Point-of-Care Quantification of H3.1-Nucleosomes in Fresh Whole Blood: A Novel Tool For NETs Measurement In Hospital Setting

Dorian Pamart[1], Brieuc Cuvelier[1], Julie Candiracci[1], Marielle Herzog[1]

[1]Belgian Volition, SRL, 22 Rue Phocas Lejeune, Parc Scientifique Crealys 5032 Isnes, Belgium

Introduction: The WHO recommendation includes the early identification of patients with acute infections who are at risk of sepsis in order to improve outcomes. There is growing evidence implicating Neutrophil Extracellular traps (NETs), composed of nucleosomes and granular-derived components, as a key component of the dysregulated host immune response in sepsis. Elevated levels of H3.1-nucleosomes have been reported in several conditions including sepsis, and are associated with an increased risk of death, renal failure, respiratory failure, multiple organ dysfunction and septic shock.

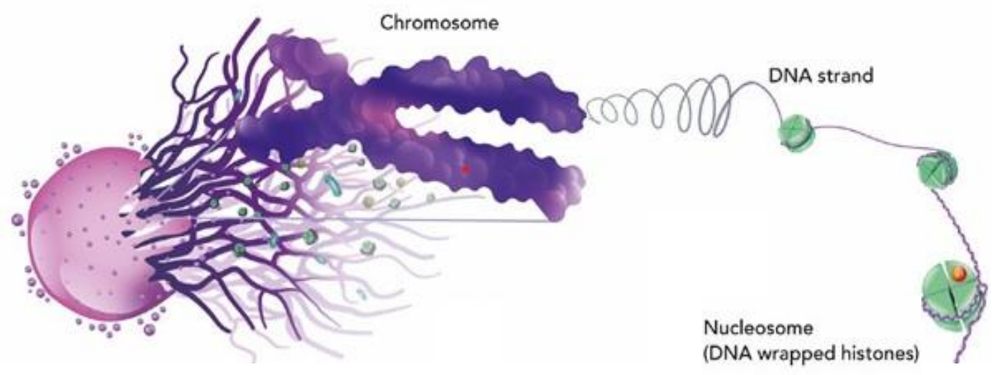


Figure 1: Chromosome and NETs are made of nucleosomes

Currently, this biomarker can be measured using an automated immunoassay: Nu.Q® NETs ChLIA, in a central laboratory.

Aim: To develop a rapid and low-cost Point-of-Care (POC) test for detecting H3.1-nucleosomes levels as an aid in the detection of the immune system activation by the measurement of NETs.

Method: The POC test, based on a Lateral Flow (LF) platform, detects H3.1-nucleosomes from a single drop of blood. Briefly, 20 µL of sample are mixed with 100 µL of buffer and applied onto the device. After 15 minutes of incubation, quantification is performed using a LF reader (*Figure 2*).

