Precision of the Methotrexate measurement

Blandine ROUGEMONT

Introduction

The toxicological analysis laboratory carries out blood tests, to measure drugs or medicines. But, besides its routine analyses, the laboratory must always check its measurement methods. In this context, I studied the precision of an anticancer medicine assay called methotrexate.
The methotrexate is measured with the EMIT (Enzyme Multiplied Immune assay Technique) test methotrexate assay, commercialized by Siemens. This test is an immune enzymatic assay, and it is made on patients' serum.
This work consisted in studying the repeatability and the reproducibility of the technique and to compare these experimental data to the data given on the kit's instructions.

Experimental Method :

The technique was adapted on an automatic machine, a spectrophotometer : the Pentra 400. The reading of the turbidimetric analysis is made in 340 nm. Every day, the method was calibrated with standards of calibrations. Next, standards of validation were used.
Two different concentrations levels were used :
Targets :

1 : 0.43µmol/L

2 : 1.31 µmol/L

This study was based on the EP5-A2 protocol so, 2 times a day each level was measured twice.

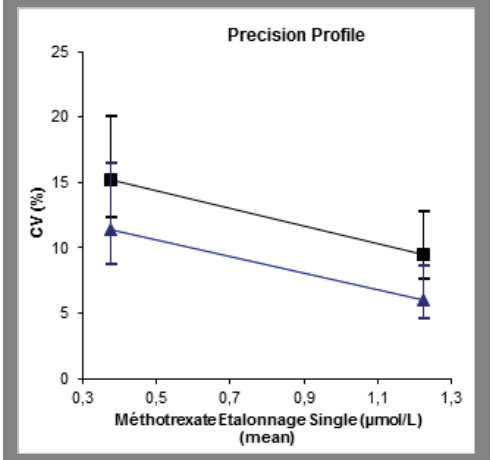
Results

Thanks to this approach, we were able to find the following precision :

		Repeatability	Reproducibility
Instructions uses	0.43µmol/L	CV <5%	CV < 10.7%
	1.31 µmol/L	CV <4%	Cv < 7.7%
experimental	0.43µmol/L	11.40%	15.20%
	1.31 µmol/L	6.00%	9.50%
	1.31 µmol/L	5.40%	9%

We can also draw the profile of precision which shows the total variability of the method (Fig 1).

Fig 1 : *Precision method of the Methotrexate measurement*



In black : the total variability of the method
In blue : the repeatability
We saw that reproducibility and repeatability increase when the level of methotrexate decreases.

Conclusion

The precision determined with the experimental method is worse than the precision specifies in the use instructions.
But, it is not going to raise problems for the laboratory. Indeed, these variations are not going to lead to modifications of the maintenance dosage, and that is what is important for the laboratory.

Université Claude Bernard Lyon 1