INSTRUCTION MANUAL

REF 3600

August 02, 2016

Anti-Intrinsic Factor

- 96 determinations -

IVD *In vitro* diagnostic device

Enzyme immunoassay for the determination of IgG autoantibodies to intrinsic factor in human serum

REF	Catalogue number	LOT	Batch code
	Consult accompanying documents		Manufactured by
	Temperature limitation		Use by
	Consult operating instruction		Biological risk

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INTENDED USE

Anti-intrinsic factor is used for the quantitative determination of IgG autoantibodies to intrinsic factor in human serum.

Intrinsic factor (IF), a glycoprotein containing sialic acid, plays an important role in the absorption of vitamin B_{12} (extrinsic factor) in the digestive tract. IF is produced by parietal cells located in the stomach mucosa. Following secretion into the stomach IF binds to vitamin B_{12} that was ingested with food. The vitamin B_{12}-IF complex is absorbed in the ileum by binding to a specific receptor to IF. After absorption vitamin B_{12} is released into the blood stream where it binds to transcobalamin.

Reduced production of IF and/or impairment of its transport function bring about a deficiency in vitamin B_{12} that leads to the development of Biermer's anemia.

The chronic atrophic gastritis of type A (5% of all forms) is characterized by autoimmune processes that lead to the destruction of parietal cells and the production of autoantibodies to both parietal cells and IF. The gastritis of type A occurs frequently in context with other autoimmune polyendocrinic disorders (e.g. Hashimoto's thyroiditis, Addison's disease, insulin-dependent diabetes mellitus). Patients suffering from gastritis of type A face an elevated risk of carcinoma.

According to their binding sites autoantibodies to IF are divided into two types. Type 1 autoantibodies interact with the binding site for vitamin B_{12} and, therefore, interfere with the binding of vitamin B_{12} to the IF in the stomach. Otherwise, type 2 autoantibodies block the binding of both IF and vitamin B_{12}-IF complexes to the specific receptor in the ileum by reacting with the corresponding site on the IF.

In contrast to RIA methods based on radioactive labeled vitamin B_{12} Anti-intrinsic factor Elisa determines both types of autoantibodies to IF and is not influenced by high concentrations of vitamin B_{12} in the sample.

Waters HM, Dawson DW, Horwarth JE, Geary CG: High incidence of type II autoantibodies in pernicious anaemia.
J Clin Pathol (1993) 46 (1) : 45-7

Waters HM, Smith C, Horwarth JE, Dawson DW, Delamore IW. New enzyme immunoassay for detecting total, type I, and type II intrinsic factor antibodies. J Clin Pathol (1989) 42 (3) : 307-12.

PRINCIPLE OF THE TEST

Anti-intrinsic factor is an enzyme immunoassay for the quantitative determination of IgG autoantibodies to intrinsic factor.

The antibodies of the calibrators, controls and diluted patient samples react with intrinsic factor immobilized on the solid phase of microtiter plates. Human recombinant intrinsic factor coated on the microtiter plate guarantees the specific binding of Anti-intrinsic factor IgG autoantibodies of the specimen under investigation. Following an incubation period of 60 min at room temperature (18…25°C), unbound serum components are removed by a washing step.

The bound antibodies react specifically with anti-human-IgG-antibodies conjugated to horse radish peroxidase (HRP). Following an incubation period of 30 min at room temperature, excessive conjugate is separated from the solid-phase immune complexes by an additional washing step.

The horse radish peroxidase 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 (H_2SO_4) 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.

PATIENT SAMPLES

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.

Samples are stable up to 3 days at 2-8°C, for extended storage freeze at -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.

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.***

TEST COMPONENTS FOR 96 DETERMINATIONS

A Ag 96	**Microtiter plate**, 12 breakable strips per 8 wells; coated with intrinsic factor (human recombinant)	1 vacuum sealed with desiccant
B BUF WASH 10x	**Concentrated wash buffer** sufficient for 1000 ml solution	100 ml concentrate capped white
C DIL	**Sample diluent**	100 ml ready for use capped black
D CONJ	**Conjugate** containing anti-human-IgG (sheep) coupled with HRP	15 ml ready for use capped red
E SOLN TMB	**Substrate** 3,3',5,5'-tetramethylbenzidine in citrate buffer containing hydrogen peroxide	15 ml ready for use capped blue
F H2SO4	**Stop solution** 0.25 M sulfuric acid 0.25M	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
N CONTROL	**Negative control** (diluted serum) conc.: see leaflet enclosed -	1 ml ready for use capped green

Materials required in addition

- micropipette 100 - 1000 µl
- micropipette 10 - 100 µl
- multi-channel pipette 50 - 200 µl
- 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
- tubes (2 ml) for sample preparation

Size and storage

Anti-Intrinsic Factor IgG 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 Anti-Intrinsic Factor 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.

