A new version of CISBIO Chromogranin A ELISA kit (#: CGA-ELISA-NG)

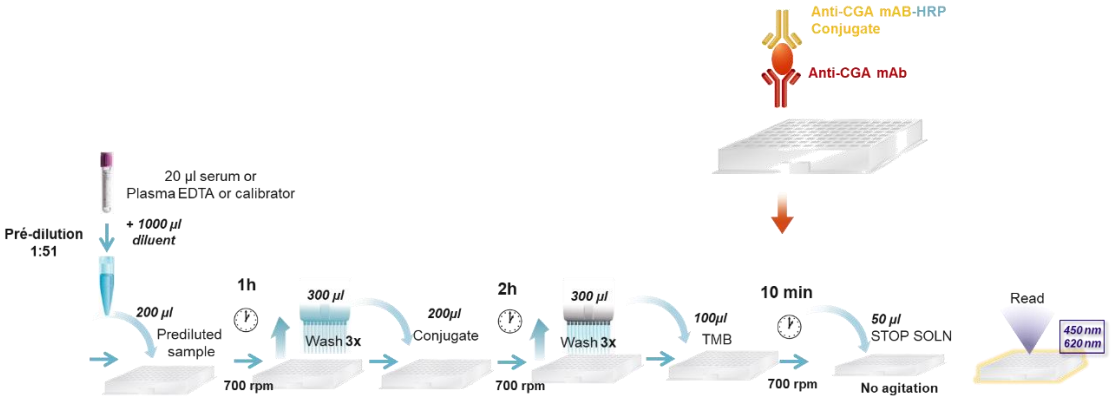
Introduction

Pioneered in the early 2000s by CISBIO, the chromogranin A assay (CGA) using RIA technology has provided a marker of choice for neuroendocrine tumors (NETs). In 2008, to follow the evolution of laboratory assays, a CGA ELISA kit was added to our diagnostic tools.

In 2022, a new version of this kit is available (CGA-ELISA-NG) and further improves the quality of the assays for this tumor marker. This version is now fully compatible with the new European regulatory rules of the IVDR 2017/746.

All other parameters have been optimized to best meet the requirements. Incubation times have been reduced, dilution and recovery tests have been improved, the robustness of the kit has been optimized. The following paragraphs support these improvements.

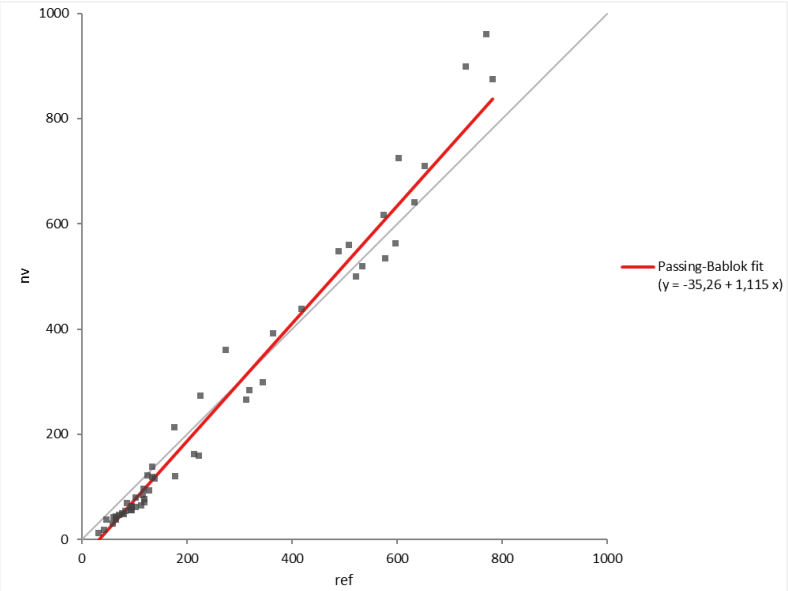
The new kit uses the same antibodies as the previous ones to maintain the same specificity. The antibodies are now produced in cell culture, thus reducing the use of animals.



Correlation between CGA-RIA-CT and CGA-ELISA-NG:

The experiment was performed on two batches of CGA-ELISA-NG and 2 batches of CGA-RIA-CT on 109 serum samples covering the whole calibration range.

