

# PRESCRIPT-ASSIST™ PROBIOTIC-PREBIOTIC TREATMENT FOR BACTERIAL-DIARRHEA: CLINICAL EXPERIENCE IN ECUADOR

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Clinical research was conducted to evaluate the efficacy of *Prescript-Assist*<sup>TM</sup> (P-A), an advanced probiotic-prebiotic formulation for treatment of human bacterial-diarrhea in two communities in Ecuador. P-A has previously demonstrated efficacy against: Canine bacterial-diarrhea in short-term studies; and Irritable-Bowel-Syndrome and related gastric diseases in both short-term and longer-term human studies (e.g., 1-year follow-up). In this study, P-A treatment was shown to result in significantly shorter diarrhea-durations than standard-antibiotics used in treating bacterial diarrhea [ $\chi^2(4) = 41.1, P < 2.6 \cdot 10^{-7}$ ]. *Prescript-Assist*<sup>TM</sup> and other advanced probiotic-prebiotic combinations have considerable promise as emerging treatments for a wide spectrum of both acute and chronic GI diseases.

## INTRODUCTION

This report describes our recent clinical experience using an advanced probiotic-prebiotic formulation for treatment of human bacterial-diarrhea in Ecuador [*Prescript-Assist*<sup>TM</sup> (P-A): Safer Medical, Inc. (SMI), Ft. Benton, MT].<sup>1</sup> The P-A complex previously was found particularly effective for the treatment of a wide variety of human GI disorders and bacterial diarrhea.<sup>[1,2,3,4]</sup> The particular breadth of efficacy – relative to earlier probiotic treatments – is attributed to the actions of a unique complex of >29 probiotic *soil-based-organisms* (SBOs) and *leonardite*, a prebiotic mix of humic substances that differentially enhance SBO proliferation.<sup>[1]</sup> Among conditions initially reported as clinically responding to the P-A probiotic-prebiotic complex were both: “Occasional diarrhea” (e.g., travelers); and “Chronic diarrhea” such as associated with Irritable Bowel Syndrome (IBS) and Colitis.<sup>[4]</sup> Early clinical reports<sup>[4]</sup> prompted a formal randomized, placebo-controlled, double-blind clinical study with regard to IBS.<sup>[3]</sup> This placebo-controlled study identified 3 relatively-independent *subsyndromic factors* of IBS: *General ill-feelings/nausea*, *Indigestion/flatulence*, and *Colitis*. More importantly, the combined probiotic-prebiotic treatment (P-A, 550mg BID) was associated with broad reductions in each of the subsyndromic factors ( $P_s < 0.04$ ) – and their associated symptoms – in a two-week double-blind study in patients with IBS.<sup>[1]</sup>

The reduction in “Colitis” – associated in the literature with a *C. difficile* infection – arguably supported the earlier indications of P-As potential as a treatment for occasional diarrhea.<sup>[5]</sup> This, together with the earlier clinical observations, encouraged both: Follow-up of P-A’s efficacy in the IBS study population<sup>[2]</sup> and Evaluation of P-A’s specific efficacy for bacterial-diarrhea in animal patients.<sup>[3]</sup> In the remainder of this section, the results of these efforts are introduced both as part of the “background” for the current effort and to set the stage for the “purpose.”

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## BACKGROUND

Bacterial diarrhea is one of the most common maladies faced by medical care-givers working in the third-world. In humans, and their canine companions, a number of bacteria have been commonly associated with diarrhea, including: *Salmonella*, *C. perfringens*, and *C. difficile*. However, these and a variety of other organisms are not uncommonly a part of “healthy intestinal microflora.” Hence, stool studies and related diagnostic approaches may be neither timely nor useful when faced with an accelerating outbreak of diarrhea in a closed population. Medical emphases consequently include: immediate treatment to limit the duration of diarrhea; and other actions to limit its possible transmission (particularly as untreated-diarrhea is associated with up to 20% deaths in the young).

