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Performance evaluation of a novel ^{14}C-urea breath test (solid scintillation) for the diagnosis of *helicobacter pylori* infection

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Abstract

^{14}C-urea breath tests (UBTs) can be used to diagnose *helicobacter pylori* (*H. pylori*) infection. This study aimed to evaluate the accuracy of a solid scintillation ^{14}C-UBT in diagnosing *H pylori* infection. This open-label, prospective multicenter study enrolled patients who underwent *H pylori* screening from January 7, 2020, to October 28, 2020, in 3 centers in China. All participants underwent solid scintillation UBT first and then gastroscopy. The rapid urease test and histological examination results were the gold standards (*H pylori*-positive was defined as the 2 tests being positive; *H pylori*-negative was defined as both tests being negative). The solid scintillation ^{14}C-UBT involves a scintillation sampling bottle and a ^{14}C-urea capsule. The sampling bottle contains a stack of carbon dioxide-absorbing and scintillation sheets. The test is read using a photomultiplier. The sensitivity, specificity, accuracy, positive predictive value, and negative predictive value for *H pylori* infection were evaluated. This study enrolled 239 participants. There were 98 males and 141 females, aged 45.8 ± 11.9 (range: 21–66) years. Thirty-four participants were excluded due to a discrepancy between the rapid urease test and immunohistochemistry examination. Finally, 205 participants were included in the analysis. According to the gold standard, 87 out of 205 (42.4%) participants were *H pylori*-positive. Compared with the gold standard, the sensitivity, specificity, accuracy, and positive and negative predictive values of the solid scintillation ^{14}C-UBT were 95.4%, 97.5%, 96.6%, 96.5%, and 96.6% for the solid scintillation UBT, respectively. One participant experienced 1 adverse event (AE) (exacerbation of chronic cholecystitis), and the AE eventually improved by itself. The investigators determined that the AE was unrelated to the study device. The noninvasive solid scintillation ^{14}C-UBT has a high diagnostic value for *H pylori* infection, comparable to the diagnostic value of the gold standard.

Abbreviations: CO_2 = carbon dioxide, *H. pylori* = *helicobacter pylori*, UBT = urea breath test.

Keywords: ^{14}C, diagnostic value, gastroscopy, *helicobacter pylori*, solid scintillation, urea breath test

1. Introduction

Helicobacter pylori (*H. pylori*) is present in the gastrointestinal tract of about 44% to 49% of individuals world wide,[1–4] but the rate of *H pylori* infection is 52% to 62% in China.[5,6] *H pylori* is classified as a Class I carcinogen by the World Health Organization.[7] Optimizing the detection methods of *H pylori* infection have great clinical significance for the diagnosis and treatment of digestive system diseases.[3,4,8]

The urea breath test (UBT) is a noninvasive method for detecting *H pylori* infection in humans; it is based on radionuclide-labeled urea and is accurate, specific, and rapid.[8–10] Various guidelines recommend UBTs as the first choice for diagnosing *H pylori* infection and reexamining after eradication therapy.[3,11–13] UBTs are the first choice because they have high accuracy, are easy to perform, are noninvasive, are unaffected by the distribution of *H pylori* in the stomach, and allow a high flow of patients being tested. During UBT, ^{13}C- or ^{14}C-labeled urea is swallowed and converted to labeled carbon dioxide (CO_2) by the urease of *H pylori*; the CO_2 is then absorbed into the bloodstream and is released by the lungs, and the labeled gas is measured in exhaled air.[4] The ^{13}C-UBT is preferred over the ^{14}C-UBT in children and pregnant females due to smaller radiation exposure, although radiation exposure with the ^{14}C-UBT is < the daily background radiation exposure.[3] Some drugs (e.g., ranitidine, pantoprazole, and vonoprazan) appear to affect the

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The study was approved by the Ethics Committee of these three centers (The Second Affiliated Hospital of Zhejiang University School of Medicine [Approval number: 2020-063]; Renji Hospital, School of Medicine, Shanghai Jiao Tong University [Approval number: 2019.04-1]; The First Affiliated Hospital of Nanchang University [Approval number: 2020-36]). All participants signed the informed consent form before any study procedures.

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accuracy of the ^{13}C-UBT,[14,15] and the ^{13}C-UBT requires mass spectrometry for measurement, making ^{14}C-UBT a more convenient approach.

