HELICO BLOT 2.1

WESTERN BLOT ASSAY

Instructions For Use

CE

REVISION DATE: 2016-06
MAP0011-ENG-4

Note: Changes Highlighted.

REF (18 tests kit) : 12420-018
(36 tests kit) : 12420-036

NAME AND INTENDED USE

The **MP Diagnostics 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, with the added indication of current infection status. 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 **MP Diagnostics 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.

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.

DESCRIPTION OF SYMBOLS USED

The following are graphical symbols used in or found on **MP Diagnostics** products and packaging. These symbols are the most common ones appearing on medical devices and their packaging. Some of the common symbols are explained in more detail in the European and International Standard EN ISO 15223: 2012.

	Use by *Synonym for this :* Expiry Date	IVD	*In vitro* diagnostic medical device
LOT	Batch Code *Synonyms for this are:* Lot Number Batch Number	REF	Catalogue Number
	Temperature Limitation		Attention. See Instructions for Use
	Manufacturer	EC REP	Authorised Representative in the European Community
Σ	Contains sufficient for <n> tests		
	Do not reuse		Consult Instructions for Use

CHEMICAL & BIOLOGICAL PRINCIPLES OF THE PROCEDURE

The **MP Diagnostics 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

	Component Description	Quantity Provided
ANTIGEN STRIPS	**NITROCELLULOSE STRIPS** Incorporated with *H. pylori* lysate, a current infection marker (CIM) and a serum control band. Keep dry and away from light.	Available in 18 or 36 strips
CONTROL —	**NON-REACTIVE CONTROL** Inactivated normal human serum negative for antibodies to *H. pylori.* Non-reactive for anti-HCV, anti-HIV 1/2, and HBsAg. Contains sodium azide and thimerosal as preservatives.	1 vial (80 µl)
CONTROL +	**REACTIVE CONTROL** Inactivated human serum with high titer antibodies to *H. pylori.* Non-reactive for anti-HCV, anti-HIV 1/2 and HBsAg. Contains sodium azide and thimerosal as preservatives.	1 vial (80 µl)
BUF STOCK 10x	**STOCK BUFFER CONCENTRATE (10x)** Tris buffer with heat inactivated normal goat serum. Contains thimerosal as preservatives.	1 bottle (20 ml)
BUF WASH 20x T	**WASH BUFFER CONCENTRATE (20x)** Tris with Tween-20. Contains thimerosal as preservative.	1 bottle (70 ml)
CONJUGATE	**CONJUGATE** Goat anti-human IgG conjugated with alkaline phosphatase. Contains sodium azide as preservative.	1 vial (120 µl)
SUBS BCIP / NBT	**SUBSTRATE** Solution of 5-bromo-4-chloro-3-indolyl-phosphate (BCIP) and nitroblue tetrazolium (NBT).	1 bottle (100 ml)
POWDER BLOTTING	**BLOTTING POWDER** Non-fat dry milk	10 packets (1g each)
	Incubation tray*	
	Instructions For Use	1 copy
	Forceps	1 pair

Note : Volume of reagents provided is sufficient for 4 separate runs.

* Incubation trays provided but packed separately from the kit.

WARNINGS AND PRECAUTIONS

1. For *in vitro* diagnostic use only.
2. For Professional use only.
3. Please refer to the product labelling for information on potentially hazardous components.

HEALTH AND SAFETY INFORMATION

⚠ **CAUTION:** This kit contains materials of human origin. No test method can offer complete assurance that human blood products will not transmit infection.

HANDLE ASSAY SPECIMENS, REACTIVE AND NON-REACTIVE CONTROLS AS POTENTIALLY INFECTIOUS AGENTS. It is recommended that kit components and test specimens be handled using good laboratory working practices. They should be disposed off in accordance with established safety procedures.

The ***Reactive Control*** and ***Non-Reactive Control*** contain Thimerosal and Sodium azide while **Stock Buffer Concentrate** and **Wash Buffer Concentrate** contain Thimerosal and Conjugate contains Sodium azide. Sodium azide can react with copper and lead used in some plumbing systems to form explosive salts. The quantities used in this kit are small, nevertheless when disposing of azide-containing materials they should be flushed away with relatively large quantities of water to prevent metal azide buildup in plumbing system.

