Anti-ds-DNA-M EIA

For the quantitative and qualitative determination of IgM antibodies against double stranded DNA (dsDNA) in human serum

For Research Use Only. Not For Use In Diagnostic Procedures.

Catalog Number: 35-DSMHU-E01
Size: 96 wells
Version: 002: 2007-08-28 – ALPCO 08/21/2012
1. Intended Use

*Anti-ds-DNA-M EIA* is a solid phase enzyme immunoassay with human recombinant double-stranded DNA (dsDNA). This kit is for the quantitative and qualitative detection of antibodies against dsDNA in human serum. Anti-dsDNA antibodies mainly recognize the phosphate units of DNA, thus these autoantibodies also bind single-stranded DNA (ssDNA). To ensure correct quantitation of anti-dsDNA antibodies, the antigen used has to be free of contamination with ssDNA. The assay is a tool in the study of systemic lupus erythematosus (SLE).

2. Application and Principle of the Assay

Antibodies binding to DNA belong to the group of anti-nuclear antibodies (ANA) that have been observed in several autoimmune diseases. Antibodies reacting with native double-stranded (ds) DNA are regarded as being specific for systemic lupus erythematosus (SLE) and have been observed in approximately 50-80% of SLE cases.

Antibodies against dsDNA are found during active phases of SLE. The amount of the serum concentration is positively correlated with the severity of the disease. Thus, detection of these autoantibodies is important in the study of SLE.

Most individuals with SLE display IgG class antibodies against dsDNA. These autoantibodies are associated with lupus nephritis. Approximately 30% of individuals with SLE additionally develop IgA class anti-dsDNA antibodies. There have been suggestions that the presence of these IgA class anti-dsDNA antibodies may define a certain subset of individuals with SLE. Indeed, studies demonstrated the association of this subclass with certain parameters of the disease activity, such as elevated erythrocyte sedimentation rate, or the consumption of complement component C3, as well as the parameters of cutaneous vasculitis, acral necrosis, and erythema. No such association was found with nephritis and arthritis.

IgM class anti-dsDNA antibodies were found in 52% of the sera from individuals with SLE. In contrast to IgG and IgA class autoantibodies, the subclass IgM antibodies do not correlate with disease activity. However, a highly significant negative correlation between IgM anti-dsDNA antibodies and lupus nephritis, including its laboratory parameters, was demonstrated. Therefore, IgM class anti-dsDNA antibodies may indicate a subset of individuals with lupus being protected against the risk of developing nephritis.

*Principle of the test*

Serum samples diluted 1:101 are incubated in the microplates coated with the specific antigen. Sample antibodies, if present in the specimen, bind to the antigen. The unbound fraction is washed off in the following step. Afterwards, anti-human immunoglobulins conjugated to horseradish peroxidase (conjugate) are incubated and react with the antigen-antibody complex of the samples in the microplates. Unbound conjugate is washed off in the following step. Addition of TMB-substrate generates an enzymatic colorimetric (blue) reaction, which is stopped by diluted acid (color changes to yellow). The rate of color formation from the chromogen is a function of the amount of conjugate bound to the antigen-antibody complex and this is proportional to the initial concentration of the respective antibodies in the sample.
3. Kit Contents

To be reconstituted:

5x Sample Buffer  1 vial, 20 ml - 5x concentrated (capped white: yellow solution)
   Containing: Tris, NaCl, BSA, sodium azide < 0.1% (preservative)

50x Wash Buffer   1 vial, 20 ml - 50x concentrated (capped white: green solution)
   Containing: Tris, NaCl, Tween, sodium azide < 0.1% (preservative)

Ready to use:

Negative Control  1 vial, 1.5 ml (capped green: yellow solution)
   Containing: Human serum (diluted), sodium azide < 0.1% (preservative)

Positive Control  1 vial, 1.5 ml (capped red: yellow solution)
   Containing: Human serum (diluted), sodium azide < 0.1% (preservative)

Cut-off Calibrator 1 vial, 1.5 ml (capped blue: yellow solution)
   Containing: Human serum (diluted), sodium azide < 0.1% (preservative)

Calibrators       6 vials, 1.5 ml each 0, 3, 10, 30, 100, 300 U/ml
   (color increasing with concentration : yellow solutions)
   Containing: Human serum (diluted), sodium azide < 0.1% (preservative)

Conjugate         1 vial, 15 ml IgM (capped green: green solution)
   Containing: Anti-human immunoglobulins conjugated to horseradish peroxidase

TMB Substrate     1 vial, 15 ml (capped black)
   Containing: Stabilized TMB/H₂O₂

