



Patient:

Order Number:

DOB:

Completed:

Sex:

Received:

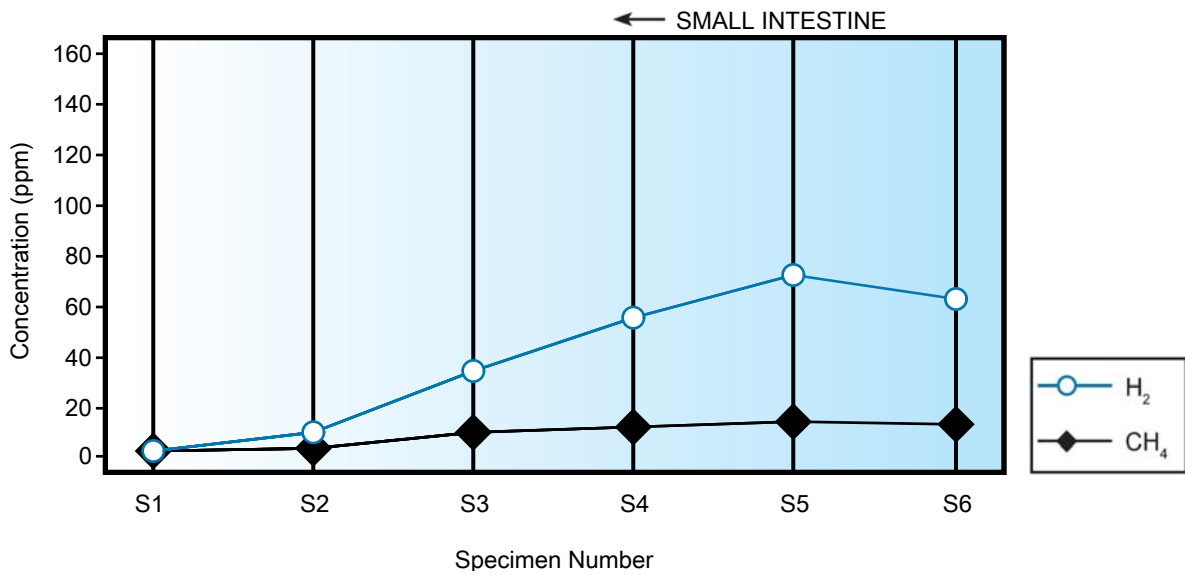
MRN:

Collected:

2306 Small Intestinal Bacterial Overgrowth (SIBO) 2 Hour- Breath

Methodology: GC-TDC/SSS

Hydrogen (H₂) and Methane (CH₄) Breath Gases



Hydrogen (H ₂), Methane (CH ₄), and Carbon Dioxide (CO ₂) (ppm)						
	Baseline 0 min (S1)	20 min (S2)	40 min (S3)	60 min (S4)	90 min (S5)	120 min (S6)
H ₂	2	9	33	55	72	62
CH ₄	<2	3	9	11	13	12
H ₂ + CH ₄	NR	12	42	66	85	74
CO ₂ **	✓	✓	✓	✓	✓	✓
Actual Collection Times						
Actual Time	9:34	9:54	10:14	10:34	11:04	11:34
Actual Interval	0 min	20 min	40 min	60 min	90 min	120 min
**CO ₂ is measured for quality assurance. ✓ indicates the CO ₂ level is acceptable. ✗ indicates room air contamination exceeding acceptable limits.						

Evaluation for Hydrogen (H ₂)		
<i>Hydrogen increase over baseline by 90 minutes</i>		
Change in H ₂	Result: 70 H	Expected Value: <20 ppm
A rise of ≥ 20 ppm from baseline in hydrogen by 90 min should be considered a positive test to suggest the presence of SIBO.		

Evaluation for Methane (CH ₄)		
<i>Peak methane level at any point</i>		
CH ₄ Peak	Result: 13 H	Expected Value: <10 ppm
A peak methane level ≥ 10 ppm at any point is indicative of a methane-positive result.		



