Evaluatie sneltesten voor blauwalgtoxines

Symposium platform blauwalgen

9 Februari 2023, Els Faassen

WAGENINGEN
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Waarom willen we iets weten over blauwalgtoxines in water?

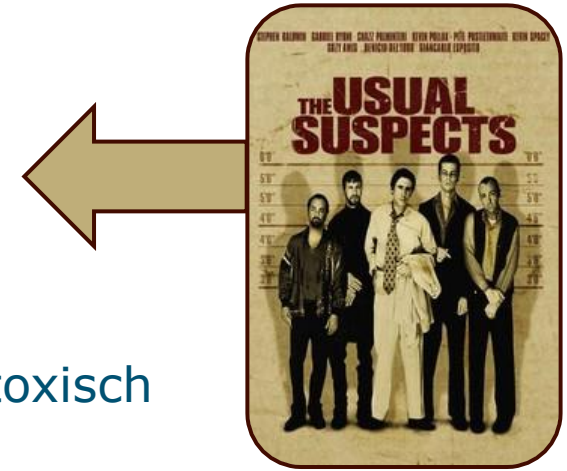
- Nu wordt de aanwezigheid van blauwalgen vaak ingeschat door middel van chlorofyl of biovolume bepalingen.
- De hoeveelheid blauwalgen is een zeer grove indicator voor het risico dat blauwalgen vormen.
- Het bepalen van de toxiciteit geeft een betere inschatting.

Hoe zit het met toxines en blauwalgen

- Sommige blauwalgen produceren gifstoffen (toxines)
- Bij een bepaalde blootstelling vormen deze gifstoffen een risico voor degene die wordt blootgesteld

Over welke gifstoffen hebben we het dan?

- Microcystines en nodularines
  - Lever, nier en neurotoxisch
  - Carcinogeen?
- Cylindrospermopsines
  - O.a. lever- en nierschade, genotoxisch
- Anatoxines
  - Acute neurotoxines
- Saxitoxines
  - Acute neurotoxines



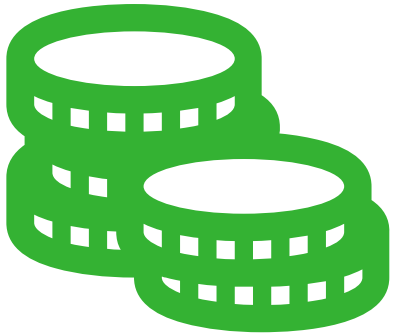
Aanleiding van het onderzoek

Waterbeheerders willen de concentratie blauwalgtoxines in oppervlaktewater kunnen bepalen, maar beschikken nu niet over snelle, relatief makkelijk uit te voeren testen.

