State of the art and the future of microbiome-based biomarkers: a multidisciplinary Delphi consensus

Julie Rodriguez, Zahra Hassani, Carolina Alves Costa Silva, Fay Betsou, Federica Carraturo, Alessio Fasano, Mads Israelsen, Anandhi Iyappan, Aleksander Krag, Amira Metwaly, Robert Schierwagen, Jonel Trebicka, Hub Zwart, Joel Doré, Magali Cordaillat-Simmons, Celine Druart*, on behalf of the Human Microbiome Action consortium*

Although microbiome signatures have been identified in various contexts (ie, pathogenesis of non-communicable diseases and treatment response), qualified microbiome-based biomarkers are currently not in use in clinical practice. The Human Microbiome Action consortium initiated a Delphi survey to establish a consensus on the needs, challenges, and limitations in developing qualified microbiome-based biomarkers. The questionnaire was developed by a scientific committee via literature review and expert interviews. To ensure broad applicability of the results, 307 experts were invited to participate; 114 of them responded to the first round of the survey, 93 of whom completed the second and final round as well. The survey highlighted the experts' confidence in the potential of microbiome-based biomarkers for several indications or pathologies. The paucity of validated analytical methods appears to be the principal factor hindering the qualification of these biomarkers. The survey also showed that clinical implementation of these biomarkers would only be possible if kitted and validated molecular assays with simple interpretation are developed. This initiative serves as a foundation for designing and implementing public-private collaborative projects to overcome the challenges and promote clinical application of microbiome-based biomarkers.

Lancet Microbe 2025; 6: 100948

Published Online September 5, 2024
https://doi.org/10.1016/j.lanmic.2024.07.011

*Contributed equally

Pharmabiotic Research Institute, Narbonne, France (J Rodriguez PhD, M Cordaillat-Simmons PhD, C Druart PhD); **KPL-Paris, Paris, France** (Z Hassani PhD); **Gustave Roussy Cancer Campus (GRCC), ClinicObiome, Villejuif Cedex, France** (C Alves Costa Silva PhD); **CRBIP, Institut Pasteur, Université Paris-Cité, Paris, France** (F Betsou PhD); **European Biomedical Research Institute of Salerno (EBRIS), Salerno, Italy** (F Carraturo PhD, Prof A Fasano MD); **Department of Biology, University of Naples Federico II, Naples, Italy** (F Carraturo); **Department of Pediatrics, Mucosal Immunology and Biology Research Center, Mass General Brigham, Harvard Medical School, Boston, MA, USA** (Prof A Fasano); **Centre for Liver Research, Department of Gastroenterology and Hepatology, Odense University Hospital, Odense, Denmark** (M Israelsen PhD, A Krag PhD); **European Molecular Biology Laboratory (EMBL), Heidelberg, Germany** (A Iyappan PhD); **Institute of Clinical Research, University of Southern Denmark, Odense, Denmark** (A Krag); **Chair of Nutrition and Immunology, School of Life Sciences, Technical University of Munich, Freising, Germany** (A Metwaly PhD); **European Foundation for the Study of Chronic Liver Failure (EF CLIF), Barcelona, Spain** (R Schierwagen PhD, Prof J Trebicka PhD); **Department of Internal Medicine B, University of Münster, Münster, Germany** (R Schierwagen, Prof J Trebicka); **Erasmus School of Philosophy, Erasmus University Rotterdam,**

Introduction

Human bodies are complex ecosystems hosting trillions of microorganisms across various sites, including the oral cavity, skin, and gastrointestinal tract.[1] These microbiomes perform several crucial functions, such as protection against pathogens, development of the immune system, maintenance of immune homoeostasis, inflammation control, and metabolic processes such as vitamin production and carbohydrate fermentation.[2–4] Disruptions in microbiome composition or function are associated with diseases of diverse nature.[5]

Biomarkers are defined by the European Medicines Agency (EMA) as "An objective and quantifiable measure of a physiological process, pathological process or response to a treatment" and by the US Food and Drug Administration (FDA) as "A defined characteristic that is measured as an indicator of typical biological processes, pathogenic processes, or biological responses to an exposure or intervention, including therapeutic interventions."[6,7]

The context of use for a biomarker is crucial and involves the biomarker's purpose, target population, and setting, influencing the development of the biomarker and qualification studies designed to guarantee the suitability and reliability of its intended use.[6,8] Biomarkers are pivotal in health care and medical research; they are used to diagnose diseases, track disease progression, predict disease risks, and possibly gauge responses to treatments. Thus, EMA and FDA have classified biomarkers into various categories, including susceptibility or risk, predictive, prognostic, diagnostic, monitoring, and response biomarkers (definitions for these are provided in appendix 2 Suppl. table 1).

The journey from discovery to qualification and use of biomarkers in clinical practice remains a key concern and involves validation of analytical methods and demonstration of clinical relevance.[9] Method validation assesses accuracy, precision, specificity, range, and robustness.[10] Numerous potential biomarkers are discovered in the microbiome field every year, but few undergo a qualification programme. To our knowledge, no microbiome-based biomarker is currently used in medical practice. However, the potential utility of biomarkers is acknowledged, notably in disease diagnostics and prediction of treatment response for multiple conditions, including inflammatory, neurological, and metabolic diseases, as well as cancer.[11,12]

For instance, in the context of metabolic or inflammatory diseases, several bacterial DNA-based (or bacterial metabolite-based) signatures have been associated with the development or progression of disease, or both, and could be considered for a predictive or diagnostic biomarker qualification programme.[12–16] The clinical benefit of immune checkpoint inhibitors has also been linked to the presence or absence of distinct intestinal commensals, leading to the discovery of microbial consortia that drive clinical response to PD-1 blockade in some cancers.[17] This finding has raised interest in microbiome-based biomarkers for qualification as response biomarkers. Similarly, medicinal product developers can also rely on safety biomarkers for demonstrating the safety or efficacy, or both, of their products. As such, a Microbiome Health Index for post-antibiotic dysbiosis has been developed with the objective of understanding and managing the risks of administering antibiotics.[18]

