LITTOISTENJÄRVEN seuranta sinilevämyrkkyjen suhteen

Date of analysis: **9.7.2021**

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).
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)

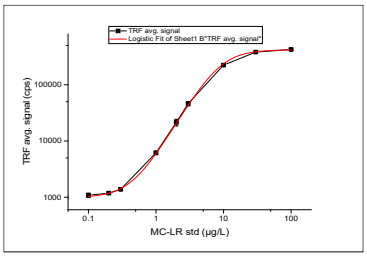
30.9.2019SA: Further working standard solution in reagent water: 100, 30, 10, 3, 2, 1, 0.3, 0.2 and 0.1 µg/L

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 (9 blank)
0	1066	970	1000	**997**	37	3.7	1108
0	1002	970	1047				
0	986	971	958				
0.1	1196	1030	1037	**1088**	94	8.6	
0.2	1148	1184	1220	**1184**	36	3.0	
0.3	1332	1380	1434	**1382**	51	3.7	
1	5776	6067	6651	**6165**	446	7.2	
2	18737	21213	24459	**21470**	2870	13.4	
3	41560	47938	49219	**46239**	4102	8.9	
10	212269	220763	238442	**223825**	13352	6.0	
30	387349	362127	392416	**380631**	16224	4.3	
100	405234	433907	433651	**424264**	16481	3.9	

	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)**
1_A_8.7.2021 raw(1X)	1368	1216	1273	**1286**	low	77	6.0	0.25	1	0.25	0.25
5_A'_8.7.2021 raw(1X)	1620	1780	1741	**1714**	low	83	4.9	0.38	1	0.38	0.38
6_D'_8.7.2021 raw(1X)	1488	1368	1343	**1400**	low	78	5.5	0.29	1	0.29	0.29

DL based on (blk+3SD) sig	1108	0.16 µg/L
DL based on true standard above (blk+3SD) signal	**1184**	0.20 µg/L

Interpretation (9.7.2021 SA)

Raw water samples (collected on 8.7.2021) were stored at +4 C until analysis on 9.7.2021.

Before analysis, samples were heated at 80 ˚C for 10 min to release cell bound toxins if any.

The results represent the total cyclic 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 and/or nodularin).
For quantification, microcystin-LR was used as standard.

Result:

In Littoistenjärvi water, the detected cyanobacterial peptide hepatotoxin (free and cell bound microcystin) concentrations (µg/L) are shown below from the following samples:

8.7.2021

A_Saarten taus: 0,25 µg/L
A'_Hiekkaranta: 0,38 µg/L
D'_Ristikallion Uimaranta: 0,29 µg/L