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Instruction For Use
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ORG 545 ASCA IgG/IgA

NAME AND INTENDED USE

ASCA IgG/IgA is an ELISA test system for the quantitative measurement of IgG and IgA class anti-Saccharomyces cerevisiae antibodies (ASCA) in human serum or plasma. This product is intended for professional in vitro diagnostic use only.

SYMBOLS USED ON LABELS

IVD	In vitro diagnostic medical device	MICROPLATE	Microplate
Manufacturer		CALIBRATOR A	Calibrator
REF	Catalogue number	CALIBRATOR B	Calibrator
96	Sufficient for 96 determinations	CALIBRATOR C	Calibrator
LOT	Batch code	CALIBRATOR D	Calibrator
Use by		CALIBRATOR E	Calibrator
Temperature limitation		CALIBRATOR F	Calibrator
Consult instructions for use		CONTROL +	Control positive
Keep away from sunlight		CONTROL -	Control negative
Do not reuse		DILUENT	Sample Buffer P
Date of manufacture		CONJUGATE G	Enzyme Conjugate
		CONJUGATE A	Enzyme Conjugate
		TMB	TMB Substrate
		STOP	Stop solution
		WASH	Wash Buffer
		RTU	Ready to use

PRINCIPLE OF THE TEST

Highly purified mannan from *Saccharomyces cerevisiae* is bound to microwells.

Antibodies against the coated antigen, if present in diluted patient sample, bind to the respective antigen. Washing of the microwells removes unbound unspecific serum and plasma components. Horseradish peroxidase (HRP) conjugated anti-human antibodies immunologically detect the bound patient antibodies forming a conjugate/antibody/antigen complex. Washing of the microwells removes unbound conjugate. An enzyme substrate in the presence of bound conjugate hydrolyzes to form a blue colour. The addition of an acid stops the reaction forming a yellow end-product. The intensity of this yellow colour is measured photometrically at 450 nm. The amount of colour is directly proportional to the concentration of antibodies present in the original sample.

SUMMARY AND EXPLANATION OF THE TEST

Accurate diagnosis of inflammatory bowel disease (IBD), in particular the differentiation between the two major IBDs ulcerative colitis and Crohn's disease, is important for treatment and prognosis. Ulcerative colitis is characterized by an inflammation and ulcers in the top layers of the lining of the colon and rectum. Crohn's disease shows a wide spread inflammation of the gastro-intestinal tract with granuloma formation extending deep into the affected tissue. Inflammation in Crohn's disease is asymmetrical and segmental, with areas of both healthy and diseased tissue, in contrast to ulcerative colitis where inflammation is symmetrical and uninterrupted from the rectum proximally [1].

To differentiate between Crohn's disease and ulcerative colitis the detection of ANCA (Anti-Neutrophil Cytoplasmic Antibody) and ASCA (Anti-Saccharomyces Cerevisiae Antibody) can be used. ASCA are directed against oligomannosidic epitopes on the cell wall mannan (phosphor-peptidomannan) of the yeast *Saccharomyces cerevisiae* [2]. IgG as well as IgA ASCA show a specificity of 95-100% for Crohn's disease. ASCA are strongly associated to Crohn's disease. Studies showed 5% positive IgG and 7% IgA class ASCA in ulcerative colitis whereas in Crohn's disease a sensitivity of 75% for IgG and 60% for IgA class ASCA could be observed [3, 4].

The occurrence of atypical ANCA (aANCA) in Crohn's disease is more infrequent than in ulcerative colitis. The prevalence of ANCA varies from 50% to 90% in ulcerative colitis and 10% to 20% in Crohn's disease [5].

The combination of both serological tests makes possible a rapid and non-invasive differential diagnosis between Crohn's disease and ulcerative colitis.

CONTENTS OF THE KIT

ORG 545	▽ 96	Sufficient for 96 determinations
MICROPLATE	1	One divisible microplate consisting of 12 modules of 8 wells each. Ready to use. Product code on module: SC
CALIBRATOR A	1x 1.5 ml	Calibrator A 0 U/ml, containing serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR B	1x 1.5 ml	Calibrator B 6.3 U/ml, containing ASCA antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR C	1x 1.5 ml	Calibrator C 12.5 U/ml, containing ASCA antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR D	1x 1.5 ml	Calibrator D 25 U/ml, containing ASCA antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR E	1x 1.5 ml	Calibrator E 50 U/ml, containing ASCA antibodies in a serum/buffer matrix (PBS, BSA, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR F	1x 1.5 ml	Calibrator F 100 U/ml, containing ASCA antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CONTROL +	1x 1.5 ml	Control positive, containing ASCA antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use. The concentration is specified on the certificate of analysis.
CONTROL -	1x 1.5 ml	Control negative, containing ASCA antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use. The concentration is specified on the certificate of analysis.
DILUENT	20 ml	Sample Buffer P, containing PBS, BSA, detergent, preservative sodium azide 0.09%, yellow, concentrate (5 x).
CONJUGATE G	15 ml	Enzyme Conjugate; containing anti-human IgG antibodies, HRP labelled; PBS, BSA, detergent, preservative PROCLIN 0.05%, light red. Ready to use.
CONJUGATE A	15 ml	Enzyme Conjugate; containing anti-human IgA antibodies, HRP labelled; PBS, BSA, detergent, preservative PROCLIN 0.05%, light red. Ready to use.
TMB	15 ml	TMB Substrate; containing 3,3', 5,5'- Tetramethylbenzidin, colorless. Ready to use.
STOP	15 ml	Stop solution; contains acid. Ready to use.
WASH	20 ml	Wash Buffer, containing Tris, detergent, preservative sodium azide 0.09%; 50 x conc.
i	1	Instruction for Use: ELISA Mini-DVD
i	1	Certificate of Analysis

