LITTOISTENJÄRVEN seuranta sinilevämyrkkyjen suhteen

Date of analysis: **24.7.2020**

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)

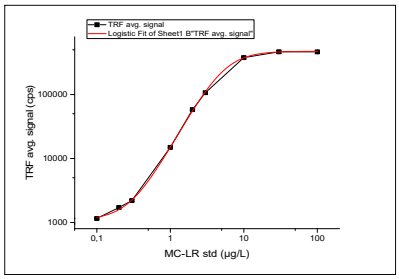
30.9.2019SA: Further working standard solution in reagent water: 100, 30, 10, 3, 2, 1, 0.3, 0.2 and 0.1 µg/L

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	1118	1086	1108				
0	1288	1009	966				
0	1120	858	942	**1055**	126	12.0	1434
0.1	1186	1116	1160	**1154**	35	3.1	
0.2	1746	1659	1736	**1714**	48	2.8	
0.3	2135	2300	2176	**2204**	86	3.9	
1	14763	15098	14933	**14931**	168	1.1	
2	58263	56356	59703	**58107**	1679	2.9	
3	111960	105871	102801	**106877**	4662	4.4	
10	379410	385636	367983	**377676**	8953	2.4	
30	441049	485999	468920	**465323**	22690	4.9	
100	482342	445498	466445	**464762**	18480	4.0	

		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	1376	1118	1200	**1231**	below blk+3SD	132	10.7	0.11	1	**0.11**	<0.2
B_Koilliselkä	2_B	1090	1150	1218	**1153**	below blk+3SD	64	5.6	--	1		<0.2
C_Luoteisselkä	3_C	1114	1104	1287	**1168**	below blk+3SD	103	8.8	--	1		<0.2
A'_Hiekkaranta	4_A'	1162	1178	1297	**1212**	below blk+3SD	74	6.1	0.10	1	**0.10**	<0.2
B'_Pirtan Laituri(1), near Littoistenjärv	5_B'	1278	1229	1170	**1226**	below blk+3SD	54	4.4	0.11	1	**0.11**	<0.2
C'_Bussilaituri(2) Rauhaniemi, bus sto	6_C'	1167	1128	1228	**1174**	below blk+3SD	50	4.3	--	1		<0.2
D'_Ristikallion Uimaranta	7_D'	1334	1174	1109	**1206**	below blk+3SD	116	9.6	--	1		<0.2
E'_Kuoviluoto	8_E'	1112	1186	1183	**1160**	below blk+3SD	42	3.6	--	1		<0.2
F'_Rantapolun laituri(3)	9_F'	1258	1237	1057	**1184**	below blk+3SD	110	9.3	--	1		<0.2

DL based on(blk+3SD) sig	1434	0.17	µg/L
DL based on true standard above (blk+3SD) signal	1714	0.2	µg/L

Interpretation (24.7.2020 SA)

Raw water samples were analyzed fresh on 24.7.2020.

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:

24.7.2020 A_Saarten taus: <0.2 µg/L
24.7.2020 B_Koilliselkä: <0.2 µg/L
24.7.2020 C_Luoteisselkä: <0.2 µg/L

24.7.2020 A'_Hiekkaranta: <0.2 µg/L
24.7.2020 B'_Pirtanlaituri , near Littoistenjärventie 109: <0.2 µg/L
24.7.2020 C'_Bussilaituri, Rauhaniemi, bus stop 6378 : <0.2 µg/L

24.7.2020 D'_Ristikallion Uimaranta: <0.2 µg/L
24.7.2020 E'_Kuoviluoto: <0.2 µg/L
24.7.2020 F'_Rantapolun laituri: <0.2 µg/L