Children’s Probiotic Combination for Pediatric Health
Disclaimer

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Introduction

Over the past several years, there has been an explosion of information regarding the role that the human microbiota plays in both health and disease. Projects such as the National Institutes of Health Human Microbiome Project, the European Meta-Hit Project, the Belgian Flemish Gut Flora Project, as well as the creation of the International Human Microbiome Consortium are collecting data on the human microbiota from large populations that will contribute to increased knowledge about the microbes inhabiting the body. These projects will also offer information on the impact of host and environmental factors on microbiota variations within average, healthy populations and provide information on microbiome-associated variables that may point to disease risks as well.

One of the growing areas of interest with regard to the microbiome is diversity across all age groups. Probiotics are being used with increasing frequency worldwide, and as such, the safety and efficacy of probiotics for use in males and females of all ages, including infants and children have been reviewed by experts in food safety. All of these reviews support the safety and sustainability of lactobacilli and bifidobacteria for use as oral probiotics, a conclusion largely based on their long history of safe use in food and as dietary supplements.

This monograph will focus on the development of the gut microbiota in infants and children and a brief introduction to general categories of use for probiotics in children. It will also introduce the UAS Labs Children’s unique probiotic strain combination containing Bifidobacterium animalis subsp. lactis UABLA-12™ and Lactobacillus acidophilus DDS®-1.

Development of the Gut Microbiota

The term “microbiome” refers to the microbiota and the habitat it colonizes as well as the collective genomes of the microbes and the human microenvironments they inhabit, or the “metagenome”. Found on the skin, oral cavity, respiratory tract, urogenital tract, gastrointestinal (GI) tract, and even the brain, the human microbiota consists of approximately 40 trillion microbes—predominantly bacteria, but also nonbacterial organisms such as viruses and fungi. Many references say this represents 10 times the number of cells in the human body, although a recent paper suggests that the ratio of microbes to human cells is closer to 1.3:1. By far the largest repository of these microbes is the GI tract which harbors between $10^{13}$ to $10^{14}$ microorganisms and each person hosts hundreds of bacterial species that have been identified by DNA sequencing.
The microbiota of the infant gut is initially a low-diversity community that gradually undergoes successive changes until it reaches high diversity similar to the adult gut by about 1 to 2 years of age. For years, the infant gut was considered to be sterile, only becoming colonized after birth from maternal microbiota, diet, and the environment. However, recent research has characterized placental microbiome which may influence the infant gut as early as gestation. Clearly the most significant influences on early gut microbiota development are mode of birth (e.g. vaginal vs. cesarean section) and early feeding (e.g. breast feeding vs. formula). Increasing evidence suggests that initial colonization influences gut maturation and immune, brain, and metabolic development.

Cesarean section rates are on the rise worldwide. According to current estimates, approximately 30% of births in the United States are by cesarean section. Compared to infants born vaginally, infants born by cesarean section have decreased bacterial diversity or “richness” in the gut. Studies have also found an increased risk of obesity, allergy, atopic dermatitis (AD), and asthma in children born by cesarean section.

Decreased bacterial richness in the gut may also occur in infants who are formula fed versus those who are breast fed. Breastfed infants typically have a bifidobacteria-dominated microbiota, most likely due to the presence of prebiotic breast milk oligosaccharides as well as probiotic organisms in breast milk.

Improper acquisition of a diverse and balanced microbiota during infancy and early childhood may have an adverse impact on health into adulthood.

Probiotics

A panel of experts convened in 2014 by the International Scientific Association for Probiotics and Prebiotics, defined probiotics as “live microorganisms that, when administered in adequate amounts, confer a health benefit on the host.” Most of the scientific and clinical research with probiotics has focused on oral use of probiotics for GI and immune health. Oral use and vaginal application of probiotics has also been studied for female genitourinary tract health. Emerging science is also exploring the use of probiotics in areas such as atopic dermatitis, hypercholesterolemia, anxiety, depression, metabolic syndrome, and autism spectrum disorder.

