Supplementary material for the article:

Dragačević, L.; Lopandić, Z.; Gavrović-Jankulović, M.; Živković, I.; Blagojević, V.; Polović, N.; Minić, R. Comparison of Enzyme-Linked Lectin Sorbent Assay and Flow Cytometry for Profiling Microbial Glycans. Appl Biochem Biotechnol 2022. https://doi.org/10.1007/s12010-021-03772-w.

In this study, precision was determined by two types of measurements: 1) repeatability and 2) reproducibility, and both are defined by two values: coefficient of variation (CV) and standard deviation (SD), for both methods ELLSA and flow cytometry.

Repeatability, describes variation of sample result in one experiment within the method, and in our laboratory, it was calculated based on the value of quadruplicate of the same sample (Table 1., Table 2., Table 3. and Table 4.), according to the formula: $CV_{repeat} = \{[(\Sigma SD(d)) : n] : \bar{a}\} \times 100$, where SD is standard deviation of quadruplicate, n is number of replicates and $\bar{a}$ is the mean of quadruplicate sample.

Table 1. Repeatability of flow cytometric detection of different quantities of BL-eGFP binding to *C. albicans.*

BL-eGFP µg	V1	V2	V3	V4	AV	SD	CV repeat %
0.3475	1947	1837	2916	1708	2102	551	7
0.695	1149	957	1138	1194	1110	105	2
1.39	4302	4337	3709	3521	3967	414	3
2.78	11075	11679	9287	10448	10622	1022	2
5.56	25333	31228	37586	22850	29249	6575	6
11.12	100816	68510	106674	71744	86936	19601	6
22.24	91395	98070	76309	72031	84451	12305	4
44.48	67557	77895	85605	38000	67264	20864	8

Table 2. Repeatability of ELLSA detection of different quantities of BanLec-B binding to *C. albicans.*

BanLec-B µg	V1	V2	V3	V4	AV	SD	CV repeat %
5	2.453	2.592	2.402	2.487	2.4835	0.0803	0.8
2.5	2.416	2.506	2.626	2.494	2.5105	0.0867	0.9
1.25	2.581	2.673	2.382	2.578	2.5535	0.1225	1.2
0.625	2.456	2.549	2.829	2.773	2.6518	0.1779	1.7
0.3125	2.456	2.549	2.321	2.549	2.4688	0.1078	1.1
0.15625	2.021	2.159	2.321	2.272	2.1933	0.1334	1.5
0.078125	1.733	1.736	1.729	1.678	1.719	0.0275	0.4
0.039063	0.964	1.088	1.133	1.1	1.0713	0.074	1.7
0.019531	0.536	0.569	0.619	0.623	0.5868	0.0418	1.8
0.009766	0.198	0.267	0.291	0.264	0.255	0.0399	3.9
0.004883	0.224	0.219	0.238	0.212	0.2233	0.011	1.2
0.002441	0.153	0.152	0.025	0.031	0.1525	0.0007	0.1

Table 3. Repeatability of flow cytometric detection of different quantities of RCA_{120}-FITC binding to *L. casei* DG.

RCA120-FITC µg	V1	V2	V3	V4	AV	SD	CV repeat %
0.3475	2800	440	3842	3229	2578	1488	14
0.695	3691	6839	4893	969	4098	2456	15
1.39	5299	6882	5860	5872	5978	659	3
2.78	8688	6755	6755	7214	7353	916	3
5.56	1634	12146	15203	13191	10544	6074	14
11.12	24703	29696	41065	23516	29745	8008	7
22.24	34466	39082	31874	25672	32774	5595	4
44.48	31617	35515	28719	25886	30434	4117	3

Table 4. Repeatability of ELLSA detection of different quantities of RCA_{120}-B binding to *L. casei* DG.

RCA120-B µg	V1	V2	V3	V4	AV	SD	CV repeat %
11.5	1.663	1.621	1.451	1.499	1.5585	0.09987	1.6
5.75	1.793	1.617	1.619	1.687	1.679	0.08267	1.2
2.875	1.712	1.654	1.702	1.756	1.706	0.04186	0.6
1.4375	1.554	1.525	1.576	1.512	1.54175	0.0288	0.5
0.71875	1.058	1.258	1.158	1.169	1.16075	0.08184	1.8
0.359375	0.821	0.848	0.888	0.864	0.85525	0.02814	0.8
0.1796875	0.473	0.484	0.491	0.492	0.485	0.00876	0.5
0.08984375	0.253	0.201	0.217	0.22	0.22275	0.02182	2.4
0.044921875	0.145	0.141	0.146	0.156	0.147	0.00638	1.1
0.022460938	0.076	0.069	0.072	0.073	0.0725	0.00289	1
0.011230469	0.077	0.076	0.078	0.076	0.07675	0.00096	0.3
0.005615234	0.064	0.061	0.058	0.061	0.061	0.00245	1

Reproducibility, describes variation of the same sample result in three different laboratories. The coefficient of variation for reproducibility was calculated based on value of quadruplicates of the same sample done in three different laboratories at our Institute (Table 5.), according to the formula: $CVrepro= \{[(SD(1) + SD(2) + SD(3)) : 3] : [(\bar{A}1 + \bar{A}2 + \bar{A}3) : 3]\} \times 100$, where SD(X) is the average SD of the mean values in different laboratories, and $\bar{A}$ is the averages of the results from different laboratories.

