LOW AND HIGH-RISK HPV TYPES DETECTED WITH HYBRID CAPTURE II (HC2) IN LIQUID-BASED CYTOLOGY (DNA CITOLIQ®) SYSTEM: EXPERIENCE FROM THE LAMS (LATIN AMERICAN SCREENING) STUDY

DETECÇÃO DE HPV DE BAIXO E ALTO RISCOS PELO MÉTODO DE CAPTURA DE HÍBRIDOS II (HC2) EM CITOLOGIA DE BASE LÍQÜIDA (SISTEMA DNA CITOLIQ®): EXPERIÊNCIA DO GRUPO DE ESTUDO LAM (RASTREIO LATINO AMERICANO)

Adhemar Longatto Filho[1], Maria Lúcia Utagawa[1], Sonia Maria M Pereira[1], Janaína Érika Pittoli[1], Luciana S Aguiar[1], Marina YS Maeda[1], Celso Di Loreto[1], Cecília Roteli-Martins[2], Temístocles P Lima[2], Janice Olivia Galvane[2], Kari J Syrjänen[3]

RESUMO

Introdução: o método de captura de híbridos associado à citologia de base líqüida tem sido advogado como um meio eficaz de se detectar lesões cervicais e aumentar a sensibilidade do rastreio de alterações precursoras do carcinoma de colo uterino. **Objetivo:** avaliar a *performance* do meio *UCM (Universal Collecting Médium)* do novo sistema DNACITOLIQ (DIGENE-Brasil) para citologia de base líqüida e ensaios biomoleculares em uma população geral submetida a rastreio de rotina para lesões de colo uterino associadas ao HPV de baixo e alto riscos. **Métodos:** esse estudo analisou a detecção de HPV de baixo e alto risco com o sistema de captura híbrida II (HC2) como parte do projeto *Latin American Screening* (LAMS), usando o novo sistema de citologia em meio líqüido DNACITOLIQ (DIGENE-Brasil). As amostras foram coletadas prospectivamente no Hospital Leonor Mendes de Barros em 357 mulheres voluntárias, participantes do estudo LAMS (INCO-DEV ICA4-CT-2001-10013) durante o ano de 2002. As amostras foram coletadas com escova do sistema DNACITOLIQ com protocolo exclusivo para citologia de base líqüida (*direct-to-vial)*, sem preparo de esfregaços convencionais, e preservado imediatamente em meio ??? Primeiramente, prepararam-se as amostras citológicas para, em seguida, desnaturar-se o material conforme o protocolo da HC2 para HPV de baixo e alto riscos. **Resultados:** o HPV de baixo risco foi positivo em 10 casos citologicamente negativos e 1 positivo (carcinoma invasivo). O sistema HC2 para HPV de alto risco, resultou em 293 casos negativos e 46 positivos. Destes 46 casos positivos, 36 apresentavam diagnóstico de citologia normal, 3 de lesão de baixo grau, 5 de atipia escamosa indeterminada, 1 de ACG e 1 de carcinoma invasor. Em 18/357 (5%) casos houve positividade para HPV de baixo e alto riscos. **Conclusão:** estes resultados mostram que o meio testado oferece possibilidades de estudo citológico e de captura de híbridos, estimulando-nos a ampliar o âmbito da pesquisa, com relação ao novo sistema DNACITOLIQ em grandes populações, e associarmos avaliações colposcópicas e biópsias.

Palavras-chave: Captura híbrida, HPV, HPV de baixo risco, HPV de alto risco, Citologia de base líquida