The primary genera of bacteria that have been used and studied as probiotics are Lactobacillus and Bifidobacterium. Table 1 lists commonly used members of these two families as well as other bacterial and yeast organisms used in probiotic supplements.


### Table 1 - Commonly Used Probiotic Species

<table>
<thead>
<tr>
<th>Genus</th>
<th>Species</th>
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<tbody>
<tr>
<td><em>Lactobacillus</em></td>
<td>rhamnosus, acidophilus, bulgaricus, casei, salivarius, \</td>
</tr>
<tr>
<td></td>
<td>johnsonii, helveticus, gasseri, brevis, plantarum, \</td>
</tr>
<tr>
<td></td>
<td>fermentum, paracasei, reuteri</td>
</tr>
<tr>
<td><em>Bifidobacterium</em></td>
<td>bifidum, longum, infantis, breve, animalis subsp. lactis</td>
</tr>
<tr>
<td><em>Other</em></td>
<td>*Saccharomyces boulardii, Streptococcus thermophilus, \</td>
</tr>
<tr>
<td></td>
<td><em>Lactococcus lactis, Bacillus coagulans, Bacillus subtilis</em></td>
</tr>
</tbody>
</table>

### HOW PROBIOTICS WORK

Probiotics beneficially affect the intestinal microbiota via local and systemic immune mechanisms as well as non-immune mechanisms. While an extensive overview is beyond the scope of this introductory article, these include:29

- Competition with microbial pathogens for nutrients and adhesion sites
- Production of bacteriocins that inhibit pathogen growth
- Alteration of local pH to create an unfavorable local environment for pathogens
- Enhancement of intestinal barrier function
- Stimulation of epithelial mucin production
- Increased production of short chain fatty acids in the colon
- Augmentation of mucosal immunity through enhanced secretory IgA production
- Reduction of systemic antigen exposure (e.g. food allergens)
- Systemic immune modulation

Over the past few years, science has also been emerging in the area of the gut-brain axis and how the GI microbiota communicates with the central nervous system and especially the hypothalamic-pituitary-adrenal axis.30 Research in this area has ramifications for the potential use of probiotics in the modulation of stress, adjunctive treatment of anxiety and depression, and possibly treatment of autism spectrum disorder.31

### PROBIOTICS FOR PEDIATRIC WELLNESS AND HEALTHCARE

Evidence continues to grow for the safe and efficacious use of selected probiotic strains in infants and children.32, 33, 34 While this monograph is not intended to extensively cover clinical studies focusing on the use of probiotics in children, Table 2 lists some of the key indications that probiotics have shown efficacy either as adjunctive or primary treatments in pediatric healthcare.
### Antibiotic Associated Diarrhea

A meta-analysis looking specifically at the use of probiotics for prevention of pediatric antibiotic associated diarrhea (AAD) included 23 randomized, double-blind, placebo-controlled trials (RDBPCTs) and 3,938 children. A priori and post hoc analyses of the data indicated that higher doses of probiotics (>5 billion CFU/day) were more effective in the reduction of AAD compared to lower doses (<5 billion CFU/day).

#### Irritable Bowel Syndrome

A 20-week RDBPCT studied the effect of *L. rhamnosus* (3 billion CFU/day) in children with IBS or functional abdominal pain. At the end of the study, children in the probiotic group had a significant reduction in the number of episodes of pain (p < 0.001) and severity of pain (p < 0.001) compared to the placebo group. Both results persisted at an 8-week post-treatment evaluation as well. Children in the probiotic group with abnormal intestinal permeability at the beginning of the study also had a significantly greater reduction in intestinal permeability at the end of the study compared to similar children in the placebo group (p < 0.03).

