

Consensus statement

Title

International consensus statement on microbiome testing in clinical practice

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ABSTRACT

There is growing interest in the potential exploitation of the gut microbiome as a diagnostic tool in medicine, but available evidence supporting its clinical utility remains limited. An increasing number of commercial providers offer direct-to-consumer microbiome diagnostic tests without any consensus on their regulation and/or any proven value in clinical practice, which may result in considerable waste of individuals' and healthcare resources, as well as potential drawbacks in the clinical management of patients. We convened an international multidisciplinary expert panel to standardise best practices of microbiome testing for clinical implementation, including recommendations on general principles and minimum requirements for their provision, indications, pre-testing protocols, methodology of analyses, reporting of results, and on their potential clinical value. We also evaluated the current knowledge gaps and future directions in this field. We aimed to establish a framework to regulate the provision of microbiome testing and minimise the use of inappropriate tests, in order to pave the way for the evidence-based development and use of human microbiome diagnostics in clinical medicine.

INTRODUCTION

The gut microbiota is a key mediator of essential human functions, including metabolism,¹ immune regulation,² colonisation resistance,³ and response to drugs.⁴ An increasing body of evidence has shown, initially via association studies but also through mechanistic lines of research, that an imbalance of the gut microbiome is associated with a broad range of intestinal and extraintestinal disorders,⁵ as well as response to therapies.^{6–8}

Manipulation of the gut microbiome, e.g. through faecal microbiota transplantation (FMT), has been explored as a therapeutic strategy. FMT is now recommended for the routine management of recurrent *Clostridioides difficile* infection (CDI) and has shown promise for a range of non-CDI indications.⁹

There is also growing interest in the potential exploitation of the gut microbiome as a diagnostic tool in clinical practice, for several applications,¹⁰ including: the diagnosis, prognostication, or risk assessment for particular disorders; the prediction of patient response to a specific therapy; the targeting of therapies aimed at modulating the gut microbiome, for example probiotics or FMT; the monitoring of the efficacy of such therapies.¹¹

Despite this enthusiasm, the application of gut microbiome research in clinical practice remains currently minimal because of different factors,¹² including the complexity of the microbiota and associated sequencing datasets, the difficulties in disentangling correlation from causation, the reliance on pre-clinical models with low generalisability to humans,¹³ the limited knowledge of most clinicians about this field, the absence of any validated test to enable therapeutic follow-up, and the lack of established regulations and framework for the clinical translation of this research.

In contrast, there is increasing expectation from patient groups for the rapid introduction of microbiome-based diagnostics and therapeutics to routine care. Because of this disparity, direct-to-consumer (DTC) microbiome testing, claimed to drive the clinical management of patients with dysbiosis-associated diseases, have proliferated worldwide in recent years. These tests are primarily based on amplicon sequencing or whole genome sequencing (WGS)¹⁴ but may also exploit other technologies (i.e. conventional PCR or culture). This trend raises several concerns due to the absence

of a standardised framework relating to the indications and methodology for their use, which limits their interpretability and applicability, with considerable waste of patient and, more generally, healthcare resources, i.e. due to the potentially inappropriate requests for medical exams, or to the prescription of supplements and medications, driven by the results of these tests. Moreover, these tests can generate false hopes in patients, often suffering from severe disorders, leading potentially to detrimental consequences. Finally, due to the absence of a formal postgraduate clinical education in microbiome science, most physicians and other healthcare professionals are neither adequately trained to interpret a microbiome test and therapeutically manipulate the gut microbiome, nor to distinguish a well conducted test from an inappropriate one.^{15,16}

For these reasons, we convened an international multidisciplinary expert panel aimed at standardising and defining best practices of microbiome testing applied to the management of human diseases, evaluating the current knowledge gaps and future directions in this field, and helping pave the way for evidence-based development of human microbiome diagnostics in clinical practice.

METHODS

Development of consensus

The development of this consensus report was based on a multi-step process that included: recruitment of the expert panel; identification of key issues and building of corresponding working groups; development of statements according to the best available evidence; development of consensus through an online Delphi process; completion of the final report. This methodological framework has been adopted successfully in previous consensus initiatives.^{9,17}

In July 2022, a steering committee of internationally acclaimed opinion leaders in gut microbiome research (AG, GC, GH, GI, HS, MS, NS, SN) invited peers to join the consensus expert panel, based on their expertise in gut microbiome assessed by their publication track record. We assembled an international, multidisciplinary group including clinicians with expertise in gut microbiome and related modulation, clinical microbiologists, microbial ecologists, computational biologists and bioinformaticians, for a total of 69 experts from 18 countries. The steering committee identified the following key issues to be addressed: 1) general principles and minimum requirements for providing microbiome testing; 2) procedural steps before testing; 3) microbiome analysis; 4) characteristics of reports; 5) relevance of microbiome testing in clinical practice: present and future.

Key issues, described in Box 1, were reviewed and approved by the whole expert panel, and five working groups (WGs), one for each key issue, were built by the steering committee, that assigned each expert to a specific WG based on her/his expertise. Each WG included 13 or 14 experts, without any overlap between them. Further details on the membership of each WG are described in the Appendix (page 1). Members of each WG nominated two coordinators to chair activities and to liaise with the steering committee. For each key issue, the steering committee developed relevant sub-issues/questions, that experts of the corresponding WG were requested to address by the release of pertinent statements. As the topic of microbiome testing is highly pioneering and innovative, statements were released as expert opinions, although they were built according to the best available evidence.

Statements and narrative comments from each WG were edited by the respective coordinators and then uploaded, together with supporting references, to an online electronic voting system accessible to the expert panel (https://ec.europa.eu/consultation/resources/documents/Delphi_Guide).

The whole expert panel (n= 69 members) was requested to evaluate the statements released by the WGs. The Delphi method was used to achieve a consensus.¹⁸ For each statement, experts were asked to rate anonymously their agreement, according to a Likert scale anchored by 1–5 (1= agree strongly; 2= agree with reservation; 3= undecided; 4= disagree; 5= disagree strongly). In the case of rating differing from ‘agree strongly’, respondents were requested to clarify their reservation or disagreement and give suggestions to ameliorate the statement. The *a priori* established threshold of consensus for each statement was reached if $\geq 80\%$ experts agreed either strongly or with reservation. All statements not reaching at least 80% of agreement were discarded or modified and rated again in a further voting round. After each round, expert responses were collected by the steering committee and shared with the whole panel. Experts had the chance to modify their answers in subsequent rounds. After multiple rounds, the Delphi method enabled achievement of the ‘correct’ response through consensus.

