

Cell Wall Lysed Probiotic Tincture Decreases Immune Response to Pathogenic Enteric Bacteria and Improves Symptoms in Autistic and Immune Compromised Children

Special Paper for Naturally Oriented Medical Doctors, Naturopaths, and Pediatricians

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Abstract

Up to 70% of children with autism spectrum disorders (ASD's) are impacted by gastrointestinal dysfunction that presents as constipation, food intolerances, diarrhea, inflammation, malabsorption and leaky gut.¹ A major issue for many parents and doctors faced with these health challenges in autistic children is that common testing methods are invasive and show limited results at best. In this study we propose using Immunobilan testing to measure IgA (Immunoglobulin A) and IgM (Immunoglobulin M) immune response to 7 pathogenic enteric bacteria; and if the levels are found to be elevated, to treat with a course of a new cell wall lysed probiotic tincture to decrease the occurrence of immune response indicators.

CytoFlora^{®2} is a natural supplement and the world's first probiotic tincture, containing beneficial bacteria that have been cell wall decimated and suspended in liquid as a tincture. The ingredients are: cell wall lysed lactobacillus rhamnosus, bifidobacterium bifidum, lactobacillus acidophilus, bifidobacterium infantis, bifidobacterium longum, streptococcus thermophilus, lactobacillus plantarum, lactobacillus salivarius, lactobacillus reuteri, lactobacillus casei, lactobacillus bulgaricus, lactobacillus acidophilus DDS-1 and lactobacillus sporogenes in a base of rice starch, ascorbic acid 5% and 18% grain neutral spirits as a preservative.³

Primary physiological effects noted in this intervention study using the cell wall lysed probiotic tincture include the correction of intestinal dysbiosis, removal of pathogens from the gastro-intestinal tract and the creation of an optimal environment for healthy intestinal flora to colonize and flourish.

The Autism Treatment Evaluation Checklist (ATEC), which is a measurement of Speech/Language Communication, Sociability, Sensory/Cognitive Awareness, and Health/Physical/Behavior, was completed on each participant. The results showed 30% improvement across the board.⁴

Key words: gut dysbiosis, ATEC, immune system, beneficial bacteria, Autism, Immunobilan

About Probiotics

Some studies and clinical observations discuss the distinctions between non-viable, or dead cell wall lysed probiotics, and live whole probiotics.^{5,6} "Comparing a live probiotic and a lysed one, in terms of immune system support, is like comparing a match to a blowtorch," said Dr. Elin Ritchie of Taos, N.M.⁷ Live probiotics have historically been recommended for balancing over growth of pathogenic bacteria in the gastrointestinal tract. However, they must be taken in billions of cells to overpower existing pathogenic bugs in the gut.

Lysing the cell wall of beneficial bacteriocin, a substance produced by the bacteria that is toxic to similar bacterial strains, releases vital immune enhancing nutrients from the cell wall and cytoplasm, and drives pathogenic bacteria out of the GI and colon.⁸ These immune system modulators exist inside the cells of healthy bacteria and act as a prebiotic, stimulating the growth and activity of beneficial bacteria in the digestive system. Furthermore, the process of tincturing the cell wall lysed bacteriocin, a traditional Chinese method of remedy preparation, reduces the surface tension and encourages the immediate absorption of its beneficial properties.

CytoFlora[®] is distinctly different from a homeopathic remedy, though it shares common ground with the work and research developed by Bach-Paterson and collaborators in the early part of this century. Their research revealed nosodes made from pathogenic intestinal bacteria to be effective in treating various intestinal maladies.⁹ In the intervention study below this tincture is derived from a host of beneficial bacteria.

