Ask the Experts:
How to use the microbiome as an innovative diagnostic tool

Speaker Profiles

Moderator: Zhengxi Danard – Marketing Director

Arnaud Beurdeley, M.Sc. Senior Regulatory Scientist
Arnaud is an accomplished HealthTech professional in the field of nonclinical and early clinical development with a strong expertise in microbiome-based products. He has more than 20 years of experience in translational research and molecular biology in Europe and the US. Throughout his extensive tenure in the industry, Arnaud has also successfully led multiple nonclinical development programs of in vitro diagnostic products in the field of women health and onco-immunology.

Anne-Charlotte Jarrige, Ph.D. Senior Regulatory Scientist
Anne-Charlotte has more than 20 years of experience in both consulting and pharmaceutical industries, involved in the development of in vitro diagnostic tests (including Companion Diagnostics (CDx)) in the field of Alzheimer's disease & oncology. Her expertise encompasses navigating the complexities of regulatory requirements and managing clinical studies as well as the constitution and maintenance of regulatory dossier.

Question: Under what regulatory environment would microbiome-based diagnostics fall?

Answer:
Before explaining under which regulatory environment microbiome-based diagnostics would fall let's come back to define what is a microbiome-based diagnostic Microbiome-based diagnostics" are tools that allow the detection of human diseases, and/or making prognoses using microbial signatures A microbial signature is a set of microbiome biomarkers that can be used as an indicator of a particular biological state, to identify subject of a particular biological state among non-particular biological states.

According to the National Institute of Health (NIH), a biomarker is "a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention".

Therefore, a diagnostic biomarker is simply a biomarker that "detects or confirms the presence of a disease or condition of interest or identifies an individual with a subtype of the disease.

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Emergence of applications in the diagnostic area are anticipated to bring high value as innovative biomarkers for many critical conditions. Microbiomes can be used in testing

applications for example: diagnostic /prognostic: susceptibility/risk biomarkers; diagnostic predictive biomarker; response biomarker; safety biomarker.

But one of the most interesting applications of microbiome biomarker discovery is companion diagnostics. Some companies are applying their microbiome-mining platforms to identify signatures in the microbiota to predict efficacy of established drugs.

Even though the emergence of microbiome biomarkers is becoming more and more frequent there is currently no single structure that regulates microbiome products.

Applicable status may vary depending on the product's intended purpose (e.g. target indication and population claims).

But based on the definition provided by the US and European authorities, microbiome-based diagnostics would therefore fall primarily under the framework of in vitro diagnostic medical devices.

Question: What important methodological considerations should be anticipated for microbiome diagnostic?

Answer: I would like to quote Werner Heisenberg who said :"What we observe is not the Nature itself, but Nature exposed to our methods of questioning." Another degree of complexity, out of technical challenges which I will address next, microbiome is a living entity influenced by many criteria.

From a technical perspective, Standardization of sample collection, nucleic extraction and data analysis are critical to ensure accurate, replicable, and comparable results. These represent the main challenges and pitfalls for any diagnostic applications. Within the metagenomic workflow those steps can be prone to error or bias.

Although the goal of a microbiome study is to get a comprehensive, unbiased profiling of microbial communities, this is not achievable in practice as specimens are often self-collected and need to be preserved adequately, requiring dedicated collection kits easy to use and transport.

Extraction of nucleic acids (DNA and/or RNA) is a second critical step in microbiome testing and can introduce considerable bias.

Library preparation becomes less complex (including fewer steps), faster (single-shift WF) and easier to automate, requiring minimal QC and qualification.

NGS is a highly sensitive methodology that will sequence all genetic material present in the sample after library preparation. Once again host nucleic acid will potentially limit the accuracy of microbiome profiling. Initiatives to provide an effective standardization of microbiome analysis technologies are growing. As an example, as described by Amos et al (2020) the development of a list of reference reagents and reporting system to conduct microbiome analysis was set by the National Institute for Biological Standards and Controls (NIBSC) and was proposed as candidate to World Health Organization international reference reagents. 2 mock communities (i.e., Gut Mix-RR and Gut Hilo RR) containing 20 gut microbiome strains with specific representation in each.

As with other genomic applications, accuracy, completeness, and quality of metagenomics and metatranscriptomics studies depend on sequence qualification algorithms and reference

sequences databases. Although NGS performance is continuously improving, completeness and quality of reference databases will determine the accuracy of microbiome diagnostic. Missing references could lead to both false negative(incorrect identification of organism) or false positive misidentification. Regular updates of databases will need to be implemented, validated and all changes documented.