Pursuant to EC regulation 1272/2008 (CLP), hazardous components are classified and labelled as follows:

Component:	**Nitrocellulose strips**
Signal Word:	**Danger**
Pictogram:	
Hazard Statements:	H228 Flammable solid
Precautionary Statements:	P210 Keep away from heat/ sparks/open flames/hot surfaces. – No smoking. P280 Wear protective gloves/protective clothing/ eye protection/face protection.
Supplemental Statements:	EUH210 Safety Data Sheet is available on request
Contains:	100% Nitrocellulose

Component:	**STOCK BUFFER CONCENTRATE (10x) WASH BUFFER CONCENTRATE (20x)**
Signal Word:	Warning
Pictogram:	
Hazard Statements:	H373 May cause damage to organs through prolonged or repeated exposure
Precautionary Statements:	P260 Do not breathe dust/ fume/gas/mist/vapours/ spray. P501 Dispose of contents/ container in accordance with local/regional/national/ international regulations.
Supplemental Statements:	EUH210 Safety Data Sheet is available on request
Contains:	0.1% Thimerosal

1. Avoid microbial contamination of reagents when opening and removing aliquots from the original vials or bottles.
2. Do not pipette by mouth.
3. Handle test specimens, nitrocellulose strips, Reactive and Non-Reactive Controls as potentially infectious agents.
4. Wear laboratory coats and disposable gloves while performing the assay. Discard gloves in bio-hazard waste-bags. Wash hands thoroughly afterwards.
5. It is highly recommended that this assay be performed in a biohazard cabinet.
6. Keep materials away from food and drink.
7. In case of an accident or contact with eyes rinse immediately with plenty of water and seek medical advice.
8. Consult a physician immediately in the event that contaminated materials are ingested or come in contact with open lacerations, or other breaks in the skin.
9. Wipe spills of potentially infectious materials immediately with absorbent paper and swab the contaminated area with 1% sodium hypochlorite solution before work is resumed. Sodium hypochlorite should not be used on acid containing spills unless the area is wiped dry with absorbent paper first. Material used (including disposable gloves) should be disposed off as potentially biohazardous material. Do not autoclave material containing sodium hypochlorite.
10. Autoclave all used and contaminated materials at 121°C at 15 p.s.i. for 30 minutes before disposal. Alternatively, decontaminate materials in 5% sodium hypochlorite solution for 30-60 minutes before disposal in biohazard waste-bags.
11. Decontaminate all used chemicals and reagents by adding sufficient volume of sodium hypochlorite to make a final concentration of at least 1%. Leave for 30 minutes to ensure effective decontamination.
12. We do not recommend re-use of incubation trays.

ANALYTICAL PRECAUTIONS

1. Optimal assay performance requires **STRICT ADHERENCE** to the assay procedure described in this Instructions For Use. Deviations from the procedure may lead to aberrant results.
2. **DO NOT MODIFY OR SUBSTITUTE REAGENTS FROM ONE KIT LOT TO ANOTHER.** Controls, conjugate and Western Blot strips are matched for optimal performance. Use only the reagents supplied with the kit.
3. Do not use kit components beyond the expiry date printed on the kit box.
4. Avoid microbial contamination of the reagents,when opening and removing aliquots from the original vials or bottles. As this will prematurely reduce the shelf life of the kits and give erroneous results. Use aseptic techniques including pipettes or disposable pipette tips when drawing aliquots from vials.
5. The kit controls should be assayed concurrently with patients' samples for each test run.
6. Use a new pipette tip for each specimen aliquot to prevent cross contamination.
7. For best results dispense all reagents while cold and return to 2°C to 8°C storage as soon as possible.
8. It is recommended that glassware to be used with the reagents should be washed with 2M hydrochloric acid and rinsed thoroughly with distilled or deionised water prior to use.
9. Use only reagent grade quality, deionised or distilled water to dilute reagents.
10. All reagents must be mixed well before use.
11. Working Conjugate solution, Wash Buffer and Blotting Buffer should be **prepared fresh prior to use.**
12. The Working Conjugate solution should be prepared using a polypropylene container or beaker.
13. Do not expose reagents or perform test in an area containing a high level of chemical disinfectant fumes (e.g. hypochlorite fumes) during storage or during incubation steps. Contact inhibits colour reaction. Also do not expose reagents to strong light.
14. The assay should preferably be performed at room temperature (25°C ± 3°C).
15. Make sure that the test strips are laid with the numbers on the strips facing upwards.
16. For Western Blot Assay, it is important to use a rocking platform shaker and not a rotary shaker. Otherwise, performance of the kit will be compromised. The recommended speed and tilt angle of the shaker are 12 to 16 cycles per minute, and 5 to 10 degrees, respectively.
17. Ensure that automated equipment if used is validated before use.
18. Ensure that the specimens are added away from the strip. Tray can be tilted and specimen added where the buffer is collected at lower end. This prevents dark spot formation due to specimen addition on the strip.
19. Avoid the use of self-defrosting freezers for the storage of reagents and samples.

