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3. Metabolic Correction
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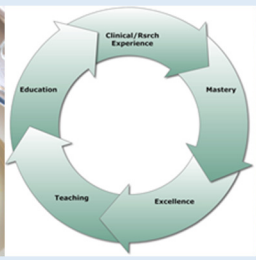

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Notable publications in 2014—❶ Lab Fraud in Functional Medicine, ❷ ISIFMC Position on HPS2-THRIVE; ❸ Unified Antiviral Strategy, ❹ Metabolic Correction: www.ichnfm.org/publications/IJHNFM_2014_review.pdf

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Assessment of the Diagnostic Accuracy of Recently Introduced DNA Stool Screening Test

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Introduction

The "gold standard" for detection of enteric pathogens in stool samples is bacterial culture using a variety of selective and differential media. However, culture methods can require several days to complete and are targeted for the detection of bacteria that can be grown in culture. There is need for qualitative and quantitative tests that are more rapid than bacterial culture. Real-time detection polymerase chain reaction (RTD-PCR) has been applied for the detection of food-borne pathogens (12), cancer (3,7,11), genetic diseases (20) and infectious diseases (6,8,10,13). This method produced a linear quantitative detection range of 7 logs, with a lower detection limit of 10^3 colony-forming units (CFU)/g tissue or a few copies per reaction. (14)

In 2007, a diagnostic testing laboratory ("Subject Laboratory") began offering a stool-screening test that uses a proprietary DNA method to identify gut microbiota including anaerobes. The Subject Laboratory claims that their DNA assessment is specific, accurate, avoids the pitfalls of sample transport, reports results as specific numbers, and is more sensitive than classic laboratory methods. Their stated cutoff for clinically significant pathogens is 1 x 10^3 organisms/gram. The purpose of this study was to assess the accuracy and specificity of this new testing modality by conducting a proficiency analysis study performed by an independent Life Sciences research organization (IIT Research Institute [IITRI], Chicago).

Materials and Methods

Stool Inoculation: Human stool was utilized as a matrix in which to spike known concentrations of various bacterial pathogens. All samples were prepared from a human stool pool that served as the consistent control matrix for all samples. This matrix also provided a background of normal stool flora and was used throughout the study. The test platforms were the Subject Laboratory's Specimen Collection Kits that were prepared as instructed by the package inserts. One gram of stool was added to each of three vials containing either C&S Medium, 10% Formalin Fixative, or Nucleic Acid Collection Solution. Each vial was subsequently spiked with 0.1mL of bacterial target concentrations at either approximately 1.0 x 10^7CFU/mL or 1.0 x 10^4 CFU/mL. All samples including the normal unaltered stool specimen were shipped to the Subject Laboratory via overnight courier the same day they were prepared with a request for stool analysis.

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Bacteria Used: Cryovials containing frozen aliquots of *Shigella sonnei, Salmonella typhi, Escherichia coli* 0157:H7, *Campylobacter jejuni, Vibrio parahemolyticus, Aeromonas caviae, Plesiomonas shigelloides, Edwardsiella tarda, Yersinia enterocolitica*, and *Clostridium difficile* were utilized. Bacterial preparations were made after aseptically inoculating bacteria into 25 mL of Trypticase Soy Broth. *S. sonnei, S. typhi, E. coli, V. parahemolyticus, A. hydrophilia, P. shigelloides, E. tarda,* and *Y. enterolytica* spiked broths were incubated overnight at 37 ± 2°C overnight. *C. jejuni* and *C. difficile* broths were cultured in anaerobic jars with BD GasPaks™ for 2-3 days at 40 ± 2°C and for 2 days at 37 ± 2°C, respectively.

Colony Counts: Each overnight incubated culture was diluted in 0.1% peptone to a concentration of approximately 1.0 x 10^7 colony forming units/mL (CFU/mL) using McFarland standardization. Serial dilutions were plated in quintuplicate to confirm the concentration of the spike-aliquots. Titer plates were incubated for the various bacteria as described.

Results

A total of 34 stool samples were sent for Stool Testing. The stool pool was tested extensively, using conventional methodologies, on two separate days and found to be free of entero pathogenic bacteria, yeast and parasites. Thirty-one specimens were spiked with bacterial pathogens at clinically significant levels that are within the sensitivity of culture based methods, and at higher levels well above the Subject Laboratory's reported lower limit for detection of pathogens. Three "control" specimens were unaltered and contained no bacterial, fungal or parasitic pathogens. All 31 stool specimens containing bacterial pathogens were reported negative for the indicated pathogens by the Subject Laboratory. Seventeen samples were reported as "Parasite present, taxonomy unavailable." Fifteen samples from the same stool specimen were reported as "No Ova or Parasites." One specimen was reported to contain *Cryptosporidium* sp. and one specimen was reported to contain *Enterobius vermicularis*. Two of the samples that were reported to contain "Parasite present, taxonomy unavailable," were also reported to contain *Cryptosporidium* sp. Complete results are shown in Table 1.

Table 1. Results of Stool analysis Conducted by Subject Laboratory: (-) bacteria not present; (+) bacteria present

Sample ID	Organism Added to Normal Stool Specimen	Quantity	Normal Stool Flora	Opportunistic Bacteria	Pathogenic Bacteria	Yeast/ Fungi	Parasites
1	*Shigella sonnei*	3.4x10^2 CFU/g	+	-	-	-	Parasite Present; taxonomy unavailable
2	*Shigella sonnei*	3.4x10^5 CFU/g	+	-	-	-	Parasite Present; taxonomy unavailable
3	Salmonella typhi	4.4x102 CFU/g	+	-	-	-	Parasite Present; taxonomy unavailable
4	Salmonella typhi	4.4x105 CFU/g	+	-	-	4+ => 1000000pg DNA/g specimen Geotricum sp.	No Ova or Parasites
5	E. coli 0157:H7	2.8x102 CFU/g	+	-	-	-	Cryptosporidium sp. Positive, Parasite Present; taxonomy unavailable
6	E. coli 0157:H7	2.8x105 CFU/g	+	-	-	-	Parasite Present; taxonomy unavailable
7	Campylobacter jejuni	2.8x102 CFU/g	+	-	-	-	Parasite Present; taxonomy unavailable

Table 1. Results of Stool analysis Conducted by Subject Laboratory—*continued*							
Sample ID	**Organism Added to Stool Specimen**	**Quantity**	**Normal Stool Flora**	**Opportunistic Bacteria**	**Pathogenic Bacteria**	**Yeast/ Fungi**	**Parasites**
8	Campylobacter jejuni	2.8x105 CFU/g	+	7.3 X 107 Bacillus sp.	-	-	No Ova or Parasites
9	Vibrio parahemolyticus	5.8x101 CFU/g	+	-	-	-	Parasite Present; taxonomy unavailable
10	Vibrio parahemolyticus	5.8x104 CFU/g	+	-	-	-	Cryptosporidium sp. Positive
11	Aeromonas caviae	3.4x102 CFU/g	+	-	-	-	Parasite Present; taxonomy unavailable
12	Aeromonas caviae	3.4x105 CFU/g	+	-	-	-	Parasite Present; taxonomy unavailable
13	Plesiomonas shigelloides	4.4x102 CFU/g	+	-	-	-	Parasite Present; taxonomy unavailable
14	Plesiomonas shigelloides	4.4x105 CFU/g	+	-	-	-	Enterobius vermicularis Positive
15	Edwardsiella tarda	9.5x102 CFU/g	+	-	-	-	Cryptosporidium sp. Positive, Parasite Present; taxonomy unavailable
16	Edwardsiella tarda	2.4x103 CFU/g	+	-	-	2+ => 1000pg DNA/g specimen Candida sp.	Parasite Present; taxonomy unavailable
17	Edwardsiella tarda	9.5x105 CFU/g	+	-	-	-	Parasite Present; taxonomy unavailable
18	Yersinia enterocolitica	5.0x102 CFU/g	+	1.0 X 108 Staphylococcus aureus	-	-	No Ova or Parasites
19	Yersinia enterocolitica	5.0x105 CFU/g	+	-	-	-	Parasite Present; taxonomy unavailable
20	Clostridium difficile	2.4x101 CFU/g	+	-	-	-	No Ova or Parasites
21	Clostridium difficile	2.4x104 CFU/g	+	-	-	-	No Ova or Parasites
22	Normal Stool Flora	N/A	+	-	-	-	No Ova or Parasites
23	Normal Stool Flora	N/A	+	-	-	-	No Ova or Parasites
24	Shigella sonnei	6.5x103 CFU/g	+	-	-	-	No Ova or Parasites
25	Shigella sonnei	6.5x106 CFU/g	+	-	-	-	No Ova or Parasites
26	Yersinia enterocolitica	9.0x103 CFU/g	+	-	-	-	No Ova or Parasites
27	Yersinia enterocolitica	9.0x106 CFU/g	+	-	-	-	No Ova or Parasites
28	E. coli 0157:H7	5.6x103 CFU/g	+	-	-	-	Parasite Present; taxonomy unavailable

Table 1. Results of Stool analysis Conducted by Subject Laboratory—*continued*							
Sample ID	**Organism Added to Normal Stool Specimen**	**Quantity**	**Normal Stool Flora**	**Opportunistic Bacteria**	**Pathogenic Bacteria**	**Yeast/ Fungi**	**Parasites**
29	E. coli 0157:H7	5.6x106 CFU/g	+	-	-	-	No Ova or Parasites
30	Vibrio parahemolyticus	9.2x102 CFU/g	+	-	-	-	Parasite Present; taxonomy unavailable
31	Vibrio parahemolyticus	9.2x105 CFU/g	+	6.1 X 107 Klebsiella pneumoniae	-	-	Parasite Present; taxonomy unavailable
32	Clostridium difficile	5.4x102 CFU/g	+	-	-	-	No Ova or Parasites
33	Clostridium difficile	5.4x105 CFU/g	+	-	-	-	No Ova or Parasites
34	Normal Stool Flora	N/A	+	-	-	-	No Ova or Parasites
35	E. coli 0157:H7	5.6x103 CFU/g	+	-	-	-	Parasite Present; taxonomy unavailable
36	E. coli 0157:H7	5.6x106 CFU/g	+	-	-	-	No Ova or Parasites
37	Vibrio parahemolyticus	9.2x102 CFU/g	+	-	-	-	Parasite Present; taxonomy unavailable
38	Vibrio parahemolyticus	9.2x105 CFU/g	+	6.1 X 107 Klebsiella pneumoniae	-	-	Parasite Present; taxonomy unavailable
39	Clostridium difficile	5.4x102 CFU/g	+	-	-	-	No Ova or Parasites

Discussion

There is a growing demand for faster results for microbiology testing and a growing demand for molecular based analyses that promise results on demand. However, molecular based testing for stool pathogens is still under development and there are currently no FDA cleared *in vitro* assay commercially available. In this study we challenged the claims of a CLIA licensed laboratory that offers a novel DNA method for identifying microorganisms in human stool samples. Our survey showed that the subject laboratory was unable to identify any of the ten enteric pathogens added to a normal stool specimen even though the quantities of microorganisms added were at levels above the stated threshold of detection for the novel assay. Furthermore, the subject laboratory reported "parasites present" in 50% of the samples tested even though no parasites were added to the survey samples and an equal number of the same stool sample were reported negative for parasites.

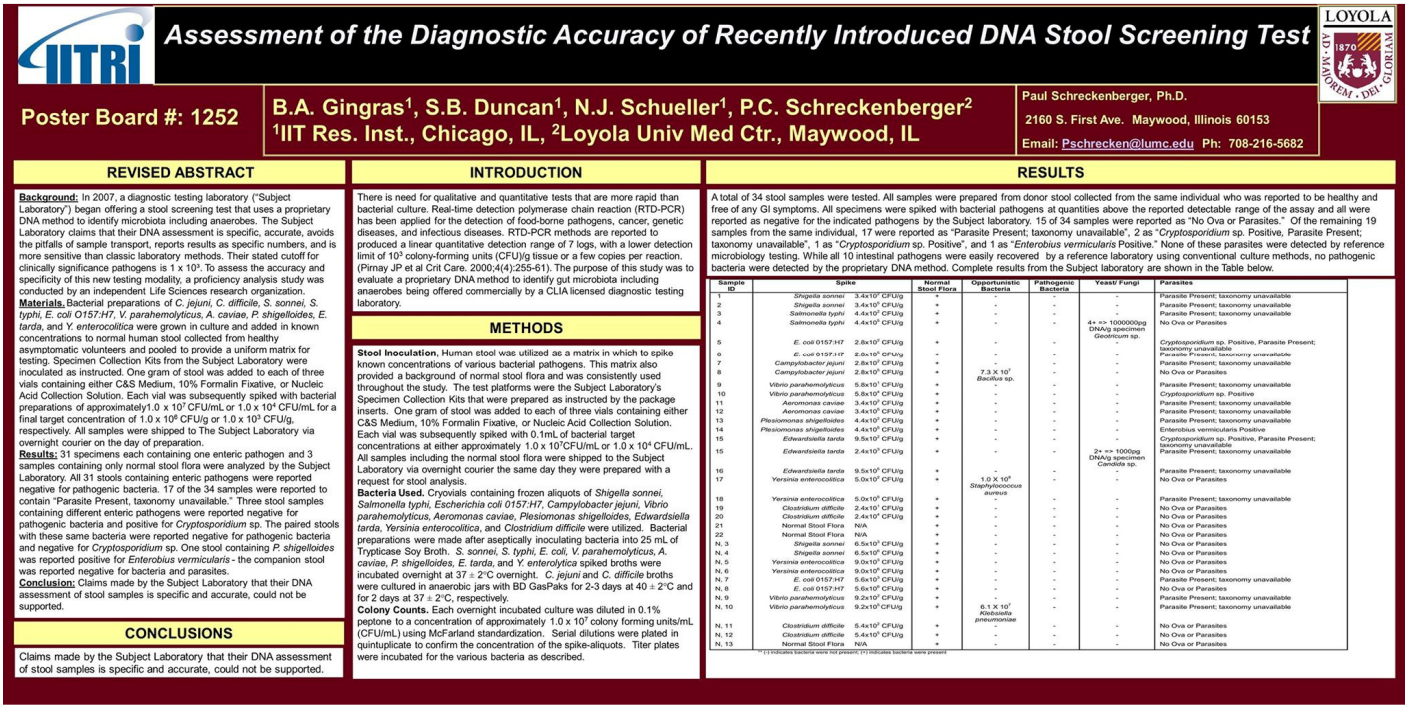
Other investigators have reported the successful application of molecular methods for detection of microorganisms from human gastrointestinal samples. Real-time PCR has been successfully applied for quantification of bacterial DNA in feces (2,9,15,19), colonic tissue (4), rumen (18), gastric tissue (5) and periodontal samples (1). Rinttilä and colleagues designed an extensive set of real-time PCR assays targeting a large group of predominant and pathogenic human gut microbial species. They demonstrated that real-time PCR using SYBR Green I chemistry has an advantage of being a very sensitive and precise technique for an extensive quantitative evaluation of the gut microbiota and is also feasible for detection of human pathogens from fecal samples. Using fecal samples spiked with various amounts of target bacteria they demonstrated detection limits could be obtained that were between 6×10^3 (*H. pylori*) and 6×10^4 (*Clostridium difficile* and *Campylobacter jejuni*) cells per gram of feces (16). In a subsequent publication, Rinttilä et al. used quantitative real-time PCR (qPCR) panel to detect 12 pathogenic microorganisms from fecal samples of irritable bowel syndrome subjects (17).

