LITTOISTENJÄRVEN seuranta sinilevämyrkkyjen suhteen

Date of analys 3.8.2022
Sample collection, immunoassay, data analyis and report by **SULTANA AKTER**
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Assay method reference:

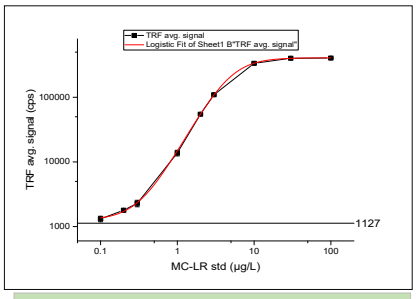
Sultana Akter, Markus Vehniäinen, Lisa Spoof, Sonja Nybom, Jussi Meriluoto, and Urpo Lamminmäki. Broad-spectrum noncompetitive immunocomplex immunoassay for cyanobacterial peptide hepatotoxins (microcystins and nodularins), Analytical Chemistry, **2016**, 88, 10080−10087. (PMID:27657987)

Assay method: Immunoassay with some modification based on concept of Akter et al., 2016

1. Prewash streptavidin coated strips (yellow,41-07 TY, KG 2109).
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)
9.6.2022: 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 242 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) s	A	B	C	avg sig	std dev	cv%	blk+3SD (9 blank)
0	1186	1126	1144	**1210**	139	11.5	1627
0	1284	1358	1332				
0	1396	1056	1004				
0.1	1466	1228	1258	**1317**	130	9.8	
0.2	1874	1672	1828	**1791**	106	5.9	
0.3	2344	2006	2521	**2290**	262	11.4	
1	12477	13685	15190	**13784**	1359	9.9	
2	53984	54944	55441	**54790**	741	1.4	
3	106491	109582	112371	**109481**	2941	2.7	
10	334971	340421	331328	**335573**	4576	1.4	
30	404629	397455	404559	**402214**	4122	1.0	
100	397982	413268	402032	**404427**	7920	2.0	

	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_Saarten taus	1385	1556	1470	**1470**	below analytical DL	86	5.8	0.14	1	0.14	below 0.2 µg/L (below analytical DL)
2_B_Koilliselkä	1674	1232	1280	**1395**	below analytical DL	243	17.4	0.11	1	0.11	below 0.2 µg/L (below analytical DL)
3_C_Luoteisselkä	1354	1224	1450	**1343**	below analytical DL	113	8.4	--	1	#VALUE!	below 0.2 µg/L (below analytical DL)
4_A'_Hiekkaranta	1248	1249	1349	**1282**	below analytical DL	58	4.5	--	1	#VALUE!	below 0.2 µg/L (below analytical DL)
5_D'_Ristikallion Uimaranta	1652	1404	1400	**1485**	below analytical DL	144	9.7	0.14	1	0.14	below 0.2 µg/L (below analytical DL)
blk+3SD (n=9)				1627				0.18			

Analytical DL (Detection limit) based on (blk+3SD) sig	1627	0.18 µg/L
set Detection Limit (based on used std signal) for reporting	**1791**	**0.20 µg/L**

Interpretation (3.8.2022 SA)

Collecttion of Raw water samples : 3.8.2022
Immunoassay analysis: 3.8.2022.
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 detects cyanobacterial peptide hepatotoxins (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 as follows:

3.8.2022 A_Saarten taus: below 0.2 µg/L (below analytical DL)
B_Koilliselkä: below 0.2 µg/L (below analytical DL)
C_Luoteisselkä: below 0.2 µg/L (below analytical DL)
A'_Hiekkaranta: below 0.2 µg/L(below analytical DL)
D'_Ristikallion Uimaranta: below 0.2 µg/L (below analytical DL)