Attachment 1.

Library

(FACE SHEET) ETHICAL REVIEW COMMITTEE, ICDDR,B. Date Sept 21, 94.

20/10/94
19/12/94

Principal Investigator Dr. Kamal Hossain Trainee Investigator (if any)

Application No. 94-017 (Revised) Supporting Agency (if Non-ICDDR,B)

Title of Study Stable Isotope Based Breath tests by the Fourier transform Infrared Spectroscopy (FTIR) method

Project status:
(X) New Study
( ) Continuation with change
( ) No change (do not fill out rest of form)

Circle the appropriate answer to each of the following (If Not Applicable write NA).

1. Source of Population:
   (a) Ill subjects (Yes) No
   (b) Non-ill subjects Yes No
   (c) Minors or persons under guardianship Yes No
2. Does the study involve:
   (a) Physical risks to the subjects Yes No
   (b) Social Risks Yes No
   (c) Psychological risks to subjects Yes No
   (d) Discomfort to subjects (Yes) No
   (e) Invasion of privacy Yes No
   (f) Disclosure of information damaging to subject or others Yes No
3. Does the study involve:
   (a) Use of records, (hospital, medical, death, birth or other) Yes No
   (b) Use of fetal tissue or abortus Yes No
   (c) Use of organs or body fluids (Yes) No
4. Are subjects clearly informed about:
   (a) Nature and purposes of study (Yes) No
   (b) Procedures to be followed including alternatives used Yes No
   (c) Physical risks (Yes) No
   (d) Sensitive questions Yes No
   (e) Benefits to be derived Yes No
   (f) Right to refuse to participate or to withdraw from study (Yes) No
   (g) Confidential handling of data Yes No
   (h) Compensation &/or treatment where there are risks or privacy is involved in any particular procedure Yes No
5. Will signed consent form be required:
   (a) From subjects Yes (No)
   (b) From parent or guardian (if subjects are minors) Yes No
6. Will precautions be taken to protect anonymity of subjects Yes (No)
7. Check documents being submitted herewith to Committee:
   ✓ Umbrella proposal - Initially submit an overview (all other requirements will be submitted with individual studies).
   ✓ Protocol (Required)
   ✓ Abstract Summary (Required)
   ___ Statement given or read to subjects on nature of study, risks, types of questions to be asked, and right to refuse to participate or withdraw (Required)
   ___ Informed consent form for subjects
   ___ Informed consent form for parent or guardian
   ___ Procedure for maintaining confidentiality
   ___ Questionnaire or interview schedule *

* If the final instrument is not completed prior to review, the following information should be included in the abstract summary:
1. A description of the areas to be covered in the questionnaire or interview which could be considered either sensitive or which would constitute an invasion of privacy.
2. Examples of the type of specific questions to be asked in the sensitive areas.
3. An indication as to when the questionnaire will be presented to the Cttee. for review.

We agree to obtain approval of the Ethical Review Committee for any changes involving the rights and welfare of subjects before making such change.

Dr. Kamal Hossain — Principal Investigator

Trainee

Title: STABLE ISOTOPE BASED BREATH TESTS BY THE FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR) METHOD

Principal Investigators: Dr. M.A. Khaled
Dr. D. Mahalanabis

Co-Principal Investigators: Dr. P.K. Bardhan
Dr. S.A. Sarker

Collaborating Investigator: Prof. K. Gyr
(University of Basel, Switzerland)

Period: One year from starting date

Budget: US$ 94,553

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Division Director
Clinical Sciences Division

## ABSTRACT SUMMARY

Breath test is a useful diagnostic procedure for many gastrointestinal diseases including the measure of malabsorption of carbohydrate, oxidation of fatty acids and protein turnover. $^{13}CO_2$ is the usual and product in the expired air measured by the isotope ratio mass spectrometry (IRMS). The IRMS is a highly sophisticated and expensive method. Third world or underdeveloped countries cannot afford to have such facilities. Simple and inexpensive methods to measure $^{13}CO_2$ is therefore highly desirable. In this study a new Fourier Transform InfraRed (FTIR) spectroscopic method will be validated. Once validated, this method may be easily available at a lower cost to measure $^{13}CO_2$ rapidly, accurately and most of all inexpensively.

