Microbiome test kits – a thorough checkup

Round 2

10/2020

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Developed the first and only standard for Microbiome friendly cosmetics and personal care products and also probiotic food supplements.

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Microbiome test kits – a thorough checkup

Overall evaluation, october 2020

Again, we did some checking on a couple of microbiome test kits. Our first evaluation round showed that general standards for analysis and testing are strongly needed to ensure comparable results. This is even more important as the clients falling back to this method hope for clarification of specific health issues (and are often disappointed by the lacking medical relevance of the outcome).

So, this time we went a bit more into detail, took into account the scientific background and worked with a test person that came with a wide range of symptoms. We compared general criteria and quality of methods. Besides, we wanted to find out if the results match the diagnose of the test person and if the dietary recommendations are useful.

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Microbiome test kits – a thorough checkup

Overall evaluation, october 2020

List of participants

Atlas Biomed, health company from London, UK (found 2016) (rerun, test 2018)

BIOMES, biotech company from Berlin, Germany (found 2017) (rerun, test winner 2018)

Elsavie, trademark of TFTAK (Center of Food and Fermentation Technologies, CFFT, microbiome tests since 2012) from Tallinn, Estonia (trademark found in 2019) (rerun, test 2018)

New in

myBioma, Start-up from Vienna, Austria (found 2018)

New in

VIOME, US-american company (found 2016)

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Microbiome test kits – a thorough checkup

Overall evaluation, october 2020

Our testperson

Our test person is male, a bit more than 50 years old and underweight. The stool type varied between Bristol 2, 4 and 6 in the days prior to testing and was type 2 on the test day.

For explanation, stool chart according to Bristoll:

Intolerances: lactose, milk protein, fructose, coffee, and alcohol

Symptoms: intestinal colic, diarrhea, flatulence, stomach ache, head ache, exhaustion, depression

Diagnose based on an allergenic and metabolic analysis:
mitochondriopathies and Leaky-Gut-Syndrome

Nutrition: wholesome organic, with a lot of vegetables, free from fruits and glutens, organic meat

What we checked

1. Sideshow assessment

- price
- order
- availability
- website languages
- handling
- shipping
- evaluation time

2. Assessment: quality of methods of analysis and evaluation

- stabilizer included?
- blank sample included?
- Method of identification
- taxonomic level (down to strain level?)
- quality of databases used
- questionnaire on personal details included?
- Disclaimer, pointing out that test is merely a lifestyle product and not meant for medical purposes

3. Ergebnisse

- Output format
- structure
- understandability
- scientific derivation
- list of literature available?
- Is there a list of all microbes identified with shares in percentage?
- Are the nutrition recommendations helpful and individual and is there an offer for personal consultancy (weighed twice)

Additional: Comparison of the outcomes / raw data regarding the test person's main issue (Leaky Gut Syndrome)

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Microbiome test kits – a thorough checkup

Overall evaluation, october 2020

The evalutation

The detailed evaluations can be downloaded here for all test kits:

PDF evaluation Atlas Biomed

PDF evaluation BIOMES

PDF evaluation Elsavie

PDF evaluation myBioma

PDF evaluation VIOME

In the following survey we compare the results of our evaluation.

Evaluation criteria:

excellent (++) | good (+) | potentail to improve (+/–) | improvement strongly recommended (-)

1. Evaluation sideshow

Usually, the client has to pay for the test him- or herself, so we thought that the price would be an important criterion apart from order, availability, website languages, shipping, and evaluation time. And because the actual testing itself is not a very pleasant thing to do, we decided to include the criterion of handling, as we did last time.