2306 Small Intestinal Bacterial Overgrowth (SIBO) 2 Hour- Breath

Methodology: GC-TDC/SSS

Commentary

NR=Non-Reportable due to analyte outside the reportable range.

This test has been developed and its performance characteristics determined by Genova Diagnostics, Inc. It has not been cleared by the U.S. Food and Drug Administration.

Commentary is provided to the practitioner for educational purposes, and should not be interpreted as diagnostic or as treatment recommendations. Diagnosis and treatment decisions are the practitioner's responsibility.

SIBO INTERPRETATION GUIDELINES

GENERAL CONSIDERATIONS FOR BREATH TESTING

Small intestinal bacterial overgrowth (SIBO) is a heterogeneous syndrome characterized by an increase in the number and/or the presence of atypical microbiota in the small intestine. Genova's SIBO breath test relies on measurement of gases produced by microbiota in the intestine - hydrogen (H₂) and methane (CH₄) - following ingestion of lactulose in a fasting state.

A normal transit time of lactulose (10 g) in healthy fasting patients from the mouth to the junction between the small and large intestine (oro-cecal transit time, or OCTT) is approximately 90 minutes. In general, transit times have been found to vary in humans. Given such findings, transit time should be taken into consideration when interpreting breath testing.

To preclude elevated breath levels of hydrogen and methane prior to the ingestion of lactulose (at baseline), impeccable patient preparation and sample collection are imperative. Falsely elevated findings may result from incomplete avoidance of high-fiber foods, residual fiber in the intestine due to delayed transit time, residual oropharyngeal (mouth and throat) bacteria, exposure to tobacco smoke, or napping during collection.

LOW BREATH GASES -A breath test finding with no CH₄ and low H₂ throughout the entire test may be due to an abundance of hydrogen sulfide (H₂S)-producing bacteria, which compete for available hydrogen for production of the H₂S gas.¹

EVALUATION FOR HYDROGEN (H₂)

In healthy humans, hydrogen gas is exclusively produced by intestinal bacteria - primarily a result of carbohydrate fermentation by anaerobic bacteria in the colon. In SIBO, fermentation of the malabsorbed lactulose substrate by bacteria residing in the small intestine results in elevated concentration of exhaled hydrogen (H₂).

A rise of H₂ of ≥ 20 ppm over baseline in the first 90 minutes of testing is positive for SIBO.¹

- The hydrogen result is the difference between the baseline hydrogen (S1) and the highest hydrogen finding among S2, S3, S4 or S5. The result does not take into account actual collection times. It is expected that the patient followed recommended collection times.
- A rise of H₂ of ≥ 20 ppm over baseline in those samples collected after 90 minutes maybe positive for SIBO in patients with slower transit time or constipation.
- **ELEVATED BASELINE** - The clinical significance of elevated baseline H₂ levels in patients reporting adherence to fasting and dietary guidelines is not known. In a symptomatic patient, some clinical groups with expertise in SIBO



Commentary

- Approximately 8 to 27% of individuals do not produce H₂ due to the presence of methanogenic microbiota which consume hydrogen molecules to produce methane gas. As a result, low H₂ findings through all time points in a symptomatic patient may reflect a false negative result. In this instance, clinical attention should be shifted to evaluation of CH₄.

EVALUATION FOR METHANE (CH₄)

Utilization of breath methane levels for SIBO assessment is controversial largely due to a lack of validation related to diagnostic specifics such as timing and magnitude of increase; however, CH₄ measurements are increasingly obtained to address other clinical questions. Recent evidence has associated CH₄ production with the pathogenesis of common clinical conditions, such as obesity, irritable bowel syndrome (IBS), and constipation.

A peak methane level > 10 ppm at any point is indicative of a methane-positive result.

