



RESEARCH

INFORMATION

BIOVaSST: Biomarkers for risk stratification of cervical disease in self-taken vaginal swab samples



AIMS

- Cervical screening programmes aim to detect human papillomavirus (HPV) infection which, in some rare cases, can cause cervical cancer. Most HPV-positive people have a very low probability of getting cervical cancer. There are no tests that can identify those people who will get cervical cancer from those who will not. Cervical screening is carried out in the UK using cervical “smear” samples usually taken in a doctor’s surgery. A recent innovation is self-taken vaginal swabs (STs), which allows people to take samples suitable for cervical screening in their own home. STs are already in use in some countries and will soon be integrated into the UK cervical screening programme to aid access to screening for those who miss appointments. Currently around 30-40% of people in the UK have not been regularly screened.
- PCR is a molecular test for detection of many infectious diseases e.g. Covid. PCR detects and quantifies genetic material, either DNA or RNA. DNA is the primary genetic material that exists in all cells, and in some viruses such as human papillomavirus. RNA is the secondary genetic material produced from DNA that templates the production of proteins, the building blocks of cells and viruses. Therefore, RNA detection and quantification can profile the molecular nature of cells. We had shown previously that certain RNAs produced in HPV-infected cells taken from “smear” samples could distinguish between cases of cervical disease with a very limited

possibility of progression to cervical cancer versus cases that may progress to cancer.

Therefore, these RNAs are biomarkers (molecules that profile disease) of cervical disease. This previous study used clinical samples from the Scottish HPV Archive Biobank. Using biobanked material avoids the need to set up a new study that would involve hiring and paying study staff and recruiting patients.

- AIM 1. We examined if STSs stored in the Scottish HPV Archive Biobank were suitable for analysis of RNA and if STSs from another source that used a different collection device performed similarly.
- AIM2. We investigated if STSs could be used in a novel PCR-based molecular test that could quantify selected RNAs (called biomarkers in the remainder of this report).
- AIM 3. We investigated if storage for a long period of time (one year) would affect the ability of STSs to be used in molecular tests.



KEY FINDINGS

- Genetic material suitable for biomarker analysis could be prepared from all self-taken vaginal swab samples (STSs) from the Scottish Archive but not from STSs taken with a different device.
- Long term storage for one year did not affect the quality of the genetic material as regards molecular testing.
- Quantification of biomarkers by PCR testing in STSs is feasible but is likely to be heavily dependent on collection and storage procedures, which may differ between testing laboratories.
- We streamlined the biomarker test we devised for “smear” samples to reduce the quantity of the clinical sample required for analysis.
- The biomarker changes associated with cervical disease in STSs did not always match those in previous study using “smear” samples. This is likely due to a much lower percentage of samples graded as having cervical disease in the STSs compare to the “smear” sample set and that STSs



contain mainly vaginal cells as opposed to cervical cells. Unique biomarker metrics are likely to be required for these samples.

- Further studies that compare the performance of our biomarker tests using matched “smear” samples and STSs is advised. Biobanks will help support this objective as will studies that recruit participants that provide both types of samples.



WHAT DID THE STUDY INVOLVE?

This was a feasibility study to determine if STSs were suitable for use in molecular biomarker tests. The study involved determining what percentage of STSs yielded sufficient genetic material for molecular testing. Then testing whether quantification of biomarkers by PCR of biobanked STSs was feasible. All samples (associated with a specific collection device) from the Scottish Archive yielded genetic material suitable for biomarker analysis. A set of biomarkers that had proven capable of differentiating between cervical disease that has a very limited possibility of progression to cancer versus cervical disease that has potential to progress to cancer were quantified in the STSs. This study was the first study to analyse cellular genetic material in STSs by PCR. This study was also the first to quantify the levels of these novel biomarkers in STSs. The results add to the field by providing proof-of-principle that genetic material can be prepared from STSs even after long term storage and that STSs are appropriate for a PCR biomarker test.



WHAT WERE THE RESULTS AND WHAT DO THEY MEAN?

- The results of the study revealed that two biomarkers showed significantly different levels between STSs where there was very limited risk of cervical disease progressing to cancer versus STSs where there was a risk of progression to cancer.
- These biomarkers could be developed as a test to determine risk of cervical cancer progression using STSs.
- Vaginal STSs should be viewed as a quite different sample type that may require different biomarkers from those that perform well in cervical “smear” samples.



