

# Evaluation of a Novel Biosensor Test System for the Specific Detection of Biomolecules

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## INTRODUCTION

- NT-proBNP biomarker to detect heart failure (<125 pg/mL healthy individual under 75)
- Prevalence ~2%, rising with age with a 50% mortality if untreated
- If treated life expectancy can be doubled
- ELISA/ECLIA detection gold standard but is time consuming (>60 min)
- According to Elionova the ElioDX method delivers a result in 10 min by monitoring real time binding

## Aims

- Develop NT-proBNP assay on ElioDX platform
- Achieve clinically relevant LOD (<125 pg/mL target)
- Validate robustness in 0-100% serum matrices
- Demonstrate inter-device reproducibility

## METHOD

### Technology:

- 635 nm laser with total internal reflection generating an evanescent field which is 200 nm depth only. Therefore only fluorophores close to surface are excited
- 3 signal cases possible:
  - Outside field → No signal (black)
  - Unbound in field → background noise (orange)
  - Bound to surface → Clear signal (green)

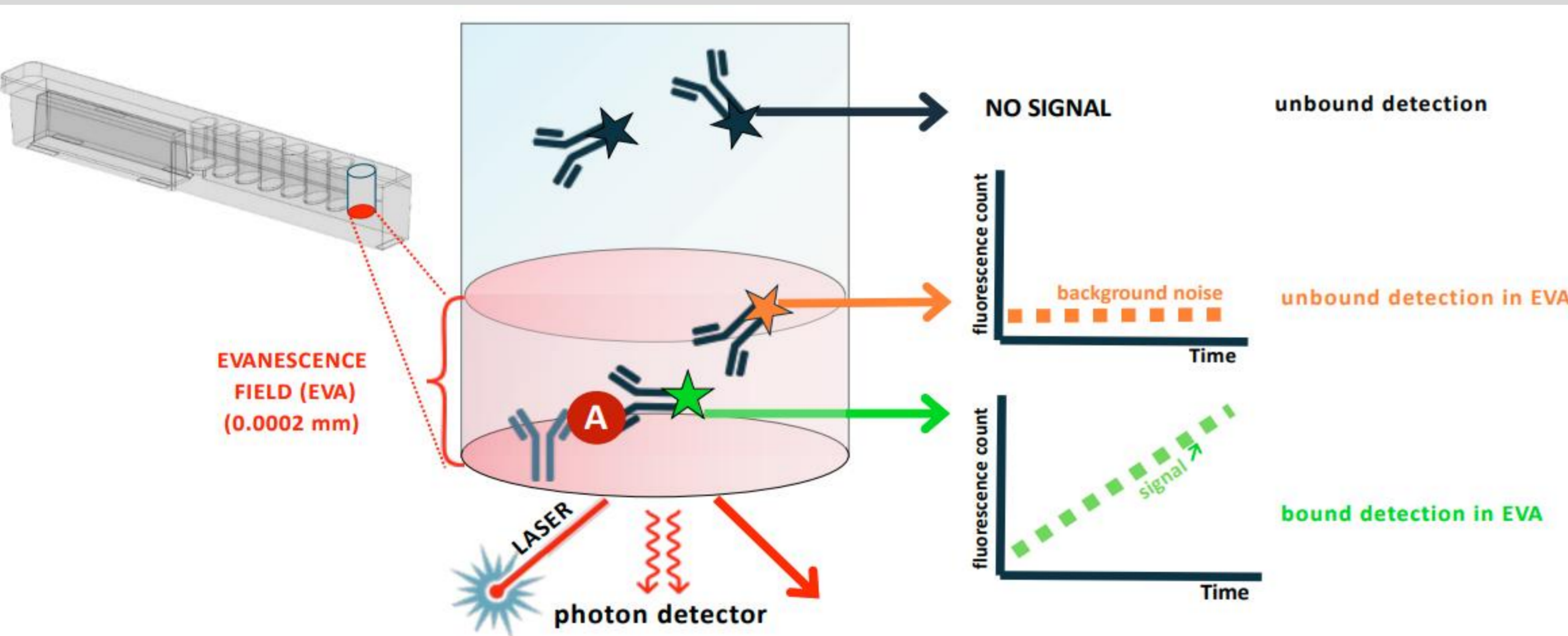


Figure 1 Illustration of the ElioDX technology

### Workflow:

- Step 1: Capture antibody (blue) coated on well surface
- Step 2: Blocking unoccupied sites (orange)
- Step 3: Pre-incubate sample (yellow) + detection antibody (red)
- Step 4: Add mix to cuvette → 10 min measurement starts

