



Adaptability of recombinant cascade reagent (rCR) to automated systems



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Abstract & Introduction

Interest in sustainable BET reagents that are free of horseshoe crab components, has been increasing year by year. Various organizations have been considering and evaluating the equivalence of recombinant reagents and conventional LAL reagents. In May 2025, recombinant reagents were officially listed in the USP as a method to perform the bacterial endotoxin test. As a result of these developments, consideration has begun to switch from LAL reagents to recombinant reagents, resulting in a growing interest in the suitability of recombinant reagents for use in automated analyzers to reduce inter-human variability in quality control using recombinant reagents and operational errors in processing multiple samples. We evaluated the suitability of our rCR reagent, PYROSTAR Neo+, for use in our fully automated, compact endotoxin measurement system, KLANOS. As a result, the measurement results obtained using the automated analyzer were found to have reduced inter-human variability and operational errors in processing multiple samples, compared to manual pipetting. In addition, comparative verification using various pharmaceutical products showed results equivalent to those obtained using manual pipetting. These results show that, like LAL reagents, recombinant reagents can also be adapted to automated systems and are effective in reducing inter-human variability and operational errors in processing multiple samples.

Reagent and Equipment Used

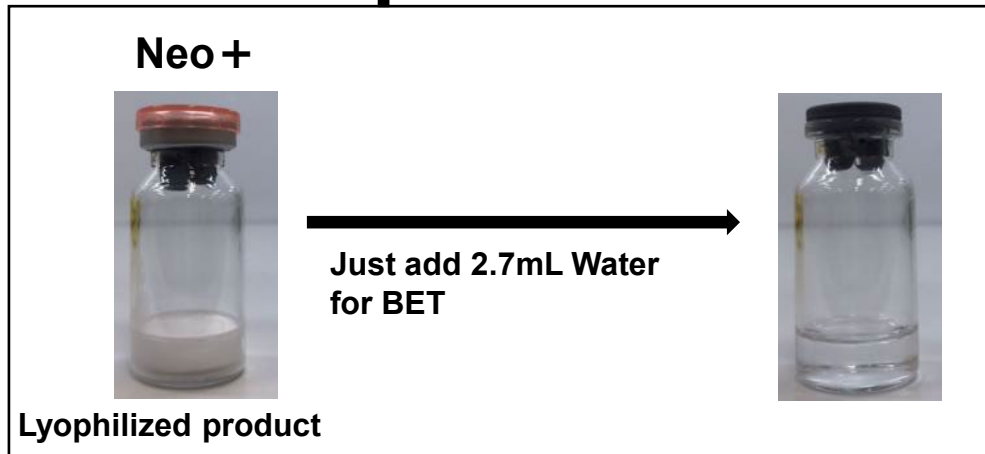
Reagent: PYROSTAR Neo+



Features:

- The same endotoxin reaction mechanism as conventional LAL
- Equivalent reactivity to conventional LAL
- Simple operation (just dissolve in test water)
- High sensitivity (high signal-to-noise ratio)

Preparation



Measurement



Equipment: KLANOS



Features:

- Automate a series of processes for endotoxin testing (Dilution and reagent addition possible)
- Support BET in accordance with Japanese/US/European pharmacopoeia
- Compact size, Desktop size automatic measuring device (W: 750 x D: 653 x H: 820 (mm))
- Simultaneous measurement 22 samples/test
- Up to 3 devices can be connected
- Supports all FUJIFILM Wako LAL reagents

Method

- Inter-operator variability

Three operators (A : beginner, B,C : expert) measured calibration curves using manual dispensing and KLANOS respectively (0.001~50 EU/mL, n=3).

- Repeatability

Endotoxin (EXT) solutions diluted to the lower (0.002 EU/mL), middle (0.1 EU/mL), and upper limits (10 EU/mL) of the standard curve range (0.001 EU/mL to 50 EU/mL), are used as samples and their ETX concentrations are measured (n=9). Three operators measured using manual dispensing and KLANOS respectively, and compared the data.

- Pharmaceutical products measurement

Six pharmaceutical products were measured using KLANOS three times and their spike recovery rates were calculated respectively.

