SUMMARY AND EXPLANATION OF THE TEST
Rheumatoid arthritis (RA) is one of the most common autoimmune diseases. The main characteristic of RA is joint inflammation that results in joint damage and loss of function. An early diagnosis of RA and an immediate beginning of an appropriate treatment is important to prevent a complete joint damage. RA is diagnosed primarily on clinical manifestations and serological support has, up to now, been mainly restricted to the determination of autoantibodies against rheumatoid factor (RF). RF is a sensitive serological marker for RA with a moderate on clinical manifestations and serological support has, up to now, been mainly restricted to the determination of autoantibodies against rheumatoid factor (RF). RF is a sensitive serological marker for RA with a moderate specificity of about 70%. In several studies it has been demonstrated that the determination of antibodies against citrullinated arginine residues in filament proteins occurs in RF negative patients. Citrullination is a peptidylarginine deiminase (PAD) catalyzed process in which the amino acid arginine (Arg) is modified to citrullin. During this conversion, the positively charged NH2-group is hydrolyzed to an oxygen group [4].

The ORGENTEC Anti-MCV® ELISA shows both a high specificity and a high sensitivity for auto-antibodies against mutated citrullinated vimentin. Vimentin is an omnipresent citrullinated protein which was observed in the rheumatoid synovial tissue of RA patients. There are recent findings of secretion and modification of vimentin by macrophages depending on pro-inflammatory signals [1, 2]. The titer of antibodies against vimentin in RA patients strongly correlates with the disease activity score (DAS).

ORG 548 Anti-MCV®
NAME AND INTENDED USE
Anti-MCV® is an ELISA test system for the quantitative measurement of IgG class autoantibodies against mutated citrullinated vimentin (MCV) in human serum or plasma. This product is intended for professional in vitro diagnostic use only.

SYMBOLS USED ON LABELS
<table>
<thead>
<tr>
<th>SYMBOL</th>
<th>USE</th>
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<tr>
<td>¥</td>
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<td>Do not reuse</td>
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PRINCIPLE OF THE TEST
Mutated citrullinated vimentin (MCV) is bound to microwells. The determination is based on an indirect enzyme linked immune reaction with the following steps:
Specific antibodies in the patient sample bind to the antigen coated on the surface of the reaction wells. After incubation, a washing step removes unbound and unspecifically bound serum or plasma components. Subsequently added enzyme conjugate binds to the immobilized antibody-antigen-complexes. After incubation, a second washing step removes unbound enzyme conjugate. After addition of substrate solution the bound enzyme conjugate hydrolyses the substrate forming a blue coloured product. Addition of an acid stops the reaction generating a yellow end-product. The intensity of the yellow color correlates with the concentration of the antibody-antigen-complex and can be measured photometrically at 450 nm.
or

Sample Buffer P: Prior to use dilute the contents (20 ml) of one vial of sample buffer 5x concentrate with distilled water for at least 30 days when stored at 2-8°C. We recommend consumption on the same day.

PROCEDURAL NOTES
• Do not use kit components beyond their expiration dates.
• Do not interchange kit components from different lots and products.
• All materials must be at room temperature (20-28°C) prior to use.
• Prepare all reagents and samples. Once started, perform the test without interruption.
• Double determinations may be done. By this means pipetting errors may become obvious.
• Perform the assay steps only in the order indicated.
• Always use fresh sample dilutions.
• Pipette all reagents and samples into the bottom of the wells.
• To avoid carryover or contamination, change the pipette tip between samples and different kit controls.
• Wash microwells thoroughly and remove the last droplets of wash buffer.
• All incubation steps must be accurately timed.
• Do not re-use microplate wells.

WARNINGS AND PRECAUTIONS
• All reagents of this kit are intended for professional in vitro diagnostic use only.
• 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 or HIV2, and so all human serum based reagents in this kit must be handled as though capable of transmitting infection.
• Bovine serum albumin (BSA) used in components has been tested for BSE and found negative.
• Avoid contact with the substrate TMB (3,3´,5,5´-Tetramethyl-benzidine).
• Stop solution contains acid, classification is non-hazardous. Avoid contact with skin.
• Control, sample buffer and wash buffer contain sodium azide 0.09% as preservative. This concentration is classified as non-hazardous. Enzyme conjugate contains ProClin 300 0.05% as preservative. This concentration is classified as non-hazardous.

