



# **HELICO BLOT 2.1**

## **WESTERN BLOT ASSAY**

**INSTRUCTION MANUAL  
FOR RESEARCH USE ONLY  
NOT TO BE USED IN DIAGNOSTIC PROCEDURES**

Revision date: 08/99

# HELICO BLOT 2.1

For the detection of antibodies to *Helicobacter pylori* in human serum or plasma, with the added indication of current infection status.

## TRADENAME AND INTENDED USE

The Genelabs Diagnostics (GLD) **HELICO BLOT 2.1** Western Blot kit assay is a qualitative assay for the detection of IgG antibodies to *Helicobacter pylori* (*H. pylori*) in human serum or plasma. It is an improved version of HELICO BLOT 2.0 where in addition to bacterial lysate, there is a recombinant antigen with high predictive value for the indication of current *H. pylori* infection. The product is intended for use as a serological test for the detection of both current and past infection with *H. pylori*. Unlike an ELISA, the HELICO BLOT 2.1 allows for the detection of antibodies to specific proteins of *H. pylori*, including antigens associated with pathology such as CagA and VacA.

This kit is supplied for research purposes only. It is not intended for the use in the diagnosis or prognosis of disease. In particular, this test cannot be used to evaluate blood specimens for the purposes of donor screening, or as a confirmatory diagnostic.

## INTRODUCTION

*H. pylori* was first isolated and characterized in 1983 by Warren and Marshall. The organism was characterized as a spiral urease-producing bacteria which colonizes the interface between the gastric epithelial cell surface and the overlying mucus layer. A high correlation has been found between the presence of this organism and gastritis, gastric ulcers and duodenal ulcers. A correlation between long term infection with *H. pylori* and gastric cancer has also been implicated. *H. pylori* is now considered a major etiological factor in the development of gastritis and peptic ulcer. Recent ulcer treatment regimens incorporating antibiotics and proton pump inhibitors have successful eradication rates of greater than 90%. The success in treatment of ulcers due to *H. pylori* and the risk of developing gastric cancer if chronic infection is left untreated point towards the need for reliable early diagnosis and appropriate treatment.

## CHEMICAL AND BIOLOGICAL PRINCIPLES OF THE PROCEDURES

The GLD HELICO BLOT 2.1 is a serological test using a Western Blot made from bacterial lysate of an ulcer causing type strain of *H. pylori* and a recombinant antigen of *H. pylori*. The proteins in the lysate are electrophoretically separated and transferred to nitrocellulose and the recombinant antigen slotted onto the nitrocellulose membrane. Individual strips are incubated with diluted serum or plasma specimens or controls. Specific antibodies to the various antigens, if present, will bind to the *H. pylori* antigens on the strips respectively. The strips are washed to remove unbound antibodies. Specifically bound antibodies are visualized using a series of reaction with the goat anti-human IgG conjugated with alkaline phosphatase and the substrate BCIP/NBT. The product allows the user to differentiate the reactivity to each of the various *H. pylori* antigens.

