Validation of Dry Self-collected FLOQSwabs® Eluted in MSwab® Medium for the Detection of Human Papillomavirus (HPV) Using Six Commercial PCR-based HPV Assays

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INTRODUCTON

Vaginal self-collection is cost effective and has been well accepted and has increased women's participation to Human Papillomavirus cervical screening programs. Published evidence demonstrates that vaginal self-collected specimens are non-inferior to clinical collected specimens for the detection of HPV. Currently, dry self-collected vaginal swabs, analysed for HPV screening with molecular assays, are eluted in variable volumes of alcohol-based cytology or other media as reported in many publications. A standardized protocol using a validated molecular medium and elution volume compatible with most clinically validated commercial PCR-based HPV assays, is necessary for the for the elution of dry self-collected vaginal swabs. MSwab® is a molecular medium that support HPV stability and compatibility with clinically validated PCR-based HPV assays.

- Is a master mix compatible molecular medium, that supports both rapid heat and traditional nucleic acids extraction methods. Self-collected vaginal samples eluted in PreservCyt™ and MSwab® were compared, demonstrating a good concordance for high-risk HPV (hrHPV) and low-risk HPV (lrHPV) detection compared to professional collected cervical samples. Excellent stability in sample preservation and concordant hrHPV detection was observed in self-collected vaginal samples eluted in 5mL MSwab™ at both 22°C and 37°C for 4 weeks and after 1 year at -20°C. Has been tested with 6 clinically validated PCR-based HPV assays using the Microbix HPV16, HPV18, HPV45, and Negative PROCEEDx™ FLOQ® Swab samples.

The objective of this validation was to examine the compatibility of MSwab® medium for the elution of dry self-collected vaginal FLOQSwabs® for the qualitative detection of HPV using six commercial clinical validated PCR-based HPV assays.

Materials

Copan 3 ml MSwab® Medium

Copa FLOQSwabs® (552C.80)

METHODS

Thirteen dual vaginal self-collected FLOQSwabs® specimens were used for this pilot study.

- One set of the swabs was eluted in 3ml PreservCyt and analysed after collection using the Seegene Anyplex II HPV28 assay.
- Testing was performed at the Clinical Microbiology and Virology Laboratory of the University of Milano-Bicocca.

The other set of swabs were analysed after dry storage for 7 to 84 days at 20^0 to 40^0C uncontrolled temperature and shipped from Italy during the summer to Australia.

- HPV 16, 18, 45 and Negative PROCEEDx™ FLOQ® swabs were used as quality controls.
- All swab samples and controls were eluted in 3ml MSwab® medium by swirling for 20-seconds before removing the swab.
- All samples were analysed for the presence of HPV genotypes and cellularity using 6 clinically validated HPV assays that are detecting beta-globin for specimen adequacy and specific HR HPV genotypes.

- Abbott Alinity m HPV Assay on the Alinity.
- Abbott Realtime HPV Assay on the m2000.
- Qiagen NeuMoDx HPV Assay on the NeuMoDx 96 system.
- Roche cobas 4800 HPV Assay on the cobas 4800 system.
- Roche cobas HPV Assay on the cobas 6800 system.
- Seegene Anyplex II HPV28 Assay on the STARlet/CFX systems

The results of the self-collected swabs eluted in the 3 ml of MSwab were compared to the results of self-collected vaginal swab eluted in 3 ml of PreservCyt, tested after collection with the Seegene Anyplex II HPV28 assay.

Results for the MicroBix PROCEEDx FLOQ Swabs were compared to manufacturer's claim Testing was performed at the VCS Pathology Molecular Microbiology Laboratory.

RESULTS

In the 13 self-collected vaginal swabs eluted in 3 ml of MSwab®:

1. **The Abbott Alinity m HPV Assay** detected 9 positive and 3 negative HR HPV genotypes.

- One specimen had a processing error, and unable to be repeated due to sample limitation.

2. **The Abbott Realtime HPV Assay** detected 10 positive and 3 negative HR HPV genotypes.
3. **The Qiagen NeuMoDx HPV Assay** detected 9 positive and 3 negative HR HPV genotypes.

- One specimen had an unresolved result and considered as invalid

4. **The Roche cobas 4800 HPV Assay** detected 10 positive and 3 negative HR HPV genotypes.

- Three specimens produced invalid result due to failure of the internal/cellularity control) but these did not change the overall result for the specimen as HPV positive results

5. **The Roche cobas 6800 HPV Assay** detected 9 positive and 3 negative HR HPV genotypes
6. **The Seegene Anyplex II HPV28 Assay** detected 10 positive and 3 negative HR HPV genotypes.

- One specimen had a negative result compared to a low HPV16 results on the initial self-collection test but was positive for other HPV genotypes.

In the 13 self-collected vaginal swabs eluted in 3 ml of MSwab®Analyzed with the 6 assay

100% Sensitivity, Specificity, Positive and Negative predictive value
Kappa Statistic =1.000 and P-value <0.0001

Proper results were obtained with the HPV positive and negative PROCEEDx™ FLOQ® Swabs controls with all 6 HPV assays.

Perfect HPV result correlation was obtained for both self-collection samples eluted in PreservCyt and MSwab® medium with all 6 HPV assays.

These validation are acceptable and statistically significant, because they all satisfies the acceptance criteria of a kappa statistic of >0.8with all 6 HPV assays.

CONCLUSIONS

Dry vaginal specimens, self-collected using the FLOQSwab® code 552C.80, eluted into 3 ml of Copan MSwab® medium, produced identical HPV results to self-collected swabs eluted into 3 ml of PreservCyt.

Abbott Alinity m HPV Assay.
Abbott Realtime HPV Assay.
Qiagen NeuMoDx HPV Assay.
Roche cobas 4800 HPV Assay.
Roche cobas HPV Assay.
Seegene Anyplex II HPV28 Assay.

No degradation affecting HPV detection or cellularity was detected in the self-collected vaginal FLOQSwab® when stored at 20^0 to 40^0C for 7 to 84 days between collection and elution in MSwab® medium.

Microbix HPV16, HPV18, HPV45 and Negative PROCEEDx™ FLOQ® Swab controls can be used to quality control the entire laboratory workflow for accurate

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