

## Standard Operating Procedure: Intestinal Permeability

### Patient instruction

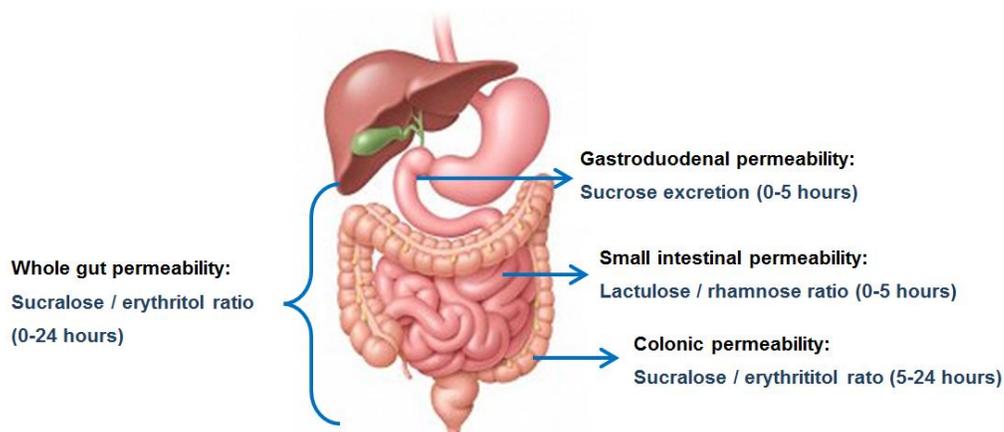
The permeability of the gut can be easily measured by drinking a sugar solution, followed by measuring the sugar excretion in the urine.

During two days prior to the examination and on the day of the examination itself you are not allowed to perform any high intensity exercises. Also, you will be instructed not to consume more than two glasses of alcoholic beverage per day on these days. The night prior to the test, you are allowed to eat until 11:00 p.m., after this you need to stay fasted. However, drinking water is allowed.

After drinking the sugar solution the next morning, you will collect all urine produced during the following 24 hours in plastic bottles provided by us. The urine should be collected in two fractions; one fraction of all urine that is produced during the first 5 hours after ingestion of the sugars, one portion from 5 to 24 hours after ingestion. During the first five hours you need to stay fasted; you are allowed to drink tea without sugar or water as much as you like. After 5 hours, you may resume eating and drinking as usual. Exactly 24 hours after ingesting the sugars you collect the last portion of urine, after which you will return the collected urine in the hospital.

### Background information

**Figure 1** [Mujagic, Ludidi *et al.* 2014]: Multi-sugar test, indicating permeability of four sites of the GI tract [van Wijck *et al* J Chromatogr B Analyt Technol Biomed Life Sci. 2011]. Sucrose (disaccharides) is hydrolyzed by the enzyme sucrase in the duodenum [Sutherland *et al.* Lancet 1994], and is therefore a good marker for gastroduodenal permeability, measured in 0-5h urine. Lactulose (disaccharides) can permeate through the paracellular route and rhamnose (mono-saccharides) through the transcellular route. The *ratio* in 0-5h urine, indicating small intestinal permeability, is used to minimize the effect of gut motility and kidney function on the result. The ratio in 5-24h and 0-24h urine of sucralose (disaccharides) and erythritol (sugar alcohol), both are not degraded by human colonic bacteria [Roberts *et al.* Toxicol 2000; Arrigoni *et al.* Br. J. Nutr. 2005], indicates the colonic and whole gut permeability respectively.



**Products for human consumption, possible suppliers:**

|             |   |
|-------------|---|
| Sucrose:    | Van Gilse, Dinteloord, the Netherlands  |
| Lactulose:  | Centrafarm, Etten-Leur, the Netherlands |
| L-rhamnose: | Danisco, Copenhagen, Denmark            |
| Sucralose:  | Brenntag, Sittard, the Netherlands      |
| Erythritol: | Now Foods, Bloomindale, USA             |

**PROTOCOL**

After an overnight fast (at least 8 hours) the test day will start.

Physical exercise and the use of alcohol or NSAIDs two days prior to the test and during the test is prohibited.

**Needed:**

- Multi-sugar mix
  - o L-rhamnose: 0.5 g
  - o Lactulose: 1.0 g
  - o Sucrose: 1.0 g
  - o Sucralose: 1.0 g
  - o Erythritol: 1.0 g
- 150 ml of cold tap water
- 2 urine jars (volume of 1.5 to 2 liters each jar) for 0-5h and 5-24h fraction (if needed, 1 extra jar).

