LITTOISTENJÄRVEN seuranta sinilevämyrkkyjen suhteen

Date of analysis: **6.8.2020**

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

Sultana Akter (sultana.akter@utu.fi), Researcher, Department of Biotechnology, University of Turku

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)

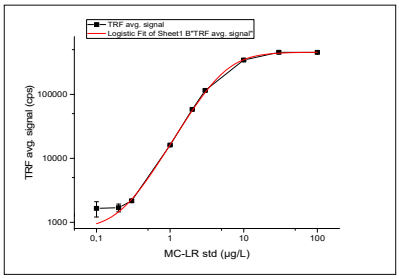
30.9.2019SA: Further working standard solution in reagent water: 100, 30, 10, 3, 2, 1, 0.3, 0.2 and 0.1 µg/L

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	B	C	avg sig	std dev	cv%	blk+3SD (n=12)
0	916	1014				
0	1137	916				
0	1014	1137				
0	1683	1541				
0	1176	984				
0	1140	990	**1137**	240	21.1	1859
0.1	1967	1340	**1654**	443	26.8	
0.2	1858	1526	**1692**	235	13.9	
0.3	2236	2097	**2167**	98	4.5	
1	16331	15947	**16139**	272	1.7	
2	59331	57237	**58284**	1481	2.5	
3	112921	117499	**115210**	3237	2.8	
10	342813	346179	**344496**	2380	0.7	
30	458506	450732	**454619**	5497	1.2	
100	449914	457437	**453676**	5320	1.2	

		TRF signal			(y)				*(x) From origin			
sample of 9.7.2020		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	1560	1270	1186	**1339**	below blk+3SD	196	14.7	0.18	1	**0.18**	<0.25
B_Koilliselkä	2_B	1267	1157	1116	**1180**	below blk+3SD	78	6.6	0.15	1	**0.15**	<0.25
C_Luoteisselkä	3_C	1040	1156	1106	**1101**	below blk+3SD	58	5.3	0.14	1	**0.14**	<0.25
A'_Hiekkaranta	4_A'	1037	1086	1329	**1151**	below blk+3SD	156	13.6	0.15	1	**0.15**	<0.25
B'_Pirtanlaituri(1), near Littoistenjärv	5_B'	2336'	1294	1380	**1337**	below blk+3SD	61	4.5	0.18	1	**0.18**	<0.25
C'_Bussilaituri(2) Rauhaniemi, bus std	6_C'	1140	1284	1208	**1211**	below blk+3SD	72	6.0	0.16	1	**0.16**	<0.25
D'_Ristikallion Uimaranta	7_D'	1244	1208	1207	**1220**	below blk+3SD	21	1.7	0.16	1	**0.16**	<0.25
E'_Kuoviluoto	8_E'	1101	1266	1275	**1214**	below blk+3SD	98	8.1	0.16	1	**0.16**	<0.25
F'_Rantapolun laituri(3)	9_F'	1108	1148	2356'	**1128**	below blk+3SD	28	2.5	0.14	1	**0.14**	<0.25

DL based on(blk+3SD) sig	1859	**0.25**	µg/L
DL based on true standard above (blk+3SD) signal	2161	0.3	µg/L

Interpretation (6.8.2020 SA)

Raw water samples were analyzed fresh on 6.8.2020.
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, the detected cyanobacterial peptide hepatotoxin concentrations (µg/L) (free and cell bound) were shown below from the following samples:
6.8.2020 A_Saarten taus: <0.25 µg/L
6.8.2020 B_Koilliselkä: <0.25 µg/L
6.8.2020 C_Luoteisselkä: <0.25 µg/L

6.8.2020 A'_Hiekkaranta: <0.25 µg/L
6.8.2020 B'_Pirtanlaituri , near Littoistenjärventie 109: <0.25 µg/L
6.8.2020 C'_Bussilaituri, Rauhaniemi, bus stop 6378 : <0.25 µg/L

6.8.2020 D'_Ristikallion Uimaranta: <0.25 µg/L
6.8.2020 E'_Kuoviluoto: <0.25 µg/L
6.8.2020 F'_Rantapolun laituri: <0.25 µg/L