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གསོ་བ་ལྷན་ཐག།
ཐིམ་ཕུ།

ROYAL GOVERNMENT OF BHUTAN
MINISTRY OF HEALTH
THIMPHU BHUTAN

Standard Operating Procedure
for
H.PYLORI ANTIGEN TESTING IN STOOL

Ministry of Health
Royal Government of Bhutan

2021

TABLE OF CONTENT

General Instructions on *H. Pylori* testing

1. Background information

As part of the government's Health Flagship project on cancer prevention in the country, cancer of the stomach is a priority. Since infection with the bacterium *Helicobacter pylori* is one of the main causes of stomach cancer, testing for *H. pylori* is taken as an important part of the screening program. Therefore, health centres all over the country will be required to perform the testing for *H. pylori*.

2. Test kits supplied

For H. pylori testing, two types of test kits are supplied by the Ministry of Health.

a. ***H. pylori* Quik Chek™**

This is a rapid qualitative test kit which can be carried out in the field or any health centre without requiring additional laboratory equipment. It can be used to test a single or small number of samples. Results can be available within 25-30 minutes after starting the test procedure.

b. ***H. pylori* Chek™**

This test kit uses an ELISA technique which can be performed manually or semi-automated. It requires additional equipment, therefore not feasible to be conducted in the field. It can test large number of samples in batches and can be completed in 60-90 minutes after starting the test procedure.

3. Who can test?

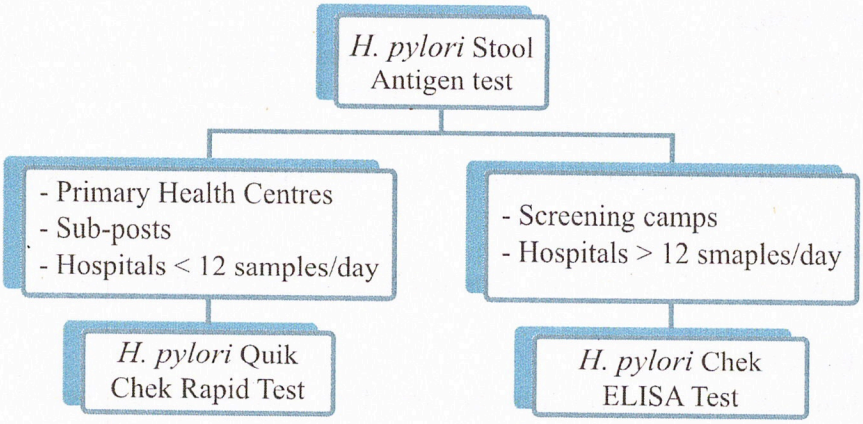
- The ***H. pylori* Quik Chek™** rapid test can be performed by a medical laboratory staff or other health workers who have received basic instructions/training on the procedure.

- The ***H. pylori* Chek™** ELISA test shall be performed only by certified laboratory professionals in a hospital laboratory setting with the required equipment.

4. Standard Operating Procedures

For the testing of stool samples for *H. pylori*, two SOPs for the two different test kits have been developed. However, staff are also required to read and follow the procedures provided in the company leaflet with the test kits.

5. **Testing algorithm**



Wherever possible, hospitals are requested to collect and store samples to be tested together with the ELISA test kit. Stool samples can be stored for 2-3 days at room temperature but best stored at 2-8°C.

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SOP No.	Title	Version No.	Total Pages
MoH/HFS/SoP/01	SOP on Qualitative detection *of Helicobacter pylori* Specific Antigen in human fecal specimen using ELISA method	1.0	7

Issue Date	Effective Date	Review Period
		Every 3 years

Function	Name	Designation / Department	Signature
Prepared by	Ms. Jamyang Wangmo Mr. Sonam Tshering Ms. Geeta Maya Bandhari Dr. Tshokey Mr. Pempa	Microbiology Lab, JDWNRH Gidakom Hospital Dechencholing Hospital Microbiologist, JDWNRH Project Coordinator, Health Flagship Project, MoH	
Verified by	Mr. Tandin Dorji	Project Director, Health Flagship Project, MoH	
Approved by	Dr. Pandup Tshering	Secretary, MoH	

Distribution:	All health facilities
Location	20 Dzongkhags

REVISION SUMMARY

Version No	Effective Date	Reason for Change	Details of the change

1. Abbreviations and Definition:

NC	Negative Control
PC	Positive Control
HRP	Horseradish peroxidase
H. pylori	Helicobacter pylori
IQC	Internal Quality Control
SOP	Standard Operating Procedure
T	Test
ml	milliliter
ul	Microliter

2. Scope

This Standard operating procedure (SOP) is intended for health professional as an aid in the diagnosis of *H. pylori* infection using ***H. PYLORI* CHEK™** (Enzyme immunoassay).