Thus, the human microbiota has great potential for biomarker research with multiple purposes. Nevertheless, myriad challenges specific to the microbiome field impair their qualification, including the paucity of standard, validated sampling and analytical methods and the need for large-scale cohorts to identify confounding factors, such as

Rotterdam, Netherlands (Prof H Zwart PhD); **Université Paris-Saclay, INRAE, MGP Metagenopolis, Jouy-en-Josas, France** (J Doré PhD); **Université Paris-Saclay, INRAE, AgroParisTech, Micalis Institute, Jouy-en-Josas, France** (J Doré)

Correspondence to: Celine Druart, Pharmabiotic Research Institute, Narbonne 11100, France
celine@pharmabiotic.org

See Online for appendix 2

environmental influences. These issues lead to biases and poor study comparability, thereby restricting the potential of biomarker qualification.[19–21]

To address these issues, the Human Microbiome Action consortium organised a Delphi survey among experts from diverse microbiome fields. The Delphi method, known for its value in driving consensus building, integrates international and interdisciplinary perspectives to achieve results that are more robust than the judgements of individual experts.[22,23] In the context of the Delphi method, consensus refers to a collective agreement or convergence of opinions among a group of experts.[24] The Delphi method involves a series of iterative rounds in which a panel of experts respond to a questionnaire and justify their responses. The Delphi method's ability to harness diverse perspectives, minimise biases, and generate reliable consensus views is valued in diverse fields, particularly in health care.

This survey aimed at arriving at a consensus on the challenges faced in the qualification of microbiome-based biomarkers, proposing future research and innovation agendas to overcome these challenges, and guiding the transition from discovery to qualification and clinical implementation of microbiome-based biomarkers as an essential step in strengthening clinical decision making and drug development.

Methods

See Online for appendix 1

Additional details are provided in appendix 1.

Panel generation and statement development

This modified Delphi survey was conducted to garner expert consensus on the current and future use of microbiome-based biomarkers, with particular focus on the discovery and qualification of such biomarkers.[25–27] Unlike the consensus building method that uses a focus group approach, the Delphi method is performed in an anonymous manner to avoid the dominance of strong personalities and to allow for the involvement of a larger number of respondents and their expertise.

The questionnaire was based on a thorough literature review followed by semistructured qualitative interviews with microbiome experts. A total of 17 statements structured into five main sections covering all the aspects and challenges in the development and qualification of microbiome-based biomarkers were drafted and validated by an expert scientific committee. All consensus statements contained six-point Likert type response categories (appendix 1 p 1).

To establish the list of potential respondents, a first list was drawn up by the research team members on the basis of their professional contacts, both in the public and private sectors. More respondents were invited to participate through social media communication and public newsletters. The chief criterion for joining the expert panel was to be a stakeholder in microbiome-based research or development.

Data collection

The Delphi survey was managed by an online platform (ClinInfo), which ensured anonymity of the respondents (by assigning a number to each respondent) and security of the collected data (General Data Protection Regulation compliant platform). The Delphi process comprised two sessions of online data collection: a first-round (R1) survey carried out from March 3 to April 11, 2023, and a second-round (R2) survey from May 23 to June 30, 2023.

Analysis plan

For each statement, consensus was declared as achieved when agreement was obtained from two-thirds (66·67%) of the respondents, a cutoff that was agreed upon a priori. Given the large number of respondents and the diversity of specialties involved, a consensus threshold of two-thirds (66·67%) was considered appropriate.[28] For the agreement analysis, responses recorded on the six-point scale were transformed into a dichotomous construct: positive consensus was defined as the sum of Points 4–6 on the scale and negative consensus as the sum of Points 1–3 (figure 1A). As in most Delphi studies, we used a per cent agreement with a median threshold of 66·67% to achieve consensus.[29]

Statements that reached consensus in the first round of voting were not included in the second round. Along with the second-round questionnaire, respondents received the aggregated responses obtained in the first round, as per the protocol of the Delphi method. Statements that did not achieve consensus after the second round were considered as non-consensual.

The purpose of the Delphi survey was to obtain feedback from diverse stakeholders. Therefore, to accommodate for the diversity of expertise in the pool of respondents, an "I don't know" option was made available for all statements.

