

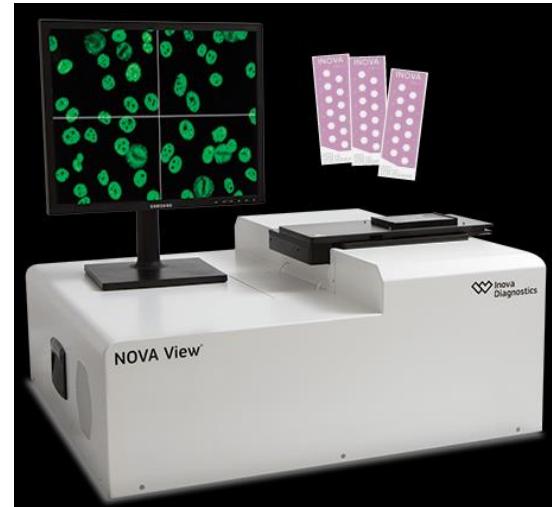
COMPARISON OF TWO ANTI-DOUBLE-
STRAND-DNA ANTIBODIES ASSAYS :
CRITHIDIA LUCILIAE SUBSTRATE READ BY
AUTOMATED FLUORESCENT MICROSCOPE
VERSUS CHEMILUMINESCENT
IMMUNOASSAY.

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Introduction



Anti-DNA: Chemiluminescent immunoassay (QUANTA Flash[®], BioFlash[®]) (CIA).



CL substrate read by an indirect immunofluorescent automated microscope (NovaView[®]) (CLAM)

Methods

- 190 routine samples, which were tested for anti-dsDNA antibodies with both assays. Equivocal CIA results, from 27 up to 35 UI/ml, were considered positive.
- Digitalized pictures linked to indeterminate CLAM results were read by trained biologists.
- CL substrates were also read in the usual way on microscope by operators (CLO).

Table 1 – Qualitative screening agreement


	CLAM vs CIA	CLAM vs CLO	CIA vs CLO
Agreement (%)	82,6 (good)	85.8 (good)	90,5 (good)
Kappa	$\kappa=0,631$ (0.523-0.740)	$\kappa=0.698$ (0.598-0.799)	$\kappa=0.770$ (0.669-0.870)

NovaView®'s software has failed to supply results in 10% of the samples.


A recent decrease of CIA assay positivity threshold by manufacturer resulted in improved qualitative agreement

Table 2 – Qualitative discrepancies analysis

	CLAM+ CIA- CLO+	CLAM+ CIA- CLO-	CLAM- CIA+ CLO-
Followed SLE	3	4	0
Suspected SLE	1	3	0
Others	5	14	3



CLAM assay reported 30 positive results discrepant with CIA. Among these, 21 (70%) were considered negative by CLO. However, 9 were confirmed by CLO, including 3 followed SLE, 1 suspected SLE and 5 other conditions.



One patient was treated for dermatomyositis, the second one had unlabeled neurological disorders, and the third one was treated by infliximab for IBD.

Figure 1: Relationship between quantitative CIA values and CLAM semiquantitative assay results

Blue spots : results confirmed by CLO
red spots : not confirmed.