Table 1: Evaluation sideshow – price, order, availability, website languages

	price	order	availability	website language
Atlas Biomed	169,00 €	website, other platforms	worlwide (with some restricitions)	GER, EN, IT, DA
BIOMES	139,00 €	Website, amazon.com, fitness centers, pharma-cies, dietitians	worldwide	GER, EN, ES, FR
Elsavie	169,00 €	Website	worldwide	ET, EN
myBioma	149,90 € (no. shipping costs)	Website	Europe wide	GER, EN
VIOME	120,95 € (varying)	Website, amazon.com (limited)	worlwide (with some restricitions)	EN

Microbiome friendly

Microbiome test kits – a thorough checkup

Overall evaluation, october 2020

Table 2: evaluation sideshow – handling, shipping, evaluation time

	Handling	Versand	Analysedauer
Atlas Biomed	Improvement strongly recommended	Pre-stamped, self-addressed envelope	5 weeks (as announced)
BIOMES	Potential to improve	Pre-stamped, self-addressed envelope, free only in Germany	3 weeks (as announced)
Elsavie	Potential to improve	Pre-stamped, self-addressed envelope	appr. 4 weeks (without notification)
myBioma	Potential to improve	Pre-stamped, self-addressed envelope	appr. 6 weeks (about the announced time)
VIOME	good	Self-addressed envelope, to be paid for by client	7 weeks (1 week longer than announced)

2. Quality of methods of analysis and evaluation

The significance of a stool test is based on the quality of the methods used for the analysis.

To prevent bacteria from growing or the genetic material from decaying within the sample on the way to the lab, for instance, the sample has to be carried in an appropriate stabilizer. If this is not the case, the result will be distorted. Also, a reasonable lab includes blank samples for quality controls to make sure, instruments, material, and sample jar were not contaminated. We have sent according inquiries directly to the tested companies.

Also, the method of identification of prevalent microbes plays a very important role for a profound evaluation of a microbiome. This includes the taxonomic level of identification.

Side note: method of sequenzing

State of the art in microbiome analysis today is the sequencing of the bacterial 16S-rRNA genome. Extended databases to compare the results with already exist. They include the precise DNA sequence it takes for alignment. In most cases, however, this method takes you only to the taxonomic level of species and we know that it is the strain level (sub-categories of the species) that causes effects in one or the other way. A gut microbiome that is only identified down to the species level may explain inconsistencies between reported effects of bacteria and observed symptoms. And this is true even more if the identification goes only down to the level of genus or family.

https://doi.org/10.1038/nmeth.3837

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Microbiome test kits – a thorough checkup

Overall evaluation, october 2020

With the identification method of 16S-rRNA genome sequencing, only bacteria are identified. But a microbiome is also home to viruses, archaea, fungi or eukaryotes. Microbiomes research is only in its first steps and we will see what the future brings.

Side note: analysis of metatranscriptions

Another interesting method, we would like to shed some light on here, is the new method of not looking exclusively at DNA sequences, but including RNA sequencing in the identification process. This gives us information on what microbial genome was expressing at the time of testing. Only the expression of a genome sets free bacterial metabolic products like proteins or enzymes with a specific (positive or negative) effect. To find out about the activity of microbes with RNA sequencing, it takes complex meta-transcription analyses that only a few labs are able to perform. Moreover, it is important to appropriately stabilize the RNA that is even more sensitive than DNA is, so that transportation and environmental impacts do not damage the sample. DOI: 10.1038/nrmicro3451

Side note: Metabolome Analysis

Metabolome analysis does not aim after identification of microbes, but after their metabolites. This complex and cost-intense way of analyzing should become standard in microbiome science, because strictly speaking it is not the microbes in our gut that have an impact on what's going on down there, but their biochemical output they produce when decomposing foods. It is very likely that we cannot identify all microbes, by far. But what we are able to, in fact, is to identify their metabolites. And sometimes the microbes are quite different, sometimes even unknown, but exercising the same function. Consequently, a metabolome analysis gives far more precise information regarding health.

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4869604/

Microbiome analyses should aim after depicting the intestinal processes with highest scientific precision. Only active microbes have an effect on their environment – in a negative or positive way. So, if you limit the identification solely to 16S-rRNA, you can only tell that an organism was present in a given point in the past. This is helpful in forensic, for instance.

