LITTOISTENJÄRVEN seuranta sinilevämyrkkyjen suhteen

Date of analysis: **11.9.2020**

Sample collection, immunoassay, data analyis 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, Lot KG1739).
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.

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)

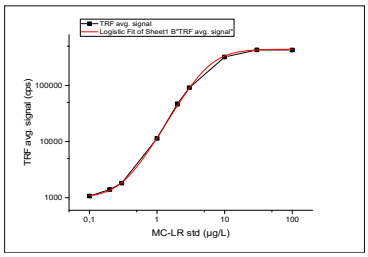
30.9.2019SA: Further working standard solution in reagent water: 100, 30, 10, 3, 2, 1, 0.3, 0.2 and 0.1 µg/L

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).



standard curve of microcystin-LR

(x)	TRF signal (counts per second)			(y)			
MC-LR (µg/L) std	A	B	C	avg sig	std dev	cv%	blk+3SD (n=9)
0	988	1054	1076				
0	1114	911	944				
0	1102	987	874	**1006**	86	8.5	1263
0.1	1131	1046	1045	**1074**	49	4.6	
0.2	1474	1376	1362	**1404**	61	4.3	
0.3	1890	1714	1863	**1822**	95	5.2	
1	11666	11598	10961	**11408**	389	3.4	
2	50216	46893	43408	**46839**	3404	7.3	
3	93409	89452	90143	**91001**	2114	2.3	
10	328484	314135	318427	**320349**	7365	2.3	
30	427007	431886	428430	**429108**	2509	0.6	
100	410765	455097	416800	**427554**	24043	5.6	

		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)
A_Saarten taus	1_A	1346	1234	1136	**1239**	close to blk+3SD	105	8.5	0.16	1	**0.16**	<0.2
B_Koilliselkä	2_B	1226	1002	1334	**1187**	close to blk+3SD	169	14.3	0.15	1	**0.15**	<0.2
C_Luoteisselkä	3_C	1212	1246	1131	**1196**	close to blk+3SD	59	4.9	0.15	1	**0.15**	<0.2
A'_Hiekkaranta	4_A'	1156	1214	1306	**1225**	close to blk+3SD	76	6.2	0.16	1	**0.16**	<0.2
B'_Pirtan Laituri(1), near Littoistenjärv	5_B'	1204	1276	1096	**1192**	close to blk+3SD	91	7.6	0.15	1	**0.15**	<0.2
C'_Bussilaituri(2) Rauhaniemi, bus sto	6_C'	1144	1210	1162	**1172**	close to blk+3SD	34	2.9	0.14	1	**0.14**	<0.2
D'_Ristikallion Uimaranta	7_D'	1269	1214	1111	**1198**	close to blk+3SD	80	6.7	0.15	1	**0.15**	<0.2
E'_Kuoviluoto	8_E'	1142	1138	1266	**1182**	below blk+3SD	73	6.2	0.14	1	**0.14**	<0.2
F'_Rantapolun laituri(3)	9_F'	1343	1404	1190	**1312**	below blk+3SD	110	8.4	0.18	1	**0.18**	<0.2

DL based on (blk+3SD) sig	1263	0.17	µg/L
DL based on true standard above (blk+3SD) signal	1404	0.2	µg/L

Interpretation (11.9.2020 SA)

Raw water samples were analyzed fresh on 11.9.2020.
Before analysis, samples were heated at 80 ˚C for 10 min to release cell bound toxins if any.
Hence, the results represent the total 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).
For quantification, microcystin-LR was used as standard.

Result:

In Littoistenjärvi water sample of 11.9.2020, the detected cyanobacterial peptide hepatotoxin concentrations (µg/L) (free and cell bound) were shown below from the following samples:

A_Saarten taus: <0.2 µg/L
B_Koilliselkä: <0.2 µg/L
C_Luoteisselkä: <0.2 µg/L

A'_Hiekkaranta: <0.2 µg/L
B'_Pirtanlaituri , near Littoistenjärventie 109: <0.2 µg/L
C'_Bussilaituri, Rauhaniemi, bus stop 6378 : <0.2 µg/L

D'_Ristikallion Uimaranta: <0.2 µg/L
E'_Kuoviluoto: <0.2 µg/L
F'_Rantapolun laituri: <0.2 µg/L