

Validation of L-shape Endo/Esocervical Flocked Swab and CyMol Transport Medium with NucliSENS® EasyQ® HPV v1.1 Assay

D. Jeantet¹, M. Bonhomme¹, F. Meynier¹, A. Giambra², A. Squassina², E. Valagussa³, P. Libretti³, M. Peroni⁴, G. Ciravolo⁴, S. Castriciano²
¹Biomerieux, Grenoble, France. ²Copan Italia Spa, Brescia, Italy.
³Fleming Lab, Brescia Italy. ⁴Department of Gynecology, Brescia University, Italy.



Abstract

Objective: NucliSENS® EasyQ® HPV v1.1 (bioMérieux) is a qualitative nucleic acid assay for the detection of E6/E7 HPV oncogene mRNA (HPV16, 18, 31, 33 and 45). The L-shape endo/esocervical flocked swab (LEC) is a device for Pap collection and CyMol is a cell and nucleic acid preservation medium (Copan Italia).

Study objectives were:

- 1) validate the performance of LEC and CyMol medium compared to the Cervex Brush (CB) (Rovers) and PreservCyt solution (Hologic) for collection and preservation of specimens for HPV detection with the NucliSENS EasyQ® HPV 1.1 assay.
- 2) Evaluate stability of specimens in CyMol medium after 3 weeks at room temperature (RT) and 6 weeks at -20°C.

Methods: Six hundred clinical specimens were collected from voluntary women during a routine visit for Pap testing at 5 Fleming Lab sites in Brescia. Two clinical specimens (300 with CB in PreservCyt and 300 with LEC in CyMol) were collected from each patient in a randomized order and classified for PAP cytology status. Aliquots of all positive specimens in CyMol medium and the same number of negative specimens were used for 3 weeks RT and 6 weeks -20°C stability study. All specimens were extracted and tested with the NucliSENS® easyMAG® and EasyQ® HPV v1.1 assay.

Results: The results of the comparison study show a concordance of 100 % between the specimens collected in CyMol and specimens collected in PreservCyt (Table 1). The invalid rates are comparable in the two transport media. Stability of specimens in CyMol stored at RT was demonstrated up to 3 weeks up to 6 weeks at -20°C.

Conclusions: No statistical difference was found between the specimens collected with cervex brush in PreservCyt solution and specimens collected with L-shape endo/esocervical flocked swab in CyMol medium when tested with NucliSENS® EasyQ® HPV v1.1.

Objective

NucliSENS® EasyQ® HPV v1.1 (bioMérieux) is a qualitative nucleic acid assay for the detection of E6/E7 HPV oncogene mRNA (HPV16, 18, 31, 33 and 45). The L-shape endo/esocervical flocked swab (LEC) is a device for Pap collection and CyMol is a cell and nucleic acid preservation medium (Copan Italia).

Study objectives were:

- 1) To evaluate, in routine conditions, the performance of NucliSENS EasyQ HPV 1.1 assay when using specimens collected with the Copan L-shape endo/esocervical flocked swab in CyMol transport medium compared to specimens collected with the Rovers cervex brush in PreservCyt transport solution.
- 2) To evaluate the stability of specimen in CyMol medium after 3 weeks storage at RT and after 6 week storage at -20°C for the detection of five HPV genotypes.

Materials & Methods

Materials:

300 Kits, pre-coded for random collection order, were prepared. Each kit was numbered from 1 to 300 and contained:

- 2 record copies (patients data and Pap results)
- One vial of PreservCyt solution (20mL)
- One tube of CyMol medium (2mL)
- One Cervex Brush
- One L-Shaped Flocked swab
- One 15 ml conical centrifuge tube

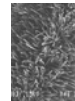
Each item inside the kit was labeled with the kit number.



PreservCyt and Cervex Brush



CyMol and L-Shaped Endo/Esocervical Swab



EM picture of the fibers of the L-Shape

Six hundred clinical specimens were collected from 300 voluntary women, after signed consent, during a routine visit for Pap testing at 5 Fleming Lab sites in Brescia.