- The peer-reviewed literature suggests an association with certain clinical conditions and methanogen overgrowth at levels as low as 3 ppm, CH₄ values between 3 and 9 may indicate the need for clinical intervention in the symptomatic patient.
- ELEVATED BASELINE** - An elevated baseline may be a more common pattern with CH₄ positive tests primarily due to the ability of methanogenic organisms to ferment in the absence of an ingested carbohydrate substrate.

Methane gas itself may slow intestinal transit, and patients with CH₄-predominant bacterial overgrowth have been found to be five times more likely to have constipation compared to individuals with H₂ - predominant overgrowth. Moreover, the severity of constipation has been found to directly correlate with the CH₄ level.

TOTAL HYDROGEN AND METHANE (H₂ + CH₄)

Prior to the 2017 North American consensus paper on breath testing, it was common to report a positive result for SIBO with a rise in the combined sum of H₂ and CH₄. The North American consensus group does not offer guidelines for a combined value. The combined values are provided for clinicians who wish to have it displayed in this manner.

CO₂

Carbon Dioxide (CO₂) is measured in every sample. CO₂ levels exceeding acceptable limits indicate room air contamination likely at the time of sample collection. If CO₂ levels exceed acceptable limits, sample integrity is questionable and results are designated as non-reportable (NR).

SAMPLE COLLECTION INFORMATION

Actual Time

The actual time of collection of samples is provided to enhance clinical interpretation. The actual times reported are utilized to determine the actual interval for comparison to the recommended interval.

Actual Interval

The actual interval can be compared to the recommended collection interval. If the recommended collection interval is not followed correctly, interpretation should be made within the context of the altered collection schedule. Generally, deviations of a few minutes will not significantly alter the interpretation.

- Rezaie A, Buresi M, Lembo A, et al. Hydrogen and Methane-Based Breath Testing in Gastrointestinal Disorders: The North American Consensus. *The American journal of gastroenterology*. May 2017;112(5):775-784.



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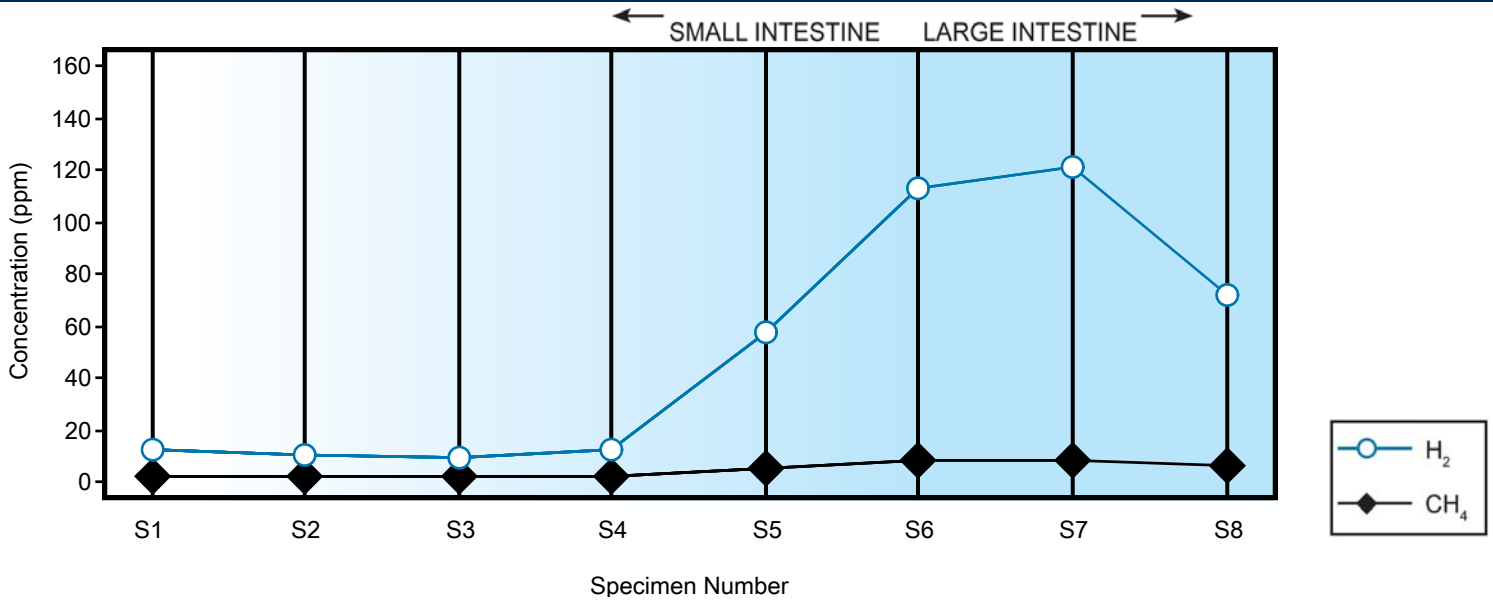
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2337 Small Intestinal Bacterial Overgrowth (SIBO) 3 Hour - Breath