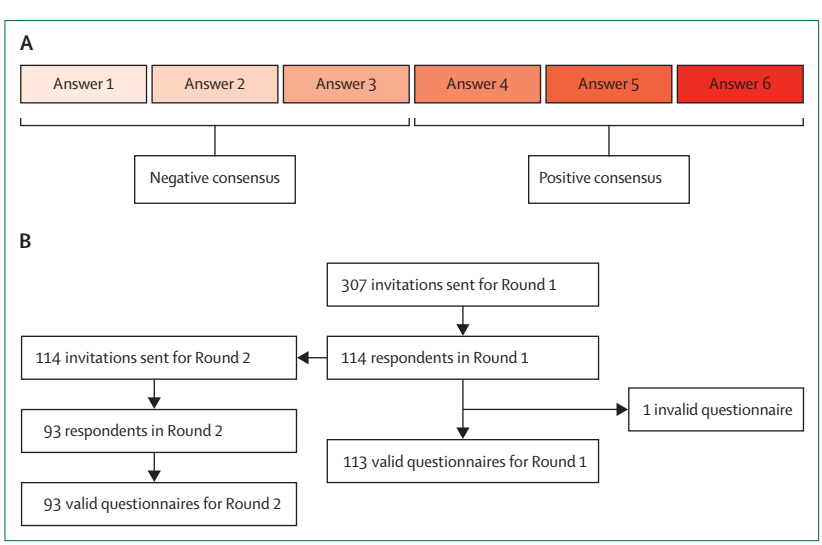
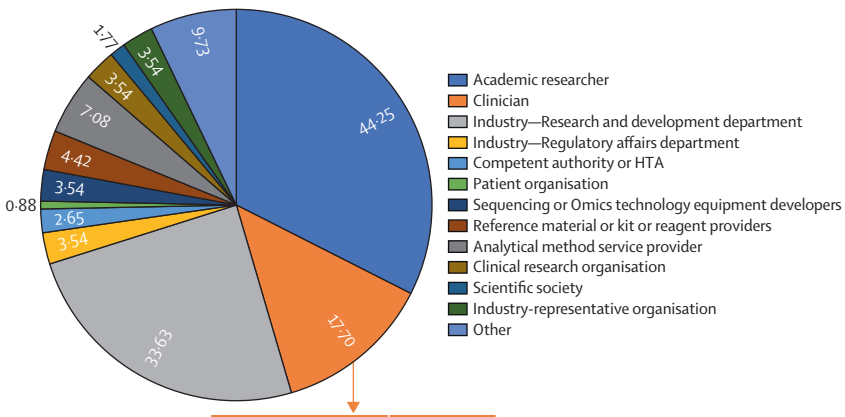


Figure 1: **Delphi survey - analysis plan and number of respondents**
(A) Representation of the conversion of answers obtained on a 6-point scale into a dichotomous construct (negative *vs* positive consensus). (B) Presentation of the number of experts to whom invitations were sent and the number of respondents in Round 1 and Round 2.

A

Country	%
France	27·43
USA	11·5
Germany	7·96
Belgium	7·96
Italy	7·08
UK	6·19
Denmark	5·31
Norway	4·42
Canada	3·54
Spain	2·65
Israel	2·65
Netherlands	3·54
Ireland	1·77
Sweden	1·77
Portugal	0·88
Estonia	0·88
United Arab Emirates	0·88
Ethiopia	0·88
Croatia	0·88
Qatar	0·88
China	0·88

B



Clinician speciality	%
Gastroenterology	40
Hepatology	30
Dermatology	5
Oncology	5
Other	20

Figure 2: **Geographical distribution and profession of the respondents to the Delphi survey**
(A) Geographical distribution of the respondents. (B) Profession declared by the respondents. The other category included the following professional activities: industry product management, clinical microbiologist, consultancy (4), solution provider (Medtech), non-profit organisation, provider of consumer microbiome test and food, government researcher or regulator, and clinical stage biotech. Within the clinical specialty sub-question, the other category included the following specialties: internal medicine and infectious diseases, microbiology, surgery, clinical pathology, and general practitioner. HTA=health technology assessment.

For the sake of clarity, levels of consensus achieved per statement and the non-consensual statements are presented in the appendix 2 Supp. table 4–7 and 9.

Results

Response rates and panel participation

A total of 307 experts received a personal invitation to access the survey platform (figure 1B). Among them, 114 (37·13% response rate) participated in R1, and one of the questionnaires was invalid (blank questionnaire). Only these 114 respondents were invited to R2. Among them, 93 (81·58%) participated in R2 (figure 1B).

Delphi panel characteristics

The characteristics of the Delphi panel participants (n=114), including demographics and professional expertise, are summarised in figure 2. Approximately 80% (n=89) of the respondents were European and 15% (n=17) were from North America. One limitation is the low number of experts from Asia and Africa, an unfortunate but well-known bias existing in microbiome studies, with the majority of human microbiome data obtained from high-income countries.[30] The top three types of professional expertise represented among the Delphi survey respondents were academic researcher (44·25%; n=50), industry research and development department (33·63%; n=38), and clinician (17·70%; n=20), indicating that the respondents had different backgrounds and came from different types of organisations within the microbiome field.

In the first part of the questionnaire, the respondents were asked to address several queries to assess their level of knowledge about biomarker definitions and qualification procedures (appendix 2 Suppl. table 2) as well as their awareness about the standards and reference materials for microbiome-based analytical methods (appendix 2 Suppl. table 3).

Among the 114 respondents, 24·78% (n=28) were not aware of both biomarker definitions and qualification procedures, whereas 40·71% (n=46) declared being aware of both. Approximately 20% (n=22) had been involved in the qualification procedure for microbiome-based biomarkers. Among the 80% (n=91) who had never been involved in such qualification procedures, the majority (44·25%; n=50) were envisaging to get involved in the future (*vs* 36·28% who were not; n=41). The main reason stated for not being involved in any qualification procedure for microbiome-based biomarkers was insufficient awareness of such procedures (32·97%; n=30), ranking well before paucity of data to scientifically support the qualification of a biomarker (21·98%; n=20). A first recommendation can be formulated on the basis of the results obtained for these survey questions: the need for more visibility of the qualification procedures for microbiome-based biomarkers and their dissemination to and training of various stakeholders (panel 1).

