R.E.D. Laboratories
Specialty Testing for Multifactorial Afflictions
FOCUS ON GASTROINTESTINAL DYSFUNCTIONS

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R.E.D. Laboratories

WHO WE ARE

- R.E.D. Laboratories is private Belgian company developing tests for patients with complex clinical picture, chronic immune diseases and intestinal dysfunctions
- We actively pursue new tests development in order to provide clinicians with updated tools

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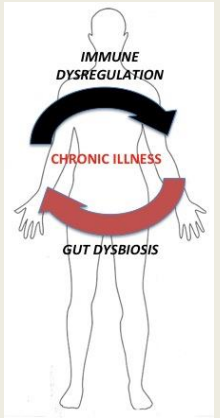
R.E.D. Laboratories

HOW WE WORK

- At R.E.D. Laboratories, we are continuously developing new tests according to the specific needs from health care providers.
- All generated benefits are used for research and development of new assays.
- We are involved in several international groups aiming to advance knowledge in biological markers of **multifactorial afflictions** that are not optimally supported by general health care systems.



R.E.D. Laboratories

OUR PHILOSOPHY

- We focus on setting up the tests that are not (or rarely) available elsewhere.
- Personalization of testing panel leads to more efficient and rapid management of patients with complex clinical picture.
- Assay development programs at R.E.D. Laboratories focus on disorders that contribute to the onset and pathogenesis of diseases such as **chronic fatigue syndrome, autism, chronic infections or autoimmune diseases**.

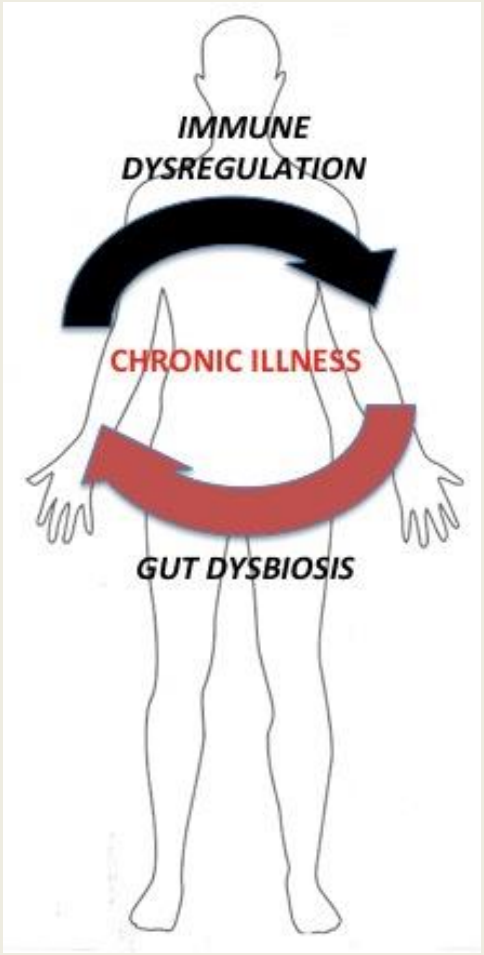
R.E.D. Laboratories

WHAT WE OFFER

- More and more evidence points towards a combination of factors (genetic, infectious, environmental, etc.) being important in the development of chronic immune dysfunctions, the cardinal finding in autistic, chronically infected and CFS patients.
- In many countries these affections are still considered as psychiatric despite clear biomedical evidence.

For better management of these multifactorial affections suffering from lack of medical recognition, we offer **SPECIALTY TESTS** focused on 3 major topics:

- **1. immune disorders,**
- **2. intestinal dysfunctions and**
- **3. infections.**


IMMUNE
DYSREGULATION
CHRONIC ILLNESS
GUT DYSBIOSIS

Intestinal dysfunctions

Regulation of immune function in the gut

- **70% of our immune cells reside in the gut**
  - Gut associated lymphoid tissue (GALT) is spread along the intestinal mucosa (Peyer's patch in the small intestine, lymphoid follicles in the colon) and hosts 70% of the body's immune cells
  - These immune cells permanently interact with mucosa-associated micro-organisms (bacteria, viruses…)
  - A delicate balance is maintained between tolerance to gut antigens (down-regulation of inflammation,…), and defense against pathogens (production of defensins,…)
- **Imbalance of gut immunity affects the whole body**
  - ***Gut barrier integrity is essential :*** Increased permeability of the mucosa causes systemic endotoxemia (chronic low grade inflammation) and abnormal immune reactions to gut antigens
  - ***Interactions host/gut flora :*** the gut microbial flora plays a major role in maintenance of host health, but can be affected by abnormal host immune function

Intestinal dysfunctions

Establishment and composition of the gut flora

- ***Human GI tract is colonized by 10^{14} microorganisms***
  - 10 times more than human cells, 1.5kg of biomass.
- ***Colonization starts immediately at birth***
  - **Establishment of specific populations** will depend on many factors, (normal birth or cesarean section, breast- vs formula feeding, hygiene conditions during the first months of life, early use of antibiotics, genetics…). At 1-2 years of age the ecosystem stabilizes.
  - **Extreme diversity** (500 to 1,000 different bacterial species)
  - **Specificity**: each individual displays a unique pattern of microbial diversity
  - **Apparent stability** over life, good resilience, but can be affected by drugs, infections, diet changes…

Intestinal dysfunctions

Gut flora and health

Germ-free mice can survive but present developmental and morphological alterations, as well as abnormalities in their immunological and nutritional functions. Although some bacteria are potentially pathogenic, host-bacterial interaction is mainly symbiotic

The microbiome contributes to the processing and metabolization of food

- digestion and absorption of nutrients

- sugar metabolism

-synthesis of short chain fatty acids (eg, synth. of butyrate by Faecalibacterium, Roseburia… is important for colon health… but gives a lot of energy -> obesity)

- synthesis of amino acids and vitamins (vit B12, vit B9, vit K)

-detoxification of pollutants and toxic molecules present in the food

- regulation of immune function

Testing offers at R.E.D. Laboratories – Intestinal dysfunctions

At R.E.D. Labs, we use the following assays to investigate intestinal dysfunctions:

- Blood-based assays
  - sCD14 in serum
  - Lactase deficiency assay
  - D-lactate quantification in serum
  - Ammonia quantification in serum
- Stool-based assays
  - sIgA ELISA test in stool samples
  - ZONULIN ELISA test in stool samples
  - EDN / EPX ELISA test in stool samples
  - Beta-Defensin-2 ELISA test in stool samples
  - Inflammation markers in stool samples
  - Infections in stool samples
  - MSA assay

