KRESSER INSTITUTE FOR FUNCTIONAL AND EVOLUTIONARY MEDICINE

Gut: Intestinal Permeability, Part 3

So here's a comparison of the lactulose-mannitol test and the antigenic permeability screen:

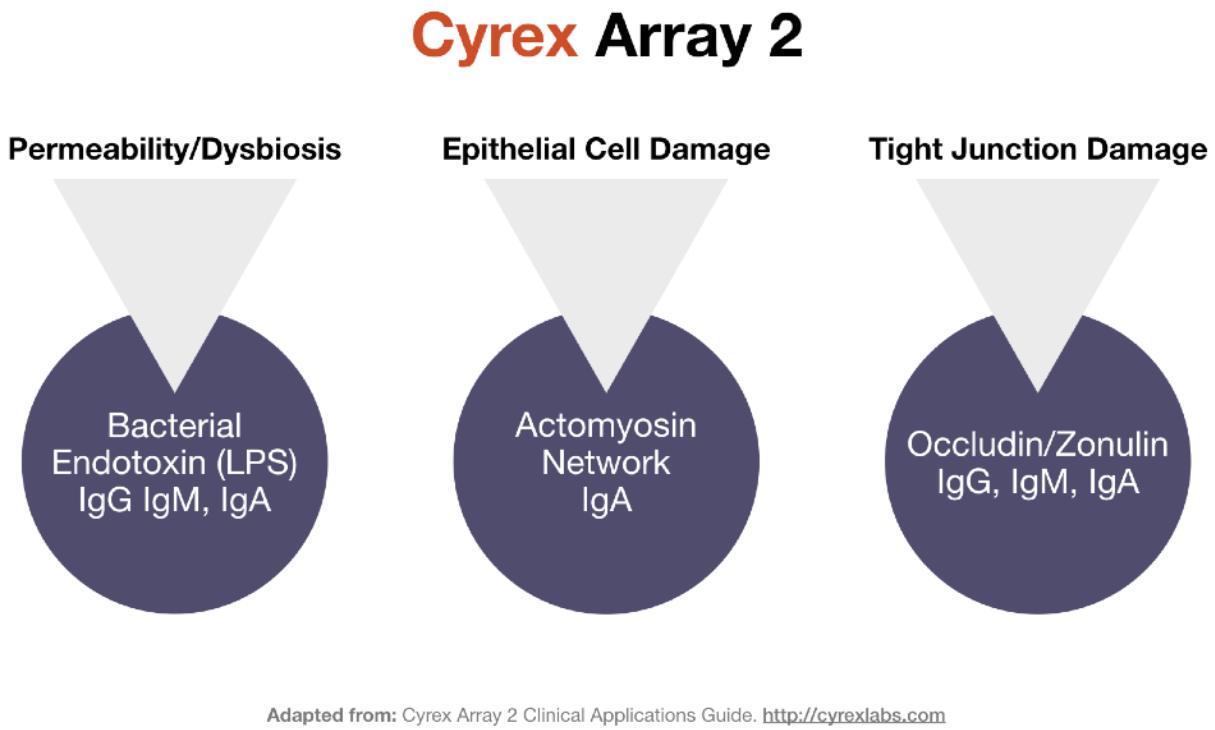
Antigenic permeability screen vs. L/M assessment