The seriousness of bacterial diarrhea and earlier reports of efficacy against “occasional diarrhea” prompted an exploratory study with regard to canine bacterial-diarrhea.<sup>[3]</sup> The P-A complex [marketed for pets as *PetFlora™* Vitality Sciences Inc, Oakland Park, FL] was administered (250mg BID) to 10 dogs suffering from bacterial diarrhea during a kennel outbreak. In response to this outbreak, kennel areas and animals were first thoroughly cleansed and *all* animals were immediately administered *PetFlora™* (as a prophylactic for those not showing symptoms). It is noteworthy that both food and water were made available to all – past experience suggesting that diarrhea could be readily controlled without restrictions. No signs of diarrhea were seen after 12 hours observation – this was certainly less-than the median of the 24-to-48 hours typical with a traditional antibiotic treatment (Neomycin generic “Biosol”, 1.1cc/10kg, with food and possibility water restrictions). This [*PetFlora™* vs. antibiotic] difference in diarrhea-duration of was very-highly significant ( $P < 0.002$ , 2-tail, *Binomial Sign-Test*).<sup>[3, 6]</sup>

Supporting these “antibiotic-like actions” were observations of complete remission of IBS symptoms at one-year follow-up of patients maintained open-label for a 2 to 4 weeks period immediately following a double-blind study (4-6 weeks total).<sup>[2]</sup> Continuing remissions were observed in 62.5% in a group – at the 1-year follow-up – without further support (5/8), and in 100% of those (6/6) choosing maintenance P-A treatment [ultimately at reduced dosing, e.g., one cap every 1-3 days]. The final group of 10 patients – to generally good effect – chose intermittent use of P-A only when signs of IBS symptoms began to reappear.<sup>2</sup>

The body of past studies altogether encouraged our initiation of clinical studies of bacterial-diarrhea in populations at risk – the present study is represents a first step in this direction.

## PURPOSE

The primary goal of this study was to evaluate clinical efficacy of *Prescript-Assist™* (P-A), an advanced probiotic-prebiotic formulation for treatment of human-diarrhea in two population centers in Ecuador. A secondary goal was to explore the multivariate nature of the presenting diarrhea symptoms – particularly toward selecting measure(s) of disease severity and efficacy.

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<sup>2</sup> No evidence of adverse-reactions or side-effects were reported either during either the formal study or at one-year follow-up.<sup>[1,2]</sup>

## METHOD AND RESULTS

The body of this section first delineates methodological aspects of the study: *Participants and Test-Instrument*. This is followed by a joint consideration of analytic methods and their principal findings. This sets the stage for the final section – Discussion and Conclusions.

### PARTICIPANTS

The study population was comprised of 21 patients (12♂ and 9♀) – presenting with persistent bacterial-diarrhea symptoms – almost equally drawn from two agrarian mountain communities located in mountainous regions about Quito, EC. *Miraflores*, served by Dra. Caicedo during the research study, is located 2.5 hours South of Quito; whereas, *Malchinqui*, about 1.25 hours North toward Cayambe, was served by Dr. De la Torre. Both communities – not surprising given continuing waves of diarrhea – were found to have bacterially contaminated public or well-water sources (e.g., *Fecal Coliforms*).<sup>3</sup> Patients – reflecting the largely *indios-mestizo* ethnicities of their communities – ranged in age between 2 to 65 years.<sup>4</sup>

### TEST-INSTRUMENT

Recorded for each participant were: *Name, Age, Community, Sex* (♂ or ♀), and *Physician Observations/Comments*. Six primary symptoms also were assessed on a daily basis: *Diarrhea, Gases, Abdominal pain, Constipation, Vomiting, and Fever*. For research purposes, these symptoms were scored according to the number of days they were observed – starting with “1” at day of clinical diagnosis. Breaks – in any symptom – were conservatively scored as if present, if the symptom subsequently returned during the observational period. Using this approach each of the six symptoms was scored for *total days duration*.

### ANALYSES AND FINDINGS

Analyses were conducted in three phases. During the Phase I, a *Principal Factor Analysis* (PFA) was conducted of the six clinical symptoms in a patients receiving P-A Treatment.<sup>[7,1]</sup> This provided for an exploratory analysis of dimensional “syndromic-factor” structure of the symptoms – akin to that reported in our earlier study of IBS.<sup>[1]</sup> In keeping with this earlier study, Analyses-of-Variance (ANOVAs) were planned for applications to any emergent sub-syndromic factors (with a focus on the main effects of Place-Community, Age, and Sex).<sup>[8,1,2]</sup> During Phase II, ANOVAs were conducted to evaluate the effects of Place (community), Sex, Age and selected interactions on the *log-transformed duration-of-diarrhea: Ln(D)*. Phase III follows this with final analyses comparing the log-transformed durations-of-diarrhea with P-A vs. community-specific experiences with standard antibiotic treatments.

