Introduction

The BreathID® Hp urea breath test (UBT) System provides several advantages over other 13C breath analyzers for the detection of H. pylori, including: higher accuracy, operator independence and immediacy of results. However, there are occasions when mailing or transporting saved breath samples may be preferable to real time analysis, especially in centers requiring a large number of automated analyses where continuous sampling from a single patient may cause a bottle neck.

Aim

To evaluate the sensitivity and specificity of a new BreathID® Hp Lab System (Exalenz Bioscience Ltd, Israel), a 13C-UBT system using breath sampling bags, for the diagnosis of H. pylori in a multi-center prospective international clinical study.

Materials & Methods

Subjects

A total of 207 subjects (age > 18 yrs) from 13 clinical sites in the USA and in Israel: 189 subjects with unknown H. pylori (Hp) status were included in the pre-therapy group and 68 subjects who had completed eradication therapy were included in the post-eradication group.

Evaluation of H. pylori status

Each subject was evaluated for H. pylori status by 3 diagnostic methods.

1. Histopathology: At least three biopsy specimens taken from each stomach location: antrum, corpus, and antrum under endoscopy (EGID). Biopsy specimens were fixed in formalin, stained with hematoxylin & eosin (H&E) and an immunohistochemistry (IHC) assay was performed at a central laboratory.
2. RUT: Additionally at least three biopsy specimens taken from each stomach location were tested on site for urease activity with an FDA-cleared rapid urease testing (RUT) (Pronto Dry®).

Histopathology and RUT interpretation

• RUT or histology (by IHC) were considered positive if at least one of the samples showed a positive result.
• If all samples were negative the patient was classified as RUT or histology (by IHC) negative.
• To define a patient as positive or negative when combining the RUT and histology results, FDA guidelines’ were used.
• For patients in the pre-therapy group only concordant results between RUT and histology were used to classify subjects as positive or negative. Patients with discordant results were considered non-evaluable.
• For patient in the post-eradication group, any positive outcome, RUT or histology or both, would render the subject’s classification as positive. Only F10 RUT and histology were found to be negative, the subject was classified as negative.

3. UBT: UBT was performed within one week before or after EGID. Antimicrobials, proton pump inhibitors (PPIs) and bismuth preparations were avoided within two weeks prior to administering the UBT. Each participant fasted for at least one hour, filled 2 bags prior to the test, then ingested a test solution containing the 13C-urea test solution (Exalenz Hp™, Two, Exalenz, Israel) and filled 2 bags within 15 to 20 min after ingesting the test solution.

UBT analysis

The BreathID® Hp Lab System, contains an auto-sampler unit that can measure up to 10 sets of bags automatically within approximately 30 min on site or at a remote location. The BreathID® Hp Lab System is based on molecular correlation spectrometry. The system calculates the 13CO2/12CO2 ratio change in the exhaled breath before and after ingestion of 13C labeled urea and produces a Delta-over-Baseline (DOB) value. DOB ≥ 5 indicates H. pylori infection.

Stability assessment of the breath samples in the breath sample bags

Each pair of breath sample bags (before and after ingestion) obtained from the pre-therapy cohort was analyzed at a different time point up to 14 days after collection. The first evaluable set of bags was used for the primary analysis. The second set of bags was used to assess the stability of the breath samples over time.

Statistical analysis

Statistical programming and analyses were performed using SAS® Version 9.4. The results are presented in two-way contingency tables. The exact binomial distribution was used to calculate the lower and upper limits of the 95% confidence intervals (CI) of the performance statistic.

Results

Comparison of 13C-UBT results to endoscopy biopsy results

Pre-therapy cohort: Evaluation of the composite results from the RUT and histological exam was performed. 176 of 178 results matched those of the first evaluable UBT resulting in an overall agreement between the breath test and the reference biopsy result of 98.3% (95% CI 97.9-98.7). 37 results were positive and 142 results were negative showing a sensitivity of 100% and specificity of 97.9%. Comparing the UBT to RUT only showed sensitivity of 88.1% and specificity of 96.2%. The sensitivity of the UBT compared to histology was 96.6% with a specificity of 98% (Tables 1 & 2).

Post-eradication therapy cohort: In 67 of 68 subjects, UBT results matched those of the Composite Reference Standard biopsy results, of which 55 were negative and 13 were positive. The overall agreement between the UBT and the biopsy was 98.5% (95% CI 98.1-100%). The sensitivity of the UBT was 92.3% and specificity 100%. Comparing the UBT to RUT only showed a sensitivity of 100% and a specificity of 97.3%. Comparing the UBT to histology demonstrated a sensitivity of 92.3% and a specificity of 100% (Tables 1 & 2).

Table 1: Comparatives results of UBT, histology, RUT and composite test results

<table>
<thead>
<tr>
<th></th>
<th>Pre-treatment</th>
<th>Post-eradication therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HP (+)</td>
<td>HP (-)</td>
</tr>
<tr>
<td>UBT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HP (+)</td>
<td>37</td>
<td>3</td>
</tr>
<tr>
<td>HP (-)</td>
<td>0</td>
<td>139</td>
</tr>
<tr>
<td>Sensitivity (%)</td>
<td>100 (90.6-100.0)</td>
<td>88.1 (75.0-94.8)</td>
</tr>
<tr>
<td>Specificity (%)</td>
<td>97.9 (94.0-99.3)</td>
<td>95.2 (90.5-97.7)</td>
</tr>
</tbody>
</table>

Table 2: Diagnostic performance of the tests

<table>
<thead>
<tr>
<th></th>
<th>Pre-treatment</th>
<th>Post-eradication therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Composite</td>
<td>RUT</td>
</tr>
<tr>
<td>Sensitivity (%)</td>
<td>100 (90.3-100.0)</td>
<td>98.3 (90.7-99.7)</td>
</tr>
<tr>
<td>Specificity (%)</td>
<td>92.3 (66.7-98.6)</td>
<td>100 (74.1-100.0)</td>
</tr>
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</table>

Conclusion

The validation studies of the BreathID® Hp Lab System described above show it is a highly accurate and reliable method for the diagnosis of H. pylori infection for both pre-treatment and for eradication confirmation. Based on the current study, the BreathID® Hp Lab System received marketing clearance from the FDA for H. pylori detection, in November 2016.

Declaration of conflict of interest

Haim Shinn is a consultant, received research grants and has ownership interest with Exalenz Bioscience Ltd, Israel.