

O. Allard¹, M. Barrieu², J. Claves¹, Y. Bouattour¹, P. Chennell²

¹CHU Clermont-Ferrand, Pharmacy- ² Clermont Auvergne University, CHU Clermont Ferrand, Clermont Auvergne INP, CNRS, ICCF, F-63000 Clermont-Ferrand, France

Introduction

Injectable preparations must be free of bacterial endotoxins.

While the **Limulus Amebocytes Lysate (LAL)** cartridge test remains the gold standard, recombinant alternatives (**Recombinant Factor C (rFC)** or **recombinant cascade reagent (rCR)**) offer new possibilities.

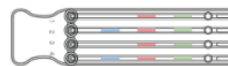
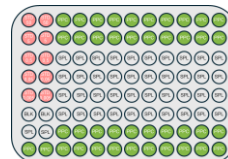
Objective



To compare these 3 assays by taking into account their **analytical** and **environmental** performance, as well as their **productivity** and **practical effectiveness**

Materials and methods

Techniques	Type	Conditions	Animals
rFC ^a	Plate	Optimal or unfavorable*	Bovine Serum Albumin
rCR ^b	Plate (rCRp)	Optimal or unfavorable*	Ø
	Cartridge (rCRc)	Not applicable	
LAL ^b	Cartridge	Not applicable	Horseshoe crab blood



*Optimal = Full plate (21 samples) and Unfavorable = one sample per plate

^abioMérieux, ^bCharles River

Comparison using the RGB method¹



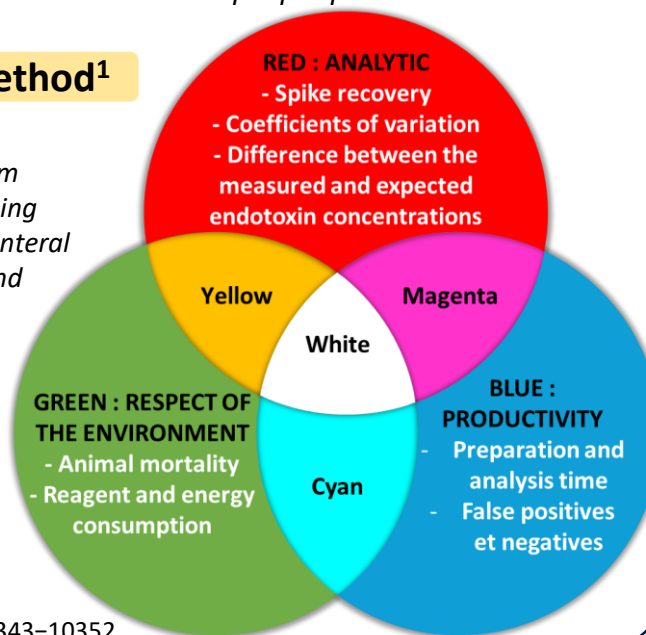
Results obtained from experimental data (using lipidic or non-lipidic parenteral nutrition samples) and literature sources

Allows the evaluation of analytical method based on 3 main criteria :

Analytics, Environment, Productivity

Overall performance =
Color + Brightness (MB)

(1) Nowak et Kościelniak, Anal. Chem. 2019, 91, 10343–10352

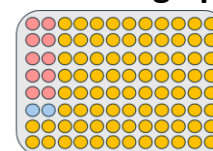


Results

All three techniques meet the established analytical criteria

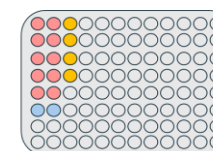
Techniques	rFC		rCRp		rCRc	LAL
	Optimal	Unfavorable	Optimal	Unfavorable		
Color						
MB (%)	65	51	78	64	89	61

Plate filling optimization :

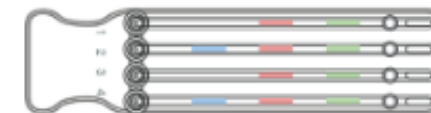


Optimal = 21 samples /21
3 minutes/ sample

>



Unfavorable = 1 sample /21
60 minutes/ sample



Cartridge-based techniques
reduce preparation time
(5 minutes/ sample)

Conclusion

rCRc technique is the most balanced based on the evaluated performance

- **Necessity to reduce reliance on animal-based resources**
- **Importance of optimizing plate filling**
- **Accounting for waste generated and the cost of analysis per sample**