International consensus statement on microbiome testing in clinical practice

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There is growing interest in the potential exploitation of the gut microbiome as a diagnostic tool in medicine, but evidence supporting its clinical usefulness is scarce. An increasing number of commercial providers offer direct-to-consumer microbiome diagnostic tests without any consensus on their regulation or any proven value in clinical practice, which could result in considerable waste of individual and health-care resources and 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, method of analyses, reporting of results, and potential clinical value. We also evaluated 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 and pave the way for the evidence-based development and use of human microbiome diagnostics in clinical medicine.

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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] Increasing evidence has shown, initially via association studies but also through mechanistic lines of research, that imbalance of the gut microbiome is associated with a broad range of intestinal and extraintestinal disorders[5] and response to treatments.[6–8]

Manipulation of the gut microbiome, eg, 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 and has shown promise for a range of other indications.[9]

There is also growing interest in the potential exploitation of the gut microbiome as a tool in clinical practice for several applications, 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 (eg, probiotics or FMT); and the monitoring of the efficacy of such therapies.[10,11]

Despite this enthusiasm, the application of gut microbiome research in clinical practice remains minimal because of a number of 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 most clinicians have about this field, the absence of any validated test to enable therapeutic follow-up, and the absence of established regulations and framework for the clinical translation of this research.

By contrast, patient groups increasingly expect the rapid introduction of microbiome-based diagnostics and therapeutics to routine care. Because of this disparity, direct-to-consumer microbiome testing (which often claims to drive the clinical management of patients with dysbiosis-associated diseases) has proliferated worldwide. These tests are primarily based on amplicon sequencing or whole-genome sequencing[14] but can also use other technologies (eg, conventional PCR or culture). This trend raises several concerns about the absence of a standardised framework relating to the indications and methods of these tests, which limits their interpretability and applicability, with considerable waste of patient and health-care system resources, (eg, due to inappropriate requests for medical exams or inappropriate subsequent prescribing of supplements and medications). Moreover, these tests can generate false hopes in patients who are often living with severe disorders, with potentially detrimental consequences. Finally, due to the absence of a formal postgraduate clinical education in microbiome science, most physicians and other health-care professionals are not adequately trained to interpret a microbiome test and therapeutically manipulate the gut microbiome or 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,

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Panel 1: Key issues of the consensus statement

1. General principles and minimum requirements for providing diagnostic microbiome testing
We outline the general principles and requirements with which commercial providers should comply for providing microbiome testing, including the acknowledgment that current evidence for their wide application in clinical practice is scarce.

2. Procedural steps before testing
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
We give recommendations on how to do the analyses of gut microbiome.

4. Characteristics of reports
We recommend items to be included (and excluded) in the microbiome testing report.

5. Relevance of microbiome testing in clinical practice: present and future
We address the relevance of microbiome testing in clinical practice and the future strategies needed to build evidence for their application in clinical practice and to expand their use within the boundaries of science.

evaluating 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

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, and completion of the final report. This 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, and SCN) 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, and 5) relevance of microbiome testing in current and future clinical practice (panel 1).

These key issues were reviewed and approved by the whole expert panel, and five working groups, one for each key issue, were built by the steering committee, that assigned each expert to a specific working group based on their expertise. Each working group included 13 or 14 experts, without any overlap. Further details on the membership of each working group are described in the appendix (p 1). Members of each working group nominated two coordinators to chair activities and to liaise with the steering committee. For each key issue, the steering committee developed relevant sub-issues or questions, which experts of the corresponding working group were requested to address by the release of pertinent statements. As the topic of microbiome testing is relatively new and rapidly evolving, statements were released as expert opinions, although they were built according to the best available evidence.

Statements and narrative comments from each working group were edited by the respective coordinators and then uploaded, together with supporting references, to an online electronic voting system accessible to the expert panel.

The whole expert panel was requested to evaluate the statements released by the working groups. The Delphi method was used to achieve a consensus.[18] For each statement, experts were asked to rate their agreement anonymously, according to a 5 point Likert scale (1=agree strongly, 2=agree with reservation, 3=undecided, 4=disagree, and 5=disagree strongly). If rating differed 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 at least 80% of experts agreeing 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 consensus response.

