INTENDED USE
EliA CTD Screen is intended for the in vitro qualitative measurement of antinuclear IgG antibodies in human serum and plasma as an aid in the clinical diagnosis of systemic lupus erythematosus (SLE), mixed connective tissue disease (MCTD), Sjögren's syndrome, scleroderma and polymyositis/dermatomyositis. EliA CTD Screen uses the EliA IgG method on the instrument Phadia 250.

SUMMARY AND EXPLANATION OF THE TEST
The determination of antinuclear antibodies (ANA) is of central importance for the clinical diagnosis of connective tissue diseases, which are systemic inflammatory diseases with a chronic course of disease. Connective tissue diseases exhibit overlapping symptomatic features that render an accurate diagnosis difficult.

For the diagnosis of systemic lupus erythematosus (SLE), dsDNA antibodies are considered to be a highly specific marker representing one of the diagnostic criteria for SLE (ACR criteria). More than 90% of sera from patients with active SLE contain dsDNA antibodies. Additionally, the determination of dsDNA antibodies is a tool to monitor the clinical course of a defined SLE patient, because a clear-cut relationship exists between anti-dsDNA titer and disease activity, in particular renal involvement.

Sm antibodies offer a highly specific, but comparatively insensitive, clinical marker for SLE. Indeed, their presence constitutes one of the revised ACR criteria for diagnosis, even though their overall prevalence ranges from 20% to 30% in SLE.

U1-snRNP antibodies typically appear in both SLE and mixed connective tissue disease (MCTD, Sharp Syndrome). In MCTD, the presence of U1-snRNP antibodies is required for diagnosis, whereas they occur in only 30 to 40% of SLE patients.

Detection of SS-A/Ro antibodies is of interest and significance for the clinical diagnosis of SLE (prevalence 40-50%) and Sjögren's syndrome (prevalence 60-75% for primary Sjögren's syndrome). They have been reported to occur in tight association with certain disease subsets, such as subacute cutaneous LE, neonatal lupus erythematosus or vasculitis in Sjögren's syndrome. As anti-SS-A/Ro may be the only antibody present in many patients with SLE or Sjögren's syndrome, failure to measure anti-SS-A/Ro leaves a diagnostic void which cannot be filled by other tests.

SS-B/La antibodies are the serological hallmark of Sjögren's syndrome but a small proportion of patients remains anti-SS-B/La negative. La antibodies are found in 6-15% of sera from SLE patients. Here, they are associated with a lower prevalence of dsDNA antibodies and renal disease. Although a strong association of neonatal lupus erythematosus (NLE) with Ro antibodies was recognized first, the majority of mothers with babies with NLE are now known to have La antibodies as well.
REAGENTS / MATERIAL

The EliA reagents are available as modular packages, each purchased separately. All packages except for the EliA ANA Positive Control 250 and the EliA IgG/IgM/IgA Negative Control 250 are required to carry out an EliA CTD Screen Test. The EliA CTD Screen Wells are packed in carriers which are stored in sealed aluminium foil bags containing a desiccant.

**EliA CTD Screen Test-Specific Reagents**

**EliA CTD Screen Wells (Art. No. 14-5596-01)**

| CTD Screen Well; short name: ctd | coated with human recombinant U1RNP (RNP 70, A, C), SS-A/Ro (60 kDa, 52 kDa), SS-B/La, Cen tromere B, ScI-70, Jo-1, Fibrillarin, RNA Pol III, Rib-P, PM-Sci, PCNA, Mi-2 proteins, Sm proteins and native purified DNA | 4 carriers (12 wells each); sufficient for 48 determinations | ready for use; store dry at 2-8 °C until expiration date |

**EliA ANA Positive Control 250 (Art. No 83-1033-01)**

| Human serum in PBS containing BSA, detergent and sodium azide (0.095 %); symbol: pos | Multiparameter control containing IgG antibodies to dsDNA, RNP, Sm, Ro, La, ScI-70, CENP and Jo-1 | 6 single-use vials (0.3 ml each); sufficient for 2 determinations per vial | Ready for use; store at 2-8 °C until expiration date |