Moreover, diagnostic application of microbiota profiling requires, as all diagnostic tests, adequate QC of sample processing, data generation, data analysis to ensure reliable diagnostic result(s). there are no accepted QC criteria yet but in practice, commonly used metrics include:

- External controls (positive and negative) to ensure performance of the entire workflow, identification of potential contaminants or artifacts.
- Minimum requirements for sequencing quality and quantity as they may fluctuate (e.g., error rate, base call quality)
- Sufficient quantity and quality of specimens, leading to sufficient nucleic acid and library yields, allowing data quality and quantity. Internal controls (whole organism easily differentiated from microorganisms of interest should be picked into specimens before the nucleic extraction phase to ensure reliable results at the end of the analysis workflow.

As also outlined in the previous question, microbiome data offers a huge therapeutic potential but remains challenging to analyse ant interpret for a broad variety of reasons including its compositional structure nature leading to negative correlation bias. The majority of existing microbiome diagnostic approaches suffers from proper validation resulting in leakage or overfitting. Nevertheless it is anticipated that future development and use of machine learning models will lead to invaluable microbiome-based diagnostic or pre-screening tools.

Question: Is there already a registered diagnostic tool?

Answer: Yes, there are some registered products under development, but we're unable to name the companies. There are a lot under development.

Question: Which therapeutic areas and diseases can be, or have already been utilized the microbiome-based diagnostics?

Answer: Changes in "normal" microbiota (normality yet to be defined), have been linked with different major groups of diseases such as cancer, CNS diseases, inflammatory bowel diseases, cardiovascular disease, systemic infections, allergic diseases.

I will mainly emphasize Cancer and IBD/IBS.

Cancer: studies showed that microbiome-associated biochemical reactions can affect cancer prognosis and proliferation as well as immunotherapy reactions Furthermore, cancer treatment can alter the host microbiome due to immune-compromising activity, enhancing infection liability and consequently cancer prognosis. Cancer microbiota has also been linked to chemotherapy resistance.

IBS/IBD: evidence suggests the existence of different pathogenic factor contribution to irritable bowel syndrome and inflammatory bowel disease, Microbiome alterations indicated that microbiota dysbiosis invoked irregular immune reaction against body cells ans, resulting in autoimmune, GI tract inflammatory diseases.

In those indications where a strong link between microbiome and its alterations has been demonstrated, the use of microbiome-based diagnosis should be under consideration.

Many other indications were demonstrated to have effect on or from microbiome. We will be pleased to discuss those directly with you if needed.

Question: Considering the microbiome is influenced by many factors such as the environment, nutrition, age, and lifestyle of the host, in addition to genetics, what are the advantages and constraints of developing microbiome-based diagnostics using microbial signatures?

Answer:
Advantages:

- Small genome size of viral, bacterial and many fungal members of the human microbiota makes it possible to determine their entire genetic makeup, at least the most abundant members of a community
- Very promising for the future since more and more research identify correlations between microbial signature and health status: opens a whole new field of opportunities to better diagnose patients

Constraints:

- As previously mentioned, the identification of a microbiome-based biomarkers for disease diagnostic, prognosis, risk profiling and even precision medicine, implies the clear definition of a healthy microbiome in all different human populations BUT there is no current/established definition of an “healthy” microbiome, as the distribution of bacteria across healthy individuals fluctuates largely. While it is increasingly admitted that a diversified microbiome is often correlated with a healthy body, growing number of articles on the subject also emphasize that changes in the composition of microbiomes correlates with numerous diseases.
- Causality between microbial signatures and disease is difficult to glean, yet in some cases microbiome signatures are useful diagnostically despite having no known causal relationship with the disease or condition in question.
- The main challenge facing microbiome diagnostics is validation, as microbial signatures change in response to many types of factors, making it difficult to establish clear-cut connections between disease course / onset and the microbiome. Furthermore, we are probably still far from finding out what the microbiome should look like (eubiosis), and conversely what the opposite (dysbiosis) means. This is always an important limitation when trying to define health and disease.
- High inter variability between individuals is a challenge to overcome to produce a robust microbiome signature.

Question: Which is the best tissue sample to perform diagnosis for microbiome, stool, saliva – which one has more research available?

Answer: There are no ‘best’ tissue samples, it depends on the indication you are working on. The stool microbiome and intestinal microbiome is the most widely used, and most widely known as there is no issue regarding the quantity you can sample. You can have a lot of sample from stools. With some new providers researching the low abundant microbiome population, I think even saliva or skin may become more and more studied.

Question: Are there any methods preferred by the regulators ?

Answer: There are no preferred methods accepted by regulators. Each used genomic analysis method has pros and cons.

