The Validity of Breath Collection Bags Using the Novel *BreathID® Hp Lab System:* A Multicenter Clinical Study in 257 Subjects

Haim Shirin[1], Jeff O Gonzalez[2], Sabine Hazan[3], Gary Gottlieb[4], Keith Friedenberg[5], David Gatof[6], Ravi Ganeshappa[7], Jorge-Shmuel Delgado[8], Dov Abramowitz[1], Robert Hardi[9], Allan Coates[10], Mahmudul Haq[11], Nilesh Mehta[12], Blake A Jones[13], Steven F Moss[14]

[1]The Kamila Gonczarowski Institute of Gastroenterology, Assaf Harofeh Medical Center, Tel-Aviv University, Israel, [2]Palmetto Research, LLC, Hialeah, FL, USA, [3]Ventura Clinical Trials, Ventura, CA, USA, [4]Del Sol Research Management, Tuscon, AZ, USA, [5]Great Lakes Medical Research, Mentor, OH, USA, [6]Innovative Clinical Research – CO, Lafayette, CO, USA, [7]Digestive Disease Center of South Texas, P.L.L.C, San Antonio, TX, USA, [8]Barzilai Medical Center, Ashkelon, Israel, [9]Chevy Chase Clinical Research, Chevy Chase, USA, [10]West Michigan Clinical Research Center, Wyoming, MI, USA, [11]Hope Clinical Research, Kissimmee, FL, USA, [12]Digestive Disease Care PC, New Hyde Park, NY, USA, [13]Innovative Clinical Research – SD, Rapid City, SD, USA, [14]Rhode Island Hospital, Brown University, RI, USA.

Introduction

The BreathID® Hp urea breath test (UBT) System provides several advantages over other ^{13}C 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 ^{13}C-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 257 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 taken from each stomach location: angularis, corpus, antrum under upper endoscopy (EGD). Biopsy specimens were fixed by 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 determine if a patient was positive or negative when combining the RUT and histology results, FDA guidelines[1] 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 if both 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 EGD. 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 ^{13}C-urea test solution (IDkit 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 $^{13}CO_2/^{12}CO_2$ ratio change in the exhaled breath before and after ingestion of ^{13}C 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.

[1]FDA Draft Guidance Document: " Establishing the Performance Characteristics of In Vitro Diagnostic Devices for the Detection of Helicobacter pylori", issued September 23, 2010. https://www.fda.gov/downloads/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/UCM227110.pdf

Results

Comparison of ^{13}C-UBT results to endoscopy biopsy results

Pre-therapy cohort: Evaluation of the composite results from the RUT and histological exam was performed. 176 of 179 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:95.2%;99.7%): 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 95.2%. The sensitivity of the UBT compared to histology was 97.6% 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:92.1%;100%). The sensitivity of the UBT was 92.3% and specificity was 100%. Comparing the UBT to RUT only showed a sensitivity of 100% and a specificity of 98.3%. Comparing the UBT to histology demonstrated a sensitivity of 92.3% and a specificity of 100% *(Tables 1 & 2)*.

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

UBT	Composite		RUT		Histology (IHC)	
	HP (+)	HP (-)	HP (+)	HP (-)	HP (+)	HP (-)
Pre-treatment						
HP (+)	37	3	37	7	41	3
HP (-)	0	139	5	140	1	144
Post-eradication therapy						
HP (+)	12	0	11	1	12	0
HP (-)	1	55	0	56	1	55

Table 2: Diagnostic performance of the tests

	Composite	RUT	Histology (IHC)
Pre-treatment			
Sensitivity (%)	100 (90.6-100.0)	88.1 (75.0-94.8)	97.6 (87.7-99.6)
Specificity (%)	97.9 (94.0-99.3)	95.2 (90.5-97.7)	98.0 (94.2-99.3)
Post-eradication therapy			
Sensitivity (%)	92.3 (66.7-98.6)	100 (74.1-100.0)	92.3 (66.7-98.6)
Specificity (%)	100 (93.5-100.0)	98.3 (90.7-99.7)	100 (93.5-100.0)

Safety

Four adverse events in the initial diagnosis cohort and one adverse event in the post-eradication therapy cohort:

- None were serious or severe.
- None were related to the *BreathID® Hp Lab System* device.
- The test procedure was found to be very **safe** and well-tolerated by all subjects.

Stability of breath samples over time

The stability of the breath samples from 191 subjects (45 positive, 146 negative) was excellent, with positive agreement in 97.8% [95% CI (88.43, 99.61)] and negative agreement in 100% [95% CI (97.44, 100)].

Conclusions

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

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