**EliA IgG/IgM/IgA Negative Control 250 (Art. No 83-1037-01)**

| Human serum in PBS containing BSA, detergent and sodium azide (0.095 %); symbol: neg | Multiparameter control containing normal sera from healthy donors | 6 single-use vials (0.3 ml each); sufficient for 2 determinations per vial | ready for use; store at 2-8 °C until expiration date |

**EliA Method-Specific Reagents (Phadia 250)**

**EliA Sample Diluent (Art. No 83-1023-01)**

| Sample Diluent (yellow colored); PBS containing BSA, detergent and sodium azide (0.095 %) | 6 bottles (48 ml each); sufficient for ≥6 x 180 dilutions | ready for use; store at 2-8 °C until expiration date |

**EliA IgG Conjugate 50 (Art. No 83-1017-01)**

| IgG Conjugate (blue colored); β-Galactosidase anti-IgG (mouse monoclonal antibodies) in PBS containing BSA and sodium azide (0.06 %); symbol: El-G | 6 wedge shaped bottles (5 ml each); sufficient for 6 x 50 determinations | ready for use; store at 2-8 °C until expiration date |

**EliA IgG Conjugate 200 (Art. No 83-1018-01)**

| IgG Conjugate (blue colored); β-Galactosidase anti-IgG (mouse monoclonal antibodies) in PBS containing BSA and sodium azide (0.06 %); symbol: El-G | 6 wedge shaped bottles (19 ml each); sufficient for 6 x 200 determinations | ready for use; store at 2-8 °C until expiration date DO NOT FREEZE DO NOT REUSE |

**EliA IgG Calibrator Strips (Art. No 83-1015-01)**

| human IgG (0, 4, 10, 20, 100, 600 μg/l); in PBS containing BSA, detergent and sodium azide (0.095 %) | 5 strips | ready for use; store at 2-8 °C until expiration date |

**EliA IgG Calibrator Well (Art. No 14-5509-01)**

| IgG Calibrator Well coated with mouse monoclonal antibodies; short name: Gcal | 4 carriers (12 wells each); sufficient for 48 determinations | ready for use; store at 2-8 °C until expiration date |

**Phadia 250 General Reagents**

**Development Solution (Art. No. 10-9440-01)**

| Development Solution 0.01 % 4-Methylumbelliferyl-β-D-galactoside, <0.0010% preservative* | 6 bottles (17 ml each); sufficient for 6 x >170 determinations | ready for use; store at 2-8 °C until expiration date DO NOT FREEZE |

**Stop Solution (Art. No. 10-9442-01)**

| Stop Solution 4 % Sodium Carbonate | 6 bottles (119 ml each); sufficient for 6 x >560 determinations | ready for use; store at 2-8 °C until expiration date |

**Dilution Plates (Art. No. 12-3907-08)**

| MicroWell™ plates with 96 wells, 0.5 ml each; Polypolypropylene | 60 plates per package; sufficient for 60 x 96 samples | ready for use DO NOT REUSE |


**Washing Solution (Art. No. 10-9422-01/10-9202-01)**

For information see separate Washing Solution package insert.
WARNINGS AND PRECAUTIONS

- For in vitro diagnostic use.
- Do not use reagents beyond their expiration dates.
- We do not recommend to pool reagents.
- Wear gloves while handling samples and reagents provided.
- Some of the reagents are manufactured from human blood components. The source materials have been tested by immunoassay for hepatitis B surface antigen, for antibodies to HIV1, HIV2 and hepatitis C virus and found negative. Nevertheless, all recommended precautions for the handling of blood derivatives should be observed. Please refer to Human Health Service (HHS) Publication No. (CDC) 93-8395 or local and national guidelines on laboratory safety procedures.

