HAL
open science

Comparative evaluation of different DNA extraction methods for HPV genotyping by Linear Array and INNO-LiPA

Maria Gabriella Dona', Maria Benevolo, Fulvia Pimpinelli, Mara Battista, Francesca Rollo, Francesca Stivali, Antonella Moscarelli, Massimo Giuliani, Aldo Di Carlo, Amina Vocaturo

To cite this version:

Maria Gabriella Dona', Maria Benevolo, Fulvia Pimpinelli, Mara Battista, Francesca Rollo, et al.. Comparative evaluation of different DNA extraction methods for HPV genotyping by Linear Array and INNO-LiPA. Journal of Medical Virology, 2011, 83 (6), pp.1042. 10.1002/jmv.22088 . hal-00633209

HAL Id: hal-00633209

https://hal.science/hal-00633209v1

Submitted on 18 Oct 2011

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Journal of Medical Virology

Comparative evaluation of different DNA extraction methods for HPV genotyping by Linear Array and INNO-LiPA

Journal:	*Journal of Medical Virology*
Manuscript ID:	JMV-10-2159.R2
Wiley - Manuscript type:	Research Article
Date Submitted by the Author:	03-Feb-2011
Complete List of Authors:	Dona', Maria Gabriella; San Gallicano Dermatological Institute, Dermatologia Infettiva Benevolo, Maria; Regina Elena Cancer Institute, Pathology Pimpinelli, Fulvia; San Gallicano Dermatological Institute, Clinical Pathology and Microbiology Battista, Mara; San Gallicano Dermatological Institute, Clinical Pathology and Microbiology Rollo, Francesca; Regina Elena Cancer Institute, Pathology Stivali, Francesca; San Gallicano Dermatological Institute, Clinical Pathology and Microbiology Moscarelli, Antonella; San Gallicano Dermatological Institute, Clinical Pathology and Microbiology Giuliani, Massimo; San Gallicano Dermatological Institute, Dermatologia Infettiva Di Carlo, Aldo; San Gallicano Dermatological Institute, Dermatologia Infettiva Vocaturo, Amina; Regina Elena Cancer Institute, Pathology
Keywords:	HPV genotyping, DNA extraction, cervico-vaginal cytology

