LABtobacillus

LABORATORY TECHNICAL PROCEDURE

HELICOBACTER PYLORI UREASE TEST

A qualitative biochemical assay used to detect the presence of the urease enzyme produced by Helicobacter pylori in gastric biopsy specimens.

PREPARED BY Reah Sheen Z. Aspacio

Helicobacter pylori Urease Test

Laboratory Technical Procedure

Overview

This technical procedure outlines the **Helicobacter pylori Urease Test,** a qualitative test designed to detect the presence of urease activity in gastric biopsy specimens. This method aids in the diagnosis of H. pylori infections in patients presenting with symptoms like dyspepsia, gastric ulcers, or chronic gastritis. It is intended for in vitro diagnostic use and should be performed by trained healthcare professionals. The test provides rapid results that, when interpreted alongside clinical findings, help guide patient management.

Description of the Procedure

The Helicobacter pylori Urease Test is a rapid diagnostic method used to detect the presence of **Helicobacter pylori bacteria** in gastric biopsy specimens. H. pylori is known for causing **peptic ulcers and chronic gastritis**. The test is based on detecting urease enzyme activity, which is a hallmark of H. pylori. The enzyme hydrolyzes urea into ammonia and carbon dioxide, increasing the pH of the surrounding medium and causing a color change in the pH indicator. This procedure is simple, quick, and effective, making it suitable for use in clinical laboratories during endoscopic examinations. The test typically yields results within a few minutes to 24 hours, allowing for prompt diagnosis and treatment decisions.

Principle of the Procedure

The Helicobacter pylori Urease Test detects the enzyme urease, which is produced by Helicobacter pylori in gastric biopsy specimens. Urease plays a crucial role in the survival of H. pylori within the acidic environment of the stomach by breaking down urea, a compound naturally present in the stomach, into ammonia and carbon dioxide. The ammonia generated during this process increases the pH (alkalinity) of the surrounding environment.

The test medium contains urea and a pH-sensitive dye, such as phenol red, which changes color in response to pH shifts. When a biopsy sample containing H. pylori is placed into the medium, the urease enzyme breaks down the urea, releasing ammonia. This release of ammonia raises the pH, causing the medium's color to shift from yellow (acidic conditions) to pink or red (alkaline conditions). The appearance of a pink or red color indicates a positive result, suggesting the presence of H. pylori, while no color change suggests a negative result.

he test is qualitative, providing a simple positive or negative outcome based on whether urease activity is detected. Rapid changes in color typically indicate a higher concentration of H. pylori, while slower changes (up to 24 hours) can occur when bacterial concentrations are lower.

Clinical Reasons for Testing

This test is used to detect H. pylori infection in the following scenarios:

- **Diagnostic:** When patients exhibit symptoms consistent with H. pylori infection, such as persistent upper abdominal pain, bloating, or history of gastric ulcers.
- **Monitoring:** In patients undergoing treatment for H. pylori, the test can confirm the eradication of the bacterium after therapy.
- **Screening:** For individuals with a history of peptic ulcer disease or those in high-risk groups for gastric cancer.

Specimen Requirements

Type of Specimen: Gastric biopsy specimen

- **Volume:** A small biopsy specimen (approximately 2-3 mm in diameter) is required for testing.
- **Collection Method:** Biopsy specimens should be collected via standard endoscopic biopsy forceps. Specimens can be taken from the antral or corpus region of the stomach and can also be collected from different areas to improve diagnostic accuracy, especially in cases with patchy infection.
- **Storage and Handling:** Place the biopsy directly into the urease test medium immediately after collection to ensure the viability of the tissue and accuracy of the results. If testing cannot proceed immediately, store the specimen at room temperature (15–30 °C) and test within 1 hour. Avoid freezing biopsy specimens, as this may degrade tissue and affect test accuracy.

