HISTO SPOT® SSO

Technical Report

Influence of different DNA extraction methods on the performance of HISTO SPOT® SSO kits

Introduction

DNA quality and concentration are crucial parameters in all PCR techniques including the HISTO SPOT® SSO system for HLA typing. The HISTO SPOT® SSO test includes an amplification of the variable exons of the respective HLA gene followed by a fully automated SSO assay (hybridisation to SSO probes and colorimetric detection of bound amplicon) on the MR.SPOT® processor. The kits were validated for CE certification using the manual protocol for the Qiagen columns.
Five commercially available and widely used DNA extraction methods were evaluated regarding their suitability for use in the HISTO SPOT® SSO system.

Material and methods

Samples were provided by different labs which use the DNA extraction methods for their routine work. The DNA samples were prepared according to the instructions of the manufacturers (Table 1). The DNA samples extracted with the Qiagen EZ1/GenoM™-6 and the Roche filter tubes were from the same blood samples and were HLA pre-typed.

Name (Manufacturer)	Method	automated	No. of samples
QuickGene (Fujifilm)	Membrane technology	yes	8
Chemagic Blood Kit (Chemagen / Perkin Elmer)	Magnetic beads	yes	8
Qiagen EZ1/GenoM™-6	Magnetic beads	yes	8
Maxwell Promega	Magnetic beads	yes	8
High Pure PCR Template Preparation Kit (Roche)	Filter tubes	no	8

Table 1: Overview of the used DNA extraction methods

The DNA concentration and purity indexes $OD_{260/280}$ and $OD_{260/230}$ were determined using a NanoDrop photometer. Since residual magnetic beads could influence the measurement and the PCR amplification, the samples isolated with the respective techniques were measured after placing them on a magnet for 10 minutes.

Samples with a DNA concentration outside the specified range of 15-30 ng/µl for the HISTO SPOT® SSO test were diluted to 15 ng/µl.

All samples were typed for HLA-A, -B and –DRB1 with the respective HISTO SPOT® SSO kits according to the instructions for use. As a measure of the quality of the test the average number of false positives or false negative probes was determined. This includes probes that were either ignored automatically by the software in the default setting or inactivated manually during the interpretation.

Results

The DNA concentration was around 20 ng/µl for the QuickGene method, the Roche filters and the Qiagen EZ1/GenoM™-6. The Chemagen method generally showed higher concentrations while the Maxwell Promega method had the highest variability (Figure 1).

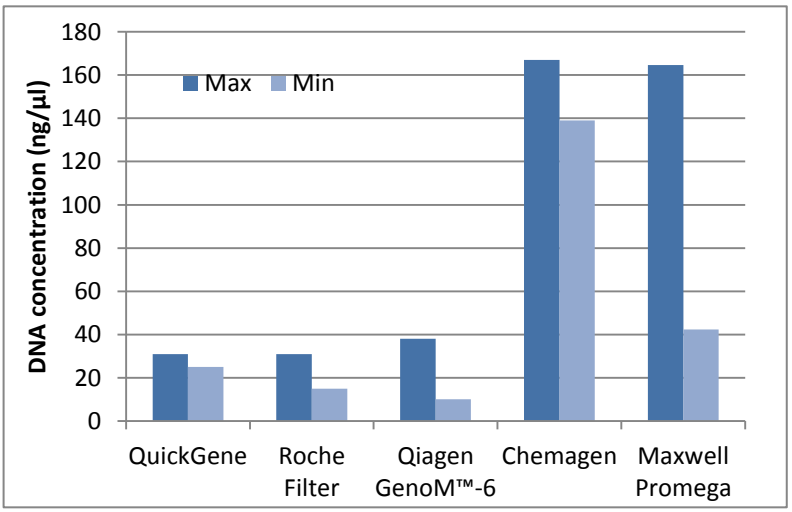

Figure 1:
Minimum and Maximum DNA concentration values for the different extraction methods

The purity index $OD_{260/280}$ ranged from 1.6 to 2.0 and all the DNA samples were in the recommended range (1.5 - 2.0). The purity index $OD_{260/230}$ was in the range between 0.8 and 2.2. 23 DNA samples had an $OD_{260/230}$ below the recommended value of at least 1.8, but this did not have a negative impact on the results.

As expected the average number of discrepant probes was generally higher for the HLA-B locus than for the HLA-A and HLA-DRB1. This is due to the different number of probes in the three tests. The HLA-B test includes more probes than the other two. There were no systematic differences in test performance between the different extraction methods (Figure 2).

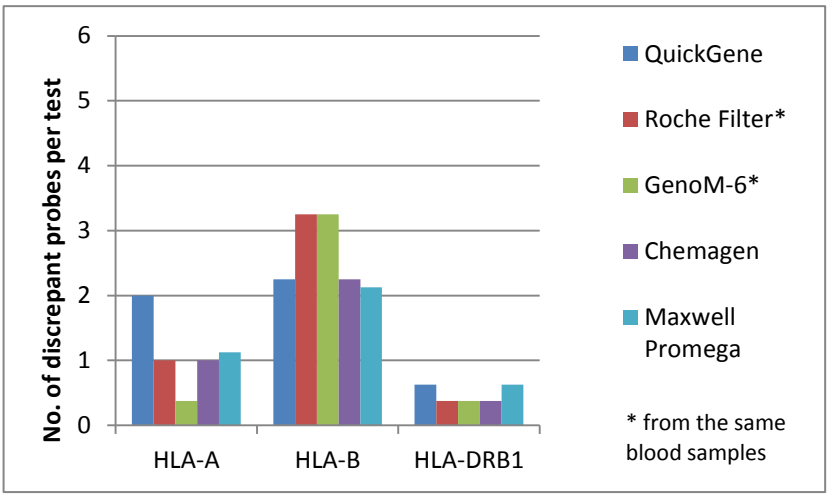

Figure 2:
Average number of ignored probes per test for the different extraction methods

Conclusion

The DNA extraction methods tested in this study are very different in regarding both their test procedure and their DNA yield. This variability nevertheless does not influence the performance of the HISTO SPOT® Kits. As shown above all DNA extraction methods evaluated in this study are suitable to be used with the HISTO SPOT® SSO system for HLA typing, if the DNA concentration is adjusted and the purity index $OD_{260/280}$ is in the recommended range.

Acknowledgements
We would like to thank the following institutions for generously providing DNA samples:
Médiane diagnostics,Plaisir, France
Leids Universitair Medisch Centrum (LUMC), Leiden, Netherlands
Universitätsklinikum Magdeburg, HLA-Labor, Germany
Med. Versorgungszentrum für Labordiagnostik, Eppelheim, Germany