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Colorimetric H_2S gas Detection Tube in Breath Testing: A Reliable Alternative?

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Abstract

Despite the simplicity, the affordability, and the wide use in clinical practice of breath tests, in some cases, monitoring only H_2 and CH_4 concentration variations can lead to incorrect interpretations of their results. Several studies, therefore, suggest that also the assessment of the presence of hydrogen sulphide in the breath samples should be included. However, breath H_2S concentration is not commonly measured, since expensive specialized equipment or expensive, time-consuming mass spectrometric techniques are required. This study explores a cost-effective, rapid, and user-friendly approach to qualitatively measure H_2S during breath tests using colorimetric tubes, normally used for environmental monitoring. With such devices, it is possible to obtain preliminary information that will implement the test results and could be used by the physician for the interpretation of the test itself. The results show that, with a single device, more than one measurement can be made. Moreover, they are not influenced by humidity and the additional gases in the human exhaled breath.

Keywords: Hydrogen sulphide; Hydrogen sulphide Detection; Exhaled breath; Breath test; Colorimetric tube; Cumulative samplings

Introduction

Breath test is a simple diagnostic tool used during the last 50 years to detect a group of conditions, characterized by symptoms attributable to an increase of carbohydrate residual fermentation in the small or large intestine [1]. A large body of evidence suggests that monitoring the modification of intestinal gas levels in exhaled breath, currently hydrogen and methane, allows a very accurate detection of carbohydrate malabsorption [2] and small intestine bacterial overgrowth [3]. Together with its simplicity, the addition of its affordability determined a large diffusion of breath test in clinical practice [4]. However, according to several studies, the interpretation of such BTs may be difficult and controversial, since evaluating only the H_2 and CH_4 concentrations could lead to an incorrect interpretation of the results [5-7]. Hydrogen sulphide (H_2S) is another gas produced in the intestine, which could be potentially considered as an additional marker for monitoring the entity of carbohydrate intraluminal fermentation [5]. Moreover, in subgroups of patients it should be stigmatized that hydrogen production may not be significant, due to the predominant activity of hydrogen-consuming bacteria in the gut, such as Sulphate Reducing Bacteria (SRB), which employ H_2 as a respiratory substate during metabolism to reduce sulphate to H_2S [5,8,9] and an increased concentration of H_2S in breath was detected in patients with ulcerative colitis [10,11].

The interaction among hydrogen, methane and sulphide is very complex and it was previously shown, with an in vitro incubation system, that methanogens outcompete sulphate reducing bacteria for hydrogen utilization in the human colon [12] and in subgroup of subjects a pathway may predominate on the other one [13]. Like hydrogen and methane, H_2S enters the bloodstream, travels to the lungs, and is ultimately expelled in breath [5,14,15] where its concentration can be measured. However, H_2S is not usually detected during breath tests, despite some studies reporting that its concentrations can be measured with very expensive specialized devices, with time-consuming mass spectrometry-based techniques or with expensive electrochemical sensors that would prevent the physician from performing the measurement himself [5,14-16]. For this reason, it was considered necessary to devise an easy, cost-effective, and "physician-friendly" approach for a qualitative screening measurement of the H_2S during breath tests. In this work, a colorimetric tube commonly used for environmental studies was used to detect H_2S in standard gas mixtures. Furthermore, the feasibility of performing cumulative replicates with a single tube was investigated.

Materials and Methods

Standard gas

A standard gas mixture of H_2S at a concentration of approximately 0.7 ppm in N_2 has been prepared and stored in a 750 ml breath collection bag (Sample 1). This concentration was chosen to stay below the H_2S concentration reported in exhaled breath in some literature studies, where measured significant concentrations range from 1.2 to 6 ppm [17-19]. Another standard gas mixture, simulating the human breath, was prepared, and stored in a breath collection bag: 78% N2, 17% O2, 5% CO2 and 0.7 ppm H_2S; an amount of water has been added to this mixture to achieve an absolute humidity of 44 mg/L (Sample 2).

H_2S measurement system

Colorimetric gas detection tubes (Kitagawa America LLC, New Jersey, USA), with a measuring range between 0.1 and 6 ppm and a sensitivity of 0.1 ppm, were used to determine the presence of H_2S in both gas mixtures. The colorimetric tube is a glass vial filled with a dehumidifying material at the top and silica gel on which a silver compound, lithium bromide monohydrate and a pH indicator are adsorbed (Material Safety Data Sheet No. KE002 Ver. 7, Kitagawa, 2013). When the silver compound reacts with gaseous H_2S, an acidic compound is produced, and the pH indicator changes color (from yellow to pink); the length of the color change typically indicates the measured concentration. A graduated scale printed on the tube itself, allows to directly determine the gas concentration. This scale is calibrated for standard temperature and atmospheric pressure (293.15 K and 1013 hPa) conditions; therefore, if these conditions are not met, correction factors should be applied (Instruction Manual Hydrogen Sulphide Detector Tube No.120U, Kitagawa). All analyses were carried out by connecting one end of the tube directly to the breath collection bag and the other end to a manual pump specifically designed for such tubes. The first 100 ml were manually aspired at a rate of about 100 ml/min (t_0). After 3 minutes (t1), an analogous measurement was performed on the same tube by aspirating another 100 ml at the same rate conditions. This process was repeated after three minutes at t2, t3 (10 minutes after t2), and t4 (30 minutes after t3).