SCHOLARONE™
Manuscripts

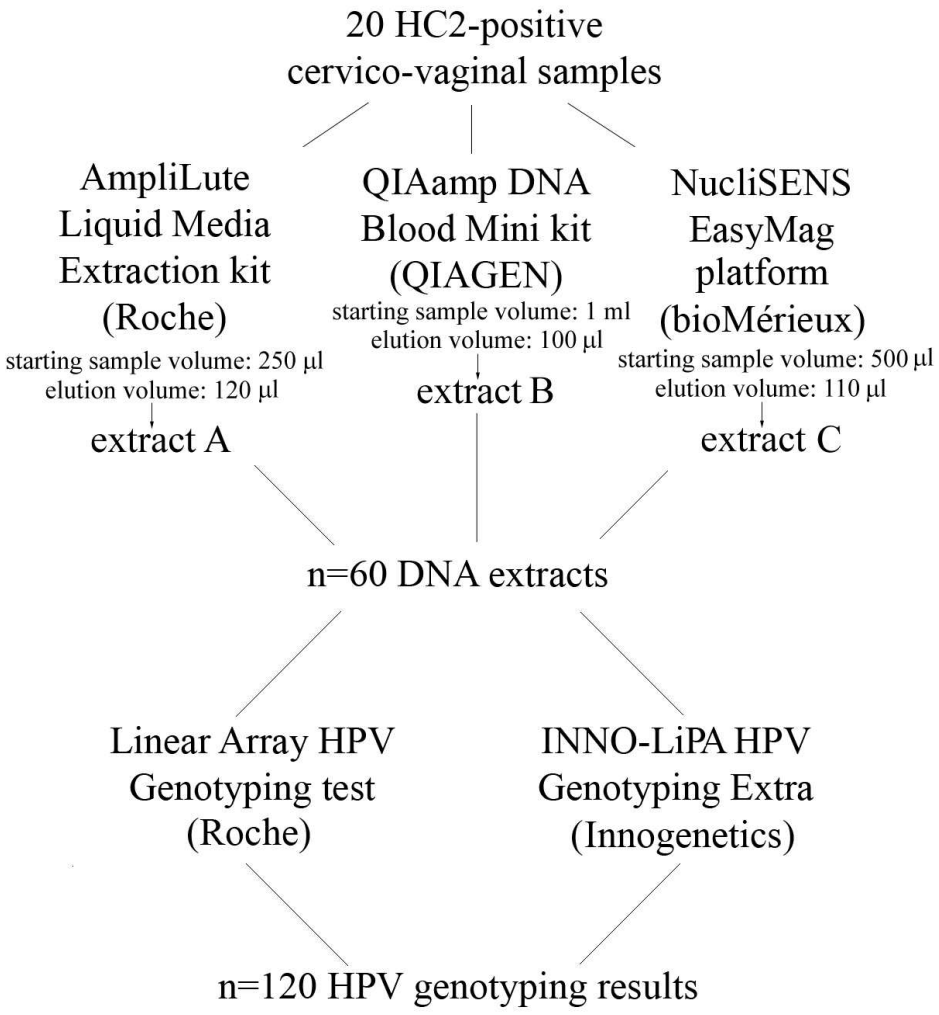

FIG. 1 Diagram of the study flow.

109x124mm (300 x 300 DPI)

TABLE I. Comparison of the results obtained by Linear Array and INNO-LiPA

Extract	No. (%) of samples		
	Concordant	Compatible	Discordant
A	8/20 (40)	11/20 (55)	1/20 (5)
B	7/20 (35)	12/20 (60)	1/20 (5)
C	8/20 (40)	12/20 (60)	0/20 (0)

NOTE. See Materials and Methods for definition of concordant, compatible and discordant results.

For Peer Review

TABLE II. HPV type-specific concordance in Linear Array and INNO-LiPA results

	No. (%) of concordant samples			
HPV Test	A vs B vs C	A vs B	A vs C	B vs C
Linear Array	11/20 (55)	15/20 (75)	13/20 (65)	13/20 (65)
INNO-LiPA	15/20 (75)	15/20 (75)	16/20 (80)	18/20 (90)

A, AmpliLute extract (Roche)
B, QIAamp DNA Blood Mini kit extract (QIAGEN)
C, NucliSENS EasyMAG extract (bioMérieux)

For Peer Review

TABLE III. Non-concordant cases among samples tested by Linear Array

Sample	Extract	HPV genotypes							
4	A	16	**42**	58	[52]				
	B	16	**42**	58	[52]				
	C	16		58	[52]				
5	A	18		42	45				
	B	18	**39**	42	45				
	C	18		42	45				
6	A	**31**	84						
	B		84						
	C	**31**	84						
7	A		42	53	56	66			
	B		42	53	56	66			
	C	**39**	42	53	56	66			
8	A	6	**42**	52	73				
	B	6	**42**	52	73				
	C	6		52	73				
10	A	6	45	51		54	59	70	
	B	6	45	51	**53**	54	59	70	
	C	6	45	51	**53**	54	59	70	
15	A	16		70					
	B	16	**66**	70					
	C	16	**66**	70					
16	A	6		[52]	58	59		73	
	B	6		[52]	58	59		73	
	C	6	**42**	[52]	58	59	**66**	73	
19	A			45	53		73	82	84 **CP6108**
	B	**16**		45	53	**56**	73	82	84
	C		**33**	45	53		73	82	84 **CP6108**

NOTE. HPV types not detected in all the three extracts of the same sample are shown in bold.
HPV types between brackets are possibly present as co-infections.

For Peer Review

TABLE IV. Non-concordant cases among samples tested by INNO-LiPA

Sample	Extract	HPV genotypes					
9	A	[52]	**53**				
	B	[52]		**58**			
	C	52					
10	A	**45**	51	**54**	**70**		
	B		51				
	C	**45**	51	**54**	**70**		
11	A	39	[52]	**58**			
	B	39	52				
	C	39	52				
12	A		**51**				
	B	**16**		**53**			
	C	**16**		**53**			
16	A	6		[52]	39	**58**	66
	B	6	**18**	52	39		66
	C	6	**18**	52	39		66

NOTE. HPV types not detected in all the three extracts of the same sample are shown in bold.
HPV types between brackets are possibly present as co-infections.

