Instruction Manual

REF **4067**

November 26th, 2015

Anti-Factor H

- 48 determinations -

CE

IVD *In vitro* diagnostic device

Enzyme immunoassay for the determination of IgG antibodies to complement factor H in human serum

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

GA GENERIC ASSAYS GmbH

Ludwig-Erhard-Ring 3

15827 Dahlewitz, Germany

Telephone: +49 (0) 33708 – 9286 - 0
Fax: +49 (0) 33708 – 9286 - 50

www.genericassays.com

INTENDED USE

Anti-Factor H is used for the quantitative determination of IgG antibodies to complement factor H in human serum for the diagnosis of atypical haemolytic-uraemic syndrome (HUS).

Hemolytic-uremic syndrome (HUS) is a disease of small blood vessels, characterized by hemolytic anemia, thrombocytopenia and acute renal failure. Most common cause is an infection with toxin forming Escherichia coli bacteria (Shiga toxin, Vero toxin). First symptom mainly is watery, sometimes bloody diarrhea, later extra intestinal manifestations are possible. Beside acute renal insufficiency neurological and cardiac complications may occur. Up to 10% of critical cases are lethal.

About 5% of HUS patients do not show diarrheal symptoms or other symptoms of E. coli infection. This so-called atypical HUS is based on a disorder of complement regulation, caused by genetic mutations or antibodies to complement factor H.

Literature:

Dragon-Durey MA, Loirat C, Cloarec S, Macher MA, Blouin J, Nivet H, Weiss L, Fridman WH, Fremeaux-Bacchi V: Anti-Factor H Autoantibodies associated with Atypical Hemolytic Uremic Syndrome. J Am Soc Nephrol 16: 555-563, 2005

Dragon-Durey MA, Sidharth KS, Bagga A, Blanc C, Blouin J, Ranchin B, Andre JL, Takagi N, Cheong HI, Hari P, Le Quintrec M, Niaudet P, Loirat C, Fridman WH, Fremeaux-Bacchi V: Clinical Features of Anti-Factor H Autoantibody-Associated Hemolytic Uremic Syndrome. J Am Soc Nephrol 21: 2180-2187, 2010

Loirat C, Fakhouri F, Ariceta G, Besbas N, Bitzan M, Bjerre A, Coppo R, Emma F, Johnson S, Karpman D, Landau D, Langman CB, Lapeyraque AL, Licht C, Nester C, Pecoraro C, Riedl M[7], van de Kar NC, Van de Walle J, Vivarelli M, Frémeaux-Bacchi V; for HUS International. An international consensus approach to the management of atypical hemolytic uremic syndrome in children. Pediatr Nephrol 31(1): 15-39, 2016

PRINCIPLE OF THE TEST

Anti- Factor H is an enzyme immunoassay for the quantitative determination of IgG antibodies to complement factor H.

The antibodies of the calibrators, controls, and diluted patient samples react with human recombinant complement factor H immobilized on the solid phase of microtiter plates. Following an incubation period of 60 min at room temperature (RT), unbound serum components are removed by a wash step.

The bound IgG antibodies react specifically with anti-human-IgG 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'-tetramethyl-benzidine (TMB) added into a blue product. The enzyme reaction is stopped by dispensing an acidic solution (HCl) into the wells after 15 min at RT 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.

PATIENT SAMPLES

Specimen collection and storage

Blood is taken by venipuncture. Serum is separated after clotting by centrifugation. Lipaemic, haemolytic or contaminated samples should not be run. 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.***

Diluted samples should be analysed instantly.

TEST COMPONENTS FOR 48 DETERMINATIONS

A Ag 48	**Microtiter plate**, 6 breakable strips per 8 wells coated with human recombinant complement factor H		1 vacuum sealed with desiccant
B BUF WASH	**Concentrated wash buffer** sufficient for 1000 ml solution	10x	100 ml concentrate capped white
C DIL	**sample diluent**		100 ml ready for use capped black
D CONJ	**Conjugate** containing anti-human-IgG- (goat) 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 sulphuric acid	0.25 M	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
- glassware
- 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
- distilled or de-ionized water

Size and storage

Anti-Factor H has been designed for 48 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-Factor H 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.

Wash buffer: Prepare a sufficient amount of wash solution by diluting the concentrated wash buffer 10 times with de-ionized 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 at 2 - 8 °C up to 30 days.

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 sample diluents 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 calibrators 0 - 4
   100 µl controls P, N (N optionally)
   100 µl diluted patient samples
   into the respective wells.
3. Incubate **60 min** at room temperature (18-25°C).
4. Decant, then wash each well **three** times using **300 µl** wash solution (made of B).
5. Add **100 µl** of conjugate (D) to each well.
6. Incubate **30 min** at room temperature (18-25°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. Incubate **15 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 OD at **450 nm** versus 620 or 690 nm within **30 min** after adding the stop solution.

