# DRG® ANA Combi (ANA-8-Profile) (EIA-3563)

CE

IVD

**Revised 27 June 2011 rm (Vers. 3.1)**

***Please use only the valid version of the package insert provided with the kit.***

## NAME AND INTENDED USE

The ANA Combi assay is an indirect solid phase enzyme immunometric assay (ELISA) for the qualitative determination of ANA autoantibodies directed against RNP-70, RNP/Sm, Sm, SS-A, SS-B, Scl-70, Centromere B and Jo-1 in human serum or plasma. The assay is intended for in vitro diagnostic use only as an aid in the diagnosis of certain systemic rheumatic diseases.

## SUMMARY AND EXPLANATION OF THE TEST

Inflammatory connective tissue diseases are characterised by idiopathic genesis along with disturbances in terms of cellular and humoral immunity, systemic organ failure and a chronic course of disease. Additionally, connective tissue diseases exhibit overlapping symptomatic features that render an accurate diagnosis difficult [1].
Considering the diversity of mixed connective tissue diseases, such disorders exhibit a common serological characteristic; the presence of antinuclear antibodies [2]. These antibodies are directed against parts of the cell nucleus and the cytoplasm, and many rheumatic diseases are characterised by the presence of one or more of these ANAs [3].
Antibodies to double-stranded DNA (dsDNA), single-stranded DNA (ssDNA), histone, nuclear ribonucleoprotein (RNP) and Smith antigen (Sm) are associated with SLE [4], while antibodies to Sjögren´s syndrome A (SS-A/Ro) and Sjögren´s syndrome B (SS-B/La) can occur in both SLE and Sjögren´s syndrome (SS) [5, 6]. Antibodies to Jo-1 may be observed in polymyositis and dermatomyositis [6], while antibodies to scleroderma-associated antigen (Scl-70) and centromere can occur in patients with progressive systemic sclerosis (PSS). Anti-histone antibodies are associated with SLE and drug-induced lupus [7], while anti-RNP antibodies are linked with mixed connective tissue disease (MCTD) and with SLE [2]. Antibodies directed against centromere are associated with CREST syndrome [3]..

## PRINCIPLE OF THE TEST

Purified antigens SS-A/Ro, SS-B/La, RNP-70, Sm, RNP/Sm, Centromere B and Jo-1 are bound to microwells in row A to H separately. Antibodies against these antigens, if present in diluted serum or plasma, bind to the respective antigens. Washing of the microwells removes unspecific serum and plasma components. Horseradish peroxidase (HRP) conjugated anti-human IgG immunologically detects the bound patient antibodies forming a conjugate/antibody/antigen complex. Washing of the microwells removes unbound conjugate. An enzyme substrate in the presence of bound conjugate hydrolyzes to form a blue colour. The addition of an acid stops the reaction forming a yellow end-product. The intensity of this yellow colour is measured photometrically at 450 nm..

## WARNINGS AND PRECAUTIONS

1. All reagents of this kit are strictly intended for in vitro diagnostic use only.
2. Do not interchange kit components from different lots.
3. Components containing human serum were tested and found negative for HBsAg, HCV, HIV1 and HIV2 by FDA approved methods. No test can guarantee the absence of HBsAg, HCV, HIV1 and HIV2, and so all human serum based reagents in this kit must be handled as though capable of transmitting infection.
4. Avoid contact with the TMB (3,3´,5,5´-Tetramethyl-benzidine). If TMB comes into contact with skin, wash thoroughly with water and soap.