For Peer Review

Comparative evaluation of different DNA extraction methods for HPV genotyping by Linear Array and INNO-LiPA

Maria Gabriella Donà,[1*] Maria Benevolo,[2] Fulvia Pimpinelli,[3] Mara Battista,[3] Francesca Rollo,[2] Francesca Stivali,[3] Antonella Moscarelli,[3] Massimo Giuliani,[1] Aldo Di Carlo,[1] and Amina Vocaturo[2]

[1]*UOC Dermatologia Infettiva, San Gallicano Dermatological Institute, Rome, Italy*

[2]*Pathology Department, Regina Elena Cancer Institute, Rome, Italy*

[3]*UOC Microbiology and Clinical Pathology, San Gallicano Dermatological Institute, Rome, Italy*

Institution at which the work was performed: Pathology Department, Regina Elena Cancer Institute, Rome, Italy

*Correspondence to:

Maria Gabriella Donà, PhD, UOC Dermatologia Infettiva, San Gallicano Dermatological Institute

Via Elio Chianesi 53, 00144, Rome, Italy.

Phone: +39 0652666905

Fax: +39 0652662920

E-mail: dona@ifo.it

Shortened title: DNA extraction and HPV genotyping

For Peer Review

ABSTRACT

In order to investigate the influence of DNA extraction on two PCR-based HPV genotyping tests (Linear Array, Roche and INNO-LiPA *Extra*, Innogenetics), three different procedures were used to purify DNA from 28 cervico-vaginal samples tested previously by the Hybrid Capture 2: the AmpliLute Liquid Media Extraction kit (Roche), the QIAamp DNA Blood mini kit (QIAGEN) and the NucliSENS EasyMAG automated platform (bioMérieux). All HC2-positive samples were found positive by both assays, independently of the extract used. Type-specific concordance (i.e. identical HPV type-specific profile in all the extracts of the same sample) was observed in 55% and 75% of the cases testing samples by the Linear Array and the INNO-LiPA, respectively. Using the DNA extracted with the two manual methods the results were concordant in 75% of the cases both for the Linear Array and the INNO-LiPA. When comparing the Linear Array results obtained on either of the two manual extracts with those obtained following automated extraction, 65% of the samples showed type-specific concordance in both cases. The INNO-LiPA results were concordant in 80% of the cases comparing the AmpliLute vs. the automated extract, while concordant results were observed in 90% of the cases when comparing the QIAGEN vs. the automated extract. In conclusion, the Linear Array and INNO-LiPA results are affected by the method of DNA extraction. Consequently, different HPV type-specific profiles may be observed using different extracts of the same sample. The use of consistent protocols for DNA purification is a priority to guarantee intra-assay reproducibility over time.

KEY WORDS: HPV genotyping, DNA extraction, cervico-vaginal cytology

For Peer Review

INTRODUCTION

Human Papillomaviruses (HPVs) are the etiological agents of cervical carcinoma, the second most common malignancy among women after breast cancer [Munoz et al., 2006; zur Hausen, 2009]. Virtually all cases of cervical carcinoma worldwide are HPV-positive, although only a limited number of HPV genotypes among the more than 100 types identified so far seem to have a role in the development of cervical cancer. In particular, 12 genotypes have been classified as "carcinogenic" or "high-risk" [Bouvard et al., 2009; Munoz et al., 2006], with HPV16 representing the most prevalent type, being present in 61% of all cervical cancer cases [de Sanjose et al., 2010]. Cervical cytology screening, which represents one of the best examples of the efficacy of secondary prevention for cancer, resulted in a reduction in cervical cancer morbidity and mortality [Kitchener et al., 2006]. Furthermore, the relatively recent introduction of HPV molecular tests has added a powerful tool to cervical cancer prevention programs [Wheeler, 2007]. These tests now represent an integral part of the management of women with equivocal cytology and have major clinical relevance as an adjunct to cytology [Snijders et al., 2003; Wright et al., 2007]. A number of assays are available for the molecular diagnosis of HPV infections [Molijn et al., 2005]. Some tests only provide a positive vs. negative result, with no individual identification of the genotypes (e.g. Hybrid Capture 2, HC2), while others allow simultaneous HPV detection and genotyping (e.g. the Linear Array HPV Genotyping Test and the INNO-LiPA). Importantly, since the oncogenic potential of the different HPV types, even in the high-risk group, varies greatly and the risk of developing high-grade cervical lesions and cancer depends on the genotype/s responsible for the infection [Munoz et al., 2003; Munoz et al., 2006], the accurate assessment of the type-profile is a major step toward a reliable evaluation of cancer risk. Although the clinical value of HPV genotyping tests is still debated, these tests are, however, useful for assessing the prevalence of specific genotypes in population-based studies and might assume an increasing importance for determining a possible variation in the prevalence of non-vaccine HPV types in the HPV vaccine era, in order to monitor

For Peer Review

type-specific viral persistence [Kjaer et al., 2010], to plan a correct management of the patient, and to predict treatment effectiveness in cervical cancer patients [Nagai et al., 2004; Nobeyama et al., 2004].

