INFORM HPV II Family 6 Probe

REF 800-2220

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IVD Σ 50

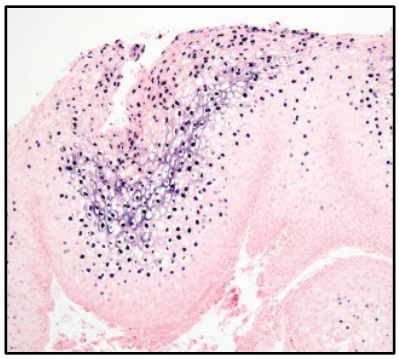

Figure 1. Condyloma tissue stained with the INFORM HPV II Family 6 Probe and the ISH *i*VIEW $_{Blue}$ Plus Detection Kit. Magnification 10x.

INTENDED USE

Ventana Medical Systems' (Ventana) INFORM HPV II Family 6 Probe is to be used in conjunction with ISH *i*VIEW $_{Blue}$ Plus Detection Kit and other accessory reagents to stain neutral buffered formalin fixed, paraffin embedded tissue sections on the BenchMark IHC/ISH instruments.

Light microscopy is used to detect the staining reaction. Positive results aid in the classification of normal and abnormal specimens, and serve as an adjunct to conventional histopathology tests. The clinical interpretation of any staining, or the absence of staining, must be complemented by morphological studies and evaluation of proper controls. Evaluation must be made by a qualified pathologist within the context of the patient's clinical history and other diagnostic tests.

This product is intended for *in vitro* diagnostic (IVD) use.

SUMMARY AND EXPLANATION

INFORM HPV II Family 6 Probe contains a cocktail of labeled human papillomavirus (HPV) genomic probes.[1] The intended targets are the common HPV genotypes not associated with cervical cancer. The probe cocktail has demonstrated positive hybridization to the low risk genotypes 6 and 11, but does not show positive hybridization to the most common high risk HPV genotypes.

Cervical cancer is the second most common cancer of women worldwide, with an estimated 529,000 new cases and 275,000 deaths globally per year.[2,3]

HPV is a double stranded DNA virus with more than 200 different genotypes containing approximately 7,900 base pairs.[4] The life cycle of HPV is tied directly to keratinocyte differentiation. One key event in the viral life cycle is the differentiation dependent escalation in viral replication. The increase in replication activity results in an amplification of the HPV genome from approximately 50 copies per cell, in basal keratinocytes during the sub clinical infection stage, to thousands of copies of viral genomes per cell in suprabasal keratinocytes during the clinical infection phase.[5] Infection of the cervical epithelium by HPV has been established as an initiating factor in the oncogenic process leading toward the development of cervical cancer.[6]

The risk of disease progression from dysplasia to cervical cancer is dependent on the HPV types. Therefore, HPV types have been grouped into different oncogenic risk groups. These groups are associated with the level of threat that a patient may develop cancer. For example, the high risk group, HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, 73 and 82, has been recognized as tumor producing, whereas low risk group, HPV 6, 11, 40, 42, 43, 44, 54, 61, 70, 72, and 81, has little chance of progressing to malignant disease.[7,8] Identification of HPV risk group by slide based *in situ* hybridization (ISH) testing in combination with conventional morphologic slide evaluation may enhance clinical decisions.[9]

Slide based ISH is an easy to perform, reliable and reproducible method for detecting HPV on paraffin embedded tissue sections.[10] The detection level of both radioisotopic and nonisotopic labels using genomic probes appears to be between 10 to 50 viral copies per cell when using formalin fixed tissue samples.[11,12]

The ISH system offers several advantages over other methods that require destruction of the target cells, for example nucleic acid capture techniques or polymerase chain reaction (PCR). The advantages may include: direct correlation with the cytologic findings; the ability to test archival specimens; and the ability to identify the HPV ISH result in the context of tissue morphology.

PRINCIPLE OF THE PROCEDURE

INFORM HPV II Family 6 Probe is formulated for use with ISH *i*VIEW $_{Blue}$ Plus Detection Kit and accessory reagents on the BenchMark XT IHC/ISH instrument.