- Marker gene analysis – 16sRNA amplification and sequencing
  - Pros:
    - Quick, simple, and non-expensive sample preparation and analysis
    - Good correlation with genomic content
  - Cons:
    - No discrimination between no live, dead samples components
    - Technology may be subject to amplification bias
    - No/Low functional information

- Whole metagenome analysis:
  - Pros:
    - No previous knowledge of microbial community requested (phage viruses, plasmids, eukaryotes…)
    - Detection for novel gene families
  - Cons:
    - Relatively expensive, complex, and laborious sample preparation and analyses
    - Microbial signature may be contaminated by contamination from host-DNA.
    - No live, dead or active microbes' discrimination

- Metatranscriptomics analysis:
  - Pros
    - Estimation of which microorganisms in a whole community are actively transcribing when associated to marker gene analysis
    - Discriminate between active live organisms versus dormant or dead microorganisms and extracellular DNA
  - Cons:
    - Most expensive, laborious, and complex sample preparation than other detailed methods
    - Host genomic material (DNA and /or RNA must be removed
    - Requires careful sample collection and storage.

Question: You mentioned diagnostic tools already registered, could you name any companies?

Answer: GMT Science has just highlighted themselves!

Question: Given that there is no clear regulatory framework, are there any other challenges for the adoption of microbiome-based diagnostics?

Answer: Some of the challenges facing the adoption of this type of diagnostic are:

- No widely accepted consensus among physicians and patients on where and how diagnostic tools should be used along the patient journey, or even if microbiome manipulation may ultimately impact human health and treat different diseases.
  - For example, as routine tests, upon presentation of specific disease symptoms, or before and after a dietary change.

Also, as stated earlier, is the lack of clear regulatory pathways for these products.

- The lack of approved microbiome-based therapies.
- Lack of reimbursement for most microbiome-based diagnostics presents another challenge.
- Developers must create a robust evidence generation strategy detailing how they will identify and address data gaps regarding a diagnostic's value proposition.

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Question: What are the best approaches & practices to ensure performance of testing in clinical labs regarding the new genomic technologies and microbial sequence data analysis?

Answer: First, I want to introduce few notions on diagnostic biomarkers: it needs to be easily measurable, minimally invasive and cost effective.

Identification of a microbiome-based biomarker for disease diagnostic or prognosis implies the clear definition of a healthy microbiome. It needs to consider different factors known to impact the microbiome constitution. I will only point out age, lifestyle, diet, ethnicity but there are plenty of potential impacting factors.

Many challenges in microbiome research are linked to standardization, reproducibility, knowledge, and sometimes financial reasons may be raised as pointed out in a survey led by the Human Microbiome Action.

If we focused only on standardization challenges, we could highlight method standardization, sample collection and storage methods. As they are the earliest step of the workflow, any deviation/differences in those step. A single bias in those early steps may lead to high variation in final analysis.

Those challenges in standardization also impact the reproducibility, the fact that knowledge in microbiome remains difficult to capture and may explain some of the cost interrogations.

To summarize I would emphasize that microbiome research requires novel strategies for both standardization and mechanistic validation of the identified microbial gene clusters.

Human microbiota will certainly pave the road to new era in biomarker research for disease diagnosis or pharmacotherapy monitoring, revolutionizing precision medicine and individualized treatment. Currently the majority of existing microbiome diagnostic approaches suffers from proper validation resulting in leakage or overfitting. However, collaborative work will be needed to develop robust, comprehensive, and open-source databases to allow researchers and clinicians to upload, explore visualize and interpret their data, to standardize methods to be able to compare results between groups and obtain reliable results from their testing methods or device.

This question opens a longer discussion, feel free to contact us if you want to discuss further on microbiome study challenges.First, I want to introduce few notions on diagnostic biomarkers: it needs to be easily measurable, minimally invasive and cost effective.

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- It is important to keep in mind that The absence of specific guidelines can delay the approval process for companies looking to bring their pioneering products => Appropriate internal and external guidance based on expert scientific knowledge is needed to facilitate the regulatory process, bring confidence to the microbiome field and ensure the safety of patients:

- The regulatory authorities and the scientific community have already started to draft action plans to reply to this unmet guidance:
- To illustrate this point, let me give you a couple of examples from the FDA and the American Academy of Microbiology:
  - FDA
    - Issued a draft guidance with recommendations for the establishment of analytical and clinical performance characteristics for NGS-based diagnostic devices for microbial identification and the detection of antimicrobial resistance and virulence markers

    - The American Academy of Microbiology

      - recently published the outcomes of a colloquium composed of subject matter experts tasked with defining the specific challenges and establishing recommendations for the transition of NGS from research to the clinical and public health laboratory setting.

Question: What would be your advice for companies developing this innovative product?

Answer: Ensure that all the earliest steps from the sample collection are standardized and then use the same way of analyzing your samples, then you will be in a good position to identify specific biomarkers for a condition or disease.

Even if you have an amazing innovative solution in mind keep in mind to discuss with the authority, they can offer support and advice on the development. The key question is the design of every investigational study you have in mind to deliver strong robust clinical evidence.

