Suporting material for

DTT protein equalization and Tryptophan protein quantification as a powerful tool in analytical proteomics.

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Contents of the supporting information

Table SM 1 – Discrimination of study population.

Healthy Individuals		
# Patient code	**Age**	**Sex**
C10	71	F
C15	78	M
C25	94	F
C29	83	F
C33	54	F
C37	90	M
C38	78	M
C41	73	M
C46	53	F
C49	67	M
C57	65	M

Table SM 2 - Quantification of the total proteome in sera samples of healthy individuals (n=11) by Bradford, BCA, and W Emission. Each sample were analyzed in duplicate.

Sample	[Total proteome]$_{Serum}$ (g/L)		
	Bradford Assay	**BCA Assay**	**W Emission**
C10	63 ± 4	66 ± 1	61.5 ± 0.4
C15	61 ± 2	79 ± 4	65 ± 3
C25	65 ± 2	64 ± 2	56 ± 5
C29	69.6 ± 0.3	73 ± 5	73.37 ± 0.03
C33	68 ± 4	79 ± 4	76 ± 5
C37	67.8 ± 0.5	70 ± 5	70 ± 2
C38	61 ± 1	62 ± 4	58 ± 3
C41	57 ± 1	69 ± 5	64 ± 4
C46	64 ± 1	72 ± 3	60 ± 4
C49	69 ± 2	81 ± 3	73 ± 5
C57	70 ± 1	78 ± 1	71 ± 2

Table SM 3 – Mean comparison statistic test of healthy sera (n=11). Each sample were analyzed in duplicate.

Table A - Mean comparison statistic test between Bradford assay and BCA assay

Sample	Bradford vs BCA		
	F	$F < F_{observed}$	
C 10	8.894757408	True	There are no statistically significant differences in variances
C 15	0.177672899	True	
C 25	0.803418241	True	
C 29	0.005184862	True	
C 33	1.130354589	True	
C 37	0.010966563	True	
C38	0.081016256	True	
C 41	0.078362001	True	
C 46	0.273448137	True	
C 49	0.487320877	True	
C 57	1.138522179	True	
$F_{observed}$ **= F (1,1)** = 647.8			

Table B - Mean comparison statistic test between Bradford assay and Tryptophan emission assay

Sample	Bradford vs Tryptophan Emission		
	F	$F < F_{observed}$	
C 10	8.89475741	True	There are no statistically significant differences in variances
C 15	0.1776729	True	
C 25	0.80341824	True	
C 29	0.00518486	True	
C 33	1.13035459	True	
C 37	0.01096656	True	
C38	0.08101626	True	
C 41	0.078362	True	
C 46	0.27344814	True	
C 49	0.48732088	True	
C 57	1.13852218	True	
$F_{observed}$ **= F (1,1)** = 647.8			

41 Table C - Mean comparison statistic test between BCA assay and Tryptophan emission assay

Sample	BCA vs Tryptophan Emission		
	F	$F < F_{observed}$	
C 10	10.97820174	True	There are no statistically significant differences in variances
C 15	2.16050745	True	
C 25	0.208322207	True	
C 29	4.77373E-05	True	
C 33	0.450559076	True	
C 37	3.962406049	True	
C38	1.894800657	True	
C 41	2.178737026	True	
C 46	0.443116333	True	
C 49	0.43569611	True	
C 57	0.184736213	True	
$F_{observed}$ **= F (1,1) =** 647.8			

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Table SM 4 – Variance comparison statistic test of healthy sera (n= 11). Each sample were analyzed in duplicate.

Table A - Variance comparison statistic test between Bradford assay and BCA assay

Sample	Bradford vs BCA		
	S^2	t	Statistic significant difference?
C 10	9.480392901	0.797698144	False
C 15	8.096777309	4.219902299	False
C 25	4.960192242	0.059983877	False
C 29	11.10235781	0.825815662	False
C 33	13.8112098	2.677224283	False
C 37	11.49337635	0.564247725	False
C38	7.986653449	0.140393103	False
C 41	15.29659221	2.866897232	False
C 46	4.843981741	1.859447032	False
C 49	7.813081068	2.885583583	False
C 57	0.968102217	2.060128701	False
Critic value = 4.3			

Table B - Variance comparison statistic test between Bradford assay and Tryptophan emission assay

Sample	Bradford vs Tryptophan Emission		
	S^2	t	Statistic significant difference?
C 10	8.609545102	0.3315	False
C 15	4.403774157	0.8482	False
C 25	15.41256636	2.1423	False
C 29	0.057794528	0.8674	False
C 33	21.71706923	1.9473	False
C 37	2.99381635	0.4825	False
C38	4.497698326	0.8880	False
C 41	7.622231209	1.7135	False
C 46	9.624422688	0.9843	False
C 49	14.61681023	0.9318	False
C 57	2.965909398	0.2649	False
Critic value = 4.3			

Table C - Variance comparison statistic test between BCA assay and Tryptophan emission assay

Sample	BCA vs Tryptophan Emission		
	S^2	t	Statistic significant difference?