PCR-based methods are used widely in clinical and research laboratories. The Linear Array HPV Genotyping Test (Roche) and the INNO-LiPA (Innogenetics) has received the CE mark certification, are registered for in *vitro* diagnostic use in Europe and, among all the available methods, have the most extensive clinical validation. The Linear Array, which is based on the amplification of a 450 bp sequence within the L1 region using PGMY primers, identifies 37 ano-genital HPV genotypes. The INNO-LiPA HPV Genotyping *Extra* is based on the use of SPF10 primers for the amplification of a 65 bp fragment within L1, and is designed for the identification of 28 different genotypes. The Linear Array is validated only with a specific, *ad hoc* developed method: cervical samples have to be collected in PreservCyt and only the AmpliLute Liquid Media Extraction kit (Roche) is validated for sample preparation. In contrast, in the case of the INNO-LiPA, a variety of media can be used for collection of cervical material (e.g. water, PBS, PreservCyt) and DNA extraction can be carried out using proteinase K or commercial kits.

Several studies have shown that the Linear Array and the INNO-LiPA often lead to different genotyping results, because of the different primer sets, reaction conditions, sensitivity, specificity, plus a number of other parameters [van Hamont et al., 2006]. However, not many studies have focused on the influence of the DNA extraction method on the genotyping results [Dunn et al., 2007].

The aim of the present study was to investigate the influence of the method used for DNA extraction from cervico-vaginal samples on HPV genotyping results obtained by the Linear Array and the INNO-LiPA. Three different procedures, two manual and one automated, were used: the Roche-recommended AmpliLute Liquid Media Extraction kit (Roche), the QIAamp DNA Blood mini kit (QIAGEN) and the NucliSENS EasyMAG automated platform (bioMérieux). The QIAamp DNA Blood mini kit was employed since it represents one of the most common manual extraction

For Peer Review

methods in clinical and research laboratories. Finally, an automated extractor was included in the present investigation as automated extraction instruments are now widely adopted in many laboratories, because they are highly flexible, allow the simultaneous processing of many different samples and they reduce the hand-on time of the technicians.

For Peer Review

MATERIALS AND METHODS

***Samples*.** Cervico-vaginal samples were obtained from women attending the Pathology Department of the Regina Elena Cancer Institute, Rome (Italy). Specimens, collected in PreservCyt (Cytyc Corp., Rome, Italy) using a cytobrush (Cytyc) and an Ayre spatula (Cytyc), were tested by the HC2 high-risk HPV DNA test (QIAGEN, Milan, Italy) according to the manufacturer's instructions. Twenty HC2-positive samples (i.e. specimen RLU/cutoff value>1) and 8 HC2-negative samples (i.e. specimen RLU/cutoff value<1) were selected.

***DNA Extraction*.** The three methods of extraction used are explained in Figure 1. The specimen preparation with the AmpliLute Liquid Media Extraction kit (Roche, Milan, Italy) was performed following the manufacturer's instruction. Briefly, cervico-vaginal samples were vortexed and 250 µl aliquots of each specimen were used for the extraction. The final elution step was performed using 120 µl of the provided elution buffer (AVE), obtaining "extract A". The "DNA purification from Blood or Body Fluids Spin Protocol" of the QIAamp DNA Blood mini kit (QIAGEN, Milan, Italy) was used to purify the DNA from 1 ml of the cervico-vaginal specimens. 100 µl of the provided elution buffer (AE) were used for the final elution step, obtaining "extract B". For the extraction with the NucliSENS EasyMAG automated platform (bioMérieux, Florence, Italy), the lysis step was performed by the instrument ("on-board protocol") and the "Specific B" protocol was then used to extract total nucleic acids from 500 µl of the cervico-vaginal samples. The elution step was carried out with 110 µl of NucliSENS Elution buffer (bioMérieux, Florence, Italy), obtaining "extract C". All extracts were stored at -20°C prior to processing.