What 16S-rRNA/ DNA does not tell us, is if the organism is right now dead or alive. And dead DNA can be traced even in the remains of a mummy, as we know from archaeology. We find that only extensive meta-transcriptomic or metabolome analyses (combined with alignment of sub-categories) lead to scientifically reliable results regarding activity and effect of a microbiome. We have put an emphasis on that point in our assessment: If a company meets both "best-of-criteria" (meta-transcriptomic or metabolome analyses; and identification of strain level), the method of analysis is rated with an "excellent" in both cases, otherwise with "good". In the total evaluation, this counts double.

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Microbiome test kits – a thorough checkup

Overall evaluation, october 2020

As we know, the variety of our microbiome and its effects is immeasurable. That's why we also ranked the question highly, on what databases the analysis is based on.

We differentiate between three databases:

1. Genome databases (e. G. 16S-rRNA-Genome databases), for taxonomic alignment;

2. Reference databases, for comparison of the outcomes;

3. Scientific databases, meaning collections of scientific evidence of clinical observations on the effects of microbes and the effects of foods.

The providers of test kits usually have their own algorithms to take the content of these databases into account.

Every person – and with him or her his microbiome – is unique. This is why we wanted to include the personal situation at the time of testing in the analysis. Especially in the case of difficulties, those should be taken into account and problems should be referred to in recommendations. This is why we took a look at the use of questionnaires and checked on how detailed they were. To some extend we also wanted to include into the assessment if the company paid attention to the personal details from the questionnaires.

Last but not least, it is clear that standardized microbiome testing is still at the very beginning. Consequently, a company offering tests has to inform the customer that the product offered is just a **lifestyle product** and cannot replace medical consultancy. And this disclaimer should ideally be placed so that is can be found before purchasing the product.

Table 3: Quality of method – stabilizer, blank sample method of identification, taxonomic level

	Stabilisiert	Leerprobe	Identifizierungsmethode	Taxonomisches Level
Atlas Biomed	yes	yes	16S-rRNA genome sequencing	Family and genus
BIOMES	yes	yes, but not always	High throughput sequencing	down to species
Elsavie	yes	yes	16S-rRNA genome sequencing	down to species
myBioma	yes	yes	16S-rRNA genome sequencing	down to species
VIOME	yes	not known	Metatranscriptom analysis	Unterart (strain level)

Table 4: Quality of method – databases, questionnaire, information lifestyle product

	Databases 1. genome 2. references 3. science	Questionnaire	Information lifestyle product
Atlas Biomed	1. company-owned (on basis of HITdb, bacteria only) 2. Company-owned (unclear amonunt) 3. compiled by company (> 20000 publications)	yes, potential to improve	yes, but very easy to miss when purchasing product
BIOMES	1. external 2. own (> 20000 data) 3. internally compiled and reviewed (> 6000 publications)	yes, potential to improve	yes, but not with the purchase
Elsavie	1. unclear 2. yes, compilation unclear 3. Science and research based literature	yes, very detailled	yes, but not with the purchase
myBioma	1. Greengenes and Silva 2. unclear 3. Internally compiled (> 3000 publications)	yes, potential to improve	yes, but not with the purchase
VIOME	1. external 2. own (amount?) 3. internally compiled (research, extent?)	yes, very detailled	yes, but not with the purchase

3. Results

The results are the core of the analysis. To meet all clients' needs equally, access to the results via computer and printable results would be good. The app is the most popular tool among customers. So, we checked on the output format (online dashboard, app, PDF file). As we are talking about a lifestyle product that also private persons can purchase, the results should be explained understandable and in a well-structured layout. Here, we took into account the layout in general, as well as the graphical presentation of the results. To tell if the company is working on a serious scientific basis, we also checked the scientific derivation, especially the open citation of sources used and if they were linked in a useful way into the presentation of the results.

The data extracted from the stool sample are a more or less long list of microbes and their share. It occurred to us that the unit (percent) is missing in some tables or scales. We surmised that percent were meant, but we want to stress that this lack of information makes a piece of lab analysis incomplete, scientifically speaking.

