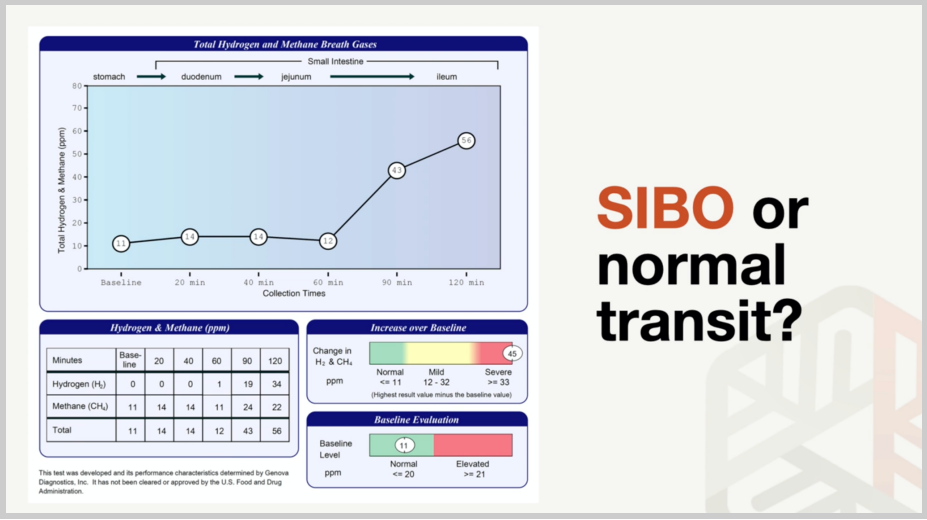
Identifying Gut Pathologies: Small Intestinal Bacterial Overgrowth Breath Test (Part 4)

We mentioned lactulose is not absorbed at all in the small intestine. It goes all the way to the colon, where it's fermented by bacteria. The advantage of this is that you can detect [small intestinal bacterial overgrowth] (SIBO) in the jejunum and ileum when you are using lactulose as a substrate. The biggest problem is that the lactulose breath test is based on the idea that oral-cecal transit time in healthy people is always longer than 90 minutes. Most labs interpret a rise in hydrogen over a certain cutoff before 90 minutes as a positive result. However, some studies have shown that oral-cecal transit in healthy people averages between 72 and 85 minutes. A study in India found a median transit time of 60 minutes. And a study in Taiwan found a median transit time of 85 minutes. This suggests that transit time may vary according to race, ethnicity, and geography.



One recent study in [the] Western population used radiolabeled lactulose and tracked the progress of lactulose through the gut while the patients were performing a SIBO test. That study showed that a high percentage of people with [irritable bowel syndrome] (IBS) had an early single peak of hydrogen before 90 minutes on the lactulose breath test. But in 88 percent of cases,

lactulose had already reached the colon, according to the radiolabel assay. That meant that 88 percent of these patients would have had a false positive on the lactulose breath test for SIBO. A 2006 study found that a hydrogen peak within the first 60 to 80 minutes significantly increases the specificity of lactulose breath testing. That suggested late single peaks that occur after this time, and like the one pictured on this slide, may often be false-positive results.

Shortly, [we'll be] talking about the new consensus on SIBO breath testing that recognizes this fact and that the 120-minute cutoff that's used by some labs and that the lactose is probably already in the colon in many cases in that time period. And this is often why that 90-minute adjustment was made for the recommendations in the North American Consensus.

