

The calibration curve can be plotted either automatically or manually as follows by plotting the anti-dsDNA autoantibody concentration on the log scale against the OD on the linear scale for each calibrator:

- Automatic - use appropriately validated software, and the curve fit that best fits the data.
- Manual - using log/linear graph paper, draw a smooth curve through the points (**not** a straight line or point to point).

4. Treatment of anomalous points

If any one point does not lie on the curve, it can be removed. If the absence of this point means that the curve has a shape dissimilar to that of the sample calibration curve, or more than one point appears to be anomalous, then the assay should be repeated.

5. Calculation of autoantibody levels in controls and diluted samples

Read the level of the anti-dsDNA autoantibody in the controls and diluted samples directly from the calibration curve. The control values should fall within the range given on the QC Certificate. **Note:** The calibrator values have been adjusted by a factor of 100 to account for a 1:100 sample dilution. No further correction is required.

6. Assay calibration

The assay is calibrated in IU/mL against the WHO reference calibrator Wo80⁶.

Limitations of the Procedure

1. This assay, by virtue of its ability to detect only high avidity anti-dsDNA antibodies, may miss some forms of SLE where patients have only antibodies of low avidity. It might not be suitable for applications where a total anti-dsDNA antibody measurement is required.
2. This kit is used to aid diagnosis only. A positive result suggests certain diseases, which must be confirmed by clinical findings and other serological tests.
3. The results obtained from this assay are not diagnostic proof of the presence or absence of disease.

Expected Values

The following ranges have been established based on the results obtained from testing samples from both healthy blood donors (n=150) and SLE patients (n = 224) on FARRZYME:

INTERPRETATION	
≤ 30 IU/mL	Negative result
> 30 IU/mL	Positive result

The 150 sera from normal blood donors all gave results below 17.0 IU/mL, with 146 (97%) of the results below 12.3 IU/mL which is the lower measuring limit of the kit. 22.8% of the SLE patient sera gave positive FARRZYME results (>30 IU/mL).

The results shown below are of a comparison study between the same 224 SLE samples of the FARRZYME and a conventional anti-dsDNA EIA assay, which measures both low and high avidity antibodies to dsDNA. Samples giving borderline results in the conventional dsDNA assay were treated as negative when calculating positive and negative percentage agreement and overall agreement.

		Conventional Anti-dsDNA ELISA		
		Positive	Borderline	Negative
FARRZYME	Positive	47	3	1
	Negative	33	51	89

Positive percent agreement = 58.8%

Negative percent agreement = 97.2%

Overall agreement = 83.5%

The incidence of high avidity anti-dsDNA antibodies detected by FARRZYME and by Farr RIA in 100 SLE patients has been reported to be 36% and 38%, respectively⁸. By contrast, in 100 patients with various connective tissue diseases and other autoimmune diseases the incidence was 4% and 5% respectively for the two assays.

Unit values obtained with the FARRZYME assay may not agree with those obtained in other assays reporting in IU/mL, as FARRZYME only measures the high avidity anti-dsDNA antibody subset.

The ranges are provided as a guide only. ELISA assays are very sensitive and capable of detecting small differences in sample populations. It is recommended that each laboratory determine its own normal range, based on the population techniques and equipment employed.

Performance Characteristics

1. Measuring Range

The measuring range of the assay is 12.3 – 1000 IU/mL.

2. Confirmation of Assay Sensitivity

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) was obtained by statistical analysis.

3. Specificity Agreement

189 samples from patients with SLE, together with 35 samples positive for dsDNA antibodies by Crithidia or ELISA and 28 samples from healthy normals were tested by FARRZYME, by *Crithidia luciliae* immunofluorescent assay and also in a conventional dsDNA ELISA.

		Crithidia IFA	
		+	-
FARRZYME	+	37	14
	-	18	183
Positive percent agreement		67.3%	
Negative percent agreement		92.9%	
Overall agreement		87.3%	

FARRZYME EIA demonstrated good negative agreement with the *Crithidia luciliae* immunofluorescent assay. Positive percent agreement was reduced because the latter assay is known to detect anti-DNA antibodies of low avidity¹¹, unlike the FARRZYME assay.

		Conventional dsDNA ELISA		
		+	Borderline	-
FARRZYME	+	47	3	1
	-	33	51	117
*Positive percent agreement		58.8%		
Negative percent agreement		97.7%		
Overall agreement		85.3%		

*For the calculation of agreement between the two assays, samples in the borderline region of the conventional dsDNA assay were classified as negative. The conventional ELISA detects antibodies of both high and low avidity, resulting in reduced positive agreement for the FARRZYME assay.

4. Intefering Substances

Various serum types were assayed to test the possible effect of interfering substances using an Interference Check A plus kit (Kokusai, Japan).