Various technologies are available for collecting ^{14}C-labeled breath test samples and measuring radioactivity, including absorbing CO_2 in a solution of methanol and a CO_2 absorbent into a scintillation bottle (i.e., liquid scintillation UBT).[16] Another method uses a card-type sampling device containing a solid CO_2 absorbent tablet (i.e., the breath card method UBT).[17] These 2 methods have disadvantages in clinical application. Indeed, liquid scintillation UBT has a high detection efficiency, but toxic organic solutions have to be used. On the other hand, the breath card method UBT uses a solid absorbent, but the radioactivity is detected using a Geiger-Müller counter, which has a low sensitivity.[4,18] Therefore, developing a new detection system is necessary to improve the shortcomings. An ideal system would avoid toxic materials, be convenient to use and process, and use sensitive radiation detection methods.

Here, we introduce a new ^{14}C-based detection system using solid scintillation technology and a sampling device that includes solid CO_2-absorbing and scintillation components. This study aimed to evaluate the accuracy of a novel ^{14}C-UBT solid scintillation sampling bottle for *H pylori* infection.

2. Materials and methods

2.1. Study design and participants

This open-label, prospective multicenter diagnostic study enrolled patients who underwent *H pylori* screening from January 7, 2020, to October 28, 2020. The study was approved by the Ethics Committee of these 3 centers (The Second Affiliated Hospital of Zhejiang University School of Medicine [Approval number: 2020-063]; Renji Hospital, School of Medicine, Shanghai Jiao Tong University [Approval number: 2019.04-1]; The First Affiliated Hospital of Nanchang University [Approval number: 2020-36]). All participants signed the informed consent form before any study procedures.

The inclusion criteria were: 18 to 65 years of age and; Indication for gastroscopy. The exclusion criteria were: Known or suspected allergy to test drugs or their components; Used antibiotics, traditional Chinese medicines with antibacterial effects, traditional Chinese medicines for treating gastrointestinal diseases, bismuth preparations, H_2 receptor antagonists, proton pump inhibitors, or sucralfate in the past month; Upper gastrointestinal bleeding within the recent 1 week; History of gastrectomy; Contraindications or influencing factors for gastroscopy; Severe cardiovascular or cerebrovascular diseases (such as class III–IV New York Heart Association functional classification, congestive heart failure, myocardial infarction, acute stroke, etc); Malignant tumors, acute infectious diseases, or severe lung diseases (such as chronic obstructive pulmonary diseases, bronchospasm, bronchial asthma, respiratory failure, etc); Neurological or mental diseases or were unable to cooperate or unwilling to cooperate, or history of mental disorders or mental abnormalities with suicidal tendencies; Suspected or genuine propensity to abuse or rely on alcohol or drugs; Pregnant or lactating women; Participated in other clinical trials within 3 months before screening, or; Any other situations that the investigators deemed not suitable for participation.

2.2. Solid scintillation ^{14}C-UBT

The test involves a scintillation sampling bottle (solid scintillation) (Shenzhen Zhonghe Headway Bio-Sci & Tech, Co., Ltd. Shenzhen, China) and a ^{14}C-urea capsule (specification: 27.8 kBq (0.75 μCi)/capsule). The scintillation sampling bottle consists of a bottle and a blowpipe. The blowpipe is made of polyvinyl chloride (PVC), connected to a saliva separator, and plugged into the air inlet of the sampling bottle when in use.

In the sample bottle, the absorbing sheet containing the CO_2 absorbent and the scintillator containing the solid scintillator are stacked and form 1 detection unit. The gap between the absorbing and scintillation sheets constitutes the airflow channel. When exhaled air flows through the airflow channel, the CO_2 in the exhaled breath is absorbed on the absorbing sheet. β particles generated by the decay of ^{14}C will cross the airflow channel and hit the scintillator. The generated scintillation light is transmitted to the photomultiplier tube of the liquid scintillation counter via the scintillation sheets and the casing for recording. In order to increase the CO_2 absorption and the detection area, many independent absorbing and scintillation sheet units are stacked (Fig. 1).

2.3. Procedures

Each participant underwent solid scintillation ^{14}C-UBT and a gastroscopy-based test as the gold standard. The interval between the UBTs and gastroscopy was > 1 day but < 7 days.