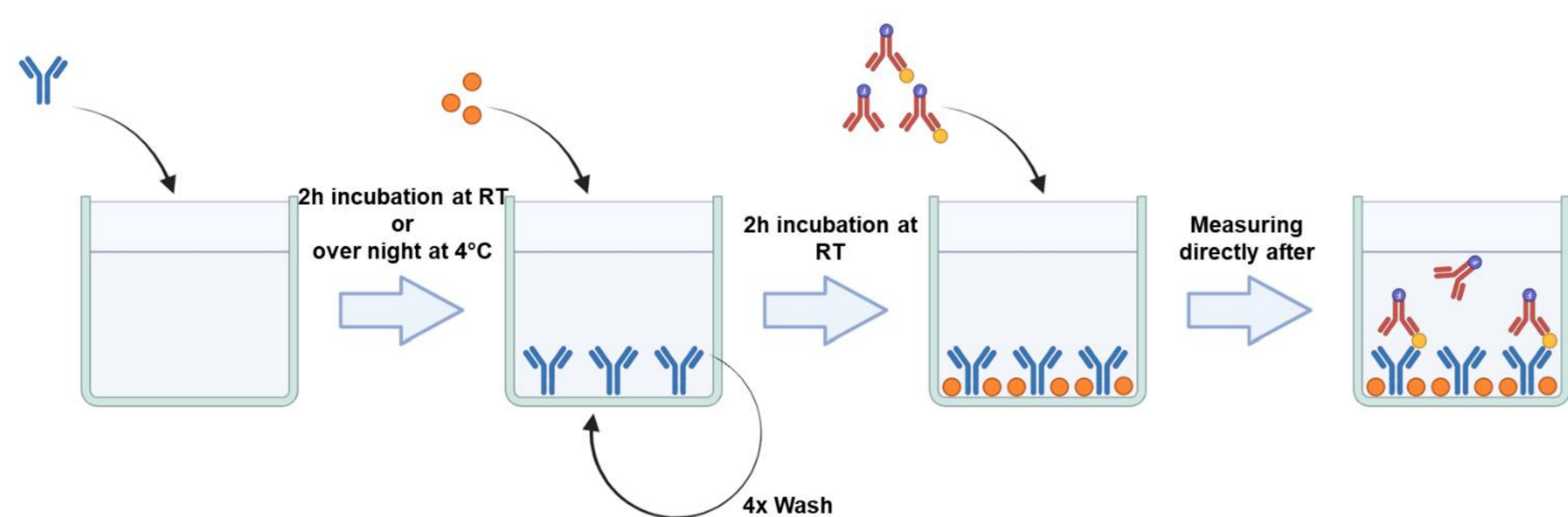


Figure 2 Illustration of the workflow to detect NT-proBNP

## Results

### Stability of NT-proBNP:

- Measured slopes in different experiments at 6'666 pg/mL for the same aliquot of NT-proBNP stored at 4°C
- A decrease of the signal could be seen between 5 and 12 days with a clear tendency after day 12

