The extended stability of cervical swabs in careHPV™ Collection Medium

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Research article

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Abstract

Background:
The careHPV™ Test is a US FDA approved, CE mark, and WHO prequalified \textit{in vitro} diagnostic test that is designed to screen for 14 HRHPV genotypes. The careHPV™ Test is the only commercial HPV tests validated to be used in the low resource settings, boasting the economy of processing a maximum of 90 samples per batch and a near point-of-care turnaround time of 3 hours. Cervical swabs stored in the careHPV™ Collection Medium are stable for 30 days when stored between 2–8 °C according to the manufacturer. However, we often had difficulty to consolidate enough samples for a full batch-test within 30 days, especially when screening women living in the low-density villages in the rural Sarawak, Malaysian Borneo.

Objectives:
The aim of this study is to determine the stability and repeatability of cervical swabs preserved in careHPV™ Collection Medium stored at 4 °C over the recommended 30 days using the careHPV™ Test.

Methods:
Two groups of confirmed HRHPV positive and HRHPV negative cervical swab samples in careHPV™ Collection Medium consisting of 4 samples each were maintained at 4 °C and tested using careHPV™ Test at day-38, -123, -131, -223 and −395.

Results:
All cervical swabs in careHPV™ Collection Medium stored at 4 °C remained stable for testing and demonstrated 100% repeatability for at least 395 days from the day of collection.

Conclusion:
The careHPV™ Test can be successfully performed on cervical swabs preserved in the careHPV Collection Medium, which are stored at 4 °C for at least 395 days.

Introduction
Cervical cancer and prevention
Cervical cancer is the fourth most frequently diagnosed cancer and the fourth leading cause of cancer death in women worldwide. Cervical cancer is responsible for approximately 570,000 cases and 311,000 deaths annually (1). It was estimated that more than 80% of cervical cancer incidences were from low- and medium-income countries (LMIC) that lack organised screening and HPV vaccination programmes (2). There is also a significant disparity between the urban and the rural populations (3, 4), mainly due to the inaccessibility of proper healthcare facilities, poverty and other cofactors (5).

Human papillomavirus (HPV) is the major factor in the development of cervical cancer (6). Currently, 198 HPV genotypes have been identified, but only about 40 genotypes are sexually transmitted. Of the 40 sexually transmitted HPVs, 14 are oncogenic and are considered high-risk (7). Since the oncogenesis from infection to the development of precancerous lesions and cancer is a long and complicated process, this opens up a window of opportunity for prevention, diagnosis, and treatment (8). Early cervical cancer screening combined with HPV vaccination will effectively reduce the incidence of cervical cancer, as has been shown in many developed countries (1). Cervical cancer screening using the conventional Papanicolaou (Pap) smear was initiated in Malaysia in 1969, with the annual cost of approximately RM3.55 million (about USD800,000) (9). Despite the investment, the national Pap smear coverage was only 23% in 2002 and 22% in 2012, far from the recommended coverage of 80% by the World Health Organisation (WHO). Pap smear has a very high specificity of 98–99%, but its sensitivity is generally accepted as 50% (10). A successful Pap smear programme with trained healthcare professionals, including smear takers, cytotechnologists, cytopathologists, colposcopists and programme managers could achieve a sensitivity of 75% (10). Nonetheless, a cross-sectional study in 2013 involving 316 eligible women in West Malaysia showed a very high non-adherence rate to the Pap-free program, revealing other problems with the Pap smear programme.

