TITLES AND LEGENDS TO SUPPLEMENTARY FIGURES AND TABLES

Figure S1. Interpretation of measured serum EGF concentrations. The robustness of the proposed methodology for sera separation and its quantification.

The curves in **Figure S1** show the kinetics of EGF release in three healthy donors whose sera were separated from blood samples pertaining to two different phlebotomies. Aliquots of four milliliters were incubated for coagulation during two and five hours (1st venipuncture) or for periods of one and four hours (2nd venipuncture), before the separation of the sera. Therefore, each phlebotomy provided two sera and two serum EGF concentrations ([EGF]): $[EGF]_{2h}$ and $[EGF]_{5h}$ or $[EGF]_{1h}$ and $[EGF]_{4h}$, corresponding to 1st and 2nd venipunctures, respectively.

The shape of the curves in **Figure S1** supports our interpretations about $[EGF]_{1h}$ and $[EGF]_{4h}$: as an estimate of the actual concentration of free EGF in blood circulation and as a good approximation to the concentration plateau achieved by the progressive EGF release by platelets, during aggregation. As shown in **Figure S1**, the major increase in [EGF] occurs between the first and the second hour after phlebotomy, the EGF rise was lower from 2h to 4h, and almost imperceptible between 4h and 5h. Thus, the plateau in the release of EGF by platelets occurs very near the 4h of coagulation. For donor 3, which had the highest [EGF], the inverted EGF pattern ($[EGF]_{5h} < [EGF]_{4h}$) clearly indicates that the plateau in the release of EGF was reached very near to 4h. In the other two donors the increase from $[EGF]_{4h}$ to $[EGF]_{5h}$ was very small, which also supports our interpretation of $[EGF]_{4h}$ as a good approximation to the concentration plateau (the whole EGF stock).

Additionally, the graphic in **Figure S1** shows the expected dependency between [EGF] and the time of sera separation. As the sigmoid curves derive from measurements corresponding to two different phlebotomies of the same donors, spaced approximately 5-6 months in time, the curves also support the robustness of the proposed methodology for sera separation and its quantification.

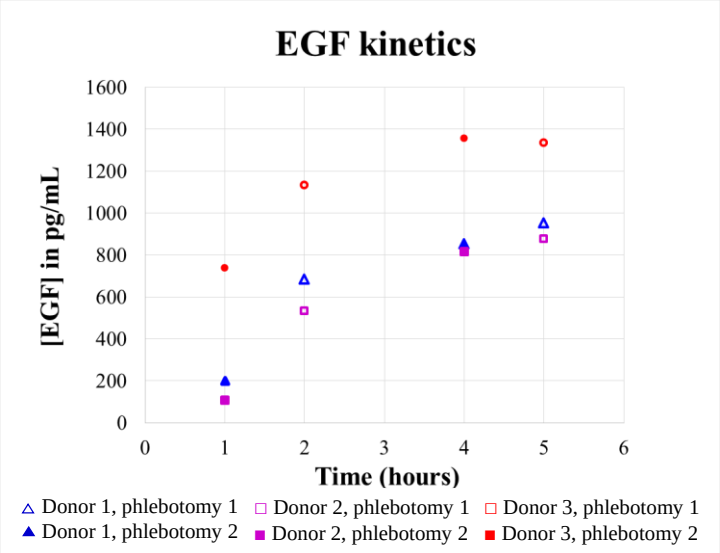
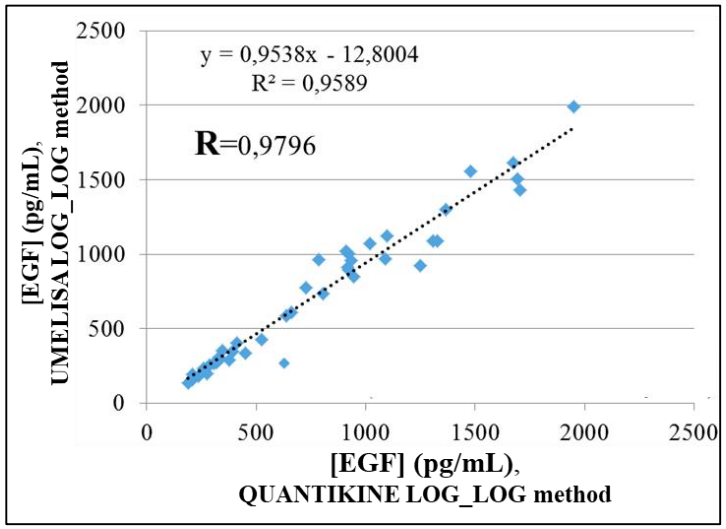


Figure S2. Agreement between UMELISA and Quantikine methods for the estimation of serum EGF concentrations.