BACKGROUND

With the introduction of the stable isotope $^{13}C$, $^{13}CO_2$ breath tests have become the main applications in the field of medical diagnosis (1-3). In these type of tests a $^{13}C$-labeled substrate is enzymatically cleaved during its passage through the stomach or gut, during its resorption, in liver microsomes etc. The amount of $^{13}CO_2$ eliminated in this process is considered as a measure of either a direct or in a consecutive step of a biochemical reaction. The total elimination rate of $^{13}CO_2$ via breath characterizes the actual function of a corresponding organ provided the biochemical reaction under consideration is a rate-limiting one. Based on this principle, various $^{13}C$ labeled substrates have been developed for diagnostic applications, some of which are described briefly below:

1. Hepatic Microsomal Biotransformation:

The carbon-13 enriched substrates, eg. [$^{13}C$] aminopyrine, [1,3,7-methyl-$^{13}C_3$] caffeine, (ethyl-$^{13}C$]phenacetin, [methyl-$^{13}C$] methacetin etc., have been used to diagnose many liver diseases in pediatrics (4,5).

2. Enterohepatic Circulation of Bile Acids

This is measured using [$^{13}C$] glycolic acid. Solomons et al(6) utilized this test for the diagnosis of jejunal bacterial overgrowth and ileal dysfunction.

3. Fat Malabsorption

Unlike unabsorbed proteins and carbohydrates, unabsorbed fatty substances are degraded by the colonic bacteria. The $^{13}C$-trioctanoin or $^{13}C$ mixed triglyceride breath test provides a rapid and convenient alternative to the classical 72 hours fecal fat measurements in the quantitation of fat malabsorption and its response to enzyme therapy (7).

4. Fatty Acid Oxidation

The use of fatty acids of different chain lengths has been the subject of great interest in clinical nutrition. [1-$^{13}C$] octanoate was used to detect both the medium and long-chain length triglyceride oxidation (8).

5. Amino acid oxidation

Growth monitoring of the malnourished children is usually accomplished by the protein synthesis as source of energy. L-[1-$^{13}C$]leucine has been used for $^{13}CO_2$ breath test for this purpose

since its oxidation rate is dependant on the carbohydrate and total energy intakes (9).

In addition to the above mentioned clinical applications, [$^{13}CO_2$] breath test has been used for the diagnosis of many other gastrointestinal dysfunctions. However, the detection of *Helicobacter pylori* [*H. Pylori*] infection by using [$^{13}C$] urea has drawn a greater attention of clinicians and researchers in recent years. At ICDDR,B the use of [$^{13}C$] urea breath test is also an ongoing medical procedure which makes it an important area for further investigations.

## 6. Gastric *H. pylori* Infection

*H. pylori*, originally known as *Campylobacter pylori*, is primarily responsible for the common form of chronic gastritis which affects over 50% of the World's population, mostly in the developing countries (10). It is also considered to cause duodenal and gastric ulcers and recently implicated in the pathogenesis of gastric cancer (11). The detection of this bacterium has therefore become a topic of great interest among the clinicians and researchers. Serum antibody to *H. pylori* can reliably be detected by the ELISA method. The antibody, however, remains over a long period after eradication, thus the method is not useful for short-term eradication. Probably the most sensitive and specific method is the culture of endoscopic biopsies. However, the method is invasive and requires very dependable laboratory experts. When the endoscopic biopsies are placed together with urea in a solution, the pH of the solution rises and $CO_2$ is produced. This is because of urea being the substrate of urease, secreted by *H. pylori*, degraded into ammonia and $CO_2$. $^{13}C$-labeled urea is usually taken by mouth and the expired air is collected for the measurement of the level of $^{13}CO_2$ in the breath. Since $^{13}C$ is non-radio-active i.e. a stable isotope and since the breath test is a non-invasive procedure the use of [$^{13}C$] urea is gaining popularity in recent years. The estimation of the $^{13}CO_2/^{12}CO_2$ (45/44) masses is accomplished by the Isotope Ratio Mass Spectrometry [IRMS]. But the IRMS is very expensive and expensive also to maintain. This makes the use of this method severely limited in the developing countries. Clinicians at the ICDDR,B collect the expired air in Vacutainer tubes and send them to some developed countries for their IRMS measurements. This costs time and money. A rapid and inexpensive method is therefore highly desirable for its use in the detection of $^{13}CO_2$ at least for the third world countries. A Fourier Transform InfraRed [FTIR] as described below may provide such a method.

