MAT IMPLEMENTATION IN THE ANTISERA PRODUCED IN BRAZIL

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FUNDAÇÃO BUTANTAN
Gestão é uma ciência

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SÃO PAULO
GOVERNO DO ESTADO
SÃO PAULO SÃO TODOS

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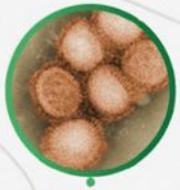
TIMELINE

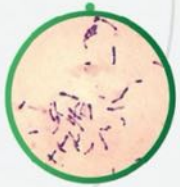
1901 — SERUM — Bubonic plague

1918 — VACCINE — Influenza

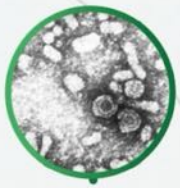
1948 — VACCINE — Influenza



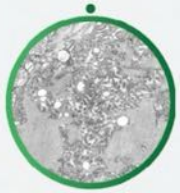
1986 — VACCINE — Diphtheria, tetanus, and pertussis



1993 — VACCINE — Hepatitis B

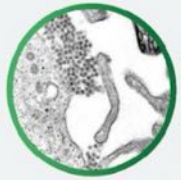


2002 — VACCINE — Rabies



2020 — VACCINE — Covid-19

2024 — VACCINE — Dengue



INSTITUTO BUTANTAN
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PRODUCTION OF SERA AND VACCINES

We are the **largest producer** of sera and vaccines in **Latin America** and a worldwide reference of **quality and efficiency**

INSTITUTO BUTANTAN
A serviço da vida

PRODUCTION OF **SERA AND VACCINES**

São Joaquim Farm

560K
Vials of serum
per year

Against **venomous animals**, **bacterial toxins**, and the **rabies virus**

In 2019, the **process of equine plasma collection** was automated through **plasmapheresis**, thus improving the quality of the sera's raw material

Antivenom

antibothropic (pentavalent)
antibothropic (pentavalent) and antilaquetic
anticrotalic
antibothropic (pentavalent) and anticrotalic
antielapid (bivalent)
antiscorpion
antiarachnid (Loxosceles, Phoneutria and Tityus)
antilonomic

Antitoxin

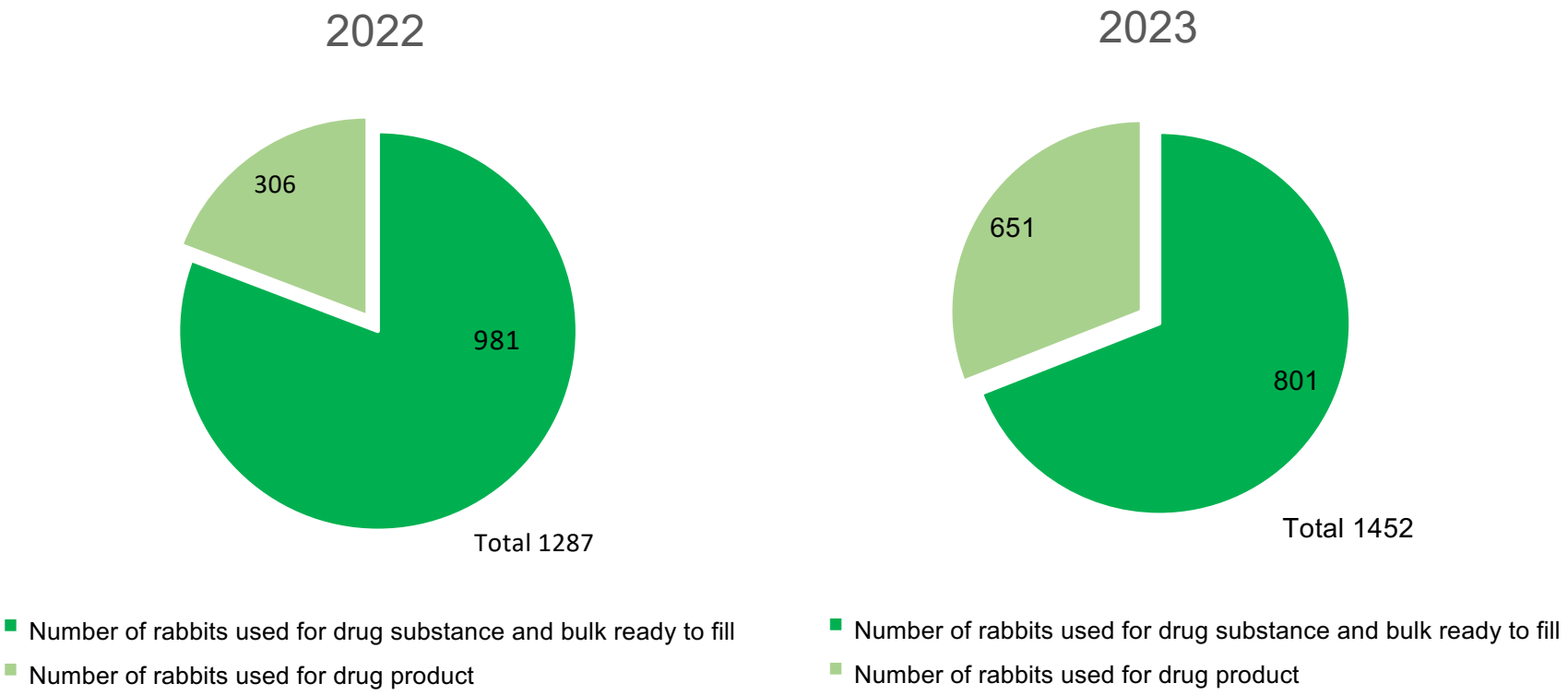
antidiphtheria
antitetanus
antibotulinum AB (bivalent)
antibotulinum E