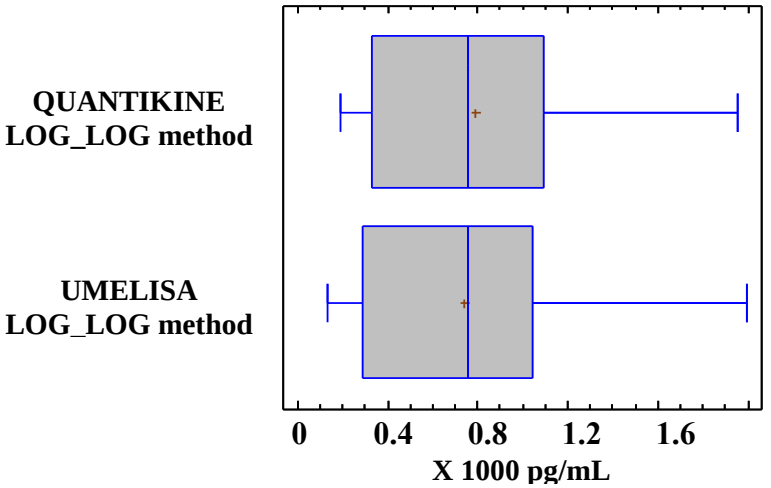
The figure includes the analysis of correlation of estimations obtained by both methods of EGF quantification **(A)** and its Box and whisker plot (**B**).

The regression curve in **Figure S2A** shows a good correlation between the estimations of both methods (QUANTIKINE LOG_LOG and UMELISA LOG_LOG). The test of significance of the intercept and slope of the regression line proved that the slope is significantly different from zero (p=0.0000) and the intercept is equal to 0 (p=0.6671), for α=0.05. The Pearson's correlation coefficient R=0.979586 was significant (p=0.0000), which indicates that with a confidence level of 95.0% there is a relatively strong statistically significant relationship between the variables.
The analysis in **Figure S2B** shows that there are no differences between the compared methods for EGF quantification. The p-values in the t-tests for means and medians were 0.6541 and 0.5933, respectively. Additionally, the Kolmogorov-Smirnov test for the comparison of both distributions of estimations, allowed to conclude about its statistical equivalence at a significance level of 5 % (p= 0.9883).

(A)



(B)



Abbreviations:

QUANTIKINE LOG_LOG method= Quantikine EGF estimations obtained by a linear regression model, applying the method of least squares to log-transformed-primary data (EGF concentrations and absorbance units).

UMELISA LOG_LOG method = UMELISA EGF estimations obtained by a linear regression model, applying the method of least squares to log-transformed-primary data (EGF concentrations and fluorescence units).

Table S1. Demographic characteristics of compared cohorts											
		NSCLC1/To		**NSCLC2/To&T1**		**HC1**		**HC2**		**HC3**	
Gender	**Parameter**	**n**	**Age**[a]	**n**	**Age**[a]	**n**	**Age**[a]	**n**	**Age**[a]	**n**	**Age**[a]
W+M	Mean	25	63	18/25	62	25	62	18/25	61	15	69
	Median		62		62		63		61		68
W	Mean	5	63	3/5	64	5	64	3/5	64	11	70
	Median		65		68		63		63		71
M	Mean	20	62	15/20	62	20	62	15/20	61	4	67
	Median		62		60		62		60		68

Abbreviations:

W: women; M: men; W+M: women plus men; HC: healthy control

Footnotes:

[a] *Rounded values in years*

Table S2 shows that there were not statistically significant differences between HC2 and HC1 by gender composition, age or measured [EGF] at the 5% significance level. However, significant differences were found between HC3 and the other two groups by gender, age and $[EGF]_{4h}$, but not by $[EGF]_{1h}$, although slightly reduced values were found in comparison with HC1.

Table S2. Comparison between HC groups, α=0.05								
Cohort	**W/M**	**p**	[d]**Age** (years)	**p**	[d]**[EGF]$_{1h}$** (pg/mL)	**p**	[d]**[EGF]$_{4h}$** (pg/mL)	**p**
HC1	5/20	[a]$p_{12=}$ 1.4573	62.32 ± 1.42	[b]$p_{12=}$ 0.6504	450.90 ± 57.25	[b]$p_{12=}$ 0.8831	1013.53 ± 62.84	[b]$p_{12=}$ 0.7063
HC2	3/15	[a]$p_{23=}$ 0.0000	61.33 ± 1.62	[c]$p_{23=}$ 0.0014	437.58 ± 70.30	[b]$p_{23=}$ 0.0926	1051.94 ± 81.37	[b]$p_{23=}$ 0.0301
HC3	11/4	[a]$p_{31=}$ 0.0000	69.33 ± 0.95	[c]$p_{31=}$ 0.0025	266.81 ± 67.04	[b]$p_{31=}$ 0.0487[*]	806.77 ± 66.30	[b]$p_{31=}$ 0.0379

Abbreviations:

W: women; M: men

Footnotes:

[a] *exact binomial two-tailed test of goodness-of-fit*

[b] *unpaired t-test*

[c] *Mann-Whitney W-test*

[d] *Mean ± SME values*

[*] *slightly reduced $[EGF]_{1h}$ in HC3, p value in the limit of decisions*