Testing Methods

Immunobilan laboratory testing, completed by VIP Dx (Viral Immune Pathology Diagnosis) Laboratory,¹⁰ measured the levels of IgA and IgM to enteric bacteria present in the participant's blood. The Immunobilan test is an antibody-screening assay for antibodies (IgA and IgM) directed against antigens from intestinal pathogens. The testing measured IgA and IgM levels of *Klebsiella pneumoniae*, *Proteus mirabilis*, *Citrobacter koseri*, *Pseudomonas aeruginosa*, *Pseudomonas putida*, *Morganella morganii*, and *Hafnia alvei*.

¹ PubMed <http://www.ncbi.nlm.nih.gov/pubmed/16685179> ² <http://www.bioraynaturaldetox.com/where-do-i-start/healthy-gut-function.aspx> ³ <http://www.bioraynaturaldetox.com/store/product-detail.aspx?id=2> ⁴ <http://www.autism.com/arilatec/index.htm> ⁵ <http://www.ncbi.nlm.nih.gov/pubmed/15702852> ⁶ http://health.ucsd.edu/news/2002/04_29_Raz.html ⁷ Bio-Medicine <http://www.bio-medicine.org/medicine-technology-1/Are-Muramyl-Peptides-the-Magic-Bullet-in-Probiotics-3F-1038-1/> ⁸ Probiotics 2, Applications and practical aspects. Ed. R. Fuller, Pub. Chapman & Hill. "Antimutagenic and antitumor activities of lactic acid bacteria" A Hosono, H. Kitazawa and T. Yamaguchi. ⁹ Intestinal Nosodes of Bach Paterson By O.A. Julian ¹⁰ VIP Dx <http://www.redlabsusa.com/>

LRT has also been successfully used in clinical studies to reduce fatigue and protect cellular and mitochondrial membranes from oxidative damage.^{45,46} For example, this dietary supplement has been used in a vitamin and mineral mixture in cancer patients to reduce the effects of cancer therapy, such as chemotherapy-induced fatigue, nausea, vomiting and other side effects associated with chemotherapy.⁴⁸ This double-blinded, cross-over, placebo-controlled, randomized trial on cancer patients receiving chemotherapy showed that LRT improved fatigue, nausea, diarrhea, impaired taste, constipation, insomnia and other quality of life indicators.⁴⁸

NTFactor[®] has been used in a study with severely chronic fatigued patients to reduce their fatigue.⁴⁵ Using the Piper Fatigue Scale⁵ we found that fatigue was reduced approximately 40.5% (P<0.0001), from severe to moderate fatigue, after eight weeks of LRT supplementation with NTFactor[®].⁴⁵ Recently we examined the effects of this form of lipid replacement therapy on fatigue in moderately and mildly fatigued subjects and to determine if their mitochondrial function improved.⁴⁶ Use of this LRP dietary supplement[®] for 8 or 12 weeks resulted in a 33% or 35.5% reduction in fatigue, respectively (P<0.001).⁴⁶ In this clinical trial there was good correspondence between reductions in fatigue and gains in mitochondrial function.

After only 8 weeks of LRT, mitochondrial function was significantly improved (P<0.001), and after 12 weeks LRT supplementation, mitochondrial function was found to be similar to that of young healthy adults.⁴⁶ After 12 weeks of supplement use, subjects discontinued the supplement for an additional 12 weeks, and their fatigue and mitochondrial function were again measured. After the 12-week wash-out period, fatigue and mitochondrial function were intermediate between the initial starting values and those found after eight or 12 weeks on supplement, indicating that continued dietary LTR is probably required to show improvements in mitochondrial function and maintain lower fatigue scores.⁴⁶ The results indicate that in moderately to severely fatigued subjects dietary LRT can significantly improve and even restore mitochondrial function and significantly improve fatigue. Similar results were found with CFS and/or Fibromyalgia Syndrome patients indicating that LRT plus antioxidants for 8 weeks reduced moderate to severe fatigue by 43.1%.⁷ 🌿

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For additional information on Mitochondrial Function, please see the back cover of this Issue

