Author's response to reviews

Title: Assessing the Contribution of Herpes Simplex Virus DNA Polymerase to Spontaneous Mutations.

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PDF covering letter
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Dear Editor:

Please accept the revised manuscript entitled "Assessing the Contribution of Herpes Simplex Virus DNA Polymerase to Spontaneous Mutations" by Duffy et al. for consideration of publication BioMedCentral Infectious Diseases.

All of the reviewers suggestions have been addressed within the modified manuscript.

Reviewer #1: Dr. Sandra Weller

Major Comments

1. The significance of percentages needs to be presented in Table 2 data to allow accurate interpretation of data.

   The Materials and Methods have been reworded to provide additional clarity regarding experimental design. Three individual experiments (data in Table 2) were performed with the test plasmids, each with an approximate total of 300,000 colonies evaluated. The mutation frequency percentages shown in Table 2 were all normalized relative to the HP66 control sample (i.e., 0.12% was subtracted from the test value). The triplicate test values for HP66 were 0.11, 0.13 and 0.12%, with an average of 0.12%.

   Providing the total number of colonies for each test virus within each experiment does not provide additional value to the reader, as each experiment evaluated between 280,000 to 305,000 colonies.

   The reviewer also requested an explanation for the high number of colonies in HP66 transfection. The process of viral infection is known to be mutagenic, and this reference and statement has been added to the text. Since HP66 is incapable of replication in Vero cells, this virus represents a control for mutagenic activity contributed by viral infection.

2. The statement pg 13, line 8 is misleading.

   We have modified this statement according to the reviewer's suggestions.

3. Were the S1 nuclease experiments properly controlled?

   Two separate plasmid preparations were examined for sensitivity to S1 nuclease, to ensure that the degree of nicking during the plasmid purification process did
not contribute to the differential in sensitivity. The M&M have been modified to address this concern.

4. *Do the type 2 and type 1 tk genes differ in GC or homopolymer content?* This is an excellent point raised by the reviewer, and the Discussion section includes a comment on the differences between these viral sequences, and the potential contribution to formation of different secondary structures.

Furthermore, we did try to generate an HSV-1 recombinant virus containing the type 2 tk gene, but were unsuccessful.

**Minor Comments**
All minor comments have been modified directly as requested by the reviewer.

**Reviewer #2: Dr. Ella Swierkosz**

**Major Comments**

1. *Reference for first statement was not included.*
The appropriate reference has now been cited within the text.

2. *Describe pUC19.*
This plasmid source is now described in M&M.

3. *Why weren't recombinant viruses obtained for SC16 and others?*
We reported data on all recombinant viruses we were able to generate. Unfortunately, attempts to generate SC16 were unsuccessful.

The typos have been modified throughout the text.

5. *Define antimutator virus.*
The PAAr5 antimutator virus with low error rate has been clarified within the text.

6. *The syntax p12, line 13 is poor.*
We have modified this statement for improved clarity and grammar.

7. *Please expand significance of S1 nuclease assay.*
DNA which can form such secondary structures have been shown to be involved in formation of triplex DNA, recombination or enhanced errors. Polymerases
often have difficulty replicating through highly ordered secondary structures, which often leads to slippage and the consequent formation of additional errors. This has been clarified within the text.

Thank you for consideration of publication

Robert Sarisky