

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO



No. 06-128821- -00008-0000

Fecha: 2008-07-23 16:02:22 Dep. 2020 NUECREACION
Ira. 2 PATENTES Eve: 1 REGDEPOSITO
Act. 449 RESPUESTA OPOSICION Folios: 34

Señora

Alix Carmenza Céspedes de Vergel

Jefe de División de Nuevas Creaciones

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

E. S. D.

Referencia: Expediente No. 06.128.821

Solicitud de Patente de Invención titulada: "Un método mediante una prueba de aliento para la detección de microorganismos"

Solicitante: Germán Antonio Campuzano Maya

**RESPUESTA A LA OPOSICION PRESENTADA POR ISOMED S.L. CON FUNDAMENTO EN LA
PUBLICACION HECHA EN LA GACETA NO.584 DEL 31 DE DICIEMBRE DE 2007**

ÁLVARO CORREA ORDÓÑEZ, mayor de edad y vecino de Bogotá D.C., identificado tal como aparece al pie de mi firma, en mi carácter de apoderado del Dr. Germán Antonio Campuzano Maya, doy respuesta a la oposición notificada por fijación en lista No. 128 del 24 de abril de 2008, presentada contra la solicitud de patente referida, en nombre de la sociedad Isomed S.L.

1. SOLICITUD

Frente a la oposición presentada por la sociedad Isomed S.L., contra la solicitud de patente del expediente de la referencia, solicito que se desestimen por parte de la Superintendencia, los argumentos elevados por la opositora y que en consecuencia, se continúe con el trámite de la solicitud de patente bajo análisis y se proceda a su concesión.

2. FRENTE A LOS FUNDAMENTOS DE LA OPOSICION

La oposición presentada enumera 18 hechos que constituyen, según la opositora, una justificación para el sustento de su pretensión. Frente a los mismos, me permito señalar lo siguiente:

Frente a los hechos primero al cuarto: Son ciertos.
Frente al hecho quinto: No es cierto.
Frente a los hechos sexto al noveno: Son ciertos.
Frente al hecho décimo: No es un hecho.
Frente al hecho décimo primero: No es un hecho.
Frente al hecho décimo segundo: No es cierto.
Frente al hecho décimo tercero: No es cierto.
Frente al hecho décimo cuarto: No es cierto.
Frente al hecho décimo quinto: No es cierto.
Frente al hecho décimo sexto: No es cierto.
Frente al hecho décimo séptimo: No es un hecho.
Frente al hecho décimo octavo: No es un hecho.

3. FUNDAMENTOS DE LA RESPUESTA

La oposición está fundamentada en falta de novedad y nivel inventivo de la solicitud 06.128.821 con base en la patente de Isomed S.L. No. 27386 que fue otorgada el 30 de mayo de 2002 para un invento denominado "Kit para la detección de *heliobacter pylori*". Expone el opositor que esta patente reclama prioridad de una patente española No. ES9602383 del 12 de noviembre de 1996.

El opositor asegura que la presente solicitud de patente carece de novedad "*puesto que está constituido por características fundamentales, que hacen que este métodos, sea conocido, y sea igual al que se utiliza en el "Kit para la detección de *heliobacter pylori*" de mi representada. (...) no proporciona características o efectos técnicos nuevos para que se considere como una solicitud nueva susceptible de patentabilidad*" y no tiene nivel inventivo "*ya que la patente de invención de mi representada "Kit para la detección de *heliobacter pylori*", ya posee todas estas características que pretenden reivindicar como nuevas en esta solicitud, objeto de la observación, donde podemos analizar que carece de novedad, puesto que está constituido por un método que hace que sea exactamente igual al que produce ISOMED S.L., todo de acuerdo a las reivindicaciones que acompañamos (...)*".

167
261

El opositor presenta argumentos adicionales relacionados con competencia desleal y registro de marcas, que se discutirán mas adelante.

3.1. La presente invención cumple con el requisito de novedad

El método mediante una prueba de aliento para la detección de microorganismos patógenos desarrollado por el Dr. Germán Antonio Campuzano cuenta con novedad frente a lo divulgado en la patente No. 27386 en la medida que presenta diferencias sustanciales en comparación con este ultimo.

En primer lugar, el método divulgado en la patente No. 27386 involucra la administración de ácido cítrico antes de administrar urea al paciente. La presente invención no administra ácido cítrico al paciente como parte del método con lo cual se cumple con el requisito de novedad establecido en el artículo 16 de la Decisión 486 frente a las enseñanzas de la patente No. 27386.

Como se explicará más adelante, esta característica del presente método –no necesita de la administración del ácido cítrico- no solo le brinda novedad a la invención, sino que además le proporciona ventajas sorprendentes al método que le aportan al mismo el nivel inventivo necesario para acceder el titulo de patente.

De otra parte, el presente método incorpora una etapa novedosa adicional al método desarrollado por Isomed S.L. correspondiente a la administración oral de una cantidad adecuada de agua al paciente, inmediatamente después de haber ingerido la solución con urea con carbón marcado.

Igualmente, el Dr. Campuzano después de haber hecho un estudio de 70 pacientes y más de 40 pacientes de referencia, con todos los rigores del método científico, estableció un listado de diferencias entre el método de Isomed S.L. y el método que el desarrolló que se pueden resumir así:

Prueba colombiana	TAU-kit de Isomed S.L. Laboratorio Farmacéutico
Utiliza baja dosis de urea marcada con carbono 13: 50 mg	Utiliza alta dosis de urea marcada con carbono 13: 100 mg

168
162

No utiliza ácido cítrico	Utiliza 3,2 g de ácido cítrico
Riesgo para el paciente: absolutamente inocua	Riesgo para el paciente: náuseas y vómito, sobretudo en niños y aumento de la hiperacidez gástrica en pacientes con síntomas de dispepsia y úlceras.
No altera la fisiología gástrica	Altera la fisiología gástrica retardando el vaciamiento gástrico y disminuyendo el pH (de alcalino a ácido en caso de estar infectado)
Con la forma de administrarla, como un “trago fuerte” que se “pasa rápidamente” con 200 mL agua se eliminan los falsos positivos relacionados con bacterias productoras de ureasa en la boca y en la orofaringe	Falsos positivos por bacterias productoras de ureasa en la boca y en la orofaringe
Estimula el contacto de la urea marcada con carbono 13 con la totalidad de la mucosa gástrica que elimina los resultados falsos negativos. Provee una solución acuosa adecuada para una hidrolisis optima de la urea marcada con carbono 13 por la ureasa del H. pylori. Minimiza interferencia de moléculas acidas (H+) excesivas entre enzima y sustrato. Provee una concentración optima de urea marcada (0.06 -0.43 mg/ml) en la solución acuosa que se forma en el estomago.	Falsos negativos por mala distribución de la urea en la mucosa gástrica
Es corta (10 minutos)	Es larga (30 minutos)
Sensibilidad: 100%	Sensibilidad: 94,3%
Especificidad: 100%	Especificidad: 96,3%
Utiliza 2 tubos	Utiliza 4 tubos
Es barata, lo cual permite masificarla	Es cara, lo cual restringe su masificación

A la luz de las mencionadas diferencias entre el documento citado por el opositor y la presente invención, es claro e cumplimiento del requisito de novedad por parte de la invención del Dr. Campuzano.

3.2. La presente invención cumple con el requisito de nivel inventivo

El invento bajo análisis cumple con el requisito de nivel inventivo en la medida que el resultado que propone no es obvio ni se hubiese podido derivar de manera evidente a partir de las enseñanzas contenidas en el estado de la técnica. EL Dr. Campuzano tuvo que realizar

163
163

un nuevo estudio con metodología científica estricta de 70 pacientes y más de 40 pacientes de referencia para presentar como validos los elementos novedosos de su invención.

Adicionalmente, hago referencia al método utilizado por la División de Nuevas Creaciones de la Superintendencia de Industria y Comercio y la Oficina Europea de Patentes para determinar la existencia del nivel inventivo llamado análisis del problema – solución en donde:

- i- Determinar el estado de la técnica más cercano;
- ii- Establecer el problema técnico a resolver; y
- iii- Considerar si la invención, comenzando del estado de la técnica más cercano y el problema técnico a resolver, sería obvia para una persona del oficio normalmente versada en la materia técnica correspondiente.

Si se estableciera que el estado de la técnica más cercano fuera la patente No. 27386, se pasaría a identificar el problema técnico a resolver por la presente invención que en concreto es:

Proporcionar un método mediante una prueba de aliento para la detección de microorganismos que evite la administración de componentes irritantes para el paciente, no tenga efectos colaterales ni empeoramiento de la enfermedad gástrica y sea altamente confiable.

Entrando en el tercer paso del método de análisis de problema - solución, es innegable que el método ahora propuesto no es de ninguna manera obvio a partir de las enseñanzas del estado de la técnica más cercano.

La anterior conclusión se sustenta en que hasta la fecha, el Laboratorio Clínico Hematológico S.A.¹ ha practicado más de 10.000 estudios utilizando los productos de Isomed S.L. y en la actualidad es el único laboratorio que realiza la prueba de aliento con carbono 13 utilizando los estuches de TAU-kit de Isomed S.L., distribuidos en la actualidad por Electrónica Digital de Colombia.

¹ Laboratorio que ofrece la prueba de aliento para la detección de microorganismos.

En particular, las pruebas de aliento con carbono 13 para el diagnóstico de microorganismos y especialmente la infección por *Helicobacter pylori*, como prueba de laboratorio, disponibles a nivel mundial se basan en la detección de la actividad de la ureasa, una enzima que desdobra la urea produciendo amonio (NH_3) y dióxido de carbono (CO_2). *Helicobacter pylori* es una de las muchas bacterias que producen esta enzima y esta reacción-. Entonces, la prueba de aliento con carbono 13, para la detección de la infección por *Helicobacter pylori*, se basa en la reproducción de esta reacción bioquímica que se presentaría en las personas infectadas, en cuyo caso el NH_3 es recuperado por la bacteria para protegerse de la acción ácida del estómago y el CO_2 restante es expelido por el pulmón, en donde puede ser medido en el aire expirado con la ayuda de un espectrofotómetro de masas como los que se han desarrollado comercialmente para tal fin.

La base bioquímica de la prueba de aliento con carbono 13 se conoce desde 1924 gracias a los estudios de J. Luck² que demostró la presencia de ureasa en la cavidad gástrica; y, segundo, que el prototipo de la prueba de aliento con carbono 13 fue descrito en 1987 por Graham y colaboradores utilizando 350 mg de urea marcada con carbono 13, tras la administración de una dieta rica en grasa para retardar el vaciamiento gástrico con dieciocho (18) muestras, una cada 10 minutos por 180 minutos, tomas de aire expirado³.

A partir de la descripción original de la prueba, se han desarrollado a nivel de la comunidad científica muchas variaciones, que tienen que ver con el número de muestras, los tiempos entre las muestras basales y las muestras postprandiales, la dosis de urea marcada con carbono 13 y las formas (en polvo, en tabletas, en cápsulas, en pudines, etc.) y las vías de administración (oral, transnasal, intragástrica, etc.) y en las "comidas" para retardar el vaciamiento gástrico con la premisa de tener el mayor tiempo posible la urea marcada en contacto con la ureasa en caso de haber infección.

Con relación a este último aspecto, una de las sustancias más utilizadas ha sido el ácido cítrico⁴, y es así como se utiliza en la mayoría de las pruebas de aliento patentadas hasta el momento, **incluida la prueba de Isomed S.L.**, en donde el paciente recibe 3,2 gramos de ácido cítrico, 10 minutos antes de tomar la muestra basal. Como evidencia, en la Tabla 1 se

² Luck, J. Gastric urease. Biochem J 18, 1227-1231 (1924).

³ Graham, D.Y. et al. *Campylobacter pylori* detected noninvasively by the 13C-urea breath test. *Lancet* 1, 1174-7 (1987).

⁴ Domínguez-Muñoz, J.E., Leodolter, A., Sauerbruch, T. & Malfertheiner, P. A citric acid solution is an optimal test drink in the 13C-urea breath test for the diagnosis of *Helicobacter pylori* infection. *Gut* 40, 459-62 (1997).

anexan las variaciones analíticas de la mayoría de las pruebas de aliento referenciadas en la literatura médica mundial.

Tras la ejecución de más de 10.000 pruebas de aliento con carbono 13 en el Laboratorio Clínico Hematológico S.A., como también se ha descrito en la literatura médica mundial (ver referencias de la tabla 1), se ha observado que la administración de ácido cítrico a pacientes infectados por *Helicobacter pylori* puede aumentar con manifestaciones adversas en algunos pacientes con riesgo de precipitar complicaciones ulcerosas o desencadenar sintomatología de difícil manejo médico; en niños se ha observado que lo toleran mal, con náuseas y vómito, en un alto porcentaje de ellos motivo por el cual la prueba puede dar resultados aberrantes.

Una complicación de mayor gravedad se presentó en el Laboratorio Clínico Hematológico S.A. cuando un paciente sufrió un paro cardiorrespiratorio, que requirió maniobras de resucitación, tras la ingestión del ácido cítrico (3,2 g), administrado de acuerdo con el protocolo de Isomed S.L. Laboratorio Farmacéutico, como claramente se evidencia en el anexo 2. Incidente.

Motivados por las dificultades del ácido cítrico, en particular las relacionadas con el incidente del paciente que presentó el paro cardiorrespiratorio, el periodo tan largo (30 minutos) que debían permanecer los pacientes en el laboratorio clínico, el alto costo de la urea marcada con carbono 13 y la necesidad de reducir los costos de la prueba, entre otras muchas circunstancias, en el mes de febrero de 2006 el Dr. Campuzano inició un protocolo de estudio en el cual se compararon dos esquemas con 50 mg de urea marcada, disuelta en 15 ml de agua administrada, como quien toma un “trago fuerte” que “pasa” con 200 ml de agua (protocolo 1 en la descripción de la patente) versus agua con 4,2 g de ácido cítrico (protocolo 2 en la descripción de la patente), seguido de movimientos circulares que agitan el líquido, en total de 210 ml, que llega a la cavidad gástrica en tres tiempos, a los 10, 20 y 30 minutos.

Para desarrollar el protocolo se convocó a 70 voluntarios a los cuales, previo consentimiento informado, se les practicó una prueba de aliento con carbono 13 estándar utilizando estuches comerciales de TAU-kit de Isomed S.L. y preparaciones manuales de urea marcada con carbono 13. Las muestras se tomaron entre el 15 de mayo y el 15 de agosto de 2006. Se hicieron los análisis estadísticos y se redactó el informe científico que fue remitido a la revista World Journal of Gastroenterology el 27 de junio de 2007, fue aceptado para

publicación el 17 de agosto de 2007 y apareció publicado en la citada revista el 7 de noviembre de 2007, bajo el título: *"An optimized 13C-urea breath test for the diagnosis of H pylori infection"*⁵ (Anexo 3).

Con relación a la evaluación del artículo, vale la pena enfatizar los aspectos más relevantes de los examinadores internacionales del artículo original, conceptos que se transcriben en su totalidad:

"Marco Teórico"

La infección por H pylori afecta cerca del 50% de la población mundial y se ha asociado con la patogénesis de enfermedades gástricas como la gastritis, la úlcera péptica y el linfoma MALT, y con una variedad de enfermedades extradigestivas, incluyendo la púrpura trombocitopénica idiopática, la anemia por deficiencia de hierro y la tiroiditis auto inmune, entre otras. El diagnóstico de la infección por H pylori se puede establecer por métodos invasivos, mediante la endoscopia, o por métodos no invasivos como la prueba de aliento con 13C-urea.

