Automated nucleic acid purification from ThinPrep® PAP samples for CT/NG detection by real-time PCR and HPV detection by a laboratory-developed test using Hologic Invader® ASRs.

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1. Abstract

ThinPrep® solution is commonly used to preserve cervical swab cells for morphological analysis. We examined automated nucleic acid purification from these samples for molecular assays. DNA isolated by Maxwell® 16 and analyzed for Chlamydia trachomatis and Neisseria gonorrhoea (CT/NG) by real-time PCR showed high correlation to replicate samples assayed by either Roche COBAS®AMPLICOR or Gen-Probe APTIMA Combo 2® Assays in independent laboratories (99% concordance). This same method did not consistently yield DNA of sufficient concentration for Invader®-based Human Papillomavirus (HPV) detection, so we developed a modified extraction method. This method gave 88% concordance to Digene Hybrid Capture II (HC2). These data show Maxwell® 16 can be used for automated nucleic acid purification from ThinPrep® PAP samples for downstream CT/NG and Invader®-based HPV detection.

2. Introduction

The ThinPrep® solution used to preserve cervical swab samples is alcohol-based and poses a challenge for extraction of nucleic acids. Extraction techniques can be time consuming and labor intensive. The Maxwell® 16 System was developed to meet the needs of low- to moderate-throughput users by providing automated purification at a scale appropriate to their workload without considerable capital investment, training or maintenance. The pre-filled cartridge design and mechanical plunger action of the Maxwell® 16 System make it simple, rapid purification of a wide variety of sample types. The Maxwell® 16 System uses paramagnetic particles that bind and move DNA through a series of wash steps prior to elution. This technology has been adapted to a range of purification technologies for DNA, RNA and protein, making Maxwell® 16 a highly versatile, integral tool in the laboratory. We developed two automated protocols for isolation of DNA from ThinPrep® PAP samples for downstream analysis in molecular assays for CT/NG and HPV. Data were found to be highly correlative to traditional detection techniques.

3. The Maxwell® 16 System

The Maxwell® 16 System uses paramagnetic particles that bind and move DNA through a series of wash steps prior to elution. This technology has been adapted to a range of purification technologies for DNA, RNA and protein, making Maxwell® 16 a highly versatile, integral tool in the laboratory. We developed two automated protocols for isolation of DNA from ThinPrep® PAP samples for downstream analysis in molecular assays for CT/NG and HPV. Data were found to be highly correlative to traditional detection techniques.


1. Centrifuge 1.8 ml ThinPrep® PAP sample at 14,000×g for 3 min.
2. Discard 1.8 ml and suspend pellet in 200 µl of Maxwell® Lysis Buffer/DTT Solution to sample and vortex.
3. Transfer to Well #1 of LEV Cell DNA Cartridge. Elute in 100 µl.

ThinPrep® samples are concentrated, then lysed and transferred directly to Well #1 of the Maxwell® 16 LEV Cell DNA Purification Kit cartridgge and processed on the Maxwell® 16 Instrument with LEV hardware. The robust chemistry makes it unnecessary to wash or heat-treat the cells before extraction. Nucleic acid is then added to a qualitative multiplexed assay for detection of Chlamydia and Gonorrhea. The number of cells, and therefore the amount of DNA, in ThinPrep® samples is highly variable. DNA must be 10-100 ng/µl for the Invader® HPV assay. A 2X ThinPrep® PAP sample input, the LEV Cell DNA method (Panel 4) only gave DNA of sufficient concentration from 6 out of 8 samples, whereas a total nucleic acid isolation method using the LEV Total RNA Purification (Panel 7) consistently gave DNA 210 ng/µl. Subsequent Invader®-based tests used this method.

5. Correlation of the Maxwell® 16/real-time PCR method and Gen-Probe APTIMA Combo 2 Assay.

A. Correlation Data

B. Discrepant Sample Analysis

6. Concentration of DNA isolated from ThinPrep® samples by two different Maxwell® 16 methods.

The table shows the concentration of DNA isolated from ThinPrep® PAP samples by two different Maxwell® 16 methods.

References


1. Centrifuge 2.0 ml ThinPrep® PAP sample at 14,000×g for 3 min.
2. Discard 1.8 ml and suspend pellet in remaining liquid.
3. Add 0.4 ml of Low-Elution Buffer Solution to sample and vortex.
4. Transfer to Well #1 of Maxwell® 16 LEV Total RNA cartridge. Elute in 100 µl.

ThinPrep® samples are concentrated then lysed and transferred directly to Well #1 of the Maxwell® 16 Tissue LEV Total RNA Purification Kit (Cat.IAS1220) cartridge modified to include an ethanol wash, then processed on the Maxwell® 16 Instrument with LEV hardware. Ten microliters of eluate is added directly to the HPV Invader®-based test (3).


ThinPrep® PAP samples were analyzed for HPV by either Digene HC2 or by extraction with Maxwell® 16 followed by an Invader®-based HPV detection method. Of 58 samples, 7 gave discordant results and 1 gave an indeterminate result. Discordance averages 10% from previous studies (2, 4) of these two detection assays (but different nuclear acid extraction methods) compared with 12% discordance from this sample set. The Invader®-based assay includes an internal control to ensure that sufficient genomic DNA is present for a valid reaction. Digene has no internal control and therefore it cannot be determined if negative results are caused by insufficient DNA from the extraction method.

9. Conclusion

Extraction of DNA from ThinPrep® PAP samples can be automated using Maxwell® 16. DNA is of suitable quality for detection of Chlamydia trachomatis and Neisseria gonorrhoea (CT/NG) by real-time PCR, and Human Papillomavirus (HPV) by an Invader®-based detection method. Data are highly correlative to traditional detection techniques, Roche COBAS®AMPLICOR / Gen-Probe APTIMA Combo 2® Assay and Digene HC2, 99% and 88%, respectively. HPV correlation data are comparable to previously published concordance data for Digene and Invader®-based detection methods (3, 4).