**INTRODUCTION**

*Helicobacter pylori* is now recognized as an etiologic agent in chronic gastritis and peptic ulcer disease. Diagnostic tests for *H. pylori* can be categorized as invasive (endoscopy, biopsy) or noninvasive (serology, urea breath test and stool antigen test). The stool antigen test has been considered as an accurate non-invasive test.

The bioNexia® *H. pylori* Ag test is a qualitative rapid test for the detection of *Helicobacter pylori* antigen in human stool samples. The test is intended to be used as an aid for diagnosis of *H. pylori* infection and for demonstration of eradication of the bacteria after treatment.

The aim of this study was to evaluate the performance of the qualitative rapid test bioNexia® *H. pylori* Ag, in terms of performance by comparison to the Meridian ELISA test (HpSA® PLUS). Other performance criteria related to the cross-reactivity of the test to gastrointestinal pathogens including bacteria and virus isolates as well as the interference with certain endogenous substances or drugs that may be present in stool were evaluated. The precision of the test was also assessed.

**MATERIAL AND METHODS**

- **bioNexia® *H. pylori* Ag test principle:**
  - bioNexia® *H. pylori* Ag test is a rapid immunochromatographic test for the qualitative detection of *H. pylori* antigen in human stool.
  - The kit uses monoclonal antibodies specific to *H. pylori* antigen. During testing, the stool sample reacts with the red latex particles coated with anti-*H. pylori* monoclonal antibody. In the case of a positive sample, the particle-antigen-antibody complexes migrate by capillary along the membrane that is pre-coated with anti-*H. pylori* monoclonal antibody in the test line region. These specific antibodies react with the complex particle-antibody-antigen and generate a red line in the test-line area. The green control line indicates that test migration has been performed correctly.

- **Content of the kit and assay procedure**
  - Each bioNexia® *H. pylori* Ag kit contains 25 cassettes inside sealed pouches and 25 stool collection tubes.
  - As an optional solution for collecting samples: bioNexia® *H. pylori* Ag PS kit (sold separately) contains a stool collection paper that constitutes an alternative solution for stool collection mainly in cases of Near Patient Testing.

- **Comparative study between bioNexia® *H. pylori* Ag test and Meridian ELISA test (HpSA® PLUS)**
  - The performance of bioNexia® *H. pylori* Ag was compared to Meridian ELISA test (HpSA® PLUS) in two different studies using human stool samples from adults and children including symptomatic patients and at-risk patients:
    - First study performed on verification step of the product using 99 stool samples
    - Second study performed on validation step of the product using 196 stool samples.

- **Cross-reactivity**
  - The cross-reactivity was evaluated on 32 gastrointestinal pathogens including bacteria and virus isolates. Positive and negative stool samples were spiked with each pathogen at a pre-established concentration.

- **Interfering substances**
  - The interference of certain endogenous substances and some drugs that may be present in stool was evaluated. Positive and negative stool samples were spiked with each substance at a pre-established concentration.

- **Precision (repeatability/reproducibility)**
  - The precision of bioNexia® *H. pylori* Ag was evaluated on 2 reagent lots, using native urine samples: one moderate positive, one low positive and one negative. These samples were tested six times on each lot at two different times of the day. Each test was read by three independent readers, generating 72 results.

- **Near Patient Testing**
  - A study was conducted by 15 non-laboratory healthcare professionals (physicians and nurses) using one panel of 16 samples per operator: 4 negative, 4 low positive (close to the assay detection limit), 4 moderate and 4 high positive samples. Each operator tested the panel randomly and blindly. Two-hundred and forty results were generated. These data were compared to the expected results for each sample.

**RESULTS**

1. **Comparative study**
   - A study was carried out using 99 stool samples. The results of the bioNexia® *H. pylori* Ag test were compared to those of an ELISA microplate (HpSA® PLUS).
   - Verification study carried out on 99 human stool samples
   - Overall percent agreement of bioNexia® *H. pylori* Ag versus the ELISA (HpSA® PLUS): 91% CI 95% [93.4; 95.7]
   - Validation study carried out on 196 human stool samples
   - Overall percent agreement bioNexia® *H. pylori* Ag versus the ELISA (HpSA® PLUS): 94.9% CI 95% [90.9; 97.2%]

2. **Cross-reactivity**
   - None of the following organisms induced a positive result with the negative samples, nor interfered with the detection of positive samples:

3. **Interfering substances**
   - None of the following endogenous substances or drugs induced a positive result with negative samples, nor interfered with the detection of positive samples:
     - **Endogenous substances:** Whole Blood 50%, Urine 50%, Human Albumin 1mg/mL, Human Hemoglobin 1mg/mL, HCG hormone 1000mIU/mL, Bovine IgG 0.5mg/mL
     - **Drugs:** Antifungal (Ketoconazole®) 5%, Protons pump inhibitor (Pantoprazole®) 4%, Antibiotic with local action (Moxalactam®) 1:20, Antacid with clay (Bismuth®) 3%, Antacid with silicone (Polysilane®) 1:20
     - However, the following substances were evaluated and did interfere with the bioNexia® *H. pylori* antigen: Stearic acid 0.48%, Palmitic acid 0.43%, Mucin 0.125%

4. **Precision (repeatability/reproducibility)**
   - The concordance rate between results obtained and expected results was 100%.

5. **Near Patient Testing**
   - The overall percent agreement between the expected results and the results obtained with non-laboratory healthcare professionals on the panel of 18 negative and positive samples at different levels was: 90.4% CI 95% [86.0 – 93.5%]

**CONCLUSIONS**

- The bioNexia® *H. pylori* Ag test shows very satisfactory performance compared to the ELISA test. This good level of performance was confirmed on the final product during clinical trials by comparison to clinical criteria (results are not yet published). No interference was detected with pathogens other than *H. pylori* or substances potentially present in human stool. The test is an aid in the diagnosis of *H. pylori* as well as in the monitoring of the eradication of the bacteria after treatment.

- This non-invasive and easy-to-use rapid test is appropriate for Near Patient Testing by non-laboratory healthcare professionals. Furthermore, a stool collection paper sold separately is an optional alternative solution that makes the stool collection by the user easier and more comfortable.