For the UBT, all participants were tested after fasting in the morning or 2 hours after breakfast. For the solid scintillation sampling bottle, 20 minutes after taking the capsule, the sample bottle and blowing pipe were removed from the packaging bag. The pipe was plugged into the air inlet of the sample bottle. The participants slowly blew into the sample bottle through the blowing pipe for about 2 to 5 minutes. The sampling was complete when the indicator's color changed from blue to white (a few blue spots were allowed). The scintillation sampling bottle (solid scintillation) was assessed using a scintillation counter (HUBT-01P, Shenzhen Zhonghe Headway Bio-Sci & Tech Co., Ltd., Shenzhen, China). When the decay events of a sample exceeded 100 dpm, the test was considered positive.

During gastroscopy, mucosal tissue specimens were obtained from the gastric antrum (2 specimens), gastric body (2 specimens), and gastric angle (1 specimen). One specimen from the gastric antrum and 1 from the gastric body were used for the rapid urease test (Shanghai Huitai Medical Technology Co., Ltd.). The other specimens were used for routine histological examination and immunohistochemistry (anti-*H pylori*). Formalin-fixed paraffin-embedded freshly cut tissue sections were used. The primary antibody was added at a concentration ratio of 1:100 and incubated at 4°C overnight. The secondary antibody was added and incubated. After coloration with the 3,3-diaminobenzidine (DAB) solution for 30 to 45 seconds, the sections were counterstained with hematoxylin and observed under a microscope. The *H pylori* showed in brown and could be spiral, spherical, or long. A pathologist unrelated to the study routinely examined the slides.

The presence of *H pylori* was determined when the rapid urease test and immunohistochemistry examination were both positive. A participant was considered negative when both tests were negative. Participants with discrepant results were not included in the analysis.[19]

2.4. Determination of the sample size

The sensitivity for diagnosing *H pylori* infection was used as an evaluation index to estimate the sample size. According to local data, the positive rate of the gold standard was 28.5% (53/186), the positive rate of the UBT test was 31.7% (59/186), and the positive consistency rate of the 2 methods was 27.4% (51/186). The sensitivity of the original preparation for diagnosing *H pylori* infection was 96% (51/53). Considering the clinical significance, the researchers and statisticians decided that the maximum allowable absolute difference |δ| between the experimental test and the gold standard

test in diagnosing *H pylori* infection was 8%. Using the bilateral test level $\alpha = 0.05$ and power = 80% (i.e., $\beta = 0.2$) and the "Equivalence Tests for 2 Correlated Proportions [Difference]" module in the PASS 11.0 software, at least 159 participants were needed. Considering 20% dropouts, at least 191 participants were enrolled.

2.5. Statistical analysis

Descriptive statistics were used. The sensitivity, specificity, accuracy, positive predictive value, and negative predictive value of *H pylori* infection were calculated and compared between the experimental test and the gold standard. The adverse events were recorded and analyzed.

3. Results

3.1. Characteristics of the participants

In this study, 239 participants were enrolled. There were 98 males and 141 females, aged 45.8 ± 11.9 (range: 21–66) years. Thirty-four participants were excluded due to discrepant results of the rapid urease test and immunohistochemistry examination. Finally, 205 participants were included in the analysis.

3.2. Diagnostic value of solid scintillation UBT

According to the gold standard, 87 out of 205 (42.4%) participants were *H pylori*-positive (Table 1). The sensitivity, specificity, accuracy values, positive predictive value, and negative predictive value of the solid scintillation ^{14}C-UBT test were 95.4%, 97.5%, 96.6%, 96.5%, and 96.6%, respectively (Table 2).

3.3. Safety

One participant experienced 1 AE. The AE was an exacerbation of chronic cholecystitis, and the AE eventually improved. The investigators determined that the AE was unrelated to the study device.

4. Discussion

^{14}C-UBTs can be used to diagnose *H pylori* infection. After collecting the breath sample with the new solid scintillation

