The proof of concept that culturomics can be superior to metagenomics to study atypical stool samples

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Dear Editor,

We read with interest the comments regarding our article [1]. We are grateful to the authors for their remarks, and we agree with the biases linked to metagenomics explaining the absence of reproducibility between the different studies about the gut microbiota repertoire and have reported this elsewhere [2–4]. Nevertheless, the aim of this study was to compare the most commonly used molecular method to explore the microbiota (pyrosequencing of 16 rRNA amplicons targeting the V6 region) used as a standard with culturomics [5]. Indeed, it would be another study to compare all the pyrosequencing techniques previously reported, which was not our goal [6].

Nevertheless, we are confident in our pyrosequencing results because, as with most of the studies, we used only one hypervariable region of the 16S rRNA amplicon, and because we chose the most hypervariable V6 region as previously described for the gut microbiota exploration [6]. In addition, we pyrosequenced the amplicons using one-half of the GS Titanium Plate, generating a reasonable total of 89,469 trimmed reads for a total of 106,000 reads. These sequences have been analyzed using both phylotypes and the operational taxonomic unit method with equivalent results, and we used willingly stringent criteria to avoid erroneous assignment. These results are consistent with the microscopic analysis showing that less than 10^4 prokaryotes per gram of stools were present. It is clear that the threshold of metagenomic

and 16S rDNA amplification cannot, because of inhibitors, go much lower than 10^3 – 10^4 bacteria per gram of stools [3].

Finally, this study is a part of the rebirth of the culture [7] that can detect a single living organism and is, therefore, indeed, more sensitive than any of the molecular techniques as reported for *Staphylococcus aureus* [5]. Nevertheless, this is the first time, because of the low number of bacteria observed in this patient, that culture yields more bacterial species than metagenomic-detected phylotypes, exemplifying the need to use a comprehensive approach based on the complementarity of the techniques rather than being blinded by the theoretical superiority of the molecular techniques.

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