# ECAT Information:
# Emicizumab pilot study

In Autumn 2020 a pilot study was performed on the laboratory testing of emicizumab, a bispecific factor IXa- and factor X-directed antibody. This antibody was developed to bring together factor IXa and factor X without the need of factor VIII. It is therefore able to restore blood coagulation in patients with haemophilia A.

This pilot study consisted of two parts. Part I investigated the inter-laboratory variability of the quantitative measurement of emicizumab, while part II investigated the interference of emicizumab in a range of different coagulation tests. Here we report on the results of part I. In the next newsletter we will report on part II of this pilot study.

In total 97 laboratories participated in this pilot study. Sixty-three (65%) laboratories returned results for either the quantitative (part I) or the interference study (part II), or both.

The samples used in this pilot study was spiked with different levels of emicizumab. The samples were obtained from $r^2$ Diagnostics (South Bend, United States).

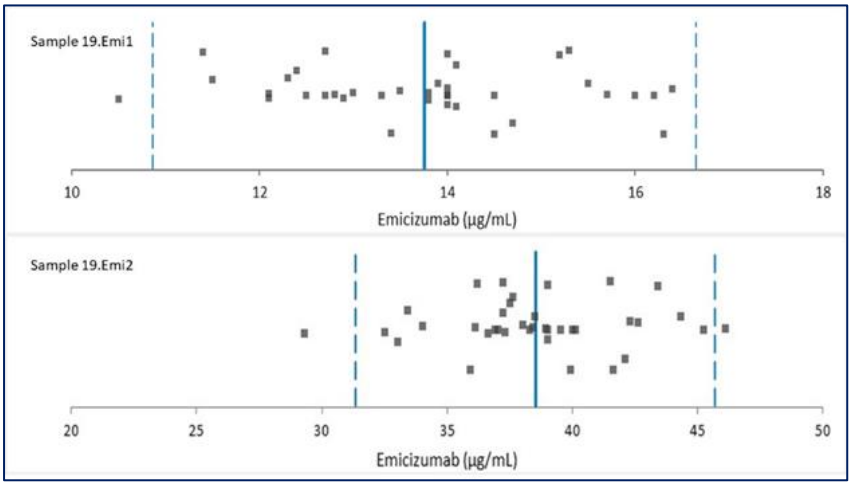
In part I the participants had the possibility of returning quantitative emicizumab measured by either a direct measurement (expressing results in emicizumab units) or an indirect measurement (expressing results in factor VIII equivalent units).

The table below shows the overall statistical evaluation of the results of the direct measurement

| Sample | Description | N | Mean (µg/mL) | SD (µg/mL) | Range (µg/mL) | Inter-laboratory CV (%) |
|---|---|---|---|---|---|---|
| Sample 19.Emi1 | Emicizumab Low | 37 | 13.8 | 1.45 | 10.5 – 16.4 | 10.5 |
| Sample 19.Emi2 | Emicizumab High | 37 | 38.5 | 3.58 | 29.3 – 46.1 | 9.3 |

Note: SD = standard deviation / CV = Coefficient of variation

The figure below shows the distribution of the results for both samples.



The majority of the participants used the calibrator of $r^2$ Diagnostics (76%). The other participants used calibrators from other sources or had prepared a home-made calibrator with emicizumab. The table below shows the result for both samples by calibrator used.

| Calibrator | N | Sample 19.Emi1<br>Mean ± SD (µg/mL) | Sample 19.Emi2<br>Mean ± SD (µg/mL) |
|---|---|---|---|
| Avant Medical | 1 | 12.1 | 32.5 |
| Cryopep | 1 | 13.8 | 38.4 |
| Homemade | 5 | 12.4 ± 1.42 | 36.8 ± 4.85 |
| ERL | 1 | 12.4 | 37.5 |
| Haemachrom | 1 | 15.3 | 41.5 |
| $r^2$ Diagnostics | 28 | 14.1 ± 1.34 | 39.0 ± 3.34 |

Note: SD = standard deviation

Because of the low numbers of participants in the different groups, besides the $r^2$ Diagnostics group, no firm conclusion can yet be drawn on the results of the different calibrators. The observed inter-laboratory variability is in the same range as recently published data (11 – 13%) [1].

A number of participants reported results for the indirect measurement of emicizumab. In total 8 participants reported results using a one-stage clotting assay and 12 participants using a chromogenic method.

The table below shows a summary of the results.

| Assay | N | Sample 19.Emi1<br>Mean ± SD (IU/mL) | Sample 19.Emi2<br>Mean ± SD (IU/mL) |
|---|---|---|---|
| One-stage clotting assay | 8 | 1.42 ± 0.32 | 3.95 ± 0.82 |
| Chromogenic assay * | 8 | 0.12 ± 0.03 | 0.26 ± 0.11 |

Note: SD = standard deviation

* four participants reported results below their lower limit of quantification.

The number of participants using a one-stage clotting assay is too low to warrant looking into potential differences between APTT reagents.

The participants who reported numerical results using a chromogenic method all used the Hyphen BioMed Biophen Factor VIII method. This method contains human factor X and IXa. The other chromogenic methods used (Coamatic FVIII, Coatest FVIII, Siemens Factor VIII) include bovine factor X and IX. These latter methods are not sensitive to the human-based bispecific factor IXa- or factor X-directed antibody.

For both the one-stage clotting and chromogenic assays the participants used plasma-based calibrators. The results are therefore expressed in factor VIII-equivalent units.

It is obvious that with the clotting-based assay much higher levels were measured than with the chromogenic method.

The ratio between sample 19.Emi1 and 19.Emi2 is similar for both the direct measurement and the one-stage clotting assay (2.79). For the chromogenic method this is slightly lower (2.17).

The results of this pilot study demonstrate that external quality assessment is feasible and a valuable addition to the ECAT's external quality assessment repertoire.

In 2021 ECAT will start a regular survey programme for the quantitative measurement of emicizumab (2 surveys / year). If you are interested in participating, please contact the ECAT office (info@ecat.nl).

In the next newsletter we will report the results of the interference part of the pilot study.

**References**

1. Lowe, A., S. Kitchen, I. Jennings, D.P. Kitchen, T.A.L. Woods and I.D. Walker, Effects of Emicizumab on APTT, FVIII assays and FVIII Inhibitor assays using different reagents: Results of a UK NEQAS proficiency testing exercise. Haemophilia, 2020; 26: 1087-1091.