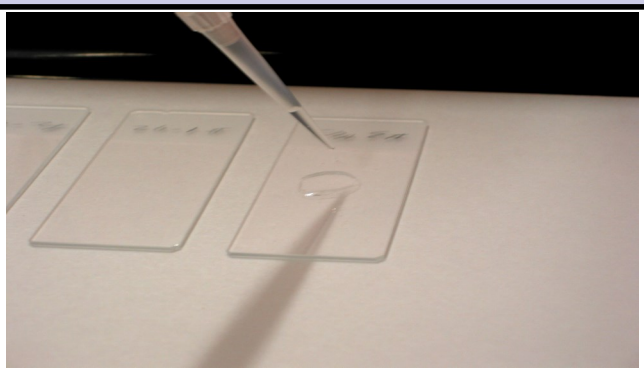
Liqui-PREP®
The Next Generation of Liquid Cytology

Technical Tips



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SUBJECT: HPV - DNA PCR Protocol using **Liqui-PREP®**

TECHNICAL TIP OVERVIEW:

This Technical Tip for HPV-DNA PCR Reflex testing is from a **Liqui-PREP®** user, in Israel. The procedure is being routinely used with good success. NOTE: This is from correspondence from Israel in 2005. This has not been verified by LGM International, Inc.

THE PROTOCOL: (After Liqui-PREP® Cytology Processing)

DNA Extraction:

- Transfer the complete Liqui-PREP® Cellular Pellet from the Liqui-PREP "Working Tube", along with the remnant fluid into a 1.5ml tube.
- Centrifuge the specimen for ~10 minutes at ~1,000xg.
- Remove the supernatant by inverting the centrifuge tube and lightly blot residual liquid from the lip of the centrifuge tube.
- Add 400µl of PCR grade water to the 1.5ml tube.
- Mix the pellet well using a vortex to break up the pellet.
- Place the centrifuge tube in a DRY HEAT BLOCK at 100^{O}C for 10 minutes. (This causes cytolysis of the cells and releases the nucleic and cytoplasmic genetic material into solution.
- Cool the specimen to room temperature.
- Add 10µl of Proteinase K (for Proteolysis) and incubate over night at 37^{O}C or incubate at 55^{O}C for 5 hours.
- To stop the proteolysis, insert the tubes into a DRY HEAT BLOCK at 100^{O}C for 10 minutes.

The supernatant now contains genetic sequences that can be identified by PCR.

- Prepare the dntp's solution for the hybridization with HPV DNA, if present.
- To 90µl of the dntp's solution, add 10µl of each sample and perform the routine PCR process.

Samples are run on the gel in parallel with known controls of HPV Types 6, 11, 16, 18, 31, 33.
Results are read as usual in the PCR gels.

Any Questions, Contact your local Liqui-PREP® Representative :

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