EVALUATION OF SAMPLE CELLULARITY AND NUCLEIC ACIDS STABILITY USING COPAN eNAT™ MEDIUM ASSOCIATED WITH FLOQSWABS™: SELF-COLLECTED VERSUS CLINICIAN-VAGINAL SAMPLING

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Background/Objectives

Vaginal self-sampling represents a promising alternative to increase women's participation to screening for HPV and sexually transmitted diseases.

Clinical specimens for nucleic acids amplification tests (NAATs) are traditionally transported in viral or bacterial culture media.

Copan developed eNAT™, a molecular medium designed for storage and transport of clinical samples for the detection infectious agents by NAATs, able to inactivate pathogens viability and preserve nucleic acids at room temperature for extended periods of time.

The objectives of this study were to validate the performance of eNAT™ medium associated with FLOQSwabs™ (Copan, Brescia, Italy) for sample cellularity and nucleic acid stability in self- and clinician collected vaginal samples.

Materials

Paired self-collected at point of care (POC) and physician administered vaginal samples using FLOQSwabs™ were randomly collected from 35 asymptomatic women attending the Cytology Unit, Synlab, Brescia, Italy. A further sample was also self-collected by all women at home.

Samples were transported in eNAT™ medium and stored at –20°C until testing at the Microbiology Laboratory of the University Milano-Bicocca.

FLOQSwabs™ and eNAT™ were kindly provided by Copan.

Methods

Sample cellularity was evaluated, following nucleic acid extraction (NucliSENS®easyMAG, bioMérieux), by means of CCR5 gene and beta-actin mRNA quantification by real-time PCR as previously described[1,2].

A separate sample aliquot was stored at -80°C and re-extracted and re-tested after 18 months to assess nucleic acids stability in eNAT™.

Samples were also tested for the presence of oncogenic HPV genotypes using AnyplexII HPV28 (Seegene) kindly provided by Arrow Diagnostics.

Results

CCR5 and beta-actin copy numbers/2 ml eNAT™ median values were found to be 12275 and 169 x 10^6.

Cellularity median values after 18 months were found to be 14464 and 43 x 10^6.

PATIENT	SELF-SAMPLING (Home and POC)	CLINICIAN-SAMPLING
#1	neg	58 ++
#2	58 ++	58 ++ 68 ++
#3	16 +	neg
#4	56 +	neg
#5	39 +	neg
#6	16+++ 56+++	16+++ 56+++
#7	16+ 35+	16+ 35++
#8	18++	18++
#9	neg	66++

Table 3: HPV-types identified among HPV positive women

Target sequence	SELF-SAMPLING (Home and POC) (copy number/2 ml)	CLINICIAN-SAMPLING (copy number/2 ml)
CCR5	12805	13333
β-actin	164*10^6	165*10^6

Table 1: CCR5 and beta-actin copy numbers/2 ml

Target sequence	SELF-SAMPLING (Home and POC) (copy number/2 ml)	CLINICIAN-SAMPLING (copy number/2 ml)
CCR5	12530	12971
β-actin	52*10^6	64*10^6

Table 2: CCR5 and beta-actin copy numbers/2 ml after 18 months

HPV was detected in 26.5% (9/34) of women; 45% were positive in both samples, 33% only in self-collected and 22% only in clinician-collected samples.

HPV-types identified are reported in Table 3.

Conclusion

Cellularity of both home and POC self- and clinician-collected vaginal samples using FLOQSwabs™ showed comparable results. Sample storage in eNAT™ medium for 18 months at -80°C showed good nucleic acid stability for both DNA and RNA. Data obtained demonstrated a good performance of both FLOQSwabs™ and eNAT™ medium in vaginal sample collection, transport and storage for NAATs as well as of AnyplexII HPV28 (Seegene) in HPV testing.

References [1] C. Jobin *et al.* The Journal of Immunology, 1997; 158:226-234. [2] F. Broccolo *et al*. Journal of Clinical Microbiology. 2002; 4652-4658