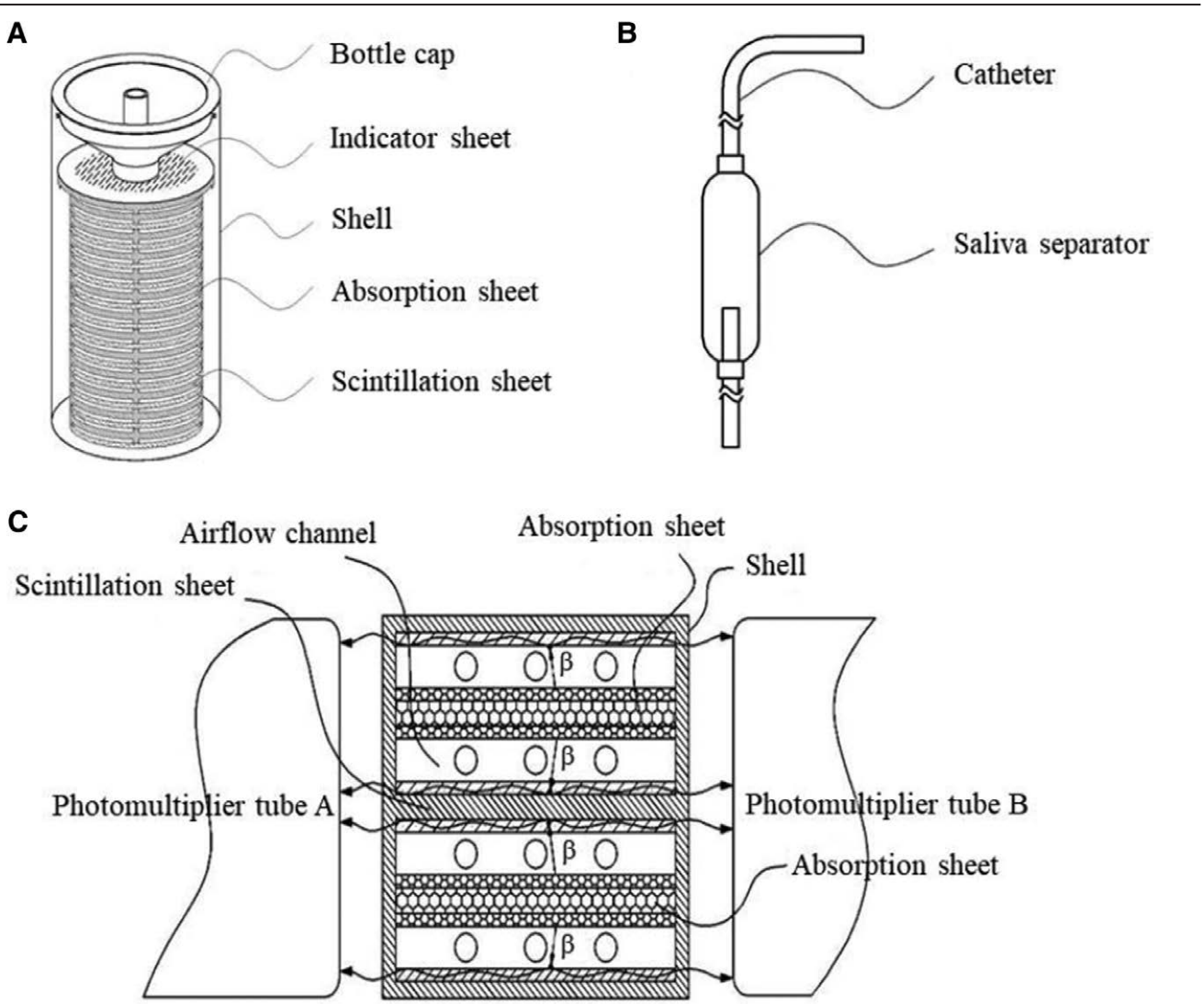

Figure 1. The bottle of the solid scintillation test. (A) The bottle consists of a bottle cap, indicator sheet, shell, absorption sheet, and scintillation sheet. (B) The blowpipe consists of a PVC tube and a saliva separator. (C) The CO_2 absorbent and scintillation material are stacked (including the airflow channel, absorption sheets, and scintillation sheets) in a shell. When placed in the photomultiplier tube, the β particles generate light measured by the measuring device. CO_2 = carbon dioxide.

Table 1

***pylori* detected by different methods.**

		Gold standard (rapid urease test and histology)		
		Positive	**Negative**	**Total**
Solid scintillation	Positive	83	3	86
	Negative	4	115	119
Total		87	118	205

Table 2

Diagnostic value for *H pylori* infection.

Products	Sensitivity	Specificity	Accuracy	Positive predictive value	Negative predictive value
Scintillation sampling bottle (solid scintillation)	95.4%	97.5%	96.6%	96.5%	96.6%

H pylori = helicobacter pylori.

sampling device, the sampling bottle is placed in a scintillation counter for measurement. This study aimed to validate the technique in *H pylori* screening. The results indicate that solid scintillation sampling ^{14}C-UBTs has a high diagnostic value for *H pylori* infection.