Antiviral

antirabies

Pyrogen test

Total number of rabbits used for the pyrogen test in serum.



Pyrogen test

National Council for the Control of Animal Experimentation (CONCEA) - Brazil

Resolution no. 45, published on October 22, 2019

- Art. 2 For the purposes of this Normative Resolution, the National Council for the Control of Animal Experimentation **recognizes the alternative method the Monocyte Activation Test for evaluating pyrogenic contamination in injectable products**.
- One single paragraph. With the recognition of the alternative method described in Art. 2 of this Normative Resolution, **a period of up to 5 (five) years is established as a limit for mandatory replacement of the original method by the alternative method**.

Deadline
10/22/2024

Pyrogen test

Brazilian Pharmacopoeia

The Brazilian Pharmacopoeia is being **updated to include MAT in chapter 5.5.2.1 *TESTS FOR EVALUATION OF PYROGENS***. This revision is currently open for public comments, and the **final version** is expected to be published in **December 2024**.

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CONSULTA PÚBLICA Nº 1.270, DE 2 DE AGOSTO DE 2024

Pyrogen test

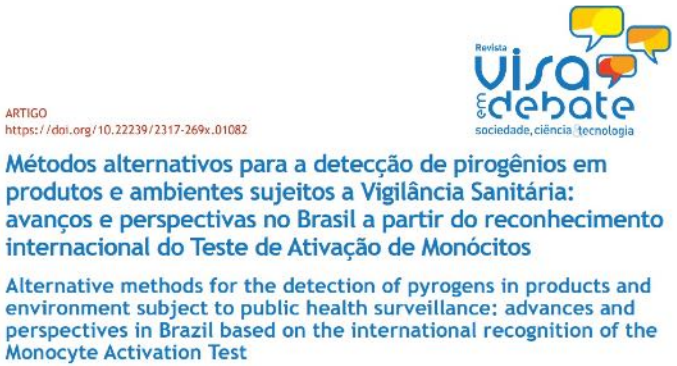
We have been looking for alternative methods to replace testing on rabbits for many years.

The beginning

Commercial kit with whole blood (PyroDetect). ✗

2017

National research group to explore other methods.



2022

Commercial kit with PBMC. ✓

MAT validation

Validation of the analytical methodology

- Selectivity;
- Limit of Detection (LOD);
- Endotoxin standard curve (linearity),
- Accuracy.

Suitability of the method for sera

- Product interference in the ELISA assay;
- Product interference in endotoxin (LPS) and non-endotoxin (HKSA) detection,
- Intermediate precision.

Chapter 2.6.30 of the European Pharmacopoeia and RDC 166/2017 of the ANVISA (Brazilian Regulatory Agency).

