The versatile performance of Seegene's HPV assays

• Monitoring HPV type in post-vaccination era • Using self-collected specimens • Expanding various specimens

Key word	Reference	
Prevalence / Evaluation	Clinical and analytical evaluation of the Anyplex II HPV HR Detection assay within the VALGENT-3 framework	*Ostrbenk et al. (2018) J. Clinical Microbiology*
	Risk of cervical dysplasia among human papillomavirus-infected women in Korea: a multicenter prospective study	*Park Y. et al. (2019) J. Gynecol Oncol.*
	Clinical performance of Anyplex II HPV28 by human papillomavirus type and viral load in a referral population	*Baasland I et al. (2019) PLoS One.*
	Age-specific HPV prevalence among 116,052 women in Australia's renewed cervical screening program: A new tool for monitoring vaccine impact	*Brotherton JM et al. (2019) Vaccine.*
	High-Risk human papillomavirus genotype distribution in the Northern region of Portugal: Data from regional cervical cancer screening program	*Sousa H et al. (2019) Papillomavirus Res.*
	Effectiveness of bivalent and quadrivalent human papillomavirus vaccination in Luxembourg	*Latsuzbaia A et al. (2019) Cancer Epidemiol.*
	Continuing global improvement in human papillomavirus DNA genotyping services: The 2013 and 2014 HPV LabNet international proficiency studies	*Eklund C et al. (2018) J. Clin. Virol.*
	Prevalence of human papillomavirus and bacteria as sexually transmitted infections in symptomatic and asymptomatic women	*Kim YK et al. (2018) Clin. Pract.*
Self-sampling device	hrHPV prevalence and type distribution in rural Zimbabwe: A community-based self-collection study using near-point-of-care GeneXpert HPV testing	*Fitzpatrick M.B et al. (2019) Int. J. Infect. Dis.*
	Comparison of urine, self-collected vaginal swab, and cervical swab samples for detecting human papillomavirus (HPV) with Roche Cobas HPV, Anyplex II HPV, and Real Time HR-S HPV assay	*Cho H.W. et al. (2019) J. Virol. Methods.*
	Safety and acceptability of human papillomavirus testing of self-collected specimens: A methodologic study of the impact of collection devices and HPV assays on sensitivity for cervical cancer and high-grade lesions	*Leinonen M.K. et al. (2018) J. Clin. Virol.*
	A comparison of cotton and flocked swabs for vaginal self-sample collection	*Viviano M. et al. (2018) Int. J. Womens Health*
	Acceptability of human papillomavirus self-sampling for cervical cancer screening in an indigenous community in Guatemala	*Gottschlich A. et al. (2017) J. Glob. Oncol.*
	Human papillomavirus prevalence and type-specific distribution of high- and low-risk genotypes among Malagasy women living in urban and rural areas	*Catarino R. et al. (2016) Cancer Epidermiol.*
	Use of swabs for dry collection of self samples to detect human papillomavirus among Malagasy women	*Vassilakos P. et al. (2016) Infect. Agent Cancer*
Specimen expansion (anal, oral and FFPE)	Evaluation of the Anyplex II HPV28 Assay in the detection of human papillomavirus in archival samples of oropharyngeal carcinomas	*Rollo F. et al. (2019) Arch. Pathol. Lab. Med.*
	HPV detection and genotyping of head and neck cancer biopsies by molecular testing with regard to the new oropharyngeal squamous cell carcinoma classification based on HPV status	*Veyer D. et al. (2019) Pathology*
	High prevalence of anal and oral high-risk human papillomavirus in human immunodeficiency virus–uninfected French men who have sex with men and use preexposure prophylaxis	*Mboumba Bouassa R.S. et al. (2019) Open Forum Infect Dis.*
	Detection of HPV in oral leukoplakia by brushing and biopsy: prospective study in an Italian cohort	*Della Vella F. et al. (2019) Clin Oral Investig.*
	Anal human papillomavirus infections in young unvaccinated men who have sex with men attending a sexual health clinic for HPV vaccination in Melbourne, Australia	*Chow E.P.F. et al. (2019) Vaccine.*
	Unusual and unique distribution of anal highrisk human papillomavirus (HR-HPV) among men who have sex with men living in the central African Republic	*Mboumba Bouassa R.S. et al. (2018) PLoS One.*
	Findings of multiple HPV genotypes in cervical carcinoma are associated with poor cancer-specific survival in a Swedish cohort of cervical cancer primarily treated with radiotherapy	*Kaliff M. et al. (2018) Oncotarget*
	Prevalence of human papillomavirus in laryngeal squamous cell carcinoma in Azerbaijan population	*Aliyev A.J. et al. (2018) Head & Neck Can. Res.*
	Detection of human papillomavirus in Korean breast cancer patients by real-time polymerase chain reaction and meta-analysis of human papillomavirus and breast cancer	*Choi J. et al. (2016) J. Pathol. Transl. Med.*
	Evaluation of HPV Genotyping Assays for Archival Clinical Samples	*Liilsunde Larsson G. et al. (2015) J. Mol. Diagn.*

