TRAINING UPDATE

Lab Location:	WAH	**Date Distributed:**	9/20/2013
Department:	Micro	**Due Date:**	10/30/2013
		Implementation:	**11/1/2013**

DESCRIPTION OF PROCEDURE REVISION

Name of procedure:

CLO Test WAH.M09.001

Description of change(s):

Added to section 8.2

With each negative test, perform the following positive control to ensure the CLOtest is working properly:

1. Peal back the label on the CLOtest slide so that the yellow gel is visible. Insert one CLOtest Positive Control sample into the gel. Re-seal the CLOtest lid over the gel.
2. After ten (10) minutes, inspect the gel for a positive color change (red or magenta).
3. If the gel turns red or magenta, the test is valid and may be reported as Negative.
4. If the gel does not turn red or magenta the test is invalid, do not report patient results. Report to supervisor and notify the manufacturer.

Record the result of the Urease Positive Control when performed (when patient result is Negative at 24 h)

Section	Reason
4, 6 , 8	Add Urease Control Tablet and instructions for use
Add. A	Add Section to record read at receipt and 24 h and QC results for confirmation of negative results

This revised SOP will be implemented on November 1, 2013

Document your compliance with this training update by taking the quiz in the MTS system.

Approved draft for training all sites (version 001)

Technical SOP

Title	**CLO Test**
Prepared by	Ron Master Date: 8/10/2009
Owner	Ron Master Date: 8/10/2009

Laboratory Approval	**Local Effective Date:**	
Print Name and Title	**Signature**	**Date**
Refer to the electronic signature page for approval and approval dates.		

Review		
Print Name	**Signature**	**Date**

Form revised 3/31/00

TABLE OF CONTENTS

1. TEST INFORMATION

Assay	Method/Instrument	Local Code
CLO test	Production of urease enzyme	CLO

Synonyms/Abbreviations
H. pylori test

Department
Microbiology

Form revised 3/31/00

2. ANALYTICAL PRINCIPLE

The CLO Test provides a rapid presumptive diagnosis for the presence of *Helicobacter pylori*. *H. pylori* has been shown to cause active chronic gastritis and is a risk factor for gastric cancer and mucosal-associated-lymphoid-type lymphoma. *H. pylori* produces large amounts of urease enzyme. Although urease primarily allows *H. pylori* to utilize urea as a nitrogen source, the breakdown of urea also produces high local concentrations of ammonia, which enable the organism to tolerate low pH. Tests for the presence of urease enable a rapid presumptive diagnosis.

3. SPECIMEN REQUIREMENTS

3.1 Patient Preparation

Component	Special Notations
Fasting/Special Diets	N/A
Specimen Collection and/or Timing	1. Biopsy the sump of the antrum at least 2 cm away from the pylorus along the lesser or greater curve. 2. Biopsy an area of normal looking tissue rather than an area affected by erosions or ulcerations as *H. pylori* may be present in smaller numbers if the epithelium is eroded or the mucus layer is denuded.
Special Collection Procedures	1. Inspect CLO test before use to ensure the well is full and yellow in color. 2. Immediately before endoscopy, the CLO test should be warmed to room temperature 7 – 10 minutes. If the CLO test can be warmed to 30° - 40° C warming will help speed the chemical reaction. 3. Insert biopsy specimen into the CLO test.
Other	1. Patients should not have taken antibiotics or bismuth salts for at least three weeks prior to endoscopy. 2. If the biopsy specimen appears to be very small, it may be worthwhile taking a second biopsy and inserting both specimens into the CLO test. 3. Deliver specimen to the Laboratory as soon as possible.

3.2 Specimen Type & Handling

Criteria		
Type -Preferred	Biopsy	
-Other Acceptable	None	
Collection Container	CLO test well	
Volume - Optimum	2-3 mm diameter biopsy	
- Minimum	1 mm diameter biopsy	
Transport Container and Temperature	CLO test well at room temperature	
Stability & Storage Requirements	Room Temperature:	Deliver to laboratory as soon as possible.

