LITTOISTENJÄRVEN seuranta sinilevämyrkkyjen suhteen

Date of analysis: **6.8.2021**

Sample collection, immunoassay, data analysis and report by **SULTANA AKTER**

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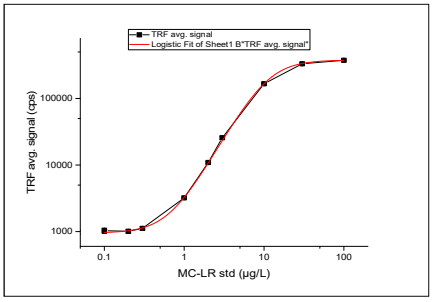
Assay method reference:

Sultana Akter, Markus Vehniäinen, Lisa Spoof, Sonja Nybom, Jussi Meriluoto, and Urpo Lamminmäki. Broad-spectrum noncompetitive immunocomplex immunoassay for cyanobacterial peptide hepatotoxins (microcystins and nodularins), Analytical Chemistry, **2016**, 88, 10080−10087. (PMID:27657987)

Assay method: Immunoassay based on Akter et al., 2016 with slight modification

1. Prewash streptavidin coated strips (yellow, normal).
2. Add blank (reagent water), MC-LR standard or sample, **50 µL**/well as Triplicate .
3. Add Reagent Mixture, **50 µL**/well
4. Incubate with slow shaking for 1 hour at RT.
5. Wash 4 x.
6. Add Enhancement solution 200 µL per well. Use the Plate Dispenser.
7. Incubate with slow shaking for 10 min at RT.
8. Measure the Time resolved fluorescence (TRF) signal with Plate fluorometer.
9. Resolve standard curve with Origin 2016 and logistic fit.



standard curve of microcystin-

microcystin-LR (MC-LR) standard

MC-LR (Enzo Life sciences, ALX350-431)

Prepared original stock of 1000 µg/L in reagent water+5%Methanol. Stored at (-20C)

30.9.2019SA: Further working standard solution in reagent water: 100, 30, 10, 3, 2, 1, 0.3, 0.2 and 0.1 µg/L

Reagent mixture in assay buffer

1 µg/mL biotinylated anti-ADDA Antibody (stock 256 µg/ml); +

1 µg/mL anti-immunocomplex scFv-AP (stock 440 µg/ml) +

0.5 µg/mL N1-Eu-anti AP pAb (stock 200 µg/ml, 16.1.2020).

(x)	TRF signal (counts per second)			(y)			
MC-LR (µg/L) std	A	B	C	avg sig	std dev	cv%	blk+3SD (9 blank)
0	999	982	1080	**987**	57	5.7	1156
0	1006	989	1020				
0	947	870	988				
0.1	1142	994	964	**1033**	95	9.2	
0.2	1082	960	988	**1010**	64	6.3	
0.3	1168	1052	1130	**1117**	59	5.3	
1	3138	3189	3307	**3211**	87	2.7	
2	10657	10723	11403	**10928**	413	3.8	
3	27113	25428	24466	**25669**	1340	5.2	
10	166278	168329	164731	**166446**	1805	1.1	
30	327184	326230	343224	**332213**	9548	2.9	
100	378043	369174	378981	**375399**	5412	1.4	

	TRF signal			(y)			*(x) From origin			
sample	A	B	C	Avg	sig comments	std dev	cv%	conc µg/L	DF	1x conc (µg/L)	**reported conc (µg/L)**
1_A_Saarten taus (1x)	1158	1054	1010	**1074**	below blk+3SD	76	7.1	0.25	1	0.25	below 0.3
2_B_Koilliselkä(1x)	1041	1091	1100	**1077**	below blk+3SD	32	3.0	0.25	1	0.25	below 0.3
3_C_Luoteisselkä(1x)	1078	1012	1050	**1047**	below blk+3SD	33	3.2	0.22	1	0.22	below 0.3
4_A'_Hiekkaranta(1x)	1110	1102	1074	**1095**	below blk+3SD	19	1.7	0.27	1	0.27	below 0.3
5_D'_Ristikallion Uimaranta(1x)	1160	1128	1074	**1121**	below blk+3SD	43	3.9	0.29	1	0.29	below 0.3

DL based on (blk+3SD) sig	1156	0.31 µg/L
DL based on true standard above (blk+3SD) signal	**1117**	0.30 µg/L

Interpretation (6.8.2021 SA)

Raw water samples (collected on 5.8.2021) were stored at +4 C until analysis on 6.8.2021.
Before analysis, samples were heated at 80 ˚C for 10 min to release cell bound toxins if any.

The results represent the total cyclic peptide hepatotoxin amount (already released toxin in water and the cell bound toxin .
The immunoassay (Akter et al., 2016) detects cyanobacterial peptide hepatotoxins (eg microcystins and/or nodularin).
For quantification, microcystin-LR was used as standard.

Result:

In Littoistenjärvi water, the detected cyanobacterial peptide hepatotoxin (free and cell bound microcystin) concentrations (µg/L) are below 0,3 µg/L from the following samples:

6.8.2021, A_Saarten taus : below 0,3 µg/L
6.8.2021 , B_Koilliselkä : below 0,3 µg/L
6.8.2021 , C_Luoteisselkä : below 0,3 µg/L
6.8.2021 , A'_Hiekkaranta : below 0,3 µg/L
6.8.2021 , D'_Ristikallion Uimaranta : below 0,3 µg/L