# Practical value of the marker MUC4 for identification of vaginal secretion in penile swabs

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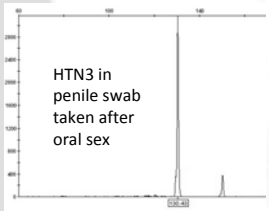
## INTRODUCTION

Consider a sexual assault involving vaginal intercourse without a condom, where no semen was detected in the vagina. In the case of a known suspect, a penile swab is needed to confirm intercourse by identification of vaginal secretion as well as to obtain the DNA profile of the victim. MUC4 is one of the prevalent mucin messenger ribonucleic acids of the human endocervix, but its expression was also detected in the oviduct and uterus, oral epithelial cells, ectocervix and vagina in low levels [1]. Some authors have reported MUC4 cross-reactivity with others body fluids [3]. According to our knowledge, MUC4 specificity and cross- reactivity, involving much more common and real forensic samples, i.e. penile swabs, has not been evaluated previously, therefore, we focus our study to evaluate the specificity of the vaginal secretion specific mRNA-marker MUC4 in different types of penile swabs after different sexual intercourses. To improve the reliability of identified MUC4 in penile swabs we also included two additional markers STATH and HTN3, specific only for saliva. In order to confirm constant expression of MUC4, also in vaginal secretion, we have investigated vaginal swabs taken from women before and after menopause.

## MATERIALS AND METHODS

Penile swabs were collected 1 hour after sexual intercourse without ejaculation, after sex, involving two heterosexuals, after oral sex between two heterosexuals and two homosexuals, and from anal sex involving two homosexuals. Penile swabs were taken from circumcised and from uncircumcised penis and smegma. Swabs (10) were taken from tonsils of men and women for specificity of MUC4. Vaginal samples (42) needed for evaluation of MUC4 mRNA marker expression were collected from 6 different women, between 25 and 50 years of age (one known medical history, HPV) in triplicates. Buccal swabs (42) were collected from the same men and women as other samples. All samples were analyzed two months after they have been collected.

- Total RNA was extracted with the RNAqueous ® -Micro Kit (with some modifications)
- For reverse transcription reaction High-Capacity cDNA reverse transcription kit and SUPERase•In™ RNase Inhibitor
- Final concentrations of the primers were as follows: MUC4 0.04 μM; STATH and HTN 0.16 μM; GAPDH 0.40 μM.
- PCR conditions used for amplification were as follows: denaturing step (95 °C, 11 min) followed by 33 cycles (94 °C, 20 s; 55 °C, 30 s; 72 °C, 40 s) and the final elongation at 72 °C for 5 min using GeneAmp® PCR System 9700.
- PCR products were detected using 3130 Genetic Analyzer and analyzed with GeneMapper® ID-X ver. 1.1.1 (Applied Biosystems).




## RESULTS

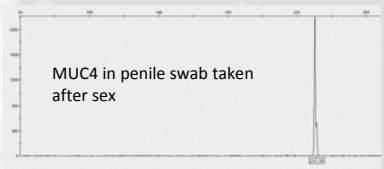
**Specificity of the mRNA markers**

| sample | marker | | | |
|---|---|---|---|---|
| | GAPDH | MUC4 | STATH | HTN3 |
| vaginal secretion | (42/42) | (33/42) | (0/42) | **(0/42)** |
| saliva | (42/42) | (3/42) | (42/42) | **(42/42)** |

**Penile swabs taken after different sexual intercourses**

| sample | marker | | | |
|---|---|---|---|---|
| | GAPDH | MUC4 | STATH | HTN3 |
| penile swab – circumcised penis [1] | (5/5) | (1/5) | (0/5) | **(0/5)** |
| penile swab – uncircumcised penis [1] | (5/5) | (5/5) | (0/5) | **(0/5)** |
| penile swab – circumcised penis [2] | (0/5) | (0/5) | (0/5) | **(0/5)** |
| penile swab – circumcised penis [3] | (5/5) | (0/5) | (5/5) | **(5/5)** |
| penile swab – uncircumcised penis [3] | (5/5) | (0/5) | (5/5) | **(5/5)** |
| penile swab – circumcised penis [4] | (5/5) | (0/5) | (5/5) | **(5/5)** |
| penile swab – uncircumcised penis [4] | (5/5) | (0/5) | (5/5) | **(5/5)** |
| smegma on penis | (5/5) | (0/5) | (0/5) | **(0/5)** |
| man tonsils | (5/5) | (0/5) | (5/5) | **(5/5)** |
| women tonsils | (5/5) | (0/5) | (5/5) | **(5/5)** |
| Negative control (DNA) | (0/5) | (0/5) | (0/5) | **(0/5)** |

Numbers in parentheses equal PCR result/number of sample tested. GAPDH, MUC4, STATH and HTN3 genes were considered as expressed if any of the samples obtained showed a PCR product for gene.




1. sex between two heterosexuals,
2. anal sex between two homosexuals,
3. oral sex between two heterosexuals,
4. oral sex between two homosexuals

## DISCUSSION

GAPDH served as a positive marker and our results are in accordance with the ones previously reported. We found out that the specific mRNA-marker MUC4 was detected in 78 % (33/42) vaginal samples. Similar findings were published by Gipson et. al. [1]. Interestingly, all 9 vaginal samples were MUC4 negative, taken from the same woman in menopause, in different time periods. The explanation for this phenomenon could be the low levels of estrogen during the menopausal period, leading to thinning of the vaginal epithelium, which can cause vaginal dryness and atrophic vaginitis. The prevalence of genitourinary symptoms increases with the menopausal transition, with up to one-third of perimenopausal and postmenopausal women experiencing vaginal dryness. It is possible that women with vaginal dryness have less MUC4 or that the women in menopause have lower levels of MUC4, because the expression of MUC4 is correlated with the levels of estrogen and progesterone in blood.

No cross-reactivity was detected, when using probes HTN3 and STATH for identification of MUC4 in penile swabs, suggesting that mentioned markers STATH and HTN3 could be used for identification of MUC4 in vaginal samples or in probative evidence of oral intercourse.Since only one sample of penile swab taken from circumcised penis after sex was positive for MUC4, we think that the reason can be the already mentioned estrogen levels and vaginal dryness or because there is no foreskin in circumcised penis, which would keep vaginal secretion on the penis.

Since it has been reported that foreskin secretion contains mucins, including MUC4, we tested smegma for presence of MUC4. The reason for negative results could be the length of the region of the investigated gene or male hygiene. The presence of MUC4 in smegma should be further investigated, because of the false positive results. We think that the reason for negative results for penile swabs taken after anal sex is the use of lubricant during sexual intercourse. Positive samples were obtained from all samples taken from circumcised and uncircumcised penis after oral sex, suggesting that markers STATH and HTN3 could be used for identification of saliva. Based on our results we think that MUC4 can be used only to determine the orientation of sexual intercourse, if isolated on penile swab. We recommend, that identification of vaginal secretion is always performed together with one of the mRNA saliva's markers. Further studies should be made to investigate MUC4 expression in women in menopause and smegma. With proper identification of MUC4 on penis we could actually confirm sexual intercourse, while negative results are much less reliable.

**Key references:**
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