Pyrogen detection on medical devices

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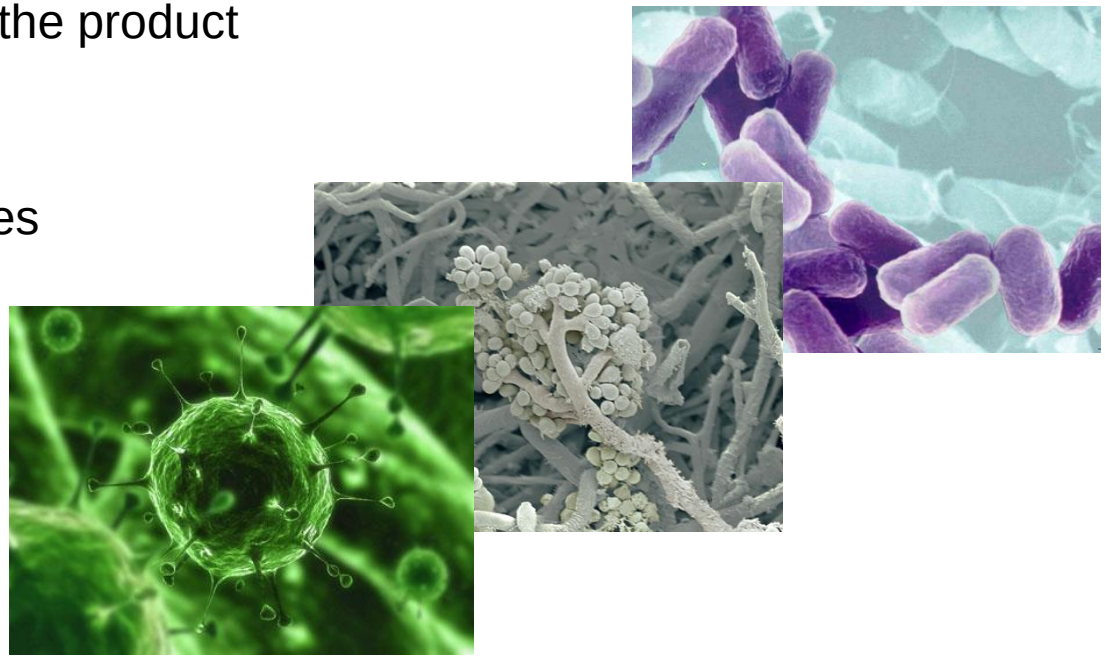
Pyrogens and their detection

Pyrogens – definition and possible origins

- Fever inducing substances
- Product / process contaminants
    - Possible origins: bacteria, yeast, viruses
- Inherent characteristics of the product
    - Vaccines / Adjuvants
    - Synthetic Lipopeptides
    - Surfaces
- Endogenous pyrogens



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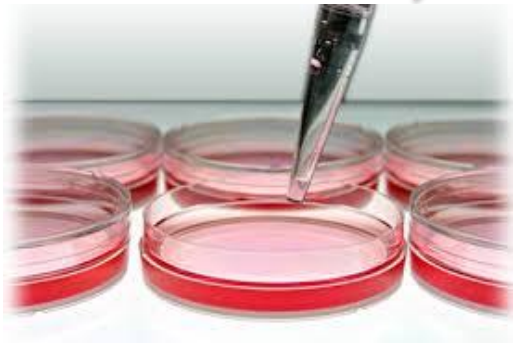
Pyrogen detection methods