Specimen Handling Criteria

- **Collection:** Collect the biopsy using sterile techniques to avoid contamination. Immediately place the specimen in the test medium to preserve the enzyme activity. Ensure proper labeling of the test container with the patient's name, identification number, date, and time of collection.

- **Labeling:** Specimen labels must include the patient's full name, unique identification number, and date and time of collection to ensure traceability.

- **Rejection Criteria:** Specimens will be rejected if they are improperly labeled, contaminated with blood, or if the biopsy specimen is too small for testing. Biopsies stored for more than 1 hour before being placed in the test medium, or those exposed to inappropriate temperatures, may be deemed unsuitable for testing.

- **Storage:** Store the biopsy specimen in the test medium at room temperature (15–30°C) if testing within 1 hour. Longer storage periods are not recommended, as they can compromise the accuracy of the results.

- **Transport:** Transport biopsy specimens in their test medium using a leak-proof container if testing is not done at the collection site. Keep specimens at room temperature for short distances to preserve enzyme activity, and follow biohazard packaging guidelines to ensure safety.

Submission to Central Labs

For the Helicobacter pylori Urease Test, if the test is not done immediately on-site, specimens can be sent to a central lab. Biopsy samples must be properly labeled, packed, and include the necessary documentation. They should be transported at room temperature (15–30°C) following biohazard guidelines, with testing performed within 1 hour of collection to maintain accuracy. The central lab will check the specimen's integrity, labeling, and transport conditions; any issues may result in rejection.

Microscopic Examination

Microscopic examination is not applicable for the Helicobacter pylori Urease Test, as it detects urease enzyme activity through a color change in the medium, not by visualizing cells. The result is based on the observed color shift, indicating the presence or absence of H. pylori due to pH changes.

Reagents, Supplies, and Equipment

Helicobacter pylori Urease Test Kit

The Helicobacter pylori Urease Test kit typically includes the following components:

- **Urease Test Medium:**
  - Contains urea as a substrate and a pH-sensitive dye (e.g., phenol red) that changes color upon hydrolysis of urea.
  - The medium is typically yellow under neutral or acidic conditions and turns pink/red upon an increase in pH due to the presence of urease activity.

- **Packaging:**
  - The urease test medium is provided in sealed, individual test containers or slides that are sterile and pre-prepared for immediate use.

- **Test Container or Slide:**
  - The test substrate is often embedded in a gel or a solidified surface on a slide.
  - Each slide or container includes an adhesive seal that must be peeled back to expose the reaction surface.

Active Ingredients of the Main Components

- **Urea:** 1–2% concentration in the medium.
- **pH Indicator:** Typically phenol red (changes from yellow to red as pH rises).
- **Sterile Gel or Membrane:** Embedded with the urease test medium.

Storage Requirements

- **Temperature:** Store the urease test kit at 2°C to 30°C. Avoid freezing.
- **Shelf Life:** The kit components are stable for 24 months under ideal storage conditions, as indicated on the packaging.
- **Light Protection:** Store the test components away from direct sunlight to avoid degradation of the reagents.
- **Use after Opening:** Once opened, use the test kit immediately. Do not use expired or damaged test kits.

Calibration

The biopsy-based urease test does not require external calibration. However, performance should be verified using positive and negative control samples periodically.

Quality Control

Internal Quality Control

The urease test device includes a built-in procedural control. The pH indicator serves as the internal control mechanism; the color change occurs only in the presence of urease, confirming the test's functionality.

External Quality Control

It is recommended to run positive and negative control samples at regular intervals, such as at the start of each new batch of test kits. If control results are inconsistent, investigate potential causes (e.g., improper storage, reagent contamination) and repeat the test using new reagents.

- **Positive Control:** An artificial sample known to contain H. pylori urease or a urease-producing bacterial culture.
- **Negative Control:** A sterile sample without urease activity.

Step-by-Step Instructions for Testing

1. **Open the Test Kit**
   Peel back the label of the urease test container or slide to expose the yellow reaction pad.