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Validation of the analytical methodology – Selectivity

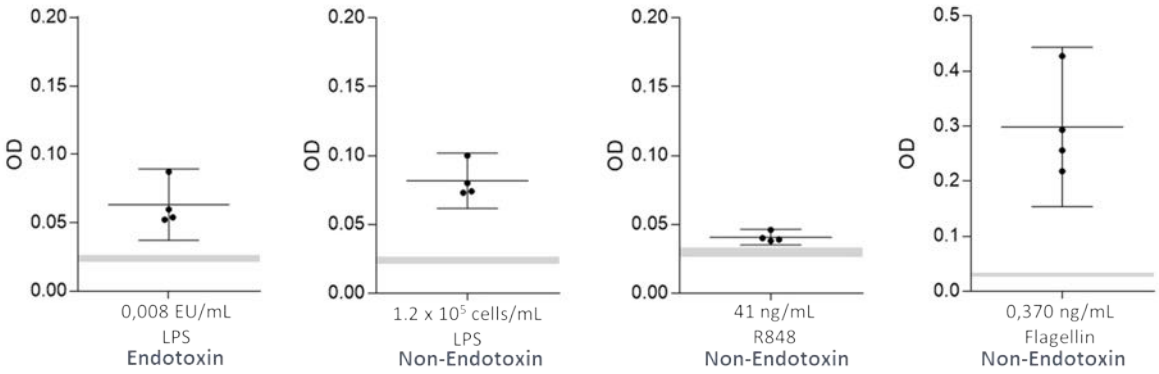
Different concentrations of **endotoxin** (LPS) and **non-endotoxin** (HKSA, R848, and Flagellin) were tested for their ability to **induce PBMCs to produce IL-6 above their basal levels** (blank).

Acceptance criteria

- At least **one of the concentrations** of each pyrogen evaluated must be capable of **inducing PBMCs to produce IL-6 above the basal levels** (blank).

Results

- O.D. values above blank were obtained from 0.008 EU/mL of endotoxin (LPS); 1.2 x10^5 cells/mL of HKSA; 41 ng/mL of R848 and 0.370 ng/mL of flagellin. ✓



Validation of the analytical methodology – LOD

The LOD was performed as described in chapter **2.6.30 of the European Pharmacopoeia**.

$$cut - off = \bar{x} + 3s$$

- $\bar{x}$: average of the O.D. responses obtained for 4 blank replicates.
- 3s: standard deviation of responses obtained from 4 replicates of the blank.

Acceptance criterion

- The LOD must be **≤ 0.016 EU/mL**.

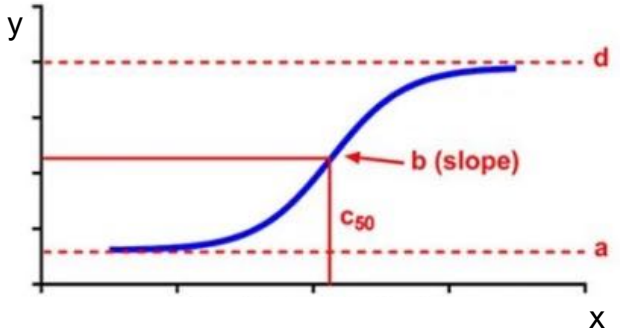
Result

- The detection limit in **all tests** performed was **≤ 0.016 EU/mL**. ✔

Validation of the analytical methodology – Endotoxin standard curve (LPS)

The **4PL regression** is fit for purpose.

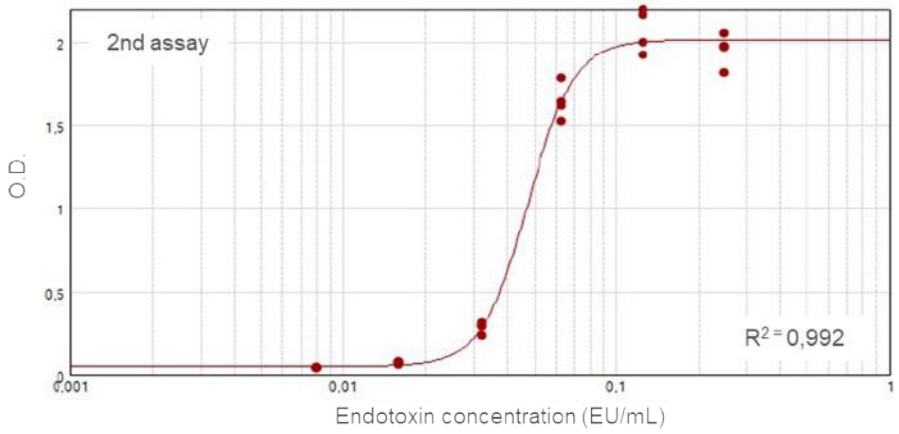
$$y = \overline{D} + \frac{(A - D)}{\left[1 + \left(\frac{x}{c}\right)^{B}\right]}$$



Acceptance criteria

- Significance of parameters B, C, and D ($p < 0.05$).
- The coefficient of determination (R^2) of each of the independent assay must be ≥ 0.975.
- Test values of homoscedasticity must be $p > 0.05$;
- The CV% between the replicates of the same concentration must be ≤ 30%.

