Original Research Article

STUDY OF THE EFFICIENCY OF ANALYSIS OF HYLICOBACTER PYLORI BY THE RESPIRATORY TEST AT ^{13}C UREA IN MONITORING TREATED PATIENTS

ABSTRACT

The detection and monitoring of H. pylori colonization of the gastric mucosa has become a usual analysis by the ^{13}C-labeled urea breath test (TRU) because it is non-invasive and now available in almost total medical analysis laboratories. The objective of this work is the validation of the TRU to verify and confirm the analytical results frequent in our laboratory. Samples were taken using the Taukit Isomed Pharma kit and analyzes were performed by infrared isotope ratio spectrometry. The results obtained show that the TRU is efficient, faithful, accurate and robust and we can apply it daily on patients with great confidence.

Keywords: H. Pylori, TRU, Validation, Efficiency.

I-Introduction

The H. pylori is mostly acquired during childhood and is usually asymptomatic [1]. This infection can lead to various disorders such as inflammation of the stomach (gastritis), peptic ulcer (10% to 20% of cases), adenocarcinoma of the distal stomach (1% to 2% of cases) and lymphoma of the lymphoid tissue associated with the mucous membranes of the stomach [2]. The prevalence of H. pylori infection in Canada is estimated at 7.1% in children aged 5 to 18 years and at 30% in adults according to the World Gastroenterology Organization [3], in Morocco this prevalence is of the order of 36% according to a study carried out recently in the region of Rabat-Salé-Zamour-Zaer [4]. H. pylori infection can be detected by noninvasive serological testing and testing for bacterial antigens in the stool as well as by the ^{14}C urea breath test (TRU ^{14}C) using a radiometric technique in nuclear medicine. The ^{13}C urea breath test (TRU ^{13}C) is more efficient than serology for the detection of H. pylori both to diagnose the infection and to confirm its post-treatment eradication. TRU ^{13}C and TRU ^{14}C are generally equally effective, but ^{14}C is radioactive and must be performed in a facility with a nuclear medicine facility, which limits its accessibility. When H. pylori is present in the stomach of an individual and that the latter ingests urea labeled with ^{13}C, the said bacteria transforms this urea

into $^{13}CO_2$ and ammonia (NH_3), thanks to the action of its abundant urease. The enzymatic reaction takes place in the mucus layer where H. pylori is found and the $^{13}CO_2$ produced diffuses into the epithelial cells and then into the blood and is eliminated by the lungs [5] (figure).

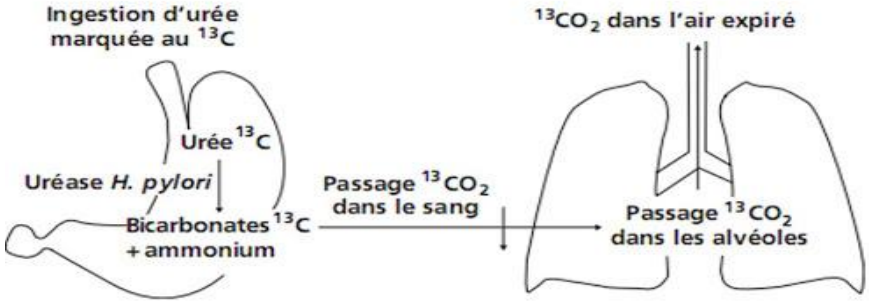

Figure 1: Principle of performing the carbon-13 urea breath test

All prospective studies demonstrate that TRU ^{13}C is a powerful non-invasive method to confirm post-treatment eradication of H. pylori [6].

The aim of this work is to validate the non-invasive method of analyzing H. pylori by the ^{13}C urea breath test, since we have observed a marked increase in the prescription of ^{13}C TRU in patients with gastric symptoms in order to guarantee the analytical results to clearly specify the diagnosis to prescribers and to verify the effectiveness of TRU in the follow-up of patients treated for gastric ulcer of H. pylori.

II- Material and method

II.1- Instrumentation

The analyzes of H. Pylori by the ^{13}C urea breath test were carried out by an Isotope Ratio Infrared Spectrometer (SIRI), IR-force (IR 3000) on samples of air exhaled by the patient in air samples 10 ml dry tubes [7].

II.2- Sampling

According to the information provided by the applicant, TRU ^{13}C is performed using the Taukit kit from Isomed Pharma. First, the patient on an empty stomach (for at least 3 hours) should drink a citric acid drink and then blow, through a straw, into two tubes. Then he should drink another citric acid drink in which the ^{13}C urea has been dissolved, then wait 30 minutes. Finally, he must blow again, with a straw, in two other tubes. In addition to fasting, the patient should prepare by following guidelines for the consumption of certain medications. The collection should be done with the tubes of the kit, which, afterwards, must be hermetically

sealed and then stored and transported at room temperature. Thus, the samples will be analyzed by SIRI (IR-force). These samples are indeed stable for long periods (5 weeks) [8].