Blood-based assays for Intestinal dysfunctions

- ***sCD14 in serum***
  - sCD14 is expressed in monocytes/macrophages and plays a critical role in the recognition of bacterial cell wall components (LPS). The extracellular part of CD14 can be cleaved and released in the plasma, where it will inactivate circulating LPS. Serum soluble CD14 levels are significantly elevated in patients with leaky gut, inflammatory bowel disease, Crohn's disease, but also in patients suffering from Brucellosis or Lyme disease.
- ***Lactase deficiency assay***
  - a polymorphism in the gene coding for lactase, an enzyme responsible for the digestion of lactose (C/T-13910 polymorphism). In affected people, production of the enzyme declines during or shortly after childhood, resulting in lactose malabsorption. Undigested lactose sugars affect the development of gut microflora, leading to dysbiosis.
- ***D-lactate in serum***
  - a product of bacterial metabolism, it is neither produced nor metabolized by mammalian cells. Typically, elevated D-lactate levels are due to bacterial infection or short bowel syndrome in humans. Due to slow metabolism and excretion, high D-lactate can cause acidosis and encephalopathy.
- ***Ammonia in serum***
  - Ammonia is derived from bacterial enzymatic action on ingested amino acids. It is absorbed from the gastrointestinal tract and delivered through the portal vein to the liver, which converts most of it into urea. Abnormally high levels of ammonia can result from colic or "enteric hyperammonemia" (combination of increased bacterial production and increased gut permeability) that occurs despite normal hepatic function. Hyperammonemia is a metabolic condition characterized by elevated levels of ammonia in the blood. Increased entry of ammonia to the brain is a primary cause of neurologic disorders, metabolic disorders and some toxic encephalopathies.

Stool-based assays for Intestinal dysfunctions

- sIgA ELISA test in stool samples

- sIgA key function is to bind to invading micro organisms and toxins and entrap them in the mucus layer or within the epithelial cells, so inhibiting microbial motility, agglutinating the organisms and neutralizing their exotoxins and then assist in their harmless elimination from the body in the fecal flow.

- The concentration of sIgA gives us information about the intestinal immune defense:

A lack of sIgA *indicates a* ***diminished activity of the intestinal immune system***

An increased *level of sIgA shows* ***intestinal inflammation****.*

- ZONULIN ELISA test in stool samples

- Zonulin is the "doorway" to leaky gut. Zonulin opens up the spaces between the cells of the intestinal lining. When leaky gut is present, the spaces between the cells open up too much allowing larger protein molecules and bacteria to get into the bloodstream where an immunologic reaction can take place. As the zonulin level rises, the seal between the intestinal cells diminishes. Zonulin is the only physiological modulator of intercellular tight junctions described so far that is involved in trafficking of macromolecules and, therefore, in tolerance/immune response balance.

- beta-Defensin-2 ELISA test in stool samples

- Defensins exert a variable degree of antimicrobial activity against bacteria, fungi, and some enveloped viruses. The expression of ß-defensins is induced by the pro-inflammatory cytokines and also through microorganisms (e.g. E. coli, H. pylori or P. aeruginosa) and by probiotic microorganisms. A ß-defensin-2 deficiency can, for example, be observed in the intestinal mucous of patients with Crohn's disease. The defense system of the mucous membrane is therefore restricted and allows an increased invasion of bacteria, which could possibly lead to a typical infection in Crohn's disease patients. Recent results imply that ß-defensin-2 is overexpressed in active intestinal inflammation, especially in ulcerative colitis.

- EDN / EPX ELISA test in stool samples

- The accumulation of EDN in the intestine is associated with inflammation and tissue damage. Fecal EDN is considered the best of the cytotoxic granule proteins for assessment of gut inflammation. Elevated levels of fecal EDN are linked to multiple inflammatory conditions, like food allergy/sensitivity, pathogenic infections (C. difficile and H. Pylori), IBS, Eosinophilic Gastrointestinal Disorders.

- Inflammation markers in stool samples

- ***Hemoglobin*** : discharged with the feces in gastrointestinal bleeding diseases
- ***Transferrin***: a blood-derived component ; a good marker for gastrointestinal bleeding
- ***Calprotectin***: a neutrophil cytosolic protein with antimicrobial properties, which is present at increased concentration in stool during bowel inflammation
- ***Lactoferrin***: a primary component of the acute inflammatory response released from fecal leukocytes; may serve as a marker of inflammation in the intestine

Consequences of the leaky gut - Chronic activation (inflammation) of the immune system

- Lipopolysaccharide (LPS) - bacterial compound that can easily make its way to the blood.
- Present in the bloodstream LPS will induce a strong pro-inflammatory response in monocytes and macrophages, involving recognition by a receptor (Toll-like receptor-4) and the subsequent secretion of cytokines such as IL-1, IL-6, TNF-alpha.
- LPS also induces the NK-kB-mediated production of nitric oxide. Because NO is increased, NK function is inhibited and opportunistic infections such as mycoplasma infections are often observed.
- Herpesviruses, which tend to reactivate in a context of immune activation, will also be frequently detected.

- ***At R.E.D. Labs, these issues are evaluated by testing for***
  - **sCD14 expression**
  - **Cytokines' expression**
  - **PGE2 expression**
  - **Virus detection in blood and intestinal biopsies**
  - **Mycoplasma infections**
  - **Oxydative stress**

Testing offers at R.E.D. Laboratories – Intestinal dysfunctions

Stool-based assays

- **Infections assessments in stool samples**

□ **C/PARAS**	Cryptosporidium/Giardia Ag
□ **HPYL**	Helicobacter Pylori Ag
□ **SHIG**	Shigella spp. Ag
□ **ADENOV**	Adenovirus Ag
□ **SALMO**	Salmonella Spp. Ag
□ **LIST**	Listeria monocitogenes Ag
□ **ENTEROV**	Enterovirus Ag
□ **HepA**	Hepatitis A virus Ag
□ **EntaHIST**	Entamoeba histolytica
□ **CampBact**	Campylobacter Ag
□ **YERSI**	Yersinia enterocolitica
□ **CLOST**	Clostridium difficile
□ **TULAR**	Tularemia (*Francisella tularensis)*

METAGENOMIC STOOL ASSAY (MSA)

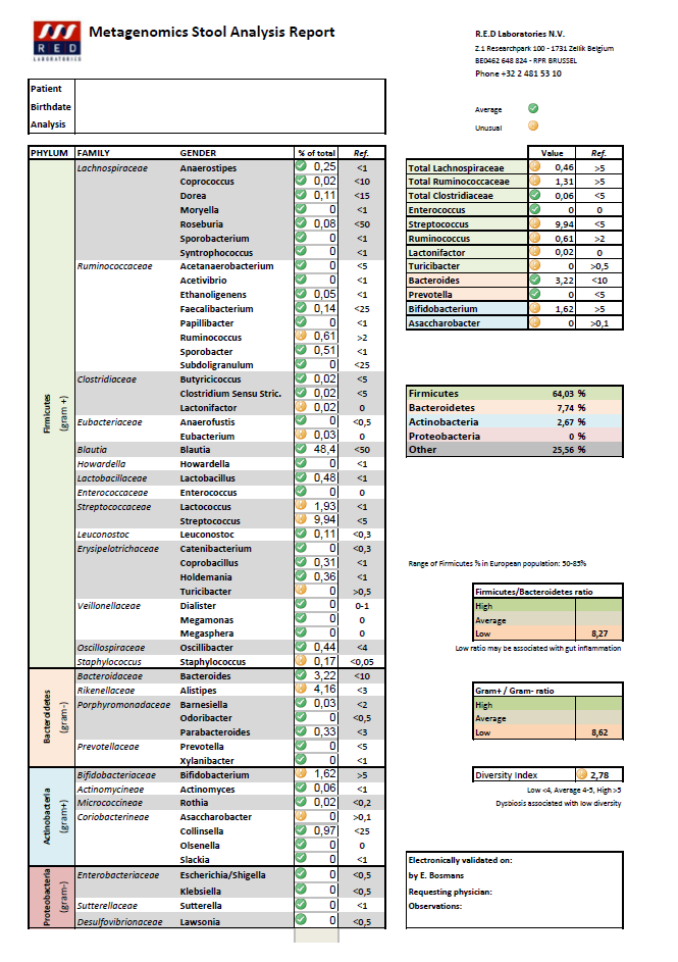
Intestinal microbiota analysis : from culture to high-throughput sequencing:

- *Until recently research into microbiota composition relied almost exclusively on culture ; 40 to 80% of gut bacteria cannot be cultured*
- *Identification of colonies can be difficult*
- *Bacteria must be alive: studies of anaerobes very difficult, major loss during collection and processing of samples*
- *Culture approach may address only a small fraction of all bacterial species (10%?)*
- *E.coli once thought to be a dominant species, is a minor member…*

R.E.D. Labs scientists have developed and validated a new procedure to analyze bacterial populations in a stool sample : MSA assay

- ***New molecular technique involving sequencing of specific regions of bacterial DNA (metagenomics)***
- Can be performed on dead organisms (exposure to oxygen, freezing are not a problem)
- Identification of each bacteria by comparing sequence with public databases: extremely precise, not subjective
- High-throughput technology allows identification of tens or even hundreds of thousends organisms in a single sample
- Bacterial DNA was extracted from stool samples, PCR amplification was performed on 16S rRNA gene regions, and PCR amplicons were sequenced Bacteria were classified by phylum, family and genus.

MSA assay template report



Metagenomics Stool Analysis Report

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Patient	
Birthdate	
Analysis	

Average
Unusual

PHYLUM	FAMILY	GENDER	% of total	Ref.
Firmicutes (gram +)	*Lachnospiraceae*	Anaerostipes	0,25	<1
		Coprococcus	0,02	<10
		Dorea	0,11	<15
		Moryella	0	<1
		Roseburia	0,08	<50
		Sporobacterium	0	<1
		Syntrophococcus	0	<1
	Ruminococcaceae	Acetanaerobacterium	0	<5
		Acetivibrio	0	<1
		Ethanoligenens	0,05	<1
		Faecalibacterium	0,14	<25
		Papillibacter	0	<1
		Ruminococcus	0,61	>2
		Sporobacter	0,51	<1
		Subdoligranulum	0	<25
	Clostridiaceae	Butyricicoccus	0,02	<5
		Clostridium Sensu Stric.	0,02	<5
		Lactonifactor	0,02	0
	Eubacteriaceae	Anaerofustis	0	<0,5
		Eubacterium	0,03	0
	Blautia	Blautia	48,4	<50
	Howardella	Howardella	0	<1
	Lactobacillaceae	Lactobacillus	0,48	<1
	Enterococcaceae	Enterococcus	0	0
	Streptococcaceae	Lactococcus	1,93	<1
		Streptococcus	9,94	<5
	Leuconostoc	Leuconostoc	0,11	<0,3
	Erysipelotrichaceae	Catenibacterium	0	<0,3
		Coprobacillus	0,31	<1
		Holdemania	0,36	<1
		Turicibacter	0	>0,5
	Veillonellaceae	Dialister	0	0-1
		Megamonas	0	0
		Megasphera	0	0
	Oscillospiraceae	Oscillibacter	0,44	<4
	Staphylococcus	Staphylococcus	0,17	<0,05
Bacteroidetes (gram-)	*Bacteroidaceae*	Bacteroides	3,22	<10
	Rikenellaceae	Alistipes	4,16	<3
	Porphyromonadaceae	Barnesiella	0,03	<2
		Odoribacter	0	<0,5
		Parabacteroides	0,33	<3
	Prevotellaceae	Prevotella	0	<5
		Xylanibacter	0	<1
Actinobacteria (gram+)	*Bifidobacteriaceae*	Bifidobacterium	1,62	>5
	Actinomycineae	Actinomyces	0,06	<1
	Micrococcineae	Rothia	0,02	<0,2
	Coriobacterineae	Asaccharobacter	0	>0,1
		Collinsella	0,97	<25
		Olsenella	0	0
		Slackia	0	<1
Proteobacteria (gram-)	*Enterobacteriaceae*	Escherichia/Shigella	0	<0,5
		Klebsiella	0	<0,5
	Sutterellaceae	Sutterella	0	<1
	Desulfovibrionaceae	Lawsonia	0	<0,5

	Value	Ref.
Total Lachnospiraceae	0,46	>5
Total Ruminococcaceae	1,31	>5
Total Clostridiaceae	0,06	<5
Enterococcus	0	0
Streptococcus	9,94	<5
Ruminococcus	0,61	>2
Lactonifactor	0,02	0
Turicibacter	0	>0,5
Bacteroides	3,22	<10
Prevotella	0	<5
Bifidobacterium	1,62	>5
Asaccharobacter	0	>0,1

Firmicutes	64,03 %
Bacteroidetes	7,74 %
Actinobacteria	2,67 %
Proteobacteria	0 %
Other	25,56 %

Range of Firmicutes % in European population: 50-85%

Firmicutes/Bacteroidetes ratio	
High	
Average	
Low	8,27

Low ratio may be associated with gut inflammation

Gram+ / Gram- ratio	
High	
Average	
Low	8,62

Diversity Index	2,78

Low <4, Average 4-5, High >5
Dysbiosis associated with low diversity

Electronically validated on:
by E. Bosmans
Requesting physician:
Observations:

Tick-borne Infections (TBI) and gastrointestinal disorders

Signs and symptoms related to the gastrointestinal tract and liver may provide important clues for the diagnosis of various tickborne diseases

Manifestation	Lyme disease	Ehrlichiosis	RMSF	Tularemia	Colorado tick fever	TBRF	Q fever	Babesiosis
Anorexia	+	++	+	+	+	+	+	+
Nausea	+	++	++	++	++	+++	++	+
Vomiting	+	++	++	++	++	+++	++	+
Abdominal pain	+	++	++ to +++	++	+	++	+	+
Diarrhea	+	++	++	++ to +++	+	+ to ++	++	+
Hepatomegaly	R	+ to ++	+	+ to ++	R	+	+	+
Splenomegaly	+	+ to ++	+	+ to ++	R	R to +	+	+
Jaundice	+	+++	+	+	+	+	+	+ to ++
Elevated bilirubin level	+	+++	+ to ++	+	+	+	+ to ++	++ to +++
Elevated ALT level	++	++++	++ to +++	++	+	++	++[a]	+

NOTE. ALT, alanine aminotransferase; R, rare; RMSF, Rocky Mountain spotted fever; TBRF, tickborne relapsing fever; +, uncommon; ++ common; +++, very common; ++++, almost always present.

