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# Nu.Q® NETs immunoassay detects Neutrophil Extracellular Traps by quantifying circulating H3.1-nucleosomes.

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## Introduction

Neutrophil extracellular traps (NETs), released by activated neutrophils in response to infectious agents, play a significant role in various diseases, including sepsis, COVID-19, and autoimmune disorders.

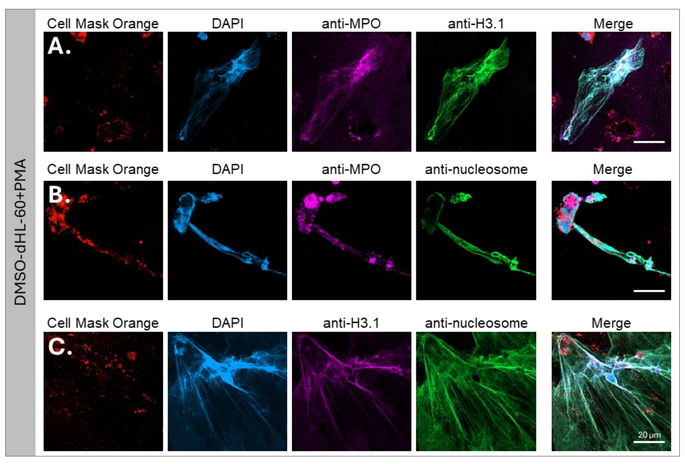
Overproduction or insufficient clearance of NETs can cause tissue damage and exacerbate disease, highlighting the importance of early detection and monitoring of NETs levels in clinical samples.

NETs detection methods like fluorescence microscopy and immunoassays targeting common NETs biomarkers (i.e.; MPO, NE, cfDNA) have limitations, prompting the need for more reliable approaches.

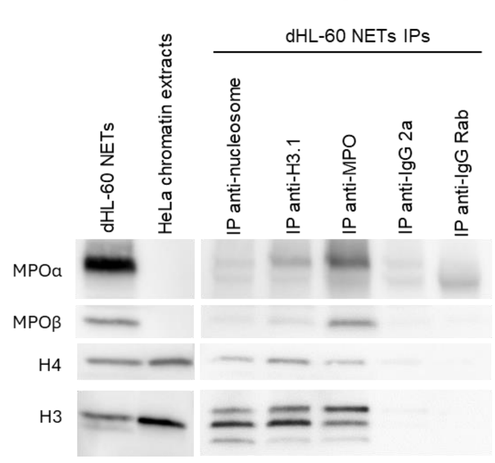
In this study, we identified circulating H3.1-nucleosome as a promising biomarker for detecting NETs and we developed a chemiluminescent immunoassay detecting circulating H3.1-nucleosomes; the **Nu.Q® NETs.**

## Confirmation of H3.1-Nucleosomes presence in NETs using the dHL-60 model

### Immunofluorescence Imaging of NETs




### Co-IP of NETs components



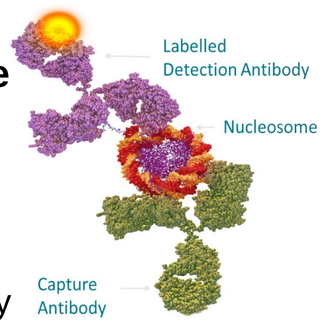

- Colocalization of H3.1-nucleosomes with both MPO and DNA, confirming the presence of H3.1-nucleosomes in NETs structures.
- Successful co-immunoprecipitation of MPO with H3.1-nucleosomes, further confirming their association in NETs.

## Nu.Q® NETs is analytically validated for H3.1-nucleosomes detection in plasma, with strong performance metrics

### Nu.Q® NETs Automated ChemiLuminescence ImmunoAssay

Aid to the detection and evaluation of diseases associated with NETosis in Human K2EDTA Plasma

- Capture: anti-histone H3.1 antibody
- Detection: conformational anti-nucleosome antibody




### Sensitivity

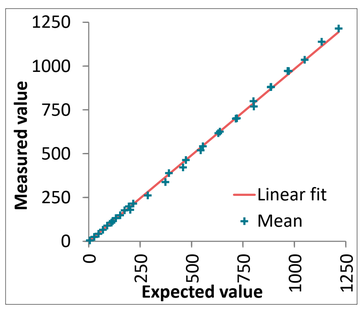
All Limit of blank (LOB), Limit of detection (LOD) and Limit of quantification (LOQ) estimated for 3 independent lots of reagents are below 3 ng/mL, defined as the assay limit.

| | n = 3 lots | SD |
|---|---|---|
| **LOB** ng/mL | 0.33 | ± 0,3 |
| **LOD** ng/mL | 0.93 | ± 0,9 |
| **LOQ** ng/mL | 2.10 | ± 2,4 |

### Linearity

Consistent and proportional response across a range from 26.3 ng/mL to 1214.4 ng/mL of H3.1-nucleosomes is demonstrated.

| | Linearity Range |
|---|---|
| Frozen K2EDTA samples | 26.3 – 1214.4ng/mL |




### Precision

Excellent repeatability and reproducibility are exhibited, with low intra-assay and inter-assay variability.

