LIMITATIONS OF THE ASSAY

1. The binding activity of the antibodies and the activity of the enzyme used are temperature-dependent. It is therefore recommended using a thermostat in all three incubation steps. The higher the room temperature (+18°C to +25°C) during the incubation steps, the greater will be the absorbance values. Corresponding variations apply also to the incubation times. However, the calibrator is subject to the same influences, with the result that such variations will be largely compensated in the calculation of the result.
2. For duplicate determination the mean of the two values should be taken. If the two values deviate substantially from one another, retesting the samples is recommended.
3. For diagnosis, the clinical picture of the patient always needs to be taken into account along with the serological findings.

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SYMBOL KEYS

Temperature Limitation	Consult Instructions for use	Date of Manufacture	LOT Batch Number / Lot Number
Manufacturer	IVD *In vitro* Diagnostic Medical Device	This side up	Σ Contains sufficient for <n> tests
Use by	REF Catalogue Number	Do not reuse	

Manufactured by:

Zephyr Biomedicals

A Division of Tulip Diagnostics (P) Ltd.

M 46-47, Phase III B, Verna Industrial Estate, Verna, Goa - 403 722, INDIA.

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0324/VER-02

Qualisa™

Microwell Enzyme Immunoassay | ANA

Anti-nuclear IgG antibody (ANA)

Enzyme Linked Immunosorbent Assay (ELISA) for semi quantitative detection of anti-nuclear IgG antibodies (ANA) in Human Serum

FOR IN VITRO DIAGNOSTIC USE ONLY

Store at 2°C to 8°C

INTENDED USE

Qualisa™ ANA- ELISA test system is an enzyme linked immunosorbent assay for semi quantitative detection of anti-nuclear IgG antibodies (ANA) in human serum. For in Vitro Diagnostic Use only.

INTRODUCTION

Anti-nuclear antibodies (ANA) are auto-antibodies that bind to several nuclear antigens including double stranded DNA (dsDNA), single-stranded DNA (SSDNA), ribonucleic-protein (RNP), Sm, SS-A and SS-B. ANA are frequently present in patients with systemic lupus erythematosus (SLE) and less commonly in other autoimmune diseases such as Rheumatoid arthritis, Collagen vascular diseases, chronic liver diseases and systemic sclerosis (scleroderma). The assay is used as a screening procedure for autoimmune diseases.

PRINCIPLE

The **Qualisa™ ANA** test kit is an enzyme linked immunosorbent assay. Diluted serum is added to microwells coated with purified nuclear antigens. If ANA IgG specific antibodies are present in diluted serum, they bind to the microwells coated with purified nuclear antigen. Washing of the microwells removes all unbound materials. Enzyme Conjugate is added to the microwells to bind to the antibody-antigen complex if present. And washing of the microwells removes the excess of conjugate. A solution of TMB substrate is added to allow hydrolysis of the substrate by the enzyme. The reaction is stopped after specified time using stop solution. The intensity of the colour generated is proportional to the amount of IgG specific antibody in the sample.

MATERIALS AND COMPONENTS

A. Materials provided with the test kit:

1. Coated Microwells: Microwells coated with Nuclear antigen
2. Calibrator: Ready to use
3. Sample Diluent: Ready to use
4. Negative Control: Ready to use
5. Positive Control: Ready to use
6. Wash Buffer Concentrate (20X)
7. Enzyme Conjugate: Ready to use.
8. TMB Substrate: Ready to use
9. Stop Solution: Ready to use

B. Materials required but not provided:

1) Precision pipettes: 10-100µl, 20-200µl, 100-1000µl
2) Disposable pipette tips
3) Distilled water
4) Disposable Gloves
5) ELISA reader
6) ELISA washer

STORAGE AND STABILITY

1. **Qualisa™ ANA** kit is stable at 2-8°C up to the expiry date printed on the label.
2. Coated Microwells should be used within one month upon opening the pouch provided that once opened, the pouch must be resealed to protect from moisture. If the colour of the desiccant has changed from blue to white at the time of opening the pouch, another coated Microwells pouch should be used.
3. Diluted Wash Buffer is stable up to one week when stored at 2-8°C.

SAMPLE COLLECTION

1. Collect Blood specimen by venipuncture according to standard procedure.
2. Only serum should be used.
3. Avoid grossly hemolytic, lipemic or turbid samples.
4. Preferably use fresh samples. However specimens can be stored upto 48 hour at 2-8°C for short duration.
5. For longer storage, specimens can be frozen at − 20°C. Thawed samples must be mixed prior to testing.
6. Do not heat inactivate before use.

7. Specimen containing precipitate or particulate matter should be clarified by centrifugation prior to use.
8. Specimen should be free from particulate matter and microbial contamination.

