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WORKS FOR ME 1

PreTect SEE

COMMENTS 0

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[1]PreTect AS

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ABSTRACT

PreTect SEE is a CE-IVD kit for the qualitative detection of E6/E7 mRNA from HPV 16, 18 and 45 in a single analysis, identifying women at risk of having or developing cervical pre-cancer or cancer related to the carcinogenic HPV types included.
The kit is based on real-time NASBA technology combining nucleic acid amplification and simultaneous detection with Molecular Beacon probes. This process requires isolated nucleic acids as starting material.An Intrinsic sample control (ISC) is included in order to assess specimen quality and reveal possible factors that may inhibit the amplification, hereby monitoring the entire test process.
NASBA is an enzymatic one-step amplification process that is able to amplify RNA under isothermal conditions (41°C). The presence of genomic dsDNA will not cause false positives, as denaturation of dsDNA does not occur at this low temperature.

Cervical cancer is the most common cancer in women under 35 years of age. The main cause of cervical cancer is the human papilloma virus (HPV). Most women are infected with HPV during their lifetime and as many as 10-30 % of a female population below 35 years of age may be infected.
PreTect SEE is focusing on the HPV types with the highest risk of cervical cancer (HPV 16, 18 and 45). To further increase the specificity, only active oncogene expression from the E6/E7 oncogenes are measured. Published research show that 90 % or more of the cervical cancer cases in younger women are caused by only the three HPV types 16, 18, and 45.

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EXTERNAL LINK

https://www.pretect.no/

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GUIDELINES

Precautions:

For in vitro diagnostic use.

Laboratory related:

1. Use routine laboratory precautions. Do not eat, drink, or smoke in designated work areas. Wear disposable, powderless gloves and laboratory coats when handling specimens and kit reagents. Wash hands thoroughly after handling specimens and kit reagents.
2. Use only supplied or specified disposable laboratory ware.
3. Use a new pipette tip for each pipetting action.
4. Worksurfaces, pipettes, and other equipment must be regularly decontaminated with 1% (w/v) (freshly prepared) sodium hypochlorite solution.
5. Collect used disposable material in a container. Close and remove container after each test run and dispose waste in accordance with local regulations.

Specimen related:

1. All human sourced materials should be considered potentially infectious. Only personnel adequately trained should be permitted to perform this procedure.
2. Buffers used for sampling and lysis of cells must be used according to the safety precautions stated by the manufacturer.
3. Maintain proper temperature conditions during specimen shipping and storage to ensure the integrity of the specimen.
4. Avoid cross-contamination during the specimen handling steps. Ensure that specimen containers do not contact one another, and discard used materials without passing over open containers. Change gloves if they come in contact with specimen.

Assay related:

1. Store reagents at the specified temperatures. Performance of the assay may be affected by use of improperly stored reagents.
2. Make sure reagents are at room temperature (18-25 °C) prior to use.
3. Avoid microbial and ribonuclease contamination of reagents.
4. Do not use kit after its expiration date.
5. The PreTect Analysis Software (PAS) must be kept free of computer viruses. Virus scan is recommended on all connected computers and portable data storage devices prior to data transfer.

MATERIALS TEXT

MATERIALS

PreTect SEE **PreTect AS Catalog #110301**

PreTect X **PreTect AS Catalog #111001**

PreTect SEE contains a PCR plate prefilled with Mastermix / assay-controls with sufficient reagents for 96 amplification reactions, a maximum of 94 samples.

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1 x 96 wells PCRP	**PCR-plate** Pre-filled with mastermix. Positive and negative controls are located in well positions A1 and B1 respectively.
1x 8 x (1 x 5,2 mg) ENZ	**Enzymes** Lyophilised sphere containing AMV-RT, RNase H, T7 RNA polymerase and BSA. 8 tubes include 1 sphere each. Each tube is enclosed in a foil pack together with silica gel desiccant. Colour code: red screw cap
1 x 0.6 ml ENZdil	**Enzyme diluent** Sorbitol in aqueous solution. Colour code: red screw cap
1 x 12 CS	**Cap strips**
1 x	**Instructions for Use**

PreTect SEE content

PreTect X is intended for the isolation (purification and concentration) of total nucleic acids (RNA/DNA) from biological specimens prior to analysis with the products PreTect SEE and PreTect HPV-Proofer.
For in vitro diagnostic use.To be operated by professionals according to instructions.