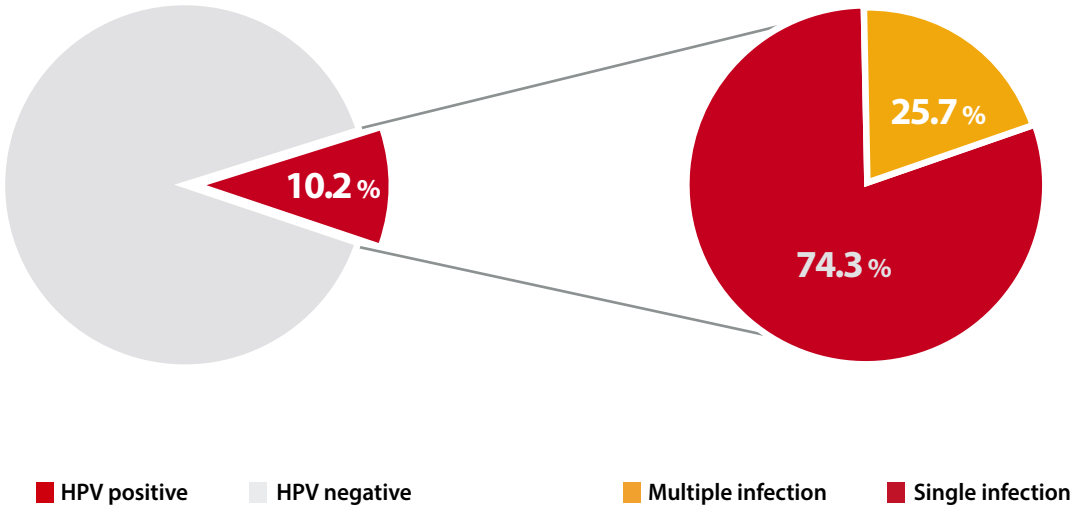
Seegene

Anyplex™ Ⅱ HPV HR Detection has been used for **more than100,000 women in cervical cancer screening program**

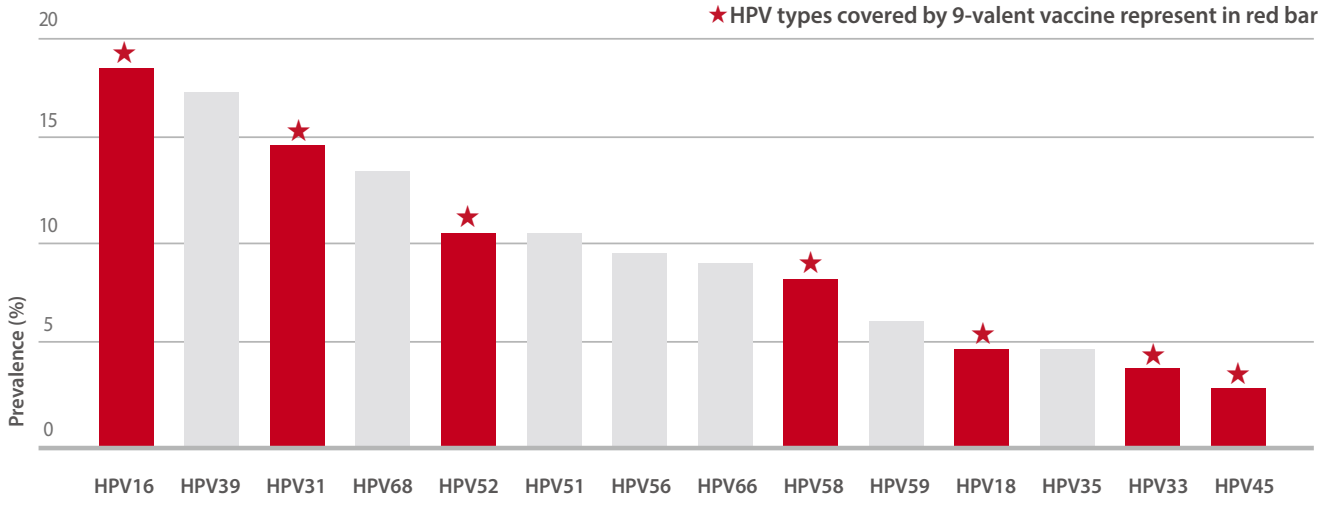
- **Implemented for the largest cervical cancer screening in European populations**

