The effects of sub-optimal sample handling on multiple complement measurements: Is there a potential signature of mishandling?

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Abstract

Introduction: The demand for complement testing has grown substantially in recent years. This demand is related to off target anaphylactoid responses, to increased rates of drug clearance, and to the advent of complement targeting therapeutics. The wider adoption and utility of complement testing has been hampered by the potential for errors caused by pre-analytic issues. For example, storage at even -20° can allow for *ex vivo* increases in activation fragments and potential drops in functional activity. The importance of assessing and mitigating these potential confounding pre-analytic issues is key to achieving consistent complement results.

Methods: To look at the sensitivity of complement draw site issues we have tested commercially sourced specimens. The potential effects were investigated for a number of complement assays including, CH50, C3a, sC5b-9, CICs among others. Multiple methods were also utilized, including two different methods for testing complement function (hemolytic and ELISA) and two different methods for activation fragment assessment (ELISA and Luminex). To test for the effects of the site handling, specimens were also collected within Exsera and the data were recorded from across the same assays. To further test for storage stability, an Exsera-sourced specimen was subjected to different storage and handling conditions prior to testing.

Results: In general, the individual activation fragments demonstrated greater differences in the mean between the two specimen sets than did the functional assays. Of all the analytes tested, the classical/lectin pathway marker C4a demonstrated the greatest difference in mean values from the specimens handled within Exsera and those processed commercially.

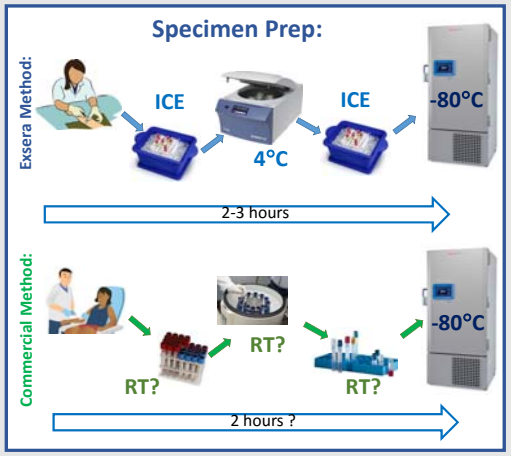
The stability testing demonstrated that some complement analytes were largely unaffected by the handling, while other analytes were profoundly affected. C4a proved to be one of the analytes most affected and even though the specimen tested was from a normal healthy individual, storage for 4 hours at room temp led to a 120% increase in the measured C4a value. Such *ex vivo* increases could be expected to be larger in patients with disease or drug-related ongoing complement activation.

Conclusion: These results expanded on previous data from the 1990 study about the temperature sensitivity of four complement measures. These data also indicate that proper handling is possible, but may require improved site management and training plans. This data suggests the possibility of being able to measure select complement analytes to investigate suspected *ex vivo* complement consumption. The potential to have such testing reveal improper specimen handling could have utility in the field.

Methods

To validate complement testing for Exsera BioLabs, specimens from normal individuals were purchased from a commercial specimen provider and tested per laboratory SOPs. After expectedly high values were found for a couple tests, separate testing was undertaken. For this second set of tests specimens were prepared at Exsera BioLabs. Under approved IRB specimens from normal individuals were drawn and those specimens were processed by the Exsera BioLabs Technical Staff. The differences in the specimen preparation methods are shown in the following figure.

	Exsera		Commercial	
	%	Age	%	Age
Females	62%	35	50%	37
Males	38%	36	50%	39
African American	0%		73%	
Caucasian	65%		21%	



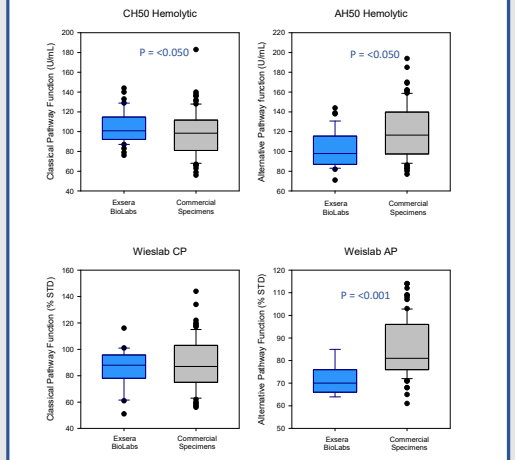


Figure 1-4: Comparison of values from four functional tests. Two hemolytic, RBC lysis assay, CH50 and AH50 were tested. In addition two ELISA methods were tested, the Weislab Classical Pathway (CP) and Alternative Pathway (AP). P values reflect t-tests statistical analysis.

