The Journal of Molecular Diagnostics, Vol. 19, No. 2, March 2017

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the Journal of Molecular Diagnostics
jmd.amjpathol.org

Development and Validation of a Preanalytic Procedure for Performing the cobas HPV Test in SurePath Preservative Fluid

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Accepted for publication October 5, 2016.

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The formation of chemical cross-links between nucleic acids and proteins in formalin-containing media presents challenges for human papillomavirus (HPV) testing of cervical samples collected in SurePath Preservative Fluid. A preanalytic process involving addition of a nucleophilic buffer and heating the sample to 120°C was developed to reverse the effects of cross-linking and improve nucleic acid accessibility for the cobas HPV Test in SurePath. Cycle threshold (C_T) values for cobas HPV detection were evaluated over time and various temperatures, and mean C_T differences between pretreated and both untreated SurePath samples and those collected in PreservCyt were assessed. Without pretreatment, low viral levels (1 × limit of detection) of HPV were no longer detectable by 7 days. For prospectively collected specimens, mean (95% CI) C_T differences between pretreated and untreated samples indicated enhanced HPV DNA recovery in all categories of treated samples: −2.58 (−3.16 to −2.01), −2.63 (−3.62 to −1.64), and −3.39 (−4.95 to −1.82), respectively, for other 12 high-risk HPV types, HPV16, and HPV18. Furthermore, mean (95% CI) C_T differences of pretreated SurePath samples were comparable to simultaneously collected PreservCyt samples: −0.48 (−0.98 to 0.02) and −0.23 (−0.93 to 0.46), respectively, for HPV16 and HPV18; a borderline significant difference [−0.35 (−0.57 to −0.13)] was observed for other 12 high-risk HPV types. This preanalytic procedure therefore ensures a validated, safe, and accurate method for cobas HPV testing in SurePath. *(J Mol Diagn 2017, 19: 288–294; http://dx.doi.org/10.1016/j.jmoldx.2016.10.003)*

Most cervical cancer cases and deaths can be prevented through early detection of precancerous changes in the cervix. Cytology has been central to cervical cancer screening programs for >50 years and has contributed to the 70% decline in rates of cervical cancer in the developed world.[1] Human papillomavirus (HPV) is now recognized as a single, necessary cause of cancer of the cervix and has been isolated from the tissue of nearly all cervical cancer cases.[2] Thirteen HPV genotypes are classified as carcinogenic or high risk (HR): 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68. An additional genotype (66) is classified as possibly carcinogenic.[3] Therefore, tests that detect infection with these HR HPV genotypes are now being used increasingly in cervical cancer screening programs. The 2007 Consensus Guidelines for the Management of Women with Abnormal Cervical Cancer Screening Tests recognized the benefit of using a combination of cervical cytology, tests for HR HPV infection, and type-specific HPV16/18 testing for women undergoing screening for cervical cancer.[4] Current guidelines in the United States now recommend the combination of cytology and HR HPV testing

Supported by Roche Molecular Systems (Pleasanton, CA).

Disclosures: M.D.K., D.H., J.P., and S.A. are employees of Roche Molecular Systems; C.M.B. is a former employee of Roche Molecular Systems who continues to consult for Roche and has received stock options.

Per the manufacturer, the BD SurePath sample medium has not been approved by the Federal Drug Administration for use with the HC2 High-Risk HPV Test and use of the SurePath sample with the HC2 test may under certain conditions provide false-negative results. False-negative results could lead to inappropriate patient management and potentially compromise patient safety.

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(cotesting) as the preferred method of screening, with HPV16/18 genotype–specific testing an added option to triage women with negative cytology to colposcopy.[5] In 2014, the Federal Drug Administration approved the use of a HR HPV test as the first-line primary screen for cervical cancer and by 2015 US professional societies issued interim guidance that supports HPV primary screening as an option.[6,7]

SurePath Preservative Fluid (SPPF; formerly AutoCyte; BD TriPath, Burlington, NC) was approved by the Federal Drug Administration in 1999 for use as a collection medium for liquid-based cytology of cervical specimens. Because of decreased cost and a lower percentage of cytology slides read as unsatisfactory (College of American Pathologists, CYP.07600 statistical records, *http://www.cap.org/apps/docs/proficiency_testing/CYP07600.pdf*, last accessed April 27, 2016), the use of SPPF has been adopted by a high number of laboratories. This change has implications for HPV testing because molecular testing is generally performed on the same liquid medium sample as the cytology specimen. However, until recently, none of the current Federal Drug Administration–approved HPV tests were approved for cervical samples collected in SPPF. To accommodate HR HPV testing in SPPF, many laboratories conducted their own internal validation for testing in SPPF, and in effect used an off-label testing method.