### FTIR Method

InfraRed (IR) spectroscopy is one of the oldest physical methods applied to detect vibrational mode of an individual functional

group in many molecules. Such vibrational energy appears as well-defined band(s) in the IR spectrum in the frequency range between 4000 to 650 $cm^{-1}$(12). The advantage of the IR spectroscopy, unlike any other spectroscopies, is that it can detect a functional group in any form of matter, i.e. either in solid, or liquid, or gaseous state. The integrated absorption intensity (A) of an IR band is defined by

$A = \int k(v)dv$ , $k(v) = (1/cl)Ln(I_o/I)$

where c= molar concentration
l= path length of the sample
$I_o$= intensity of the incident light
I= intensity of the transmitted light

A quantitative analysis is therefore possible if a sample is examined in an IR cell with a fixed path length.

The main interest of this project is the detection of $CO_2$ in expired air (breath). The atmospheric $^{12}CO_2$ has been found to appear between 2375 to 2355 $cm^{-1}$ in the IR spectrum(13). The isotopic shift for $^{13}CO_2$ band is estimated theoretically to appear between 2280 to 2260 $cm^{-1}$. In fact several years ago Hirano et al (14) measured the $^{13}CO_2/^{12}CO_2$ ratio in the breath of animals showing the $^{13}CO_2$ and $^{12}CO_2$ bands at 2270.0 and 2360.2 $cm^{-1}$ respectively. They used a long IR cell and a large volume of expired air since the natural abundance of $^{13}C$ is 1.1% which is difficult to observe in a single-scan dispersive IR spectrometer. This is, however, impractical for clinical uses. Fortunately, the recent IR instrumental development includes the use of interferometry in which multiple scans are collected and transformed into an IR spectrum applying Fourier Transform mathematics, hence the FTIR (15). An FTIR is now capable of detecting functional groups at very low concentrations including the naturally occurring isotopes. We (MAK & DM) have already used an FTIR instrument to detect $^{13}CO_2$ in human breath at 2278.6 $cm^{-1}$ and $^{12}CO_2$ at 2361.8 $cm^{-1}$ which correspond very well with the earlier report (14) as mentioned above.

Since it is now possible to use FTIR method for quantitative determination of $^{13}CO_2$ and $^{12}CO_2$ in expired air we propose to develop further and validate this method for clinical uses, using it initially for the detection of H. pylori infection.

SPECIFIC AIMS

1. To measure $^{13}CO_2$ and $^{12}CO_2$ ratio in patients with H. pylori infection by using FTIR method.

2. To validate the data obtained by the FTIR method with the data of the same test samples measured by the IRMS method.

RATIONALE

As mentioned earlier, breath test has become an invaluable diagnostic modality in medical science. However, the prohibitive cost of IRMS instrument limits its application in the developing countries. Development of a low cost and rapid FTIR method, as described above, is extremely important. Once established this method could turn out to be a valuable diagnostic tool for numerous medical applications.

