

LITTOISTENJÄRVEN seuranta sinilevämyrkköjen suhteen

Date of analysis 15.9.2022

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

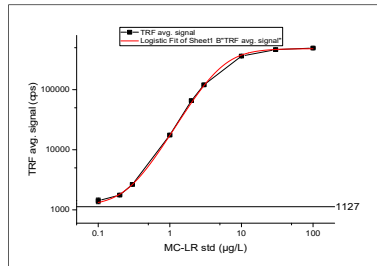
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Assay method reference:

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 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.



standard curve of microcystin-LR

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).

(x)	TRF signal (counts per second)			(y)	std dev	cv%	blk+3SD (9 blank)
MC-LR (µg/L)	A	B	C	avg sig			
0	1386	1256	1296	1345	81	6.0	1589
0	1486	1312	1306				
0	1338	1270	1458				
0.1	1583	1278	1398	1420	154	10.8	
0.2	1769	1714	1802	1762	44	2.5	
0.3	2683	2592	2663	2646	48	1.8	
1	16900	18172	17667	17580	640	3.6	
2	63722	64593	68916	65744	2782	4.2	
3	125738	117607	117665	120337	4678	3.9	
10	367242	355515	363548	362102	5996	1.7	
30	487048	446136	460620	464601	20745	4.5	
100	497203	484209	497264	492892	7520	1.5	

Date	sample	TRF signal			(y)	sig comments	std dev	cv%	*(x) From origin		1x conc (µg/L)	Reported conc (µg/L)
		A	B	C	Avg				conc µg/L	DF		
18.8.2022	1_A_Saarten laus	1412	1304	1260	1325	below analytical DL	78	5.9	1	1	#VALUE!	below 0.2 µg/L (below analytical DL)
	2_B_Koilliselkä	1516	1336	1535	1462	below analytical DL	110	7.5	0.14	1	0.14	below 0.2 µg/L (below analytical DL)
	3_C_Luoteiselkä	1518	1446	1360	1441	below analytical DL	79	5.5	0.13	1	0.13	below 0.2 µg/L (below analytical DL)
	4_A'_Hiekkaranta	1408	1392	1440	1413	below analytical DL	24	1.7	0.12	1	0.12	below 0.2 µg/L (below analytical DL)
	5_D'_Ristikallion Uimaranta	1531	1426	1372	1443	below analytical DL	81	5.6	0.13	1	0.13	below 0.2 µg/L (below analytical DL)
31.8.2022	1_A_Saarten laus	1354	1258	1312	1308	below analytical DL	48	3.7	1	1	#VALUE!	below 0.2 µg/L (below analytical DL)
	2_B_Koilliselkä	1418	1416	1483	1439	below analytical DL	38	2.6	0.13	1	0.13	below 0.2 µg/L (below analytical DL)
	3_C_Luoteiselkä	1346	1415	1288	1350	below analytical DL	64	4.7	0.11	1	0.11	below 0.2 µg/L (below analytical DL)
	4_A'_Hiekkaranta	1342	1455	1428	1408	below analytical DL	59	4.2	0.12	1	0.12	below 0.2 µg/L (below analytical DL)
	5_D'_Ristikallion Uimaranta	1356	1332	1418	1369	below analytical DL	44	3.2	0.11	1	0.11	below 0.2 µg/L (below analytical DL)
15.9.2022	1_A_Saarten laus	1359	1280	1466	1368	below analytical DL	93	6.8	0.11	1	0.11	below 0.2 µg/L (below analytical DL)
	2_B_Koilliselkä	1480	1444	1529	1484	below analytical DL	43	2.9	0.14	1	0.14	below 0.2 µg/L (below analytical DL)
	3_C_Luoteiselkä	1438	1475	1344	1419	below analytical DL	68	4.8	0.13	1	0.13	below 0.2 µg/L (below analytical DL)
	4_A'_Hiekkaranta	1552	1630	1432	1538	below analytical DL	100	6.5	0.15	1	0.15	below 0.2 µg/L (below analytical DL)
	5_D'_Ristikallion Uimaranta	1372	1480	1582	1478	below analytical DL	105	7.1	0.14	1	0.14	below 0.2 µg/L (below analytical DL)
blk+3SD (n=9)				1589							0.16	

Analytical DL (Detection limit) based on (blk+3SD) sig **1589** **0.16 µg/L**
 set Detection Limit (based on used std signal) for reporting **1762** **0.20 µg/L**

Interpretation (15.9.2022 SA)

Collection of raw water samples : 18.8.2022, 31.8.2022 and 15.9.2022

Immunoassay analysis: 15.9.2022.

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:

18.8.2022	A_Saarten laus:	below 0.2 µg/L (below analytical DL)
	B_Koilliselkä:	below 0.2 µg/L (below analytical DL)
	C_Luoteiselkä:	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)
31.8.2022	A_Saarten laus:	below 0.2 µg/L (below analytical DL)
	B_Koilliselkä:	below 0.2 µg/L (below analytical DL)
	C_Luoteiselkä:	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)
15.9.2022	A_Saarten laus:	below 0.2 µg/L (below analytical DL)
	B_Koilliselkä:	below 0.2 µg/L (below analytical DL)
	C_Luoteiselkä:	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)