After providing information on the international standards and reference materials currently available for microbiome-based analytical methods in a dedicated section of the questionnaire, the awareness of the respondents about them was assessed in two survey

Panel 1: **List of recommendations to promote qualification of microbiome-based biomarkers**

- Enhance awareness of biomarker definitions, context of use, and qualification procedures.
- Establish dialogue to support qualification of microbiome-based biomarkers to improve clinical practice in several indications or pathologies.
- Support research projects tackling the challenges in the qualification of microbiome-based biomarkers. These challenges include poor reproducibility of clinical data, interindividual variation in microbiomes, paucity of metadata, ambiguity in thresholds for healthy or unhealthy microbiomes, and scarcity of validated analytical methods.
- Raise awareness of existing standards and reference materials in the field of microbiomes and encourage their adoption as requirements by scientific journals and funding agencies, so as to expedite validation of the analytical methods.
- Establish dialogue between standardisation bodies (such as International Organization for Standardization [ISO] and European Committee for Standardization [CEN]) and the reference material developers, academic researchers, and clinical end-users, to align the development of the standards and reference materials in line with the expectations of the microbiome field.
- Establish a dialogue between clinicians, clinical laboratories, and scientists for effectively promoting the use of microbiome-based biomarkers in clinical practice.

questions (appendix 2 Suppl. table 3). About 30·09% (n=34) of the respondents did not know the standards for microbiome-based analytical methods and 33·63% (n=38) had only heard about them. This result clearly highlights insufficient awareness about the standards within the microbiome field. Of note, only 4·42% (n=5) of the respondents used standards in their practice and only 8·85% (n=10) were in the process of implementing them into their practice. As these standards are quite recent, a higher percentage of respondents were expected to be currently in the process of implementing them into their research practice.

With respect to the reference material for microbiome-based analytical methods, 18·30% (n=17) of the respondents were not aware of it; 9·68% (n=9) had heard of it but did not know its exact content; and 5·30% (n=5) knew of the reference material but had never used it. About 38·58% (n=36) of the respondents were not using the reference material for various reasons, including a total (18·30%; n=17) or partial (9·68%; n=9) lack of awareness about its availability, the preference for their own internal controls (5·30%; n=5), or for no given reason (5·30%; n=5). These numbers highlight the fact that a substantial proportion of the respondents had not used the reference material in their analytical pipelines, which is not compatible with a biomarker qualification procedure. In contrast, 41·60% (n=38) of the respondents used the reference material in their analytical pipelines: 28·30% (n=26) used multiple reference materials and 13·30% (n=12) used at least one type of reference material in their analytical pipelines. Furthermore, 3·30% (n=3) of the respondents were in the process of implementing reference material in their analytical pipelines. These results are encouraging and will certainly bolster confidence in analytical methods, in addition to promoting the use of validated analytical methods, a necessary step for qualification of microbiome-based biomarkers.

Delphi survey results and analysis

The results of the Delphi survey are presented here in five consecutive sections.

Section 1. The promise of microbiome-based biomarkers. The data on the association between microbiome modification and diseases that are currently available in the literature make the experts confident (positive consensus) about the use of microbiome-based biomarkers that are currently in the discovery stage in the following indications or pathologies: inflammatory bowel disease (Crohn's disease and ulcerative colitis), cancer, metabolic diseases, and liver diseases (metabolic dysfunction-associated fatty liver disease, alcohol-related diseases, or cirrhosis). In contrast, the experts are not confident (negative consensus) of microbiome-based biomarkers that are currently in the discovery stage in the following indications or pathologies: autism, attention-deficit hyperactivity disorder, emotional and behavioural disorders, and lung diseases (asthma, cystic fibrosis, chronic obstructive pulmonary disease, and chronic lung inflammation). Finally, the most promising context of use for microbiome-based biomarkers within an area of expertise includes the response susceptibility or risk, monitoring, predictive, diagnostic, or prognostic biomarkers (see appendix 2 Suppl. table 4 for a detailed description of the results).

The high level of positive consensus achieved for microbiome-based biomarkers currently in the discovery stage for several indications or pathologies validates the importance of their qualification and clinical implementation. However, the negative consensus revealed the scepticism of the experts about the promise of microbiome-based biomarkers in some medical contexts and clearly highlights the need for generating more data in these areas. The high level of positive consensus achieved in the context of use of microbiome-based biomarkers in the first round suggested a broad-based context of use of these biomarkers.

The high level of consensus achieved for microbiome-based biomarkers in the discovery stage for several indications and in their broad-based context of use reinforces the importance of working on the qualification of microbiome-based biomarkers to improve their application in clinical practice in several indications or pathologies. Thus, the hurdles in expediting qualification of microbiome-based

Panel 2: **Reasons for the current absence of qualified microbiome-based biomarkers**

Statements that achieved consensus in this section:

Microbiome-based biomarkers can be different from other types of biomarkers owing to the following challenges or reasons:

- High complexity in analysis and interpretation of results
- High interindividual variability of microbiomes
- No consensus on the definition of a healthy microbiome and dysbiosis
- Quantification of microbiome-based biomarkers being challenging
- High susceptibility to preanalytical variation
- High complexity of microbiome analytical methods
- Microbiome analysis methods being expensive and time-consuming

Factors challenging the qualification of microbiome-based biomarkers are:

- Relevant metadata required for the interpretation of microbiome data being missing or not available in numerous clinical studies
- Poor reproducibility of clinical data
- Interindividual variability of microbiomes
- Scarcity of quantitative data or threshold for defining a healthy or unhealthy microbiome
- Underpowered clinical studies
- Insufficient validation of analytical methods
- Dearth of knowledge about the biomarker qualification programme
- Insufficient resources (time, budget, and human resources)
- Scarcity of incentive for companies to pursue qualification of biomarkers, since qualified biomarkers eventually end up in the public domain

The following studies would be an appropriate qualification programme for microbiome-based biomarkers:

- Large multicentre studies
- Confirmation by independent clinical studies
- Prospective interventional studies
- Prospective observational studies
- Studies with increased confidence for analytical methods (in place of increasing cohort size)

biomarkers currently in the discovery stage should be addressed in future collaborative projects.