PreTect X is based on Booms chemistry using magnetic silica particles as a solid phase.
Any cellular matter or viral particles in cervical specimen will be disrupted in the presence of PreTect X Lysis buffer containing GuanidineThiocyanate and Triton x-100, releasing nucleic acids and inactivating any RNases and DNases present. Nucleic acids will bind to magnetic silica particles under the high salt concentrations. Non-nucleic acid components will be removed by several washing steps. Subsequently the nucleic acids are eluted from the solid phase thereby making DNA/RNA available for use in amplification and detection procedures.

SAFETY WARNINGS

All human sourced materials should be considered potentially infectious. Dispose of all specimens and materials used

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to perform the test as if they contain infectious agents and in accordance with local regulations.
Laboratory reagents should always be handled with care. Chemical spillage (e.g. lysis buffer) should be dealt with promptly and in an appropriate manner.

BEFORE STARTING

Specimen collection and preservation:

Liquid based media e.g. PreTect TM, PreservCyt/ThinPrep, SurePath have been validated for the use with the PreTect SEE assay.
Users must follow the manufacturer`s instructions for collecting and storage of cervical cell specimens.

The NASBA process requires isolated nucleic acids as starting material, several protocols may be used. Completion of the isolation of Nucleic acids (Total nucleic acids or RNA) from the specimens allows for amplification and detection of HPV mRNA E6/E7 types 16, 18, 45 and ISC

1 **Sample preparation and lysis of material:**

Mix each sample thoroughly and pipette 1ml (PreservCyt) to a 10 ml sterile centrifuge tube.
Centrifuge cells for 12 minutes at 2500 rpm (1125g)
Discard the supernatant with a Pasteur-pipette or by vacuum suction
Add 1 ml lysis buffer (PreTect X) to the cells and homogenize by vortexing.
Incubate at room temperature for minimum 10 minutes until isolation procedure starts.
Lysed material may be stored at 2-30°C for 24 hours prior to isolation

2 **Automated Isolation of total nucleic acids (can be done manually using magnetic rack)**

Hamilton StarLET and PreTect X kit; based on Booms technology.
1 ml lysed material is added 100µl magnetic silica suspension, incubated at room temperature for 5 min.
3 different Wash steps (Wash buffer 1, 2 and 3) prior to elution.
Elution in 80 µl buffer at 60`C for 5 minutes.

3 **PreTect SEE assay: Amplification and detection of HPV mRNA E6/E7**

Bring all kit reagents and samples to room temperature prior to processing.
Allow the prefilled PCR plate to thaw at room temperature for 10 min protected from light.
Centrifuge the PCR plate for 10 seconds at 500g prior to addition of samples.
Please note that positive control is pre-filled into well A1 and negative control into well B1.

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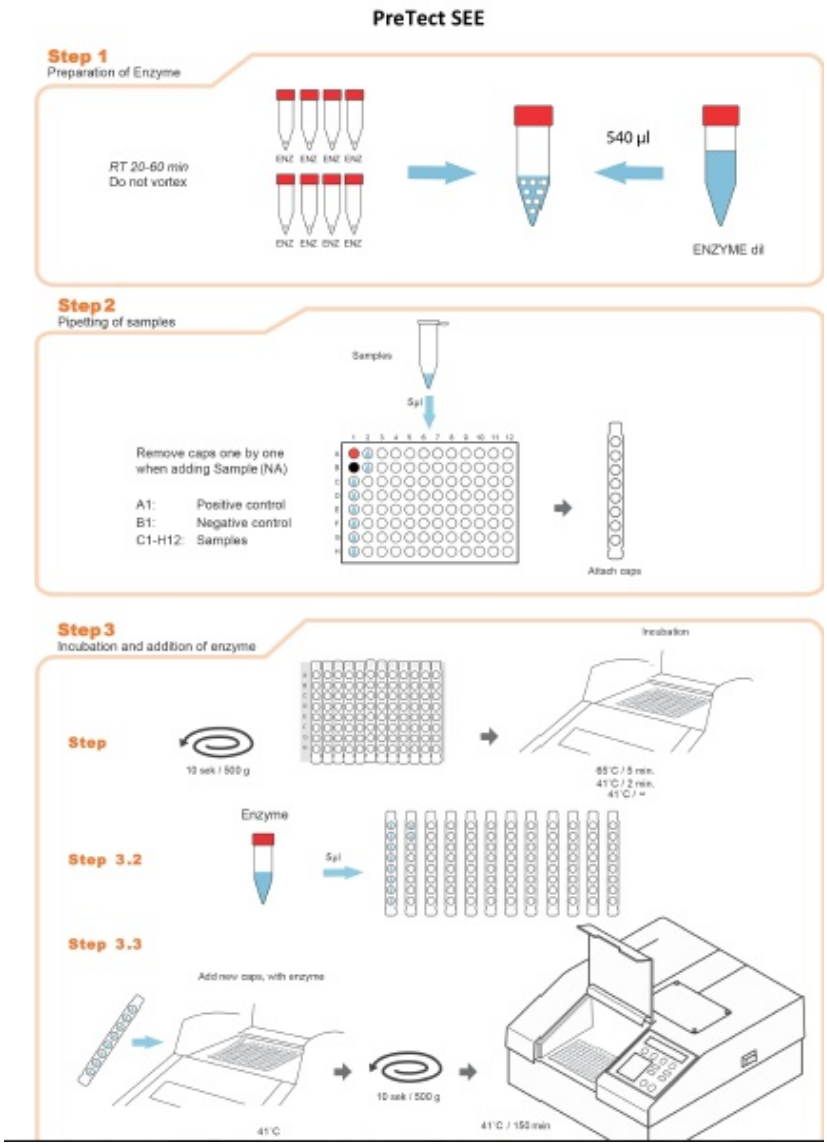

