

LITTOISTENJÄRVEN seuranta sinilevämyrkykjen suhteen

Date of analysis: **23.6.2021**

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).
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)

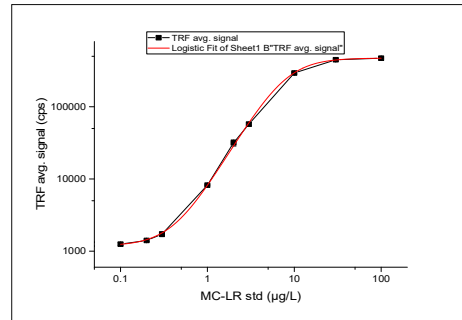
30.9.2019SA: Further working standard solution in reagent water: 100, 30, 10, 3, 2, 1, 0.3, 0.2 and 0.1 µg/L

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% |
| 0 | 1298 | 1285 | 1172 | 1254 | | 62 |
| 0 | 1357 | 1222 | 1289 | | | 4.9 |
| 0 | 1272 | 1178 | 1212 | | | |
| 0.1 | 1322 | 1217 | 1218 | 1252 | | 60 |
| 0.2 | 1458 | 1396 | 1371 | 1408 | | 45 |
| 0.3 | 1875 | 1642 | 1654 | 1724 | | 131 |
| 1 | 8257 | 8071 | 8331 | 8220 | | 134 |
| 2 | 30509 | 29375 | 35086 | 31657 | | 3024 |
| 3 | 54897 | 55892 | 61506 | 57432 | | 3563 |
| 10 | 300567 | 295025 | 283734 | 293109 | | 8579 |
| 30 | 455370 | 457434 | 430177 | 447660 | | 15176 |
| 100 | 478609 | 471995 | 458800 | 469801 | | 10085 |

| sample | TRF signal | | | (y) | | | *(v) From origin | | DF | 1x conc (µg/L) | reported conc (µg/L) |
|--------------------|------------|--------|--------|---------------|--------------|---------|------------------|-----------|----|----------------|----------------------|
| | A | B | C | Avg | sig comments | std dev | cv% | conc µg/L | | | |
| 1_A_11.5.2021 (1X) | 1353 | 1310 | 1163 | 1275 | below DL | 100 | 7.8 | 0.12 | 1 | 0.12 | <0.2 |
| 2_A_10.6.2021 (1X) | 41943 | 41181 | 40712 | 41279 | high | 621 | 1.5 | 2.44 | 1 | 2.4 | 2.4 |
| 3_B_10.6.2021 (1X) | 78794 | 78479 | 78539 | 78604 | high | 167 | 0.2 | 3.52 | 1 | 3.5 | 3.5 |
| 4_C_10.6.2021 (1X) | 72526 | 82713 | 84558 | 79932 | high | 6480 | 8.1 | 3.55 | 1 | 3.6 | 3.6 |
| 5_A_10.6.2021 (1X) | 20903 | 20102 | 18218 | 19741 | high | 1378 | 7.0 | 1.63 | 1 | 1.6 | 1.6 |
| 6_D_10.6.2021 (1X) | 22890 | 21597 | 23501 | 22663 | high | 972 | 4.3 | 1.76 | 1 | 1.8 | 1.8 |
| 7_A_22.6.21 (1X) | 383327 | 380340 | 385716 | 383128 | very high | 2694 | 0.7 | 15.90 | 1 | 15.9 | 15.9 |
| 8_B_22.6.21 (1X) | 443241 | 392899 | 415395 | 417178 | very high | 25218 | 6.0 | 20.98 | 1 | 21.0 | 21.0 |
| 9_C_22.6.21 (1X) | 387742 | 390749 | 385986 | 388159 | very high | 2409 | 0.6 | 16.47 | 1 | 16.5 | 16.5 |
| 10_A_22.6.21 (1X) | 361205 | 367879 | 363048 | 364044 | very high | 3447 | 0.9 | 14.10 | 1 | 14.1 | 14.1 |
| 11_D_22.6.21 (1X) | 368516 | 378058 | 373085 | 373220 | very high | 4772 | 1.3 | 14.91 | 1 | 14.9 | 14.9 |

| | | |
|--|------|-----------|
| DL based on (blk+3SD) sig | 1439 | 0.20 µg/L |
| DL based on true standard above (blk+3SD) signal | 1724 | 0.30 µg/L |

Interpretation (23.6.2021 SA)

Raw water samples were stored at -20 C until analysis on 23.6.2021. 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:

11.5.2021

A'_Hiekkaranta: <0.2 µg/L

10.6.2021

A_Saarten taus: 2,4 µg/L

B_Koilliselkä: 3,5 µg/L

C_Luoteisselkä: 3,6 µg/L

A'_Hiekkaranta: 1,6 µg/L

D'_Ristikallion Uimaranta: 1,8 µg/L

22.6.2021

A_Saarten taus: 15,9 µg/L

B_Koilliselkä: 21,0 µg/L

C_Luoteisselkä: 16,5 µg/L

A'_Hiekkaranta: 14,1 µg/L

D'_Ristikallion Uimaranta: 14,9 µg/L

