University of Colorado Anschutz Medical Campus

ICW Poster 052

What is Normal for Complement? Testing of 30 Complement Analytes across 100 Normal Individuals

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Abstract

With complement as a treatment target, it is important to understand what constitutes normal levels of the components. Without knowing what is normal and how different analytes compare, we don't truly know how a disease or a treatment is affecting those levels. With new reagents, techniques and components being quantified it is also necessary to update previous data to reflect these changes. To address this need we tested over 100 self-described "normal" adults using 36 tests each. These tests represent at least five different assay modalities including most of the common methods for measuring complement. Specifically, we tested hemolytic function, nephelometry and ELISA as well as the newer Luminex multiplex. The specimens utilized were aliquoted for single-use vials so the testing was not influenced by the number of freeze/thaw cycles. The population tested was 54% female, 46% male. The mean age was 37 overall with a range of 19 to 78 years of age. The populations was 54% African American, 46% Caucasian, 5% Hispanic and 4% Asian. We found the number of individuals with low levels of MBL was 17% which is in line with previously reported percentages. With this population and testing we were able to review not only the normal values and distribution, but we were also able to compare complement levels across gender and age. In addition it allowed comparison of the interplay between different complement components. Comparisons of particular interest include comparisons of C3 total with C3 intact, as well as with the C3a and iC3b activation products. Similarly we were able to look at correlation of Factor B with both Bb and Ba fragments, as well as comparing terminal pathway activation markers C5a and sC5b-9. Comparing C5a and sC5b-9 also offers an opportunity to investigate sC5b-9 as a surrogate marker for C5a production since C5a clears rapidly. This testing of normal values is of foundational utility for the interpretation of patient or study specimens.

Demographics

	Number	Percent
Females	63	54%
Males	54	46%
Age	19y -78y	37y
African American	58	50%
Caucasian	41	35%
Biracial	9	8%
Hispanic	6	5%
Asian	5	4%

All specimens collected in full compliance with applicable regulations and local IRB.

Methods

Specimens were commercially purchased or collected in compliance with approved IRB protocol by Exsera. The collected specimens complied with laboratory requirements, including freezing at -70°C or lower within two hours of draw. Before freezing all specimens were aliquoted, so all testing could be performed on the first thaw. Function testing was performed on serum; other testing utilized EDTA plasma. The exception is C1-INH function which was run on citrate plasma. All tests were optimized and validated within Exsera, including those that involved commercially available reagents. The Exsera optimizations include but are not limited to: standard curve adjustments to Millipore Luminex reagents, creation and characterization of standard curves for the Wieslab AP and MP assays, and a number of adjustments to specimen dilutions and incubations times. These changes were made to improve performance, make tests comparable with international levels or to better meet USA regulatory requirements for clinical testing..