- Tested 105,458 women in Northern Portugal
- Provides critical data which impact program management and vaccine policy

Prevalence of high-risk HPV and multiple infection[1)]



Distribution of high-risk HPV types



1) Sousa H et al. (2019) Papillomavirus Research 8:100179

Anyplex™Ⅱ HPV HR Detection is

clinically validated assay for primary cervical cancer screening[1)]

Anyplex™Ⅱ HPV HR Detection meets the international consensus validation metrics for HPV DNA tests for cervical cancer screening[1) 2)]

Clinical sensitivity & specificity of Anyplex™Ⅱ HPV HR Detection

Category		Clinical sensitivity	Clinical specificity
Test population & number		60 samples with ≥ CIN2	816 samples with < CIN2
Result	Reference Test (GP5+/6+-PCR)	98.3% (59/60)	94.1% (768/816)
	Anyplex™Ⅱ HPV HR	98.3% (59/60)	93.6% (764/816)
Relative analysis to reference test		100%	99.5%
Requirements		≥ 90%	≥ 98%

▶ **The clinical sensitivity and specificity for CIN2+ of Anyplex™Ⅱ HPV HR Detection were non-inferior to those of GP5+/6+-PCR.**

Inter-lab agreement & intra-lab reproducibility of Anyplex™Ⅱ HPV HR Detection

Category		Intra-laboratory reproducibility	Inter-laboratory agreement
Test population & number		505 samples	505 samples
Result	Agreement (95% CI)	96.0% (94.3~97.4)	96.8% (95.3~98.1)
	kappa value	0.91	0.93
Requirements	Lower 95% CI	≥ 87%	≥ 87%
	kappa value	≥ 0.5	≥ 0.5

▶ **Anyplex™Ⅱ HPV HR Detection displayed sufficient intra-laboratory reproducibility and inter-laboratory agreement.**

1) Hesselink AT *et al*. (2016) *Journal of Clinical Virology* 76:36-39
2) Meijer CJLM *et al*. (2009) *Int J Cancer* 124(3):516~20

Seegene's HPV assays can be applied with extra-cervical & FFPE specimens

- Studies with a variety of specimen types (Anal, Oral, FFPE, etc.) have been published.
- It is a powerful tool for etiology related to 14 individual genotyping including HPV16/18.

Anal & Oral specimens in HIV-negative MSM group in France[1)]

Category	Anal specimens	Oral specimens
Sample no.	61	56
HPV Positive	57 / 61 (93.4%)	19 / 56 (33.9%)
Predominant type	-33 (31%)	-33 (16%)
prevalence ranking	-33, -42, -53, -51, -6, -70	-33, -66, -43, -44, -61, -6

All HPV prevalence were detected 93.4 % and 33.9%, in Anal and Oral specimen, respectively. HPV 33 was most detected in both specimens (31% in Anal; 16% in Oral).

1) RSM Bouassa et al. (2019) Open Forum Infectious Diseases 6(9):ofz291

The summary of peer-reviewed journals using formalin-fixed, paraffin-embedded(FFPE) specimens with Seegene's HPV assays

Country	FFPE type	FFPE No. (N)	Seegene's HPV assays	Other HPV assays	Result	
Italy[2)]	Oropharyngeal squamous cell carcinoma	160	Anyplex™II HPV28	INNO-LiPA	Good agreement	Cohen's κ = 0.75
				Xpert		Cohen's κ = 0.80
France[3)]	Head and neck squamous cell carcinoma	55	Anyplex™II HPV28	INNO-LiPA	Good agreement (Cohen's κ = 0.78)	
Sweden[4)]	Cervical cancer	209	Anyplex™II HPV28	not applied	84% positivity (n=176)	
Azerbaijan[5)]	Cervical cancer infected HPV16/18	10	Anyplex™II HPV HR	not applied	100% positivity (8 HPV16, 1 HPV18, 1 HPV16/18/58)	
Korea[6)]	Breast cancer, Intraductal papillomas	132	Anyplex™II HPV HR	not applied	18% positivity (n=24)	
Sweden[7)]	Cervical lesions, Penile, Head & neck carcinoma etc.	99	Anyplex™II HPV HR	CLART HPV2, Sequencing	97% agreement with reference assay	

Seegene's HPV assays showed comparable outcomes with other HPV assays for HPV genotyping in FFPE.

