PROTEA biopharma
Press Conference
Ritz Hotel London, Thursday 28th of May 2009

Unravelling the Origin of Myalgic Encephalomyelitis: Gastrointestinal Dysfunction, Production of Neurotoxins and Environmental Exposure

Prof. Kenny De Meirleir, M.D, Ph.D
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Marc Frémont, Ph.D

PROTEA biopharma

Myalgic encephalomyelitis: A highly prevalent debilitating disease

- **Persistent, debilitating fatigue associated with numerous physical and neurocognitive symptoms**

Disease severity can range from moderate to extremely severe: patients bedridden for years, totally caregiver dependent

- **Prevalence estimates: 0,3 to 0,6%; one million patients in the USA, two million patients in Europe**

This may just be the tip of the iceberg

- **High socio-economic cost**

Cost to the society estimated as approximately $16 billion in the USA, €20 billion in Europe

Intestinal disorders in ME patients

- **Patients usually present with multiple intestinal symptoms including:**

Nausea	Abdominal pain
Poor appetite	Abnormal bowel motility
Gastric reflux	Bloating

- **Inflammation of the gastrointestinal tract**

- **Marked alteration of the intestinal microbial flora**

Alterations of intestinal microflora (aerobes)

• ***Enterococcus*** **and** ***Streptococcus*** **species are strongly over-represented in ME patients :**

Organisms	Control	ME patients	*p*-value
E.coli	1.0 x 10^8	4.26 x 10^7	p=0.98
Enterococcus spp.	5.0 x 10^6	3.5 x 10^7	**p<0.001**
Streptococcus spp.	8.9 x 10^4	9.8 x 10^7	**p<0.001**

Henry Butt, University of Melbourne

Alterations of intestinal microflora (anaerobes)

• **Among anaerobic bacteria, *Prevotella* is the most consistently overgrown bacteria :**

Organisms	Control	ME patients	*p*-value
Bacteroides spp.	3.2×10^{11}	1.6×10^{11}	$p=0.39$
Prevotella spp.	1.0×10^{8}	9.0×10^{9}	**$p< 0.001$**
Bifidobacterium spp.	6.0×10^{8}	5.5×10^{9}	**$p=0.001$**
Lactobacillus spp.	2.7×10^{7}	1.8×10^{8}	**$p=0.002$**

Henry Butt, University of Melbourne

Bacterial overgrowth correlates with symptoms severity

- ***Enterococcus* spp. counts correlate with symptom expression :**

Symptoms	r and *p*-values
Headache	r=.17, ***p*<0.01**
Arm pain	r=.20, ***p*<0.003**
Shoulder pain	r=.15, ***p*<0.04**
Myalgia	r=.20, ***p*<0.003**
Palpitations	r=.16, ***p*<0.02**
Sleep disturbance	r=.20, ***p*<0.004**

Henry Butt, University of Melbourne

Bacterial overgrowth correlates with symptoms severity

- ***Streptococcus* spp. counts correlate with symptom expression :**

Symptoms	r and *p*-values
Post Exertional fatigue	r=.15, ***p*<0.03**
Photophobia	r=.14, ***p*<0.04**
Mind going blank	r=.17, ***p*<0.01**
Cervical gland lymphodynia	r=.14 ***p*<0.04**
Palpitations	r=.15, ***p*<0.03**
Dizziness/Faintness	r=.14, ***p*<0.05**

Henry Butt, University of Melbourne

Hydrogen sulfide produced by bacteria works as a potent toxin for the body

- **Hydrogen sulfide (H_2S) has important physiological functions…**

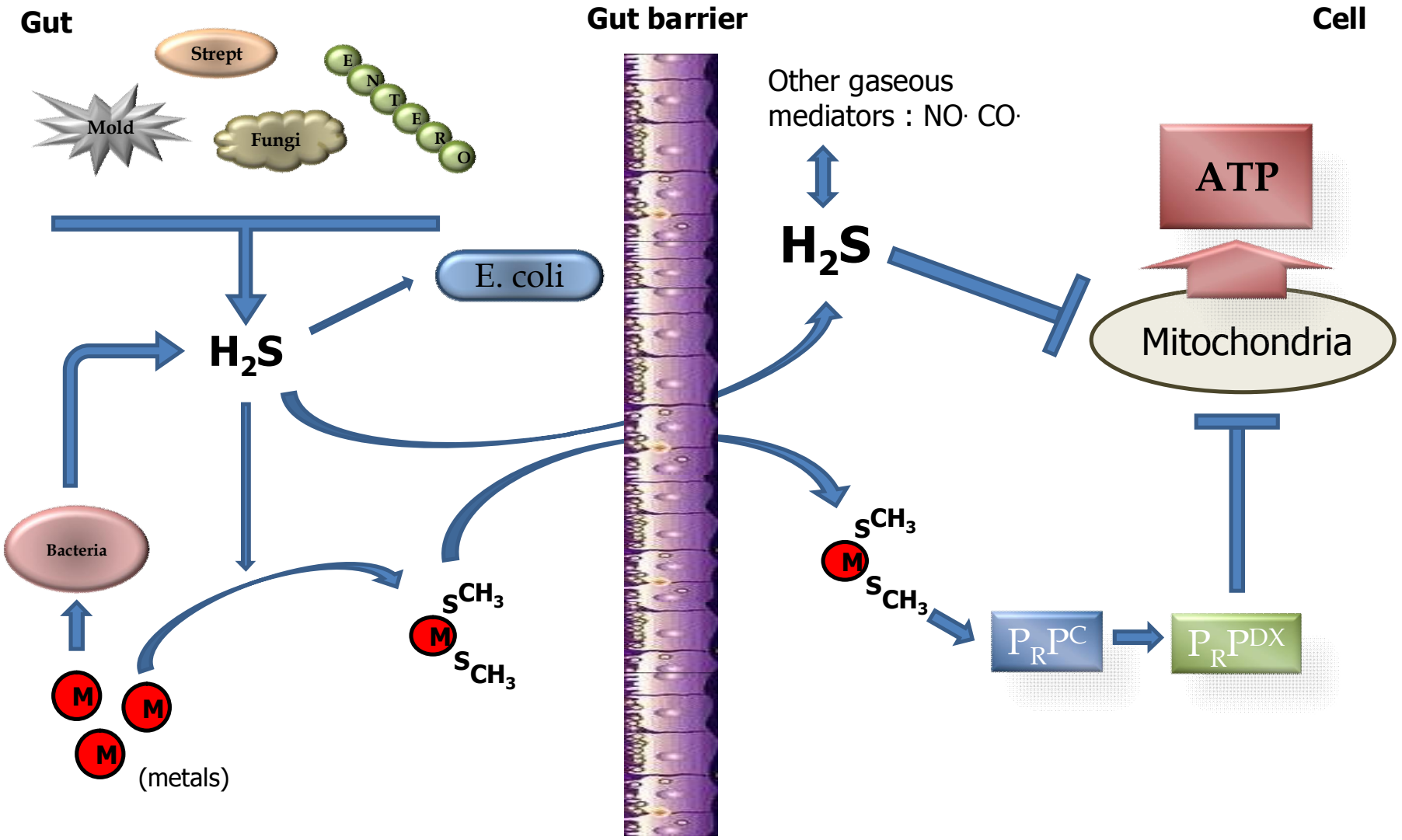
H_2S is produced by the cells and is an important gaseous signal molecule, involved in regulation of blood pressure, neurotransmission, muscle relaxation and regulation of inflammation