ABSTRACT

Introduction: hybrid Capture assay associated to liquid-based cytology is believed to be more effcient than conventional smars alone and able to improve the sensitivity of uterine cervix lesions in screening programs. **Objective:** the objective of this study was to analyze the detection of low and high risk HPV infection with Hybrid Capture II (HC2) assay in Latin American Screening study (LAMS) using the new system of liquid-based cytology DNACITOLIQ® (DIGENE-Brasil) designed for both cytology and molecular studies. **Method:** the samples were collected prospectively from 357 voluntary women examined at Hospital Leonor Mendes de Barros participating in the ongoing LAMS study (INCO-DEV ICA4-CT-2001-10013) during 2002. Samples were collected with the brush of DNA CITOLIQ® system (Digene-Brasil) in direct-to-vial protocol, immediately preserved in the UCM (*Universal Collecting Medium*), a universal conservation medium of the system. The material was firstly used for the preparation of the cytological slides. Subsequently, the material was denatured and the HC2 assay for both low- and high-risk HPV types was performed. **Results:** in the 11 low-risk HPV-positive cases, cytology was negative for intraepithelial lesions and malignancy in 10 cases and 1 was an invasive carcinoma. HC2 assay for the high-risk HPV types resulted in negative findings in 293 cases, whereas 46 cases were HPV-positive. Of these 46 positive cases, 36 presented with normal cytology, 3 were consistent with LSIL, 5 with ASC-US, 1 was ACG and another one was an invasive carcinoma. Altogether, 18/357 (5.0%) cases showed HC2 positivity for both the low- and high-risk HPV types. **Conclusions:** the results encourage us to explore the new DNACITOLIQ system in larger series of women with colposcopy and biopsy data available.

Keywords: Hybrid Capture II, HPV, Low-Risk HPV, High-Risk HPV, Liquid- Based cytology, HSIL

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[1]Pathology Division of Adolfo Lutz Institute· São Paulo, Brazil; Hospital Leonor Mendes de Barros[2], São Paulo, Brazil; [3]Unit of Cytopathology, Laboratory of Epidemiology and Biostatistics, National Health Institute (ISS), Rome, Italy.

INTRODUCTION

The use of liquid-based cytology has been advocated as a preferential means to prepare cytology samples, because of its evident advantages as compared with the conventional smears. These proven benefits include the clean background, devoid of leukocyte infiltration, haemorrhage and debris, as well as the ideal conditions for molecular biological tests provided by this novel technique[1, 2]. Conventional smears have limitations in individual test sensitivity and specificity[3]. Additional slides can be readily prepared in case that the quality of the previous preparations is insufficient, thus avoiding the recall of the patients for new sampling, leading to considerable cost savings using this technology[1].

Using the currently available liquid-based cytology systems, there is no need to modify the sample collection practices. Using well designed cytobrushes, both ecto and endocervical cells are adequately present in the samples. In fact, the number of metaplastic and columnar cells in the liquid-based cytology is usually superior to those in the conventional smears[4, 5]. Another major innovation is the preservation liquid of these systems, which provides, in addition to perfect cell preservation, an ideal medium for doing molecular biology (HPV testing, PCR, immunocytochemistry), due to the superb preservation of DNA, RNA and cellular proteins.[1, 6, 7]

According to the recent trends in many countries, combining cytology (mainly liquid-based) and molecular tests for identifying HPV infection is a frequently recommended algorithm in patient triage and cervical cancer screening[8] The potential cost-effectiveness of such an approach was recently demonstrated in a series of 9.000 women, where high-grade lesions and cancer were correctly diagnosed in 100% of cases.[7]

According to the Bethesda 2001 guidelines, the use of HPV testing for ASC smears is justified, because of the potential prognostic value of this combination, particularly in predicting the benign behavior of HPV-negative cases. They only recommend testing for the high-risk HPV, however, neglecting the low-risk HPV types in the test panel, because of the missing evidence on the impact of the latter in routine HPV testing[8].

The present study was conducted to correlate the cytological findings in the liquid-based system (DNA CITOLIQ) with the results of HPV testing by HC2, performed in the residual material of the Digene new medium, UCM (*Universal Collection Medium*). This work is part of the ongoing LAMS (Latin American Screening) study, funded by the European Commission (INCO-DEV ICA4-CT-2001-10013) with partner clinics in three cities in Brazil (São Paulo, Campinas and Porto Alegre), Buenos Aires in Argentina, and from three European countries: Italy, Slovenia and Finland. The goal of this project is to assess the biology of HPV infections in Latin America as well as to test six different screening tools in a cohort of 12,000 women. The objective of this work was to evaluate the relevance of including the low-risk HPV hybrid capture assay in our algorithm and to compare these results with the cytological diagnosis performed with the new medium (Digene, UCM).

