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alegria

Instruction for use

03/2008



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NAME AND INTENDED USE

The respective SMC[®] test device is an ELISA based test system intended for in vitro determination of serum or plasma samples. Used in the Alegria[®] -a fully automated Random Access Analyser - this test device is a diagnostic aid for the detection of autoimmune diseases.

PRINCIPLE OF THE TEST

The SMC[®] test device is based on an indirect enzyme immune reaction. It consists of a barcoded 8-well-strip. Each test strip is designed for one determination. The test strip is equipped with a complete set of reagents, which includes enzyme conjugate, enzyme substrate, dilution buffer and a test specific control. Each test strip consists of two coated wells which act as reaction wells for the control and the patient sample. Depending on the test, the wells are coated with the respective antigen.

The reaction includes the following steps: Autoantibodies in positive samples bind to the respective antigen bound to the surface of the wells. After incubation a first washing step removes unspecific bound molecules. Conjugate binds to the immobilized antibody-antigen complex. After incubation a second washing step removes unbound conjugate. Addition of substrate solution results in hydrolysis and color development during incubation. The concentration of the blue color correlates with the concentration of the antibody-antigen-complex and can be measured photometrically at 650 nm.

WARNINGS AND PRECAUTIONS

1. All reagents of this kit are strictly intended for in vitro diagnostic use with the Alegria[®] system only.
2. Human serum used in components has been 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. Also BSA used in components has been tested on BSE and found negative.
3. Avoid contact with the TMB (3,3',5,5'-Tetramethyl-benzidine). If TMB comes into contact with skin, wash thoroughly with water and soap.
4. Avoid contact with the System Fluid which contains acid. If it comes into contact with skin, wash thoroughly with water and seek medical attention.
5. Some kit components (i.e. controls, sample buffer and buffered wash solution) contain sodium azide as preservative. Sodium azide (NaN₃) 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 7., 8., 9.)
6. 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.
7. Wear disposable gloves while handling specimens or kit reagents and wash hands thoroughly afterwards.
8. Do not pipette by mouth.
9. Do not eat, drink, smoke or apply makeup in areas where specimens or kit reagents are handled.

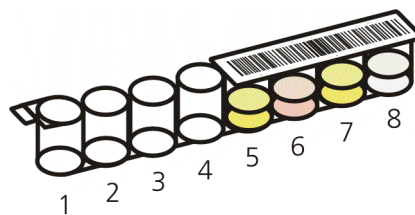
10. Avoid contact between the buffered peroxide solution and easily oxidized materials; extreme temperature may initiate spontaneous combustion.
11. For disposal of laboratory waste the national or regional legislation has to be observed.

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

CONTENTS OF THE KIT

Qty. 12 or 24

figure 1: Composition of the test strip



- 1-2: empty, uncoated
- 3-4: empty, coated with specific antigen
- 5: control
- 6: enzyme conjugate
- 7: sample buffer
- 8: TMB substrate

2 wells coated with the respective antigen; 2 empty, not coated wells; 4 wells containing the following test specific reagents: control (yellow; consisting of the disease relevant marker in a serum/buffer matrix: PBS, BSA, $\text{NaN}_3 < 0,01\%$ (w/w)), enzyme conjugate (light red; containing anti-human antibodies, labelled with horseradish peroxidase; PBS, PROCLIN 300 < 0,5% (v/v)), sample buffer (yellow; Tris, $\text{NaN}_3 < 0,01\%$ (w/w)), TMB substrate solution (3,3', 5,5'-Tetramethylbenzidin)

1 vial, 20 ml Wash Solution (PBS, $\text{NaN}_3 < 0,1\%$ (w/w)), concentrate (50x)

1 vial, 2,5 ml System Fluid contains acid

Qty. 1 Quality Control Certificate

Qty. 1 Product Specifications Sheet

STORAGE AND STABILITY

1. Store the kit at 2-8 °C.
2. Keep test strips sealed at a dark place.
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 wash buffer is stable for at least 30 days when stored at 2-8 °C.
6. Diluted system fluid is stable until the expiration date printed on the label when stored at 2-8 °C.

MATERIALS REQUIRED

- Vortex mixer
- Pipets for 10 µl or 20 µl in case of the Anti-GBM test (ORG 250)

- measuring cylinder for 1000 ml and 2500 ml
- distilled or deionized water

INSTRUMENT

The SMC[®] test devices are intended for use with the diagnostic instrument Alegria[®] only. Please make sure that the latest software version has been installed. All steps apart from loading the instrument with the prepared SysTrays will be done automatically. The run of a performance is completed when results will be printed. Detailed information about the instrument settings can be taken from the user manual Alegria[®].

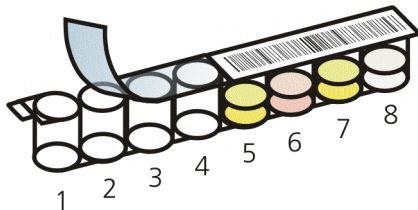
SPECIMEN COLLECTION, STORAGE AND HANDLING

1. Collect whole blood specimens using acceptable medical techniques to avoid hemolysis.
2. Allow blood to clot and separate the serum or plasma by centrifugation.
3. Test serum should be clear and non-hemolyzed. Contamination by hemolysis or lipemia should be 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 or plasma samples. This may result in variable loss of autoantibody activity.
6. Testing of heat-inactivated sera is not recommended.