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

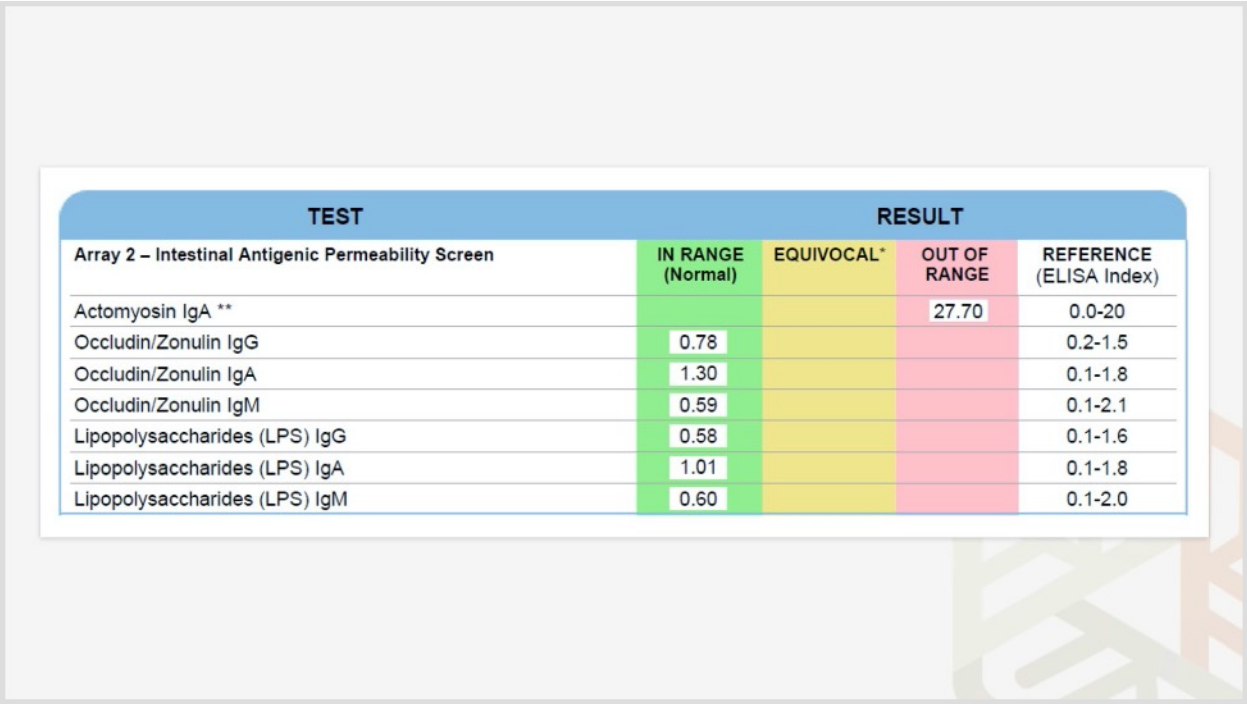
The lactulose-mannitol assessment is looking at mostly the upper small intestine, where the antigenic permeability screen looks at the entire length of the small intestine as well as the large intestine. The lactulose-mannitol assay uses small sugar molecules, under 500 daltons, even under 350 daltons, actually, in size, and they're not antigenic, they don't provoke an immune response in the body. The antigenic permeability screen uses large molecules that are bigger than 10,000 daltons and that are strongly antigenic. The small sugar molecules in the lactulose-mannitol assessment don't always correlate with uptake with larger dietary and microbial antigens, whereas with the antigenic permeability screen, there is a positive correlation between large molecules and dietary and microbial antigens. With the lactulose-mannitol assessment, permeability of small molecules is not always pathological, so you get a higher risk of false positives, whereas the antigenic permeability screen, with the permeability to large molecules, that does indicate damage to tight junctions and pathological permeability, so there's a lower risk of false positive. In the lactulose-mannitol assessment, the small openings in tight junctions can be repaired in hours, so you could get a high risk of false negative, whereas with the antigenic permeability screen, large openings in tight junctions take much longer for repair, so there's a low risk for false negative. And finally, lactulose-mannitol is affected by GI motility, renal clearance, variations in gastric emptying, smoking, and alcohol, whereas the antigenic permeability screen is not affected by those factors.

So overall, I'd say that 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. The lactulose-mannitol can tell you about malabsorption due to blunting of the intestinal villi and decreased absorptive surface area, which you don't get that information from the antigenic permeability screen, and that can be valuable.



Adapted from: Cyrex Array 2 Clinical Applications Guide. http://cyrexlabs.com

Cyrex Array 2 measures immune reactivity and thus permeability to three molecules. The first is lipopolysaccharide, which is an endotoxin present in the cell membrane of gram-negative bacteria. The detection of antibodies to LPS, lipopolysaccharide, indicates infiltration of large endotoxins through the intestinal barrier into the systemic circulation. The second molecule is occludin/zonulin. Occludin is a main component of proteins that hold tight junctions together. Antibodies to occludin indicate the tight junction is breaking down, whereas zonulin is a protein that regulates the opening and closing of tight junctions. Antibodies to zonulin indicate regulation of tight junctions has been compromised, and it suggests an ongoing mechanism that is triggering damage to the gut barrier. And finally, the third molecule is actomyosin, and this is a protein that regulates the plasticity of tight junctions. Antibodies to actomyosin indicate transcellular permeability and movement of molecules through the cells rather than between cells, as zonulin/occludin and LPS would indicate. Antibodies to actomyosin are very common in patients with celiac disease; in fact, one study found that 98 percent of celiac patients have antibodies to actomyosin.