Section 2. Reasons for the current absence of qualified microbiome-based biomarkers. The statements that achieved consensus in this section are presented in panel 2 (see appendix 2 Suppl. table 5 for a detailed description of the results). The consensus achieved on the challenges or reasons why microbiome-based biomarkers can be different from other types of biomarkers will help to design and implement robust studies in qualification programmes (clinical studies with appropriate analytical methods). Identification of and awareness about these challenges will help regulatory or competent authorities to develop appropriate guidelines to support the evaluation and qualification of microbiome-based biomarkers. Indeed, owing to these challenges, the qualification dossier of microbiome-based biomarkers can be different from that of a classic biomarker, to which the regulators are more accustomed. Furthermore, identification of these challenges underscores the importance of collaborative projects aimed at addressing the challenges to increase the likelihood of more qualified microbiome-based biomarkers emerging in the future (panel 1)

Of note, a wide range of factors can hinder the qualification of microbiome-based biomarkers, including shortage of resources, insufficient awareness about biomarker qualification programmes, and issues in clinical studies such as reproducibility challenges, insufficient metadata, absence of criteria for defining a healthy microbiome, underpowered studies, and absence of validated analytical methods (appendix 2 Suppl. table 5). In discussing the suitable qualification programme for microbiome-based biomarkers, the emphasis is once again placed on the need for enhancing confidence in analytical methods. This crucial aspect should be addressed before initiating extensive multicentre studies. Therefore, careful consideration is essential in selecting and implementing analytical methods for microbiome analysis, ensuring that appropriate standards and reference materials are kept in view throughout the analytical procedures.

Section 3. Analytical methods used to measure potential microbiome-based biomarkers. Based on the opinion of the experts (positive consensus), the current analytical methods that can be considered appropriate for microbiome-based biomarker discovery are shotgun metagenomic sequencing, shotgun metagenomic sequencing coupled with metabolomics, other multiomics, targeted metabolomics, amplicon sequencing combined with metabolomics, untargeted metabolomics, metaproteomics, and shotgun metagenomic sequencing combined with cell counting by flow cytometry. The experts also concluded that the current analytical methods that cannot be considered

Panel 3: **Validation of analytical methods (standards and reference materials)**

Statements that reached consensus in this section:

The following points are missing or problematic, or both, in the adoption of such international standards within the microbiome field:

- Insufficient awareness about these standards
- Recommendations for their use not being detailed enough or missing practical details
- Poor perception of the benefit that adoption of such standards represents for the community
- The process of developing standards not involving all types of stakeholders who could benefit from them (academia, research and development, service providers, medical laboratories, etc)
- The need for frequent updates to standards so as to follow technological developments

The following points are missing or problematic in the adoption of reference material within the microbiome field:

- Insufficient awareness about these reference materials
- Suitability of the reference material for the specimen (gut, vaginal, oral, skin etc)
- Poor perception of the benefit that the implementation of such reference material could bring to the reliability of the analytical methods
- Suitability of the reference material for analytical methods

appropriate (negative consensus) for microbiome-based biomarker discovery are cell counting by flow cytometry and enumeration by plate count or most-probable number (colony-forming unit enumeration).

For biomarker qualification, positive consensus was achieved for the following analytical methods: targeted metabolomics, shotgun metagenomic sequencing combined with metabolomics, shotgun metagenomic sequencing, quantitative PCR (qPCR), and other multiomics. Only enumeration by plate count or most-probable number (colony-forming unit enumeration) was seen by the experts as a current analytical method that cannot be considered appropriate (negative consensus) for this purpose (see appendix 2 Suppl. table 6 for a detailed description of the results).

Based on these results, fewer analytical methods (only five) achieved positive consensus in the context of biomarker qualification, in contrast to those (nine) that achieved positive consensus in the context of biomarker discovery (appendix 2 Suppl. table 6). Of note, the qPCR method achieved positive consensus in the context of qualification, but not in the context of discovery, which is not surprising given that qPCR is a targeted technique, necessitating knowledge of the target for the design of suitable primers. Furthermore, in the context of biomarker discovery, both targeted and untargeted metabolomics achieved positive consensus, whereas only targeted metabolomics achieved positive consensus in the context of qualification. Finally, the amplicon sequencing analytical method failed to achieve consensus in both discovery and qualification contexts. This observation aligns with the ongoing controversy regarding amplicon sequencing.[31,32]

Section 4. Validation of analytical methods (standards and reference materials). Statements that achieved consensus in this section are presented in panel 3.

The responses to survey questions (presented in the Delphi panel characteristics section) revealed that a low percentage of respondents were aware of or using, or both, the standards and reference materials in the field of microbiome research. Therefore, these consensus statements are important to understand the deficiencies or challenges in incorporating such standards and reference materials in the field of microbiome research (see appendix 2 Suppl. table 7 for a detailed description of the results).

The consensus of the experts shows that the inadequate implementation of the available international standards in the microbiome field is mainly linked to insufficient awareness and the development process of the standards, rather than technical issues or burdens linked to the implementation of such standards (appendix 2 Suppl. table 7). To date, four international standards are available (appendix 2 Suppl. table 8),[33–36] and a practical recommendation could be to enhance communication and promote awareness about them and their implementation. A more important recommendation would be that scientific journals require better documentation of the standards used, ultimately ensuring their enforcement, as is the case with ethics approval in the context of clinical trials (panel 1). Another recommendation could be to ensure that all stakeholders (including researchers and the industry) are included in the process of drafting these standards such that they are comprehensive and meet the needs of all stakeholders (panel 1).

The collective response of the experts highlights a similar dearth of awareness and insufficient utilisation of reference material in the field of microbiome research, together with the poor perception of the benefit of using such reference material, which are essentially the same limiting factors as those for standards (appendix 2 Suppl. table 7). Indeed, different reference materials have been recently developed (appendix 2 Suppl. table 8). Some of these materials resulted from collaborative efforts and have even been evaluated and endorsed by WHO.[37–39] As for the available standards, a practical recommendation would be to enhance communication about the available reference material and its value for both the research community and industry (panel 1). Another proposal could be to insist on the necessity for those engaged in microbiome-based

biomarker discovery and qualification studies to necessarily use and acknowledge the reference material. Another key suggestion would be to mandate evaluation of all research projects as well as microbiome studies submitted for publication in the light of implementation of existing standards and documentation of the reference material used (panel 1).

