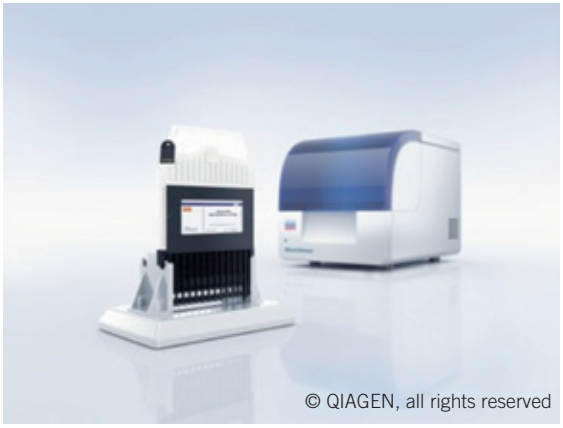
OLERUP SSP®
GENOMIC HLA TYPING

Application Note:

QIAxcel® Advanced System and the QIAxcel DM80 Method for Olerup SSP® Data Generation and Automatic Transfer into the Olerup SSP® version of Helmberg-SCORE™

Introduction

Olerup SSP AB and QIAGEN GmbH have in a Joint Improvement Program of the QIAxcel Advanced System for Olerup SSP® data generation and automatic data transferal into Olerup SSP® version of Helmberg-SCORE™, optimized QIAxcel analysis setting for PCR-SSP fragment analysis of 96 reactions in 24 minutes.



The QIAxcel Advanced System replaces traditional, labor-intensive gel analysis – streamlining your workflow and reducing time to result. The QIAxcel Advanced System fully automates sensitive, high-resolution capillary electrophoresis of up to 96 samples per run. DNA fragment analysis of 96 samples can be performed in as little as 24 minutes. Ready-to-run gel cartridges allow 96 samples to be analyzed with a minimum of hands-on interaction, reducing manual handling errors and eliminating the need for tedious gel preparation. User-friendly QIAxcel ScreenGel software ensures convenient analysis and documentation of data.

In 2008, QIAGEN GmbH and Olerup SSP AB jointly launched the QIAxcel DM190 separation method for automation of Olerup SSP® typing kit analysis and result interpretation. In this new study, performed at Olerup SSP AB during 2011, optimized analysis settings for the DM80 method have been evaluated to enable a run time of 24 minutes compared to 45 minutes for the DM190 method. Furthermore, this evaluation also investigated the impact of the extended shelf life of the QIAxcel DNA Fast Analysis cartridges on the SSP results.

OLERUP SSP®
GENOMIC HLA TYPING

Method

Data generation from HLA-C low resolution SSP (24 wells) and HLA-DR low resolution SSP (24 wells) typings for 10 different samples at four time points was performed. Each sample was run with 3 different gel cartridges at 4 different time points of gel cartridge life (0 months, 2 months, 4 months and 6 months). All samples were run on 2 instruments with the DM80 method and default rise time.

This means that data of 120 complete 96-well plates was generated (10 donors x 3 gel cartridges x 4 time points x 2 instruments / 2 donors per plate). Raw data was processed several times with different analysis settings to find the optimal parameters for peak detection and subsequent automatic data transfer into SCORE.

Result

Based on the result of the DM80 method evaluation, the recommended analysis settings to serve as a universal setting for use of the DM80 method for Olerup SSP® data generation and automatic transfer into SCORE is presented in Table 1.

Table 1. Recommended QIAxcel analysis settings for use of the DM80 method for Olerup SSP® data generation and automatic transfer into SCORE. All other analysis settings should be as in the Default Fast Analysis Profile defined within QIAxcel ScreenGel software.

QIAxcel Method:	**DM80**
Positive Threshold:	0,14 RFU
Minimum Distance:	1,5 s
Data Smoothing Filter:	0 pts

A comparison of the results with the recommended analysis settings generated in the DM80 evaluation with the results from the DM190 evaluation performed at QIAGEN GmbH and Olerup SSP AB in 2008 is shown in Table 2.

Table 2. Result comparison of the DM80 (T = 0 – 6) and DM190 methods. Results shown considering the 96-well plates and the total number of wells run respectively.