[a] Elevated alkaline phosphatase level is the predominant abnormality.

From: Gastrointestinal and Hepatic Manifestations of Tickborne Diseases in the United States
Syed Ali Zaidi & Carol Singer, Clin Infect Dis. 2002;34(9):1206-1212. doi:10.1086/339871

Lyme disease and gastrointestinal disorders

- **Patients with Lyme and TBDs may present primarily with GI manifestations.**
- **2015 ILADS conference, Dr. Farshid Rahbar: These patients may have complex or persistent GI symptoms involving upper, mid, or lower GI tract and have already been treated for GI issues**

  Bloating/Gas: in 76% of patients

  Abdominal Pain: in 48% of patients

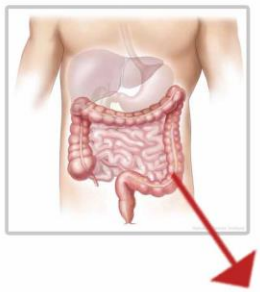
  Constipation: in 42% of patients

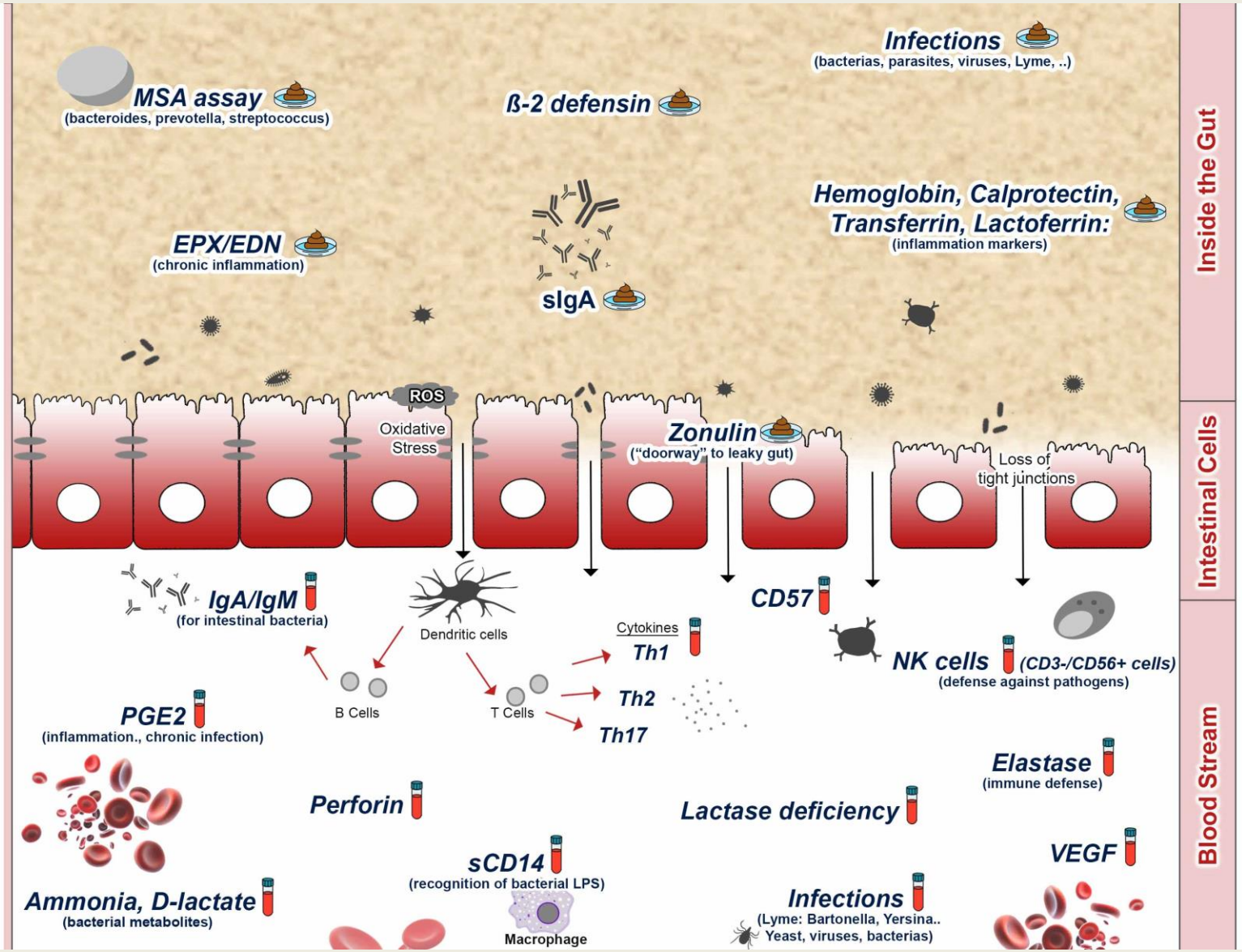
  Food Intolerance: in 42% of patients

  Irregular Bowel Movements: in 37% of patients

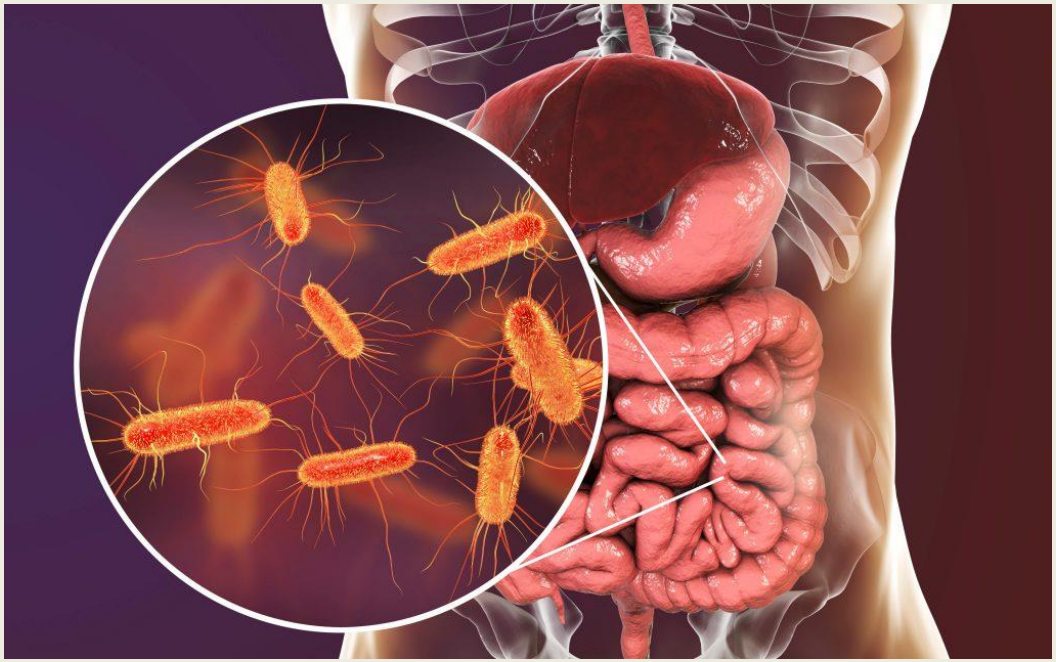
- **The number of patients presenting with such symptoms is probably reaching epidemic proportions.**
- **Testing for gastrointestinal problems need to be included**
- **Useful assays to investigate intestinal dysfunctions:**
  - **BLOOD-BASED Tests: Ammonia in serum, Lactase deficiency assay, D-lactate, sCD14**
  - **BIOPSY-BASED Tests: PCR-based detection of viral and bacterial infections**
  - **STOOL-BASED Tests:**
    - **Intestinal Inflammation: sIgA, Beta-2 Defensin, EPX / EDN, Inflammation markers in stool samples**
    - **Intestinal Infections : immunochromatography antigenic testing for intestinal infections**
    - **Leaky gut: ZONULIN ELISA test in stool samples**
    - **Dysbiosis: MSA assay (metagenomic stool test)**

