DRG® ASCA IgA ELISA (EIA-4279)

REVISED 29 DEC. 2009 RM (VERS. 4.0) **RUO IN THE USA**

Please use only the valid version of the package insert provided with the kit.

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 bowl diseases: **ASCA IgA** and **ASCA IgG**. Both 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 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 calibrators 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 60 min at room temperature, unbound sample components are removed by a wash step.

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

HRP converts the colorless substrate solution of 3,3',5,5'-tetramethylbenzidine (TMB) added into a blue product. The enzyme reaction is stopped by dispensing an acidic solution into the wells after 15 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. The standard curve is established by plotting the antibody concentrations of the calibrators (x-axis) and their corresponding OD values (y-axis) measured. The concentration of antibodies of the specimen is directly read off the standard curve. Evaluating the test by a semi-quantitative method is also possible.

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.

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

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 (C), prior to assay.

4 TEST COMPONENTS FOR 96 DETERMINATIONS

A **(Ag 96)**	**Microtiter plate** 12 breakable strips per 8 wells (total 96 individual wells) coated with mannan (*Saccharomyces cerevisiae*)	1 vacuum sealed with desiccant; 2 foils
B **(BUF WASH)** **(10X)**	**Concentrated wash buffer** sufficient for 1000 ml solution each	100 ml concentrate capped white
C **(DIL)**	**Sample diluent**	100 ml ready for use capped black
D	**Conjugate**	15 ml

(CONJ)	containing anti-human-IgA - (sheep) coupled with HRP	ready for use capped purple
E **(SOLN TMB)**	**Substrate** 3,3',5,5'-tetramethylbenzidine in citrate buffer containing hydrogen peroxide	15 ml ready for use capped blue
F **(H2SO4)** **(0.25M)**	**Stop solution** 0.25 M sulfuric acid	15 ml ready for use capped yellow
0 – 4 **(CAL)**	**Calibrators** (diluted serum) conc.: 1, 10, 30, 100, 300 U/ml	1 ml each ready for use capped white
P **(CONTROL)** **(+)**	**Positive control** (diluted serum) conc.: see leaflet enclosed	1 ml ready for use capped red

4.1 Materials required in addition

- 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
- 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!

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 °C - 25 °C) before use. Mix gently without causing foam.
2. Dispense
   100 µl calibrators 1 – 4 (CAL 0 optionally, quantitative) or
   100 µl calibrator 1 (semi-quantitative)
   100 µl positive control (P)
   100 µl diluted patient samples into the respective wells.
3. Cover plate, incubate **60 min** at room temperature (18 °C - 25 °C).
4. Decant, then wash each well **three** times using **300 µl** wash solution (made of B).
5. Add **100 µl** of conjugate (D) solution to each well.
6. Cover plate, incubate **30 min** at room temperature (18 °C - 25 °C).
7. Decant, then wash each well **three** times using **300 µl** wash solution (made of B).
8. Add **100 µl** of substrate (E) to each well.
9. Cover plate, incubate **15 min protected from light** at room temperature (18 °C - 25 °C).
10. Add **100 µl** of stop solution (F) 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 calibrators 1 - 4 (CAL 0 optionally) on the ordinate, y-axis, (lin. scale) versus their respective ASCA IgG-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.

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 calibrator 1 (**10 U/ml**) multiplied by a **factor 2**.

$$BI = OD_{sample} / (OD_{calibrator\ 1\ (10\ U/ml)} \times 2)$$

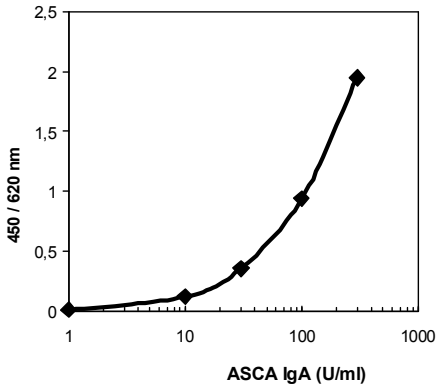
Both evaluation variants of ASCA IgA may be achieved also with computer assisted analysis software intergrated in the photometers.

Example of typical assay results (quantitative)

well	OD (a)	OD (b)	OD (mean)	U/ml
Calibrator 0	0.007	0.011	0.009	1
Calibrator 1	0.112	0.128	0.120	10
Calibrator 2	0.351	0.363	0.357	30
Calibrator 3	0.928	0.948	0.938	100
Calibrator 4	1.935	1.965	1.950	300
Patient 1	1.192	1.204	1.198	140

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

TYPICAL STANDARD CURVE (example)



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

6.3 Test validity

The test run is valid if:

- the mean OD of the calibrator 1 is ≤ 0.5
- the mean OD of the calibrator 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.

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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 the ASCA IgA is calibrated in arbitrary units (U/ml).