Methodology: GC-TDC/SSS

Hydrogen (H₂) and Methane (CH₄) Breath Gases



Hydrogen (H ₂), Methane (CH ₄) and Carbon Dioxide (CO ₂) (ppm)								
	Baseline 0 min (S1)	20 min (S2)	40 min (S3)	60 min (S4)	90 min (S5)	120 min (S6)	150 min (S7)	180 min (S8)
H ₂	12	10	9	12	57	112	121	71
CH ₄	2	2	<2	2	5	8	8	6
H ₂ + CH ₄	14	12	NR	14	62	120	129	77
CO ₂ **	✓	✓	✓	✓	✓	✓	✓	✓
Actual Collection Times								
Actual Time	7:55	8:15	8:35	8:55	9:25	9:55	10:25	10:55
Actual Interval	0 min	20 min	40 min	60 min	90 min	120 min	150 min	180 min
**CO ₂ is measured for quality assurance. ✓ indicates the CO ₂ level is acceptable. ✗ indicates room air contamination exceeding acceptable limits.								

Evaluation for Hydrogen (H ₂)		
<i>Hydrogen increase over baseline by 90 minutes</i>		
Change in H ₂	Result: 45	Expected Value: <20 ppm
A rise of ≥ 20 ppm from baseline in hydrogen by 90 min should be considered a positive test to suggest the presence of SIBO.		

Evaluation for Methane (CH ₄)		
<i>Peak methane level at any point</i>		
CH ₄ Peak	Result: 8	Expected Value: <10 ppm
A peak methane level ≥ 10 ppm at any point is indicative of a methane-positive result.		



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LOW BREATH GASES -A breath test finding with no CH₄ and low H₂ throughout the entire test may be due to an abundance of hydrogen sulfide (H₂S)-producing bacteria, which compete for available hydrogen for production of the H₂S gas.¹

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consume hydrogen molecules to produce methane gas. As a result, low H₂ findings through all time points in a symptomatic patient may reflect a false negative result. In this instance, clinical attention should be shifted to evaluation of CH₄.

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1. Rezaie A, Buresi M, Lembo A, et al. Hydrogen and Methane-Based Breath Testing in Gastrointestinal Disorders: The North American Consensus. *The American journal of gastroenterology*. May 2017;112(5):775-784.

CHECKLIST (PRIOR TO SHIPPING)

1. All Tubes

- Label tubes you've been instructed to collect by your clinician
- Label tubes with patient's first and last name, date/time of collection, and the tube number – (6 tubes for 2 hour collection – discard the additional tubes) or (8 tubes for the 3 hour collection)
- All tubes placed in Bubblewrap bags

2. Test Requisition Form with Payment

- Test Requisition Form is complete **Test is marked, patient's first and last name, date of birth, gender, and date of collection** are recorded
- Collection table** on requisition has filled out with collection times
- Payment** is included or pay online at www.gdx.net/prc
- Complete** survey online at www.gdx.net/prc

3. Return to the Laboratory

- Be sure to return the specimen within the original package and place that inside the included mailing material(s)

SHIP THE SPECIMEN(S) TO THE LAB

Specimen(s) must be returned in the Genova Diagnostics specimen collection pack.
Please refer to the shipping instruction insert found in your specimen collection pack.



