



# INSTRUCTION MANUAL

REF 5300

March 3<sup>rd</sup>, 2008

# Anti-Yersinia Blot IgG

- 27 determinations -



Immunoblot for the determination of  
IgG antibodies to *Yersinia enterocolitica*  
in human serum or plasma

<b>REF</b>	Catalogue number	<b>LOT</b>	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**

**Telefon: +49 (0) 33708-9286-0**  
**Fax: +49 (0) 33708-9286-50**

**www.genericassays.com**

## INTENDED USE

**Anti-Yersinia Blot IgG is used for the qualitative determination of IgG antibodies to *Yersinia enterocolitica* in human serum.**

*Yersinia* are gram negative rod bacteria belonging to the family of enteric bacteria. The genus *Yersinia* comprises three human pathogenic species: *Y. pestis*, *Y. pseudotuberculosis* and *Y. enterocolitica*. Up to 60 different serotypes are known of *Y. enterocolitica*. The incubation period is about 2 to 7 days, at most 10 days. The pathogens are mainly transferred indirectly through contaminated food (meat and sausages) and more infrequently through direct contact. The vector reservoir includes many mammalian species, whereas rodents, domestic, and farm animals are of special epidemiological importance.

The clinical symptoms of an infection with *Y. enterocolitica* and *Y. pseudotuberculosis* are comparable with each other. In infants an infection induces a self limited gastroenteritis frequently accompanied by vomiting and fever. Juveniles often react with a lymphadenitis characterized by acute abdominal pain often mistaken for appendicitis. Furthermore, yersiniosis in adults may cause ileitis and colitis. Immunopathologic complications often occur in the form of **reactive arthritis** (HLA-B27 associated) and erythema nodosum. The detection of specific antibodies is very important in such late stages of infection.

All human pathogenic *Yersinia* show a 70 kB plasmide coding for virulence factors including the genetic information for cytosolic, exterior, and membrane proteins. The main virulence markers are the high immunogenic *Yersinia* outer proteins (Yop). IgA, IgG and IgM antibodies are synthesized as a consequence of contact with these virulence factors. After a few months the titer of IgM and IgA antibodies decreases whereas IgG antibodies may persist for many years. In the case of immune complications and chronic yersiniosis IgA antibodies may be detectable over a long period.

Heesemann J: Entero-pathogene Yersinien: Pathogenitätsfaktoren und neue diagnostische Möglichkeiten. *Immun. Infekt.* 1990, 6, 186-191  
Fendler C, Laitko S, Sorensen H, Gripenberg-Lerche C, Groh A, Uksila J, Granfors K, Braun J, Sieper J: Frequency of triggering bacteria in patients with reactive arthritis and undifferentiated oligoarthritis and the relative importance of the tests used for diagnosis. *Ann Rheum Dis.* 2001, 60, 337-43

## PRINCIPLE of the TEST

Anti-Yersinia Blot IgG is a sensitive immunoblot for the determination of IgG antibodies to *Yersinia* outer proteins (Yops).

Purified Yops of *Yersinia enterocolitica* (Serotype O:3) were separated electrophoretically according to their molecular weight using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to a nitro-cellulose membrane. After blocking of free binding sites the membrane was cut into ready-for-use strips, provided in the kit.

Patient sera and strips are incubated in the test tray. During the first incubation *Yersinia* specific antibodies of the patient samples bind to the Yops immobilized on the solid phase of the strips. Following an period of 45 min unbound serum components are removed by a washing step.

The bound antibodies react specifically with anti-human-IgG conjugated to horse radish peroxidase (HRP). Following an incubation period of 45 min excessive conjugate is separated from the solid-phase immune complexes by an additional washing step.

Horse radish peroxidase converts the colorless substrate solution of 3,3'.5.5'-tetramethylbenzidine (TMB) added into a dark blue precipitating product. Different patterns of lines become visible defined by the antibodies in the individual serum samples. Strips are interpreted using the reference pattern provided in the kit and the cut-off line.

## 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.

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. The use of lipemic or hemolytic samples increases the background reaction and can lead to false positive results.

### 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:** *Neat patient samples have to be used and are diluted (1 + 100) in the incubation tray.*

### Size and Storage

Anti-Yersinia Blot IgG has been designed for 27 determinations.

