

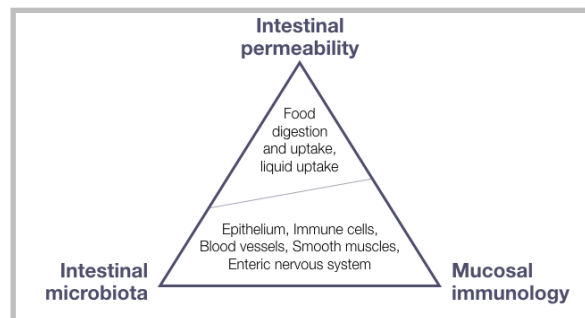
Gut Diagnosis Intestinal Permeability Review

Gut barrier consists of multiple layers:

1. External physical barrier.
2. Inner, functional physiological barrier.

Successful interaction of these two barriers allows appropriate permeability to be maintained, and when either system malfunctions, permeability becomes inappropriate, and this is what we refer to as leaky gut.

The intestine should be leaky to some degree because that is how we absorb nutrients. If the intestine wasn't at all leaky, we couldn't get any of the nutrition that we need, so leaky gut is actually referring to intestinal permeability to the wrong molecules at the wrong times.



Possible **causes of impairment** of intestinal barrier

Nutrition	e.g. Western diet, lack of fermentable carbohydrates and fermented foods
Infections and toxins	e.g. bacterial, viral, parasitic infections; fungal overgrowth; heavy metals; mycotoxins
Medications	e.g. PPIs, antibiotics, NSAIDs
Lifestyle	e.g. chronic stress, sleep deprivation, inappropriate physical activity
“Hygiene hypothesis”	Inadequate immune stimulation during crucial developmental period
Endogenous factors	Chronic inflammation, SIBO, gut-brain dysfunction, low MSH
Genetic susceptibility	e.g. 70% of asymptomatic relatives of CD patients positive for ↑ intestinal permeability

Diseases associated with intestinal permeability

Intestinal	Extra-intestinal
Gastric ulcers	Allergies
Infectious diarrhea	Autoimmune disease
Irritable Bowel Syndrome (IBS) & other functional GI diseases	Arthritis and other inflammatory conditions
Inflammatory Bowel Disease (IBD)	Obesity and metabolic disease
Celiac disease	Parkinson's, Alzheimer's, and other neurological conditions
Cancer (esophageal, colorectal)	Autism Spectrum Disorder, ADHD and other behavioral disorders

Adapted from: Bischoff et al. BMC Gastroenterology 2014, 14:189

A combination of genetic susceptibility and environmental triggers cause the mucosal barrier to become permeable, and this leads to enlarged spaces between the cells of the gut wall and disassociation of tight junction proteins.

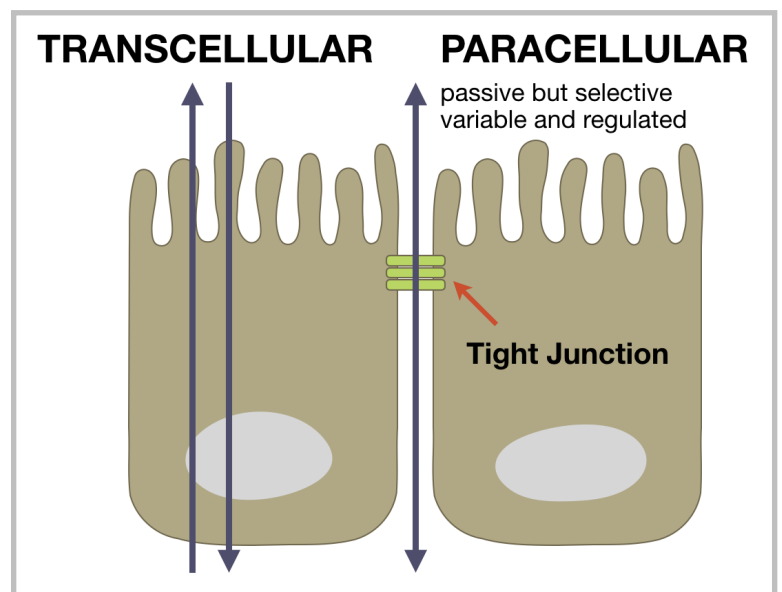
There are two key transport mechanisms of antigens through the gut barrier:

Transcellular means through the cell, and that's where the antigen passes through the cell itself.

Paracellular means between cells, where the antigen passes between the cells.