- **Prior to implementation in laboratories, self-validation is recommended, as it has not included intended use in package insert**
- **Either QIAamp DNA mini kit or QIAamp DNA FFPE kit was used for DNA extraction**
- **Please contact your regional manager if you have any question/suggestion regarding validation with FFPE specimens**

Seegene's HPV assays were validated in multiple studies with self-collection devices

- **Anyplex™ II HPV28 Detection** shows high concordance between self-collected and expert-collected specimens for HPV detection
- Compared to other HPV assays, **Seegene's HPV assays** show excellent agreements of high-risk HPV positive results using self-collected specimens.

The summary of peer-reviewed journals using self-collection devices with Seegene's HPV aassays

Country	Self-collection device	Patient (No.)	Result	Note
Zimbabwe[8)]	Cervex-brush®	108	78% agreement with Xpert HPV	- QIAGEN DNA mini elute kit*
South Korea[9)]	Vaginal brush (Flocked)	101 (87 HSIL)	In HPV 16/18, 94.1% concordance with expert-collected cervical sample	- QIAamp DNA blood minikit*
Norway[10)]	Evalyn®Brush	232 (≥ pre-cancer)	In high-risk HPV, 94.0% agreement with expert-collected specimens using Evalyn®Brush	- Self-collected specimens were stable up to 4 weeks - Equivalent to Cobas 4800 HPV & Xpert HPV assay/ - NucliSENS easyMag*
Switzerland[11)]	FLOQSwab (Flocked)	119	38.1% positivity	- NIMBUS IVD*
Guatemala[12)]	Eve HerSwab	178	21% positivity	- 17% positivity of high-risk HPV - 67.7% was multiple infections
Madagascar[13,14)]	Cotton swab (Dry swab	1,020	39% positivity	
		397	38% positivity	- Comparable to Cobas HPV Test (89.9% agreement) - The specimen stability maintained up to 2 weeks

• Prior to implementation in laboratories, self-validation is recommended, as it has not included intended use in package insert.
• Please contact your regional manager if you have any question/suggestion regarding validation with self-collected specimens.
*** Nucleic acid extraction method**

2) Rollo F. et al. (2019) Arch. Pathol. Lab. Med.
3) Veyer D. et al. (2019) Pathology
4) Kaliff M. et al. (2018) Oncotarget
5) Aliyev A.J. et al. (2018) Head & Neck Can. Res.
6) Choi J. et al. (2016) J. Pathol. Transl. Med.
7) Lillsunde Larsson G. et al. (2015) J. Mol. Diagn.
8) Fitzpatrick M.B et al. (2019) Int. J. Infect. Dis.
9) Cho H.W. et al. (2019) J. Virol. Methods.
10) Leinonen M.K. et al. (2018) J. Clin. Virol.
11) Viviano M. et al. (2018) Int. J. Womens Health
12) Gottschlich A. et al. (2017) J. Glob. Oncol.
13) Catarino R. et al. (2016) Cancer Epidermiol.
14) Vassilakos P.. et al. (2016) Infect. Agent Cancer

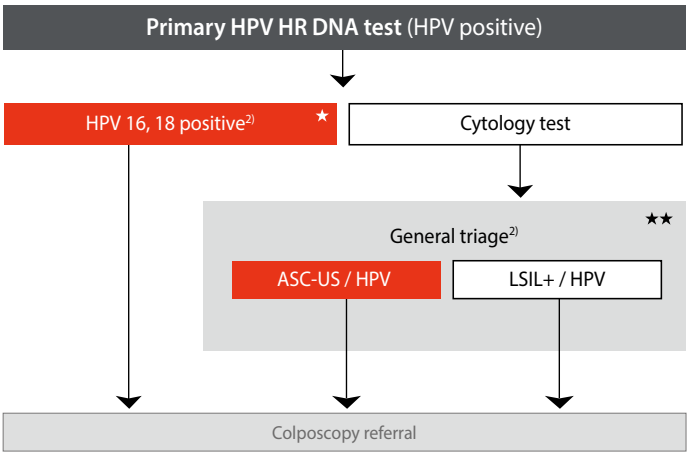
Optimizing HPV-based primary screening program and triage algorithm

