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Stability of Cervical Specimens in SurePath Medium for Human Papillomavirus Testing with the Roche cobas 4800 Assay

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The stability of cervical specimens in SurePath preservative fluid for human papillomavirus (HPV) testing with Roche cobas 4800 was determined using a panel of 308 pooled specimens from a colposcopy referral population. The SurePath specimens appeared to be stable for up to 10 weeks at ambient temperature for HPV testing with cobas 4800.

Detection of human papillomaviruses (HPV) in cervical specimens is of clinical importance based on the established causal relationship between high-risk (HR) HPV genotypes, cervical cancer precursors, and invasive cervical carcinomas. The growing body of knowledge over the past decades has led to the introduction of molecular tests for HR-HPV types into routine patient care, generally as an adjunct to Pap cytology (1–6). The cobas 4800 HPV test (Roche Molecular Systems Inc., Pleasanton, CA) is a fully automated PCR assay for the detection of 14 high-risk HPV types. This test also simultaneously differentiates genotypes 16 and 18, which account for about 70% of cervical cancers worldwide. The cobas 4800 HPV test has been approved by the U.S. Food and Drug Administration as an adjunct to cytology in primary screening and as a reflex test for the triage of atypical squamous cells of undetermined significance (ASCUS) cytology results. It is also CE (Conformité Européenne) marked for use as a primary screen for cervical cancer and its precursors. Cervical specimens collected in either cobas PCR cell collection medium or PreservCyt medium (Hologic Inc., Bedford, MA) are stable at 2 to 30°C for up to 6 months for testing with the cobas 4800 HPV test (7). While cervical specimens collected in SurePath preservative fluid (Becton Dickinson Co., Franklin Lakes, NJ) are not yet approved for testing with the cobas 4800 HPV test in the United States, in Canada the intended use of the test includes SurePath specimens. However, the specimens were required to be refrigerated at 2 to 8°C after collection and tested within 4 weeks at the time of this study (7). As the need for continuous refrigeration of specimens can pose practical difficulties in many routine clinical settings, it would be helpful to determine the stability of specimens collected in SurePath preservative fluid and maintained at ambient temperature for an extended period for use with the cobas 4800 HPV test. Therefore, we carried out a study to determine the stability of cervical specimens collected in SurePath preserva-
Negative 1 56

Control samples were refrigerated at 2 to 8°C, and test samples were stored at 21 to 26°C. Treatment was with preanalytic sample preparation buffer.

Both control samples were refrigerated at 2 to 8°C.

tive fluid for up to 10 weeks at ambient temperature for testing with the cobas 4800 HPV test. To counteract any chemical linkages between proteins and nucleic acids generated by formalin present in SurePath preservative fluid during extended storage, the use of an experimental preanalytic sample preparation buffer to treat specimens was also assessed (8, 9). Cervical specimens were collected in SurePath preservative fluid from women referred to undergo colposcopy at the Health Sciences Centre, St. John’s, as per the routine clinical protocol as part of standard care for cervical screening. The specimens were collected using the Cervex-Brush broom-type brush (Rovers Medical Devices, BV, Netherlands) and suspended into SurePath medium as per the manufacturer’s instructions. SurePath specimens were submitted to the cytology laboratory on the same day of collection at ambient temperature. SurePath liquid-based cytology (LBC) was performed in accordance with the manufacturer’s instructions. Following cytology, the postgradient residual samples were held at 2 to 8°C at the cytology laboratory and forwarded to the Public Health Laboratory (PHL) within a week of collection. At the PHL, the HPV status was determined based on routine ASCUS-HPV triage testing. Depending on the amount of residual sample (1 to 2 ml) in the SurePath vial, 4 to 6 specimens were pooled at random to obtain a sufficient volume to allow for multiple testing for the study. A total of 355 pooled specimens, each containing 6 to 7 ml, were prepared for the study.

The pooled samples were aliquoted in three sets of test tubes (Fig. 1). The first set of tubes was stored at 2 to 8°C and used for baseline testing to serve as the study control (control 1). At baseline, these samples were tested with the cobas 4800 HPV test as well as the Hybrid Capture 2 HPV test (HC2; Qiagen, Gaithersburg, MD). The second set of tubes was stored refrigerated at 2 to 8°C for 10 weeks, to serve as a control for 10-week postcollection (control 2). The third set of tubes was stored at ambient temperature ranging from 21 to 26°C for 10 weeks. One aliquot from the third set of the samples was tested after treatment with the preanalytic sample preparation buffer (test 1), and a second aliquot was tested without the treatment (test 2; Fig. 1). For specimen treatment, 0.5 ml of the SurePath samples was mixed with an equal volume of the buffer (190 mM Tris, 0.4% SDS [wt/vol], 0.09% sodium azide [wt/vol], pH 8.5), vortexed for 5 to 10 s, and heated at 120°C for 20 min. This is based on personal communication with researchers at Roche Molecular Systems, Inc., who have developed a potential solution that could counteract chemical linkages, as stated above. All tests were carried out according to manufacturers’ instructions, and samples from each condition were tested independently of one another.

Of the total of 355 pooled samples obtained for the study, results from the above-described four testing matrices (controls 1 and 2 and tests 1 and 2) were available for 308 samples. The baseline agreement for the presence of any high-risk HPV between the cobas 4800 HPV test (control 1) and HC2 was 85.1% (kappa value, 0.593). The agreement between baseline cobas 4800 HPV test results (control 1) and results from 10-week postcollection (control 2) was 94.2% (Table 1). The results of cobas 4800 HPV testing at 10 weeks postcollection at ambient temperature with treatment (test 1) showed an agreement of 96.1% and 96.8%, respectively, compared with control 1 and control 2 (Table 2). The results of cobas 4800 HPV at 10 weeks postcollection at ambient temperature without treatment (test 2) showed an agreement of 93.2% and 95.8%, respectively, compared with control 1 and control 2 (Table 2). For the above-described sets of results, kappa values ranged from 0.802 to 0.909, which is excellent agreement based on Fleiss’s magnitude guidelines and ranges from substantial to almost perfect agreement based on Landis and Koch interpretation guidelines. All internal controls were satisfactory for our panel with the exception of three, which were excluded from the study analyses.