Some laboratories have developed in-house

assays and offer them commercially with the nomenclature of Lab Developed Tests (LDTs). They offer these assays under the banner of a CLIA licensed laboratory and provide a disclaimer on the patient report stating that the "Assay is not FDA cleared and results should not be used for patient diagnosis." Such is the case for the laboratory that is the subject of this study. The results from the stool analysis are labeled with the following disclaimer: "These test results are not for the diagnosis of disease. They are intended to provide nutritional guidelines to qualified healthcare professionals with full knowledge of patient history and concerns to assist in their design of an appropriate healthcare program." However, when a sample of physicians who use the Subject laboratory for stool analysis were asked if they use the results from the Subject laboratory for patient diagnosis they all said yes and pointed to the fact the laboratory was CLIA licensed so they concluded that the test results must be valid. We should point out that there is no proficiency testing survey available for the assay that is performed by the Subject laboratory, the method being used is proprietary and has not been published and the laboratory is not willing to provide their verification study data to their clients.

Although there is a need to develop rapid molecular testing assays for characterization of the gut microbiome, physicians and patients need to be aware that all stool analysis assays may not be valid and users of these assays should demand to see verification study data in order to discern the claims of the commercial entity offering the lab developed assay. The claims made by the Subject Laboratory that their DNA assessment of stool samples is specific and accurate, could not be supported by this independently conducted proficiency challenge.

Editor's note: The poster presentation from these authors is presented immediately below; readers can magnify the image for better viewing of details.



Assessment of the Diagnostic Accuracy of Recently Introduced DNA Stool Screening Test

Poster Board #: 1252

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REVISED ABSTRACT

Background: In 2007, a diagnostic testing laboratory ("Subject Laboratory") began offering a stool screening test that uses a proprietary DNA method to identify microbiota including anaerobes. The Subject Laboratory claims that their DNA assessment is specific, accurate, avoids the pitfalls of sample transport, reports results as specific numbers, and is more sensitive than classic laboratory methods. Their stated cutoff for clinically significance pathogens is 1 x 10^3. To assess the accuracy and specificity of this new testing modality, a proficiency analysis study was conducted by an independent Life Sciences research organization.
Materials. Bacterial preparations of *C. jejuni, C. difficile, S. sonnei, S. typhi, E. coli O157:H7, V. parahemolyticus, A. caviae, P. shigelloides, E. tarda,* and *Y. enterocolitica* were grown in culture and added in known concentrations to normal human stool collected from healthy asymptomatic volunteers and pooled to provide a uniform matrix for testing. Specimen Collection Kits from the Subject Laboratory were inoculated as instructed. One gram of stool was added to each of three vials containing either C&S Medium, 10% Formalin Fixative, or Nucleic Acid Collection Solution. Each vial was subsequently spiked with bacterial preparations of approximately1.0 x 10^7 CFU/mL or 1.0 x 10^4 CFU/mL for a final target concentration of 1.0 x 10^6 CFU/g or 1.0 x 10^3 CFU/g, respectively. All samples were shipped to The Subject Laboratory via overnight courier on the day of preparation.
Results: 31 specimens each containing one enteric pathogen and 3 samples containing only normal stool flora were analyzed by the Subject Laboratory. All 31 stools containing enteric pathogens were reported negative for pathogenic bacteria. 17 of the 34 samples were reported to contain "Parasite Present, taxonomy unavailable." Three stool samples containing different enteric pathogens were reported negative for pathogenic bacteria and positive for *Cryptosporidium* sp. The paired stools with these same bacteria were reported negative for pathogenic bacteria and negative for *Cryptosporidium* sp. One stool containing *P. shigelloides* was reported positive for *Enterobius vermicularis* - the companion stool was reported negative for bacteria and parasites.
Conclusion: Claims made by the Subject Laboratory that their DNA assessment of stool samples is specific and accurate, could not be supported.

CONCLUSIONS

Claims made by the Subject Laboratory that their DNA assessment of stool samples is specific and accurate, could not be supported.

INTRODUCTION

There is need for qualitative and quantitative tests that are more rapid than bacterial culture. Real-time detection polymerase chain reaction (RTD-PCR) has been applied for the detection of food-borne pathogens, cancer, genetic diseases, and infectious diseases. RTD-PCR methods are reported to produced a linear quantitative detection range of 7 logs, with a lower detection limit of 10^3 colony-forming units (CFU)/g tissue or a few copies per reaction. (Pirnay JP et al Crit Care. 2000;4(4):255-61). The purpose of this study was to evaluate a proprietary DNA method to identify gut microbiota including anaerobes being offered commercially by a CLIA licensed diagnostic testing laboratory.

METHODS

Stool Inoculation. Human stool was utilized as a matrix in which to spike known concentrations of various bacterial pathogens. This matrix also provided a background of normal stool flora and was consistently used throughout the study. The test platforms were the Subject Laboratory's Specimen Collection Kits that were prepared as instructed by the package inserts. One gram of stool was added to each of three vials containing either C&S Medium, 10% Formalin Fixative, or Nucleic Acid Collection Solution. Each vial was subsequently spiked with 0.1mL of bacterial target concentrations at either approximately 1.0 x 10^7CFU/mL or 1.0 x 10^4 CFU/mL. All samples including the normal stool flora were shipped to the Subject Laboratory via overnight courier the same day they were prepared with a request for stool analysis.
Bacteria Used. Cryovials containing frozen aliquots of *Shigella sonnei, Salmonella typhi, Escherichia coli 0157:H7, Campylobacter jejuni, Vibrio parahemolyticus, Aeromonas caviae, Plesiomonas shigelloides, Edwardsiella tarda, Yersinia enterocolitica*, and *Clostridium difficile* were utilized. Bacterial preparations were made after aseptically inoculating bacteria into 25 mL of Trypticase Soy Broth. *S. sonnei, S. typhi, E. coli, V. parahemolyticus, A. caviae, P. shigelloides, E. tarda,* and *Y. enterolytica* spiked broths were incubated overnight at 37 ± 2°C overnight. *C. jejuni* and *C. difficile* broths were cultured in anaerobic jars with BD GasPaks for 2-3 days at 40 ± 2°C and for 2 days at 37 ± 2°C, respectively.
Colony Counts. Each overnight incubated culture was diluted in 0.1% peptone to a concentration of approximately 1.0 x 10^7 colony forming units/mL (CFU/mL) using McFarland standardization. Serial dilutions were plated in quintuplicate to confirm the concentration of the spike-aliquots. Titer plates were incubated for the various bacteria as described.

RESULTS

A total of 34 stool samples were tested. All samples were prepared from donor stool collected from the same individual who was reported to be healthy and free of any GI symptoms. All specimens were spiked with bacterial pathogens at quantities above the reported detectable range of the assay and all were reported as negative for the indicated pathogens by the Subject laboratory. 15 of 34 samples were reported as "No Ova or Parasites." Of the remaining 19 samples from the same individual, 17 were reported as "Parasite Present; taxonomy unavailable", 2 as "*Cryptosporidium* sp. Positive, Parasite Present; taxonomy unavailable", 1 as "*Cryptosporidium* sp. Positive", and 1 as "*Enterobius vermicularis* Positive." None of these parasites were detected by reference microbiology testing. While all 10 intestinal pathogens were easily recovered by a reference laboratory using conventional culture methods, no pathogenic bacteria were detected by the proprietary DNA method. Complete results from the Subject laboratory are shown in the Table below.

Sample ID	Spike	Normal Stool Flora	Opportunistic Bacteria	Pathogenic Bacteria	Yeast/ Fungi	Parasites
1	*Shigella sonnei* 3.4x10^7 CFU/g	+	-	-	-	Parasite Present; taxonomy unavailable
2	*Shigella sonnei* 3.4x10^6 CFU/g	+	-	-	-	Parasite Present; taxonomy unavailable
3	*Salmonella typhi* 4.4x10^6 CFU/g	+	-	-	-	Parasite Present; taxonomy unavailable
4	*Salmonella typhi* 4.4x10^6 CFU/g	+	-	-	4+ => 1000000pg DNA/g specimen *Geotricum* sp.	No Ova or Parasites
5	*E. coli* 0157:H7 2.8x10^5 CFU/g	+	-	-	-	*Cryptosporidium* sp. Positive, Parasite Present; taxonomy unavailable
6	*E. coli* 0157:H7 2.8x10^6 CFU/g	-	-	-	-	Parasite Present; taxonomy unavailable
7	*Campylobacter jejuni* 2.8x10^6 CFU/g	+	-	-	-	Parasite Present; taxonomy unavailable
8	*Campylobacter jejuni* 2.8x10^6 CFU/g	+	7.3 X 10^7 *Bacillus* sp.	-	-	No Ova or Parasites
9	*Vibrio parahemolyticus* 5.8x10^5 CFU/g	+	-	-	-	Parasite Present; taxonomy unavailable
10	*Vibrio parahemolyticus* 5.8x10^6 CFU/g	+	-	-	-	*Cryptosporidium* sp. Positive
11	*Aeromonas caviae* 3.4x10^5 CFU/g	+	-	-	-	Parasite Present; taxonomy unavailable
12	*Aeromonas caviae* 3.4x10^6 CFU/g	+	-	-	-	Parasite Present; taxonomy unavailable
13	*Plesiomonas shigelloides* 4.4x10^5 CFU/g	+	-	-	-	Parasite Present; taxonomy unavailable
14	*Plesiomonas shigelloides* 4.4x10^6 CFU/g	+	-	-	-	Enterobius vermicularis Positive
15	*Edwardsiella tarda* 9.5x10^5 CFU/g	+	-	-	-	*Cryptosporidium* sp. Positive, Parasite Present; taxonomy unavailable
15	*Edwardsiella tarda* 2.4x10^5 CFU/g	+	-	-	2+ => 1000pg DNA/g specimen *Candida* sp.	Parasite Present; taxonomy unavailable
16	*Edwardsiella tarda* 9.5x10^6 CFU/g	+	-	-	-	Parasite Present; taxonomy unavailable
17	*Yersinia enterocolitica* 5.0x10^5 CFU/g	+	1.0 X 10^6 *Staphylococcus aureus*	-	-	No Ova or Parasites
18	*Yersinia enterocolitica* 5.0x10^6 CFU/g	+	-	-	-	Parasite Present; taxonomy unavailable
19	*Clostridium difficile* 2.4x10^5 CFU/g	+	-	-	-	No Ova or Parasites
20	*Clostridium difficile* 2.4x10^6 CFU/g	+	-	-	-	No Ova or Parasites
21	Normal Stool Flora N/A	+	-	-	-	No Ova or Parasites
22	Normal Stool Flora N/A	+	-	-	-	No Ova or Parasites
N, 3	*Shigella sonnei* 6.5x10^5 CFU/g	+	-	-	-	No Ova or Parasites
N, 4	*Shigella sonnei* 6.5x10^6 CFU/g	+	-	-	-	No Ova or Parasites
N, 5	*Yersinia enterocolitica* 9.0x10^5 CFU/g	+	-	-	-	No Ova or Parasites
N, 6	*Yersinia enterocolitica* 9.0x10^6 CFU/g	+	-	-	-	No Ova or Parasites
N, 7	*E. coli* 0157:H7 5.6x10^5 CFU/g	+	-	-	-	Parasite Present; taxonomy unavailable
N, 8	*E. coli* 0157:H7 5.6x10^6 CFU/g	+	-	-	-	No Ova or Parasites
N, 9	*Vibrio parahemolyticus* 9.2x10^5 CFU/g	+	-	-	-	Parasite Present; taxonomy unavailable
N, 10	*Vibrio parahemolyticus* 9.2x10^6 CFU/g	+	6.1 X 10^7 *Klebsiella pneumoniae*	-	-	Parasite Present; taxonomy unavailable
N, 11	*Clostridium difficile* 5.4x10^5 CFU/g	+	-	-	-	No Ova or Parasites
N, 12	*Clostridium difficile* 5.4x10^6 CFU/g	+	-	-	-	No Ova or Parasites
N, 13	Normal Stool Flora N/A	+	-	-	-	No Ova or Parasites

** (-) indicates bacteria were not present; (+) indicates bacteria were present

Citations to Research:

1. Asai, Y., T. Jinno, H. Igarashi, Y. Ohyama, and T. Ogawa. 2002. Detection and quantification of oral treponemes in subgingival plaque by real-time PCR. *J. Clin. Microbiol.* 40:3334–3340.
2. Bélanger, S.D., M. Boissinot, N. Clairoux, F.J. Picard, and M.G. Bergeron. 2003. Rapid detection of *Clostridium difficile* in feces by real-time PCR. *J. Clin. Microbiol.* 41:730–734.
3. Bieche, I., M. Olivi, M.H. Champeme, D. Vidaud, R. Lidereau, and M. Vidaud. 1998. Novel approach to quantitative polymerase chain reaction using real-time detection: application to the detection of gene amplification in breast cancer. *Int. J. Cancer.* 78:661–666.

4. Fujita, H., Y. Eishi, I. Ishige, K. Saitoh, T. Takizawa, T. Arima, and M. Koike. 2002. Quantitative analysis of bacterial DNA from *Mycobacteria* spp., *Bacteroides vulgatus*, and *Escherichia coli* in tissue samples from patients with inflammatory bowel diseases. *J. Gastroenterol*. 37:509–516.

5. He, Q., J.-P. Wang, M. Osato, and L.B. Lachman. 2002. Real-time quantitative PCR for detection of *Helicobacter pylori*. *J. Clin. Microbiol*. 40:3720–3728.

6. Kimura, H. M. Morita, Y. Yabuta, K. Kuzushima, K. Kato, S. Kojima, T. Matsuyama, and T. Morishima. 1999. Quantitative analysis of Epstein–Barr virus load by using a real-time PCR assay. *J Clin Microbiol*. 37:132–136.

7. Laurendeau, I., M. Bahuau, N. Vodovar, C. Larramendy, M. Olivi, I. Bieche, M. Vidaud, and D. Vidaud. 1999. TaqMan PCR-based gene dosage assay for predictive testing in individuals from a cancer family with INK4 locus haploinsufficiency. *Clin. Chem*. 45:982–986.

8. Lewin, S.R., M. Vesanen, L. Kostrikis, A. Hurley, M. Duran, L. Zhang, D.D. Ho, and M. Markowitz. 1999. Use of real-time PCR and molecular beacons to detect virus replication in human immunodeficiency virus type 1- infected individuals on prolonged effective antiretroviral therapy. *J. Virol*. 73:6099–6103.