- **…but exogeneous exposure can be extremely toxic**

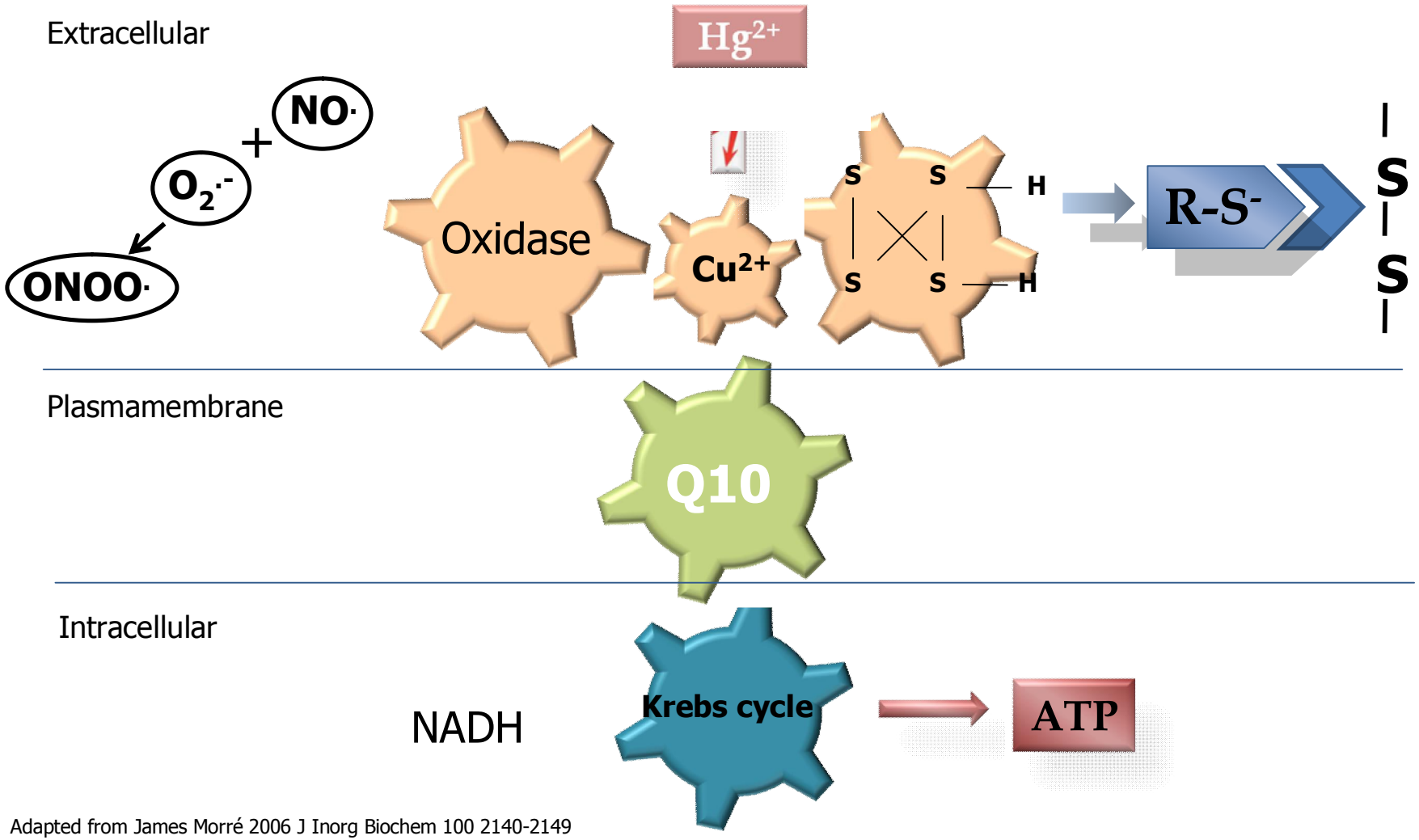
In excess, H_2S acts as a mitochondrial poison. It can directly inhibit enzymes involved in the cellular production of energy. H_2S also interferes with oxygen transport by blocking hemoglobin in the red blood cells.