Through identifying major high-risk HPV including vaccine-covered types,

Anyplex™ II HPV HR detection can help :

- **Setting risk threshold**
- **Considering new alternative triage**
- **Proposing better algorithm**

General primary HPV screening with cytology triage vs. Alternative triage based on the HPV genotype



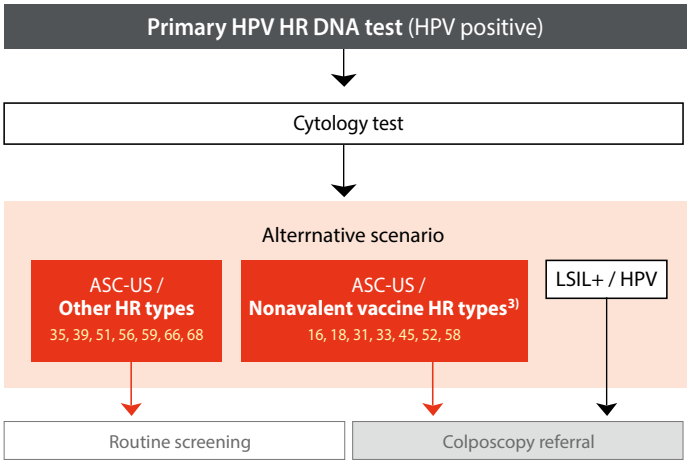
General example of triage algorithm for primary HPV screening[2)]

1. HPV genotyping for HPV16, HPV18 and cytology in US
2. HPV positive and cytology in Europe

: Women with ASC-US or higher are referred to colposcopy

★ The primary HPV screening program in USA.

★★ The primary HPV screening program in the Netherlands.



A new screening approach is required[2)]

1. **Vaccination effect** : An increase of HPV vaccination coverage is likely to leading lower prevalence
2. **Low specificity** : Referring HPV+ women with ASC-US to colposcopy is not efficient, because the large number of women do not have precancer or anything related to cervical cancer
3. **Management trend** : Risk thresholds* rather than individual results

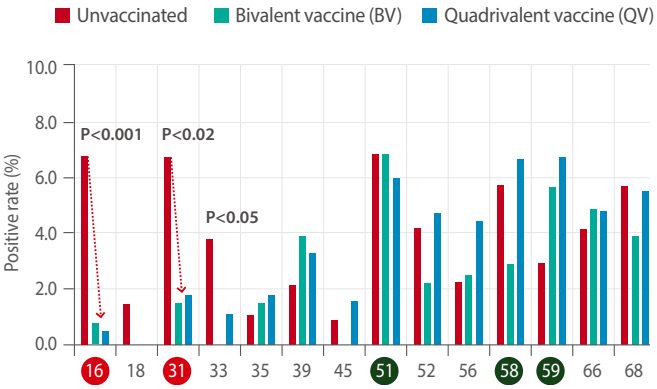
*It depend on many factors including a societal perception of risk, established clinical practice, different weight on benefits and harms, health economic considerations, and health infrastructure
The approach to setting risk thresholds differs

2) *Wentzensen N. et al. Triage of HPV positive women in cervical cancer screening (2016)*
3) *A nonavalent vaccine targets seven carcinogenic types (HPV16/18/31/33/45/52/58) that contribute to 90% of cervical cancer cases*

The impact of Seegene's HPV assay in the post-vaccination era

- **Monitoring changes of HPV types in a vaccinated population**
- **Evaluating the prevalence of HPV vaccine types**
- **Measuring the efficacy and cross-protection of vaccine**

HPV prevalence and vaccine efficacy 8 years following the implementation of the vaccination program in Luxembourg[4)]

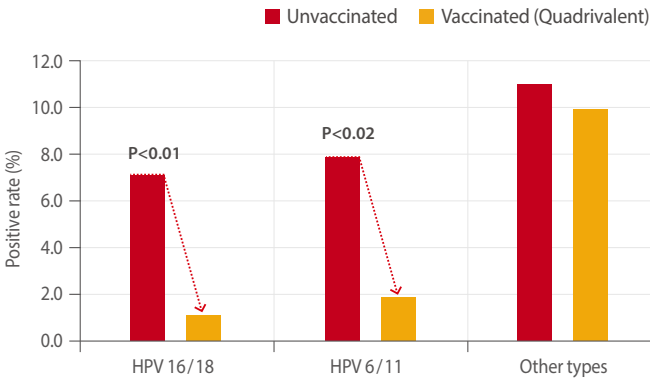


18~29 Yrs women (participant no.: 401)	Unvaccinated	Vaccinated
HPV positive rate	53.5%	46.4%
		42% (BV), 49% (QV)
Most prevalent type	16, 31, 51	51, 58, 59

