



# Quantification of integrated HIV DNA by repetitive sampling Alu-HIV PCR and Poisson statistics

De Spiegelaere Ward<sup>a</sup>, Malatinkova Eva<sup>a</sup>, Van Nieuwerburgh Filip<sup>b</sup>, O'Doherty Una<sup>c</sup>, Vandekerckhove Linos<sup>a</sup>



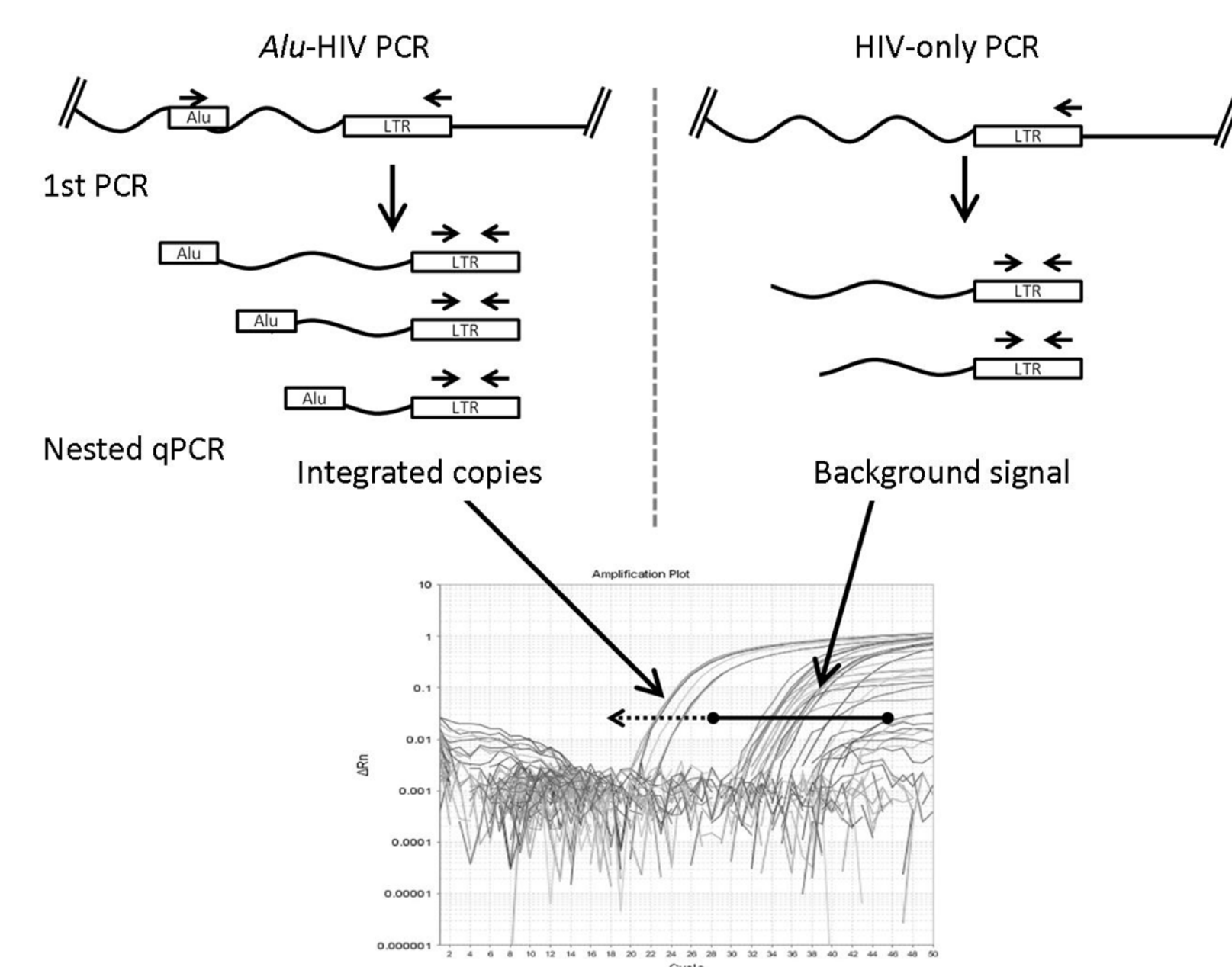
Affiliations: a) HIV Translational Research Unit, Department of Internal Medicine, Ghent University, Belgium; b) Department of Pharmaceutics, Ghent University, Belgium; c) Department of Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia, USA