The diagnostic methods for *H pylori* include invasive and noninvasive methods.[8–10] Gastroscopy is performed only when there are specific indications because it is unsuitable for repeated testing and is difficult for patients to accept. Noninvasive detection methods are relatively simple and more acceptable to patients, among which UBT is the most used method in the clinical setting. The principle of the UBT is based on the presence of urease in the stomach, of which *H pylori* is the only possible source. When *H pylori* is present, the labeled urea will be degraded to CO_2, and the CO_2 will be absorbed in the bloodstream and expelled by the lungs in exhaled air.[8–10] Therefore, the UBTs are highly specific, but the sensitivity of the UBTs relies on the ability of the devices to capture the expelled CO_2 and detect the isotopes. ^{13}C is a stable isotope and has no radioactivity-related safety issues, and it can be applied to any population, but ^{13}C-UBTs are more expensive because of the need for mass spectrometry or infrared spectrometry to quantify the ^{13}C. On the other hand, the ^{14}C-based UBTs only require a benchtop scintillometer, which is relatively inexpensive, widely available, and do not require special skills. Still, the dose of ^{14}C-urea used in UBTs is extremely small (about 27.8 kBq), and the radiation dose is about 1.59 μSv, which is only 1/630 of the annual effective dose limit of 1 mSv stipulated in the basic standards for protection against ionizing radiation and for the safety of radiation sources.[20] Therefore, there are no special protection requirements for the patients and staff conducting the tests.

At present, ^{14}C-UBTs commonly used in China and other countries include 2 methods: liquid scintillation UBT and breath card UBT. The above 2 methods have shortcomings in clinical application. Liquid scintillation UBT has high detection efficiency during measurement, but toxicity issues are encountered due to the use of methanol and toluene in the solution, posing potential safety issues for the patient and the environment. Although breath card UBTs use a solid absorbent, the detectors are based on a Geiger-Müller counter, which has low sensitivity, especially when the radioactivity in the sample is lower than the ambient background radioactivity.[4,18] Therefore, an ideal system would answer those shortcomings, that is, avoid using toxic solutions, be convenient to use and process, and use sensitive radiation detection methods.

The ^{14}C-UBT introduced in this paper is based on solid scintillation technology, and the sampling device includes a stack of absorbent and scintillation sheets and is read using a photomultiplier, improving sensitivity. Compared with the breath card method UBT, the stacked absorbent and scintillation sheets increase the absorption and detection areas. Hence, the signal is stronger, and the sensitivity of diagnosis might be greatly improved. No other breath test based on this technology has been reported so far. The higher sensitivity of the photomultiplier compared with a Geiger-Müller counter should decrease the risk of false-negative results, making more patients eligible for eradication therapy when needed. Still, the solid scintillation ^{14}C-UBT should be compared with other UBTs available on the market.

The values obtained in this study are near those determined by a meta-analysis of UBTs, at 96% for sensitivity and 93% for specificity.[21] Similar results were found specifically for ^{14}C-UBTs, with 96% sensitivity and 93% specificity.[22] A recent study revealed a sensitivity of 96.9% for ^{14}C-UBT but a specificity of only 54.7% or 76.9% after adjusting the cutoff values.[23] In the present study, the cutoff values were those recommended by the manufacturer, and the observed sensitivity and specificity were close to that of the 2 meta-analyses.[21,22]

In terms of safety, during the trial, 1 participant experienced 1 AE (incidence rate of 0.4%). The AE was the exacerbation of chronic cholecystitis, which improved after the test. The investigators ruled it to be unrelated to the study device.

Despite its strengths, this study has some limitations. The UBT and gastroscopy were performed at different time points, possibly introducing some bias in measurement. Besides, only 3 centers in China were included, and generalizability is unknown. Further large sample studies are warranted to confirm our results.

In conclusion, solid scintillation ^{14}C-UBTs have a high diagnostic value for *H pylori* infection. Solid scintillation ^{14}C-UBT is safe, and its clinical operation is simple.

Author contributions

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Writing – review & editing: Wei Zhang, Yu-Ting Wang, Zhi-Juan Xiong, Qin Du, Yong Xie, Hong Lu.

References

[1] Zamani M, Ebrahimtabar F, Zamani V, et al. Systematic review with meta-analysis: the worldwide prevalence of helicobacter pylori infection. Aliment Pharmacol Ther. 2018;47:868–76.