Two clinical specimens were collected in a randomized order from each patient, one with Cervex Brush placed in a vial of PreservCyt and another with L-Shaped endo/esocervical flocked swab placed in a CyMol tube.

After collection, the samples were delivered to the Fleming Cytology center. Five ml of PreservCyt sample were transferred to the pre-labeled 15 ml conical tube and the left-over of the sample was used to prepare the Pap test smear using the ThinPrep Processor. Pap test smear were stained and read by a professional pathologist of the Fleming center.

The 5 ml PreservCyt specimen tubes and CyMol sample tubes were placed in a specimen box and transported at RT to Copan for HPV testing.

After recording, the PreservCyt tubes were centrifuged and the supernatant was removed to the last 1 mL fluid above the pellet. Resuspended pellets were added to a pre-labeled tube with Lysis Buffer. The CyMol tubes were vortexed, 0.5 mL was added to pre-labeled tube with Lysis Buffer®, 0.6 mL was dispensed in a cryovial for -20°C frozen storage and the left-over samples were stored at RT up to 3 weeks.



Sample preparation
30 min



Automatic extraction
60 min



Amplification and detection
150 min

All samples in Lysis Buffer were extracted in batches of 24 with the NucliSENS® easyMAG®. Less than 24, samples in Lysis Buffer were stored at -80°C until extracted.

All extracted samples were tested with the NucliSENS® EasyQ® HPV v1.1 assay. If unable to same day test, extracted samples were stored at -20°C until testing.

Aliquots of all positive specimens in CyMol medium and the same number of negative specimens were used for 3 weeks RT and 6 weeks -20°C stability study. Unresolved results were repeated in the next run.

Results

Results of the comparison study

	Positive	Negative	Invalid	Total
PreservCyt & CB	26	271	3*	300
CyMol & LEC	26	271	3*	300
Concordant	26	269	NA**	295

*One same patient, 2 different patients.

**NA: concordance is calculated on valid samples only.

HPV types in both PreservCyt and CyMol media

HPV 16	HPV 18	HPV 31	HPV 45	HPV 33	HPV 18 HPV 33	Total
14	2	5	1	3	1	26

Pap Test versus NucliSENS® EasyQ® HPV results (N=300)

Cytology & HPV positive	Cytology normal & HPV positive	Cytology positive & HPV negative	Cytology normal & HPV negative	Cytology inadequate & HPV negative
21	5	16	255	3
1 ASCUS	3 HPV 31	8 ASCUS		
2 ASC-H	1 HPV 16	4 ASC-H		
3 LSIL	1 HPV 45	4 LSIL		
15 HSIL				

Stability results:

After 3 weeks storage at RT

- 25 / 26 were positive (1 HPV16 becomes negative)
- 26 / 26 were negative

→ 96.2% concordance between T0 and T3 weeks

After 6 weeks storage at -20°C

- 23 / 26 were positive (1 HPV16, 1 HPV31 and 1 HPV 45 become negative)
- 25 / 26 were negative (1 HPV33 becomes positive)

→ 84.6% concordance between T0 and T6 weeks

Conclusions

A good correlation was found between the samples collected with the Cervex Brush in PreservCyt solution and the samples collected with the L-Shape endo/esocervical flocked swab in CyMol medium when tested with the NucliSENS® EasyQ® HPV v1.1.

Cervical specimen stability in CyMol was demonstrated up to 3 weeks when stored at room temperature and up to 6 weeks when stored at -20°C.

Copan L-shape endo/esocervical flocked swab in CyMol medium for cervical specimens collection/preservation and the bioMérieux NucliSENS® EasyQ® HPV v1.1 for the detection of high risk HPV genotypes are an excellent combination for improving women health by predicting the risk of developing cervical cancer and thus reduce the number of unnecessary colposcopies.