5. Avoid contact with the Stop Solution which is acid. If it comes into contact with skin, wash thoroughly with water and seek medical attention.
6. Some kit components (i.e. Controls, Sample buffer and Buffered Wash Solution) contain Sodium Azide as preservative. Sodium Azide ($NaN_3$) is highly toxic and reactive in pure form. At the product concentrations (0,09%), though not hazardous. Despite the classification as non-hazardous, we strongly recommend using prudent laboratory practices (see 8., 9., 10.)
7. Some kit components contain Proclin 300 as preservative. When disposing reagents containing Proclin 300, flush drains with copious amounts of water to dilute the components below active levels.
8. Wear disposable gloves while handling specimens or kit reagents and wash hands thoroughly afterwards.
9. Do not pipette by mouth.
10. Do not eat, drink, smoke or apply makeup in areas where specimens or kit reagents are handled.
11. Avoid contact between the buffered Peroxide Solution and easily oxidized materials; extreme temperature may initiate spontaneous combustion.

Observe the guidelines for performing quality control in medical laboratories by assaying controls and/or pooled sera. During handling of all kit reagents, controls and serum samples observe the existing legal regulations.

## CONTENTS OF THE KIT

Package size: 12 x 8 determinations

| | |
|---|---|
| Qty.1 | Divisible **microplate** consisting of 12 modules of 8 wells each, coated with highly purified antigens<br>RNP-70 (row A), RNP/Sm (row B),<br>Sm (row C), SS-A (row D),<br>SS-B (row E), Scl-70 (row F),<br>Centromer B (row G), Jo-1 (row H).<br>Ready to use. |
| 2 vials, 2.5 ml each | Anti-ANA **controls** in a serum/buffer matrix (PBS, $NaN_3$ <0.1% (w/w)).<br>Negative Control (NC), Calibrator control (CC).<br>Ready to use. |
| 1 vial, 20 ml | **Sample buffer** (Tris, $NaN_3$ <0,1% (w/w)), yellow,<br>concentrate (5x). |
| 1 vial, 15 ml | **Enzyme conjugate** solution<br>(PBS, Proclin 300 <0.5% (v/v)), (light red) containing polyclonal anti-human IgG; labelled with horseradish peroxidase.<br>Ready to use. |
| 1 vial, 15 ml | **TMB substrate solution**.<br>Ready to use. |
| 1 vial, 15 ml | **Stop solution** (contains acid).<br>Ready to use. |
| 1 vial, 20 ml | **Wash solution** (PBS, $NaN_3$ <0.1% (w/w)),<br>concentrate (50x). |

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## STORAGE AND STABILITY

1. Store the kit at 2-8 °C.
2. Keep microplate wells sealed in a dry bag with desiccants.
3. The reagents are stable until expiration of the kit.
4. Do not expose test reagents to heat, sun or strong light during storage and usage.
5. Diluted sample buffer and wash buffer are stable for at least 30 days when stored at 2-8 °C.

## MATERIALS REQUIRED

### Equipment

- Microplate reader capable of endpoint measurements at 450 nm
- Multi-Channel Dispenser or repeatable pipet for 100 µl
- Vortex mixer
- Pipets for 10 µl, 100 µl and 1000 µl
- Laboratory timing device
- Data reduction software

### Preparation of reagents

- Distilled or deionized water
- Graduated cylinder for 100 and 1000 ml
- Plastic container for storage of the wash solution

## SPECIMEN COLLECTION, STORAGE AND HANDLING

1. Collect whole blood specimens using acceptable medical techniques to avoid hemolysis.
2. Allow blood to clot and separate the serum by centrifugation.
3. Test serum should be clear and non-hemolysed. Contamination by hemolysis or lipemia is best avoided, but does not interfere with this assay.
4. Specimens may be refrigerated at 2-8 °C for up to five days or stored at -20 °C up to six months.
5. Avoid repetitive freezing and thawing of serum samples. This may result in variable loss of autoantibody activity.
6. Testing of heat-inactivated sera is not recommended.