Fronteras con la investigación

La prueba de aliento con 13C-urea se basa en la capacidad de una enzima (la ureasa), producida por H pylori en el estómago, de metabolizar la urea administrada. Los pacientes ingieren la urea marcada con un isótopo no radiactivo (13C). Después de unos minutos, el dióxido de carbono marcado con el isótopo (13C02) es expirado en el aliento si hay presencia de la ureasa de H pylori en el estómago. La diferencia entre los valores del 13C02 antes y después de la ingestión de la urea marcada determinará la presencia o ausencia de la infección.

Innovaciones y descubrimientos

Numerosas investigadores han tratado de disminuir el costo de la prueba de aliento con 13C-urea, sin comprometer la exactitud diagnóstica de la prueba. Para ello, se han modificado las dosis, la vía y la forma de administrar la urea, los tiempos de recolección de las muestras y las "comidas" para retardar el vaciamiento gástrico.

⁵ Campuzano-Maya, G. An optimized 13C-urea breath test for the diagnosis of H pylori infection. World Gastroenterology 13, 5454-64 (2007).

Aplicaciones

Una prueba de aliento con 13C-urea de bajo costo para la detección de la infección por H pylori, antes y después del tratamiento de erradicación, haría más accesible esta prueba no invasiva para los pacientes, especialmente en países en vía de desarrollo.

Terminología

13C-UBT: prueba de aliento que utiliza urea marcada con 13C, un isótopo no radiactivo.

DOB: "Delta over baseline", unidades para expresar la cantidad de 13C02 en la muestra de aliento.

Opinión de los árbitros

Este es un artículo bien escrito y bien referenciado. La metodología está descrita en forma apropiada y los resultados son presentados de manera clara. Las conclusiones son una interpretación justa de los resultados."

A partir de las observaciones antes expuestas y comentarios de los revisores del artículo – expertos en la materia técnica bajo análisis- es claro que la nueva prueba de aliento, aparte de ser una nueva prueba, introduce innovación, reduce costos y hace posible que llegue a países pobres, que como Colombia, tienen las más altas tasas de infección por *Helicobacter pylori* del mundo como claramente lo demuestran estudios realizados en el medio⁶.

Además, en el mes de diciembre de 2007 -un mes después de haber salido la publicación original-, en un artículo titulado "*Breath tests for gastrointestinal disease: The real deal or just a lot of hot air?*" (Anexo 4) en una sección titulada "Imaging and advanced technology" de la revista *Gastroenterology* de la Sociedad Americana de Gastroenterología⁷, en donde analiza el uso de las pruebas de aliento en gastroenterología, hace referencia a las pruebas de aliento con carbono 13 en el diagnóstico y manejo de la infección por *Helicobacter pylori* en tres (3) oportunidades y una de ellas es a la prueba colombiana, a la cual se refiere el autor en los siguientes términos:

⁶ Duque, J.J. & Gutiérrez, F. Presencia de *Helicobacter pylori* en la mucosa gástrica de cadáveres. *latreia* 8, 96-97 (1995) y Duque, J.J. *Helicobacter pylori* en la mucosa gástrica de cadáveres de niños. *latreia* 12, 135-138 (1999).

⁷ Saad, R.J. & Chey, W.D. *Breath tests for gastrointestinal disease: the real deal or just a lot of hot air?* *Gastroenterology* 133, 1763-6 (2007)

“Un estudio reciente reportó que un protocolo simplificado de la 13C-UBT en el cual se utiliza una dosis menor de 13C-urea (50 mg), sin comida y con una recolección de una muestra única a los 10 minutos tenía una exactitud del 100% para el diagnóstica de H pylori”. (ver página 1763 columna 2).

Ante los anteriores hechos es incuestionable la existencia de nivel inventivo del método y kit que se proponen en la solicitud bajo análisis. Los anteriores hechos claramente documentados, controvierten de manera contundente los argumentos presentados por el opositor en contra del patentamiento del presente invento.

Adicionalmente, es bien importante tener en cuenta que la presente solicitud de patente colombiana reivindica solamente el kit el cual, por supuesto, es novedoso e inventivo. Hago notar que el oponente para nada hace referencia al kit lo cual es claro indicio de que no existen argumentos que afecten la patentabilidad del mismo. Es también importante anotar que en la solicitud colombiana no se reivindica el método mencionado y objetado (sin fundamento) por el oponente.

3.3. Contrato de distribución - Buena fe

El opositor hace mención del contrato de distribución que celebraron con Laboratorio Clínico Hematológico S.A. (terminado el 11 de agosto de 2005) como evidencia de la mala fe del Dr. Campuzano al querer proteger por medio de patente el mismo producto que había sido objeto del contrato de distribución.

Sobre este hecho, el solicitante solo desea mencionar que el 20 de abril de 2004, en vista de la falta de representación efectiva en Colombia en cabeza de Isomed Colombia Limitada y como una manera de introducir la prueba en el mercado colombiano, el Laboratorio Clínico Hematológico S.A. accedió a firmar un contrato de distribución de la prueba con la condición de que se consumirían al menos dos mil (2.000) estuches por año y como contraprestación, Isomed S.L. Laboratorio Farmacéutico, haría la publicidad y el mercadeo para lograr la penetración de la misma en el mercado colombiano y más allá de sus fronteras (al menos en el mercado del Pacto Andino).

Por razones ajenas al Laboratorio Clínico Hematológico, Isomed S.L. Laboratorio Farmacéutico se limitó a hacer las ventas al Laboratorio y no avanzó en el compromiso de la publicidad y el mercadeo, situación por la cual no fue posible cumplir con la meta impuesta por Isomed S.L. Laboratorio Farmacéutico, suspendiéndose la representación el 11 de agosto de 2005, y las compras, de ahí en adelante, fueron directas con Isomed S.L. Laboratorio Farmacéutico o actualmente con su representante comercial en Bogotá, Electrónica Digital de Colombia a partir del 22 de junio de 2006.

De esta manera, la mención de este contrato de distribución por cada una de las partes ha sido transparente y por lo tanto, respetuosamente considero que no hay lugar a mencionar este hecho dentro del presente análisis técnico de la patentabilidad del invento y mucho menos que este contrato y su posterior terminación den lugar a actuaciones por fuera de la buena fe. En definitiva de lo que se trata aquí es de demostrar la novedad, el nivel inventivo y la aplicación industrial y no de ninguna otra cosa que no viene al caso.

3.4. Competencia desleal

De manera respetuosa considero que el hecho detallado en el punto 3.17 de la oposición relacionado con los posibles actos de competencia desleal que se configurarían si la SIC otorgara el título de patente a esta invención, no deben ser presentados dentro del marco de una oposición de patente por cuanto: En primer lugar, el tema a evaluar por la División de Nuevas Creaciones es la patentabilidad de una invención a la luz de los tres requisitos básicos de patentabilidad y segundo, el opositor no presenta pruebas que demuestren que el Dr. Campuzano ha realizado actos de competencia desleal en contra de la sociedad Isomed SL.

3.5. Relación con las causales de irregistrabilidad de marcas.

Finalmente, sobre la mención por parte del opositor a las causales para irregistrabilidad de marcas, respetuosamente manifiesto que tampoco es de recibo este argumento por cuanto no son aplicables las causales de irregistrabilidad de marcas al tema de patentes.

Tabla 1. Selección de estudios que evalúan las características de la prueba aliento con ^{13}C -urea.

Autor(es)	País	Año	Patrón de referencia *	Indicación	Pacientes	Dosis (mg)	Comida	Tiempo posura (min)	Punto de corte ($\delta^{13}\text{C}_2$)	Sensibilidad	Especificidad
Graham y colaboradores ¹		1987	H, C		26	5 mg/kg de peso	Sustacal de Mead Johnson [®]	Cada 10 minutos por 180 minutos	De acuerdo con la curva tiempo/ecceso de $^{13}\text{C}_2$		
Good y colaboradores ²	Alemania	1991	H	p	50	100	Comida líquida no definida	30	5	94%	93%
Braden y colaboradores ³	Alemania	1994	UBT	p	217	75		20	5	99%	100%
Labenz y colaboradores ⁴	Alemania	1996	H, C	P	70	75	Jugo naranja	30	4	98%	100%
Klein y colaboradores ⁵	Estados Unidos	1996	H	pp	120	125	Leche	30	2,4	100%	100%
Klein y colaboradores ⁵	Estados Unidos	1996	H	PP	120	250	Leche	30	4,2	100%	98%
Malaty y colaboradores ⁶	Estados Unidos	1996	RUT, H, C	p		125	-----	20	2,4	96%	100%
Taniguchi y colaboradores ⁷	Japón	1996	H	p		100	-----	15	?	98%	74%
Bielanski y colaboradores ⁸	Polonia	1996	RUT, H	p	114	38	-----	-----	5	97%	95%
Dominguez-Muñoz y colaboradores ⁹	Alemania	1997	RUT, H, C	p	80	75	Ácido cítrico	30	4	100%	100%
Ellenrieder y colaboradores ¹⁰	Alemania	1997	RUT, H, C	p	145	75	Jugo de manzana	30	3,5	91%	89%
Epple y colaboradores ¹¹	Alemania	1997	H	pp	77	75	Ácido cítrico	30	1,3	96	100
Kato y colaboradores ¹²	Japón	1998	RUT, H, C	P	151	100	-----	10	3,5	99%	100%
Kkato y colaboradores ¹²	Japón	1998	RUT, H, C	post	151	100	-----	10	3,5	87%	99%
Miwa y colaboradores ¹³	Japón	1998	H	pp		100	-----	20	5	96%	97%
Ohara y colaboradores ¹⁴	Japón	1998	RUT, H, C	p		100	-----	20	2,5	98%	98%
Tanahashi y colaboradores ¹⁵	Japón	1998	H, C	pp		100	-----	20	2,5-3	98%	100%
Leodolter y colaboradores ¹⁶	Alemania	1999	RUT, H, C	p	50	75	Ácido cítrico	30	4	100%	100%
Leodolter y colaboradores ¹⁶	Alemania	1999	RUT, H, C	p	50	75	Jugo naranja	30	4	88%	100%
Leodolter y colaboradores ¹⁷	Alemania	1999	RUT, H, C	p		75	Ácido cítrico	30	4	92%	99%
Leodolter y colaboradores ¹⁷	Alemania	1999	RUT, H, C	p		75	Ácido cítrico	30	4	95%	98%
Leodolter y colaboradores ¹⁷	Alemania	1999	RUT, H, C	post		75	Ácido cítrico	30	4	95%	100%
Coelho y colaboradores ¹⁸	Brasil	1999	RUT, H, 14C-UBT	p	73	75	Jugo naranja	30	4	100%	100%
Savarino y colaboradores ¹⁹	Italia	1999	RUT, H	p		75	Ácido cítrico	15	5	98%	100%
Savarino y colaboradores ¹⁹	Italia	1999	RUT, H	p		75	Ácido cítrico	30	5	100%	100%
Savarino y colaboradores ¹⁹	Italia	1999	RUT, H	p		75	Ácido cítrico	15	5	98%	93%
Savarino y colaboradores ¹⁹	Italia	1999	RUT, H	p		75	Ácido cítrico	30	5	97%	95%
Hamlet y colaboradores ²⁰	Suecia	1999	RUT, H, C	p	134	100	Ácido cítrico	10	1,8	95%	100%
Riepl y colaboradores ²¹	Alemania	2000	H, C	p		75	Jugo naranja	15	6,5	92%	94%
Peng y colaboradores ²²	China	2000	RUT, H, C	p		100	Leche	15	4,8	94%	89%
Gisbert y colaboradores ²³	España	2000	UBT	p	53	100	Ácido cítrico	30	3,3-3,9	100%	100%
Wong y colaboradores ²⁴	Hong Kong	2000	RUT, H	p		50	Ácido cítrico	10	3,5	96%	99%

172

Wong y colaboradores ²⁴	Hong Kong	2000	RUT,H	p		50	Ácido cítrico	15	3,5	96%	99%
Wong y colaboradores ²⁴	Hong Kong	2000	RUT,H	p		75	Ácido cítrico	30	5	96%	98%
Wong y colaboradores ²⁴	Hong Kong	2000	RUT,H	p		75	-----	30	5	95%	98%
Savarino y colaboradores ²⁵	Italia	2000	RUT,H	pp		75	Ácido cítrico	30	5	98%	97%
Savarino y colaboradores ²⁵	Italia	2000	RUT,H	pp		75	Ácido cítrico	30	5	98%	97%
Sheu y colaboradores ²⁶	Taiwan, China	2000	H,C	p		100	-----	15	4	97%	97%
Sheu y colaboradores ²⁶	Taiwan, China	2000	H,C	p		100	-----	15	4	97%	97%
Sheu y colaboradores ²⁷	Taiwan, China	2000	H,C	post	177	50	-----	15	2,5	96,4%	98,7%
Sheu y colaboradores ²⁷	Taiwan, China	2000	H,C	p		50	Ácido cítrico	15	3,5	96%	99%
Wong y colaboradores ²⁸	Hong Kong	2001	RUT,H	pp		50	-----	20	7	100%	96%
Wong y colaboradores ²⁸	Hong Kong	2001	RUT,H	pp		50	-----	30	3	100%	100%
Wong y colaboradores ²⁸	Hong Kong	2001	RUT,H	pp		50	Ácido cítrico	20	3	100%	98%
Wong y colaboradores ²⁸	Hong Kong	2001	RUT,H	pp		50	Ácido cítrico	30	2,5	100%	98%
Wong y colaboradores ²⁸	Hong Kong	2001	RUT,H	pp		75	-----	30	3,5-4,5	100%	100%
Wong y colaboradores ²⁸	Hong Kong	2001	RUT,H	pp		75	-----	20	4,5	100%	92%
Ng y colaboradores ²⁹	Hong Kong	2002	RUT,H	p		75	Ácido cítrico	30	5	97%	96%
Ng y colaboradores ²⁹	Hong Kong	2002	RUT,H	p		75	-----	30	3,5	96%	94%
Ng y colaboradores ²⁹	Hong Kong	2002	RUT,H	p		75	Ácido cítrico	30	5,5	93%	97%
Chua y colaboradores ³⁰	Sinagapur	2002	RUT,H,S (1 de las tres)	p	100	100	Ácido cítrico	30	3,5	94,2%	100%
Chang y colaboradores ³¹	Taiwan, China	2002	RUT,H,C,SA	p	62	20	-----	20	3,5	100%	100%
Liao y colaboradores ³²	Taiwan, China	2002	RUT,H	p		50	Leche	10	2,5	99%	95%
Liao y colaboradores ³²	Taiwan, China	2002	RUT,H	p		50	Leche	15	2,5	99%	97%
Liao y colaboradores ³²	Taiwan, China	2002	RUT,H	p		50	Leche	30	2,5	99%	97%
Gisbert y colaboradores ³³	España	2003	RUT,H	pre	36	100	Ácido cítrico	30	4,6	96%	100%
Gisbert y colaboradores ³³	España	2003	RUT,H	post	85	100	Ácido cítrico	30	4,6	100%	97%
Gisbert y colaboradores ³⁴	España	2003	RUT,H	pp	41	100	Ácido cítrico	30	11	100%	89%
Wong y colaboradores ³⁵	China	2003	RUT,H	pp		50	Ácido cítrico	10	1,2	100%	90%
Wong y colaboradores ³⁵	China	2003	RUT,H	pp		50	Ácido cítrico	20	2,1	100%	100%
Wong y colaboradores ³⁵	China	2003	RUT,H	pp		50	Ácido cítrico	30	1,2	100%	98%
Gatta y colaboradores ³⁶	Italia	2003	RUT,H,C	p	200	100	Ácido cítrico	10	1,5-3	100%	100%
Gatta y colaboradores ³⁶	Italia	2003	RUT,H,C	post	200	100	Ácido cítrico	10	1,4-7,4	100%	100%
Gatta y colaboradores ³⁶	Italia	2003	RUT,H,C	p	200	50	Ácido cítrico	10	1,6-3,1	100%	100%
Gatta y colaboradores ³⁶	Italia	2003	RUT,H,C	post	200	50	Ácido cítrico	10	1,5-1,6	100%	100%
Gatta y colaboradores ³⁶	Italia	2003	RUT,H,C	p	200	75	Ácido cítrico	10	3,1-6,8	100%	100%
Gatta y colaboradores ³⁷	Italia	2003	RUT,H,C	post	200	75	Ácido cítrico	10	4,9-7	100%	100%
Kato y colaboradores ³⁷	Japón	2003	H,C	post	190	100	-----	20	3,5	67%	99%
Kato y colaboradores ³⁷	Japón	2003	H,C	Post	190	100	-----	20	2,5	100%	96%