- The reason for this is that STSs will contain mostly vaginal cells with some cervical cells rather than “smear” samples that will contain mainly cervical cells.
- The results provide data for the first time on the quantity and quality of genetic material that can be prepared from STSs. Further, they indicate that optimal STS collection/storage protocols are key to development of future tests for cervical disease.



WHAT IMPACT COULD THE FINDINGS HAVE?

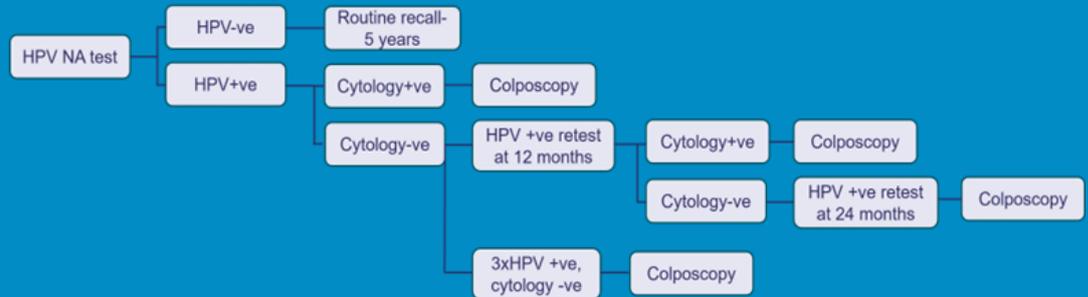
First, these findings reveal that STSs are suitable for RNA based molecular biomarker tests. Second, the data show that performance of STSs in molecular tests is unaffected by long term storage. However, the self-sampling device/method of collection and storage conditions (e.g. temperature) may adversely affect the potential downstream use of STSs. Based on our study, an optimised international protocol for collecting and storing STSs in a way that supports biomarker investigation and testing is required.

Our study has demonstrated feasibility of quantifying biomarkers by PCR in STSs as a means of triaging HPV-positive individuals with cervical disease that has a very limited possibility of progression to cancer, while identifying individuals whose cervical disease could progress to cancer. Due to the large amount of natural variability in biomarker levels between individuals, we have designed a triple biomarker matrix with a high specificity in identifying cervical disease. Development of this biomarker matrix test for use in clinical testing laboratories could reduce the time frame of cervical screening leading to reduced anxiety for patients and reduced time and costs for the NHS (see diagram below).

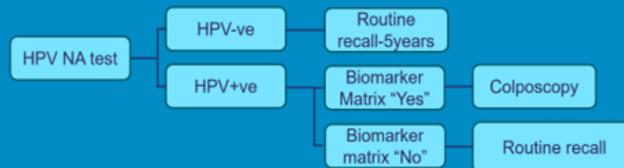


A diagram showing (A) the current cervical screening strategy in Scotland compared to (B) a possible novel screening strategy if an RNA biomarker PCR triage test is developed

A. Current Scottish cervical screening strategy



B. Cervical screening strategy with a high-performance PCR test.



HOW WILL THE OUTCOMES BE DISSEMINATED?

- The results have been presented at two international scientific meetings: EUROGIN 2024 and the International Papillomavirus Conference 2024.
- Two papers describing the outcomes of the work are published:
 - Scott et al. Journal of Medical Virology 2025 97:e7073 doi.10.1002/jmv.70737.
 - Scott et al. Journal of Virological Methods 2026 339:115268 doi: 10.1016.j.jviromet.2025.115268.
- A third paper will be submitted pending further statistical analysis of the data.
- The next piece of research will be to compare the performance of the biomarkers in STSs with their performance in traditional “smear” samples. We will apply for ethical permission to collect and analyse matched samples (“smear” and STSs) from patients called for cervical screening to enable a direct comparison of the two biospecimen types with respect to detection and quantification of the biomarkers.



CONCLUSION

- This study has shown that self-taken vaginal swab samples can be used in PCR-based tests to detect RNA biomarkers.
- Optimal and standardised collection and storage protocols will need to be determined to ensure good performance of these samples.
- There are some differences in RNA biomarker levels associated with disease in STSs compared to those from clinician-taken “smear” samples. Therefore, unique quantification thresholds and/or novel biomarkers are likely to be required for STSs.
- This work provides essential insight into the credibility of RNA based biomarkers as a means to detect significant cervical disease in STSs.



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