

Technical Note

TN6: Use of EZ1 Advanced XL (Qiagen) with MycAssay™ *Aspergillus* Serum:

Summary

Evaluation of the utility of the EZ1 Advanced XL (EZ1) (Qiagen) automated DNA extraction system for extracting cell-free *Aspergillus* DNA from serum for use with the MycAssay™ *Aspergillus* kit involved:

- Repeat extraction of negative samples
- Repeat extraction of <10 genome equivalent samples
- Serial dilution experiment to assess the ability of the EZ1 system to accurately extract and discriminate various concentrations of DNA

The EZ1 extraction system is simple to use and performs well with good and reproducible extraction efficiency.

Background

The ability to use automated DNA extraction systems with MycAssay™ real-time PCR kits is desirable to simplify the laboratory workflow, reduce hands on time and meet the requirements of higher through-put laboratories. Various automated extraction systems are commercially available to meet these demands. The EZ1 uses magnetic bead technology to allow separation of nucleic acids and has the advantage of utilising individual sealed reagent cartridges which reduces both the need for human manipulation and therefore the likelihood of cross-contamination.

Methods

Free *Aspergillus* DNA template (100 copies/μL) was added to aliquots of commercially available serum (Invitrogen) as necessary. The blank or spiked serum samples were run on the EZ1 instrument using the following kit and protocol:

EZ1 Virus mini kit v2.0*

Sample volume = 400μL

Elution volume = 60μL

** The use of carrier RNA is optional and was not used in this protocol*

- Negative extracts
 - 10 Negative water extractions
 - 10 Negative serum extractions
 - PCR run in duplicate on SmartCycler II (Cepheid)
- <10 genome equivalent extracts

TN6: Use of EZ1 Advanced XL (Qiagen) with MycAssay™ *Aspergillus* Serum

Technical Note

- 8.1 genome equivalents were tested in 10 serum extractions
- PCR run in duplicate on SmartCycler II
- Serial dilution
 - 3 x High *Aspergillus* (Asp) DNA template concentration
 - 3 x Medium Asp DNA template concentration
 - 3 x Low Asp DNA template concentration
 - PCR run in duplicate on SmartCycler II

Results

Negative extracts	19/20 negative results obtained (Ct >39.0 or 0) for 10 water extractions tested in duplicate
	16/18 negative results obtained for 10 valid negative serum extractions tested in duplicate
<10 genome equivalent extracts	17/19 positive results obtained (Ct <39.0) for serum spiked with <i>Aspergillus</i> template DNA at 8.1 genome equivalents
	Average Ct = 37.3 (±1.3) for 8.1 genome equivalents

Serial Dilution	High Asp template concentration	Medium Asp template concentration	Low Asp template concentration
Genome equivalents	270	135	27
Average Ct	31.7 (± 0.63)	32.7 (± 0.48)	35.2 (± 0.50)
Expected Ct difference compared to High Asp template concentration	-	1.0	2.3
Actual Ct difference compared to High Asp template concentration	-	1.0	2.5

Conclusion

The EZ1 is a fast, efficient method of extracting free DNA from serum for use with the MycAssay™ *Aspergillus* Serum assay. The serial dilution data indicates that it is able to accurately discriminate between differing concentrations of DNA yielding reliable results. The EZ1 system is very simple to operate and to maintain and therefore effectively streamlines the sample preparation step of the real-time PCR procedure.

TN6: Use of EZ1 Advanced XL (Qiagen) with MycAssay™ *Aspergillus* Serum