173

Peng y colaboradores ³⁶	Taiwan, China	2003	RUT, H, C		100	100				100%	100%
Peng y colaboradores ³⁸	Taiwan, China	2003	RUT, H, C		100	50				96,4	100%
Gatta y colaboradores ³⁹	Italia	2004	RUT, H, C	p	72	50	Acido cítrico	10	1,5	100%	-----
Gatta y colaboradores ³⁹	Italia	2004	RUT, H, C	p	72	75	Acido cítrico	30	4,5	100%	-----
Gatta y colaboradores ³⁹	Italia	2004	RUT, H, C	p	72	75	Jugo naranja	30	4,5	100%	-----
Gatta ⁴⁰	Italia	2006	RUT, H	pp	100	25	Acido cítrico (1 g)	30	4,40-6,26	100%	100%
Gatta ⁴⁰	Italia	2006	RUT, H	pp	100	15	Acido cítrico (1 g)	30	4,30-6,19	96,1%	100%
Gatta ⁴⁰	Italia	2006	RUT, H	pp	100	10	Acido cítrico (1 g)	30	1,67-262	89,1%	100%
Yang y colaboradores ⁴¹	China	2007	UBT, SAT	p	153	25 (1 mg/kg)		30	3,5	88,9%	95%
Ortiz-Olvera y colaboradores ⁴²	México	2007	H	p	88	50	Acido cítrico	30	4,22	90,2%	93,3%
Campuzano ⁴³	Colombia	2007	UBT		70	50	No	10	1-2	100	100
Campuzano ⁴³	Colombia	2007	UBT		70	50	Acido cítrico	10	1-2	100	100

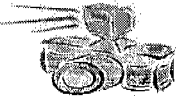
Convenciones: RUT: prueba de ureasa rápida; H: histología; C: cultivo; ¹³C-UBT: prueba aliento con ¹³C-urea; ¹⁴C-UBT: prueba aliento con urea marcada con carbono ¹⁴; SAT: antígeno de *Helicobacter pylori* en materia fecal; S: serología; UBT: prueba de aliento.

Referencias

- Graham DY, Klein PD, Evans DJ, Jr., et al. *Campylobacter pylori* detected noninvasively by the ¹³C-urea breath test. *Lancet* 1987;1:1174-7.
- Good DJ, Dill S, Mossi S, et al. [Sensitivity and specificity of a simplified, standardized ¹³C-urea breath test for the demonstration of *Helicobacter pylori*]. *Schweizerische medizinische Wochenschrift* 1991;121:764-6.
- Braden B, Duan LP, Caspary WF, Lembcke B. More convenient ¹³C-urea breath test modifications still meet the criteria for valid diagnosis of *Helicobacter pylori* infection. *Z Gastroenterol* 1994;32:198-202.
- Labenz J, Barsch G, Peitz U, et al. Validity of a novel biopsy urease test (HUT) and a simplified ¹³C-urea breath test for diagnosis of *Helicobacter pylori* infection and estimation of the severity of gastritis. *Digestion* 1996;57:391-7.
- Klein PD, Malaty HM, Martin RF, Graham KS, Genta RM, Graham DY. Noninvasive detection of *Helicobacter pylori* infection in clinical practice: the ¹³C urea breath test. *Am J Gastroenterol* 1996;91:690-4.
- Malaty HM, el-Zimaity HM, Genta RM, Klein PD, Graham DY. Twenty-minute fasting version of the US ¹³C-urea breath test for the diagnosis of *H. pylori* infection. *Helicobacter* 1996;1:165-7.
- Taniguchi Y, Kimura K, Sohara H, et al. Simple ¹³C-urea breath test with infra-red spectrophotometer. *J Gastroenterol* 1996;31 Suppl 9:37-40.
- Bielanski W, Konturek SJ. New approach to ¹³C-urea breath test: capsule-based modification with low-dose of ¹³C-urea in the diagnosis of *Helicobacter pylori* infection. *J Physiol Pharmacol* 1996;47:545-53.
- Dominguez-Muñoz JE, Leodolter A, Sauerbruch T, Malfertheimer P. A citric acid solution is an optimal test drink in the ¹³C-urea breath test for the diagnosis of *Helicobacter pylori* infection. *Gut* 1997;40:459-62.
- Ellenrieder V, Glasbrenner B, Stoffels C, et al. Qualitative and semi-quantitative value of a modified ¹³C-urea breath test for identification of *Helicobacter pylori* infection. *Eur J Gastroenterol Hepatol* 1997;9:1085-9.
- Epple HJ, Kirstein FW, Bojarski C, et al. ¹³C-urea breath test in *Helicobacter pylori* diagnosis and eradication. Correlation to histology, origin of 'false' results, and influence of food intake. *Scand J Gastroenterol* 1997;32:308-14.
- Kato M, Asaka M, Kudo T, et al. Ten minute ¹³C-urea breath test for the diagnosis of *Helicobacter pylori* infection. *J Gastroenterol* 1998;33 Suppl 10:40-3.
- Miwa H, Murai T, Ohkura R, et al. Usefulness of the [¹³C]-urea breath test for detection of *Helicobacter pylori* infection in fasting patients. *J Gastroenterol Hepatol* 1998;13:1039-43.
- Ohara S, Kato M, Asaka M, Toyota T. Studies of ¹³C-urea breath test for diagnosis of *Helicobacter pylori* infection in Japan. *J Gastroenterol* 1998;33:6-13.
- Tanahashi T, Kodama T, Yamaoka Y, et al. Analysis of the ¹³C-urea breath test for detection of *Helicobacter pylori* infection based on the kinetics of delta-¹³CO₂ using laser spectroscopy. *J Gastroenterol Hepatol* 1998;13:732-7.
- Leodolter A, Dominguez-Munoz JE, Von Arnim U, Malfertheimer P. Citric acid or orange juice for the ¹³C-urea breath test: the impact of pH and gastric emptying. *Aliment Pharmacol Ther* 1999;13:1057-62.

17. Leodolter A, Dominguez-Munoz JE, von Arnim U, Kahl S, Peitz U, Malfertheiner P. Validity of a modified ^{13}C -urea breath test for pre- and posttreatment diagnosis of *Helicobacter pylori* infection in the routine clinical setting. *Am J Gastroenterol* 1999;94:2100-4.
18. Coelho LG, Reber M, Passos MC, et al. Application of isotope-selective non-dispersive infrared spectrometry for the evaluation of the ^{13}C -urea breath test: comparison with three concordant methods. *Brazilian journal of medical and biological research = Revista brasileira de pesquisas medicas e biologicas / Sociedade Brasileira de Biofisica* [et al 1999;32:1493-7.
19. Savarino V, Mela GS, Zentilin P, et al. Comparison of isotope ratio mass spectrometry and nondispersive isotope-selective infrared spectroscopy for ^{13}C -urea breath test. *Am J Gastroenterol* 1999;94:1203-8.
20. Hamlet A, Stage L, Lonroth H, Cahlin C, Nystrom C, Pettersson A. A novel tablet-based ^{13}C urea breath test for *Helicobacter pylori* with enhanced performance during acid suppression therapy. *Scand J Gastroenterol* 1999;34:367-74.
21. Riepl RL, Folwaczny C, Otto B, et al. Accuracy of ^{13}C -urea breath test in clinical use for diagnosis of *Helicobacter pylori* infection. *Z Gastroenterol* 2000;38:13-9.
22. Peng NJ, Hsu PI, Lee SC, et al. A 15-minute ^{13}C -urea breath test for the diagnosis of *Helicobacter pylori* infection in patients with non-ulcer dyspepsia. *J Gastroenterol Hepatol* 2000;15:284-9.
23. Gisbert JP, Vazquez MA, Jimenez I, et al. ^{13}C -urea breath test for the diagnosis of *Helicobacter pylori* infection before treatment: is citric acid necessary? *Dig Liver Dis* 2000;32:20-4.
24. Wong WM, Wong BC, Wong KW, et al. ^{13}C -urea breath test without a test meal is highly accurate for the detection of *Helicobacter pylori* infection in Chinese. *Aliment Pharmacol Ther* 2000;14:1353-8.
25. Savarino V, Landi F, Dulbecco P, et al. Isotope ratio mass spectrometry (IRMS) versus laser-assisted ratio analyzer (LARA). A comparative study using two doses of ^{13}C Urea and two test meals for pre and posttreatment diagnosis of *Helicobacter pylori* infection. *Dig Dis Sci* 2000;45:2168-74.
26. Sheu BS, Lee SC, Yang HB, et al. Selection of lower cutoff point of ^{13}C urea breath test is helpful to monitor *H. pylori* eradication after proton pump inhibitor-based triple therapy. *Dig Dis Sci* 2000;45:1330-6.
27. Sheu BS, Lee SC, Yang HB, et al. Lower-dose ^{13}C -urea breath test to detect *Helicobacter pylori* infection-comparison between infrared spectrometer and mass spectrometry analysis. *Aliment Pharmacol Ther* 2000;14:1359-63.
28. Wong WM, Wong BC, Li TM, et al. Twenty-minute 50 mg ^{13}C -urea breath test without test meal for the diagnosis of *Helicobacter pylori* infection in Chinese. *Aliment Pharmacol Ther* 2001;15:1499-504.
29. Ng FH, Lai KC, Wong BC, et al. ^{13}C -urea breath test without prior fasting and without test meal is accurate for the detection of *Helicobacter pylori* infection in Chinese. *J Gastroenterol Hepatol* 2002;17:834-8.
30. Chua TS, Fock KM, Teo EK, Ng TM. Validation of ^{13}C -urea breath test for the diagnosis of *Helicobacter pylori* infection in the Singapore population. *Singapore Med J* 2002;43:408-11.
31. Chang MC, Chang YT, Sun CT, Wu MS, Wang HP, Lin JT. Quantitative correlation of *Helicobacter pylori* stool antigen (HpSA) test with ^{13}C -urea breath test (^{13}C -UBT) by the updated Sydney grading system of gastritis. *Hepatogastroenterology* 2002;49:576-9.
32. Liao CC, Lee CL, Chiang TC, et al. The ^{13}C -urea breath test to detect *Helicobacter pylori* infection: a validated simple methodology with 50 mg ^{13}C -urea. *Aliment Pharmacol Ther* 2002;16:787-92.
33. Gisbert JP, Ducons J, Gomollón F, et al. Validation de la prueba de aliento con ^{13}C -urea para el diagnóstico inicial de la infección por *Helicobacter pylori* y la confirmación de su erradicación tras el tratamiento. *Rev Esp Enferm Dig* 2003;95:121-6, 15-20.
34. Gisbert JP, Gomollon F, Dominguez-Munoz JE, et al. [Comparison between two ^{13}C -urea breath tests for the diagnosis of *Helicobacter pylori* infection: isotope ratio mass spectrometer versus infrared spectrometer]. *Gastroenterol Hepatol* 2003;26:141-6.
35. Wong WM, Lam SK, Lai KC, et al. A rapid-release 50-mg tablet-based ^{13}C -urea breath test for the diagnosis of *Helicobacter pylori* infection. *Aliment Pharmacol Ther* 2003;17:253-7.
36. Gatta L, Vakil N, Ricci C, et al. A rapid, low-dose, ^{13}C -urea tablet for the detection of *Helicobacter pylori* infection before and after treatment. *Aliment Pharmacol Ther* 2003;17:793-8.
37. Kato C, Sugiyama T, Sato K, et al. Appropriate cut-off value of ^{13}C -urea breath test after eradication of *Helicobacter pylori* infection in Japan. *J Gastroenterol Hepatol* 2003;18:1379-83.
38. Peng NJ, Lai KH, Liu RS, et al. Capsule ^{13}C -urea breath test for the diagnosis of *Helicobacter pylori* infection. *World J Gastroenterol* 2005;11:1361-4.
39. Gatta L, Vakil N, Ricci C, et al. Effect of proton pump inhibitors and antacid therapy on ^{13}C urea breath tests and stool test for *Helicobacter pylori* infection. *Am J Gastroenterol* 2004;99:823-9.
40. Gatta L, Ricci C, Tampieri A, et al. Accuracy of breath tests using low doses of ^{13}C -urea to diagnose *Helicobacter pylori* infection: a randomised controlled trial. *Gut* 2006;55:457-62.
41. Yang YJ, Sheu BS, Lee SC, Wu JJ. More economic 25 mg (^{13}C) urea breath test can be effective in detecting primary *Helicobacter pylori* infection in children. *J Gastroenterol Hepatol* 2007;22:335-9.
42. Ortiz-Olvera Nayeli NX, Moran Villota S, Gallardo Wong L, Blancas Valencia JM, Cabrera Munoz L. [Validation of a simplified ^{13}C -urea breath test method for the diagnosis of *Helicobacter pylori* infection]. *Rev Esp Enferm Dig* 2007;99:392-7.
43. Campuzano-Maya G. An optimized ^{13}C -urea breath test for the diagnosis of *H pylori* infection. *World J Gastroenterol* 2007;13:5454-64.

176 182 182

 Laboratorio Clínico Hematológico	ACCIONES CORRECTIVAS y/o PREVENTIVAS		Fecha: <u>8 de febrero/2007</u>
ACCIÓN CORRECTIVA ☐ No. _____		ACCIÓN PREVENTIVA ☒ No. <u>10</u>	
CALIDAD: ☒	AMBIENTAL: ☐	S. & S.O.: ☐	Proceso: <u>Toma de muestras</u>
Acción Solicitada por: <u>Blanca E. Escobar</u>		Cargo: <u>Coordinadora del laboratorio</u>	
DESCRIPCIÓN DEL PROBLEMA: <u>El protocolo diseñado para la prueba de aliento para Helicobacter pylori contiene ácido cítrico y es la única disponible en el mercado (Ver informe anexo)</u>			
Se había presentado antes este tipo de problema ☒ Si ☐ No			
CAUSAS (S): <u>Mano de obra:</u> <u>Método: la usuaria ingiere ácido cítrico que hace parte de la prueba, el cual desencadena la complicación descrita en el formato anexo. Es frecuente la intolerancia del ácido cítrico produciendo vómito y dolor abdominal, especialmente en niños.</u> <u>Máquina:</u> <u>Medición:</u>			
ACCIÓN PROPUESTA PARA ELIMINAR LA CAUSA: <u>Buscar alternativas que eliminen el ácido cítrico de la prueba de aliento para Helicobacter pylori.</u>			
Fecha de Implementación: _____		Responsable de Ejecutar la Acción: <u>Dirección Científica</u>	
SEGUIMIENTO A LA EFICACIA DE LA ACCIÓN CORRECTIVA:			
LA ACCIÓN CORRECTIVA y/o PREVENTIVA ☒ Sigue Abierta: ☒ Se cierra: ☐			
Seguimiento Realizado Por: _____		Fecha Seguimiento: _____	

486
22
180

MATERIALS AND METHODS

Subjects

The study population included 70 volunteers with no gastrointestinal symptoms. The volunteers were informed about the study and the tests, and signed an informed consent in accordance with the Helsinki Declaration^[30]. The study was classified as a research study with no biological, physiological, psychological or social risks by the Health Ministry of Colombia^[31]. Because of the nature of the study in healthy volunteers, it was considered non-ethical to perform invasive tests such as biopsy, culture or endoscopy.