9. Malinen, E., A. Kassinen, T. Rinttilä, and A. Palva. 2003. Comparison of real-time PCR with SYBR Green I or 5′-nuclease assays and dot-blot hybridization with rDNA-targeted oligonucleotide probes in quantification of selected faecal bacteria. *Microbiology* 149, 269–277.

10. Martell, M., J. Gomez, J.I. Esteban, S. Sauleda, J. Quer, B. Cabot, R. Esteban, and J. Guardia. 1999. High-throughput real-time reverse transcription-PCR quantitation of hepatitis C virus RNA. *J. Clin. Microbiol*. 37: 327–332.

11. Mensink, E., A. van de Locht, A. Schattenberg, E. Linders, N. Schaap, A. Geurts van Kessel, and T. De Witte. 1998. Quantitation of minimal residual disease in Philadelphia chromosome positive chronic myeloid leukemia using real-time quantitative RT-PCR. *Br. J. Haematol*. 102:768–774.

12. Oberst, R.D., M.P. Hays, L.K. Bohra, R.K. Phebus, C.T. Yamashiro, C. Paszko-Kolva, S. J. Flood, J.M. Sargeant, and J.R. Gillespie. 1998. PCR-based DNA amplification and presumptive detection of *Escherichia coli* O157:H7 with an internal fluorogenic probe and the 5′″′ nuclease (TaqMan) assay. *Appl. Environ. Microbiol.* 64:3389–3396.

13. Pahl, A., U. Kuhlbrandt, K. Brune, M. Rollinghoff, and A. Gessner. 1999. Quantitative detection of *Borrelia burgdorferi* by real-time PCR. *J. Clin. Microbiol*. 37:1958–1963.

14. Pirnay, J.P., D. De Vos, L. Duinslaeger, P. Reper, C. Vandenvelde, P. Cornelis, and A. Vanderkelen. 2000. Quantitation of *Pseudomonas aeruginosa* in wound biopsy samples: from bacterial culture to rapid 'real-time' polymerase chain reaction. *Crit. Care.* 4:255-61.

15. Requena, T., J. Burton, T. Matsuki, K. Munro, M.A. Simon, R. Tanaka, K. Watanabe, and G.W. Tannock. 2002. Identification, detection, and enumeration of human *Bifidobacterium* species by PCR targeting the transaldolase gene. *Appl. Environ. Microbiol*. 68:2420–2427.

16. Rinttilä, T., A. Kassinen, E. Malinen, L. Krogius, and A. Palva. 2004. Development of an extensive set of 16S rDNA-targeted primers for quantification of pathogenic and indigenous bacteria in faecal samples by real-time PCR. *J Appl. Microbiol*. 97:1166-77.

17. Rinttilä, T., A. Lyra, L. Krogius-Kurikka, and A. Palva. 2011. Real-time PCR analysis of enteric pathogens from fecal samples or irritable bowel syndrome subjects. *Gut Pathogens* 3:6.

18. Tajima, K., R.I. Aminov, T. Nagamine, H. Matsui, M. Nakamura, and Y. Benno. 2001. Diet-dependent shifts in the bacterial population of the rumen revealed with real-time PCR. *Appl. Environ. Microbiol*. 67:2766–2774.

19. Tenover, F.C., S. Novak-Weekley, C.W. Woods, L.R. Peterson, T. Davis, P. Schreckenberger, F.C. Fang, A. Dascal, D.N. Gerding, J.H. Nomura, R.V. Goering, T. Akerlund, A. S. Weissfeld, E. J. Baron, E. Wong, E. M. Marlowe, J. Whitmore, and D. H. Persing. 2010. Impact of strain type on detection of toxigenic *Clostridium difficile*: comparison of molecular diagnostic and enzyme immunoassay approaches. *J Clin Microbiol*. 48:3719-3724.

20. Von Ahsen, N., E. Schutz, V.W. Armstrong, and M. Oellerich. 1999. Rapid detection of prothrombotic mutations of prothrombin (G20210A), factor V (G1691A) and methylenetetrahydrofolate reductase (C677T) by real-time fluorescence PCR with the LightCycler. *Clin Chem*. 45:694–696.

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Clinical Model of Health and Disease • Review • Nutritional Science • Biochemical Individuality

Chronic Diseases as Inborn Errors of Metabolism: The Metabolic Correction Therapy Approach

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Introduction

The term inborn error of metabolism was coined by British physician, Archibald Garrod (1857-1936), in the early 20th century (1908). He is known for the "one gene, one enzyme" hypothesis, which arose from his studies on the nature and inheritance of alkaptonuria. His seminal text, Inborn Errors of Metabolism was published in 1923 (**1**).

Inborn errors of metabolism comprise a large number of genetic diseases which involves disorders of metabolism. The majority are due to defects of single genes that code for enzymes. Inborn errors of metabolism are inherited disorders. These disorders may be caused by the altered activity of essential enzymes, deficiencies of the substances that activate the enzymes, or faulty transport of important metabolic compounds.

Inborn errors of metabolism often require dietary changes. The particular enzyme absence or inactivity for each inborn error of metabolism dictates which components are restricted and which should be supplemented. The goals of nutrition therapy are to correct the metabolic imbalance and promote growth and development by providing the adequate needed nutrition, while also restricting (or supplementing) one or more nutrients or dietary components. These restrictions and/or supplementations are specific for each disorder. Inborn errors of metabolism, if subtle can accumulate incomplete metabolic products that can give rise to chronic degenerative diseases.

Chronic degenerative diseases are diseases in which the function or structure of affected tissues or organs will progressively deteriorate over time, whether due to normal bodily wear or lifestyle choices such as exercise or eating habits. These long-lasting diseases are characterized by a slow, progressive deterioration. Degenerative diseases are major causes of morbidity and death (**2**).

We believe that the main human degenerative diseases are divided into four groups: cardiovascular, neoplastic, structural and diseases of the nervous system.

This paper will discuss some relevant aspects (i.e., genetics, biochemical, nutritional and patho-/physiological) in order to answer the question whether CDD should be considered inborn errors of metabolism.

Why we should consider chronic degenerative diseases as inborn errors of metabolism?

The idea of considering chronic degenerative diseases as inborn errors of metabolism is supported by a large amount of evidence concerning the hereditary and biochemical aspects of diseases.

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Some genetic disorders are inherited, while other genetic diseases are caused by acquired changes or mutations in a preexisting gene or group of genes. Mutations occur either randomly or due to some environmental exposure. Any change that affects the quantity or quality of metabolic enzymes predispose to an adverse physiological condition. Even though many conditions *per se* are not inherited, the predisposition to suffer or to be at risk from the condition is. This is the main reason we consider chronic degenerative diseases as inborn errors of metabolism.

Chronic diseases may be caused by genetic factors and environment (lifestyle) and their interaction (i.e. epigenetics) play an important role, and may cause genes to (or fail to manifest) in particular ways. In spite of this, if we submit two non-related individuals to the same conditions why one develops the condition and the other one does not Clearly, genetic mutations are not the only components at work in the body, the genetic predisposition is relevant as is the biochemical individuality of each individual.

Degenerative diseases can manifest themselves in the human body when the body is out of physical and chemical balance. Degenerative diseases are not a local condition just like cancer is not just a tumor, they are chronic, systemic, metabolic dysfunctions, usually characterized by specific dietary deficiencies or insufficiencies, a host of pathological conditions and a series of chemical, physical, mental and energy imbalances.

The concept underlying an individualized, integrated metabolic program is that of biochemical individuality which addresses the patient's deficiency and excess levels, biochemical function, energy level, and psychological factors. Certain individuals have a greater need than that supplied by the diet (even a good dietary regime). Their needs may vary from 10 to 1,000 times the physiological requirement. This could be caused by: Digestive problems, poor absorption, food sensitivities, difficulty in the metabolism of certain amino acids, fatty acids, complex carbohydrates, levels in the precursors of neurotransmitters, etc.

This lack of needed cofactors has the problem that it shows no specific symptoms. Some vague symptoms such as lethargy, irritability, insomnia and difficulty in concentrating may be present. Also it affects the body's ability to resist disease and infection, its ability to recover from exercise, surgery, disease, the ability of the brain to function at a high level. Detecting and treating disease at its earliest stages of cellular biochemical abnormality, rather than waiting for clear clinical symptoms is a cost effective measure and of benefit to the patient. We must have very clear in our minds that nutrient deficiency diseases are the end product of a long and complex series of nutrient depletion reactions.

Enzyme Control of Metabolic Reactions

Enzymes are often linked in multistep pathways, such that the product of one reaction becomes the substrate for another. In addition, the multiple steps provide additional levels of regulation, and intermediates can be shunted into other pathways to make other products. When all the enzymes in a pathway are functioning properly, intermediates rarely build up to high concentrations. This is the basis of the Metabolic Correction concept.

Metabolism and the Metabolic Correction Concept

The Metabolic Correction Concept provides the biochemical explanation of how to use nutrients for prevention and therapeutic purposes against disease. Metabolic Correction is a functional biochemical/physiological concept that explains how improvements in cellular biochemistry help the body achieve metabolic or physiological optimization. Impaired or incomplete cellular biochemical reactions are amended with Metabolic Correction.

Enzyme Defects Cause Metabolic Disorders

It has been documented that the main cause of enzyme defects are genetic mutations that affect the structure or regulation of the enzyme or that create problems with the transport, processing, or binding of enzymatic cofactors. In general, the consequences of an enzyme deficiency are due to perturbations of the cellular biochemistry, because of either a reduction in the amount of an essential product, the buildup or production of a toxic intermediate or side product (**3**) All these tribulations are probably due to a lack or limitation of necessary enzymatic cofactors and coenzymes.

Polymorphisms, Nutrigenomics and Genetic Nutritioneering

The enzymopathy (disturbances of enzyme function) present in these conditions are determinant in the further development of chronic degenerative diseases. are generally characterized by uncertain etiology, multiple risk factors, a long latency period, a prolonged course of illness, non-contagious origin, functional impairment or disability, and incurability. Nevertheless we believe that with proper metabolic correction; these polymorphic inborn errors of metabolism can be made functional through proper metabolic corrections in the patient's physiology as a more effective manner to successfully treat or prevent disease. In order to fully understand this idea, the concept that we first have to embrace is biochemical individuality. Biochemical individuality refers to the unique nutritional needs each person has,

based on their genetics, lifestyle, and environmental exposure to various stresses.

Dr. Roger Williams contributed to the understanding of the molecular origin of disease with the development of the concept of biochemical individuality (**4**). He described anatomical and physiological variations among people and how they related to their individual responses to the environment. He was the first to gain recognition for the term biochemical individuality and how this related to differing nutritional needs for optimal function among different people. He pointed out that even identical twins could be different in their needs for optimal function based upon the fact that they developed in different environments in utero. Although identical twins share the same genes, their differing nutrition and developmental environments can result in different expression of the genes as they grow older. The second important concept we need to understand is the recognition that nutritional status can influence the expression of genetic characteristics. It is now well recognized that our genotype gets transformed into our phenotype as a consequence of nutritional, lifestyle and environmental factors which are important in determining our eventual health patterns.

Dr. Williams coined the term genetotrophic disease to describe diseases which resulted from genetically determined nutritional metabolic needs not being met by the individual and which result in faulty gene expression. Motulsky explained that many the common degenerative diseases are the result of the imbalance nutritional intake with genetically determined needs for good health (**5**).

The principle can explain some of these discrepancies since every individual organism has a distinctive genetic background and therefore distinctive nutritional needs. Although all human beings operate on the same general physical mechanisms and the same metabolic processes, the individual physical structures and genetically determined enzyme efficiencies vary sufficiently between individuals so that the effect of all the combined reactions in one body may be completely different from that in another individual, even if of the same age, sex, and body size (**4**). These concepts can irreversibly change the way medicine is practiced and may result in the extension of both life expectancy and health span, or disease-free years of life.

A person's particular genetics influences on how much of a specific nutrient they need. For example, folic acid is a B-vitamin that is relevant for cardiovascular and neurological health. One important role of folic acid is to decrease the amount of homocysteine that may accumulate as a normal part of metabolism. Homocysteine is an amino acid by product of methionine that plays a role in the development of heart disease, osteoporosis, dementia, and cancer. Folic acid is required to break down homocysteine. In order for folic acid to do this, it must be activated by the enzyme methylenetetrahydrofolate reductase (MTHFR). MTHFR is produced by the body and coded for by a specific gene. People can have different variations of this gene, which slightly changes the structure of MTHFR. This structural change can reduce its function by 30–65%, meaning that it may not be able to activate folic acid as easily. People who have the gene that de- creases the MTHFR activity require higher doses of folic acid or an activated form of the vitamin to effectively push the reaction forward and decrease homocysteine. The requirement for folic acid is greater in people with this genetic variation.

G6PD (Glucose-6-phosphate dehydrogenase) human polymorphism, is a cytosolic enzyme in the pentose phosphate pathway, a metabolic pathway that supplies reducing energy to cells (such as erythrocytes) by maintaining the level of the co-enzyme nicotinamide adenine dinucleotide phosphate (NADPH). G6PD deficiency is the most common human enzyme defect. . Individuals with the disease may exhibit non-immune hemolytic anemia in response to a number of causes, most commonly infection or exposure to certain medications or chemicals. The NADPH in turn maintains the level of glutathione in these cells that helps protect the red blood cells against oxidative damage. Patients with this deficiency should not receive vitamin C infusions because it can cause hemolytic anemia. The regulation of gene expression gives the cell control over the versatility and adaptability of any organism and serves as a substrate for evolutionary change. This is profound since our diet has impact on our genetic code which is passed on to the next generation. The more nutritious our diet, the stronger will be the gene pool.

The Km concept

Approximately 50 different human genetic diseases are due to a poor binding affinity (Km) of the mutant enzyme for its coenzyme. This can be remedied by feeding high-dose B vitamins, which raise levels of the corresponding coenzyme. Many polymorphisms also result in a lowered affinity of the enzyme for the coenzyme (**6**).This should be of interest since it seems that a considerable percentage of the population is affected by polymorphisms (**6**).

The Weakest Link

Every element of your physiology must be addressed in order for your body to perform at peak efficiency. Michael Zumpano coined the term Metabolic Optimization in the early eighties to describe his systematic approach to training and nutrition. You can view the metabolic processes as links in a chain. The strength of the entire chain can be compromised by only one weak link.

A significant fraction of the American population appears to not obtain even the Recommended Daily Allowance (RDA) of some critical nutrients from their food (**7,8**). Levels of deficiencies that fall between the RDA and the levels that produce recognized deficiency diseases (Subclinical Deficiencies) can have serious health consequences. Supplementation with specific nutrients has been estimated to be cost effective in preventing disease. Food alone may not provide sufficient micronutrients for preventing deficiency (**9**). A large proportion of older adults do not consume sufficient amounts of many nutrients. Supplements compensate to some extent, but only an estimated half of this population uses them daily.