3. Responsibilities

It is the responsibility of authorized laboratory staff to perform the procedure as per the SOPs.

4. Principle

1.1 The *H. PYLORI* CHEK enzyme immunoassay uses antibodies specific to *H. pylori* antigen. The microassay plate in the kit contains immobilised capture antibodies against *H. pylori* antigen. The, conjugate consist of antibodies specific to *H. pylori* antigen conjugated to Horse Radish Peroxidase (HRP). In the assay, an aliquot of a diluted faecal specimen is transferred to a microassay well containing the conjugate. If the antigen is present in the specimen, it will bind to the conjugate and to the immobilised capture antibody during the incubation phase. Any unbound material is removed during the washing steps. Following the addition of substrate, a colour is detected due to the enzyme-antibody-antigen complexes that formed in the presence of antigen.

5. Requirement

5.1. Material provided with the kit

- Microassay plates (10)
- Conjugate (7 ml)
- Diluent (40 ml)
- Positive control (3.5 ml)
- Substrate (14 ml)
- Wash buffer (50 ml)
- Stop solution (7ml)
- Disposable plastic transfer pipette – graduated at 50, 100, 200 and 300 ul.
- Wooden applicator sticks
- Plastic adhesive sheets

5.2. **Sample**

- Fresh stool specimen
- Frozen stool sample
- Stool specimen stored/transported in Cary Blair transport medium.

5.3. **Equipment**

- Vortex (if available)
- Timer
- ELISA reader (if available), if not use visual interpretation provided with the kit
- ELISA strip washer (if available)
- Centrifuge
- Water bath

5.4. **Lab consumables**

- Clean screw-capped leak proof container
- Clean disposable gloves (no need of sterile gloves)
- Surgical mask
- Sample rack
- Marker

5.5. **Associated Documents**

- ELISA worksheet
- IQC record sheet

6. Precaution/safety

6.1. Always read the instructional manual provided by the manufacturer in the test kit

6.2. Check test kit components for any signs of leakage or breakage.

6.3. Store the kit at 2-8°C and do not freeze

6.4. Bring the test kit to room temperature before carrying out the test.

6.5. Do not use stool specimen collected in fixatives such as formalin or alcohol based solutions

6.6. Use basic PPE (surgical gloves, apron/lab coat and face mask) while handling specimens and perform hand hygiene frequently

6.7. Treat all specimens as potentially infectious

6.8. Avoid contact of stop solution with skin. If accidental contact is made, wash thoroughly with running water immediately

7. Procedure

7.1. Bring the test kits to room temperature before use.

7.2. Set up and label the test tube/cryovial for each specimens and controls.

7.3. Preparation of Assay Strips

- Considering the number of samples to be tested, determine the number of test strips (one strip has 8 wells) to be utilized.
- Avoid contact with the base of the well
- Unused strips should be put back to the foil pouch and carefully resealed for future use

7.4. **Pre-preparation of reagents and samples**.

- **Wash buffer**
  - Prepare 1X wash solution by mixing 950ml of distilled water with 50 ml of 20X wash buffer (supplied).
  - This freshly prepared 1X solution can be stored at 2-8°C for up to the expiry date of the test kit

- **For liquid/semi-solid specimen**
  - Using a graduated transfer pipette, add 200ul of diluent and 50ul of specimen to the labelled tube/cryovial and mix thoroughly by vortexing or inverting several times

- **For formed/solid specimen**
  - Add 200ul of diluent into the labelled tube and using a wooden applicator stick, transfer a small portion of faecal specimen approximately 0.05g or 3 mm diameter into the diluent tube and mix thoroughly as above

For stool specimen collected in transport medium (Cary Blair):

- Add 100 ul of diluent to each tube and100ul of specimen, mix thoroughly as above.

