

LITTOISTENJÄRVEN seuranta sinilevämyrkyjen suhteen

Date of analysis: 20.8.2020

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

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Assay method reference:

Sultana Akter, Markus Vehniäinen, Lisa Spoo, 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)

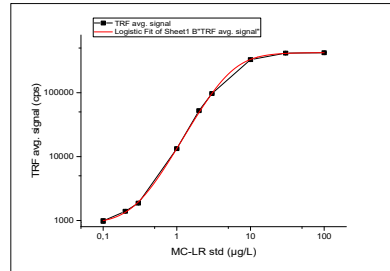
30.9.2019SA: Further working standard solution in reagent water: 100, 30, 10, 3, 2, 1, 0.3, 0.2 and 0.1 µg/L

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	886	928	891				
0	916	871	870				
0	890	882	860	888	22		954
0.1	1062	904	988	985	79		8.0
0.2	1441	1406	1337	1395	53		3.8
0.3	1815	1930	1858	1868	58		3.1
1	13586	13163	13194	13314	236		1.8
2	52217	51090	53761	52356	1341		2.6
3	96511	96043	98835	97130	1495		1.5
10	327939	331979	329122	329680	2077		0.6
30	415678	419225	414585	416496	2426		0.6
100	422284	420723	423808	422272	1543		0.4

sample of 9.7.2020	TRF signal			(y)	sig comments	std dev	cv%	*(x) From origin			
	A	B	C	Avg				conc µg/L	DF	1x conc (µg/L)	reported conc (µg/L)
A_Saarten taus	1_A	1024	1005	996	1008	14	1.4	0.11	1	0.11	<0.2
B_Koilliselkä	2_B	1100	1116	1008	1075	58	5.4	0.13	1	0.13	<0.2
C_Luoteisselkä	3_C	1118	1069	991	1059	64	6.0	0.13	1	0.13	<0.2
A^Hiekkaranta	4_A'	1002	1086	1160	1083	79	7.3	0.13	1	0.13	<0.2
B^Pirtan Laituri(1), near Littoistenjärvi	5_B'	1287	1096	1045	1143	128	11.2	0.15	1	0.15	<0.2
C^Bussilaituri(2) Rauhaniemi, bus stop	6_C'	1127	1097	1064	1096	32	2.9	0.14	1	0.14	<0.2
D^Ristikallion Uimaranta	7_D'	1096	1056	1206	1119	78	6.9	0.14	1	0.14	<0.2
E^Kuoviluo	8_E'	955	908	912	925	26	2.8	--	1	#VALUE!	<0.2
F^Rantapolun laituri(3)	9_F'	850	895	934	893	42	4.7	--	1	#VALUE!	<0.2
DL based on (blk+3SD) sig				954							µg/L
DL based on true standard above (blk+3SD) signal				1395				0.2			µg/L

Interpretation (20.8.2020 SA)

Raw water samples were analyzed fresh on 20.8.2020.

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:

20.8.2020 A_Saarten taus: <0.2 µg/L

20.8.2020 B_Koilliselkä: <0.2 µg/L

20.8.2020 C_Luoteisselkä: <0.2 µg/L

20.8.2020 A^Hiekkaranta: <0.2 µg/L

20.8.2020 B^Pirtanlaituri, near Littoistenjärventie 109: <0.2 µg/L

20.8.2020 C^Bussilaituri, Rauhaniemi, bus stop 6378 : <0.2 µg/L

20.8.2020 D^Ristikallion Uimaranta: <0.2 µg/L

20.8.2020 E^Kuoviluo: <0.2 µg/L

20.8.2020 F^Rantapolun laituri: <0.2 µg/L