Call 800.522.4762 or visit our website at www.gdx.net

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IS-1051,g,pi,b-128-b,0818-SIBO

PATIENT BREATH COLLECTION INSTRUCTIONS

Small Intestinal Bacterial Overgrowth (SIBO)

GASTROINTESTINAL

The following test(s) can be collected using these instructions:

Small Intestinal Bacterial Overgrowth (SIBO) 2 hour #2306
Small Intestinal Bacterial Overgrowth (SIBO) 3 hour #2337

Test may not be processed without this information.

Test Requisition Form

Please Label:

- Patient's first/last name
- Date of Birth
- Gender
- Date of Collection

All Tubes

Please Label:

- Patient's first/last name
- Date/time of Collection
- Tube number

VERY IMPORTANT
PATIENT MUST FILL IN COLLECTION TIMES ON THE TABLE LOCATED ON THE FRONT OF THE REQUISITION. PLEASE LOCATE AND HAVE DOCUMENT AVAILABLE DURING COLLECTION!

Specimen

- Breath

Collection Materials

Additional Materials

- Labels
- 2 Bubblewrap Bags
- Test Requisition Form
- Mailing Envelope



Vacuum-sealed Tubes
(6 for 2 hour collection) or
(8 for 3 hour collection)*

Lactulose
Solution
(10gm)

Mouthpiece/
Plastic Bag**

* The white rubber seal will often be either convex or concave. Either is okay.
** There is an intentional small hole in the plastic bag to keep from overinflating.

This test is not appropriate for children under 25 pounds.

Watch the collection video at www.gdx.net/tests/prep

IMPORTANT PREP BEFORE PATIENT TAKES TEST

2-4 WEEKS BEFORE THE TEST:



- ❑ Wait 4 weeks from your last dose of antibiotics, colonoscopy or barium enema.
- ❑ Wait at least 2-4 weeks from your last dose of antifungals, Pepto-Bismol™ or herbal/natural antimicrobial products.

7 DAYS BEFORE THE TEST:



- ❑ **Avoid the use of laxatives**, stool softeners, and/or stool bulking agents as well as antacids containing aluminum or magnesium hydroxide.

24 HOURS BEFORE THE TEST:



- ❑ **Foods you CAN eat before you start your 12 hour fast:** Baked or broiled chicken, fish or turkey (salt and pepper only), white bread (only), plain steamed white rice, eggs, clear chicken or beef broth with no vegetable pieces, water, plain coffee, or tea (no sugar/artificial sweeteners or cream).
- ❑ **Vegetarians** may have tofu with salt and pepper.
- ❑ **Do not take probiotics.**

12 HOURS BEFORE THE TEST:



- ❑ **Fast for 12 hours prior to the test.** Do not eat or drink anything other than water for 12 hours prior and during the test.
- ❑ **Do not take non-essential medications or supplements** until the test is completed, unless your physician has advised you otherwise. Do not chew gum, eat candy, or use mouthwash until the test is completed.

1 HOUR BEFORE THE TEST:



- ❑ **No smoking**, including second-hand smoke, for at least 1 hour before or at any time during the breath test.
- ❑ **No sleeping** or vigorous exercise for at least 1 hour before or at any time during the breath test.
- ❑ **Do Not Use Toothpaste.**

Important Things To Know And Consider:

This test uses lactulose; since lactulose contains galactose and lactose, it is not recommended for individuals who have had allergic reactions to lactulose, or who are on a galactose/ lactose-restricted diet. It should be used with caution in diabetics.