Two rounds of electronic voting were needed to reach consensus. The outcomes of the whole Delphi process, including the rate of agreement 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 (pp 2–6). Finally, the whole expert panel approved the final version of released statements (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

	Agreement	Text
Working group 1: 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 scarce 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 or health-care professionals
Statement 4	100%	Laboratories that provide microbiome testing should guarantee high quality standards and protection of patient data, and be accredited, registered, and 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
Working group 2: procedural steps before testing		
Statement 6	80·4%	As there is little 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 that which might influence gut microbiome characteristics, should be collected; essential information to be captured should at least include age, gender, BMI, dietary habits, smoking and alcohol status, gut transit time, 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
Working group 3: microbiome analysis		
Statement 12	98%	Appropriate methods 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 before 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 timepoints might be useful in specific clinical scenarios
Statement 20	90%	Metabolomic analysis of biofluids is not recommended in clinical practice. Inference of the patient microbiome “metabolic potential” by its taxonomic profile is discouraged
Working group 4: 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 and known microbial pathogens. The report of specific health-relevant taxa and clusters, regardless of their abundance, might be of interest, despite the scarce 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 warrant 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—eg, 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 can be provided to the patient upon request (eg, for a second-opinion analysis) in form of amplicon or metagenomic reads (based on the sequencing method)

(Table 1 continues on next page)

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description of approved statements. The figure summarises the resulting recommended framework and characteristics of microbiome testing in clinical practice.

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 might also participate in research protocols under strict investigative conditions, with the final aim of generating evidence for this emerging 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

	Agreement	Text
(Continued from previous page)		
Working group 5: relevance of microbiome testing in clinical practice: present and future		
Statement 32	90%	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 might 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, and 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 1: Summary of statements