During handling of all reagents, controls and serum samples observe the existing regulations for laboratory safety regulations and good laboratory practice:
• First aid measures: In case of skin contact, immediately wash thoroughly with water and soap. Remove contaminated clothing and shoes and wash before reuse. If system fluid comes into contact with skin, wash thoroughly with water. After contact with the eyes carefully rinse the opened eye with running water for at least 10 minutes. Get medical attention if necessary.
• Personal precautions, protective equipment and emergency procedures:
  • Exposure controls / personal protection: Wear protective gloves of nitril rubber or natural latex. Wear protective glasses. Used according to intended use no dangerous reactions known.
• Conditions to avoid: Since substrate solution is light-sensitive. Store in the dark.
• For disposal of laboratory waste the national or regional legislation has to be observed.

Observe the guidelines for performing quality control in medical laboratories by assaying control sera.

PREPARATION OF REAGENTS

Dilute the contents of one vial of the buffered wash solution concentrate (50x) with distilled or deionised water to a final volume of 1000 ml prior to use.

Sample Buffer P: Prior to use dilute the contents (20 ml) of one vial of sample buffer 5x concentrate with distilled or deionised water to a final volume of 1000 ml prior to use.

MATERIALS REQUIRED
• Microplate reader capable of endpoint measurements at 450 nm; optional: reference filter at 620 nm
• Data reduction software
• Multi-channel dispenser or repeatable pipette for 100 µl
• Vortex mixer
• Pipettes for 10 µl, 100 µl and 1000 µl
• Laboratory timing device
• Distilled or deionised water
• Measuring cylinder for 1000 ml and 100 ml
• Plastic container for storage of the wash solution

This ELISA assay is suitable for use on open automated ELISA processors. Each assay has to be validated on the respective automated system. Detailed information is provided upon request.

STORAGE AND STABILITY
• Store test kit at 2-8°C in the dark.
• Do not expose reagents to heat, sun, or strong light during storage and usage.
• Store microplate sealed and dessicated in the clip bag provided.
• Shelf life of the unopened test kit is 18 months from day of production.
• Unopened reagents are stable until expiration of the kit. See labels for individual batch.

• Diluted Wash Buffer and Sample Buffer are stable for at least 30 days when stored at 2-8°C.

We recommend consumption on the same day.

SPECIMEN COLLECTION, STORAGE AND HANDLING
• Collect whole blood specimens using accepted medical techniques to avoid hemolysis.
• Allow blood to clot and separate the serum or plasma by centrifugation.
• Test serum should be clear and non-hemolyzed. Contamination by hemolysis or lipemia should be avoided, but does not interfere with this assay.
• Specimens may be refrigerated at 2-8°C for up to five days or stored at -20°C up to six months.
• Avoid repetitive freezing and thawing of serum or plasma samples. This may result in variable loss of antibody activity.
• Testing of heat-inactivated sera is not recommended.

Wash microwells thoroughly and remove the last droplets of wash buffer.

All incubation steps must be accurately timed.

Do not re-use microplate wells.

• Enzyme conjugate contains ProClin 300 0.05% as preservative. This concentration is classified as non-hazardous.

During handling of all reagents, controls and serum samples observe the existing regulations for laboratory safety regulations and good laboratory practice:

• Data reduction software
• Multi-channel dispenser or repeatable pipette for 100 µl
• Vortex mixer
• Pipettes for 10 µl, 100 µl and 1000 µl
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deionised water to a final volume of 100 ml.

Preparation of samples
Dilute patient samples 1:100 before the assay: Put 990 µl of prediluted sample buffer in a polystyrene tube and add 10 µl of sample. Mix well. Note: Calibrators / Controls are ready to use and need not be diluted.