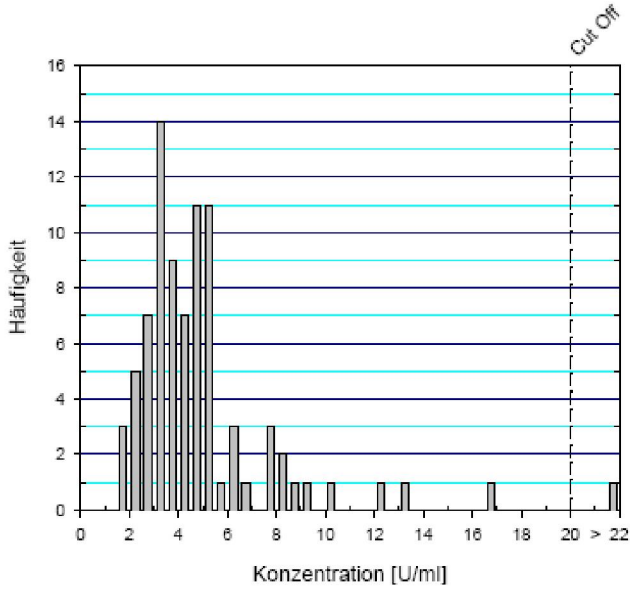
8.2 Diagnostic sensitivity and specificity

The diagnostic sensitivity and specificity of ASCA IgA and IgG (EIA-4280), were determined by testing 82 patients with Chrohn`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%

8.3 Frequency distribution

84 normal sera (without clinical symptoms) were tested ASCA IgA. Only 1 serum was found with concentration above the cut-off (20 U/ml). This corresponds to a specificity of 98.8 %.



8.4 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 INCUBATION SCHEME

Dilute patients sample★	**10 µl serum + 1.0 ml sample diluent (C)**

★This dilution can also be used in the ASCA IgG (EIA-4280)

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1	Bring all ready for use reagents to room temperature (18 °C - 25 °C) before use.			
		calibrators	control	sera
2	Pipette Calibrators (0 - 4) or Calibrator 1	100 µl		
	Positive Control (P)		100 µl	
	prediluted 1 + 100 patient sera			100 µl
3	Incubate	60 minutes at room temperature (18 °C - 25 °C)		
4	Wash	Decant, Dispense 3 x 300 µl (made of B)		
5	Pipette conjugate (D)	100 µl	100 µl	100 µl
6	Incubate	30 minutes at room temperature (18 °C - 25 °C)		
7	Wash	Decant, Dispense 3 x 300 µl (made of B)		
8	Pipette substrate (E)	100 µl	100 µl	100 µl
9	Incubate protected from light	15 minutes at room temperature (18 °C - 25 °C)		
10	Pipette stop solution (F)	100 µl	100 µl	100 µl
11	Measure 450 nm versus 620 (690) nm			

10 SAFETY PRECAUTIONS

- **This kit is for in vitro 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 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.

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Symbols used with DRG Assays

Symbol	English	Deutsch	Français	Español	Italiano
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las instrucciones de uso	Consultare le istruzioni per l'uso
CE	European Conformity	CE-Konfirmitäts-kennzeichnung	Conformité aux normes européennes	Conformidad europea	Conformità europea
IVD	In vitro diagnostic device	In-vitro-Diagnostikum	Usage Diagnostic in vitro	Para uso Diagnóstico in vitro	Per uso Diagnostica in vitro
RUO	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
REF	Catalogue number	Katalog-Nr.	Numéro de catalogue	Número de catálogo	Numero di Catalogo
LOT	Lot. No. / Batch code	Chargen-Nr.	Numéro de lot	Número de lote	Numero di lotto
	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Storage Temperature	Lagerungstemperatur	Température de conservation	Temperatura de conservación	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeits-datum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
Distributed by	Distributor	Vertreiber	Distributeur	Distribuidor	Distributore
Content	Content	Inhalt	Conditionnement	Contenido	Contenuto
Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Quantité	Volumen/Número	Volume/Quantità

Symbol	Portugues	Dansk	Svenska	Ελληνικά
	Consulte as instruções de utilização	Se brugsanvisning	Se bruksanvisningen	Εγχειρίδιο χρήστη
CE	Conformidade com as normas europeias	Europaeisk overensstemmelse	Europeisk överensstämmelse	Ευρωπαϊκή Συμμόρφωση
IVD	Diagnóstico in vitro	In vitro diagnostik	Diagnostik in vitro	in vitro διαγνωστικό
RUO				
REF	Catálogo n.º	Katalognummer	Katalog nummer	Αριθμός καταλόγου
LOT	No do lote	Lot nummer	Batch-nummer	Αριθμός Παρτίδος
		Indeholder tilsttrækkeligt til "n" test	Innehåller tillräckligt till "n" tester	Περιεχόμενο επαρκές για «n» εξετάσεις
	Temperatura de conservação	Opbevarings-temperatur	Förvaringstempratur	Θερμοκρασία αποθήκευσης
	Prazo de validade	Udløbsdato	Bäst före datum	Ημερομηνία λήξης
	Fabricante	Producent	Tillverkare	Κατασκευαστής
Distributed by				
Content	Conteúdo	Indhold	Innehåll	Περιεχόμενο
Volume/No.	Volume/Número	Volumen/antal	Volym/antal	Όγκος/αριθ..