HELICO BLOT 2.1

WESTERN BLOT ASSAY

INSTRUCTION MANUAL

FOR RESEARCH USE ONLY

NOT TO BE USED IN DIAGNOSTIC PROCEDURES

Revision date: 08/99

Trial Version

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 evaluated
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.

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KIT COMPONENTS

1. **NITROCELLULOSE STRIPS** Available in
Western blot strips containing *H. pylori* lysate, 18 or 36 strips
a current infection marker band and a serum
addition control band .
2. **REACTIVE CONTROL** 1 vial
Inactivated human serum with IgG antibodies to (80 ul)
H. pylori. Non-reactive for anti-HCV, anti-HIV1/2
and HBsAg. Contains sodium azide and
thimerosal as preservatives.
3. **NON-REACTIVE CONTROL** 1 vial
Normal human serum negative for antibodies to (80 ul)
H. pylori. Non-reactive for anti-HCV, anti-HIV1/2
and HBsAg. Contains sodium azide and
thimerosal as preservatives.
4. **STOCK BUFFER CONCENTRATE (10X)** 1 bottle
Tris buffer with heat inactivated animal and (20 ml)
non-animal proteins. Contains thimerosal as preservative.
5. **WASH BUFFER CONCENTRATE (20X)** 1 bottle
Tris buffer with Tween-20. Contains thimerosal (70 ml)
as preservative.

6. **CONJUGATE** 1 vial
Goat anti-Human IgG conjugated with (120 ul)
alkaline phosphatase.
7. **SUBSTRATE** 1 bottle
Solution of 5-bromo-4-chloro-2-indolyl-phosphate (100 ml)
(BCIP) and nitroblue tetrazolium (NBT)
8. **BLOTTING POWDER** 10 packets
Non-fat dry milk (1g each)
9. Incubation Trays, 36 wells each 1 tray
or 9 wells each 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.

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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)

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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.

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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.

AMOUNT OF REAGENTS REQUIRED FOR VARIOUS NUMBERS OF STRIPS

	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

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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

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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.
S 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%

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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.

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of *H.pylori* infection. Gastroenterol. (116): A235.

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as visualized with
a) Non-reactive Control
b) Reactive Control
CagA (116kD)
VacA (89kD)
Urease B (61kD)
Heat shock protein B (58kD)
37kD
35kD

Urease A (30kD)
19.5kD
Serum control band
Current infection marker
A B