II.3- Experimental protocol

The IR-force analyzer designed for the determination of ^{13}C labeled CO_2 in exhaled air. The analysis focuses on the measurement and determination of the $^{12}CO_2$ / $^{13}CO_2$ quotient in the air exhaled by the patient.

II.3.1- Instrument calibration

We blow into the exhaled air sampling bag, which we place in the device's adapter before launching the automatic calibration of the device which is necessary to exclude any impact of the $^{12}CO_2$ concentration on the value. delta during measurement.

II.3.2- Analysis of samples

The analysis is carried out on the 2 sampling tubes (T_0 and T_{30}) which are placed on the adapter of the device. Before starting the analysis, the patient identification must be entered on the list of samples to be analyzed, launch the test, after a few minutes the device displays the result of the analysis of each sample which is the delta (Δ), expressed in part per thousand (‰), which represents the relative difference between the isotope ratio of the sample and that of a reference substance. The difference between the deltas of the samples (T = 30 and T = 0) from the same patient represents the delta over base-line (DOB). The final test result is generally considered positive when the BOD is greater than 5 ‰ [9].

II.4- Validation parameters

The ^{13}C urea breath test is considered semi-quantitative therefore the validation parameters that we have verified are; repeatability, intermediate precision, inter-operator variability, uncertainties and comparison of methods.

II.5-Verification of the effectiveness of the ^{13}C urea breath test in post-treatment control

The verification of the effectiveness of the TRU in monitoring patients on treatment against H. Pylori was carried out on a number of 170 patients in equitability between the two sexes (83 males and 82 females) of an age group divided between 17 and 65 years by the determination of the delta over base-line (BOD) before and after the treatment.

III- Results and Discussion

The analysis of H. pylori by infrared spectrometry with isotopic relation gives results in delta of difference of isotopic rations between T_{30} and T_0 expressed in part per thousand of the same sample.

T30 -T0 in delta / 1000: <3: Negative result.

$3 \leq R \leq 5$: Undetermined result.

> 5: Positive result [10].

III.1- Repeatability

The repeatability of this test was determined on 2 levels of controls (6 Negative and 6 Positive). The coefficients of variation (CVr) calculated are lower than the limits retained by the Laboratory [11], see table 1.

Table 1: Result of the repeatability study

Determination	**Control 1 (Negatif) Delta ‰**			**Control 2 (Positif) Delta ‰**		
	T_0	**T_{30}**	**T_{30}-T_0**	**T_0**	**T_{30}**	**T_{30}-T_0**
1	-25.43	-25.16	0.30	-25.65	26.74	52.40
2	-25.58	-25.41	0.20	-25.98	25.35	51.30
3	-25.30	-25.16	0.10	-25.15	25.49	50.60
4	-25.00	-24.64	0.40	-25.58	25.04	50.60
5	-25.88	-25.76	0.10	-25.68	25.30	51.00
6	-25.43	-25.02	0.20	-25.64	26.28	54.90
Average	-25,44	-25,19	0.217	-25,61	25,61	51.80
Standard deviation	0,33	0,38	0.117	0,27	0,51	1.658
CVr (%)	**1.29**	**1.51**	-	**1.05**	**1.99**	-
CV (%) reference	**< 3%**			**< 3%**		

III.2- Intermediate reliability

Reproducibility was evaluated at 2 control levels (6 Negative and 6 Positive). The calculated coefficients of variation (CVR) are lower than the limits retained by the Laboratory [11], see table 2.

Table 2: Result of the reproducibility

Determination	**Control 1 (Négatif) Delta ‰**			**Control 2 (Positif) Delta ‰**		
	T_0	**T_{30}**	**T_{30}-T_0**	**T_0**	**T_{30}**	**T_{30}-T_0**
1	-25.80	-25.33	0.5	-25.92	22.54	48.5
2	-25.16	-24.68	0.5	-25.79	26.01	51.8
3	-25.10	-24.91	0.2	-25.76	24.19	50.0
4	-25.71	-25.58	0.1	-25.35	27.07	52.4
5	-25.01	-24.57	0.2	-25.01	26.81	51.8
6	-25.80	-25.33	0.4	-25.64	26.17	51.8
Average	-25,43	-25,07	0.317	-25,58	25,46	51.05
Standard deviation	0,38	0,41	0.172	0,34	1,75	1.491
CVR (%)	**1.48**	**1.62**	**-**	**1.33**	**-**	**-**
CV (%) reference	**< 3%**			**< 3%**		

III.3- Inter-operator variability

Each control was run 8 times by all operators with the same lot of reagent and the same instrument. The results of this inter-operator correlation are consistent (Table 3).

