LITTOISTENJÄRVEN seuranta sinilevämyrkkyjen suhteen

Date of analysis: 9.7.2020
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.
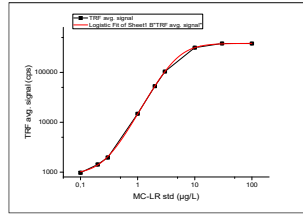
microcystin-LR (MC-LR) standard

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	983	900	943					
0	970	878	918					
0	922	905	884	923	36	4.0		1032
0.1	1040	938	943	974	58	5.9		
0.2	1488	1459	1325	1424	87	6.1		
0.3	2094	1932	1870	1965	116	5.9		
1	14449	15228	14786	14821	391	2.6		
2	51030	53956	53629	52872	1603	3.0		
3	103664	104103	103964	103910	224	0.2		
10	313455	312099	314579	313378	1242	0.4		
30	382237	378185	379441	379954	2074	0.5		
100	380006	377796	380242	379348	1349	0.4		

		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	1202	1169	1164	1178	low	21	1.8		0.15	1	0.15	<0.2
B_Koilliselkä	2_B	1123	1165	1188	1159	low	33	2.8		0.15	1	0.15	<0.2
C_Luoteisselkä	3_C	1184	1196	1118	1166	low	42	3.6		0.15	1	0.15	<0.2
A'_Hiekkaranta	4_A'	1166	1218	1164	1183	low	31	2.6		0.15	1	0.15	<0.2
B'_Pirtan Laituri(1), near Littoistenjär	5_B'	1192	1202	1081	1158	low	67	5.8		0.15	1	0.15	<0.2
C'_Bussilaituri(2) Rauhaniemi, bus std	6_C'	1085	1086	1237	1136	low	87	7.7		0.14	1	0.14	<0.2
D'_Ristikallion Uimaranta	7_D'	1123	1168	1074	1122	low	47	4.2		0.14	1	0.14	<0.2
E'_Kuoviluoto	8_E'	1149	1072	1152	1124	low	45	4.0		0.14	1	0.14	<0.2
F'_Rantapolun laituri(3)	9_F'	1176	1225	1142	1181	low	42	3.5		0.15	1	0.15	<0.2

(blk+3Sd) sig	1032	0.11	µg/L
DL based on true standard signal	1424	0.2	µg/L

Interpretation (9.7.2020 SA)

Raw water samples were analyzed fresh on 9.7.2020.
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:

9.7.2020 A_Saarten taus: <0.2 µg/L
9.7.2020 B_Koilliselkä: <0.2 µg/L
9.7.2020 C_Luoteisselkä: <0.2 µg/L

9.7.2020 A'_Hiekkaranta: <0.2 µg/L
9.7.2020 B'_Pirtan laituri , near Littoistenjärventie 109: <0.2 µg/L
9.7.2020 C'_Bussilaituri, Rauhaniemi, bus stop 6378 :<0.2 µg/L

9.7.2020 D'_Ristikallion Uimaranta: <0.2 µg/L
9.7.2020 E'_Kuoviluoto: <0.2 µg/L
9.7.2020 F'_Rantapolun laituri: <0.2 µg/L
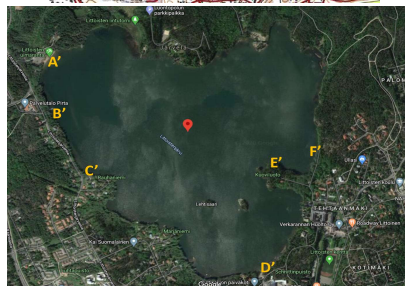
(A') Hiekkaranta, Littoistenjärvi,
Kuva: Sultana Akter 9.7.2020

(B') Pirtan Laituri, Littoistenjärvi,
Kuva: Sultana Akter 9.7.2020

(C') Bussilaituri, Rauhaniemi, Littoistenjär...
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(D') Ristikallion uimaranta, Littoistenjärvi,
Kuva: Sultana Akter 9.7.2020

(E') Kuoviluoto, Littoistenjärv...
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(F') Rantapolun laituri, Littoistenjärvi,
Kuva: Sultana Akter 9.7.2020