⁵ Piper BF, Linsey AM, Dodd MJ. Fatigue mechanism in cancer. *Oncol Nursing Forum* 1987; 14:17-23. ⁷ Nicolson, G.L. and Ellithorpe, R. Lipid replacement and antioxidant nutritional therapy for restoring mitochondrial function and reducing fatigue in chronic fatigue syndrome and other fatiguing illnesses. *J Chronic Fatigue Syndr* 2006; 13(1): 57-68. ⁴⁵ Ellithorpe RR, Settineri R, Nicolson GL. Pilot Study: Reduction of fatigue by use of a dietary supplement containing glycopospholipids. *J Am Nutraceut Assoc* 2003; 6(1):23-28. ⁴⁶ Agadjanyan M, Vasilevko V, Ghochikyan A, Berns P, Kesslak P, Settineri R, Nicolson GL. Nutritional supplement (NTFactor) restores mitochondrial function and reduces moderately severe fatigue in aged subjects. *J Chronic Fatigue Syndr* 2003; 11(3):23-26. ⁴⁸ Colodny L, Lynch K, Farber C, Papish S, et al. Results of a study to evaluate the use of Propax to reduce adverse effects of chemotherapy. *J Am Nutraceut Assoc* 2000; 2:17-25.

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IgA are secreted from intestinal cells, while immune cells in the blood produce IgM. In healthy individuals pathogenic bacteria are found only in low quantities in the gut, and antibody titers are very low in the blood. All Immunobilan bacteria are strictly associated with the gut, with the exception of *Klebsiella*, which is also associated with respiratory and urinary tract infections.¹¹ In the case of bacterial overgrowth however, large quantities of IgA are produced and some will be found in the bloodstream. In case of leaky gut, bacterial proteins may make their way to the bloodstream, and specific IgM will be produced. Essentially, high titers of IgM for intestinal bacteria are an indicator of increased intestinal permeability.

Enteric Bacteria Tested

Pseudomonas aeruginosa is a Gram-negative bacterium that is noted for its environmental versatility, its ability to cause disease in particular susceptible individuals with immune system deficiencies, and its resistance to antibiotics. *P. aeruginosa* can produce a number of toxic proteins, which not only cause extensive tissue damage, but also interfere with the human immune system's defense mechanisms. These proteins range from potent toxins that enter and kill host cells at or near the site of colonization to degradative enzymes that permanently disrupt the cell membranes and connective tissues in various organs. This bacterium is also noted for its resistance to many antibiotics.¹²

Klebsiella pneumoniae (*K. pneumoniae*) is one of the most commonly occurring *gram-negative bacteria* studied worldwide. It is a facultative anaerobic, meaning that it has a characteristic feature of becoming both aerobic (survives in the presence of oxygen) and anaerobic (survives in the absence of oxygen) depending upon the situation. It is found naturally in the soil, water and vegetables. In humans, it can be found in the skin, pharynx and gastrointestinal tract. It can cause urinary tract infections and abdominal infections. In fact, it is the second only to *E. coli* as a pathogenic cause of UTI. It normally affects persons with low immune systems such as hospital patients, *diabetes* patients and people with chronic lung disease.¹³

Proteus mirabilis is a bacterium that can cause kidney stones, as well as hard-to-treat urinary tract infections. An enzyme produced by *P. mirabilis*, which breaks down urea in the urinary tract, reduces the acidity of urine and leads to the formation of kidney or bladder stones. Once a stone begins to form, bacteria stick to the stone and live within its layers, where they are protected from antibiotics.¹⁴

Citrobacter koseri (formerly called *Citrobacter diversus*) is best known as the cause of sepsis and meningitis leading to central nervous system (CNS) abscesses in neonates and young infants. The majority of cases are sporadic, with no clear source of infection.¹⁵

Pseudomonas putida thrives in moist environments. Its isolation from clinical specimens, especially non-sterile sites, is an unusual event and is considered to have uncertain pathogenic significance. In immune-compromised patients, *P. putida* has been found to cause septicemia and septic arthritis.¹⁶