Table 5. Reproducibility of BanLec-B binding to 21 different microorganisms. Experiments were done in three different laboratories, with preparing new plates each time.

	LAB 1 AV	LAB1 SD	LAB 2 AV	LAB 2 SD	LAB 3 AV	LAB3 SD	CV repro (%)
L. reuteri DSM 17938	0.2955	0.0134	0.232	0.0368	0.206	0.017	9.2
L. plantarum WCFS1	1.273	0.017	0.925	0.017	0.9875	0.046	2.5
L. rhamnosus LA68	0.653	0.0255	0.468	0.0014	0.5465	0.0078	2.1
L. rhamnosus LB64	0.804	0.0085	0.6115	0.0035	1.3505	0.0813	3.4
L. rhamnosus LGG	0.502	0.0141	0.4585	0.0007	0.5405	0.0219	2.4
L. helveticus LAFTI	0.78	0.0453	0.42	0.0042	0.6175	0.012	3.4
L. casei DG	0.609	0.0297	0.641	0.017	0.624	0.0354	4.4
S. agalactiae ATCC 13813	0.7295	0.0163	0.534	0.0269	0.62	0.0113	2.9
Streptococcus sp. CI group B	0.8155	0.0078	0.6235	0.0219	0.732	0.0014	1.4
Streptococcus sp. CI group A	1.338	0.0014	1.0685	0.0106	0.914	0.0184	0.9
E. fecalis CI	0.5885	0.0615	0.5645	0.0177	1.351	0.017	3.8
S. aureus CI	0.695	0.0057	0.528	0.0226	0.259	0.0141	2.9
P. mirabilis CI	0.594	0.0198	0.3995	0.029	0.494	0.0028	3.5
E. coli CI	0.258	0.0071	0.169	0.0099	0.4195	0.046	7.4
S. enteritidis CI 12	4.021	0.0212	4.0755	0.0912	3.8305	0.1039	1.8
K. pneumoniae ATCC 13883	0.846	0.058	0.755	0.0368	0.475	0.0255	5.8
P. aeruginosa ATCC 27853	0.43	0.0014	0.7065	0.0544	0.386	0.0269	5.4
P. houseri ATCC 13315	0.4645	0.0049	0.2805	0.0078	0.203	0.0071	2.1
S. flexneri ATCC 12022	0.5415	0.0304	0.3235	0.0276	0.2025	0.0233	7.6
E. coli ATCC 25922	0.566	0.0297	0.7535	0.0021	1.1025	0.0559	3.6
C. albicans ATCC 10231	3.8715	0.0219	4.0455	0.0233	3.939	0.0226	0.6

Since the coefficients of variation obtained in our tests for ELLSA method for repeatability are lower than 5%, assay is considered to be highly precise, and since reproducibility coefficients are lower than 10%, assay is considered highly reproducible.(Murray and Lawrence 1993, Biddle-combe 1996).

One of characteristic for method validation is linearity, and here it refers to the range of lectin dilutions that provide the linear segment of the curve. The range in which ELLSA can be used to quantitate the obtained absorbance is determined by the linear part of the binding curve.

Linearity was determined by using BanLec with *C.albicans* coated on plate, and RCA_{120} with *L.casei* DG coated on plate, as those two lectins show high binding values towards those two microorganisms. Serial twofold dilutions were prepared and gave linear part of the curve that was used to calculate parameters *a* and *b* from linearity equation $y=a+b*x$ of the linear curve.

Binding of both lectins resulted in very high coefficient of determination (R^2), 0.98 for BanLec-B, Figure 1. and 0.99 for RCA_{120}, Figure 3. in the set range. Linearity for RCA_{120} was obtained in range from 0.01 to 0.3 μg, while for BanLec-B it was in range from 0,003 to 0.079 μg. This shows that testing binding of the tested lectins in this range of dilution can be objectively

measured. In addition, flow cytometry parallel testing of linearity in the set range provided lower coefficient of determination for both lectins, Figure 2. and Figure 4..