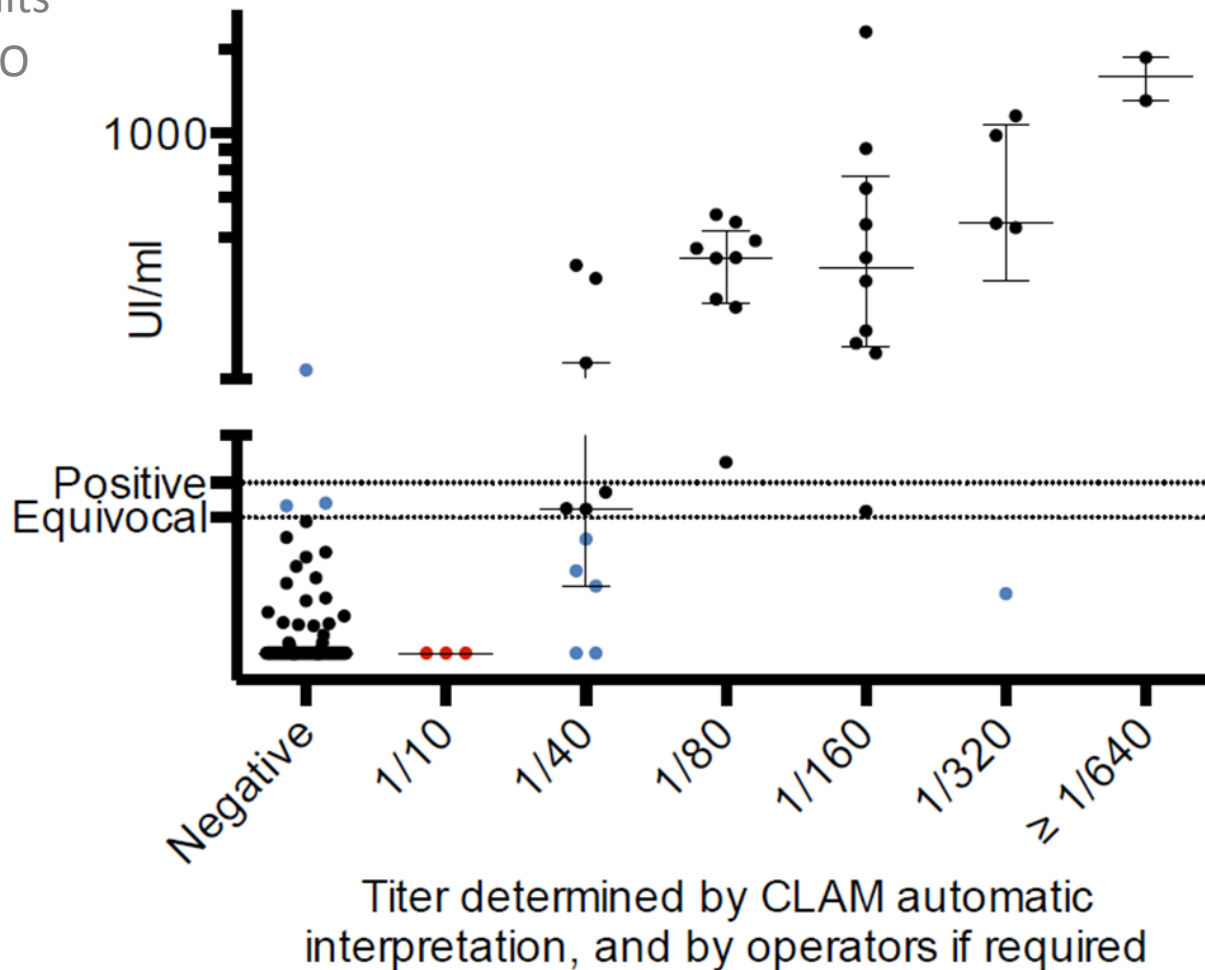


Table 3 – Quantitative agreement, evaluated by Spearman analysis.

	CLAM vs CIA	CLAM vs CLO	CIA vs CLO
Spearman	0.756 (0.675-0.819)	0,967 (0,954-0,976)	0.742 (0.667-0.802)
	p<0.0001	p<0.0001	p<0.0001
N	N=148	N=145	N=186

Spearman analysis demonstrated a good correlation between CIA results and CLAM titers, and between CIA and CLO.

Interestingly, correlation between CLAM and CLO titers was excellent.

Conclusion

- We found good qualitative and quantitative agreements between each couple of studied assays.
- Even if discrepancies were expected between assays based on different analytical principles, we encountered an unexpected high rate of isolated positive CLAM results, suggesting a too low CLAM positivity threshold.
- In order to improve consistency, especially for the CLAM positive specimens showing significant titers below 1:40, we suggest to elevate threshold.
- CIA assay demonstrated good performances and technical advantages (speed and reduced needs for trained personnel).