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1 INTENDED USE

ASCA IgA is used for the quantitative and qualitative determination of IgA antibodies to *Saccharomyces cerevisiae* in human serum.

Non-specific inflammatory bowel diseases including Crohn's disease (Enteritis regionalis) and ulcerative colitis (UC) are characterized by unknown etiology as well as chronic-remitting inflammatory processes of the intestine. Whereas the inflammation of ulcerative colitis is restricted to the mucosa and submucosa of colon and rectum, Crohn's disease (CD) shows a wide spread inflammation of the gastro-intestinal tract with granuloma formation.

The risk developing one of these diseases is strongly influenced by immunologic, genetic, infectious and environmental factors.

The differential diagnosis of inflammatory bowel diseases to chronic diarrhea, recurrent abdominal dolor, infectious colitis, anorexia as well as the differentiation of CD to ulcerative colitis is still a high challenge.

The determination of IgA and IgG antibodies to *Saccharomyces cerevisiae* (baker's yeast) has been described as one important serological marker for the differential diagnosis of Crohn's disease recently. Up to 70 % of patients with CD show antibody levels to *Saccharomyces cerevisiae*. Although the cause for their occurrence has been unclear, antibodies to *Saccharomyces cerevisiae* (ASCA) are strongly associated with inflammatory processes of the intestine.

In combination with the detection of autoantibodies to atypical anti-neutrophil cytoplasmic antigens (aANCA) which are mainly found in patients with ulcerative colitis, ASCA are a valid parameter for the differentiation of Crohn's disease and ulcerative colitis.

DRG[®] offers two innovative serological markers for inflammatory bowel diseases: ASCA IgA and ASCA IgG. Both assays employ the same assay scheme and predilution maximizing laboratory efficiency.

2 PRINCIPLE OF THE TEST

ASCA IgA is an enzyme immunoassay for the quantitative determination of IgA antibodies to *Saccharomyces cerevisiae* in human serum.

Autoantibodies of the diluted patient samples, the control, and standards react with mannan (cell surface component of baker's yeast) immobilized on the solid phase of a microtiter plate. ASCA IgA guarantees the specific binding of anti-*Saccharomyces cerevisiae* IgA antibodies of the specimen under investigation by employing purified mannan of *Saccharomyces cerevisiae* for coating. Following an incubation period of 30 min at 37°C, unbound serum components are removed by a washing step.

The bound antibodies react specifically with anti-human-IgA-antibodies conjugated to horseradish peroxidase (HRP). Within the incubation period of 30 min at 37°C, excessive conjugate is separated from the solid-phase immune complexes by the following washing step.

HRP converts the colorless substrate solution of 3,3',5,5'-tetramethylbenzidine (TMB) added into a blue product. This enzyme reaction is stopped by dispensing an acidic solution (H₂SO₄) into the wells after 15 min at 37°C turning the solution from blue to yellow.

The optical density (OD) of the solution at 450 nm is directly proportional to the amount of specific antibodies bound.

The standard curve is established by plotting the concentrations of the antibodies of the standards (x-axis) and their corresponding OD values (y-axis) measured. The concentration of antibodies of the specimen is directly read off the standard curve.



REVISED 18 MAY 2006



3 PATIENT SAMPLES

3.1 Specimen collection and storage

Blood is taken by venipuncture. Serum is separated after clotting by centrifugation. Lipemic, hemolytic and contaminated samples should not be used.

Repeated freezing and thawing should be avoided. If samples are to be used for several assays, initially aliquot samples and keep at - 20 °C.

3.2 Preparation before use

Allow samples to reach room temperature prior to assay. Take care to agitate serum samples gently in order to ensure homogeneity.

**Note: Patient samples have to be diluted 1 + 100 (v/v),
e.g. 10 µl sample + 1.0 ml sample diluent , prior to assay.**

The samples may be kept at 2 - 8 °C for up to two days. Long-term storage requires - 20 °C.

