A Novel Lateral Flow Assay that Detects Acetylcholine Receptor Ligand Toxin Anatoxin-a

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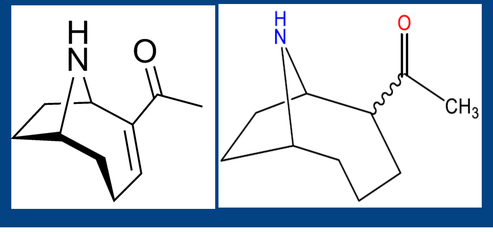
Introduction

Anatoxin-a (ATX) and its lesser known (but more deadly) counterpart dihydroananatoxin-a (dhATX) are neurotoxins produced by freshwater algal blooms. The most toxic of the congeners when taken orally is dhATX, making it particularly worrisome. The ATX and dhATX toxins exert their effects by acting as potent pre- and post-synaptic depolarizing agents that bind with very high affinity to the nAChR of motor neurons, stimulating muscle cell contraction and depolarization of neurons in the central nervous system. Outbreaks of dhATX-producing cyanobacteria have led to the death of animals in waterways throughout the world, leading to closing of potable and recreational waters and are often not detected because of limited detection techniques. Thus, developing a detection system for ATX/dhATX is essential for the recreational and water industries as well as pet owners and farmers worldwide.

Attogene has developed a lateral flow-based receptor binding assay to detect ATX/dhATX. Using this assay we demonstrate that in the presence of anatoxin-a and dihydroanatoxin-a, nAChR bound gold specifically reduces its ability to associate with the nicotine conjugate (test line), resulting in a diminished test line signal. We have evaluated this response to be both concentration dependent and isomer specific. On the other hand, the test line was not diminished by microcystin or other non-nAChR ligands. Attogene has also demonstrated this test is able to detect nAChR ligand toxins present in Oscillatoria brevis (UTEX B 1567), Anabaena flos-aquae (UTEX 2557), Anabaena subcylindrical (UTEX LB2382), and UTEX 2497, UTEX 1611, UTEX 3013 Anabaena algae cultures. The test is stable, robust and is now offered in a field deployable format for customers to perform rapid screening and easy to use analysis of anatoxin-a in the low ppb range.

Attogene first of its kind ATX lateral flow based receptor binding assay kit. Cat#AU2062 for recreational water – Field Based.



Chemical structure of anatoxin-a (ATX, left) and dihydroanatoxin-a (dhATX, right).

Assay Principle

Attogene has developed a unique, robust, stable, and scalable competitive lateral flow assay capable of detecting the acetylcholine receptor ligand toxin anatoxin-a. We have developed methods to stably assemble a functional recombinantly expressed surrogate to the human α4β2 and α7 nicotinic acetylcholine receptor (nAChR) onto colloidal gold particles. The complex of AChR colloidal gold was embedded into a release matrix pad and dried. The nAChR-conjugated gold release matrix pads were applied to backing cards containing nitrocellulose sprayed with a specially designed nicotine conjugate (test line) and an independent control line. A wick and sample pad were applied to the backed cards and then cut into strips.

Results

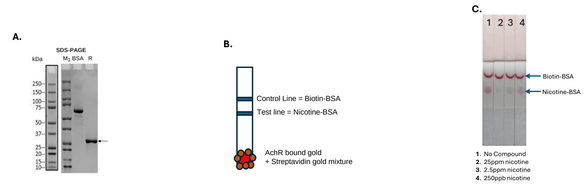
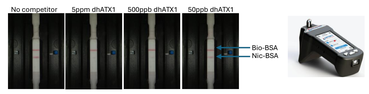


Figure 1. Competitive Lateral Flow Receptor Binding Assay. Expression and purification of recombinant nAChR made in insect cells (A). The architecture of the lateral flow assay is depicted in (B). The data generated from the lateral flow architecture was evaluated in a competitive lateral flow assay using a nicotine-BSA conjugate with free nicotine (C). The conclusion of these studies indicate that nicotine can competitively bind with nAChR bound gold.