### Atopic Dermatitis

A primary area of clinical interest is the potential for probiotics to reduce the incidence of atopic dermatitis (AD) as well as other atopic diseases such as asthma. A 2013 meta-analysis published in *Pediatrics* included 25 studies with a total of 4,031 children. Conclusions included the following:

- Probiotics were effective in reducing total IgE and the reduction was more pronounced with longer follow-up.
- Probiotics significantly reduced risk of atopic sensitization when administered prenatally (to mothers) and postnatally (to mothers or infants).
- Probiotics did not significantly reduce the incidence of asthma or wheeze.

Prenatal and postnatal administration of probiotics has been shown in several RDBPCTs to significantly reduce the risk of AD in at-risk infants (e.g. those with a parent or sibling with a history of atopy).
Acute Respiratory Infection Prevention
A meta-analysis published in 2016 looked at studies examining the effect of probiotics in the prevention of acute respiratory infections (ARIs). The paper included 23 clinical trials involving 6,269 children. The age range of children was from newborn to 18 years of age. Probiotics were shown to significantly decrease the number of days with ARIs and days absent from daycare or school.

UAS Labs Children’s Probiotic Supplement
UAS Labs Children’s is a unique, proprietary, probiotic blend, specifically formulated with a combination of Bifidobacterium animalis subsp. lactis UABLA-12™ and Lactobacillus acidophilus DDS®-1 for children ages 1 to 12 years. The formulation offers digestive, immune, respiratory, and skin health benefits.

The Bifidobacterium animalis subsp. lactis UABLA-12™ probiotic accounts for the majority of the combined potency in UAS Labs Children’s with total potency being 5 billion CFU/serving, and a small amount of fructooligosaccharide is included as well. The product can be delivered as an easy-to-mix powder with a neutral flavor that quickly dissolves in liquid or cool foods or as a chewable tablet.

Children’s Probiotic Strains

BACKGROUND AND ORIGIN

Bifidobacterium lactis was originally described by Meile et al. and was re-classified as Bifidobacterium animalis subsp. lactis. B. animalis subsp. lactis UABLA-12™ is a strain originally isolated from a human intestinal isolate and has been deposited at the National Collection of Industrial Food and Marine Bacteria (NCIMB). B. animalis subsp. lactis UABLA-12™ has been genetically characterized and properly classified as B. animalis subsp. lactis using modern phenotypic and genotypic methods, including 16S rRNA gene sequencing, whole genome sequence alignment, and PCR using species-specific primers. For the remainder of this Dossier, the strain will be referred to as B. lactis UABLA-12™ for the purpose of simplicity.

Lactobacillus acidophilus DDS®-1 is a strain originally isolated from a human intestinal isolate and has also been deposited NCIMB. L. acidophilus DDS®-1 has been genetically sequenced and properly classified as L. acidophilus using modern phenotypic and genotypic methods, including whole genome sequence alignment, 16S rRNA gene sequencing and hybridization to a species-specific probe.
Toxicology
Based on the long-history of safe use of lactobacilli and bifidobacteria species in food, strain specific animal toxicology studies are not required to support safety. Moreover, microorganism host interactions are species and/or strain specific, and the reader is directed to the clinical studies, which report on the safety, tolerability and efficacy of Children’s.

Antibiotic Resistance
Antibiotic resistance testing of probiotic organisms is advisable to ensure that antibiotic resistance determinants are not introduced into a context where these genes are at risk of being transferred to pathogenic organisms. The minimum inhibitory concentrations (MIC) of various antibiotics against B. lactis UABLA-12™ and L. acidophilus DDS®-1 were determined using the microdilution method in accordance with ISO 10932 guidelines (ISO 10932 IDF 223). Assessment of the antimicrobial resistance pattern of B. lactis UABLA-12™ and L. acidophilus DDS®-1 are shown in Tables 3 and 4, and was determined by comparing the observed MIC’s with the most recent European Food Safety Agency (EFSA) breakpoint values. With the exception of tetracycline, B. lactis UABLA-12™ was shown to be susceptible to all antibiotics tested at a concentration below the cut-off MIC established by EFSA. Tetracycline resistance is consistent with other strains of B. animalis subsp. lactis and has previously been shown to correlate directly with the presence of a single gene, tetW. L. acidophilus DDS®-1 was shown to be susceptible to all antibiotics tested at a concentration below the cut-off MIC established by EFSA.