In vivo rabbit test – 1995 - 2010

Ex vivo Endotoxin test – since 1999

MAT – since 2008



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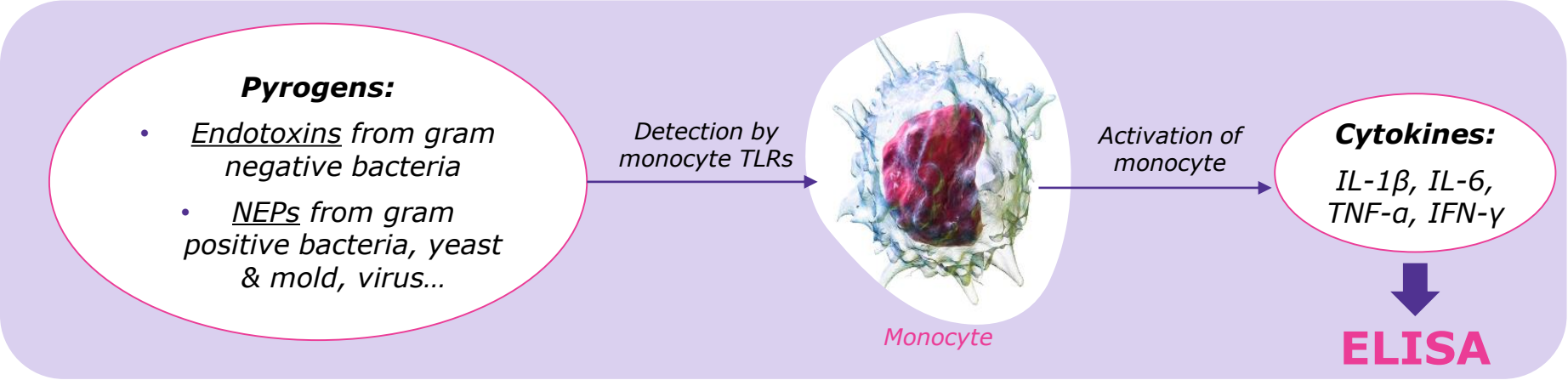
Detection of pyrogens on medical devices

Method	Advantage	Disadvantage
Rabbit pyrogen test after extraction of the device	All pyrogens detectable Mixtures of pyrogens detectable	Extraction dependent on solvent used (polar / non-polar) Extraction conditions may influence test result
Bacterial endotoxin test after extraction of the device	Sensitivity limited to endotoxin	Solvents for efficient extraction of endotoxin are well defined
Monocyte activation test after extraction	All pyrogens detectable Mixtures of pyrogens detectable	Extraction dependent on solvent used (polar / non-polar) Extraction conditions may influence test result
Monocyte activation test in direct contact	All pyrogens detectable Mixtures of pyrogens detectable	Test conditions reflect in-use situation

Monocyte activation test



- Test based on the human reaction to pyrogens
- Pyrogens are recognized by monocytic cells, which produce cytokines
- The resulting reaction is measured in ELISA
- **Able to detect all substances pyrogenic to humans**

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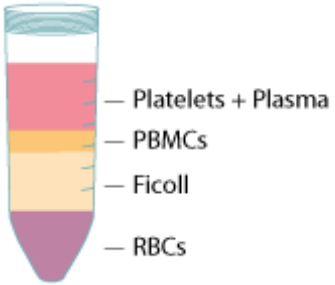
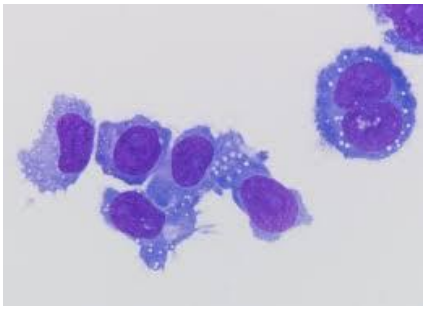
Our experience with different methods





Blood based	PBMC based	Cell line based
Pooled human blood	Cellular fraction of human blood	Origin of the Cell line: human
Range 0.2 to 1.0 EU/mL	Range 0.05 to 5.0 EU/mL	Range 0.05 to 5.0 EU/mL
Reaction not standardized	**Reaction not standardized**	**Reaction standardized**
Limited quantity of raw material	Limited quantity of raw material	Unlimited quantity of cells