The fact that lactulose has a laxative effect complicates this issue further because lactulose itself accelerates transit. If a patient's fasting and they consume lactulose, that would be expected to move more quickly through the small intestine to the colon, which again could raise the risk of a false positive. The final note here is that patients with lactose allergies should probably not do the lactulose breath test. However, most patients who have lactose intolerance rather than a true allergy, I think, are able to do the lactulose breath test. They may experience discomfort while they're doing it, though. For that matter, a lot of people with SIBO experience discomfort with the lactulose breath test because lactulose is not absorbed. And it causes fermentation if the bacteria [are] in the small intestine. That can cause gas, bloating, and a lot of other symptoms for people who have SIBO. So you might want to warn your patients about that before they do the test. Overall, the takeaway is that we have to be cognizant of the impact that oral-cecal transit time may have on the test results. But this is the reason why we treat the whole patient, symptoms, [and] history, and not just the test result.

Comparison of glucose vs. lactulose as substrate for breath test

Substrate	Advantage	Disadvantage	Risk
Glucose	More specific	Greater risk of *false negative*	Under-treatment
Lactulose	More sensitive	Greater risk of *false positive*	Over-treatment

I know that this might be a little overwhelming or confusing, so let me bring it all together for you. The glucose breath test favors specificity over sensitivity. So if you choose glucose breath testing, you're accepting a greater possibility of [a] false negative in the case of SIBO that's happening in the middle or lower parts of the small intestine. And you're erring toward under-treatment rather than over-treatment. But overall, glucose breath testing does have more diagnostic accuracy than lactulose breath testing. On the other hand, lactulose breath testing favors sensitivity over specificity. So if you choose lactulose breath testing, there's a greater possibility of a false positive, especially if the patient has normal or faster than normal transit time. So here, you are erring toward treatment overall. And there's less diagnostic accuracy, maybe, than the glucose breath test. So, with all this in mind, what do we use?

The answer is the lactulose breath test. And that might be somewhat surprising, given what we've covered in the previous slides and the overall lower level of diagnostic accuracy compared to [the] glucose breath test. But here's the thing: I think the risk of under-treating SIBO is higher than over-treating [it]. And this is true, especially because the treatments for SIBO, whether you're doing a botanical antimicrobial protocol or whether you're using pharmaceutical treatments for SIBO, are both remarkably safe and aren't likely to cause a lot of side effects and don't have a lot of complications or risks. So we're always making calculations as clinicians. We're weighing [the] benefits versus consequences. And in the case of SIBO, I think the potential benefits of treatment far outweigh the potential consequences because of the safety of the treatment interventions. I sometimes order glucose breath testing, all that said, if I think it will clarify the clinical picture. But to be honest, I really haven't done this very much because I found that if glucose breath testing is negative, it still doesn't rule out SIBO because it's so insensitive, and it won't stop me from doing a therapeutic trial if I really suspect the patient has SIBO. So I haven't found it to be all that useful even as a clarifying test.

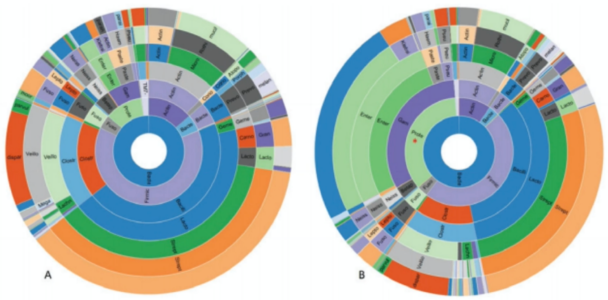
SIBO using lactulose and GBT in IBS patients and controls

		SIBO	Non-SIBO
Lactulose	Controls (n=150)	45 (30%)	105 (70%)
	Patients (n=175)	60 (34.3%)	115 (65.7%)
Glucose	Controls (n=150)	1 (0.66%)	149 (99.3%)
	Patients (n=175)	11 (6.2%)*	164 (93.8%)

* $p < 0.01$ for glucose between controls and patients

Adapted from: Rana et al. Digestion. 2012;85(3):243-7

There are few studies that have compared lactulose breath testing with glucose breath testing directly in both IBS patients and controls. What they found was that SIBO was present in 34 percent of IBS patients if you use the lactulose breath test, but only 6 percent of patients using the glucose breath test. The numbers in controls were even more dramatic. Using the lactulose breath test, 30 percent of healthy controls had SIBO vs. only 0.7 percent with glucose breath testing. SIBO was not statistically different in patients with lactulose breath testing, but it was statistically different in IBS patients and controls with glucose breath testing. Put another way, only glucose breath testing was able to distinguish between IBS patients and controls, whereas lactulose breath testing was not able to distinguish between them.