Table 3: Results of the inter-operator correlation

Operator	**Date**	**Control 1 (Négatif) Delta ‰**	**Control 2 (Positif) Delta ‰**	**Variation between operators**	
				C_1	**C_2**

P1	18/05/2017	0.8	42.0	0.0	0.0
P2	18/05/2017	0.9	40.3	0.1	1.7
P3	19/05/2017	1.4	43.1	0.5	2.7
P4	19/05/2017	1.1	38.5	0.3	4.6
P1	22/05/2017	1.3	41.9	0.2	3.4
P2	22/05/2017	1.1	41.4	0.2	0.5
P3	23/05/2017	1.6	34.8	0.5	5.6
P4	23/05/2017	1.9	36.5	0.3	1.7
Average bias		-	-	**0,3**	**2,88**
Average standard deviation		-	-	**0,15**	**1,65**

III.4- Uncertainty

2 levels of controls (7 Negative and 7 Positive) were analyzed internally at our Laboratory (CIQ) and externally in a foreign laboratory (CEQ) (Table 4 and 5).

Table 4 : Results of the uncertainty calculation (Control Negatif)

Control code	Result CIQ	Result CEQ	Bias
21812100279	0.23	0.51	0.28
21912100348	0.37	0.65	0.28
21902270158	0.03	0.59	0.56
21902270200	0.25	0.48	0.23
21903150387	0.44	0.84	0.40
21903150460	0.24	0.63	0.39

21903180523	0.09	0.44	0.35
21903180559	0.50	0.11	0.39
CIQ average	**0.269**	**-**	**0.36**
CEQ Average	**-**	**0.531**	
Variance	**0.103**		
Absolute uncertainty (U)	**0.963**		
Relative uncertainty (U%)	**3.58 %**		

Table 5: Results of the uncertainty calculation (Positive Control)

Control code	**CIQ result**	**CEQ result**	**Bias**
21812100033	8.99	9.97	0.98
21812100352	60.25	64.97	4.72
21902270487	60.13	67.08	6.95
21902150134	11.16	11.58	0.42
21903180023	62.98	70.09	7.11
21903180137	33.11	42.11	9.00
21903190194	44.59	43.85	0.74
CIQ average	**40.173**	**-**	**4.27**
Average CEQ	**-**	**44.236**	
Variance	**0.115**		
Absolute uncertainty (U)	**9.017**		
Relative uncertainty (U%)	**22.45%**		

III.5- Method comparison

A volume of 13 control samples were analyzed by the IR-force machine and by The HeliFANplus machine (table 6). The results found are consistent with the comparison interval (CI).

Table 6: Result of the method comparison

Code Control	Result IR-force	Result HeliFANplus	Bias
21812100033	8.99	9.97	0.98
21812100279	0.23	0.51	0.28
2191210348	0.37	0.65	0.28
2181210352	60.25	64.97	4.72
21902270200	0.25	0.48	0.23
21902270487	60.13	67.08	6.95
21903150134	11.16	11.58	0.42
21903150387	0.44	0.84	0.40
21903150460	0.24	0.63	0.39
21903180023	62.98	70.09	7.11
21903180523	0.09	0.44	0.35
21903180559	0.50	0.11	0.39
21903190194	44.59	43.85	0.74
Average Bias	**1.61**		
Standard deviation	**2.73**		

Interval CI Bias (95%)	**-4.43 à 8.71**

IV- Determination of the effectiveness of the ^{13}C urea breath test after monitoring processing

The results of the TRU analysis of the patients with H. pylori infection in the study before the treatment were all positive, after the treatment 97.65% of the results of these patients became negative with the TRU (Table 7), this compatibility of the results of the patients by the TRU with the monitoring of the treatment is higher than the limit of conformity of the results which is 95% [12]. As a result, the TRU is truly effective both for the diagnosis of gastric ulcer by H. pylori and for the monitoring of patients during medical treatment.

Table 7: Distribution of TRU results before and after treatment

Effective	**Analysis before treatment**	**Analysis after treatment**	**Compatibility with treatment**
87 Male Patients	Positive Results	85 Negative Results	97.70%
		2 Positive Results	
83 Female Patients	Positive Results	81 Negative Results	97.59%
		1 Positive Results	
Average	**97.65%**		

Conclusion

The non-invasive analysis of H. pylori by the ^{13}C urea breath test is efficient compared to other serological or even classical bacteriological techniques for diagnosing infection, faithful, accurate and robust. It's applied directly to samples of patient exhaled air without any pre-treatment and is also used to confirm post-treatment eradication of H. Pylori with very satisfactory efficiency, since the compatibility of the results of the TRU with the condition of the patients before and after the treatment is very satisfactory.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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