

September 25<sup>th</sup>, 2015

### Point-by-point Answers to the Comments of the Editor and Referee

We thank the Editor and Reviewer for their useful suggestions, which have helped us in the improvement of the manuscript.

We modified our manuscript according to the request of the Editor and Referee.

We added the acknowledgments just before the references section (page 14): “

#### « Acknowledgments

The authors thank Ms. Virginia Eskridge (Pittsburgh, Pennsylvania, USA) for her invaluable assistance in preparing this manuscript. »

*1) In the introduction, the authors discuss the several potential techniques providing the metabolomics analysis. It would be important to have a comparative table summarizing the advantages and disadvantages of the different techniques at a glance*

We added in the manuscript the **table 1** (in the introduction, page 5, line 137) to provide rapid view with advantages/strength or disadvantages/weakness of both technics:

**Table 1: Advantages and Disadvantages of NMR and Mass spectroscopy.**

	NMR	MS
<b>Sensitivity (detection limit)</b>	Usually micromolar (nanomolar with cryosonde)	picomolar
<b>Reproducibility</b>	High	Low
<b>Detected Metabolite</b>	Non targeted approach Detect metabolite Only if contain proton on the molecule	Targeted approach Need specific preparation to well detected some metabolites (Lipids...)
<b>Metabolite identification</b>	Easy, using 1D and/or 2D spectra and databases	More difficult, need sometime complementary analysis
<b>Number of know identifiable metabolites</b>	More than 200	More than 4000
<b>Sample</b>	Simple preparation (minimal add	Preparation more complex

	of D2O, Buffer and sometime reference) Non destructive method Need 400 microliters (less than 10 microliters with microprobe)	(protein extraction...)  Destructive method Need few microliters
<b>Type of sample</b>	Liquid (urine, whole blood, serum, plasma...) and intact tissue	Liquid
<b>Cost of machine</b>	Very High	High
<b>Cost of sample analysis</b>	Lower	Higher
<b>Signal acquisition time</b>	5 to 15 min for 1D spectra More longer for 2D spectra (few hours)	Around 10 minutes

2) *The authors do not discuss the results in terms of superiority compared with standard analysis...How does metabolomics compare with the standard analytical approach in these examples ?*

Some studies compared metabolomics approach and usual biomarkers. For instance and as noted page 11, Wen and coll. find that diagnostic performance of metabolomics approach to diagnose cholangiocarcinome was better than conventional markers (Wen and coll., J Hepatology, 2010 Feb;52(2):228-33).

Nevertheless, that will be confusing to oppose conventional and metabolomics approach. More than superiority, metabolomics approach is a complementary approach helping to diagnose or follow treatment efficacy.

We added in the manuscript :

“Metabolomics approach showed sometime better diagnosis performance than usual biomarkers. Nevertheless, at the beginning, metabolomics approach should be considered more than complementary tool, helping clinician to diagnose disease, evaluate disease stage or follow treatment that substitution technic.”

3) *How much is the cost of a single analysis?*

NMR based metabolomics approach is a rapid and cost-effective screening tool. In a single experiment, hundred of metabolites could be detected and quantified (or semi-quantified). The cost of the analysis is very low in France (less than \$20 per sample, and around \$100 with interpretation). In USA, the cost (using company like metabolon®, North Carolina : [www.metabolon.com](http://www.metabolon.com)) is higher around \$1000/sample with analysis and interpretation. We didn't find in the litterature cost analysis or comparaisn with

conventional analysis. We added in the comparative table (table 1) a line with the “range” of cost (low/high).

4) *How long does it take from bed to benchside and back?*

Signal acquisition is very fast (10 to 20minutes in 1D and around 4hours in 2D added in table 1). In a near future, complete results will be available in less than 1 day. We add in the manuscript (in the part: current limitation of metabolomics and future clinical application) the follow sentence (p13 line 349).

“Only after that and probably in a near future, results from metabolomics approach (with analysis and interpretation) will be available to the clinician in less than 1 day.”



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