References

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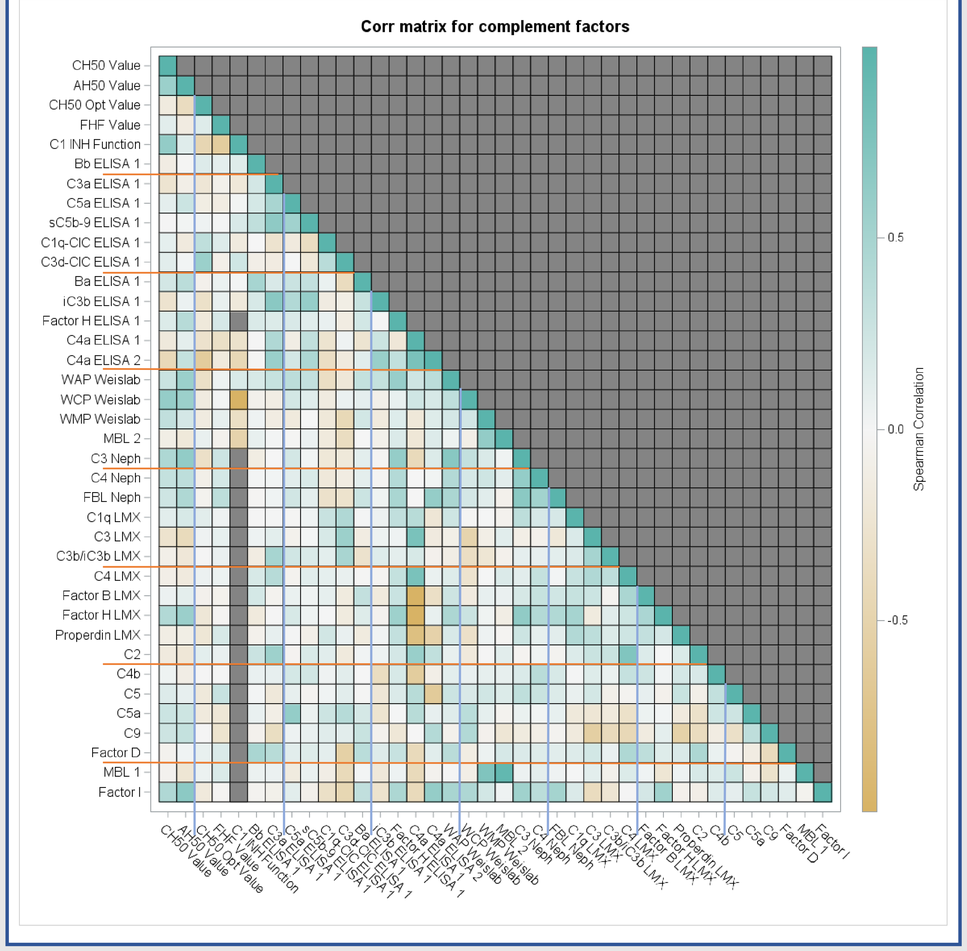
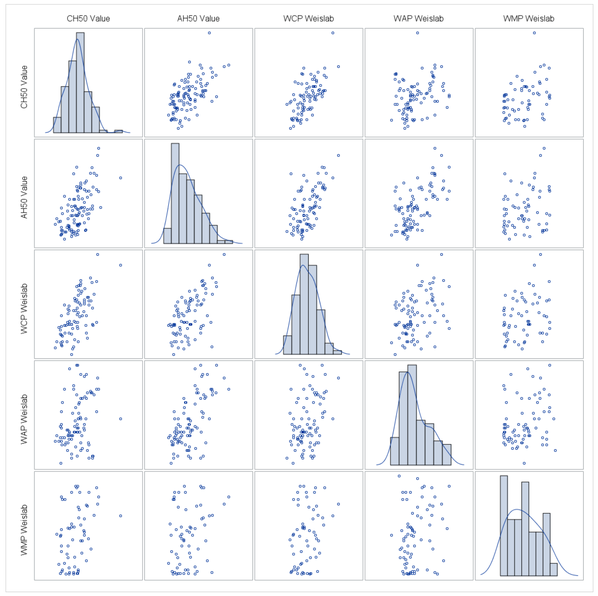


