Reviewer’s report

Title: Safety and performance analysis of acriflavine and methylene blue for in vivo imaging of precancerous lesions using fibered confocal fluorescence microscopy (FCFM): an experimental study

Version: 4 Date: 6 December 2014

Reviewer: Antoni Rosell

Reviewer’s report:

The authors present a comprehensive and elegant experimental study that compares the diagnostic capability and discriminative power, as well as the genotoxicity, of two exogenous fluorophores used topically to diagnose preneoplastic epithelial lesions in vivo by fibered confocal fluorescence microscopy.

• Major Compulsory Revisions

None

• Minor Essential Revisions

Text:

p. 3 line 21: describe what can be seen when using the 660nm laser stimulation without exogenous fluorophores

p. 7 line 8: ImageJ (together), add that it’s an open software and specify source (www.imagej.nih.gov)

When comparing cellular diameter and density of different premalignant lesions, it would be nice to show tendency a part of confirming there’s a difference between groups.

In the discussion, I’d appreciate some lines to describe availability, costs, storage requirements and potential effects for health workers of both fluorophores. The GHS (Globally Harmonised System) of classification and labelling of chemicals adds the H340 and H350 for acriflavine. This can be an issue when considering the clinical practice.

The conclusion paragraph needs to be stronger and more convincing, like “The experiment demonstrates equal of even better diagnostic power for methylene blue in relation to acriflavine, without being genotoxic in the tested conditions. For that reason…….”

Figures:

- Figure 2. Comet or COMET?
- Figure 3 and 7. Better resolution would be welcome
- Figure 6. Clearer if not using 3D graphs

• Discretionary Revisions
  Figure 1. Consider focusing the image to the hamster cheek pouch without showing the rat’s paws
  p. 3 line 7: specify that is a contact mode test
  p. 4 line 4: I’d rather write the objectives in consecutive sentences
  p. 6 line 7 w/v: consider specify that it corresponds to mass/volume
  p.10 line 2: not need to abbreviate Se and Sp if used only once

**Level of interest:** An article of outstanding merit and interest in its field

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

'I declare that I have no competing interests'