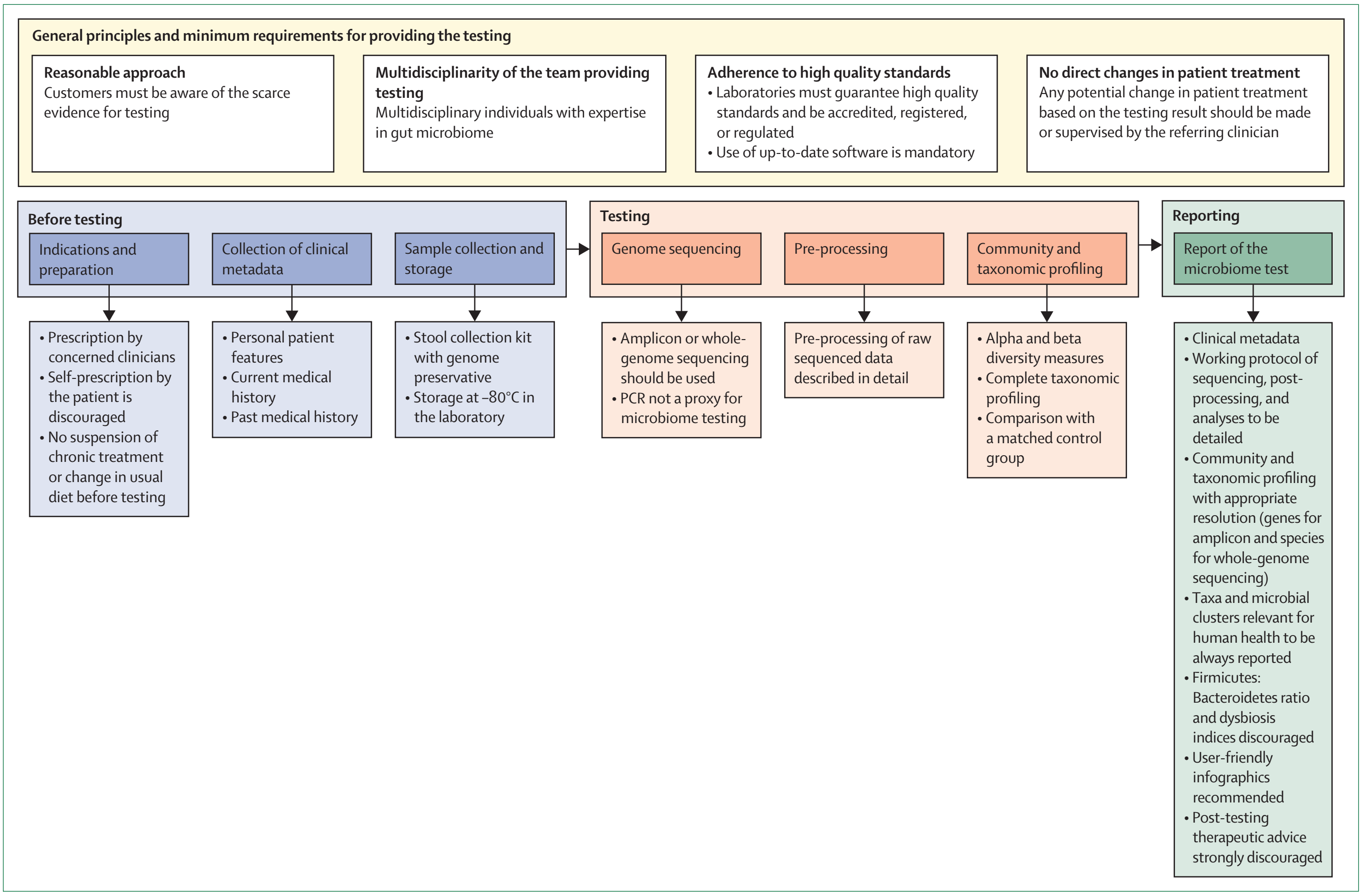

Figure: Suggested framework and characteristics of microbiome testing in clinical practice release of an interpretable report. Therefore, providers of microbiome testing should include experts with multidisciplinary competences (statement 2).

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

The working group agreed that any change in the clinical management of the patients based on microbiome testing should be made only by their referring physicians or 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 or health-care 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 agreed by the working group was that laboratories that provide microbiome testing should guarantee high quality standards, as well as protection of patient data, and be accredited, registered, and regulated (statement 4). This regulation should be provided at a national level. Microbiome testing providers should also guarantee the protection of the patient data reported in the testing, as discussed in panel 2 (appendix p 7).[19]

Panel 2: Use and protection of data generated by microbiome testing

There are several legal implications regarding personal data related to microbiome testing, focused on protection of the patient undergoing the testing. Some of these legal principles are as similar to those for other more established forms of medical testing (informed consent, anonymisation of stored data). However, the absence of defined regulatory standards or authorities for microbiome 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 (eg, selling of data to commercial entities), and the possibility that different aspects of an overall microbiome test might be done 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 or being done; this would include the General Data Protection Regulation (GDPR) in the UK or EU 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, 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 when personal data is being transferred between countries or 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 or discussion with an informed member of the testing provision team before 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 for indications that the patient might not reasonably expect, including selling on of data to commercial entities, potential data mining in future research studies, or training of machine learning models in any context. As with provision of any similar 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) might need to cease use of that data.

Providers could be required to undertake a data protection impact assessment before 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 or shares (a subject access request), and the right to ask for their data to be deleted.

Similar to what would be expected for data from other medical testing, providers of microbiome testing should anonymise data wherever possible and should ensure appropriate retention periods are in place for ensuring that data are not retained for longer than necessary.

Given well-documented cyberattacks focused around gaining access to health data,[19] providers of microbiome testing are expected to use robust safety and technical protocols related to mitigating ransomware attacks and other unwarranted access to their stored data.

Providers of microbiome testing might subcontract some aspects (eg, particular elements of laboratory testing) to a third party; in this case, a contract is needed 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 might retain any of the generated data. Providers might 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.

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	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 (within 3 months of testing)

*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 challenging. †Gut transit time, a key factor that can influence gut microbiome,[20] is usually assessed by complex assays, but can be inferred even through simple proxies, including stool frequency or stool consistency (eg, Bristol stool scale). Moreover, other proxies of gut transit are under investigation.[21] ‡Although 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 probably continue to expand, therefore all medications should be recorded, including prebiotics, probiotics, symbiotics, and food supplements.

Table 2: **Essential data to be collected before microbiome testing**

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Working group 1's final statement was that 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 (p 7). All the steps should include a panel of checkpoints or quality controls for 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

Regarding workflows to be followed before testing, working group 2's first statement was that, 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 health-care professionals (eg, dietitians). Moreover, non-licensed professional figures, such as personal trainers, coaches, homeopaths, and 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, BMI, 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 thereby influence the interpretation of the test results. For example, diet can be a major modifier of the gut microbiome, so the patient's food habits should be recorded. The effect of these variables on gut microbiome is often complex, with marked inter-individual variability, making them hard to interpret directly at the individual level. However, future accumulation of pertinent evidence might 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. 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 challenging.[20–23]

The panel recommended 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 recommended 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, suspending a drug 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 (eg, 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. First, collection of stool samples should avoid any environmental contamination and ensure genome preservation" (statement 9). Second, 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). Lastly, 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).