Enterococcus, Streptococcus, Prevotella **are strong H_2S producers**

Cumulative effects of H_2S and heavy metals

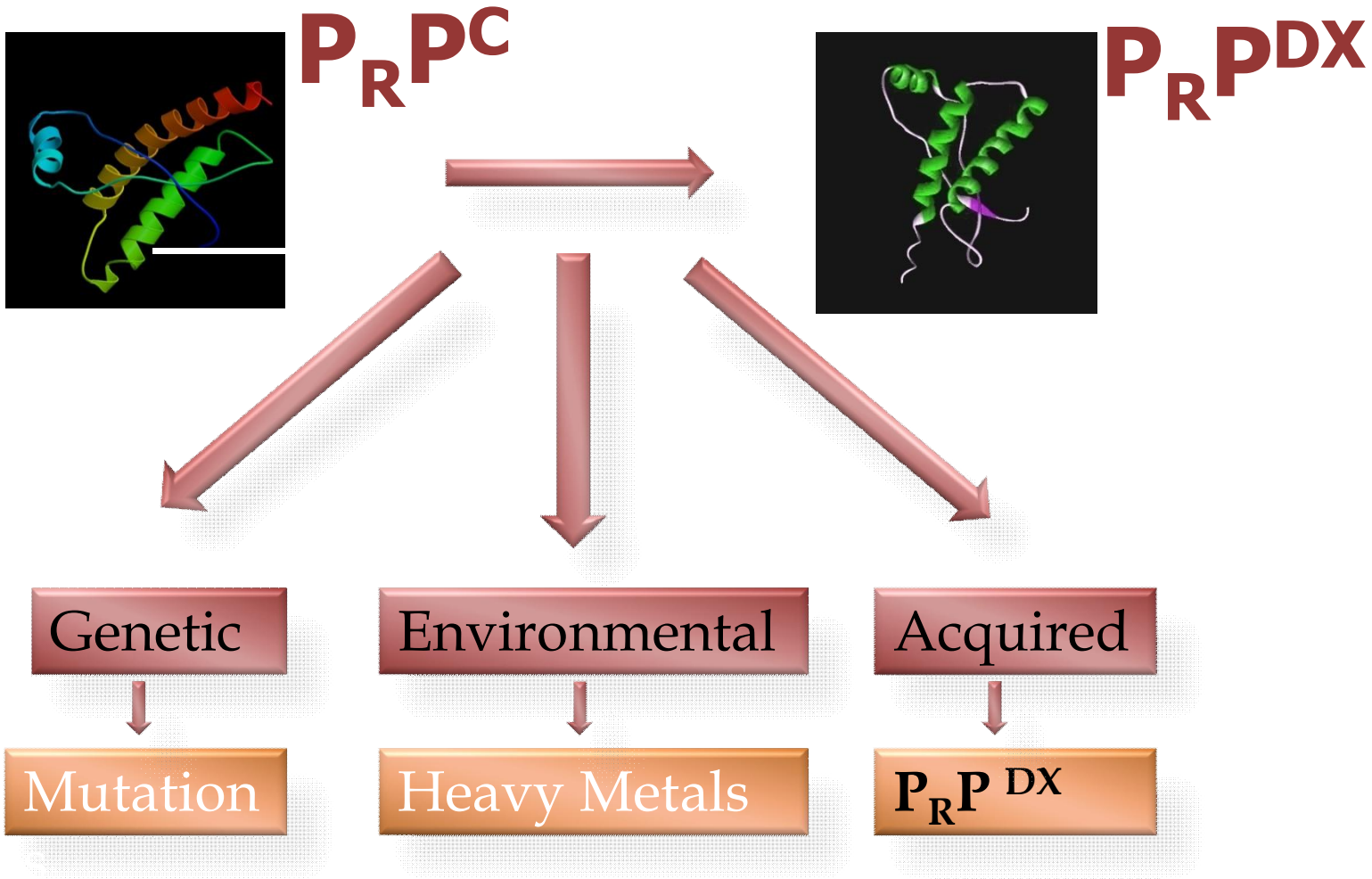


Heavy metals interfere directly with energy production

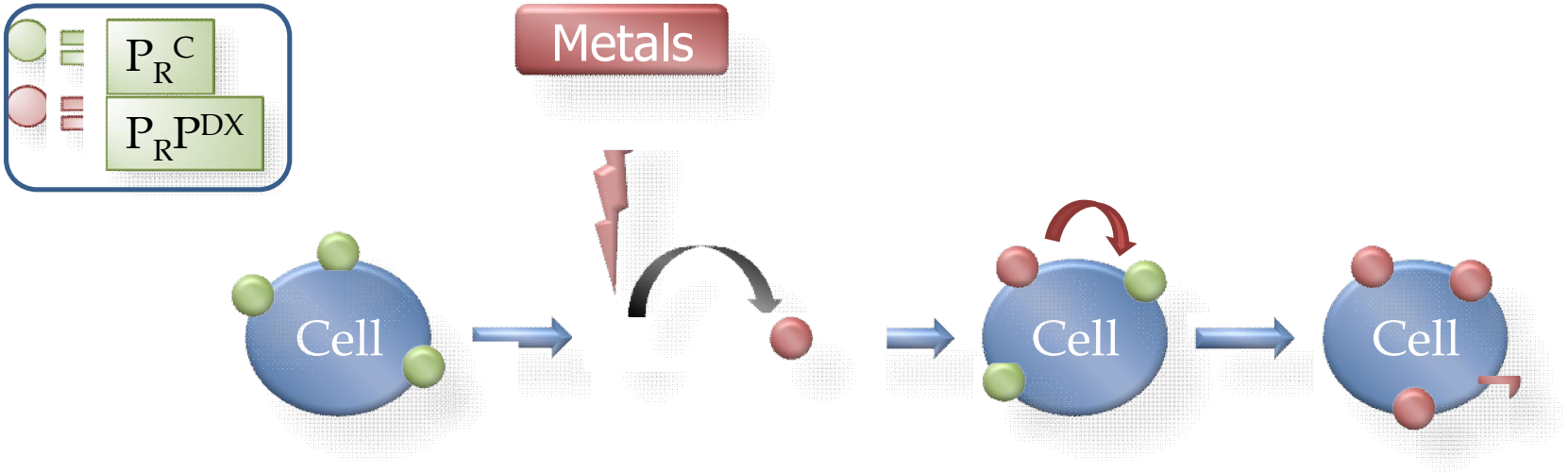


Adapted from James Morré 2006 J Inorg Biochem 100 2140-2149

Genetic and environmental factors contribute to aberrant protein conformation



Abnormal conformation can be transmitted from one cell to another

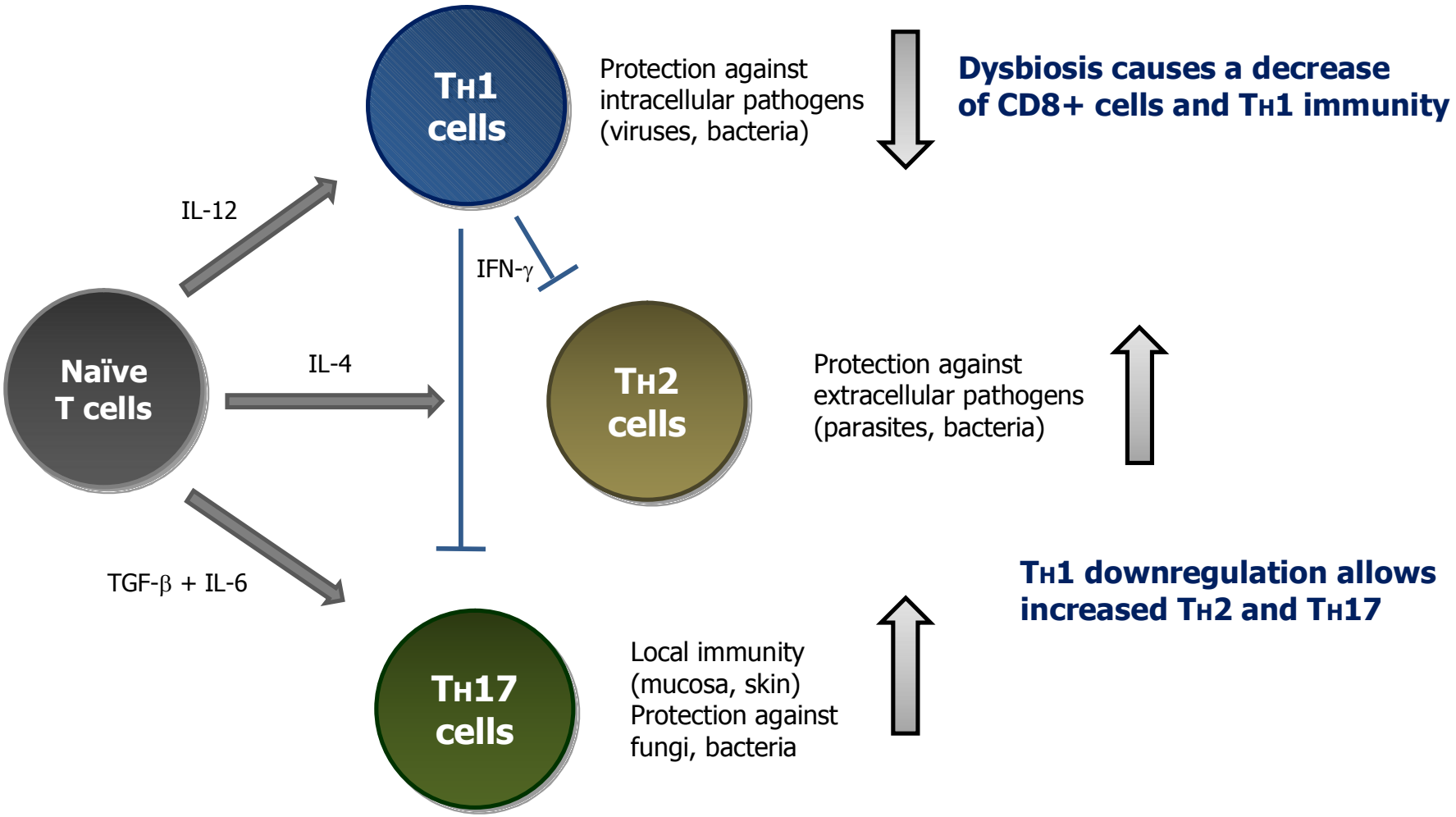


Disease severity in ME is associated with different physiological dysfunctions

	I "Pre-ME"	II Moderate disease	III Severe disease
Dysfunctions	Abnormal faecal test, high H_2S	Abnormal faecal test, high H_2S, exposure to heavy metals	Abnormal faecal test, high H_2S, exposure to heavy metals that has caused aberrant protein conformation (APD)
Symptoms	No fatigue, possible gastro-intestinal symptoms. Low VO_2, slow recovery. May be asymptomatic	Fatigue, gastro-intestinal symptoms	Strong fatigue, multiple symptoms
Treatment	Restore the gut: probiotics	Restore the gut: probiotics, enterocoated antibiotics. Metal chelation, Zinc supplementation	Difficult. Gut restoration, metal chelation. Treatment of associated dysfunctions (opportunistic infections). Treatment of APD is still experimental

