STATISTICAL MODELING TO ASSESS VARIATION BETWEEN TESTS IN SINGLE ANTIGEN BEAD ANALYSIS

- **The study shows proof of concept that statistical modeling can be employed to facilitate auto-reporting of SAB I/II data.**
- **Analysis of the distribution of the top 20 bead MFI's in a new test result compared to two prior results can be used to auto-result negative tests.**

Hypothesis: We hypothesize statistical modeling can be employed to determine the result of a new single antigen bead test in comparison to two prior tests. The results of new tests can be auto-resulted if statistical modeling identifies no change in bead strength and specificity as compared to historic results.

Methods: For this quality improvement project, a data set of SAB I/II (n=280; One Lambda, Class I lot 15, Class II Lot 16) were analyzed to determine the percentage of negative tests, and frequency/characteristics of spuriously reactive beads defined as up to 2 positive beads >1000 MFI in the absence of CREG or broad group reactivity, and considered false positive. Data are acquired from sera batched in a 96-well plate, with Fusion acquisition software, and data analysis is performed in Histotrac. A second set of SAB I/II data was selected for negative (n=9 class I/II) or 'spurious' (n=21 class I/II) reactivity. Statistical models were used to assess whether the variation in distribution of bead strength and specificity in a new result were significantly different (outside 1 or 2 standard deviations; SD) from the two prior results.

Results and Figures:

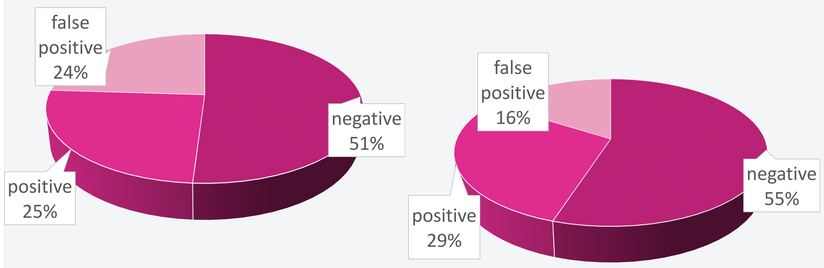

Figure 1. A large proportion of SAB test results are reported negative after review. SAB results are negative in 51% and 55% of class I or II tests, respectively (positive threshold, 1000 MFI). An additional 24% of class I and 16% of class II are false positive due to spurious reactivity, and reported negative after expert review.

Figure 2. Certain beads contribute to false positive reactions with high frequency. For example, B*15:12 or DPB1*01:01/DPA1*02:01 were above 1000 MFI more than 10 times, without other B or DP locus positive beads, affecting more than 7% of tests.

			MFI >1000	Review Type	Final Result
Patient 1	Test 1	Class I	none	Expert Reviewed	Negative
		Class II	none		Negative
	Test 2	Class I	none	Expert Reviewed	Negative
		Class II	none		Negative
	Test 3	Class I	none	Statistical Model	Negative
		Class II	none		Negative
Patient 2	Test 1	Class I	A11	Expert Reviewed	Negative
		Class II	none		Negative
	Test 2	Class I	A11	Expert Reviewed	Negative
		Class II	none		Negative
	Test 3	Class I	A11	Statistical Model	Negative
		Class II	none		Negative
Patient 3	Test 1	Class I	B75	Expert Reviewed	Negative
		Class II	DP9 DP6		Negative
	Test 2	Class I	B75 B37	Expert Reviewed	Negative
		Class II	DP9		Negative
	Test 3	Class I	B75	Statistical Model	Negative
		Class II	DP9		Negative
Patient 4	Test 1	Class I	none	Expert Reviewed	Negative
		Class II	none		Negative
	Test 2	Class I	none	Expert Reviewed	Negative
		Class II	DP1		Negative
	Test 3	Class I	none	Statistical Model	Negative
		Class II	DP1		Negative
Patient 5	Test 1	Class I	none	Expert Reviewed	Negative
		Class II	none		Negative
	Test 2	Class I	none	Expert Reviewed	Negative
		Class II	DQ9		Negative
	Test 3	Class I	none	Statistical Model	Negative
		Class II	DQ8 DQ9		Negative

Table 1. Examples of longitudinal SAB results from 5 of 10 patients. Tests 1 and 2 were analyzed by a trained expert. Test 3 was analyzed in comparison to the two prior tests using statistical modeling. All results were reported negative.