When one component in the metabolic micronutrient network is inadequate, repercussions are experienced in a specific biochemical process or even in a large number of processes and can lead to diseases. Many of the carriers of 50 human genetic diseases that are due to defective enzymes can be remedied or ameliorated by the administration of high doses of the vitamin component of the corresponding needed coenzyme, which raises the levels of the coenzyme and at least may partially restore the needed enzymatic activity (**10**).

Conclusion

In most cases, disease results when the individual elects a lifestyle or diet that alters the expression of the genes in such a way that the weakness or uniqueness of inheritance factors result in a phenotype we call disease. That is why we can consider chronic degenerative diseases as inborn errors of metabolism. Metabolic Correction seems as a very logical approach toward attaining the healthy state.

References:

1. Garrod AE. Inborn error of metabolism. 1923, Oxford University Press. London, UK.
2. Doll R. Chronic and degenerative disease: major causes of morbidity and death. *Am J Clin Nutr* 1995; 62: 1301S-1305S.
3. http://medicine.jrank.org/pages/2512/Metabolic-Disease-Enzyme-Defects-Cause-Metabolic-Disorders.html#ixzz2abE7pncM
4. Williams RJ. Biochemical Individuality and Its Implications. *Chem Eng News*, 1947,25(16), 1112-3
5. Motulsky A. Nutrition and genetic susceptibility to common diseases. *Am J Clin Nutrition* 1992; 55:1244S-1245S.
6. Ames BN, Elson-Schwab I, Silver EA. High-dose vitamin therapy stimulates variant enzymes with decreased coenzyme-binding affinity (increased Km): Relevance to genetic disease and polymorphisms. *Am J Clin Nutr* 2002;75:616-58.
7. Davis DR, Williams RJ. Potentially useful criteria for judging nutritional adequacy. *Am J Clin Nutrition* 1976; 29:710-715.
8. http://www.health.gov/dietaryguidelines/ Accessed 2014 Jan
9. Misner B. Food alone may not provide sufficient micronutrients for preventing deficiency. *J Int Soc Sports Nutr* 2006;3:51-5.
10. Gonzalez MJ, Miranda-Massari JR. Metabolic Correction: A Functional Explanation of Orthomolecular Medicine. *J Orthomolec Med* 2012;27: 13-20

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Position Paper • Nutritional Science • Cardiology & Cardioprotection

ISIFMC* Position Paper on the HPS2-THRIVE Study

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Introduction

In this brief paper, we review data from the study known as "HPS2-Thrive"[1] and establish our position in refutation of this work.

Summary of Data

HPS2-THRIVE[1] is a recent study of an investigational drug (Tredaptive, Merck) containing both extended release niacin (Niaspan, ERN) and the drug laropiprant, a selective antagonist of the prostaglandin D2 receptor subtype 1 (DP1R), which partially blocks the dermal flushing response to niacin.[2,3] HPS2-THRIVE randomized 25,673 high-risk patients who could tolerate niacin to either placebo or extended-release niacin (ERN) plus laropiprant (ERNL). The study subjects were all on simvastatin 40 mg/day. The primary endpoint was the time to first major vascular event, defined as the composite of non-fatal myocardial infarction (MI) or coronary death, stroke, or any arterial revascularization.[1]

The primary composite endpoint of major vascular events (MVE) was not significantly reduced (risk ratio 0.96, 95% CI: 0.90-1.03, p=0.3) in the active arm. “Serious adverse events” were found in 3% more subjects in the active arm, although most were “minor hyperglycemic problems.” Myopathy generally was uncommon (0.34% per year), but was 4-fold higher overall in the active arm, and 10-fold higher among Chinese subjects.[1,4]

The study subjects had excellent baseline control of serum lipids on statin therapy (simvastatin 40 mg/day) with an average LDL-C of 63 mg/dl, HDL of 44 mg/dl, and triglycerides of 125 mg/dl. In March 2013, the National Lipid Association (NLA) published their position paper[4] stating that in HPS2-THRIVE, “niacin was clinically irrelevant in the average study subject" and "there was substantial subgroup heterogeneity" and concluded that the investigators “tested a drug in patients who, on average, had no indication to take it." MVE reduction with ERNL was strongly predicted by baseline LDL-C (heterogeneity p=0.02), with apparent net benefit if LDL-C was above 58 mg/dl at study entry. Therefore and importantly, this study population was not likely to have any significant CVD reduction. Niacin studies, such as the Coronary Drug Project (CDP)[5,6], have shown significant reductions in cardiovascular events with niacin monotherapy in known CHD. For patients in whom LDL-C or triglycerides are increased, niacin in combination with statins improves both the lipid profile and decreases CV events.

Several clinical benefits of ERNL were noted, including reductions in weight, blood pressure, lipoprotein(a), a significant reduction in arterial vascularization procedures (p= 0.03) and significant reduction in CV risk in the subgroup with the higher baseline LDL cholesterol level (p=0.02). The adherence rate was poor at one year and at the completion of the

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study, and this noncompliance may have altered hard CV outcomes. The average age was 64.9 years, and the study population was mostly male. Thus, the data cannot be confidently extrapolated to a younger population nor perhaps to females.

Position

The claim that HPS2-THRIVE proved that niacin induced more harm than the statin arm of the study is not supported by the data. To evaluate this paper, one must consider ❶ the participants' risk at entry, ❷ their demographics (especially the Chinese population), ❸ known and measured benefits of ERNL, ❹ potential harm of laropiprant, ❺ research support for the benefits of niacin, and ❻ whether the flushing response to niacin correlates with and/or mediates part of its benefit. Unlike other studies using statins and niacin in combination, this study showed increases in serious adverse events (ADE) (3.7% absolute excess adverse events) including:

- Myalgia (0.7%, p<0.001)
- New-onset diabetes (NOD) (1.3%, p<0.001)
- Gastrointestinal problems (1.0%, p<0.001)
- Skin problems (0.3%, p<0.003)
- Infections (1.4%, p<0.001)
- Bleeding (0.7%, p<0.001)

The dose of niacin was high and fixed resulting in dose-related adverse effects. About 43% of the study population were of Chinese descent; this influenced many of the adverse effects, especially the myopathy and skin eruptions.[1,4,7] As noted in the original paper, "the absolute risk of myopathy in the placebo group was higher in China than in Europe and the relative risk with ERNL versus placebo was 5.2 in China, as compared to 1.5 in Europe. This is 10x greater in China participants with 50 cases per 10,000 versus 3 cases per 10,000 in Europe." Overall, the absolute risk of ADEs was low.

Laropiprant may cause adverse effects with either increased or decreased risk of thrombosis.[8-14] Laropiprant with aspirin or clopidogrel induces a prolongation of bleeding time and an inhibitory effect on platelet aggregation ex vivo in healthy subjects and in patients with dyslipidemia.[13] Stimulation of the prostaglandin D receptors as well metabolites of prostaglandin D2 to prostaglandin J2 must be considered as part of the beneficial effects of niacin and the propensity for flushing which may be beneficial.[14-27]

In a recent meta-analysis of niacin-CHD studies, definitive benefit from niacin was demonstrated for CVD and CHD.[28] This included eleven trials of 9,959 patients showing a reduction in composite endpoints of any CVD by 34% and a reduction of major CHD event by 25%. There was no change in CVA. The magnitude of on-treatment HDL difference between treatment arms was not significantly associated with the magnitude of the effect of niacin on CVD outcomes; thus, niacin's reduction in CVD events may occur through a mechanism not reflected by changes in HDL or other lipid parameters.[29-33] Niacin use over three years increased glucose levels by 5 mg % compared to placebo. There was no increased DM risk.[34] Niacin significantly reduced CHD progression and stenosis and other major CV events in 407 subjects in other major clinical trials, including FATS, HATS, AFREGS and CPC clinical trials.[3] Also, analysis of the AIM HIGH trial by Guyton et al indicated a CV benefit by niacin in patients with baseline HDL < 32 mg/dl and triglyceride > 200 mg/dl (American Heart Association 2012 Scientific Sessions. November 3-7, 2012; Los Angeles, California). The data from HPS 2 THRIVE does not support harm resulting from the addition of ERNL alone. The data from previous studies as well as the improvement in revascularization procedures in HPS2-THRIVE support CV benefit of ERNL as monotherapy and as additive therapy with statins and other lipid-lowering agents.

Conclusion & Summary of Major Points

1. Niacin remains an efficacious agent for the treatment of dyslipidemia and prevention of CVD as single therapy, and with statins and other lipid-lowering agents with a relatively low side effect profile. Neither the HPS2-THRIVE nor the AIM HIGH studies provide any convincing evidence against the use of niacin in the appropriate clinical situation.
2. The vast majority of clinical trials with niacin alone or niacin with other anti-lipid agents show significant reductions in CVD, CHD and carotid atherosclerosis.
3. In patients not taking statins or those with high LDL levels at baseline (over about 85 mg/dl), high TG over 200 mg/dl and HDL-C < 32 mg/dl, HPS2-THRIVE study results are not likely to be applicable.
4. Laropiprant may have actually been the culprit in the increased incidence of adverse effects; the data do not show that niacin alone was the cause. In addition, Laropiprant may have actually reduced the efficacy of niacin in this trial as noted above.
5. If a patient does not have goal LDL-C, triglycerides, or HDL, then niacin may provide additional

efficacy in LDL reduction, LDL particle number reduction, increase in LDL size, increase in HDL, HDL 2b, HDL particle number, HDL function, reverse cholesterol transport and triglyceride reduction.

6. Patients with CVD and dyslipidemia with HDL< 32 mg/dl and triglyceride > 200 mg/dl may benefit from extended–release niacin added to intensive statin based LDL-C lowering therapy.
7. Niacin may have non-lipoprotein actions that are clinically important to prevent and treat CVD and CHD.
8. Niacin remains in important agent for the treatment of dyslipidemia and the prevention and treatment of CVD, CHD, and carotid atherosclerosis.

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Citations to literature:

1. Effects of Extended-Release Niacin with Laropiprant in High-Risk Patients. The HPS2-THRIVE Collaborative Group. *N Engl J Med* 2014; 371:203-212
2. Lai E, De Lepeleire I, Crumley TM, Liu F, Wenning LA, Michiels N, Vets E, O'Neill G, Wagner JA, Gottesdiener K. Suppression of niacin-induced vasodilation with an antagonist to prostaglandin D2 receptor subtype 1. *Clin Pharmacol Ther*. 2007 Jun;81(6):849-57
3. Lai E, Wenning LA, Crumley TM, De Lepeleire I, Liu F, de Hoon JN, Van Hecken A, Depré M, Hilliard D, Greenberg H, O'Neill G, Metters K, Gottesdiener KG, Wagner JA. Pharmacokinetics, pharmacodynamics, and safety of a prostaglandin D2 receptor antagonist. *Clin Pharmacol Ther*. 2008 Jun;83(6):840-7
4. NLA Statement on Use of Niacin in Light of HPS2-THRIVE Presentation on March 9, 2013. lipid.org/nla/nla-statement-use-niacin-light-hps2-thrive-presentation-march-9-2013
5. Coronary Drug Project report on clofibrate and niacin. *Atherosclerosis*. 1978 Jul;30(3):239-40
6. Canner PL, Berge KG, Wenger NK, Stamler J, Friedman L, Prineas RJ, Friedewald W. Fifteen-year mortality in Coronary Drug Project patients: long-term benefit with niacin. *J Am Coll Cardiol*. 1986 Dec; 8(6):1245-55
7. Yang YL, Hu M, Chang M, Tomlinson B. A high incidence of exanthematous eruption associated with niacin/laropiprant combination in Hong Kong Chinese patients. *J Clin Pharm Ther*. 2013 Dec;38(6):528-32
8. HPS2-THRIVE Collaborative Group. HPS2-THRIVE randomized placebo-controlled trial in 25 673 high-risk patients of ER niacin/laropiprant: trial design, pre-specified muscle and liver outcomes, and reasons for stopping study treatment. *Eur Heart J*. 2013 May;34(17):1279-91
9. Ong KL, Barter PJ, Waters DD. Cardiovascular drugs that increase the risk of new-onset diabetes. *Am Heart J*. 2014;167:421-8
10. Waters DD, Ho JE, DeMicco DA, Breazna A, Arsenault BJ, Wun CC, Kastelein JJ, Colhoun H, Barter P. Predictors of new-onset diabetes in patients treated with atorvastatin: results from 3 large randomized clinical trials. *J Am Coll Cardiol*. 2011 . 5;57(14):1535-45
11. Sattar N, Preiss D, Murray HM, Welsh P, Buckley BM, de Craen AJ, Seshasai SR, McMurray JJ, Freeman DJ, Jukema JW, Macfarlane PW, Packard CJ, Stott DJ, Westendorp RG, Shepherd J, Davis BR, Pressel SL, Marchioli R, Marfisi RM, Maggioni AP, Tavazzi L, Tognoni G, Kjekshus J, Pedersen TR, Cook TJ, Gotto AM, Clearfield MB, Downs JR, Nakamura H, Ohashi Y, Mizuno K, Ray KK, Ford I. Statins and risk of incident diabetes: a collaborative meta-analysis of randomised statin trials. *Lancet*. 2010. 27;375(9716):735-42
12. Doneen A, Bale B. Niacin: Throwing the baby out with the bathwater? *Journal of Arteriology*. March 2, 2013. vol 2. baledoneen.com
13. De Kam PJ, Luo WL, Wenning L, Ratcliffe L, Sisk CM, Royalty J, Radziszewski W, Wagner JA, Lai E. The effects of laropiprant on the antiplatelet activity of co-administered clopidogrel and aspirin. *Platelets*. 2013 Nov 8
14. Labrecque P, Roy SJ, Fréchette L, Iorio-Morin C, Gallant MA, Parent JL. Inverse agonist and pharmacochaperone properties of MK-0524 on the prostanoid DP1 receptor. *PLoS One*. 2013 Jun 10;8(6):e65767

15. Gibson CR, Lu P, Maciolek C, Wudarski C, Barter Z, Rowland-Yeo K, Stroh M, Lai E, Nicoll-Griffith DA. Using human recombinant UDP-glucuronosyltransferase isoforms and a relative activity factor approach to model total body clearance of laropiprant (MK-0524) in humans. *Xenobiotica*. 2013 Dec;43(12):1027-36. doi: 10.3109/00498254.2013.791761. Epub 2013 May 3

16. Hovsepian E, Penas F, Goren NB. 15-deoxy-Delta12,14 prostaglandin GJ2 but not rosiglitazone regulates metalloproteinase 9, NOS-2, and cyclooxygenase 2 expression and functions by peroxisome proliferator-activated receptor gamma-dependent and -independent mechanisms in cardiac cells. *Shock*. 2010 Jul;34(1):60-7

17. Yokoyama Y, Masaki T, Kiribayashi K, Nakashima A, Kokoroishi K, Ogawa T, Kohno N, Yorioka N. 15-Deoxy-Delta12,14-prostaglandin J2 inhibits angiotensin II-induced fibronectin expression via hepatocyte growth factor induction in human peritoneal mesothelial cells. *Ther Apher Dial*. 2010 Feb;14(1):43-51

18. Kansanen E, Kivelä AM, Levonen AL. Regulation of Nrf2-dependent gene expression by 15-deoxy-Delta12,14-prostaglandin J2. *Free Radic Biol Med*. 2009 Nov 1;47(9):1310-7

19. Hwang J, Lee HI, Chang YS, Lee SJ, Kim KP, Park SI. 15-deoxy-Delta12,14-prostaglandin J2-induced down-regulation of endothelial nitric oxide synthase in association with HSP70 induction. *Biochem Biophys Res Commun*. 2007 May 25;357(1):206-11. Epub 2007 Mar 30

20. Lin YC, Huang GD, Hsieh CW, Wung BS. The glutathionylation of p65 modulates NF-κB activity in 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2-treated endothelial cells. *Free Radic Biol Med*. 2012 May 1;52(9):1844-53

21. Migita H, Morser J. 5-deoxy-Delta12,14-prostaglandin J2 (15d-PGJ2) signals through retinoic acid receptor-related orphan receptor-alpha but not peroxisome proliferator-activated receptor-gamma in human vascular endothelial cells: the effect of 15d-PGJ2 on tumor necrosis factor-alpha-induced gene expression. *Arterioscler Thromb Vasc Biol*. 2005 Apr;25(4):710-6.

