Optimization and Validation of Single Chain Recombinant Antibody Based ELISA to Detect Microcystins from Fresh Water Bodies in Sri Lanka

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Microcystins (MCs) and nodularins (NODs) are produced by different species of cyanobacteria. MCs are heptapeptides composed of varying combinations of seven amino acids. Nodularin also has a structure similar to those of MCs. Over 90 different variants of identified MCs, MC-LR is the most abundantly found MC. MCs are known to cause liver damage, and reproductive toxicity. Detection of these toxins need to follow extraction, purification and detection using sophisticated instruments such as HPLC-UV and LC-MS/MS. Though these techniques offer a good sensitivity and accuracy, they are highly expensive and not readily available in Sri Lanka. Therefore, we optimized and validated single chain recombinant antibody (Nat.1.scAbs) based indirect competitive enzyme linked immunosorbent assay (ic-ELISA) to detect MCs from freshwater bodies, well below the WHO recommended value of 1 µg/L. The standard curve was constructed by plotting the binding percentage against the concentration of the ten calibrators on a logarithmic scale. To determine the accuracy (recovery percentage), precision (Coefficient of variation (CV)) and matrix effect, three blank samples were spiked at four concentration levels (2.5 µg/L, 1.25 µg/L, 0.8 µg/L, and 0.35 µg/L). The working range of an ic- ELISA is between IC80- IC20 and the range was determined by the calibrators. Cross reactivity was determined by testing the binding ability of the scAb's against seven microcystin analogues and nodularin. The optimum sub-saturation concentration of MC-LR specific Nat.1.scAbs was 0.0781 µg/L. The Limit of detection (LOD) was 0.255 µg/L with the range of 11.133– 0.353 µg/L. The ic-ELISA showed recoveries in the range of 88.80% to 139.20% for all three tested matrixes namely de-ionized water, tap water and water obtained from freshwater bodies. The precision of the ic-ELISA was also high where the coefficient of variation (CV) was less than 10% for all the matrixes. There was no significant matrix effect noted. The cross reactivity was within the range of 44.88% to 96.62% for MC-RR, MC-YR, MC-WR, MC-LW and NOD. ELISA with Nat.1.scAb proved to be a sensitive and a reliable method to detect MCs from fresh water.

Keywords: Microcystins, Nodularin, ic-ELISA, Fresh water

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