Overall microbiome distribution of the duodenum-associated microbiome of subjects without SIBO (n=104) and subjects with SIBO (n=45).

A. Overall microbial relative abundance distribution the duodenum-associated microbiome of subjects without SIBO detected by microbial culture on MacConkey agar.

B. Overall microbial relative abundance distribution the duodenum-associated microbiome of subjects with SIBO detected by microbial culture on MacConkey agar.

Source: Leite G., et. al. (2019). 4 – First Large Scale Study Defining the Characteristic Microbiome Signatures of Small Intestinal Bacterial Overgrowth (SIBO): Detailed Analysis from the Reimagine Study. Gastroenterology, 156(6), S-1–S-2. doi:10.1016/S0016-5085(19)36776-9

There have since been more studies evaluating SIBO in IBS patients using 16S ribosomal RNA gene sequencing. These small cohort studies generally show lower microbial diversity in the

duodenum of subjects with IBS when compared with subjects without IBS and higher levels of *Pseudomonas* in the small bowel of subjects with IBS when compared to healthy cohorts. So we are seeing altered microbial diversity in IBS patients, and in the REIMAGINE study, sequencing was able to validate SIBO as greater than or equal to 10 to the power of three by culture on MacConkey agar based on correlation to symptoms, sequencing, and breath testing results. In the same study, using a cutoff of greater than or equal to 10 to the power [of] three also correlated with a positive hydrogen breath test at 90 minutes and also correlated with the clinical symptoms of bloating and urgency. There was one study, though, that showed duodenal aspirates using culture and [polymerase chain reaction] of 16S RNA genes found no significant correlation between the glucose breath test results and bacterial levels. Overall, what we're seeing is a pattern of disrupted small bowel microbial diversity in IBS and SIBO patients that seem to correlate with breath tests. Although the rate of SIBO and IBS is debated, meta-analysis suggests that up to 78 percent of IBS subjects suffer from SIBO. Although there remains a question of cause or effect in IBS, there is little controversy that a subset of subjects with IBS also have SIBO, although large-scale studies are probably still needed and in the works.

Relationships between H_2 levels: area under the curve (AUC) during breath testing, detection of SIBO, relative abundances of microbial taxa, and other parameters

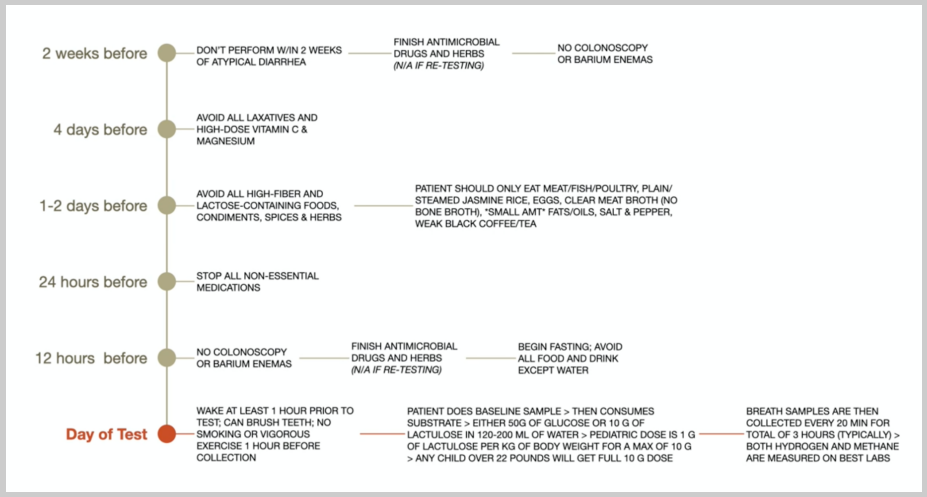
Timepoint (minutes)	SIBO detection		H_2 AUC	Gammaproteobacteria abundance		Firmicutes abundance		Excess of gas		Energy metabolism (H_2 production)	
	Sensitivity (%)	Specificity (%)		Spearman r	P-value	Spearman r	P-value	Spearman r	P-value	Spearman r	P-value
60	14.28	92.3	16.72	0.488	**0.021**	-0.318	0.149	0.355	0.105	0.272	0.221
75	57.14	84.61	24.06	0.516	**0.014**	-0.371	0.089	0.39	0.073	0.313	0.156
90	57.14	84.61	35.16	0.63	**0.002**	-0.508	**0.016**	0.408	0.059	0.457	**0.033**
105	57.14	69.23	49.06	0.689	**3.88E-04**	-0.583	**0.004**	0.444	**0.039**	0.527	**0.012**
120	57.14	61.53	52.97	0.781	**1.80E-05**	-0.651	**0.001**	0.368	0.092	0.602	**0.003**