Criteria	
	Refrigerated: Unacceptable
	Frozen: Unacceptable
Timing Considerations	N/A
Unacceptable Specimens & Actions to Take	Reject frozen or refrigerated samples.
Compromising Physical Characteristics	N/A
Other Considerations	Allow gel to reach room temperature (7-10 minutes) before inserting biopsy.

4. REAGENTS

Refer to the Material Safety Data Sheet (MSDS) supplied with the reagents for complete safety hazards. Refer to the section in this procedure covering "SAFETY" for additional information.

4.1 Reagent Summary

Reagents / Kits	Supplier & Catalog Number
CLOtest	Kimberly-Clark CLO test 104379 (Quick Req)
Urease Control Tablets, 50 Each	Kimberly-Clark 60407

4.2 Reagent Preparations and Storage

NOTES: Date and initial all reagents upon opening. Each container must be labeled with (1) substance name, (2) lot number, (3) date of preparation, (4) expiration date, (5) initials of tech, (6) any special storage instructions; check for visible signs of degradation.
Refer to the Material Safety Data Sheet (MSDS) for a complete description of hazards. If a specific hazard is present, it will be noted in this procedure when the hazard is first encountered in a procedural step.

Reagent	CLO Test well
Stability	Shelf life 18 months 2° C - 8° C
Preparation	Allow gel to reach room temperature 7-10 minutes before use.

5. CALIBRATORS/STANDARDS

N/A

6. QUALITY CONTROL

6.1 Controls Used

Controls
Escherichia coli - negative
Proteus mirabilis - positive
Urease Control Tablets, Positive Control

Form revised 3/31/00

6.2 Control Preparations and Storage

NOTE: Date and initial all controls upon opening. Each container should be labeled with (1) substance name, (2) lot number, (3) date of preparation, (4) expiration date, (5) initials of tech, and (6) any special storage instructions; check for visible signs of degradation.

External Controls:

- Control organisms are stored on nutrient ag ar slants or plates. They m ay be purchased in a lyophilized form.
- Prepare a fresh in-house contro1 from stock cultures of *E. coli* (ATCC 25922) and *P. mirabilis*, or equivalent strains, as follows:
  1. From culture plates or slants or a lyophilized stock of *E. coli* and *P. mirabilis*, inoculate a loop full of each onto sheep blood agar plates.
  2. Incubate for 24 hours and examine to assure a pure culture.
  3. Label the tubes with the date of preparation and date of expiration (one month from the preparation date). Store at 2° - 8° C.
  4. Prepare these aliquots every 2 weeks or as needed, according to the volume of testing.

6.3 Frequency

To ensure the integrity of agar slides QC with *E. coli* and *Proteus mirabilis* is run with each new lot number of slides and from each shipment.

With each negative test, perform the following positive control test using a Urease Control Tablet to ensure the CLOtest is working properly.

6.4 Tolerance Limits

Control	Expected Reaction
Escherichia coli	Negative - yellow
Proteus mirabilis	Positive - red

IF ….	THEN…
If both organism controls produce the expected reaction and the patient result is positive	Patient results can be reported.

Form revised 3/31/00

If the patient test is negative, perform a positive control test using a Urease Control Tablet	If the gel turns red or magenta after adding the Urease Control Tablet, report the patient result as "Negative." If the gel does not turn red or magenta after adding the Urease Control Tablet, the test is invalid. The QC failure must be investigated and no patient results can be reported. Confirm the activity of the Urease Control, Tablet by repeating the QC testing with a fresh Urease tablet. If that does not resolve the problem, contact Kimberly-Clark Technical Support for assistance.
If the *Proteus mirabilis* does not turn red	The test is invalid. The QC failure must be investigated and no patient results can be reported. Repeat the QC testing with a fresh isolate. If that does not resolve the problem, contact Kimberly-Clark Technical Support for assistance.
If the *E. coli* turns red	The test is invalid. The QC failure must be investigated and no patient results can be reported. Repeat the QC testing with a fresh isolate. If that does not resolve the problem, contact Kimberly-Clark Technical Support for assistance..

6.5 Review Patient Data

Review patient results for unusual patterns, trends or distributions in patient results, such as an unusually high percentage of abnormal results. Computer aided tools should be used when available.