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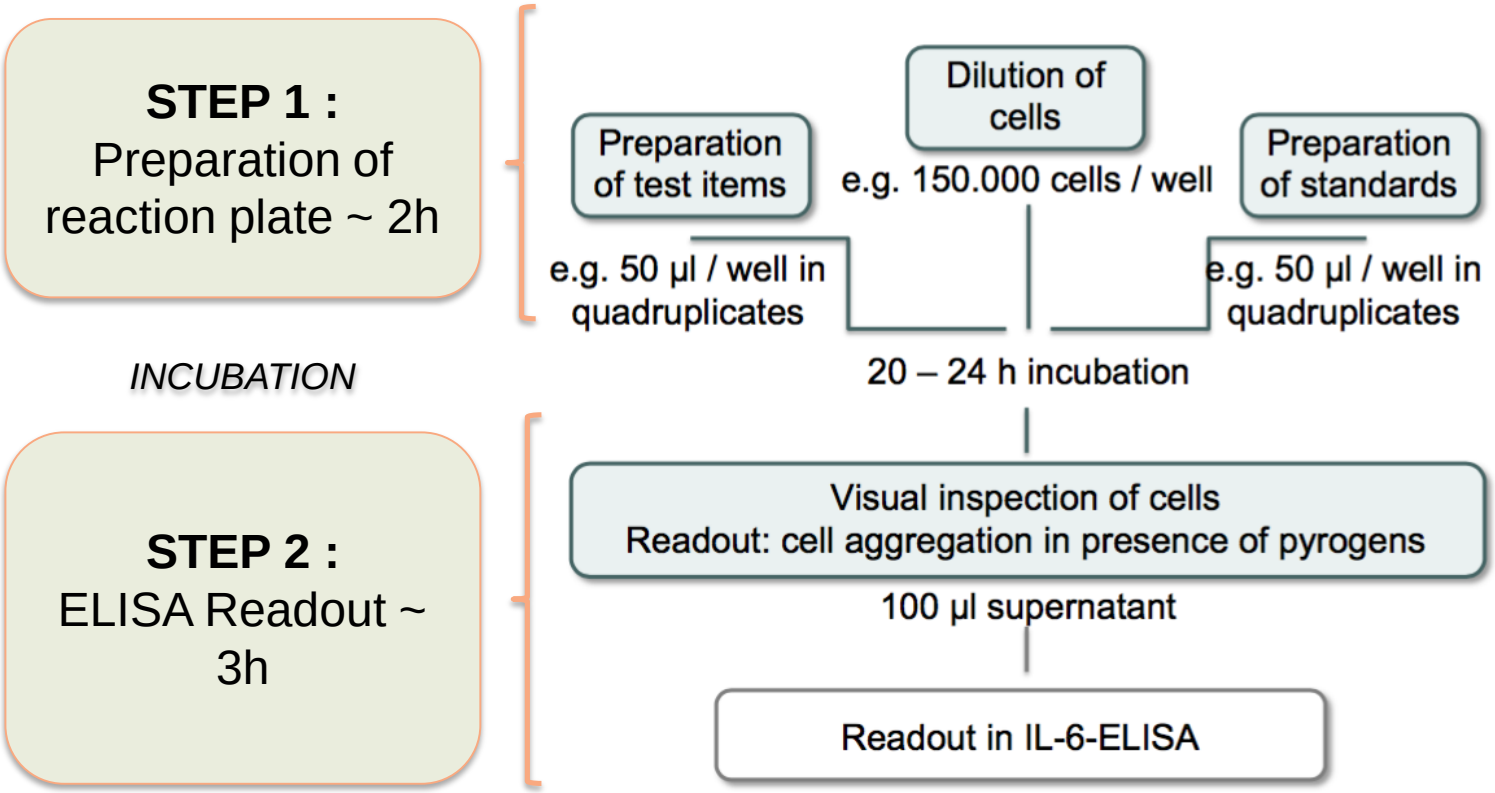
Assay layout