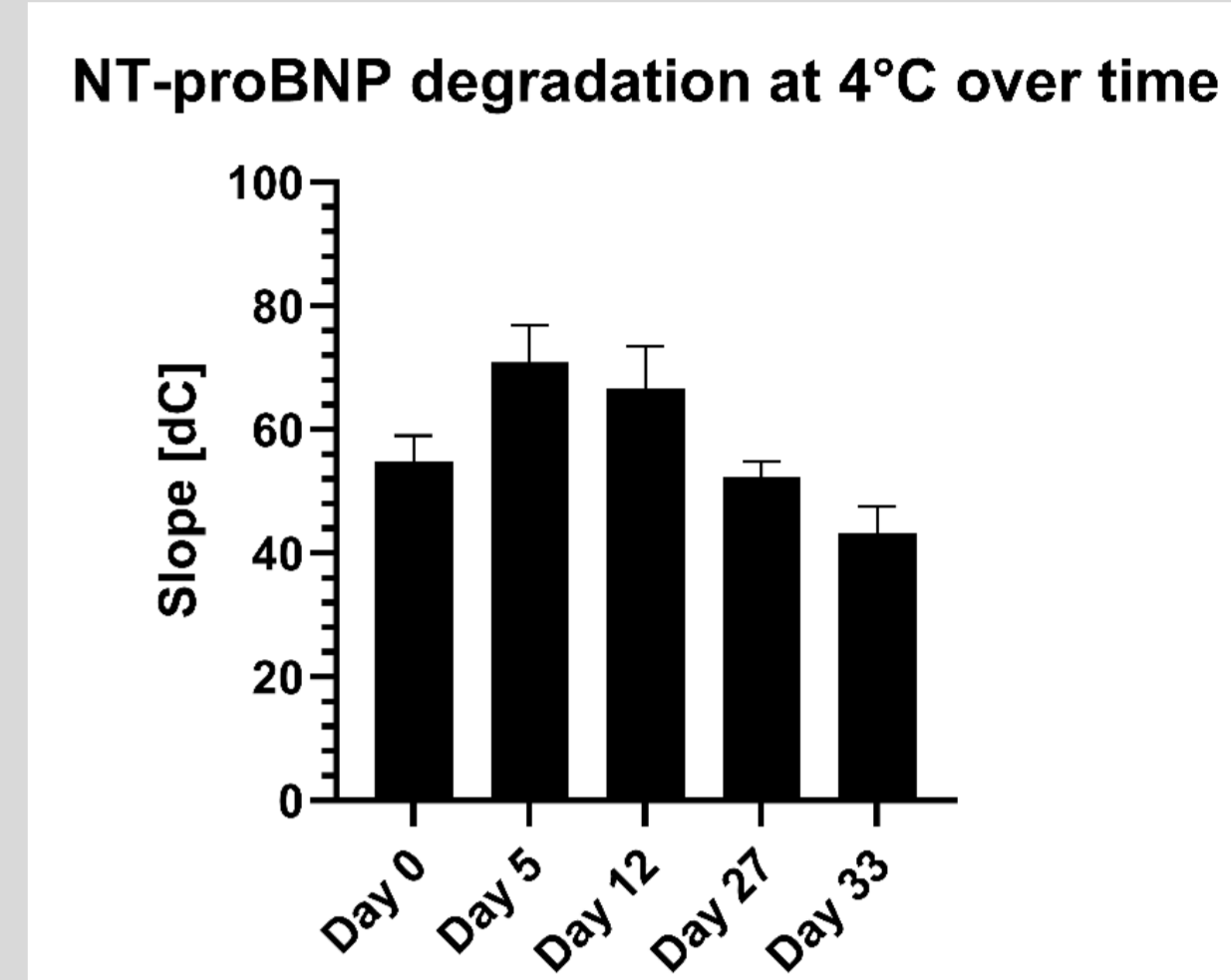


Figure 3 Illustration of stability of NT-proBNP at 4°C

### Inter-device reproducibility:

- A binding curve ranging from 741 pg/mL to 60'000 pg/mL was performed
- The same solution was measured on two devices P12 (black) and P9 (blue)
- In red the area is marked where the clinically relevant concentration (125 – 3'000 pg/mL)