**HPV test**

HPV tests can only be performed using molecular techniques, as the virus is not readily culturable nor elicits any meaningful immune response to the infecting virus (11). Numerous commercial molecular tests have been developed based on either the template amplification or the signal amplification technique. Digene® Hybrid Capture 2 (Digene HC2) (QIAGEN) is one of the commercial HPV tests that employs the signal amplification technique. Digene® HC2 is currently the most widely used HPV test in the United States and remains the gold standard in HPV diagnostics (12). A simplified version of Digene® HC2 known as the careHPV™ Test has been developed for use in the low-resource settings, with portability, economy, and a turnaround time of approximately 3 hours, ideal for use in the rural areas, as long as reliable power is available (13). The careHPV™ Test screens for 14 known HRHPV genotypes (HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68) with the semiquantitative positive cutoff value that correlates with cervical intraepithelial neoplasia 2 (CIN2) or worse (14, 15). The careHPV™ Test is a closed-batch system running on a 96-well plate format and handling up to 90 samples per batch (16). Cervical swabs obtained using the careBrush (QIAGEN) and stored in the corresponding careHPV Collection Medium (Qiagen) are stated to be stable at 15-30°C and 2-8°C for 14 days and 30 days, respectively (17,
18), a property that is crucial for the transportation and consolidation of specimens for batching purposes.

**Screening Women in the Rural**

Sarawak, Malaysian Borneo has a population of > 2.47 million population based on the 2010 census (19) with a low population density of 23/km². About half of the population lives in rural areas, many of which are still inaccessible by road (20). The rate of cervical cancer in Sarawak is currently the highest in Malaysia with an age-standardised rate (ASR) of 12.1/100,000 compared to 3.8/100,000 in Kelantan, West Malaysia (National mean ASR = 6.5, 2011) (21). This is not surprising as women living in low-resource settings are often at higher risk of developing cervical cancer and with poorer prognosis due to the inaccessibility to a proper healthcare facility, poverty, lack of awareness, and the presence of other cofactors (5, 21).

Our team conducts monthly cervical cancer screening in rural Sarawak using the careHPV™ Test and visual inspection using acetic acid (VIA) as part of the capacity building effort towards the Screen and Treat Strategy as recommended by WHO (22). As much as the same day careHPV™ result is desired to triage HRHPV positive women for VIA and treatment, we found it to be a challenge to achieve the maximum batch capacity of the careHPV™ Test. It would be sensible to consolidate samples collected from a few outreach programmes in order to achieve the maximum economy of the batch capacity, but this is limited by the recommendation that samples can only be stored for no more than 30 days if stored at 2–8 °C. The purpose of this paper is therefore to study the stability and repeatability of the clinician-sampled cervical swabs stored under refrigeration in the careHPV™ Collection Medium over a period of 1 year.

**Materials And Methods**

The clinician-collected cervical swabs preserved in careHPV™ Collection Medium were residual volumes from the careHPV™ test performed on the 22nd April 2019 in Bario, Sarawak, Malaysian Borneo (13). The samples were transported back to the Faculty of Medicine and Health Sciences, Universiti Malaysia Sarawak, at room temperature and then stored at 4°C until use. Four HRHPV positive (Pos-1-4) and four HRHPV negatives (Neg-1-4) samples were randomly selected and consistently tested together with new batches of samples in the subsequent careHPV™ tests at Day-38, -123, -131, -223, -395 using the protocol recommended by the manufacturer. The careHPV™ test done on the 22nd April, 2019 represents Day-0. Test days follow batch-test and not scheduled as a separate study, filling the blank wells that would otherwise be wasted.

**Results**

All four previously tested positive samples (Pos1-4) yielded positive results while all four previously negative samples (Neg-1-4) yielded negative results when tested at Day-38, -123, -131, -223, -395 demonstrating 100% stability and repeatability as compared to their initial results from Day-0 (Table 1).
Table 1
Detection of HRHPV DNA from the confirmed positive and negative specimens stored in careHPV™ Collection Medium at 4°C at day-0, -38, -123, -131, -223, -395 using the careHPV™ Test.

| Specimens | Day-0 | Day-38 | Day-123 | Day-131 | Day-223 | Day-395 |
|-----------|-------|--------|---------|---------|---------|---------|
| Pos-1     | +     | +      | +       | +       | +       | +       |
| Pos-2     | +     | +      | +       | +       | +       | +       |
| Pos-3     | +     | +      | +       | +       | +       | +       |
| Pos-4     | +     | +      | +       | +       | +       | +       |
| Neg-1     | -     | -      | -       | -       | -       | -       |
| Neg-2     | -     | -      | -       | -       | -       | -       |
| Neg-3     | -     | -      | -       | -       | -       | -       |
| Neg-4     | -     | -      | -       | -       | -       | -       |

