INTRODUCTION

The oral polio vaccine (OPV) is a live attenuated poliovirus vaccine made of serotypes 1, 2 and 3 Sabin strains. Easily administered by mouth, OPV vaccination can however result on rare occasions in a case of vaccine-associated paralytic polio in recipients, due to the accumulation of revertants during manufacture of the vaccine. Monitoring consistency of genetic composition of OPV is therefore an important part of its quality control. This genetic stability is routinely checked by a molecular assay "Mutant Analysis by PCR and Restriction Enzyme Cleavage" (MAPREC) used to quantify neurovirulent revertants in the viral genome. For the Sabin 3 strain, a U→C mutation at nucleotide 472 was shown to be directly related with neurovirulence and a vaccine batch fails the test if its proportion of C revertants (%472C) exceeds 0.9% [1]. However MAPREC suffers from some shortcomings including the need for radioactive isotopes and various technical challenges at multiple steps of its complex protocol. As the French Officinal Medicines Control Laboratories (OMCL), we routinely perform quality control and batch release of human vaccines in addition to the manufacturer’s ones prior to marketing batches in Europe. In this context we developed an NGS-based assay as an alternative method to MAPREC serotype 3 for monitoring genetic consistency of OPV vaccines.

METHODS

A 697bp PCR amplicon [figure 2] covering the 472 nucleotide position was used for library preparation with Nextera XT DNA Library Preparation Kit. Reads were generated on an Illumina MiSeq sequencer with a minimum sequencing depth 10 000X to obtain significant revertant counts to 0.1% level (corresponding to 10 readings /10 000) on paired-end reads of 101 nt in length. Data analysis was conducted with CLC Genomics Workbench software. Reads were aligned to the vaccinal strain reference genome with the Low frequency Variant Detection algorithm to quantify the proportion of revertants at position 472 (%472C).

RESULTS

Accuracy

Accuracy was verified on pass (WHO 96/572) and fail (WHO 96/578) international MAPREC viral reference preparations. %472C results obtained on 7 independent sequencing runs were within MAPREC historical limits for both preparations (figure 3), indicating that the preparations could be used as quality controls in a control chart.

CONCLUSION

These results show that our NGS-based assay can be used as an alternative method to the conventional MAPREC type 3 assay. A WHO collaborative study will be set up in 2017 in order to validate NGS for monitoring molecular consistency of OPV vaccines so it can be introduced for route regulatory use.