Note: If using semi-automated ELISA washer, after dilution, specimen must be centrifuged at 5000g for 10 minutes to avoid blockage in washing probe.

- Test samples within 2 hrs of mixing in the diluent

7.5. **Test Procedure**

- Add one drop (50ul) of conjugate to each tube including the tubes for positive and negative controls (**Note: Gently mix the conjugate bottle by inverting several time prior to addition)**

- **For quality control testing**
  - **For external positive control:**
    - Add one drop (50ul) of positive control (black cap) to the positive control well.
  - **For external negative control:**
    - Add 100 ul of diluent to the negative control well.

- Using a new transfer pipette, transfer 100ul of diluted specimen (or supernatant if using automated washing) to the respective sample wells.
- Cover micro assay plate with plate sealer.
- Mix/tap gently
- Incubate for 50 minutes at 37°C ±2°C (in Water bath)
- Decant the content into a discard pan/sink

- **Washing steps**
  - Manual washing – add diluted wash buffer solution (1X) with a fine-tipped nozzle to the bottom of each well with force, shake the wells and discard the solutions into a discard pan. Tap the plate on a dry absorbent paper in each washing until no particles are seen in the wells. Repeat this washing steps for 5 times.
  - Semi-automated washer – Add 350ul of diluted wash buffer solution (1X) and wash for 5 times

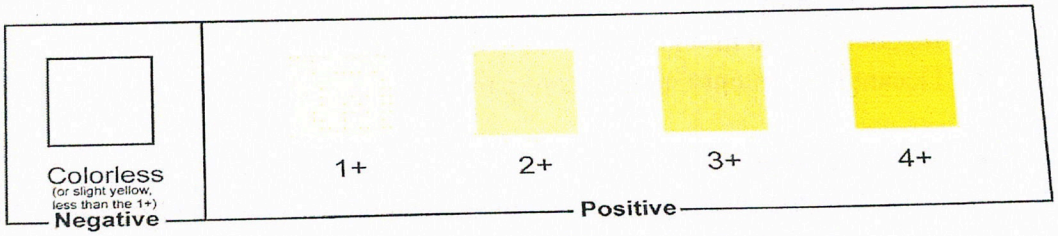
- Tap the wells into absorbent paper to remove any residual liquids in the wells completely
- Add 2 drops (100ul) of substrate (blue cap) to each wells and tap gently to mix well
- Incubate at room temperature for 10 minutes (Gently tap the wells at 5 minutes for further mixing during incubation)
- Add 1 drop (50ul) of stop solution (Yellow cap) into each wells and tap gently for mixing.
- Wait for 2 minutes before reading.
- Visual reading should be done in presence of good light against a white background using **"Visual Interpretation for *H. Pylori Chek*"** chart/guide.
- Read within 10 minutes after adding the stop solution. Any colour change after 10 minutes should not be considered.
- Using ELISA reader read the microplate with single/dual wavelength (450/620/630 nm).

8. Results interpretation

Visual interpretation

Visual Interpretation for H. PYLORI CHEK™

1. Compare the color in the well with the color in the chart below.
2. Read the control wells first. The negative control well should be colorless (< 0.120 at OD_{450} or < 0.080 at $OD_{450/620 \text{ or } 450/630}$).
   The positive control well should be $\geq$ the 2+ color (≥ 0.500 at OD_{450} or $OD_{450/620 \text{ or } 450/630}$).
3. **Positive** results will have a color intensity $\geq$ the 1+, 2+, 3+, or 4+ color (≥ 0.120 at OD_{450} or ≥ 0.080 at $OD_{450/620 \text{ or } 450/630}$).
4. **Negative** results will be colorless or slight yellow, less than the 1+ color.



< 0.120 at OD_{450} or < 0.080 at $OD_{450/620 \text{ or } 450/630}$

≥ 0.120 at OD_{450} or ≥ 0.080 at $OD_{450/620 \text{ or } 450/630}$

Refer to the Package Insert for complete details.

Any equivocal results in ELISA may be re-tested using the rapid H. Pylori test kit.

9. Quality control/Validation/Calculations

9.1. Run the commercially provided known positive and negative controls along with samples all the time

9.2. QC and validation should take place with special focus in the following situations:

- Conducting the test for the first time
- New test kit is received
- New staff is performing the test
- With changes in storage temperature of the test kits
- With change in lot number of the test kits.

