Assay Panel for Diagnosis and Monitoring Treatment of TA-TMA in HCT Recipients

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INTRODUCTION

Transplant Associated Thrombotic Microangiopathy (TA-TMA) is a complication in hematopoietic cell transplant (HCT) patients due to overactivation of the complement cascade. TA-TMA can result in injury to multiple organs including the kidneys and requires rapid initiation of treatment. Markers used to diagnose and monitor treatment of TA-TMA include a high-risk injury marker sC5b-9, the soluble form of terminal complement complex (TCC), and CH50 that measures potential complement activity. Eculizumab is a monoclonal antibody drug that inhibits cleavage of C5 in the complement cascade and has been used to treat TA-TMA.

An assay to measure sC5b-9 was developed and validated, and an assay for CH50 was validated, to aid in early diagnosis and treatment of TA-TMA. These assays are performed rapidly and results provided the same day. An assay to measure free eculizumab concentration in the blood was developed and validated to assist in monitoring patient treatment.

MATERIALS AND METHODS

The sC5b-9 ELISA assay measures the soluble TCC in EDTA plasma using a monoclonal antibody specific for the complex. The CH50 total complement assay is a liposomal turbidimetric assay that measures CH50 in serum. The Eculizumab ELISA measures levels in serum and is specific for the free form of eculizumab, not bound to endogenous C5.

In validation, the assays demonstrated exceptional accuracy, precision, and sensitivity in accordance with guidelines recommended by the New York State Department of Health, College of American Pathologists (CAP), and Clinical and Laboratory Standards Institute (CLSI) to establish the analytical specificity, linearity and dynamic range, analytical sensitivity (limit of detection and lower limit of quantification), intra- and inter-assay precision (reproducibility), and analytical accuracy of the test method.

RESULTS

- Validation of the eculizumab ELISA indicated an LLOQ of 4 µg/mL, 100% accuracy, and precision CVs ≤ 10%.
- The sC5b-9 lower limit of quantification (LLOQ) was 81ng/mL, the reference range was ≤ 256 ng/mL correlating with the published normal of ≤ 244 ng/mL and the CVs for precision were ≤ 19%.
- The CH50 LLOQ was 13 U/mL, the reference range was 36-95 U/mL and the CVs for precision were ≤ 5%.

Clinical samples previously analyzed for sC5b-9 were also analyzed for eculizumab and CH50 to determine correlation of the results. Samples with sC5b-9 levels > 256 ng/mL had eculizumab levels that were below the recommended trough level of 99 µg/mL. These results indicate excessive complement activation, and eculizumab treatment had not controlled the activation. Subject 4-10 with sC5b-9 ≤256 ng/mL had detectable eculizumab >99 µg/mL. These results suggest the patients were being treated and the complement activation was controlled.

References:

1. Modification of the Eculizumab Dose to Successfully Manage Intravascular Breakthrough Hemolysis in Patients with Paroxysmal Nocturnal Hemoglobinuria. Kelly et al. Blood. 2008. https://doi.org/10.1182/blood.V112.11.3441.3441
2. Terminal Complement Inhibitor Eculizumab in Atypical Hemolytic–Uremic Syndrome. Legendre CM et al, NEJM 2013. DOI: 10.1056/NEJMoa1208981
3. The Complement Inhibitor Eculizumab in Paroxysmal Nocturnal Hemoglobinuria. Hillmen P et al, NEJM 2006. DOI: 10.1056/NEJMoa061648
4. Variable Eculizumab Clearance Requires Pharmacodynamic Monitoring to Optimize Therapy for Thrombotic Microangiopathy after Hematopoietic Stem Cell Transplantation. Jodele et al. 2015. ASBMT. http://dx.doi.org/10.1016/j.bbmt.2015.10.002
5. Basic Method Verification, 3rd Edition. JO.Wesgard, Ph.D. Westgard QC, Inc. Madison, WI. 2008.
6. CLIA Interpretive Guidelines 493.1252. CDC, DHHS. CLIA Current Regulations.01/24/2004.
7. EP12-A2, Vol.28 No.3. User Protocol for Evaluation of Qualitative Test Performance; approved Guideline, Second Edition, Clinical and Laboratory Standards Institute. Wayne, PA. 2008.
8. Guidance for Industry, Bioanalytical Method Validation. U.S. Department of Health and Human Services, FDA Food and Drug Administration, Center for Drug Evaluation and Research Center for Veterinary Medicine, May 2018 BP.