***HPV Genotyping tests*.** HPV amplification, amplicons hybridization and detection were performed in parallel on each extract following the Roche and Innogenetics instructions for the Linear Array and the INNO-LiPA, respectively. The volume of each extract used to perform the PCR was calculated in order to correspond to the same volume of the original cervico-vaginal sample and, whenever required, adjusted to 50 µl for the Linear Array and 10 µl for the INNO-LiPA. The Gold-

For Peer Review

plated 96-Well GeneAmp® PCR System 9700 (Applied Biosystems, Milan, Italy) was used for both the Linear Array and INNO-LiPA amplification steps. All hybridization steps up to color development were carried out with a Profiblot T48 instrument (Tecan, Männedorf, Switzerland) for samples tested by the Linear Array and in an *Auto*-LiPA (Innogenetics, Pomezia, Italy) for samples analyzed by the INNO-LiPA.

The Linear Array results were interpreted visually. The Linear Array testing algorithm, which is unable to discriminate HPV 52 infection in case of HPVs 33, 35, or 58 infections, was used. HPV 52 infection was not ascertained by HPV 52-specific PCR. The LiRAS® for LiPA HPV software was used for the interpretation of the INNO-LiPA results. Visual interpretation was adopted to confirm the automatic reading.

Results were considered valid if successful amplification of the human DNA control, β-globin for the Linear Array and a fragment of HLA-DPB1 gene for the INNO-LiPA, was observed.

For the Linear Array and INNO-LiPA comparison, results were defined as concordant when identical assay-common genotypes were detected by both assays, compatible when one or more assay-common genotypes were not detected by either of the assays and discordant when no similarities in the assay-common genotypes were found between the two assays. When comparing the genotyping results obtained by either assay on the three different extracts of the same cervico-vaginal sample, type-specific concordance was defined as an identical HPV type-specific profile in the extracts compared.

For Peer Review

RESULTS

For the present study three different nucleic acids extracts were obtained from 20 HC2-positive and 8 HC2-negative cervico-vaginal samples using commercially available methods, as explained in Materials and Methods. Subsequently, all extracts were tested both by the Linear Array and the INNO-LiPA (Figure 1). Successful amplification of the human DNA control was obtained for all the extracts in both assays, proving the efficiency of each extraction technique employed and the quality of the sample, i.e. inadequate specimen processing and the presence of inhibitors can be excluded.

Among the 8 HC2-negative samples examined, seven were consistently negative both by the Linear Array and the INNO-LiPA, independently of the extract used. Therefore, these samples were not useful for comparing the HPV type-specific profiles obtained on different extracts. One sample was negative by the Linear Array and positive for HPV 74 by the INNO-LiPA in all the three extracts. HPV74 is not included in either the HC2 nor in the Linear Array. Thus, this sample was not taken into consideration in our analysis.

Results obtained by the Linear Array and INNO-LiPA on HC2-positive samples were compared considering only the assay-common genotypes. The number of concordant, compatible and discordant results obtained using the same extract for both assays are shown in Table I.

All HC2-positive samples were positive by both assays, independently of the extract used to perform the PCR. As far as the Linear Array genotyping results are concerned, type-specific concordance in all the three extracts was observed in 11/20 cases (55%), while INNO-LiPA results were concordant in 15/20 cases (75%; Table II). When comparing the results of the HPV genotyping tests performed on the DNA extracted with the two manual methods (extracts A and B), 15/20 cases (75%) showed type-specific concordance both by the Linear Array and the INNO-LiPA. When comparing the Linear Array results obtained using either of the two manually extracted DNA (extract A or B) with those obtained on the DNA isolated by the automated extractor (extract C), 13/20 samples (65%) showed type-specific concordance in both cases. On the

For Peer Review

other hand, the INNO-LiPA results were concordant in 16/20 cases (80%) when extract A (AmpliLute) was compared with extract C (automated extract), while concordant results were observed in 18/20 cases (90%) when comparing extract B (QIAGEN) with extract C. The sample size was too limited to perform any statistical analysis to determine if the observed differences were statistically significant.