Stool samples should be collected through a stool catcher or any suitable stool collection kit, using devices with genome preservative media. Collection kits or devices should contain proper instructions for the recommended amount of stool (minimum and maximum volumes) to be collected; an appropriate sample container; and proper instructions for labelling, packaging, short-term storage, and waste disposal. Faecal samples should be collected at home by all participants, using tubes containing genome preservative media. The time and temperature of collection and 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] The timeframe and conditions of transfer from the patient to the laboratory should be reported, and the storage temperature at the laboratory once the samples have arrived should be traced.[25] The panel also acknowledged that there is the chance to ship faeces collected without genome preservatives within 24 h from collection on ice or dry ice, but this solution is less straightforward and conveys a greater risk of analysis biases due to the potential variability in the different steps.

The panel also agreed that the analysis of microbiome from extraintestinal body sites is a promising field of research[26] but needs further development before being applied to clinical practice. Processing recommendations should follow the available evidence and should concern sampling locations and time, number of swabs, swabbing methods and shipping method for swab samples, while for saliva and breastmilk samples the recommendations should cover time of sampling, volume of samples, and shipping modalities.

Working group 3: microbiome analysis

Members of working group 3, focused on recommendations for microbiome analysis, agreed that appropriate modalities for gut microbiome community profiling include amplicon sequencing and whole genome sequencing (statement 12) and 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 sequencing[27] are reliable options for community-based profiling of microbiomes, albeit with strengths and drawbacks (table 3). Defined positive controls (eg, mock community or spiked-in bacteria) and negative controls (eg, DNA extraction kit components and library preparation components with no DNA template) should accompany sequencing to minimise biases; development of defined positive and negative controls has already been attempted by the National Institute for Biological Standards and Control and WHO.[28,29] The assessment of non-bacterial microbiome communities might also be relevant. Evaluation of the gut mycobiome, for example, might be performed through specific analyses (eg, internal transcribed spacer region, 18S rRNA gene sequencing, or by whole-genome sequencing). The expert panel also acknowledged growing interest in virome sequencing and its potential usefulness in clinical practice,[30] making it an area for future development. These sequencing methods are probably appropriate for other sample types, such as mucosal surfaces or biofluids, assuming enough DNA from the microbiome has been retrieved. Other sequencing methods (eg, single molecule sequencing technologies or full-length 16S rRNA gene sequencing or Nanopore sequencing) could have a future role but are too nascent to be recommended in clinical practice now.

	Amplicon sequencing (eg, 16S rRNA)	Whole genome sequencing
Sample requirements	Lower amount of biological sample required	Higher amount of biological sample required
Risk of contamination by host DNA	Hardly affected by host DNA	Can be affected by host DNA (in particular for low-biomass or highly host-contaminated sample types)
Target of sequencing	Specific gene (eg, 16S rRNA) or portion (eg, 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

Table 3: **Advantages and drawbacks of amplicon sequencing versus whole genome sequencing**

Conventional microbial cultures or molecular techniques (eg, 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 (appendix p 7).[32]

After defining sequencing methods, the panel recommends that the pre-processing of raw sequenced data should be detailed before analysis (statement 14). Key variables of amplicon sequencing should include the number of reads per sample, the reference database used (with version), the bioinformatic analysis approach used, 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 (eg, KneadData or operational modal analysis[34]) and should also be briefly mentioned in the final report.

Finally, the task force considered the analyses to be done after genome sequencing. They agreed that the microbiome analysis should include alpha diversity metrics, including richness and evenness (statement 15) and that beta diversity measures should be included in the microbiome analysis (statement 16). Alpha diversity, an ecological measure of the complexity and variety of an ecosystem that might associate with clinical response, should always be calculated within testing. However, further studies are needed to clarify its defined positioning into clinical practice (appendix p 8).[35–46] Beta diversity, 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 (appendix pp 8–9).[37–40]

Additionally, the panel agreed that a complete taxonomic profiling of gut microbial communities is an essential component of microbiome testing

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See **Online** for appendix

For more on **KneadData** see http://huttenhower.sph.harvard.edu/kneaddata (statement 17). Taxa should be identified at all possible levels, from phylum to genus or species for amplicon sequencing and to species or strain for whole-genome sequencing, with their estimated relative contribution to the whole community.[47] For whole-genome sequencing, both marker gene-focused sequence mapping and de novo assembly with reconstruction of metagenomic assembled genomes can be used.[33,48]

The panel stated that 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 such as the curated MetagenomicData repository,[49] should be used to guarantee a sufficient size of the control, and potential confounding factors (eg, biogeography, age, gender, 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 (or methods used to factor in potential confounders as part of statistical comparison) should also be described.