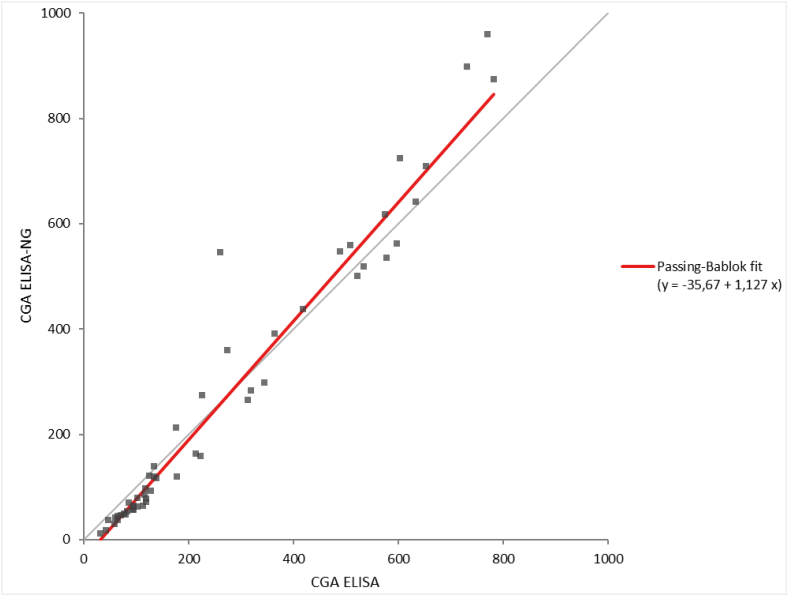
The results below show a good correlation between the kits with a slope close to 1.0 which will facilitate the switch from previous ELISA version to the CGA-ELISA-NG version.



Correlation - r	0,988

Correlation between CGA-ELISA and CGA-ELISA-NG:

Similar correlation was performed using 53 samples covering the whole range of concentrations. The little gap on the slope is linked to the adjustment of the new kit to obtain optimal dilution performances along the calibration curve. Nevertheless, the correlation is good.



Correlation - r	0,977

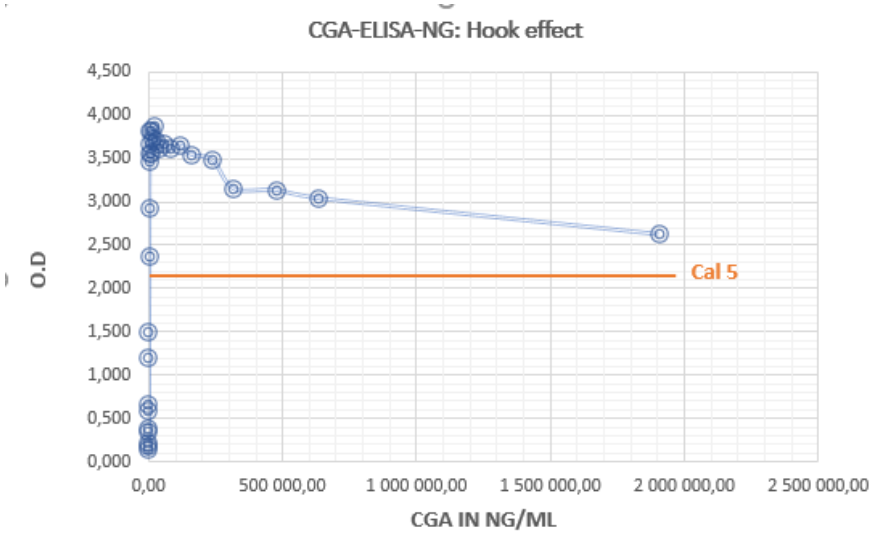
Essential analytical characteristics of the CGA-ELISA-NG kit:

Hook effect:

In this experiment, CGA antigen is diluted in normal human serum to cover a range of CGA concentration from 2 million ng/mL to 0 ng/mL.

We can see on the graph below that up to 2 million ng/mL, no hook effect appears making this kit perfectly compatible with very high serum concentrations of CGA avoiding problems of false

negatives. In the opinion of clinicians, the highest values that can be encountered are below one million ng/mL (L. Chardon HCL Lyon 2021internal meeting).



Recovery and Dilution:

To control the similarity of the assay response between exogenous CGA and serum CGA, recovery tests were performed. The results show a perfect similarity of the assays whatever the origin of the antigen hence meeting Cisbio internal standards in force (90%-110%).

	Recoveries		
	[CGA] ng /mL	% Recovery Min	% Recovery Max
Sample 1	200	98,1%	105,2%
Sample 2	401	100,8%	106,2%
Sample 3	770	97,5%	100,1%
Sample 4	170	96,4%	105,2%

As NETs can be associated with very high concentrations of CGA, out-of-range samples must be diluted. To ensure that dilutions do not compromise the accuracy of the results provided, cascade dilution tests were performed (1/2; 1/4, 1/8, 1/16).