10. Documentation and reporting

10.1. Enter the patient details as per the *H. pylori* register/worksheet

10.2. Record the results of IQC in a QC log file.

10.3. Record the following details in the ELISA worksheet.

- Test date
- Name of the kit used.

- Manufacturer, Lot number and expiry date of the kit.
- Initials of the technologist/technician/health personnel who performs the test and supervisor who verifies the results

11. Limitations and interferences

11.1. The test procedure, precaution and interpretation of results sections for this test kit must be followed strictly

11.2. Refer test kit insert

12. Infection control and waste management

1.1 Handle and dispose all fecal specimen and the used laboratory articles as infectious wastes

1.2 Any accidental spillage of materials containing antigen or antibody should also be handled like an infectious material

13. Annexure

13.1. ELISA worksheet

13.2. IQC record sheet

14. Reference

14.1. Test kit leaflet of *H. pylori* Chek

H. pylori ELISA Protocol sheet

Date performed: .....

Analyst:

Kit Used: **Manufacturer:**

Lot Number: **Expiry Date:**

Equipment Used:

- ☐ EIA washer
- ☐ Water Bath
- ☐ EIA Reader

Plate Map

No: .....

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

H. pylori ELISA Worksheet

Trade Name: .....

Date of Performed: ..... Lot No : .....

Analyst: ..... Exp. Date: .....

Numbers of Sample: ..... Manufacturer.....

Room Temperature.....

Date	Sl. No.	Name	Age/ Sex	PID/CID	Test Result	Remarks

Note: .....

Date: ..... Verified By:

Copy No. [ ]

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SOP No.	Title	Version No.	Total Pages
MoH/HFS/SoP/02	SOP on the Qualitative Detection of *Helicobacter pylori* Specific Antigen in Human Faecal Specimen using rapid membrane enzyme immunoassay method	1.0	7

Issue Date	Effective Date	Review Period
		3 yearly

Function	Name	Designation / Department	Signature
Prepared by	Ms. Jamyang Wangmo Mr. Sonam Tshering Ms. Geeta Maya Bandhari Dr. Tshokey Mr. Pempa	Microbiology Lab, JDWNRH Gidakom Hospital Dechencholing Hospital Microbiologist, JDWNRH Project Coordinator, Health Flagship Project, MoH	
Verified by	Mr. Tandin Dorji	Project Director, Health Flagship Project, MoH	
Approved by	Dr. Pandup Tshering	Secretary, MoH	

Distribution:	All health facilities
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1. Abbreviations and Definition:

C	Control
HRP	Horseradish peroxidase
H. pylori	Helicobacter pylori
IQC	Internal Quality Control
SOP	Standard Operating Procedure
T	Test
ml	milliliter
ul	microliter

2. Scope

This Standard operating procedure (SOP) is intended for health professional as an aid in the qualitative detection of *H. pylori* in faecal sample using the ***H. PYLORI* QUIK CHEK™** (A rapid membrane enzyme immunoassay) rapid test kit.

3. Principle

This test utilizes antibodies specific for the *H. Pylori* antigen. The membrane contains immobilized antibodies whereby the test line (T) contains antibodies specific for *H. pylori* antigen and the control line (C) contains antibodies to horseradish peroxidase (HRP). The conjugate which contains antibodies to *H. pylori* antigen bind with HRP to form antigen-antibody-peroxidase complexes. The complex migrates through a membrane filter where they are captured by the immobilized anti-*H. pylori* antigen antibodies in the test line.

4. Responsibility

It is the responsibility of authorized laboratory/other authorized health staff to perform the procedure as per the SOPs.

5. Requirement

5.1. Material provided with the kit

- Membrane device: 25 pack size, each pouch contains one test device
- Conjugate (2.5 ml): containing antibody specific to *H. pylori* antigen coupled to HRP in a buffered protein solution.
- Diluent (22 ml): Buffered protein solution with black graduated dropper assembly.
- Positive control (2 ml): *H. pylori* antigen in a buffered protein solution
- Substrate (3.5 ml): Solution containing tetramethylbenzidine
- Wash buffer (12 ml): buffered solution with white graduated dropper assembly
- Disposable plastic transfer pipette (50 nos): graduated at 25, 100, 200, 300,400 & 500 ul
- Wooden applicator sticks (50 pieces)

5.2. **Sample**

- Fresh stool specimen
- Frozen stool specimen
- Stool specimen stored/transported in Cary Blair transport medium.