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.

SPECIMEN COLLECTION, STORAGE AND HANDLING

- Collect whole blood specimens using acceptable 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.

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 desiccated 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.

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:
Observe laboratory safety regulations. Avoid contact with skin and eyes. Do not swallow. Do not pipette by mouth. Do not eat, drink, smoke or apply makeup in areas where specimens or kit reagents are handled. When spilled, absorb with an inert material and put the spilled material in an appropriate waste disposal.
- 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

WASH

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.

DILUENT

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 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.

- 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.
- 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.
- Dispense **100 µl** of TMB substrate solution into each well.
Incubate for **15 minutes** at room temperature.
- 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.
The developed colour is stable for at least 30 minutes. Read during this time.

Example for a pipetting scheme:

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	P1	A	P1								
B	B	P2	B	P2								
C	C	P3	C	P3								
D	D	P4	D	P4								
E	E	P5	E	P5								
F	F	P6	F	P6								
G	C+	P7	C+	P7								
H	C-	P8	C-	P8								

IgG IgG IgA IgA

P1, ... patient sample A-F calibrators C+, C- controls

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 lin-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 IgG: 0 - 100 U/ml IgA: 0 - 100 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 IgG: 10 U/ml IgA: 10 U/ml

Interpretation of results

Negative: IgG < 10 U/ml IgA < 10 U/ml
Positive: ≥ 10 U/ml ≥ 10 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 lin-log coordinates.

Sample	Dilution	Observed U/ml	Expected U/ml	O/E [%]
IgG 1	1:100	64.2	64.2	100
.	1:200	34.1	32.1	106
.	1:400	16.9	16.1	105
.	1:800	7.6	8.0	95
IgG 2	1:100	53.9	53.9	100
.	1:200	26.4	27.0	98
.	1:400	12.9	13.5	96
.	1:800	7.1	6.7	106
IgG 3	1:100	48.3	48.3	100
.	1:200	25.9	24.2	107
.	1:400	13.3	12.1	110
.	1:800	6.8	6.0	113
IgA 1	1:100	64.2	64.2	100
.	1:200	34.1	32.1	106
.	1:400	16.9	16.1	105
.	1:800	7.6	8.0	95
IgA 2	1:100	53.9	53.9	100
.	1:200	26.4	27.0	98
.	1:400	12.9	13.5	96
.	1:800	7.1	6.7	106
IgA 3	1:100	48.3	48.3	100
.	1:200	25.9	24.2	107
.	1:400	13.3	12.1	110
.	1:800	6.8	6.0	113

Limit of detection

Functional sensitivity was determined to be: IgG: 0.5 U/ml IgA: 0.5 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.

Intra-Assay IgG		
Sample	Mean U/ml	CV %
1	9.6	4.3
2	19.3	6.6
3	76.5	8.8

Inter-Assay IgG		
Sample	Mean U/ml	CV %
1	10.5	7.1
2	31.2	3.8
3	67.3	7.5

Intra-Assay IgA		
Sample	Mean U/ml	CV [%]
1	5.1	5.2
2	26.8	6.1
3	66.4	6.1

Inter-Assay IgA		
Sample	Mean U/ml	CV [%]
1	5.9	6.6
2	29.1	6.0
3	81.2	6.4

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

Study population	n	Pos IgG	%	Pos IgA	%
Crohn's disease	72	54	75.0	45	62.5
Normal human sera	130	4	3.1	3	2.3

	Clinical Diagnosis				Clinical Diagnosis		
	Pos	Neg	202		Pos	Neg	202
ORG 545 IgG	54	4	18	45	3	27	
	72	130	202	72	130	202	
Sensitivity:	75.0 %			62.5 %			
Specificity:	96.9 %			97.7 %			
Overall agreement:	89.1 %			85.1 %			

LIMITATIONS OF THE PROCEDURE

This assay 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 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

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