WARNING! Reagents 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 and national guidelines. Waste Bottle and ImmunoCAP/EliA Well Waste Container may be contaminated by potentially infectious material. Use appropriate safety measures and wear gloves.

Indication of Instability
Phadia 250 Instrument Software has built-in acceptance limits for the calibration curve and the curve control. EliA Wells are moisture sensitive. An activity loss that might occur due to inappropriate handling can be detected using the appropriate EliA Control. For more information see Phadia 250 User’s Guide/Reference Manual.

INSTRUMENT
EliA reagents are to be used with the Phadia 250 Instrument Software version 1.05 or higher. The Phadia 250 Instrument processes all steps of the test. For further information regarding test set-up, instrumentation and software etc. see Phadia 250 User’s Guide/Reference Manual.

SPECIMEN COLLECTION, HANDLING AND PREPARATION
The procedure can be performed with serum or plasma specimens. Lipemic, hemolyzed or microbially contaminated samples may give poor results and should not be used. Avoid repeated freezing and thawing. Samples should be stored in aliquots at -20 °C (-4 °F) or below for repeated measurements.

Sample Dilution
Samples must be diluted with EliA Sample Diluent. A 1:10 dilution of the samples is required for the EliA CTD Screen Test. Samples can be diluted manually, but instrument dilution is recommended.

PROCEDURE
Handling of EliA CTD Screen Well
In the Phadia 250 storage chamber, carriers are stable for up to 14 days. If you are not expecting to use them up within this time, the carriers should be loaded via the Phadia 250 Loading Tray and, for stability reasons, must be put back into the desiccant-containing foil bag directly after the run. Because it is important to store the wells in dry conditions at 2-8°C, the bag must be properly resealed. If stored under these conditions, the shelf-life from the date of first opening is 6 months, if not limited by the expiry date stated on the carrier and foil bag.

Lot specific barcode
Use the built-in barcode reader to enter the lot specific information of EliA CTD Screen Well, EliA IgG Calibrator Well and EliA IgG Conjugate. In case of manual handling make sure to enter the characters below the barcode.

On-board stability of reagents
- **EliA Wells**
  - EliA Well carriers can be stored on-board for 14 days at 2-8°C or 24 hours at room temperature.
- **EliA Calibrator Strips, EliA Curve Control Strips**
  - Can be stored on-board for 28 days. During longer interval between usage (>3 days) it is recommended to unload the strips from the instrument and store them at 2-8°C.
- **EliA Sample Diluent**
  - Can be stored on-board for 7 days at room temperature. Re-cap bottles every night.
- **EliA Conjugate**
  - Can be stored on-board at 2-8°C until expiry date. Single use reagent, open vials must not be stored.
- **Development Solution, Stop Solution**
  - Can be stored on-board for a total of 40h at room temperature. Can be used 5 times during shelf life and be stored at room temperature for 8 hours on each occasion. Re-cap bottles every night. During weekends or longer interval between instrument usage it is recommended to store bottles at 2-8°C.
- **Wash Solution**
  - Prepared solution can be stored on-board for 7 days at room temperature. During 1 week the same bottle can be refilled. Discard what’s left after last run on Friday and start with freshly prepared Wash Solution in cleaned bottle on Monday.

Volumes per determination

<table>
<thead>
<tr>
<th>Reagent volumes per determination</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Calibrator</td>
<td>90 µl</td>
</tr>
<tr>
<td>EliA IgG Conjugate</td>
<td>90 µl</td>
</tr>
<tr>
<td>Development Solution</td>
<td>90 µl</td>
</tr>
<tr>
<td>Stop Solution</td>
<td>200 µl</td>
</tr>
</tbody>
</table>

Sample volumes per determination

| Manual dilution: | 90 µl of diluted sample |
| Instrument dilution (1:10): | 9 µl of non diluted sample |


Reagent volumes per 200 determinations

<table>
<thead>
<tr>
<th>Reagent volumes per 200 determinations</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Washing Solution</td>
<td>5-7 l*</td>
</tr>
<tr>
<td>Rinse Solution</td>
<td>5-6 l*</td>
</tr>
</tbody>
</table>

* The residual volume depends on the number of samples and dilution method used.
Procedural comments
• When using software default, samples are run in single determination.
• Washing Solution must be at room temperature when used.
• The first result is available after approx. 2 hours and further results at one minute intervals afterwards. Up to 5 x 10 samples can be loaded continuously and are processed by random access.
• Incubations are automatically performed at 37 °C (98.6 °F).