Morganella morganii (*M. morganii*) has been associated with CNS infections, urinary tract infections, *sepsis*, *pneumonia*, musculoskeletal infections, pericarditis, chorioamnionitis, *endophthalmitis*, empyema, and *spontaneous bacterial peritonitis*.¹⁷

Hafnia alvei is a member of the family Enterobacteriaceae, strains may be isolated from the faeces of man and other animals. They may also be found in sewage, soil, water and dairy products. It's an environmental bug, primarily. Some strains carry the genes encoding the ability to cause "attaching and effacing" lesions of intestinal cells, similar to EPEC (enteropathic *E. coli*). It is associated with chronic underlying illnesses.¹⁸

A TEC – Autism Treatment Evaluation Checklist

Participant benchmarks in this intervention study included parental completion of the Autism Treatment Evaluation Checklist (ATEC), as well as Immunobilan laboratory testing. The ATEC was developed by Bernard Rimland and Stephen M. Edelson of the Autism Research Institute, and features a one-page form to be completed by parents, teachers, or caretakers. It consists of 4 subtests: Speech/Language Communication, Sociability, Sensory/ Cognitive Awareness, and Health/Physical/Behavior.

Study Participants

The test group consisted of ten autistic and immune-deficient children, ranging in age from 4 to 15 years old. All pre-existing supplements and medications remained unchanged and no new supplements or medications were added during a 35 day test period, with the exception of the cell wall lysed probiotic tincture, which was added after the first blood draw for Immunobilan testing, ATEC scoring, and a comprehensive health questionnaire were completed.

Baseline Test Results

Immunobilan testing indicated most enteric bacteria levels elevated above 2.0, or normal range, with a mean IgA level of 4.22 and a mean IgM level of 1.86. Reference range <2.0.

ATEC testing showed eight of the ten children scoring in moderate to severe ranges, with a mean total score of 42.10.

¹¹ VIP Dx http://www.redlabsusa.com/intestinal_dysbiosis/ ¹² Pseudomonas Database. http://www.pseudomonas.com/p_aerug.jsp ¹³ Buzzle – Intelligent Life on the Web <http://www.buzzle.com/articles/klebsiella-pneumoniae.html> ¹⁴ Science Daily <http://www.sciencedaily.com/releases/2006/05/060524125023.htm> ¹⁵ Bio News Online http://www.bionewsonline.com/5/2/citrobacter_a.htm ¹⁶ Canada Communicable Disease <http://www.phac-aspc.gc.ca/publicat/ccdr-rmtc/00vol26/dr2621eb.html> ¹⁷ eMedicine <http://emedicine.medscape.com/article/222443-overview> ¹⁸ R Podschun – 2001 jmm.sgmjournals.org/cgi/reprint/50/3/208.pdf

In addition, parents reported a variety of symptoms on their child's health questionnaire at the start of the study, including food sensitivities, yellow teeth, hyperactivity/lethargy, irrational tempers/moods, constipation/diarrhea, anxiety, violent/destructive behaviors, distant and disconnected attitudes, lack of concern/interest in others or their environment, and an inability and disinclination to communicate, both verbally and nonverbally.

First Test Markers

The cell wall lysed probiotic tincture was administered to the children twice a day, at 5 droppers full per dose for a period of 21 days, at which point another blood test and ATEC was completed. Immunobilan testing revealed that 100% of the IgA markers had a statistically significant decrease, with a mean IgA level decrease of 1.79, 42.53%. IgM markers, however, seemed relatively unaffected, with no significant decrease recorded.

21-day ATEC scores also showed statistically significant decreases across the board, with a mean total score of 29.4, indicating an overall 30.17% improvement. Results revealed a 14.12% improvement in Speech/Language

Communication, 27.14% improvement in Sociability, 20% improvement in Sensory/ Cognitive Awareness, and a 45.03% improvement in Health/Physical/Behavior.