TEST	RESULT			
Array 2 – Intestinal Antigenic Permeability Screen	IN RANGE (Normal)	EQUIVOCAL*	OUT OF RANGE	REFERENCE (ELISA Index)
Actomyosin IgA **			27.70	0.0-20
Occludin/Zonulin IgG	0.78			0.2-1.5
Occludin/Zonulin IgA	1.30			0.1-1.8
Occludin/Zonulin IgM	0.59			0.1-2.1
Lipopolysaccharides (LPS) IgG	0.58			0.1-1.6
Lipopolysaccharides (LPS) IgA	1.01			0.1-1.8
Lipopolysaccharides (LPS) IgM	0.60			0.1-2.0

So here's what Cyrex Array 2 looks like. You can see the antigens we've discussed: actomyosin, occludin/zonulin, LPS. With actomyosin, they only test IgA antibodies. With occludin and zonulin and LPS, they run IgA, IgG, and IgM. In general, IgG antibodies indicate previous exposure of that antigen to serum, so they would signal a past issue. IgM and IgA are more indicative of a current problem, and when the patient has antibodies to actomyosin alone, as is the case on this slide, 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 **		20.83		0.0-20
Occludin/Zonulin IgG	0.70			0.2-1.5
Occludin/Zonulin IgA		1.36		0.1-1.8
Occludin/Zonulin IgM	0.79			0.1-2.1
Lipopolysaccharides (LPS) IgG	0.95			0.1-1.6
Lipopolysaccharides (LPS) IgA	1.29			0.1-1.8
Lipopolysaccharides (LPS) IgM	0.59			0.1-2.0

Here, actomyosin is marked as equivocal; however, anything above 10 for actomyosin is clinically significant. Cyrex uses the actomyosin reference range established by the kit manufacturer. This reference range is based on a disease model. Most functional medicine providers, myself included, tend to consider equivocal results positive. The laboratory reference range is, again, indicative of a disease state, and we're trying to prevent this disease state from occurring in the first place. That's one of the key differences between functional and conventional medicine. So you could say that equivocal ranges may be catching problems earlier than they would otherwise be caught in the conventional model, before disease occurs. Notice that occuldin and zonulin IgA are also elevated in the equivocal range. So together, these two results would indicate a breakdown of the gut barrier via both the paracellular and transcellular pathways.

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

Here we see equivocal or out-of-range antibodies to actomyosin IgA, to occludin/ zonulin IgA, and to LPS, both IgG and IgA. This indicates both paracellular and transcellular breakdown of the gut barrier.

TEST	RESULT			
Array 2 – Intestinal Antigenic Permeability Screen	IN RANGE (Normal)	EQUIVOCAL*	OUT OF RANGE	REFERENCE (ELISA Index)
Actomyosin IgA **	3.23			0.0-20
Occludin/Zonulin IgG		1.15		0.2-1.5
Occludin/Zonulin IgA	0.39			0.1-1.8
Occludin/Zonulin IgM	1.24			0.1-2.1
Lipopolysaccharides (LPS) IgG	0.54			0.1-1.6
Lipopolysaccharides (LPS) IgA	0.62			0.1-1.8
Lipopolysaccharides (LPS) IgM	1.07			0.1-2.0

