AUTOMATION AND VALIDATION OF COMPLEMENT ELISA TESTS ON THE DS2 DYNEX INSTRUMENT

CLASSICAL PATHWAY / ALTERNATIVE PATHWAY / LECTINE PATHWAY

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ABSTRACT

This paper describes a validation of total complement activity ELISA tests on the DS2 ELISA analyzer. Euro Diagnostica has developed diagnostic tests for the functional assessment of all three pathways of the complement system. These functional assays are supplied in an ELISA format.

Since 2011, Euro Diagnostica is a partner of Dynex and adapts all ELISA tests to the Dynex automated systems DSX and DS2. Dr. Kristel Boonen of the General Clinical Laboratory of the Catharina Hospital in Eindhoven, The Netherlands, has performed a validation of 3 complement ELISA tests (classical, alternative and lectin pathway) from Euro Diagnostica on the DS2 system.

The validation shows that the ELISA tests on the DS2 meet the demands regarding reproducibility and agreement with other tests and linearity. The tests are now run at routine-base, after the clinicians were informed about the changed reference values.

INTRODUCTION

Mrs. Dr. Kristel Boonen is clinical chemist in training at the general clinical laboratory of the Catharina Hospital in Eindhoven. The Catharina hospital is a peripheral hospital with a strong regional function in the South East of The Netherlands. Around 100 persons are working in this lab; it is active (amongst others) in the field of Clinical Chemistry, Hematology and Immunology. For Immunology they perform a wide arrangement of test including ANA, ENA, anti-CCP and anti-tTG tests. Methods used for these tests are ImmunoCap (Phadia), Immunofluoresence and ELISA. For ELISAs they are using the DS2 system from Dynex.

Functional complement tests were formerly outsourced, but in 2011 the laboratory management decided to investigate the possibility to perform these tests at their own laboratory. The requirements were cost-efficiency and possibility of automation on the DS2. Furthermore, reproducibility and agreement with other tests and linearity had to be acceptable.

The Euro Diagnostica complement test system consists of functional assessments of all three pathways in a simple and accurate ELISA format. Assessment of three pathways (classical, alternative and lectin) can be carried out with separate ELISA kits or in a combined kit. Euro Diagnostica, as a partner of Dynex, has harmonized all ELISA tests to the DSX and DS2 platform. The Euro Diagnostica complement ELISA tests are gaining popularity in The Netherlands and more and more laboratories are starting to automate these tests. Reasons for this automation are: decreased hands-on-time, increased reliability and reproducibility, direct processing of information to the LIS system and cost savings. The internal cost analysis performed at the Catharina Hospital showed cost savings in performing the complement tests in house. It was therefore decided to perform a validation of the tests.
VALIDATION COMPARISON

The results of samples send to Sanquin Laboratory in Amsterdam were compared to the results of the ELISA tests performed on the DS2.

At Sanquin the activity of the alternative (AP50) and classical (CH50) pathway is measured with labeled rabbit- and sheep erythrocytes. The free label is measured and is linear to the complement activity. Mannose binding lectin (MBL) is measured quantitatively in an ELISA (mg/L).

In the Euro Diagnostica ELISA tests, the wells of the microtiter strips are coated with specific activators of the classical, lectin, or alternative pathway. Patient serum is diluted with diluents containing specific blockers to ensure that only the desired pathway is activated. During the incubation of the patient’s serum, complement is activated by the specific coating. Finally the neoepitope formed by C5b-9 is detected. The amount of this neoepitope generated is proportional to the functional activity of the complement pathway and the result is calculated as a ratio to the positive control.

In Table 1 the comparison is shown for 19 samples tested for the classical pathway (CP), 17 samples for the alternative pathway (AP) and 15 samples for the lectin pathway (MP). The classifications of the results (deficient versus non-deficient) are for the most part similar for all three tests. Furthermore, 6 test samples of the Dutch Foundation for Quality Assessment in Medical Laboratories (SKML) were tested and compared to the consensus results of SKML. Despite a negative bias in the high levels, the classifications of the samples were equal.

| Table 1: Classifications of the results (deficient vs normal) |
|----------------------------------|----------------|---------|
|                                  | Normal (old)   | Deficient (old) |
| AP                               |                |         |
| Normal (Wieslab)                 | 17             | 0       |
| Deficient (Wieslab)              | 0              | 0       |
| CP                               |                |         |
| Normal (Wieslab)                 | 17             | 0       |
| Deficient (Wieslab)              | 1              | 1       |
| MP                               |                |         |
| Normal (Wieslab)                 | 9              | 3       |
| Deficient (Wieslab)              | 0              | 3       |

REPRODUCIBILITY

Samples were measured as intra- or inter-run duplicates. The coefficient of variation (CV) is calculated from the complement activity of the inter-run results and from OD values of the intra-run results.

Table 2 shows the obtained results. The average CV values met the criteria. There were some relatively high CV values in the inter run assay; but these samples showed equal classifications (deficient/non-deficient).

<table>
<thead>
<tr>
<th>Table 2: Intra-run and inter-run CVs</th>
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<tbody>
<tr>
<td>Inter-run CV average and (max)</td>
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<tr>
<td>CP</td>
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<tr>
<td>AP</td>
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<td>MP</td>
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LINEARITY
To determine linearity of all three pathways a sample with a normal complement activity was diluted with inactivated serum. The results of these experiments are shown in Figure 1. All pathways partly show linearity until a certain point when probably some component of the pathways is exhausted.

CONCLUSION
The ELISA tests for all three complement pathways run on the DS2 analyzer showed acceptable results for reproducibility, agreement to other tests and linearity. In order to use these tests in everyday practice the clinicians were informed by the Clinical Chemists about the changed reference values. The tests are running now for several months in the laboratory to the satisfaction of the personnel, responsible clinical chemists, lab management and clinicians.

Figure 1: Linearity of the three Complement pathways:
AP = Alternative pathway
CP = Classical pathway
MBL = Lectin pathway
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EURO DIAGNOSTICA

Euro Diagnostica offers automated solutions for autoimmunity testing and diagnosis. Our diagnostic kits and reagents are harmonized on Dynex automated systems and also available on the majority of the instruments platforms available worldwide. Our tests are developed for clinical autoimmunology, microbiology and immunochemistry. Thanks to the long experience and continuous development of our personnel and production processes, Euro Diagnostica offers top quality cost-effective customized coating services.

Our Laboratory Testing Unit, Wieslab Laboratory Services, offers clinical testing and disease assessment within autoimmunity by qualified diagnostic specialists every day of the year. Wieslab Laboratory Services offers a comprehensive range of individual and panel tests, and ensures accurate and fast results. Testing is available for most autoimmune diseases.

LITERATURE

Euro Diagnostica. A unique diagnostic solution for functional assessment of the complement system. E-028-BG00
Euro Diagnostica. All-in-one Diagnostic Solution. E-025-G0-00