The panel also stated that a longitudinal assessment of the patient microbiome at different time points might be useful in specific clinical scenarios (statement 19). The longitudinal evaluation of the patient microbiome can increase robustness of the measurement[50] and be useful in several clinical scenarios (eg, to assess the effects of a treatment or diet), or to evaluate the microbiome composition after a stressful event (eg, a gastrointestinal infection; appendix p 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 (appendix p 9).[51–54]

Working group 4: characteristics of reports

Members of working group 4, who set out to define the items to be included (and excluded) in microbiome testing reports, 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 could ease the interpretation of the testing by the referring physician, if the patient has consented to it, and protecting their privacy, as detailed in panel 2. The stool collection protocol (eg, buffers for DNA preservation and details of sample storage) should also be reported in addition to the characteristics of DNA extraction, as these variables could influence the outcome of the analysis.[55–61] The main features of sequencing methods (eg, amplicon-based methods *vs* whole-genome sequencing), amplicon region if applicable, and the depth of sequencing (expressed as gigabytes or megabytes of DNA) should be provided, as they provide different taxonomical and functional findings.[62]

Moreover, details of sequencing machines and software, libraries, and pipelines used for computational analysis should be given, with software versions stated and the identity and version of the taxonomic reference database used. For whole-genome sequencing, 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 or species level for 16S rRNA gene sequencing data[63] and species level for shotgun sequencing data (appendix p 10).[33,47,63] 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, might 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. Additionally, the presence of known pathogens (eg, *C difficile*, Salmonella spp, Shigella spp, or pathogenic *Escherichia coli* strains) should be reported.

Finally, although the evidence for a causal connection between the abundance of specific microbes and human diseases is still scarce, the report of other health-relevant taxa and clusters (eg, at least *Akkermansia* spp, *Bifidobacterium spp*, Enterobacteriaceae, *Fusobacterium* spp, *Lactobacillus* spp, and short-chain fatty acid-producers), regardless of their abundance, could help the clinical management of patients.

The panel then 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 deserve further research (statement 27). Evidence suggests that phylum-level descriptors are insufficient to capture the whole spectrum of variation in the gut microbiota and can give deceiving results—eg, a high relative *Bacteroides* spp abundance can both mean a healthy *Bacteroides*-high community, an altered ecosystem, and 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 this cannot be used in clinical practice and requires future research.

Additionally, the panel stated that generally there is not enough information to report strict healthy reference ranges of species relative abundance (statement 28). By contrast with 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—eg, 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).

The panel strongly discouraged the reporting of any post-testing therapeutic advice by the testing provider (statement 30). Post-testing therapeutic advice on how to modulate the patient microbiota on the basis of the testing results might be tempting, due to the scarce 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 requested the testing.

Finally, the panel agreed that raw data can be provided to the patient upon request (eg, 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 diagnoses[71] and reducing the number of unnecessary diagnostic exams,[72] with relevant consequences for health-care systems. Laboratory-related second opinions and interactions between clinical laboratories and practicing physicians have been encouraged for decades.[73] As post-sequencing analyses require complex skills,[32] in some situations (eg, need for information on specific taxa), a further analysis of metagenomic reads from computational biologists or microbiologists might be required by the physician who manages the patient. This approach could be more convenient than repeating the microbiome analysis later, 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

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.

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 the 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 encephalopathy[77] and irritable bowel syndrome without constipation.[78] International guidelines recommend probiotics for infectious or antibiotic-associated diarrhoea,[79] as coadjuvants of *Helicobacter pylori* eradication regimens,[80] in the management of ulcerative colitis,[81] and for other disorders. FMT has become an established treatment option for recurrent *C difficile* infection. These therapeutic approaches were recommended for their target disorders after being shown to be clinically effective.[82–85] The introduction of microbiological endpoints, beyond clinical outcomes, in clinical trials of therapeutic microbiome modulators has been recommended.[86]

However, there is still no consolidated and direct evidence that microbiome-based diagnostics benefit the clinical management of gastrointestinal or extraintestinal disorders, either 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 might 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 could be useful in driving the management of different disorders associated with gut microbiome imbalance at several levels (appendix pp 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 observational studies, preferably those that 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 (eg, 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 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 the microbiome is of interest to physicians, most do not have the knowledge base required to interpret and exploit a microbiome report.