CALIBRATION AND REFERENCE MATERIAL

The calibration curve is obtained with EliA IgG Calibrators which are run in duplicate. The curve is stored and subsequent tests are evaluated against the stored curve using only the EliA IgG Curve Control (run in duplicate).

A new calibration curve must be run when:
• the last calibration was made more than one month ago or
• a new lot of EliA IgG Conjugate is introduced or
• when the EliA IgG Curve Control is outside the specified limits (defined in Phadia 250 Instrument Software).

For EliA CTD Screen results are given in Ratio.

QUALITY CONTROL

Control Specimens
Good laboratory practice requires that quality control specimens should be included in every run. Any material used should be assayed repeatedly to establish mean values and acceptance ranges.

EliA Controls are available for the quality control of the measurements.

CALCULATION AND INTERPRETATION OF RESULTS

Presentation of Results
Phadia 250 measures specific IgG concentrations in μg/l. By using a conversion factor given by the lot-specific code of the EliA CTD Screen Well, the results are automatically converted to Ratio.

Interpretation of Test Results
The ranges (negative, equivocal, positive) recommended for the evaluation of the results are given in the table below.

<table>
<thead>
<tr>
<th>Test</th>
<th>Unit</th>
<th>negative</th>
<th>equivocal</th>
<th>positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>EliA CTD Screen</td>
<td>Ratio</td>
<td>&lt; 0.7</td>
<td>0.7 - 1.0</td>
<td>&gt; 1.0</td>
</tr>
</tbody>
</table>

Good laboratory practice requires that each laboratory establishes its own range of expected values.

LIMITATIONS

A definitive clinical diagnosis should not be based on the results of a single diagnostic method, but should only be made by the physician after all clinical and laboratory findings have been evaluated.

EXPECTED VALUES

Antibody prevalence in autoimmune patients varies widely depending on disease area. The proportion of sera from a normal population found positive for the antinuclear antibodies covered by the EliA CTD Screen test is below 1 %. Expected values may vary depending on the population tested.\(^\text{1,2,3,4,5}\)

Results Obtained for Healthy Subjects

The frequency distribution for antinuclear antibodies was investigated on the instrument Phadia 250 in a group of apparently healthy subjects equally distributed by age and gender, using sera from a Caucasian population obtained from a blood bank. The results are given in the table below.\(^\text{(1)}\)

<table>
<thead>
<tr>
<th>Test</th>
<th>Unit</th>
<th>No.of Samples</th>
<th>Mean Value</th>
<th>95%-percentile</th>
<th>99%-percentile</th>
</tr>
</thead>
<tbody>
<tr>
<td>EliA CTD Screen</td>
<td>Ratio</td>
<td>400</td>
<td>0.2</td>
<td>0.5</td>
<td>0.8</td>
</tr>
</tbody>
</table>

PERFORMANCE CHARACTERISTICS

Measuring Range
The measuring range (detection limit, upper limit) for EliA CTD Screen is from 0.03 to ≥ 32 Ratio. No hook effects could be observed for concentrations up to 4 fold above the measuring ranges.\(^\text{(1)}\) Only values above the Detection Limit can be regarded as valid results. The upper limit of the reported results can vary due to a lot-specific conversion from μg/l to Ratio. Results above the upper limit are reported as “above”.

Specificity
The EliA CTD Screen Test permits the determination of IgG antibodies directed against the antigens as described in section “Reagents”.

1,2,3,4,5
REFERENCES