2. Clinical Studies

Clinical studies were carried out at the Gastroenterology Departments of two Chilean Hospitals. In these studies, the efficiency of the **GOLD Hpdry** and the **HE-PY TEST**[MR] were compared. For that purpose, two biopsy samples were obtained per each patient both from the same area of the stomach, one for **HE-PY TEST**[MR] and the other for **GOLD Hpdry**. In the case of **GOLD Hpdry**, results were observed at 10, 30 and 60 minutes. In the case of **HE-PY TEST**[MR], results were observed at 24 hours. The results are shown in the following table.

	GOLD Hpdry			**HE-PY TEST**[MR]
Samples	10 minutes	30 minutes	60 minutes	24 hours
Positives	40	44	47	43
Negatives	28	24	21	25
Total	68	68	68	68

This Table shows that that results obtained with **GOLD Hpdry** at 30 minutes are comparable with those obtained with **HE-PY TEST**[MR] after 24 hours of reaction.

VII. ASSAY LIMITATIONS.

1. False Negative results

- You may observe false negative results with **GOLD Hpdry** when very low number of bacteria are present or when the bacterium has a patchy distribution.

- When the biopsy is too small.

- There are some patients (1 to 5%) in which the bacterium is present in the body of the stomach but not in the antrum and vice versa. This tends to happens in patients under Omeprazole treatment.

- In patients with extense intestinal metaplasia an area of intestinal epithelium may be biopsied. As *H. pylori* does not colonize intestinal mucosa, a false negative result may be obtained.

2. False Positive results

False-positive are uncommon, and could be obtained with any assay measuring urea metabolization, included the breath air assays. This could occur in patients who have hypochlorhydria, or achlorhydria which favour superficial colonization of the gastric mucosa layer with other urease-producing organisms. The gastric acidity and peristalsis prevents bacterial growing, but when these functions are altered by the prolonged use of Omeprazole, previous gastric surgery or pernicious anemia, the gastric mucosa turns into in an adequate substrate for bacterial overgrowth, which causes chronic gastritis that could be associated to a higher risk of gastric adenocarcinoma (11-13).

Slow, positive results in patients with prolonged use of proton pump inhibitors or with of the gastric mobility abnormalities, without other evidence of *H. pylori* infection (serology, culture or histology), could be an indication of over infection of the gastric mucosa and should be considered by the physician. Other representatives of the *Helicobacter* genus (*H. helmanii, H. felis*) have been described in human gastric mucosa. These bacteria, whose natural hosts are domestic animals, as *H. pylori,* are related with gastritis and ulcers. The *H. helmanii* infection prevalence in humans is approximately 0.5% in endoscopy patients and could be considered a zoonosis. The infection with these bacteria could give positive results in urease test, even when the results could be slower than usual (2, 14).

VIII. TROUBLESHOOTING

Problem	**Posible Cause**
The reaction starts quickly, but after a while the color change diminishes or turns yellow	Lack of water: - Just one small biopsy was used - The sample dried during the process - The slide was not sealed properly
The color change only affects one filter side	The biopsy was not centered in the filter
The reaction is positive and does not correlate with other techniques results	Probably an over infection due to hypochlorhydria or achlorhydria (see 7.2).

SYMBOL EXPLANATION

IVD	In vitro Diagnostic Device
	Use by
	Temperature limitation
LOT	Lot number
REF	Catalog number
	Manufacturer
EC REP	European Authorized Representative

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Santiago, Chile

EC REP EMERGO EUROPE
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2513 BH, The Hague
The Netherlands
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CE

GOLD Hpdry

CE

Read entire protocol before use

GOLD Hpdry is a rapid assay for the detection of *Helicobacter pylori* in gastric mucosal biopsies, based on the detection of urease activity.

Only for *in vitro* diagnostic.