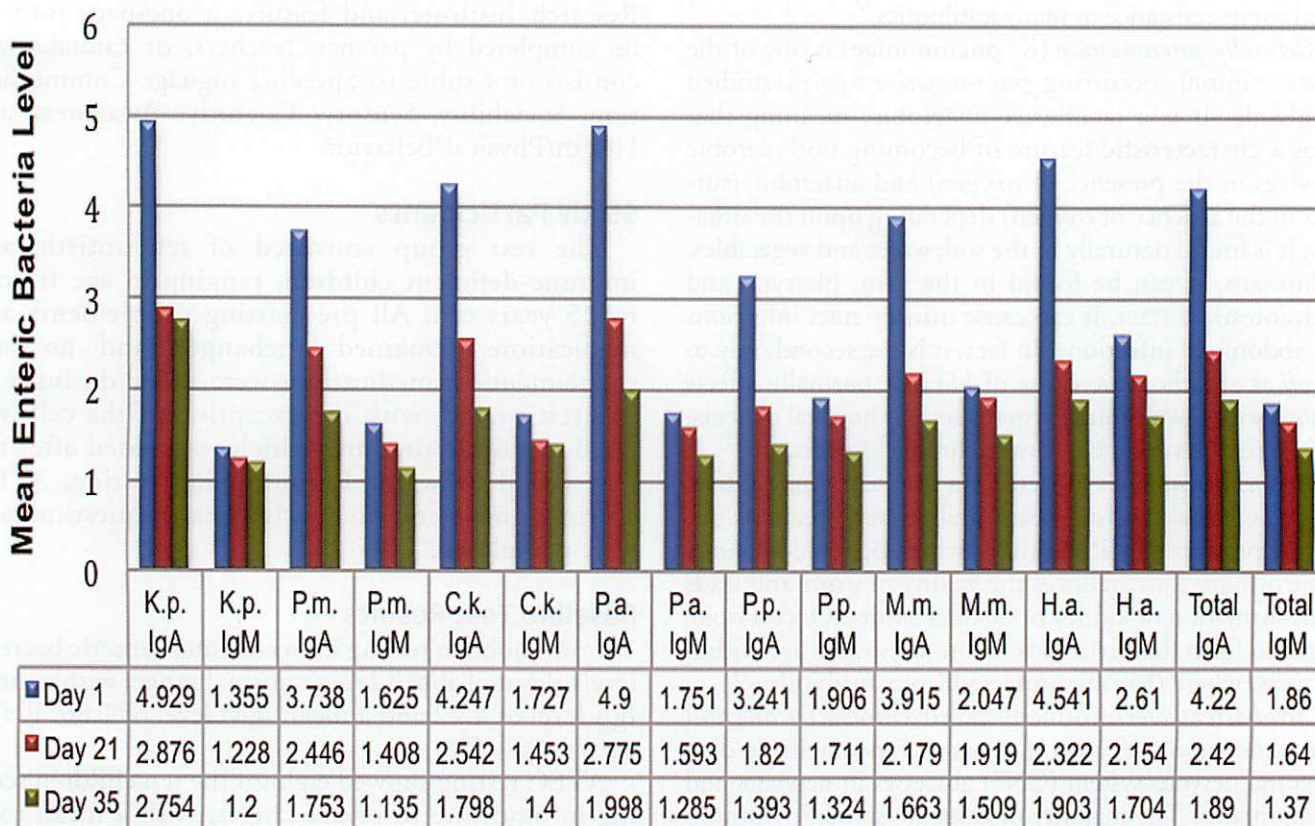
Washout Period

Following the 21-day testing, the participants were monitored during a two-week washout period. The cell wall lysed probiotic tincture was discontinued at this time and no new supplements or medications were added to the child's protocol. All parents reported mild to full return of previous symptoms. At the end of the washout period, a final blood test was administered.