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<sup>3</sup>Contaminated food(s), perhaps not unrelated to water issues, were occasionally mentioned as the source of illness/diarrhea in some patient reports.

<sup>4</sup> SMI’s IRB had approved the study protocol, which provided for incrementally extending treatment of outside the initial 5-65 year age-limits – if/as clinical experience supported efficacy and safety of P-A. No patients <2 years were treated for diarrhea during the study – this was reportedly due to: the relative absence of the disease in infants (purportedly due to their being breast-fed till 2 years-of-age).

**Phase I Analysis Findings**

PFA revealed two factors that accounted for 68.8% of the total symptom variation. Table 1 presents the rotated factor matrix where it may be seen that the first factor “F1-Diarrhea-Vomiting” is essentially defined by loadings on *Diarrhea*(0.83) and *Vomiting* (0.88) with lesser though significant contributions by *Fever* (0.62) and *Nausea* (0.52). The second factor “Gas/Abdominal-Pain” is defined by almost identical loadings on these symptoms: *Gas* (0.92), *Abdominal-Pain* (0.92) with no significant contributions by other variables.

Table 1. Rotated Factor Matrix\*

<b>FACTORS</b>		
<b>SYMPTOMS</b>	<b>F1</b>	<b>F2</b>
<i>Diarrhea</i>	<b>0.828</b>	-.352
<i>Vomiting</i>	<b>0.884</b>	-.095
<i>Fever</i>	<b>0.620</b>	0.221
<i>Nausea</i>	<b>0.524</b>	-.228
<i>Gases</i>	0.039	<b>0.919</b>
<i>Abdominal Pain</i>	-.288	<b>0.918</b>

\*PFA with Varimax-Rotation.<sup>[7]</sup>

Table 2 summarizes the individual variable results of an analysis evaluating potential relationships with the first factor variable: *F1-Diarrhea-Vomiting*. Examining this table, a very highly significant difference may be seen to be associated with Place ( $t(17) = 6.07$ ,  $P < 10^{-5}$  2-tailed) – indicating that disease severity differed between the two communities. There is also a marginal indication ( $P < 0.05$ , 1-tailed) that disease (*Diarrhea-Vomiting*) is more severe in younger patients ( $AgeDel = Age - 24.4$ ). This suggestion also has a parallel in the analysis of the second factor (Gas/Abdominal-Pain) which appears ( $t(17) = -1.80$ ;  $P < 0.05$ , 1-tailed) to be most severe in younger patients (no other terms approach 0.05-significance nor did the overall model,  $R^2(3, 17) = .213$ ,  $P = 0.24$ ). These results altogether point to an essentially unitary disease, largely defined by Diarrhea-Vomiting and associated with marked community differences in intensity.

Table 2. ANOVA Model Summary for F1-Diarrhea-Vomiting\*

<b>MODEL-COEFFICIENTS</b>					
<b>VARIABLE</b>	<b>B-Wt (Unstd. Coeff.)</b>	<b>STD. ERROR</b>	<b>BETA (Std. Coeff.)</b>	<b>t STATISTIC</b>	<b>SIGNIFICANCE LEVEL (P)</b>
<i>Place</i>	0.744	0.123	0.762	6.067	$<10^{-5}$
<i>AgeDel</i>	-.012	0.006	0-.223	-1.827	0.085**
<i>Sex</i>	0.178	0.124	0.180	1.432	NS

\* $R^2(3, 17) = 0.749$  ( $P < 2.4 \cdot 10^{-5}$ ) \*\*  $P < .05$  1-tailed.