6.6 Documentation

Quality Control Results are recorded on the appropriate QC sheet.

6.7 Quality Assurance Program

Refer to National and local policies and procedures for other quality assurance activities applicable to this procedure.

7. EQUIPMENT and SUPPLIES

7.1 Assay Platform

N/A

7.2 Equipment

N/A

7.3 Supplies

N/A

Form revised 3/31/00

8. PROCEDURE

NOTE: For all procedures involving specimens, buttoned lab coats, gloves, and face protection are required minimum personal protective equipment. Report all accidents to your supervisor.

The package insert for a new lot of kits must be reviewed for any changes before the kit is used. A current Package Insert is included in the Addendum to this procedure.

8.1	Specimen / Reagent Preparation
1.	CLO test specimens are collected by the gastroenterologist, when indicated, during esophogastroduodenoscopy.
2.	Immediately before endoscopy, the CLO test should be warmed to room temperature. Warming at 30 – 40° C will help speed the chemical reaction.
3.	Inspect CLO test before use to ensure the well is full and yellow in color.
4.	Insert biopsy specimen into the CLO test.
5.	The CLO tests are delivered to the laboratory by endoscopy with a CLO test record sheet. Specimens may be picked up on rounds by Histology.

8.2	Test Run
1.	CLO test order in placed in LIS by the laboratory. The GI physicians name should be entered as the ordering physician.
2.	The CLO test is read immediately upon receipt then incubated at room temperature for the remainder of the 24 hour test time.
3.	Readings are taken upon receipt and 24 hours after inoculation.
4.	If the patient test is negative after 24 h, perform a positive control test using a Urease Control Tablet 1. Peal back the label on the patient CLOtest slide so that the yellow gel is visible. Insert one CLOtest Positive Control sam ple into the gel. Re-seal the CLOtest lid over the gel. 2. After ten (10) minutes, inspect th e gel f or a positive color change (red or magenta). 3. If the gel turns red or m agenta, the test is v alid and may be reported as Negative. 4. If the gel does not turn red or m agenta the test is invalid, do not report patient results. Report to supe rvisor and notif y Kimberly-Clark (Ballard Medical Products) at 1-800-528-5591.
5.	The technologist reading each phase of the test will initial their result.

9. CALCULATIONS

N/A

10. REPORTING RESULTS AND REPEAT CRITERIA

10.1 Interpretation of Data

Form revised 3/31/00

11. If *H. pylori* is present in the tissue sample the resulting degradation of urea causes the pH to rise and the color of the gel turns from yellow to bright magenta (pink to red).
12. When first inserted in the gel, specimens may have a very slight pink tinge, particularly if blood or alkaline bile is present. Only if the pink area is deepening in color and expanding in size will the CLO test be called positive.
13. If urease is present in the tissue, an expanding pink zone will be noted around the biopsy specimen, or the CLOtest gel will gradually change to a deep orange, then magenta color. If only a small number of *H. pylori* organisms are present in the specimen, instead of seeing a red color around the biopsy, the color of the whole CLOtest gel gradually changes. An orange gel at 3 hours is likely to be positive and usually turns to a magenta color overnight.
14. A negative result is indicated when th e CLOtest gel is st ill yellow 24 hours after insertion of the specimen. Report as Negative. Subsequent color changes may occur although in most cases a stab le magenta or yellow color will be present.
15. Positive results are reported as soon as the gel turns pink to red.

10.2 Rounding

N/A

10.3 Units of Measure

N/A

10.4 Clinically Reportable Range (CRR)

N/A

10.5 Repeat Criteria and Resulting

IF the result is …	THEN…
Red, magenta, pink or deep orange	Positive: *H. pylori* is present
Yellow	Negative: *H. pylori* is not present

Patient results are reported in the LIS via function MDE (Micro Direct Exam).

16. EXPECTED VALUES

11.1 Reference Ranges

N/A

11.2 Priority 1 & 2 Limits

None established

11.3 Priority 3 Limit(s)

None established

Form revised 3/31/00

17. CLINICAL SIGNIFICANCE

The CLO test detects 75% of *H. pylori* infections within 20 minutes with no false positives.
By one hour 85% of positive patients will be detected.
Between 3 and 24 hours CLO test will detect another 5% of patients.