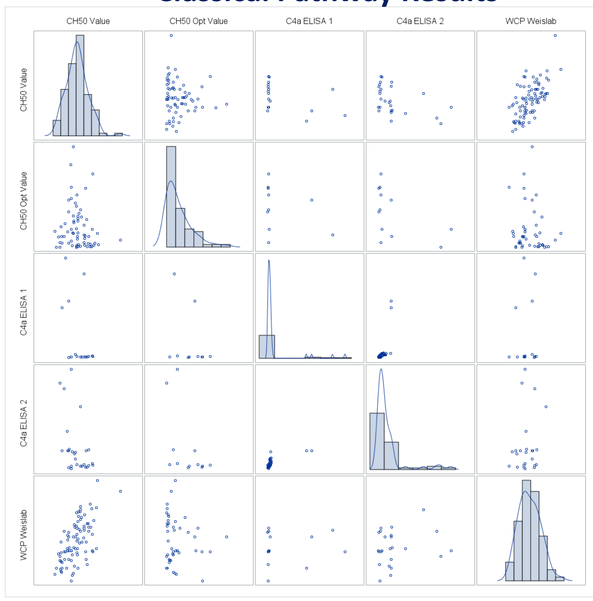
Data for individual Assay Results

Test	Units	Method	Supplier	Overall Mean	Overall St Dev	Males Mean	Males St Dev	Females Mean	Females St Dev
CH50	U/mL	Hemolytic	In House	99.8	21.0	92.5	18.2	104.7	21.3
AH50	U/mL	Hemolytic	In House	114.1	25.3	106.0	22.2	120.2	25.2
Factor H Function	% Lysis	Hemolytic	In House	20.3	12.1	20.2	10.8	20.1	12.3
C1-INH Function	% Std	Chromogenic	DiaPharma	105.6	18.3	97.0	16.2	104.0	18.3
Bb	mcg/mL	ELISA	Quidel	0.9	0.3	1.0	0.3	0.9	0.3
C3a	ng/mL	ELISA	Quidel	171.8	201.8	184.7	102.4	234.6	206.4
C5a	ng/mL	ELISA	Quidel	10.4	9.6	10.3	4.7	13.2	9.8
sC5b-9	ng/mL	ELISA	Quidel	232.4	111.2	261.3	116.4	254.0	111.3
Ba	ng/mL	ELISA	Quidel	593.5	200.7	638.1	222.3	658.6	182.1
iC3b	ng/mL	ELISA	Quidel	17.7	18.2	22.9	17.0	22.9	18.4
Factor H ELISA	mcg/mL	ELISA	Quidel	205.2	47.2	203.0	48.3	207.4	46.5
C4a I	ng/mL	ELISA	Quidel	462.7	1044.8	1896.9	2076.7	98.1	1044.8
C4a	ng/mL	ELISA	BD Pharmingen	1450.9	2089.6	1418.7	727.1	2992.1	2164.7
Wieslab AP	% Std	ELISA	Eurodiagnostica	82.3	12.9	83.3	12.7	83.2	12.6
Wieslab CP	% Std	ELISA	Eurodiagnostica	88.2	19.2	80.6	14.7	94.6	18.8
Wieslab MP	% Std	ELISA	Eurodiagnostica	64.9	32.4	67.5	34.9	59.8	32.0
MBL	mcg/mL	ELISA	BioPorto	1505.8	1074.0	1680.4	1089.1	1294.2	1058.2
C3 Level	mg/dL	Nephelometry	Beckman	121.6	23.7	115.8	23.1	127.4	24.4
C4 Level	mg/dL	Nephelometry	Beckman	31.9	9.9	31.9	11.3	32.0	9.9
Factor B Level	mg/dL	Nephelometry	Beckman	49.1	12.0	45.7	11.9	52.3	12.2
C1q Level	mcg/mL	Luminex	Millipore	103.5	22.8	104.5	24.4	102.6	22.7
C3 Level	mcg/mL	Luminex	Millipore	692.3	245.0	838.5	240.7	546.2	240.2
C3b&iC3b	mcg/mL	Luminex	Millipore	30.4	45.2	23.7	18.2	37.0	46.9
C4 Level	mcg/mL	Luminex	Millipore	216.9	53.7	231.1	47.5	202.7	54.5
Factor B Level	mcg/mL	Luminex	Millipore	150.8	34.1	160.3	34.6	141.2	32.1
Factor H Level	mcg/mL	Luminex	Millipore	231.7	39.3	217.6	36.5	245.8	39.1
Properdin Level	mcg/mL	Luminex	Millipore	23.4	5.0	25.6	5.0	21.1	5.1
C2 Level	mcg/mL	Luminex	Millipore	51.8	85.1	43.9	54.1	59.8	88.1
C4b	mcg/mL	Luminex	Millipore	7.0	5.7	7.5	5.7	6.5	5.9
C5 Level	mcg/mL	Luminex	Millipore	79.4	14.0	85.6	11.7	73.2	14.4
C5a	ng/mL	Luminex	Millipore	11.0	3.3	10.5	3.3	11.6	3.4
C9 Level*	mcg/mL	Luminex	Millipore	61.6	33.0	44.1	24.7	79.1	34.0
Factor D	mcg/mL	Luminex	Millipore	2.3	1.0	2.5	1.2	2.1	0.9
MBL	mcg/mL	Luminex	Millipore	1.4	1.5	1.8	1.7	1.0	1.6
Factor I	mcg/mL	Luminex	Millipore	39.3	10.8	35.2	8.4	43.4	11.0