The ISH *i*VIEW $_{Blue}$ Plus Detection Kit detects specific DNP labeled probes and antibodies bound to a target sequence or antigen in paraffin-embedded tissue sections. The labeled probe or antibody is located by an anti-DNP antibody, then by an enzyme-labeled secondary antibody or a biotin-conjugated secondary antibody. This step is followed by the addition of a Streptavidin-AP (alkaline phosphatase) enzyme conjugate which binds to the biotin present on the secondary antibody. The complex is then visualized with 5-Bromo-4-chloro-3-indolyl phosphate (BCIP) and nitro blue tetrazolium (NBT) chromogen, which produces a blue precipitate that is readily detected by light microscopy. Figure 2 illustrates the Blue ISH reaction.

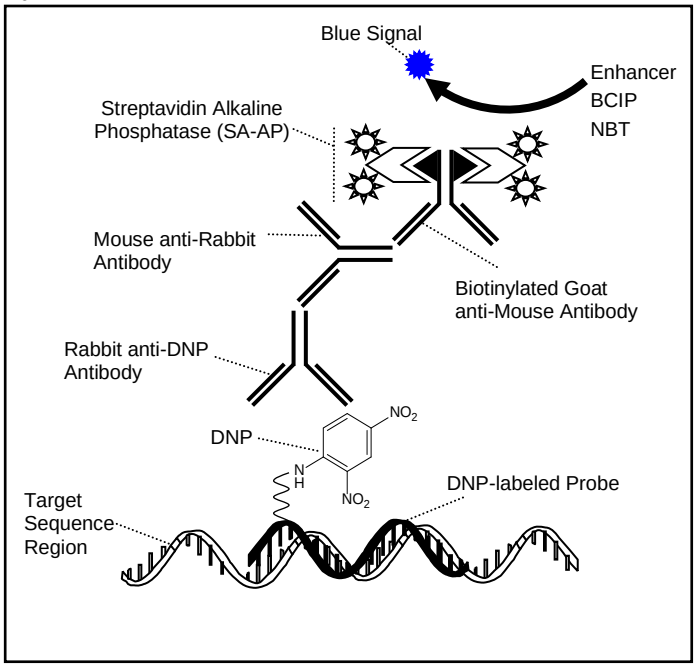

Figure 2. ISH *i*VIEW $_{Blue}$ Plus Detection Reaction

MATERIALS AND METHODS

Reagent Provided

INFORM HPV II Family 6 Probe contains sufficient reagent for 50 tests.

One 10 mL dispenser of INFORM HPV II Family 6 Probe contains approximately 0.5 µg/mL of the probe cocktail labeled with DNP in a formamide based hybridization buffer.

Refer to the appropriate VENTANA detection kit package insert for detailed descriptions of: (1) Principles of the Procedure, (2) Materials and Reagents Needed but Not Provided, (3) Specimen Collection and Preparation for Analysis, (4) Quality Control Procedures, (5) Troubleshooting, (6) Interpretation of Results, and (7) General Limitations.

Reconstitution, Mixing, Dilution, Titration

No reconstitution, mixing, dilution, or titration is required. Further dilution may result in loss of staining specificity. The user must validate any such changes.

MATERIALS REQUIRED BUT NOT PROVIDED

Staining reagents, such as VENTANA detection kits and ancillary components, are not provided.

The following reagents and materials may be required but are not provided with the kit:

1. VENTANA HPV System Control Slides

2. VENTANA Alu Positive Control Probe II
3. VENTANA ISH Negative Control
4. VENTANA ISH *i*VIEW $_{Blue}$ Plus Detection Kit
5. VENTANA ISH Protease 1, 2 or 3*
6. VENTANA Red Counterstain II
7. VENTANA Reaction Buffer (10X)
8. VENTANA SSC (10X)
9. VENTANA EZ Prep (10X)
10. VENTANA Cell Conditioning 2 (Pre-dilute)
11. VENTANA Liquid Coverslip (High Temperature)
12. BenchMark IHC/ISH instrument
13. Bar code labels (appropriate labels for control and probe being tested)
14. 10% neutral buffered formalin
15. Microtome
16. Microscope slides, appropriate for ISH
17. Positive and negative control slides
18. Xylene, histological grade
19. Ethanol or reagent alcohol, histological grade
20. Acetone
21. Deionized or distilled water
22. Mounting medium and cover glass or automated cover slipper
23. Staining jars or baths
24. Timer
25. Light microscope

* As needed for specific applications.