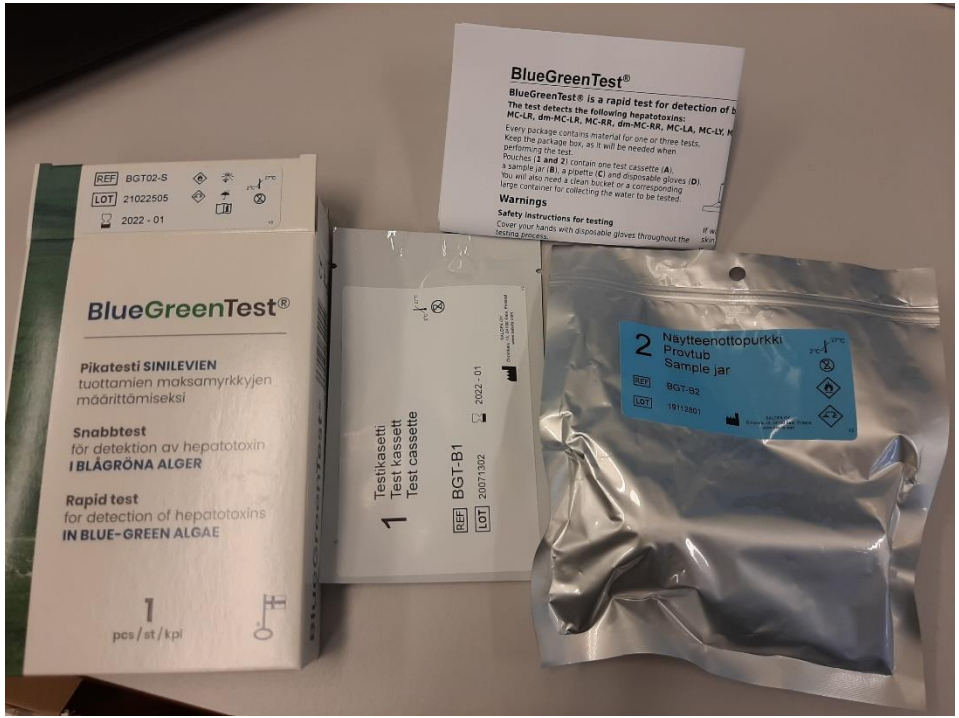
Foto: Maarten van Schijndel

Doel van het onderzoek

Inzicht te krijgen over de werking, kosten en betrouwbaarheid van commercieel verkrijgbare veld- en labtesten voor het meten van blauwalgtoxines in oppervlaktewater.



Veldtesten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1
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REF BGT02-S
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Testikasetti
Test kassett
Test cassette
1
REF BGT-B1
LOT 20071302
2022 - 01
2
Näytteenottopurkki
Provtub
Sample jar
REF BGT-B2
LOT 19112301


UITZETINSTRUCTIE
• Vul de emmer met het (besmette) water (1) en dompel het kokertje voor 15 seconden schuin onder (2).
• Zet nu het met water gevulde kokertje in het gat in de verpakking (3). Hier begint een chemisch proces waarbij het water verhit wordt.
• Laat het kokertje 10 minuten in de verpakking staan (4) en afkoelen.
• Vul het pipetje met wat water uit de koker (5) en laat drie druppels op het kleine gaatje bij de testplaat vallen (6).
• Wacht 8-10 minuten. Staat er een streepje bij de C? Dan is er geen blauwalg. Bij een streepje bij de C én de T is er blauwalg in het water. Verschijnt er helemaal geen streepje, dan is de test mislukt.
Handschoentjes aan!
15 seconden
Wacht 15 minuten.
3 druppels
Veilig water
Overal in Nederland test de rijksoverheid eens in de vier weken op blauwalg. Risicogebieden komen eens in de twee week aan de beurt.
Meer weten? Scan de QR-code om direct verder te lezen: zwemwater.nl
Wel blauwalg
Geen blauwalg

Veldtesten

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TEST TERRAIN MICROCYSTINES
EAU DE SURFACE (seuil 1µg/l)

1. Recueil de l'échantillon

Prélever 1 à 2 ml d'eau

2. Transfert-1ère Etape de Lyse*

À l'aide de la pipette graduée fournie, transférer 1 ml de l'échantillon dans le tube de lyse (contient des réactifs déshydratés).

* *Brevet en cours*

2 min. 8 min.

Fermer et agiter 2 minutes.

Laisser reposer 8 minutes.

3. Seconde étape de lyse

À l'aide de la pince fournie, ajouter un réactif-papier au tube de lyse.

2 min. 8 min.

Fermer et agiter 2 minutes.

Laisser reposer 8 minutes.

4. Transfert

Transférez 7 gouttes d'échantillon lysé au tube conique avec la pipette de transfert fournie.

(contient des réactifs déshydratés)

5. Agiter et incuber

Boucher le tube conique et agiter durant 30 s.

Incuber 20 minutes à température ambiante (10-30°C)

La coloration passe au mauve.

6. Test

Introduire la bandelette-test dans le tube conique.
Flèche vers le bas.