Stop Solution     1 vial, 15 ml (capped white: colorless solution)
   Containing: 1M Hydrochloric Acid

Microtiterplate   12x8 well strips with breakaway microwells
   Coating see paragraph 1

Materials required but not provided:

Microtiter plate reader 450 nm reading filter and optional 620 nm reference filter (600-690 nm).
Glassware (cylinder 100-1000 ml), test tubes for dilutions. Vortex mixer, precision pipettes (10, 100,
200, 500, 1000 µl) or adjustable multipipette (100-1000 ml). Microplate washing device (300 µl
repeating or multichannel pipette or automated system), absorbent paper.
Our tests are designed to be used with purified water according to the definition of the United
States Pharmacopeia (USP 26 - NF 21) and the European Pharmacopeia (Eur.Ph. 4th ed.).

4. Storage and Shelf Life

Store all reagents and the microplate at 2-8°C/35-46°F, in their original containers. Once
prepared, reconstituted solutions are stable for 1 month at 4°C, at least. Reagents and the
microplate shall be used within the expiry date indicated on each component, only. Avoid intense
exposure of TMB solution to light. Store microplates in designated foil, including the desiccant,
and seal tightly.
5. Precautions of Use

5.1 Health hazard data

**THIS PRODUCT IS FOR RESEARCH USE ONLY. Not for use in diagnostic procedures.**

Although this product is not considered particularly toxic or dangerous in conditions of normal use, refer to the following for maximum safety:

**Recommendations and precautions**

This kit contains potentially hazardous components. Though kit reagents are not classified as being irritants for eyes and skin we recommend avoiding contact with eyes and skin and wearing disposable gloves.

**WARNING!** Calibrators, Controls, and Buffers contain sodium azide (NaN₃) as a preservative. NaN₃ may be toxic if ingested or absorbed by skin or eyes. NaN₃ may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide build-up. Please refer to decontamination procedures as outlined by CDC or other local/national guidelines.

Do not smoke, eat, or drink when manipulating the kit.

Do not pipette by mouth.

All human source material used in kit reagents (e.g., controls, standards) has been tested by approved methods and found negative for HBsAg, Hepatitis C, and HIV 1. However, no test can guarantee the absence of viral agents in such material completely. Thus, handle kit controls, standards, and samples as if capable of transmitting infectious diseases and according to national requirements.

5.2 General Directions for Use

Do not mix or substitute reagents or microplates from different lot numbers. This may lead to variation in the results.

Allow all components to reach room temperature (20-32°C/68-89.6°F) before use, mix well, and follow the recommended incubation scheme for an optimum performance of the test.

**Incubation: We recommend test performance at 30°C/86°F for automated systems.**

Never expose components to higher temperature than 37°C/98.6°F.

Always pipet substrate solution with only brand new tips. Protect this reagent from light. Never pipette conjugate with tips used previously with other reagents.

6. Sample Collection, Handling, and Storage

It is preferable to use newly collected serum samples. Do not use icteric, lipemic, hemolysed, or bacterially contaminated samples. Sera with particles should be cleared by low speed centrifugation (< 1000 x g). Blood samples should be collected in clean, dry, and empty tubes. After separation, the serum samples can be used immediately, stored at 2-8°C/35-46°F for up to three days, or frozen at -20°C/64°F for longer periods.

7. Assay Procedure

7.1 Preparations prior to pipetting

Dilute concentrated reagents:

Dilute the concentrated sample buffer 1:5 with deionized water (e.g., 20 ml plus 80 ml).

Dilute the concentrated wash buffer 1:50 with deionized water (e.g., 20 ml plus 980 ml).
Samples
Dilute serum samples 1:101 with sample buffer (1x),
e.g., 1000 µl sample buffer (1x) + 10 µl serum. Mix well!

Washing
Prepare 20 ml of diluted wash buffer (1x) per 8 wells or 200 ml for 96 wells,
e.g., 4 ml concentrate plus 196 ml deionized water.

Automated washing:
Consider excess volumes required for setting up the instrument and dead volume of robot pipette.

Manual washing:
Discard liquid from wells by inverting the plate. Knock the microwell frame vigorously with wells facing down on clean absorbent paper. Pipette 300 µl of diluted wash buffer into each well, wait for 20 seconds. Repeat the whole procedure twice again.

Microplates
Calculate the number of wells required for the test. Remove unused wells from the frame, place and store in the provided plastic bag, together with desiccant, and seal tightly (2-8°C/35-46°F).

