AṄALEMMA WATER
RESEARCH REPORT

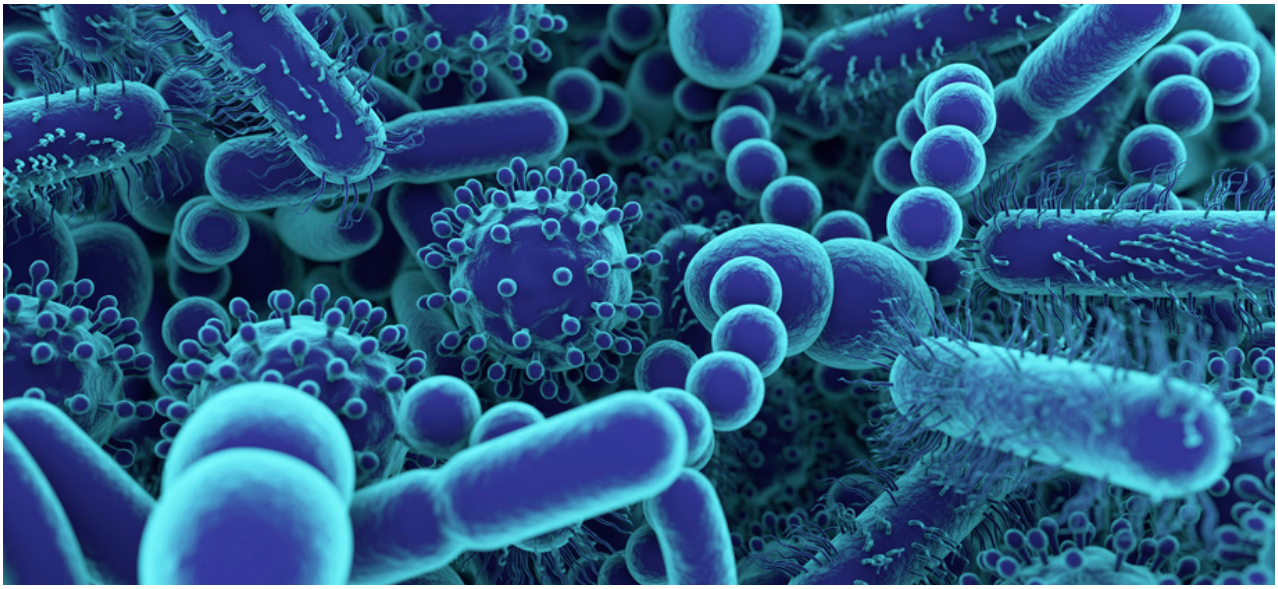
*AUGUST 2023*

# EFFECTS ON GUT MICROBIOME

*2022 double-blind placebo-controlled study*

ANALEMMA

THE ARCHITECT OF LIFE



# The human microbiome study (2022)

The aim of the study was to measure the effect of prolonged Aṅalemma water consumption on the human gut microbiota.

The **gut microbiome**, also known as the gut flora, is the collection of trillions of microorganisms living inside the human digestive tract.

The gut microbiome helps us break down food, absorb nutrients, and produce short-chain fatty acids, which can be used as an energy source for the body. Through complex interplay with immune system pathways, **the gut microbiome protects the body from harmful pathogens and helps prevent infections**.

Studies have also shown that changes in the gut microbiome can **affect our mood and behavior**, and that negative changes of the gut microbiome play a role in conditions such as anxiety and depression.

As part of this study, human participants were instructed to consume Aṅalemma or Placebo water daily for 3 months.

The change in the state of their microbiome was assessed by profiling different beneficial and potentially pathogenic bacteria before and after the water consumption period.

The results of this preliminary study indicate that **consuming Aṅalemma water daily has a positive effect on the state of the human gut microbiota**, affecting both the beneficial and the pathogenic bacteria.

RESEARCH REPORT
*AUGUST 2023*

# The human microbiome study (2022)

## EXPERIMENTAL DESIGN

**OBJECTIVE:** To assess the effect of prolonged Aȟalemma water consumption on the microbiome of adult humans.

**PARTICIPANTS:** 16 adult human subjects, aged 18-70.

**STUDY DESIGN**: The study was designed as a **double blind, placebo-controlled, randomized, parallel group clinical study**. Participants were randomly divided into two groups and given Wands producing either Aȟalemma Water (n=8) or non-treated water (Placebo Control, n=8). The setup was double blind, with neither the participants nor the clinical investigators aware of how the subjects were distributed into groups. All participants were instructed to consume at least 1.5 L of the water treated with their assigned Wand for ~100 days, with regular compliance assessment check-ups. No other changes in diet, exercise or lifestyle regimes were made. Participants' stool samples were taken prior to treatment and on the last day of treatment for a comprehensive microbiome analysis.