Incuber 10 minutes à température ambiante (10-30°C)

7. Retirer la bandelette, poser à plat et laisser la coloration se développer encore 5 minutes.

8. Lecture du Test

µg/l
5.0
2.5
1.0
0.5
0

Bande Contrôle Bande Test

Interprétation

Bande Contrôle	Bande Test	Interprétation
ABSENCE	ABSENCE	INVALIDE
PRÉSENCE	ABSENCE	> 5 µg/l (ppb)
PRÉSENCE	PRÉSENCE	ENTRE 0 ET 5 µg/l
	INTENSITÉ VARIABLE	SELON INTENSITÉ

Fabriqué par :
ABRAXIS, LLC 54 Steamwhistle Drive, Warminster, PA 18974
Phone: 215-357-3911
Fax: 215-357-5232
www.abraxiskits.com

Distribué par :
NOVAKITS, 40 boulevard Jean Ingres - 44100 NANTES
Tél. : 09 61 58 14 40
info@novakits.com
www.novakits.com

Références : *1520019 (5 tests) et 1520020 (20 tests)*

Labtesten

A. Reagents and Materials Provided

1. Microliter plate coated with a secondary antibody (anti-mouse), in a re-sealable aluminum pouch
2. Lyophilized Anatoxin-a-HRP Enzyme Conjugate, 3 vials
3. Conjugate Diluent, 12 mL
4. Lyophilized Anti-Anatoxin-a Antibody, 3 vials
5. Antibody Diluent, 12 mL
6. Empty clear and amber HDPE bottles for combining reconstituted Enzyme Conjugate and Antibody (if necessary)
7. (+)Anatoxin-a Standards (6): 0, 0.15, 0.40, 1.0, 2.5, 5.0 ppb, 1.5 mL each
8. Control at 0.75 ± 0.185 ppb, 1.5 mL
9. Sample Diluent (10X) Concentrate, 2 X 25 mL
10. Wash Buffer (5X) Concentrate, 100 mL, must be diluted before use, see Test Preparation (Section D)
11. Substrate (Color) Solution (TMB), 12 mL
12. Stop Solution, 12 mL (handle with care)

B. Additional Materials (not delivered with the test kit)

1. Micro-pipettes with disposable plastic tips (10-200 and 200-1000 µL)
2. Multi-channel pipette (10-300 µL), stepper pipette (10-300 µL), or electronic repeating pipette with disposable plastic tips (capable of delivering 50-300 µL)
3. Microliter plate washer (optional)
4. Microliter plate reader (wave length 450 nm)
5. Deionized or distilled water
6. Container with 500 mL capacity (for diluted 1X Wash Buffer, see Test Preparation, Section D)
7. Paper towels or equivalent absorbent material
8. Timer

C. Sample Collection and Handling

Collect water samples in amber glass sample containers. Drinking water samples should be treated with ascorbic acid (up to 1 mg/mL) immediately after collection to remove residual chlorine. *Do not use sodium thiosulfate. Sodium thiosulfate will degrade Anatoxin-a.*

Immediately upon collection, fresh water samples must be preserved using the Sample Diluent (10X) Concentrate (1 mL of 10X Sample Diluent Concentrate per 9 mL of water sample), to prevent degradation of Anatoxin-a. Samples must be adjusted to between pH 5 and pH 7 and protected from exposure to natural and artificial light, as exposure to light and/or high pH will cause degradation of Anatoxin-a. Store samples refrigerated (up to 28 days). For storage periods greater than 28 days, samples should be stored frozen. Seawater samples do not need to be preserved but the same pH and storage conditions should be applied.

Drinking water samples treated with ascorbic acid (0.1 mg/mL) and sodium bisulfate (1 mg/mL) according to EPA Method 545 do not need to be preserved with Sample Diluent (10X) Concentrate. Samples must be adjusted to between pH 5 and pH 7. The same storage conditions (light, temperature, and duration) apply as described above.

Anatoxin-a is an intracellular, as well as extracellular, toxin. Therefore, to measure total Anatoxin-a, cell lysing will be required. Once the sample is preserved, three freeze/thaw cycles are recommended for cell lysing. This procedure using the three freeze/thaw cycles will not degrade Anatoxin-a.

