NEXT GENERATION SEQUENCING (NGS) FOR MONITORING ULTRA-LOW LEVEL OF GENETIC MUTATION AS A QUALITY CONTROL TEST OF LIVE POLIOVIRUS VACCINE

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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%** (Figure 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 Official 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.

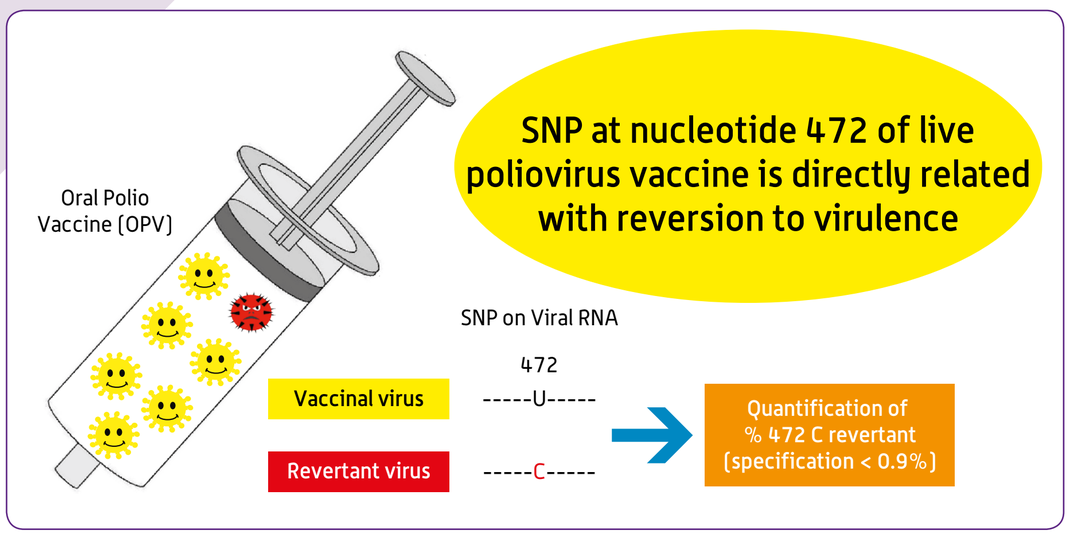

Figure 1: U→C mutation at nucleotide 472 of live poliovirus vaccine serotype 3 directly related with neurovirulence

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).

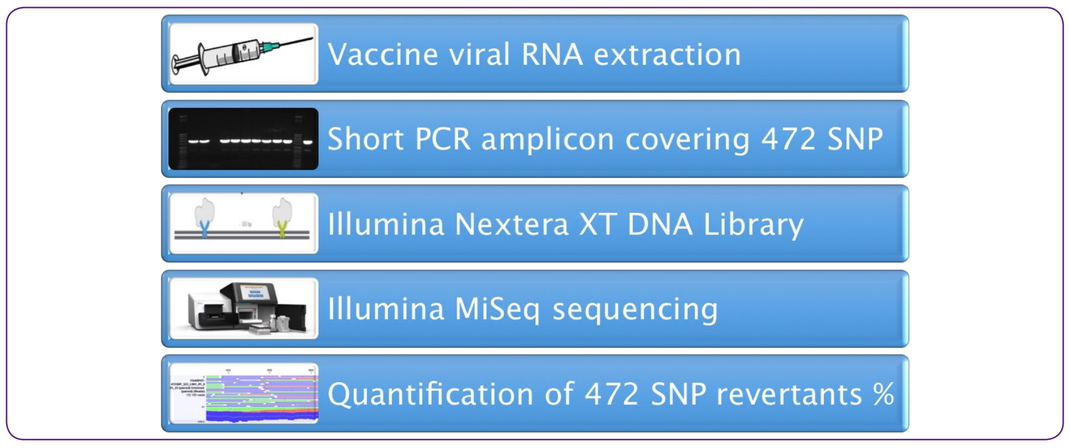

Figure 2: Protocol for quantification of revertants at position 472 by NGS

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.

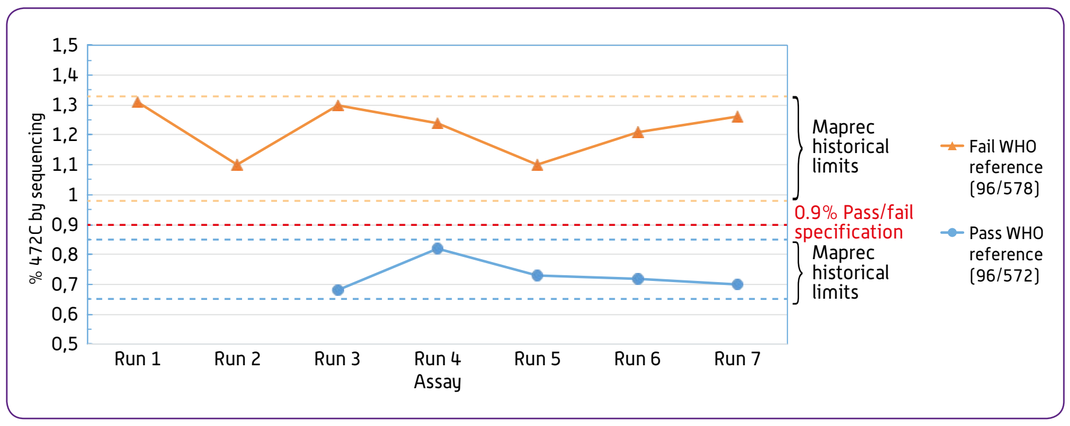

Figure 3: Follow-up of fail WHO 96/572 and pass 96/578 viral references % 472C reversion

Linearity

Linearity of the sequencing step was confirmed on artificial plasmid PCR amplicons mixtures in the range 0.25-2% 472C covering the 0.9% vaccine specification (figure 4). Comparison between experimental/theoretical results on 2 independent experiments showed a good correlation (slope ≈1).