The expiry date of each component is reported on its respective label. That of the complete kit on the box label.

Upon receipt, all components of the Anti-Yersinia Blot IgG have to be kept at 2...8°C, preferably in the original kit box.

After opening all kit components are stable for 4 weeks, provided proper storage conditions.

### Preparation before use

Allow all components to reach room temperature prior to use in the assay and take blot strips with a plastic pincer.

Prepare a sufficient amount of buffer solution by diluting the concentrated buffer 5 times (1 + 4) with deionized or distilled water. For each blot strip 11 ml of buffer solution is required. For example, dilute 15 ml of the concentrate with 60 ml of distilled water per strip. The buffer solution prepared is stable at 2...8°C up to 30 days.

Avoid exposure of the TMB substrate solution to light.

## TEST COMPONENTS for 27 determinations

<b>A</b>	<b>Blot strips</b>	27 Blot strips
<b>Ag</b>	27 numbered strips coated with Yops from <i>Yersinia enterocolitica</i>	
<b>B</b>	<b>Buffer, 5 fold</b>	2 x 35 ml
<b>BUF</b>	sufficient for 350 ml solution	concentrate
<b>WASH</b>		capped black
<b>5x</b>		
<b>D</b>	<b>Conjugate</b>	50 ml
<b>CONJ</b>	anti-human-IgG (goat), conjugated with horse radish peroxidase	ready to use
<b>G</b>		capped red
<b>E</b>	<b>Substrate</b>	50 ml
<b>SOLN</b>	3.3'.5.5'-tetramethylbenzidine in citrate buffer containing hydrogen peroxide (black bottle)	ready to use
<b>TMB</b>		capped blue
<b>F</b>	<b>Incubation tray for 9 strips</b>	2 x
<b>G</b>	<b>Lot specific blot pattern</b>	1 x

### Materials required

- micropipette 100 - 1000 µl
- micropipette 10 - 100 µl
- pipette tips
- multi-channel pipette
- trough for multi-channel pipette
- graduated cylinders
- distilled or de-ionized water
- 2 ml tubes
- horizontal plate shaker
- plastic pincers

## ASSAY PROCEDURE

**Avoid any time shift during pipeting of reagents or neat samples.**

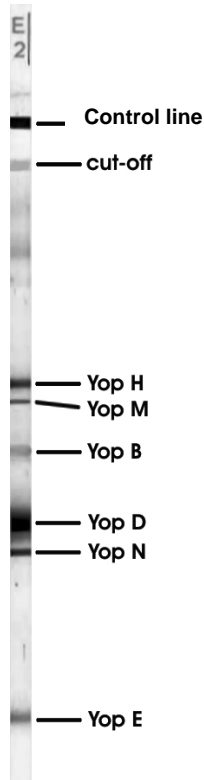
1. Bring all reagents to room temperature (RT) (20...25°C) before use. Mix gently without causing foam.
2. Place the strips (A) with the reactive side up (labels on top) Note number of serum sample and strips as well as lot number and antibody isotype (IgG/ IgA). Dispense **1.5 ml** of buffer solution (made of B) into the respective wells.
3. Seal plate, incubate 5 min on an horizontal shaker.
4. Add **15 µl** neat patient serum to the respective wells. Cover tray and incubate 45 min while shaking at RT (20...25°C).
5. Decant or aspirate, wash each well three times 5 min with **1.5 ml** buffer solution (made of B) while shaking. (Discard the solution contained in the wells by slowly inverting the plate. Dry the edges of the tray with absorbent paper.)
6. Add **1.5 ml ready to use conjugate** anti-human IgG POD (D) to each well.
7. Cover tray, incubate 45 min at RT (20...25°C) while shaking.
8. Decant or aspirate, wash each well three times 5 min with **1.5 ml** buffer solution (made of B) while shaking. (Discard the solution contained in the wells by slowly inverting the plate. Dry the edges of the tray with absorbent paper.)
9. Add **1.5 ml** of substrate (E) to each well.
10. Cover plate, incubate 10 min on an horizontal shaker at RT (20...25°C) till a blue color develops.
11. Decant or aspirate, wash each well three times with 2 ml distilled water to stop the reaction.\*
12. Collect the strips from the wells and dry the membrane by pressing briefly the reactive side of the strip on the absorbent paper. After approximately 30 min the strips are interpreted by means of the lot specific pattern and the cut-off line.