## BACKGROUND

Proviral HIV is a candidate virological marker to monitor the HIV reservoir in patients. Recent data revealed that repetitive sampling Alu-HIV PCR on patient samples correlates with quantitative coculture assays, indicating that integrated HIV DNA correlates to the amount of replication competent HIV. Although promising, the workflow and data analysis of the assay is complex and hinders a wide use. Here, we provide an improved data analysis based on binomial and Poisson statistics analogous to digital PCR. This method enables absolute quantification without a standard dilution curve and includes confidence interval estimation.

## Methods

Poisson statistics were used to assess the binomial data of positive to negative reactions from the 42 replicate Alu-HIV PCR. Confidence intervals were calculated using a modified Poisson method, designed for data from ≥40 replicates. The raw data of Alu-HIV PCRs on standard dilutions and on samples of HIV infected patients (N=47) were analyzed with the modified Poisson method and with the classical method of analysis (the % positive method) and these data were compared.

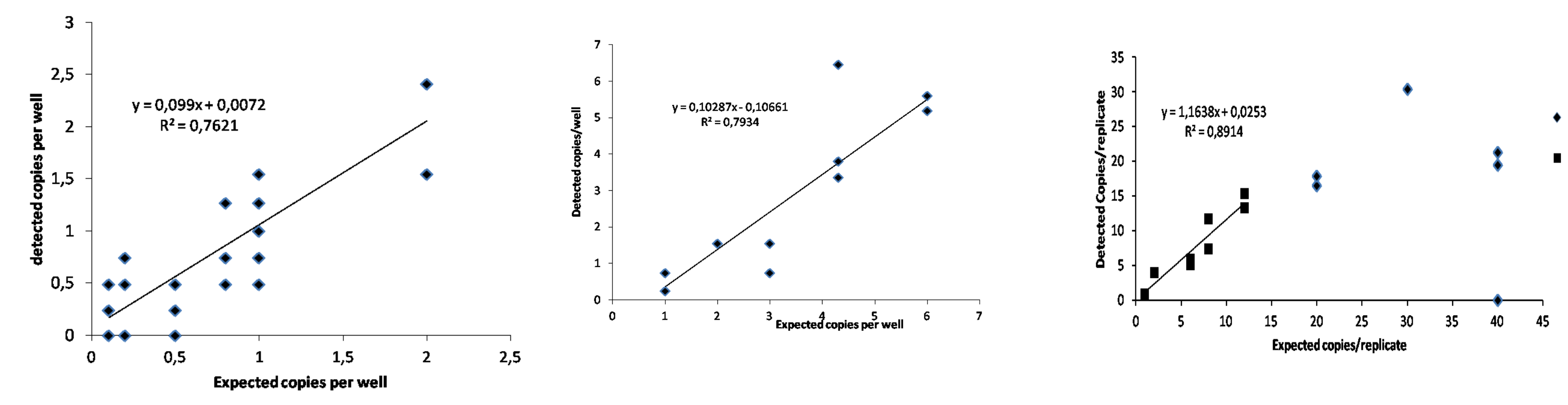


$$\lambda = -\ln(1 - \hat{p})$$

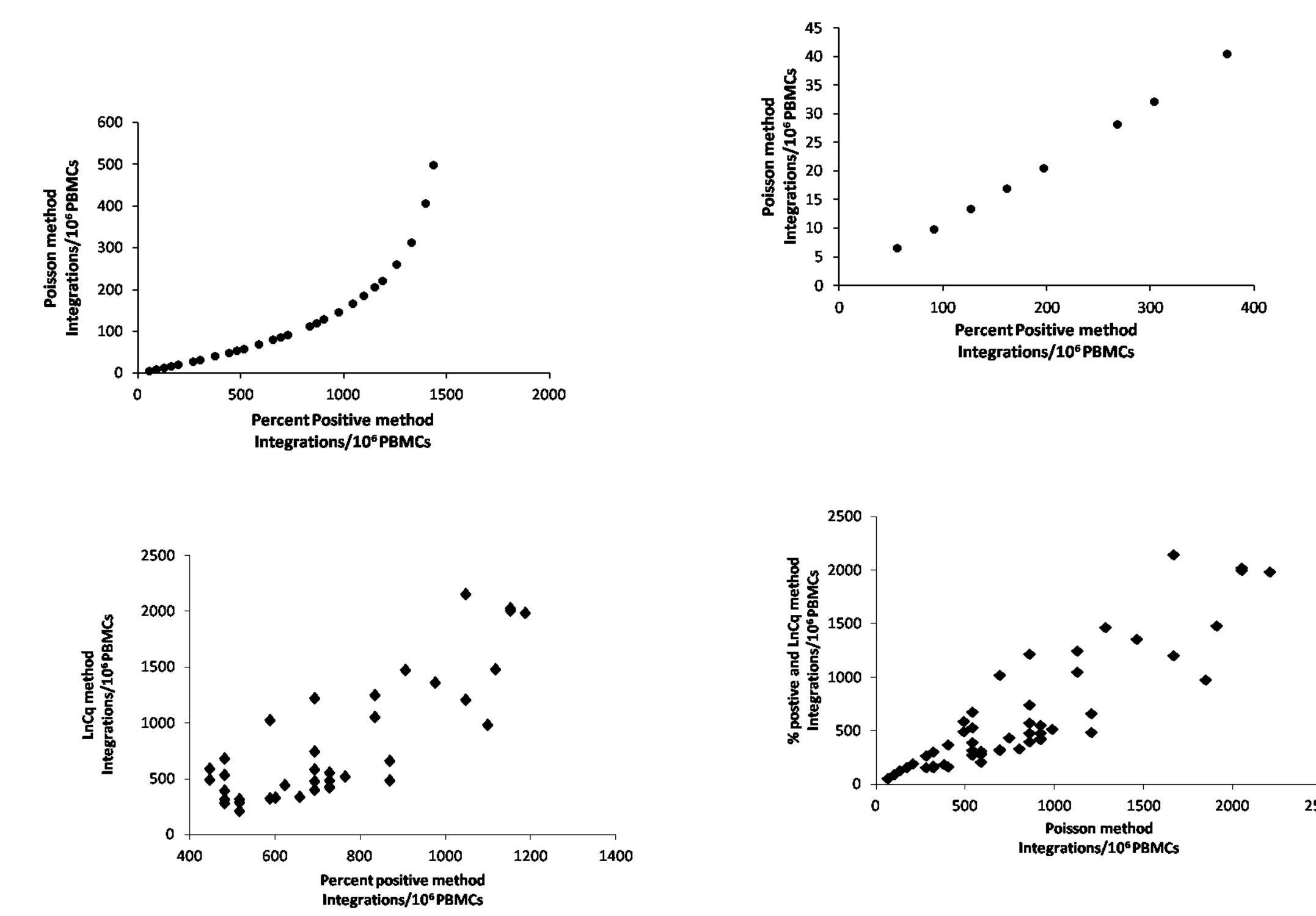
Table 1: Primers and probes used for real-time PCR detection of HLA-B\*57:01.

name	sequence	Ta °C	nM
HLA 57:01 193F exon 2	forward CGCGAGTCCGAGGATGGC	61	500
HLA 57:01 319R exon 2	reverse GGAGCGCATCCGAGTTCT	61	500
HLA 57:01 exon 2 probe	/5'FAM/AACATGAAGCCTCCGCGCAGACTTA/3'IABkFQ/	61	20
HLA 57:01 345F exon 3	forward GTCTCACATCATCCAGGTGACGT	61	500
HLA 57:01 419R exon 3	reverse GACTGCTCATGCCCGGGAG	61	500
HLA B57:01 exon 3 probe	/5'FAM/ACGTGCGCAGCCATACACCTGGATGATG/3'IABkFQ/	61	20
RPP30	forward AGATTGGACCTGCGAGCG	61	500
RPP30	reverse GAGCGGCTGTCTCCACAAGT	61	500
RPP30 probe	/5'HEX/TTCTGACCTGAAGGCTCTGCGCG/3'IABkFQ/	61	200