Two rounds of electronic voting were needed to reach consensus. The outcomes of the whole Delphi process, including the rate of agreement achieved for proposed statements at each round, and subsequent removal or modification of the statements which did not meet the threshold for acceptance, are available in the Appendix (pages 2-6).

Finally, the whole expert panel approved the final version of released statements (summarised in Table 1) and comments.

WORKING GROUP STATEMENTS

All statements are provided, along with their rate of agreement, in Table 1. Here we provide a narrative description of approved statements.

WORKING GROUP #1: GENERAL PRINCIPLES AND MINIMUM REQUIREMENTS FOR PROVIDING DIAGNOSTIC MICROBIOME TESTING

The expert panel recommends that “providers of microbiome testing should communicate a reasonable, reliable, transparent, and scientific representation of the test, making customers and prescribing clinicians clearly aware of the currently limited evidence for its applicability in clinical practice” (Statement #1). Moreover, these entities may also participate in research protocols under strict investigative conditions, with the final aim of generating evidence on this pioneering field, and to promote the knowledge and culture of the human microbiome field.

The panel also acknowledges that “the provision of a microbiome test involves a complex framework, from the collection of biological samples to the sequencing of the microbial genome and computational analyses, to the release of an interpretable report. Therefore, providers of microbiome testing should include experts with multidisciplinary competences” (Statement #2).

The expert team that provides the microbiome testing should include multidisciplinary members with different expertise, e.g., next-generation sequencing, computational biology, microbial ecology, and clinical microbiology. Physicians may also be involved as consultant to support the referring physicians in the interpretation of the microbiome testing. As the training in gut microbiome is still not defined by a core curriculum or embedded in an official educational pathway, the expert panel has preferred not to identify specific professional figures, but rather focus on defined skills in pertinent areas.

Importantly, the task force agreed that “any change in the clinical management of the patients based on microbiome testing should be made only by their referring physicians/healthcare professionals” (Statement #3). Clinical decisions are the result of a complex process that evaluates all aspects of

the patient history rather than a single test. So, only the referring physician/healthcare professional who has requested the testing should oversee any modification of the clinical management of the patient, based on the results of the microbiome testing.

Also, “laboratories that provide microbiome testing should guarantee high quality standards, as well as protection of patient data, and be accredited/registered/regulated” (Statement #4). Entities which provide microbiome testing should also be accredited, registered, or regulated, that applies to other laboratories that provide diagnostic services. This regulation should be provided at a national level. They should also guarantee the protection of the patient data reported in the testing, as discussed in Box 2 (<https://gdpr-info.eu/recitals/no-35/>).¹⁹ Further details are provided in the Appendix (page 7).

As final statement of the WG #1, “validated and up-to-date computational software pipelines and databases aimed at delineating microbial taxonomy are required to provide microbiome testing” (Statement #5). Examples of databases to align specific data against for the identification of microbes are provided in the Appendix (page 7). All the steps should include a panel of checkpoints or quality controls (QCs) to perform sequence enumeration, quality of the sequences, denoising, rarefaction curves, and alignment with the database for assignment to the different taxonomic levels. The use of proprietary protocols that cannot be externally validated is discouraged.

WORKING GROUP #2: PROCEDURAL STEPS BEFORE TESTING

In this working group, statements concerning the methodological workflow to be followed before the actual testing are presented. The first approved statement was “As there is currently limited evidence for the applicability of gut microbiome testing in clinical practice, the direct request by patients for microbiome testing without a clinical recommendation is discouraged” (Statement #6). To limit inappropriate requests that come directly from patients, which could be done without a clear clinical indication and without awareness of the limitations, we suggest testing to be requested only by physicians or other licensed healthcare professionals (i.e. dietitians). Also, non-licensed professional figures, like personal trainers, coaches, homeopaths, osteopaths, are discouraged to prescribe any microbiome testing. Also, the panel agreed that “before testing, key clinical data of the patient, including those that may influence gut microbiome characteristics, should be collected. Essential information to be captured should include at least age, gender, body mass index, dietary habits, smoking and alcohol status, gut transit time, current comorbidities and medications, and past medical history” (Statement #7). Host factors can influence the composition and functions of the gut microbiome and may thereby influence the interpretation of the test results. Often the effect of these variables on gut microbiome is complex, with marked inter-individual variability, making them hard to directly interpret at the individual level at present. However, future accumulation of pertinent evidence may allow more nuanced interpretation of microbiome reports that include this information. The panel suggested that a minimum set of data should be captured, as detailed in Table 2.^{20–23}

Notably, the panel recommends that “patients should not suspend their therapy or change their usual diet before testing, unless recommended by the referring physician” (Statement #8). As diet and individual drugs can change gut microbiome composition,²³ the panel recommends avoiding any drug suspension or change in the patient’s usual diet before testing, for several reasons. Altering usual diet and therapy could present a false picture of the patient’s gut microbiome. Moreover, drug suspension could be clinically contraindicated. Finally, drug adherence is required to evaluate its

effect on the microbiome. Drug suspension and dietary changes should only be initiated if required by the referring physician to address specific clinical questions (e.g., the effect of drug removal or dietary changes on gut microbiota) and under clinical supervision.

The panel also dealt with the collection, shipping and storage of samples, by three statements:

“collection of stool samples should avoid any environmental contamination and ensure genome preservation (Statement #9); “collected samples should be shipped to testing laboratories with assurance standards for microbiome sequencing within recommended timeframes and conditions described in the instructions of the collection kits. Once arrived, samples should be stored at -80°C until further processing” (Statement #10); “the analysis of the microbiome from biological samples other than from faeces, including vaginal, skin, and oral swabs, saliva and breastmilk samples, should be processed according to existing scientific evidence and clinical indications (Statement #11).