Illustration: Procedure at a glance

3.1 Reconstitution of Enzyme

Open the 8 foil packs (ENZ) containing 1 enzyme sphere each and collect all in one tube.
Add 540 ml ENZdil (Enzyme diluent) to the tube containing 8 enzyme spheres.
Leave the enzyme solutions at room temperature for 20-60 minutes until they come to a homogenous solution.
Spin down tubes briefly. Do not vortex.

3.2 Addition of samples and preincubation

Transfer 5 µl of each sample (Nucleic Acid) in the positions according the defined plate layout.
Spin the plate (minimum 500g, 10 seconds).
Incubate the 96 well PCR plate for 5 minutes at 65 ± 1 °C and 2 minutes at 41 ± 0.5 °C using a standard PCR cycler.

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3.3 Addition of enzyme

During incubation step, place 12 new cap strips upside down and add 5µl enzyme solution to the inner side of each cap strip.
After completion of incubation step, remove the cap strips one by one and replace with new cap strips prefilled with enzyme.
Use a Centrifuge with 96 well plate adapter (minimum 500g, 10 seconds) to spin the enzymes directly into each well.

3.4 Real time measurement (Analyzer)

Place the PCR-plate immediately into the pre-heated (41ºC) Analyzer and follow the instructions in the PreTect Analysis Software Operator Manual to start, review and accept a run.
Leave the Analyzer running for 150 minutes. The PAS software writes the results to the nc1-file continuously.

PAS supports the following instruments:

· PreTect Analyser (BioTek FLx800 BIE)
· NucliSENS EasyQ® Analyzer (BioMerieux)
· CFX96 (BioRad)
· Quant Studio 5(Applied BioSystems)

4 PreTect Analysis Software (PAS)

PAS is a dedicated application software package for planning the layout of the PreTect SEE assay setups on a fluorescence reader, retrieve measurement data and interpret, present, and finally report the complete analysis data and sample results.The PreTect Analysis Software performs all assay validation.
For details on how to operate the PreTect Analysis Software, refer to the dedicated PAS Operator Manual.
The PreTect Analysis Software package is available in several languages.

The real-time measurement data are analysed and the final results are presented as shown.
All sample results are presented in detail for both the internal control (ISC) and the HPV types, presented in columns for identification of Sample ID, well positions and result.