Protocols

Reference protocol: *H. pylori* infection status of individuals was determined by the ^{13}C -UBT, according to the European protocol described before^[29] and using commercial kits (TAU-KIT, Isomed SL, Madrid, Spain) that provide both a sensitivity and specificity close to 100%^[25]. This protocol was standardized and was validated for our region, with over 15 000 assays performed, and used as the gold standard. The ^{13}C -UBT was analyzed by means of continuous flow-isotope ratio mass spectrometry (ABCA, SerCon, Cheshire, UK) at the Laboratorio Clínico Hematológico® in Medellín, Colombia.

The reference protocol was performed as follows: After fasting for at least 8 h, individuals were given 4.2 g of citric acid dissolved in 200 mL of water. Ten minutes later, a duplicate basal breath sample was collected. Immediately after, individuals were given 100 mg of ^{13}C -urea dissolved in 125 mL of water. After 30 min, a duplicate post-urea breath sample was collected. Results over 2.5 delta-over-baseline (DOB) were considered positive for *H. pylori* infection.

Protocol 1: After fasting for at least 8 h, a first basal breath sample was collected. Individuals were given 50 mg of ^{13}C -urea (99%, Isotec, Miamisburg, Ohio, USA) dissolved in 10 mL of water, taken as a single swallow. Immediately after, individuals were given 200 mL of water (pH 6.0). Volunteers, with a final volume of 210 mL, were asked to make a circular motion around the waistline for a few times to homogenize the aqueous solution and allow contact of the ^{13}C -urea with the entire gastric mucosa. Additional breath samples were collected afterwards at 10, 20 and 30 min.

Protocol 2: Same as Protocol 1, except that 4.2 g of dehydrated citric acid were added to the 200 mL of water.

The performance of both protocols was analyzed at various cut-off values from 0.5 to 5.5, at the different time intervals (10, 20 and 30 min).

Statistical analysis

The χ^2 test was used to analyze associations between qualitative variables. For quantitative variables, the Wilcoxon's signed rank sum tests and Student's *t*-test were applied. Normality of the distribution of the data was assessed with the Wilk-Shapiro test. Sensitivity, specificity, positive predictive value, negative predictive value, accuracy,

Youden index, likelihood ratios for a positive (LR+ve) or negative (LR-ve) test were calculated against the defined gold standard. The effectiveness of each protocol was evaluated by ROC analysis. Processing and analysis of data were done with the SPSS (Statistical Product for Service Solutions) version 12.0 and EPIDATE Version 3.0. A value of $P < 0.05$ was considered statistically significant.

RESULTS

This study included 70 individuals, 24 (34.3%) males and 46 (65.7%) females, with an average age of 39.63 (SD $\pm$ 12.58) years for males and 34.33 (SD $\pm$ 10.17) years for females. There were no significant differences between the mean age for males and females ($P = 0.061$). According to the reference protocol, 46 (65.7%) individuals were positive for *H. pylori* infection and 24 (34.3%) were negative. When assessed by gender, 17 (70.8%) males and 29 (63%) females were positive for *H. pylori*; this association was not statistically significant ($P = 0.515$).

Table 1 shows the performance of the protocols in terms of sensitivity, specificity, accuracy, positive and negative predictive values, Youden index and likelihood ratios for a positive (LR+ve) or (LR-ve) test with the different DOB cut-off values at 10, 20 and 30 min. Only $\times$ Protocol 1 (with no test meal) achieved accuracy, sensitivity, specificity, positive and negative predictive values of 100%. The highest values achieved by Protocol 2 were 98.57%, 97.83%, 100%, 100% and 100%, respectively.

There were no significant differences in the ability of both protocols to correctly identify positive and negative *H. pylori* individuals at 10 ($P = 0.32$), 20 ($P = 0.32$) and 30 min ($P = 0.32$). These results were confirmed by ROC analysis (Figure 1). The areas under the ROC curves for both protocols were as follows: for Protocol 1, 1.0 at 10, 20 and 30 min; for Protocol 2, 0.9837 at 10 and 30 min, and 0.9873 at 20 min. Although these results were not statistically different, Protocol 1 shows the maximum optimal values for an assay.

Table 2 shows the distribution of the DOB values at 10, 20, 30 min for *H. pylori* positive and negative individuals for Protocols 1 and 2. For Protocol 1, the median DOB for *H. pylori* infected individuals at 10 min was 13.64, while for Protocol 2 was 12.02. There was no statistically significant difference between these 2 values (Wilcoxon, $P = 0.121$). In contrast, median DOB values at 20 and 30 min for both protocols showed significant differences ($P = 0.006$ and $P = 0.001$, respectively). In addition, for non-infected individuals there were no statistically significant differences in the median DOB values at 10, 20 and 30 min ($P = 0.710$, $P = 0.440$ and $P = 0.346$, respectively) between both protocols.

DISCUSSION

The ^{13}C -UBT has become the gold standard of the non-invasive tests for diagnosing *H. pylori* infection, before and after eradication treatment. Recently, The Maastricht III Consensus Report has recommended the ^{13}C -UBT as the best option to establish the diagnosis of *H. pylori* infection, especially in patients in whom endoscopy is not indicated^[22].

Table 1 Performance of protocols (P1 and P2) in terms of sensitivity, specificity, accuracy, positive and negative predictive values, Youden Index and likelihood ratios for a positive (LR+ve) or (LR-ve) test with the different DOB cut-off values at 10, 20 and 30 min

Time (min)	DOB	Sensitivity		Specificity		Accuracy		Positive predictive value		Negative predictive value		Youden Index		LR+ve		LR-ve	
		P1	P2	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2
10	0.5	100	97.83	45.83	62.5	81.43	85.71	77.97	83.33	100	93.75	0.46	0.6	1.85	2.61	1	0.03
	1.0	100	97.83	83.33	79.17	94.29	91.43	92	90.0	100	95	0.83	0.77	6.00	4.7	1	0.03
	1.5	100	97.83	95.83	95.83	98.57	97.14	97.87	97.83	100	95.83	0.96	0.94	24.00	23.48	1	0.02
	2.0	100	97.83	100	100	100	98.57	100	100	100	96	1.00	0.98	2	2	1	0.02
	2.5	100	95.65	100	100	100	97.14	100	100	100	92.31	1.00	0.96	2	2	1	0.04
	3.0	97.83	95.65	100	100	98.57	97.14	100	100	96.0	92.31	0.98	0.96	2	2	0.02	0.04
	3.5	97.83	95.65	100	100	98.57	97.14	100	100	96.0	92.31	0.98	0.96	2	2	0.02	0.04
	4.0	95.65	95.65	100	100	97.14	97.14	100	100	92.31	92.31	0.96	0.96	2	2	0.04	0.04
	4.5	93.48	95.65	100	100	95.71	97.14	100	100	88.89	92.31	0.93	0.96	2	2	0.07	0.04
	5.0	86.96	95.65	100	100	91.43	97.14	100	100	80	92.31	0.87	0.96	2	2	0.13	0.04
	5.5	84.78	95.65	100	100	90	97.14	100	100	77.42	92.31	0.85	0.96	2	2	0.15	0.04
20	0.5	100	97.83	66.67	62.5	88.57	85.71	85.19	83.33	100	93.75	0.67	0.6	3.00	2.61	1	0.03
	1.0	100	97.83	95.83	75	98.57	90	97.87	88.24	100	94.74	0.96	0.73	24.00	3.91	1	0.03
	1.5	100	97.83	100	83.33	100	92.86	100	91.84	100	95.24	1.00	0.81	2	5.87	1	0.03
	2.0	100	97.83	100	100	100	98.57	100	100	100	96	1.00	0.98	2	1	1	0.02
	2.5	100	95.65	100	100	100	97.14	100	100	100	92.31	1.00	0.96	2	1	1	0.04
	3.0	97.83	95.65	100	100	98.57	97.14	100	100	96	92.31	0.98	0.96	2	1	0.02	0.04
	3.5	95.65	95.65	100	100	97.14	97.14	100	100	92.31	92.31	0.96	0.96	2	1	0.04	0.04
	4.0	89.13	95.65	100	100	92.86	97.14	100	100	82.76	92.31	0.89	0.96	2	1	0.11	0.04
	4.5	84.78	95.65	100	100	90	97.14	100	100	77.42	92.31	0.85	0.96	2	1	0.15	0.04
	5.0	82.61	95.65	100	100	88.57	97.14	100	100	75	92.31	0.83	0.96	2	1	0.17	0.04
	5.5	82.61	93.48	100	100	88.57	95.71	100	100	75	88.89	0.83	0.93	2	1	0.17	0.07
30	0.5	100	97.83	54.17	45.83	84.29	80	80.7	77.59	100	91.67	0.54	0.44	2.18	1.81	1	0.05
	1.0	100	97.83	91.67	70.83	97.14	88.57	95.83	86.54	100	94.44	0.92	0.69	12.00	3.35	1	0.03
	1.5	100	97.83	100	83.33	100	92.86	100	91.84	100	95.24	1.00	0.81	2	5.87	1	0.03
	2.0	95.65	97.83	100	91.67	97.14	95.71	100	95.74	92.31	95.65	0.96	0.89	2	11.74	0.04	0.02
	2.5	91.3	97.83	100	100	94.29	98.57	100	100	85.71	96	0.91	0.98	2	2	0.09	0.02
	3.0	84.78	95.65	100	100	90	97.14	100	100	77.42	92.31	0.85	0.96	2	2	0.15	0.04
	3.5	82.61	95.65	100	100	88.57	97.14	100	100	75	92.31	0.83	0.96	2	2	0.17	0.04
	4.0	78.26	95.65	100	100	85.71	97.14	100	100	70.59	92.31	0.78	0.96	2	2	0.22	0.04
	4.5	78.26	95.65	100	100	85.71	97.14	100	100	70.59	92.31	0.78	0.96	2	2	0.22	0.04
	5.0	76.09	95.65	100	100	84.29	97.14	100	100	68.57	92.31	0.76	0.96	2	2	0.24	0.04
	5.5	71.74	93.48	100	100	81.43	95.71	100	100	64.86	88.89	0.72	0.93	2	2	0.28	0.07

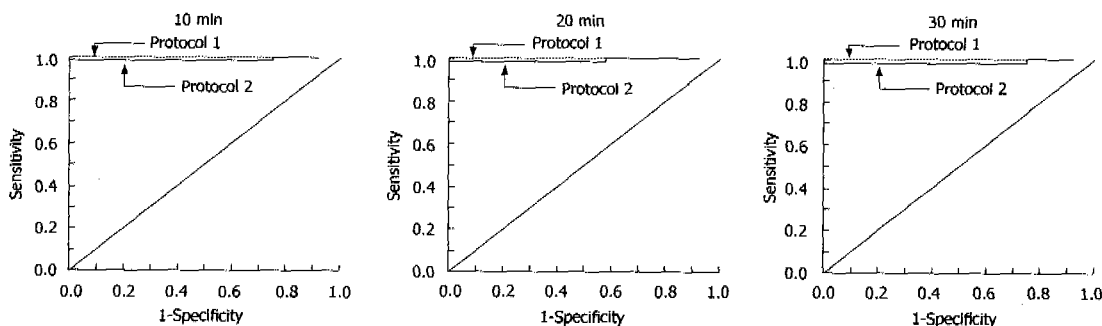
DOB: Delta-over-baseline; 1: = 0; 2: Φ +.**Figure 1** ROC curves for protocols 1 and 2 at 10, 20 and 30 min to establish the diagnosis of *H. pylori* infection.

Table 3 shows a selection of 40 studies from the literature where relevant variations to the original ^{13}C -UBT protocol^[28] have been implemented. From each study, the protocol with the best diagnostic performance was selected^[22-71]. Of these, 12 (30%) yielded sensitivities and specificities of 100%^[33,37,43,45,47,56,61,63,65,67-69].

Based on the results after reviewing the literature, the present study introduced several variations to simplify even further the technique and make it more cost-efficient,

without compromising the high standards of sensitivity, specificity, positive and negative predictive values of the test. Below is a brief review of the evolution of the assay, since its first description, which led to the designing of the protocols evaluated in the present study.

Urea dose

Originally, the ^{13}C -UBT was described with a dose of ^{13}C -urea of 5 mg/kg of bodyweight^[28]. Later on, doses of

Table 2 Distribution of DOB values in *H. pylori* positive and negative individuals for both protocols at 10, 20 and 30 min

<i>H. pylori</i> -positive individuals	10 min		20 min		30 min	
	Protocol 1	Protocol 2	Protocol 1	Protocol 2	Protocol 1	Protocol 2
Mean	17.38	17.69	18.25	22.32	15.44	22.08
Median	13.64	12.02	12.63	17.07	10.98	17.50
SD	14.47	12.68	22.80	15.01	17.75	13.00

<i>H. pylori</i> -negative individuals	10 min		20 min		30 min	
	Protocol 1	Protocol 2	Protocol 1	Protocol 2	Protocol 1	Protocol 2
Mean	0.32	0.33	0.11	0.45	0.21	0.62
Median	0.51	0.32	0.28	0.34	0.38	0.59
SD	0.91	0.8	0.77	0.86	0.84	0.91

DOB: Delta-over-baseline.

125^[35,72] and 100 mg^[36,40,47,48,51,54,57,60,62,65,73-83] were validated by several American, European and Asian groups, and more recently 75 mg^[32,37,43-45,49,50,55,72,84-93], 50 mg^[52,56,58,94,95], 38 mg^[96], 25 mg and even 10 mg^[69] of ^{13}C -urea have proved to be sufficient.

Test meal

From the beginning, there has been a belief that a delay in gastric emptying is necessary for optimal performance of the test, to allow enough time for the *H. pylori*-urease to react in the gastric mucosa, if present. Initially individuals were given a meal consisting of one can of "Sustacal" pudding or 120 mL of 25% glucose polymer, followed 10 min later by a polycose solution containing the ^{13}C -urea^[28]. Through the years there have been numerous modifications to the test meal, including the use of citric acid alone before administering the urea^[37,62,67,79,84,85,97], or mixed with the ^{13}C -urea at the time of administration^[43-45,88,91,98], or as a presentation in combination with the ^{13}C -urea^[42,61,72,94,99]. Several alternatives to citric acid have also been tested, including orange juice^[43,49,67,90,95] and apple juice^[100], as well as other types of food such as milk^[48,58,101,102] and a pudding test meal^[54,46], and even water^[41,103]. As shown in Table 3, the majority of the test meals have provided reliable results. Even the complete absence of a test meal has shown little, if any, variation in the diagnostic performance of the assay^[35,40,47,53,56,59,60,102,104].

Via of administration and formulation of ^{13}C -urea

Another issue that has been addressed by different groups is the interference of other urease-producing bacteria in the oral cavity and oropharynx^[105,106], leading to an increase in false positive values. As a result, there have been different approaches in the formulation and way of administration of the labeled urea, including the development of ^{13}C -urea tablets^[42,61,63,64,95], capsules^[68,96,99,107], and even the intragastric instillation of the urea through the endoscope^[80,108,109]. Some have also suggested mouth rinsing before and after urea administration^[39,41,48,51,52,54,58,60,68,110].

Sample collection times

The ^{13}C -UBT was originally described with a basal sample and 18 post-urea samples taken during the following

180 min^[28]. Rapidly the assay was modified and currently only 2 samples are obtained: pre and post-urea. Sampling times, although shorter than initially, have differed among protocols.

