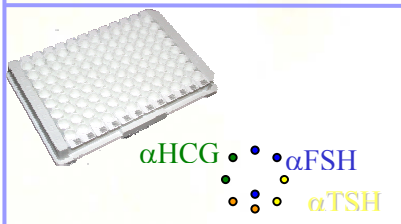
ILISA™: Immuno-Linked Sorbent Array for protein detection and quantification

Perrin,[1] A., Duracher,[2] D., Cleuziat,[2] P. and Mandrand,[1] B.

A low complexity antibody microarray for the detection and quantification of human hormones associated to fertility is described. Four parameters (FSH, TSH, LH, HGC) covering a wide range of concentration in women blood were chosen. Antibodies were spotted in 96-well microtiter plates for a final complexity of 8+1 spots/well. After sample incubation, wells were exposed to alkaline phosphatase labelled antibodies. The addition of a precipitating substrate induced spots coloration those density was correlated to analyte concentration. Specificity was investigated by adding consecutively each purified diluted hormone. Sensitivities were studied with solutions of known protein concentration. Also, comparisons were made with these reached on VIDAS™ immunoassay automate for the same serum dilutions. Testing several tens of human blood samples allowed obtaining convincing correlations between ILISA™ and VIDAS™ for each parameter. The complete range of physiological variations was covered. ILISA™ dynamic range was also enhanced by exploiting enzymatic revelation kinetic and by taking into account spot diameter at the issue of the reaction. Reproducibility, function of spot density, was in all cases below 10%.

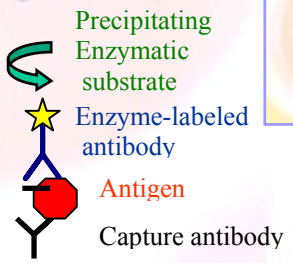
Array Design



Four antibodies spotted in each well (duplicates)

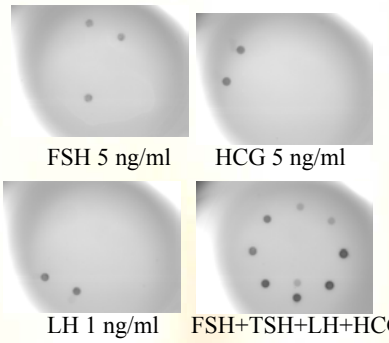
Studied hormonal parameters

TSH (Thyroid Simulating Hormon)
LH (Lutein Hormon)
FSH (Follicule Stimulating Hormon)
HCG (Human Chorionic Gonadotropin)

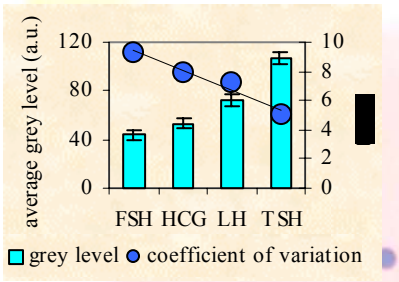


Results

Assay specificity

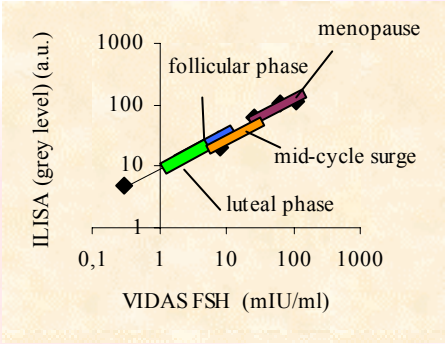


Well-to-well reproducibility vs spot density



Inter-wells variation coefficients increases while spot density decreases (CV<10% in any case)

Correlation to physiological values



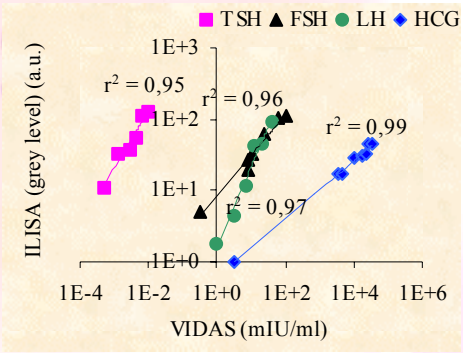
Testing several tens of human sera allows to ensure that ILISA covers the full range of studied physiological parameters

ILISA™ detection threshold/comparison to VIDAS™

[1] pg/ml [2] mIU/ml	TSH	FSH	LH	HCG
ILISA[1]	9	100	26	30
ILISA[2]	0.00013	0.39	0.21	1.7
VIDAS[2]	0.00005	0.1	0.1	2

Sensitivity threshold obtained with ILISA™ on the four parameters with diluted solutions of purified proteins, in comparison to these published for VIDAS™

Correlation between VIDAS™ and ILISA™



Human sera assayed with ILISA™ and with a commercial immunoassay automate (VIDAS™, bioMérieux)

[1] UMR 2142 46 Allée d'Italie 69364 Lyon 07 Tél: + 33 (0)4 72 72 83 60 Fax: + 33 (0)4 72 72 85 33 Email : agnes.perrin@ens-lyon.fr

[2]Apibio15 rue des Martyrs - Zone ASTEC - F - 38054 Grenoble cedex 9 - Tel: + 33 (0)4 38 78 62 33 Fax: + 33 (0)4 38 78 53 Email : apibio@cea.fr