Validation of ATILA AmpFire HPV16/18/HR

according to Meijer criteria

Criteria 1: At least 90% of the sensitivity for CIN2 + of an established and validated HPV test and at least 98% of the specificity for CIN2 + of an established and validated HPV test in women over the age of 30 years or older.

Non-inferiority vs comparator assay (p-value for non-inferiority test should be lower than 0,05)

Relative sensitivity for CIN2+ cases

	Cobas HPV positive	**Cobas HPV negative**
ATILA AmpFire HPV pos	69	0
ATILA AmpFire HPV neg	2	6

	Result	**Meijer criteria**
Test-statistic	2,017529	
p-value non-inferiority	0,02182	< 0,05
Clinical sensitivity ATILA AmpFire HPV	89,6%	
Clinical sensitivity Cobas HPV	92,2%	
Relative clinical sensitivity	0,97	>= 0,90

P-value is lower than 0,05 meaning non-inferior vs comparator assay Cobas4800 HPV.

Relative specificity for < CIN2 cases (HPV53 included)

	Cobas HPV positive	**Cobas HPV negative**
ATILA HPV pos	456	139*
ATILA HPV neg	74	3969

*62/139 samples: HPV53

	Result	**Meijer criteria**
Test-statistic	1,17	
p-value non-inferiority	0,120907	< 0,05
Clinical specificity ATILA AmpFire HPV	87,2%	
Clinical specificity Cobas HPV	88,6%	
Relative clinical specificity	0,98	>= 0,98

P-value is higher than 0,05 meaning inferior vs comparator assay Cobas4800 HPV. This is related to the fact that ATILA HPV AmpFire assay detects also HPV53 which is not detected by Cobas4800 HPV assay.

The group of samples detected as positive with ATILA HPV AmpFire but negative with Cobas 4800 HPV (n=139) includes 62 HPV53 samples.

Relative specificity for < CIN2 cases (HPV53 excluded)

	Cobas HPV positive	Cobas HPV negative
ATILA HPV pos	456	77
ATILA HPV neg	74	3969

	Result	Meijer criteria
Test-statistic	5,79	
p-value non-inferiority	0,000000004	< 0,05
Clinical specificity ATILA HPV AmpFire	88,4%	
Clinical specificity Cobas HPV	88,4%	
Relative clinical specificity	0,999	>= 0,98

When excluding the HPV53 samples, relative clinical specificity equals 1 meaning that ATILA HPV AmpFire is perfect aligned with the comparator assay Cobas4800 HPV.

P-value is quasi 0 meaning perfect, non-inferior vs comparator assay Cobas4800 HPV.

Criteria 2: At least detection of high-risk HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68

ATILA HPV 16/18/HR detects HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68

Criteria 3: CE marking

ATILA AmpFire HPV16/18/HR is a CE-marked IVD.

Criteria 4: inter- and intra-laboratory reproducibility should be not less than 87%

The inter- and intra-laboratory reproducibility of the AmpFire HPV Assay is evaluated using Thin-Prep cervical brush samples. All tests (intra-lab and inter-lab tests) were performed from the original Thin-Prep samples stored at -20°C.

Samples were previously test with Cobas HPV assay giving 122/500 positive samples. Cobas test gave fewer positive numbers because it doesn't detect HPV53.

Intra-lab tests were performed with a time difference of 3 weeks.

Inter-laboratory result		**ATILA HPV 1st test**		
		positive	**negative**	**Total**
ATILA HPV 2nd test	**positive**	135	1	136
	negative	3	361	364
	Total	138	362	500

	Result	**Meijer criteria**
Lower confidence bound for agreement	0,98	Not less than 87%
Kappa	0,98	at least 0,5

Intra-laboratory result		**ATILA HPV 1st test**		
		positive	**negative**	**Total**
ATILA HPV 2nd test	**positive**	136	1	137
	negative	2	358	360
	Total	138	359	497*

	Result	**Meijer criteria**
Lower confidence bound for agreement	0,98	Not less than 87%
Kappa	0,98	at least 0,5

**3 samples failed on one of the tests in the intra-lab study. These samples were excluded from the data analysis. Even if these 3 samples are included in worse case scenario as discrepant results, the ATILA assay still applies to the requirement of at least 87% reproducibility.*

The inter- and intra-run reproducibility of the AmpFire HPV Assay is evaluated using plasmid DNA of each of the 15 HPV genotypes at 200 copies/reaction. The assay was tested across an equal distribution of three lots of reagents over 6 days.

The data are summarized in table below.

					Inter-Run		Intra-Run		Total	
HPV Genotype	**Conc**	**% Correct**	**95% Confidence Interval**	**Mean Ct**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**
HPV16	200	100% (90/90)	(97.29% – 100%)	18.15	0.15	0.82	0.17	0.94	0.35	1.93%
HPV18	200	100% (90/90)	(97.29% – 100%)	21.84	0.35	1.60	0.43	1.97	0.48	2.20
HPV31	200	100% (90/90)	(97.29% – 100%)	40.17	1.02	2.54	0.49	1.22	1.12	2.79
HPV33	200	100% (90/90)	(97.29% – 100%)	34.40	0.62	1.80	0.85	2.76	0.84	2.44
HPV35	200	100% (90/90)	(97.29% – 100%)	32.36	0.85	2.62	0.83	2.56	0.30	0.93
HPV39	200	100% (90/90)	(97.29% – 100%)	31.94	0.51	1.60	0.43	1.34	0.53	1.66
HPV45	200	100% (90/90)	(97.29% – 100%)	38.8	1.67	4.30	1.30	3.35	1.63	4.2
HPV51	200	100% (90/90)	(97.29% – 100%)	26.38	0.26	0.99	0.20	0.76	0.46	1.74
HPV52	200	100% (90/90)	(97.29% – 100%)	30.85	0.50	1.62	0.55	1.78	0.83	2.69
HPV53	200	100% (90/90)	(97.29% – 100%)	37.94	0.93	2.45	1.02	2.69	1.40	3.69
HPV56	200	100% (90/90)	(97.29% – 100%)	29.86	0.40	1.34	0.53	1.77	0.56	1.88
HPV58	200	100% (90/90)	(97.29% – 100%)	39.70	1.23	3.10	1.44	3.63	1.24	3.12
HPV59	200	100% (90/90)	(97.29% – 100%)	30.47	0.20	0.66	0.27	0.88	0.26	0.85
HPV66	200	100% (90/90)	(97.29% – 100%)	30.88	0.40	1.30	0.55	1.78	0.54	1.75
HPV68	200	100% (90/90)	(97.29% – 100%)	28.59	0.48	1.68	0.47	1.64	0.41	1.43