Not all products listed in the package insert may be available in all geographies. Consult your local support representative.

STORAGE

Upon receipt and when not in use, store at 2–8 C. Do not freeze. The user must validate any storage conditions other than those specified in the package insert.

To ensure proper reagent delivery and stability of the probe after every run, replace the dispenser cap after every use and immediately place the dispenser in the refrigerator in an upright position.

Every dispenser is expiration dated. When properly stored, the reagent is stable to the date indicated on the label. Do not use reagent beyond the expiration date for the prescribed storage method.

There are no definitive signs to indicate instability of this product; therefore, positive and negative controls should be run simultaneously with unknown specimens. Your local Roche office should be contacted immediately if there is an indication of reagent instability.

SPECIMEN PREPARATION

Routinely processed, formalin-fixed, paraffin embedded tissues are suitable for this reagent. Tissue sections should be cut to the appropriate thickness for the proper staining.

WARNINGS AND PRECAUTIONS

1. For *in vitro* diagnostic (IVD) use.
2. For professional use only.
3. Clinical sensitivity of the assay must be validated in the user laboratory.
4. Warning, Product Contains Formamide. Formamide is toxic by inhalation and moderately toxic by ingestion. It is an irritant to skin, eyes, and mucous membranes and is absorbed through the skin. It may cause harm to the unborn child. Take precautions when handling reagents. Use disposable gloves and wear suitable protective clothing when handling suspected carcinogens or toxic materials.
5. Ensure that the waste container is empty prior to starting a run on the instrument. If this precaution is not taken, the waste container may overflow and the user risks a slip and fall.
6. Materials of human or animal origin should be handled as potentially biohazardous and disposed of with proper precautions.
7. Avoid contact of reagents with eyes, skin, and mucous membranes. If reagents come in contact with sensitive areas, wash with copious amounts of water. Avoid inhalation of reagents.
8. Avoid microbial contamination of reagents as this may produce incorrect results.
9. The reagents have been optimally diluted, and further dilution may result in loss of staining. The user must validate any such change.
10. Consult local and/or state authorities to determine the recommended method of disposal.
11. For supplementary safety information, refer to the product Safety Data Sheet and the Symbol and Hazard Guide located at www.ventana.com.

INSTRUCTIONS FOR USE

Post-run Processing

After the run is complete, please follow the following steps for proper dehydration of stained slides. (Proper dehydration will help ensure optimal signal visualization).

Dehydration Procedure

1. To remove coverslip solution, wash the slides in 2 sequential solutions of a mild dishwashing detergent (do not use detergent designed for automatic dishwashers).
2. Rinse slides well with distilled water, about 1 minute. Shake off excess water.
3. Transfer the slides to an 80% ethanol bath for approximately 1 minute.
4. Transfer the slides to a 90% ethanol bath for approximately 1 minute.
5. Transfer the slides to a 100% ethanol bath for approximately 1 minute.
6. Transfer the slides into a second bath of 100% ethanol for approximately 1 minute.
7. Dip slides 10 times into 100% acetone (one time use only; replace acetone after each staining run). Do not leave slides in acetone.
8. Transfer the slides into the first xylene bath for approximately 30 seconds.
9. Transfer the slides into a second xylene bath for approximately 30 seconds.
10. Place coverslip on slide.

Note: To ensure complete dehydration, ethanol baths need to be changed frequently and a third 100% ethanol bath may be added.

