

Magtration® System 12GC: Application data----Microbiology

DNA extraction of *Neisseria gonorrhoea* using Magtration®-MagaZorb® DNA Common Kit-200

Theodore Chiu and Linda Chui. Provincial Laboratory for Public Health (Microbiology), Edmonton, Alberta, Canada

Introduction

Magtration® System 12GC is a fully automated DNA/RNA isolation system coupled with an extraction kit, Magtration®-MagaZorb® DNA or RNA. To determine the applicability of this instrument in a diagnostic Microbiology laboratory, *Neisseria gonorrhoea* (NG) was the first organism selected for the study. The objective of this study was to evaluate the efficiency of extraction as compared with established routine extraction protocol.

Materials and Methods

Sensitivity

Two clinical isolates of NG were standardized to a 30% transmittance and 10 fold dilutions were made from 10^{-3} to 10^{-7} . Five μ L from each dilution was plated onto Mueller Hinton plate and incubated for 48h before performing a plate count. A 200 μ L volume of each dilution was used for DNA extraction.

Swab and urine samples

A total of 32 swabs and urine samples randomly selected from routine submission for NG testing were included in this study. One milliliter of saline was added to the tube containing the swab, mixed by vortex for 30 sec and 200 μ L was removed for DNA extraction. For urine samples, the specimen was vortexed and 200 μ L of the specimen was used for DNA extraction.

DNA Isolation and PCR

DNA was extracted with Magtration®-MagaZorb® DNA Common Kit-200 by Magtration® System 12GC. A 200 μ L sample volume was applied to the instrument and DNA was eluted with 170 μ L and 5 μ L was used for real-time PCR (LightCycler®, Roche Diagnostics) with primers directed to the 16s rRNA sequence. Samples with negative results were further tested for Human Factor V by PCR to check for inhibition. Routine protocol for DNA extraction was by MagNA Pure LC® (Roche Diagnostics)

Cross contamination study

An aliquot of 200 μ L from 6 clinical samples (urine or swab) previously confirmed positive for NG along with 6 negative clinical samples (urine or swab) were extracted by Magtration® System 12GC. The

negative samples were placed in between the positive samples for extraction as well as for real-time PCR.

Results

Sensitivity

The sensitivity of real-time PCR detection with primers directed to 16s rRNA sequence by PCR endpoint titration was at 1 CFU/PCR assay.

Swab and urine samples

The results are shown in the following table

Magtration		Clinical	
		Positive	Negative
Swab	Pos.	6	0
	Neg.	0	10
Urine	Pos.	7	0
	Neg.	0	9

There was 100% correlation between previously reported clinical data and PCR results generated from DNA using 12GC extraction. No inhibition was observed in all the negative samples tested by Human Factor V PCR.

Cross contamination study

No cross contamination was observed when positive samples were placed between the negative samples.

Conclusion

The PCR results using DNA extracted from swabs and urine samples by Magtration® System 12GC with Magtration®-MagaZorb® DNA kit-200 for *Neisseria gonorrhoeae* showed 100% correlation with previous clinical data. There was no cross contamination between samples and no inhibition was observed. The isolated DNA can be directly applied for down stream application such as PCR using Roche LightCycler®. Since Magtration® System 12GC uses reagent- prefilled cartridges, it can minimize hands-on-time and handling error caused by manual reagent dispensing.

References

1. Magtration®-MagaZorb® DNA kit-200 Protocol Revision 4.0, PSS Bio Instruments.