22. Katsumata Y, Shinmura K, Sugiura Y, Tohyama S, Matsuhashi T, Ito H, Yan X, Ito K, Yuasa S, Ieda M, Urade Y, Suematsu M, Fukuda K, Sano M. Endogenous prostaglandin D2 and its metabolites protect the heart against ischemia-reperfusion injury by activating Nrf2. *Hypertension*. 2014 Jan;63(1):80-7

23. Martínez AE, Sánchez-Gómez FJ, Díez-Dacal B, Oeste CL, Pérez-Sala D. 15-Deoxy-Δ(12,14)-prostaglandin J2 exerts pro- and anti-inflammatory effects in mesangial cells in a concentration-dependent manner. *Inflamm Allergy Drug Targets*. 2012;11:58-65

24. Arima M, Fukuda T. Prostaglandin D_2 and T(H)2 inflammation in the pathogenesis of bronchial asthma. *Korean J Intern Med*. 2011 Mar;26(1):8-18. doi: 10.3904/kjim.2011.26.1.8. Epub 2011 Mar 2

25. Rubic T, Trottmann M, Lorenz RL. Stimulation of CD36 and the key effector of reverse cholesterol transport ATP-binding cassette A1 in monocytoid cells by niacin. *Biochem Pharmacol*. 2004 Feb 1;67(3):411-9

26. Morrow JD, Parsons WG 3rd, Roberts LJ 2nd. Release of markedly increased quantities of prostaglandin D2 in vivo in humans following the administration of nicotinic acid. *Prostaglandins*. 1989;38(2):263-74

27. Franceschini G, Favari E, Calabresi L, Simonelli S, Bondioli A, Adorni MP, Zimetti F, Gomaraschi M, Coutant K, Rossomanno S, Niesor EJ, Bernini F,Benghozi R. Differential effects of fenofibrate and extended-release niacin on high-density lipoprotein particle size distribution and cholesterol efflux capacity in dyslipidemic patients. *J Clin Lipidol*. 2013;7(5):414-22

28. Lavigne PM, Karas RH. The current state of niacin in cardiovascular disease prevention: a systematic review and meta-regression. *J Am Coll Cardiol*. 2013 29;61(4):440-6

29. Otvos JD. The surprising AIM-HIGH results are not surprising when viewed through a particle lens. *J Clin Lipidol*. 2011;5(5):368-70

30. Taylor AJ, Villines TC, Stanek EJ, Devine PJ, Griffen L, Miller M, Weissman NJ, Turco M. Extended-release niacin or ezetimibe and carotid intima-media thickness. *N Engl J Med.* 2009;361(22):2113-22

31. Hochholzer W, Berg DD, Giugliano RP. The facts behind niacin. *Ther Adv Cardiovasc Dis*. 2011;5(5):227-40

32. Lee JM, Robson MD, Yu LM, et al. Effects of high-dose modified-release nicotinic acid on atherosclerosis and vascular function: a randomized, placebo-controlled, magnetic resonance imaging study. *J Am Coll Cardiol*. 2009;54(19):1787-94

33. Al-Mohaissen MA Pun SC, Frohlich JJ Niacin: from mechanisms of action to therapeutic uses. *Mini Rev Med Chem*. 2010;10(3):204-17

34. Phan BA, Muñoz L, Shadzi P, Isquith D, Triller M, Brown BG, Zhao XQ Effects of niacin on glucose levels, coronary stenosis progression, and clinical events in subjects with normal baseline glucose levels (<100 mg/dl): a combined analysis of the Familial Atherosclerosis Treatment Study (FATS), HDL-Atherosclerosis Treatment Study (HATS), Armed Forces Regression Study (AFREGS), and Carotid Plaque Composition by MRI during lipid-lowering (CPC) study. *Am J Cardiol*. 2013;111(3):352-5

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  - The videos: viewing + online exam = continuing education credits
  - The exam: online exam = continuing education credits
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Estrategia antiviral unificada para médicos y el público

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Historia y perspectivas

Como médicos lo que aprendemos en la escuela de medicina acerca de las infecciones virales se resume en los siguientes títulos de cursos: 1) Microbiología, 2) Patología, y 3) Farmacología. Siguiendo estas instrucciones, los tratamientos que usamos son 1) saneamiento, 2) vacunas y 3) medicamentos antivirales, respectivamente. Basado en la formación médica y mi experiencia con otros médicos, les sugiero aquí que más la mayoría de los médicos capacitados son — al menos por su entrenamiento formal — incapaces de ver más allá de las opciones limitadas a las que fueron expuestos. Lo que me gustaría hacer en el presente artículo es ampliar los horizontes conceptuales y terapéuticos mediante una estrategia estructurada antiviral que incluye el saneamiento, vacunación y medicamentos antivirales previamente mencionados, pero que se extiende más allá de estas opciones limitadas. Los datos clínicos (por ejemplo, dosificación y contraindicaciones) de esta estrategia, apoyo y referencias adicionales están disponibles en formato digital constantemente actualizado [1]; el propósito de este artículo es proveer una estrategia para cambiar el paradigma actual de la estructura.

El hecho de que la mayoría de médicos no se les enseña acerca de la ciencia de la nutrición en la Facultad de medicina es conocido públicamente.[2] Por lo general, la mayoría de los estudiantes de medicina leen solamente un capítulo sobre patologías causadas por deficiencias nutricionales extremas, pero aprenden esencialmente nada acerca de nutrición terapéutica y cómo puede ser aplicada en la prevención y tratamiento de la enfermedad. ¿Ignorando nutrición obliga a médicos por desconocimiento a confiar demasiado en medicamentos y cirugía? ¿Sería la salud pública mejor servida si se distribuye información sobre la prevención de infecciones virales y beneficios nutricionales para que los pacientes y médicos por igual tengan más opciones

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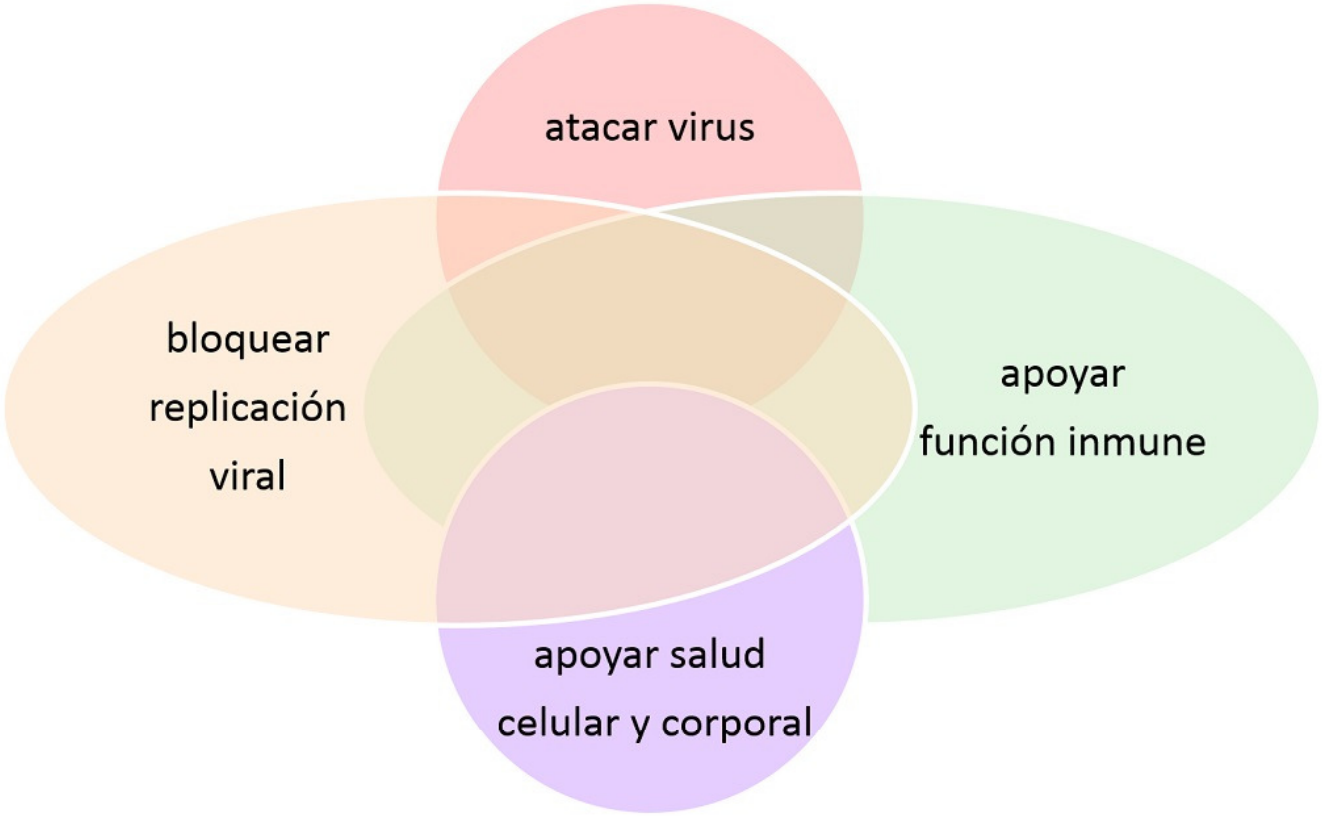
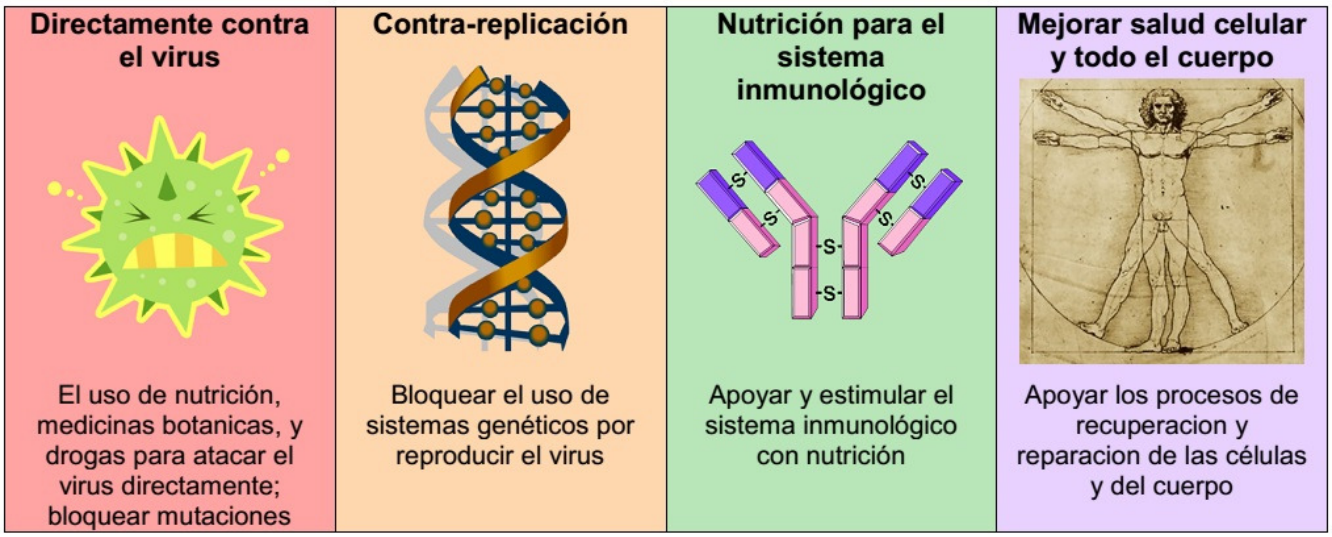
terapéuticas? ¿Estamos tratando insuficiencias nutricionales con medicamentos?

Lo que me he dado cuenta a través de los diversos programas de doctorado que he asistido es que la capacitación clínica en el tratamiento de infecciones virales sigue siendo en su mayoría fenomenalista y enigmática, en lugar de descifrada y estructurada. Como educador, investigador y escritor, he aprendido a través de la experiencia que para estructurar efectivamente la información de tal manera que la accesibilidad y la retención de la información se ve reforzada por los estudiantes/lectores (por ejemplo el acrónimo MYBESTPLAIDFIG para la inmunomodulación nutricional [3] y FINDSEX ® por tratamientos integrativos contra inflamación [4]). Mi propósito principal al escribir este ensayo es demostrar una estrategia única y estructurada antiviral y proporcionar ejemplos representativos de su aplicación práctica.

En lugar de ver las infecciones virales de una manera que es fenomenalista y enigmática y por lo tanto, difícil de manejar, llevando a estrategias de prevención y tratamiento inefectivos, nosotros debemos disminuir la complejidad del proceso infeccioso. Hacerlo – al menos en la forma que he descrito – en la cual nos da cuatro áreas en las cuales podemos enfocar nuestros esfuerzos: 1) contra el virus directamente, 2) bloqueando la replicación viral, 3) apoyando la función inmune y 4) apoyando la salud celular y de todo el cuerpo. Estos son ilustrados en el diagrama adjunto y brevemente descritos y ejemplificados en los cuatro apartados respectivos que siguen.