Increasing immune dysregulations (depressed T and NK cells, Th17 activation, opportunistic infections…)

Immune alterations resulting from intestinal dysfunction



Consequences of altered immunity

- **TH1 decrease favors the development of opportunistic viral infections**

HHV-6, Epstein-Barr, parvovirus B19, enteroviruses are found in ME patients. Gastro-intestinal mucosa is a major site of infection


in vivo 23: 209-214 (2009)

Detection of Herpesviruses and Parvovirus B19 in Gastric and Intestinal Mucosa of Chronic Fatigue Syndrome Patients

MARC FRÉMONT[1], KRISTINE METZGER[1], HAMADA RADY[2], JAN HULSTAERT[3] and KENNY DE MEIRLEIR[4]

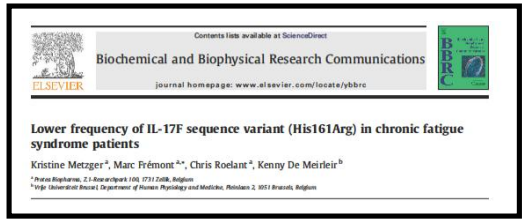
[1]Protea Biopharma, Zellik;
[2]Vrije Universiteit Brussel, [4]Department of Human Physiology and Medicine, Brussels;
[3]General Hospital Jan Portaels, Department of Gastroenterology, Vilvoorde, Belgium

- **TH2 increase favors the development of allergies**

- **TH17 increase promotes inflammation, autoimmunity, blood-brain barrier disruption**

Genetic background plays a role in TH17 upregulation

Polymorphisms of IL-17F, IL-6, TLR4, TGF-β genes are associated with ME and other intestinal diseases (Crohn's disease, UC, IBS)


Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

ELSEVIER

Lower frequency of IL-17F sequence variant (His161Arg) in chronic fatigue syndrome patients

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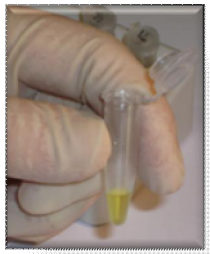
Patient evaluation

- Urine test for marker associated with H_2S production
- Intestinal microflora evaluation
- Heavy metals analysis
- Presence of proteins with abnormal conformation
- Assays evaluating subsequent immune dysfunctions (immune dysregulations, opportunistic infections...)

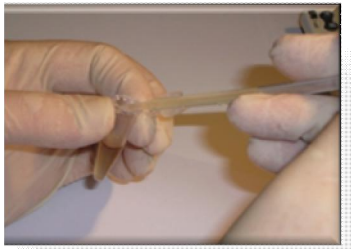
A marker associated with H_2S production can be measured with a simple urine test

1. Collect urine

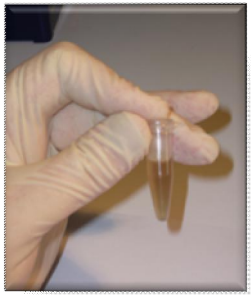
2. Open tube containing test reagent



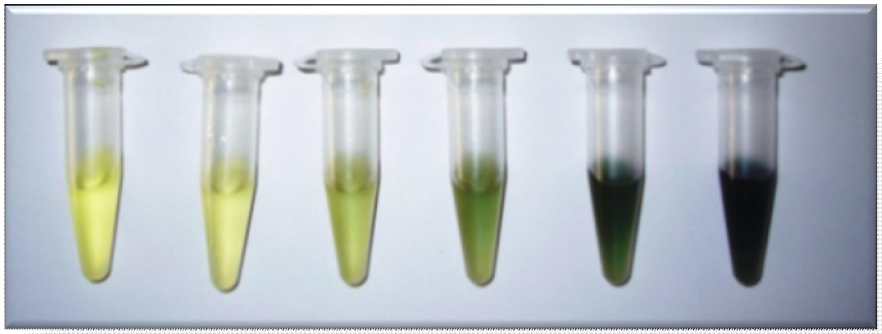
3. Add a few drops of urine to the test reagent



4. Mix by shaking gently. Wait for two minutes



5. Observe color changes. Dark color = positive sample



Negative or Pre-ME

Moderate disease

Severe disease

A specific microbiological assay can determine gut microflora populations

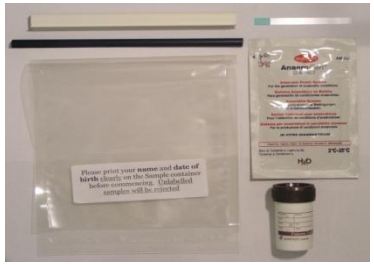
- **Investigation of the microbial flora of the intestinal tract**