Beyond accumulating data aimed at consolidating the evidence for the use of a microbiome test in clinical practice, several short-term initiatives (eg, dissemination courses) and long-term actions (eg, the introduction of microbiome research into the official educational programmes of medical schools) are advocated to disseminate greater understanding of the microbiome in disease and potential usefulness of testing, and to allow more physicians to understand microbiome testing reports.

Conclusion

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]

Our initiative represents consensus from a multidisciplinary and international consortium of experts in human microbiome research. We acknowledge that low-income and middle-income countries are not represented in this group, and that this could represent a limitation in broad implementation of 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 issue.

Statements were presented as expert opinions, and a 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 applicability of our statements.

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. The provision of direct-to-consumer genetic health risk testing, which encompasses similar issues to the microbiome testing, has been regulated by the USA Food and Drug Administration (FDA) for years. The FDA allowed the marketing of these tests only under certain conditions, which are similar to our recommendations (eg, by defining criteria to assure the tests' accuracy, reliability, and clinical relevance by recommending a clear and understandable communication of results and consultation with a health-care professional about the test results). Moreover, the FDA distinguishes genetic tests that are needed for major clinical decisions (eg, *BRCA* testing) 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 little evidence for the use of 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 modes 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 treatments within the report (a common feature of available tests).

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

Our initiative was focused on standardising procedures for the release of microbiome testing in clinical practice. However, we are also aware that there is no direct evidence that the use of such diagnostics improves the

Search strategy and selection criteria

We searched PubMed from database inception up to June 12, 2024, without date limits, using the following terms: "microbiota", "microbiome", "amplicon", "whole genome sequencing", "microbial ecology", "diversity", "taxonomy", and "profiling". We searched for all types of articles published in English.

management of patients. We recognise that our effort could have little use if further studies do not evaluate the value of microbiome testing in human disorders. However, preliminary data (mostly but not exclusively in cancer) support this hypothesis,[96–98] and the use of microbiome testing has been advocated for in international guidelines.[99] A similar development pathway has already been seen in the field of genetic testing for cancer (eg, *BRCA* testing), which is now widely used in medical practice for clinical decision-making.[100] The consolidation of such evidence is needed to allow microbiome testing to move from being non-specific health tests (eg, direct-to-consumer genetic health risk tests) to diagnostic tests applicable in clinical medicine (eg, in human cancer genomics).

Therefore, another crucial, long-term objective of our project was to guide future research on the application of human microbiome diagnostics in clinical practice. We discussed the 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.

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 diagnostics[96–98] and the increase in advanced microbiome therapeutics[101] should 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.

Contributors

GI conceived and designed the project. GC, NS, AG, and GI identified the members of the steering committee. SCN, 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 their working group). SPo and GI coordinated the Delphi process. All panel members participated to the Delphi process. SPo, BHM, FA, SCN, LZ, GH, LP, JM, GC, AG, and GI wrote the initial draft of the manuscript. SPo, BHM, FA, and GI drafted tables, figures, and panels. All authors revised the manuscript for important intellectual content and approved the final manuscript.