- Measurement devices and conditions

<devices>

Manual : ELx808IU Microplate Reader
Automated : KLANOS

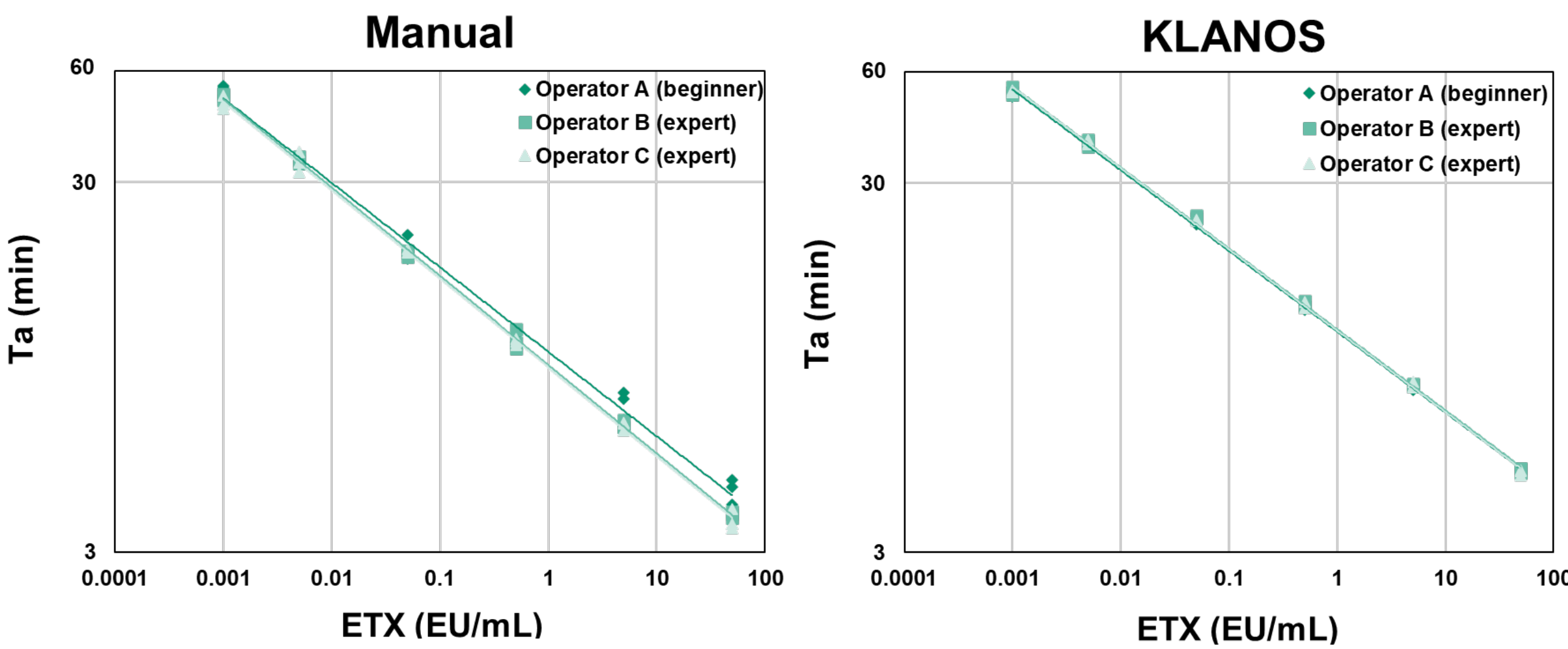
<conditions>

Measurement wavelength: main 405nm, reference 630nm
Measurement temperature: 37 °C
Measurement interval: 40s

Result

Inter-operator variability

A. calibration curve (Manual vs KLANOS)



Using KLANOS, well-matched calibration curves were obtained for all three individuals.

B. CV of BCC ※ and Accuracy of BCC for each concentration on the calibration curve (Manual vs KLANOS)

Operator A (beginner)				
ETX (EU/mL)	CV (%)		Accuracy (%)	
	Manual	KLANOS	Manual	KLANOS
0.001	4.4	6.1	75	112
0.005	13.7	5.5	109	96
0.05	25.4	3.0	122	91
0.5	8.1	7.6	145	98
5	33.7	4.6	87	99
50	30.0	4.4	91	106

Operator B (expert)				
ETX (EU/mL)	CV (%)		Accuracy (%)	
	Manual	KLANOS	Manual	KLANOS
0.001	6.9	9.6	94	115
0.005	8.2	8.5	98	100
0.05	6.1	3.3	111	83
0.5	19.5	8.1	108	99
5	8.5	3.3	95	98
50	6.8	5.7	98	110

Operator C (expert)				
ETX (EU/mL)	CV (%)		Accuracy (%)	
	Manual	KLANOS	Manual	KLANOS
0.001	13.6	3.1	106	114
0.005	20.9	4.8	99	97
0.05	4.9	0.9	99	88
0.5	6.9	8.3	104	99
5	9.9	6.7	92	95
50	20.2	6.1	109	111

BCC ※ : back calculated concentration

- The CV obtained from KLANOS yielded lower results. This was particularly evident in beginner results.