7.2 Work flow
See Annex A for pipetting scheme. We recommend pipetting samples and calibrators in duplicate. Cut-off calibrator should be used for qualitative testing only.

- Pipette 100 µl of each diluted serum sample into the designated microwells.
- Pipette 100 µl of the calibrators, OR cut-off calibrator, and negative and positive controls into the designated wells.
- Incubate for 30 minutes at 20-32°C/68-89.6°F.
- Wash 3x with 300 µl washing buffer (diluted 1:50).
- Pipette 100 µl of conjugate into each well.
- Incubate for 30 minutes at 20-32°C/68-89.6°F.
- Wash 3x with 300 µl washing buffer (diluted 1:50).
- Pipette 100 µl of TMB substrate into each well.
- Incubate for 30 minutes at 20-32°C/68-89.6°F, protected from intense light.
- Pipette 100 µl of Stop solution into each well, using the same order as pipetting the substrate.
- Incubate 5 minutes minimum.
- Agitate plate carefully for 5 sec.
- Read absorbance at 450 nm (optionally 450/620 nm) within 30 minutes.

8. Quantitative and Qualitative Interpretation

For quantitative interpretation establish the standard curve by plotting the optical density (OD) of each calibrator (y-axis) with respect to the corresponding concentration values in U/ml (x-axis). For best results we recommend log/lin coordinates and a 4-Parameter Fit. From the OD of each sample, read the corresponding antibody concentrations expressed in U/ml.

<table>
<thead>
<tr>
<th>Normal Range</th>
<th>Equivocal Range</th>
<th>Positive Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 12 U/ml</td>
<td>12-18 U/ml</td>
<td>&gt; 18 U/ml</td>
</tr>
</tbody>
</table>
**Example of a standard curve**

We recommend pipetting calibrators in parallel for each run.

<table>
<thead>
<tr>
<th>Calibrators IgM</th>
<th>OD 450/620 nm</th>
<th>CV % (Variation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 U/ml</td>
<td>0.036</td>
<td>2.9</td>
</tr>
<tr>
<td>3 U/ml</td>
<td>0.176</td>
<td>2.3</td>
</tr>
<tr>
<td>10 U/ml</td>
<td>0.314</td>
<td>2.9</td>
</tr>
<tr>
<td>30 U/ml</td>
<td>0.618</td>
<td>2.9</td>
</tr>
<tr>
<td>100 U/ml</td>
<td>1.312</td>
<td>0.1</td>
</tr>
<tr>
<td>300 U/ml</td>
<td>2.076</td>
<td>0.7</td>
</tr>
</tbody>
</table>

**Example of calculation**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Replicate (OD)</th>
<th>Mean (OD)</th>
<th>Result (U/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S 01</td>
<td>0.799/0.744</td>
<td>0.772</td>
<td>40.3</td>
</tr>
<tr>
<td>S 02</td>
<td>1.404/1.393</td>
<td>1.399</td>
<td>119.5</td>
</tr>
</tbody>
</table>

For lot specific data, see enclosed quality control leaflet. Medical laboratories might perform an in-house Quality Control by using own controls and/or internal pooled sera, as foreseen by EU regulations.

*Do not use this example for interpreting your results!*

Each laboratory should establish its own normal range based upon its own techniques, controls, equipment, and sample population according to its established procedures.

For **qualitative interpretation** read the optical density of the cut-off calibrator and the samples. Compare the sample’s OD with the OD of the cut-off calibrator. For qualitative interpretation, we recommend considering sera within a range of 20% around the cut-off value as equivocal. All samples with higher ODs are considered positive, samples with lower ODs are considered negative.

- **Negative:** $\text{OD}_{\text{sample}} < 0.8 \times \text{OD}_{\text{cut-off}}$
- **Equivocal:** $0.8 \times \text{OD}_{\text{cut-off}} \leq \text{OD}_{\text{sample}} \leq 1.2 \times \text{OD}_{\text{cut-off}}$
- **Positive:** $\text{OD}_{\text{sample}} > 1.2 \times \text{OD}_{\text{cut-off}}$

**9. Technical Data**

- **Sample Material:** Serum
- **Sample Volume:** 10 μl of sample diluted 1:101 with 1x sample buffer
- **Total Incubation Time:** 90 minutes at 20-32°C/68-89.6°F
- **Calibration Range:** 0-300 U/ml
- **Analytical sensitivity:** 1.0 U/ml
- **Storage:** At 2-8°C/35-46°F use original vials only
- **Number of determinations:** 96 tests
10. Performance Data

10.1 Analytical sensitivity
Testing sample buffer 30 times on this kit gave an analytical sensitivity of 1.0 U/ml.

10.2 Specificity and Sensitivity
The microplates are coated with recombinant human dsDNA. No crossreactivities to other autoantigens have been found. Antibodies targeting dsDNA show sensitivity of 85% for SLE thus allowing the differentiation from other inflammatory rheumatic diseases. Combining all three immunoglobulin subclasses results in sensitivity of 90% for the dsDNA test. This data was acquired with the former version of this kit that involved a 30-15-15 incubation scheme.