Consequences of TBI





Testing for Intestinal dysfunction in autism



Although ASD primarily impacts the brain, over recent years, links with other systems have become clear — in particular, gastrointestinal (GI) issues seem to occur more often in individuals with ASD than in the rest of the population.

The GI issues that come with ASD might be due to two factors: firstly, inappropriate immune activation, causing inflammation of the tract; and, secondly, differences in the types of gut bacteria that are present.

Gut-Brain Axis in Autism

Autism-Open Access

Siniscalco, Autism-Open Access 2014, 4:3
http://dx.doi.org/10.4172/2165-7890.1000e124

Editorial Open Access

Gut Bacteria–Brain Axis in Autism

Dario Siniscalco[*1,2,3]

Starting from an early idea of Nobel laureate Luc Montagnier that metabolites from gut bacteria end up in the plasma and could trigger damage to the brain [3], we can now coin the term "BBB" in autism as bacteria-brain-behavior influence that we see in autistic children. It is noteworthy to consider that nowadays almost all the autistic patients suffer from gastrointestinal symptoms and show an altered intestinal barrier, such as an impaired gut barrier function [4,5]. This impaired gut barrier permits the passage of dietary-derived non-self antigens and has a dramatic consequence on the immune system responses of the autistic child [6].

Intestinal dysfunctions in autism

- Gut flora and gastrointestinal status in children with autism correlate with autism severity
  - **Adams et al. (BMC Gastroenterology 2011) reported that gastrointestinal symptoms were strongly correlated with the severity of autism. From four types of beneficial bacteria that were investigated, the children with autism had much lower levels of Bifidobacterium (-45%), slightly lower levels of Enterococcus (-16%), and much higher levels of Lactobacillus (+100%).**
  - **Finegold et al. (Anaerobe 2010) reported that in the control children's stools, Firmicutes accounted for 63.6% of the total flora but only 38-39% of the flora of autistic children's stools. Bacteroidetes accounted for 30% of the stool flora in controls and for 51% in the flora of stools of autistic children. Actinobacteria made up 1.8% of stool flora of control children and between 0.4 and 0.7% of the flora of autistic children. Proteobacteria made up 0.5% of the flora of control children and between 2.3 and 3.1% of the flora of autistic children. In summary, the fecal flora of autistic children was statistically significantly different from the fecal flora of healthy children.**

➢ ***R.E.D. Laboratories are offering specialty test to deeply analyze gut microbiota: MSA assay***

ASD child MSA

Prevotella: strong hydrogen sulfide (H_2S) producers. In excess, H_2S acts as a mitochondrial poison and a potent neurotoxin. It can directly inhibit enzymes involved in the cellular production of energy. H_2S also interferes with oxygen transport by blocking hemoglobin in the red blood cells. Finally, H_2S is lowering gut pH preventing the growth of many beneficial bacteria,

			JAN 2019		Oct 2018	JAN 2018
PHYLUM	FAMILY	GENUS	% of total	Ref.	% of total	% of total
Firmicutes (gram +)	*Lachnospiraceae*	Anaerostipes	0.01	<1	0.01	0.13
		Coprococcus	4.06	<10	1.97	1.72
		Dorea	4.24	<15	3.92	4.55
		Moryella	0.01	<1	0	0.03
		Roseburia	3.81	<50	3.7	3.31
		Sporobacterium	0	<1	0	0
		Syntrophococcus	0	<1	0	0
	Ruminococcaceae	Acetanaerobacterium	0	<5	0	0
		Acetivibrio	0	<1	0	0
		Ethanoligenens	0	<1	0	0
		Faecalibacterium	22.95	<25	18.55	9.08
		Papillibacter	0	<1	0	0
		Ruminococcus	0.52	>2	0.24	0.01
		Sporobacter	0	<1	0	0
		Subdoligranulum	2.65	<25	0.08	0.02
	Clostridiaceae	Butyricicoccus	5.71	<5	2.59	2.67
		Clostridium Sensu Stric.	0.05	<5	0.04	3.78
		Lactonifactor	0.01	0	0.02	0
	Eubacteriaceae	Anaerofustis	0	<0,5	0	0
		Eubacterium	0	0	0.03	0
	Blautia	Blautia	20	<50	17.15	3.68
	Howardella	Howardella	0	<1	0	0
	Lactobacillaceae	Lactobacillus	0.08	<1	0.01	0.42
	Enterococcaceae	Enterococcus	0	0	0	0
	Streptococcaceae	Lactococcus	0.01	<1	0.02	0.03
		Streptococcus	1.12	<5	4.75	18.35
	Leuconostoc	Leuconostoc	0	<0,3	0.01	0.19
	Erysipelotrichaceae	Catenibacterium	0	<0,3	0	0
		Coprobacillus	0.07	<1	0.05	0.23
		Holdemania	0.02	<1	0.01	0.01
		Turicibacter	0	>0,5	0	0
	Veillonellaceae	Dialister	0	0-1	0	0
		Megamonas	0	0	0	0
		Megasphera	0	0	0	0
	Oscillospiraceae	Oscillibacter	2.47	<4	1.05	0.17
	Staphylococcus	Staphylococcus	0	<0,05	0.05	0.01
Bacteroidetes (gram -)	*Bacteroidaceae*	Bacteroides	7.3	<10	8.53	4.42
	Rikenellaceae	Alistipes	1.19	<3	0.65	0.11
	Porphyromonadaceae	Barnesiella	1.3	<2	0.95	0.11
		Odoribacter	0.13	<0,5	0.15	0.01
		Parabacteroides	0.66	<3	1.77	0.23
	Prevotellaceae	Prevotella	15.61	<5	26.07	42.02
		Xylanibacter	0	<1	0	0.03
Actinobacteria (gram+)	*Bifidobacteriaceae*	Bifidobacterium	0	>5	0.01	0
	Actinomycineae	Actinomyces	0.37	<1	1.29	0.62
	Micrococcineae	Rothia	0.27	<0,2	1.92	1.37
	Coriobacterineae	Asaccharobacter	0	>0,1	0	0
		Collinsella	0.1	<25	0.14	0.35
		Olsenella	0	0	0	0
		Slackia	0.39	<1	0.16	0.15
Proteobacteria (gram-)	*Enterobacteriaceae*	Escherichia/Shigella	0	<0,5	0	0.02
		Klebsiella	0	<0,5	0	0
	Sutterellaceae	Sutterella	0	<1	0	0
	Desulfovibrionaceae	Lawsonia	0	<0,5	0	0