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!

ASSAY PROCEDURE

- **Dilute patient sera with sample diluent (C) 1 + 100 (v/v), e.g. 10 µl serum + 1.0 ml of 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 calibrators (0 optional) 1 - 4
   100 µl control P (N optional)
   100 µl diluted patient samples
   into the respective wells.
3. Seal plate, incubate **60 min** at room temperature.
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. Seal plate, incubate **30 min** at room temperature.
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. Incubate **15 min** protected from light at room temperature.
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.

DATA PROCESSING

The standard curve is established by plotting the mean OD-values of the calibrators 0 (optional and 1 - 4 on the ordinate, y-axis, (lin. scale) versus their respective antibody concentrations on the abscissa, x-axis, (log. scale). Anti-Intrinsic Factor concentrations of the unknown samples are directly read off in U/ml against the respective OD values.

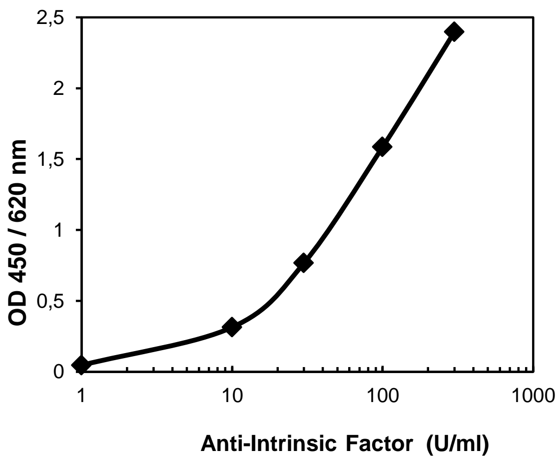
Using the recommended dilution of 1 + 100 (v/v) for patient's sera, no correction factor is necessary, as all other components of the kit are supplied accordingly.

The evaluation of Anti-Intrinsic Factor may be achieved also with computer assisted analysis software integrated in the photometers.

Example of typical assay results

well	OD (a)	OD (b)	OD(mean)	U/ml
Calibrator 0	0.047	0.045	0.046	**1**
Calibrator 1	0.320	0.308	0.314	**10**
Calibrator 2	0.770	0.764	0.767	**30**
Calibrator 3	1.579	1.593	1.586	**100**
Calibrator 4	2.397	2.396	2.397	**300**
Patient 1	1.208	1.190	1.202	**60**

TYPICAL STANDARD CURVE



Test validity

The test run is valid if:

- the mean OD of the calibrators 4 is ≥ 1.2
- Concentration of Control P see leaflet enclosed
- Control N is negative

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.

REFERENCE VALUES

Anti-Intrinsic Factor	U/ml
negative	< 10
positive	> 15
grey zone	10 – 15

Specimens with concentrations detected in the grey zone should be retested.

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

Limitations of Method

Healthy individuals should be tested negative by the Anti-Intrinsic Factor IgG. However, asymptomatic patients might attain positive results.

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.

CHARACTERISTIC ASSAY DATA

Calibration

No international reference material for this parameter is available thus the assay is calibrated in arbitrary units.

Linearity

Selected positive 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.

Sensitivity

The analytical sensitivity of this assay was determined at 1.0 U/ml.

Specificity

No cross reactivity to other autoantigens have been found.

Precision

Intraassay variability (n = 20)

sample	mean U/ml	CV (%)
1	181.3	4.2
2	60.3	3.3
3	18.9	3.6
4	5.1	3.7

Interassay variability (n = 10x5)

sample	mean U/ml	CV (%)
1	190.9	4.7
2	62.8	3.7
3	19.6	2.4
4	5.2	3.8

INCUBATION SCHEME

Anti-Intrinsic Factor (3600)

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

1	Bring all ready for use reagents to room temperature (18…25°C) before use.				
			calibrators	control	sera
2	Pipette	Calibrators (0 - 4) Controls (P, N) prediluted 1 + 100 patient sera	100 µl	100 µl	100 µl
3	Incubate		60 minutes at room temperature		
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		
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		
10	Pipette stop solution (F)		100 µl	100 µl	100 µl
11	Measure 450 nm versus 620 (690) nm				

SAFETY PRECAUTIONS

- **This kit is for in vitro use only**. Follow the working instructions carefully. GA GENERIC ASSAYS GmbH 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 Neolone M10 (< 0.05 % w/v) as preservative. 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.