Correlation:
The comparability of the performance data was assessed with at least 30 sera tested on both the current version of this kit with the 30-30-30 incubation scheme, and the former version with the 30-15-15 incubation scheme. A linear regression analysis of the two products showed the two products are equivalent. Data can be provided upon request.

10.3 Linearity
Chosen sera have been tested with this kit and found to dilute linearly. However, due to the heterogeneous nature of human autoantibodies there might be samples that do not follow this rule.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Dilution Factor</th>
<th>measured concentration (U/ml)</th>
<th>expected concentration (U/ml)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1/100</td>
<td>42.9</td>
<td>43.2</td>
<td>99.3</td>
</tr>
<tr>
<td></td>
<td>1/200</td>
<td>20.4</td>
<td>21.6</td>
<td>99.4</td>
</tr>
<tr>
<td></td>
<td>1/400</td>
<td>9.3</td>
<td>10.8</td>
<td>86.1</td>
</tr>
<tr>
<td></td>
<td>1/800</td>
<td>4.9</td>
<td>5.4</td>
<td>90.7</td>
</tr>
<tr>
<td>2</td>
<td>1/100</td>
<td>179.4</td>
<td>176.0</td>
<td>101.9</td>
</tr>
<tr>
<td></td>
<td>1/200</td>
<td>86.4</td>
<td>88.0</td>
<td>98.2</td>
</tr>
<tr>
<td></td>
<td>1/400</td>
<td>41.8</td>
<td>44.0</td>
<td>95.0</td>
</tr>
<tr>
<td></td>
<td>1/800</td>
<td>19.8</td>
<td>22.0</td>
<td>90.0</td>
</tr>
</tbody>
</table>

10.4 Precision
To determine the precision of the assay, the variability (intra and inter-assay) was assessed by examining its reproducibility on three serum samples selected to represent a range over the standard curve.

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Mean (U/ml)</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>&gt; 300.0</td>
<td>2.1</td>
</tr>
<tr>
<td>2</td>
<td>138.0</td>
<td>2.4</td>
</tr>
<tr>
<td>3</td>
<td>26.4</td>
<td>4.7</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Mean (U/ml)</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>463.3</td>
<td>2.6</td>
</tr>
<tr>
<td>2</td>
<td>171.6</td>
<td>2.3</td>
</tr>
<tr>
<td>3</td>
<td>58.2</td>
<td>4.6</td>
</tr>
</tbody>
</table>
10.5 Calibration
Due to the lack of international reference calibration this assay is calibrated in arbitrary units (U/ml).

11. Literature


ANNEX A: Pipetting Scheme

We suggest pipetting calibrators, controls, and samples as follows:
For **quantitative interpretation**, use calibrators to establish a standard curve.
For **qualitative interpretation**, use cut-off calibrator.

<table>
<thead>
<tr>
<th></th>
<th>For quantitative interpretation, use calibrators to establish a standard curve</th>
<th>For qualitative interpretation, use cut-off calibrator</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A: CalA, CalE</td>
<td>7: NC, S2</td>
</tr>
<tr>
<td></td>
<td>B: CalA, CalE</td>
<td>8: NC, S2</td>
</tr>
<tr>
<td></td>
<td>C: CalB, CalF</td>
<td>9: CC, S3</td>
</tr>
<tr>
<td></td>
<td>D: CalB, CalF</td>
<td>10: CC, S3</td>
</tr>
<tr>
<td></td>
<td>E: CalC, PC</td>
<td>11: PC</td>
</tr>
<tr>
<td></td>
<td>F: CalC, PC</td>
<td>12: PC</td>
</tr>
<tr>
<td></td>
<td>G: CalD, NC</td>
<td>1: S1</td>
</tr>
<tr>
<td></td>
<td>H: CalD, NC</td>
<td>2: S1</td>
</tr>
</tbody>
</table>

CalA-CalF: Calibrators A-F
PC: Positive control
NC: Negative control
CC: Cut-off calibrator
S1: sample 1, S2: sample 2, S3: sample 3