ASD child MSA

1st visit April 2017:

High both Bacteroides & Prevotella

2nd visit December 2017:

Still high Bacteroides but normalized Prevotella

3rd visit December 2018:

Normalized both Bacteroides and Prevotella

PHYLUM	FAMILY	GENUS	% of total	Ref.	% of total	% of total
Firmicutes (gram +)	*Lachnospiraceae*	Anaerostipes	0.05	<1	0.03	0,01
		Coprococcus	19.75	<10	13.52	8,83
		Dorea	3.96	<15	9.5	5,38
		Moryella	0	<1	0	0
		Roseburia	8.02	<50	0.97	13,26
		Sporobacterium	0	<1	0	0
		Syntrophococcus	0.01	<1	0	0
	Ruminococcaceae	Acetanaerobacterium	0	<5	0	0
		Acetivibrio	0	<1	0	0
		Ethanoligenens	0	<1	0.01	0,01
		Faecalibacterium	2.96	<25	18.87	25,32
		Papillibacter	0	<1	0	0
		Ruminococcus	0.01	>2	8.06	0,19
		Sporobacter	0.01	<1	0	0
		Subdoligranulum	0.01	<25	0.01	0,02
	Clostridiaceae	Butyricicoccus	1.07	<5	5.16	3,68
		Clostridium Sensu Stric.	0.64	<5	0.04	0
		Lactonifactor	0	0	0.07	0
	Eubacteriaceae	Anaerofustis	0.05	<0,5	0.01	0
		Eubacterium	0.05	0	0	0,03
	Blautia	Blautia	6.69	<50	5.88	19,01
	Howardella	Howardella	0.07	<1	0	0
	Lactobacillaceae	Lactobacillus	0.1	<1	0.01	0
	Enterococcaceae	Enterococcus	0	0	0.15	0
	Streptococcaceae	Lactococcus	0	<1	0.01	0,01
		Streptococcus	2.27	<5	1.44	4,42
	Leuconostoc	Leuconostoc	0.01	<0,3	0.39	0
	Erysipelotrichaceae	Catenibacterium	0	<0,3	0	0
		Coprobacillus	0.02	<1	0.01	0
		Holdemania	0	<1	0	0
		Turicibacter	0.07	>0,5	0	0
	Veillonellaceae	Dialister	2.69	0-1	0	0
		Megamonas	0	0	0	0
		Megasphera	0	0	0	0
	Oscillospiraceae	Oscillibacter	0.98	<4	3.65	0,53
	Staphylococcus	Staphylococcus	0	<0,05	0	0,01
Bacteroidetes (gram-)	*Bacteroidaceae*	Bacteroides	13.8	<10	20.21	6,88
	Rikenellaceae	Alistipes	1.66	<3	0.55	0,23
	Porphyromonadaceae	Barnesiella	0.25	<2	0.27	0,04
		Odoribacter	0.1	<0,5	0.17	0,02
		Parabacteroides	2.49	<3	0.67	0,3
	Prevotellaceae	Prevotella	21.71	<5	3.85	3,77
		Xylanibacter	0	<1	0	0
Actinobacteria (gram+)	*Bifidobacteriaceae*	Bifidobacterium	2.21	>5	0.11	0
	Actinomycineae	Actinomyces	0.16	<1	0.08	0,23
	Micrococcineae	Rothia	0.01	<0,2	0.05	1,14
	Coriobacterineae	Asaccharobacter	0	>0,1	0	0
		Collinsella	2.01	<25	0.57	1,57
		Olsenella	0	0	0	0
		Slackia	0.58	<1	0.28	0,19
Proteobacteria (gram-)	*Enterobacteriaceae*	Escherichia/Shigella	0.05	<0,5	0	0
		Klebsiella	0	<0,5	0	0
	Sutterellaceae	Sutterella	0.81	<1	0	0
	Desulfovibrionaceae	Lawsonia	0	<0,5	0	0

ASD adult 28y

Streptococcus: well known in ASD. Strep antibodies might interact with the part of the brain known as the basal ganglia. This is believed to cause the sudden onset of tics or obsessive compulsive behaviors.

PHYLUM	FAMILY	GENUS	% of total	Ref.
Firmicutes (gram +)	*Lachnospiraceae*	Anaerostipes	0	<1
		Coprococcus	0,02	<10
		Dorea	1,61	<15
		Moryella	0	<1
		Roseburia	0,23	<50
		Sporobacterium	0	<1
		Syntrophococcus	0	<1
	Ruminococcaceae	Acetanaerobacterium	0	<5
		Acetivibrio	0	<1
		Ethanoligenens	0	<1
		Faecalibacterium	0,27	<25
		Papillibacter	0	<1
		Ruminococcus	0,02	>2
		Sporobacter	0	<1
		Subdoligranulum	0,07	<25
	Clostridiaceae	Butyricicoccus	0,05	<5
		Clostridium Sensu Stric.	0,01	<5
		Lactonifactor	0,02	0
	Eubacteriaceae	Anaerofustis	0	<0,5
		Eubacterium	0	0
	Blautia	Blautia	0,6	<50
	Howardella	Howardella	0	<1
	Lactobacillaceae	Lactobacillus	4,47	<1
	Enterococcaceae	Enterococcus	8,54	0
	Streptococcaceae	Lactococcus	0,02	<1
		Streptococcus	40,61	<5
	Leuconostoc	Leuconostoc	0,01	<0,3
	Erysipelotrichaceae	Catenibacterium	0	<0,3
		Coprobacillus	0	<1
		Holdemania	0,04	<1
		Turicibacter	0	>0,5
	Veillonellaceae	Dialister	0,35	0-1
		Megamonas	0	0
		Megasphera	0	0
	Oscillospiraceae	Oscillibacter	0,3	<4
	Staphylococcus	Staphylococcus	0	<0,05
Bacteroidetes (gram-)	*Bacteroidaceae*	Bacteroides	3,91	<10
	Rikenellaceae	Alistipes	0,05	<3
	Porphyromonadaceae	Barnesiella	0	<2
		Odoribacter	0,02	<0,5
		Parabacteroides	0,18	<3
	Prevotellaceae	Prevotella	0,01	<5
		Xylanibacter	0	<1
Actinobacteria (gram+)	*Bifidobacteriaceae*	Bifidobacterium	35,39	>5
	Actinomycineae	Actinomyces	0,09	<1
	Micrococcineae	Rothia	0,24	<0,2
	Coriobacterineae	Asaccharobacter	0	>0,1
		Collinsella	0	<25
		Olsenella	0	0
		Slackia	0	<1
Proteobacteria (gram-)	*Enterobacteriaceae*	Escherichia/Shigella	1,03	<0,5
		Klebsiella	0,02	<0,5
	Sutterellaceae	Sutterella	0	<1
	Desulfovibrionaceae	Lawsonia	0	<0,5