**\* Some sera show unspecific background staining of the strips. The reaction has to be stopped quickly by rinsing the strips three times with 2 ml aqua dest..**

## DATA PROCESSING

### Evaluation criteria

Results should be interpreted only if the specific control line is clearly and the cut-off line weakly visible.



### Evaluation

Only dried blot strips should be interpreted. The interpretation of the blot strips is based upon the comparison of the developed protein lines with the lot specific pattern and the cut-off line. The relevance of the diagnostic important proteins is mentioned in the table below.

	Anti-Yersinia IgG
negative	<ul style="list-style-type: none"> <li>• no line</li> <li>or</li> <li>• lines with intensity &lt; cut-off</li> <li>or</li> <li>• one line or YopD line with intensity &gt; cut-off <b>but</b> &lt; control line</li> </ul>
borderline	<ul style="list-style-type: none"> <li>• two lines with intensity &gt; cut-off</li> <li>or</li> <li>• intensity YopD &gt; cut-off <b>but</b> &lt; control line <b>and</b> one line with intensity &gt; cut-off</li> </ul>
positive	<ul style="list-style-type: none"> <li>• intensity YopD <math>\geq</math> control line</li> <li>or</li> <li>• at least three lines with intensity <math>\geq</math> cut-off</li> </ul>

### Limits of the method

The in vitro results should always be interpreted in context with the clinical status of the patient. Repeated testing over several weeks is recommended in order to discriminate an active infection from long term persistent antibody titer without clinical implication.

In all immunologic methods bacteriological or fungal contaminations of the kit components and samples as well as cross-reactivity may cause false results.

Insufficient washing or time management may lead to false negative results.

## CHARACTERISTIC ASSAY DATA

### Relevance of the IMMUNOBLOT lines

Nomenclature of antigens	Antibody relevance
48 kD, Yop H	all listed proteins (Yops) are highly specific for human pathogenic Yersiniae
45 kD, Yop M	
42 kD, Yop B	
36 kD, Yop D	
34 kD, Yop N	
27 kD, Yop E	

The nomenclature of the lines concerning molecular weight, specificity and validity in different disease stages is interpreted consistently in literature.

### Diagnostic sensitivity and specificity

For the determination of the diagnostic sensitivity and specificity 180 sera of patients suspected to Yersiniosis were investigated in the Anti-Yersinia IgG Blot and another commercial assay.

		comparative assay		
		positive	borderline	negative
GA blot	positive	88	42	14
	borderline	1	12	11
	negative	1	2	9

Remarks:

## INCUBATION SCHEME

# Anti-Yersinia Blot IgG (5300)

Blot strips, buffer solution and substrate can be used for the **Anti-Yersinia Blot IgA (5200)**

1.	Bring all reagents and the requested number of strips to RT (20...25°C)
2.	Place the strips with the reactive side upside in the tray and dispense <b>1.5 ml of buffer solution</b> (made of B) into the respective wells
3.	Seal plate and incubate while shaking <span style="float: right;">5 min, RT (20...25°C)</span>
4.	Pipette <b>15 µl neat patient serum</b>
5.	Incubate while shaking <span style="float: right;">45 min, RT (20...25°C)</span>
6.	Decant, wash strips while shaking <span style="float: right;">3 x 5 min with 1.5 ml (made of B)</span>
7.	Pipette <b>1.5 ml ready to use anti-human IgG conjugate (D)</b>
8.	Incubate while shaking <span style="float: right;">45 min, RT (20...25°C)</span>
9.	Decant, wash strips while shaking <span style="float: right;">3 x 5 min with 1.5 ml (made of B)</span>
10.	Pipette <b>1.5 ml substrate (E)</b>
11.	Incubate while shaking <span style="float: right;">10 min, RT (20...25°C)</span>
12.	Decant, wash strips with aqua dest. <span style="float: right;">3 x with 2 ml</span>

## 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 Kathon (1.0% v/v) as preservative. They must not be swallowed or allowed to come into contact with skin or mucosa.
- 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.