4 TEST COMPONENTS FOR 96 DETERMINATIONS

Microtiter plate 12 breakable strips per 8 wells (total 96 individual wells) coated with mannan (<i>Saccharomyces cerevisiae</i>)	1 vacuum sealed with desiccant; 2 foils
Concentrated wash buffer sufficient for 1000 ml solution each	100 ml Concentrate, capped white
Sample diluent	100 ml ready for use, capped black
Conjugate containing anti-human-IgA - (sheep) coupled with HRP	15 ml ready for use, capped red
Substrate 3,3',5,5'-tetramethylbenzidine in citrate buffer containing hydrogen peroxide	15 ml ready for use, capped blue
Stop solution 0.25 M sulfuric acid	15 ml ready for use, capped yellow
Standards (diluted serum) conc.: see leaflet enclosed	1 ml each ready for use



REVISED 18 MAY 2006

RUO IN THE USA

Control

(diluted serum

conc.: see leaflet enclosed

1 ml

ready for use

4.1 Materials required

- micropipette 100 - 1000 µl
- micropipette 10 - 100 µl
- multi-channel pipette 50 - 200 µl
- trough for multi-channel pipette
- 8-channel wash comb with vacuum pump and waste bottle or microplate washer
- incubator (37 °C)
- microplate reader with optical filters for 450 nm and 620 nm or 690 nm
- graduated cylinders
- distilled or de-ionized water

4.2 Size and storage

ASCA IgA has been designed for 96 determinations.

The expiry date of each component is reported on its respective label that of the complete kit on the box labels.

Upon receipt, all components of the ASCA IgA have to be kept at 2 - 8 °C, preferably in the original kit box.

After opening all kit components are stable for at least 2 months, provided proper storage.

4.3 Preparation before use

Allow all components to reach room temperature prior to use in the assay.

The microtiter plate is vacuum-sealed in a foil with desiccant. The plate consists of a frame and strips with breakable wells. Allow the sealed microplate to reach room temperature before opening. Unused wells should be stored refrigerated and protected from moisture in the original cover carefully resealed.

Prepare a sufficient amount of wash solution by diluting the concentrated wash buffer 10 times (1 + 9) with de-ionized or distilled water. For example, dilute 8 ml of the concentrate with 72 ml of distilled water.

The wash solution prepared is stable up to 30 days at 2 - 8 °C.

Make sure the soak time of the wash buffer in the wells is at least 5 seconds per wash cycle.

Avoid exposure of the TMB substrate solution to light!

**REVISED 18 MAY 2006****RUO IN THE USA****5 ASSAY PROCEDURE**

- * **Dilute patient sera with sample diluent (C) 1 + 100 (v/v), e.g. 10 µl serum + 1.0 ml sample diluent (C).**
 - * **Avoid any time shift during pipetting of reagents and samples**
1. Bring all reagents to room temperature (18-25°C) before use. Mix gently, avoid foam.
 2. Dispense
100 µl Standards (1 - 4)
100 µl Positive control
100 µl diluted patient samples into the respective wells.
 3. Seal plate, incubate 30 min at 37 °C.
 4. Decant, then wash each well three times using 300 µl diluted wash solution.
 5. Add 100 µl of conjugate solution to each well.
 6. Seal plate, incubate 30 min at 37 °C.
 7. Decant, then wash each well three times using 300 µl diluted wash solution.
 8. Add 100 µl of substrate to each well.
 9. Incubate 15 min in the dark at 37°C.
 10. Add 100 µl of stop solution to each well and mix gently.
 11. Read the OD at 450 nm versus 620 or 690 nm within 30 min after adding the stop solution.

6 DATA PROCESSING

ASCA IgA allows both the quantitative and semi-quantitative evaluation of the results.

6.1 Quantitative evaluation

We recommend log / lin processing for best results.

The standard curve is established by plotting the mean OD-values of the Standards 1 - 4 on the ordinate, y-axis, (lin. scale) versus their respective ASCA IgA-concentrations on the abscissa, x-axis, (log. scale).

Anti-Saccharomyces cerevisiae concentrations of the unknown samples are directly read off in U/ml against the respective OD values.

Using the recommended dilution of 1 + 100 (v/v) for patient's sera, no correction factor is necessary, as all other components of the kit are supplied accordingly.



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6.2 Semi-quantitative evaluation

Results can be calculated semi-quantitatively calculating the binding index BI (ratio) between the optical density of an unknown sample and the optical density of standard 1 (20 U/ml) used as cut-off control.

$$BI = OD_{sample} / OD_{standard\ 1\ (20\ U/ml)}$$

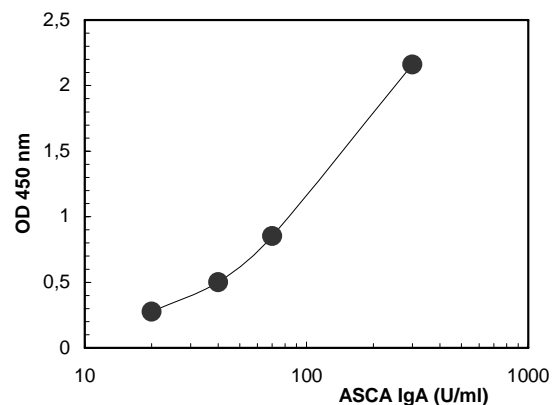
Both evaluation variants of ASCA IgA may be achieved also with computer assisted analysis software intergrated in the photometers.

