Universidade Federal de São Paulo
Departamento de Morfologia e Genética
Disciplina de Genética

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Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 2198-Review.doc).

Title: Reference genes for quantitative RT-PCR data in gastric tissues and cell lines

Author: Fernanda Wisnieski, Danielle Queiroz Calcagno, Mariana Ferreira Leal, Leonardo Caires dos Santos, Carolina de Oliveira Gigek, Elizabeth Suchi Chen, Thaís Brilhante Pontes, Paulo Pimentel Assumpção, Mônica Barauna de Assumpção , Sâmia Demachki, Rommel Rodríguez Burbano, Marília de Arruda Cardoso Smith

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 2198

A - Answers to the reviewer:

1) Why did choose DNMT1 as the candidate gene in this study?

DNMT1A was chosen as the candidate gene in this study because it has been studied in our laboratory and mainly because its expression was evaluated by using the same RT-qPCR runs for assessment of the candidate reference genes expression levels. Consequently, all samples were analyzed in the same run for both target gene and reference genes, which avoid technical run-to-run variation between the samples.

2) You have described that the probes were purchased from Life Technologies, but you did provided the detailed information about them, such as, the sequences.

In this study, we used TaqMan® inventoried Assays-on-Demand probes from Life Technologies. The identification of each assay (primers and probe) used is described on table 2, for example the assay Hs03023943_g1 was used for the evaluation of ACTB expression (see link http://bioinfo.invitrogen.com/genome-database/details/gene-expression/Hs03023943_g1). Unfortunately the company does not provide the sequence of inventoried probes. The description of the probes in material and methods item was improved.

3) For the stability comparisons of the candidate reference genes, you analyzed the data with four software. So, What is their standard value or threshold for cut-off?

All programs used in this study to compare the stability of the candidate reference genes estimate the variation of gene expression. Since ideal reference genes should not vary in the samples under investigation, the selection of

the best reference gene is based on the smallest value of variation for each analysis program. As the stability values depend on the variation of each sample, there is not a standard value or threshold for cut-off.

4) Where are the Figures? I cannot see them.

The figures were attached as individual files when resubmitted the revised paper, as recommended at http://www.wjgnet.com/esps/ModifyManuscript.aspx?UserId=pNGQswk9qhuIr8Tkl5ygJ%2fBufbjsmaBnCpm%2biaaaU9c%3d&id=NOHpqWi1RNzXFEYB%2bdP17w%3d%3d&UserNumId=. To avoid the same problem, I attached the figures bellow.

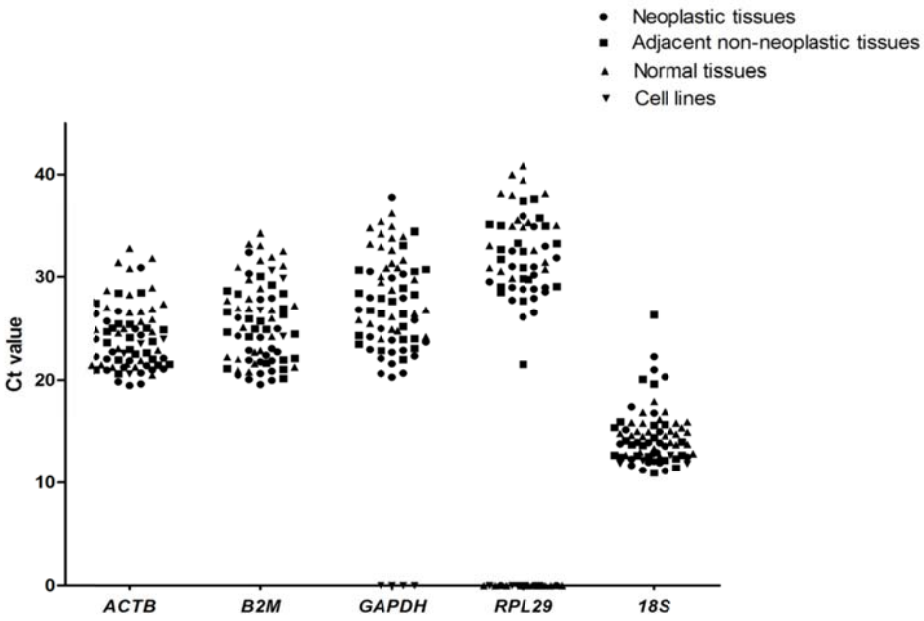

Figure 1 **Expression level of five candidate reference genes detected by quantitative real-time polymerase chain reaction.** A lower cycle threshold (Ct) value indicates higher gene expression.

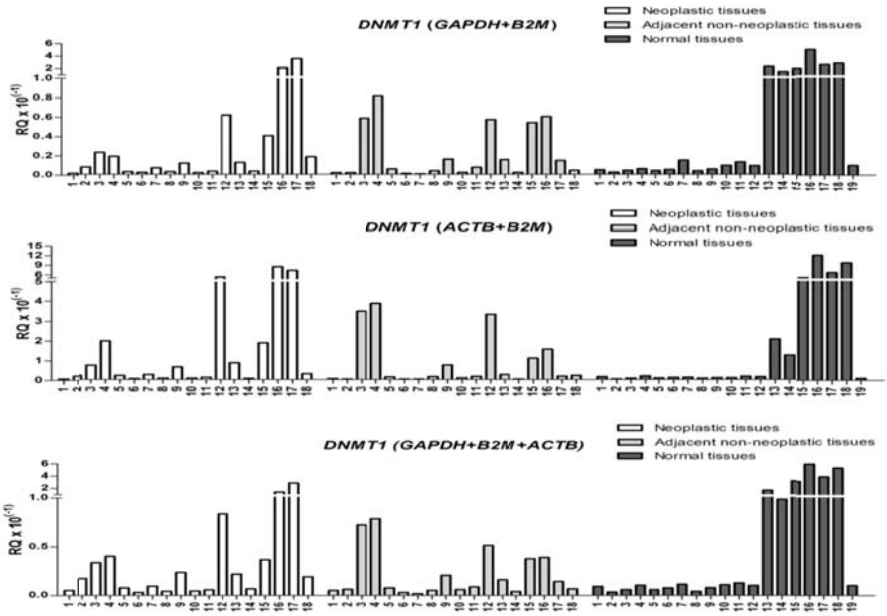

Figure 2 Relative quantification of *DNMT1*, as normalized by *GAPDH+B2M*, *ACTB+B2M*, and *GAPDH+B2M+ACTB* in gastric tissues.

B - The manuscript was improved (with marks in green in the body of the text)

1) In the Material and methods, and Introduction itens, the sentence was changed, where …"Twenty-one matched pairs of adjacent non-neoplastic and neoplastic gastric tissues…" was replaced by "… Twenty-one matched pairs of neoplastic and adjacent non-neoplastic gastric tissues…";

2) In material and methods item, a better description of the TaqMan® probes was included;

3) In Material and methods item, ND-100 was corrected by ND-1000;

4) At the note under the Table 1, AJJC was corrected by AJCC.

Thank you again for publishing our manuscript in the *World Journal of Gastroenterology*.

Sincerely yours,

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