4) *Latsuzbaia et al. Caner Epider. (2019) 63:101593*

The overall prevalence of HPV showed a very similar rate between the two groups, however, the type distribution was dramatically changed in certain types covered by HPV vaccine and other types assuming cross-protection. For instance, HPV 16, 31, and 33 were significantly decreased in vaccinated women, but not in the unvaccinated group. Instead, other types such as HPV 51, 58, and 59 were found as the most frequent types in vaccinated women.

Prevalence of vaccine type HPV in vaccinated and non-vaccinated women in Switzerland[5)]



18~31 Yrs women (participant no.: 409)	Unvaccinated	Vaccinated
HPV 16/18 positive rate	7.2%	1.1%
HPV 6/11 positive rate	8.3%	2.1%
Other HR-HPV prevalent type	11.2%	10.3%

5) *Jeannot et al. Int. J. Environ. Res. Public Health (2018) 15:1447*

The prevalence of four types, HPV6/11 and HPV 16/18, covered by the quadrivalent vaccine was significantly lower in vaccinated women, whereas cross-protection was not observed in this study.

Anyplex™ II HPV28 Detection was proved its excellent performance in WHO evaluation

- **Excellent genotype detection even in multiple infections**
- **Great sensitivity, specificity and inter-lab reproducibility**

Percent proficient results of HPV types as claimed to be detected by test[1)]

Type of HPV assay	Number of data sets	100% proficient	99-90% proficient	89-80% proficient	<80% proficient	Not proficient
All assays	**148**	**89**	**14**	**9**	**5**	**31**
Linear Array (Roche)	14	7	0	1	0	6
HPV Direct Flow-chip (Master Diagnostica)	14	9	0	0	0	5
GenoFlow HPV array (DiagCor)	14	13	0	0	0	1
Anyplex™ II HPV28 Detection (Seegene)	**11**	**11**	**0**	**0**	**0**	**0**
In-house PCR Luminex	8	3	1	1	0	3
In-house realtime PCR	8	4	0	1	1	2
In-house PGMY-CHUV	6	4	0	0	0	2
In-house blot	6	2	0	2	0	2
Papillocheck (Greiner)	5	4	0	1	0	0
Onclarity (Becton Dickinson)	**5**	**5**	**0**	**0**	**0**	**0**
CLART HPV 2 / 4 (Genomica)	4	0	1	1	2	0
Cobas 4800 (Roche)	**4**	**4**	**0**	**0**	**0**	**0**
InnoLiPA (Fujirebio)	4	1	2	0	0	1
PANA Realtyper 1001 (Panagene)	3	0	3	0	0	0
PANArray Genotyping Chip (Panagene)	**3**	**0**	**3**	**0**	**0**	**0**
Hybribio 21 HPV (Hybribio)	3	3	0	0	0	0
RealTime HPV (Abbott)	**3**	**1**	**0**	**2**	**0**	**0**
In-house sequencing	3	0	0	0	0	3
HPV SPF10-LiPA25	2	0	0	0	0	2
HPV XpressMatrix™ (DNA laboratories)	2	2	0	0	0	0
Ampliquality (Analitica)	2	0	1	0	0	1
Hybribio 13 HR (Hybribio)	2	2	0	0	0	0
Hybribio 14 HR (Hybribio)	2	2	0	0	0	0
PANA Realtyper 1002 (Panagene)	2	0	2	0	0	0
Optiplex (DIAMEX)	2	2	0	0	0	0
Other Commercial assays	14	9	1	0	1	3
Other In-house assays	2	1	0	0	1	0

100% proficiency in all tests performed by participants (11 labs worldwide)

▸ Information of participants

- Total number of participants : 121 laboratories
  - Distributions : Europe (70), America (14), Western Pacific (25), South East Asia (8), Africa (3), Eastern Mediterranean (1)
- Total number of datasets : 148

Reference 1) Carina EKlund *et al.* J CLIN VIROL (2018) 101:74-85

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