Significant changes (P<0.05) are indicated in bold
- Darker gold color represents greater sensitivity and specificity
- Darker red color represents higher correlation

Source: https://doi.org/10.1371/journal.pone.0234906.t002

In addition to the decreased duodenal microbial diversity that was seen in the IBS and SIBO patients, studies have also compared [the] lactulose SIBO breath test with SIBO diagnosis via duodenal aspirates. So 20 of 140 subjects had undergone standard of care breath testing. Of these 20 subjects, 7 had SIBO based on the threshold of greater than or equal to 10 to the third [power] on MacConkey agar. Four of those seven SIBO subjects also had a positive hydrogen breath test at 90 minutes, defined as greater than or equal to 20 parts per million from baseline, indicating a 57.14 percent sensitivity for the detection of SIBO in IBS patients. Thirteen subjects of the 20 did not have SIBO based [on those] criteria. And of those, two out of 13 had a positive

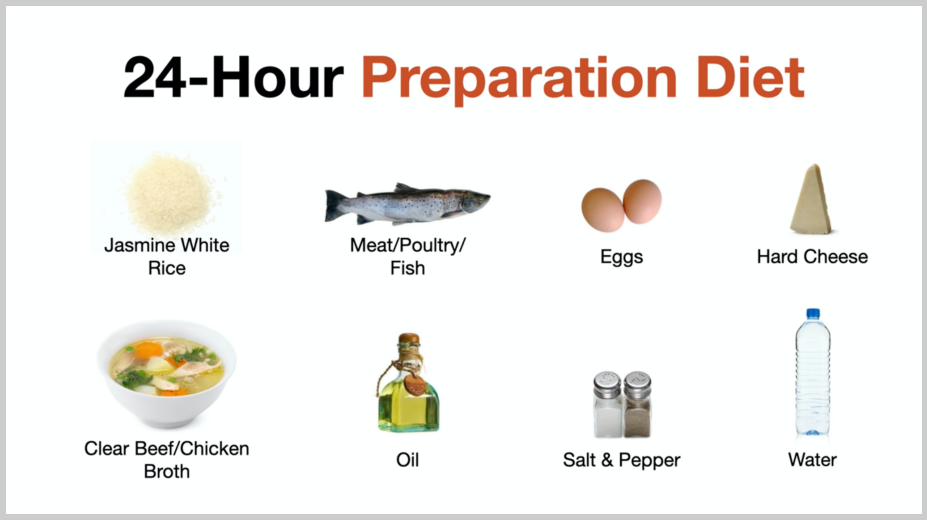
hydrogen breath test at 90 minutes, indicating an 84.61 percent specificity for the detection of SIBO.