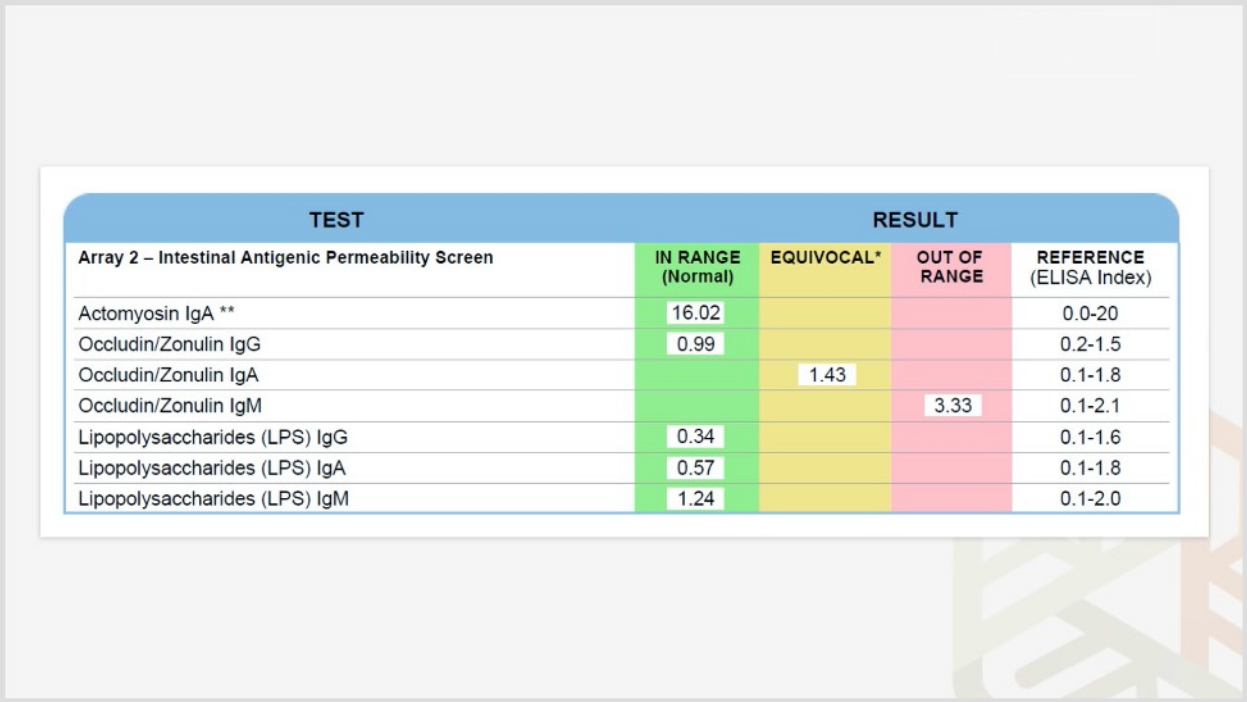
In this case, we see equivocal IgG antibodies to occludin and zonulin only, so it suggests previous tight junction malfunction with paracellular permeability, but nothing currently, so this is someone that had gut barrier permeability in the past, but it's no longer an issue.

TEST	RESULT			
Array 2 – Intestinal Antigenic Permeability Screen	**IN RANGE (Normal)**	**EQUIVOCAL***	**OUT OF RANGE**	**REFERENCE** (ELISA Index)
Actomyosin IgA **	13.76			0.0-20
Occludin/Zonulin IgG			1.58	0.2-1.5
Occludin/Zonulin IgA	1.04			0.1-1.8
Occludin/Zonulin IgM	1.40			0.1-2.1
Lipopolysaccharides (LPS) IgG	0.87			0.1-1.6
Lipopolysaccharides (LPS) IgA	0.89			0.1-1.8
Lipopolysaccharides (LPS) IgM	0.75			0.1-2.0

This test result shows occludin/zonulin IgG elevated; it indicates a past gut barrier problem that has since resolved.

TEST	RESULT			
Array 2 – Intestinal Antigenic Permeability Screen	**IN RANGE (Normal)**	**EQUIVOCAL***	**OUT OF RANGE**	**REFERENCE** (ELISA Index)
Actomyosin IgA **	4.60			0.0-20
Occludin/Zonulin IgG	0.47			0.2-1.5
Occludin/Zonulin IgA	0.52			0.1-1.8
Occludin/Zonulin IgM			2.44	0.1-2.1
Lipopolysaccharides (LPS) IgG	0.34			0.1-1.6
Lipopolysaccharides (LPS) IgA	0.73			0.1-1.8
Lipopolysaccharides (LPS) IgM	0.95			0.1-2.0

This one is a strong positive for occludin/zonulin IgM. It's a 63-year-old female with constipation, rheumatoid arthritis, and significant memory and cognitive issues. She was eating gluten, and Cyrex Array 3 testing revealed non-celiac gluten sensitivity. She also had other intolerances, food intolerances, fungal overgrowth, and dysbiosis, and after treatment, this marker, occludin/zonulin, dropped into the normal range.