Discussion

The careHPV™ Test is US FDA approved, CE mark, and WHO prequalified *in vitro* diagnostic test that has been extensively evaluated in numerous countries such as China (23), Thailand (16) Nigeria (24), India (25), and Ghana (26). The careHPV™ Test has a sensitivity of 87.76%; CI = 81.69–92.34% and a specificity of 85.37%; CI = 83.19–87.38% in detecting HRHPV infection, which correlates to CIN2 or worse. It has a positive predictive value (PPV) and negative predictive value (NPV) of 44.64% and 98.11%, respectively. The lower PPV may be attributed to the prevalence of HRHPV in a population as values decrease when testing populations with low prevalence or individuals with no risk of infections.

Most literature on the use of careHPV had described the permanent installation of their careHPV™ instruments in the established public healthcare facilities serving a high-density population such as in Drum Tower Hospital, Nanjing, China (27) within the second largest city in China, Moi Teaching and Referral Hospital, Eldoret, Kenya (28) the largest referral hospital in West Kenya, Barretos Cancer Hospital (BCH), Barretos, São Paulo, Brazil(29), Maternal and Child Health Hospital in Bachu County, Xinjiang, China(30), and the Institute of Cytology and Preventive Oncology, Uttar Pradesh, India(25) outskirt of New Delhi. They have used the opportunistic sampling method, recruiting patients attending their facilities for consultation that may not be related to cervical cancer. Therefore, the collection of a sufficient number of specimens for a complete batch-test during the recommended storage period may not be a matter of concern. However, our targeted population in rural Sarawak is of low-density and may not readily have access to proper healthcare facilities whereby bringing healthcare to them through medical outreach programmes may be the best option.
The careHPV™ Test protocol involves seven manual stages offering a realistic turnaround time of 3 hours. Despite its robust design, the careHPV™ Test System cannot tolerate power interruption. It will reboot itself back to the first stage, a default response that effectively voids the batch and wastes the careHPV™ Test Kit (16). Nevertheless, the high repeatability of samples in the careHPV™ Collection Medium allows storage, further consolidation and retest at a future date to be carried out with confidence. Furthermore, samples with confirmed results can be used as positive and negative in-house controls.

This manuscript mainly discusses the extended stability of samples in storage for the benefit of consolidation in order to achieve the economy of a full batch test. However, the careHPV™ Test Kit with a lower capacity of 18 samples per batch in 24-well format is available (17) and would be ideal to be used as the primary cervical cancer screening method in the low-density population such as in Sarawak. However, the 24-well format is not available in Malaysia at the time of writing.

Although the repeatability of the results shown using a small number of verified samples is high, we do not recommend prolonged storage of untested samples beyond the manufacturer's recommendation as delayed results may not have a significant clinical benefit for women. However, in an unforeseen event where stored samples are tested outside the recommended storage duration, such as during the laboratory shut down due to the coronavirus disease-19 pandemic may still be valid if resampling is not feasible.

**Conclusion**

The careHPV™ Test can be successfully performed on cervical swabs preserved in the careHPV Collection Medium, which are stored at 4 °C for at least 395 days.

**Abbreviations**

- HPV human papillomavirus
- HR high-risk
- VIA visual inspection using acetic acid
- Pos positive
- Neg negative

**Declarations**

**Ethical Approval and Consent to participate**
The study was approved by Universiti Malaysia Sarawak Medical Ethics Committee UNIMAS/NC-21. 02/03 – 02 Jld. 3 (17).

**Consent for publication**

Samples were from consenting women published elsewhere(13).

**Competing interests**

The author declares no competing interests.

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**Authors' contributions.**

CST performed the careHPV DNA tests and wrote the manuscript.

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