LITTOISTENJÄRVEN seuranta sinilevämyrkkyjen suhteen

Date of analysis: **24.9.2020**

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)

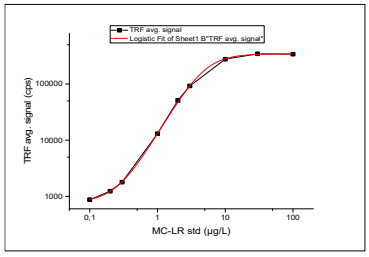
30.9.2019SA: Further working standard solution in reagent water: 100, 30, 10, 3, 2, 1, 0.3, 0.2 and 0.1 µg/L

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				avg sig	std dev	cv%	blk+3SD (n=18)
0	782	801	726	**780**	38	4.9	894
0	706	766	777				
0	767	804	772				
0	764	801	847				
0	820	728	823				
0	832	768	760				
0.1	854	854	910	**873**	32	3.7	
0.2	1266	1230	1222	**1239**	23	1.9	
0.3	1776	1746	1851	**1791**	54	3.0	
1	12792	12766	13565	**13041**	454	3.5	
2	48974	49357	54844	**51058**	3284	6.4	
3	89499	91540	95785	**92275**	3207	3.5	
10	270031	283930	274831	**276264**	7059	2.6	
30	334776	344622	354412	**344603**	9818	2.8	
100	339333	328662	350279	**339425**	10809	3.2	

		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	887	876	860	**874**	below blk+3SD	14	1.6	--	1		<0.2
B_Koilliselkä	2_B	840	895	860	**865**	below blk+3SD	28	3.2	--	1		<0.2
C_Luoteisselkä	3_C	858	918	844	**873**	below blk+3SD	39	4.5	--	1		<0.2
A'_Hiekkaranta	4_A'	891	934	886	**904**	close to blk+3SD	26	2.9	0.11	1	**0.11**	<0.2
B'_Pirtan Laituri(1), near Littoistenjärv	5_B'	818	906	795	**840**	below blk+3SD	59	7.0	--	1		<0.2
C'_Bussilaituri(2) Rauhaniemi, bus sto	6_C'	884	868	931	**894**	close to blk+3SD	33	3.7	0.10	1	**0.10**	<0.2
D'_Ristikallion Uimaranta	7_D'	949	939	916	**935**	close to blk+3SD	17	1.8	0.12	1	**0.12**	<0.2
E'_Kuoviluoto	8_E'	922	1022	895	**946**	close to blk+3SD	67	7.1	0.12	1	**0.12**	<0.2
F'_Rantapolun laituri(3)	9_F'	865	883	882	**877**	below blk+3SD	10	1.2	--	1		<0.2

DL based on (blk+3SD) sig	894	0.10	µg/L
DL based on true standard above (blk+3SD) signal	1239	0.2	µg/L

Interpretation (24.9.2020 SA)

Raw water samples were analyzed fresh on 24.9.2020.

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 24.9.2020, 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