Other limiting factors are the unsuitability of the reference material for the specimen (gut, vaginal, oral, skin, etc) and the analytical methods. This consensus view serves as a crucial message to be conveyed to developers of reference material, urging them to align their work with the expectations of the end users.

In the end, the costs associated with both standards and reference materials do not seem to be a limiting factor.

Section 5. Clinical implementation of microbiome-based biomarkers in the future. The experts' opinion converged on the view that medical biology or clinical laboratories would be able to implement microbiome-based biomarker analysis only if kitted and validated molecular assays with simple, straightforward interpretation are developed and made available (appendix 2 Suppl. table 9). This view is an important point that should be conveyed to both the research community and assay developers, as microbiome-based biomarkers can only be fully exploited in clinical practice when analysis can be performed routinely in accredited and qualified laboratories (see appendix 2 Suppl. table 9 for the detailed description of the results).

Furthermore, the experts also agreed by consensus that medical biology or clinical laboratories do not have the appropriate protocols, bioinformatics expertise, or expertise for integration of sequencing data, statistics, and clinical data (appendix 2 Suppl. table 9) at the moment. If medical biology laboratories are to be the natural actors implementing microbiome-based biomarkers, appropriate protocols and support should be provided to them; hence, for some time at least, they might have to rely on data science expertise. To expedite clinical implementation of microbiome-based biomarkers, one possibility would be the establishment of specialised centres capable of performing these analyses on behalf of clinical laboratories. However, their analytical capacity should be aligned with the demand, to avoid bottlenecks and long delays in analysis, especially for microbiome-based biomarkers that are to be used in clinical routine (panel 1). Health-care professionals would expect tests that can provide results within hours or a few days.

Discussion and conclusions

This Delphi survey highlighted that microbiome-based biomarkers are eagerly awaited and are most likely to become highly relevant for the field in the near future. The experts are confident about the microbiome-based biomarkers currently in the discovery stage for several indications or pathologies but also agree that their context of use will be broad-based, thereby highlighting the importance of identifying the factors that are hindering the qualification of microbiome-based biomarkers and finding appropriate solutions through collaborative projects and public precompetitive funding.

Regarding the identification of these limiting factors, the Delphi survey provides first insights by detailing the numerous reasons why microbiome-based biomarkers can differ from other biomarkers. Indeed, the qualification of microbiome-based biomarkers itself faces various challenges. Some of these challenges, such as underpowered clinical studies, inadequate knowledge about the qualification programme, and scarcity of resources and incentives, are of concern for the classic biomarkers as well. However, the other factors are specific to microbiome-based biomarkers, such as poor reproducibility of clinical data, interindividual variation of the microbiomes, scarcity of metadata to properly identify and validate biomarkers, ambiguity about the thresholds of a healthy or unhealthy microbiome, and paucity of validated analytical methods. Owing to the specificity of microbiome-based biomarkers, the field should find solutions to overcome these challenges through the collective efforts of public or private precompetitive projects.

One specific problem in the microbiome field is the unavailability of validated analytical methods. In addition to hindering the qualification of microbiome-based biomarkers, this issue has also been mentioned in the literature as being partly responsible for poor reproducibility and comparability of microbiome data.[19,21,37] In this Delphi survey, we assessed the appropriateness of various analytical methods for the discovery and qualification of microbiome-based biomarkers. The consensus results considered various analytical methods relevant for discovery of microbiome-based biomarkers, but only five remained relevant for their qualification. Some methods, such as untargeted techniques, appear useful in the discovery step but were not considered relevant for the qualification step. Indeed, in these two different phases, different attributes are sought for analytical methods and only techniques that can be fully validated are relevant in the context of qualification.[10]

Implementation of international standards and reference materials in analytical pipelines is also essential for the standardisation and validation of analytical methods. The Delphi survey revealed that the primary barriers to adopting these tools in microbiome research are a dearth of awareness and an inadequate understanding of their benefits. To mitigate this issue, communication about these standards and reference materials should be enhanced among scientists both in academia and industry, and awareness should be raised. In addition, scientific journals and funding agencies should be encouraged, if not mandated, to enforce the implementation of these tools, to promote their adoption and effects in the field.

In addition, standardisation efforts have already been initiated in the past or are underway, such as collaborative and international research projects (International Human Microbiome Coordination and Support Action; International Human Microbiome Standards) or open consortia

(International Microbiome and Multi'Omics Standards Alliance); collaborative efforts for the development of reference material and products (as examples, two reference works were endorsed by WHO[37–39] and one FDA de novo authorised collection device for microbiomes has been developed[40]); interlaboratory studies allowing for multicentre evaluation of bioinformatic pipelines;[32,41] and development of specific standards for microbiome studies by international standardisation bodies (International Organization for Standardization [ISO] and European Committee for Standardization [CEN]).[33–36]

As in the case of recommendations for the adoption and use of standards and reference materials, other limiting factors such as their suitability for the specimen (eg, availability of reference material for different microbiomes: gut, vaginal, oral, skin, etc) should be brought to the attention of standardisation bodies (such as ISO and CEN) and developers of reference material, to align standards and product development with the expectations of the field. Indeed, only a wide adoption of these tools will bring a real added value for the microbiome field.