STORAGE

1. Store MP Diagnostics HELICO BLOT 2.1 kit and its components at 2°C to 8°C when not in use.
2. All test reagents and strips when stored at 2°C to 8°C, are stable until the expiry date given on the kit. Do not freeze reagents.

A. **Antigen strips**
- Avoid unnecessary exposure of antigen strips to light.

B. **Reagents**
- Store reagents in their original vials or bottles, and they should be capped for storage.
- Dispense all reagents while cold and return to 2°C to 8°C storage as soon as possible.
- Precipitates may form when the Substrate is stored at 2°C to 8°C. This will not affect the performance of the kit.

CAUTION: Avoid unnecessary exposure of substrate to light.

SPECIMEN COLLECTION, TRANSPORT AND STORAGE

Samples should be stored at 2°C to 8°C if the test is to be run within 7 days of collection or frozen at -20°C or colder if the test is to be delayed for more than 7 days. Clear, non-hemolyzed samples are preferred. Lipemic, icteric or contaminated (particulate or bacterial) samples should be filtered (0.45µm) or centrifuged before testing.

Samples can be inactivated but this is not a requirement for optimal test performance.

Inactivate as follows:
1. Loosen cap of sample container.
2. Heat-inactivate sample at 56°C for 30 minutes in a water bath.
3. Allow sample to cool down before retightening cap.
4. Sample can be stored frozen until analysis.

Repeated freeze-thawing of sample is not recommended.

ADDITIONAL MATERIALS REQUIRED BUT NOT PROVIDED

- Deionized or distilled water
- Disposable gloves
- Rocking platform (designed with a rocking speed range of 12 to 16 cycles per minute, and which moves through a 5° to 10° tilt to wash membranes evenly)
- Pipettors and tips of appropriate volume
- Aspirator with sodium hypochlorite trap
- 56°C water bath (optional)
- Sodium hypochlorite for decontamination

PREPARATION OF REAGENTS

1. DILUTED WASH BUFFER
(a) DILUTED WASH BUFFER should be **prepared fresh prior to use.**
(b) 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 1 g of BLOTTING POWDER to every 20 ml of the diluted STOCK BUFFER prepared in step 2(b) above. Stir to ensure powder dissolves completely.
(d) Stir again before dispensing.

3. WORKING CONJUGATE SOLUTION
Note : Prepare solution in polypropylene container / beaker.
(a) WORKING CONJUGATE SOLUTION should be **prepared fresh prior to use.**
(b) Prepare WORKING CONJUGATE SOLUTION by diluting CONJUGATE 1:1000 into BLOTTING BUFFER, for example, 5 µl CONJUGATE to 5 ml BLOTTING BUFFER.

4. SUBSTRATE SOLUTION (ready to use)
(a) Dispense directly the required volume from the bottle. Use a clean pipette. Cap tightly after use.

ASSAY PROCEDURE

Note: a) Aspirate all used chemicals and reagents into a trap containing Sodium hypochlorite.

b) All incubations are to be carried out on a rocking platform.