RESULTS

Table 1. Eculizumab positive and negative analytical accuracy sample data.
10 accuracy positive samples were spiked with Eculizumab in C5 depleted human serum.
10 accuracy negative samples were normal healthy donors.
100% accuracy was observed with positive samples ≤21% Relative Error with an average RE=2%.
100% accuracy was observed with negative samples qualitatively resulting negative as <4 µg/mL.

Positive Eculizumab Accuracy				Negative Eculizumab Accuracy			
Sample	Obs µg/mL	Exp µg/mL	%RE	Sample	Obs µg/mL	Exp µg/mL	Pass/Fail
ACC1	297	246	21%	ACC11: 195	<4	<4	Pass
ACC2	144	142	1%	ACC 12: 762	<4	<4	Pass
ACC3	66	62	6%	ACC 13: 786	<4	<4	Pass
ACC4	168	204	-18%	ACC 14: 656	<4	<4	Pass
ACC5	94	109	-14%	ACC 15: 728	<4	<4	Pass
ACC6	59	55	7%	ACC 16: 486	<4	<4	Pass
ACC7	208	193	8%	ACC 17: 616	<4	<4	Pass
ACC8	102	97	5%	ACC 18: 720	<4	<4	Pass
ACC9	173	174	-1%	ACC 19: 670	<4	<4	Pass
ACC10	89	87	2%	ACC 20: 234	<4	<4	Pass

Table 2. Eculizumab Sensitivity, linearity, and dynamic range.
Reference Range and LOD were determined to be equivalent to the LLOQ equal to 3.91 µg/mL
Dilutional linearity for samples is acceptable at a maximum of a 1 to 128,000 dilution.
Comparing linearity of the sensitivity data set had a R^2=1.00

Eculizumab LLOQ, LOD, and Dilutional Linearity									
Sample	Rep1	Rep2	Rep3	Stdev	Average µg/mL	CV%	Dilution	Expected µg/mL	%RE
Sens1	452	401	453	29.7	435	7%	1to1000	435	0%
Sens2	188	192	204	8.3	195	4%	1to2000	218	-11%
Sens3	92	91	93	1.0	92	1%	1to4000	109	-15%
Sens4	43	44	45	1.0	44	2%	1to8000	54	-19%
Sens5	23	23	24	0.6	23	2%	1to16000	27	-14%
Sens6	12	12	12	0.0	12.0	0%	1to32000	13.6	-12%
Sens7	6	7	6	0.6	6.3	9%	1to64000	6.8	-7%
Sens8	4	4	4	0.0	4.0	0%	1to128000	3.4	18%

Table 3. Eculizumab Intra- and inter-assay precision.
Intra-Assay precision was exceptional with the highest CV≤8%.
Inter-assay across different days, different operators, and with different equipment had a CV≤10%.

Eculizumab Intra-Assay Precision µg/mL					Eculizumab Inter-Assay Precision µg/mL				
Donor	Val-1	Val-1	Val-1	%CV	Donor	Val-1	Val-3	Val-4	%CV
Prec1	190	205	194	4%	Prec1	190	178	158	9%
Prec2	143	131	128	6%	Prec2	143	116	130	10%
Prec3	89	79	77	8%	Prec3	89	86	82	4%
Prec4	<4	<4	<4	PASS	Prec4	<4	<4	<4	PASS
Prec5	<4	<4	<4	PASS	Prec5	<4	<4	<4	PASS

Clinical Significance

Figure 1. Pediatric Subject 1 CH50, sC5b-9, and Eculizumab concentration trends at week 2 showed a spike in sC5b-9 requiring dosage of eculizumab observed increase in week 3 to bring sC5b-9 ≤256 ng/mL. Week 7 increase in high sC5b-9 led to week 8 decrease in eculizumab <99 µg/mL recommending dosage to maintain sC5b-9 ≤256 ng/mL at week 9.