Example of typical assay results (quantitative)

well	OD (a)	OD (b)	OD (mean)	U/ml
Standard 1	0.279	0.275	0.277	20
Standard 2	0.501	0.503	0.502	40
Standard 3	0.850	0.854	0.852	70
Standard 4	2.175	2.145	2.160	300
Patient	1.334	1.328	1.331	120

The above mentioned standard concentrations are only an example for a typical standard curve. They can change from lot to lot.

TYPICAL STANDARD CURVE



Specimens with an OD > Standard 4 should be retested in a greater sample dilution. The results have to be multiplied with the chosen dilution factor.

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The test run is valid if:

- the mean OD of the standard 1 is ≤ 0.5
- the mean OD of the standard 4 is ≥ 1.2

If the above mentioned quality criteria are not met, repeat the test and make sure that the test procedure is followed correctly (incubation times and temperatures, sample and wash buffer dilution, wash steps etc.). In case of repeated failure of the quality criteria contact your supplier

7 REFERENCE VALUES

ASCA IgA	U/ml	BI
positive	≥ 20	≥ 1.0
negative	< 20	< 1.0

It is recommended that each laboratory establishes its own normal and pathological reference ranges for serum ASCA IgA antibody levels as usually done for other diagnostic parameters, too. Therefore, the above mentioned reference values provide a guide only to values which might be expected.

7.1 Limitations of Method

Healthy individuals should be tested negative by the ASCA IgA. However, ASCA IgA antibody positive apparently healthy persons do occur.

Any clinical diagnosis should not be based on the results of in vitro diagnostic methods alone. Physicians are supposed to consider all clinical and laboratory findings possible to state a diagnosis. In the United States, this kit is intended for Research Use Only.

8 CHARACTERISTIC ASSAY DATA**8.1 Calibration**

Due to the lack of an international reference material the ASCA IgA is calibrated in arbitrary units (U/ml).

8.2 Linearity

Dilutions of positive specimens in anti-Saccharomyces cerevisiae free human serum are determined according to their expected theoretical values with ASCA IgA.



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8.3 Sensitivity

The analytical sensitivity of the ASCA IgA is 10 U/ml.

8.4 Diagnostic sensitivity and specificity

The diagnostic sensitivity and specificity of ASCA IgA and IgG were determined by testing 82 patients with Crohn's disease, 65 patients with ulcerative colitis, 101 patients with celiac disease, 33 patients with PBC, 44 patients with SLE, and 250 apparently healthy blood donors.

Diagnostic sensitivity: 50%

Diagnostic specificity: 94%

8.5 Precision

Intraassay Variance (n=8)		Interassay Variance (n=3 x16)	
U/ml	CV (%)	U/ml	CV (%)
205	2.39	209	11.38
167	3.56	174	8.28
115	2.23	113	7.20
76	2.00	72	5.92

9 SAFETY PRECAUTIONS

- This kit is for in vitro use only. In the United States, this kit is intended for Research Use Only.
- Follow the working instructions carefully. DRG[®] and its authorized distributors shall not be liable for damages indirectly or consequentially brought about by changing or modifying the procedure indicated. The kit should be performed by trained technical staff only.
- The expiration dates stated on the respective labels are to be observed. The same relates to the stability stated for reconstituted reagents.
- Do not use or mix reagents from different lots.
- Do not use reagents from other manufacturers.
- Avoid time shift during pipetting of reagents.
- All reagents should be kept at 2 - 8 °C before use in the original shipping container.
- Some of the reagents contain small amounts of Thimerosal (< 0.1 % w/v) and Kathon (1.0 % v/v) as preservatives. They must not be swallowed or allowed to come into contact with skin or mucosa.

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- Source materials derived from human body fluids or organs used in the preparation of this kit were tested and found negative for HBsAg and HIV as well as for HCV antibodies. However, no known test guarantees the absence of such viral agents. Therefore, handle all components and all patient samples as if potentially hazardous.
- Since the kit contains potentially hazardous materials, the following precautions should be observed:
 - Do not smoke, eat or drink while handling kit material,
 - Always use protective gloves,
 - Never pipette material by mouth,
 - Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.

REFERENCES

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2. Vermeire S:
Serological Diagnosis in IBD. IBDM 2002 3:82-89