Since the cobas 4800 HPV test provides type-specific identification for the two most significant HPV genotypes, 16 and 18, we also determined the agreements based on these two types in comparison with other high-risk HPV types across the four testing matrices. The agreement for types 16 and 18 ranged from 95.1% to 98.1% between the four matrices. There was a trend of a higher rate of detection for types 16 and 18 between the controls and those treated than among the specimens which were not treated (Table 3).

The SurePath LBC system is one of the commonly used platforms in Pap cytology, and cervical samples collected in SurePath medium allow for HPV reflex testing in cervical cancer screening. However, the requirements and stability of cervical specimens collected in SurePath medium for HPV testing vary between different HPV test kits. The cobas 4800 HPV test provides an appealing feature, in that it is fully automated with a simultaneous identification of HPV types 16 and 18 and is approved for cervical cancer

**TABLE 1 Agreement between cobas 4800 at baseline (control 1) and at 10 weeks postcollection (control 2)**

<table>
<thead>
<tr>
<th>Test Result</th>
<th>Control 1 (baseline)</th>
<th>Control 2 (10 weeks postcollection)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of positive results</td>
<td>No. of negative results</td>
</tr>
<tr>
<td>Positive</td>
<td>234</td>
<td>17</td>
</tr>
<tr>
<td>Negative</td>
<td>1</td>
<td>56</td>
</tr>
</tbody>
</table>

**TABLE 2 Agreement between cobas 4800 control results and test results at 10 weeks postcollection with and without treatment**

<table>
<thead>
<tr>
<th>Test Result</th>
<th>Control 1 (baseline)</th>
<th>Control 2 (10 weeks postcollection)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of positive results</td>
<td>No. of negative results</td>
</tr>
<tr>
<td>Test 1 (10 weeks postcollection with treatment)</td>
<td>Positive</td>
<td>239</td>
</tr>
<tr>
<td>Test 2 (10 weeks postcollection without treatment)</td>
<td>Positive</td>
<td>230</td>
</tr>
</tbody>
</table>

a Control samples were refrigerated at 2 to 8°C; and test samples were stored at 21 to 26°C. Treatment was with preanalytic sample preparation buffer.
screening in many countries, including the United States. While cervical specimens collected in SurePath are approved for testing in Canada with the cobas 4800 HPV test, it was validated only for samples refrigerated up to 4 weeks at the time of this study. Since we completed the study, the manufacturer’s guidelines have been revised with indications for storing SurePath specimens at 15 to 30°C for up to 14 days and at 2 to 8°C for up to 6 months (10). In this regard, our study provides useful data on the stability of SurePath specimens at ambient temperature up to 10 weeks postcollection. Our data showed no significant differences in cobas 4800 HPV test results between samples tested soon after collection at baseline and those stored at ambient temperature up to 10 weeks postcollection. We also assessed the usefulness of the treatment protocol using a preanalytic sample buffer, and this did not show any significant difference (P < 0.05). However, our testing used pooled HPV-positive specimens from a colposcopy referral population. These specimens are likely to have a higher viral concentration and, hence, are unlikely to drop to an undetectable level with longer storage at ambient temperature. Therefore, our study samples may not be representative of routine cytological specimens collected in SurePath medium. Also, based on our findings, we can make no conclusion with regard to the use of the preanalytic sample preparation buffer treatment in routine settings. Further research should be conducted to determine the usefulness of the treatment protocol for routine cytological specimens that may have a lower viral concentration. Since HC2 is the most commonly used HPV test and recommended as a standard, we included this test at baseline with cobas 4800 HPV testing simply to obtain comparative data. The agreement of 85.1% that we obtained was lower than expected, and, thus, treatment with preanalytic sample preparation buffer was required at baseline with cobas 4800 HPV testing simply to have a lower viral concentration. Since HC2 is the most commonly used HPV test, our results can be compared with those obtained with HC2.

ACKNOWLEDGMENT
This study was supported by Roche Molecular Diagnostics, Laval, Quebec, Canada.

REFERENCES

### TABLE 3 Detection of HPV types 16 and 18 between cobas 4800 control and at 10 weeks postcollection with and without treatment

<table>
<thead>
<tr>
<th>Test</th>
<th>HPV type</th>
<th>Result</th>
<th>Control 1 (baseline)</th>
<th>Control 2 (10 weeks postcollection)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HPV16</td>
<td>No. detected</td>
<td>No. not detected</td>
<td>% agreement</td>
</tr>
<tr>
<td>Test 1 (10 weeks postcollection with treatment)</td>
<td>HPV16</td>
<td>Detected</td>
<td>115</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>HPV18</td>
<td>Not detected</td>
<td>4</td>
<td>185</td>
</tr>
<tr>
<td></td>
<td>HPV16</td>
<td>Detected</td>
<td>108</td>
<td>4</td>
</tr>
<tr>
<td>Test 2 (10 weeks postcollection without treatment)</td>
<td>HPV16</td>
<td>Not detected</td>
<td>11</td>
<td>185</td>
</tr>
<tr>
<td></td>
<td>HPV18</td>
<td>Detected</td>
<td>32</td>
<td>2</td>
</tr>
</tbody>
</table>

*Control samples were refrigerated at 2 to 8°C, and test samples were stored at 21 to 26°C. Treatment was with preanalytic sample preparation buffer.*