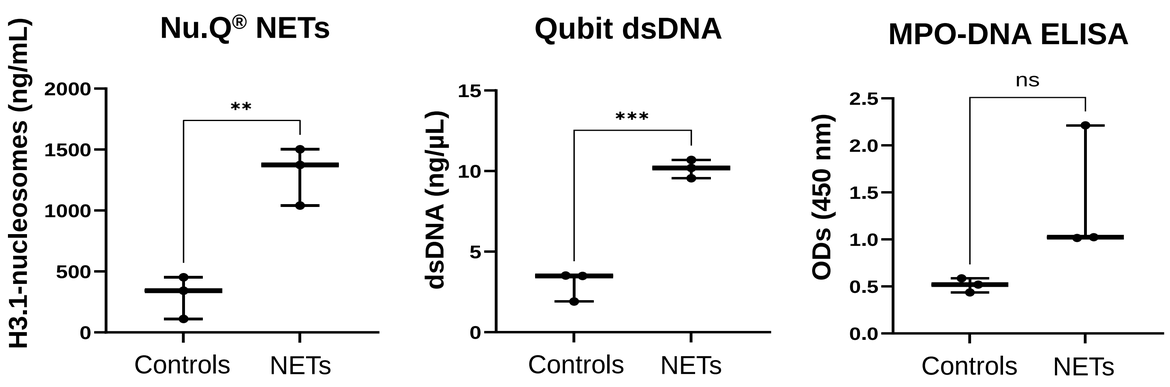
| | Precision Ranges |
|---|---|
| Within-run (% CV) | 1.4 – 2.8 % |
| Within-lot (% CV) | 2.9 – 6.4 % |
| Within-laboratory (% CV) | 3.1 – 9.2 % |

### Spike recovery

Recovery rates of known quantities of spiked analytes in the sample matrix indicate no matrix effect, confirming the accuracy of the assay.

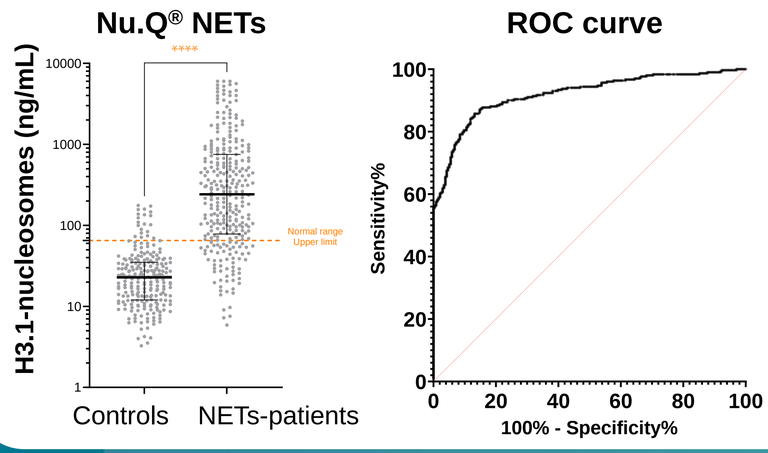
| | Spike recovery Range |
|---|---|
| K2EDTA samples spiked with H3.1-nucleosomes | 89 – 105 % |

## NETs Quantification in dHL-60 NETs (n=3) with Nu.Q® NETs compared with other quantification methods




Although all three tests show a higher signal in the presence of NETs, the Nu.Q® NETs assay significantly distinguishes Control and NETs groups, with the advantage of requiring simple sample processing.

## Nu.Q® NETs assay detects diseases associated with NETs in blood samples




- Significant elevation of H3.1-nucleosomes levels in patients suffering of NETs-related diseases.
- ROC curve analysis demonstrates a 91% accuracy in distinguishing NETs patients from controls.

## Methods

**NETosis Induction in dHL-60 cells:** HL-60 cells were differentiated into neutrophil-like cells using 1.5% DMSO for 5 days, then treated with 100nM PMA for 5 hours to induce NETosis.
**Immunofluorescence (IF) Staining:** use of antibodies against MPO, histone H3.1, or nucleosome. DNA and membranes were stained with DAPI and Cell Mask Orange. Confocal microscopy was used for imaging.
**NETs Immunoprecipitation (IP) and Analysis:** IP of NETs involved magnetic beads coated with specific antibodies, followed by western blot analysis.
**dsDNA:** DNA was extracted from dHL-60 NETs samples with the QIAamp® DSP Circulating NA kit (Qiagen), then quantified using the Qubit™ 1X dsDNA HS assay Kit (ThermoFisher).
**MPO-DNA ELISA:** following instructions in Rada, 2019. Methods Mol Biol. 2019 ; 1982: 517–528.
**Clinical data:** Retrospective study performed on K2EDTA plasma samples from 236 healthy donors and 302 patients with diseases associated with NETosis

## Conclusions

- **We confirmed the presence of H3.1-nucleosomes in NETs, using the *in vitro* dHL-60 NETs model.**
- **We developed and analytically validated a chemiluminescent immunoassay for the detection of circulating H3.1-nucleosomes, the Nu.Q® NETs assay.**
- **We demonstrated the potential of the Nu.Q® NETs assay as a groundbreaking tool in clinical diagnostics.**

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