Evaluation of the EliGene® Spondylitis HLA-B27 C6 Assay on the cobas 6800 System

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Table of Contents

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Introduction

The cobas omni Utility Channel allows users to establish and run laboratory developed tests on the cobas 6800 system. This feature can also be used by third party vendors to produce commercial test systems for the cobas 6800. Recently, ELISABETH PHARMACON launched an assay for detection of HLA-B27 (EliGene® Spondylitis HLA-B27 C6). To my knowledge this is the first commercial CE-IVD marked assay for the cobas 6800 not developed and supplied by Roche. In this evaluation, I compared the characteristics of the EliGene® Spondylitis HLA-B27 C6 assay to a laboratory developed HLA-B27 test.

Material and Methods

Sample preparation

Samples for the EliGene® Spondylitis HLA-B27 C6 (EliGene HLA-B27) assay were diluted 1:3, 1:5 or 1:7 with water or PBS (manufacturer´s instructions). Blood samples for the laboratory developed HLA-B27 assay (HLA-B27 LDT) were diluted 1:10 with water.

Reagent cassettes

Reagent cassettes for the EliGene® Spondylitis HLA-B27 C6 assay were prepared according to the manufacturer´s instructions. Briefly, 480 µl of the provided HLA-B27 Mix were mixed with 8 ml of UC MMx-R2. Thereafter 7 ml of this master mix were transferred into row 2 of the reagent cassette. For the laboratory developed HLA-B27 assay, 300 µl of Primer/Probe-Mix were mixed with 5 ml of UC MMx-R2. The master mix was completely transferred into row 2 of the reagent cassette.

Assay profiles (UCAPS)

HLA-B27 LDT:

PCR profile:	generic profile
Sample volume:	200 µl
Channel 2	
Target name:	B27
Sensitivity (RFI min):	1.5
Channel 3	
Target name:	GAPDH
Sensitivity (RFI min):	1.5

EliGene HLA-B27:

PCR profile	assay specific profile
Sample volume:	200 µl

Channel 2

Target name:	B27
Sensitivity (RFI min):	2.5

Channel 3

Target name:	SYPL2
Sensitivity (RFI min):	2.5

EliGene HLA-B27 profile:

Pre-PCR step		
55°C	120 s	
94°C	5 s	
1st Measurement	5 cycles	
95°C	5 s	
62°C	30 s	Data collection
2nd Measurement	45 cycles	
91°C	5 s	
62°C	62°C	Data collection

Data analysis

Results were obtained automatically by the cobas 6800 system using the criteria defined in UC analysis packages. In addition, all results were reanalyzed by using the Lab Developed Test Curve Viewing Tool or Utility Channel Optimization Tool.

Results

In a first experiment, I tested the behavior of the EliGene HLA-B27 assay on the cobas 6800 system. For this, three HLA-B27 positive and two negative samples were diluted 1:3, 1:5 and 1:7 in water or PBS (Figure 1). These dilutions were recommended by the manufacturer and there was no significant difference in the performance of the EliGene HLA-B27 assay whether using water or PBS. The three positive samples were clearly positive by the EliGene HLA-B27 assay regardless to the grade of dilution. The negative samples showed a non-specific amplification but due to the RFI min threshold of 2.5, none of the dilutions were called false positive. In addition, the human control gene SYPL2 could be detected in all samples and dilutions.