## Results



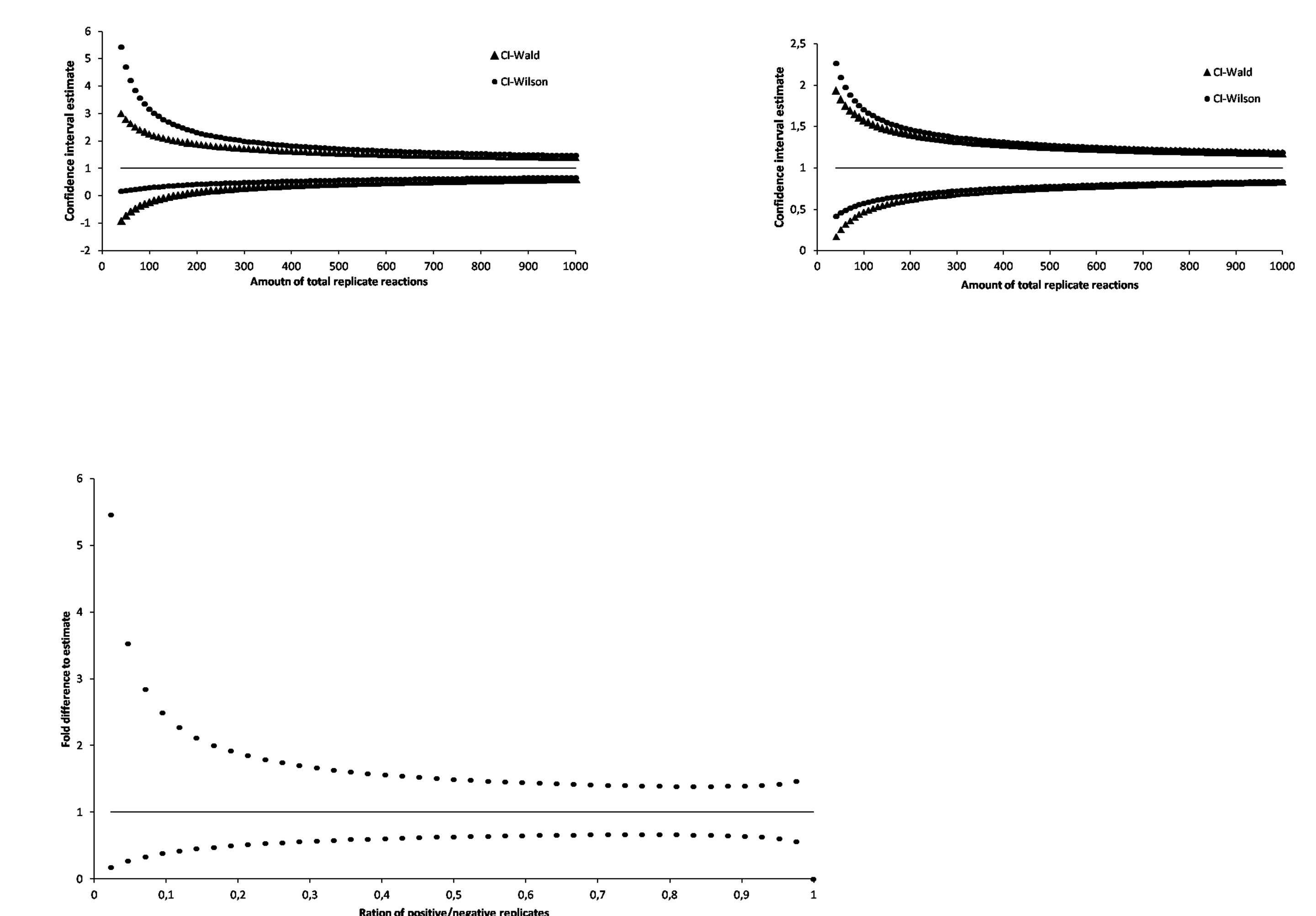
blablabla



blablabla

$$CI_{Wald} = \hat{p} \pm k \sqrt{\frac{\hat{p}\hat{q}}{n}}$$

$$CI_{Wilson} = \frac{X + k^2/2}{n + k^2} \pm \frac{k}{n + k^2} \sqrt{(\hat{p}\hat{q} + \frac{k^2}{4n})}$$



## CONCLUSIONS:

The proposed methodology provides a more efficient workflow as preparation of the standard curve in the classical Alu-HIV is time consuming and requires frequent validation. In addition, the implementation of confidence intervals permits an improved qualitative analysis of the data and provides a statistical base for the required number of technical replicates.

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