Assessment of Inter-Laboratory Variability for Flow Cytometric Crossmatch Testing: Lessons Learned from Proficiency Testing Surveys

On Behalf of the PT committee
Mary Carmelle Philogene[1], Olga Timofeeva[2], Idoia Gimferrer[3], Reut Hod Dvorai[4]
[1] Virginia Commonwealth University, [2] UCLA, [3] BloodWorksNW [4], SUNY Upstate Medical University

Introduction

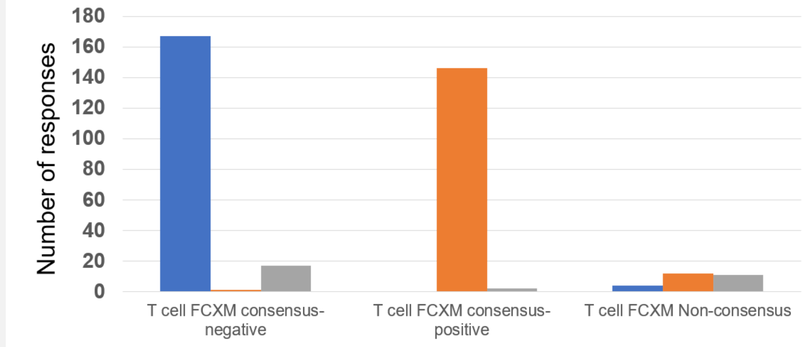
Detection of antibody directed against human leukocyte antigens (HLA) using a combination of flow cytometric crossmatch (FCXM) and antibody tests, is an important responsibility of Histocompatibility laboratories. Proficiency testing (PT) surveys utilize the results of these assays to assess concordance across multiple laboratories. We aimed to evaluate the degree and nature of inter-laboratory FCXM and antibody assay variability by analyzing ASHI PT antibody and crossmatching (AC) survey results over a 6-year period.

Methods

FCXM results were graded based on 80% consensus. Results that did not agree with consensus were graded "Discrepant". Results that did not reach a consensus were not graded. HLA class I and HLA class II antibody specificities were graded separately. An antibody specificity reported by ≥90% of participants, either positive or negative, reached consensus. For the analysis of FCXM-DSA correlation (DSA defined as antibodies against "donor" cells HLA antigen), DSA were considered consensus-positive if the antibody specificity was reported by ≥90% of participating laboratories. DSA were considered consensus-negative if the antibody specificity was reported by ≤10% of participating laboratories. A T cell FCXM result was correlated with presence of consensus class I DSA, while a B cell FCXM was correlated with presence of consensus class I and/or class II DSA. Kit bias, defined as specificities that reach a consensus (≥90%) but display less than 60% concordance among participants using one specific kit, was assessed .

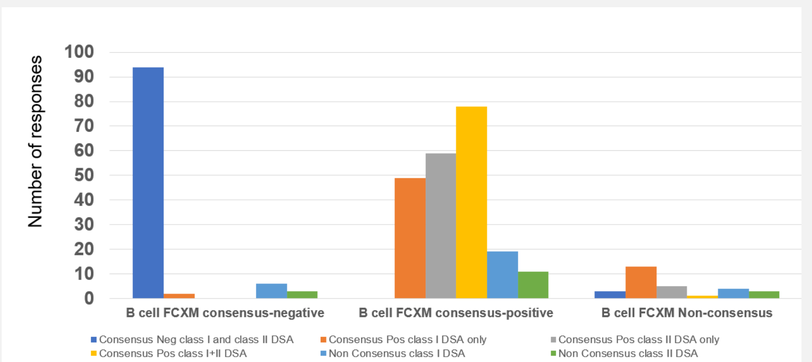
Results

Figure 1: Correlation between HLA Class I DSA and T cell FCXM



DSA status	T cell FCXM Consensus-Neg (≥80%)	T cell FCXM Consensus-Pos (≥80%)	T cell FCXM non-consensus
Consensus (≥ 90%)Neg class I DSA	167	0	4
Consensus (≥ 90%)Pos class I DSA	1	146	12
Non consensus class I DSA	17	2	11

Figure 2: Correlation between HLA Class I and Class II DSA and B cell FCXM



DSA status	B cell FCXM consensus-negative	B cell FCXM consensus-positive	B cell FCXM Non-consensus
Consensus Neg class I and class II DSA	94	0	3
Consensus Pos class I DSA only	2	49	13
Consensus Pos class II DSA only	0	59	5
Consensus Pos class I+II DSA	0	78	1
Non Consensus class I DSA	6	19	4
Non Consensus class II DSA	3	11	3

Figure 3: DSA distribution in AC survey between 2018 and 2023

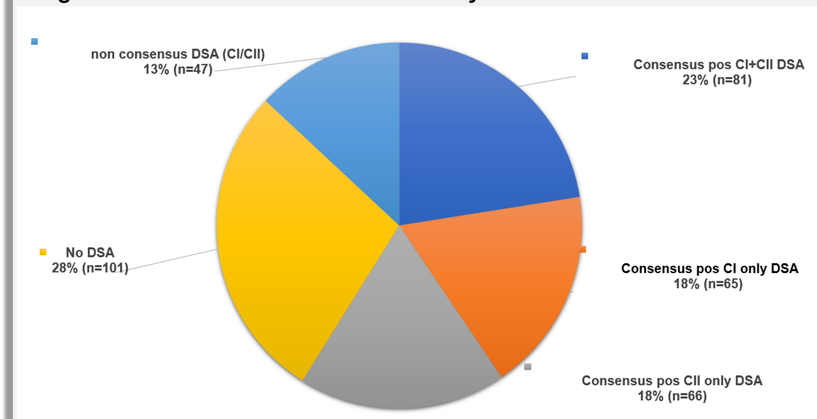


Table 1: Summary of FCXM grading between 2018-2023

Flow crossmatch	Total combinations	100% consensus Negative	80-99% Consensus Negative	100% consensus Positive	80-99% Consensus Positive	Non consensus
T cell	353	129 (36.5%)	52 (14.7%)	100 (28.3%)	44 (12.5%)	28 (7.9%)
B cell	341	85 (24.9%)	14 (4.1%)	158 (46.3%)	51 (15.0%)	33 (9.7%)

Conclusions

There is a great consensus (>90%) for the FCXM test among laboratories (Table 1).

Discrepant grades may be linked to laboratory's performance, whereas non-consensus results may reflect the variability of the assays. Some of the non-consensus results were associated with DSA that did not reach consensus or with DSA against low expression loci.

While PT surveys may not allow investigation of outcome, PT data provides laboratories with relevant information to evaluate their laboratory performance and enhance their ability to provide accurate results that impact transplant outcome.

Acknowledgements

Proficiency testing committee members who perform evaluation and review of data

Cheryl Hartman, PT Program Administrator

Contact: Mary.Philogene@vcuhealth.org

Authors have no conflict of interest.