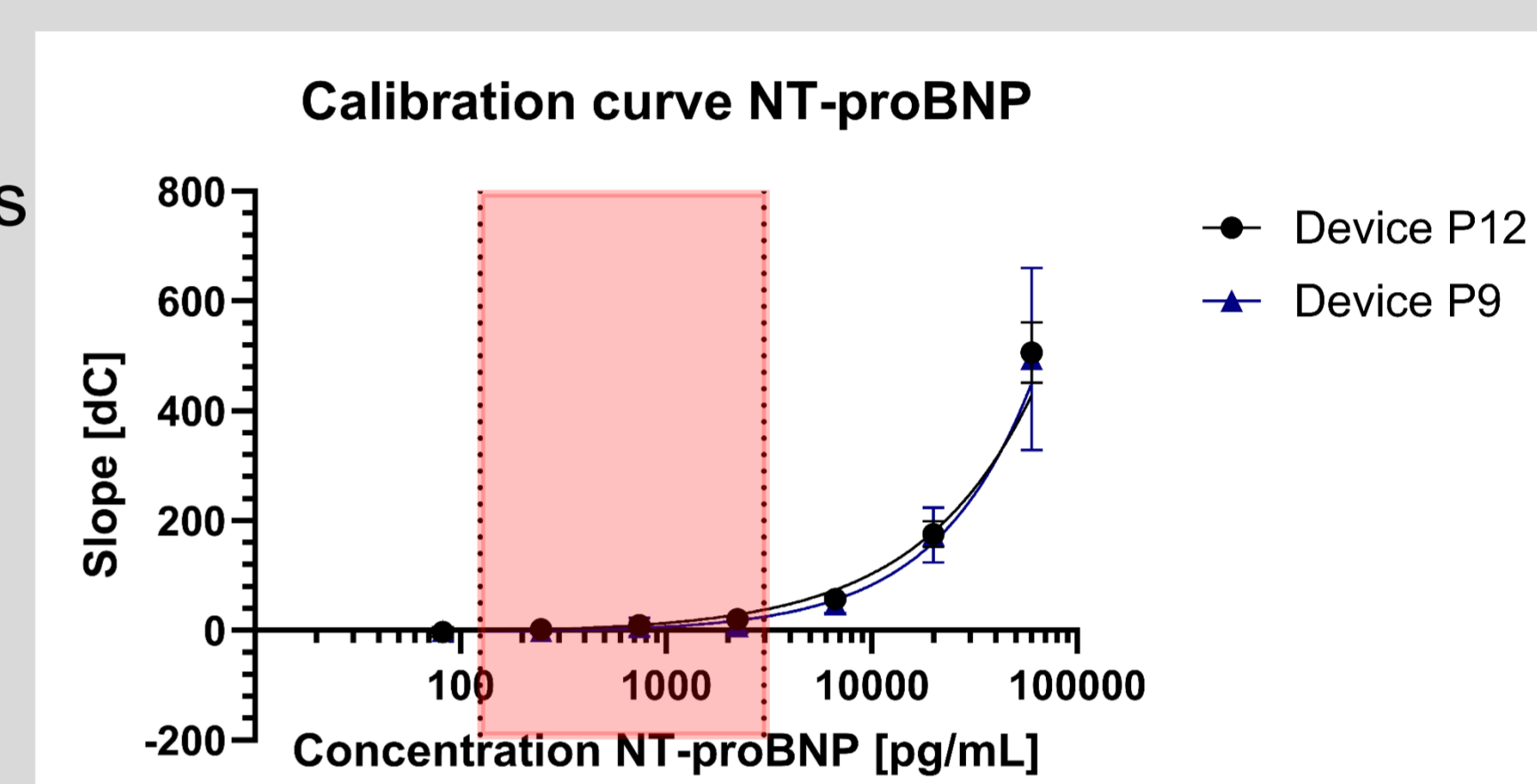


Figure 4 Illustration of inter-device reproducibility

- Outcome: Very comparable results at both devices

### Different serum concentrations compared to buffer:

- Several serum amounts (100%, 50%, 10% and 0%) were tested by spiking defined NT-proBNP concentrations ranging from 2'222 pg/mL to 540'000 pg/mL
- Quenching effect visible at 100% and 50% serum but not at 10% serum
- Special is that the ratio between measured slope in buffer and serum in the linear range was stable for 50% (around 1.3)
- Has to be investigated if still true for different serum