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Microbiome test kits – a thorough checkup

Overall evaluation, october 2020

It is up to the testing company to interpret the raw data and the client has to rely on the company in doing so in an appropriate way – this is probably the bottleneck of every testing kit, because the underlying studies differ a lot from each other and are also discussed controversially. **So, in our opinion, it would be only fair to present the raw data to the client openly, so that he or she or at least any independent consultancy can use these data.**

Side note: Microbiome research With all the hundreds on studies on the matter, we must not forget one point: The simple correlation between the composition of the microbiome and certain diseases does not automatically mean a causality.

The technical and methodical progress in the field of microbiome research has turned out many general assumptions to be true. This goes, for instance, for the fact that our microbiome and the food we give to it are important and vital helpers within us. It is also considered true that our way of life and the intake of antibiotics have some serious consequences for our gut.

Scientist started revealing possible correlations between civilization diseases and the composition of the gut bacteria. However, this field of research is still too young to make definite statements and often, correlations are turned into causalities without checking further.

The general direction that science is going to, is certainly true and future-bound. Still, the results have to be reviewed, bigger studies have to be conducted, and above all, the interpretations have to be seen critically.

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6520976/

This is why we find it also important to double-check the sources of literature the results of the studies are based on and to check if the underlying literature is communicated openly. Most companies providing test kits also come up with nutrition recommendations or tips for dietary supplements.

We took a closer look at those – not only regarding practicability and possible further personal consulting, but we also checked if the symptomatic characteristics of our test person match with the recommendations given.

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Microbiome test kits – a thorough checkup

Overall evaluation, october 2020

Table 5: Results – Output format, structure, understandability

	Output format	Structure of layout	Understandability for laypersons
Atlas Biomed	Dashboard online, App (EN only), PDF after request only	confusing	potential to improve
BIOMES	Dashboard online, PDFs, no app	Very well structured	good
Elsavie	PDF (en) only	Well structured	potential to improve
myBioma	no Dashboard for computer, but for app and PDF	Very well structured	good
VIOME	Dashboard online + PDFs +App	Very well structured	potential to improve

Table 6: Results – scientific derivation, sources of literature, list of microbes, nutrition recommendations, personal consultation

	Scientific derivation	Sources of literature	List of microbes (%-share)	Nutrition recommendations	Personal consultation
Atlas Biomed	good	yes	yes	Dashboard confusing, but personal consultation	yes
BIOMES	potential to improve	yes	yes	potential to improve (no personal consultation)	Facebook group
Elsavie	potential to improve	yes	On request, without percentage share	Improval strongly recommended	yes
myBioma	good	yes	On request	Many recommendations regarding symptoms, personal consultation	yes
VIOME	potential to improve	Yes, but not linked	Yes, but no percentage	potential to improve, automated consultation with chat window	AI Vie

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Microbiome test kits – a thorough checkup

Overall evaluation, october 2020

Comparison of some of the results

There is no standardized procedure in microbiome analysis, so far, so it is up to every company itself to set up criteria on which to sort the results and how to categorize the output. Therefore, a direct comparison is pretty hard. As we did in first evaluation round, we picked a few criteria and compared the companies with. The stool testing was done at the same time, so actually, the results should all be pretty similar.

DIVERSITY

Atlas Biomed	high (295 bacteria; Chao-Index 9)
BIOMES	online: conspicuous; PDF: good (!???, no value given)
Elsavie	good (111 bacteria; Shannon-Index 3,4)
myBioma	excellent (Shannon-Index 5,05)
VIOME	no indication

The high diversity is understandable, as our test person keeps an eye on diverse nutrition.

ENTEROTYPE

Atlas Biomed	1, *Bacteroides* dominate
BIOMES	3, *Ruminococcus* dominate
Elsavie	1, *Bacteroides* dominate
myBioma	1, *Bacteroides* dominate
VIOME	no indication

Taking into account the diet or our test person (wholesome, many vegetables), and his under-weight, we expected the outcome to be enterotype 2 (*Prevotella* dominate).

INFLAMMATION INDICATORS

Atlas Biomed	hard to compare
BIOMES	good
Elsavie	good
myBioma	hard to compare
VIOME	action required

The test person's symptoms hint at inflammatory processes in the gut. We had expected this to refelect in the inflammation indicators.