In details, stool samples should be collected through a stool catcher or any suitable stool collection kit, using devices with genome preservative media. Collection kits/devices should contain; 1) proper instructions of recommended amount of stool (minimum and maximum volumes) to be collected; 2) appropriate sample container; and 3) proper instructions for labelling, packaging, short-term storage, and waste disposal. Faecal samples should be collected at home by all subjects using tubes containing genome preservative media. The time and temperature of collection, as well as the temperature of storage, should be recorded by the patient. The Bristol Stool Chart should be used to record the consistency of stool samples.²⁴ Timeframe and conditions of transfer should be reported, and the storage temperature should be traced.²⁵ The panel also acknowledges that there is also the chance to ship faeces collected without genome preservatives within 24 hours from collection on ice or dry ice, but this solution is less straightforward for the higher risk of analysis biases due to the potential variability in the different steps of this workflow.

The panel also agreed that the analysis of microbiome from extraintestinal body sites is a promising field of research²⁶ but needs further development before being applied to clinical practice.

Processing recommendations should follow the available evidence and should concern, specifically: sampling locations and time, number of swabs, swabbing methods and shipping modalities for swab samples, while time of sampling, volume of samples, and shipping modalities, for saliva, breast milk, and other body sites.

WORKING GROUP #3: MICROBIOME ANALYSIS

In this WG the panel gathered recommendations for microbiome analysis.

First, experts agreed that “appropriate modalities for gut microbiome community profiling include amplicon sequencing and whole genome sequencing” (Statement #12), as well as that “multiplex PCR and bacterial cultures, although potentially useful, neither can be considered microbiome testing nor can be used as a proxy for microbiome profiling.” (Statement #13) Currently, both amplicon sequencing and shotgun metagenomic sequencing²⁷ are reliable options for community-based profiling of microbiomes, although with their strengths and drawbacks (detailed in Box 3). Defined positive controls (i.e., mock community, spiked-in bacteria) and negative controls (i.e., DNA extraction kit components and library preparation components with no DNA template) should accompany sequencing to minimise biases, as already attempted by the NIBSC and World Health Organisation.^{28,29} Also the assessment of non-bacterial microbiome communities may be relevant. The evaluation of the gut mycobiome may be performed through specific analyses, i.e., internal transcribed spacer (ITS) region, or 18S rRNA gene sequencing, or by WGS. The expert panel also acknowledges growing interest in virome sequencing and its potential usefulness in clinical practice,³⁰ making it an area for future development. These sequencing modalities are likely appropriate for other sample types, such as mucosal surfaces or biofluids, assuming enough DNA from the microbiome has been retrieved. Other sequencing modalities (i.e., single molecule sequencing technologies or full-length 16S rRNA gene sequencing/Nanopore sequencing) may play a future role but are too nascent to be currently recommended in clinical practice.

Conventional microbial cultures or molecular techniques i.e., multiplex PCR, are extremely useful in several clinical contexts, mainly in the identification of specific pathogens,³¹ but they are not appropriate to evaluate the composition of microbial communities, and therefore can neither be considered microbiome testing nor be used as a proxy for microbiome profiling. Further details are provided in the Appendix (page 7).³²

After defining sequencing modalities, the panel recommends that “ the pre-processing of raw sequenced data should be detailed prior to analysis (Statement #14). Key variables of amplicon sequencing should include the number of reads per sample, the reference database (with version) used, the bioinformatic analysis approach employed and any quality-control step undertaken. Pre-processing of shotgun metagenomic data include trimming and filtering reads based on their length and average sequencing quality and the removal of the host DNA as a potential contaminant.³³ Optimised approaches for standardised pre-processing have been described (e.g., KneadData – <http://huttenhower.sph.harvard.edu/kneaddata> – or OMA³⁴), and should also be briefly mentioned in the final report.

Finally, the task force handled the analyses to be performed after genome sequencing. They agreed that “The microbiome analysis should include alpha diversity metrics, including richness and evenness” (Statement #15) as well as that “beta diversity measures should be included in the microbiome analysis” (Statement #16). More in details, alpha diversity, which is an ecological measure of the complexity and variety of an ecosystem, and may associate with clinical response, should always be calculated within testing. However, further studies are needed to clarify its defined positioning into clinical practice. Further details are provided in the Appendix (page 8).^{35–46} Beta diversity, which is an ecological measure of the similarity between the composition of two (here microbial) communities, should be calculated within the testing when longitudinal samples or multiple samples from different sites are compared or when they are contextualised with other “normal” or “pathological” results. Additional evidence is advocated to identify a clear role for beta diversity measures in clinical practice. Further details are provided in the Appendix (pages 8-9).^{37–40} Additionally, the panel agreed that “a complete taxonomic profiling of gut microbial communities is an essential component of microbiome testing” (Statement #17). Taxa should be identified at all possible levels, from phylum to genus/species for amplicon sequencing and to species/strain for WGS, with their estimated relative contribution to the whole community.⁴⁷ For WGS, both marker

gene-focused sequence mapping and de novo assembly with reconstruction of metagenomic assembled genomes (MAGs) may be used.^{33,48}

Also, “appropriate comparison to a matched healthy control group should be included in microbiome testing to aid the interpretation of patient taxonomic and diversity profile” (Statement #18). Publicly available metataxonomic and metagenomic data, accessible in resources e.g. the curated MetagenomicData repository,⁴⁹ should be used to guarantee a sufficient size of the control, and potential confounding factors (e.g. biogeography, age, sex, BMI, medication intake, diet, technical confounders as preservatives, methods of DNA extractions, or read depth) should be considered. Statistical tests used to compare patient and the control group – and/or methods used to factor in potential confounders as part of statistical comparison – should also be described.

Additionally, “a longitudinal assessment of the patient microbiome at different time points may be useful in specific clinical scenarios” (Statement #19). The longitudinal evaluation of the patient microbiome can increase robustness of the measurement⁵⁰ and be useful in several clinical scenarios, i.e., to assess the effects of a therapy and/or of a diet, or to evaluate the microbiome composition after a stressful event (e.g. a gastrointestinal infection). Further details are provided in the Appendix (page 9).