18. PROCEDURE NOTES

- **FDA Status:** Exempt
- **Validated Test Modifications:** None

19. LIMIATIONS OF METHOD

14.1 Analytical Measurement Range (AMR)

N/A

14.2 Precision

The CLO test detects 75% of *H. pylori* infections within 20 minutes with no false positives. By one hour 85% of positive patients will be detected. Between 3 and 24 hours CLO test will detect 5% of patients.

14.3 Interfering Substances

Antibiotics, bismuth, proton pump inhibitors, or sucralfate which can inhibit the organism.

14.4 Clinical Sensitivity/Specificity/Predictive Values

Possible causes for false negative CLO test:

- Very low numbers of *H. pylori* in the tissue sample.
- Patchy *H. pylori* distribution so that the organism is not captured in the tissue sample.
- A sample of intestinal metaplasia (*H. pylori* does not colonize intestinal mucosa).
- Formalin contamination of the sample.

Possible causes for false positive CLO test:

- When the test is properly performed, false positives are relatively rare. Late false positive reactions (>12 hours) may be caused by failure to completely insert the biopsy into the gel so that contaminating organisms can grow in tissue.
- Theoretically, a false positive could occur in patients who have achlorhydria from bacterial overgrowth. This could be the result of the following conditions: pernicious anemia, previous gastric surgery, or recent use of proton pump inhibitor drugs. However, other bacterial produce much less urease than *H. pylori* and should not cause a rapid color changes.

Form revised 3/31/00

20. SAFETY

You, the employee, have direct responsibility to avoid injury and illness at work. Nearly all-harmful exposures to infectious substances and chemicals, and other injuries, can be avoided with effective training and consistent safe work practices.

Become familiar with the Safety Manual to learn requirements on working safely and protecting the environment from harm. Although lab work typically focuses on the hazards of working with specimens and chemicals, we must also control other important hazards.

- Slips, trips, and falls cause many serious injuries. Please ensure that spills are cleaned quickly (to avoid slippery floors) and that you can see and avoid obstacles in your path.
- Ergonomic injuries result from performing tasks with too much repetition, force, or awkward position. Ergonomic injuries include strains and back injuries. Learn about ergonomic hazards and how to prevent this type of injury.
- Scratches, lacerations, and needle sticks can result in serious health consequences. Attempt to find ways to eliminate your risk when working with sharp materials.
- Warnings of other specific hazards are noted in this procedure. Please comply with the requirements to reduce your risk of injury."

Report all accidents and injuries to your supervisor or Safety Officer.

21. RELATED DOCUMENTS

None

22. REFERENCES

CLO test package insert, 2003.

23. REVISION HISTORY

Version	Date	Section	Reason	Reviser	Approval
		Supersedes	SOP M012.002		
000	8/15/13	4, 6 , 8	Add Urease Control Tablet and instructions for use	R. Master	R. Master
000	8/15/13	Add. A	Add Section to record read at receipt and 24 h and QC results for confirmation of negative results	R. Master	R. Master

24. ADDENDA

Addendum	Title
A	CLO Test Record

Addendum A

Washington Adventist Hospital
Takoma Park, MD

CLO Test Record

Date: _____ Time Implanted: _____

R.N. Signature: _____

G.I. Physician Signature: _____

FOR LABORATORY USE ONLY

Time of Receipt in Laboratory: _____

Evaluation on Receipt in Laboratory:

POSITIVE Tech Code: _____
(If positive, report results immediately, test complete)

NEGATIVE Tech Code: _____
(If negative, incubate in 25C incubator for 24h)

24-Hour Evaluation:

POSITIVE Tech Code: _____

NEGATIVE Tech Code: _____
(If negative @24h, confirm negative test with Urease Control Tablet)

Urease Control Tablet result: Yellow (Invalid test, Do not report patient test) / Red-Magenta (OK, report negative patient result)

Tech Code: _____

Revised 8/15/13

Form revised 3/31/00