**MICROBIOME ANALYSIS:** The microbiome analysis produced two main outputs. The first was the **dysbiosis index**, which is a comprehensive measure of the health of the human gut based on the presence of different bacteria. The second parameter measured the **pathogenic bacteria index**. This parameter was calculated based on the presence of 11 different bacterial species with potentially negative (pathogenic) effects.

**For easier reading, both the dysbiosis index and the pathogenic bacteria index are expressed as percentage of change, with a positive score indicating improvement and a negative score indicating worsening.**


INSTITUTION

**Clinical phase: Den Hoek** (De Bilt, The Netherlands)
**C. de Gooijer-Kant**; **P. Voshol**, Medical Physiologist, PhD; **G. Roozemond**, MSc., neurotherapist; **R. Steinmann**, MD; **T. van Elst**, MD ; **L. Barnhoorn, BA**, neurotherapist

**Microbiome analysis: Biovis Diagnostik MVZ GmbH** (Limburg an der Lahn, Germany)

# The human microbiome study (2022)

## HOW WAS THE DYSBIOSIS INDEX MEASURED

The **dysbiosis index** is a comprehensive measure of the health of the human gut. The index is calculated after obtaining measurements of a number of different factors, including the presence of different bacteria with known positive or negative effects on human gut health. The list of parameters measured as part of the dysbiosis index analysis performed by Biovis Diagnostik GmBH:

- pH
- Enterotype (a classification of living organisms based on the bacteriological composition of their gut microbiota)
- Bacterial diversity
- Firmicutes/Bacteroidetes ratio
- presence of butyrate-producing bacteria
- presence of *A. muciniphila*
- presence of *F. prausnitzii*
- presence of environment stabilizing bacteria
- presence of immunogenic bacteria
- Clostridia germcount
- presence of Clostridia cluster I
- presence of Fusobacteria
- presence of H2S-forming bacteria
- presence of potentially pathogenic bacteria
- Candidiasis

**For easier reading, the dysbiosis index was expressed as percentage of change, with a positive score indicating improvement and a negative score indicating worsening.**

# The human microbiome study (2022)

**RESULTS**: Improvement in the dysbiosis index and pathogenic bacteria index indicates a positive effect of Aȟalemma Water!

On average, participants in the Aȟalemma group had a **10.7% improvement** in the dysbiosis index, while participants in the Placebo group had **5.3% worsening** the dysbiosis index (**Figure 1, left panel**) amounting to an overall **16% improvement in the Aȟalemma group over Placebo**.

Similarly, on average, participants in the Aȟalemma group had an **11% improvement** in the pathogenic bacteria index, compared to a striking **35% worsening** in the Placebo group (**Figure 1, right panel**). Although this amounts to a 46% increase in the Aȟalemma group over Placebo, this large difference is highly contributed to by the worsening in the Placebo group. Therefore, in this case, it is more informative to look at this data separately to avoid overinterpretation.