Finally, the panel agreed that “metabolomic analysis of biofluids is not currently recommended in clinical practice. Inference of the patient microbiome “metabolic potential” by its taxonomic profile is presently discouraged” (Statement #20). Metabolomics is a highly valuable tool for gaining insights into host-microbiome interactions, but evidence for its use in clinical practice is too preliminary at present. Further details are provided in the Appendix (page 9).^{51–54}

WORKING GROUP #4: CHARACTERISTICS OF REPORTS

In this working group items to be included (and not to be included) in the report of the microbiome testing are described. The panel agreed that “data concerning the patient medical history should appear in the final report” (Statement #21), and that “the report should briefly detail the test protocol, including methods of stool collection and storage, DNA extraction, amplification, sequencing, and post-sequencing analyses” (Statement #22). The reporting of clinical metadata may ease the interpretation of the testing by the referring physician, as long as the patient has consented to it, and protecting her/his privacy, as already detailed in Box 2. Also the stool collection protocol, (i.e. buffers for DNA preservation, details of sample storage) should be reported, as well as the characteristics of DNA extraction, as these variables may influence the outcome of the analysis.^{55–61} The main features of sequencing methods (e.g., amplicon-based methods vs WGS), if applicable amplicon region, and the depth of sequencing should be provided (expressed as gigabytes or megabytes of DNA), as they provide different taxonomical and functional findings.⁶²

Moreover, details of sequencing machines, as well as softwares, libraries and pipelines used for computational analysis, with software versions stated as well as the identity and version of the taxonomic reference database used, should be given. For WGS, the use of marker gene-focused sequence mapping or of a de novo assembly approach should be reported.

Concerning microbiome characteristics, the panel agreed that “alpha and beta diversity measures assessed in the testing phase should be included in the final report” (Statement #23), as they are a potentially valuable information for clinicians, and that “microbiome composition should be described with the deepest possible taxonomic resolution” (Statement #24). The report should describe the composition of the patient’s microbiome at the deepest possible taxonomic resolution according to different techniques, specifically genus/species level for 16S rRNA gene sequencing data⁶³ and species level for shotgun sequencing data.³³ More details are provided in the Appendix (page 10).^{33,63,47} Moreover, regardless of the approach used, the reported taxonomic profile should

provide at least a degree of reference to the percentage of sequencing data that could not be assigned to a particular taxonomy.

The panel also recommended that “the report should include all taxa that shift significantly from healthy matched controls as well as known microbial pathogens. Also, the report of specific health-relevant taxa and clusters, regardless of their abundance, may be of interest, despite the limited evidence for a causal connection with human diseases” (Statement #25). To ease the interpretation of the testing, and to provide complete landscape of the patient microbiome, all taxa that diverge significantly from matched health ranges tailored to the patient population should be reported. Also the presence of known pathogens (i.e. *Clostridioides difficile*, *Salmonella* spp, *Shigella* spp, pathogenic *E. coli* strains, etc.) should be reported.

Finally, although the evidence for a causal connection between the abundance of specific microbes and human diseases is still limited, the report of other health-relevant taxa and clusters (i.e. at least *Akkermansia*, *Bifidobacteria*, Enterobacteriaceae, Fusobacteria, *Lactobacilli*, short-chain fatty acid-producers, and others), regardless of their abundance, may help the clinical management of patients. Then, the panel dealt with items not to be included in the text. They agreed that “the reporting of Firmicutes-to-Bacteroidetes ratio in the microbiome testing is discouraged” (Statement #26) and that “there is insufficient evidence to include any dysbiosis index in the report of microbiome testing, but these metrics deserves further research” (Statement #27). Current evidence suggests that phylum-level descriptors are insufficient to capture the whole spectrum of variation in the gut microbiota and can give deceiving results – e.g., a high relative Bacteroides abundance can both mean a healthy Bacteroides-high community, an altered ecosystem as well as a Prevotella-dominant ecosystem.⁶⁴ Moreover, although several indices have been proposed to identify dysbiosis,^{65,66} a common definition of dysbiosis is not available, therefore cannot be used in clinical practice, and deserves future research.

Additionally, the panel approved that “generally, there is not enough information to report strict healthy reference ranges of species relative abundance” (Statement #28). In contrast to other typically reported health biomarkers, sequence-based quantifications of microbial taxa are relative.⁶⁷ To avoid relative abundances being wrongly interpreted as absolute numbers, reporting them as percentages is recommended. Statistics on the magnitude of the change together with the direction for each taxon displaying significant differences should be reported, as recommended in the reporting guidelines for human microbiome research data.⁶⁸ We currently lack sufficient knowledge to report strict healthy reference ranges for the relative abundances of bacterial taxa.

Focusing on the presentation of the report, the task force proposed that “the use of a user-friendly infographic, i.e., barplots or boxplots displaying the relative abundances of key taxa, is recommended to make the report easily interpretable, while simple ordinations of taxa should be avoided” (Statement #29).

Importantly, the panel discouraged the reporting of any post-testing therapeutic advice by the testing provider (Statement #30). Post-testing therapeutic advices on how to modulate the patient microbiota based on the testing results may be tempting, due to the limited knowledge of average clinicians on gut microbiota and its modulation.⁶⁹ However, as previously stated, the panel firmly believes that the therapeutic management of these patients is a complex process that cannot rely on a single test and must be charged to the referring physician (who has requested the testing). Therefore, the reporting of post-testing therapeutic advice is strongly discouraged.

Finally, the panel agreed that “raw data may be provided to the patient upon request (e.g., for a second-opinion analysis) in form of amplicon or metagenomic reads (based on the sequencing method)” (Statement #31). The request for a second opinion is a common strategy in medicine, particularly among pathologists and radiologists, and in the management of specific disorders such as cancers.⁷⁰ This approach has shown to be effective in improving rates of correct diagnoses⁷¹ and to reduce the number of unnecessary diagnostic exams,⁷² with relevant consequences for healthcare systems. Laboratory-related second opinions, as well as interactions between clinical laboratories

and practicing physicians have been advocated for decades.⁷³ As post-sequencing analyses require complex skills,³² in some situations (e.g., need for information on specific taxa), a further analysis of reads from computational biologists or microbiologists may be required by the physician who manages the patient. This approach could be more convenient than repeating the microbiome analysis at a later time, due to the variability of the gut microbiome.⁷⁴ The sharing of microbial genome data implies specific ethical aspects,^{75,76} therefore the panel recommends that, in case of a second-opinion for post-sequencing microbiome analysis, the patient should sign a written informed consent, and data should be anonymised.

WORKING GROUP #5: RELEVANCE OF MICROBIOME TESTING IN CLINICAL PRACTICE: PRESENT AND FUTURE

Here the expert panel addressed the current relevance of microbiome testing in clinical practice and the future strategies that are needed to build evidence for their application in clinical practice and to expand their use within the boundaries of science.