35-Day Test Markers

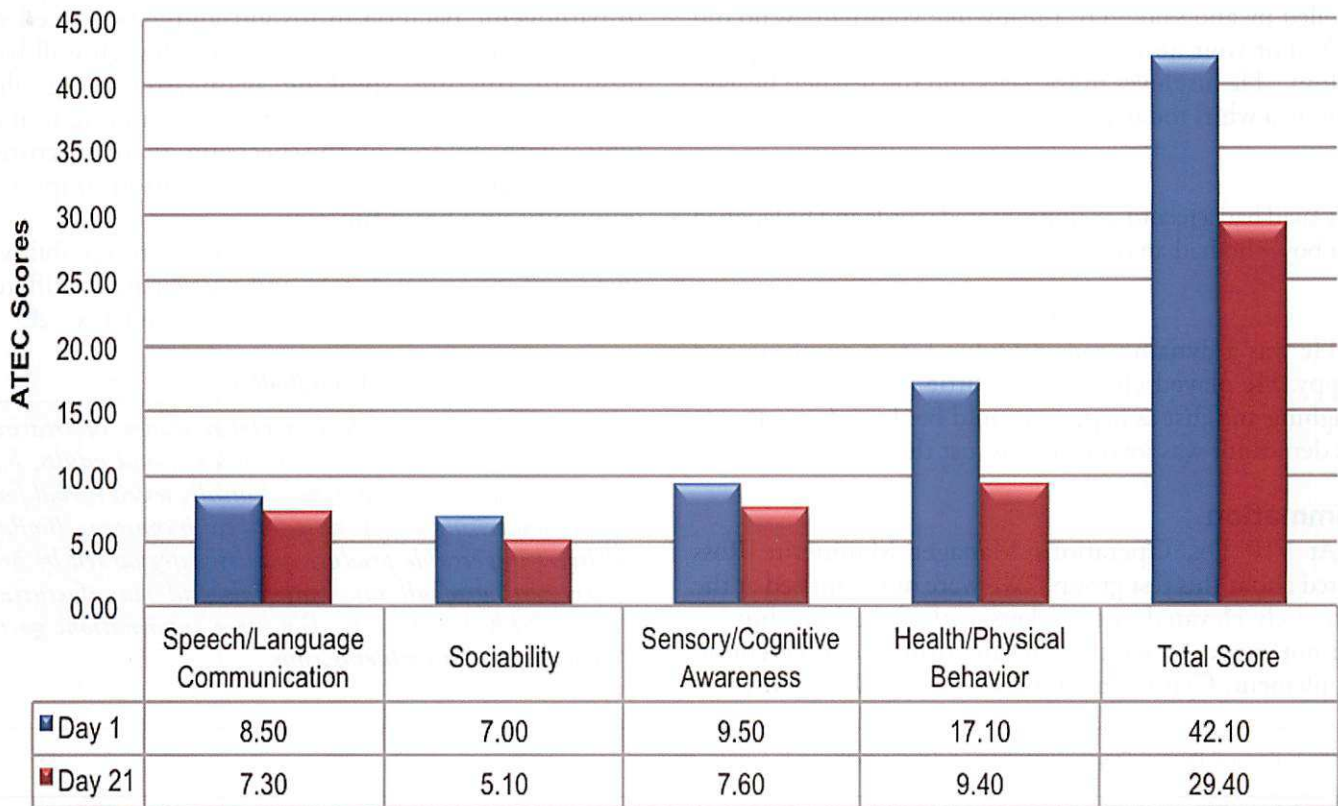
35-day Immunobilan testing revealed a further decrease in enteric bacteria levels, with 12 of the 14 markers showing statistically significant results. All IgA markers continued to significantly decline, with a mean IgA level of 1.89, indicating an overall drop of 2.32, a 55.06% decrease. All IgM markers also declined; with a mean IgM level was 1.37, indicating an overall drop of .49, a 26.60% decrease.