2. **Insert the Biopsy Specimen**
   Using a sterile blunt instrument or the biopsy forceps, transfer the gastric biopsy specimen onto the reaction pad. Ensure that the biopsy is in full contact with the urease test medium.

3. **Re-Seal the Test Container**
   Re-seal the container or slide immediately by pressing the label back over the reaction pad. Press lightly to ensure the biopsy specimen is in contact with the test surface.

4. Record the Patient Information

Label the test container with the patient's information, including the name, date, and time of the test initiation.

5. Monitor the Test

Place the test container on a flat surface at room temperature. Observe the test for color changes at **5 minutes**, **30 minutes**, and **1 hour**. For the final reading, check for a color change after 24 hours for cases where no immediate result is visible. Discard the test after 24 hours, as any results beyond this point may be inaccurate due to contamination.

Quantitative Testing

Not applicable. This is a qualitative test and does not provide quantitative measurements of H. pylori concentration.

Qualitative Testing

Results are interpreted as **positive** or **negative** based on the color change in the test medium. A shift from **yellow to pink or red** indicates a **positive result**, while **no color change** indicates a **negative result**.

Interpretation of Results

- **Positive Result:**

A visible color change from yellow to pink or red indicates the presence of urease enzyme activity, suggesting an H. pylori infection. The degree of color change can vary depending on the bacterial load, with more intense color indicating a higher concentration of urease.

Interpretation Timeframes:

- **5 minutes:** Rapid urease activity, indicating a high bacterial load.
- **30 minutes – 1 hour:** Moderate bacterial presence.
- **24 hours:** Low bacterial concentration.

- **Negative Result:**

If no color change occurs after 24 hours, the test is considered negative, indicating no detectable urease activity and likely the absence of H. pylori.

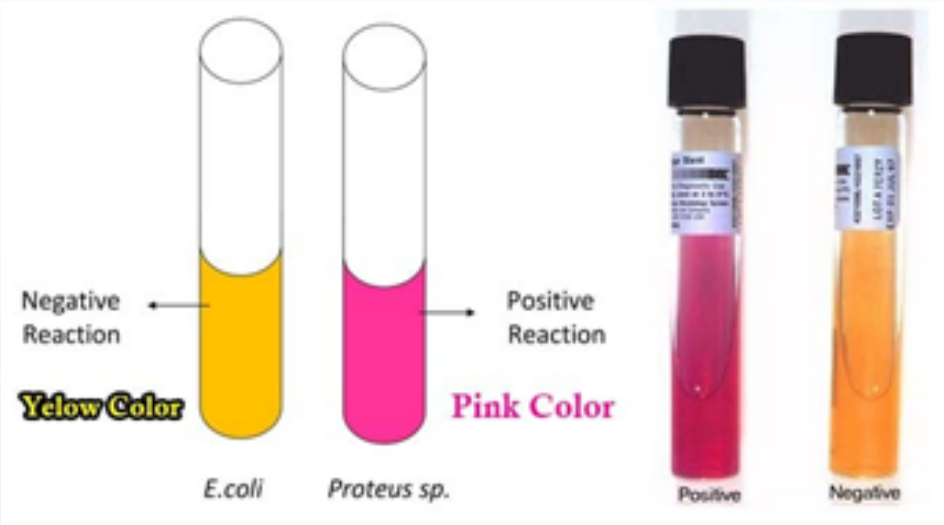

Image Source: microbenotes.com

Image Source: ADC Education and Practice

Reporting of Results

- **Positive Result:** Report as "Positive for H. pylori urease activity."
- **Negative Result:** Report as "Negative for H. pylori urease activity."
- **Invalid Result:** Repeat testing and report if repeated results remain invalid.

Procedure Notes

- **Special Precautions:**

- **a)** Treat all biopsy specimens as potentially infectious, as they come from the human gastrointestinal tract. Wear appropriate personal protective equipment (PPE), including gloves and lab coats, when handling specimens and test reagents.