Do not open, remove, or loosen tops of collection tubes—this will break the vacuum and make it impossible to perform your test. **Do not stick your finger** into the mouthpiece/plastic bag – there is a sharp needle inside.

We do not suggest collecting during an acute infectious illness.

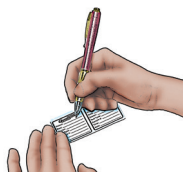
This collection is extremely time-sensitive. You need to carefully plan the morning around these timed collections. An uninterrupted 2 or 3 hours is ideal.

Use the Breath Collection Schedule Table on the front of the Requisition to help you schedule your collection times. **Be sure you fill in the Table as the test progresses.**

Special Instructions for patients weighing 100 pounds or less:
Follow the instructions on the blue bag for rolling and stapling the bag in accordance with weight. (Note: stapling will not damage the bag or affect the results).

BREATH COLLECTION

- 1** **To ensure accurate test results you must provide the requested information.** Write patient's first/last name and the tube number (1 - 6 (or 8) in order of collection) on the tube labels provided. Write patient's first/last name, date of birth, gender and date of collection on the Test Requisition Form.
- 2** Stir the Lactulose solution into 8 ounces of water. Set aside until after you have completed your first breath collection. **Brush your teeth and tongue** (including the back of your tongue) **without using toothpaste or mouthwash.** Rinse your mouth with water.
- 3** Take the first collection tube and apply the label to the tube. Be sure to **record the time and date of collection to the label** and **the collection time** on the **Breath Collection Table** (located on the requisition).



For full details refer to: www.gdx.net/tests/prep (collection video available)

- 4** Pick up the mouthpiece/plastic bag in one hand and Tube 1 in your other hand.
- 5** Breathing normally, inhale and hold for 5 seconds. Then close your mouth tightly around the mouthpiece and exhale normally into the plastic bag until it fills completely. **Do not blow hard.**
- 6** Continue to exhale normally with the bag expanded, and press the specimen tube into the side part of the mouthpiece. The needle will puncture the tube's self-sealing membrane allowing air to fill the tube. **Do not inhale at any point.**
- 7** Remove the tube within 2 seconds of puncturing. **You may stop exhaling** into the mouthpiece. **Do not unscrew** the cap on the collection tube. **Place** the tube in the bubblewrap bag.
- 8** Immediately drink the Lactulose solution before continuing with the rest of the breath test. **Drink** the entire amount within 5 minutes. **Do not drink water** for 1 hour after you drink the solution.



- 9** Repeat steps 3-7 for each breath collection, using the remaining tubes and labels 2-6, in order and according to the collection schedule. **Record** times on Collection Schedule found on the Test Requisition. (see image) →

Please Record Your Collection Times in the Blank Fields

Specimen Interval	RECORD COLLECTION TIMES	RECORD	EXAMPLE
SAMPLE 1 @ 0 min	8:05	AM or PM	8:00 AM
SAMPLE 2 @ 20 min	8:25	AM or PM	8:20 AM
SAMPLE 3 @ 40 min	8:45	AM or PM	8:40 AM
SAMPLE 4 @ 60 min	9:05	AM or PM	9:00 AM
SAMPLE 5 @ 80 min	9:25	AM or PM	9:20 AM
SAMPLE 6 @ 100 min	10:05	AM or PM	10:00 AM
ONLY COLLECT/RECORD TIMES BELOW IF USING THE 2 HOUR TEST			
SAMPLE 7 @ 120 min	10:35	AM or PM	10:30 AM
SAMPLE 8 @ 140 min	11:05	AM or PM	11:00 AM

- STOP HERE IF USING THE 2 HOUR COLLECTION!**
Discard the remaining tubes.

- 10** **CONTINUE TO STEP 10 IF USING THE 3 HOUR COLLECTION**
Repeat steps 3-7 for each breath collection, using the remaining tubes and labels 7-8 in order and according to the collection schedule.