Preserved fresh water or seawater samples may be filtered following cell lysing and prior to analysis using any of the following syringe filters: Environmental Express 0.2 µm PES (PN SF020E), Pall Acrodisc® 0.2 µm PVDF (PN 4450), Supor® membrane syringe filters (PN 4612), or Environmental Express 1.2 µm Glass Fiber (PN SF012G). *Note: Fresh water samples must be preserved (and lysed) prior to filtration or Anatoxin-a may bind to the filter, removing it from the sample, and producing falsely low sample results.*

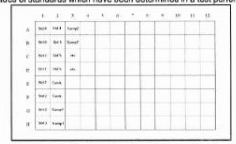
D. Test Preparation

Micro-pipetting equipment and pipette tips for pipetting the standards and the samples are necessary. A multi-channel, stepping, or electronic repeating pipette is recommended for adding the enzyme conjugate, antibody, substrate, and stop solutions in order to equalize the incubation periods across the entire microliter plate. Please only use the reagents and standards from one package lot in one test, as they have been adjusted in combination.

1. Allow the microliter plate, reagents, and samples to reach room temperature before use.
2. The enzyme conjugate and antibody need to be reconstituted prior to use. Add 3 mL of the appropriate diluent to the appropriate vial and vortex well. Let sit for at least 10 minutes and re-vortex prior to use. ***If more than one vial is required for testing, combine the reconstituted enzyme conjugate vials in the amber HDPE bottle and the reconstituted antibody vials in the clear HDPE bottle prior to use.*** The solutions are stable for up to 2 weeks if stored at 2-8°C and up to 1 month if stored frozen.
3. Remove the number of microliter plate strips required from the foil bag. The remaining strips are stored in the foil bag and zip-locked closed.
4. The standard solutions, substrate and stop solutions are ready to use and do not require any further dilutions.
5. Dilute the Wash Buffer (5X) Concentrate at a ratio of 1:5. If using the entire bottle (100 mL) add to 400 mL of deionized or distilled water.
6. Dilute the Sample Diluent (10X) Concentrate at a ratio of 1:10 with deionized or distilled water (i.e. 1 mL of Sample Diluent (10X) Concentrate into 9 mL of deionized water) as needed for sample dilutions.
7. The stop solution must be handled with care as it contains diluted H_2SO_4.
8. After analysis, store the remaining kit components in the refrigerator (2-8°C).

E. Working Scheme

The microliter plate consists of 12 strips of 8 wells, which can be used individually for the test. The standards must be run with each test. Never use the values of standards which have been determined in a test performed previously.



Std 0-Std5: Standards
Contr.: Control
Samp1, Samp2, etc: Samples

F. Assay Procedure

1. Add **50 µL of the standard solutions, control, or samples** into the wells of the test strips according to the working scheme given. Analysis in duplicate or triplicate is recommended.
2. Add **50 µL of the reconstituted enzyme conjugate solution** to the individual wells successively using a multi-channel, stepping, or electronic repeating pipette.
3. Add **50 µL of the reconstituted antibody solution** to the individual wells successively using a multi-channel, stepping, or electronic repeating pipette. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a circular motion on the benchtop for 60 seconds. Be careful not to spill the contents. Incubate the strips for **60 minutes** at room temperature.
4. After incubation, remove the covering, decant the contents of the wells into a sink, and blot the inverted plate on a stack of paper towels. Wash the strips **four times** using the diluted wash buffer. Use at least a volume of **250 µL of 1X wash buffer** for each well and each washing step. ***Blot the inverted plate after each wash step*** on a stack of paper towels. After the last wash/blot, check the wells for any remaining buffer in the wells, and if necessary, remove by additional blotting.
5. Add **100 µL of substrate (color) solution** to the individual wells successively using a multi-channel, stepping, or electronic repeating pipette. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a circular motion on the benchtop for 30 seconds. Be careful not to spill the contents. Incubate the strips for **20-30 minutes** at room temperature. Protect the strips from sunlight.
6. Add **100 µL of stop solution** to the wells in the same sequence as for the substrate (color) solution using a multi-channel, stepping, or electronic repeating pipette.
7. Read the absorbance at 450 nm using a microtiter plate ELISA photometer within 15 minutes after the addition of the stopping solution.