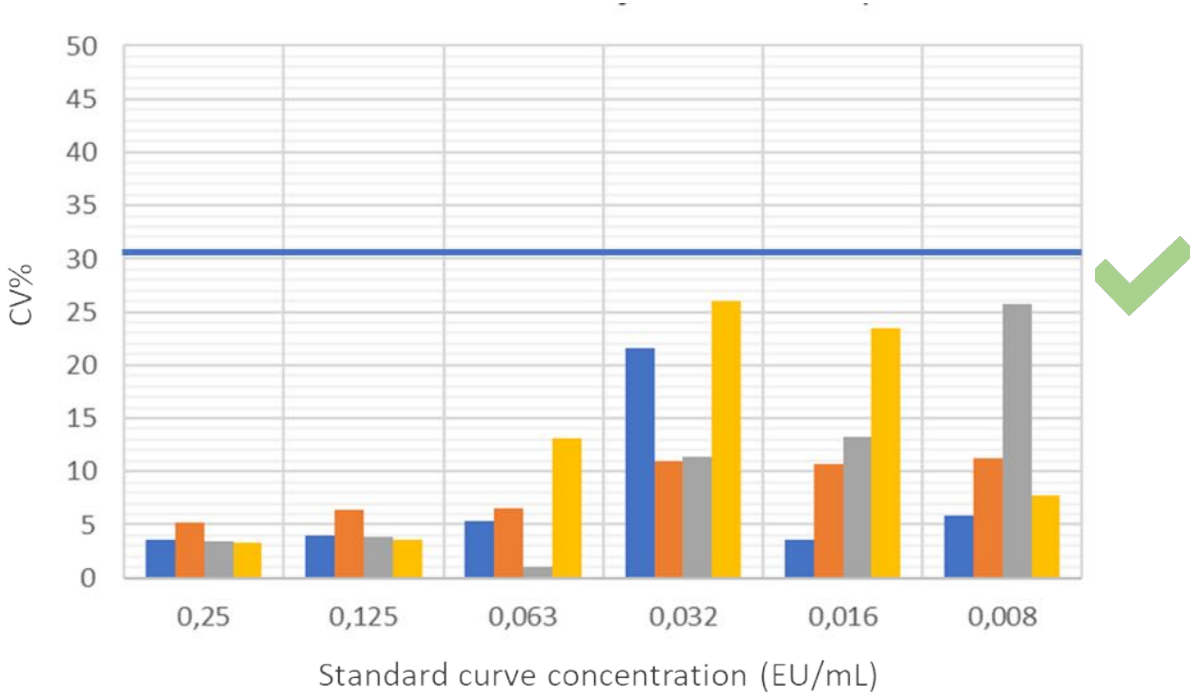
Validation of the analytical methodology – Endotoxin standard curve (LPS)



Result

- Significance of parameters B, C, and D: $p < 0.05$ (t Student test).
- $R^2 \geq 0.975$.
- Lack-of-fit test performed using ANOVA and F test:
  - p-value ≥ 0.005
  - R^2 > 95% (F test)
- Homoscedasticity (Breusch-Pagan test) - $p > 0.05$.

Validation of the analytical methodology – Endotoxin standard curve (LPS)



Analysis of the coefficient of variation (CV%)

- 1rst assay
- 2nd assay
- 3rd assay
- 4th assay

Validation of the analytical methodology - Accuracy

Standard endotoxin results (**LPS**) prepared in **three different concentrations**:

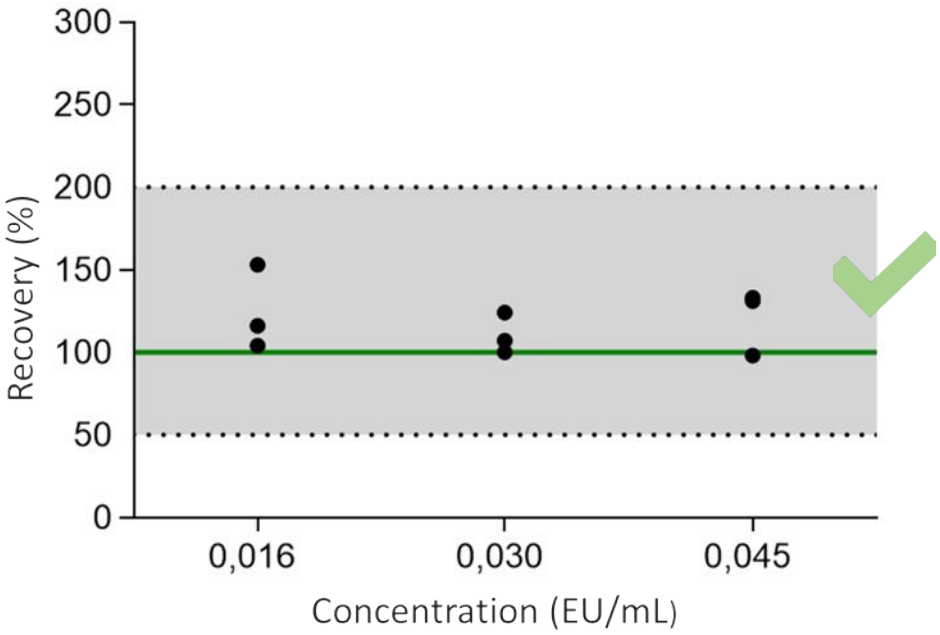
- Low - 0.016 EU/mL
- Medium - 0.030 EU /mL
- High - 0.045 EU/mL

$$\text{Recovery (\%)} = \frac{LPS\ experimental\ concentration}{LPS\ theoretical\ concentration}\ x\ 100$$

Acceptance criterion

- **Recovery** must be within the range of **50 to 200%**.

Result



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Antivenom

antibothropic (pentavalent)

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antilonomic

Antitoxin

antidiphtheria

antitetanus

antibotulinum AB (bivalent)

antibotulinum E

Antiviral

antirabies

Product interference in the ELISA assay

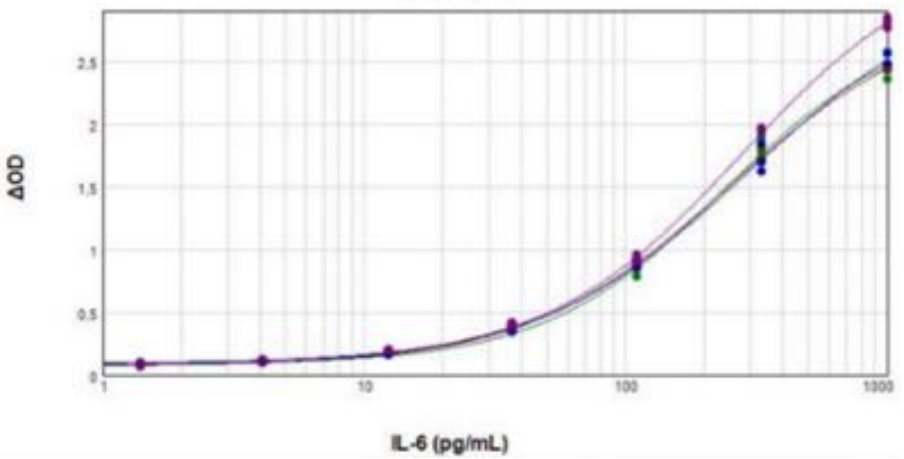
An **IL-6 curve** was constructed with the **ELISA buffer** and **with each type of serum** diluted 1:20.

Acceptance criterion

- The **coefficient of variation** between the IL-6 curve prepared in the buffer and the serum must be **≤ 20%**.

Result

- The **coefficient of variation** between the O.D. of the IL-6 curve prepared in the buffer and each serum **was ≤ 20%.** ✔



Product interference in endotoxin (LPS)

Each serum was tested at three dilutions (1:20, 1:40, and 1:80), in quadruplicate for each dilution, **with and without the addition of 0.03 EU/mL of LPS**.

LPS recovery:

$$Recovery\ (\%) = \frac{(Experimental\ product\ concentration + LPS) - Experimental\ product\ concentration}{LPS\ experimental\ concentration}\ x\ 100$$

Acceptance criterion

- **LPS recovery**, in at least one of the dilutions evaluated (1:20, 1:40, and/or 1:80), must be **50 to 200%**.