Hands on time : ~5 hours
Total time to result : 1,5 days

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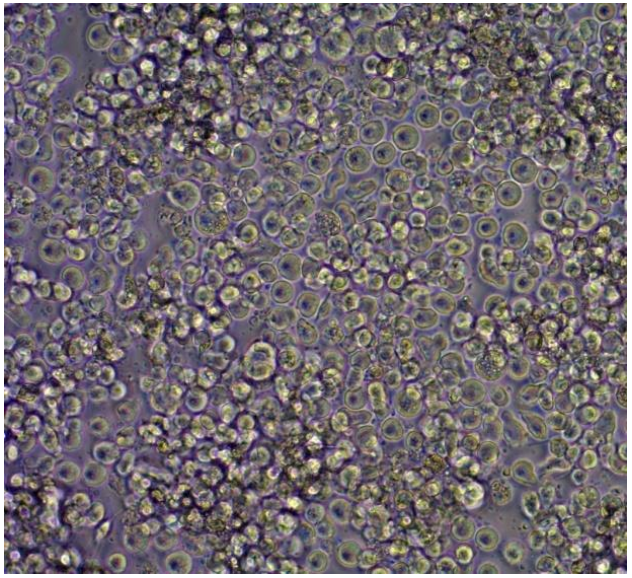
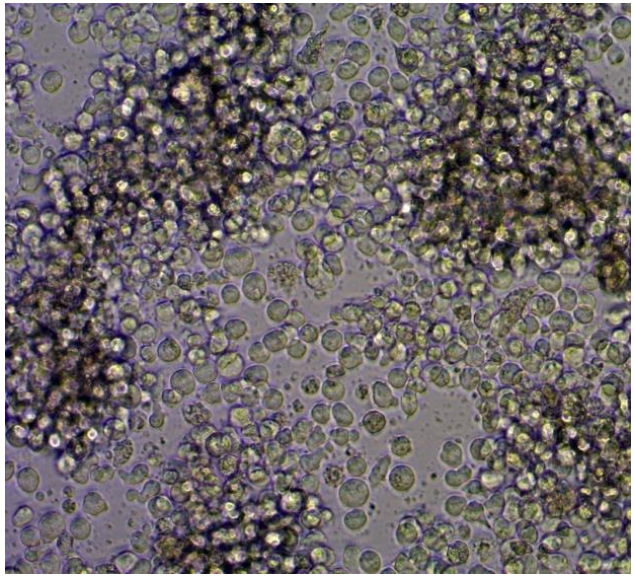
Reaction of Mono Mac 6 to pyrogens