- Quantifies major aerobic and anaerobic bacterial groups and yeast

- Focuses on dysbiosis (imbalance of the intestinal ecosytem) rather than digestive analysis to ascertain gut integrity

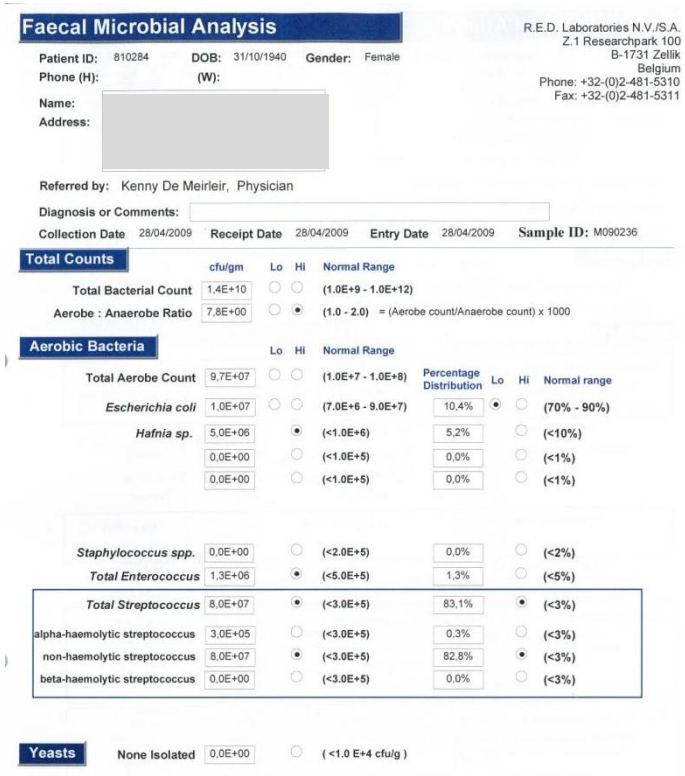
- **Challenge: keep anaerobic bacteria viable for analysis**

- Validated oxygen-free, temperature controlled collection and shipping system



Microbiological assay : sample result

- **Patient presents increased *Streptococcus*, *Enterococcus*, and *Prevotella***



Faecal Microbial Analysis

R.E.D. Laboratories N.V./S.A.
Z.1 Researchpark 100
B-1731 Zellik
Belgium
Phone: +32-(0)2-481-5310
Fax: +32-(0)2-481-5311

Patient ID: 810284 DOB: 31/10/1940 Gender: Female
Phone (H): (W):
Name:
Address:
Referred by: Kenny De Meirleir, Physician
Diagnosis or Comments:
Collection Date 28/04/2009 Receipt Date 28/04/2009 Entry Date 28/04/2009 Sample ID: M090236

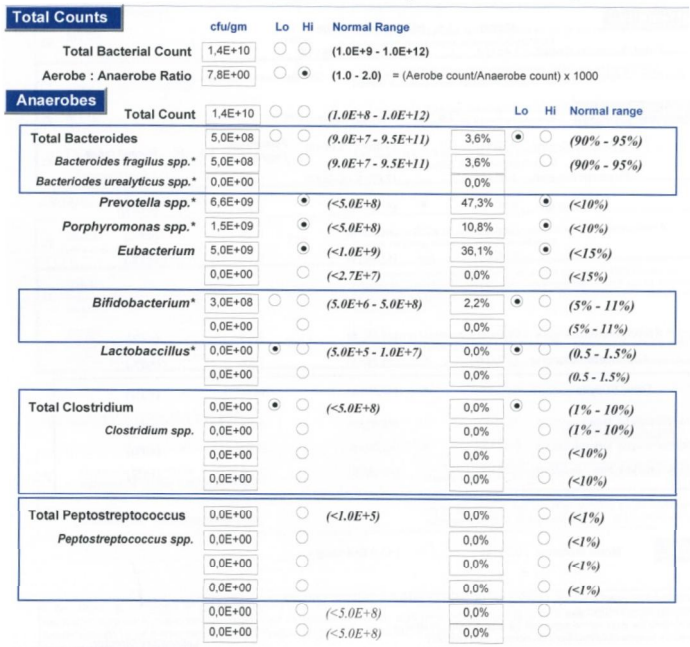
Total Counts

	cfu/gm	Lo	Hi	Normal Range
Total Bacterial Count	1,4E+10	○	○	(1.0E+9 - 1.0E+12)
Aerobe : Anaerobe Ratio	7,8E+00	○	●	(1.0 - 2.0) = (Aerobe count/Anaerobe count) x 1000

Aerobic Bacteria

		Lo	Hi	Normal Range	Percentage Distribution	Lo	Hi	Normal range
Total Aerobe Count	9,7E+07	○	○	(1.0E+7 - 1.0E+8)				
Escherichia coli	1,0E+07	○	○	(7.0E+6 - 9.0E+7)	10,4%	●	○	(70% - 90%)
Hafnia sp.	5,0E+06		●	(<1.0E+6)	5,2%		○	(<10%)
	0,0E+00		○	(<1.0E+5)	0,0%		○	(<1%)
	0,0E+00		○	(<1.0E+5)	0,0%		○	(<1%)
Staphylococcus spp.	0,0E+00		○	(<2.0E+5)	0,0%		○	(<2%)
Total Enterococcus	1,3E+06		●	(<5.0E+5)	1,3%		○	(<5%)
Total Streptococcus	8,0E+07		●	(<3.0E+5)	83,1%		●	(<3%)
alpha-haemolytic streptococcus	3,0E+05		○	(<3.0E+5)	0,3%		○	(<3%)
non-haemolytic streptococcus	8,0E+07		●	(<3.0E+5)	82,8%		●	(<3%)
beta-haemolytic streptococcus	0,0E+00		○	(<3.0E+5)	0,0%		○	(<3%)