For all the 9 samples with a discordant Linear Array type-specific profile and for 4 out of the 5 samples with a discordant INNO-LiPA type-specific profile, there was at least one HPV type in common in all the three extracts (TABLE III-IV). More specifically, the lack of type-specific concordance in the Linear Array results concerned 9 HPVs, 2 low-risk (HPVs 42 and CP6108) 5 high-risk (HPVs 16, 31, 33, 39, 56) and 2 possible high-risk types (HPVs 53 and 66), as shown in Table III. In the case of the 5 samples with a discordant INNO-LiPA type-specific profile, lack of concordance was observed for 7 high-risk (HPVs 16, 18, 45, 51, 53, 58, 70) and 1 low-risk HPV types (HPV 54), as shown in Table IV.

For Peer Review

DISCUSSION

The present study compared the HPV genotyping results obtained by the Linear Array HPV Genotyping Test and the INNO-LiPA HPV Genotyping *Extra* performed on three different nucleic acids extracts of the same cervico-vaginal samples.

The comparison between the Linear Array and the Inno-LiPA results on HC2-positive samples showed that most, if not all, the genotyping results were either concordant or compatible, as already demonstrated in previous studies performed on much larger sets of samples [Castle et al., 2008; van Hamont et al., 2006]. The data also confirmed that the Linear Array is able to detect more multiple infections and a greater number of HPV types per multiple infection (data not shown) [Castle et al., 2008]. The differences observed between the two methods may be due to the fact that these assays differ in several ways, such as the length of the amplicons and the amplicon detection. Neither the detailed genotyping results obtained for each sample nor a detailed discussion concerning the direct comparison between the two assays were considered in this study, since studies aimed at comparing results obtained by these two methods have been reported extensively.

A variability in the HPV type-specific profiles obtained both by the Linear Array and the INNO-LiPA on different extracts of the same samples was found, suggesting that both assays are affected by the DNA isolation method used. These data are consistent with the results of a previous study that showed the influence of the extraction protocol on the Linear Array performance [Dunn et al., 2007]. However, the present study seems to indicate that the INNO-LiPA is less affected than the Linear Array by the DNA purification technique used.

Interestingly, the inclusivity level of the Linear Array for most of the 9 HPV types discordant among the several extracts is medium to high (900-30,000 copies/ml), i.e. this assay detects consistently these genotypes across replicates and different runs only at high concentration, with the exception of HPVs 16, 53 and 66 for which the 95% positive hit rate concentration level predicted by Probit analysis is 195, 256 and 250 copies/ml, respectively, as reported in the Roche Linear Array Genotyping test manual. The most frequent discordant HPV type among the three extracts

For Peer Review

when tested by Linear Array was HPV 42, which has an inclusivity level of 30,000 copies/ml. In fact, among the 6 samples that tested positive for HPV 42 in at least one of the extracts, only in 3 cases was HPV 42 detected in all the three extracts of the same sample (data not shown). On the contrary, only in 1 out of 6 cases was HPV 16 not detected in all the three extracts (data not shown). These data indicate that the reproducibility of the results obtained on different extracts is lower for HPV types with a higher inclusivity level. Although the reliable detection of both low-risk and high-risk genotypes is important to assess the prevalence of individual genotypes in epidemiological studies, failure in detecting low-risk types, such as HPV 42, might not have a major clinical implication. On the contrary, failure in detecting high-risk HPVs, such as HPVs 16 and 33, is significant because of the clinical relevance of infections by these high-risk types. In fact, HPVs 16 and 33 are among the 5 high-risk types which are found most frequently in high-grade cervical lesions and cervical cancer cases [Bosch et al., 2008; de Sanjose et al., 2010] and HPV 16-positive atypical squamous cells of undetermined significance (ASCUS)/low-grade squamous intraepithelial lesions (LSIL) have an increased risk to progress to high-grade lesions [Castle et al., 2005].

In the case of the INNO-LiPA, it was more difficult to interpret the discordant cases. In fact, while in the Linear Array each hybridization band corresponds to one genotype (with the exception of HPV 33, 35 and 58 that require the presence of a type-specific band together with a cross-reactive band), by the INNO-LiPA samples are scored positive for certain genotypes only when a combination of two or more hybridization lines is observed. Thus, discordant type-specific profiles were due to the absence of one or more lines that form the specific hybridization pattern of a certain genotype.