- The results in KLANOS showed that all three operators' Accuracy fell within a range of ± 20%.

Conclusion

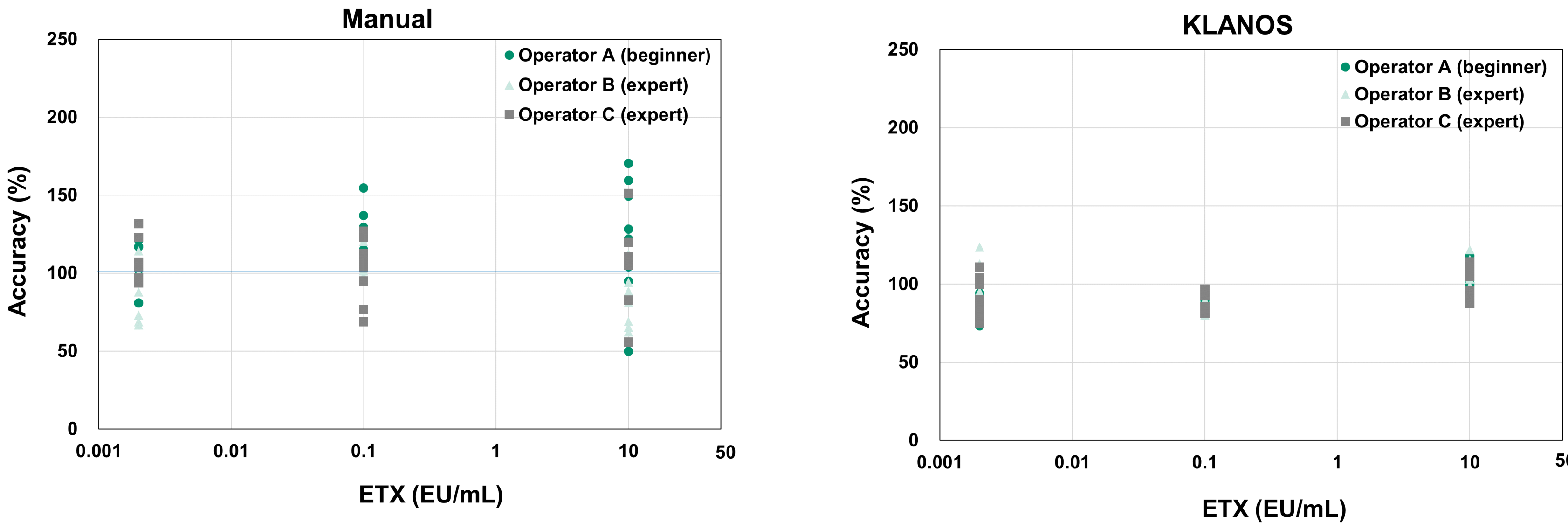
PYROSTAR Neo+ and KLANOS were effective in reducing inter-human variability and operational errors in processing multiple samples. These results demonstrate that recombinant cascade reagents can be adapted to automated systems.

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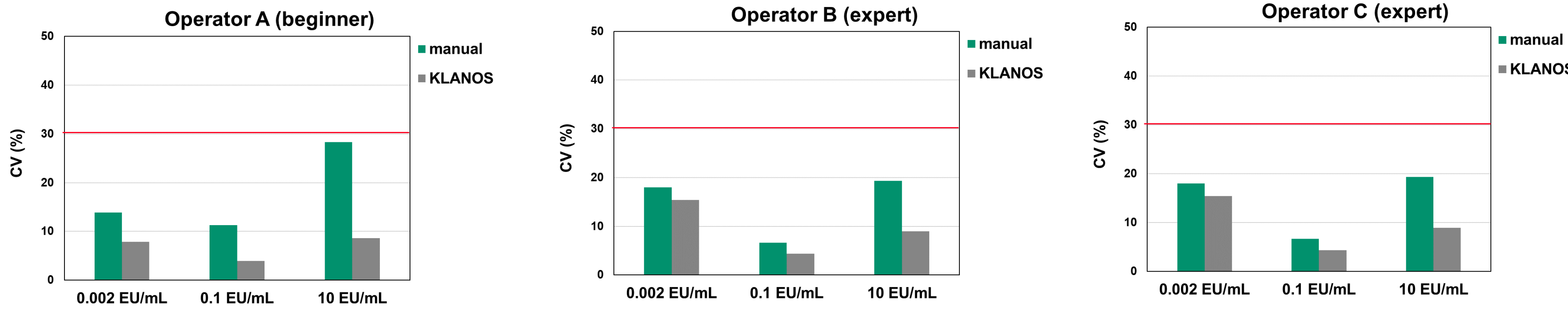
Repeatability