Ways of reducing the cost of the ^{13}C -UBT could include decreasing the amount of ^{13}C -urea used, reducing the duration of the test, and improving the ease with which the test can be administered and tolerated. The conventional European ^{13}C -UTB protocol used in our region is sensitive and specific enough (values close to 100%), but it takes 40-45 min to complete and is performed using 100 mg of ^{13}C -urea. For the present study we decided to use 50 mg of ^{13}C -urea to reduce the cost of the assays by half, a dose that has proved to be as accurate as higher doses^[52,56,58,61,63,68]. The ^{13}C -urea was administered diluted in 10 mL of water and taken as a single swallow, to try to avoid cross-contamination with urease-producing oropharyngeal bacteria. Immediately after 200 mL of water (pH 6.0) with 4.2 g of citric acid (Protocol 2) and without citric acid (Protocol 1) were administered, and volunteers were asked to make a circular motion around the waistline for a few times to homogenize the aqueous solution and allow contact of the ^{13}C -urea with the entire gastric mucosa. It has been shown that *H. pylori* urease operates in a pH range from 3.1 to 10, with an optimal activity at pH 6.0^[111,112]. By utilizing water at pH 6.0, activity of the *H. pylori* urease was optimized for Protocol 1, where no citric acid was used. Acid solutions have been used by many to delay gastric emptying and to provide a higher acidic environment to induce *H. pylori*-urease activity^[43,98], although it has been demonstrated by Pantoflickova *et al.*^[100] that the emptying is determined by the caloric density of the test meal rather than by its pH. Finally, in order to reduce the duration of the test, both protocols were tested at different sampling times: 10, 20 and 30 min.

This study included 70 individuals, 34.3% males and 65.7% females, with an average age of 39.63 ± 12.58 years for males and 34.33 ± 10.17 years for females. According to the reference protocol, 46 (65.7%) individuals were positive for *H. pylori* infection. No statistically significant association was found between gender and presence of *H. pylori* infection ($P = 0.515$).

There were no significant differences in the ability of both protocols to correctly identify positive and negative *H. pylori* individuals at the various sampling times. However, only Protocol 1, with no test meal, yielded a test with sensitivity, specificity, positive and negative predictive values, and accuracy of 100% when compared to the gold standard, when using a DOB cut-off value between 2 and 2.5 at 10 and 20 min, and a DOB cut-off value of 1.5 at 20 and 30 min. For Protocol 2, with citric acid, the highest accuracy (98.57%) was achieved at 10 min using a DOB cut-off value of 2.0, at 20 min a DOB cut-off value of 2.0, and at 30 min with a DOB of 2.5.

Median DOB for *H. pylori* infected individuals at 10 min was 13.64, while for Protocol 2 was 12.02. There was no statistically significant difference between these 2 values (Wilcoxon, $P = 0.121$). However, median DOB values at 20 and 30 min for both protocols showed significant differences ($P = 0.006$ and $P = 0.001$, respectively). These results are in accordance with those by Atherton *et al.*^[113]

Table 3 ^{13}C -UBT protocol with best diagnostic performance from each study with samples obtained within 30 min of ^{13}C -urea administration: Review of literature

First author (reference)	Year	Measuring equipment	Gold standard	n	Pre-analytical	^{13}C -urea dose (mg)	^{13}C -urea formulation and via of administration	Test meal	Additional information related to ^{13}C -urea administration	r	Cut-off point (DOB)	Sens. (%)	Spec. (%)	PPV (%)	NPV (%)	Acc. (%)
Braden ^[32]	1994	IRMS	^{13}C -UBT	217	Overnight fasting	75	NA	None		20	5	99	100			
Koletzko ^[33]	1995	IRMS, NDIRS	^{13}C -UBT	51	Overnight fasting	75	Powder in 150 mL 0.033 mol/L citric acid solution	Taken with ^{13}C -urea		15	5	100	100			
Klein ^[34]	1996	IRMS	H	465	NA	125	Powder in 90 mL sterile water (Meretek kit)	Ensure		30	2.4	95.4	87.9			94.8
Malaty ^[35]	1996	IRMS	H, RUT, C	66	Overnight fasting	125	Powder in 100 mL water	None		20	2.4	96	100			
Taniguchi ^[36]	1996	NIRS	H	153	Overnight fasting	100	Powder in 30 mL water	None		15	1	97.8	74			
Dominguez-Muñoz ^[37]	1997	IRMS	H, RUT, C	80	Overnight fasting	80	Powder in 50 mL water	200 mL 0.1 mol/L citric acid solution		30	4	100	100			
Epple ^[38]	1997	IRMS	H	77	Overnight fasting	75	NA	Citric acid		30	1.3	96	100			
Kato ^[39]	1998	IRMS	H, C, RUT	133	Overnight fasting	100	Powder in 100 mL of water	None	Mouth rinsing after ^{13}C -urea	10	3.5	99	100			
Miwa ^[40]	1998	IRMS	H	409	8 h fasting	100	Powder	None	Mouth rinsing before and after ^{13}C -urea	20	5	97	97			
Ohara ^[41]	1998	IRMS	H, RUT, C	248	Overnight fasting	100	Powder in 100 mL tap water	None	Mouth rinsing after ^{13}C -urea	20	2.5	98	98			98
Hamlet ^[42]	1999	IRMS	^{13}C -UBT, H, RUT, C	134	Overnight fasting	100	Two tablets (Diabact UBT) with 50 mg of ^{13}C -urea + 456 mg of anhydrous citric acid swallowed with 200 mL of water	Taken with ^{13}C -urea		10	1.8	95	100			
Leodolter ^[43]	1999	IRMS	H, RUT, C	50	NA	75	Powder in 200 mL 0.1 mol/L citric acid solution	Taken with ^{13}C -urea		30	4	100	100			
Leodolter ^[44]	1999	IRMS	H, RUT, C	233	NA	75	Powder in 200 mL citric acid solution	Taken with ^{13}C -urea		30	4	95	98			97
Savarino ^[45]	1999	IRMS	H, RUT	134	Overnight fasting	75	Powder in 150 mL 0.033 mol/L citric acid solution	Taken with ^{13}C -urea		30	5	100	100			
Van der Hulst ^[46]	1999	LARA	H, C	544	NA	100	Powder in 50 mL sterile water	Ensure		30	6.3-7.5	93-95	94-96	95-98	86-94	
Gisbert ^[47]	2000	IRMS	^{13}C -UBT, H	53	Overnight fasting	100	Powder in 50 mL water (TAU-KIT)	None		30	3.3-3.9	100	100			
Peng ^[48]	2000	IRMS	H, RUT, C	136	6 h fasting	100	Powder in 50 mL sterile water	100 mL milk	Mouth rinsing after ^{13}C -urea and laid on their sides, changing sides every 5 min	15	4.8	94	89			
Riepl ^[49]	2000	NIRS	H, C	100	Overnight fasting	75	Powder in 200 mL orange juice	Taken with ^{13}C -urea		15	6.5	92	94	89	94	

183

APC
26
184

Savarino ^[59]	2000	IRMS	H, RUT	117	Overnight fasting	75	Powder in 150 mL 0.033 mol/L citric acid solution	Taken with ¹³ C-urea	30	5	98	97	98	97	98	
Sheu ^[61]	2000	IRMS	H, C	441	Overnight fasting	100	NA	100 mL of fatty test meal	Mouth rinsing before and after ¹³ C-urea	15	4	98	97			
Sheu ^[62]	2000	IRMS, NDIRS	H, C	177	Overnight fasting	50	NA	100 mL citric acid solution	Mouth rinsing after ¹³ C-urea	15	3.5	96	99	99	97	
Wong ^[63]	2000	IRMS	H, RUT	202	Overnight fasting	75	Powder in 50 mL distilled water	2.4 g of citric acid		30	5	96	98	98	96	97
Yoshida ^[64]	2000	LARA	H, C, PCR	104	Overnight fasting	100	Powder in 50 mL distilled water	None	Mouth rinsing after ¹³ C-urea	20	2.7	98	100			99
Mana ^[65]	2001	NDIRS	H	223	Overnight fasting	75	Powder	20 mL 0.1 mol/L citric acid solution		10		100	95	94	100	
Wong ^[66]	2001	IRMS	H, RUT	101	Overnight fasting	75	Powder in 50 mL water	None		30	3.5-4.5	100	100			100
Chua ^[67]	2002	IRMS	H, RUT, S	100	NA	100	Powder in solution containing citric acid and ¹³ C-urea		Patients laid on their left side for 30 min	30	3.5	94	100	100	89	
Liao ^[68]	2002	IRMS	H, RUT	152	Overnight fasting	50	Powder in 50 mL sterile water	200 mL full-cream cow's milk	Patients gargled with water 3 times after ¹³ C-urea and laid on their sides, changing sides every 3 min	15	2.5-3.0	99	97	99	97	99
Ng ^[69]	2002	IRMS	H, RUT	123	Regular meal within 2 h of the ¹³ C-urea	75	Powder in 50 mL water	2.4 g citric acid in 200 mL solution		30	5.5	93	97	100	97	
Chen ^[60]	2003	NDIRS	H, RUT, C, SAT	586	Overnight fasting	100	Powder in 100 mL of water	None	Patients gargled with water 3 times after ¹³ C-urea and laid down on the left side for 5 min	20	3.5	98	97			98
Gatta ^[61]	2003	IRMS	H, RUT, C	200	Overnight fasting	50	Tablet (Diabact UBT) with 50 mg of ¹³ C-urea and 456 mg of anhydrous citric acid swallowed with 200 mL of water	Citric acid		10	1.65-3.15	100	100			
Gisbert ^[62]	2003	IRMS	H, RUT	36	Overnight fasting	100	Powder in 50 mL water (TAU-KIT)	200 mL solution with 4.2 g citric acid		30	5	96	100	100	91	
Wong ^[63]	2003	IRMS	H, RUT	150	Overnight fasting	50	Tablet (Diabact UBT) with 50 mg of ¹³ C-urea and 456 mg of anhydrous citric acid swallowed with 200 mL of water	Citric acid		20	2.1	100	100			
Ohara ^[64]	2004	IRMS	¹³ C-UBT, H, C, RUT	254	Overnight fasting	100	Film-coated tablet swallowed with 100 mL of water	None		20	2.5	98	98			98

Urita ^[60]	2004	IRMS	H, S	129 Overnight fasting	100	Powder in 100 mL tap water	None	Sample taken 20 through nostril	2.5	100	100					
Beiki ^[66]	2005	NDIRS	¹⁴ C-UBT, H, RUT	76 Overnight fasting	75	Powder in 200 mL orange juice			30	3.5	100	97	98	100	99	
Kopacova ^[67]	2005	IRMS	¹³ C-UBT	27 Overnight fasting	100	Powder in 50 mL distilled water with 1 g citric acid	150 mL distilled water with 3 g citric acid, orange juice or distilled water		10	3.5	100	100				100
Peng ^[68]	2005	IRMS	H, RUT, C	50.6 h fasting	100	Capsule with water	None	Mouth rinsing before and after ¹³ C-urea and laid on their sides, changing sides every 5 min	15	4-5	100	100				100
Gatta ^[69]	2006	IRMS	H, RUT	100 Overnight fasting	25	Dissolved in water	Citric acid (1 g)		30	4.4-6.26	100	100				
Mauro ^[70]	2006	IRMS	H, C	176 Overnight fasting	75	Powder in 100 mL citric acid solution	Taken with ¹³ C-urea		30	3	100	99	95-98	100		
Mauro ^[71]	2006	IRMS	H, C	67 Overnight fasting	75	Powder in 100 mL citric acid solution	Taken with ¹³ C-urea		10	3	100	96	95-98	99-100		
Present study	2007	IRMS	¹³ C-UBT	70 Overnight fasting	50	Powder in 10 mL sterile water immediately followed by 200 mL sterile water	None	Patients made a circular motion around the waistline for a few times	10	2.0-2.5	100	100	100	100	100	100

n: Participating individuals; t: Sampling time; PPV: Positive predictive value; NPV: Negative predictive value; Acc: Accuracy; UBT: Urea breath test; H: histology; C: Culture; RUT: Rapid urease test; S: Serology; NA: Not available; IRMS: Isotope ratio mass spectrometry; NDIRS: Non-dispersive infrared spectrometry; LARA: Laser assisted ratio analyser; DOB: Delta-over-baseline.

and Gisbert *et al*^[17], who showed that the test meal did not affect ¹³C-UBT results at 10 min, but increased the values thereafter.

In conclusion, an optimal laboratory test should be non-invasive, easy to perform, highly reproducible, cost-efficient and with a sensitivity and specificity close to 100%. When compared to other protocols published in the literature, the present conditions of Protocol 1 have further optimized the ¹³C-UBT assay, as this is the only protocol with a sampling time of 10 min, a ¹³C-urea dose of 50 mg and no test meal that can yield a test with 100% accuracy for the diagnosis of *H. pylori* infection. These variations provide a protocol that can reduce the cost of the ¹³C-UBT assay, is innocuous, well tolerated, has no restrictions and could be implemented for all patients in whom endoscopy is not an indication^[21,22] and as a screening test for *H. pylori* epidemiological studies. Further studies are underway to try to decrease the ¹³C-urea to an even lower dose, using biopsy as the gold standard.

ACKNOWLEDGMENTS

To all volunteers that willingly participated in the study and to the medical technologists at Laboratorio Clínico Hematológico, in particular to Gloria Elsy Escobar-Gallo

and Luz Marina Valencia-Zuluaga. To Victor Calvo-Betancur for his assistance with the statistical analysis. To Ana I. Toro, for her insightful discussions and help with the English translation.

COMMENTS

Background

H. pylori infection is present in around 50% of the world population and has been associated with the pathogenesis of gastric disorders such as gastritis, gastric ulcer and MALT lymphoma, and a variety of extradigestive diseases, including idiopathic thrombocytopenic purpura, iron deficiency anemia and autoimmune thyroiditis, among others. Diagnosis of *H. pylori* infection can be established by either invasive techniques, by means of endoscopy, or non-invasive techniques such as the ¹³C-urea breath test.

Research frontiers

The ¹³C-urea breath test relies upon the ability of an enzyme (urease), produced by *H. pylori* in the stomach, to break down the administered urea. Patients swallow the urea labelled with a non-radioactive isotope (¹³C). After a few minutes, the isotope-labelled carbon dioxide (¹³CO₂) is exhaled in the breath if there is presence of *H. pylori* urease in the stomach. The difference in the ¹³CO₂ values before and after ingestion of the labelled urea will determine the presence of infection.

Innovations and breakthroughs

Many have attempted to lower the high cost of the ¹³C-urea breath test, while preserving excellent diagnostic accuracy. For this purpose, modifications in the

186

dose, formulation and way of administration, sample collection times and test meals have been evaluated.

Applications

A low cost ¹³C-urea breath test for the detection of *H pylori* infection before and after eradication treatment will make this non-invasive assay more accessible for patients, especially in developing countries.

Terminology

¹³C-UBT: Breath test that includes urea labelled with ¹³C, a non-radioactive isotope. DOB: Delta over base line, units used to express the amount of ¹³CO₂ contained in the breath sample.

Peer review

This is a well written and comprehensively referenced article. The methods section is adequately described and the results clearly presented. The conclusions are a fair interpretation of the results.