<table>
<thead>
<tr>
<th>B. lactis UABla-12™</th>
<th>Minimum Inhibitory Concentration (MIC)</th>
<th>EFSA Cut-Off</th>
</tr>
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<tbody>
<tr>
<td>Ampicillin</td>
<td>0.125 ug/ml</td>
<td>2 ug/ml</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>0.500 ug/ml</td>
<td>2 ug/ml</td>
</tr>
<tr>
<td>Gentamycin</td>
<td>64.00 ug/ml</td>
<td>64 ug/ml</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>64.00 ug/ml</td>
<td>128 ug/ml</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>0.125 ug/ml</td>
<td>1 ug/ml</td>
</tr>
<tr>
<td>Clindamycun</td>
<td>&lt;0.032 ug/ml</td>
<td>1 ug/ml</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>32.00 ug/ml</td>
<td>8 ug/ml</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>04.00 ug/ml</td>
<td>4 ug/ml</td>
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**Table 4 - Minimum Inhibitory Concentration Against L. acidophilus DDS\textsuperscript{®}-1**

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<td>02.00 ug/ml</td>
<td>16 ug/ml</td>
</tr>
<tr>
<td>Streptomycin</td>
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</tr>
<tr>
<td>Erythromycin</td>
<td>0.125 ug/ml</td>
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**Other Data**

B. lactis UABLA-12\textsuperscript{™} and L. acidophilus DDS\textsuperscript{®}-1 have also been tested for the following:

- **D-Lactic Acid Production** - B. lactis UABLA-12\textsuperscript{™} is not a D-lactic acid producing strain and L. acidophilus DDS\textsuperscript{®}-1 ferments lactose to a racemic mixture of D(-)- and L(+)-lactic acid in a ratio of 40:60%.
- **Gastrointestinal Survival** – acid and bile resistance. Both strains are extremely resistant to low pH conditions in the stomach and extremely resistant to bile salts at the physiological concentrations present in the duodenum.
- **Adherence to Human Intestinal Cells** – The individual strains in Children’s have demonstrated very good adhesion in vitro to the two human intestinal cell lines, Caco-2 and HT-29.

Details on the results of this testing are available upon request from UAS Labs.
Clinical Studies

Two gold-standard, RDBPCTs provide evidence that Children’s is safe and efficacious in the treatment of AD and the prevention of ARIs. Children included in these clinical trials ranged from 1 to 12 years of age.

Atopic Dermatitis

In a randomized, double-blind, placebo-controlled clinical trial, 90 children ages 1 to 3 years old with moderate to severe AD were treated with either a mixture of *B. lactis* UABLA-12™ and *L. acidophilus* DDS®-1 at a dosage of 5 billion CFU twice daily or placebo for 8 weeks. The primary outcome measure was the percentage change in the Scoring of Atopic Dermatitis (SCORAD) value. Secondary outcome measures included changes in the Infant Dermatitis Quality of Life (IDQOL) and Dermatitis Family Impact (DFI) scores, frequency of topical corticosteroid used, and lymphocyte subsets (CD3, CD4, CD8, CD16, CD22, and CD25) in the peripheral blood were measured by laser flow cytometry. Clinical measures of SCORAD, IDQOL, and DFI were completed at weeks, 0, 2, 4, and 8. Lymphocyte subsets were measured at baseline and week 8.