TEST PROCEDURE
Prepare enough microplate modules for all calibrators / controls and patient samples.

1. Pipette 100 µl of calibrators, controls and prediluted patient samples into the wells.
   Incubate for 30 minutes at room temperature (20-28 °C).
   Discard the contents of the microwells and wash 3 times with 300 µl of wash solution.

2. Dispense 100 µl of enzyme conjugate into each well.
   Incubate for 15 minutes at room temperature.
   Discard the contents of the microwells and wash 3 times with 300 µl of wash solution.

3. Dispense 100 µl of TMB substrate solution into each well.
   Incubate for 15 minutes at room temperature.

4. Add 100 µl of stop solution to each well of the modules
   Incubate for 5 minutes at room temperature.
   Read the optical density at 450 nm (reference 600-690nm) and calculate the results.

Example for a pipetting scheme:

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<tr>
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<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
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<td>P13</td>
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<td>P16</td>
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VALIDATION
Test results are valid if the optical densities at 450 nm for calibrators / controls and the results for controls comply with the reference ranges indicated on the Certificate of Analysis enclosed in each test kit.
If these quality control criteria are not met the assay run is invalid and should be repeated.

CALCULATION OF RESULTS
For quantitative results plot the optical density of each calibrator versus the calibrator concentration to create a calibration curve. The concentration of patient samples may then be estimated from the calibration curve by interpolation.
Using data reduction software a 4-Parameter-Fit with ln-log coordinates for optical density and concentration is the data reduction method of choice.

PERFORMANCE CHARACTERISTICS

CALIBRATION
This assay system is calibrated in relative arbitrary units, since no international reference preparation is available for this assay.

Measuring range
The calculation range of this ELISA assay is 0 - 1000 U/ml

Expected values
In a normal range study with samples from healthy blood donors the following ranges have been established with this ELISA assay: Cut-off 20 U/ml

Interpretation of results
Negative: < 20 U/ml
Positive: ≥ 20 U/ml

Linearity
Patient samples containing high levels of specific antibody were serially diluted in sample buffer to demonstrate the dynamic range of the assay and the upper / lower end of linearity. Activity for each dilution was calculated from the calibration curve using a 4-Parameter-Fit with ln-log coordinates.

Limit of detection
Functional sensitivity was determined to be: 1 U/ml

Reproducibility
Intra-assay precision: Coefficient of variation (CV) was calculated for each of three samples from the results of 24 determinations in a single run. Results for precision-within-assay are shown in the table below.
Inter-assay precision: Coefficient of variation (CV) was calculated for each of three samples from the results of 6 determinations in 5 different runs. Results for run-to-run precision are shown in the table below.

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

Study results

<table>
<thead>
<tr>
<th>Study population</th>
<th>n</th>
<th>n Pos</th>
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<tbody>
<tr>
<td>Rheumatoid Arthritis</td>
<td>490</td>
<td>398</td>
<td>81.2</td>
</tr>
<tr>
<td>Other diseases</td>
<td>522</td>
<td>14</td>
<td>2.7</td>
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**LIMITATIONS OF THE PROCEDURE**

This assay is a diagnostic aid. A definitive 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 concerning the entire clinical picture of the patient. Also every decision for therapy should be taken individually.

The above pathological and normal reference ranges for antibodies in patient samples should be regarded as recommendations only. Each laboratory should establish its own ranges according to ISO 15189 or other applicable laboratory guidelines.

**REFERENCES**


1. Pipet 100 µl calibrator, control or patient sample
   → Incubate for 30 minutes at room temperature
   → Discard the contents of the wells and wash 3 times with 300 µl wash solution

2. Pipet 100 µl enzyme conjugate
   → Incubate for 15 minutes at room temperature
   → Discard the contents of the wells and wash 3 times with 300 µl wash solution

3. Pipet 100 µl substrate solution
   → Incubate for 15 minutes at room temperature

4. Add 100 µl stop solution
   → Leave untouched for 5 minutes
   → Read at 450 nm