As anticipated before, the panel suggested that “at the present time, there is insufficient evidence to widely recommend the routine use of microbiome testing in clinical practice, which should be supported by dedicated studies”(Statement #32). The key role played by gut microbiome in influencing human health and disease is supported by a growing body of evidence and increasingly accepted by the scientific community. Moreover, several modulators of gut microbiome are commonly used in clinical practice. Rifaximin is recommended to treat hepatic encephalopathy⁷⁷ and irritable bowel syndrome (IBS) without constipation.⁷⁸ International guidelines recommend probiotics for infectious and/or antibiotic-associated diarrhoea,⁷⁹ as coadjuvants of *H. pylori* eradication regimens,⁸⁰ in the management of ulcerative colitis,⁸¹ and for other disorders. More recently, FMT has rapidly become an established treatment option for recurrent CDI. These therapeutic approaches have been recommended for their target disorders after being shown to be clinically effective.⁸²⁻⁸⁵ Recently, the introduction of microbiological endpoints, beyond clinical outcomes, in clinical trials of therapeutic microbiome modulators has been advocated.⁸⁶

To date, however, there is still no consolidated and direct evidence that microbiome-based diagnostics benefit the clinical management of gastrointestinal or extraintestinal disorders, neither via an increase of clinical efficacy nor in a reduction of side effects.

The task force also stated that “qualitative or quantitative data retrievable from microbiome reports may be helpful in clinical practice, although there is still insufficient evidence to apply them in clinical practice” (Statement #33). Based on current evidence, several parameters described in microbiome reports may be useful in driving the management of different disorders associated with gut microbiome imbalance, at several levels, as detailed in the Appendix (pages 10-11).^{43,44,87-90}

Finally, experts agreed that “studies aimed at evaluating the value of microbiome profiling in different disorders are needed to enable testing to enter clinical practice” (Statement #34) and that “disclosure of the potential benefits and pitfalls of microbiome testing, as well as training on the basics of microbiome science and on the interpretation of microbiome reports, are advocated to foster and disseminate their use in clinical practice” (Statement #35). Large and well-sized observational studies, that preferably follow the STARD guidelines for diagnostic accuracy studies,⁹¹ are needed to generate direct evidence of the potential usefulness of microbiome-based diagnostics in clinical practice, i.e., to confirm if a microbiome test can be a reliable tool to make an early diagnosis of disorders or to reliably predict the response to therapeutic interventions by the identification of clear and reproducible signatures. Moreover, interventional studies, preferably with a randomised design, should compare the effectiveness of a targeted modulation of gut microbiome (according to the results of microbiome testing) over the standard one-size-fits-all approaches, with probiotics or other microbiome modulators. The training and education of the medical community is another essential milestone for the introduction of microbiome testing in clinical practice. Although this topic is of wide interest to physicians, most do not have the knowledge base presently required to interpret and exploit a microbiome report.

Therefore, beyond accumulating data aimed at consolidating the evidence for the use of a microbiome test in clinical practice, several short-term initiatives (e.g., dissemination courses) and long-term actions (e.g., the introduction of microbiome research into the official educational programs of medical schools) are advocated to disseminate greater understanding of the microbiome in disease and potential utility of testing, as well as to allow a wide range of physicians to understand a microbiome testing report.

CONCLUSIONS

Our initiative aimed to establish ethical, organisational and technical rules for the development, commercial use and clinical implementation of microbiome testing, as advocated by several voices in the scientific community.^{92–94}

Figure 1 summarises the recommended framework and characteristics of microbiome testing in clinical practice.

Our initiative represents consensus from a multidisciplinary and international consortium of experts in human microbiome research. We acknowledge that low- and middle-income Countries (LMICs) are not represented in this group, and that this may represent a potential limitation in broadly implementing the recommendations. However, the progressive decrease in costs related to the microbiome sequencing, along with the increasing dissemination of microbiome knowledge, are likely to help overcome this glitch.

Statements were presented as expert opinions, and a GRADE (Grading of Recommendations Assessment, Development and Evaluation) approach, aimed at evaluating the quality of evidence and the strength of recommendations, could not be applied because of their intrinsically conceptual or technical content. We acknowledge this is another potential limitation for the concrete applicability of our statements, but we are also aware of the pioneering nature of our initiative.

We are also aware that the practical application of our recommendations by regulatory agencies, clinicians, and patients, represents a further challenge in this area, and will deserve additional efforts beyond this initiative. Interestingly, the provision of DTC genetic health risk testing, which encompass similar issues to the microbiome testing, has been regulated by the USA Food and Drug Administration (FDA) some years ago. The FDA allowed the marketing of those testing only at certain conditions, which are similar to our recommendations. i.e. by defining criteria to assure the tests' accuracy, reliability and clinical relevance, by recommending a clear and understandable communication of results as well as the consultation with a health care professional concerning the test results. Moreover, the FDA has also distinguished clearly genetic tests used as diagnostic tests,

that are needed for major clinical decisions (e.g. the BRCA assessment, which positivity may lead to a pre-emptive removal of ovaries or breast) from those that provide information on an overall genetic health risk.⁹⁵ We expect that similar regulatory interventions will be applied also to microbiome diagnostics, if supported by pertinent evidence.

The expert panel identified clear criteria and standards to adhere to when providing microbiome testing, pointing out that there is still limited evidence for using such diagnostics in clinical practice. Moreover, we devised recommendations on different steps of the testing process, from the retrieval of clinical metadata to the collection and shipping of faecal samples, the modalities of analysis and the characteristics of the report. To avoid patients going outside the boundaries of evidence-based clinical medicine, we discouraged the suggestion of therapies within the report (which is a common feature of currently available testing).

We recognise that, due both to the advancement of technologies and the increase in pertinent evidence, our current recommendations may become outdated in the upcoming future, but we are also confident that our guidance framework will remain reliable over time.