REFERENCES

- 1 Crowe SE. Helicobacter infection, chronic inflammation, and the development of malignancy. *Curr Opin Gastroenterol* 2005; **21**: 32-38
- 2 Weaver LT. Royal Society of Tropical Medicine and Hygiene Meeting at Manson House, London, 16 February 1995. Aspects of Helicobacter pylori infection in the developing and developed world. Helicobacter pylori infection, nutrition and growth of West African infants. *Trans R Soc Trop Med Hyg* 1995; **89**: 347-350
- 3 Suzuki H, Marshall BJ, Hibi T. Overview: Helicobacter pylori and extragastric disease. *Int J Hematol* 2006; **84**: 291-300
- 4 DuBois S, Kearney DJ. Iron-deficiency anemia and Helicobacter pylori infection: a review of the evidence. *Am J Gastroenterol* 2005; **100**: 453-459
- 5 Kaptan K, Beyan C, Ural AU, Cetin T, Avcu F, Gulsen M, Finci R, Yalcin A. Helicobacter pylori—is it a novel causative agent in Vitamin B12 deficiency? *Arch Intern Med* 2000; **160**: 1349-1353
- 6 Gupta V, Eden AJ, Mills MJ. Helicobacter pylori and autoimmune neutropenia. *Clin Lab Haematol* 2002; **24**: 183-185
- 7 Cecchi R, Torelli E. Schonlein-Henoch purpura in association with duodenal ulcer and gastric Helicobacter pylori infection. *J Dermatol* 1998; **25**: 482-484
- 8 Franchini M. Thrombotic thrombocytopenic purpura: proposal of a new pathogenic mechanism involving Helicobacter pylori infection. *Med Hypotheses* 2005; **65**: 1128-1131
- 9 Campuzano-Maya G. Proof of an association between Helicobacter pylori and idiopathic thrombocytopenic purpura in Latin America. *Helicobacter* 2007; **12**: 265-273
- 10 Zentilin P, Serio B, Dulbecco P, Caratto E, Iritano E, Fasciolo D, Bilardi C, Mansi C, Testa E, Savarino V. Eradication of Helicobacter pylori may reduce disease severity in rheumatoid arthritis. *Aliment Pharmacol Ther* 2002; **16**: 1291-1299
- 11 El Miedany YM, Baddour M, Ahmed I, Fahmy H. Sjogren's syndrome: concomitant H. pylori infection and possible correlation with clinical parameters. *Joint Bone Spine* 2005; **72**: 135-141
- 12 de Luis DA, Varela C, de La Calle H, Canton R, de Argila CM, San Roman AL, Boixeda D. Helicobacter pylori infection is markedly increased in patients with autoimmune atrophic thyroiditis. *J Clin Gastroenterol* 1998; **26**: 259-263
- 13 Boixeda de Miquel D, Vazquez Romero M, Vazquez Sequeiros E, Foruny Olcina JR, Boixeda de Miquel P, Lopez San Roman A, Aleman Villanueva S, Martin de Argila de Prados C. Effect of Helicobacter pylori eradication therapy in rosacea patients. *Rev Esp Enferm Dig* 2006; **98**: 501-509
- 14 Federman DG, Kirsner RS, Moriarty JP, Concato J. The effect of antibiotic therapy for patients infected with Helicobacter pylori who have chronic urticaria. *J Am Acad Dermatol* 2003; **49**: 861-864
- 15 Mendall MA, Goggin PM, Molineaux N, Levy J, Toosy T, Strachan D, Camm AJ, Northfield TC. Relation of Helicobacter pylori infection and coronary heart disease. *Br Heart J* 1994; **71**: 437-439
- 16 Kowalski M, Pawlik M, Konturek JW, Konturek SJ. Helicobacter pylori infection in coronary artery disease. *J Physiol Pharmacol* 2006; **57** Suppl 3: 101-111
- 17 Bytzer P. Cost-effectiveness of gastroscopy. *Ital J Gastroenterol Hepatol* 1999; **31**: 749-760
- 18 Nelson DB. Infectious disease complications of GI endoscopy: Part I, endogenous infections. *Gastrointest Endosc* 2003; **57**: 546-556
- 19 Nelson DB. Infectious disease complications of GI endoscopy: part II, exogenous infections. *Gastrointest Endosc* 2003; **57**: 695-711
- 20 McCloy R. Asleep on the job: sedation and monitoring during endoscopy. *Scand J Gastroenterol Suppl* 1992; **192**: 97-101
- 21 Talley NJ, Vakil N. Guidelines for the management of dyspepsia. *Am J Gastroenterol* 2005; **100**: 2324-2337
- 22 Malfertheiner P, Megraud F, O'Morain C, Bazzoli F, El-Omar E, Graham D, Hunt R, Rokkas T, Vakil N, Kuipers EJ. Current concepts in the management of Helicobacter pylori infection: the Maastricht III Consensus Report. *Gut* 2007; **56**: 772-781
- 23 Thijs JC, van Zwet AA, Thijs WJ, Oey HB, Karrenbeld A, Stellaard F, Luijt DS, Meyer BC, Kleibeuker JH. Diagnostic tests for Helicobacter pylori: a prospective evaluation of their accuracy, without selecting a single test as the gold standard. *Am J Gastroenterol* 1996; **91**: 2125-2129
- 24 Logan RP. Urea breath tests in the management of Helicobacter pylori infection. *Gut* 1998; **43** Suppl 1: S47-S50
- 25 Gisbert JP, Pajares JM. Review article: C-urea breath test in the diagnosis of Helicobacter pylori infection — a critical review. *Aliment Pharmacol Ther* 2004; **20**: 1001-1017
- 26 Gisbert JP, Pajares JM. Stool antigen test for the diagnosis of Helicobacter pylori infection: a systematic review. *Helicobacter* 2004; **9**: 347-368
- 27 Fry LC, Curioso WH, Rickes S, Horton G, Hirschowitz BI, Monkemuller K. Comparison of 13C- urea blood test to 13C-breath test and rapid urcase test for the diagnosis of Helicobacter pylori infection. *Acta Gastroenterol Latinoam* 2005; **35**: 225-229
- 28 Graham DY, Klein PD, Evans DJ Jr, Evans DG, Alpert LC, Opekun AR, Boutton TW. Campylobacter pylori detected noninvasively by the 13C-urea breath test. *Lancet* 1987; **1**: 1174-1177
- 29 Logan RP, Dill S, Bauer FE, Walker MM, Hirschl AM, Gummert PA, Good D, Mossi S. The European 13C-urea breath test for the detection of Helicobacter pylori. *Eur J Gastroenterol Hepatol* 1991; **3**: 915-921
- 30 World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. *J Int Bioethique* 2004; **15**: 124-129
- 31 República de Colombia, Salud Md. Resolución No. 008430 de 1993 (4 de octubre de 1993), por la cual se establecen las normas científicas, técnicas y administrativas para la investigación en salud. Available from: URL: <http://www.unal.edu.co/dib/normas/etica.html>
- 32 Braden B, Duan LP, Caspary WF, Lembcke B. More convenient 13C-urea breath test modifications still meet the criteria for valid diagnosis of Helicobacter pylori infection. *Z Gastroenterol* 1994; **32**: 198-202
- 33 Koletzko S, Haisch M, Seeboth I, Braden B, Hengels K, Koletzko B, Hering P. Isotope-selective non-dispersive infrared spectrometry for detection of Helicobacter pylori infection with 13C-urea breath test. *Lancet* 1995; **345**: 961-962
- 34 Klein PD, Malaty HM, Martin RF, Graham KS, Genta RM, Graham DY. Noninvasive detection of Helicobacter pylori infection in clinical practice: the 13C urea breath test. *Am J Gastroenterol* 1996; **91**: 690-694
- 35 Malaty HM, el-Zimaity HM, Genta RM, Klein PD, Graham DY. Twenty-minute fasting version of the US 13C-urea breath test for the diagnosis of H. pylori infection. *Helicobacter* 1996; **1**: 165-167
- 36 Taniguchi Y, Kimura K, Sohara H, Shirasaki A, Kawada H, Satoh K, Kihira K, Wang XM, Takimoto T, Goto Y, Takatori K,

- Iida K, Kajiwara M. Simple 13C-urea breath test with infra-red spectrophotometer. *J Gastroenterol* 1996; **31** Suppl 9: 37-40
- 37 **Dominguez-Munoz JE**, Leodolter A, Sauerbruch T, Malfertheiner P. A citric acid solution is an optimal test drink in the 13C-urea breath test for the diagnosis of *Helicobacter pylori* infection. *Gut* 1997; **40**: 459-462
 - 38 **Epple HJ**, Kirstein FW, Bojarski C, Frege J, Fromm M, Riecken EO, Schulzke JD. 13C-urea breath test in *Helicobacter pylori* diagnosis and eradication. Correlation to histology, origin of 'false' results, and influence of food intake. *Scand J Gastroenterol* 1997; **32**: 308-314
 - 39 **Kato M**, Asaka M, Kudo T, Katagiri M, Komatsu Y, Sato F, Sukegawa M, Kobayashi T, Kagaya H, Nishikawa K, Kudo M, Hokari K, Hige S, Watanabe M, Takeda H, Sugiyama T. Ten minute 13C-urea breath test for the diagnosis of *Helicobacter pylori* infection. *J Gastroenterol Hepatol* 1998; **33** Suppl 10: 40-43
 - 40 **Miwa H**, Murai T, Ohkura R, Nagahara A, Watanabe H, Terai T, Watanabe S, Sato N. Usefulness of the [13C]-urea breath test for detection of *Helicobacter pylori* infection in fasting patients. *J Gastroenterol Hepatol* 1998; **13**: 1039-1043
 - 41 **Ohara S**, Kato M, Asaka M, Toyota T. Studies of 13C-urea breath test for diagnosis of *Helicobacter pylori* infection in Japan. *J Gastroenterol* 1998; **33**: 6-13
 - 42 **Hamlet A**, Stage L, Torroth H, Cahlin C, Nystrom C, Pettersson A. A novel tablet-based 13C urea breath test for *Helicobacter pylori* with enhanced performance during acid suppression therapy. *Scand J Gastroenterol* 1999; **34**: 367-374
 - 43 **Leodolter A**, Dominguez-Munoz JE, Von Arnim U, Malfertheiner P. Citric acid or orange juice for the 13C-urea breath test: the impact of pH and gastric emptying. *Aliment Pharmacol Ther* 1999; **13**: 1057-1062
 - 44 **Leodolter A**, Dominguez-Munoz JE, von Arnim U, Kahl S, Peitz U, Malfertheiner P. Validity of a modified 13C-urea breath test for pre- and posttreatment diagnosis of *Helicobacter pylori* infection in the routine clinical setting. *Am J Gastroenterol* 1999; **94**: 2100-2104
 - 45 **Savarino V**, Mela GS, Zentilin P, Bisso G, Pivari M, Mansi C, Mele MR, Bilardi C, Vigneri S, Celle G. Comparison of isotope ratio mass spectrometry and nondispersive isotope-selective infrared spectroscopy for 13C-urea breath test. *Am J Gastroenterol* 1999; **94**: 1203-1208
 - 46 **Van Der Hulst RW**, Lamouliatte H, Megraud F, Pounder RE, Stolte M, Vaira D, Williams M, Tytgat GN. Laser assisted ratio analyser 13C-urea breath testing, for the detection of *H. pylori*: A prospective diagnostic European multicentre study. *Aliment Pharmacol Ther* 1999; **13**: 1171-1177
 - 47 **Gisbert JP**, Vazquez MA, Jimenez I, Cruzado AI, Carpio D, Del Castillo E, Martin MJ, Morales A, Pajares R, Rodriguez A, Pajares JM. 13C-urea breath test for the diagnosis of *Helicobacter pylori* infection before treatment: is citric acid necessary? *Dig Liver Dis* 2000; **32**: 20-24
 - 48 **Peng NJ**, Hsu PI, Lee SC, Tseng HH, Huang WK, Tsay DG, Ger LP, Lo GH, Lin CK, Tsai CC, Lai KH. A 15-minute [13C]-urea breath test for the diagnosis of *Helicobacter pylori* infection in patients with non-ulcer dyspepsia. *J Gastroenterol Hepatol* 2000; **15**: 284-289
 - 49 **Riepl RL**, Folwaczny C, Otto B, Klausner A, Blendinger C, Wiebecke B, Konig A, Lehnert P, Heldwein W. Accuracy of 13C-urea breath test in clinical use for diagnosis of *Helicobacter pylori* infection. *Z Gastroenterol* 2000; **38**: 13-19
 - 50 **Savarino V**, Landi F, Dulbecco P, Ricci C, Tessieri L, Biagini R, Gatta L, Miglioli M, Celle G, Vaira D. Isotope ratio mass spectrometry (IRMS) versus laser-assisted ratio analyzer (LARA): a comparative study using two doses of. *Dig Dis Sci* 2000; **45**: 2168-2174
 - 51 **Sheu BS**, Lee SC, Yang HB, Kuo AW, Wang YL, Shiesh SC, Wu JJ, Lin XZ. Selection of lower cutoff point of [13C]urea breath test is helpful to monitor *H. pylori* eradication after proton pump inhibitor-based triple therapy. *Dig Dis Sci* 2000; **45**: 1330-1336
 - 52 **Sheu BS**, Lee SC, Yang HB, Wu HW, Wu CS, Lin XZ, Wu JJ. Lower-dose (13)C-urea breath test to detect *Helicobacter pylori* infection-comparison between infrared spectrometer and mass spectrometry analysis. *Aliment Pharmacol Ther* 2000; **14**: 1359-1363
 - 53 **Wong WM**, Wong BC, Wong KW, Fung FM, Lai KC, Hu WH, Yuen ST, Leung SY, Lau GK, Lai CL, Chan CK, Go R, Lam SK. (13)C-urea breath test without a test meal is highly accurate for the detection of *Helicobacter pylori* infection in Chinese. *Aliment Pharmacol Ther* 2000; **14**: 1353-1358
 - 54 **Yoshida H**, Hirota K, Ogura K, Maeda S, Shiratori Y, Sasaki Y, Omata M. Determination of the optimal cut-off value for the [13C]-urea breath test based on a *Helicobacter pylori*-specific polymerase chain reaction assay. *J Gastroenterol Hepatol* 2000; **15**: 155-160
 - 55 **Mana F**, Franken PR, Ham HR, Urbain D. Cut-off point, timing and pitfalls of the 13C-urea breath test as measured by infrared spectrometry. *Dig Liver Dis* 2001; **33**: 30-35
 - 56 **Wong WM**, Wong BC, Li TM, Wong KW, Cheung KL, Fung FM, Xia HH, Lam SK. Twenty-minute 50 mg 13C-urea breath test without test meal for the diagnosis of *Helicobacter pylori* infection in Chinese. *Aliment Pharmacol Ther* 2001; **15**: 1499-1504
 - 57 **Chua TS**, Fock KM, Teo EK, Ng TM. Validation of 13C-urea breath test for the diagnosis of *Helicobacter pylori* infection in the Singapore population. *Singapore Med J* 2002; **43**: 408-411
 - 58 **Liao CC**, Lee CL, Chiang TC, Lee SC, Huang SH, Tu TC, Chen TK, Wu CH. The 13C-urea breath test to detect *Helicobacter pylori* infection: a validated simple methodology with 50 mg 13C-urea. *Aliment Pharmacol Ther* 2002; **16**: 787-792
 - 59 **Ng FH**, Lai KC, Wong BC, Wong WM, Wong SY, Chow KC, Yuen ST, Leung SY, Lam SK. [13C]-urea breath test without prior fasting and without test meal is accurate for the detection of *Helicobacter pylori* infection in Chinese. *J Gastroenterol Hepatol* 2002; **17**: 834-838
 - 60 **Chen TS**, Chang FY, Chen PC, Huang TW, Ou JT, Tsai MH, Wu MS, Lin JT. Simplified 13C-urea breath test with a new infrared spectrometer for diagnosis of *Helicobacter pylori* infection. *J Gastroenterol Hepatol* 2003; **18**: 1237-1243
 - 61 **Gatta L**, Vakil N, Ricci C, Osborn JF, Tampieri A, Perna F, Miglioli M, Vaira D. A rapid, low-dose, 13C-urea tablet for the detection of *Helicobacter pylori* infection before and after treatment. *Aliment Pharmacol Ther* 2003; **17**: 793-798
 - 62 **Gisbert JP**, Ducons J, Gomollon F, Dominguez-Munoz JE, Borda F, Mino G, Jimenez I, Vazquez MA, Santolaria S, Gallego S, Iglesias J, Pastor G, Hervas A, Pajares JM. Validation of the 13C-urea breath test for the initial diagnosis of *Helicobacter pylori* infection and to confirm eradication after treatment. *Rev Esp Enferm Dig* 2003; **95**: 121-126, 115-120
 - 63 **Wong WM**, Lam SK, Lai KC, Chu KM, Xia HH, Wong KW, Cheung KL, Lin SK, Wong BC. A rapid-release 50-mg tablet-based 13C-urea breath test for the diagnosis of *Helicobacter pylori* infection. *Aliment Pharmacol Ther* 2003; **17**: 253-257
 - 64 **Ohara S**, Kato M, Saito M, Fukuda S, Kato C, Hamada S, Nagashima R, Obara K, Suzuki M, Honda H, Asaka M, Toyota T. Comparison between a new 13C-urea breath test, using a film-coated tablet, and the conventional 13C-urea breath test for the detection of *Helicobacter pylori* infection. *J Gastroenterol* 2004; **39**: 621-628
 - 65 **Urita Y**, Hike K, Torii N, Kikuchi Y, Kanda E, Kurakata H, Sasajima M, Miki K. Breath sample collection through the nostril reduces false-positive results of 13C-urea breath test for the diagnosis of *Helicobacter pylori* infection. *Dig Liver Dis* 2004; **36**: 661-665
 - 66 **Beiki D**, Khalaj A, Dowlatabadi R, Eftekhari M, Al-Sayed Hossein MH, Fard A, Fallahi B, Khoshayand MR. Validation of 13C-urea breath test with non dispersive isotope selective infrared spectroscopy for the diagnosis of *Helicobacter pylori* infection: a survey in Iranian population. *DARU* 2005; **13**: 52-55
 - 67 **Kopacova M**, Bures J, Vorisek V, Konstacky M, Rejchrt S, Zivny P, Douda J, Palicka V. Comparison of different protocols for 13C-urea breath test for the diagnosis of *Helicobacter pylori* infection in healthy volunteers. *Scand J Clin Lab Invest* 2005; **65**: 491-498
 - 68 **Peng NJ**, Lai KH, Liu RS, Lee SC, Tsay DG, Lo CC, Tseng HH, Huang WK, Lo GH, Hsu PI. Capsule 13C-urea breath test for the diagnosis of *Helicobacter pylori* infection. *World J*