Results

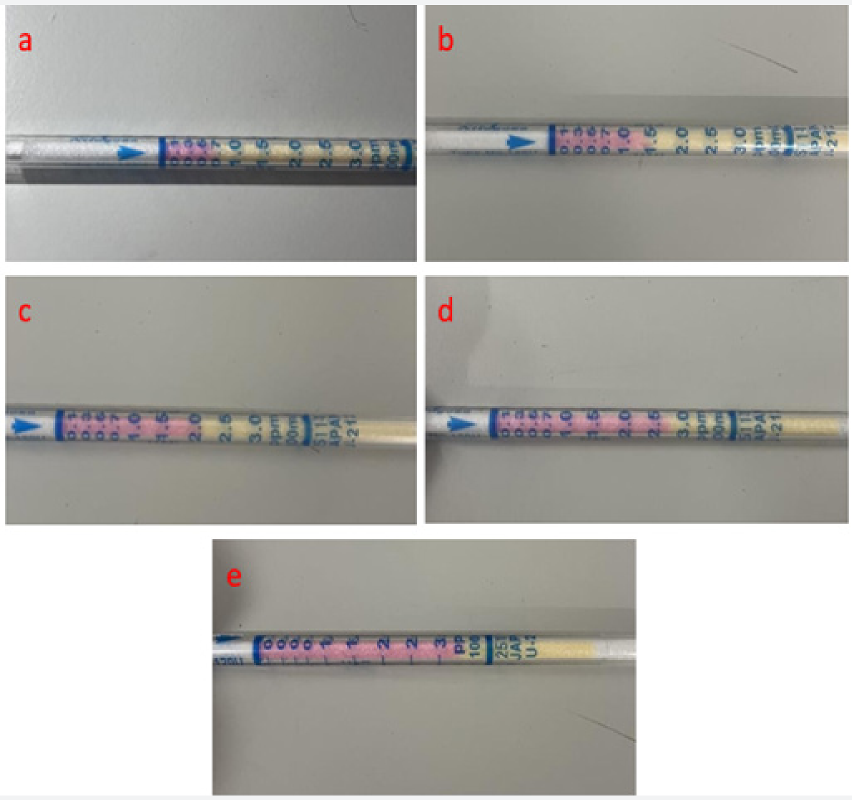

Figure 1: H2S concentration measured at t0 (a), t1 (b), t2 (c), t3 (d), t4 (e) in a gas mixture containing H2S (0.7 ppm) in N2.

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First of all, the bag containing N_2 and H_2S (Sample 1) was analyzed. As shown in Figure 1a, after the first manual pump stroke, the concentration read on the graduated scale is approximately 0.7 ppm (Figure 1a). After the second, the third, the fourth and the fifth replications the concentrations read are respectively: about 1.4 ppm (Figure 1b), 2.1 ppm (Figure 1c), 2.8 ppm (Figure 1d) and 3.5 ppm (Figure 1e). It was, therefore, found that the analyses performed on the tube are cumulative. This procedure was repeated with two other tubes, obtaining the same results. The performance of the tubes was then assessed in relation to the impact of the main components (O_2, CO_2, and H_2O) in human exhaled breath. Therefore, Sample 2 was analyzed carrying out the same procedure used for Sample 1, repeating the pump stroke at the same time intervals (t_0, t_1, t_2, t_3, t_4). Also in this case, it was observed that the analyses performed on the tube are cumulative. This procedure was repeated with two other tubes, obtaining the same results as the first one.

Conclusions

A device for measuring the concentration of H_2S, normally used in a different field of application, was tested with two different gas mixtures: one containing only N_2 and H_2S (0.7 ppm concentration) and another one containing these two same components (H_2S at the same concentration of 0.7 ppm) plus the other most abundant gases present in the human exhaled breath. According to the results from both types of samples, these devices could provide a preliminary measurement, that will allow the physician to be able to discriminate between H_2S- producing and H_2S-nonproducing patients, and to include this information in the interpretation of the breath tests results. It is possible to perform cumulative samplings with a single device, with an immediate verification of any H_2S production. Such a simple test could be performed directly by physicians even during an outpatient consultation. In this way, they would have the opportunity to verify immediately if H_2S production occurs or not, and then decide to proceed with further investigations. Among other advantages of such devices, we include first the low cost and, second, the immediacy of sample determination, without sample storing necessity, before the analysis is carried out, avoiding possible losses or contaminations of the sample. This is just a preliminary study: we are aware that, being breath a very complex matrix, it is necessary to assess the impact of all its components on such devices, and to develop a detailed and rigorous sampling protocol. For this reasons, next steps in the implementation of the method will cover the use of such devices on patients with conditions associated to severe bloating due to increase of intraluminal fermentation, to identify a H_2S concentration trend, in correspondence of the sampling points established by the BTs protocol usually followed in clinical practice. In case of positive preliminary results, the following experiment will fall the requirement of the IVDR (In Vitro Medical Device Regulation - Regulation (EU) 2017/746).

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