The AmpFire® HPV Screening 16/18/HR assay has been assessed according to the study design as in Figure 1 and the laboratory workflow in Figure 2. A panel of study samples was compiled from a biobank containing residual material remnant after cervical cancer screening. As instructed in validation guidelines, 30% of the specimens were hrHPV+ determined by the RIAOL-qPCR assay. General agreement and Cohen’s kappa (κ) were computed. The assay should demonstrate a lower 95% CI bound around the general reproducibility exceeding 87% and a κ ≥ 0.50. The literature search targeted references included in previous review—completed with references published until July 4, 2023. Relevant data from studies were extracted to estimate the relative clinical accuracy and to assess the non-inferiority compared to a standard comparator. Ninety-five percent CIs were calculated for matched proportions and statistical significance was set at p<0.05. These two criteria are fulfilled when the left 90% CI around the relative sensitivity is ≤0.90 and the relative specificity is ≥0.98.

RESULTS

Validity of the sample study set: In 555 of 556 samples, the beta-globin gene was amplified in all the three testings. One sample was excluded from analysis, resulting in 555 valid samples.

Overall hrHPV reproducibility: The testing 1 vs 2 comparison revealed 96.4% intra-laboratory reproducibility (95% CI: 94.5% – 97.8%, κ = 0.920) (Table 1). The testing 3 vs 1 comparison revealed 95.3% inter-laboratory reproducibility (95% CI: 92.3% – 96.9%, κ = 0.897) (Table 1).

Genotype-specific reproducibility: For HPV 16, 18 and the 12 other hrHPV types, the general agreement ranged from 95.3% to 99.5% with x between 0.821 and 0.903 (intra-laboratory), and from 95.3% to 99.9% with x between 0.891 and 0.940 (inter-laboratory) (Table 2).

Literature review: One study with relevant data was found: Chinese multi-centre screening trial—completed with references published until July 4, 2023. Relevant data from studies were extracted to estimate the relative clinical accuracy and to assess the non-inferiority compared to a standard comparator. Ninety-five percent CIs were calculated for matched proportions and statistical significance was set at p<0.05. These two criteria are fulfilled when the left 90% CI around the relative sensitivity is ≤0.90 and the relative specificity is ≥0.98.

CONCLUSIONS

The reproducibility of the AmpFire® assay was assessed according to the study design as in Figure 1 and the laboratory workflow in Figure 2. A panel of study samples was compiled from a biobank containing residual material remnant after cervical cancer screening. As instructed in validation guidelines, 30% of the specimens were hrHPV+ determined by the RIAOL-qPCR assay. General agreement and Cohen’s kappa (κ) were computed. The assay should demonstrate a lower 95% CI bound around the general reproducibility exceeding 87% and a κ ≥ 0.50. The literature search targeted references included in previous review—completed with references published until July 4, 2023. Relevant data from studies were extracted to estimate the relative clinical accuracy and to assess the non-inferiority compared to a standard comparator. Ninety-five percent CIs were calculated for matched proportions and statistical significance was set at p<0.05. These two criteria are fulfilled when the left 90% CI around the relative sensitivity is ≤0.90 and the relative specificity is ≥0.98.

CONFLICTS OF INTERESTS

The authors declare that they have no personal conflict of interests. This study is an extension of the VALCENT (VALIDation of HPV Genetyping tests) project in the framework of validating new HPV assays. VALCENT is an independent researcher-led project where manufacturers can have their HPV assays evaluated, under the condition that they provide equipment, kits and other costs for laboratory work and statistical analysis. Manufacturers cannot influence publication of manuscripts. Dkt8 and Y1 are employed by AML (Antwerp, Belgium), one of the HPV National Reference Centres, a private lab performing routine cervical cytology and HPV testing.