no Pyrogen



High level of Pyrogen



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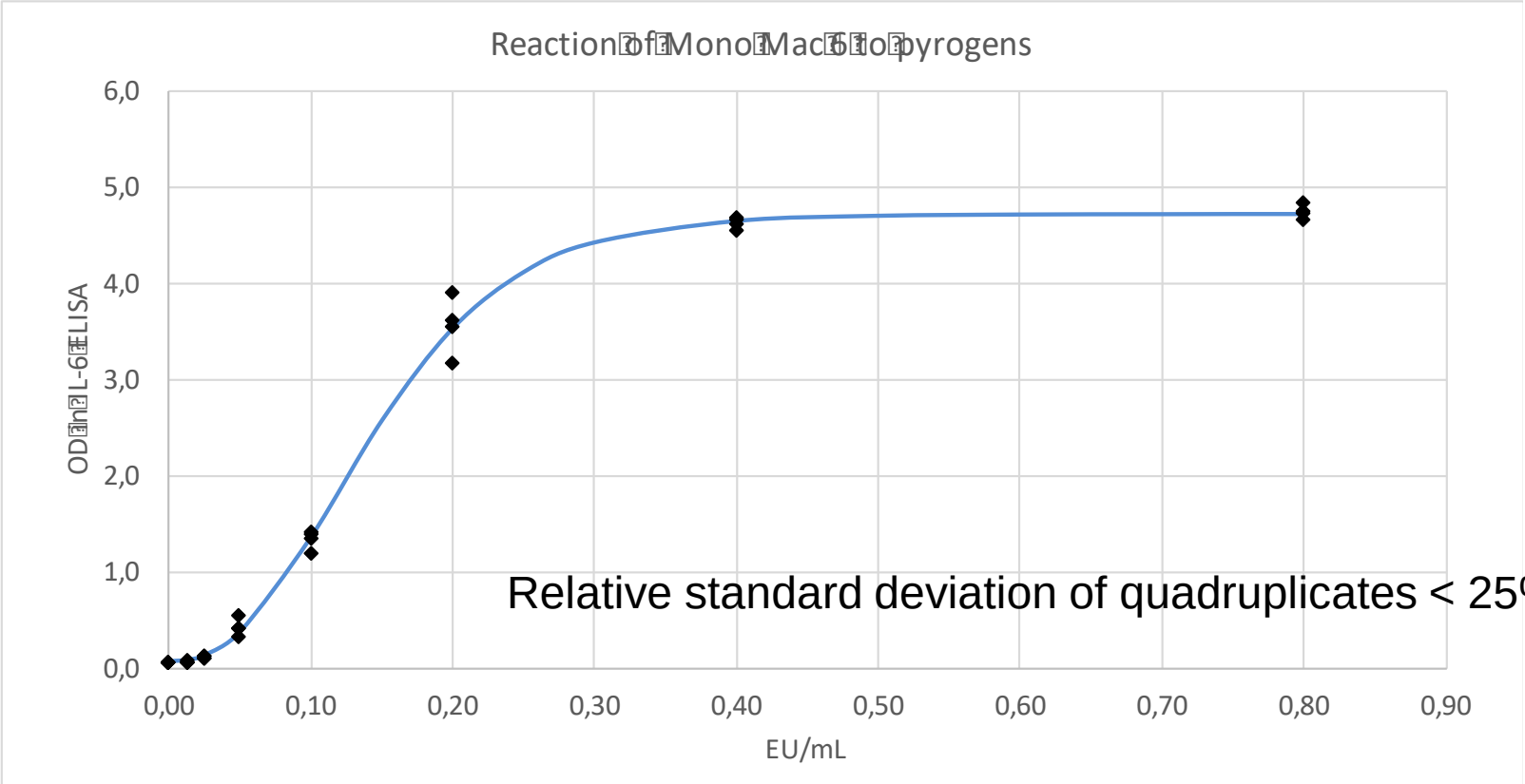
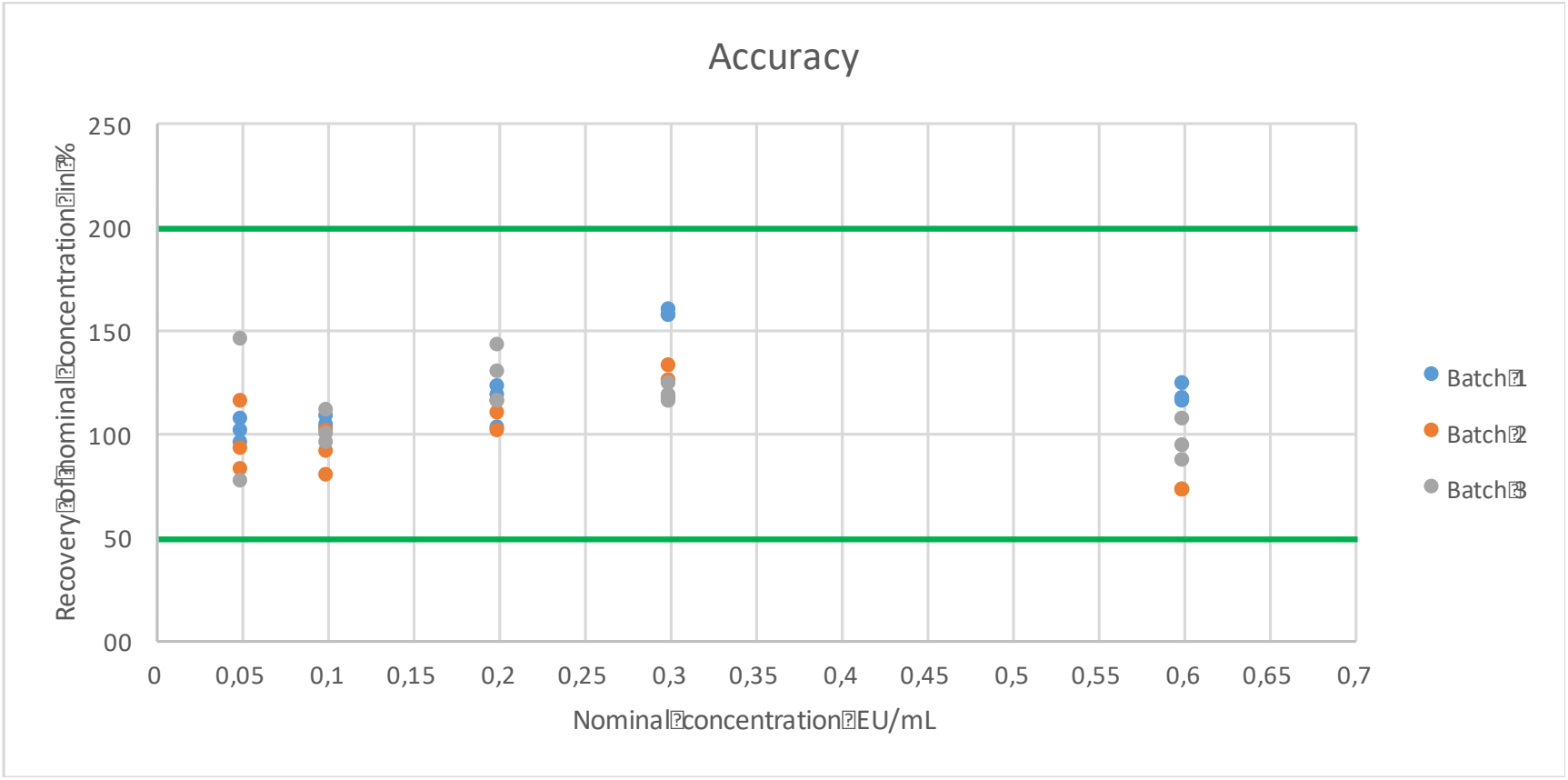
Reaction of Mono Mac 6 to pyrogens