	Value	Ref.
Total Lachnospiraceae	1,86	>5
Total Ruminococcaceae	0,36	>5
Total Clostridiaceae	0,08	<5
Enterococcus	8,54	0
Streptococcus	40,61	<5
Ruminococcus	0,02	>2
Lactonifactor	0,02	0
Turicibacter	0	>0,5
Bacteroides	3,91	<10
Prevotella	0,01	<5
Bifidobacterium	35,39	>5
Asaccharobacter	0	>0,1

Firmicutes	57,24	%
Bacteroidetes	4,17	%
Actinobacteria	35,72	%
Proteobacteria	1,05	%
Other	1,82	%

Range of Firmicutes % in European population: 50-85%

Firmicutes/Bacteroidetes ratio	
High	
Average	13,73
Low	

Low ratio may be associated with gut inflammation

Gram+ / Gram- ratio	
High	
Average	17,81
Low	

Diversity Index	2,79

Low <4, Average 4-5, High >5

Dysbiosis associated with low diversity

Electronically validated on: 24/09/2018

by E. Bosmans

Requesting physician: Himmunitas

Observations:

Intestinal inflammation in autism

- Autism and gastrointestinal inflammation
  - **Several reports have revealed a high prevalence of gastrointestinal symptoms, inflammation, and dysfunction in children with autism (reviewed by Horvath and Perman, Curr Gastroenterol Rep. 2002).**
  - **Mild to moderate degrees of inflammation were found in both the upper and lower intestinal tract. In children with ASD, the presence of GI dysfunction is often associated with increased irritability, tantrums, aggressive behavior, and sleep disturbances (reviewed by Critchfield et al., Gastroenterol Res Pract. 2011).**

Leaky gut in ASD children

Involvement of Dietary Bioactive Proteins and Peptides in Autism Spectrum Disorders

Dario Siniscalco[1,2,3,*] and Nicola Antonucci[4]

Current Protein and Peptide Science, 2013, *14*,

permeability [34]. ASD pathogenesis could be affected by this altered permeability. The impaired gut barrier function is the basis of the proposed leaky-gut hypothesis: ASD children show an elevated tight junctions-mediated intestinal permeability that allows the passage and absorption of dietary-derived incompletely digested peptides in the intestinal lamina propria. The intestinal barrier defects predispose autistic children to sensitization to environmental antigens. These

CFS-related disorders subgroups: *intestinal dysfunctions*

Evidence for GI dysfunction in CFS (Chronic Fatigue Syndrome)

- ***Gastro-intestinal symptoms in patients***
  - More than 90% of CFS patients will present IBS symptoms during their life
  - co-occurrence of CFS and IBS is associated with increased levels of inflammatory cytokines *[Aaron et al., Arch Internal Med 2000; Scully et al., Am J Gastroenterol 2010]*
- ***Endoscopic evidence***
  - Endoscopic examination of duodenum or stomach almost systematically reveal the existence of inflammed areas of the mucosa
- ***Inflammatory markers in stools***

CFS-related disorders subgroups: *intestinal dysfunctions*

Dysbiosis in CFS

A frequent disorder of intestinal function is dysbiosis, i.e. the overgrowth of pathogenic bacteria in the intestine.

- ***Several published studies suggest that CFS is associated with dysbiosis***

  - Culture-based assays revealed **increased levels of *Streptococcus* and *Enterococcus spp*.** in faecal samples of ME/CFS patients *[Sheedy et al., In Vivo 2009]*

  - **Probiotic supplementation** (*L. casei* in one study, *L. paracasei* + *L. acidophilus* + *B. lactis* in another study) **resulted in improved** emotional symptoms and neurocognitive functions *[Rao et al., Gut Pathog 2009; Sullivan et al., Nutr J 2009]*

CFS-related disorders subgroups: *intestinal dysfunctions*

We use the following assays to investigate intestinal dysfunctions:

- ***sCD14***
- ***Lactase deficiency assay***
- ***sIgA ELISA tests in stool samples***
- ***Zonulin ELISA tests in stool samples***
- ***Beta-2 defensin ELISA tests in stool samples***
- ***EPX / EDN ELISA tests in stool samples***
- ***Inflammation markers in stool samples***
- ***MSA assay***
- ***PCR-based detection of viral infections***

CFS-related disorders subgroups: *intestinal dysfunctions*

Viral infections : Several viruses have been associated with CFS

- Human Herpesvirus 6 and 7 [Chapenko et al., J Clin Virol 2006]
- Enteroviruses [Chia et al., J Clin Pathol 2010]
- Parvovirus B19 [Kerr et al., J Gen Virol 2010]
- Bornavirus [Nakaya et al., FEBS Lett 1996]
- Epstein-Barr virus [Lerner et al., In Vivo 2004]
- *Not specific for CFS; none of these viruses found in all CFS patients*
- Absence of detection could in some cases be explained by viral localization.
- **Persistent viral infections can affect intestinal immunity**
  - HHV-6 is immunosuppressive, causes depletion of CD4 cells, down-regulation of CD3 in infected T cells, alteration of cytokine expression (TNFa, IL-1b, IL-10, IL-12). Parvovirus infection associated with altered IFNg response.
- **Consequences on intestinal health**
  - Immunosuppression may favor development of other viruses or pathogens; alteration of gut immunity can also affect the gut flora.