The Delphi survey envisages marked improvement in clinical practice with implementation of microbiome-based biomarkers in clinical laboratories on a regular basis. The main results also show that clinical implementation of microbiome-based biomarker analysis would only be possible if kitted and validated molecular assays with simple interpretation are developed, given the perceived lack of expertise (mainly expertise in bioinformatics and integration of sequencing data, statistics, and clinical data) in medical biology or clinical laboratories. The need for kitted molecular assays with simple interpretation is indeed highly relevant for future research projects and research and development pipelines of assay developers. Furthermore, this observation underlines a primordial concept for research: the most advanced research can help to transform clinical practice only if it is initially aligned with the needs of clinicians in their routine practice right from the initial stages. This concept reminds us of the need to take translational aspects into account in research projects, to ensure that research findings are moved from the researcher's bench to the patient's bedside and community.[42]

In conclusion, the Delphi survey stands as a pivotal resource, involving international stakeholders of the microbiome field from both the research and industrial sectors. The survey offers a comprehensive consensus on the existing challenges within this field and presents practical recommendations for improvement (panel 1). Furthermore, the Delphi survey is not intended to target a specific category of biomarkers but addresses broad and generic questions for all microbiome-based biomarkers; thus, the recommendations are applicable to all biomarker categories. These suggestions aim to pave the way for the future qualification of microbiome-based biomarkers. The insights gained from this survey serve as a valuable foundation for academia, industry, regulators, and policy makers. The stakeholders can use these findings to develop and fund public-private collaborative projects aimed at meeting or overcoming challenges, to improve the clinical application of microbiome-based biomarkers. In addition to clinical benefits, microbiome-based biomarkers can be associated with economic benefits by allowing for early and improved diagnosis of diseases, enabling better prediction of disease progression and therapeutic responses, and supporting the development of precision medicine.[11,43]

Contributors

All authors conceptualised the study. CD, JR, ZH, and MC-S performed the formal analysis and visualisation. JD was in charge of funding acquisition. CD, JR, ZH, and MC-S conducted the investigation. CD, JD, and MC-S were in charge of the project administration. CD, JR, and ZH wrote the original draft. All authors critically reviewed and edited the manuscript. CD, JR, ZH, and MC-S had full access to and verified all the data in the study and had final responsibility for the decision to submit for publication.

Declaration of interests

JD is a cofounder and scientific adviser of GMT Science and MaaT Pharma. All other authors declare no competing interests.

Acknowledgments

This paper is the result of a Delphi survey performed in the context of a project titled International Human Microbiome Coordination and Support Action, which receives funding from EU's Horizon 2020 research and innovation programme, under grant agreement no. 964590. The contribution of JD was partly supported by the European Commission (ERC-2017-AdG no. 788191 and MetaGenoPolis grant ANR-11-DPBS-0001). The authors are grateful to Joseph Simmons for proofreading the language of the paper.

References

1 Belizário JE, Napolitano M. Human microbiomes and their roles in dysbiosis, common diseases, and novel therapeutic approaches. *Front Microbiol* 2015; **6:** 1050.

2 Berg G, Rybakova D, Fischer D, et al. Microbiome definition re-visited: old concepts and new challenges. *Microbiome* 2020; **8:** 103.

3 Scotti E, Boué S, Sasso GL, et al. Exploring the microbiome in health and disease: implications for toxicology. *Toxicol Res Appl* 2017; **1:** 2397847317741884.

4 Round JL, Mazmanian SK. The gut microbiota shapes intestinal immune responses during health and disease. *Nat Rev Immunol* 2009; **9:** 313–23.

5 Lynch SV, Pedersen O. The human intestinal microbiome in health and disease. *N Engl J Med* 2016; **375:** 2369–79.

6 FDA-NIH Biomarker Working Group. BEST (Biomarkers, EndpointS, and other Tools) Resource. 2016. https://www.fdanews.com/ext/resources/files/2020/11-24-20-BEST.pdf?1606261388 (accessed Aug 9, 2022).

7 European Medicines Agency. Glossary - Regulatory terms: biomarker. https://www.ema.europa.eu/en/glossary/biomarker (accessed Jan 10, 2024).

8 Usher-Smith JA, Sharp SJ, Griffin SJ. The spectrum effect in tests for risk prediction, screening, and diagnosis. *BMJ* 2016; **353:** i3139.

9 Davis KD, Aghaeepour N, Ahn AH, et al. Discovery and validation of biomarkers to aid the development of safe and effective pain therapeutics: challenges and opportunities. *Nat Rev Neurol* 2020; **16:** 381–400.

10 The International Council for Harmonisation of technical requirements for pharmaceuticals for human use (ICH). Validation of analytical procedures. Q2(R2). March 24, 2022. https://database.ich.org/sites/default/files/ICH_Q2-R2_Document_Step2_Guideline_2022_0324.pdf (accessed Jan 12, 2024).

11 Hajjo R, Sabbah DA, Al Bawab AQ. Unlocking the potential of the human microbiome for identifying disease diagnostic biomarkers. *Diagnostics (Basel)* 2022; **12:** 1742.

12 Metwaly A, Reitmeier S, Haller D. Microbiome risk profiles as biomarkers for inflammatory and metabolic disorders. *Nat Rev Gastroenterol Hepatol* 2022; **19:** 383–97.

13 Canfora EE, Meex RCR, Venema K, Blaak EE. Gut microbial metabolites in obesity, NAFLD and T2DM. *Nat Rev Endocrinol* 2019; **15:** 261–73.

14 Ruuskanen MO, Erawijantari PP, Havulinna AS, et al. Gut microbiome composition is predictive of incident type 2 diabetes in a population cohort of 5,572 Finnish adults. *Diabetes Care* 2022; **45:** 811–18.

15 Pittayanon R, Lau JT, Leontiadis GI, et al. Differences in gut microbiota in patients with vs without inflammatory bowel diseases: a systematic review. *Gastroenterology* 2020; **158:** 930–46.e1.

16 Radhakrishnan ST, Alexander JL, Mullish BH, et al. Systematic review: the association between the gut microbiota and medical therapies in inflammatory bowel disease. *Aliment Pharmacol Ther* 2022; **55:** 26–48.