## KIT COMPONENTS

- |     |                                                                                                                                                                                                          |                                 |
|-----|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------|
| 1.  | <b>NITROCELLULOSE STRIPS</b><br>Western blot strips containing <i>H. pylori</i> lysate, a current infection marker band and a serum addition control band .                                              | Available in<br>18 or 36 strips |
| 2.  | <b>REACTIVE CONTROL</b><br>Inactivated human serum with IgG antibodies to <i>H. pylori</i> . Non-reactive for anti-HCV, anti-HIV1/2 and HBsAg. Contains sodium azide and thimerosal as preservatives.    | 1 vial<br>(80 ul)               |
| 3.  | <b>NON-REACTIVE CONTROL</b><br>Normal human serum negative for antibodies to <i>H. pylori</i> . Non-reactive for anti-HCV, anti-HIV1/2 and HBsAg. Contains sodium azide and thimerosal as preservatives. | 1 vial<br>(80 ul)               |
| 4.  | <b>STOCK BUFFER CONCENTRATE (10X)</b><br>Tris buffer with heat inactivated animal and non-animal proteins. Contains thimerosal as preservative.                                                          | 1 bottle<br>(20 ml)             |
| 5.  | <b>WASH BUFFER CONCENTRATE (20X)</b><br>Tris buffer with Tween-20. Contains thimerosal as preservative.                                                                                                  | 1 bottle<br>(70 ml)             |
| 6.  | <b>CONJUGATE</b><br>Goat anti-Human IgG conjugated with alkaline phosphatase.                                                                                                                            | 1 vial<br>(120 ul)              |
| 7.  | <b>SUBSTRATE</b><br>Solution of 5-bromo-4-chloro-2-indolyl-phosphate (BCIP) and nitroblue tetrazolium (NBT)                                                                                              | 1 bottle<br>(100 ml)            |
| 8.  | <b>BLOTTING POWDER</b><br>Non-fat dry milk                                                                                                                                                               | 10 packets<br>(1g each)         |
| 9.  | Incubation Trays, 36 wells each<br>or 9 wells each                                                                                                                                                       | 1 tray<br>2 or 4 trays          |
| 10. | Instruction Manual                                                                                                                                                                                       | 1 copy                          |
| 11. | Forceps                                                                                                                                                                                                  | 1 pair                          |

N.B. Volume of reagents provided are sufficient for 4 separate runs.

## PRECAUTIONS TO USERS

**Caution:** HANDLE ALL ASSAY SPECIMENS, POSITIVE AND NEGATIVE CONTROLS AS POTENTIALLY INFECTIOUS AGENTS

1. Do not interchange reagents between kit lots.
2. For *research use only not to be used in diagnostic procedures*.
3. Do not use kit components beyond the expiry date.
4. Avoid microbial contamination of reagents when opening and removing aliquots from the original vials or bottles.
5. Gloves and laboratory coats must be worn.
6. Do not pipette by mouth.
7. Wipe any spills quickly and thoroughly with sodium hypochlorite solution.
8. Autoclave all used and contaminated materials at 121°C at 15 p.s.i. for 30 minutes before disposal.
9. It is highly recommended that this assay be performed in biohazard cabinet.
10. Decontaminate all used chemicals and reagents in sodium hypochlorite solution.
11. We do not recommend re-use of trays supplied with the kit.

## STORAGE INSTRUCTIONS

1. Antigen strips
  - Avoid unnecessary exposure of antigen strips to light.
2. Reagents
  - Store all reagents at 2-8°C.
  - For best results, dispense all reagents while cold and return to 2-8°C storage as soon as possible.

**CAUTION :** Avoid unnecessary exposure of substrate to light.

## MATERIALS REQUIRED BUT NOT PROVIDED

- Rocker platform
- Pipettors and tips
- Aspirator with sodium hypochlorite trap
- 56°C water bath (optional)

## **SPECIMEN HANDLING AND STORAGE (OPTIONAL)**

Patients' sera can be inactivated but this is not a requirement for optimal test performance.

Inactivate as follows:

1. Loosen caps of serum containers.
2. Heat sera to 56°C for 30 minutes in water bath.
3. Allow sera to cool before retightening caps.
4. Sera can be stored frozen until analysis.

We recommend that the patients' sera should not undergo repeated freeze-thaw cycles prior to testing.

## **PREPARATION OF REAGENTS**

### **1. DILUTED WASH BUFFER**

- a) Dilute 1 volume of WASH BUFFER CONCENTRATE (20X) with 19 volumes of reagent grade water. Mix well.

### **2. BLOTTING BUFFER**

- a) BLOTTING BUFFER should be prepared fresh prior to use.
- b) Dilute 1 volume of STOCK BUFFER CONCENTRATE (10X) with 9 volumes of reagent grade water. Mix well.
- c) Add 1g of BLOTTING POWDER to every 20 ml of DILUTED STOCK BUFFER prepared in step 2(b) above. Mix well.