[2] Hooi JKY, Lai WY, Ng WK, et al. Global prevalence of helicobacter pylori infection: systematic review and meta-analysis. Gastroenterology. 2017;153:420–9.
[3] Chey WD, Leontiadis GI, Howden CW, et al. ACG clinical guideline: treatment of helicobacter pylori infection. Am J Gastroenterol. 2017;112:212–39.
[4] McColl KE. Clinical practice. helicobacter pylori infection. N Engl J Med. 2010;362:1597–604.
[5] Shi R, Xu S, Zhang H, et al. Prevalence and risk factors for helicobacter pylori infection in Chinese populations. Helicobacter. 2008;13:157–65.
[6] Wang W, Jiang W, Zhu S, et al. Assessment of prevalence and risk factors of helicobacter pylori infection in an oilfield Community in Hebei, China. BMC Gastroenterol. 2019;19:186.
[7] International Agency for Research on Cancers. Monographs on the Evaluation of Carcinogenic Risks to Humans. Geneva: World Health Organization; 1994.
[8] Dore MP, Pes GM. What is new in helicobacter pylori diagnosis. an overview. J Clin Med. 2021;10.
[9] Makristathis A, Hirschl AM, Megraud F, et al. Review: diagnosis of helicobacter pylori infection. Helicobacter. 2019;24(Suppl 1):e12641.
[10] Sabbagh P, Mohammadnia-Afrouzi M, Javanian M, et al. Diagnostic methods for helicobacter pylori infection: ideals, options, and limitations. Eur J Clin Microbiol Infect Dis. 2019;38:55–66.
[11] Sugano K, Tack J, Kuipers EJ, et al. Kyoto global consensus report on Helicobacter pylori gastritis. Gut. 2015;64:1353–67.
[12] Malfertheiner P, Megraud F, O'Morain CA, et al. Management of helicobacter pylori infection-the maastricht V/florence consensus report. Gut. 2017;66:6–30.
[13] Liu WZ, Xie Y, Lu H, et al. Fifth Chinese national consensus report on the management of helicobacter pylori infection. Helicobacter. 2018;23:e12475.
[14] Takimoto M, Tomita T, Yamasaki T, et al. Correction to: effect of vonoprazan, a potassium-competitive acid blocker, on the (13) C-urea breath test in helicobacter pylori-positive patients. Dig Dis Sci. 2021;66:2472.
[15] Dulbecco P, Gambaro C, Bilardi C, et al. Impact of long-term ranitidine and pantoprazole on accuracy of [13 C] urea breath test. Dig Dis Sci. 2003;48:315–21.
[16] Balon H, Gold CA, Dworkin HJ, et al. Procedure guideline for carbon-14-urea breath test. society of nuclear medicine. J Nucl Med. 1998;39:2012–4.
[17] Radnia A, Farahani MH, Yousefzadeh HC, et al. Introducing the heliguide: urea breath tests system for detecting helicobacter pylori. Front Biomed Technol. 2015;2:115–7.
[18] Ozturk E. Diagnostic methods of helicobacter pylori infection. Gulhane Tip Dergisi. 2008;50:60–4.
[19] U.S. Food & Drug Administration. Establishing the Performance Characteristics of in Vitro Diagnostic Devices for the Detection of Helicobacter Pylori. Atlanta: U.S. Food & Drug Administration; 2010.
[20] International Atomic Energy Agency. International basic safety standards for protection against ionizing radiation and for the safety of radiation sources. Available at: https://www.iaea.org/publications/6900/international-basic-safety-standards-for-protection-against-ionizing-radiation-and-for-the-safety-of-radiation-sources-cd-rom-edition-2003. International Atomic Energy Agency; 2003.
[21] Ferwana M, Abdulmajeed I, Alhajiahmed A, et al. Accuracy of urea breath test in Helicobacter pylori infection: meta-analysis. World J Gastroenterol. 2015;21:1305–14.
[22] Zhou Q, Li L, Ai Y, et al. Diagnostic accuracy of the (14) C-urea breath test in helicobacter pylori infections: a meta-analysis. Wien Klin Wochenschr. 2017;129:38–45.
[23] Wang X, Zhang S, Chua EG, et al. A re-testing range is recommended for (13) C- and (14) C-urea breath tests for helicobacter pylori infection in China. Gut Pathog. 2021;13:38.