Cytotoxic crossmatching method variability – a view from UK NEQAS for H&I sample testing

Deborah Singleton, Susan Corbin, Judith Worthington & Chris Darke

Welsh Blood Service
Gwasanaeth Gwaed Cymru

UKNEQAS for HISTOCOMPATIBILITY and IMMUNOGENETICS
UNITED KINGDOM EXTERNAL QUALITY ASSESSMENT SERVICE

WELSH TRANSPLANTATION AND IMMUNOGENETICS LABORATORY

Introduction

The UK NEQAS for H&I's Cytotoxic Crossmatching scheme assesses participants' ability to determine cell/serum cytotoxic crossmatch (XM) status. We have analysed - from participant information provided during 2013 - the considerable variation continually seen in XM methodology.

Scheme material

Labs receive 10 blood samples and 40 sera/year and can register for PBL/T-cell and/or B-cell XM assessment. In 2013 there were 63 participants; all used PBL/T-cells, 49 also used B-cells.

Results

Of the 63 PBL/T-cell labs, 39 used T-cells, 18 used PBLs and 6 used both. Pre-complement (C) incubation times ranged from 20-60 min for PBL/T-cells and 30-60 min for B-cells (Table 1). Post-C incubation times ranged from 30-120 min for both PBL/T-cells and B-cells (Table 1). There were 15 different PBL/T-cell and 13 different B-cell combinations of pre- and post-C incubation times, with 30, 60 min being the most frequent combination for both cell populations (n=24 and 17), respectively.

Table 1:CDC XM Pre and post complement incubation times

Incubation Time (mins)	Pre-Complement		Post-Complement	
	PBL/T Cells	B Cells	PBL/T Cells	B Cells
20	1	–	–	–
30	30	22	2	1
35	1	1	–	–
40	2	2	1	1
45	10	9	3	3
60	17	11	36	26
75	–	–	–	2
90	–	–	11	10
105	–	–	1	–
120	–	–	6	2

3 of the 49 labs reporting both PBL/T-cell and B-cell results used different incubation times for PBL/T-cells compared to B-cells. All 3 decreased their post-C incubation time for B-cells, and 1 also decreased their pre-C incubation time.

6 different methods were used to visualise cell death; ethidium bromide was the most common (n=26).

Performance assessment was based on 75% consensus pos/neg reporting for all serum-cell combinations, and was performed separately for PBL/T-cells and B-cells. UK and non-UK labs sometimes receive different blood samples, this created a total of 72 cell-serum combinations. There were 7/72 (9.7%) PBL/T-cell and 11/72 (12.5%) B-cell results that could not be assessed (Table 2). The consensus range was 50-74% - further emphasizing test variability.

Table 2: Non-assessed CDC crossmatch results

Sample	Number of non-assessed crossmatch results (<75% consensus)	
	PBL/T-Cell (% Consensus)	B-Cell (% Consensus)
2A01	0	0
2A02	1 (67%)	0
2A03	1 (60%)	1 (68%)
2A04	0	2 (65%, 74%)
2A05	0	0
2A06	0	2 (65%, 69%)
2A07	1 (59%)	1 (56%)
2A08	1 (62%)	1 (56%)
2A09	2 (57%, 70%)	2 (50%, 70%)
2A10	1 (64%)	0

Comment

Cytotoxic crossmatching has been used by H&I laboratories for transplant compatibility assessment for several decades. Despite this, considerable variation in methodology still exists. Thus, 'technical variation' is likely to contribute to the number of non-assessed crossmatches.

Further information

Full information on all UK NEQAS for H&I schemes is available at **www.neqashandi.org** or contact the Scheme Manager - Deborah Singleton
E-mail: **ukneqashandi@wbs.wales.nhs.uk**