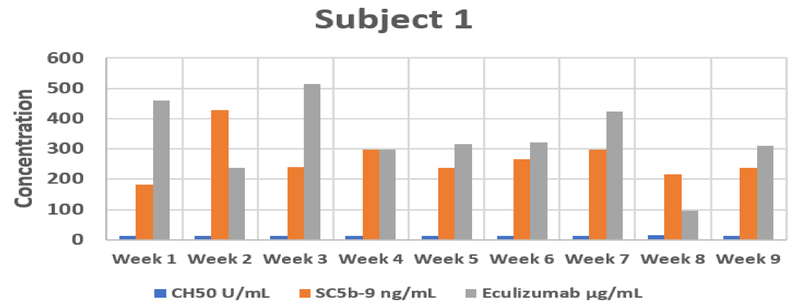


Figure 2. Subject 2 & 3 CH50, sC5b-9, and Eculizumab concentration trends. Subject 2 week 1 showed high sC5b-9 detection requiring dosage of eculizumab to reduce the patient's sC5b-9 levels ≤256 ng/mL. Subject 3 sC5b-9 was maintained ≤256 ng/mL with Eculizumab >99 µg/mL.

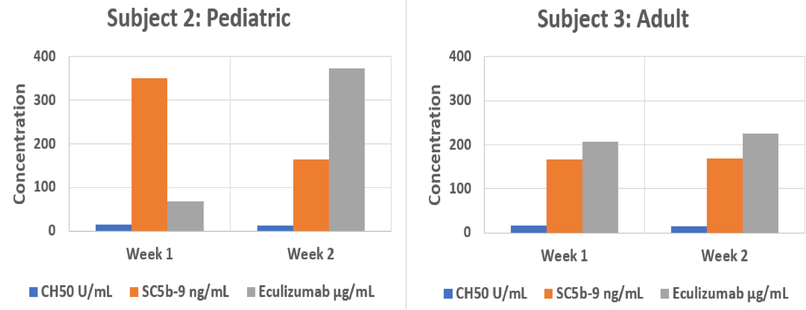
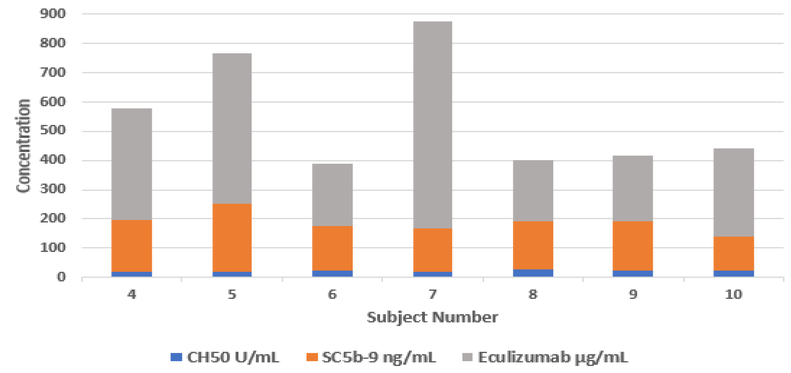


Figure 3. Pediatric subjects 4-10 sC5b-9 ≤256 ng/mL with Eculizumab >99 µg/mL



CONCLUSIONS

Assays measuring sC5b-9 and CH50 with same day turnaround time have been validated so that timely results can be provided to physicians with patients at risk of organ damage due to TA-TMA within 24 hours.

An Eculizumab assay was validated to aid in monitoring patient Eculizumab treatment trough concentrations.