Generic Study Protocol – HPV detection using Roche cobas® 4800 and 68/8800 systems

INTRODUCTION

This generic study protocol describes the steps to be followed for collection, stabilization and detection of Human Papilloma Virus (HPV) with the Roche cobas® 4800 HPV test or the Roche cobas® HPV test on the 68/8800 systems using first-void urine samples collected with (1) Colli-Pee® containing UCM, FV-5000 series or Colli-Pee® Small Volumes containing UCM .

CLINICAL BACKGROUND

HPV is the most common sexually transmitted infection (STI) and a major cause of cervical cancer. Estimates are that the lifetime probability of ever encountering HPV is as high as 80-90%[1]; moreover, HPV was responsible for more than 604 000 cervical cancer cases and 341 000 cervical cancer deaths in 2020[2]. Routine screening is critical for the prevention and control of STIs, particularly since many infections do not present symptoms. However, current screening methods present limitations. They can be invasive, time intensive, and require a clinician to perform. Also, many individuals often feel embarrassed or uncomfortable discussing their sexual activity and are reluctant to visit a clinic for testing[3].

As an alternative to traditional screening methods, HPV testing in first-void urine has been proposed to reach women that are not participating in cervical cancer screening programs. First-void urine has been shown to contain higher concentrations of HPV DNA, and therefore collection of this fraction is important to increase sample sensitivity[4].

In addition, several studies reported that first-void urine sampling, using a first-void urine collection device was preferred over a physician-collected cervical sample[5]. Thus, the use of first-void urine can increase the participation rates in HPV-based cervical cancer screening programs[6].

First-void urine can be collected in a user-friendly, volumetric and standardized way, using Colli-Pee® containing UCM to also allow for instant mixing with the preservative and thus stabilization of the urinary analytes.

Moreover, Colli-Pee® Small Volumes containing UCM has been designed to be fully compatible with postal service transport, and as such this collection device is ideally suited for home-based first-void urine collection.

Furthermore, the sample collection tube is directly compatible with the Roche cobas® 4800 system, removing the need for manual and error-prone sample preparation steps, and thereby reducing the overall cost, and decreasing the turn-around time from sample to result. Proof-of-concept studies run by Novosanis and the University of Antwerp clearly show that the cobas® HPV tests, run on both the Roche cobas® 4800 and 68/8800 systems are fully compatible with urine collected with Colli-Pee® containing UCM[7,8]. Furthermore, a recent publication proved that results from Colli-Pee® collecting 20mL and 10 mL were fully comparable[9].

Next to screening, urine-based HPV testing can also be used for monitoring the impact of vaccination[10,11,12]. HPV testing also offers possibilities for the follow-up of cervical cancer treatment[13,14]. Currently, different studies are ongoing to validate the use of Colli-Pee collected first-void urine for the follow-up of cervical cancer[15,16]. In the remainder of this document, a generic study protocol is described that outlines the steps to be followed for HPV detection and analysis, using Colli-Pee® containing UCM, FV-5000 series or Colli-Pee® Small Volumes containing UCM for sample collection, and the Roche cobas® 4800 HPV test or the Roche cobas® HPV test for use on the 68/8800 systems, for sample analysis.

MATERIALS AND METHODS

Sample collection

Samples can be collected with either one of the following Colli-Pee® devices

- **Colli-Pee® containing UCM, FV-5000 series.** The volume of the samples collected using Colli-Pee® containing UCM, FV-5000 series, lie in the range of 18-22 mL (20 mL nominal volume), and include a prefilled volume of 7 mL UCM preservative.

- **Colli-Pee® Small Volumes containing UCM**. The volume of the samples collected using Colli-Pee® Small Volumes containing UCM lie in the range of 8-12 mL (10 mL nominal volume), and include a prefilled volume of 3.4 mL UCM preservative.

