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Completing the international validation status of the AmpFire® HPV Screening 16/18/HR assay

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INTRODUCTION

Currently, there are >250 commercially available HPV assays but most have not been evaluated with tracks in the peer-reviewed literature[1]. Only clinically validated assays should be used in screening programmes. International guidelines for validation of new HPV assays involve demonstration of non-inferior clinical sensitivity and specificity relative to a standard comparator test as well as demonstration of sufficient intra- and inter-laboratory reproducibility.

So far, the AmpFire assay has been evaluated for the clinical accuracy, and the assay's reproducibility (within a laboratory and between laboratories) was not assessed yet.

AIM

We investigated the AmpFire® HPV Screening 16/18/HR assay [Atila, Sunnyvale, California, US] (abbreviated as AmpFire assay) for its **reproducibility** within a laboratory and between two laboratories. The evaluated AmpFire assay targets HPV16, HPV18 and an aggregate of 12 other hrHPV types (HPV31/33/35/39/45/51/52/56/58/59/66/68), but does not target HPV53.

Additionally, we performed a **literature review** of published data regarding the assay's performance.

CONCLUSIONS

- The AmpFire® HPV Screening 16/18/HR assay shows excellent intra- and inter-laboratory reproducibility not only for the hrHPV identification but also for the identification of HPV16 and HPV18 separately and for the aggregated 12 other hrHPV types.
- Therefore, we conclude that the AmpFire assay fulfils the third criterion of the international validation guidelines.
- The large Chinese study[4] compared the clinical accuracy of a prototype AmpFire assay - that targeted also HPV53 - with a validated comparator. Including HPV53 in the cocktail of targeted types was responsible for inferior specificity of the AmpFire assay. By removing HPV53, non-inferior specificity of the AmpFire® HPV Screening 16/18/HR assay could be demonstrated.

METHOD

The reproducibility of the AmpFire assay has been assessed according to the study design as in Figure 1 using 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 RIATOL-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[2]. The literature search targeted references included in previous review[3] – 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.

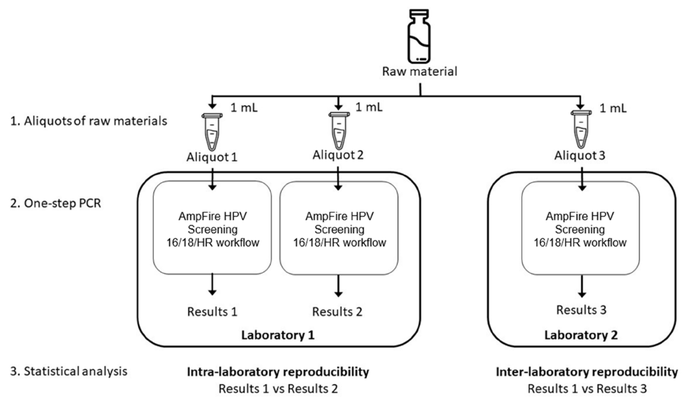

Figure 1. Reproducibility study design.

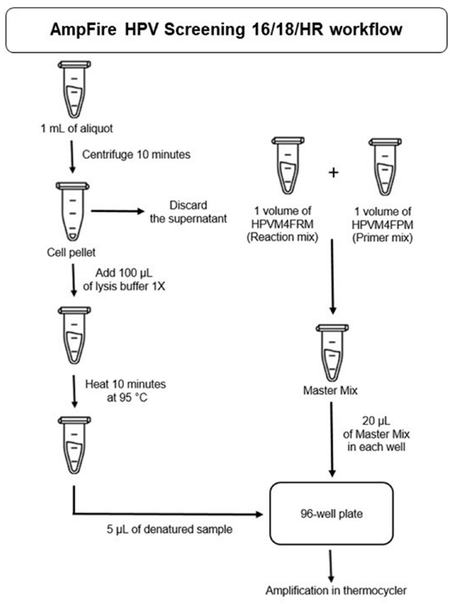

Figure 2. Laboratory workflow of the AmpFire® HPV Screening 16/18/HR assay.

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: 93.2% – 96.9%, κ = 0.897) (Table 1).

Genotype-specific reproducibility: For HPV16, HPV18 and the 12 other hrHPV types, the general agreement ranged from 95.9% to 99.5% with κ between 0.821 and 0.903 (intra-laboratory), and from 95.3% to 99.8% with κ between 0.891 and 0.940 (inter-laboratory) (Table 2).

Literature review: One study with relevant data was found: Chinese multi-centre screening trial[4] which evaluated AmpFire assay version targeting also HPV53 was compared with Cobas 4800 (Roche). The relative sensitivity for CIN2+ was 1.034 (95% CI: 0.961 – 1.113) and the relative specificity for <CIN2 as 0.997 (95% CI: 0.993 – 1.001), both with a non-inferiority $p<0.001$. The relative sensitivity for CIN3+ was 1.000 (95% CI: 1.000 – 1.000, non-inferiority $p=0.021$). The specificity criterion was fulfilled when excluding single HPV53 infections from analyses.

Intra-laboratory reproducibility

		Testing 1 in Lab1: Positive	Negative	Total	
Testing 2 in Lab1	Positive	181	13	194	Reproducibility: 96.4% (95% CI: 94.5% - 97.8%)
	Negative	7	354	361	
	Total	188	367	555	Kappa: 0.920

Inter-laboratory reproducibility

		Testing 1 in Lab1: Positive	Negative	Total	
Testing 3 in Lab2	Positive	179	17	196	Reproducibility: 95.3% (95% CI: 93.2% - 96.9%)
	Negative	9	350	359	
	Total	188	367	555	Kappa: 0.897

Table 1. Intra- and inter-laboratory reproducibilty: overall hrHPV results.

HPV type	-[a]/-[b]	+[a]/+[b]	-[a]/+[b]	+[a]/-[b]	General agreement (95% CI)	Kappa
Intra-laboratory reproducibility						
HPV 16	530	18	3	4	98.7% (95% CI: 97.4% - 99.5%)	0.831
HPV 18	545	7	2	1	99.5% (95% CI: 98.4% - 99.9%)	0.821
Other hrHPV	371	161	13	10	95.9% (95% CI: 93.8% - 97.4%)	0.903
Inter-laboratory reproducibility						
HPV 16	530	22	3	0	99.5% (95% CI: 98.4% - 99.9%)	0.933
HPV 18	546	8	1	0	99.8% (95% CI: 99.0% - 100.0%)	0.940
Other hrHPV	369	160	15	11	95.3% (95% CI: 93.2% - 96.9%)	0.891

Table 2. Intra- and inter-laboratory reproducibilty: gennotype-specific results. [a]: Testing 1. [b]: Testing 2 for intra-laboratory reproducibility and testing 3 for inter-laboratory reproducibility.

CONFLICT OF INTERESTS

The authors declare that they have no personal conflict of interests. This study is an extension of the VALGENT (VALidation of HPV GENotyping tests) project in the framework of validating new HPV assay[27]. VALGENT is an independent researcher-induced research project where manufacturers can have their HPV assays evaluated, under the condition that they provide equipment, kits and cover costs for laboratory work and statistical analysis. Manufacturers cannot influence publication of manuscripts. DvdB and YT are employed by AML (Antwerp, Belgium), one of the HPV National Reference Centres, a private lab performing routine cervical cytology and HPV testing.

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