Stable reaction of cells to endotoxin



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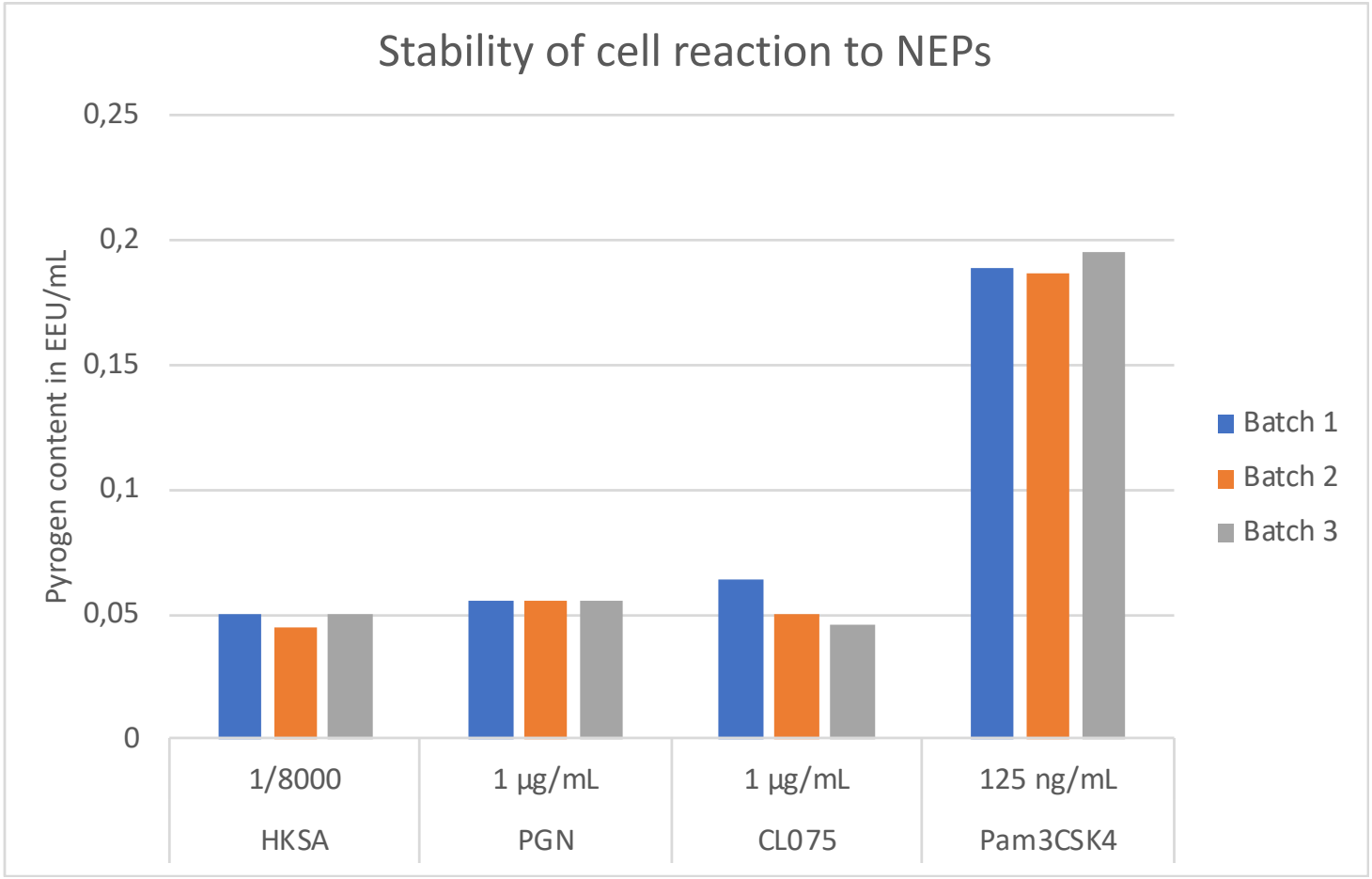
Stable reaction of cells to various pyrogens