- **b)** Dispose of used biopsy samples, test containers, and any other materials that come into contact with the specimen in biohazard waste containers to prevent contamination and ensure safety.

- **c)** Use sterile instruments for handling each biopsy specimen to prevent cross-contamination. Clean and disinfect the workspace before and after each test. A fresh test kit must be used for each patient to avoid cross-contamination.

- **d)** Patients should discontinue antibiotics, proton pump inhibitors (PPIs), and bismuth compounds at least two weeks before testing to reduce the risk of false-negative results. Avoid testing inflamed or eroded areas of the stomach, as H. pylori may be present in lower concentrations in these regions, which can also lead to false negatives.

- **e)** Perform the test in a room with a stable temperature between 15–30°C. Avoid direct sunlight, high humidity, or excessive heat, as these can affect the stability of the test medium and the accuracy of results.

- **f)** Handle reagents carefully, ensuring they are stored as per the manufacturer's instructions. Avoid using expired reagents, as this can lead to inaccurate results.

- **Possible Sources of Error:**

- **a)** Improper placement of the biopsy specimen on the test medium or delayed testing can result in false-negative results.

- **b)** Use of expired reagents or failure to store the urease test medium correctly may compromise test performance, leading to inaccurate results.

- **c)** Contamination with blood or other substances may interfere with the reaction, potentially leading to false-positive or false-negative results.

- **Answers to Common Problems:**

- If no color change is observed and the control indicates an invalid result, the test must be repeated using a new biopsy sample and a fresh test medium. Ensure that all steps were followed correctly before re-testing.

Limitations of Methods

1. **False Negatives:**
   - Can occur if the patient has taken antibiotics, PPIs, or bismuth-containing compounds recently. These medications reduce the bacterial load and inhibit urease activity.
   - False negatives may also occur in patients with patchy H. pylori distribution or severe gastric atrophy.

2. **False Positives:**
   - Rare but can occur if the specimen is contaminated with non-H. pylori urease-producing organisms such as Proteus spp., though these are uncommon in the acidic environment of the stomach.

3. **Test Duration:**
   - Tests read after 24 hours are prone to false positives due to non-H. pylori urease activity, and should not be considered reliable.

References

- Uotani, T., & Graham, D. Y. (2015). Diagnosis of Helicobacter pylori using the rapid urease test. Annals of Translational Medicine, 3(1), 10.3978/j.issn.2305-5839.2014.12.04. https://atm.amegroups.org/article/view/5299

- Graham, D. Y., & Yamaoka, Y. (2000). Helicobacter pylori: Current Status in Gastroenterology. Journal of Clinical Gastroenterology, 30(2), 117-122. https://www.ncbi.nlm.nih.gov/books/NBK534233/

- Rimbara, E., Sasatsu, M., & Graham, D. Y. (2011). PCR Detection of Helicobacter pylori DNA in Feces and Use in Diagnosis of Infection. Journal of Clinical Microbiology, 49(1), 166-170. https://www.sciencedirect.com/science/article/pii/S0167701201002251

- CLOtest and Accutest Product Insert (2015). Accutest H. pylori Urease Test. Jant Pharmacal Corporation. https://www.jantdx.com/wp-content/uploads/2017/11/UreaseTest1.pdf

- Microbenotes. (n.d.). Urease test: Principle, procedure, and result. Retrieved from https://microbenotes.com/urease-test-principle-procedure-and-result/

- ADC Education and Practice. (n.d.). Urease test interpretation. Retrieved from https://ep.bmj.com/content/98/1/18

Prepared by:

REAH SHEEN Z. ASPACIO
Assistant Clinical Pathologist

Approved by:

MARISTELLA URIELLE E. CORTEZ
Laboratory Director

EFFECTIVE IMMEDIATELY UPON APPROVAL