The results show very good dilution performances with a recovery close to 1.0, a slope interpolation close to 1.0 and a correlation coefficient also close to 1.0

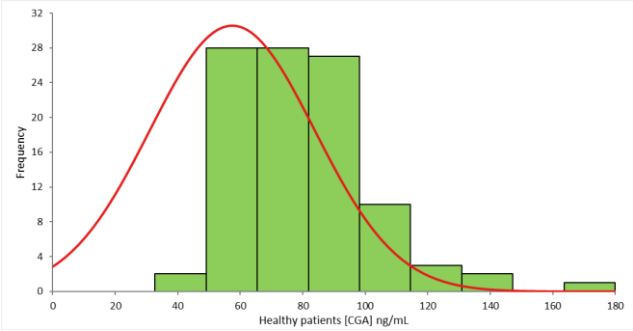
	Dilutions			
	[CGA] ng /mL	Average % Recovery	Interp. Slope	Correl Coef
Sample 1	615	93,8%	1,003	0,999
Sample 2	405	98,7%	1,006	1,000
Sample 3	845	96,0%	1,000	1,000
Sample 4	902	88,5%	0,999	0,999

Biotin interferences:

Due to the resurgence of high serum biotin levels as a result of treatments or dietary supplements, the kit has been evaluated and no significant interference up to biotin concentrations of 600 ng/mL was shown

Normal Values:

Based on a serum library of 101 healthy samples, the distribution of normal values is shown below. The 95th percentile value that can be considered as a cut-off value is estimated at 101 ng/mL



Clinical validation of the kit:

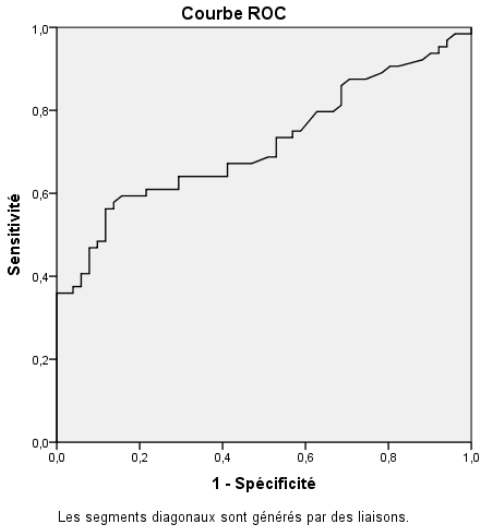
A retrospective study was carried out in partnership with the medical biology laboratory of the Hospices Civils de Lyon under the aegis of Pr Thomas Walter, specialist in neuroendocrine tumors of the digestive system (Report available on request).

The table below provides the precise type of populations studied (positive and negative groups) in terms of pathology or treatments administered.

Variables	NEN patients, n=229		« Control », n=100		p
	n available		n available		
Female, n (%)	229	107 (47%)	100	52 (52%)	0.38
Age in years, median (range)	229	65 (19-93)	100	61 (18-85)	<0.001
Chronic gastritis, n (%)	229	3 (1%)	100	3 (3%)	0.29
Chronic congestive heart failure, n (%)	229	0 (0%)	100	1 (1%)	0.13
Renal deficiency, n (%)	222	39 (18%)	93	17 (18%)	0.88
Inflammatory syndrome, n (%)	147	57 (39%)	63	42 (67%)	<0.001
Concurrent PPI, n (%)	229	44 (19%)	100	40 (40%)	<0.001
MEN1 syndrome, n (%)	227	14 (6%)	100	0 (0%)	<0.001
Concurrent somatostatin analogs, n (%)	229	123 (54%)	100	0 (0%)	<0.001
Biological parameters, median (range)					
CRP in mg/mL	147	3 (1-162)	63	11 (1-130)	0.003
GFR (CKD-EPI) in mL/mn	221	88 (7 to>90)	92	>90 (8 to>90)	0.56
Median CgA by RIA in ng/mL (range)	229	176 (20-431,846)	23	102 (35-2,365)	0.58
Median CgA by ELISA (range)	229	155 (0-227,586)	100	82 (18-1,995)	0.09
CgA ELISA	229		100		<0.001
< 1 ULN (≤94 ng/mL)		87 (38%)		56 (56%)	
1-2 ULN (95-188 ng/mL)		33 (14%)		20 (20%)	
> 2 ULN (>188 ng/mL)		109 (48%)		24 (24%)	

NEN, neuroendocrine neoplasm; GFR, glomerular filtration rate; CRP, C-reactive protein; CgA, chromogranin A; PPI, proton pump inhibitors.

At the end of this study, a ROC curve was performed excluding patients with at least 1 false positive risk factor (heart failure, renal failure, PPI, chronic gastritis and NEM 1) and those on somatostatin analog treatments (risk of false negative). A total of 64 NET patients and 51 Controls were taken into consideration. Other classical parameters were also calculated (Specificity, Sensitivity, AUC, PPV, NPV



- Power of the study: > 80%
- Significance of the study: p<0,05
- Specificity=0,78
- Sensitivity=0,61
- AUC=0,72 (0,62-0,81)
- PPV=0,59
- NPV=0,80

These data confirm those of the international recommendations demonstrating the interest of the assay in the follow-up of GEP-NET (prognostic biomarker and early marker of response to a given treatment)

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