

SECTION 7:

OPERATIONAL ASPECTS OF THE LABORATORY

KEY MESSAGES

- HPV tests need to be processed in a centralized laboratory, using the processing equipment associated with the particular test.
- HPV testing procedures need to be integrated into a pathology laboratory.

OPERATIONAL ASPECTS OF THE LABORATORY

A centralized pathology laboratory, with qualified personnel and quality assurance procedures, will be necessary to process HPV tests. Laboratory software is essential to record women's information including the HPV test results, as well as any additional test results (if applicable) such as colposcopy, biopsy and treatment.

HPV test results are used to identify two groups of women:

- a) Women who are HPV negative, which, given the test's high negative predictive value, do not need to be re-screened for at least 5 years; and
- b) Women who are HPV positive, and will require follow-up care or treatment. In this regard, timely and close communication between the pathology laboratory and health facilities will be important to communicate results.

The physical space in the laboratory

A bright room is needed to enable HPV test processing, that requires microplate techniques. The room should be enclosed, with no fast moving air, dust, or other substances that could contaminate the results. The room temperature should be constant at 20-22 °C.

The countertops should be approximately 70 cm wide and long enough to hold the different HPV testing components. Since the homogenization process makes the countertop vibrate, it is advisable to have one countertop for the various instruments; another countertop for the hot-water bath area, preferably close to a water faucet and sink; and a countertop for the luminometer. A central island is an extremely useful space for carrying out sample preparation, transferring reagents to pipettes, and various other operations, before taking samples to the testing instruments.

HPV test samples can be stored for two weeks at room temperature, an additional week in the laboratory at 2-8 °C, and up to 3 months at -20 °C. Therefore, there should be a refrigerator and freezer with capacity to store HPV test samples. Since refrigerators increase the temperature in the room where they are in use, due to heat given off by their motors, they should be kept in a location physically separated from the room where HPV processing is done.

Personnel working in the HPV testing room should observe stringent hygiene standards and always work with talcum powder-free gloves, because it could contaminate the samples. No smoking, eating, or drinking should be allowed in the work area. Unauthorized laboratory personnel should not be permitted in the processing room.

Considerations in processing HPV tests

The specific methods used to process the HPV tests will depend on the particular test used, and the manufacturer's recommendations for processing tests should always be followed. A general description of HPV test processing, its limitations and precautions is described below.

a) General considerations and limitations of HPV testing:

- The HPV test is designed to detect high-risk HPV types, typically including HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68.
- The test may not detect low-risk HPV types, such as HPV 6, 11, 42, 43, and 44.
- The test cannot differentiate between infection with one HPV type and infection with several HPV types; nor can the test identify a new infection from a re-infection.
- An HPV negative test result does not completely rule out presence of HPV, since there may be a small chance of sampling errors, or from very low levels of HPV infection (that is, less than 5,000 copies per sample).
- Samples need to be obtained using the cervical brush that accompanies the specific HPV test.
- At the time the HPV test sample is taken, if there is presence of anti-mycotic creams, contraceptive gel, or vaginal hygiene products, these may contaminate the sample and the HPV test result may be a false negative.
- The brush should not be used in pregnant women.
- There is a small chance of cross-hybridization with HPV strains 6, 11, 40, 42, 53, 54, 55, 56, MM4, MM7, MM8, and MM9, which may affect test results.
- Cross-reaction with the plasmid pBR322 may occur, which could result in false positives, since it has some analogous sequences, especially if concentrations of this bacterial plasmid are high.

b) Precautions to maximize quality of HPV test processing:

- Mouth pipetting should not be done.
- No smoking, eating, or drinking should be permitted in areas where reagents are being used.
- Talcum powder-free latex gloves should be used in all steps of the procedure.
- Spills should be cleaned up immediately, using disinfectants such as sodium hypochlorite.

- HPV test reagents should not be used after their expiration date.
- Processing tests that have passed their expiration date, or processing tests in conditions outside the recommended temperature range can lead to invalid test results.
- Quality control procedures, test calibration and verification criteria, and interpretation of test results should be carefully carried out.
- Pipetting exact amounts of reagents specified in the manufacturer's instructions and ensuring the correct blend of the various reagents are important for quality testing.
- False positives can be produced by inadequate pipetting if aliquots are not properly transported, and in the process of hybridization, the pipette should not touch the sides of the tube or the contents of the probe.
- Nucleic acids are very sensitive to degradation by environmental nucleases and nucleases on human skin. Therefore, surfaces should be covered, with no dust, and talcum powder-free gloves should be used in the processes.
- The denaturation step should be done immediately after opening the equipment. Failure to observe this precaution may produce false negative results.
- Improperly done homogenization steps and inverting or inadequately shaking tubes can result in false positives. The technique should be done meticulously and systematically, following manufacturer instructions.
- Contamination of the microplate with bacteria, saliva, hair, or skin oils should be prevented.
- Samples can be kept at room temperature for a maximum of two weeks.
- Unauthorized laboratory personnel should not be allowed to enter the processing room and untrained personnel should never be allowed to participate in the process.