G. Evaluation

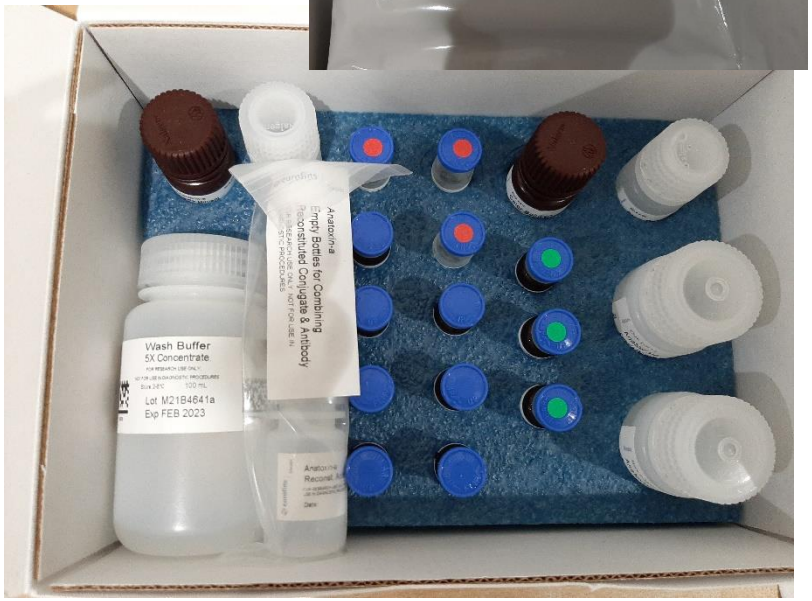
The evaluation of the ELISA can be performed using commercial ELISA evaluation programs (4-Parameter (preferred) or Logit/Log). For a manual evaluation, calculate the mean absorbance value for each of the standards. Calculate the %B/B_0 for each standard by dividing the mean absorbance value for each standard by the Zero Standard (Standard 0) mean absorbance. Construct a standard curve by plotting the %B/B_0 for each standard on a vertical linear (y) axis versus the corresponding Anatoxin-a concentration on horizontal logarithmic (x) axis on graph paper. %B/B_0 for the control and samples will then yield levels in ppb of Anatoxin-a by interpolation using the standard curve. Results can also be determined using a spreadsheet macro available from Eurofins Abraxis upon request.

Results for fresh water samples which have been preserved with Sample Diluent (10X) Concentrate as described in Sample Collection and Handling (section C) must be multiplied by a factor of 1.1 to account for the initial dilution.

The concentrations of the samples are determined using the standard curve run with each test. Samples showing a lower concentration of Anatoxin-a than standard 1 (0.15 ppb) should be reported as containing < 0.15 ppb of Anatoxin-a (< 0.165 ppb for preserved water samples). Samples showing a higher concentration than standard 5 (5.0 ppb) should be reported as containing > 5.0 ppb of Anatoxin-a (> 5.5 ppb for preserved water samples) or must be diluted using 1X Sample Diluent to obtain accurate results. The concentration of the positive control provided should be 0.75 ± 0.185 ppb.

Semi-quantitative results can be derived by simple comparison of the sample absorbances to the absorbances of the standards. Samples with lower absorbances than a standard will have concentrations of Anatoxin-a greater than that standard. Samples which have higher absorbances than a standard will have concentrations of Anatoxin-a less than that standard.

As with any analytical technique (GC/MS, HPLC, etc.), positive results requiring regulatory action should be confirmed by an alternative method.



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Selectie testen

	Veldtesten	Labtesten
Microcystines	3	4
Anatoxines	1	1
Cylindrospermopsines	1	1
Saxitoxines	1	1

Experimenten

	Veldtest	Labtest
Cross reactivity		V
Aantoonbaarheid (1 µg/l)	V	V
Juistheid, reproduceerbaarheid		V
Herhaalbaarheid		V
Effect extractie		V
Effect zoutgehalte	V	V
Effect algendichtheid	V	V