Here our initiative was focused on standardising procedures for the release of microbiome testing in clinical practice. However, we are also clearly aware that currently there is no direct evidence that the use of such diagnostics may improve the management of patients in any human disease. Then, we recognise that our effort could have limited utility if further studies do not evaluate the value of microbiome testing in human disorders, although preliminary data, coming mostly, but not only, from the cancer field, support this hypothesis,⁹⁶⁻⁹⁸ and the use of microbiome testing has also been recently advocated by international guidelines.⁹⁹ Moreover, a similar development pathway has already been experienced in the field of genetic testing for cancer, i.e. BRCA1, which are now widely used in medical practice for clinical decision-making.¹⁰⁰ Importantly, the consolidation of such evidence is needed to make the microbiome testing move from being non-specific health tests (like the DTC genetic health risk tests discussed above) to becoming diagnostic tests applicable in clinical medicine (as observed e.g. in human cancer genomics).

Therefore, another critical long-term objective of our project was to boost and guide future research on the application of human microbiome diagnostics in clinical practice. We discussed the current challenges that prevent the application of microbiome testing in clinical practice and highlighted the need for both specifically designed studies and educational pathways to advance this field.

Thus, this working group also aims to promote a gradual mindset shift of clinicians towards the importance of the gut microbiome. The strengthening of evidence for microbiome diagnostics⁹⁶⁻⁹⁸ and the recent bloom of advanced microbiome therapeutics¹⁰¹ should, therefore, be paired with concomitant educational efforts, with the definition of formal training pathways to build a dedicated functional class of “microbiome clinicians”, with expertise in microbiome assessment and modulation.

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SEARCH STRATEGY AND SELECTION CRITERIA

We have searched PubMed, without date limits, using the following terms: microbiota, microbiome, amplicon, whole genome sequencing, microbial ecology, diversity, taxonomy, profiling.

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AUTHOR CONTRIBUTIONS

GI conceived and designed the project. GC, NS, AG, and GI, identified the members of the steering committee. SN, GH, MS, HS, NS, GC, AG, and GI, selected the expert panel and established the main topics. SCN, LZ, GH, LP, BHM, OK, JM, GC, AG, and GI, coordinated the working groups. All panel members developed the statements (each member only developed statements pertinent to her/his working group). SP and GI coordinated the Delphi process. All panel members participated to the Delphi process. SP, BHM, FA, SN, LZ, GH, LP, JM, GC, AG, and GI, wrote the initial draft of the manuscript. SP, BHM, FA, and GI, drafted tables, figures and boxes. All panel members revised the manuscript for important intellectual content and approved the final manuscript.

COMPETING INTERESTS

J.A. received research support from Pfizer, Janssen and Merck and has been speaker for BMS, Abbvie, Janssen and reported consultancy with Janssen, Pfizer, Abbvie, Seres Therapeutics, Ferring, GSK, Merck, Bristol Myer Squibb Roivant, and Adiso. J.B. institution gets grants from Bausch, Grifols, Mallinckrodt, Cosmo, and Sequana and he received personal fees for acting as consultant for Merz and Novo Nordisk. P.D.C was co-founder of The Akkermansia Company and Enterosys. W.M.DV. was co-founder and shareholder of The Akkermansia Company (Belgium), Caelus Pharmaceuticals (The Netherlands) and Alba Health (Copenhagen-Stockholm). E.E. is a scientific cofounder of DayTwo and BiomX and is an advisor to Purposebio, Aposense, Zoe, and MyGutly. F.G. has received personal fees for acting as speaker and consultant from Biocodex, Danone, BioGaia, Menarini and Sanofi. C.L.H. received lecture honoraria from Baxter, Jansse, BMS and Tillotts. S.K. received research support from Rebioitx / Ferring, Vedanta, Finch, Seres and Pfizer and served as consultant for ProbioTech, Takeda and Rise. O.K. is a co-founder of Shela Accurate Diagnosis LTD

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FIGURES, BOXES AND TABLES

Figure 1. Suggested framework and characteristics of microbiome testing in clinical practice.

