Department of Microbiology
CLO Test Procedure

I. Purpose and Test Principle

The CLOtest rapid urease test detects the urease enzyme of *Helicobacter pylori* in gastric mucosal biopsies. Its use is intended for the presumptive diagnosis of *H. pylori* infection. The CLOtest is a well of urease indicator gel sealed inside a plastic slide. The gel contains urea, phenol red (pH indicator), buffers, and a bacteriostatic agent to prevent the growth of contaminating urease-positive organisms. If the urease from *H. pylori* is present in the tissue sample, it changes the gel from yellow to bright magenta.

II. Specimen Information & Device Inoculation

A. Biopsy

The recommended gastric area to biopsy is at least 2 cm away from the pylorus along the lesser or greater curve of the antrum. Tissue that appears normal should be excised. Tissue that appears eroded or ulcerated should be avoided as *H. pylori* may be present in lower numbers around those areas.

B. Inoculation

After removing the CLOtest slide from refrigeration, lift the label far enough to expose the yellow gel. With a clean applicator device, push the entire sample from the collection forceps into the gel and beneath the surface of the gel. Make sure the biopsy specimen is completely immersed in the gel. Reseal the label on the slide and record the patient name, date, and time.

III. Reagent & Materials

- A CLOtest plastic slide is submitted pre-inoculated by the physician.
- Aerobic (non-CO_2) incubator set at 35 ± 2°C

IV. Procedure

A. Upon receipt in the laboratory, check to be sure the slide is labeled with the patient's name, the date and time of inoculation.

B. Place an accession label on log sheet and record the time the device was placed into the incubator.

C. Incubate the CLOtest at 37°C in the non-CO_2 incubator for 3 h.

D. Examine the slide for a color change from yellow to magenta pink after 3 h of incubation. If a positive reaction is noted at or before 3 h, record on the log sheet and report in computer.

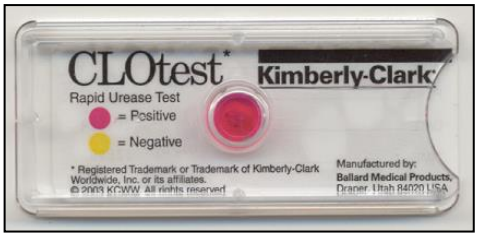
E. After 3h incubation, if the test is still negative, the specimen processing personnel should remove the slide from the incubator, and leave it at room temperature on the designated bench to be reported in the computer the following day. Record the 24 h result on the log sheet.

V. Interpretation

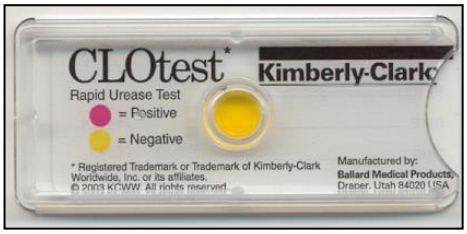
A. A specimen contaminated with blood may stain the gel around the edge of the tissue. This is NOT a positive test. If the biopsy contains urease, the change first appears around the sample and eventually colors all of the gel.

B. Positive
The pH change in a positive test is first seen at the interface of the gel and the biopsy. If a significant amount of urease is present, the visible change is rapid. Any color change of the whole gel to a shade other than yellow (e.g., red, magenta, pink, deep orange) indicates the presence of *H. pylori*.

C. Negative
A negative test remains yellow after the tissue is imbedded in the gel. If the color of the gel is yellow at 24 h, the test is negative. If the test is not examined at 24 h, the test will remain valid for 72 h after insertion of the biopsy into the gel medium.



Positive



Negative

VI. Reporting

A. A final report will be issued at the time a positive CLOtest is entered in computer.
B. Devices held for 24 h will be given a final report 24 h after the insertion of the biopsy.
C. Report a positive CLO test as: POSITIVE
D. Report a negative CLO test as: NEGATIVE

VII. Quality Control

A. Quality control should be performed upon receipt of each new lot number or shipment.
B. *Proteus mirabilis* ATCC 43504 is used to inoculate one of the test devices and serves as a positive control. Another device is incubated without inoculation to serve as a negative control.
C. If controls do not display expected results, quality control must be repeated and the supervisor notified.

VIII. Limitations

A. Before use, the CLOtest should be inspected to ensure that the agar well is full and is a yellow color. If a CLOtest is an orange color, it should be discarded because it may yield a false positive result.
B. False negative CLOtests may occur when very low numbers of *H. pylori* are present or if the bacteria are focally distributed.
C. False positive CLOtests can occur in patients with achlorhydria.
D. Commensal organisms such as *Proteus* spp. that also produce urease will grow in the absence of acid.

IX. References

A. Package Insert. Kimberly-Clark. CLOtest Rapid Urease Test. 2003.

Document Control
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