A. Accuracy of calculated ETX concentrations



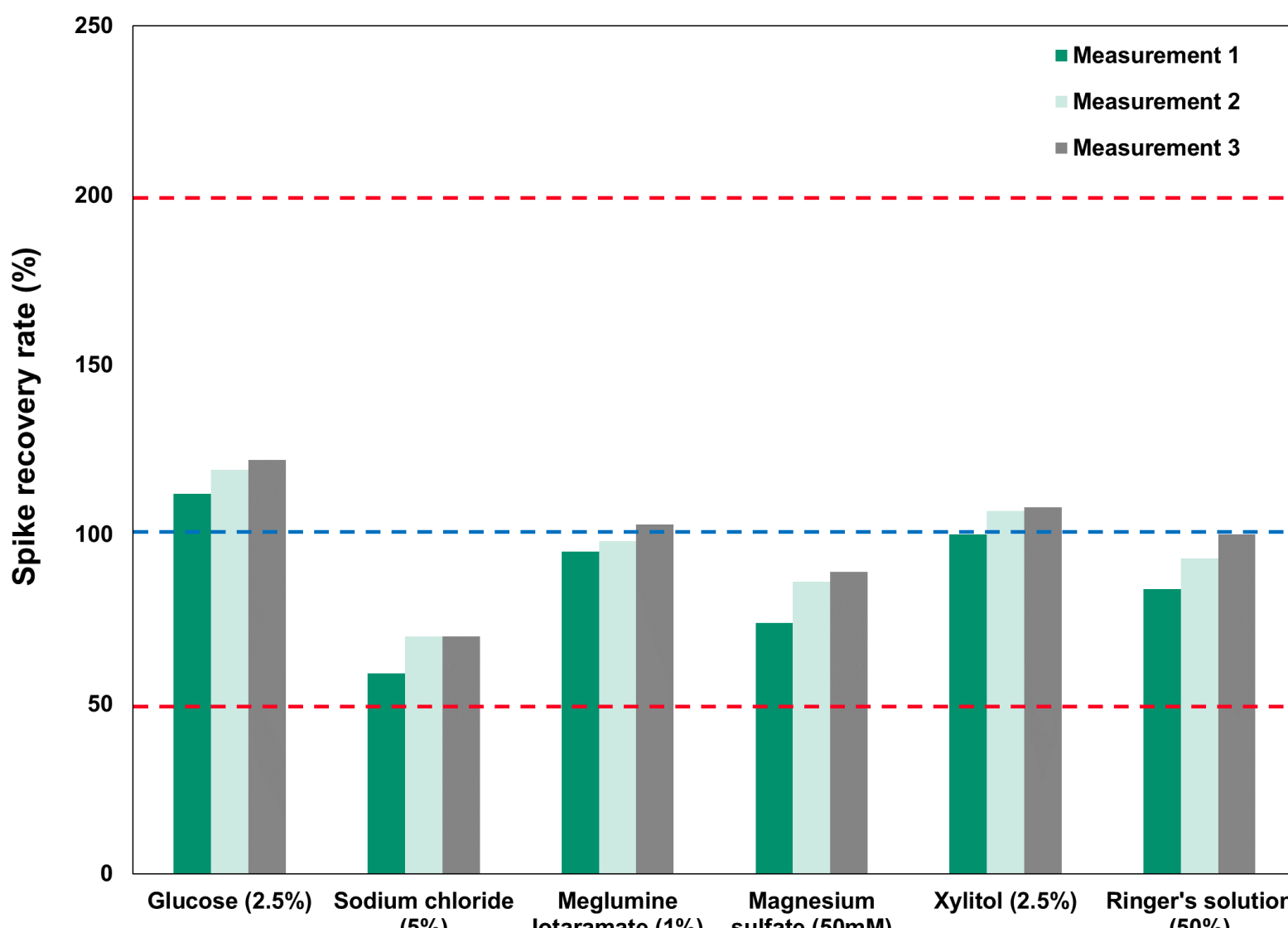
The results with KLANOS showed no significant variation among individuals, yielding nearly 100% results across both low and high concentrations.

B. CV of calculated ETX concentrations (Manual vs KLANOS)



The results measured with KLANOS showed a decrease in CV values across the range from low to high concentrations.

Pharmaceutical products measurement



The spike recovery rate of pharmaceutical products could be measured without issue using KLANOS.