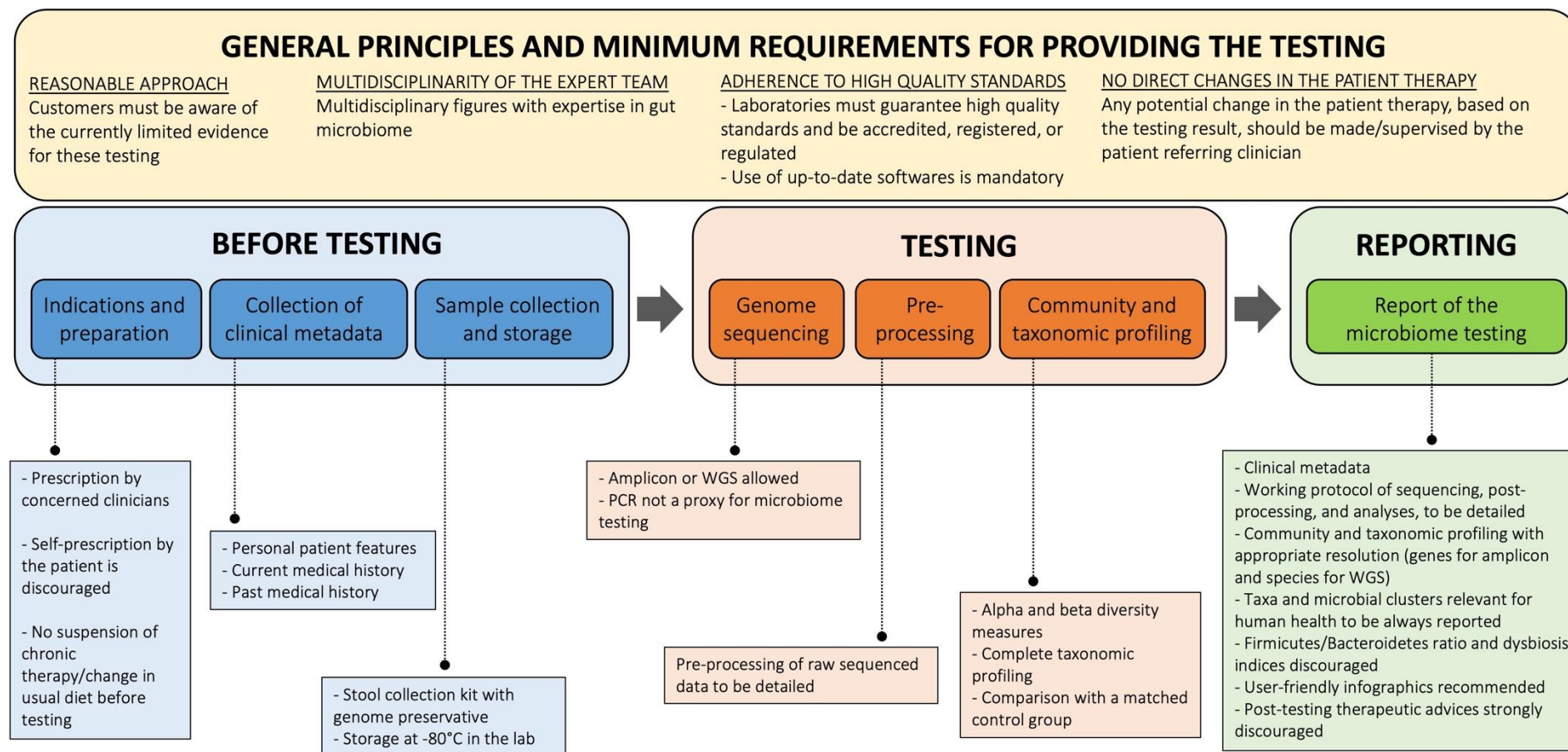


Table 1. Summary of statements

Statement	Agreement	Text
WG1 - GENERAL PRINCIPLES AND MINIMUM REQUIREMENTS FOR PROVIDING MICROBIOME TESTING		
Statement 1	100%	Providers of microbiome testing should communicate a reasonable, reliable, transparent, and scientific representation of the test, making customers clearly aware of the currently limited evidence for its applicability in clinical practice.
Statement 2	96%	The provision of a microbiome test involves a complex framework, from the collection of biological samples to the sequencing of the microbial genome and computational analyses, to the release of an interpretable report. Therefore, providers of microbiome testing should include experts with multidisciplinary competences.
Statement 3	100%	Any change in the clinical management of the patients based on microbiome testing should be made only by their referring physicians/healthcare professionals.
Statement 4	100%	Laboratories that provide microbiome testing should guarantee high quality standards, as well as protection of patient data, and be accredited/registered/regulated.
Statement 5	96%	Validated and up-to-date computational software pipelines and databases aimed at delineating microbial taxonomy are required to provide microbiome testing.
WG2 - PROCEDURAL STEPS BEFORE TESTING		
Statement 6	80•4%	As there is currently limited evidence for the applicability of gut microbiome testing in clinical practice, the direct request by patients for microbiome testing without a clinical recommendation is discouraged.
Statement 7	87%	Before testing, key clinical data of the patient, including those that may influence gut microbiome characteristics, should be collected. Essential information to be captured should include at least age, gender, body mass index, dietary habits, smoking and alcohol status, gut transit time, current comorbidities and medications, and past medical history.
Statement 8	100%	Patients should not suspend their therapy or change their usual diet before testing, unless recommended by the referring physician.
Statement 9	98%	Collection of stool samples should avoid any environmental contamination and ensure genome preservation.
Statement 10	97•5%	Collected samples should be shipped to testing laboratories with assurance standards for microbiome sequencing within recommended timeframes and conditions described in the instructions of the collection kits. Once arrived, samples should be stored at -80°C until further processing.
Statement 11	97•5%	The analysis of the microbiome from biological samples other than from faeces, including vaginal, skin, and oral swabs, saliva and breastmilk samples, should be processed according to existing scientific evidence and clinical indications.
WG3 – MICROBIOME ANALYSIS		
Statement 12	98%	Appropriate modalities for gut microbiome community profiling include amplicon sequencing and whole genome sequencing.
Statement 13	90%	Multiplex PCR and bacterial cultures, although potentially useful, neither can be considered microbiome testing nor can be used as a proxy for microbiome profiling.
Statement 14	100%	The pre-processing of raw sequenced data should be detailed prior to analysis.
Statement 15	92%	The microbiome analysis should include alpha diversity metrics, including richness and evenness.
Statement 16	92%	Beta diversity measures should be included in the microbiome analysis.

Statement 17	98%	A complete taxonomic profiling of gut microbial communities is an essential component of microbiome testing.
Statement 18	88%	Appropriate comparison to a matched healthy control group should be included in microbiome testing to aid the interpretation of patient taxonomic and diversity profile.
Statement 19	80%	A longitudinal assessment of the patient microbiome at different time points may be useful in specific clinical scenarios.
Statement 20	90%	Metabolomic analysis of biofluids is not currently recommended in clinical practice. Inference of the patient microbiome “metabolic potential” by its taxonomic profile is presently discouraged.
WG4 - CHARACTERISTICS OF REPORTS		
Statement 21	94%	Data concerning the patient medical history should appear in the final report.
Statement 22	94%	The report should briefly detail the test protocol, including methods of stool collection and storage, DNA extraction, amplification, sequencing, and post-sequencing analyses.
Statement 23	90%	Alpha and beta diversity measures assessed in the testing phase should be included in the final report.
Statement 24	96%	Microbiome composition should be described with the deepest possible taxonomic resolution
Statement 25	80•5%	The report should include all taxa that shift significantly from healthy matched controls as well as known microbial pathogens. Also, the report of specific health-relevant taxa and clusters, regardless of their abundance, may be of interest, despite the limited evidence for a causal connection with human diseases.
Statement 26	86%	The reporting of Firmicutes-to-Bacteroidetes ratio in the microbiome testing is discouraged.
Statement 27	90%	There is insufficient evidence to include any dysbiosis index in the report of microbiome testing, but these metrics deserves further research.
Statement 28	90%	Generally, there is not enough information to report strict healthy reference ranges of species relative abundance
Statement 29	92%	The use of a user-friendly infographic, i.e., barplots or boxplots displaying the relative abundances of key taxa, is recommended to make the report easily interpretable, while simple ordinations of taxa should be avoided.
Statement 30	98%	The panel discourages the reporting of any post-testing therapeutic advice by the testing provider.
Statement 31	87•8%	Raw data may be provided to the patient upon request (e.g., for a second-opinion analysis) in form of amplicon or metagenomic reads (based on the sequencing method)
WG5 - RELEVANCE OF MICROBIOME TESTING IN CLINICAL PRACTICE: PRESENT AND FUTURE		
Statement 32	90%	At the present time, there is insufficient evidence to widely recommend the routine use of microbiome testing in clinical practice, which should be supported by dedicated studies.
Statement 33	92%	Qualitative or quantitative data retrievable from microbiome reports may be helpful in the management of several disorders, although there is still insufficient evidence to apply them in clinical practice.
Statement 34	94%	Studies aimed at evaluating the value of microbiome profiling in different disorders are needed to enable testing to enter clinical practice.
Statement 35	96%	Disclosure of the potential benefits and pitfalls of microbiome testing, as well as training on the basics of microbiome science and on the interpretation of microbiome reports, are advocated to foster and disseminate their use in clinical practice.