30
188

- Gastroenterol 2005; 11: 1361-1364
- 69 Gatta L, Ricci C, Tampieri A, Osborn J, Perna F, Bernabucci V, Vaira D. Accuracy of breath tests using low doses of ¹³C-urea to diagnose *Helicobacter pylori* infection: a randomised controlled trial. *Gut* 2006; 55: 457-462
- 70 Mauro M, Radovic V, Zhou P, Wolfe M, Kamath M, Bercik P, Croitoru K, Armstrong D. ¹³C urea breath test for (*Helicobacter pylori*): determination of the optimal cut-off point in a Canadian community population. *Can J Gastroenterol* 2006; 20: 770-774
- 71 Mauro M, Radovic V, Wolfe M, Kamath M, Bercik P, Armstrong D. ¹³C urea breath test for (*Helicobacter pylori*): evaluation of 10-minute breath collection. *Can J Gastroenterol* 2006; 20: 775-778
- 72 Graham DY, Malaty HM, Cole RA, Martin RF, Klein PD. Simplified ¹³C-urea breath test for detection of *Helicobacter pylori* infection. *Am J Gastroenterol* 2001; 96: 1741-1745
- 73 Thijs WJ, Thijs JC, Kleibeuker JH, Elzinga H, Stellaard F. Evaluation of clinical and home performance of the ¹³C-urea breath test for the detection of *Helicobacter pylori*. *Eur J Gastroenterol Hepatol* 1995; 7: 603-607
- 74 Perez Garcia JI, Pajares Garcia JM, Jimenez Alonso I. C13 urea breath test in the diagnosis of *Helicobacter pylori* infection in the gastric mucosa. Validation of the method. *Rev Esp Enferm Dig* 1996; 88: 202-208
- 75 Kato M, Asaka M, Ohara S, Toyota T. Clinical studies of ¹³C-urea breath test in Japan. *J Gastroenterol* 1998; 33 Suppl 10: 36-39
- 76 Lee HS, Gwee KA, Teng LY, Kang JY, Yeoh KG, Wee A, Chua BC. Validation of [¹³C]urea breath test for *Helicobacter pylori* using a simple gas chromatograph-mass selective detector. *Eur J Gastroenterol Hepatol* 1998; 10: 569-572
- 77 Ohara S, Kato M, Asaka M, Toyota T. The UBT-100 ¹³CO₂ infrared analyzer: comparison between infrared spectrometric analysis and mass spectrometric analysis. *Helicobacter* 1998; 3: 49-53
- 78 Tanahashi T, Kodama T, Yamaoka Y, Sawai N, Tatsumi Y, Kashima K, Higashi Y, Sasaki Y. Analysis of the ¹³C-urea breath test for detection of *Helicobacter pylori* infection based on the kinetics of delta-¹³CO₂ using laser spectroscopy. *J Gastroenterol Hepatol* 1998; 13: 732-737
- 79 Gisbert JP, Benito LM, Lara S, Vazquez A, Jimenez I, Pajares JM. ¹³C-urea breath test for the diagnosis of *Helicobacter pylori* infection: are basal samples necessary? *Eur J Gastroenterol Hepatol* 2000; 12: 1201-1205
- 80 Suto H, Azuma T, Ito S, Ito Y, Miyaji H, Yamazaki Y, Kohli Y, Kuriyama M. Endoscopic [¹³C]-urea breath test for quantification of *Helicobacter pylori* infection. *J Gastroenterol Hepatol* 2000; 15: 161-167
- 81 Kubota K, Shimoyama S, Shimizu N, Noguchi C, Mafune K, Kaminishi M, Tange T. Studies of ¹³C-urea breath test for diagnosis of *Helicobacter pylori* infection in patients after partial gastrectomy. *Digestion* 2002; 65: 82-86
- 82 Kato M, Saito M, Fukuda S, Kato C, Ohara S, Hamada S, Nagashima R, Obara K, Suzuki M, Honda H, Asaka M, Toyota T. ¹³C-Urea breath test, using a new compact nondispersive isotope-selective infrared spectrophotometer: comparison with mass spectrometry. *J Gastroenterol* 2004; 39: 629-634
- 83 Gisbert JP, Olivares D, Jimenez I, Pajares JM. Long-term follow-up of ¹³C-urea breath test results after *Helicobacter pylori* eradication: frequency and significance of borderline delta¹³CO₂ values. *Aliment Pharmacol Ther* 2006; 23: 275-280
- 84 Lotterer E, Ramaker J, Ludtke FE, Tegeler R, Geletneký JV, Bauer FE. The simplified ¹³C-urea breath test—one point analysis for detection of *Helicobacter pylori* infection. *Z Gastroenterol* 1991; 29: 590-594
- 85 Lotterer E, Ludtke FE, Tegeler R, Lepsius G, Bauer FE. The ¹³C-urea breath test—detection of *Helicobacter pylori* infection in patients with partial gastrectomy. *Z Gastroenterol* 1993; 31: 115-119
- 86 Labenz J, Barsch G, Peitz U, Aygen S, Hennemann O, Tillenburg B, Becker T, Stolte M. Validity of a novel biopsy urease test (HUT) and a simplified ¹³C-urea breath test for diagnosis of *Helicobacter pylori* infection and estimation of the severity of gastritis. *Digestion* 1996; 57: 391-397
- 87 Ellenrieder V, Glasbrenner B, Stoffels C, Weiler S, Bode G, Moller P, Adler G. Qualitative and semi-quantitative value of a modified ¹³C-urea breath test for identification of *Helicobacter pylori* infection. *Eur J Gastroenterol Hepatol* 1997; 9: 1085-1089
- 88 Leodolter A, Dominguez-Munoz JE, von Arnim U, Manes G, Malfertheiner P. ¹³C-urea breath test for the diagnosis of *Helicobacter pylori* infection. A further simplification for clinical practice. *Scand J Gastroenterol* 1998; 33: 267-270
- 89 Perri F, Clemente R, Pastore M, Quitadamo M, Festa V, Bisceglia M, Li Bergoli M, Lauriola G, Leandro G, Ghos Y, Rutgeerts P, Andriulli A. The ¹³C-urea breath test as a predictor of intragastric bacterial load and severity of *Helicobacter pylori* gastritis. *Scand J Clin Lab Invest* 1998; 58: 19-27
- 90 Coelho LG, Reber M, Passos MC, Aguiar RO, Casaes PE, Bueno ML, Yazaki FR, Castro FJ, Vieira WI, Franco JM, Castro LP. Application of isotope-selective non-dispersive infrared spectrometry for the evaluation of the ¹³C-urea breath test: comparison with three concordant methods. *Braz J Med Biol Res* 1999; 32: 1493-1497
- 91 Mock T, Yatscoff R, Foster R, Hyun JH, Chung IS, Shim CS, Yacyshyn B. Clinical validation of the Helikit: a ¹³C urea breath test used for the diagnosis of *Helicobacter pylori* infection. *Clin Biochem* 1999; 32: 59-63
- 92 Mana F, Franken PR, Ham HR, Reynaert H, Urbain D. ¹³C urea breath test with nondispersive isotope-selective infrared spectrometry: reproducibility and importance of the fasting state. *Helicobacter* 2000; 5: 104-108
- 93 Niv Y, Niv G, Koren R. ¹³C-urea breath test for diagnosis of *Helicobacter pylori* infection in the elderly. *Dig Dis Sci* 2004; 49: 1840-1844
- 94 Parente F, Bianchi Porro G. The (¹³)C-urea breath test for non-invasive diagnosis of *Helicobacter pylori* infection: which procedure and which measuring equipment? *Eur J Gastroenterol Hepatol* 2001; 13: 803-806
- 95 Gatta L, Vakil N, Ricci C, Osborn JF, Tampieri A, Perna F, Miglioli M, Vaira D. Effect of proton pump inhibitors and antacid therapy on ¹³C urea breath tests and stool test for *Helicobacter pylori* infection. *Am J Gastroenterol* 2004; 99: 823-829
- 96 Bielanski W, Konturek SJ. New approach to ¹³C-urea breath test: capsule-based modification with low-dose of ¹³C-urea in the diagnosis of *Helicobacter pylori* infection. *J Physiol Pharmacol* 1996; 47: 545-553
- 97 Gisbert JP, Gomollon F, Dominguez-Munoz JE, Borda F, Jimenez I, Vazquez MA, Gallego S, Iglesias J, Pastor C, Pajares JM. Comparison between two ¹³C-urea breath tests for the diagnosis of *Helicobacter pylori* infection: isotope ratio mass spectrometry versus infrared spectrometer. *Gastroenterol Hepatol* 2003; 26: 141-146
- 98 Graham DY, Runke D, Anderson SY, Malaty HM, Klein PD. Citric acid as the test meal for the ¹³C-urea breath test. *Am J Gastroenterol* 1999; 94: 1214-1217
- 99 Yong CS, Kim YI, Park SM, Kwon R, Han HH, Park JG, Yang CY, Kim JA, Yoo BK, Rhee JD, Choi HG. Trials of novel ¹³C-urea-containing capsule for more economic and sensitive diagnosis of *Helicobacter pylori* infection in human subjects. *Arch Pharm Res* 2006; 29: 879-883
- 100 Pantoflickova D, Scott DR, Sachs G, Dorta G, Blum AL. ¹³C urea breath test (UBT) in the diagnosis of *Helicobacter pylori*: why does it work better with acid test meals? *Gut* 2003; 52: 933-937
- 101 Wang WM, Lee SC, Ding HJ, Jan CM, Chen LT, Wu DC, Liu CS, Peng CF, Chen YW, Huang YF, Chen CY. Quantification of *Helicobacter pylori* infection: Simple and rapid ¹³C-urea breath test in Taiwan. *J Gastroenterol* 1998; 33: 330-335
- 102 Wang WM, Lee SC, Wu DC, Chen LT, Liu CS, Peng CF, Ding HJ, Chen CY, Jan CM. Simplified ¹³C-urea breath test for the diagnosis of *Helicobacter pylori* infection—the availability of without fasting and without test meal. *Kaohsiung J Med Sci* 2000; 16: 607-613
- 103 Casellas F, Lopez J, Borruel N, Saperas E, Vergara M, de Torres I, Armengol JR, Malagelada JR. The impact of delaying gastric emptying by either meal substrate or drug on the

Breath Tests for Gastrointestinal Disease: The Real Deal or Just a Lot of Hot Air?

RICHARD J. SAAD and WILLIAM D. CHEY

Division of Gastroenterology, University of Michigan Health System, Ann Arbor, Michigan

Although endoscopy provides an accurate means by which to identify structural or histologic abnormalities of the gastrointestinal (GI) tract, it is invasive, expensive, and carries a small risk of complications. As such, there remains a need for simple, noninvasive, less expensive tests that can provide similar or supplemental information to endoscopy. Several breath tests have been developed to fill this void. This review discusses the rationale and evidence available to support the most commonly utilized breath tests in clinical practice.

Urea Breath Test for *Helicobacter pylori* Infection

The urea breath test (UBT) is the most accurate and best validated of the currently available breath tests. The UBT is an accurate noninvasive method of identifying active *Helicobacter pylori* (*H. pylori*) infection before and after antibiotic therapy. This test relies upon the oral administration of urea labeled with ^{13}C or ^{14}C to identify urease, an enzyme produced in large quantities by *H. pylori*. Urease cleaves the labeled urea to ammonia and labeled bicarbonate, which is subsequently converted to labeled carbon dioxide that is then measured in exhaled breath (Figure 1).

The earliest versions of the UBT utilized small amounts (1–10 μCi ; 1 μCi is less than typical daily background radiation exposure) of the radioactive isotope, ^{14}C , to label urea. The exhaled $^{14}\text{CO}_2$ can be measured using a scintillation counter. Sensitivity and specificity of the ^{14}C -UBT for *H. pylori* has consistently been reported to be >90%.¹

An alternative UBT utilizing the nonradioactive isotope, ^{13}C , is the most widely used version of this test. Given the absence of radiation exposure, the ^{13}C -UBT is most appropriate for children and pregnant women. A recent multicenter study that evaluated the ^{13}C -UBT in children reported a sensitivity of 96%–98% and specificity of 96%–99% for *H. pylori* compared with biopsy-based testing.² The greatest limitations of the ^{13}C -UBT are the high cost of the substrate and the isotope ratio mass spectrometer needed for breath analysis. Newer analyzers offering greater convenience and lower cost include a tabletop instrument utilizing molecular correlation spectrometry³ and an infrared spectrometer.⁴ A recent study reported that a simplified

^{13}C -UBT protocol that utilized a smaller dose of ^{13}C -urea (50 mg), no test meal, and a single, 10-minute breath collection yielded 100% accuracy for the diagnosis of *H. pylori*.⁵ Similar results have been reported with simplified ^{14}C -UBT protocols.⁶

The sensitivity of the UBT can be reduced by the recent use of proton pump inhibitors (PPIs), bismuth-containing compounds, or antibiotics. Most experts suggest that PPIs should be withheld for 1–2 weeks and bismuth and antibiotics should be withheld for ≥ 4 weeks before the UBT. Standard doses of histamine-2 receptor antagonists do not affect the sensitivity of the UBT.⁷

Carbohydrate Breath Tests

A number of carbohydrate breath tests have been utilized to assess orocecal transit, carbohydrate malabsorption, or identify patients with small intestinal bacterial overgrowth (SIBO; Table 1). This breath testing modality relies on the ability of intestinal bacteria to metabolize various carbohydrate substrates producing hydrogen and/or methane and leading to the release of measurable levels of these gases in exhaled air from the lungs (Figure 2).