Yeasts None Isolated 0,0E+00 ○ (<1.0 E+4 cfu/g)



Total Counts

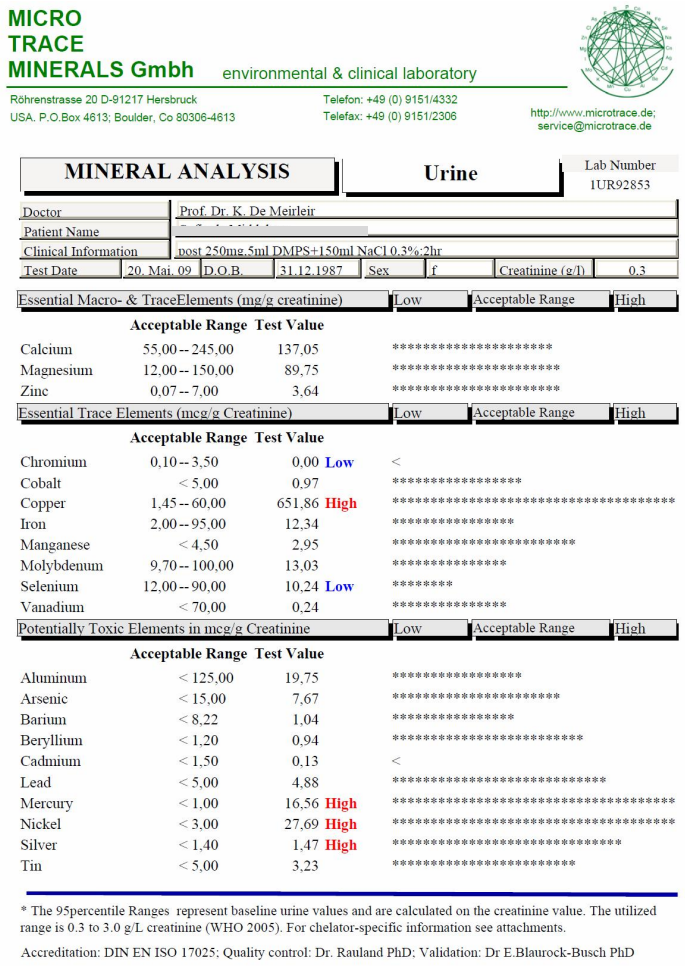
	cfu/gm	Lo	Hi	Normal Range
Total Bacterial Count	1,4E+10	○	○	(1.0E+9 - 1.0E+12)
Aerobe : Anaerobe Ratio	7,8E+00	○	●	(1.0 - 2.0) = (Aerobe count/Anaerobe count) x 1000

Anaerobes

Total Count	1,4E+10	○	○	*(1.0E+8 - 1.0E+12)*		Lo	Hi	Normal range
Total Bacteroides	5,0E+08	○	○	*(9.0E+7 - 9.5E+11)*	3,6%	●	○	*(90% - 95%)*
*Bacteroides fragilus spp.**	5,0E+08		○	*(9.0E+7 - 9.5E+11)*	3,6%		○	*(90% - 95%)*
*Bacteriodes urealyticus spp.**	0,0E+00				0,0%			
*Prevotella spp.**	6,6E+09		●	*(<5.0E+8)*	47,3%		●	*(<10%)*
*Porphyromonas spp.**	1,5E+09		●	*(<5.0E+8)*	10,8%		●	*(<10%)*
Eubacterium	5,0E+09		●	*(<1.0E+9)*	36,1%		●	*(<15%)*
	0,0E+00		○	*(<2.7E+7)*	0,0%		○	*(<15%)*
*Bifidobacterium**	3,0E+08	○	○	*(5.0E+6 - 5.0E+8)*	2,2%	●	○	*(5% - 11%)*
	0,0E+00		○		0,0%		○	*(5% - 11%)*
*Lactobaccillus**	0,0E+00	●	○	*(5.0E+5 - 1.0E+7)*	0,0%	●	○	*(0.5 - 1.5%)*
	0,0E+00		○		0,0%		○	*(0.5 - 1.5%)*
Total Clostridium	0,0E+00	●	○	*(<5.0E+8)*	0,0%	●	○	*(1% - 10%)*
Clostridium spp.	0,0E+00		○		0,0%		○	*(1% - 10%)*
	0,0E+00		○		0,0%		○	*(<10%)*
	0,0E+00		○		0,0%		○	*(<10%)*
Total Peptostreptococcus	0,0E+00		○	*(<1.0E+5)*	0,0%		○	*(<1%)*
Peptostreptococcus spp.	0,0E+00		○		0,0%		○	*(<1%)*
	0,0E+00		○		0,0%		○	*(<1%)*
	0,0E+00		○		0,0%		○	*(<1%)*
	0,0E+00		○	*(<5.0E+8)*	0,0%		○	
	0,0E+00		○	*(<5.0E+8)*	0,0%		○	

Heavy metal analysis : sample result

- **Patient presents mercury and nickel intoxication**



MICRO TRACE MINERALS Gmbh environmental & clinical laboratory

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USA. P.O.Box 4613; Boulder, Co 80306-4613