5.3. **Equipment**

- Vortex (optional)
- Timer

5.4. **Lab consumables**

- Clean screw-capped leak proof sample container
- Clean disposable gloves (no need of sterile gloves)
- Surgical mask
- Sample rack
- Marker

5.5. **Associated Documents**

- Worksheet to record test results
- IQC record sheet

6. Precaution

6.1. Always read the instructional manual provided in the test kit by the manufacturer
6.2. Check test kit components for expiry date and any signs of leakage or damage
6.3. The kit should be stored between 2-8°C and not frozen
6.4. Bring the test kit to room temperature before carrying out the test
6.5. Do not use stool specimen collected in fixatives such as formalin or alcohol based solutions
6.6. Wear disposable gloves while handling specimens and perform proper hand hygiene

7. Procedure

7.1. Bring the test kits to room temperature before use.
7.2. Set up and label the test tube/cryovial for each specimens and controls depending on the number of samples
7.3. **Preparation of diluent-conjugate mixture**

7.3.1. For fresh and frozen stool specimen

- Add 750 ul of diluent (black capped bottle) to each test tube

7.3.2. For stool specimen collected in transport medium (Cary Blair)

- Add 650 ul of diluent (Black capped bottle) to each tube

7.3.1. To either of the above, add one drop of conjugate (red capped bottle) to each tube **(Note: Gently mix the conjugate bottle by inverting several time prior to addition)**

7.4. **Preparation and addition of sample**

7.4.1. For liquid/semi-solid specimen

- Using a graduated transfer pipette, mix specimen thoroughly and add 25 ul to the diluent/conjugate mixture tube

7.4.2. For formed /solid specimen

- Using wooden applicator stick, mix specimen thoroughly and transfer a small portion of approximately 2 mm diameter into the diluent/conjugate mixture tube

7.4.3. For specimen collected in transport medium

- Using a graduated transfer pipette, add 100 ul of specimen in to the diluent/conjugate mixture tube.

7.5. **For quality control testing**

- **For external positive control:**
  - Add one drop of positive control (Gray capped bottle) to the positive control tube

- **For external negative control:**
  - Add 25 ul diluent to the negative control tube

7.6. Mix thoroughly using vortex mixer or by inverting the tube several times.
7.7. Open the device/card for each test and control
7.8. Transfer 500 ul of the mixture to the small sample well on the device/card
7.9. Incubate 15 minutes at room temperature
7.10. Using the graduated transfer pipette, add 300 ul of wash buffer to central reaction window
7.11. Allow the wash buffer to be absorbed completely
7.12. Add 2 drops of substrate (white capped bottle) to the central reaction window
7.13. Incubate for 10 minute at room temperature
7.14. Read immediately after the incubation. A positive result may be seen anytime **during** the 10 minute incubation or **immediately** after 10 minutes.

H. PYLORI QUIK CHEK™ Test Procedure

1 Add to a test tube:

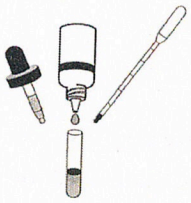
For fresh or thawed frozen specimens:
- 750 µL *Diluent*
- 1 drop *Conjugate*
- 25 µL of specimen* (1st graduation)

OR

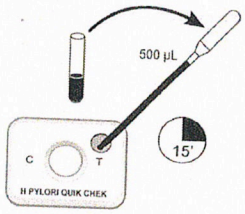
For specimens in transport media:
- 650 µL *Diluent*
- 1 drop *Conjugate*
- 100 µL of specimen (2nd graduation)

Mix thoroughly.

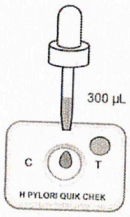
*For solid specimens, use applicator stick to transfer approximately 2mm diameter of specimen.



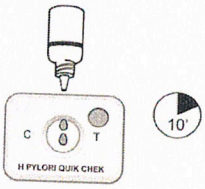
2 Transfer 500 µL (highest graduation) from tube to small *Sample Well*. Keep the cassette at room temperature and wait 15 minutes.