QUALITY CONTROL PROCEDURES

Positive Specimen Control

A positive specimen control (system level control) must be run with every staining run performed. The system level control is used to confirm that the probe was applied and the instrument functioned properly. This specimen should contain both positive and negative staining cells or tissue components. Control specimens should be biopsy or surgical specimens prepared or fixed in a manner identical to patient specimens. Such controls should monitor all steps of the procedure, from specimen preparation through staining. Use of a specimen fixed or processed differently from the patient specimen provides an appropriate control to confirm the reagents and instrument functioned properly. A specimen with weak positive staining, i.e. demonstrating a punctate staining pattern (which may require microscopic examination at magnifications as high as 20x or 40x) is more suitable than one with strong positive staining for optimal quality control and for detecting minor levels of reagent degradation. If the positive control fails to demonstrate positive staining, results with the test specimens should be considered invalid.

Negative Specimen Control

A negative specimen control must be run with every staining procedure performed. The purposes is to monitor unintended probe and antibody cross reactivity to cellular components. The same specimen used for the positive specimen control may also be used as the negative specimen control. The variety of different cell types present in most specimens offers internal negative control sites, but this should be verified by the user. The non-staining components should demonstrate absence of specific staining and provide an indication of background staining. If unacceptable staining occurs in the negative specimen control sites, result with the patient specimens should be considered invalid.

Positive Reagent Control

A positive reagent control should be run during assay verification and troubleshooting since DNA accessibility may vary depending on fixation method and pretreatment of the specimen. Ventana Alu Positive Control Probe II can be used as a positive control for this assay.

Negative Reagent Control

A negative reagent control must be substituted for the ISH probe with every specimen stained to aid in interpretation of each patient result. This provides an indication of nonspecific background staining for each slide. In place of the ISH probe, stain the slide

with Ventana ISH Negative Control. The incubation period for controls should correspond to that of the probe.

The negative control is especially important with the finding that the intestinal form of alkaline phosphatase may be found in cells other than the brush border of intestinal epithelial cells. Additionally, enzymes capable of reducing nitro blue tetrazolium may be preserved during fixation.

Unexplained Discrepancies

Unexplained discrepancies in controls should be referred to your local support representative immediately. If quality control results do not meet specifications, patient results are invalid. See the Troubleshooting section of this insert. Identify and correct the problem, then repeat the patient samples.

Assay Verification

Prior to initial use of a reagent in a diagnostic procedure, the performance of the reagent should be verified by testing it on a series of specimens with known ISH performance characteristics. These quality control assessments should be repeated for each new lot of reagents, or whenever there is a change in assay parameters.

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INTERPRETATION OF RESULTS

A qualified pathologist experienced in the microscopic interpretation of anatomic pathology specimens, ISH procedures and the recognition of *in situ* hybridization results (which require microscopic examination using 20x, 40x, and/or 60x objectives) must evaluate controls before interpreting results.[13] The presence of a blue reaction product in cervical epithelial nuclei in either a homogeneous or punctate staining pattern is indicative of positive reactivity. The homogeneous pattern appears as a large, uniform, globular dark blue precipitate within the epithelial cell nucleus. It is generally prominent in the superficial keratinized region of the epithelium often within koilocytotic cells. The punctate pattern is a discreet, stippled dark blue nuclear pattern. It is more often present in groups of cells in a more basal location within the epithelium. In some cases, the punctate pattern may require high magnification (40x objective) to visualize.

It is imperative that only epithelial cell nuclear staining be considered positive if a false positive interpretation is to be avoided. Non-cellular stromal precipitates and cytoplasmic staining of neutrophils and plasma cells represent artifactual nonspecific staining.

The morphology of each sample should also be examined utilizing an H&E stained section when interpreting any ISH result. The patient's morphologic findings and pertinent clinical data must be interpreted together with the ISH results.

Note: Use of 100x objective is not recommended. All of the design verification and validation testing was performed using 20x, 40x, and/or 60x objectives.

Controls

The stained positive reagent and specimen controls should be examined first to ascertain that all reagents are functioning properly. The presence of a discrete, blue colored, precipitated reaction product within the target cell nuclei is indicative of positive reactivity.

The negative reagent and specimen controls should be examined after the positive control to verify the specificity of the reaction. There should be no specific staining in the negative control. If staining occurs, it may indicate nonspecific cross reactivity to cells or cellular components. Intact cells should be used for interpretation of staining results since necrotic or degenerated cells often stain nonspecifically.

If the positive or negative controls fail to demonstrate appropriate staining, any results with the test specimens should be considered invalid.