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HPV E6/E7 mRNA resultater														
Prøve ID (brønn)	ISC	HPV 16	HPV 18	HPV 45	Prøve ID (brønn)	ISC	HPV 16	HPV 18	HPV 45	Prøve ID (brønn)	ISC	HPV 16	HPV 18	HPV 45
Control+ (A1)	Pos	Pos	Pos	Pos	E40113 (A5)	Pos				E40145 (A9)	Pos			
Control- (B1)					E40114 (B5)	Pos				E40146 (B9)	Pos			
E40083 (C1)	Pos				E40115 (C5)	Pos				E40147 (C9)	Pos			
E40084 (D1)	Pos				E40116 (D5)	Pos				E40148 (D9)	Pos			Pos
E40085 (E1)	Pos				E40117 (E5)	Pos				E40149 (E9)	Pos			
E40086 (F1)	Pos				E40118 (F5)	Pos				E40150 (F9)	Pos			
E40087 (G1)	Pos			Pos	E40119 (G5)	Pos				E40151 (G9)	Pos			
E40088 (H1)	Pos				E40120 (H5)	Pos				E40152 (H9)	Pos			
E40089 (A2)	Pos				E40121 (A6)	Pos				E40153 (A10)	Pos	Pos		
E40090 (B2)	Pos				E40122 (B6)	Pos				E40154 (B10)	Pos	Pos		
E40091 (C2)	Pos				E40123 (C6)	Pos				E40155 (C10)	Pos			
E40092 (D2)	Pos				E40124 (D6)	Pos				E40156 (D10)	Pos			
E40093 (E2)	Pos				E40125 (E6)	Pos				E40157 (E10)	Pos			
E40094 (F2)	Pos				E40126 (F6)	Pos				E40158 (F10)	Pos			
E40095 (G2)	Pos				E40127 (G6)	Pos				E40159 (G10)	Pos			
E40096 (H2)	Pos				E40128 (H6)	Pos				E40160 (H10)	Pos		Pos	
E40097 (A3)	Pos				E40129 (A7)	Pos				E40161 (A11)	Pos			
E40098 (B3)	Pos				E40130 (B7)	Pos				E40162 (B11)	Pos	Pos		
E40099 (C3)	Pos				E40131 (C7)	Pos				E40163 (C11)	Pos			
E40100 (D3)	Pos				E40132 (D7)	Pos				E40164 (D11)	Pos	Pos		
E40101 (E3)	Pos				E40133 (E7)	Pos				E40165 (E11)	Pos			
E40102 (F3)	Pos				E40134 (F7)	Pos				E40166 (F11)	Pos			
E40103 (G3)	Pos				E40135 (G7)	Pos				E40167 (G11)	Pos			
E40104 (H3)	Pos				E40136 (H7)	Pos				E40168 (H11)	Pos			
E40105 (A4)	Pos		Pos		E40137 (A8)	Pos				E40169 (A12)	Pos			

4.1 Assay Controls/Error messages

PAS checks and validates all positive and negative controls for HPV 16, 18, 45 and ISC.
All controls have to be valid in order to report HPV mRNA results.
If all positive/negative controls are valid a statement is added to the Messages section of the sample report.

4.2 Intrinsic sample control

All specimen ISC results are checked and validated by PAS.
ISC monitors the isolation, amplification and detection steps of the assay, revealing possible factors that may inhibit the amplification. The ISC must give a positive result for a negative test result to be valid.
If an ISC is invalid, an error message is given for the relevant samples and an explanation is found in the Messages section of the sample report.

5 Interpretation of results

Clinical interpretation of the results should be made in conjunction with all other clinical findings for the patient and patient history.
PreTect SEE detects the presence of oncogenic activity and genotype individually HPV types 16, 18 and / or 45. These three HPV types cause more than 90 % of cervical cancer in younger women.

5.1 Negative test result

Oncogene activity caused by HPV types 16, 18 or 45 has not been detected.

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A negative PreTect SEE test does not exclude the possibility of an existing HPV infection, but merely indicates the absence of detectable HPV oncogene mRNA from HPV types 16, 18 and 45.
Results depend on adequate specimen collection, absence of inhibitors and sufficient mRNA to be detected.

5.2 Positive test result

Oncogenic activity of one or more of the HPV types 16, 18 and 45 has been detected.
It leads to increased risk for high grade cervical dysplasia, even if the coinciding Pap smear is normal.
A positive PreTect SEE test result is not a diagnosis of cervical cancer or high-grade cervical lesions.
Governmental instructions and recommendations for the monitoring of HPV status should be followed carefully.

5.3 Invalid test result

It has not been possible to obtain a valid test result. Improper specimen collection, storage or specimen processing may have caused the invalidity.
It is recommended repeat isolation or to collect a new sample from the patient, following adequate procedures to ensure high quality and a reliable test result.

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