Caution:

Some samples cause dark patches on the spot of the strip where they are added. To avoid this problem, one should ensure the following:-

i. Sample should be added only after BLOTTING BUFFER is added.

ii. Tilt the tray slightly by elevating either the top or bottom end of the tray. The Blotting Buffer will flow to the lower end of the tray. Add the sample where the Blotting Buffer is collected. When all the samples are added, return the tray back to its original flat position. Always ensure that the strips are kept wet during the process.

iii. Alternatively, if tilting the tray is not desired, the samples may be added to the top or bottom end of the well. This way if dark patches showed, the reading of the strip results will not be affected.

Procedure:

1. Using forceps, carefully remove required number of STRIPS from the tube and place numbered side up into each well. Include strips for Reactive and Non-Reactive controls.
2. Add 2 ml of DILUTED WASH BUFFER to each well. **2 ml**
3. Incubate the strips for at least 5 minutes at room temperature (25 ± 3°C) on a rocking platform (speed of 12 to 16 cycles per minute). Remove buffer by aspiration. **5 minutes**
4. Add 2 ml of BLOTTING BUFFER to each well. **2 ml**
5. Add 20 µl each of patients' sera or controls to appropriate wells. **20 µl**
6. Cover the tray with the cover provided and incubate for 1 hour at room temperature (25 ± 3°C) on the rocking platform. **60 minutes**
7. Carefully uncover the tray to avoid splashing or mixing of samples. Tilt the tray to aspirate the mixture from the wells. Change aspirator tips between samples to avoid cross-contamination.
8. Wash each strip 3 times with 2 ml of DILUTED WASH BUFFER allowing 5 minutes soak on the rocking platform between each wash. **3 x 2 ml**
9. Add 2 ml of WORKING CONJUGATE SOLUTION to each well. **2 ml**
10. Cover tray and incubate for 1 hour at room temperature (25 ± 3°C) on the rocking platform. **60 minutes**
11. Aspirate CONJUGATE from the wells. Wash as in step 8. **3 x 2 ml**
12. Add 2 ml of SUBSTRATE SOLUTION to each well. **2 ml**
13. Cover tray and incubate for 15 minutes on the rocking platform. **15 minutes**
14. Aspirate the SUBSTRATE and rinse the strips at least three times with reagent grade water to stop the reaction (A dark background can result if washing is insufficient at this step). **3 x 2 ml**
15. Using forceps, gently remove strips onto paper towels. Cover with paper towels and dry. Alternatively, allow strips to dry in the wells of the tray.
16. Mount strips on worksheet (non-absorbent white paper). Do not apply adhesive tape over the developed bands. Observe the bands (See Interpretation of Results) and grade the results. For storage, keep the strips in the dark.

SUMMARY OF ASSAY PROTOCOLS

Reagents	Qty	Duration
Nitrocellulose strip	1	-
Wash Buffer	2 ml	5 mins
Blotting Buffer	2 ml	-
Specimen	20 µl	60 mins
Wash Buffer	3 x 2 ml	3 x 5 mins
Conjugate	2 ml	60 mins
Wash Buffer	3 x 2 ml	3 x 5 mins
Substrate (Ready to use)	2 ml	15 mins
Distilled Water	3 x 2 ml	-

AMOUNT OF REAGENTS REQUIRED FOR VARIOUS NUMBER OF STRIPS

Reagents	NUMBER OF STRIPS TO BE USED						
	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 (µl)	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

QUALITY CONTROL

The Non-Reactive & Reactive Controls should 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 MP Diagnostics HELICO BLOT 2.1. The bands are 116kD(CagA), 89kD(VacA), 61kD(Ure B), 58kD(HSp B), 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

INTERPRETATION OF RESULTS

Record patient's identity number, sample number, clinical status and other appropriate information. 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 controls for the assay. Use the REACTIVE control strip to identify bands on the patients' strips. Record the appropriate bands on the patients' strips.

RECOMMENDED INTERPRETATION:

The recommended criteria for MP Diagnostics 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, patients 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)] x 100 where TP=number of true positives, FP=number of false positives.

LIMITATION OF THE METHOD

Optimal assay performance requires strict adherence to the assay procedure described. Deviation from the 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 geographical origins of the cases. The strain of *H. pylori* which has been used in the blot is useful for diagnosis of cases worldwide.