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

No interference was observed with free or conjugated bilirubin, haemoglobin, lipid or rheumatoid factor. In a separate study 6 IgG myeloma sera were tested on the FARRZYME assay and none of these gave a positive result.

5. Precision

The intra- and inter-assay precision was measured using six samples covering the range of the calibration curve. The mean and % C.V. for each sample are given below:

Intra-assay precision was measured using 20 replicates in one assay.

INTRA-ASSAY PRECISION		
n = 20	Concentration (IU/mL)	% C.V.
Sample 1	25.1	5.4
Sample 2	39.0	4.8
Sample 3	74.5	2.2
Sample 4	205.5	3.3
Sample 5	361.2	4.3
Sample 6	528.6	5.1

Inter-assay precision was measured by testing samples in duplicate in 6 assays performed over 3 days.

INTRA-ASSAY PRECISION		
n = 6	Concentration (IU/mL)	% C.V.
Sample 1	24.6	13.5
Sample 2	42.3	3.9
Sample 3	61.3	11.6
Sample 4	123.2	4.9
Sample 5	272.4	7.0
Sample 6	474.1	6.9

Plate template

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

Summary of Procedure

1. Add 100 μ L of each calibrator, control and 1:100 diluted sample to the appropriate wells.
Incubate for 30 minutes.
Wash.
2. Add 100 μ L of conjugate to each well.
Incubate for 30 minutes.
Wash.
3. Add 100 μ L of substrate to each well.
Incubate for 30 minutes.
4. Add 100 μ L of stop solution to each well.
Measure the absorbance at 450nm.

QUANTA Lite and INOVA Diagnostics are registered trademarks. Copyright 2011 All Rights Reserved. ©

References

1. Isenberg D and Smeenk R. Clinical laboratory assays for measuring anti-dsDNA antibodies. Where are we now? *Lupus*, 2002; **11**: 797-800.
2. Ceppelini R, Polli E and Celada F. A DNA-reacting factor in serum of a patient with lupus erythematosus diffuses. *Proc Soc Exp Biol Med*, 1957; **96**: 572-574.
3. Tan *et al.* The 1982 revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheumatism*, 1982; **25**:1271-7.
4. Riboldi P *et al.* Anti-DNA antibodies: a diagnostic and prognostic tool for systemic lupus erythematosus? *Autoimmunity*. 2005; **38**: 39-45.
5. Werle E *et al.* The clinical significance of measuring different ant-dsDNA antibodies by using the Farr assay, an enzyme immunoassay and a crithidia luciliae immunofluorescence test. *Lupus*, 1992; **1**: 369-377.
6. Isenberg D. Anti-dsDNA antibodies: still a useful criterion for patients with systemic lupus erythematosus? *Lupus*, 2004; **13**: 881-885.
7. Nossent H C and Rekvig O P. Is closer linkage between systemic lupus erythematosus and anti-double-stranded DNA antibodies a desirable and attainable goal? *Arthritis Res Ther*, 2005; **7(2)**: 85-7.
8. Jaekel HP *et al.* Anti-dsDNA antibody subtypes and anti-C1q antibodies: toward a more reliable diagnosis and monitoring of systemic lupus erythematosus and lupus nephritis. *Lupus*, 2006; **15**: 1-11.
9. Renaudineau Y *et al.* Association of α -actinin-binding anti-dsDNA antibody Protein Reference Unit Handbook of Autoimmunity (3rd Edition) 2004 Ed A with lupus nephritis. *Arthritis Rheumatism*, 2006 (in press)
10. Protein Reference Unit Handbook of Autoimmunity (3rd Edition) 2004 Ed A Milford Ward. J. Sheldon, GD Wild. Publ. PRU Publications, Sheffield. 14.
11. Smeenk R, van der Lelij G and Aarden L. Measurement of low avidity anti-dsDNA by the Crithidia luciliae test and the PEG assay. *Clin Exp Immunol*, 1982; **49**: 603-610.
12. Biosafety in Microbiological and Biomedical Laboratories. Center for Disease Control/National Institute of Health, 2007, Fifth Edition.

Manufactured By:

INOVA Diagnostics, Inc.
9900 Old Grove Road
San Diego, CA 92131
United States of America

Technical Service (U.S. & Canada Only) : 877-829-4745
Technical Service (Outside the U.S.) : 00+ 1 858-805-7950
support@inovadx.com

Authorized Representative in the EU:

Medical Technology Promedt Consulting GmbH
Altenhofstrasse 80
D-66386 St. Ingbert, Germany
Tel.: +49-6894-581020
Fax.: +49-6894-581021
www.mt-procons.com

624615ENG

May 2011
Revision 2