**METHODS**

Subject selection:

Many patients are referred to the Travellers clinic at the ICDDR,B for routine endoscopy because of ulcer-like symptoms usually characterized by epigastric pain related to food and relieved by milk or antacids. Dr. P. Bardhan, the co-investigator, usually performs the endoscopic examinations. Fifty three patients, aged above 20 years, will be recruited from this outpatient clinic who are referred for diagnostic upper GI endoscopy. Informed consents will be obtained from these subjects.

Sample Size

The prevalence of *H. pylori* in patients undergoing upper G.I. Endoscopy in the Traveller's Clinic, ICDDR,B is about 85%. Expecting that the accuracy of the FTIR method will not be less than 85% compared to the IRMS method, the sample size is calculated at 53 (significance at 5% and with 80% power).

Endoscopy and Biopsy

The patients will be asked to fast for at least 8 hours before the examination by Dr. Bardhan by using Olympus GIF-P2 fibreoptic panendoscope. Before reuse, the endoscope and the biopsy forceps will be washed, cleaned and soaked in glutaraldehyde solution for 15 minutes and thoroughly washed again with sterilised water. Three antral biopsy specimens will be collected for quick urease (CLO) test (16), bacterial cultures and a direct Gram stain.

CLO Test

One specimen applied on a quick urease test kit, available commercially, will be kept at 30°C for 3 hours and then at room temperature for one day. The kit will be examined every hour after 24 hours to see if a pink colour has been developed which is considered positive for H. pylori.

## Bacteria Culture and Gram Staining

Two other specimens will be processed in a microbiological laboratory within two hours after their collection. Each specimen will be cultured on chocolate-blood agar containing lincomycin, colistin, amphotericin B, trimethoprim and 1% vitox. The plates will be incubated at 37°C for 7 days in an anaerobic jar in which a microaerophilic atmosphere will be maintained. Growth will be monitored and identified by morphological and biochemical tests for H. pylori. Smears prepared from the specimen will be tested for spiral Gram negative organisms.

## [$^{13}$C] Urea Breath Test

Once the H. pylori infection is diagnosed, as described above, the breath test will be conducted within 5 days of the endoscopic test. The patients will be asked to fast again overnight. In the morning baseline breath samples will be collected into 20 ml Vacutainer tubes in triplicate. Thereafter the subjects will ingest 120 ml of a 31% (w/v) solution of nutrient-dense glucose in order to delay gastric emptying. Fifteen minutes later a dose of 100 mg [$^{13}$C]-urea (99.0%) dissolved in 20 ml water will be given to each patient to swallow. This dose of urea is believed to be adequate since Graham and Klein (17) suggested a dose range of 75 to 250 mg per patient. Thirty minutes after taking the [$^{13}$C] urea another breath sample will be collected in 20 ml Vacutainer tubes again in triplicate. A 30-minute waiting period has been shown to be optimum for elimination of $^{13}CO_2$ in the breath of H. pylori infected patients (17). One baseline sample and one post-dose sample for each patient will be sent to Prof. Klaus Gyr's lab in Basle, Switzerland for the estimation of $^{13}CO_2$ and $^{12}CO_2$ by using the IRMS method. The rest of the breath samples will be analyzed by the FTIR at the ICDDR,B.

## Analysis

The breath test results will be expressed as the Atom Percent Excess (APE) of the post-dose sample relative to the baseline sample. The APE thus obtained by both the IRMS and FTIR methods will be analyzed statistically and a relative accuracy of the newly developed FTIR method will be established.