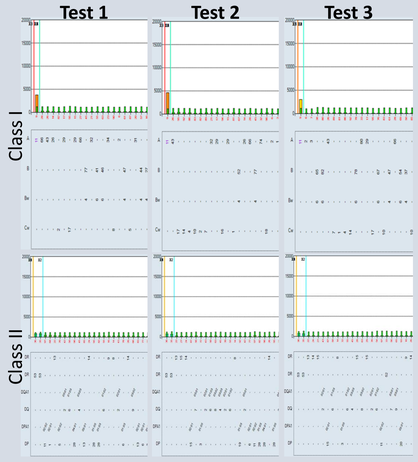

Figure 3. Examples of test results with spurious reactivity. Example histograms are from Patient 2 shown in Table 1.

Model	
Positive	Did the positive beads change in the most recent test as compared to the two previous tests?
Positive_strength	Are the MFIs for positive beads in the most recent test within 2 SD (95% CI) of the MFI from the two previous tests?
Top20	Are the top 20 beads in the most recent test different from the two previous tests?
Top20_MFI	Are the MFIs of beads in the top 20 in the most recent test within 2 SD (95% CI) of the MFI from the two previous tests?
Top20_version1_2sd	Are the distribution of MFIs/strengths for the top 20 beads in the most recent test within 2 SD (95% CI) of the distribution from the two previous tests? Calculated the 95% CI for each of the top 20 beads individually and plot the 95% CI distribution.
Top20_version1_1sd	as above, but using only 1 SD (68% CI).
Top20_version2_2sd	same as 'top20_version1_2sd', but defined the distribution by calculating the 95% CI for the top beads, and plot a 95% CI distribution.
Top20_version2_1sd	as above, but using only 1 SD (68% CI).

Table 2. Statistical Models Tested. multiple statistical models were tested to determine the best at identifying variation in distribution of bead strength and specificity between a new test, and two prior tests

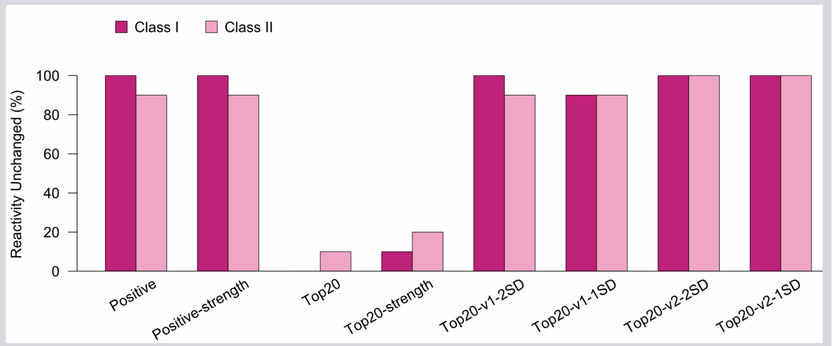

Figure 4. Percentage of samples defined as having unchanged strength (MFI) and specificity compared with the two prior results for Class I and Class II, respectively. Individual model definitions of unchanged are described in Table 2. Plotting the distributions of the 95% confidence intervals over the top 20 bead MFI's was most robust showing the majority of new results were within 1 SD of the two prior results, and all were within two SD.

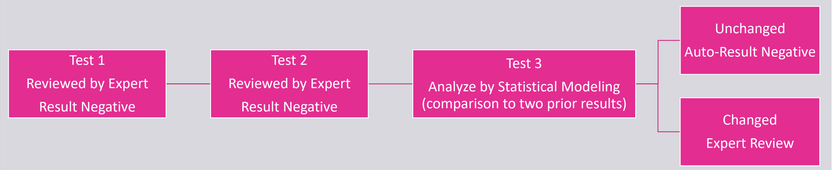

Figure 5. Proposed flow chart for auto-resulting or expert review of longitudinal Single Antigen Bead tests. At least 2 samples are reviewed by a histocompatibility expert to establish a baseline. The third test may be analyzed by statistical modeling, and if the result is statistically unchanged from the prior two results, it may be auto-resulted as Negative. However, if statistical modeling shows a change from the two prior tests, the result must be reviewed and resulted by an expert.

Harry Pickering[1], Elaine F. Reed[1], Michelle J. Hickey[1]

[1]UCLA Immunogenetics Center, Department of Pathology & Laboratory Medicine

David Geffen School of Medicine at Los Angeles, CA, USA