Patient Specimen

Patient specimens should be examined last. Positive staining intensity should be assessed within the context of any background staining of the negative reagent control. A negative result means that the DNA sequence in question was not detected, not necessarily that the sequence is absent in the cells assayed. The morphology of each sample should also be examined utilizing a hematoxylin and eosin stained section when interpreting any ISH result. The patient's morphologic findings and pertinent clinical data must be interpreted by a qualified pathologist.

LIMITATIONS

1. ISH is a multiple step diagnostic process that requires specialized training in the selection of the appropriate reagents, specimen selection, processing, preparation of the ISH slide, and interpretation of the results.
2. Tissue staining is dependent on the handling and processing of the tissue prior to staining. Improper fixation, freezing, thawing, washing, drying, heating, sectioning, or contamination with other tissues or fluids may produce artifacts, false positive or false negative results.
3. Inconsistent results may result from variations in fixation and embedding methods, or from inherent irregularities within the tissue.
4. Excessive or incomplete counterstaining may compromise proper interpretation of results.
5. The clinical interpretation of any positive staining, or its absence, must be evaluated within the context of clinical history and morphology, and must be complemented by proper controls and other diagnostic tests. It is the responsibility of a qualified pathologist to be familiar with the probes, reagents and methods used to produce the stained preparation. Staining must be performed under the supervision of a pathologist who is responsible for reviewing the stained slides and assuring the adequacy of positive and negative controls.
6. Reagents may demonstrate unexpected reactions in previously untested specimens. The possibility of unexpected reactions even in tested specimen groups cannot be completely eliminated due to biological variability of targeted sequence in biological specimens. Contact your local Ventana office with documented unexpected reactions.
7. Due to variation in specimen processing it may be necessary to increase or decrease the ISH protease treatment time on individual specimens. Such changes must be validated by the user.

SUMMARY OF EXPECTED RESULTS

1. Ventana INFORM HPV II Family 6 Probe, ISH *i*VIEW $_{Blue}$ Plus Detection Kit, and accessory reagents in conjunction with a BenchMark IHC/ISH instrument will result in blue precipitate at the target sequence sites as located by the ISH probes.
2. Specificity and sensitivity of target sequence detection has been optimized and tested by Ventana. The probe cocktail has demonstrated positive hybridization to the HPV genotypes 6 and 11 in cervical biopsy specimens. However, each ISH probe must be validated by user to ensure desired staining.
3. Intra run reproducibility of staining with INFORM HPV II Family 6 Probes was determined by staining 10 cervical tissue slides on a BenchMark XT IHC/ISH instrument. All slides stained with comparable intensity. Users should verify intra run (within run) reproducibility results by staining several sets of serial sections with low, medium, and high target sequences in a single run.
4. Inter run reproducibility of staining with INFORM HPV II Family 6 Probes was determined by staining triplicate slides of positive cervical tissues on 2 BenchMark and 2 BenchMark XT instruments for 3 runs on each instrument. Ninety-seven percent of slides stained with comparable intensity. Users should verify inter run (between run) reproducibility results by staining several sets of serial sections with low, medium, and high target sequences on different runs.

TROUBLESHOOTING

1. If the positive control is negative, it should be checked to ensure that the slide has the proper bar code label.
2. If the positive control is negative or exhibits weaker staining than expected, any additional positive controls (e.g. positive tissue controls on the patient specimen slide) stained on the same staining run should be checked to determine if the failure is due to the control slide or reagents used. Specimens that have been improperly collected, fixed, stored, or deparaffinized may not exhibit appropriate staining.
3. If the specimen is washed off the slide, slides should be checked to ensure that they are positively charged.
4. If the slides demonstrate whiskering artifact from the detection chemistry, dehydration is not complete. Refer to the Dehydration Procedure section.
5. If nonspecific staining of the glass or nonspecific staining of areas of tissue (particularly areas other than the squamous epithelium) are observed, an additional section of the patient specimen should be stained.
6. For corrective actions, refer to the Step By Step Procedure section, the BenchMark IHC/ISH Instrument Operator's Manual or contact your local Roche office.

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