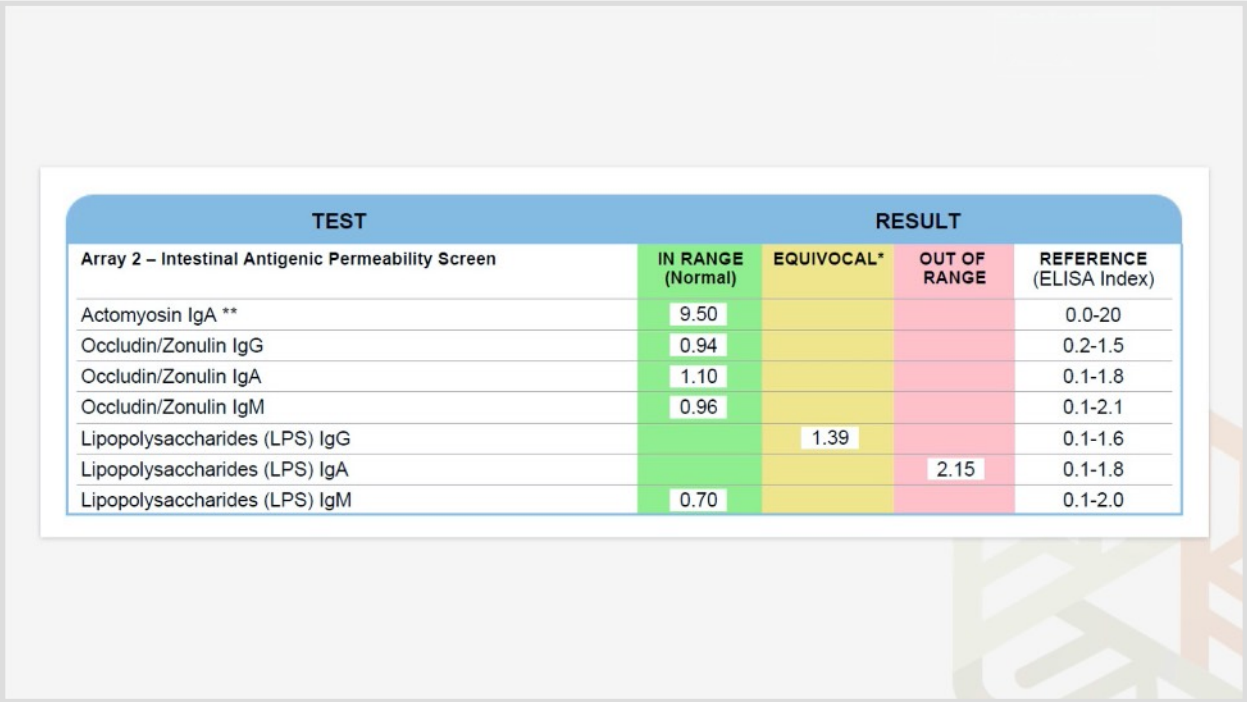


TEST	RESULT			
Array 2 – Intestinal Antigenic Permeability Screen	IN RANGE (Normal)	EQUIVOCAL*	OUT OF RANGE	REFERENCE (ELISA Index)
Actomyosin IgA **	16.02			0.0-20
Occludin/Zonulin IgG	0.99			0.2-1.5
Occludin/Zonulin IgA		1.43		0.1-1.8
Occludin/Zonulin IgM			3.33	0.1-2.1
Lipopolysaccharides (LPS) IgG	0.34			0.1-1.6
Lipopolysaccharides (LPS) IgA	0.57			0.1-1.8
Lipopolysaccharides (LPS) IgM	1.24			0.1-2.0

Here, both occludin/zonulin IgA and IgM are significantly elevated, and IgM is very elevated, 3.3 in the range of 0.1 to 2.1. This was a 40-year-old male with muscle aches, insomnia, frequent colds and flus, and one of the highest mercury levels I've ever seen. He had equivocal gluten intolerance with multiple food intolerances on Cyrex Array 10.

