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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 a 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 (1). The pre-filled cartridge design and mechanical plunger action of the Maxwell® 16 System make it ideal for 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 Instrument



The Maxwell® LEV format pre-dispensed cartridge

The Maxwell® 16 instrument is used with optimized reagents predispensed into disposable cartridges to yield optimal performance. The unique design of the cartridge allows the direct processing of a variety of liquid and solid sample types often without the need for preprocessing. The low elution volume (LEV) format (shown) allows elution of nucleic acid in as little as 30 µl.

References

1. Kephart, D. et al. (2006) *Promega Notes* 92, 20–3.
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3. Ginocchio, C.C. et al. (2008) *J. Clin. Microbiol.* 46(5), 1641-6.
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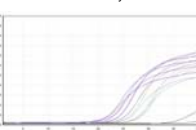
4. Method for automated purification and detection of Chlamydia and Gonorrhea in ThinPrep® PAP samples.

1. Centrifuge 1.0 ml ThinPrep® PAP sample at 14,000xg for 3 min.
2. Discard 0.8ml and suspend pellet in remaining liquid.
3. Add 0.4ml Lysis Buffer/DTT Solution to sample and vortex.
4. Transfer to Well #1 of LEV Cell DNA Cartridge. Elute in 100µl.



Automated nucleic acid extraction by Maxwell® 16 in ~30 minutes.

Real-time PCR analysis of eluate.



ThinPrep® samples are concentrated, then lysed and transferred directly to Well #1 of the Maxwell® 16 LEV Cell DNA Purification Kit (Cat.#AS1130) cartridge 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 (2).

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

A. Correlation Data

COBAS AMPLICOR or APTIMA Combo 2 Assays	N=243	Maxwell® 16/real-time PCR				Indeterm
		CT+/NG-	CT-/NG+	CT+/NG+	CT-/NG-	
CT+ NG-		19			1	
CT- NG+			1		2	
CT+ NG+				1		
CT- NG-					217	1
Indeterm						1

B. Discrepant Sample Analysis

COBAS/APTIMA	Maxwell/PCR	Retest
CT+ NG-	CT- NG-	Weak CT+ (by PCR)
CT- NG+	CT- NG-	NG- (by APTIMA)
CT- NG+	CT- NG-	Not enough material
CT- NG-	Indeterm	Not enough material

ThinPrep® PAP samples were analyzed for Chlamydia and Gonorrhea (CT/NG) by either the Roche COBAS® AMPLICOR or Gen-Probe APTIMA Combo 2® Assays or by extraction with Maxwell® 16 followed by a qualitative real-time multiplex PCR assay (2). Of 240 samples, 3 gave discordant results and 1 was an indeterminate result. Upon retest, one sample gave a different, concordant result by APTIMA and a second gave a weak concordant result by PCR. There was not sufficient material in 2 samples to retest.

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

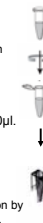
Sample ID	LEV Cell DNA	LEV Total RNA
A	29.28	71.63
B	6.64	13.09
C	11.71	51.41
D	14.08	35.25
E	9.78	22.24
F	20.06	73.39
G	17.67	20.96
H	22.02	60.71
I	7.1	35.18

The number of cells, and therefore the amount of DNA, in ThinPrep® samples is highly variable. DNA must be 10-100ng/µl for the Invader® HPV Assay. At 2ml ThinPrep® PAP sample input, the LEV Cell DNA method (Panel 4) only gave DNA of sufficient concentration from 6 out of 9 samples, whereas a total nucleic acid isolation method using the LEV Total RNA Purification (Panel 7) consistently gave DNA ≥10ng/µl. Subsequent Invader®-based tests used this method.

7. Method for automated purification and detection of Human Papillomavirus in ThinPrep® PAP samples.

1. Centrifuge 2.0 ml ThinPrep® PAP sample at 14,000xg for 3 min.
2. Discard 1.8ml and suspend pellet in remaining liquid.
3. Add 0.4ml Lysis/Dilution Buffer Solution to sample and vortex.
4. Transfer to Well #1 of Tissue LEV Total RNA cartridge. Elute in 100µl.

* Replace the buffer in Well #6 with 0.4ml 80% ethanol.



Automated nucleic acid extraction by Maxwell® 16 in ~30 minutes.

HPV Invader®-based Detection.

Sample ID	HPV Invader Result	HPV Results Summary			
		HPV 16	HPV 18	HPV 31	HPV 33
Sample A	Weak	1.0	1.0	1.0	1.0
Sample B	Weak	1.0	1.0	1.0	1.0
Sample C	Weak	1.0	1.0	1.0	1.0
Sample D	Weak	1.0	1.0	1.0	1.0
Sample E	Weak	1.0	1.0	1.0	1.0
Sample F	Weak	1.0	1.0	1.0	1.0
Sample G	Weak	1.0	1.0	1.0	1.0
Sample H	Weak	1.0	1.0	1.0	1.0
Sample I	Weak	1.0	1.0	1.0	1.0

ThinPrep® samples are concentrated then lysed and transferred directly to Well #1 of the Maxwell® 16 Tissue LEV Total RNA Purification Kit (Cat.#AS1220) 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).

8. Concordance of the Maxwell® 16/Invader®-based method and Digene HC2.

Digene HC2	N=59	TWT Invader Result		
		Positive	Negative	Indeterm
Positive		25	2	1
Negative		5	26	0
Indeterm		0	0	0

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 59 samples, 7 gave discordant results and 1 gave an indeterminate result. Discordance averages 18% from previous studies (3, 4) of these two detection assays (but different nucleic 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).

Disclaimer: The Maxwell® 16 instrument and associated reagent kits are intended for the upstream workflow of isolating DNA, RNA, or protein. Promega makes no claims as to the suitability of the Maxwell® 16 System with regard to clinical determinations related to any specific disease or disorder. Maxwell is a registered trademark of Promega Corporation. Products may be covered by existing or pending patents, please visit www.promega.com for more information.