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Microbiome test kits – a thorough checkup

Overall evaluation, october 2020

DOMINATING BACTERIAL STRAIN IN THE HUMAN GUT

Actinobacteria/Bacteroidetes/Firmicutes/Proteobacteria/Verrucomicrobia

Atlas Biomed	only family and genus indicated
BIOMES	0,98/19,13/74,84/2,95/0,75
Elsavie	1,9/10,0/84,0/0,8/0,3 (old report, strains not explicitly indicated in new report)
myBioma	0,29/11,05/47,37/34,61/6,07
VIOME	no indication

The highest share of bacteria in the human gut are *Bacteroidetes* and *Firmicutes*. It is striking that myBioma found a very high share in proteobacteria. Those are said to boost inflammation, what would match the test person's symptoms.

We are at least becoming suspicious by the striking differences between the raw data of the analysis (of the very same stool sample, not to forget). Would it be necessary to stir the sample in advance? This would be rather difficult for stool types Bristol 1 to 3.

Also, the hint that stool has to be taken from three different sites, was only given by Elsavie and myBioma. But also, the tests of those three site samples come with a few inconsistencies regarding the symptoms of the test person (underweight, diarrhea, and Leaky-Gut-Syndrome). Especially the latter should result in a critical value of Butyrate synthesizing bacteria, bifidobacteria, *Akkermansia muciniphila*, *Faecalibacterium prausnitzii*. Those are responsible for building up a healthy mucosa, which our test person apparently does not have, so we expected an according outcome of the tests.

ASSESSMENT GUT MUCOSA

Atlas Biomed	high rate in Butyrate synthetizing bacteria, apart from that no explicit information on health of gut mucosa ▸ does not match test person
BIOMES	Butyrate synthetizing bacteria noticeable, bifidobacteria low, share of other mucosa protecting bacteria rated good, but low compared to reference group ▸ matches with test person very well
Elsavie	low rate of Butyrate synthetizing bacteria, no indication of Bifidobakteria, apart of that no explicit information on mucosal health ▸ matches test person
myBioma	Butyrate synthetizing bacteria not explicitly identified, rate of mucosa protecting bacteria identified excellent ▸ does not match test person
VIOME	average rate of Butyrate synthesis, bifidobacteria not listed, average mucosal health ▸ matches test person

Microbiome friendly

Microbiome test kits – a thorough checkup

Overall evaluation, october 2020

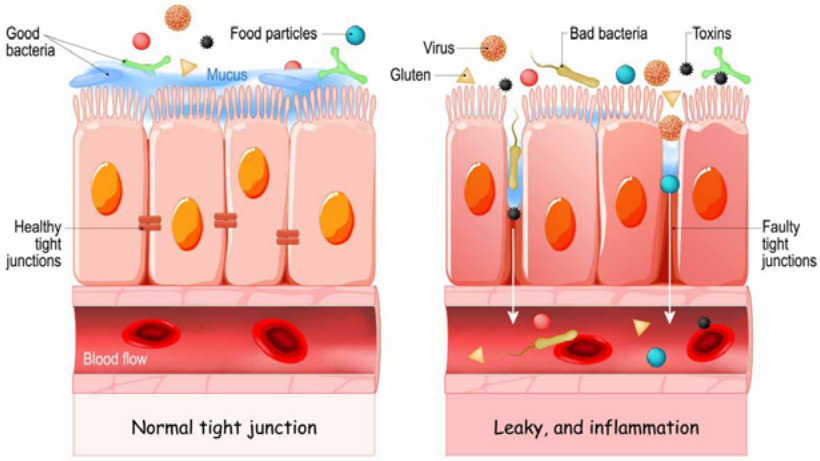
The result of BIOMES was one to match best with the test person's main issue, second best were Elsavie and Viome. Atlas Biomed and myBioma did not meet our expectations, however, we would like to stress that a single stool sample is just a snapshot.