DNA concentration in the several extracts was not evaluated in the current study. However, it is plausible that the overall amount of DNA recovered from each extraction procedure and then used for PCR was different. Nevertheless, it is also possible that the observed differences in the genotyping results depend on a differential recovery of different HPV genotypes achieved using different methods. The preferential amplification of certain types, present at higher concentration,

For Peer Review

may play an important role in determining non identical genotyping results for all the extracts of the same sample. Dunn et al. [2007] have shown that even small variations in the extraction protocol, for instance in the centrifuge speed used to process the PreservCyt cervico-vaginal samples, determine a difference in the Linear Array performance.

The Linear Array results showed higher concordance when the two manual extraction protocols were compared. Roche-recommended protocol of extraction (A) is based on a 2-step lysis followed by DNA purification on a QIAGEN vacuum manifold. The QIAGEN protocol (B) is based on a 4-step procedure (lyse, bind, wash and elute), which is similar to the Roche protocol. The automated nucleic acids extraction method (C) also includes similar steps, but is based on the use of magnetic silica particles instead of silica columns and entails multiple washes. It is possible that the effectiveness of the two manual methods in yielding DNA samples suitable for amplification was comparable in terms of quantity and quality of the DNA sample, while the automatic procedure might have had an overall different performance.

Published data indicate that the use of an automated DNA extraction system prior to amplification may increase the detection of microbiological agents from several clinical samples when compared with manual techniques, possibly because of a higher DNA yield or fewer inhibitors [Loens et al., 2007; Pillet et al., 2009]. The present results, although obtained from a limited number of patients, also suggest a superior performance of the automated procedure (C) compared to the Roche-recommended method (A). In fact, considering the seven cases that showed discordant Linear Array results between extracts A and C, in five cases one or more HPV types were found in extract C in comparison with extract A, while only in two cases did the Linear Array fail to detect in extract C an HPV type present in extract A (HPV 42, see TABLE III). On the other hand, when comparing the automated extraction (C) with the QIAGEN method (B), extra genotypes were detected in extracts C and B in an equal number of cases.

Possible variability introduced by the use of different volumes of the original cervico-vaginal samples for DNA extraction and other possible, random sources of variations need to be taken into

For Peer Review

account. In spite of these limits, the present study confirms previous results that showed that changes in the DNA extraction method modify HPV genotyping tests performance [Dunn et al., 2007]. Thus, the consistent use of the same DNA isolation protocol is recommended, in order not to have an extraction-dependent variability.

In conclusion, these preliminary data suggest that DNA extraction is a critical step for HPV genotyping tests, although a larger sample size is needed in order to evaluate thoroughly how different DNA extraction protocols affect these assays and to obtain a statistical validation of the results.

Acknowledgments

This study was supported by the Italian Ministry of Health and Lega Italiana per la Lotta contro i Tumori (LILT).

For Peer Review

REFERENCES

Bosch FX, Burchell AN, Schiffman M, Giuliano AR, de Sanjose S, Bruni L, Tortolero-Luna G, Kjaer SK, Munoz N. 2008. Epidemiology and natural history of human papillomavirus infections and type-specific implications in cervical neoplasia. Vaccine 26 Suppl 10:K1-16.

Bouvard V, Baan R, Straif K, Grosse Y, Secretan B, El Ghissassi F, Benbrahim-Tallaa L, Guha N, Freeman C, Galichet L, Cogliano V. 2009. A review of human carcinogens--Part B: biological agents. Lancet Oncol 10:321-322.

Castle PE, Porras C, Quint WG, Rodriguez AC, Schiffman M, Gravitt PE, Gonzalez P, Katki HA, Silva S, Freer E, Van Doorn LJ, Jimenez S, Herrero R, Hildesheim A. 2008. Comparison of two PCR-based human papillomavirus genotyping methods. J Clin Microbiol 46:3437-3445.