The SCORAD questionnaire includes an assessment of the extent and intensity of the rash, pruritus (itching of the skin), and sleep disturbance. The IDQOL and DFI questionnaires each included ten questions specific to infant or family activity. CD4 and CD25 lymphocytes are often elevated in AD while CD8 is reduced.

At week 8, the percentage decrease in SCORAD was 33.7% for the probiotic group compared to 19.4% in the placebo group (*p* = 0.001; see Figure 1). At week 8, children in the probiotic group also showed a greater decrease in the mean [SD] SCORAD score compared to those in the placebo group (-14.2 [9.9] vs. -7.8 [7.7], respectively; *p* = 0.001). IDQOL and DFI scores decreased significantly from baseline by 33% and 35.2% in the probiotic group compared to 19% and 23.8% in the placebo group (*p* = 0.013 and *p* = 0.010, respectively. Use of topical corticosteroids during the 8-week study period averaged 7.7 g less in the probiotic group (*p* = 0.006) [Figure 2].

The percentage of CD4, and the percentage and absolute count of CD25 lymphocyte subsets decreased while the absolute count of CD8 increased significantly in the probiotic group compared to the placebo group at week 8 (*p* < 0.007). There was a significant correlation between CD4 percentage, CD25 percentage, CD25 absolute count, and SCORAD values in the probiotic group at week 8 (*p* < 0.05). The investigators suggest this may represent a positive influence on the balance of T helper-1/T-helper-2 ratio in the probiotic group.

There were no clinically significant adverse events in either group.
Evidence continues to grow for the safe and efficacious use of selected probiotic microbes. "Strong scientific evidence" is defined in the QPS (Qualified Presumption of Safety) system and especially the hypothalamic-pituitary-adrenal axis.30 Research in this area has also shown that probiotics can influence systemic immune modulation and mechanisms as well as non-immune mechanisms. While an extensive overview is beyond the scope of this manuscript, the reader is directed to the clinical studies, which report on the safety, tolerability and efficacy of Children’s.53

Human Food 52 as well as the list of Microorganisms with documented history of use in current food and feed uses do not present cause for safety concern, and QPS safe use by the food industry, and is based on four essential pillars of information: beneficial use.53

To learn more visit www.uaslabs.com or contact a Probiotic Expert at UAS Labs has been committed to enhancing wellness and quality of life by providing scientifically-proven probiotic solutions for over 35 years. Their dedication to quality has been recognized by regulatory agencies across the globe, including Health Canada, the FDA, the EFSA, and the Codex Alimentarius. UAS Labs is continually innovation-driven, focusing on the latest research and development in the field of probiotics and prebiotics. Their mission is to support healthy and active lifestyles through probiotics that are safe, effective, and of the highest quality.5

Figure 1 - SCORAD Percentage Decrease

33% Reduction in SCORAD Severity p = 0.001

Figure 2 - Corticosteroid Use

30% Reduction in Corticosteroid Use p = 0.006
Acute Respiratory Infections

In a RDPCT, 240 children ages 3 to 12 years old were enrolled to assess the short-term use of probiotics in ARIs. On the first day of appearance of a sick household member, the otherwise healthy subject was randomized to receive a mixture of B. lactis UABLA-12™ and L. acidophilus DDS®-1 at a dosage of 5 billion CFU per day or placebo. Supplementation and follow-up lasted for 14 days or until the end of the secondary ARI. The primary outcome was the incidence of ARIs. The secondary outcomes were time to resolution and severity of the ARIs.

Upon exposure to a sick household member, there was no significant difference between the probiotic and placebo groups in terms of the percentage of children developing ARIs (57% vs. 65%, respectively; p = 0.261). However, time to resolution of the secondary ARI was significantly shorter in the probiotic group (5 vs. 7 days, respectively; p < 0.001 [see Figure 3]). The median severity of ARIs was significantly less in the probiotic group compared to the placebo group (p < 0.001). Comparing the probiotic and placebo groups (see Figure 4), there was a 2-day decrease in the median number of days or daycare/school missed 7 vs. 9, respectively; p < 0.001) or workdays missed by a caregiver (5 vs. 7, respectively; p < 0.001).