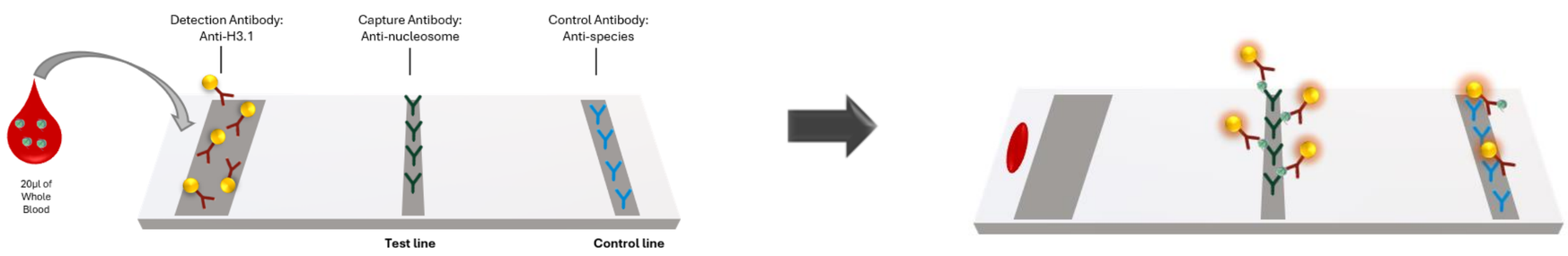


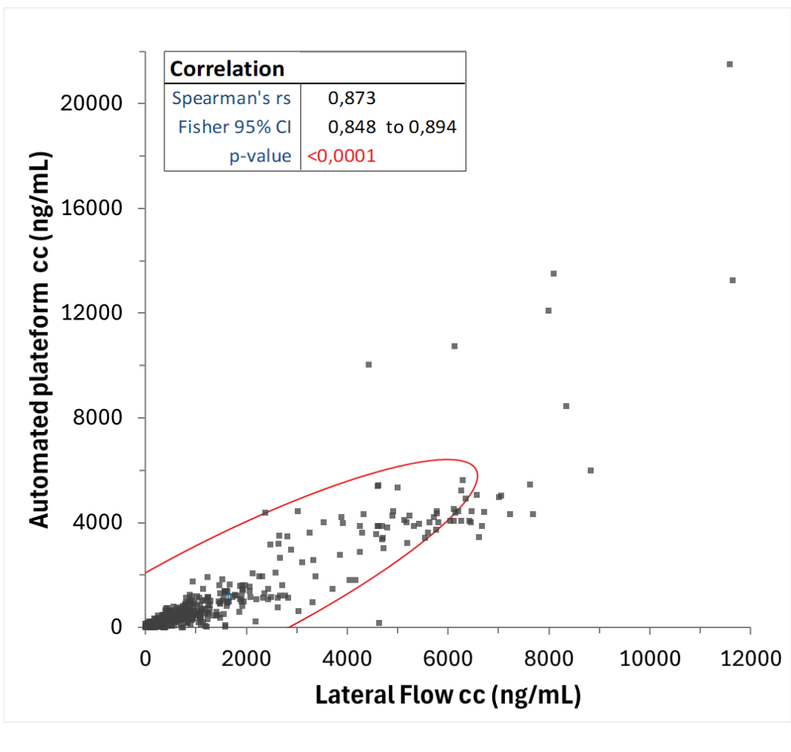
Figure 2: Lateral Flow assay components

Results:

Strong correlation for the quantification of H3.1-nucleosomes on plasma samples using Nu.Q® ChLIA and new Nu.Q® POC test

Figure 3: Correlation plot comparing the results obtained with our Lateral Flow assay to those from the automated reference platform (n=432).

The initial analytical correlation was conducted using plasma K2EDTA samples from a cohort of 432 individuals, including healthy donors as well as patients with inflammatory or infectious conditions, with either suspected or confirmed sepsis. A strong positive correlation was observed between the two methods, with a concordance rate of 87.3% ($p < 0.0001$). These findings indicate that the Nu.Q® POC test provides measurements consistent with those obtained using the laboratory-based reference platform.



Robust concordance of Point-of-Care whole blood and plasma H3.1-nucleosome quantification by Lateral Flow