Estrategia antiviral multicomponente

1. **Ataque directo al virus**: Atacar directamente el virus ha sido el foco de los esfuerzos de salud pública y la práctica médica a través de saneamiento, vacunación y – más recientemente – el uso de medicamentos antivirales específicos. Varios nutrientes y productos botánicos también son muy efectivos para atacar directamente las infecciones virales, y daré dos ejemplos aquí. El mineral selenio tiene un amplio margen de seguridad y proporciona beneficios antivirales a través de varios mecanismos, dos de los cuales bloquean la replicación viral y también bloquean la mutación viral; beneficios antiinfecciosos clínicos son probados en seres humanos con VIH/SIDA.[5] La medicina botánica y té de hierbas Glycyrrhiza glabra ha demostrado eficacia antiviral en estudios experimentales y ensayos clínicos en humanos contra varios patógenos virales diferentes, incluyendo el virus de la hepatitis B (VHB), virus de la hepatitis C (VHC), virus del herpes simple (VHS), un virus de influenza, virus de inmunodeficiencia humana (VIH-1), el síndrome respiratorio agudo severo (SARS)-relacionados con el coronavirus, virus respiratorio sincitial, arbovirus, virus de la vaccinia y virus de la estomatitis vesicular [6]; este botánico tiene una excelente historia de seguridad que abarca varios miles de años, con pocos efectos adversos incluyendo un efecto de pseudoaldosterona (agotamiento de potasio y retención de sodio) y un descenso de testosterona, efecto y mecanismo de acción incluyendo vía la unión del virus, inhibición de la replicación viral, mejora de la inmunidad, la inhibición de la inflamación y el bloqueo de actividad de enzimas específicas. Botánicos y nutrientes antivirales pueden utilizarse solos, en combinación y junto con medicamentos para beneficios aditivos y sinérgicos.
2. **Bloqueo de la replicación viral**: Inhibición de la replicación viral es el objetivo terapéutico de muchos fármacos antivirales, mientras varios nutrientes también pueden proporcionar un efecto similar. Debido a que los virus son incapaces de replicar por si solos y por lo tanto deben contar con una maquinaria genética y de síntesis de su anfitrión humano para su replicación, nutrientes que modulan la expresión genética pueden tener valor terapéutico, es decir mediante la metilación del ADN y bloqueo del factor de transcripción NFkB. Los pocos nutrientes que promueven la metilación del ADN y que también han demostrado eficacia clínica contra las infecciones virales incluyen el ácido fólico [7] (ahora utilizado clínicamente en las formas de ácido folínico y metilo y 5 metil folato), vitamina D3 [8], betaína y S-adenosil-metionina.[9] inhibición del NFkB como mecanismo efectivo antiviral ha sido probada, con dos ejemplos: NAC (acetil-l-cisteina) contra gripe [10] y el ácido lipoico contra hepatitis viral y el VIH.[11]
3. **Apoyo a la función inmune**: El funcionamiento y regulación del sistema inmune es fuertemente dependiente del estado nutricional óptimo y sin una nutrición adecuada, el sistema inmunitario está inclinado simultáneamente hacia hipoactividad (inmunodepresión inducida por deficiencia o insuficiencia) y la hiperactividad que se manifiesta con inflamación y autoinmunidad.[12] Las carencias son muy comunes en la población general y contribuyen a epidemias de enfermedades infecciosas e inflamatorias. Ensayos clínicos en humanos usando nutrientes solos o en combinación para apoyar la función inmune en general han demostrado eficacia contra las enfermedades infecciosas y con una seguridad excepcional, especialmente el uso de glutamina, proteína, vitamina A, vitamina D, zinc y aceite de pescado.[13] Ha sido demostrado en varios casos que los suplementos nutricionales mejoran la respuesta inmunológica a las vacunas; por ejemplo, fue observado que cistina y teanina aumentan la seroconversión de vacunación contra la influenza en las personas mayores. [14]
4. **Apoyo a la salud celular y corporal**: Las infecciones virales tienen numerosos efectos adversos sobre la salud celular y todo el cuerpo. Consecuencias intracelulares de infecciones virales incluyen la disfunción mitocondrial [15] y estrés del retículo endoplasmático [16], que se manifiesta clínicamente como inflamación prolongada, la fatiga y – probablemente – en el caso de infecciones por herpes simple, la enfermedad de Alzheimer.[17] Entre las más de 30 intervenciones para mejorar la función mitocondrial y aliviar el estrés del retículo endoplasmático, vemos que el ejercicio, las dietas bajas en carbohidratos, ácido lipoico, coenzima Q-10 y acetil-l-carnitina son preeminentes por su seguridad, eficacia y beneficios colaterales.[18] La manipulación osteopatíca, quizás mediante la promoción del mejoramiento de la respiración y el flujo linfático y la distribución de las quimiocinas, también ha demostrado beneficio en el mejoramiento no farmacológico de las enfermedades infecciosas.[19]

En resumen, mediante el uso de una estrategia estructurada antiviral, las intervenciones farmacológicas y no farmacológicas pueden aplicarse con mayor eficacia clínica y de salud pública, aliviando las cargas de estas enfermedades infecciosas clínicas, sociales, financieras y políticas.

Conclusión y aplicación

Los brotes recientes internacionales de infecciones virales han hecho una cosa muy clara: necesitamos una nueva estrategia antiviral en los tiempos modernos para combatir estos nuevos flagelos virales en curso; la pandemia de propagación de estas infecciones en 2014 es prueba de que las medidas médicas habituales y las de salud pública de saneamiento, la vacunación y medicación son insuficientes. Para la mayoría de médicos y funcionarios de salud pública, éstas han sido las herramientas utilizadas contra las infecciones virales con la más reciente adición de fármacos antivirales molecularmente orientados específicamente para cada virus. Bajo esta premisa la estrategia antiviral ideal sería tanto en general y específicamente eficaz, ampliamente disponible, de bajo costo y con pocos o insignificantes efectos adversos e interacciones. Mi propósito de escribir este ensayo no es discutir, ni debatir el saneamiento ni vacunas, ni medicamentos, sino señalar otras estrategias de intervención que pueden beneficiar el paciente además de la salud pública. Estas intervenciones basadas en evidencia han demostrado seguridad, eficacia y rentabilidad con amplia e inmediata disponibilidad internacional y generalmente insignificantes efectos adversos y no interacciones con medicamentos y enfermedades.

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Editor's note: Given the international viral crises occurring in late 2014, publication and distribution of this article is a priority; the fact that these viral-infection health crises exist is prima facie evidence of the failure of current systems and the need—not for new treatments within the same model—for a new model better suited for international distribution, disease prevention, and broad-spectrum effectiveness.

Unified Antiviral Strategy published by ICHNFM

Alex Vasquez DC ND DO FACN in Bogota, Colombia

History and Perspectives

What we as doctors learn in medical school about viral infections is summarized within the following course titles: Microbiology, Pathology, and Pharmacology. Following this instruction, the treatments that we use are sanitation, vaccination, and antiviral drugs, respectively. Based on training and my experience with other doctors, I suggest here that most medically-trained doctors are—at least per their formal training—unable to see beyond these blinders and limited options. My intention in writing this article is to broaden those conceptual and therapeutic horizons via the outlining of a structured antiviral strategy that includes the previously mentioned sanitation, vaccination and antiviral drugs but extends well beyond those limited options. Additional citations, support, and clinical details (e.g., dosing and contraindications) for this strategy are available in a digital format constantly updated[1]; the purpose of this article is to structure the strategy, to shift the paradigm.

The fact that most doctors learn nothing about the science of Nutrition in medical school is well known publicly and within medical school academics.[2] Typically, most medical students read one chapter about pathologies caused by extreme nutritional deficiencies, but they learn essentially nothing about therapeutic nutrition and how it can be applied in the prevention and treatment of disease. Does ignoring Nutrition force doctors *by default* to over-rely on drugs and surgery? Would not public health be better served if information were distributed on the nutritional prevention of viral infections, so that patients and doctors alike would have more options?

What I have noticed through the various doctorate programs I have attended is that clinical training in the management of viral infections remains mostly

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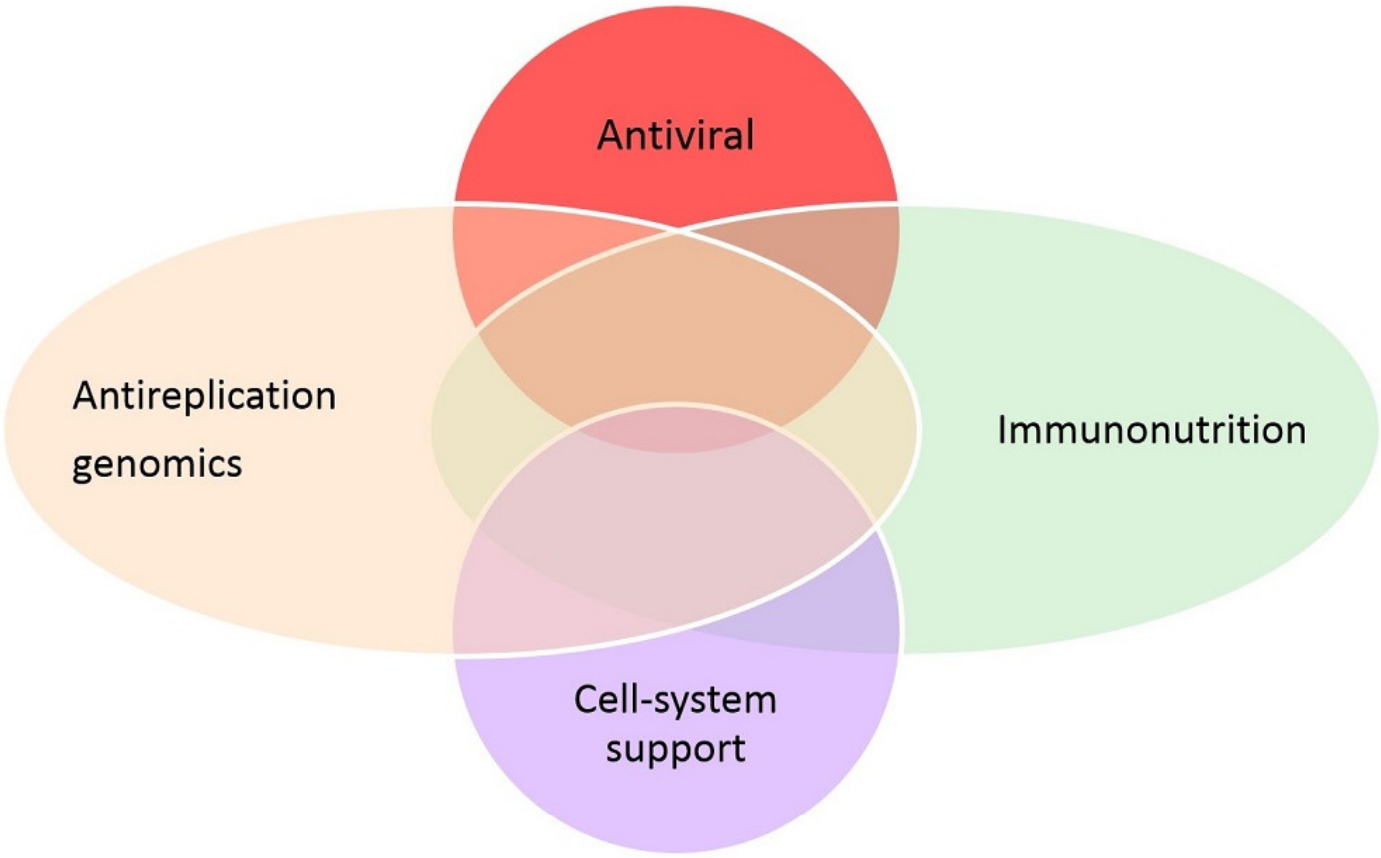
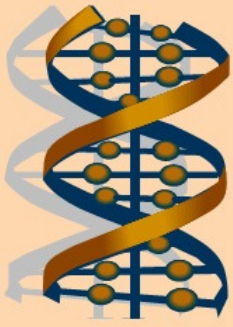
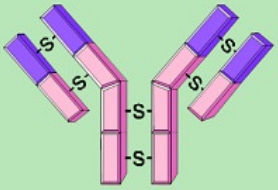
phenomenalistic and enigmatic, rather than deciphered and structured. As an educator, and researcher and writer, I have learned through experience to structure information in such a way that the accessibility and retention of the information is enhanced by students/readers (e.g. the DDIRRT for risk management [e.g., defensive mindset, duration of treatment, interactions, referral, return visit, treatment plan], MYBESTPLAIDFIG for nutritional immunomodulation[3], and FINDSEX® acronyms[4]). My purpose in writing this essay is to demonstrate a unique and structured antiviral strategy and to provide representative examples of its practical application.



Antiviral	Antireplication	Immunonutrition	Cell-system support
			
Direct action against the virus itself, using nutrients and botanicals and drugs, targeting the machinery and blocking viral mutations	Inhibition of viral use of human DNA and replicative machinery; viruses can only replicate by "hijacking" human genetic process	Support and occasional stimulation of humoral (antibody, immunoglobulin) cell-mediated, and cytokine-mediated immunity	Supporting the intracellular systems (mitochondria and endoplasmic reticulum) and whole-body health to optimize immune response, limit damage, promote recovery, prevent recurrence

Multicomponent Antiviral Strategy

Rather than viewing viral infections in a manner that is phenomenalistic and enigmatic, and therefore unwieldy, leading to clumsy prevention and treatment strategies, we should deconstruct the complexity of the infectious process. Doing so – at least in the manner that I have described – gives us four areas upon which we can focus our efforts: 1) targeting the virus directly, 2) blocking viral replication, 3) supporting immune function, and 4) supporting cellular and whole-body health. These are illustrated in the accompanying diagram and briefly described and exemplified in the four respective paragraphs that follow.