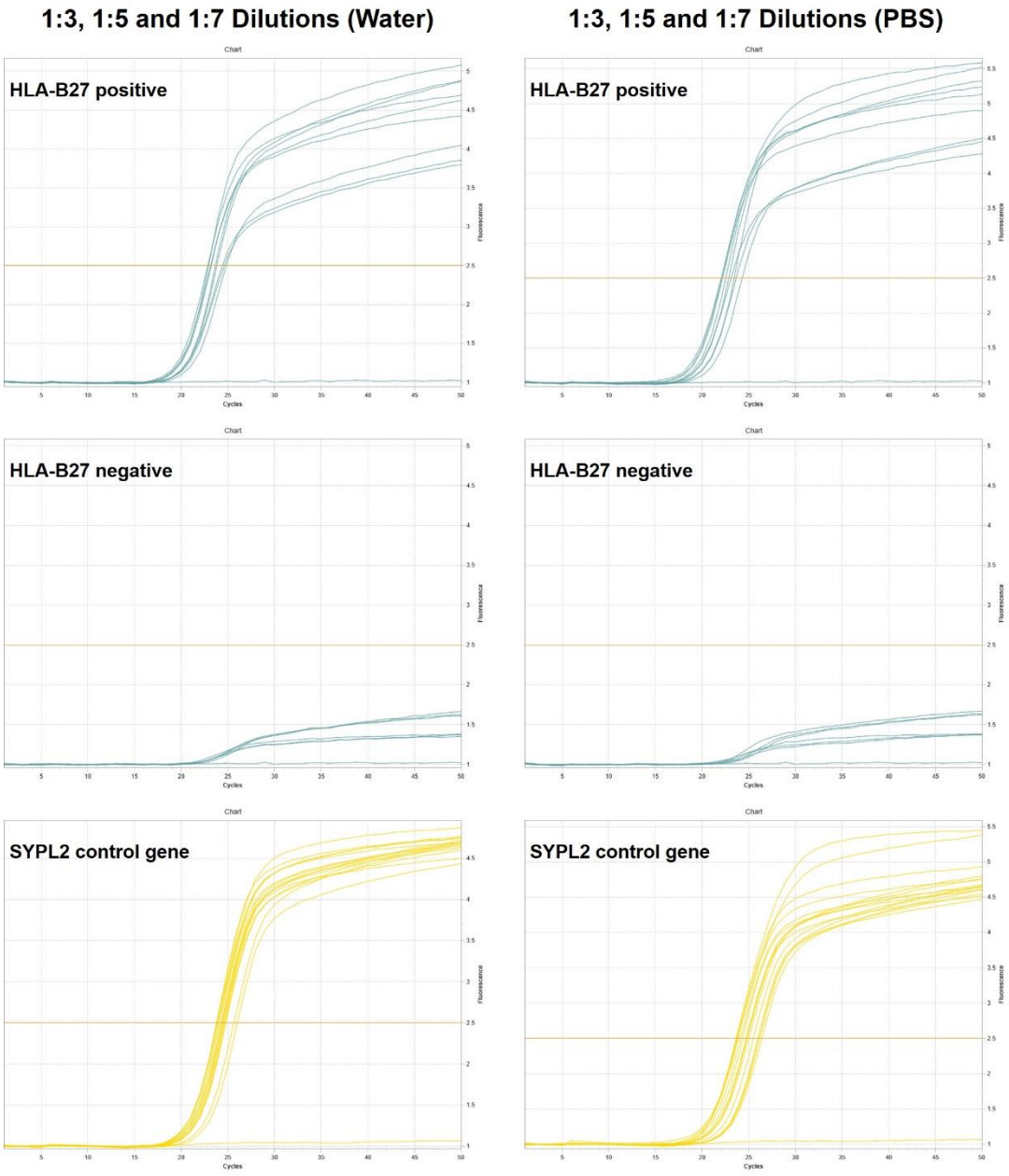

Figure 1: 1:3, 1:5 and 1:7 dilutions of three HLA-B27 positive and two negative samples in water or PBS. All samples were correctly analyzed by the EliGene HLA-B27 assay regardless to the grade of dilution or diluent.

Based on these results, I decided that the test is functional and started the evaluation with a larger number of samples. I usually use water to dilute blood samples because dilutions in PBS tend to form a sediment of blood components after 30 minutes. For this, all further experiments with the EliGene HLA-B27 assay were performed with 1:7 dilutions in water. For the evaluation, 100 HLA-B27 positive and 70 negative blood samples were blinded and retested with the EliGene HLA-B27 assay (Figure 2). I used this high amount of negative blood samples to check whether the amplification of non HLA-B27 alleles could be a problem. Again, all positive HLA-B27 samples were resolved without any problems and the internal control showed constant and reproducible results. This was in complete consistency with the results of the HLA-B27 LDT. The majority of negative samples showed non-specific signals by the EliGene HLA-B27 assay but most of them were far below the RFI min setting of 2.5. Therefore, all samples were correctly called HLA-B27 negative.