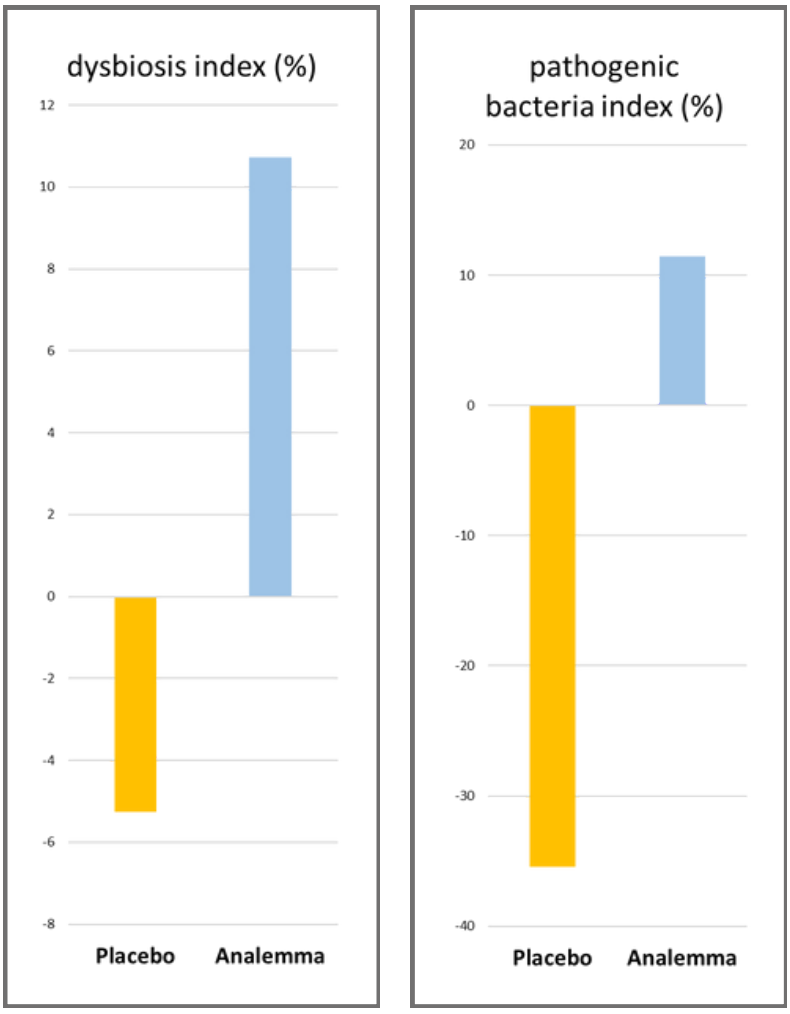



**Figure 1**. The changes in the dysbiosis index (left panel) and the pathogenic bacteria index (right panel) calculated by comparing the results before and after prolonged consumption of Aȟalemma water (blue bars) or Placebo (yellow bars).

The values are expressed as percentage of change, with positive values indicating improvement, and negative values indicating worsening. The values are based on averaged scores of 8 participants per group.

# The human microbiome study (2022)

**RESULTS**: There was a greater number of participants with improved dysbiosis index in the Aὴalemma group compared to Placebo.

The human microbiome can change in response to a variety of factors, and inter-individual differences play an important role in that process. While one person may experience a significant change in the gut microbiome in response to a certain factor, another person might not be as strongly affected by it, resulting in large differences between individuals.

The following illustrations show the number of participants who experienced **improvement**, **no change** or **worsening** in the dysbiosis index after consuming Aὴalemma Water vs. Placebo. This schema shows the general effect of the treatment, without focusing on the actual scores. From this viewpoint, it is evident that in the Aὴalemma group, in contrast to Placebo, the vast majority of participants experienced a general improvement in the dysbiosis index.

## PLACEBO GROUP


In the Placebo group, **38% of participants** experienced an **improvement** in the dysbiosis index, while **the remaining 62%** experienced either a **worsening or no change at all.**

## AỊ̀NALEMMA GROUP


In the Aὴalemma group, **the majority (88%) of participants** experienced **an improvement in the dysbiosis index**.

* average percentage of change

# The human microbiome study (2022)

**RESULTS**: There was a greater number of participants with improved pathogenic bacteria index in the Aὴalemma group compared to Placebo.

The following illustrations show the number of participants who experienced either **improvement**, **no change** or **worsening** in the pathogenic bacteria index after consuming Aὴalemma Water vs. Placebo.

Similar to the dysbiosis index, in the Aὴalemma group, the majority of participants experienced either an improvement or stability in this parameter.

## PLACEBO GROUP


In the Placebo group, **25% of participants** experienced **an improvement in the pathogenic bacteria index,** while **50%** experienced **a worsening in this index**.

## AỊNALEMMA GROUP


In the Aὴalemma group, **50% of participants** experienced **an improvement in the pathogenic bacteria index**.

* average percentage of change

## CONCLUSION

Taken together, the results of this preliminary double-blind placebo-controlled study indicate that Aȟalemma water has a positive effect on human gut health.

In only 3 months of everyday consumption of Aȟalemma water, participants experienced, on average, more improvement in the dysbiosis index compared to Placebo. The dysbiosis index describes the state of the gut by measuring the presence of relevant bacterial species with known roles in affecting the function and overall health of the gut. While the scores differed between individuals, **the majority of participants in the Aȟalemma group experienced an improvement in the dysbiosis index**.

Moreover, in the Aȟalemma group, **a greater percentage of participants experienced a decline or stability in the number of pathogenic bacteria** compared to Placebo, indicating a protective effect of Aȟalemma water on the human gut.

These exciting early results have been tremendously important for the organization of our upcoming research study on the effects of Aȟalemma water on human gut health using the latest advancements in human gut microbiome analysis.

