Reviewer's report

Title: Minisequencing mitochondrial DNA pathogenic mutations

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Reviewer: Veronique Paquis-Fluckinger

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« MINISEQUENCING MITOCHONDRIAL DNA PATHOGENIC MUTATIONS »

The authors used a minisequencing multiplex strategy to detect selected mtDNA pathogenic mutations in 15 patients presenting with Leber phenotype (11/14) or neurological symptoms (4/15). One assay, including 13 amplicons, allows the screening of 25 different mtDNA mutations. The authors have detected 9 m.11778G>A and 2 m.14484T>C mutations which were then confirmed by conventional sequencing.

The approach is interesting, mainly for routine diagnosis laboratories searching for a set of deleterious mutations. Nevertheless, there are several mistakes or imprecisions, and the quality of the manuscript needs to be highly improved.

Major comments:

1. In the background section, the paragraph which describes the different methods available for mtDNA analysis is not complete. The difference between the methods allowing a complete screening of mtDNA and those which only detect selected mutations is not clearly exhibited. Other techniques than sequencing should be listed.

2. For a better comprehension, a paragraph should be added to give an overview of minisequencing method using SNaPshot kit.

3. The nomenclature is false and need to be corrected (m.11778G>A)

4. Figure 2, the sequence electrophoregrams corresponding to SNaPshot assays must be added for each pattern. Furthermore, the different percentages of heteroplasmy obtained by mixing 2 types of mtDNA must be indicated.

5. Among the 25 selected mutations, several are frequently identified (m.3243A>G, for example). It should be interesting to show that this method can detect other mutations than m.11778G>A and m.14484T>C variations.

6. There are a lot of grammatical and spelling mistakes (minisequening,
minisiquencing…)

Minor comments
1. The pages of the manuscript should be numbered
2. Page 5, lane 24, Table 1 should be replaced by Table 2
3. Page 6, lanes 12-13, « primers are sizes (?) between 19 and 67 bps », should be replaced by « the length of primers is between 25 and 76 bp »
4. Page 6, lanes 14 and 17, Table 2 should be replaced by Table 3
5. Page 6, lanes 21 and 22, SAP should be replaced by ExoSAP-IT.
6. Page 7 : What do the authors mean by «a clear suspicion of mtDNA disease »?
7. Page 10, lanes 23, 86-197 should be replaced by 89-196 as in Table 2

In conclusion : Acceptance is not possible until the authors have responded to the major comments