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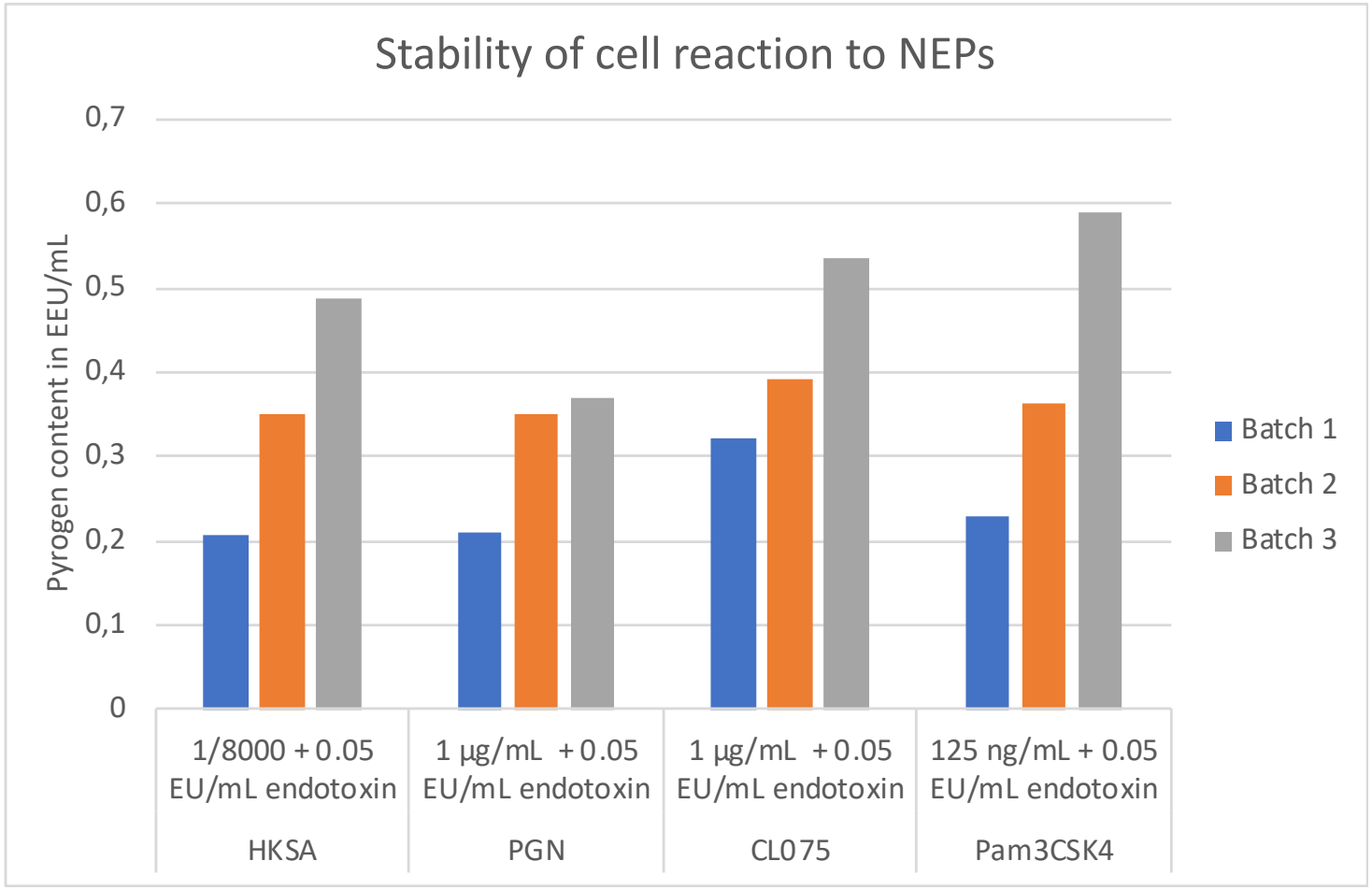
Stable reaction of cells to combinations of pyrogens