A

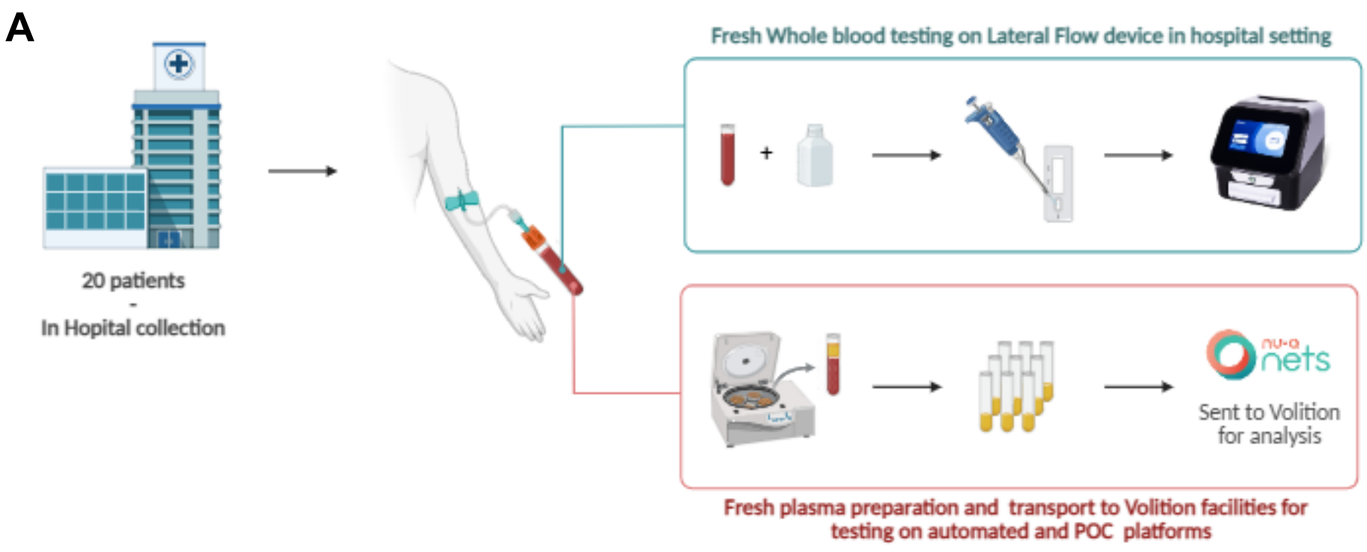


Figure 4A: Design of the Clinical Study conducted in a hospital setting.

B

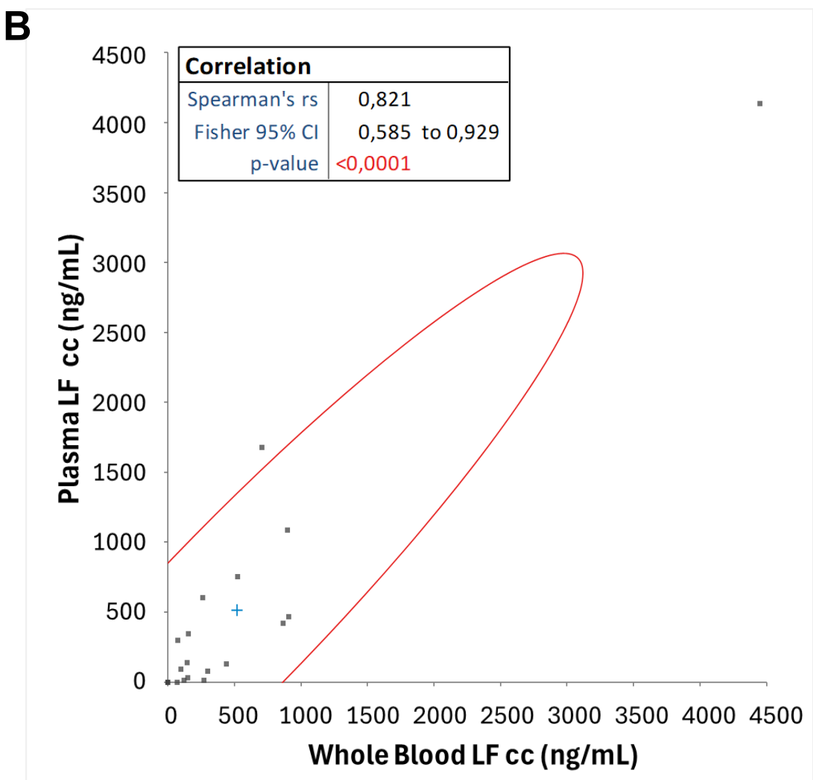


Figure 4B: Correlation plot comparing the performance of our lateral flow assay across two biological fresh matrices, plasma and whole blood (n=20).

Strong correlation between the two matrices tested on the Nu.Q® POC with a concordance rate of 82.1% ($p < 0.0001$).

Conclusion:

For the first time, we were able to quantify circulating nucleosome from Whole Blood at the point-of-care using a Lateral Flow device. This is the first real-world hospital use of the test under conditions reflecting potential intended clinical application.

Further larger-scale studies, particularly using capillary samples, are planned.

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Conflict of interest: All the authors are employees of Belgian Volition, SRL. DP and MH hold shares in VolitionRx Limited.