Sample collection using either one the above listed Colli-Pee® variants should be performed as outlined in the Instructions for Use (IFU), which can be found on the Novosanis website. As stated, the Colli-Pee® Small Volumes is fully compatible with postal services and is therefore ideally suited for use in home-based sample collection. To this end, Novosanis developed a postal kit solution that is fully customizable.

For more information on the Colli-Pee® postal kit solution, please visit novosanis.com/home-collection, or read our white paper on self-sampling at home, for the detection of sexually transmitted infections (Mehta et al., 2019).

Sample storage and transportation

Due to the presence of the UCM preservative, collected samples can be stored at room temperature (21°C – 25°C) for a period of maximum of 7 days, before downstream processing. Prolonged storage of the urine samples is possible at 4°C for up to 14 days, and at -20°C for up to 90 days. Prolonged sample storage at -80°C is also possible for a period of up to 12 months; however, in this scenario, urine samples should be transferred and/or aliquoted into dedicated cryovials prior to storage. For more information, please read our white paper on sample storage and transportation on the Novosanis website (Meers et al., 2021).

Sample preparation

Samples collected with Colli-Pee® containing UCM, FV-5000 series

For the 20 mL urine samples collected with Colli-Pee® containing UCM, FV-5000 series, no special sample preparation is needed. The required input volume (see Table 1, next page) needs to be transferred into the correct tube to allow for loading onto the Roche cobas® analyzer.

Samples collected with Colli-Pee® Small Volumes containing UCM

The collector tube of Colli-Pee® Small Volumes is compatible with several high-throughput instruments like the Roche cobas® 4800 system. Therefore, the sample can be placed directly into the rack of the Roche cobas® system, without any further sample preparation needs. For the Roche cobas® 68/8800 systems, Novosanis is currently developing a Colli-Pee® variant that is directly compatible with the proprietary Roche 10 mL collection tube. In the meantime, as is the case for the samples collected with the FV-5000 series Colli-Pee®, the required input volume (see Table 1, next page) needs to be transferred into the correct tube to allow for loading onto the Roche cobas® 68/8800 analyzer.

Sample analysis

The Roche cobas® 4800 HPV test and Roche cobas® HPV test for use on cobas® 68/8800 systems are HPV detection tests that are clinically validated, CE-IVD registered and FDA approved. They utilize amplification of target DNA by the polymerase chain reaction (PCR) and nucleic acid hybridization for the detection of 14 high-risk HPV (hrHPV) types in a single analysis (see Table 1). HPV DNA tests have extensive longitudinal data to support the safety of a negative result. To ensure confidence in a negative result, each cobas® HPV test also includes appropriate controls to verify human cells are present in the sample.

Table 1. List of analytical method and associated parameters

Analytical method	Volume input	Analytical endpoints
Roche cobas® 4800 HPV test and Roche cobas® HPV test for use on cobas® 68/88000 systems	400 µL (min. 1 µL)	Detection and cycle numbers of HPV16, HPV18 and other HR HPV (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68)

Roche cobas® 4800 HPV test

A sample volume of 400 µL is used in the automated extraction step. Extracted nucleic acids are eluted in a volume of 125 µL, of which 25 µL is used in the PCR reaction. A net volume of 80 µL of sample is used for PCR. For more information, please read the proof-of-concept study poster by Vorsters et al. (2016) on the Novosanis website. The Roche cobas® 4800 HPV test package insert can be retrieved from the Roche website.

Roche cobas® HPV test for use on cobas® 68/8800 systems

A sample volume of 400 µL is used in the automated extraction step. Extracted nucleic acids are eluted in a volume of 50 µL, of which 27 µL used for PCR reaction. A net volume of 216 µL of sample is used for PCR. The increased net sample volume processed in this qPCR reflects in an increased analytical sensitivity compared to the Cobas® 4800 HPV test[8].

For more information, please read the proof-of-concept study poster by Vorsters et al. (2017) on the Novosanis website. The Roche cobas® HPV test for use on cobas® 68/8800 systems package insert can be retrieved from the Roche website.

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