PRECAUTIONS

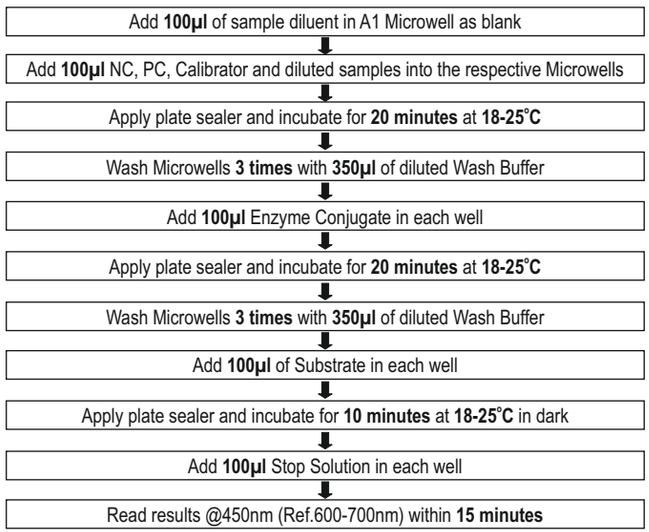
(1) Bring all reagents and specimen to room temperature before use.
(2) Do not pipette any material by mouth.
(3) Do not eat, drink or smoke in the area where testing is done.
(4) Use protective clothing and wear gloves when handling samples.
(5) Use absorbent sheet to cover the working area.
(6) Immediately clean up any spills with sodium hypochlorite.
(7) All specimens and controls should be considered potentially infectious and discarded appropriately.
(8) Neutralize acid containing waste before adding hypochlorite.
(9) Do not use kit after the expiry date.
(10) Do not mix components of one kit with another.
(11) Always use new tip for each specimen and reagent.
(12) Do not allow liquid from one well to mix with other wells.
(13) Do not let the strips dry in between the steps.

REAGENT PREPARATION

1. All reagents should be brought to room temperature (18-25°C) and mixed by gently inverting or swirling prior to use. Do not induce foaming.
2. Dilute wash buffer 20 times (for example add 5ml concentrated buffer to 95ml of distilled or deionized water). Mix well before use.

TEST PROCEDURE

1. Place the desired number of coated strips into the holder.
2. Prepare 1:21 dilutions by adding **10µl** of the test samples to **200µl** of sample diluent. Mix well.
   Please Note: The Negative control, Positive control and the calibrator are ready to use, DO NOT dilute.
3. Dispense **100µl** of diluted serum samples, negative control, positive control and calibrator into the appropriate wells. For the reagent blank, dispense **100µl** of sample diluent in A1 well position. Shake the holder to remove air bubbles from the liquid and mix well. Incubate at room temperature **(18-25°C) for 20 minutes.**
4. After incubation, empty the microtitre wells and wash the plate 3 times with **350µl** of diluted wash buffer. Strike the microtitre plate sharply onto the absorbent paper towel to remove all residual water droplets.
5. Dispense **100µl** of Enzyme Conjugate to each well and incubate at room temperature **(18-25°C) for 20 minutes.**
6. After incubation, empty the microtitre wells and wash the plate 3 times with **350µl** of diluted wash buffer. Strike the microtitre plate sharply onto the absorbent paper towel to remove all residual water droplets.
7. Dispense **100µl** of TMB Substrate into each well and incubate at room temperature **(18-25°C) for 10 minutes in dark**.
8. Stop the reaction by adding **100µl** of Stop Solution to each well. Gently mix for 10 seconds until the blue color completely changes to yellow.
9. Read the optical density at 450/630 nm with a microtiter plate reader within **15 minutes**.



RUN CRITERIA

1) The O.D of the Calibrator should be greater than 1.0
2) The ANA Index of the Negative control should be 0.9 or less.
3) The ANA index of the Positive control should be in the range as stated on the label.
If any of these criteria are not met, the results are invalid and the test should be repeated.

CALCULATION OF RESULTS

1) To obtain the Cut off Value (COV): Multiply the OD of Calibrator by Calibrator Factor (CF) (which is lot specific & will be printed on the label of the calibrator vial).
2) Calculate the ANA Index of each determination by dividing the OD values of each sample by obtained OD of Cut off value.

For example
If the Calibrator Factor (CF) on the label is 0.38
Calibrator mean OD = 2.001
Cut-off Value = 2.001 × 0.38 = 0.760
Patient sample OD = 1.594
ANA Index = 1.594/0.760 = 2.09 (Positive result)
Patient Sample OD = 0.232
ANA Index = 0.232/0.760 = 0.30 (Negative result)

INTERPRETATION OF THE RESULT

Negative: ANA Index of 0.90 or less. No detectable ANA IgG by ELISA.
Equivocal: ANA Index of 0.91 - 1.1 are equivocal. Follow up testing is recommended if clinically indicated.
Positive: ANA Index of 1.11 or above. Detectable ANA IgG by ELISA.

PERFORMANCE CHARACTERISTICS

1) Sensitivity and Specificity

233 patient sera were tested by this ELISA and a reference ELISA method. 2 sera were positive and 220 sera were negative by both methods. The correlation between the two methods was 95.2%. The results are summarized as below:

Qualisa ANA IgG ELISA					
		+	+/-	-	Total
Reference ELISA Kit	+	2	2	6	10
	+/-	0	0	2	2
	-	1	0	220	221
Total		3	2	228	233

2) Precision:

Intra Assay study

Sample	No of replicates	Mean	Standard Deviation	Coefficient of variation (%)
1	16	1.79	0.08	4.60
2	16	1.36	0.043	3.21
3	16	0.37	0.042	11.4

Inter Assay study

Sample	No of replicates	Mean	Standard Deviation	Coefficient of variation (%)
1	10	1.94	0.115	5.94
2	10	1.32	0.078	5.89
3	10	0.41	0.046	10.96

IMPORTANT NOTE

1. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
2. It is recommended to use the multi-channel pipettes to avoid time effect. A full plate of 96 wells may be used if automated pipetting is available.
3. Duplication NC, PC, Calibrator & samples is not mandatory but may provide information on reproducibility & application errors.