DATA PROCESSING

We recommend log / lin processing for best results.

The standard curve is established by plotting the mean OD-values of the calibrators 0 - 4 on the ordinate, y-axis, (lin. scale) versus their respective anti-factor H concentrations on the abscissa, x-axis, (log. scale).

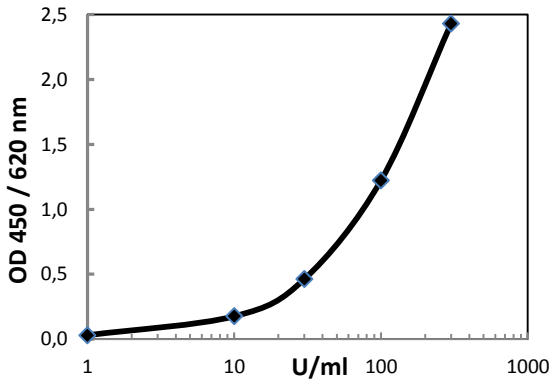
Antibody concentrations of the unknown samples are directly read off in U/ml against the respective OD values. Using the recommended dilution of 1 + 100 (v/v) for patient's sera, no correction factor is necessary, as all other components of the kit are supplied accordingly.

Anti-Factor H may be used also with Computer Assisted Analysis using software able to plot log/lin curves with four-parameter fit.

Example of Typical Assay Results

well	OD (a)	OD (b)	OD (mean)	U/ml
Calibrator 0	0.030	0.030	0.030	**1**
Calibrator 1	0.180	0.172	0.176	**10**
Calibrator 2	0.466	0.457	0.462	**30**
Calibrator 3	1.244	1.202	1.223	**100**
Calibrator 4	2.444	2.416	2.430	**300**
Patient 1	0.833	0.791	0.812	**59**

TYPICAL STANDARD CURVE



Specimens with an OD > calibrator 4, should be diluted with antibody negative serum and tested again. Results are multiplied with the dilution factor chosen.

Do not use this example for interpreting results.

Test validity

The test run is valid if:

- the mean OD of the calibrator 4 is ≥ 1.2
- the value of Control P is in the range indicated on the leaflet.
- 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-Factor H	(U/ml)
negative	**< 10**
positive	**≥ 10**

It is recommended that each laboratory establishes its own normal and pathological reference ranges for serum anti-factor H 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.

Limitations of Method

Healthy individuals should be tested negative by the Anti-Factor H. However, autoantibody 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.

CHARACTERISTIC ASSAY DATA

Calibration

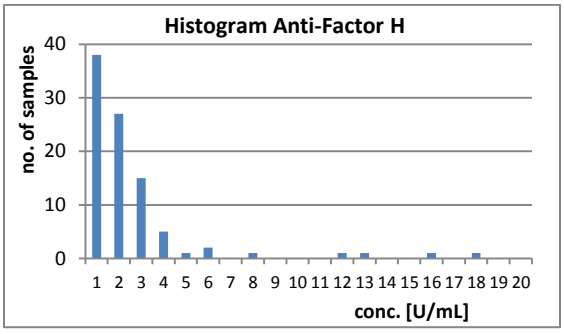
No international reference material for this parameter is available thus the assay is calibrated in arbitrary units U/ml.

Detection limit

The analytical sensitivity (lower detection limit, mean zero sample + 3 SD) of this assay was determined at 1.0 U/ml. The limit of quantitation (mean zero sample + 10 SD) has been found at 2 U/ml.

Specificity

Analyzing 93 sera of healthy donors, 4 samples have been found above the cut-off, leading to a specificity of 95.7%.



Sensitivity

From 13 sera of patients suffering from atypical HUS and positive result in a reference assay, 12 have been detected also positive with Anti-Factor H Elisa. The relative sensitivity is 92.3%.

Precision

Intra-assay (n = 20)		Inter-assay (n = 5 x 10)	
mean (U/ml)	**CV %**	**mean (U/ml)**	**CV %**
186.6	4.7	189.1	3.4
64.4	3.5	65.0	4.9
20.5	3.0	21.3	8.6

INCUBATION SCHEME

Anti-Factor H (4067)

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.				
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 (18-25°C)		
4	Wash		Decant, 3 x 300 µl (made of B)		
5	Pipette conjugate (D)		100 µl	100 µl	100 µl
6	Incubate		30 minutes at at room temperature (18-25°C)		
7	Wash		Decant, 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 (18-25°C)		
10	Pipette stop solution (F)		100 µl	100 µl	100 µl
11	Measure 450 nm versus 620 (690) nm within 30 min.				

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 (≤ 1.0 % v/v) and 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.