### Phase II Analysis Findings

ANOVAs were initially conducted to first evaluate the effects of Place (community), Sex, Age and selected interactions on the *log-transformed duration-of-diarrhea: Ln(D)*. Results of this analysis were very highly significant ( $R^2(5,14) = 0.868, P=10^{-5}$ ). Table 3 summarizes the individual variable results, where it may be seen that the most prominent effects are associated with *Place* ( $t(14) = 8.10, P < 10^{-6}$ ) and its age-related interaction “*PxA*” ( $t(14) = -2.31, P < 0.02$ ). At the same time, the significant *SxA* interaction ( $t(14) = 2.87, P < 0.02$ ) points to a modulation of place result – depending on Sex and AgeDel. Evaluating these interactions in terms of “Days”, it is clear that duration-of-diarrhea is less at the first community; 1.28 days for mean age (~25 years) patients across Sexes vs. 3.28 days in the second community.<sup>5</sup> This relative community advantage is somewhat increased in younger patients, but lessened as patients age (e.g., with 55 year-olds estimated as requiring 1.66 recovery days in the first community and only 2.55 days in the second).

Table 3. ANOVA Model Summary for Ln-Transformed Days [Ln (D)]\*

<b>MODEL-COEFFICIENTS</b>					
<b>VARIABLE</b>	<b>B-Wt. (Unstd. Coeff.)</b>	<b>STD. ERROR</b>	<b>BETA (Std. Coeff.)</b>	<b>t STATISTIC</b>	<b>SIGNIFICANCE LEVEL (P)</b>
<i>Constant</i>	0.722	0.059	–	–	–
<i>Place (P)</i>	0.474	0.058	0.814	8.10	$<10^{-6}$
<i>AgeDel (A)</i>	-.004	0.004	-.137	-1.12	NS
<i>Sex (S)</i>	0.061	0.060	0.103	1.43	NS
<i>PxA</i>	0.009	0.004	-.280	-2.31	$<0.040$
<i>SxA</i>	0.010	0.004	0.340	2.87	$<0.013$

\* $R^2(5, 14) = 0.868 (P < 1.1 \cdot 10^{-5})$

The *SxA* interaction also overlays the above cross gender-results suggesting some advantages for males, particularly before the end of adolescence (18 years). This appears to be increasingly offset by the tendency for males to experience longer bouts of diarrhea as they become older (increasingly so >25 years).

### Phase III Analysis Findings

Compared in this final results section are the Log-transformed durations-of-diarrhea [Ln(D)] with *P-A* vs. “*Past Antibiotic Experience*.” Based on the medical experiences of the authors in their respective communities, diarrhea durations with traditional antibiotic treatment were estimated to be between respectively 48-72 hours and 72-96 hours (with either 7-day courses of *Amoxicillin or Ampicillin*, or the *Sulfamethoxazole-Trimethoprim* combination *Clotrimoxazol*).

<sup>5</sup>This requires the back (inverse) transformation of “log-transformed days” using the Exp-transformation. It should be noted that the back transformed days estimates would be maximum likelihood estimates (MLEs)– assuming analyses with Ln-Days resulted in MLE model coefficient estimates.

Comparison was first conducted for the community with an estimated 1.28 Days diarrhea-duration after P-A. Noting that 48-72 hours corresponds to diarrhea secession no-earlier-than Day 3, we conservatively choose to employ this as the standard for comparison [after appropriate Ln-transformations and Std-Errors drawn from Table 3). This comparison revealed a very highly significant statistical difference – with P-A treatment shortening diarrhea-duration to <43% of that experience with antibiotics ( $t(14) = -10.28, P < 10^{-7}$ ). Subsequently, a comparison was conducted for the community with an estimated 3.28 Days with P-A. Noting that 72-96 hours corresponds to secession no earlier than Day 4, we again conservatively tested with this as the standard for comparison (again with Ln-transformations and Std-Errors drawn from Table 3). This also revealed a significant difference – with P-A treatment shortening diarrhea-duration to <82% of that experience with antibiotics ( $t(14) = -2.26, P < 0.04$ ).

Our final analysis was aimed at estimating the overall statistical significance of P-A vs. Antibiotic results by combining the significance levels from the two communities using the Pearson  $P_{\lambda}$ -Test.<sup>[8]</sup> This basic meta-analysis broadly supported our findings of significant reductions in the duration of diarrhea with *Prescript-Assist*<sup>TM</sup> (P-A) vs. Antibiotics Treatment in Ecuador ( $\chi^2(4) = 41.1, P < 2.6 \cdot 10^{-7}$ ).