Endotoxins that pass through the gut barrier into the blood and **elicit an autoimmune response**.

Bacterial toxins act as superantigens to T-lymphocytes or provoke a response through **molecular mimicry**. Many bacteria have antigenic sites that are similar to human tissue antigens, so the body will attack both the endotoxins and the self tissue during the defensive response.



Strong connection between autoimmune disease and intestinal permeability.

Dr. Alessio Fasano, discoverer of zonulin, one of the proteins that regulates tight junction function, believes that an individual cannot develop an autoimmune disease without having intestinal permeability.

Leaky gut can also result in chronic systemic inflammation, which is the root of virtually all modern diseases.

Taking a functional medicine approach to intestinal permeability:

- We find that it is almost always caused by something else: poor diet, gut infections, chronic stress, etc.
- We want to remove all these triggers before addressing intestinal permeability specifically.
- In many cases, we found that once you remove the triggers, the barrier integrity is restored without additional intervention because the intestinal epithelium regenerates itself every five days.

In clinical practice, there are two primary methods of assessing leaky gut

1. Lactulose/mannitol permeability assay (Genova)
2. Antigenic permeability screen (Cyrex Array 2)

The **lactulose/mannitol** test measures levels of both sugars in a patient's urine after oral ingestion. Lactulose and mannitol are both oligosaccharides.

Their differing molecular weights help determine if permeability is transcellular or paracellular.

- Mannitol
 - Small enough to pass through the cell (transcellular).
 - Weighs 182 daltons with a molecular radius up to 0.4 nanomicros.
- Lactulose
 - Larger size means it passes between cells (paracellular).
 - Weighs 342 daltons with a molecular radius of 0.42 nanomicros.

In a healthy person, the smaller mannitol molecules are expected to be measured in larger amounts, normally from 10 to 30 percent of the orally ingested dose. Lactulose would be expected to be recovered at only about 1 percent of the oral dose due to its larger size.

Gut barrier integrity depends on the proper functioning of the **intercellular tight junctions** and should, therefore, limit transit via the paracellular route. A higher lactulose-to-mannitol ratio is indicative of **intestinal permeability**.

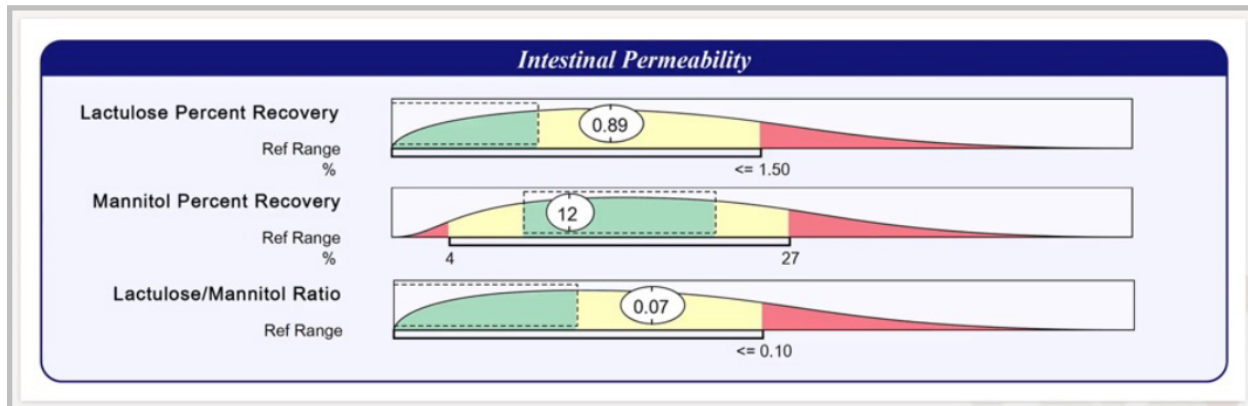
The changed ratio can have several causes:

- Increased lactulose absorption via the paracellular route. This may be due to decreased villous height or impaired function of tight junctions.
- Decreased mannitol absorption due to decreased surface areas of gut villi.