# The human microbiome study (2022)

**1. INVESTIGATORS AND ADMINISTRATIVE STRUCTURE**

The study was designed and performed with the following contributions:

Screening & planning – Performed by R. Steinmann
Clinical phase – conducted by R. Steinmann, G. Roozemond, C. de Gooijer-Kant and T. van Elst
Analysis – performed by R. Steinmann, G. Roozemond and P. Voshol

**2. BASELINE ACTIVITIES**

Baseline activities were comprised of: obtaining study-specific written informed consent, completing a questionnaire for collecting demographic data, handout of requirements for feces analysis and handout of Water Wands including instructions on how to use the device. The Wands were filled with either Aṅalemma water or Placebo (tap water).

The participants were instructed on how to collect their baseline stool sample. The timeline of stool sample delivery, see **Table A1**.

**3. RANDOMIZATION AND BLINDING**

20 adult participants were included in this pilot study.

They were blindly randomized to consume either Aṅalemma water or Placebo in a 1:1 ratio during a period of 100 days. The only person who had access to the randomization process and schedule was in no other way was involved in the study.

One participant dropped out due to personal reasons. Furthermore, three participants finished the study, but their scores were omitted from the analysis presented here due to use of probiotics and/or antibiotics during the water intake period.

This was the distribution of the remaining 16 participants into groups.

Subjects in the Aṅalemma group: 13, 20, 26, 31, 35, 43, 52, 53

Subjects in the Placebo group: 1, 7, 8, 14, 25, 34, 38, 44

**4. ACTIVITIES DURING THE WATER INTAKE PERIOD**

The participants were instructed to consume a minimum of 1.5 L of water daily after treating it with the assigned Water Wand as per the instructions. The participants were discouraged from making any other changes to their lifestyle during the study period.

All participants received a short questionnaire every 3-4 weeks where they could fill in relevant lifestyle changes that had happened over this period of time (e.g. illness, vacation, etc.).

The participants were instructed to collect a stool sample at the end of the study period. The timeline of stool sample delivery, see **Table A1**.

**Table A1.** Stool sample collection details.

| | Aǹalemma group | Placebo group |
|---|---|---|
| Subject number | 13, 20, 26, 31, 35, 43, 52, 53 | 1, 7, 8, 14, 19, 25, 34, 36, 38, 44 |
| Microbiome analysis #1 | Participants sent their stool samples to the laboratory during the first week of July 2022, with the exception of participant 52 who sent their stool sample to on August 10th 2022. | Participants sent their stool samples to the laboratory during the first week of July 2022 |
| Microbiome analysis #2 | Participants sent their stool samples to the laboratory during the week before October 15th 2022, with the exception of participant 52 who sent their stool sample to on November 22nd 2022. | Participants sent their stool samples to the laboratory during the week before October 15th 2022. |

**5. MICROBIOME ANALYSIS**

Two main parameters were measured as part of the study.

The dysbiosis index (DI) is an index based on a proprietary formula developed by Biovis Diagnostik GmbH, which describes the degree of deviation in the microbiome, taking into account different bacterial phyla and species and their weighting factor. It does not take into the degree the amount of inflammation. Its value can range from 0 to 30, with lower score indicating a healthier gut microbiome. For simplicity, the change in this parameter between the baseline measurement and end measurement was expressed as percentage of change, in which a positive value indicates a positive change (improvement), and a negative value indicates a negative change (worsening).

The number of potentially pathogenic bacteria is a parameter based on measuring the presence of 11 different potentially pathogenic bacterial species. The score can range from 0 to 11, where 0 indicates "no highly abundant presence of pathogenic/potentially pathogenic bacteria" and any other number indicates "the number of pathogenic bacteria are present in higher abundance than normal". For simplicity, the change in this parameter between the baseline measurement and end measurement was expressed as percentage of change and referred to as the pathogenic bacteria index, in which a positive value indicates a positive change (improvement), and a negative value indicates a negative change (worsening).

**6. REFERENCES**

Clapp M, Aurora N, Herrera L et al. (2017) Gut microbiota's effect on mental health: The gut-brain axis. Clin Pract 7:987.
Flint H, Scott K, Louis P et al. (2012) The role of the gut microbiota in nutrition and health. Nat Rev Gastroenterol Hepatol 9, 577–589.
Lazar V, Ditu L, Pircalabioru GG, Gheorghe I et al. (2018) Aspects of gut microbiota and immune system interactions in Infectious diseases, immunopathology, and cancer. Front Immunol 9:1830.