SPECIFICITY AND SENSITIVITY

Patients sera were tested using the MP Diagnostics 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 subjects to be considered positive, at least 2 out of these 3 tests have to be positive. For negative subjects, all of the performed tests, 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%

LIMITED EXPRESSED WARRANTY DISCLAIMER

The manufacturer makes no warranty other than that the test kit will function as an *in vitro* diagnostic assay within the specifications and limitations described in the product Instructions For Use 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.

TECHNICAL PROBLEMS / COMPLAINTS

Should there be a technical problem / complaint, please do the following :

1. Note the kit lot number, the expiry date and the strip lot number.
2. Retain the kits and the results that were obtained.
3. Contact the nearest MP Biomedicals office or your local distributor.

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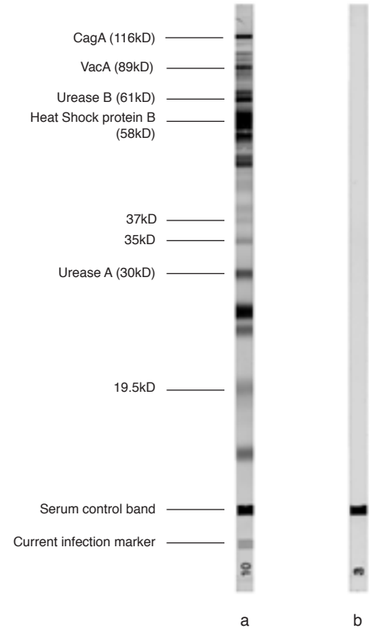
FIGURE 1



H. pylori specific bands as visualized with
a) Reactive Control
b) Non-reactive Control

TROUBLE SHOOTING CHART

Dark spots develop on strips

1. Bacterial or fungal contamination of test sample.
2. Precipitation of immune complexes in aged test sample.
3. Bacterial or fungal contamination on strip due to improper storage.
4. Strips physically damaged, cracked or scratched.
5. Strips not properly washed between assay steps.

White patches develop on strips

1. Strips was flipped over during assay.
2. Trays not properly washed before use.
3. Poor dissolution of Blotting Powder.
4. Electrotransblot interference during manufacturing.

Expected bands do not develop or are of weak intensity.

Check positive control

- Positive control weak → The problem is probably caused by the reagents.
  1. Reagents not properly prepared.
  2. Wrong conjugate dilution.
  3. Unstable reagents due to improper temperature exposure.
  4. Conjugate contaminated with human IgG.
  5. Incorrect substrate pH due to exposure to strong UV light or reducing agent.
  6. Trays, reagent(s) or water having high phosphate concentration.
  7. Rotary platform used instead of Rocking platform.
- Positive control OK → The problem is probably caused by test sample.
  1. Wrong test sample dilution.
  2. Test sample contaminated with conjugate.
  3. Test samples severely immune-complexed.
  4. Test sample IgG deteriorated or denatured due to repeated freeze- thaw or improper storage.
  5. Rotary platform used instead of Rocking platform.
  6. Test sample may be an ELISA “false” positive.

Strong Background develops on strip in the absence or presence of positive bands.

1. Overdeveloped strips (stop reaction sooner).
2. Incomplete washing.

Absence of Serum Control Band

1. Serum not added.
2. Strips flipped over during assay.
3. Conjugate not added.
4. Substrate not added.

Bands other than the Serum Control band develops on negative control

Tray wells or Control may have been crossed contaminated.

Non-specific bands and/or dark background develop on strips

1. Wrong test sample or conjugate dilution.
2. Test sample/reagent incubation too long.
3. Incomplete washing during assay.
4. Incubation temperature greater than 30˚C.

Strips are defective

1. They are cracked.
2. They contain air bubbles which cause the appearrance of white spots in reactive zones big enough to prevent and detection.
3. They show dark spots due to fungal growth upon initial opening of the strip tubes. However, if dark spots develop sometime later after initial opening of the tube then the problem is due to improper strip storage conditions at the user’s site.

Watery marks on developed strips

1. Strips left to dry after pre-soaking step prior to adding Blotting Buffer.