Reviewer's report

Title: Why are results on the prognostic value of the methylation status in colon cancers conflicting? The role of the preservation method

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Reviewer: Shuji Ogino

Reviewer's report:

This is a great study which compared DNA methylation results from FFPE vs. frozen tissue. This is a substantial contribution. This comparison has not systematically been done, although bisulfite method assessed is only Qiagen kit method and no other method has been examined. This paper provides very useful data on Qiagen kit which is widely used. Thus, this paper is of particularly high interest.

Major compulsory revisions:

One critical reference is missing. Data presented are comparable to LINE-1 methylation precision data by Irahara et al. (J Mol Diagn 2010). This reference should be compared to the authors' data.

There is a critical difference between Irahara et al. (and Ogino et al. J Mol Diagn 2006) and this current study in terms of bisulfite methods. As the authors discussed, Ogino et al. actually showed that MLH1 methylation and MGMT methylation data from FFPE correlated well with loss of expression by immunohistochemistry. So the authors' data simply indicate that Qiagen kit is not suitable to FFPE.

Actually, we recently performed a comparison study (on bisulfite methods) which compared manual method (Ogino et al. J Mol Diagn 2006) with Qiagen EpiTect Bisulfite kit, using FFPE. Indeed, Qiagen kit method not only resulted in more failed cases than the manual method, but also yielded considerably higher LINE-1 methylation level than the manual method. We have not published data. But the authors can quote our findings as personal communication with me.

MLH1 amplicon of 181 bp is definitely not good for bisulfite-based methylation assay.

Page 15. bottom sentence “... primers have to hybridize out of the CpG islands,...” implies methylation-independent PCR. It is different from methylation-specific reaction. Please see Sepulveda et al. (J Mol Diagn 2009) for comparing these two different strategies for methylation analysis.

There are also numerous associations which have been discovered and confirmed by the use of FFPE. For example, in the quoted reference, Shima et al. showed a strong relationship between MGMT methylation and loss of
expression. Thus, the authors’ recommendation of exclusive use of frozen tissue for methylation research is an overstatement.

Abstract, Discussion and Conclusion need to be rewritten.

The main point is that the authors beautifully assessed Qiagen EpiTect kit and it’s questionable validity on FFPE, but the authors did not assess any other bisulfite methods including some manual method.

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

**Quality of written English:** Acceptable

**Statistical review:** Yes, and I have assessed the statistics in my report.

**Declaration of competing interests:**

'I declare that I have no competing interests'