Telefon: +49 (0) 9151/4332
Telefax: +49 (0) 9151/2306

http://www.microtrace.de;
service@microtrace.de

MINERAL ANALYSIS | **Urine** | Lab Number 1UR92853

Doctor	Prof. Dr. K. De Meirleir
Patient Name	
Clinical Information	post 250mg,5ml DMPS+150ml NaCl 0,3%;2hr

Test Date	20. Mai. 09	D.O.B.	31.12.1987	Sex	f	Creatinine (g/l)	0.3

Essential Macro- & TraceElements (mg/g creatinine)	Acceptable Range	Test Value	Low	Acceptable Range	High
Calcium	55,00 -- 245,00	137,05		**********	
Magnesium	12,00 -- 150,00	89,75		**********	
Zinc	0,07 -- 7,00	3,64		**********	

Essential Trace Elements (mcg/g Creatinine)	Acceptable Range	Test Value	Low	Acceptable Range	High
Chromium	0,10 -- 3,50	0,00	Low	<	
Cobalt	< 5,00	0,97		**********	
Copper	1,45 -- 60,00	651,86	High	**********	
Iron	2,00 -- 95,00	12,34		**********	
Manganese	< 4,50	2,95		**********	
Molybdenum	9,70 -- 100,00	13,03		**********	
Selenium	12,00 -- 90,00	10,24	Low	********	
Vanadium	< 70,00	0,24		**********	

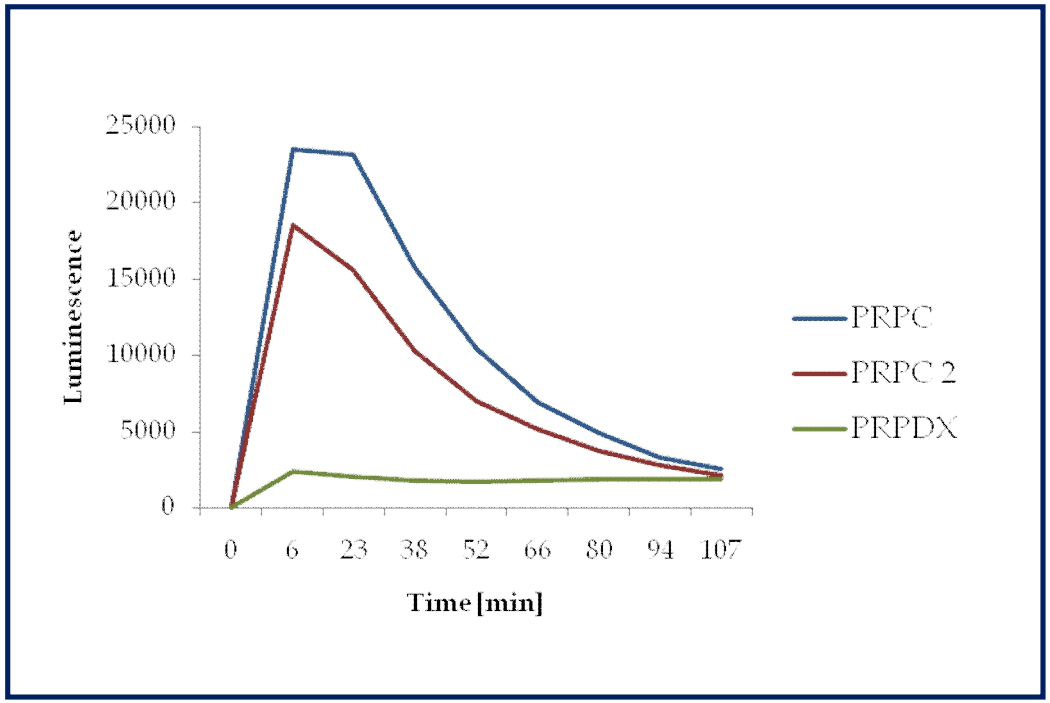
Potentially Toxic Elements in mcg/g Creatinine	Acceptable Range	Test Value	Low	Acceptable Range	High
Aluminum	< 125,00	19,75		**********	
Arsenic	< 15,00	7,67		**********	
Barium	< 8,22	1,04		**********	
Beryllium	< 1,20	0,94		**********	
Cadmium	< 1,50	0,13		<	
Lead	< 5,00	4,88		**********	
Mercury	< 1,00	16,56	High	**********	
Nickel	< 3,00	27,69	High	**********	
Silver	< 1,40	1,47	High	**********	
Tin	< 5,00	3,23		**********	

* The 95percentile Ranges represent baseline urine values and are calculated on the creatinine value. The utilized range is 0.3 to 3.0 g/L creatinine (WHO 2005). For chelator-specific information see attachments.

Accreditation: DIN EN ISO 17025; Quality control: Dr. Rauland PhD; Validation: Dr E.Blaurock-Busch PhD

Abnormal protein conformation assay

- **Aberrant luminescence response indicates abnormal conformation**



CONCLUSIONS

- **Gastro-intestinal dysfunctions play a central role in the pathogenesis of ME**

- **Dysbiosis detrimental effect mediated by increased production of H_2S**

- **Immune dysfunctions and opportunistic infections arise as a consequence of pre-existing intestinal problems**

Once established, infections will contribute to the maintenance/aggravation of the disease

Acknowledgements

- **Henry Butt at the Bio21 Institute, University of Melbourne**

- **Marian Dix Lemle, Independent Researcher, Washington DC**

Med Hypotheses. 2009 Jan;72(1):108-9. Epub 2008 Sep 16. Hypothesis: chronic fatigue syndrome is caused by dysregulation of hydrogen sulfide metabolism. Lemle MD.