Declaration of interests

JA received research support from Pfizer, Janssen, and Merck; has been a speaker for BMS, AbbVie, and Janssen; and reports consultancy with Janssen, Pfizer, AbbVie, Seres Therapeutics, Ferring, GSK, Merck, Bristol Myer Squibb Roivant, and Adiso. JB received grants to institution from Bausch, Grifols, Mallinckrodt, Cosmo, and Sequana and received personal fees for acting as has consultant for Merz and Novo Nordisk. PDC was co-founder of The Akkermansia Company and Enterosys. WMdV was co-founder and shareholder of The Akkermansia Company (Belgium), Caelus Pharmaceuticals (Netherlands) and Alba Health (Copenhagen-Stockholm). EE is a scientific cofounder of DayTwo and BiomX and is an advisor to Purposebio, Aposense, Zoe, and MyGutly. FG has received personal fees for acting as speaker and consultant from Biocodex, Danone, BioGaia, Menarini, and Sanofi. CLH received lecture honoraria from Baxter, Janssen, BMS, and Tillotts. SK received research support from Rebioitx/Ferring, Vedanta, Finch, Seres, and Pfizer and served as consultant for ProbioTech, Takeda, and Rise. OK is a co-founder of Shela Accurate Diagnosis (Israel). JKu received travel support and speaker fees from Ferring, AbbVie, KRKA, Takeda, Janssen, Pfizer, and Ipsen. RL received research funding from Celltrion, Shire, Janssen, Takeda, Gastroenterological Society of Australia, NHMRC, Gutsy Group, Pfizer, Joanna Tiddy grant, and McKusker Charitable Foundation and is an advisory board member for AbbVie, Aspen, BMS, Celgene, Celltrion, Chiesi, Ferring, Glutagen, Hospira, Janssen, Lilly, MSD, Novartis, Pfizer, Prometheus Biosciences, and Takeda. PM received speaker honoraria from Aboca, Alfasigma, Allergosan, Bayer, Biocodex, and Menarini and is a member of the advisory board of Aboca, Alfasigma, Allergosan, Bayer, Biocodex, and Menarini. JM has received consultancy fees from Cultech and EnterioBioti. SCN received personal fees for acting as speaker for Ferring, Tillotts, Menarini, Janssen, AbbVie, and Takeda; receives patent royalties through her affiliated institutions and is named inventors of patent applications held by The Chinese University of Hong Kong and Microbiota I-Center that cover the therapeutic and diagnostic use of microbiome; received research grants through her affiliated institutions from Olympus, Ferring, and AbbVie; is a founder member and shareholder of GenieBiome; and has served as an advisory board member for Pfizer, Ferring, Janssen, and AbbVie. SPa reports consultancy for Vedanta Biosciences and received personal fees for acting as speaker and for acting as advisory board member for AbbVie, Dr Falk Pharma, Ferring, Janssen, and Takeda. FRP received personal fees for acting as speaker or consultant for AbbVie, Gilead, Roche, Astra Zeneca, Ipsen MSD, Eisai, Kedrion, Bayer, and Alfasigma and is an advisory board member of AbbVie, Gilead, Roche, Astra Zeneca, Ipsen MSD, Eisai, Kedrion, Bayer, and Alfasigma. MRS received personal fees for acting as speaker or advisory board member for Hemofarm, Abela Pharm, and ADOC Pharma. HS reports lecture fee, board membership, or consultancy from Amgen, Fresenius, Ipsen, Actial, Astellas, Danone, THAC, Biose, BiomX, Eligo, Immusmol, Adare, Nestle, Ferring, MSD, Bledina, Pfizer, Biocodex, BMS, Bromatech, Gilead, Janssen, Mayoli, Roche, Sanofi, Servier, Takeda, and AbbVie; has stocks from Enterome bioscience; and is co-founder of Exeliom Biosciences. HTu is a named inventor of patent applications held by the CUHK and MagIC that cover the therapeutic and diagnostic use of microbiome. RKW received unrestricted research grants from Takeda, Johnson & Johnson, Tramedico, and Ferring; received speaker's fees from MSD, AbbVie, and Janssen Pharmaceuticals; and acted as consultant for Takeda Pharmaceuticals. GZ is named inventor on a patent (EP2955232A1) and received personal fee as member of the scientific advisory board of Alpha Biomics. FZ conceived the concept of GenFMTer and trasnendoscopic enteral tubing and the devices related to them (FMT Medical) and is an advisory board participant for Ferring and Seres. NS reports consultancy or SAB contracts with Zoe, Roche, Ysopia, and Freya, and Alia Therapeutics; speaker fees by Illumina; and is cofounder of PreBiomics. AG reports personal fees for consultancy for Eisai, 3PSolutions, Real Time Meeting, Fondazione Istituto Danone, Sinergie Board MRGE, and Sanofi; personal fees for acting as a speaker for Takeda, AbbVie, and Sandoz; and personal fees for acting on advisory boards for VSL3 and Eisai. GC has received personal fees for acting as advisor for Ferring Therapeutics. GI has received personal fees for acting as speaker for Biocodex, Danone, Sofar, Malesci, Metagenics, Illumina, and Tillotts Pharma and for acting as consultant or advisor for Ferring Therapeutics, Giuliani, Metagenics, and Tillotts Pharma. All other authors declare no competing interests.

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