I. SUMMARY

Helicobacter pylori is a spiral bacillus, Gram negative, neutrophil and microaerophilic bacteria. It was first isolated in 1983 from gastric mucosal biopsies from patients having chronic gastritis (1).
Epidemiological studies have demonstrated a close relationship between the presence of *Helicobacter pylori* in the stomach and chronic gastritis type B, gastroduodenal ulcers and the risk of acquiring some types of gastric cancers. The eradication of *H. pylori* by means of a proper treatment, reverts the clinical symptoms and heals the physiologic alterations of the gastric epithelium, being the indicated treatment for infected patients that suffer peptic ulcers and gastric mucosa-associated lymphomas in early stages (2-4).

H. pylori infection can be detected in gastric biopsies samples by using classical histological techniques, by culture, by PCR or by its characteristic of producing large amounts of urease The urease detecting assays are the most common because of their simplicity, speed and low cost (2,5). Moreover, they have demonstrated its effectiveness in monitoring the eradication treatment when 3 biopsies per assay are used (6).

Urease is an enzyme that hydrolyzes urea, producing ammonium and bicarbonate, which causes a general raise of the pH according to the following reaction:

$$(NH_2)_2CO + 2H_2O + H^+ \xrightarrow{\text{UREASE}} 2NH^+_4 + HCO^-_3$$

Urea — Water — Ion Ammonia — Ion Bicarbonate

The urease from *H. pylori* show high specific activity compared to other microorganisms (7). Also this bacterium produces large amounts of the enzyme, part of which localizes on the outer membrane. This unique characteristic allows it to quickly metabolize the urea present in the gastric mucosa, thus creating an appropriate pH micro ambient for stomach colonization (8).

The urea hydrolyzing ratio for *H. pylori* can be up to 1000 times greater than the ones for other bacteria present in the digestive tract. In addition, the fact that urease does not exist in mammals' stomach makes urease activity based tests highly sensitive and specific for *H. pylori*.

II. PRODUCT DESCRIPTION

GOLD Hpdry consists of a polystyrene sealed slide holding a cellulose support disc containing urea, a pH indicator, bacteriostatic agents and a buffer solution.

The cellulose support comprises two yellow areas, a disc (sample layer) and a ring (indicator layer). If the tissue sample being placed in the disc contains *Helicobacter pylori*, it will metabolize the urea present in the media producing ammonia, an increase in pH and a change in color of the ring layer which will turn from yellow to an intense magenta. If there is no presence of urease activity, the indicator layer will maintain its original yellow color.

The assay should be performed at room temperature (20-25°C) and the results are obtained in one hour.

III. STORAGE AND TRANSPORTATION CONDITIONS

GOLD Hpdry should be storage between 8° and 42°C in a dry place and away from volatile reagents. No special transportation conditions are required.

IV. PRECAUTIONS

- For professional use only
- For *in vitro* diagnostic only.
- Before using **GOLD Hpdry** verify that the cellulose membranes has its characteristic yellow color.
- Handle all biopsies as potentially infectious samples
- Wear gloves when handling forceps to remove the biopsy.
- Use a new, sterile needle for taking the biopsy from the forceps.
- Do not drink, eat, smoke or manipulate contact lenses in the work area.
- Discard all contaminated material in containers dedicated to this purpose.

V. PROCEDURE

1. PATIENT PREPARATION

Patients should not have been taken antibiotics, bismuth salts or Omeprazole for at least four weeks prior to endoscopy procedure to diminish the possibility of a false negative result.

2. BIOPSY

The biopsy sample for the urease assay may be obtained immediately after the endoscopist has examined the stomach. It is recommended to obtain the biopsy from the sump antrum, along the greater curve (2,9).

In post-eradication treatment patients, it is recommended to take an extra biopsy from the antrum or from the gastric angle. A standard biopsy forceps will provide a specimen of sufficient size for urease assay (2-3 mm diameter). Take the biopsies from an area of normal looking tissue rather than from an area affected by erosions, ulcerations or with blood contamination. *Helicobacter pylori* could be present in smaller numbers when the epithelium is eroded or if the mucosa layer is denuded. Blood contamination in the sample can prevent the assay color change.

Note: place the biopsies immediately over the reaction filter and seal the plate as soon as you have finished the procedure, securing the label seal. Samples dehydration could lead to false negative results.