Castle PE, Solomon D, Schiffman M, Wheeler CM. 2005. Human papillomavirus type 16 infections and 2-year absolute risk of cervical precancer in women with equivocal or mild cytologic abnormalities. J Natl Cancer Inst 97:1066-1071.

de Sanjose S, Quint WG, Alemany L, Geraets DT, Klaustermeier JE, Lloveras B, Tous S, Felix A, Bravo LE, Shin HR, Vallejos CS, de Ruiz PA, Lima MA, Guimera N, Clavero O, Alejo M, Llombart-Bosch A, Cheng-Yang C, Tatti SA, Kasamatsu E, Iljazovic E, Odida M, Prado R, Seoud M, Grce M, Usubutun A, Jain A, Suarez GA, Lombardi LE, Banjo A, Menendez C, Domingo EJ, Velasco J, Nessa A, Chichareon SC, Qiao YL, Lerma E, Garland SM, Sasagawa T, Ferrera A, Hammouda D, Mariani L, Pelayo A, Steiner I, Oliva E, Meijer CJ, Al-Jassar WF, Cruz E, Wright TC, Puras A, Llave CL, Tzardi M, Agorastos T, Garcia-Barriola V, Clavel C, Ordi J, Andujar M, Castellsague X, Sanchez GI, Nowakowski AM, Bornstein J, Munoz N, Bosch FX. Human papillomavirus genotype attribution in invasive cervical cancer: a retrospective cross-sectional worldwide study. Lancet Oncol 11:1048-1056.

Dunn ST, Allen RA, Wang S, Walker J, Schiffman M. 2007. DNA extraction: an understudied and important aspect of HPV genotyping using PCR-based methods. J Virol Methods 143:45-54.

For Peer Review

Kitchener HC, Castle PE, Cox JT. 2006. Chapter 7: Achievements and limitations of cervical cytology screening. Vaccine 24 Suppl 3:S3/63-70.

Kjaer SK, Frederiksen K, Munk C, Iftner T. Long-term Absolute Risk of Cervical Intraepithelial Neoplasia Grade 3 or Worse Following Human Papillomavirus Infection: Role of Persistence. J Natl Cancer Inst 102:1478-1488.

Loens K, Bergs K, Ursi D, Goossens H, Ieven M. 2007. Evaluation of NucliSens easyMAG for automated nucleic acid extraction from various clinical specimens. J Clin Microbiol 45:421-425.

Molijn A, Kleter B, Quint W, van Doorn LJ. 2005. Molecular diagnosis of human papillomavirus (HPV) infections. J Clin Virol 32 Suppl 1:S43-51.

Munoz N, Bosch FX, de Sanjose S, Herrero R, Castellsague X, Shah KV, Snijders PJ, Meijer CJ. 2003. Epidemiologic classification of human papillomavirus types associated with cervical cancer. N Engl J Med 348:518-527.

Munoz N, Castellsague X, de Gonzalez AB, Gissmann L. 2006. Chapter 1: HPV in the etiology of human cancer. Vaccine 24 Suppl 3:S3/1-10.

Nagai Y, Toma T, Moromizato H, Maehama T, Asato T, Kariya K, Kanazawa K. 2004. Persistence of human papillomavirus infection as a predictor for recurrence in carcinoma of the cervix after radiotherapy. Am J Obstet Gynecol 191:1907-1913.

Nobeyama H, Sumi T, Misugi F, Okamoto E, Hattori K, Matsumoto Y, Yasui T, Honda K, Iwai K, Ishiko O. 2004. Association of HPV infection with prognosis after neoadjuvant chemotherapy in advanced uterine cervical cancer. Int J Mol Med 14:101-105.

Pillet S, Bourlet T, Pozzetto B. 2009. Comparative evaluation of a commercially available automated system for extraction of viral DNA from whole blood: application to monitoring of epstein-barr virus and cytomegalovirus load. J Clin Microbiol 47:3753-3755.

Snijders PJ, van den Brule AJ, Meijer CJ. 2003. The clinical relevance of human papillomavirus testing: relationship between analytical and clinical sensitivity. J Pathol 201:1-6.

For Peer Review

van Hamont D, van Ham MA, Bakkers JM, Massuger LF, Melchers WJ. 2006. Evaluation of the SPF10-INNO LiPA human papillomavirus (HPV) genotyping test and the roche linear array HPV genotyping test. J Clin Microbiol 44:3122-3129.

Wheeler CM. 2007. Advances in primary and secondary interventions for cervical cancer: human papillomavirus prophylactic vaccines and testing. Nat Clin Pract Oncol 4:224-235.

Wright TC, Jr., Massad LS, Dunton CJ, Spitzer M, Wilkinson EJ, Solomon D. 2007. 2006 consensus guidelines for the management of women with abnormal cervical cancer screening tests. Am J Obstet Gynecol 197:346-355.

zur Hausen H. 2009. Papillomaviruses in the causation of human cancers - a brief historical account. Virology 384:260-265.

For Peer Review