Labtesten: Betrouwbaarheid

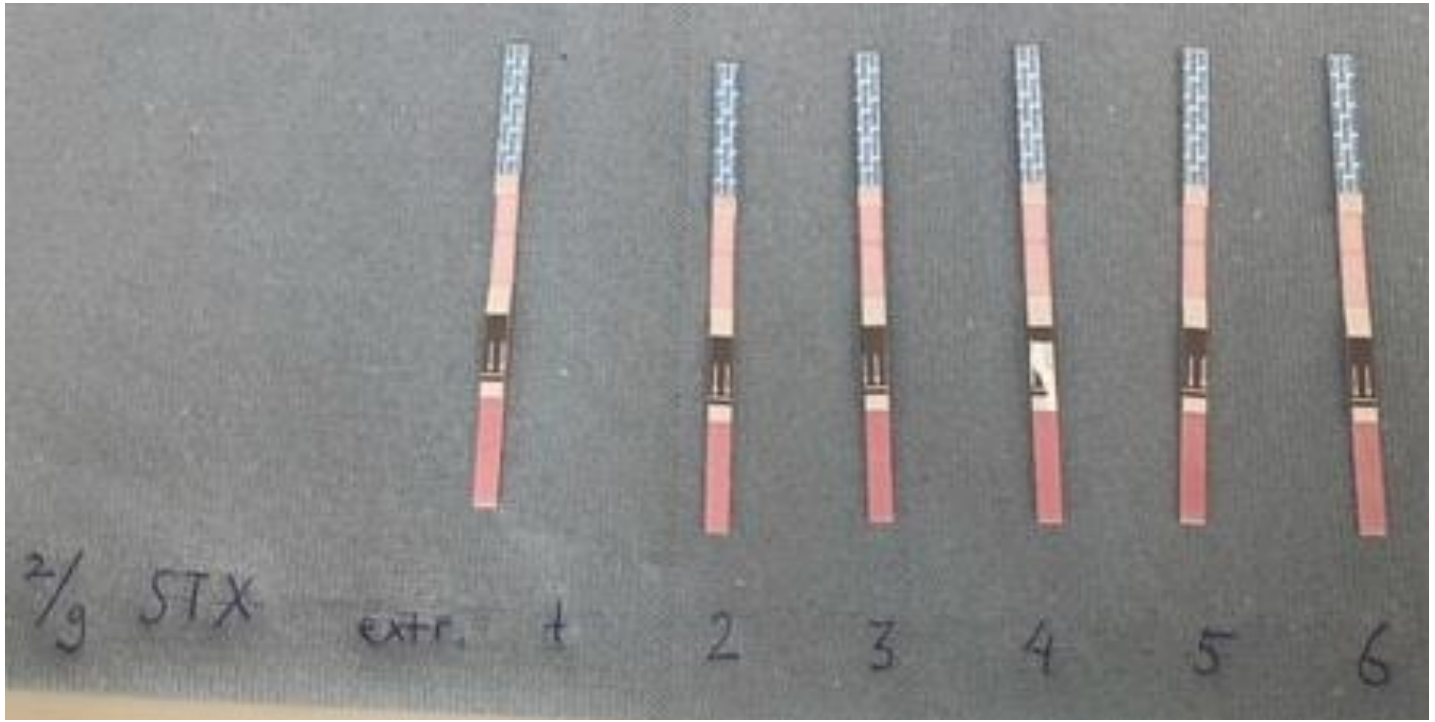
Juistheid ✓
Precisie ✗

Juistheid ✗
Precisie ✓

Juistheid ✗
Precisie ✗

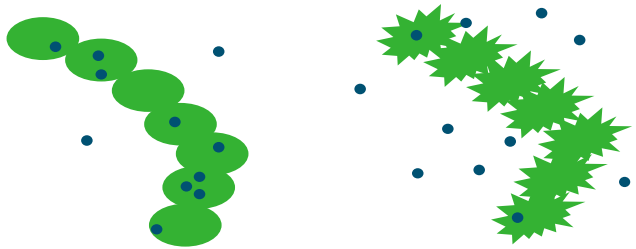
Juistheid ✓
Precisie ✓

Resultaten veldtesten

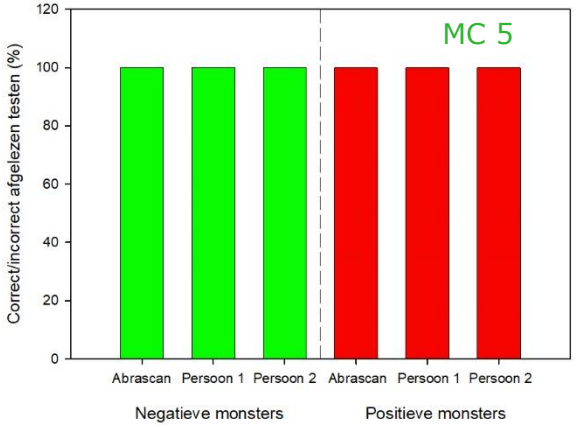