Side note: Leaky Gut Syndrome

The Leaky Gut Syndrome is a disease that is until today not fully understood and discussed controversially. It is about the barrier between the gut and the inner parts of the body. That barrier is comprised of numerous microbes dwelling withing it, and of epithelial cells that are held together by so-called Tight Junctions (membrane proteins). Countless bacteria contribute to the stability of the barrier. The permeability of the intestinal wall is regulated biochemically. Good things may enter, bad stuff is kept outside. The regulatory activity can be impaired by many factors, resulting in an increased permeability of the intestinal wall. This comes with a number of problems, such as intolerances, or inflammatory processes, as the substances entering the body unwanted cause immunologic processes. An additional disbalance of the intestinal microbiome can foster that effect (for example an additional lack of mucosa protecting bacteria)

https://www.mikrooek.de/seminare/fachtagungen/das-mikrobiom-in-der-praxis/

Leaky gut syndrome



(Picture: © designua - stock.adobe.com)

With that background, the repetition of testing that every provider recommends does indeed make sense. This makes sure that both correct and questionable results are reproduced and their informative value is strengthened or potential mistakes of the analysis are revealed.

For the informative value regarding the main issue Leaky Gut Syndrome, we awarded some additional points: two points go to BIOMES. Elsavie and Viome earned one additional point each.

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Microbiome test kits – a thorough checkup

Overall evaluation, october 2020

Summary

Pretty much all of the companies can be counted as "young", so they might go through developmental processes. In almost every case, the communication with MyMicrobiome was fantastic. Only for VIOME, we have to say that customer communication was not existent. Apart from VIOME, all providers were interested in our test runs, replied to our questions and it turned out that they all go through processes of constant improvement. We took our time with the evaluations and checked for improvements after a while, which were partly delivered promptly.

Each test kit and every analysis come with strengths and weaknesses. In total, we set up 21 criteria that we evaluation with "excellent", "good", "potential for improve" or "improve strongly recommended". For the final evaluation, we took the assessment with the highest share and weighed every "excellent" double. The number of total credits regarding informative value was added to the general points. If companies tied for the highest rank, we chose to rank the company first that reached an "excellent" evaluation. Regarding the ranking, we awarded the places according to the worst evaluation achieved when companies tied. (Example: Atlas Biomed and VIOME added up to 11x "good" each, so we took into account the number of "improve strongly recommended" to align the total rank).

	Rank 1: MyBioma	Rank 2: BIOMES	Rank 3: Atlas Biomed	Rank 4: VIOME	Rank 5: Elsavie
Excellent (++)	0	0	0	2	0
Good (+)	16	14	13	8	10
Potential to improve (+/-)	7	9	6	9	10
Improvement strongly recommended (-)	0	0	4	3	4
Grade	**1,30**	**1,39**	**1,61**	**1,63**	**1,75**

Microbiome friendly

Microbiome test kits – a thorough checkup

Overall evaluation, october 2020

Conclusively, we think about what might have caused the different results of raw data, as almost every company is working with the same method of identification. First, the way of taking the sample might be improved. Second, for a standardized microbiome analysis, the focus would have to put on the underlying databases and algorithms.

And only after a thorough redesign and extended analysis by more custom-fit algorithms and artificial intelligence (which we are no experts for, though), it would make sense to expect individual specifications from the results. Because also the nutrition recommendation did not seem to have any correlation to the answers given in the questionnaire. Often, the personal constitution regarding intolerances was not taken into account. Besides, the test person does indeed follow the very recommendations for several years, already (apart from foods covered by the intolerance) and has not experienced any relief in his symptoms, so far. **This seems to prove that for serious illnesses like the Leaky-Gut-Syndrome, only integral diagnostics can help.**

> **Last, but not least, we would like to emphasize the importance of the scientific method in terms of health improve offers on the free market. If you want to benefit from the test without further medical assistance, you should definitely pay attention to that before spending money on it.**

One step into the direction of integral diagnostics would be, for instance, a combination of metagenome, meta-transcriptomic and metabolom-analyses with emphasis on the last one, as this can deliver the most information. We hope to be able to tell you about such a test soon, besides Viome already using meta-transcriptome analysis.

The ongoing processes of microbiome research are very exciting and future-bound to us. This is why we stick to our mission of assessing tests and reporting on it on a regular basis.