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## PROCEDURAL NOTES

1. Do not use kit components beyond their expiration dates.
2. Do not interchange kit components from different lots.
3. All materials must be at room temperature (20-28 °C).
4. Have all reagents and samples ready before start of the assay. Once started, the test must be performed without interruption to get the most reliable and consistent results.
5. Perform the assay steps only in the order indicated.
6. Always use fresh sample dilutions.
7. Pipette all reagents and samples into the bottom of the wells.
8. To avoid carryover contamination, change the tip between samples and different kit controls.
9. It is important to wash microwells thoroughly and remove the last droplets of wash buffer to achieve best results.
10. All incubation steps must be accurately timed.
11. Control sera or pools should routinely be assayed as unknowns to check performance of the reagents and the assay.
12. Do not re-use microplate wells.

For all controls, the respective concentrations are provided on the labels of each vial. Using these concentrations a calibration curve may be calculated to read off the patient results semi-quantitatively.

## PREPARATION OF REAGENTS

### Preparation of sample buffer

Dilute the contents of each vial of the sample buffer concentrate (5x) with distilled or deionised water to a final volume of 100 ml prior to use.
Store refrigerated: stable at 2-8 °C for at least 30 days after preparation or until the expiration date printed on the label.

### Preparation of wash solution

Dilute the contents of each vial of the buffered wash solution concentrate (50x) with distilled or deionised water to a final volume of 1000 ml prior to use.
Store refrigerated: stable at 2-8 °C for at least 30 days after preparation or until the expiration date printed on the label.

### Sample preparation

Dilute all patient samples **1:100** with sample buffer before assay.
Therefore combine 10 µl of sample with 990 µl of sample buffer in a polystyrene tube. Mix well.

Controls are ready to use and need not be diluted.

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## TEST PROCEDURE

1. Prepare a sufficient number of microplate modules to accommodate controls and prediluted patient samples.
2. Pipette **100 µl** of calibrators, controls and prediluted patient samples in duplicate into the wells.

| | | 1 | 2 | 3 | 4 | 5 | 6 | |
|---|---|---|---|---|---|---|---|---|
| RNP70 | **A** | CC | NC | P1 | P2 | P.. | | CC Calibrator control<br>NC: negative control<br>P1, P2 patient samples |
| RNP/Sm | **B** | CC | NC | P1 | P2 | P.. | | |
| Sm | **C** | CC | NC | P1 | P2 | P.. | | |
| SS-A | **D** | CC | NC | P1 | P2 | P.. | | |
| SS-B | **E** | CC | NC | P1 | P2 | P.. | | |
| Scl-70 | **F** | CC | NC | P1 | P2 | P.. | | |
| Centromer B | **G** | CC | NC | P1 | P2 | P.. | | |
| Jo-1 | **H** | CC | NC | P1 | P2 | P.. | | |

3. Incubate for 30 minutes at room temperature (20-28 °C).
4. Discard the contents of the microwells and wash 3 times with **300 µl** of wash solution.
5. Dispense **100 µl** of enzyme conjugate into each well.
6. Incubate for 15 minutes at room temperature.
7. Discard the contents of the microwells and wash 3 times with **300 µl** of wash solution.
8. Dispense **100 µl** of TMB substrate solution into each well.
9. Incubate for 15 minutes at room temperature.
10. Add **100 µl** of stop solution to each well of the modules and incubate for 5 minutes at room temperature.
11. Read the optical density at 450 nm and calculate the results. Bi-chromatic measurement with a reference at 600-690 nm is recommended.

**The developed colour is stable for at least 30 minutes. Read optical densities during this time.**

## INTERPRETATION OF RESULTS

### *Quality Control*

This test is only valid if the optical density at 450 nm for Negative Control (NC) and Calibrator Control (CC) complies with the respective range indicated on the Quality Control Certificate enclosed to each test kit!
If any of these criteria is not fulfilled, the results are invalid and the test should be repeated.

### *Calculation of results*

Evaluation of the ANA Combi test is carried out by comparison of the optical densities of the patient sample with the optical density of the Calibrator Control.