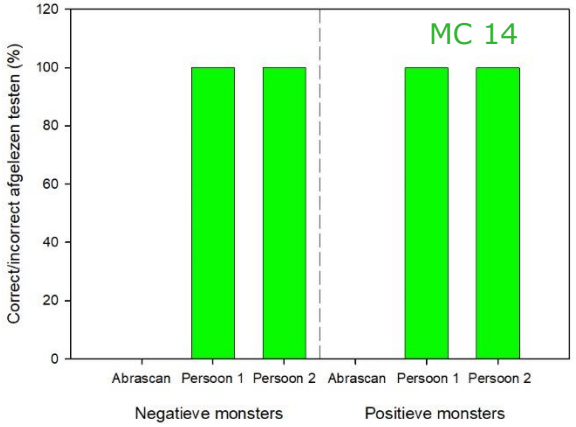
Geëvalueerde testen

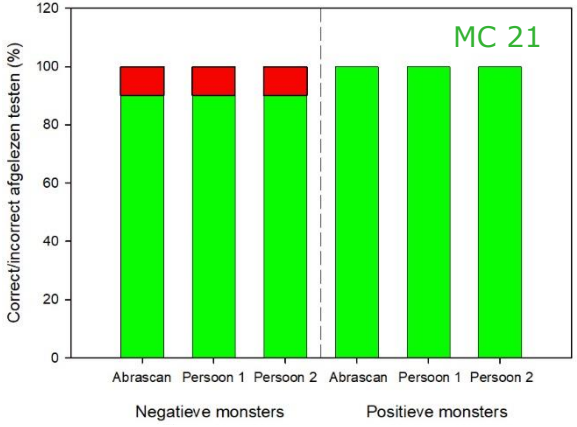
	Producent	Opmerkingen
MC 5	[redacted]	Recreatie water
MC 14	[redacted]	
MC 21	[redacted]	Drinkwater
ANA 3	[redacted]	Geen lyseerstap
CYN 4	[redacted]	
STX 2	[redacted]	Geen lyseerstap