There were no clinically significant adverse events in either group.

Regulatory Status
Products containing *L. acidophilus* DDS®-1 and *B. lactis* UABLA-12™ have been issued a product license by Health Canada’s Natural Non-prescription Health Products Directorate (NNHPD). Products with a license have been assessed by Health Canada and found to be safe, effective and of high quality under their recommended conditions of use.

In the European Union (EU), the *L. acidophilus* DDS®-1 and *B. lactis* UABLA-12™ probiotic species each have Qualified Presumption of Safety (QPS) status. This assessment process recognizes that many microorganisms have long-histories of safe use by the food industry, and is based on four essential pillars of information: established identity, body of knowledge, possible pathogenicity, and end use. Following a review of the current uses of *L. acidophilus* and *B. lactis* in food and feed products, the European Food Safety Authority (EFSA) concluded that the current food and feed uses do not present cause for safety concern, and QPS status was granted for each species.\(^5\)

In Australia, the *L. acidophilus* DDS®-1 and *B. lactis* UABLA-12™ probiotic species are each on the Therapeutic Goods Administration’s (TGA) approved list of medical ingredients.\(^5\)

The *L. acidophilus* DDS®-1 and *B. lactis* UABLA-12™ probiotic species are each listed in the Inventory of Microorganisms With Documented History of Use in Human Food 52 as well as the list of Microorganisms with technological beneficial use.\(^5\)
About UAS Labs

UAS Labs has been committed to enhancing wellness and quality of life by providing scientifically-proven probiotic solutions for over 35 years. Their dedication to excellence has brought together a team of professionals with over 100 years of combined probiotic experience. Together, they drive the industry forward with new product development and continual investment in gold-standard clinical trials. To ensure quality, UAS Labs manufactures in a GMP and organic certificated facility that is dedicated solely to probiotics. This singular focus creates the optimal environment and processes for probiotic viability and global distribution. With this experience and dedication plus two trademarked superstrains, *Lactobacillus acidophilus* DDS®-1 for digestive and immune health and patented *Lactobacillus reuteri* NCIMB 30242 (LRC™) for cholesterol support, it is easy to see how UAS Labs has earned their name as The Probiotic Company®.

To learn more visit www.uaslabs.com or contact a Probiotic Expert at 800-422-3371 or info@uaslabs.com.
Other Data

L. acidophilus extensively cover clinical studies focusing on the use of probiotics in children, Table

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References

44. EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP). Guidance on the assessment of bacterial susceptibility to antimicrobials of human and veterinary importance. EFSA J 2012; 10:2740.
51. Therapeutic Goods Administration, Australian Government. Substances that may be used in Listed medicines in Australia. 2007.
UABLA-12™ and Other Data

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- Decreased bacterial richness in the gut may also occur in infants who are formula

- The majority of the combined potency in UAS Labs Children’s with total potency being

- A meta-analysis published in 2016 looked at studies examining the effect of

- Atopic Dermatitis

- Beneficial use.53

- List of probiotics and prebiotics:

- UAS Labs has been committed to enhancing wellness and quality of life by

- The majority of the combined potency in UAS Labs Children’s with total potency being

- The microbiota of the infant gut is initially a low-diversity community that gradually

- UABLA-12™ for the purpose

- Improper acquisition of a diverse and balanced microbiota during infancy and early

- Many references say

- But also nonbacterial organisms such as viruses and fungi. Many references say

- The largest repository of these microbes is the GI tract which harbors between 10^{13}

- By far

- Ef_f_iacious in the treament of AD and the prevention of ARIs. Children included in

- A priori

- Acute Respiratory Infections

- of simplicity.

- Acute Respiratory Infections

- pro bi otics in areas such as atopic dermatitis,