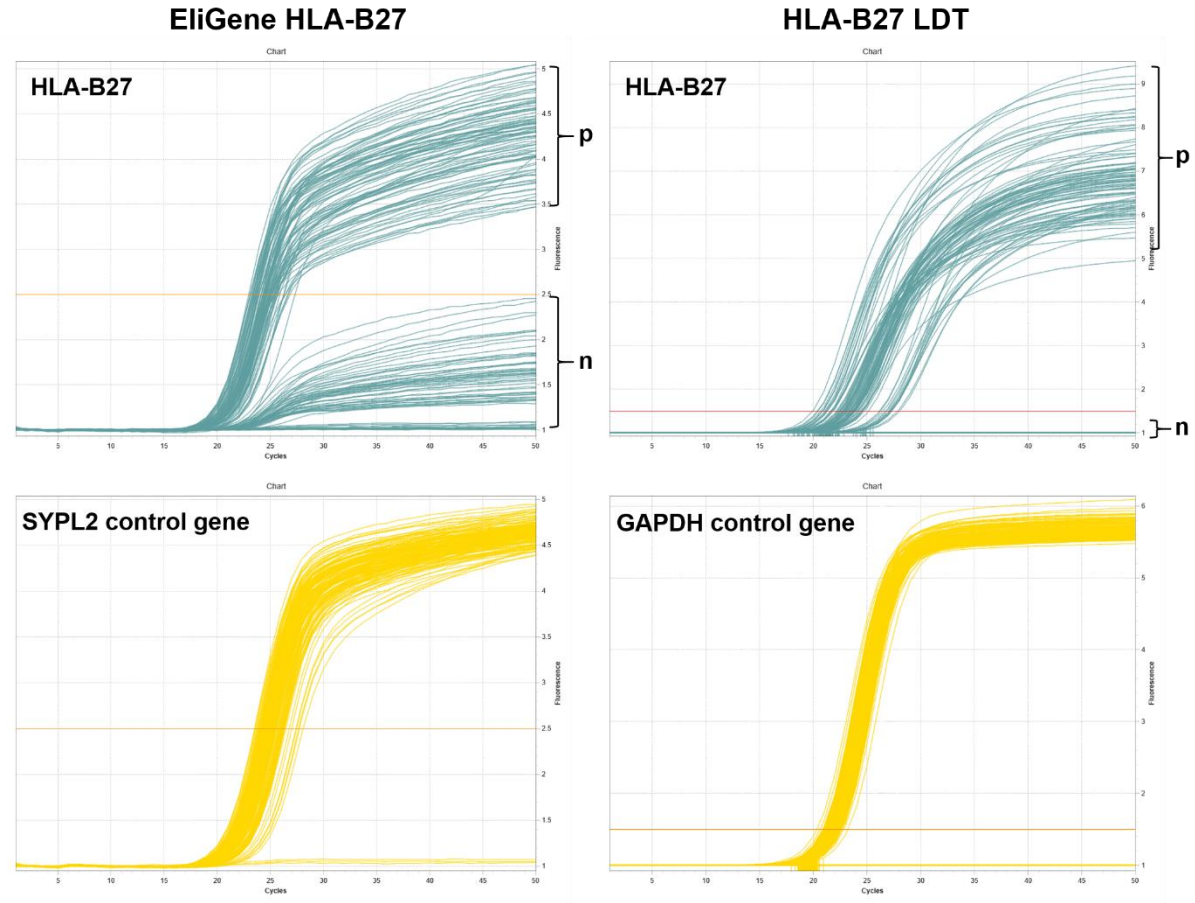

Figure 2: Comparison of the EliGene HLA-B27 assay with the HLA-B27 LDT. One hundred positive and seventy negative samples were correctly analyzed by the EliGene HLA-B27 assay. Most of the negative samples showed a tendency for non-specific amplification but only four samples with signals near the RFI min value of 2.5. Abbreviations are p = HLA-B27 positive and n = HLA-B27 negative.

Discussion

In this evaluation, I compared the performance of the EliGene HLA-B27 assay to a HLA-B27 LDT. The EliGene HLA-B27 assay was easy to use, showed constant results in different runs and was in complete agreement with the HLA-B27 LDT. Compared to the laboratory developed test the EliGene HLA-B27 assay showed the tendency for non-specific amplification in negative samples. I usually consider non-specific amplification as a serious problem, but the overall risk of false positive results is very low. Of the 70 HLA-B27 negative samples, only four showed signal intensities near the RFI min value of 2.5. As long as the results of the cobas 6800 system are rechecked by the cobas omni Utility Channel Optimization Tool software, no problems will occur. A minor drawback of the EliGene HLA-B27 assay is the PCR profile which differs from the generic profile. Since all of my cobas omni Utility Channel assays were validated with the generic profile, the EliGene HLA-B27 test is standalone and cannot be combined with other lab developed tests.

In conclusion, I found that the use of the EliGene HLA-B27 assay allows the rapid and reliable identification of HLA-B27 in a full automated way. Primers and probes are ready to use and due to the simplicity of the cobas 6800 system, any diagnostic laboratory can adapt the EliGene HLA-B27 assay in a short time.