This approach to molecular testing in SPPF may, however, raise patient safety issues. It has been documented for nearly a decade that there are limitations in the recovery of nucleic acids from SPPF.[8,9] For DNA, the reduction in recovery was observed to be up to 1000-fold in cell-spiked samples in SPPF.[9] Recent reports of false-negative HPV results in clinical specimens collected in SPPF have also raised safety concerns.[10]

Although the exact formulation for SPPF is proprietary, the Medical Safety Data Sheet from the manufacturer states that formaldehyde (formalin) is present in the medium. As early as the 1950s, it had been shown that formaldehyde induces cross-linkages between protein and nucleic acids.[11] Awareness of this chemical reactivity and its potential to cause poorer recovery of HPV DNA from SPPF led to the development of a preanalytic treatment in which a nucleophilic reagent is added to the sample and then heated to ensure release of trapped DNA from SPPF. In July 2016, the Federal Drug Administration granted approval for the cobas HPV Test to be performed in samples collected in SPPF, provided that they are subjected to the preanalytic treatment to reverse the effects of cross-linking and maximize DNA accessibility. The description of this preanalytic procedure and the supporting analytic data will be presented in this study. In addition, comparisons of the cycle threshold (C_T) values generated with the cobas HPV Test between pretreated and untreated clinical samples collected in SPPF and between pretreated SPPF and PreservCyt (PC) samples will also be described.

Materials and Methods

The cobas HPV Test Performed on the 4800 System

The cobas HPV Test is a fully automated real-time PCR DNA assay that qualitatively detects the presence of 14 HR HPV genotypes (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68) in cervical specimens. Results are simultaneously reported as positive or negative for the pooled 12 HR HPV types (HPV 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68) from channel 1 and HPV16 and HPV18 genotypes read individually from channels 2 and 3, respectively. The C_T values determined to be the cutoff for positive results are 40.0, 40.5, and 40.0 for channels 1, 2, and 3, respectively. A fourth channel detects the human β-globin gene as a control that serves to confirm that cellular DNA is present or to identify the presence of an inhibitory substance.[12]

Specimens

For the analytic studies, all cervical specimens were procured through clinics using appropriate patient consent and institutional review board approval. Cervical specimens collected in SPPF were maintained at 2°C to 8°C during shipping, storage, and processing. For the comparison of clinical samples collected in SPPF (untreated and pretreated) and PC, specimens were collected during year 3 of the Addressing The Need for Advanced HPV Diagnostics (ATHENA) study follow-up, as described previously.[13] Briefly, women aged ≥21 years presenting for routine screening were enrolled and received both cytology and HPV testing performed in PC medium (ThinPrep; Hologic, Bedford, MA). Those who screened positive for either cytology (aged ≥21 years) or HR HPV (aged ≥25 years) were referred for baseline colposcopy and biopsy. The follow-up phase of the ATHENA study included those women who had been referred to colposcopy and were found not to have high-grade cervical disease (cervical epithelial neoplasia grade 2 or greater) on biopsy. During this longitudinal phase, women were seen annually for cytology and HPV testing and were referred to colposcopy if they had abnormal cytology. As in the baseline phase, those having cervical epithelial neoplasia grade less than 2 on colposcopic biopsy continued to be followed up; at year 3, an exit colposcopy was performed on all consenting women. All women presenting at year 3 were also offered the option of cocollection with a second cervical sample in SPPF. The ATHENA study protocol was approved by institutional review boards of all study sites, and written informed consent was obtained.

SurePath Preservative Fluid Preanalytic Treatment

Cervical specimens collected in SPPF were subjected to the preanalytic process involving addition of the cobas sample preparation buffer (CSPB; Roche Molecular Systems,