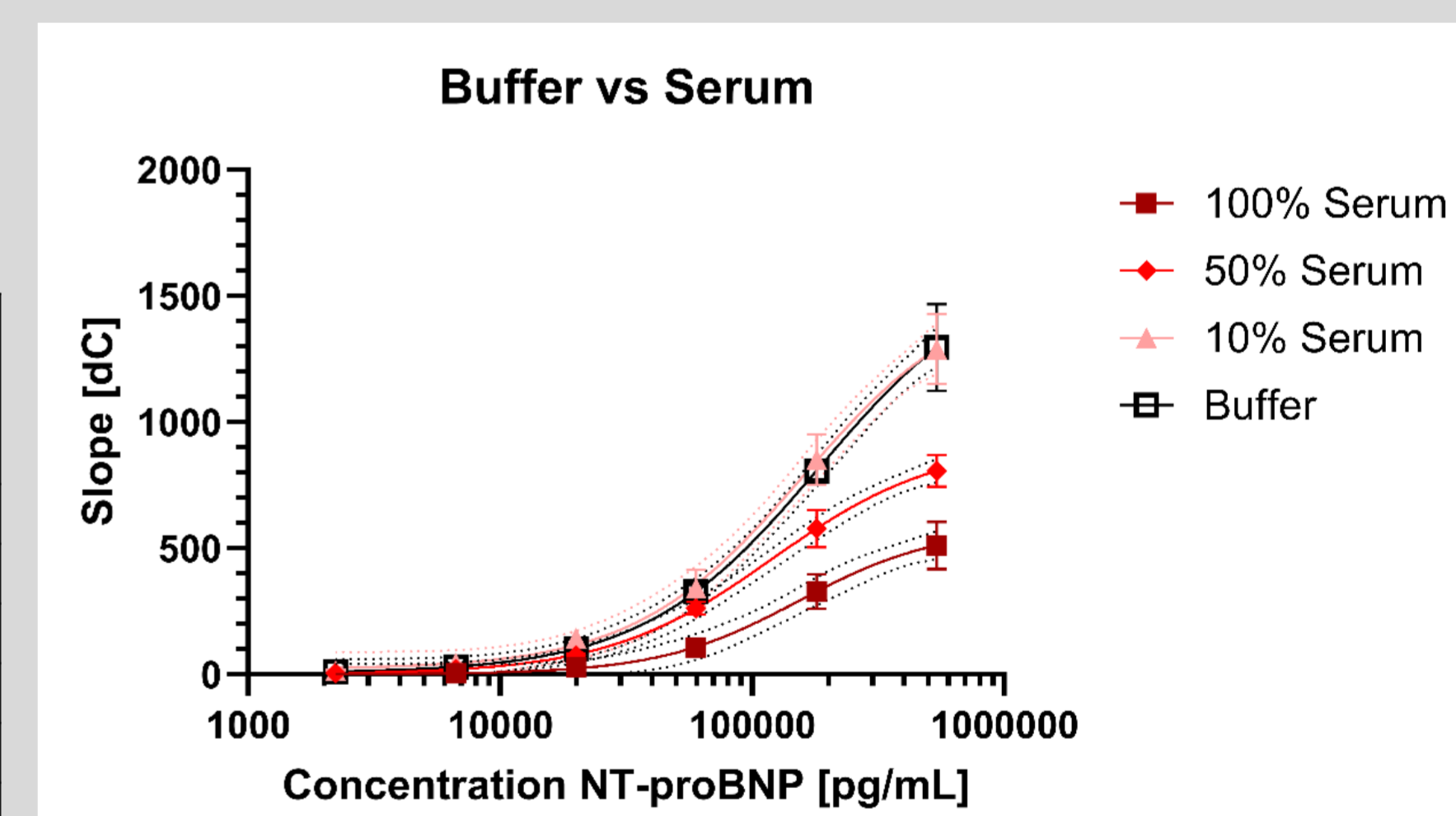


Figure 5 Illustration of spiking experiment in sera

Table 1 Ratio between slopes in buffer and several serum amounts

Concentration [pg/mL]	Ratio slope (Buffer/100% Serum)	Ratio slope (Buffer/50% Serum)	Ratio slope (Buffer/10% Serum)
540'000	2.2	1.4	0.9
180'000	2.5	1.4	0.9
60'000	3.1	1.2	1.0
20'000	3.5	1.3	0.7
6'667	7.7	1.5	0.7
2'222	-9.8	1.8	0.7

## Discussion and conclusion

- Too low sensitive (LOQ 1'075 pg/mL) to detect in a clinically relevant concentration area (125-3'000 pg/mL) but near which means it is possible with a more sensitive antibody pair
- The method looks quite robust against serum effect
- The inter-device comparability could be shown
- The advantages against ELISA could be shown (shorter analysis time, no washing steps and similar results)
- Method has to be further developed with more sensitive antibodies
- ElioDX platform has a high potential to enable fast detection of biomarkers

## Reference

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- [3] G. Colom, J.-P. Salvador, G. Acosta, F. Albericio, M. Royo, and M.-P. Marco, 'Competitive ELISA for N-terminal pro-brain natriuretic peptide (NT-proBNP) determination in human plasma', *Ana-lyst*, vol. 145, no. 20, pp. 6719-6727, Oct. 2020, doi: 10.1039/D0AN00650E.