1. **Targeting the virus directly**: Targeting the virus directly has been the focus of medical practice and public health efforts through sanitation, vaccination, and –more recently– the use of disease-specific antiviral drugs. Several nutrients and botanicals are also very effective for directly targeting viral infections, and I will provide two examples here. The mineral selenium has a wide margin of safety and provides antiviral benefits through several mechanisms, two of which are blocking viral mutation and also blocking viral replication; anti-infectious clinical benefits are proven in humans with HIV/AIDS.[5] The botanical medicine and common herbal tea licorice (*Glycyrrhiza glabra*) has demonstrated antiviral effectiveness in experimental studies and human clinical trials against several different pathogenic viruses, including hepatitis B virus (HBV), hepatitis C virus (HCV), herpes simplex virus (HSV), influenza A virus, human immunodeficiency virus (HIV-1), severe acute respiratory syndrome (SARS)-related coronavirus, respiratory syncytial virus, arboviruses, vaccinia virus, and vesicular stomatitis virus[6]; this botanical has a an excellent history of safety spanning several thousand years, with adverse/beneficial effects including a pseudoaldosterone effect (sodium retention and potassium depletion) and a testosterone-lowering effect, and mechanism of action including via direct virus binding, inhibition of viral replication, enhancement of immunity, inhibition of inflammation, and blocking the activity of specific enzymes. Antiviral nutrients and botanicals can be used alone, in combination, and alongside medications for additive and synergistic benefits.
2. **Blocking viral replication**: Inhibition of viral replication is the therapeutic goal of many antiviral drugs, while several nutrients can also provide a similar effect. Because viruses are unable to self-replicate and must therefore rely on host/human genetic and synthetic machinery for their replication, nutrients that modulate genetic expression can have therapeutic value here, namely via DNA methylation and blockade of the transcription factor NFkB. The few nutrients which promote DNA methylation and which also have proven clinical effectiveness against viral infections include folic acid[7] (now used clinically in the forms of folinic acid and methyl-folate), vitamin D3[8], betaine and S-adenosyl-methionine.[9] Inhibition of the NFkB pathway for antiviral effectiveness is well-documented, with two examples being with NAC against influenza[10] and lipoic acid against viral hepatitis and HIV.[11]
3. **Supporting immune function**: The performance and regulation of the immune system is heavily dependent on optimal nutritional status, and without proper nutrition, the immune system is slanted simultaneously toward underactivity (deficiency-induced immunosuppression) and hyperactivity manifesting as inflammation and autoimmunity.[12] Nutritional deficiencies are very common in the general population and thereby contribute to epidemics of infectious and inflammatory diseases. Human clinical trials using nutrients alone or in combination to support immune function in general have shown outstanding safety and efficacy against infectious diseases, especially use of glutamine, whey protein isolate, vitamin A, vitamin D, fish oil, and zinc.[13] Nutritional supplementation has been shown in several instances to improve immunological response to vaccinations; for example, cystine and theanine were noted to increase seroconversion of influenza vaccination in elderly persons.[14]
4. **Supporting cellular and whole-body health**: Viral infections have numerous adverse effects on cellular and whole-body health. Intracellular consequences of viral infections include mitochondrial dysfunction[15] and endoplasmic reticulum stress[16], manifesting clinically as prolonged inflammation, fatigue and – likely – in the case of herpes simplex infections, Alzheimer's disease.[17] Among the more than 30 interventions to improve mitochondrial function and alleviate endoplasmic reticulum stress, we see that exercise, low-carbohydrate diets, coenzyme Q-10, lipoic acid, and acetyl-L-carnitine are preeminent in their safety, effectiveness, and collateral benefits.[18] Osteopathic manipulative medicine, perhaps via promotion of improved respiration and lymphatic flow and distribution of chemokines, has also shown benefit in the nonpharmacologic amelioration of infectious disease.[19]

In summary, via the use of a structured antiviral strategy, pharmacologic and nonpharmacologic interventions can be applied with greater clinical and public health effectiveness, thereby alleviating the clinical, social, financial, and political burdens of these infectious diseases.

Conclusion and Application

The recent international outbreaks of viral infections have made one thing very clear: we need a new antiviral strategy in modern times to combat ongoing scourges of viral infections; pandemic spread of these infections in late 2014 is proof that the usual medical and public health measures of sanitation, vaccination, and medication are insufficient. The ideal antiviral strategy would be both generally and specifically effective, widely available, low-cost, with few or negligible adverse effects and drug/disease interactions. For most of medical and public health history, the tools used against viral infections have been sanitation and vaccination, with the more recent addition of molecularly-targeted antiviral drugs specific for each virus. My purpose in writing this essay is not to discuss or debate sanitation nor vaccination nor medication, but rather to point out several other intervention strategies that can be used additionally and to great patient and public health benefit. These evidence-based interventions have proven safety, effectiveness, and cost-effectiveness with wide and immediate international availability and generally negligible adverse effects and drug/disease interactions.

Publication history, author disclosures, citation format: The primary goal of this article is to outline a more complete strategy to counter the personal and population-wide impacts of viral infections; representative citations supporting these concepts are provided. This article underwent legitimate peer-review by an international interdisciplinary team of professionals; *IJHNFM* Editorial Board is listed online ichnfm.org/publications). Dr Vasquez has authored several of the books and articles cited in this article. Dr Vasquez has served as a Lecturer and Researcher for Biotics Research Corporation. Because this is a conceptual essay, citations to literature have been compiled together for efficiency.

Citations:

1. Digital clinical protocol updated regularly: Vasquez A. *Antiviral Nutrition: Against Colds, Flu, Herpes, AIDS, Hepatitis, Ebola, Dengue, and Autoimmunity: A Concept-Based and Evidence-Based Handbook and Research Review for Practical Use*. International College of Human Nutrition and Functional Medicine, 2014. (ASIN: B00OPDQG4W) http://www.amazon.com/dp/B00OPDQG4W. Printed in book format: Vasquez A. *Antiviral Strategies and Immune Nutrition*. CreateSpace Publishing, 2014. (ISBN: 1502894890)
2. Vetter et al. What do resident physicians know about nutrition? An evaluation of attitudes, self-perceived proficiency and knowledge. *J Am Coll Nutr*. 2008 Apr;27(2):287-98. Halsted CH. The relevance of clinical nutrition education and role models to the practice of medicine. *Eur J Clin Nutr*. 1999 May;53 Suppl 2:S29-34. Raman M, Violato C, Coderre S. How much do gastroenterology fellows know about nutrition? *J Clin Gastroenterol*. 2009 Jul;43(6):559-64
3. Vasquez A. *Functional Inflammology: Volume 1: Introduction to Clinical Nutrition, Functional Medicine, and Integrative Pain Management for Disorders of Sustained Inflammation*. International College of Human Nutrition and Functional Medicine, 2014. (ISBN: 9780990620402)
4. Vasquez A. *F.I.N.D. S.E.X. The Easily Remembered Acronym for the Functional Inflammology Protocol*. CreateSpace Independent Publishing, 2013. (ISBN: 1484046765)
5. Beck MA. Antioxidants and viral infections: host immune response and viral pathogenicity. *J Am Coll Nutr.* 2001 Oct;20(5 Suppl):384S-388S. Beck MA. Nutritionally induced oxidative stress: effect on viral disease. *Am J Clin Nutr.* 2000 Jun;71(6 Suppl):1676S-81S. Beck MA. Selenium and vitamin E status: impact on viral pathogenicity. *J Nutr.* 2007 May;137(5):1338-40. Beck MA. Selenium and host defence towards viruses. *Proc Nutr Soc.* 1999 Aug;58(3):707-11. Hurwitz et al. Suppression of human immunodeficiency virus type 1 viral load with selenium supplementation. *Arch Intern Med.* 2007 Jan 22;167(2):148-54
6. Fiore et al. Antiviral effects of Glycyrrhiza species. *Phytother Res.* 2008 Feb;22(2):141-8. Matsumoto et al. Antiviral activity of glycyrrhizin against hepatitis C virus in vitro. *PLoS One.* 2013 Jul 18;8(7):e68992. Ming LJ, Yin AC. Therapeutic effects of glycyrrhizic acid. *Nat Prod Commun.* 2013 Mar;8(3):415-8. Bean P. The use of alternative medicine in the treatment of hepatitis C. *Am Clin Lab.* 2002 May;21(4):19-21. Pompei et al. Glycyrrhizic acid inhibits virus growth and inactivates virus particles. *Nature*. 1979;281(5733):689-90. Feng Yeh et al. Water extract of licorice had anti-viral activity against human respiratory syncytial virus in human respiratory tract cell lines. *J Ethnopharmacol.* 2013 Jul 9;148(2):466-73. Cinatl et al. Glycyrrhizin, an active component of liquorice roots, and replication of SARS-associated coronavirus. *Lancet*. 2003 Jun 14;361(9374):2045-6. Ikeda et al. Prevention of disease progression with anti-inflammatory therapy in patients with HCV-related cirrhosis: a Markov model. *Oncology*. 2014;86(5-6):295-302. van Rossum et al. Intravenous glycyrrhizin for the treatment of chronic hepatitis C: a double-blind, randomized, placebo-controlled phase I/II trial. *J Gastroenterol Hepatol.* 1999 Nov;14(11):1093-9
7. Butterworth et al. Improvement in cervical dysplasia associated with folic acid therapy in users of oral contraceptives. *Am J Clin Nutr*. 1982 Jan;35(1):73-82. See also Butterworth et al. Folate deficiency and cervical dysplasia. *JAMA*. 1992 Jan 22-29;267(4):528-33. "About 20 percent of women taking contraceptive hormones manifest mild megaloblastic changes on Papanicolaou smears of the cervicovaginal epithelium which disappear after folic acid therapy. The current evidence, however, would not indicate that any significant benefit would ensue from routine folate supplementation in women on oral contraceptives." Lindenbaum et al. Oral contraceptive hormones, folate metabolism, and the cervical epithelium. *Am J Clin Nutr*. 1975 Apr;28(4):346-53. Knowing what we know now about "folic acid" as a synthetic and pro-oxidative form of the vitamin, these studies should be performed again using folinic acid or methyl-folate. Note negative studies including Childers et al. Chemoprevention of cervical cancer with folic acid: a phase III Southwest Oncology Group Intergroup study. *Cancer Epidemiol Biomarkers Prev*. 1995 Mar;4(2):155-9.
8. Vasquez A, Manso G, Cannell J. The clinical importance of vitamin D (cholecalciferol). *Altern Ther Health Med.* 2004 Sep-Oct;10(5):28-36 http://antiviralnutrition.com/pdf/vasquez_2004_vitamindmonograph-athm.pdf. Roth et al. Acute lower respiratory infections in

childhood: reducing the global burden through nutritional interventions. *Bull World Health Organ.* 2008 May;86(5):356-64. Yamshchikov et al. Vitamin D for Treatment and Prevention of Infectious Diseases: A Systematic Review of Randomized Controlled Trials. *Endocr Pract.* 2009 Jun 2:1-29. White JH. Vitamin D signaling, infectious diseases, and regulation of innate immunity. *Infect Immun.* 2008 Sep;76(9):3837-43. Cannell et al. Epidemic influenza and vitamin D. Epidemiol Infect. 2006;134(6):1129-40. Autier P, Gandini S. Vitamin D supplementation and total mortality: a meta-analysis of randomized controlled trials. *Arch Intern Med.* 2007 Sep;167(16):1730-7. Grant WB. Hypothesis—ultraviolet-B irradiance and vitamin D reduce the risk of viral infections and thus their sequelae, including autoimmune diseases and some cancers. *Photochem Photobiol.* 2008 Mar-Apr;84(2):356-65. Fetahu et al. Vitamin D and the epigenome. *Front Physiol.* 2014 Apr 29;5:16. Carlberg C. Genome-wide (over)view on the actions of vitamin D. *Front Physiol.* 2014 Apr 29;5:167. Abu-Mouch et al. Vitamin D supplementation improves sustained virologic response in chronic hepatitis C (genotype 1)-naïve patients. *World J Gastroenterol.* 2011 Dec 21;17(47):5184-90. Nimer A, Mouch A. Vitamin D improves viral response in hepatitis C genotype 2-3 naïve patients. *World J Gastroenterol.* 2012 Feb 28;18(8):800-5

9. Feld et al. S-adenosyl methionine improves early viral responses and interferon-stimulated gene induction in hepatitis C nonresponders. *Gastroenterology*. 2011 Mar;140(3):830-9. Filipowicz et al. S-adenosyl-methionine and betaine improve early virological response in chronic hepatitis C patients with previous nonresponse. *PLoS One*. 2010 Nov 8;5(11):e15492

10. De Flora S, Grassi C, Carati L. Attenuation of influenza-like symptomatology and improvement of cell-mediated immunity with long-term N-acetylcysteine treatment. *Eur Respir J*. 1997 Jul;10(7):1535-41

11. Suzuki et al. Alpha-lipoic acid is a potent inhibitor of NF-kappa B activation in human T cells. *Biochem Biophys Res Commun*. 1992 Dec 30;189(3):1709-15. Fuchs et al. Studies on lipoate effects on blood redox state in human immunodeficiency virus infected patients. *Arzneimittelforschung*. 1993 Dec;43(12):1359-62. Jariwalla et al. Restoration of blood total glutathione status and lymphocyte function following alpha-lipoic acid supplementation in patients with HIV infection. *J Altern Complement Med.* 2008 Mar;14(2):139-46. Baur et al. Alpha-lipoic acid is an effective inhibitor of human immuno-deficiency virus (HIV-1) replication. *Klin Wochenschr.* 1991 Oct 2;69(15):722-4. Kim et al. α-Lipoic acid attenuates coxsackievirus B3-induced ectopic calcification in heart, pancreas, and lung. *Biochem Biophys Res Commun*. 2013 Mar 8;432(2):378-83

12. "Deficiency in vitamin D is associated with increased autoimmunity and an increased susceptibility to infection." Aranow C. Vitamin D and the immune system. *J Investig Med.* 2011 Aug;59(6):881-6. Harbige LS. Nutrition and immunity with emphasis on infection and autoimmune disease. *Nutr Health.* 1996;10(4):285-312. Vasquez A. *Naturopathic Rheumatology v3.5*. International College of Human Nutrition and Functional Medicine, 2014. (ISBN: 0990620425)

13. Micke et al. Oral supplementation with whey proteins increases plasma glutathione levels of HIV-infected patients. *Eur J Clin Invest.* 2001 Feb;31(2):171-8. Micke et al. Effects of long-term supplementation with whey proteins on plasma glutathione levels of HIV-infected patients. *Eur J Nutr.* 2002 Feb;41(1):12-8. Moreno et al. Features of whey protein concentrate supplementation in children with rapidly progressive HIV infection. *J Trop Pediatr.* 2006 Feb;52(1):34-8. Linday et al. Lemon-flavored cod liver oil and a multivitamin-mineral supplement for the secondary prevention of otitis media in young children: pilot research. *Ann Otol Rhinol Laryngol*. 2002 Jul;111(7 Pt 1):642-52. Linday et al. Effect of daily cod liver oil and a multivitamin-mineral supplement with selenium on upper respiratory tract pediatric visits by young, inner-city, Latino children: randomized pediatric sites. *Ann Otol Rhinol Laryngol.* 2004 Nov;113(11):891-901. The glutamine dose in this study was "a total of 26 g/day" administered in four divided doses. CONCLUSION: "The results of this prospective randomized clinical trial show that enteral G reduces blood culture positivity, particularly with P. aeruginosa, in adults with severe burns and may be a life-saving intervention." Garrel et al. Decreased mortality and infectious morbidity in adult burn patients given enteral glutamine supplements: a prospective, controlled, randomized clinical trial. *Crit Care Med.* 2003 Oct;31(10):2444-9

14. Miyagawa et al. Co-administration of l-cystine and l-theanine enhances efficacy of influenza vaccination in elderly persons: nutritional status-dependent immunogenicity. *Geriatr Gerontol Int.* 2008 Dec;8(4):243-50

15. El-Bacha et al. Virus-induced changes in mitochondrial bioenergetics as potential targets for therapy. *Int J Biochem Cell Biol.* 2013 Jan;45(1):41-6. Anand et al. Viruses as modulators of mitochondrial functions. *Adv Virol.* 2013;2013:738794. Saffran et al. Herpes simplex virus eliminates host mitochondrial DNA. *EMBO Rep.* 2007 Feb;8(2):188-93. For clinical contexualization of mitochondrial dysfunction in primary care, see: Vasquez A. Mitochondrial medicine arrives to prime time in clinical care: nutritional biochemistry and mitochondrial hyperpermeability ("leaky mitochondria") meet disease pathogenesis and clinical interventions. *Altern Ther Health Med.* 2014 Winter;20 Suppl 1:26-30 http://inflammationmastery.com/reprints/vasquez_2014_mitochondrial_medicine_editorial.pdf