**Preparation of the multi-sugar mix and the urine jars:**

- Prepare the sugar-mix no longer than 2 hours before start of the test day, in a food grade lab.
- Mix the 5 sugars on an accurate balance and put them in a clean cup (with a cover).
- Note the exact weight of each sugar on the test form and make sure that the weight does not deviate more than 2% of the needed mass.
- Weigh the empty urine jars and note the weight on the test form.

**Test day:**

The sugar drink can be ingested at home or at the lab. The 24-hour urine collection can be done at home.

- After an overnight fast (at least 8 hours) the subject empties the urinary bladder in the toilet.
- After that the sugar-mix has to be dissolved into the cup in 150 ml of cold tap water and the subject drinks the multi-sugar drink (time 0). Note that all the sugar has to be ingested, if still some sugar is left in the cup, add some extra water, dissolve the sugar and drink it.
- For the next 5 hours the subject has to collect the urine in 'Jar 1' (fraction 0-5 hours) and has to stay fasted, with the exception of drinking water or tea without sugar. Clear water can be consumed *ad libidum*.
- The jars with urine have to be stored at a cool place, if possible in a fridge.
- At the end of this period (time 5 hours) the subject has to urinate one last time in 'Jar 1', to make sure that the bladder is empty at the end of the 0-5h period.
- After that the subject can consume food and drinks again and has to collect urine for the rest of the 24-hour period in 'Jar 2' (fraction 5-24 hour).
- At the end of the 24-hour period, at the same time in the morning the next day, the subject has to urinate in 'Jar 2', to make sure that the bladder is empty at the end of the 5-24h period.

- The urine jars have to be brought back to the investigator on the same day for further processing.

**Processing of the urine:**

- Fill in the test form (date and time of multi-sugar intake and urine collection).
- Weigh the full urine jars and note the weight on the test form.
- Shake or stir the urine in the jar.
- Aliquot 3 x 1 ml per urine jar and fill 3 Eppendorf tubes per urine fraction (0-5 and 5-24h fraction). Make sure the tubes are labeled correctly.
- Store the tubes at -80 °C until analysis and note the location on the test form.

**Analysis of sugar probes:**

- The urinary excreted sugars will be analyzed by High-performance liquid chromatography - mass spectrometry (HPLC-MS) as described by van Wijck *et al.* 2011 [van Wijck *et al.* Novel analytical approach to a multi-sugar whole gut permeability assay. J Chromatogr B Analyt Technol Biomed Life Sci. 2011].

**Analysis of data:**

- 0-5h fraction = Absolute volume of Jar 1 (full volume – baseline volume)
- 5-24h fraction = Absolute volume of Jar 2 (full volume – baseline volume)
- 0-24h fraction = Absolute volume of Jar 1 + 2
- Urinary sugar excretion = urine sugar concentration (HPLC) multiplied by urine volume
- Sugar permeability ratio = urine sugar excretion of larger sugar probe / urine sugar excretion of smaller sugar probe

**GUT PERMEABILITY MEASUREMENT**

**Study:**.....

**TEST FORM**

**Subject number:** .....

**Date:** .....

**Investigator:**.....

**Signature investigator:**.....

Sugars: Real weight of the sugars:

L-rhamnose (0.5g) .....

Lactulose (1g) .....

Sucrose (1g) .....

Sucralose (1g) .....

Erythritol (1g) .....

**Intake / processing**

*date*

*time*

Date / Time intake sugars: |\_|\_| |\_|\_| |\_|\_|\_|\_|\_| |\_|\_| :  
|\_|\_| u

Date / Time urine received: |\_|\_| |\_|\_| |\_|\_|\_|\_|\_| |\_|\_| :  
|\_|\_| u

Date / Time urine processed: |\_|\_| |\_|\_| |\_|\_|\_|\_|\_| |\_|\_| :  
|\_|\_| u

**Baseline weight of jars:**

Jar 1 (0-5h fraction): .....gr.

Jar 2 (5-24h): .....gr.

Extra Jar 3 (5-24h): .....gr.

**Weight of jars with collected urine:**

Jar 1 (0-5h fraction): .....gr.

Jar 2 (5-24h): .....gr.

Extra Jar 3 (5-24h): .....gr.

**Number of samples (tubes):** (3x1ml / fraction)

Urine 0-5h:

Urine 5-24h:

**Location storage -80 °C freezer:**