MATERIAL AND METHODS

The material of the present study comprises a series of 357 consecutive cervical samples taken from women examined during 2002 at Leonor Mendes de Barros Hospital, Sao Paulo, Brazil, as a part of the ongoing LAMS (Latin American Screening) study, funded by the European Commission (INCO-DEV ICA4-CT-2001-10013). The samples were collected using the brush of the DNA CITOLIQ® System (Digene-Brasil) and immersed in the UCM (Universal Collecting Medium, Digene) vials.

The sample processing followed the manufacturer's instructions. The sample in the tube was homogenized in high-speed vortex for 20 seconds, and 200-µl aliquot is placed on a polycarbonate membrane, 25 mm in diameter and 5µm of porosity, with uniform distribution over the total area of the membrane. Slides and membranes are disposed in a system with the capacity to process twelve samples simultaneously (Lamigene ®, Digene Brasil). In practice, the slides mounted in the Lamigene are placed into a metal box equipment (Prepgene), in which the cover is closed for 10 seconds with a constant pressure. The end result of this procedure is a slide with a homogeneous "imprint" of the sample. The slides are fixed in absolute ethanol and stained with conventional Papanicolaou method.

The residual material of each sample was used for the Hybrid Capture II test (Digene, USA), including both the low- and high-risk HPV types, following the standard protocol of the HC2 procedure[9].

The reading of the cytological samples was done in a blinded manner, the cytologists being unaware of the HC2 test results. The cytological results were reported according to the 2001 Bethesda System[10]. Positive cases (including ASC and ACG) were defined as consensus diagnosis between the participating cytologists.

RESULTS

The HC2 test results and cytology are correlated in **Table 1.** Of the 357 tested samples, 328 were negative and 11 were positive for the low-risk HPV types. Of the 11 HPV-positive cases, cytology was negative for intraepithelial lesions and malignancy in 10 cases and 1 was an invasive carcinoma.

HC2assay for the high-risk HPV types resulted in negative findings in 293 cases, whereas 46 cases were HPV-positive. Of these 46 positive cases, 36 presented with normal cytology, 3 were consistent with LSIL, 5 with ASC-US, 1 were ACG and 1 was an invasive carcinoma.

Altogether, 18/357 (5.0%) cases showed HC2positivity for both the low- and high-risk HPV type; such cases included both HSIL and carcinoma samples, as shown in the **Table 1**.

HC2 results include 10 cases (21.7%) with high-risk HPV in intraepithelial lesions (SIL) and cancer and 36 cases (78.3%) with negative cytology; in one cases (9.1%), these samples were also positive for the low-risk HPV. Considering the low-risk HPV HC2 testing alone, only one positive case showed an intraepithelial lesion. The Fisher's exact test of these data showed a p value of 0.165, which is not significant.

DISCUSSION

DNA-CITOLIQ System (DCS) was recently developed for liquid-based cytology and molecular studies, including HPV tes-

TABLE 1 - Correlation of cytological diagnosis and Hybrid Capture II results

DNACITOLIQ DIAGNOSIS	**HC2-LOW RISK**		**HC2-HIGH RISK**	
	negative	positive	negative	Positive BOTH
Negative	295	10	269	36 8
LSIL	5	-	2	3 2
HSIL	1	-	1	- 3
ASC-US	25	-	20	5 3
AGC	2	-	1	1 2
Carcinoma	-	1	-	1 -
Total	328 (91.9%)	11 (3.1%)	293 (82.1%)	46 18 (12.9%) (5.0%)

ting by Hybrid Capture assay. The system uses a Universal Collecting Medium (UCM) that preserves cytological morphology and DNA, RNA and cytoplasmic proteins for complementary investigations, e.g., such as usually done in Reflex test. The system is entirely manual, of low-cost and does not require any special adaptations in the structure of the laboratory. An imprint-like device is used to prepare the slides. After 20 seconds in the vortex, a 200 µl aliquot is transferred from the UCM tube (1 ml of total volume) onto a membrane, mounted in a support with the slide. The cover of the PrepGene is closed, imprinting the sample deposited on the membrane against the slide. The result of this protocol is a preparation of a slide with thin-layer appearance and clear background. Previous studies have shown its potential usefulness in both cytology and molecular applications, with results encouraging enough to prompt us testing new laboratory algorithms in the detection of intraepithelial lesions and cancer[11, 12].