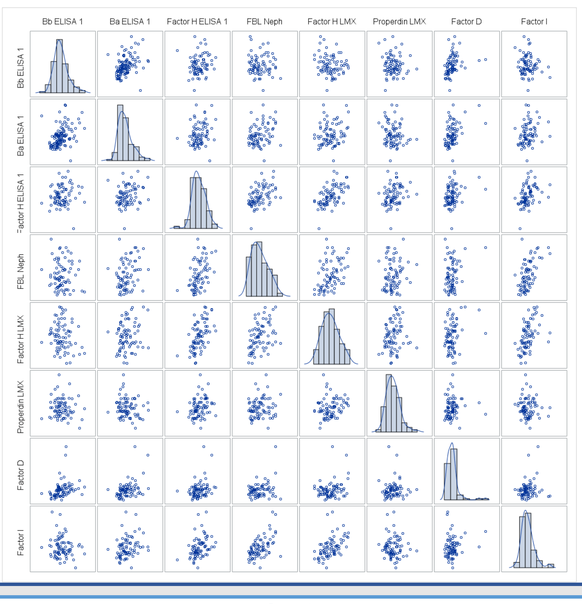
Functional Assays



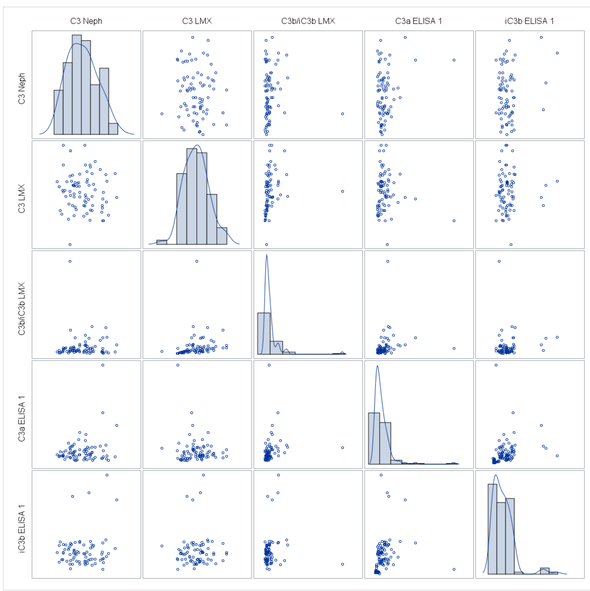
Classical Pathway Results



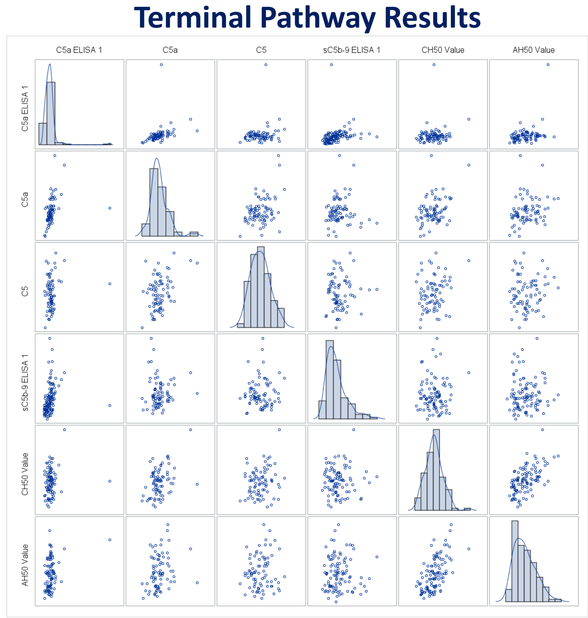
Alternative Pathway Results



C3 Related Results



Terminal Pathway Results



Conclusions

While individual or related collections of analytes have been measured and compared across populations, analysis of the relative levels and correlations is limited. The limit of this analysis is only 120 normals were measured, but this study still adds to the information base. The strongest positive correlation was seen for the two methods of measurement of Mannose Binding Lectin. The Wieslab classical pathway assay had a strong negative correlation with C1-INH function while the correlation was in the reverse direction with the hemolytic CH50, an interesting discordance between the two measurements of classical pathway function. It is unclear if this difference is true and reflects a difference in the assays, but it needs to be confirmed by a larger study. More unexpected is the correlation seen between Factor B level and C4a level as measured by Elisa 2. It was also unforeseen that there would be a negative correlation between the alternative pathway Bb and Ba fragment levels.

Overall the correlations were not as strong as might have been expected, but there are some interesting insights that might allow for further insight into connections between the pathways. Further testing and a larger specimen set would be necessary to determine the nature of these correlations. This is a relatively small study and contains just normals; expanded specimen pool and inclusion of disease state specimens would be important next steps.

Abbreviations

LMX, Luminex	Neph, Nephelometry/Turbidity	WCP, Wieslab Classical Pathway
ELISA 1, Quidel Corp	MBL 2, BioPorto	WAP, Wieslab Alternative Pathway
ELISA 2, BD Pharmingen .	MBL 1, Millipore Luminex .	MP, Wielsab Lectin/MBL Pathway