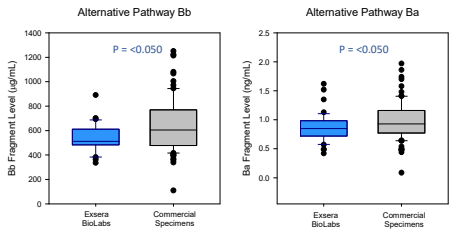


Figure 5 &6: Comparison of measurement for the alternative pathway markers Bb and Ba. Both fragments are produced from Factor B cleavage by Factor D.

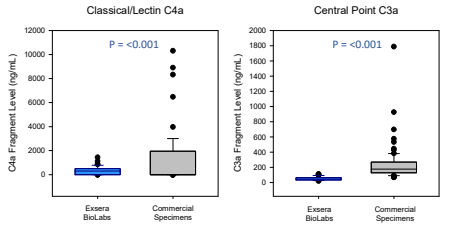


Figure 7 & 8: Comparison of measurement for the classical/lectin pathway fragment C4a and the central point C3. Both demonstrated increases in the commercially prepared specimens

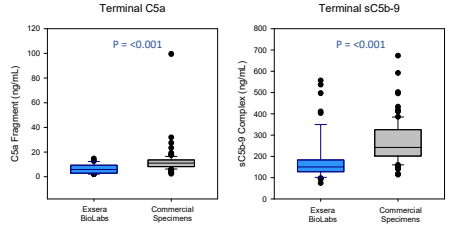


Figure 9 & 10: Comparison of measurement of the terminal pathway markers C5a and sC5b-9. The C5a levels are additionally important because C5a is an anaphylatoxin and is strongly pro-inflammatory. The soluble membrane attack complex was also increased in the commercial method for specimen preparation.

Data Table

Analytes	Source	Mean	St Dev	High	Low
CH50	Exsera	104	16	144	76
	Commercial	97	22	183	56
AH50	Exsera	101	18	144	71
	Commercial	120	26	194	77
Wieslab CP	Exsera	86	15	116	51
	Commercial	89	15	116	51
Wieslab AP	Exsera	72	7	85	64
	Commercial	85	13	114	61
C4a	Exsera	504	279	1,443	125
	Commercial	3,334	2,435	10,312	300
C3a	Exsera	53	23	113	24
	Commercial	239	225	1,788	69
C5a	Exsera	6.4	3.6	14.7	2.0
	Commercial	12.6	11.0	99.5	2.4
sC5b-9	Exsera	183	106	557	74
	Commercial	266	102	673	114

Conclusions:

While these data sets were from separate subjects, it was still possible to compare the results at the population level. Ten different complement tests were performed on both populations by the same methods and only one test failed to demonstrate a statistical difference. The activation fragments, C4a, C3a and C5a demonstrated the greatest difference, but importantly the complex sC5b-9 also demonstrated a difference between the specimen sets. Interestingly, the two tests of alternative function demonstrated an unexpected increase in function for the commercial specimens prepared without wet ice. It is generally believed that sub-optimal specimen handling leads to *ex vivo* activation of complement which is expected, in turn, to lead to a decrease in function measurement. What may be occurring in these tests is likely related to both the way the alternative pathway is activated and how it is controlled. Alternative pathway activity can start from C3b created by classical/lectin activation, which appears to be created during sub-optimal handling. Secondarily, Factor H is key for control of the alternative pathway, so the possibility exists that the increased alternative pathway function may reflect dissociation of Factor H.

The dramatic increase in C4a was the greatest (mean 504 to 3,334 ng/mL). This suggests that a measurement of C4a could be utilized to gauge the quality of specimen preparation.

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