These preliminary data with DNA CITOLIQ® System have shown preparations with excellent preservation of cell morphology, and providing ample of residual material ideally prepared for the HC2 assay. No artifacts were observed in cytological preparations, and e.g. the cytopathic alterations of HPV were very similar to those described in the conventional smears[11] . The DNA CITOLIQ® System showed that the UCM medium offers the same level of diagnostic performance than does the Standard Transfer medium (STM), concerning the identification of infectious agents[13]. Preliminary results have shown that the UCM and STM medium provide significantly concordant results, with Kappa analysis superior to 0.9 [13].

So far, few studies for HPV testing have been performed using the DNA CITOLIQ® technique (DCS). The first study evaluating the diagnostic performance of DCS showed that it improves the diagnosis of HSIL in 100% when compared with a conventional split Pap smear sample[14]; these data were similar to the reports using another liquid-based cytology systems[5, 15]. Another study using DCS analyzed the microbiological agents presents in vagina concluding that all infectious agents were routinely found and well recognized[15].

The present results suggest that Bethesda recommendations to perform HPV testing only for the high-risk group are justified. This is because in the present series, the HC2assay positive only for the low-risk types showed only one positive case with ASC-US cytology, but no higher-grade abnormalities. Indeed, the HSIL and carcinoma cases positive for the low-risk HPV types were also positive for the high-risk HPV types. The known cross-reactivity inherent to HC2 test is not significant enough to link high-grade lesions with the low-risk HPV, which would be contradictory to the known association of HSIL and cancer with the high-risk HPV types, shown by other techniques, i.e., PCR[17]. Hybrid Capture III test is underway to the commercial use, designed to be more sensitive and specific than HC2 and devoid of the cross-reaction problems[17].

In the present series, 5 ASC-US and 1 ACG cases tested positive for the high-risk HPV types (**Table 1**). This is consonant with the 2001 Bethesda suggestions[8] to review these undetermined cases, with the options to recommend the clinician a special follow-up, or to attempt a re-interpretation of the ASC-US and ACG cases for possible re-classification. The results obtained by ALTS study strongly suggest that the inclusion of high risk HPV testing with liquid-based cytology to enhance the sensitivity of HSIL (CIN3) and cancer detection[18].

Another point of interest in this study is related to the ACG cases. According to the 2001 Bethesda System, HPV testing for these cases is encouraged, but until now, literature does not provide sufficient evidence to justify a formal recommendation. Our 3 cases of ACG were all positive for HC2 ® high-risk HPV types, indicating that the aroused suspicion of these cases was in fact important. Despite the low number of ACG cases, we consider this finding very important, because glandular lesions are uncommon in routine series. Perhaps HPV testing in the future might prove to be of value in the cytological diagnosis of glandular lesions, particularly due to its high negative predictive value.

To conclude, the present results encourage us to continue the testing of DNACITOLIQ® System in a larger series of patients and correlate the results with colposcopy and biopsy data. The ongoing LAMS study offers excellent possibilities for this type of testing, while comparing six optional diagnostic tools in attempting to establish the cost-effective strategies for cervical cancer detection in the low-resource settings in Latin America. The inclusion of HPV testing based on HC2 high-risk assay can represent an important strategy to improve the intraepithelial detection of cytological examination mainly if performed with liquid-based systems. Moreover, the impact of negative predictive results of both negative cytology and HC2 tests should provide reassurance for extending the screening interval among the low-risk women[19].

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Address for Corresponce:
ADHEMAR LONGATTO FILHO
Adolfo Lutz Institute
Pathology Division 7º floor
Dr. Arnaldo Avenue, 355, Cerqueira Cezar
01246-902, São Paulo, SP, Brazil
Tel: +55 - 11 - 3068-2875
Fax: +55 - 11- 3068-2871
E-mail: longatto16@hotmail.com

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