



**Figure S2. Analytical validation of the VHIO-YWBC protocol for NGS.** (A) Three technical replicates were performed for each commercial standard used, SeraSeq 1% MAF and SeraSeq 0.5% MAF. Expected allelic frequency (MAF%) is in red, in blue the values detected by the VHIO-YWBC protocol. Median of the triplicates, with its 95% interval confidence (CI) was also plotted. (B) Accuracy of the detected MAF % was analyzed as  $(X-X_0/X_0)$ , where X is the detected value,  $X_0$  is the expected value (theoretical) and  $X-X_0$  the absolute error. Median of the triplicates and 95% CI was plotted. Theoretical 100% accuracy is depicted with a dotted line at 0 value. (C) Analytical sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated for each triplicate and the average for each condition. True positive (TP) were considered those variants detected by SeraSeq and VHIO-YWBC protocol, True Negatives (TN) were considered those variants not detected by any method, False Positives (FP) were those variants only detected by the VHIO-YWBC method; False negatives (FN) were the variants only detected by SeraSeq.