17 Derosa L, Iebba V, Silva CAC, et al. Custom scoring based on ecological topology of gut microbiota associated with cancer immunotherapy outcome. *Cell* 2024; **187:** 3373–89.e16.

18 Blount K, Jones C, Walsh D, Gonzalez C, Shannon WD. Development and validation of a novel microbiome-based biomarker of post-antibiotic dysbiosis and subsequent restoration. *Front Microbiol* 2022; **12:** 781275.

19 Hornung BVH, Zwittink RD, Kuijper EJ. Issues and current standards of controls in microbiome research. *FEMS Microbiol Ecol* 2019; **95:** fiz045.

20 Nearing JT, Comeau AM, Langille MGI. Identifying biases and their potential solutions in human microbiome studies. *Microbiome* 2021; **9:** 113.

21 Schloss PD. Identifying and overcoming threats to reproducibility, replicability, robustness, and generalizability in microbiome research. *mBio* 2018; **9:** e00525-18.

22 Hsu C-C, Sandford BA. The Delphi technique: making sense of consensus. *Pract Assess Res Eval* 2019; **12:** 10.

23 Niederberger M, Spranger J. Delphi technique in health sciences: a map. *Front Public Health* 2020; **8:** 457.

24 Nasa P, Jain R, Juneja D. Delphi methodology in healthcare research: how to decide its appropriateness. *World J Methodol* 2021; **11:** 116–29.

25 Linstone HA, Turoff M, eds. The Delphi method: techniques and applications. Reading, MA: Addison-Wesley, 1975.

26 Gupta UG, Clarke RE. Theory and applications of the Delphi technique: a bibliography (1975–1994). *Technol Forecasting Soc Change* 1996; **53:** 185–211.

27 Rattray J, Jones MC. Essential elements of questionnaire design and development. *J Clin Nurs* 2007; **16:** 234–43.

28 Orozco-Beltrán D, Carratalá-Munuera C, Arriero JM, et al. Management and referral of patients with severe and poorly controlled asthma in primary care. *Fam Pract* 2016; **33:** 678–83.

29 Diamond IR, Grant RC, Feldman BM, et al. Defining consensus: a systematic review recommends methodologic criteria for reporting of Delphi studies. *J Clin Epidemiol* 2014; **67:** 401–09.

30 Abdill RJ, Adamowicz EM, Blekhman R. Public human microbiome data are dominated by highly developed countries. *PLoS Biol* 2022; **20:** e3001536.

31 Wensel CR, Pluznick JL, Salzberg SL, Sears CL. Next-generation sequencing: insights to advance clinical investigations of the microbiome. *J Clin Invest* 2022; **132:** e154944.

32 Roume H, Mondot S, Saliou A, Le Fresne-Languille S, Doré J. Multicenter evaluation of gut microbiome profiling by next-generation sequencing reveals major biases in partial-length metabarcoding approach. *Sci Rep* 2023; **13:** 22593.

33 European Committee for Standardization. CEN/TS 17626:2021. Molecular in vitro diagnostic examinations — Specifications for pre-examination processes for human specimen — isolated microbiome DNA. May 25, 2021. https://standards.iteh.ai/catalog/standards/sist/35481def-db5c-4e33-b8c0-e70e28a52f05/sist-ts-cen-ts-17626-2021 (accessed June 7, 2022).

34 International Organization for Standardization. ISO 20397-1:2022. Biotechnology — massively parallel sequencing — Part 1: Nucleic acid and library preparation. March, 2022. https://www.iso.org/standard/74054.html (accessed June 7, 2022).

35 International Organization for Standardization. ISO 20397-2:2021. Biotechnology — massively parallel sequencing — Part 2: Quality evaluation of sequencing data. https://www.iso.org/standard/67895.html (accessed June 7, 2022).

36 International Organization for Standardization. ISO/TS, 2023. Biotechnology — massively parallel DNA sequencing — General requirements for data processing of shotgun metagenomic sequences. May, 2023. https://www.iso.org/standard/78741.html (accessed June 10, 2023).

37 Amos GCA, Logan A, Anwar S, et al. Developing standards for the microbiome field. *Microbiome* 2020; **8:** 98.

38 Sergaki C, Anwar S, Fritzsche M, et al. Developing whole cell standards for the microbiome field. *Microbiome* 2022; **10:** 123.

39 Sergaki C, Anwar S, Hassall J, et al. A WHO collaborative study to evaluate the candidate 1st WHO International Reference Reagents for Gut Microbiome analysis by Next-Generation Sequencing. March 3, 2022. https://cdn.who.int/media/docs/default-source/biologicals/bs-documents-(ecbs)/2022-documents/who_bs_2022.2416-microbiome_bs_report_2022.pdf?sfvrsn=f7c9d6ed_3&download=true (accessed March 7, 2022).

40 FDA. FDA de novo authorization letter for the Class II device OMNIgene•GUT Dx (DEN200040). Nov 3, 2021. https://www.accessdata.fda.gov/cdrh_docs/pdf20/DEN200040.pdf (accessed June 13, 2024).

41 Forry SP, Servetas SL, Kralj JG, et al. Variability and bias in microbiome metagenomic sequencing: an interlaboratory study comparing experimental protocols. *Sci Rep* 2024; **14:** 9785.

42 Rubio DM, Schoenbaum EE, Lee LS, et al. Defining translational research: implications for training. *Acad Med* 2010; **85:** 470–75.

43 Panel for the Future of Science and Technology. Health and economic benefits of microbiomes (online workshop). May, 2021. https://www.europarl.europa.eu/stoa/en/events/details/health-and-economic-benefits-of-microbio/20210409WKS03441 (accessed June 13, 2024).