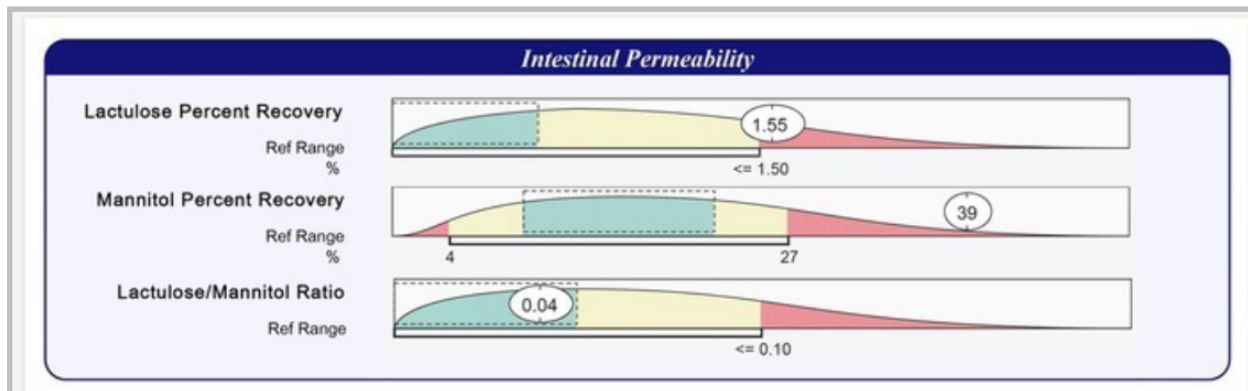
Lactulose mannitol test interpretation matrix

Analyte	Result	Analyte	Result	Analyte	Result	Indication
Lactulose	High	Mannitol	Normal	L/M Ratio	High	Increased IP
Lactulose	High	Mannitol	Low	L/M Ratio	High	Increased IP + malabsorption
Lactulose	High	Mannitol	High	L/M Ratio	Normal	Increased IP d/t insulin resistance
Lactulose	Normal	Mannitol	Normal	L/M Ratio	Normal	Normal IP
Lactulose	Normal	Mannitol	Low	L/M Ratio	High	Malabsorption
Lactulose	Normal	Mannitol	High	L/M Ratio	Low	↑ transcellular permeability
Lactulose	Low	Mannitol	High	L/M Ratio	Low	↑ trans ↓ para permeability
Lactulose	Low	Mannitol	Normal	L/M Ratio	Low	↓ paracellular permeability
Lactulose	Low	Mannitol	Low	L/M Ratio	Normal	Malabsorption?

GENOVA'S INTESTINAL PERMEABILITY ASSAY:



In this example, lactulose is borderline high, and mannitol is normal. This creates a borderline lactulose-to-mannitol ratio. This patient is a 30-year-old female with interstitial cystitis and vulvodynia, and she had this test prior to starting her work with us. We identified cryptosporidium and SIBO; we treated these and then retested her intestinal permeability markers, and her results normalized.



In the above example, both lactulose and mannitol are above the reference range, so one possibility for this is metabolic dysfunction. In this case, we're seeing an increase in intestinal permeability, but the cause is likely to be metabolic dysfunction rather than gut pathology.

- Another possibility, and one of the criticisms of lactulose-mannitol testing, is that transport of lactulose and mannitol through the barrier is not necessarily an indicator of tight junction malfunction. Many factors can influence the uptake of these sugars, including GI motility, use of medications like NSAIDs, smoking, alcohol intake, variations in gastric emptying, surface area of the intestine, mucosal blood flow, and renal clearance.
- In addition, only molecules larger than 5,000 daltons can change permeability of the intestinal epithelial cells and result in T-cell response and production of cytokines and antibodies. Lactulose and mannitol are below 500 daltons, so they may not be appropriate as challenge molecules to determine pathological intestinal permeability.

ANTIGENIC PERMEABILITY SCREEN: (CYREX ARRAY 2)

Measures immune reactivity and permeability to three molecules:

Antibodies:

- **IgG:** Indicates **previous exposure** of that antigen, but alone does not indicate a current problem.
- **IgM & IgA:** **Indicative of a current problem.**

1. Lipopolysaccharides (LPS)

- An endotoxin present in the cell membrane of Gram-negative bacteria
- Measures IgG, IgM, and IgA
- Detection of antibodies indicates infiltration of large endotoxins through the intestinal barrier into systemic circulation

2. Occludin/Zonulin

- Occludin is the main component of proteins that hold tight junctions together. Antibodies can indicate breakdown of these tight junctions.
 - Measures IgG, IgM, and IgA
- Zonulin regulates the opening and closing of tight junctions. Antibodies suggest that regulation of these tight junctions may be compromised.

