

COMPARISON BETWEEN CYTOTOXICITY, FLOW CYTOMETRY AND TWO ELISA KITS FOR RENAL PATIENT HLA-CLASS I AND II ANTIBODY SCREENING



WELSH BLOOD SERVICE
GWASANAETH GWAED CYMRU
WELSH TRANSPLANTATION AND
IMMUNOGENETICS LABORATORY



R WALTERS, H WILLIAMS
J DOWNING, L HAMMOND, H BASS,
S WINKLER AND C DARKE

Correlation between the four assays

In comparisons between the four techniques the highest correlation coefficient (r) was between QS and CDC ($r=0.74$), followed by: FC/QS ($r=0.72$) and FC/CDC ($r=0.70$); r -values between LAT and FC, QS and CDC were 0.60, 0.57 and 0.56, respectively:

R values between:	LAT ELISA	Flow cytometry	QS ELISA
Flow cytometry	0.60		
QS ELISA	0.57	0.72	
Cytotoxicity	0.56	0.70	0.74

Introduction

As part of our evaluation of solid phase ELISA to detect IgG HLA-Class I and II antibodies we have compared the findings from two cell-based assays with the results from two ELISA kits.

Methods

Sera from 170 consecutive patients on our local renal transplant waiting list were tested by complement dependent cytotoxicity (CDC), flow cytometry (FC), and QuikScreen™ together with B-Screen (QS) (GTI) and Lambda Antigen Tray (LAT™) (One Lambda) ELISA kits.

CDC testing was done with dithiothreitol treated sera against class-II positive cells (ostensibly B-cells) prepared from 36 random blood donors using Dynabeads (Dyna). Test incubation was 30 minutes both pre- and post-complement addition, at 22oC. CDC positive was $\geq 40\%$ cytotoxicity against at least two donors.

Assays by FC (Coulter EPICS XL-MCL) used two pools of 10 EBV-transformed B-cell lines covering all the major HLA-A,B,C,DRB1,DQB1 specificities. Cells were incubated with sera for 15 minutes at 37oC. F(ab')₂ fragment of goat anti-human IgG FITC conjugate was used to identify bound antibody. FC positive was $\geq 5\%$ positive fluorescence over the negative control.

ELISA kits were tested exactly according to the manufacturers' instructions.

Results

Note: Reference to LAT and QS ELISA data includes HLA-Class I and/or Class II antibodies

Test "sensitivity"

The most "sensitive" technique was LAT, which detected 48.8% class-I and/or class-II positive sera, followed by: FC (37.7%), QS (class-I/II positive-25.3%) and CDC (22.9%):

Method	Positive sera identified (n=170)
LAT ELISA	48.8% (n=83)
Flow cytometry	37.7% (n=64)
QS ELISA	25.3% (n=43)
Cytotoxicity	22.9% (n=39)

Of the 170 random sera tested 46.5% (n=79) were negative by all methods.

The LAT ELISA detected: 87.5% of the FC+ sera and of the 8 it failed to detect 7 were FC+ only; all but 1 of the QS+ and all the CDC+ sera. However, 26 (15.3%) sera were positive by LAT only. **Flow cytometry detected:** 67.5% of the LAT+ sera, 97.7% (42/43) of the QS+ sera and all 39 CDC+ sera. Of the 27 LAT+/FC- sera 26 were LAT+ only. Seven sera (4.1%) were FC+ only. **The QS ELISA detected:** 50.6% of the LAT+ sera, 65.6% of the FC+ and 84.6% of the CDC+ sera. No sera reacted by CDC or QS ELISA only.

Detection of HLA-Class I antibodies by ELISA

LAT class-I detected 40 (23.5%) and the QS class-I 25 (14.7%) positive sera; 92.0% of the QS class-I+ were LAT class-I+ ($r=0.67$):

	QS ELISA positive	QS ELISA negative
LAT ELISA positive	23	17
LAT ELISA negative	2	128

Detection of HLA-Class II antibodies by ELISA

LAT class-II detected 72 (42.4%) and the QS class-II 34 (20.0%) positive sera; 94.1% of the QS class-II+ were LAT class-II+ ($r=0.52$):

	QS ELISA positive	QS ELISA negative
LAT ELISA positive	32	40
LAT ELISA negative	2	96

Comment

These findings show considerable variability in both the sensitivity and equivalence of the four methods.

Our flow cytometry assay detected most or all positive sera identified by the other techniques with the exception of the highly sensitive LAT ELISA.

However, the LAT ELISA (class-I/II) and FC techniques were comparable, since "outsider" positives were largely confined to technique specific reactions; LAT giving the greatest number of these.

The significance of the LAT ELISA only positive reactions remains to be identified.

These findings show that our antibody screening method using B-cells lines and flow cytometry is still the method of choice for the initial detection of likely IgG HLA antibodies.