### **3. WORKING CONJUGATE SOLUTION**

- a) Prepare WORKING CONJUGATE SOLUTION by diluting CONJUGATE 1:1000 in BLOTTING BUFFER, for example, 10 ul Conjugate to 10 ml blotting buffer.
- b) WORKING CONJUGATE SOLUTION should be prepared fresh prior to use.

### **4. SUBSTRATE SOLUTION (Ready to use)**

- a) Dispense directly the required volumes from the bottle. Use a clean pipette. Cap tightly after use.

## RECOMMENDED ASSAY PROCEDURE

**NOTE:**      **Aspirate all used chemicals and reagents into a trap containing sodium hypochlorite.**

1. Using forceps, carefully remove the required number of STRIPS from the tube and place numbered side up into each well. Include strips for one reactive and one non-reactive control.
2. Add 2 ml of DILUTED WASH BUFFER to each well.
3. Incubate the strips for at least 5 minutes at room temperature (25±3°C) on a rocking platform. Remove buffer by aspirations.
4. Add 2 ml of BLOTTING BUFFER to each well followed by 20 ul each of patients' sera or controls to appropriate wells.
5. Cover the tray with the cover provided and incubate for 1 hour at room temperature (25±3°C) on a rocking platform.
6. Carefully uncover the tray to avoid splashing or mixing of samples. Aspirate the mixtures from the wells. Change aspirator tips between the samples to avoid cross contamination.
7. Wash each strip 3 times with 2 ml of DILUTED WASH BUFFER, allowing 5 minutes soak on the rocking platform between each wash.
8. Add 2 ml of WORKING CONJUGATE SOLUTION to each well. Cover tray and incubate for 1 hour at room temperature (25±3°C) on a rocking platform.
9. Remove Conjugate from the wells. Wash as in step 7.
10. Add 2 ml of SUBSTRATE SOLUTION to each well. Cover tray and incubate for 15 minutes on the rocking platform.
11. Remove the substrate and rinse the strips several times with reagent grade water to stop the reaction.
12. Using forceps, gently remove strips onto the paper towels. Cover with paper towels and dry.
13. Mount strips on worksheet (non-absorbent white paper) for visual reading or on appropriate template for automated reading (i.e. AutoScan). For storage, keep the strips in the dark. Do not apply adhesive tape over the developed bands.

<b>AMOUNT OF REAGENTS REQUIRED FOR VARIOUS NUMBERS OF STRIPS</b>							
NUMBER OF STRIPS TO BE USED							
REAGENTS	3	6	9	15	20	27	36
1X WASH BUFFER (ml)	60	100	140	240	300	400	520
1X BLOTTING BUFFER (ml)	20	40	60	80	100	120	160
CONJUGATE (ul)	11	17	23	35	45	59	77
SUBSTRATE (ml)	11	17	23	35	45	59	77
BLOTTING POWDER (g)	1	2	3	4	5	6	8

## REFERENCE STANDARDS

The Non-Reactive and Reactive controls must be run with every assay. In order for the results obtained from any assay to be considered valid, the following conditions must be met.

### 1. NON-REACTIVE CONTROL

The non-reactive control must not react with any proteins used in the criteria for interpretation. There may be bands or a broad band appearing in the 60kD region, but reactivity with proteins in this region alone is not specific for *H.pylori* (see Figure 1).

### 2. REACTIVE CONTROL

All relevant molecular weight bands must be evident. Figure 1 provides a guide to the relative positioning of bands visualized on the Genelabs Diagnostics HELICO BLOT 2.1. The bands are 116kD(CagA), 89kD(VacA), 37kD, 35kD, 30kD(UreA) and 19.5kD. The current infection marker band (below the serum control band) must be evident.

### 3. The serum addition control band must be present on all strips. This band serves as an internal control for sample and reagent additions.

## IDENTIFICATION OF BANDS

Record sample's identity number, and other appropriate information onto the provided report sheet. A NON-REACTIVE control and a REACTIVE control must be run with each assay.

The serum control band serves as a check for serum and reagent (conjugate and substrate) additions in the assay. Absence of this band on a strip would indicate that no test serum or conjugate or substrate has been added, or other operational errors.