QIAxcel Method **Recommended analysis settings** **(Positive Threshold; Minimal distance):**	**DM80** **0,14RFU; 1,5s**	**DM190** **0,14RFU; 3,0s**
Time point for evaluation (year):	2011	2008
Number of 96-well plates run and evaluated:	120 plates	22 plates
Number of processed wells in total:	11520	2112
Runtime/96-well plate	24 min	45 min
RESULT (% of 96-well plates):		
A.Direct transfer into SCORE:	**89,2%**	**90,9%**
of which:		
A1. No editing needed	52,5%	45,5%
A2. Editing in SCORE needed (non-alignment corrections)	36,7%	45,5%
of which:		
A2.1. Average number of edits/plate	1,9 edits	5,5 edits
B. Edit in electropherogram prior to transfer into SCORE (alignment corrections):	**10,8%**	**9,1%**
B1. Average number of electropherogram edits/plate	1,5 edits	1,0 edit
RESULT (% of wells):		
C. Direct transfer into SCORE	**98,9%**	**97,1%**
D. Edit in SCORE needed (non-alignment corrections):	0,9%	2,8%
E. Edit in electropherogram prior to transfer into SCORE needed (alignment corrections):	0,2%	0,1%

Conclusion and Recommendation

The comparison clearly shows the benefit of the shortened run time per 96-well plate and otherwise comparable results for the two different separation methods. However, it should be noted that the number of plates run in 2008 was significantly smaller compared to the evaluation of the DM80 method now performed. This is most likely to influence the results and might make a straightforward comparison biased. The method comparison should therefore be considered as a means to compare trends more than to compare detailed percentages. We conclude that the result trends of these two methods are comparable.

Furthermore, our conclusion is that when using the QIAxcel Advanced System for SSP data generation, which is a much more sophisticated data analysis method than visual inspection of traditional agarose gels, run- and time dependent fluctuations in PCR-SSP data that are not visually detectable on a traditional gel may have an effect on the result. We believe that this is one major factor influencing result fluctuation leading to the noted need of corrections in SCORE and/or in the electropherograms prior to data transferal into SCORE. However, this does not rule out that other factors may also be of importance for generating the need for corrective actions. One additional factor that also may influence results is center-dependent result fluctuations. The DM80 method evaluation is a single center study performed at Olerup SSP AB and the magnitude and influence of center-dependent fluctuations are therefore at this point difficult to foresee. Hence, inspection of transferred data into SCORE is an important part of the process to ensure correct interpretation and this action is also in a user-friendly way facilitated in SCORE.

Based on the outcome of the evaluation of the DM80 method, Olerup SSP AB recommends the QIAGEN QIAxcel Advanced System, the QIAxcel Fast Analysis Kit, the QIAxcel ScreenGel software and the QIAxcel DM80 method for customers who wish to automate their Olerup SSP® typing kit data generation and transferal into the Olerup SSP® version of Helmberg-SCORE™. Moreover, we also conclude that the prolongation of the QIAxcel DNA Fast Analysis cartridge shelf life from 3 to 6 months upon production show no impact on the result for the application of SSP data generation and subsequent automatic transfer into SCORE.

For more information on the QIAGEN QIAxcel Advanced System, please go to the QIAGEN webpage: **www.qiagen.com/products/qiaxceladvanced.aspx**

Manufactured by:

OLERUP SSP AB
Box 12283
SE-102 27 Stockholm, Sweden
Tel: +46 8 717 88 27. Fax: +46 8 717 88 18
Visitors address: Franzéngatan 5
E-mail: info-ssp@olerup.com

Distributed by:

OLERUP GmbH
Address: Loewengasse 47/6,
1030 Vienna, Austria
Orders and support: support-at@olerup.com
Tel: +43-1-710 15 00 00.
Fax: +43-1-710 15 00 10

OLERUP SSP GENOMIC HLA TYPING

Manufactured by Olerup SSP AB
www.olerup.com

OLERUP

Distributed by Olerup
www.olerup.com

CareSSP-180308-2

CareDx®