3. Actomyosin

- A protein that regulates plasticity of tight junctions.
- Measures IgA
- Antibodies can indicate transcellular permeability and movement of molecules through cells.
 - Very common in celiac.
- Antibodies to actomyosin alone indicate autoimmunity against the cell epithelium or other tissues of the gut barrier.
- Any result above 10 is clinically significant, even if it's marked as equivocal.

CYREX ARRAY 2 EXAMPLES:

When the patient has antibodies to actomyosin alone, that suggests autoimmunity against mucosal epithelium and other tissue cell cytoskeleton of the gut barrier.

TEST	RESULT			
Array 2 – Intestinal Antigenic Permeability Screen	IN RANGE (Normal)	EQUIVOCAL*	OUT OF RANGE	REFERENCE (ELISA Index)
Actomyosin IgA **		20.55		0.0-20
Occludin/Zonulin IgG	0.75			0.2-1.5
Occludin/Zonulin IgA		1.58		0.1-1.8
Occludin/Zonulin IgM	0.66			0.1-2.1
Lipopolysaccharides (LPS) IgG			2.53	0.1-1.6
Lipopolysaccharides (LPS) IgA			4.42	0.1-1.8
Lipopolysaccharides (LPS) IgM	0.26			0.1-2.0

In this next test result, we see equivocal or out-of-range antibodies to actomyosin, occludin/zonulin IgA, and LPS, both IgG and IgA. This indicates both paracellular and transcellular breakdown of the gut barrier. Equivocal IgG antibodies to occludin and zonulin only suggests previous tight junction malfunction with paracellular permeability but nothing currently, so this is someone that had gut barrier permeability in the past, but it's no longer an issue.

TEST	RESULT			
Array 2 – Intestinal Antigenic Permeability Screen	IN RANGE (Normal)	EQUIVOCAL*	OUT OF RANGE	REFERENCE (ELISA Index)
Actomyosin IgA **	9.50			0.0-20
Occludin/Zonulin IgG	0.94			0.2-1.5
Occludin/Zonulin IgA	1.10			0.1-1.8
Occludin/Zonulin IgM	0.96			0.1-2.1
Lipopolysaccharides (LPS) IgG		1.39		0.1-1.6
Lipopolysaccharides (LPS) IgA			2.15	0.1-1.8
Lipopolysaccharides (LPS) IgM	0.70			0.1-2.0

On this test, LPS IgG was equivocal, and IgA was out of range. We would treat as a positive given the IgA being out of range.

Antibodies to LPS only is not as significant as it would be if you also had antibodies to occludin/zonulin and/or actomyosin elevated because those would indicate a breakdown of the gut barrier via transcellular and paracellular pathway, respectively, whereas when you only see the elevated lipopolysaccharide antibodies, that's just evidence of dysbiosis and passage of endotoxins into the bloodstream.

**Interpretation of antibodies against LPS,
occludin / zonulin and actomyosin network**

LPS IgA, IgG, IgM	+	+	-	+	-
Occludin/Zon. IgA, IgG, IgM	-	+	+	-	-
Actomyosin IgA	-	-	-	+	+
Clinical indication	Gut dysbiosis	Bacterial paracellular permeability	Non-bacterial paracellular permeability	Bacterial transcellular permeability	Autoimmunity against epithelium/ cell cytoskeleton

Adapted from: [Cyrex Array 2 Clinical Applications Guide. http://cyrexlabs.com](http://cyrexlabs.com)

Antigenic permeability screen vs. L/M assessment

Lactulose mannitol assessment	Antigenic permeability screen
Upper small intestine	Entire length of small intestine and large intestine
Small sugar molecules <350 Da in size; not antigenic	Large molecules >10,000 Da; strongly antigenic
Small sugar molecules don't always correlate with uptake of larger dietary and microbial antigens	Positive correlation between large molecules and dietary and microbial antigens
Permeability to small molecules not always pathological; high risk of false positives	Permeability to large molecules indicates damage to tight junctions and pathological permeability; low risk false positive
Small openings in tight junctions can be repaired in hours; high risk of false negative	Large openings in tight junctions take longer for repair; low risk of false negative
L/M affected by GI motility, renal clearance, variations in gastric emptying, smoking, alcohol, etc.	Permeability to large molecules is not affected by these factors

Adapted from: [Vojdani Altern Ther Health Med.](#) 2013 Jan-Feb;19(1):12-24

Overall, the lactulose-mannitol screen has a higher risk of false positive. It's not reliable on its own but may be worthwhile in conjunction with the antigenic permeability screen.