TEST	RESULT			
Array 2 – Intestinal Antigenic Permeability Screen	**IN RANGE (Normal)**	**EQUIVOCAL***	**OUT OF RANGE**	**REFERENCE** (ELISA Index)
Actomyosin IgA **	5.92			0.0-20
Occludin/Zonulin IgG	0.48			0.2-1.5
Occludin/Zonulin IgA	0.46			0.1-1.8
Occludin/Zonulin IgM	1.42			0.1-2.1
Lipopolysaccharides (LPS) IgG	0.44			0.1-1.6
Lipopolysaccharides (LPS) IgA	0.63			0.1-1.8
Lipopolysaccharides (LPS) IgM			2.05	0.1-2.0

In this test result, the only marker that's out of range is lipopolysaccharide IgM. IgM does indicate current antibody production, so that is something we would consider a positive active result. This patient is a 29-year-old male with significant dysbiosis and SIBO. His main complaint was rosacea and brain fog, also had high cholesterol. Interestingly enough, LDL plays an antimicrobial role, and it's produced to clear endotoxins in cases of sepsis.



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, and this is evidence of dysbiosis and passage of endotoxins into the bloodstream, so when you see only antibodies to LPS, it's not as significant as it would be if you had antibodies to occludin/zonulin and/or actomyosin also are 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.

TEST	RESULT			
Array 2 – Intestinal Antigenic Permeability Screen	IN RANGE (Normal)	EQUIVOCAL*	OUT OF RANGE	REFERENCE (ELISA Index)
Actomyosin IgA **	6.22			0.0-20
Occludin/Zonulin IgG	0.66			0.2-1.5
Occludin/Zonulin IgA	0.57			0.1-1.8
Occludin/Zonulin IgM	0.75			0.1-2.1
Lipopolysaccharides (LPS) IgG			1.87	0.1-1.6
Lipopolysaccharides (LPS) IgA	0.67			0.1-1.8
Lipopolysaccharides (LPS) IgM			2.05	0.1-2.0

In this test, we have both LPS IgG and IgM out of range, indicating an active problem and no other markers out of range, so again, this is indicative of dysbiosis and passage of endotoxins into the blood, but not necessarily disruption of paracellular or transcellular permeability. This was a 26-year-old female who'd recently returned from the Peace Corps in Africa. She had three parasites and really significant dysbiosis, fatigue, muscle aches, and GI distress.

TEST	RESULT			
Array 2 – Intestinal Antigenic Permeability Screen	IN RANGE (Normal)	EQUIVOCAL*	OUT OF RANGE	REFERENCE (ELISA Index)
Actomyosin IgA **	4.04			0.0-20
Occludin/Zonulin IgG	0.79			0.2-1.5
Occludin/Zonulin IgA	0.30			0.1-1.8
Occludin/Zonulin IgM			2.13	0.1-2.1
Lipopolysaccharides (LPS) IgG	0.60			0.1-1.6
Lipopolysaccharides (LPS) IgA	0.47			0.1-1.8
Lipopolysaccharides (LPS) IgM			2.06	0.1-2.0