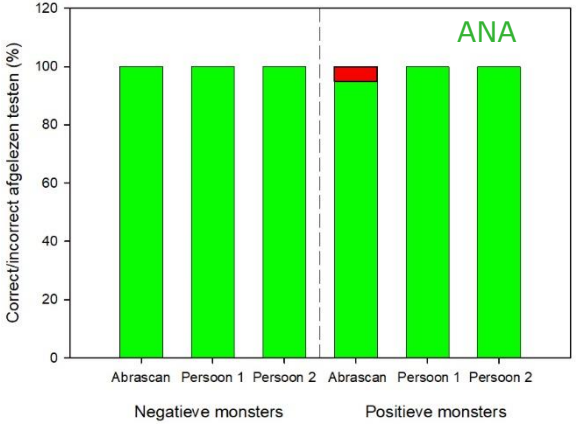


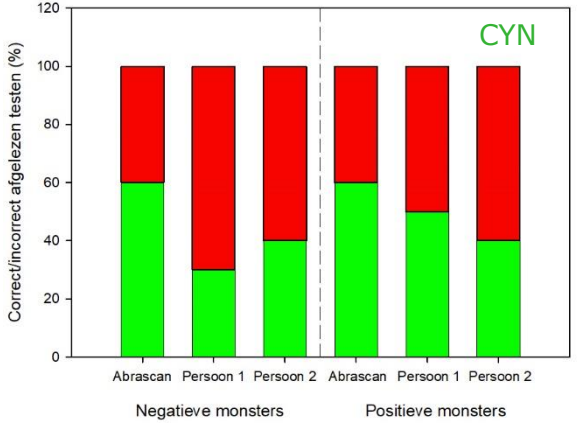
Veldtesten: aantoonbaarheid

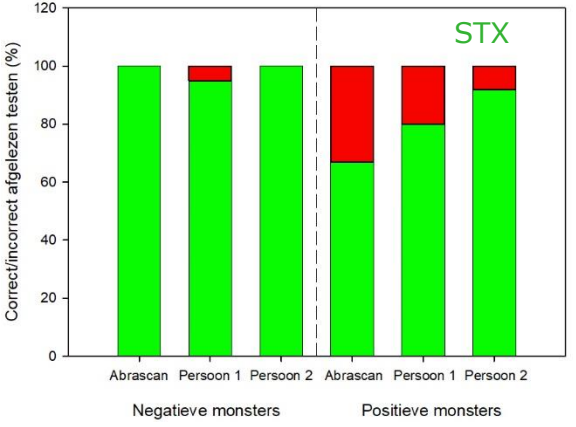












Samenvatting resultaten veldtesten

	Prestatie
Microcystines	☺
Anatoxines	☹
Cylindrospermopsines	☹ ☹
Saxitoxines	☹

- Eén microcystine test was betrouwbaar, snel en goedkoop
- Anatoxine and saxitoxine testen hebben geen lyseerstap
- Cylindrospermopsine testen kunnen toxine slecht aantonen
- De meeste testen duren te lang en zijn te ingewikkeld om routinematig in te zetten in het veld

Resultaten labtesten

Geëvalueerde testen

	Producent	Omschrijving
MC 1	[redacted]	Quantiplate kit for microcystins
MC 9	[redacted]	ADDA ELISA
MC 10	[redacted]	Monoclonal ELISA
MC 16	[redacted]	ADDA ELISA, Gevoeliger dan MC 9 en zouttolerant
ANA 6	[redacted]	Anatoxin ELISA
CYN 8	[redacted]	Cylindrospermopsin ELISA
STX 12	[redacted]	Saxitoxin ELISA

Samenvatting resultaten labtesten

	Prestatie
Microcystines	
Anatoxines	
Cylindrospermopsines	
Saxitoxines	

- Microcystines: Verschillende sterke en zwakke punten per test, gemiddeld genomen acceptabel
- Anatoxine test presteert vrij goed
- Cylindrospermopsine test geeft veel vals positieve resultaten, protocol moet aangepast worden
- Saxitoxine test is niet erg nauwkeurig
- 1 extractiemethode voor meerdere toxines is mogelijk
- Sommige testen kunnen beter tegen zout water dan andere

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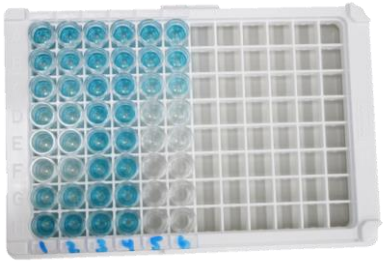
Vergelijking technieken

	Veldtest	Labtest	LC-MS/MS
Meest geschikt voor	Individuele monsters	Monsterseries	Monsterseries
Uitkomst	Aan/afwezigheid toxinegroep	Schatting concentratie per toxinegroep of aan/afwezigheid	(Schatting) concentratie per toxine
Doorlooptijd	30-60 minuten	1 dag	1,5 dag
Expertise	Niet nodig	Labpersoneel	Gespecialiseerd labpersoneel
Beschikbaar voor	MC (ANA, STX)	MC, ANA, CYN, STX	MC, ANA, CYN, STX
Kosten per monster per toxinegroep (euro)	50-100	Ca 40 (screening) Ca 90 (schatting concentratie)	Ca 120

Vervolg

- Geplande implementatie van de labtesten in de waterschapslaboratoria in 2023
    - Alle vier de toxinegroepen
    - Voor het meten van pelagische (zwevende) en eventueel benthische (aan substraat gehechte) blauwalgen
    - Initiatief nu bij Aquon en WFSR
    - Plan van aanpak eind februari bespreken met betrokken waterbeheerders en labs



Bedankt