Now, let's talk a little bit about preparation for the test. There are a lot of things to be aware of in terms of trying to get as accurate a result as possible, and I've listed them here on this slide. We also created a handout that has this test preparation matrix for you. Two weeks before the test, you'd want to make sure that the patient finishes any antimicrobials they're taking, drugs or botanicals or nutrients. I think we mentioned this, that in practice, we've even stretched this out to four weeks for antibiotics or antimicrobials for retests. So you'd want to make sure that they're not having any atypical diarrhea [and] that they haven't had a colonoscopy or barium enema two weeks prior to the SIBO test. So, four weeks before the breath test, you'd want to make sure that the patient finishes any antimicrobials or antibiotics that they may be on prior to taking this test. As I mentioned previously, we've jumped a little bit between two and four weeks for antibiotics or antimicrobial protocols. I think we really landed on four weeks. That's also the North American Consensus. So then two weeks before, we want to make sure that the patient doesn't have any atypical diarrhea and there's no colonoscopy or barium enemas. Four days before the breath test, you would instruct the patient to avoid all laxatives and high-dose vitamin C and magnesium. I have a lot of patients that take magnesium for bowel regularity or sleep, and I generally don't have a problem with lower doses like 200 to 400 milligrams of magnesium impacting the results of the test. So if they can stop, then it's probably ideal. But if not, use your judgment or contact the lab directly to ask. One or two days before the test, you would tell them to avoid all high-fiber and lactose-containing foods, condiments, spices, and herbs. During this time, the patient should only eat meat, fish or poultry, plain steamed jasmine rice, eggs and clear meat broth, no bone broth or no vegetable broth, I should say, small amounts of fats and oils, salt and pepper, and maybe weak black coffee or tea. And don't worry about writing all this down. I'm going to provide

you with a SIBO prep procedure handout so that you can brand it with your own clinic logo and color schemes and be able to give it to your patients. I'm just going through it now to familiarize you with it before we get into the testing itself.

I typically recommend the diet change 24 hours or one day prior to taking the test. I think it can be tricky for people to do it for two days, depending on how restrictive a diet they are already on, and I haven't seen it impact the results terribly that I know of. So 24 hours before the test, you'd have them stop all non-essential medications; 12 hours before, you'd [have them] begin a water fast, so [they'd] avoid everything except for water on the day of the test. [They'd] wake at least one hour prior to the test. They can brush their teeth, but no smoking or vigorous exercise for one hour before the collection, and then the patient does the baseline breath sample. Then they consume the substrate, whether using glucose, it would be 50 grams of glucose or 10 grams of lactulose, with about 120 to 20 milliliters of water. The pediatric dose is 1 gram of lactulose per kilogram of body weight, up to a maximum of 10 grams. So you can do the math here. Any child over 22 pounds would get a full 10 gram dose. And then the breath samples are collected every 15 to 20 minutes for a total of two to three hours, depending on the lab. Hydrogen methane and hydrogen sulfide are measured during these 20-minute intervals. So again, we've included a handout on proper test preparation that you can generate in a PDF generator and give to your patients. [It's] really, really important that they follow the proper procedures. If someone doesn't think they're going to be able to do it, then I often have them wait and prepare until they're able to.



As I mentioned, the proper test prepping requires this 24-hour preparation diet and overnight fast. So we talked about, this is a quick little version of what's included in that diet. You've got

jasmine white rice, meat, poultry or fish, eggs, hard cheese, clear broth without vegetables, oil, salt and pepper, and water. And the purpose is to avoid fermentable carbohydrates or proteins [to] prevent that residual fiber in the gut so that you don't have elevations in gases during your baseline readings. In some cases, like constipation, the prep diet of two days may be required to reduce the baseline gases to zero. So that's one consideration in extending that preparation diet, and, of course, that four-week period before the test making sure that the patient is off of antibiotics or antimicrobials.