If the biopsy sample is too small, insert an extra biopsy.

3. ASSAY

3.1. Record the patient data on the label.

3.2. Peel back the label covering the **GOLD Hpdry** slide just enough to expose the cellulose substrate.

3.3. Once the biopsies are obtained, extract them immediately from the forceps with a sterile needle and place them in the center of the cellulose membrane. Spread the tissue over the disc surface in order to get the maximum contact. Proceed the same way with the second sample. All samples should be in the center of the cellulose membrane.

3.4. Immediately re-seal the slide with the same label. Make sure the plastic cover adhered to the inner part of the label fits inside the well.

3.5. Put the slide over a table, and press firmly over the plastic cover in order to squeeze the gastric fluid from the biopsy. Do not press the slide against a soft surface like an examining couch, because the slide could break.

3.6. Record the time the sample was inserted in the sample layer and observe the slide at 2, 10, 30 and 60 minutes thereafter.

4. REACTION FOLLOW-UP

4.1. Urease activity (*H. pylori*) is present in the tissue sample:

- An expanding red zone will be formed starting from the inner border of the indicator layer. The color begins to spread across the layer until the entire ring has changed. The colour will gradually change from dark orange to magenta.
- Once equilibrium is reached, the color will stay stable, ranging from light pink to magenta. The sample layer (center of the ring) will not change its color. Only very minor changes could be noticeable, due to small amounts of blood from the sample.
- The speed of the change and the final color would vary in direct proportion to the urease activity present on the sample.

4.2. Urease activity (*H. pylori*) is not present in the tissue sample:

- The indicator layer will not change its color; it will remain yellow over time.
- The sample layer (center of the ring) will not change its color, except for minor changes that could occur due to blood from the sample.

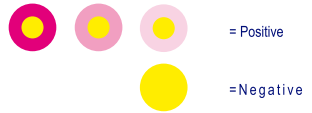
5. RESULTS INTERPRETATION

5.1 Positive Results

Results will be positive when the indicator sheet, i.e. the membrane with the hole in the center, changes its color in one hour or less after sample addition. The color change could be visible between 5 to 60 minutes, depending on the *Helicobacter pylori* amount in the biopsies.

5.1 Negative results

the result will be negative when the indicator sheet, i.e. the membrane with the hole in the center, maintains its yellow color one hour after sample addition (see 4.2).



VI. ASSAY CHARACTERISTICS

1. Sensitivity Studies

Studies were carried out in GrupoBios S.A. using a commercial urease and an *H. pylori* strain CH-CTX1 (4, 10) grown *in vitro*. In these studies the sensitivity of the **HE-PY TEST**[MR] (agar based urease test) and the **GOLD Hpdry** were compared.

1.1. Commercial urease sensitivity determination

The slides were loaded with 2 uL of different urease dilutions from *Canavalia ensiformis* and the reaction was followed over time. The minimal amount of urease capable to develop a change in color, are shown in the following table.

	GOLD Hpdry	HE-PY TEST[MR]
C. ensiformis urease	2,0 mUI in 10 minutes at room temperature.	3,0 mUI in 24 hours at 37°C.

V1.2. *H.pylori* urease sensitivity determination

H. pylori was grown in *Brucella* agar medium supplemented with horse blood in a microaerobic environment. The colonies were resuspended in NaCl 0,9% to an approximate OD of 0.2 and serially diluted with the same solution. 3 uL of every dilution were loaded on the tests. **The HE-PY TEST**[MR] was incubated at 37°C and the **GOLD Hpdry** was kept at room temperature. The required time for observing color changes for a given number of bacteria are shown in the following table:

Nº of bacteria	GOLD Hpdry	HE-PY TEST[MR]
210.000	2 minutes	30 minutes
23.000	10 minutes	24 hours
8.000	60 minutes	Negative at 24 hours

The table shows that the results obtained using *in vitro* cultured *H. pylori* at 24 hours with regular **HE-PY TEST**[MR], is obtained in 10 minutes with **GOLD Hpdry**.