## DISCUSSION AND CONCLUSIONS

The primary goal of this study was to comparatively evaluate the clinical efficacy of *Prescript-Assist*<sup>TM</sup> (P-A), an advanced probiotic-prebiotic formulation for treatment of human-diarrhea in two communities in Ecuador. As seen in the previous section, diarrhea durations with traditional antibiotic treatment were estimated to be 48-72 hours and 72-96 hours in the respective study communities (with 7-day courses of either *Amoxicillin*, *Ampicillin*, or *Clotrimoxazol*). Comparisons against clinical experience – as in the present study – may be classed as a form of a *pre-post quasi-experimental research*.<sup>[9]</sup> Ordinarily, the *pre-post* design – particularly for single evaluative tests – is viewed as facing a number of potential validity challenges. These, however, may be largely offset by replications at different sites and in different populations – this in part motivated the two-community approach employed herein, and the earlier canine diarrhea study; as well as, our background presentation of supporting exploratory and formal clinical research.<sup>[3,1-2,4]</sup> The present *pre-post* study also may be strengthened by assuming a very conservative approach to establishing statistical significance – focusing on effects arguably too-large to be due to small validity challenges.<sup>6</sup> In the present study, *P-A vs. Antibiotic* comparisons for each community were constructed very conservatively, i.e., assuming the lower-bound of respective antibiotic treatment experiences [i.e., most rapid recovery]. Nonetheless, each of these comparisons proved statistically significant – with P-A associated with marked proportional reductions in duration in both communities (i.e., <43% to <86%). Overall statistical significance of the P-A vs. Antibiotic comparisons, computed across the individual communities, was found to be very-highly significant [ $\chi^2(4) = 41.1, P < 2.6 \cdot 10^{-7}$ ]. The body of findings – reflected herein and in earlier research – broadly supports the efficacy of *Prescript-Assist*<sup>TM</sup> (P-A) as a treatment for bacterial diarrhea.

A secondary goal – of the present study – was to explore the multivariate nature of the presenting diarrhea symptoms, particularly toward selecting measure(s) of disease severity and efficacy. As seen in the Phase I PFA results, some progress was made toward this end. Our findings essentially identified a unitary factor with significant loadings by all but *Gas* and *Abdominal-Pain* (which defined a second factor without much in the way of significant relationships with the experiment variables of interest). The dominant first factor “Diarrhea-Vomiting” was: (a) Found related to variables of interest; and (b) Broadly defined by loadings on *Diarrhea* (0.83) and *Vomiting* (0.88) with lesser though significant contributions by *Fever* (0.62) and *Nausea* (0.52). The prominent *Diarrhea* (0.83) loading – as well as its primary clinical significance – supported a focus on this variable during the analyses of Phase II.

Comparative efficacy and structural analysis goals of this study were largely met. However, as in all research, new opportunities and questions are suggested by the emergent findings. Our future interest will be toward more fully exploring the potential of advanced probiotic-prebiotic treatments for GI-track infections and conditions. The considerable promise seen in the present results certainly recommends clinical explorations of the emerging opportunities represented by *Prescript-Assist*<sup>TM</sup>.

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<sup>6</sup> This approach arguably compromises statistical power, but this is likely to not be an issue when effect sizes are very large – as anticipated in the present case from the earlier canine and clinical reports.

## CONCLUSIONS

Three broad conclusions may be drawn from the body of reviewed and research findings presented in this report. These include:

- *Advanced probiotic-prebiotic combination “Prescript-Assist™”* has previously demonstrated efficacy against IBS and related gastric diseases in both short- and longer-term studies (e.g., 1-year follow-up).
- *Advanced probiotic-prebiotic “Prescript-Assist™”* demonstrated a comparative advantage – reduced duration – over some standard-antibiotics typically used to address bacterial diarrhea in humans.
- *Prescript-Assist™* – and other *advanced probiotic-prebiotic combinations* – have considerable promise as emerging treatments for a wide spectrum of acute and chronic GI diseases.

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