3 Add 300 µL *Wash Buffer* to large *Reaction Window*. Allow to completely absorb.

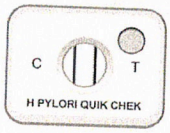


4 Add 2 drops *Substrate* to large *Reaction Window*. Keep the cassette at room temperature and wait 10 minutes. Read results.

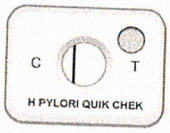


Interpretation

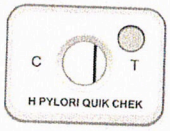
A positive result may be interpreted at any time during the 10 minute incubation.
A test cannot be ruled negative or invalid until 10 minutes have passed.



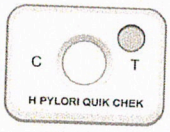
POSITIVE



NEGATIVE



INVALID



INVALID

If there is no line at C (control) the test is invalid. See package insert for additional interpretation of results.

Developed and Manufactured by:
TECHLAB®
2001 Kraft Drive
Blacksburg, VA 24060-6358 USA
Made in the USA

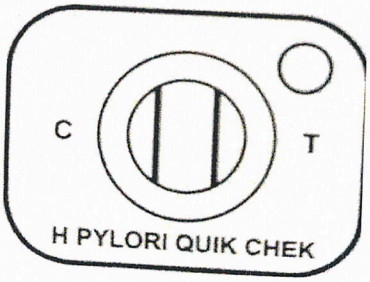
This chart does not contain the complete instructions for use of the *H. PYLORI QUIK CHEK*™ test. For proper use of the assay, please read the package insert.

Part No. 92-050-02
Issued: 01/2021

8. Results interpretation

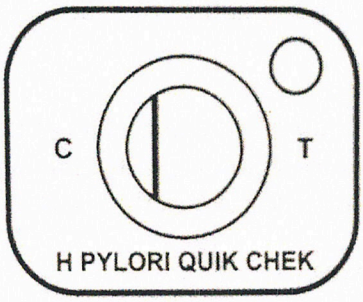
8.1. Positive results

- Two vertical blue lines are visible, the control line on the "C" side and test line on the "T" side of the reaction window. A positive results indicates the presence of *H. pylori* antigen.



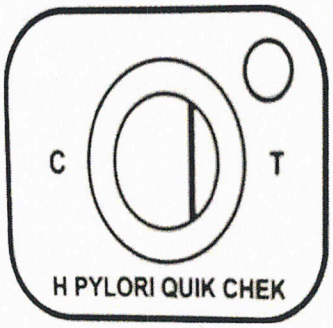
8.2. Negative results

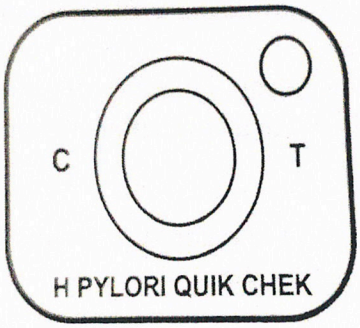
- A single vertical line is visible on the "C" side and no test line on the T side of the reaction window.



8.3. Invalid results

- A single line is visible on the "T" side or no lines are visible on both the C and T sides in the reaction window





9. Quality control/ Validation/ Calculations

9.1. Run the commercially provided known positive and negative controls along with samples in the following situation:

- When using the kit for the first time
- New test kit is received
- New staff is performing the test
- With changes in storage temperature of the test kits
- With change in lot number of the test kits.

H. pylori RAPID Kit Worksheet

Trade Name: .....

Date of Performed: ..... Lot No : .....

Analyst: ..... Exp. Date: .....

Numbers of Sample: ..... Manufacturer.....

Room Temperature.....

Date	Sl. No.	Name	Age/ Sex	PID/CID	Test Result	Remarks

Note: .....

Date: ..... Verified By:

Daily QC log

DATE	LOT NO.	EXPIRY DATE	POSTIVIE KIT CONTROL (PC)	NEGATIVE KIT CONTROL (NC)	PERFORMED BY	SUPERVISORY SIGN

NOTE: QC MUST BE DONE EACH DAY OF USE. IF QC IS NOT FILLED IN FOR A SPECIFIC DATE; THIS INDICATES PATIENT TEST WAS NOT PERFORMED.