	Intercept (a)		**Slope (b)**		**Statistics**
	Value	**Standard Error**	**Value**	**Standard Error**	**R^2**
BanLec/*C.albicans*	0.12498	0.049642	21,18137	1.2605	0.98254
RCA_{120}/*L.casei*	0.041	0.01064	2.29239	0.06784	0.99477

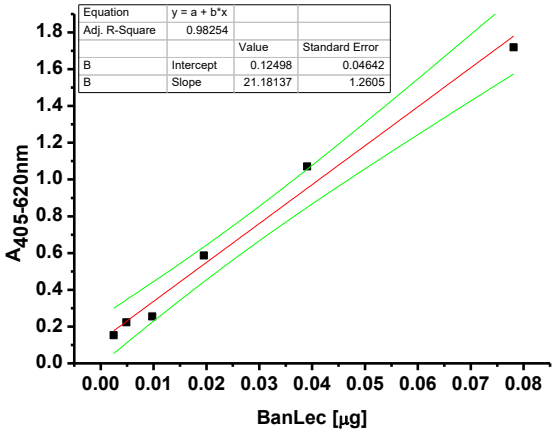

Fig. 1 Determining linearity of ELLSA method, by measuring binding between *C.albicans* and different quantity of BanLec-B.

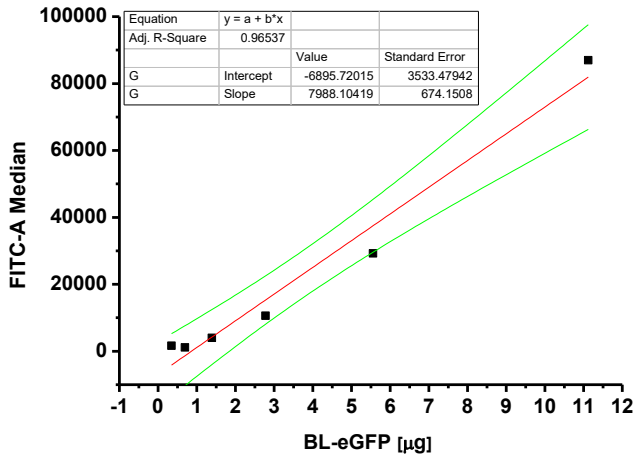

Fig. 2 Determining linearity of flow cytometry, by measuring binding between *C.albicans* and different quantity of BanLec-eGFP.

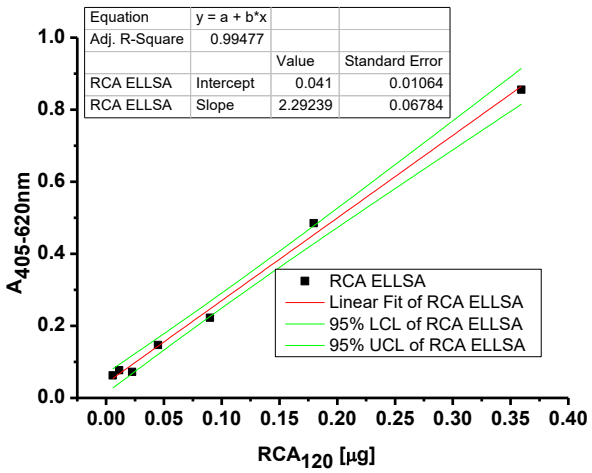

Fig. 3 Determining linearity of ELLSA method, by measuring binding between *L.casei* DG and different quantity of RCA_{120}.

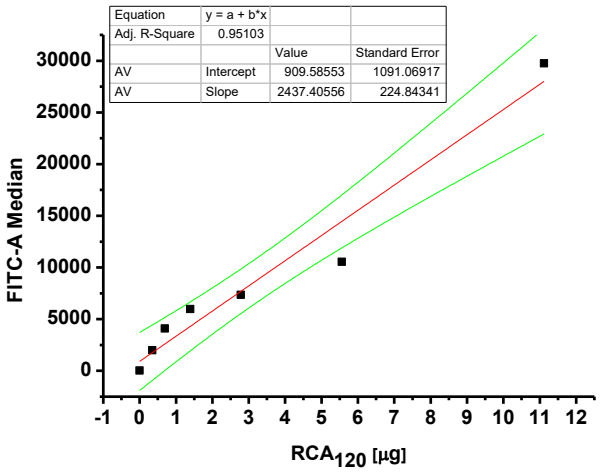

Fig. 4 Determining linearity of flow cytometry, by measuring binding between *L.casei* DG and different quantity of RCA_{120}.

SDS-PAGE showing:
Lane 1 - purified banana lectin – BanLec;
Lane2 - chimera of banana lectin and enhanced green fluorescent protein BanLec-eGFP;
Lane 3 – MW - molecular weight markers.