For each antigen the OD-Cut-off value should be calculated according to the following equation:

$$OD_{Cut\text{-}Off} = OD_{CalibratorControl} \times Spezific\ Factor$$

For each patient sample 8 Index-values should be calculated according to the following equation:

$$Index\text{-}Value = OD_{Sample} / OD_{Cut\text{-}Off}$$

**Calculation Example**

The chart below shows typical results for ANA Combi. These data are intended for illustration only and should not be used to calculate results from another run.

| Sample No | Anti- | Row | OD Calibrator | Factor | OD $_{Cut\text{-}Off}$ | OD $_{Sample}$ | Result |
|---|---|---|---|---|---|---|---|
| 1 | RNP-70 | A | 0.48 | 1.10 | 0.53 | 0.083 | Negative |
| 1 | RNP/Sm | B | 0-50 | 1.00 | 0.50 | 0.420 | Negative |
| 1 | Sm | C | 0.40 | 1.25 | 0.50 | 0.138 | Negative |
| 1 | SS-A | D | 0.42 | 0.85 | 0.36 | 0.133 | Negative |
| 1 | SS-B | E | 0.53 | 0.75 | 0.39 | 0.505 | Positive |
| 1 | Scl-70 | F | 0.57 | 1.00 | 0.57 | 0.820 | Positive |
| 1 | Centromer B | G | 0.49 | 0.80 | 0.39 | 0.212 | Negative |
| 1 | Jo-1 | H | 0.50 | 0.80 | 0.40 | 0.189 | Negative |

***Qualitative Interpretation***

A sample is positive for one ANA parameter if OD-Sample is > OD Cut-off:

| | ANA Combi (OD-Sample) |
|---|---|
| Negative: | < OD Cut-off |
| Positive: | ≥ OD Cut-off |

For detailed quantitative analyses we recommend to use the individual ANA-ELISA test systems.

***Semi-quantitative interpretation***

A sample is positive for one ANA parameter if its Index-value is ≥ 1.2:

| | ANA Combi (Index-value) |
|---|---|
| Negative: < | 1.0 |
| Borderline: | 1.0 - 1.2 |
| Positive: | ≥ 1.2 |

***Expected Values***

The approximate incidence of positive ANA is 5% in the general normal population, 40% in normal old age and 25% in healthy relatives of SLE patients.

ANA positivity has been reported in:

| | |
|---|---|
| SLE (systemic Lupus erythemotosus) | >95% |
| SS (Sjogren´s sysndrome) | 50-65% |
| PSS (progressive systemic sklerosis) | 40-60% |
| RA (rheumatoid arthritis) | 12-24% |
| juvenile RA (juvenile rheumatoid arthritis) | 20% |

## PERFORMANCE CHARACTERISTICS

***Specificity***

The microplate is coated with the antigens RNP-70, RNP/Sm, Sm, SS-A, SS-B, Scl-70, Centromere B and Jo-1, respectively. All antigen preparations, native and recombinant, are highly purified by affinity chromatography. The ANA Combi test is specific only for autoantibodies directed to these antigens. No cross reactivities have been observed.

***Calibration***

The assay system is calibrated against the internationally recognised reference sera from CDC, Atlanta USA, since no other international standards are available.

## LIMITATIONS OF PROCEDURE

1. The ANA Combi ELISA is a diagnostic aid and by itself is not diagnostic. A definite clinical diagnosis should not be based on the results of a single test, but should be made by the physician after all clinical and laboratory findings have been evaluated.
2. Positive ANA may be found in apparently healthy people.
3. SLE patients undergoing steroid therapy may have negative test results.
4. Commonly prescribed drugs may induce ANA.

## INTERFERING SUBSTANCES

No interference has been observed with haemolytic (up to 1000 mg/dL), lipemic (up to 3 g/dL triglycerides) or bilirubin (up to 40 mg/dL) containing sera.
Nor have any interfering effects been observed with the use of anticoagulants.
However for practical reasons it is recommended that grossly hemolysed or lipemic samples should be avoided.

**REFERENCES / Literature**

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