PROCEDURAL NOTES

1. Do not use kit components beyond their expiration dates.
2. All materials must be at room temperature (20-28 °C) prior to use.
3. Pipet samples at the bottom of the wells.
4. To avoid carryover contaminations change the tip between samples.
5. Remove the foil covering empty wells 1 to 4 only from required strips (see figure 2).
6. Don't remove barcoded foil covering wells 5 to 8.

figure 2: Removing the test strip foil



PREPARATION OF REAGENTS

Wash solution

Dilute the content of the buffered wash solution concentrate (50x) with distilled or deionized water to a final volume of 1000 ml prior to use. Transfer the diluted wash buffer into the reagent container. The wash solution is stable at room temperature for at least one week after preparation or until the expiration date printed on the label.

System Fluid

Dilute the content of the System Fluid concentrate (1000x) with distilled or deionized water to a final volume of 2500 ml prior to use. Transfer the diluted System Fluid into the reagent container. The System Fluid solution is stable at room temperature for at least one week after preparation or until the expiration date printed on the label.

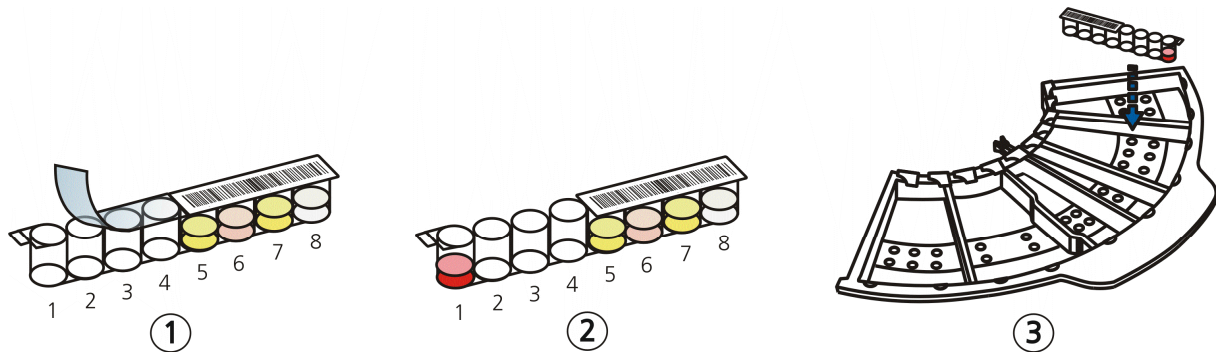
Test strips

Remove foil covering the empty wells of the required test strips. Prior to remove the foil please consider that the test strips have to reach room temperature. Store the remaining test strips at 2-8 °C in a refrigerator.

TESTPROCEDURE

1. Remove the foil from the empty wells of the test strip.
2. Pipet 10 µl undiluted patient sample at the bottom of well 1.
Exception: In case of the Anti-GBM test (ORG 250) pipet 20 µl undiluted sample!
3. Insert the test strip into the SysTray.
4. Place loaded SysTrays into the correct position in the Alegria®.

figure 3: Test procedure



Further information about operating the Alegria® are described in the user manual.

INTERPRETATION OF RESULTS AND PERFORMANCE CHARACTERISTICS

Calculation and interpretation of results will be performed automatically. Each kit contains a Quality Control Certificate as well as a Product Specifications Sheet. These documents provide product specific information used for the interpretation of results and describe the relevant performance characteristics.

The following symbols are used in the Product Specifications:

Ⓐ	Interpretation of Results	Ⓜ	Measuring range	Ⓑ	Technical Data
-	negative	Ⓣ	lower limit	Ⓑ.1	Intra-Assay
+	positive	Ⓢ	upper limit	Ⓑ.2	Inter-Assay
+/-	borderline			Ⓑ.3	Linearity

LIMITATIONS OF PROCEDURE

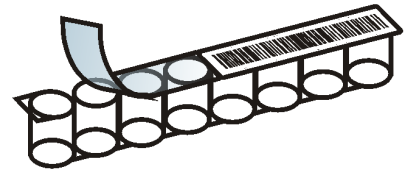
The result is a diagnostic aid. A definite clinical diagnosis should not be based on the results of a single test, but should be made by the physician after all clinical and laboratory findings have been evaluated.

INTERFERING SUBSTANCES

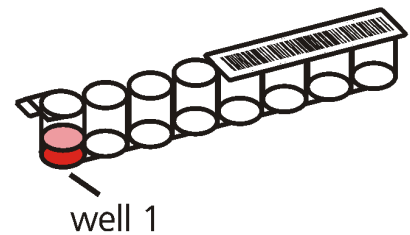
No interference has been observed with haemolytic (up to 1000 mg/dL), lipemic (up to 3 g/dL triglycerides) or bilirubin (up to 40 mg/dL) containing sera or plasma. Nor have any interfering effects been observed with the use of anticoagulants. However for practical reasons it is recommended that grossly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

PROCEDURE SCHEME

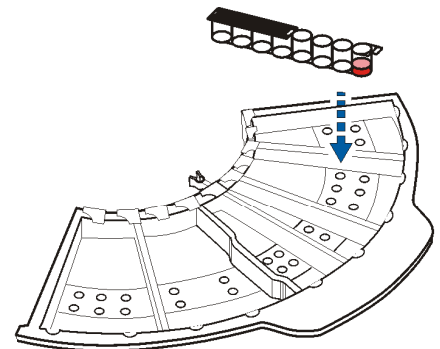
- 1** Remove foil covering the empty wells of the test strip.



- 2** Pipet 10 μ l undiluted sample at the bottom of well 1.
Exception: In case of the Anti-GBM test (ORG 250) pipet 20 μ l undiluted sample!



- 3** Insert the test strip into the SysTray.



- 4** Load the instrument minding the correct SysTray position (SysTray A in position A, SysTray B in position B, SysTray C in position C).