Table 2. Essential data to be collected prior to the microbiome testing.

KEY FIELD	DATA
Personal patient features	Age Gender BMI Smoking status Alcohol consumption Dietary habits* Gut transit time**
Current medical history	Current comorbidities Current medications***
Past medical history	Previous diseases Previous relevant surgical interventions Previous drugs (at least within three months of testing)

BMI= Body Mass Index.

*The expert panel acknowledges that a dedicated dietary questionnaire to address gut microbiome composition has not been validated yet, and that this task could be currently challenging.

**Gut transit time, a key factor that can influence gut microbiome,²⁰ is usually assessed by complex assays, but may be inferred even through simple proxies, including stool frequency or stool consistency (e.g. Bristol Stool Scale). Moreover, other proxies of gut transit are currently under investigation.²¹

***While the effect of certain drugs on gut microbiome is well defined,^{22,23} the list of medications associated with microbiome changes is wide and will likely continue expanding to a broader range, therefore all medications, including prebiotics, probiotics, symbiotics and food supplements, deserves to be recorded.

Box 1. Key issues of the consensus statement

KEY ISSUE	Description
#1 - General principles and minimum requirements for providing diagnostic microbiome testing	In this key issue we outline the general principles and requirements that commercial providers should comply with for providing microbiome testing, including the acknowledgement that current evidence for their wide application in clinical practice is limited
#2 - Procedural steps before testing	In this key issue we discuss the procedural steps to be followed before testing, including the indications, the collection of samples and clinical metadata, and shipping of samples
#3 – Microbiome analysis	In this key issue we give recommendations on how to perform the analyses of gut microbiome for the testing
#4 – Characteristics of reports	In this key issue we recommend items to be included (and not to be included) in the report of the microbiome testing
#5 - Relevance of microbiome testing in clinical practice: present and future	In this key issue we address the current relevance of microbiome testing in clinical practice and the future strategies that are needed to build evidence for their application in clinical practice and to expand their use within the boundaries of science

Box 2. Use and protection of data generated by microbiome testing

There are several legal implications regarding the personal data related to microbiome testing, focused on protection of the patient undergoing the testing. Some of these legal principles are closely comparable to microbiome testing as they are to other more established forms of medical testing (including regarding informed consent, anonymisation of data where it is stored, etc). However, the lack of defined regulatory standards/ authorities regarding this testing brings some additional issues, including: the legal framework regarding personal data use within the country where testing is occurring; the potential for generated data to be used beyond provision of a microbiome report for the patient (e.g. selling on of data to commercial entities); and the possibility that different aspects of an overall microbiome test may be performed in different laboratories, each with their own policies related to personal data management.

All management of personal data related to microbiome testing should be handled within the legal framework of the territory in which it is collected and/or being undertaken; this would include the GDPR where testing is occurring in UK/Europe, and that of the state, province or other entity when testing takes place in North America. Health data is considered a “special category” of data under GDPR (<https://gdpr-info.eu/recitals/no-35/>) which affords to it a greater level of protection than other basic personal data such as contact details. This reflects increased enforcement, publicity and fines from the data protection regulators for incorrect use, sharing or loss. The relevant laws require particular consideration in the circumstance where personal data is being transferred between countries/ legal entities.

Patients who are undergoing testing should be informed of the provider’s policies related to use of their personal data within an information sheet/ discussion with an informed member of the testing provision team prior to consent, with these issues revisited at the time of consent. The informed consent process should again make clear to the patient how their data will be handled and used; this will be of particular pertinence should this potentially be for indications that the patient may not reasonably expect, including selling on of data to commercial entities, potential data mining in

future research studies, training of machine learning models in any context, etc. As with provision of any comparable medical test, consent must be freely given, and individuals must be able to withdraw that consent, at any time; if consent is withdrawn, the provider (and third parties) may need to cease use of that data.

Providers could be required to undertake a ‘data protection impact assessment’ prior to collecting microbiome data, especially if this involves a large number of people. Patients have rights under GDPR, including the right to ask for a copy of their personal data which the provider holds/shares (a ‘subject access request’), and the right to ask for their data to be deleted.

Comparable to what would be expected for data from other medical testing, providers of microbiome testing should, wherever possible, anonymise data, and should ensure appropriate retention periods are in place for storing that data so that it is not retained for longer than necessary. Given well-documented recent cyberattacks focused around gaining illegal access to health data,¹⁹ providers of microbiome testing are expected to employ robust safety and technical protocols related to mitigating ransomware attacks and other unwarranted access to their stored data.

Certain providers of microbiome testing may subcontract certain aspects (e.g. particular elements of laboratory testing) to a third party; in this case, there is a need for a contract between the main provider and third party to define processes of transferring data between them, for how long, and for what means that the third party may be retaining any of the generated data. Providers may be responsible for any breach by their subcontractors, including being subject to penalties from regulators for the subcontractor’s misuse. This is regardless of the terms of the contract with the third party.

Box 3. Advantages and drawbacks of amplicon sequencing vs whole genome sequencing

	Amplicon Sequencing (e.g., 16S rRNA)	Whole genome sequencing
Sample requirements	Lower amount of biologic sample required	Higher amount of biologic sample required
Risk of contamination by host DNA	Hardly affected by host DNA	Can be affected by host DNA (in particular for low-biomass/highly host contaminated sample types)
Target of sequencing	Specific gene (e.g., 16S rRNA) or portion (e.g., specific 16S rRNA variable region)	Whole DNA content of the sample
Sequencing Costs	Lower cost per sample	Higher cost per sample
Taxonomic Resolution	Up to the genus taxonomic level	Strain-level resolution
Functional Analysis	Not available	Identifies genes and functions of microbial communities