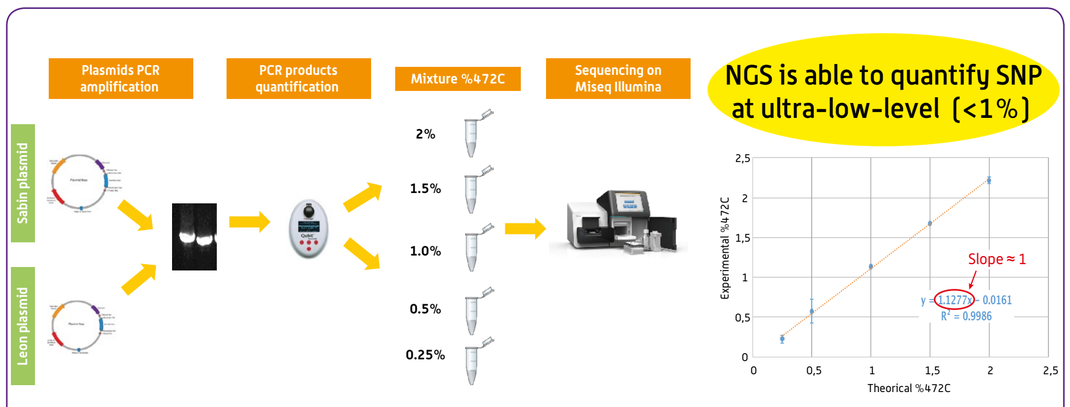

Figure 4: Linearity of the sequencing step on artificial plasmid PCR amplicons mixtures in the range 0.25-2% 472C (mean and standard deviation on 2 independent experiments)

Correlation between conventional MAPREC method and sequencing

Methods correlation was studied on a panel of 11 vaccine production materials (monovalent and working seed lots) in the range 0.30-0.99 % 472-C. A statistically significant linear relation was obtained between both methods ($r = 0.96$, $p < 0.0001$, confidence level 95%) (figure 5). The estimated slope does not significantly differ from 1 (0.708 - 1.081) and the estimated y-intercept (0.056 - 0.211) does not significantly differ from 0, indicating equivalence between conventional MAPREC method and sequencing.

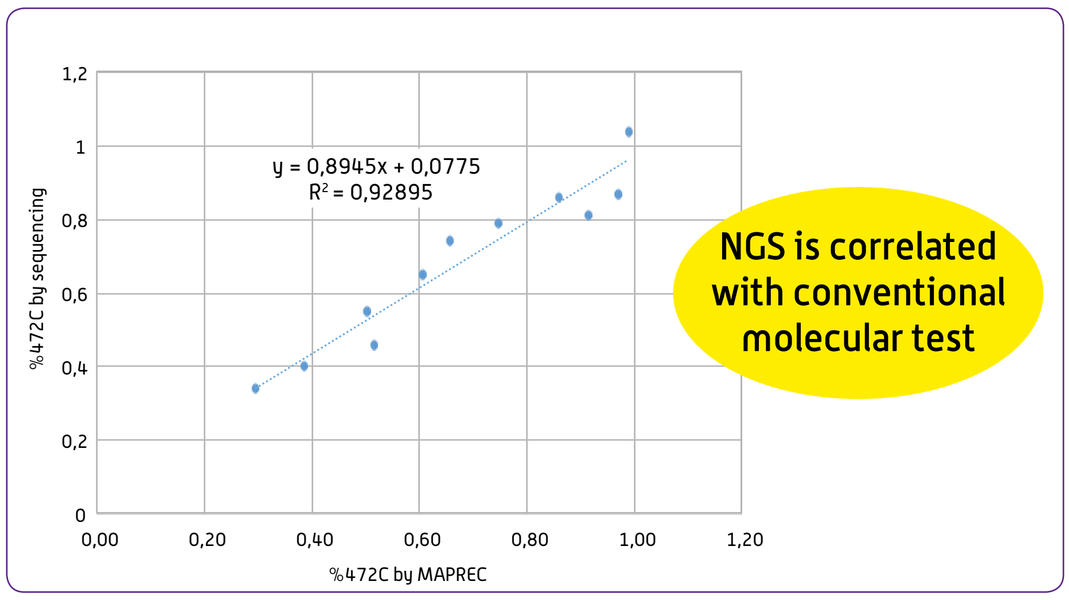

Figure 5: Correlation between conventional MAPREC method and sequencing on a panel of vaccine production materials

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 routine regulatory use.