EACH strip is compared to the strips used with the NON-REACTIVE and the REACTIVE control for the assay. Use the REACTIVE control strip to identify bands on the patients' strips. Record the appropriate bands on the patients' strips onto the provided report sheet.

### RECOMMENDED CRITERIA:

The recommended criteria for Genelabs HELICO BLOT 2.1 has been designed such that all of the bands which are used have high specificity and are easy to locate within the pattern. The recommended criteria for determining a sample as *H. pylori* seropositive is any one of the following conditions:

1. 116kD (CagA) positive, where CagA has to be present with one or more of the following bands: 89kD (VacA), 37kD, 35kD, 30kD (UreA) and 19.5kD together, OR with current infection marker.
2. Presence of any one band at 89kD, 37kD or 35kd, with or without current infection marker.
3. Presence of both 30kD and 19.5kD with or without current infection marker.

In general, individuals which have infections with *H. pylori* would have reactivity to several of these and other bacterial proteins on the blot. In addition, individuals who have current infections with *H. pylori* will most likely have reactivity to the current infection marker band. In several populations

tested, the positive predictive value\* of the current infection marker band compared against urea breath test (UBT) or other invasive tests (histology, culture, urease test) is 85-94%. The high predictive value for the current infection marker therefore serves as a quick reference for the current infection status.

Samples which meet the above criteria for positive should be reported as POSITIVE. Samples which have no reactivity to any bands except serum control band or reactivity which does not meet the criteria for positive should be reported as NEGATIVE.

\* Positive predictive value =  $TP / (TP+FP)$  where TP=number of true positives, FP=number of false positives.

## LIMITATIONS OF THE PROCEDURE

Optimal assay performance requires strict adherence to the recommended assay procedure described. Deviation from the recommended procedure may lead to aberrant results. A NEGATIVE result does not exclude the possibility of exposure to or infection with *H. pylori*. A NEGATIVE result for the current infection marker does not exclude the possibility that one is currently infected due to variations in the prevalence of antibody response to the current infection marker in different geographical populations.

There is much heterogeneity among various isolates of *H. pylori* for levels of protein expression of the various antigens, and for sequence homology as well. The antibody response of various individuals can be quite diverse. There is very good sensitivity and specificity for the 7 bacterial proteins used in the criteria for interpretation with infection, regardless of clinical status and the strain of *H. pylori* which has been used in the blot is useful for diagnosis of cases worldwide.

## PERFORMANCE

Patients were tested using the HELICO BLOT 2.1 test kit. Results were compared against gold standard tests for active infection (histology, culture, rapid urease test or urea breath test [UBT]). For gold standard positives to be considered positive, at least 2 out of the 3 tests for the above have to be positive. For gold standard negatives, all of the performed test, if performed, should be negative.

Combined total number of positive subjects = 48

Combined total number of negative subjects = 56

Compared against histology, culture, rapid urease test and/or urea breath test(UBT):

Sensitivity = 96%

Specificity = 95%

Positive predictive value for current infection marker:

Compared against gold standard active infection tests = 94%

Compared against UBT only = 91%

## TECHNICAL INQUIRIES

Should there be a technical inquiry, please do the following:

1. Note the kit lot number, strip lot number and their expiry dates.
2. Retain the kit and the results that were obtained.
3. Contact the nearest Genelabs office or your local distributor.

## LIMITED EXPRESSED WARRANTY DISCLAIMER

The manufacturer makes no warranty other than that the test kit will function as a research use only assay within the specifications and limitations described in the Product Instruction Manual when used in accordance with the instructions contained therein. The manufacturer disclaims any warranty, expressed or implied, including such expressed or implied warranty with respect to merchantability, fitness for use or implied utility for any purpose. The manufacturer is limited to either replacement of the product or refund of the purchase price of the product. The manufacturer shall not be liable to the purchaser or third parties for any damage, injury or economic loss howsoever caused by the product in the use or in the application thereof.

## REFERENCES

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FIGURE 1