CFS-related disorders subgroups: *intestinal dysfunctions*

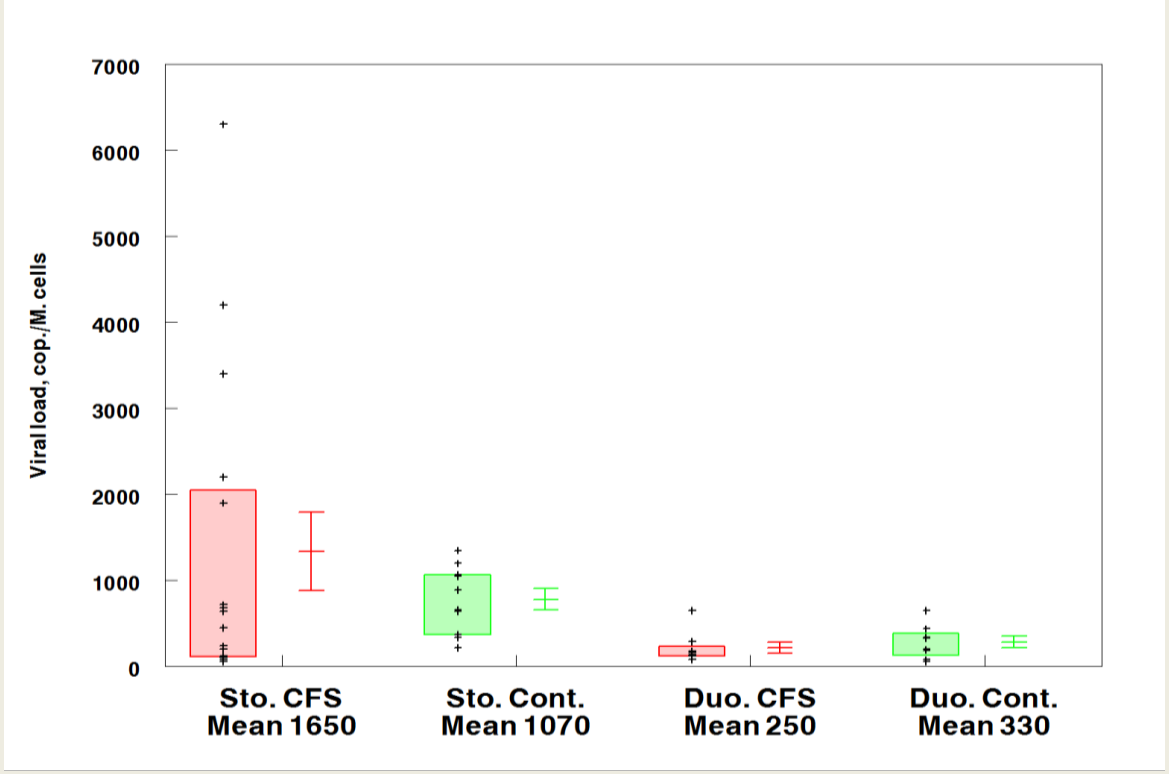
Viral infections : Search for viruses in the gut mucosa : rationale and experimental approach

- The gastro-intestinal mucosa is a known reservoir for several viruses
  - HHV-6, HHV-7, CMV are found in intestinal biopsies of HIV patients and transplant recipients;
  - EBV is found in the gastric mucosa, associated with gastritis and gastric cancer; chronic
  - enteroviral infections have been found in the stomach of CFS patients.
- A study has been conducted at RED Laboratories to investigate the presence of specific viral infections in the GI tract of CFS patients
- ***Experimental approach***
- Determination of HHV-6, EBV and parvovirus B19 viral loads in gastric and intestinal biopsies of CFS patients and non-CFS controls, by real-time quantitative PCR. 48 patients, 35 controls.
  - *Published in:* In Vivo 2009; Mar-Apr;23(2):209-13.*Detection of herpesviruses and parvovirus B19 in gastric and intestinal mucosa of chronic fatigue syndrome patients. By* Frémont M, Metzger K, Rady H, Hulstaert J, De Meirleir K.

CFS-related disorders subgroups: *intestinal dysfunctions*

Viral infections : HHV-6 viral loads in positive biopsies

- Several highly positives in the gastric mucosa of CFS patients.
- Higher loads in stomach than in duodenum.

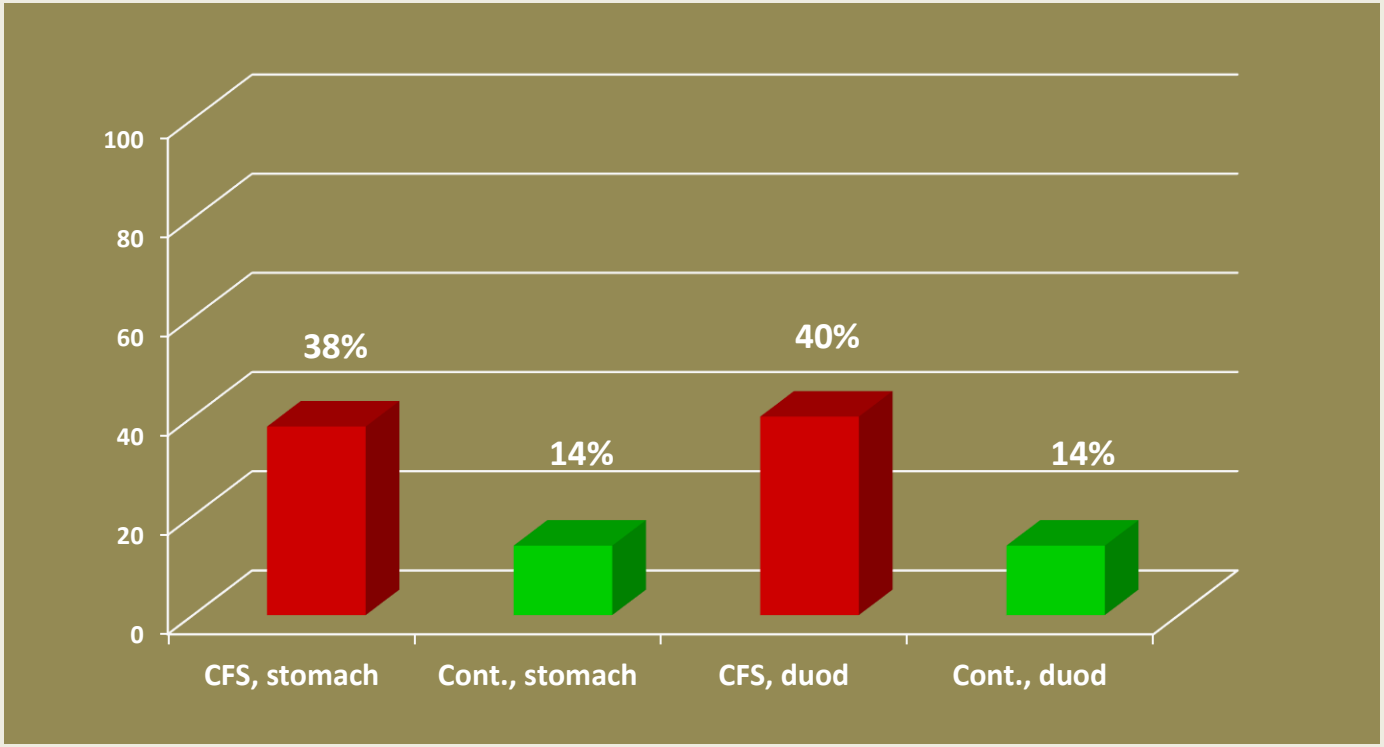


Frémont et al., In vivo 2009

CFS-related disorders subgroups: *intestinal dysfunctions*

Viral infections : Parvovirus B19 in stomach and duodenum biopsies

- **Higher frequency of Parvovirus B19** in both gastric and duodenal mucosa of patients compared to controls.



Frémont et al., In vivo 2009

Questions and Contacts

- **Material available on the website (www.redlabs.com)**
- Check regularly our website (www.redlabs.com) for the updates
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