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## 1 INTENDED USE

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

**For in vitro diagnostic use only. In the United States, this kit is intended for Research Use Only.**

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 innovative assays for the differential diagnosis of inflammatory bowel diseases: ASCA IgA, ASCA IgG and ASCA combi. All assays employ the same assay scheme and predilution maximizing laboratory efficiency

- Conrad K, Schmechta H, Klafki A, Lobeck G, Uhlig HH, Gerdi S, Henker J: Serological differentiation of inflammatory bowel diseases. *Eur J Gastrol & Hepatol.* 2002 14:129-135
- Vermeire S: Serological Diagnosis in IBD. *IBDM* 2002 3:82-89

## 2 PRINCIPLE OF THE TEST

ASCA combi is an enzyme immunoassay for the qualitative determination of both IgG and IgA antibodies to *Saccharomyces cerevisiae* in human serum.

Autoantibodies of the diluted patient samples and controls react with mannan (cell surface component of baker's yeast) immobilized on the solid phase of a microtiter plate. ASCA combi guarantees the specific binding of anti-*Saccharomyces cerevisiae* IgG as well as IgA antibodies of the specimen under investigation by employing purified mannan of *Saccharomyces cerevisiae* for coating. Following an incubation period of 60 min at 37°C, unbound serum components are removed by a washing step.

The bound antibodies react specifically with anti-human-IgG and 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 (H<sub>2</sub>SO<sub>4</sub>) into the wells after 10 min at room temperature 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.



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### 3 PATIENT SAMPLES

#### 3.1 Specimen collection and storage

Blood is taken by venipuncture. Serum is separated after clotting by centrifugation. Lipaemic, 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 + 50** (v/v),  
e.g. 10 µl sample + 0.5 ml sample diluent (C), **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

|                             |   |   |
|-----------------------------|---|---|
| <b>A</b><br><b>Ag 96</b>    | <b>Microtiter plate,</b><br>12 breakable strips per 8 wells (total 96 individual wells)<br>coated with mannan ( <i>Saccharomyces cerevisiae</i> ) | 1<br>vacuum sealed with<br>desiccant    |
| <b>B</b><br><b>BUF WASH</b> | <b>Concentrated wash buffer</b><br>sufficient for 1000 ml solution<br><b>10x</b>  | 100 ml<br>concentrate<br>capped white   |
| <b>C</b><br><b>DIL</b>      | <b>Sample diluent</b>   | 50 ml<br>ready for use<br>capped black  |
| <b>D</b><br><b>CONJ</b>     | <b>Conjugate</b><br>containing anti-human-IgG and IgA (sheep) coupled with<br>HRP   | 15 ml<br>ready for use<br>capped red    |
| <b>E</b><br><b>SOLN TMB</b> | <b>Substrate</b><br>3,3',5,5'-tetramethylbenzidine in citrate buffer containing<br>hydrogen peroxide  | 15 ml<br>ready for use<br>capped blue   |
| <b>F</b><br><b>H2SO4</b>    | <b>Stop solution</b><br>0.25 M sulfuric acid <b>0.25M</b>   | 15 ml<br>ready for use<br>capped yellow |



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|                             |   |                            |
|-----------------------------|---|----------------------------|
| <b>P</b><br><b>CONTROL</b>  | <b>Positive Control</b><br>(diluted serum),  | 1 ml<br>ready for use      |
| <b>CO</b><br><b>CONTROL</b> | <b>Cut-off</b><br>(diluted serum),           | 1 ml each<br>ready for use |
| <b>N</b><br><b>CONTROL</b>  | <b>Negative Control</b><br>(diluted serum),  | 1 ml<br>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
- distilled or de-ionized water

#### 4.2 Size and storage

ASCA combi 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 combi 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 deionized or distilled water.

For example, dilute 8 ml of the concentrate with 72 ml of distilled water per strip.

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!



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**5 ASSAY PROCEDURE**

- Dilute patient sera with sample diluent (C) 1 + 50 (v/v),  
e.g. 10 µl serum + 0.5 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 without causing foam.
  2. Dispense  
 100 µl Cut-off Control (CO)  
 100 µl Controls (P, N))  
 100 µl diluted patient samples into the respective wells.
  3. Seal plate, incubate **60 min** at 37°C.
  4. Decant, then wash each well **five times** using **300 µl** wash buffer (made of B).
  5. Add **100 µl** of conjugate (D) solution to each well.
  6. Seal plate, incubate **30 min** at 37°C.
  7. Decant, then wash each well **five times** using **300 µl** wash buffer (B).
  8. Add **100 µl** of substrate (E) to each well.
  9. Incubate **10 min** protected from light at room temperature (18-25°C).
  10. Add **100 µl** of stop solution (F) to each well and mix gently.
  11. Read the optical density at **450 nm** versus 620 or 690 nm **within 30 min** after adding the stop solution.

**6 DATA PROCESSING**

**6.1 Qualitative evaluation**

Results are interpreted by calculating the binding index (BI)

$$BI = OD_{\text{sample}} / OD_{\text{cut-off control}}$$

This calculation can be done by the integrated evaluation software of the microplate reader used, too.

**6.2 Example of typical assay results**

| wells            | OD (a) | OD (b) | OD (mean) | BI             |
|------------------|--------|--------|-----------|----------------|
| Positive control | 1.965  | 1.977  | 1.971     |                |
| Cut-off control  | 0.353  | 0.371  | 0.362     |                |
| Negative control | 0.047  | 0.048  | 0.048     |                |
| Patient 1        | 0.941  | 0.932  | 0.937     | 2.6 - positive |
| Patient 2        | 0.264  | 0.260  | 0.262     | 0.7 - negative |
| Patient 3        | 0.369  | 0.377  | 0.373     | 1.0 - positive |



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### 6.3 Test validity

The test run is valid if:

- the mean OD of the negative control is  $\leq 0.150$
- the mean OD of the positive control is  $\geq 1.200$
- the mean OD of the cut-off control is  $\geq 0.200$

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

| <u>ASCA combi</u> | <u>BI</u>  |
|-------------------|------------|
| positive          | $\geq 1.0$ |
| negative          | $< 1.0$    |

It is recommended that each laboratory establishes its own normal and pathological reference ranges for serum ASCA combi 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 combi. However, ASCA IgG and 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.

## 8 CHARACTERISTIC ASSAY DATA

### 8.1 Calibration

Due to the lack of an international reference material for ASCA results are interpreted by calculating a BI (ratio).

### 8.2 Linearity

Positive selected serum samples have been tested by this assay and found to dilute linearly. However, due to the heterogeneous nature of human autoantibodies there might be sera that do not follow this rule.

### 8.3 Sensitivity

The analytical sensitivity of this assay was determined at 0.3.



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**8.4 Diagnostic sensitivity and specificity**

The diagnostic sensitivity and specificity of ASCA IgA and IgG antibodies, 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 |      | Interassay |      |
|------------|------|------------|------|
| mean BI    | CV % | mean BI    | CV % |
| 1.2        | 6.4  | 1.6        | 9.5  |
| 2.2        | 3.9  | 2.9        | 10.5 |
| 5.9        | 6.6  | 5.6        | 10.9 |

**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.
- Source materials derived from human body fluids or organs used in the preparation of this kit were tested and found negative for HBsAg and for HIV as well as 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.