Mean Immunobilan Lab Results



Mean Immunobilan Lab Results show a 55.06% overall decrease in IgA bacteria levels and a 26.60% overall decrease in IgM bacteria levels after 35 days. Normal Range is considered to be below 2.0 and an extreme positive/high range is considered to be above 3.0.

Mean ATEC Scores



Mean scores from the Autism Treatment Evaluation Checklist (ATEC) show a 30.17% overall improvement after 21 days.

Case Study

David Y., a six-year-old boy diagnosed with autism spectrum disorder. Had full vocabulary until age 3 when he lost all ability to speak. Presented at beginning of study with 2 years of Speech Therapy with no improvement. What is interesting about this case is that his speech therapy aide supplied the notes. She did not know he was a part of this study.

David begins intake of the intervention supplement

Day 5

We had a great day today, although he really seemed to lose his steam after lunch. During Spanish he got upset when we asked him to get up and sit between two kids. He just seemed tired, so we got out his napping blanket and let him lounge on that for a few minutes.

Day 14

I just want to tell you he was an angel on Monday and a little devil on Tuesday, running and running, not listening. His behavior seems to fluctuate from one day to the other. I am doing the same routine, same discipline, even demeanor, etc. Can you link this to anything?

Day 21

The whole school is abuzz about him talking. It is amazing the rate he is going at!

Wash Out Period

Day 24

I just thought you ought to know that he was a little off when he came in yesterday. Not bad, just not himself. More scattered and irritable.

End of Wash Out Period

Day 38

Lately he hasn't been able to retrieve words he could 10 days ago. Things that motivated him before, no longer do. Maybe they are losing their appeal or maybe he's just not feeling great. I thought this worth mentioning because it might be related to medications too.

David resumes intake of the intervention supplement

Day 40

He whined and clung and even cried yesterday. Didn't seem to be able to handle the workload though his words have jumped back up.

Day 46

He's had some great days since last I wrote. He had a record high of 19 unprompted communications today! When he came in with his hair slicked up, another teacher strolled by and said, "Hi, I really like your hair! Who did it? You or your mom?" As cool as a cucumber he replied, "Mom." He just loves music class and the teacher; he gave singing a whirl today too!

Day 49

David had a record 28 unprompted words and he laughed at a boy who had an orange section in his mouth.

Day 65

He was a dynamo today! Didn't forget anything and happy. He played chase with a little girl and they were laughing and just as happy as could be. I can honestly say his demeanor was joyous. It was just the best.

Summation

At VIP Dx, Operations Manager Marguerite Ross noted about this test group, "We were not surprised at the extremely elevated gut markers with these kids, but we did not expect to see these results from the intervention supplement, CytoFlora®, in only 21 days. We are used to

seeing some positive results from a course of antibiotics and probiotics in 8 months. We ran the tests again to make sure that what we were seeing was real. It was."¹⁹

David Y. had one of the highest immune responses to pathogenic bacteria in his gut and experienced significant improvement after ingesting the cell wall lysed probiotic tincture, speaking again after being silent for three years. While we observe that no one result is typical with children on the spectrum, there is a correlation between decreasing gut inflammation, improving immunity, and brain function.

Children in this study experienced a greater ability to empathize, be joyful, laugh, and participate in different environments with more ease and independence. ❁

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¹⁹ VIP Dx Research Partners Program http://www.redlabsusa.com/research_partners_program/

**“WE HAVE WORDS...”**

Four weeks ago my daughter's speech clarity was at 25%, I would say now it is at 80%. She is mimicking words and filling in words to songs. I have been making videos of her singing. I wasn't sure what was responsible for the speech clarity until we ran out of Cytoflora. I WILL NEVER EVER RUN OUT OF CYTOFLORA AGAIN!!” -Wendy, mom of Chloe almost 4yrs. ASD



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