C 10	1.045397847	1.12922332	False
C 15	10.05746579	3.3717164	False
C 25	15.95325254	2.08235513	False
C 29	11.04561781	-0.0415784	False
C 33	20.87197303	0.72995752	False
C 37	14.23784155	0.08175047	False
C38	11.28723979	1.02837291	False
C 41	20.69568949	1.1533703	False
C 46	12.38810333	2.84374969	False
C 49	17.30997726	1.9537928	False
C 57	2.903200846	1.79519566	False
Critic value = 4.3			

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Table SM 5 – Proteins list identified in depletion heatmap from raw sera, supernatant and pellet fractions in healthy individual's (n=11).

Differential Proteins	A0A075B6I0	A0A286YEY4	C9JF17	O75636	P01782	P04217	P0DJI8	P43251-4
	A0A075B6P5	A0A286YFJ8	CON__P00761	O75882-3	P01817	P04264	P0DOY3	P43652
	A0A0C4DH68	A0A8V8TQS1	CON__P02768-1	O95445	P01834	P04275	P10412	P48740
	A0A075B6S5	A0A3B3ISR2	CON__P13647	P00450	P01871	P04433	Q3LGB0	P51884
	A0A087WT59	A0A494C018	CON__P15636	P00734	P01876	P0DUB6	P10643	P55058-3
	A0A087WXI2	C9J0D1	CON__P19001	P00738	P02042	P05154	P10909-4	P60709
	A0A087X0M8	A0A4W8ZXM2	CON__P35527	P00739	P02647	P05160	P11226	P61769
	A0A096LPE2	K7EMV3	CON__P35908v2	P00742	P02649	P05543	P12111-4	P62269
	P01857	A0A6Q8PF87	CON__Q32PJ2	P00747	V9GYM3	P05546	P13671	P62805
	A0A0A0MS09	A0A6Q8PFJ0	D6RF35	P01008	P02671-2	P06276	P15169	P68871
	A0A0A0MS15	A0A7I2V2D2	D6RGG3	P01009	P02675	P06310	P18065	P69905
	A0A0A0MSI0	A0A7P0T8D1	E5RH81	P01011	P02741	P06312	P18428	P80108
	A0A0A0MTS7	A0A7P0TAI0	E7END6	P01023	P02743	P06396	P19652	P80748
	A0A0B4J1U7	A0A8I5KRV3	G3XAM2	P01024	P02745	P06727	P19823	P98160
	A0A0B4J1X5	A0A8I5KW61	P27918	P01042-2	P02749	P07358	P19827	Q03591
	A0A0B4J231	A0A8I5KWT8	E9PHK0	P01591	P02750	P07360	P20742	Q04756
	Q5T4F6	A0A8Q3SI33	P11142	P01594	P02751-14	Q5JP53	P20851	Q06033-2
	A0A0C4DH31	A0A8Q3SI95	F2Z3N2	P01599	Q5VY30	X6RBG4	P22352	Q08380
	A0A0C4DH38	P01031	G3V2W1	P01602	P02760	P07996	P22792	Q13790
	A0A0C4DH41	A0A8Q3SIZ0	G3XAK1	P01619	P02763	P08185	P22891	Q14520-2
	A0A0C4DH72	A0A8Q3WKW0	G3XAP6	P01624	P02765	P08238	P23142-4	Q14624
	A0A0G2JMB2	A0A8Q3WL25	H0YAC1	P01700	P02787	P08519	P25311	Q15848
	A0A0G2JSC0	A0A8Q3WL79	H3BPS8	P01701	P02790	P08571	P27169	Q16610-4
	A0A0J9YY99	P07355-2	H3BRJ9	P01706	P04003	P08603	P29622	Q8TF30
	A0A3B3ISJ1	B0YIW2	K7ER74	P01714	P04004	P08697	P33151	Q92736
	P49908	B0YJC4	K7ERI9	P01715	P04114	P09871	P35858	Q92954-3
	A0A1B0GVI3	B1AH94	O43866	P01717	P04180	P0C0L4	P36955	Q96FE7-4
	A0A286YES1	B4E1Z4	Q99879	P01780	P04196	P0C0L5	P41222	Q96IY4
	Q96KN2	Q96PD5	Q96RW7-2	Q9NZP8	Q9UGM5			