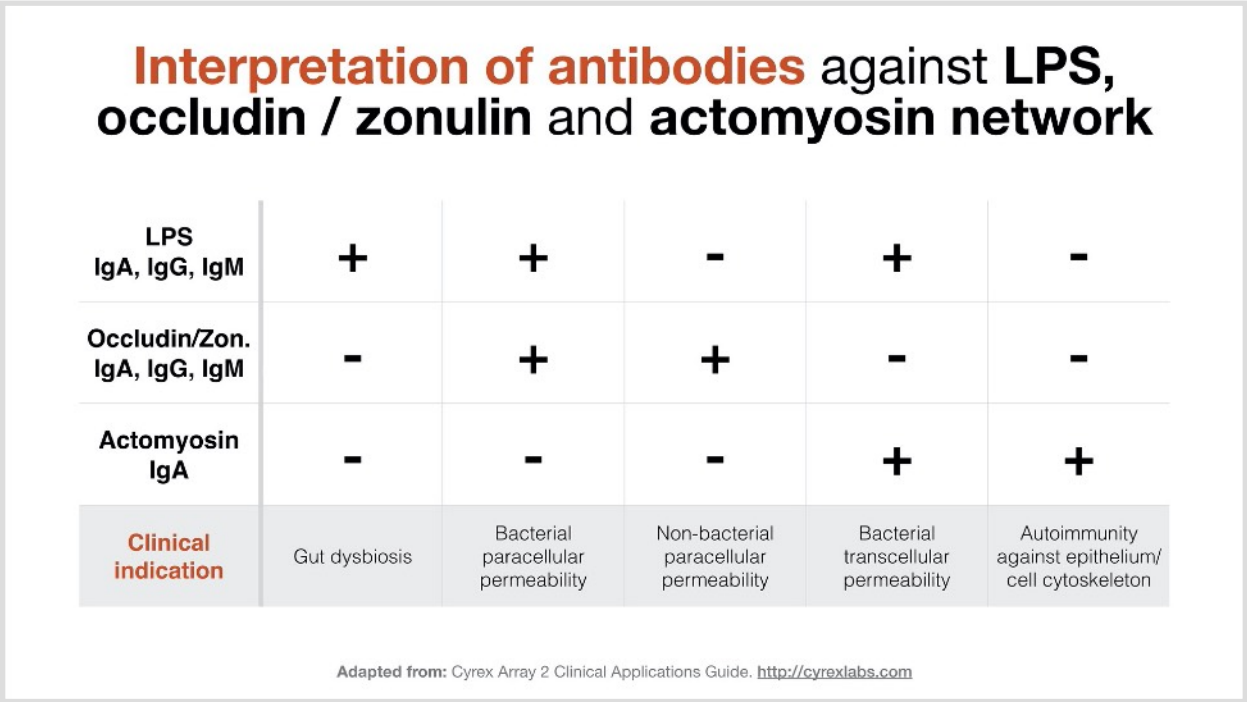
This result shows elevations in IgM, occludin/zonulin, and also LPS IgM, so this is an active problem, indicates the breakdown of the gut barrier by bacterial antigens, that's the LPS part, by the paracellular pathway, which is the occludin/zonulin part. So this is a 42-year-old female with celiac disease; she was not eating gluten regularly, but she wasn't strictly avoiding it either, and that can be enough to cause intestinal permeability in someone with celiac.

TEST	RESULT			
Array 2 – Intestinal Antigenic Permeability Screen	IN RANGE (Normal)	EQUIVOCAL*	OUT OF RANGE	REFERENCE (ELISA Index)
Actomyosin IgA **	7.96			0.0-20
Occludin/Zonulin IgG			1.71	0.2-1.5
Occludin/Zonulin IgA	0.56			0.1-1.8
Occludin/Zonulin IgM	0.68			0.1-2.1
Lipopolysaccharides (LPS) IgG		1.44		0.1-1.6
Lipopolysaccharides (LPS) IgA	1.01			0.1-1.8
Lipopolysaccharides (LPS) IgM	1.22			0.1-2.0

Here's an example of someone with gut barrier dysfunction that has resolved, so you see IgG antibodies to occludin/zonulin, you also see IgG antibodies to lipopolysaccharide, but nothing for IgA or IgM, so this again is an issue that was a problem at one point but is likely not a problem now.

TEST	RESULT			
Array 2 – Intestinal Antigenic Permeability Screen	IN RANGE (Normal)	EQUIVOCAL*	OUT OF RANGE	REFERENCE (ELISA Index)
Actomyosin IgA **			32.15	0.0-20
Occludin/Zonulin IgG	0.66			0.2-1.5
Occludin/Zonulin IgA			2.07	0.1-1.8
Occludin/Zonulin IgM			2.86	0.1-2.1
Lipopolysaccharides (LPS) IgG			1.68	0.1-1.6
Lipopolysaccharides (LPS) IgA	1.25			0.1-1.8
Lipopolysaccharides (LPS) IgM		1.60		0.1-2.0

So here you see an extreme result. All three antibody classes are out of range: actomyosin is sky high, the range is 0 to 20, and it's 32.15; and you have occludin/zonulin IgA is elevated, occludin/zonulin IgM is elevated; you've got lipopolysaccharide IgG is 1.68, so that's elevated, and then you have LPS IgM in the equivocal range. So this indicates both transcellular and paracellular intestinal permeability, so actomyosin indicating transcellular, occludin/zonulin indicating paracellular, and then LPS indicating bacterial antigens entering the circulation.



Okay, here is an interpretation matrix that I created, and since we just went over several results that cover this in detail, I'm not going to go through each square of this matrix, but we will include it as a handout and you can use it as a quick reference in your clinic. Okay, so that's it for the intestinal permeability presentation. Hope you got a lot out of it, and I'll be back next time.