## BUDGET

Duration of Proposal : 1 year

| Item | Time(%) | Amount($) |
|---|---|---|
| **Personnel Local: Salaries** | | |
| Co-PI - Dr. Bardhan | 20 | 2,953 |
| S. Sarker | 20 | 2,827 |
| Research Fellow | 50 | 3,000 |
| Lab Technician | 100 | 4,000 |
| Secretarial service (Mr. Patwari) | 20% | 1,148 |
| Sub-total | | 7,000 |
| **Personnel International:** | | |
| **Salaries** | | |
| Principal Investigators (Dr. Khaled) | 20 | UAB |
| Consultant (Dr. Mahalanabis) | | 2,000 |
| Sub-total | | 15,928 |
| **Bacteriology tests** | | 2,000 |
| **Supplies & Materials** | | |
| $13_C$ Urea | | 1,500 |
| Chemicals | | 500 |
| Others | | 250 |
| Sub-total | | 2,250 |
| **Equipment** | | 50,000 |
| **Miscellaneous** (Communication, printing, fax, repairs etc) | | 2,000 |
| Sub-total | | 1,000 |
| Total Operating Cost | | 72,178 |
| Overhead (31% of direct cost) | | 22,375 |
| Total Project Cost | | 94,553 |

19/12/94

## REFERENCES


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## Abstract Summary for the ERC

Breath test is a useful diagnostic procedure for many gastrointestinal diseases including the measure of malabsorption of carbohydrate, oxidation of fatty acids and protein turnover. $^{13}CO_2$ is the usual end product in the expired air after ingestion of $^{13}C$-urea and can be measured by the isotope ratio mass spectrometry (IRMS) and is now a non-invasive method to detect *H. pylori* infection in human. The IRMS is a highly sophisticated and expensive method. Third world or under-developed countries cannot afford to have such facilities. Simple and inexpensive methods to measure $^{13}CO_2$ is, therefore, highly desirable. A new and alternative method involving Fourier Transform Intra-red (FTIR) spectroscopy in estimating $^{13}CO_2$ in breath may have a potential field in identifying *H. pylori* infection in the developing world.

1. The protocol aims to validate the new FTIR method against (IRMS) in breath samples in identifying *H. pylori* infection in patients with peptic ulcer diseases.
2. The investigation will not cause any risk to the patients.
3. Informed consent will be obtained from the patient.
4. A short interview will be taken to obtain clinical tests.
5. There will be immediate benefit to the patient by intervention, investigation visits, medical treatment.
6. No blood will be drawn.
7. Patients record will be receiving for data analysis.

# CONSENT FORM

[Will be read and explained clearly before consent is obtained]

**Title: Stable isotope based breath tests by the Fourier Transform Intra-red (FTIR) spectroscopy method**

You are suffering probably from peptic ulcer diseases. Your doctor has sent you here for confirmation of diagnosis by endoscopic procedure. *H. pylori* bacteria is very often associated with peptic ulcer diseases. The identification of the bacteria needs collection of tissue or samples from stomach by endoscopic procedures. International Centre for Diarrhoeal Disease Research, Bangladesh is planning to develop a new and simple test to identify the presence of the bacteria in the stomach with application of breath test techniques.

We request you to participate in this study. If you agree, the following procedures will be followed:

1. A small tissue sample from your stomach will be taken for culture and microscopic examination upon enrolment in the study by endoscopic procedures (for which your doctor has sent you here) .

2. A breath test will be done after 5 days of endoscopic procedure.

   — You will be kept fast overnight for the breath test.

   — In the morning a breath sample of 20 ml will be collected when you blow through a tube.

   — You will then be allowed to drink 200 ml of milk.

   — 100 mg of $^{13}$C-urea will then be given to you with a glass of water to drink.

   — 30 minutes after the $^{13}$C-urea drink, another breath sample of 20 ml will be collected in similar way.

Your breath samples will be analyzed by two methods namely: Isotope Ratio Mass Spectrometer and Fourier Transform Intra-red (FTIR) spectroscopy in order to detect presence of *H. pylori* infection in your stomach.

There is no risk involved in the study. We will maintain confidentiality of the result. If you want to know the results of the tests we shall be happy to let you know as soon as the results are available. If you do not agree to participate in the study normal treatment will be given by us. You may withdraw your name from the study at any moment after enrolment in the study. In that case usual treatment for the disease will be continued. We will also advise you appropriate medication if your samples indicate presence of *H. pylori* in the stomach.

If you agree please sign on this consent form.

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Signature of PI/Co-PI

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Signature or thumb impression of the patient

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Date:_____

Witness:_____