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Example 1 – Testing of syringes

Test item and assay description

- Injection needles for insulin pens
  - Composite devices with metal needle glued into plastic support
  - Contaminant limit concentration at 20 EEU/device

- Test 1:
  - Spiking of the test item with 0.32 EU/mL standard endotoxin directly onto the needle, with 1h at 37°C for drying (simulation of a real contamination, spike level chosen to be in the standard curve range)
  - MAT assay run according to the 12-well protocol with incubation with Mono Mac 6 cells for 22h and IL-6 ELISA
- Test 2:
  - Spiking of the test item with 20 EU/mL standard endotoxin directly onto the needle, with 1h at 37°C for drying
  - Extraction of the complete material in 10 mL of water, followed by dilution 1/20 (dilution chosen to bring spike level into the standard curve range)
  - MAT assay run according to the 96-well protocol with incubation with Mono Mac 6 cells for 22h and IL-6 ELISA
  - Each sample run with contamination after extraction to evaluate interference of the extract with the assay

Test results

Method	Test item	Qualitative Result	Quantitative Result	Recovery of conta-mination
Test 1 - direct contact	Injection needle	Contamination detected; positive control at 100%	0.124 EEU/needle	39%
Test 2 - extraction	Injection needle	Contamination detected; positive control at 84%	24 EEU/needle	120%

- The artificial contamination was dectected on the devices
- Quantitative recovery is better with extraction protocol

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Example 2 – Testing of hyaluronic acid syringes

Test item and assay description

- Hyaluronic acid preparations of 3 qualities
  - Low viscosity gels
  - High viscosity gels
  - Highly reticulated gels
  - Contaminant limit concentration for all qualities : 0.5 EEU/mL

- Test 1:
  - Spiking of the test item with 1 EU/mL standard endotoxin directly into the syringe (simulation of a real contamination)
  - Distribution of the gels to 24-well plates
  - MAT assay run according to the 24-well protocol with incubation with Mono Mac 6 cells for 22h and IL-6 ELISA
- Test 2:
  - Spiking of the test item with 5 EU/mL standard endotoxin directly into the syringe (simulation of a real contamination)
  - Extraction of the complete material in 10 mL of water at 37°C
  - MAT assay run according to the 96-well protocol with incubation with Mono Mac 6 cells for 22h and IL-6 ELISA
  - Each sample run with contamination after extraction to evaluate interference of the extract with the assay

Example 2 results of test 1

Method	Test item	Qualitative Result	Quantitative Result	Recovery of conta-mination
Test 1 - direct contact	Low viscosity gel	Contamination detected positive control at 71.5%	0.75 EEU/syringe	75%
	High viscosity gel	Contamination detected; positive control at 103%	0.25 EEU/syringe	25%
	Reticulated gel	Contamination detected; positive control at 81.5%	0.26 EEU/syringe	26%

- The artificial contamination was detected in all gels
- Quantitative recovery is better with low viscosity gels

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Example 2 results of test 2

Method	Test item	Qualitative Result	Quantitative Result	Recovery of conta-mination
Test 2 - extraction	Low viscosity gel	Contamination detected; positive control at 85%	2.2 EEU/syringe	44%
	High viscosity gel	Contamination detected Positive control at 81%	1.3 EEU/syringe	26%
	Reticulated gel	**Contamination not detected Positive control at 83%**	N/A	N/A

- The contamination of the reticulated gel cannot be detected after extraction, altough the extract does not interfere with the assay

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