Result

- The **three sera had the LPS recovery between 50% and 200%**, in three independent tests. ✓

Product interference in non-endotoxin (HKSA)

The **three types of serum were tested** at the lowest dilution established for the product (1:20), in technical quadruplicate, **with and without the addition of 6x105 cells/mL of HKSA**.

HKSA recovery:

$$Recovery\ (\%) = \frac{Experimental\ product\ concentration + HKSA}{HKSA\ experimental\ concentration}\ x\ 100$$

Acceptance criterion

- **HKSA recovery** must be in the range of **50 to 200%.**

Result

- The **three sera had HKSA recovery between 50% and 200%**, in three independent tests.

Intermediate precision

Two operators performed the intermediate precision assessment by applying the proposed method to the release routine.

Result

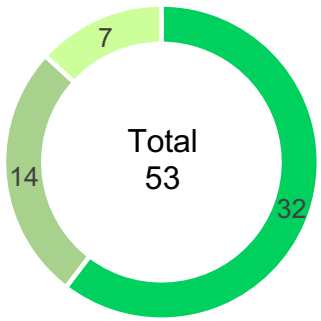
Serum	Assay	Operator 1			Operator 2		
		Dilution	Reportable result (EEU/mL)	Specification ≤ 1.75 EEU/mL	Dilution	Reportable result (EEU/mL)	Specification ≤ 1.75 EEU/mL
antibothropic	1	1:20	< 0.16	Approved	1:20	< 0.16	Approved
	2	1:20	< 0.16	Approved	1:20	< 0.16	Approved
	3	1:20	< 0.16	Approved	1:20	< 0.16	Approved
antitetanus	1	1:20	< 0.16	Approved	1:20	< 0.32	Approved
	2	1:20	< 0.16	Approved	1:20	< 0.16	Approved
	3	1:80	< 0.64	Approved	1:20	< 0.16	Approved
antirabies	1	1:20	< 0.32	Approved	1:20	< 0.16	Approved
	2	1:20	< 0.16	Approved	1:20	< 0.16	Approved
	3	1:40	< 0.32	Approved	1:20	0.40	Approved

Conclusion

The **MAT can be considered validated** and **suitable** for implementation in the Quality Control routine **for pyrogen analysis in sera** (drug product).

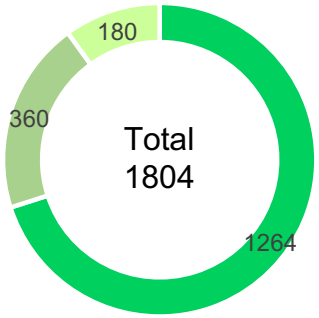
Monocyte Activation Test (MAT)

Number of tests



- Analytical development
- Validation of the analytical methodology
- Suitability of the method

Analyst hours



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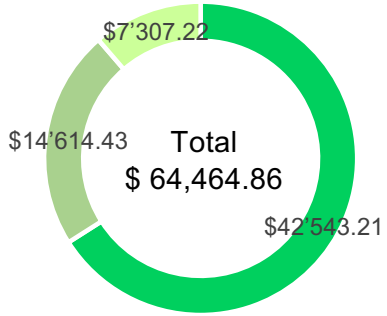
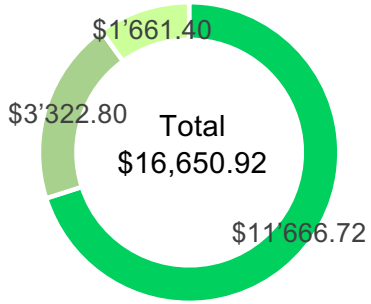
- Validation of the analytical methodology
- Suitability of the method

Investment (consumables)



- Analytical development
- Validation of the analytical methodology
- Suitability of the method

Labour costs



- Analytical development
- Validation of the analytical methodology
- Suitability of the method

Challenges

Implementing a new method is always challenging.

- **Definition of some test parameters**, like the regression model used for the LPS standard curve.
- During the development, we found that the **drug substance interfered with MAT**, so we decided to test **only bulk and drug product on this method**. For **drug substance**, we will use the validated **LAL method**.
- **Define a specification for the product** considering that we have products with different dosages.

Research and Analytical Development Team

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THANK YOU!