16. Smith JA. A new paradigm: innate immune sensing of viruses via the unfolded protein response. *Front Microbiol.* 2014 May 16;5:222

17. Carbone et al. Herpes virus in Alzheimer's disease: relation to progression of the disease. *Neurobiol Aging.* 2014 Jan;35(1):122-9. Mancuso et al. Titers of herpes simplex virus type 1 antibodies positively correlate with grey matter volumes in Alzheimer's disease. *J Alzheimers Dis.* 2014;38(4):741-5. Lövheim et al. Herpes simplex infection and the risk of Alzheimer's disease-A nested case-control study. *Alzheimers Dement.* 2014 Oct 7. pii: S1552-5260(14)02770-8

18. Vasquez A. *Mitochondrial Nutrition and Endoplasmic Reticulum Stress in Primary Care, 2nd Edition*. CreateSpace Publishing, 2014. (ISBN: 1502952505)

19. Creasy et al. Thoracic and abdominal lymphatic pump techniques inhibit the growth of S. pneumoniae bacteria in the lungs of rats. *Lymphat Res Biol.* 2013 Sep;11(3):183-6. Hodge LM, Downey HF. Lymphatic pump treatment enhances the lymphatic and immune systems. *Exp Biol Med* (Maywood). 2011 Oct;236(10):1109-15. Noll et al. Adjunctive osteopathic manipulative treatment in the elderly hospitalized with pneumonia: a pilot study. *J Am Osteopath Assoc.* 1999 Mar;99(3):143-6, 151-2. Noll et al. Efficacy of osteopathic manipulation as an adjunctive treatment for hospitalized patients with pneumonia: a randomized controlled trial. *Osteopath Med Prim Care.* 2010 Mar 19;4:2. Knott et al. Increased lymphatic flow in the thoracic duct during manipulative intervention. *J Am Osteopath Assoc.* 2005 Oct;105(10):447-56

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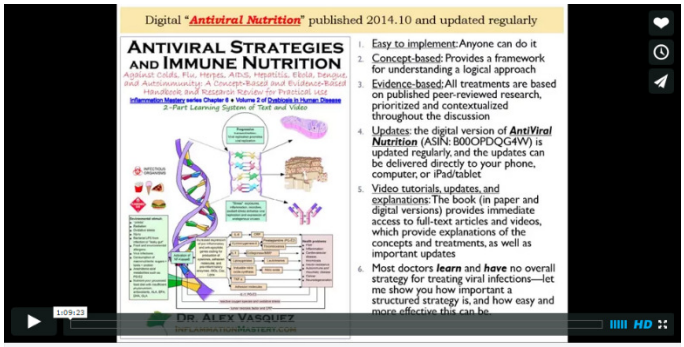

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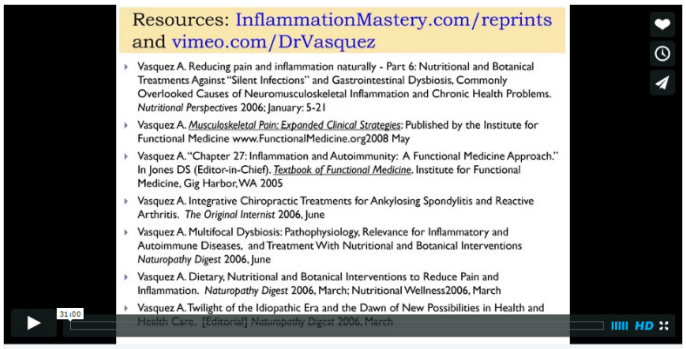

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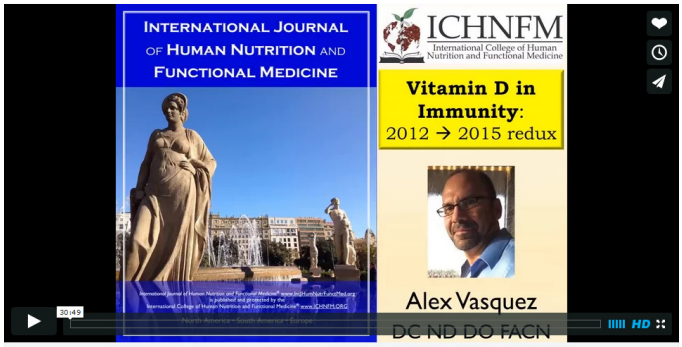

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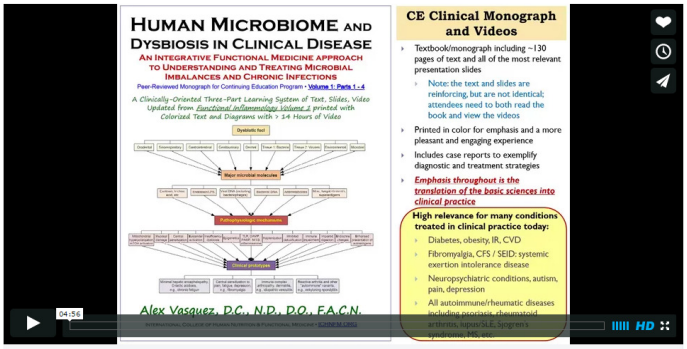

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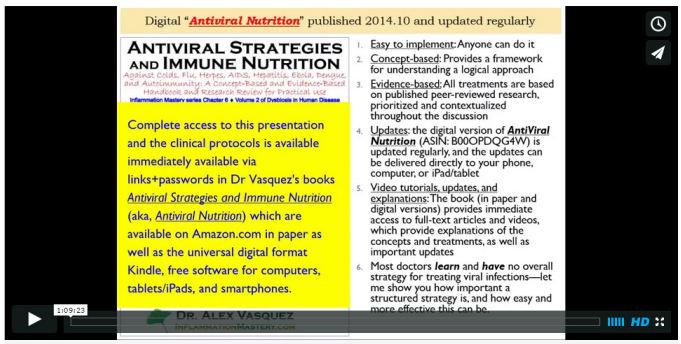

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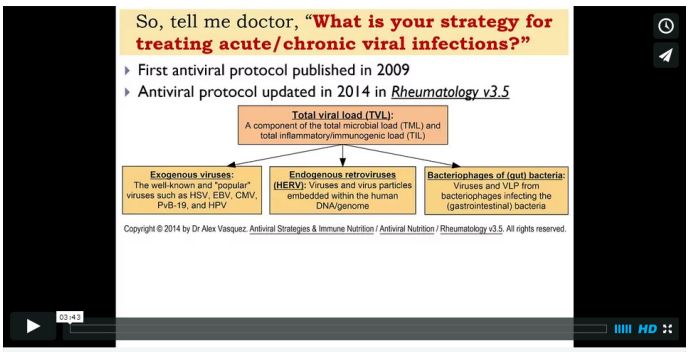

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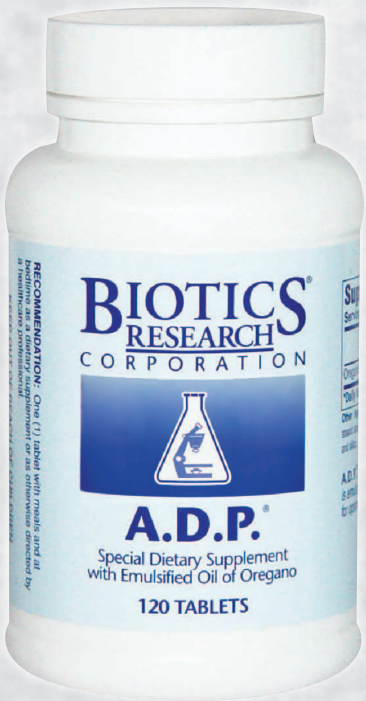

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Emulsified Oil of Oregano - Sustained Release

Patent #5,955,086

Science is making rapid progress in unlocking Nature's basic secrets, especially in the area of plants and food-stuffs, which supply more than just nutrients. One promising field is the variety of spices that have been used for centuries to add zest to a wide range of foods. Interestingly, long before the advent of refrigeration it was recognized that herbs and culinary spices could slow food spoilage–natural antimicrobial principles were obviously at work.

Food scientists have become more interested in essential oils and aromatic plants. Recent studies have focused on the effects of spices and associated oils on food-borne organisms in the context of food safety and spoilage. Attempts have been made to identify the active components of essential oils from spices, among them the oil of oregano. Analysis revealed two phenolic compounds, thymol and carvacrol, as primary constituents of oregano extracts, and their antioxidant properties were reported.

Biotics Research Corporation is widely recognized for its emulsified fat-soluble nutrients and other lipids, microemulsified with the practical objective of increased absorption and bioavailability. This technology has now been applied to the oil of oregano. By emulsifying oregano extracts, the effective surface area of the oil is dramatically increased. An additional step was to apply a sustained release mechanism to **A.D.P.®**, assuring a slow release throughout the digestive tract. The combined effect of emulsification and sustained release is to optimize intestinal exposure to the essential oil.

1. Svensen AB, Scheffer JJC. Essential Oils and Aromatic Plants. *Martinus Nijoff/W. Junk Publishers (1985).*
2. Akgul A., Kivane M. Inhibitory effects of selected Turkish spices and oregano components on some food borne fungi. *Int. J. Food Microbiology (1988) 6;263.*
3. Conner DE, Beauchat LD. Effects of essential oils from plants on growth of food spoilage yeasts. *J. Food Science (1984) 49:429.*
4. Deighton N, Glidewell SM, Deasn SG, Goodman BA. Identification by EPR spectroscopy of cavacrol and thymol as the major sources of free radicals in the oxidation of plant essential oils. *J. Science Food Agriculture (1933) 63:221.*
5. Sezik E., Tumen G., *et al.* Essential Oil Composition of Four Organum Vulgare Subspecies of Anatolian Origin. *J. Essent. Oil Res. (1933) 5:425-31.*

Recommended additional supplementation:

BioDoph-7 Plus™

Lactozyme™ - Providing lactobacillus acidophilus and bifidum, one tablet with each meal daily.

BioDophilus-FOS™ - One to three (1/2 teaspoon) servings in a glass of water between meals to help normalize intestinal bacteria.

Supplement Facts

Serving Size: 1 Tablet

	Amount Per Serving
Oregano (Origanum vulgare) (standardized extract from leaf)	50 mg*

*** Daily Value not established**

Other ingredients: Cellulose, methylcellulose, potassium sorbate, stearic acid (vegetable source), emulsifier base (water and gum arabic), silica and modified cellulose gum.

A.D.P.® supplies a standardized oregano extract which is emulsified and processed in a sustained release form for optimal effectiveness.

Patent #5,955,086

RECOMMENDATION: One (1) tablet with meals and at bedtime as a dietary supplement or as otherwise directed by a healthcare professional.

KEEP OUT OF REACH OF CHILDREN

Caution: Not recommended for pregnant or lactating women.

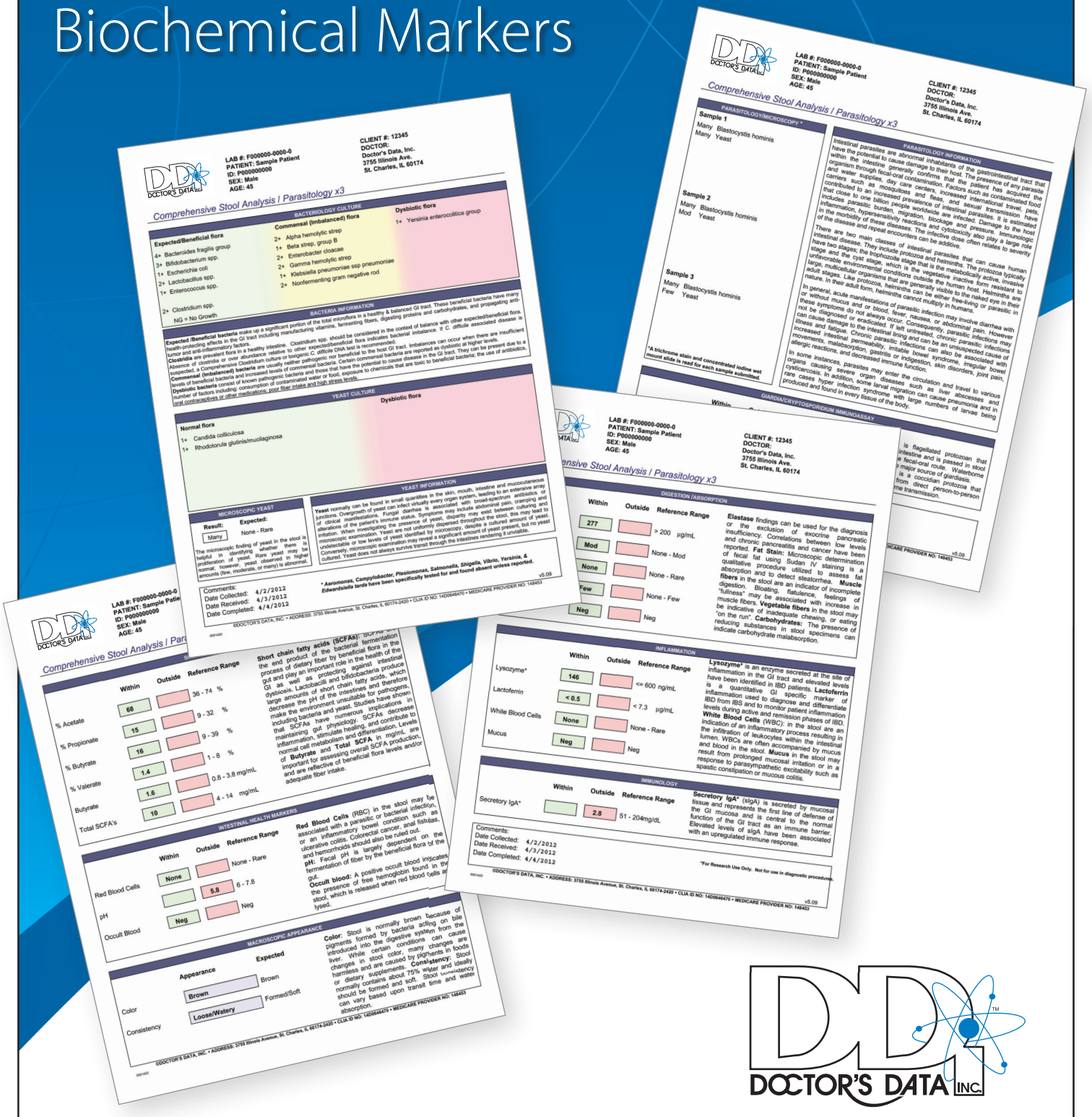
Store in a cool, dry area.
Sealed with an imprinted safety seal for your protection.

NDC #55146-01101 Rev. 09/11

LIT-002 Rev. 06/12

Comprehensive Stool Analysis with Parasitology

Breakthrough Assessment of Bacteria, Yeast, Parasites and Biochemical Markers



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SCIENCE + INSIGHT


"The purpose of life is to live it, to taste experience to the utmost, to reach out eagerly and without fear for newer and richer experience."
Eleanor Roosevelt (1884 - 1962)
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