Before testing, it is important to instruct patients to avoid fiber and unfermentable carbohydrates such as pasta or bread on the evening before their test.⁸ False-positive results can occur in patients with poor oral hygiene. This can be minimized by rinsing with an antibacterial mouthwash before ingestion of test substrate. Smoking can lead to false-positive results and physical exertion can lead to hyperventilation and false-negative results; both activities should be avoided for >2 hours before testing.

Given the need for specialized equipment, dedicated space, and technical support, hydrogen breath testing tends to be confined to larger centers. Because up to one third of individuals have microflora that do not produce hydrogen, it is mandatory for breath analyzers to measure hydrogen and methane. It is also useful for analyzers to have a “self-correction” feature that accounts for carbon dioxide in the

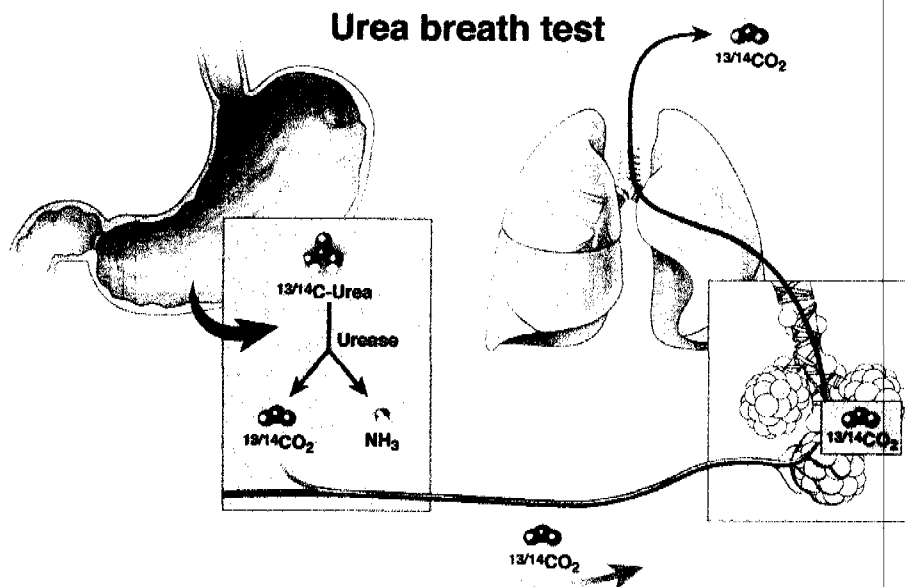


Figure 1. Schematic for urea breath testing.

breath sample. This helps to minimize errors in the event that a breath sample was imperfectly collected.

Several portable devices capable of use in virtually any office setting have been developed offering comparable accuracy to the traditional laboratory gas analyzer equipment. A novel commercially available portable breath analyzer (E-Z-EM H₂) has been compared with the standard laboratory breath analyzer in SIBO and found to have a high correlation of measured gas values at all time points (*r*-value range 0.87–0.97; *P* > .001).⁹ An earlier portable breath analyzer (Micro H₂) was also shown to closely correlate with the standard laboratory model in the diagnosis of

lactose malabsorption [overall agreement of 91% (95% confidence interval [CI], 78%–97%) and yielded an identical diagnosis in 100% (95% CI, 92%–100%) of cases].¹⁰

Breath Tests for Carbohydrate Malabsorption

Hydrogen breath testing can be useful in the assessment of carbohydrate malabsorption. Lactose is a disaccharide metabolized by the brush border enzyme lactase to glucose and galactose. Reduction or absence of this enzyme leads to excessive exposure of the colon to lactose, where fermentation can result in symptoms such as bloating, excessive flatus, cramping, and loose stools in a subset of

Table 1. Commonly Utilized Carbohydrate Breath Tests

Indication	Substrate	Recommended Challenge Dose	Positive Findings
Suspected carbohydrate malabsorption	Lactose	25 grams	Rise in H ₂ or CH ₄ > 20 ppm Rise in H ₂ or CH ₄ > 20 ppm Or Successive incremental increase >5 ppm in 3 consecutive samples
	Fructose	25 grams	
Suspected small intestinal bacterial overgrowth	Lactulose	10 grams	Baseline H ₂ or CH ₄ >20 ppm Or A double peak in H ₂ or CH ₄ levels Or Early rise in H ₂ or CH ₄ >20 ppm Or Incremental increase in H ₂ or CH ₄ >10 ppm in consecutive samples Baseline H ₂ or CH ₄ >20 ppm Or Rise in H ₂ or CH ₄ by 12-15 ppm
	glucose	50-75 grams	

H₂, hydrogen gas; CH₄, methane gas; ppm, parts per million

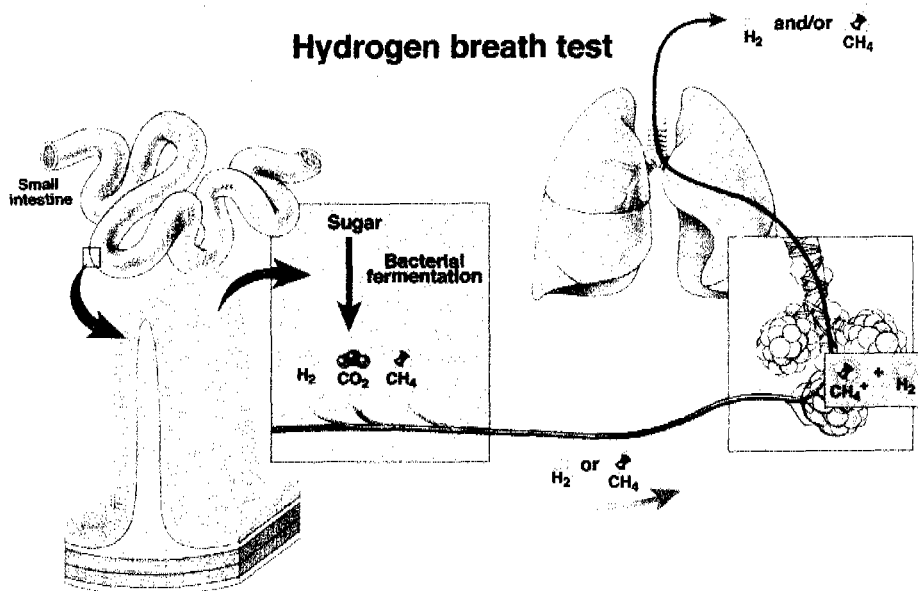


Figure 2. Schematic for carbohydrate breath testing.

affected persons.¹¹ Breath testing for lactose intolerance has been used by gastroenterologists for many years. Most centers give 25 g of lactose and collect breath samples over 2–4 hours. A positive test is typically defined as a rise in breath hydrogen or methane excretion of >20 parts per million (ppm).

Fructose is a monosaccharide used as a commercial sweetener and is also found in a number of fruits. Another important source of fructose is sucrose, a disaccharide composed of fructose and glucose. Fructose malabsorption has increasingly been identified as a potential cause of GI symptoms.^{12,13} A recent study attempted to identify the proper dosage of fructose for hydrogen breath testing.¹⁴ In this study, 20 healthy volunteers received 15–50 g of fructose in a double-blinded fashion. Based on hydrogen and methane measurements and a symptom questionnaire, a 25-g dose was found to most reliably identify patients with fructose malabsorption. The authors defined a positive test as a rise in breath hydrogen or methane excretion of >20 ppm or a rise of >5 ppm on consecutive breath samples as a positive test.

Breath Tests for SIBO

There has been a recent surge of interest in SIBO related to its possible clinical and physiologic overlap with irritable bowel syndrome (IBS).^{15,16} SIBO has traditionally been defined as $>10^5$ cfu/mL on quantitative culture of a small intestinal aspirate. Unfortunately, pitfalls including technical difficulty, frequent contamination by oral flora, high cost, and the ability to sample only from the proximal small bowel make quantitative culture an imperfect gold standard for SIBO. In clinical practice, lactulose or glucose hydrogen breath testing is often used as a means of identifying

patients with SIBO. Each test has advantages and disadvantages.

Under normal circumstances, lactulose is not absorbed by the small intestine. Once lactulose reaches the colon, it is fermented by bacteria to short-chain fatty acids as well as hydrogen and/or methane gas. Based on this physiology, the lactulose breath test was originally developed as a surrogate measure of orocecal transit time. Because bacteria anywhere in the GI tract can ferment lactulose, this test has also been suggested to identify patients with SIBO.

One issue that makes the literature difficult to interpret is the lack of standardization of the lactulose breath test protocol. Most centers use 10 g of lactulose and a 2- to 4-hour breath sampling period. A variety of findings have been used to define a positive test including a fasting breath hydrogen level of >20 ppm, the presence of a “double peak” (the first peak representing lactulose metabolism by small intestinal bacteria and the second peak corresponding to lactulose reaching the cecum), an early rise in breath hydrogen concentration of >20 ppm, or >10 ppm on consecutive samples.^{8,17}

Unfortunately, the classic double peak is rarely seen in clinical practice. More commonly, a single peak in breath hydrogen and/or methane is found, which can result from fermentation occurring in the small bowel and colon as would be expected in the setting of SIBO or simply colonic fermentation as would be the case in an individual without SIBO. The latter scenario holds particular relevance to patients with IBS and diarrhea who tend to have accelerated ileocecal transit.¹⁸ Acknowledging that there is currently no gold standard for the diagnosis of SIBO, the sensitivity and specificity of the lactulose breath test has been reported to

be 68% and 44%, respectively, relative to quantitative small bowel culture.¹⁹ A recent study in IBS patients reported that 35% and 45% of IBS patients and controls had a positive lactulose hydrogen breath test, whereas only 4% of patients and controls had SIBO by jejunal aspiration for quantitative culture. Interestingly, 43% of IBS patients versus 12% of controls had bacterial counts of $>5 \times 10^3/\text{mL}$ on jejunal culture, raising numerous questions about differences in microflora and fermentation between those with and without IBS.²⁰

The other substrate used to evaluate for SIBO is glucose, which is avidly absorbed in the proximal small intestine. Most centers administer 50–75 g of glucose and collect breath samples for 2–3 hours. A positive test is defined as a baseline breath hydrogen or methane excretion of >20 ppm and/or a rise of >12 –15 ppm after glucose ingestion. Because glucose typically does not reach the distal small bowel, the glucose breath test may theoretically be less sensitive than the lactulose breath test, although the literature is not clear on this issue. On the other hand, the glucose breath test is likely more specific than the lactulose breath test.¹⁹

Breath tests using D-xylose, ¹³C-xylose, and cholyglycine have been described in the literature,⁸ but have yielded conflicting results and are not widely available. Thus, these tests do not appear to offer clear advantages over the more conventional tests.

In summary, breath tests have become an integral part of clinical gastroenterology. The UBT is a validated means by which to identify *H pylori* infection before or after antibiotic therapy. A variety of breath tests can aid in the identification of carbohydrate maldigestion and malabsorption. However, the presence of an abnormal carbohydrate breath test does not prove cause and effect with GI symptoms. Lactulose and glucose breath tests are being used to identify patients with SIBO. Unfortunately, the sensitivity and specificity of these tests for SIBO is inadequate for routine clinical use. At present, it is not clear that a positive test predicts clinical outcomes after treatment for SIBO. Clearly, further work to improve upon the current generation of breath tests is warranted.

References

1. Chey WD. Accurate diagnosis of *Helicobacter pylori*. 14C-urea breath test. *Gastroenterol Clin North Am* 2000;29:895–902.
2. Elitsur Y, Tolia V, Giger M, et al. Urea breath testing in pediatrics: the multicenter USA study. *Gastroenterology* 2007;(abstract); 132(4 Suppl 2):A-111.
3. Israeli E, Ilan Y, Meir SB, et al. A novel 13C-urea breath test device for the diagnosis of *Helicobacter pylori* infection: continuous online measurements allow for faster test results with high accuracy. *J Clin Gastroenterol* 2003;37:139–141.
4. Chen TS, Chang FY, Chen PC, et al. Simplified 13C-urea breath test with a new infrared spectrometer for diagnosis of *Helicobacter pylori* infection. *J Gastroenterol Hepatol* 2003;18:1237–1243.
5. Campuzano-Maya G. An optimized 13C-urea breath test for the diagnosis of *H pylori* infection. *World J Gastroenterol* 2007;13:5454–5464.
6. Gatta L, Ricci C, Stanghellini V, et al. Best cut-off values for [14C]-urea breath tests for *Helicobacter pylori* detection. *Scand J Gastroenterol* 2003;38:1144–118.
7. Chey WD, Wong BC. American College of Gastroenterology guideline on the management of *Helicobacter pylori* infection. *Am J Gastroenterol* 2007;102:1808–1825.
8. Romagnuolo J, Schiller D, Bailey RJ. Using breath tests wisely in a gastroenterology practice: an evidence-based review of indications and pitfalls in interpretation. *Am J Gastroenterol* 2002;97:1113–1126.
9. DiBaise JK, Malagon I, Haywood M, et al. A comparison of a portable breath hydrogen analyzer with the Quintron SC breath analyzer in measuring small intestinal bacterial overgrowth. *Am J Gastroenterol* 2006;101(Suppl 2):s489.
10. Peuhkuri K, Poussa T, Korpela R. Comparison of a portable breath hydrogen analyser (Micro H2) with a Quintron MicroLyzer in measuring lactose maldigestion, and the evaluation of a Micro H2 for diagnosing hypolactasia. *Scand J Clin Lab Invest* 1998;58:217–224.
11. Hermans MM, Brummer RJ, Ruijters AM, et al. The relationship between lactose tolerance test results and symptoms of lactose intolerance. *Am J Gastroenterol* 1997;92:981–984.
12. Skoog SM, Bharucha AE. Dietary fructose and gastrointestinal symptoms: a review. *Am J Gastroenterol* 2004;99:2046–2050.
13. Choi YK, Johlin FC Jr., Summers RW, et al. Fructose intolerance: an under-recognized problem. *Am J Gastroenterol* 2003;98:1348–1353.
14. Rao SS, Attaluri A, Anderson L, et al. Ability of the normal human small intestine to absorb fructose: evaluation by breath testing. *Clin Gastroenterol Hepatol* 2007;5:959–963.
15. Lupascu A, Gabrielli M, Lauritano EC, et al. Hydrogen glucose breath test to detect small intestinal bacterial overgrowth: a prevalence case-control study in irritable bowel syndrome. *Aliment Pharmacol Ther* 2005;22:1157–1160.
16. Nucera G, Gabrielli M, Lupascu A, et al. Abnormal breath tests to lactose, fructose and sorbitol in irritable bowel syndrome may be explained by small intestinal bacterial overgrowth. *Aliment Pharmacol Ther* 2005;21:1391–1395.
17. Simren M, Stotzer PO. Use and abuse of hydrogen breath tests. *Gut* 2006;55:297–303.
18. Hutchinson R, Notghi A, Smith NB, et al. Scintigraphic measurement of ileocaecal transit in irritable bowel syndrome and chronic idiopathic constipation. *Gut* 1995;36:585–589.
19. Corazza GR, Menozzi MG, Stocchi A, et al. The diagnosis of small bowel bacterial overgrowth. Reliability of jejunal culture and inadequacy of breath hydrogen testing. *Gastroenterology* 1990;98:302–309.
20. Posserud I, Stotzer PO, Bjornsson ES, et al. Small intestinal bacterial overgrowth in patients with irritable bowel syndrome. *Gut* 2007;56:802–808.

Address requests for reprints to: William D. Chey, AGAF, FACP, Professor of Medicine, Director – GI Physiology Laboratory, University of Michigan Health System, 3912 Taubman Center, SPC 0362, Ann Arbor, Michigan 48109-0362. e-mail: wchey@umich.edu; fax: (734) 936-7392.