	Control	Test	Ratio	%
No Compound	531477	473190	0.89	100%
5ppm dhATX 1	505001	45275	0.09	10%
500ppb dhATX 1	540209	79455	0.15	16%
50ppb dhATX 1	495518	296476	0.6	67%

Figure 2. nAchR receptor binding assay is responsive to cis-dhATX. nAChR was applied to colloidal gold and tested in a competitive lateral flow assay using a nicotine-BSA (B). A control line was generated using streptavidin conjugated to colloidal gold. Lateral flow results show that dhATX1 can compete for the ability of the nAChR gold binding to the nicotine conjugate (C).

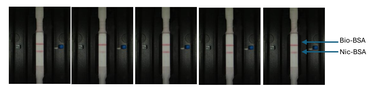


Figure 3. Specificity analysis of the nAChR receptor lateral flow binding assay. Lateral flow strips sprayed with nicotine-BSA conjugate and biotinylated BSA with AChR and streptavidin bound gold were added into wells containing the indicated amount of compound and 150ul of assay running buffer.

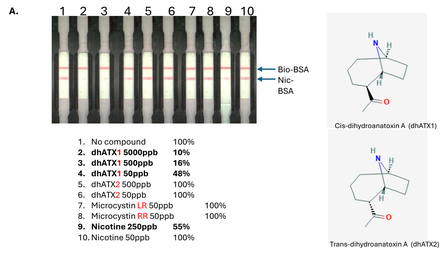


Figure 4. Assessment of lateral flow assay's ligand and isomer specificity. (A) dhATX-cis (dhATX1) is detectable at 50ppb which is >10-fold more than dhATX-trans (dhATX2) sensitivity. Microcystin LR or RR congeners do not bind to AchR while (±)-nicotine has a 10-fold lower sensitivity than dhATX in this test. (B) Cis and Trans dhATX.

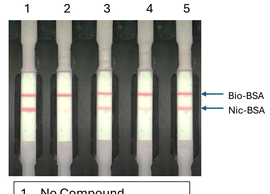
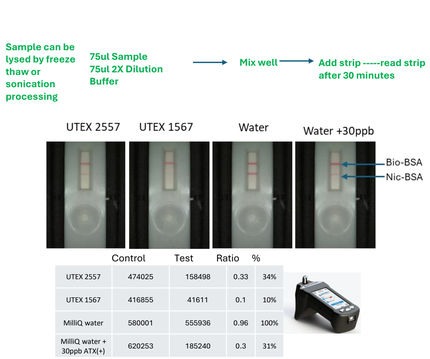


Figure 5. Evaluation of detection limit for isomer purified and mixed isomer ATX. This data demonstrates clearly that the + isomer of ATX is about 20-fold less sensitive in the assay. The relative binding activity to nAChR correlates with its toxicity. The ATX (+) is more toxic than its (-).



	Control	Test	Ratio	%
UTEX 2557	474025	158498	0.33	34%
UTEX 1567	416855	41611	0.1	10%
MilliQ water	580001	555936	0.96	100%
MilliQ water + 30ppb ATX(+)	620253	185240	0.3	31%

Figure 6. Strips in cassettes used to evaluate levels of Anatoxin-a in algae cultures. Algae cultures were extracted using sonication, centrifugation and then diluted into the lateral flow sample dilution buffer. Signals were analyzed using the RDS-2500.

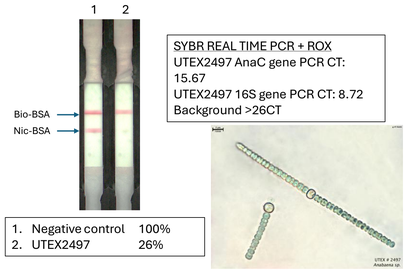
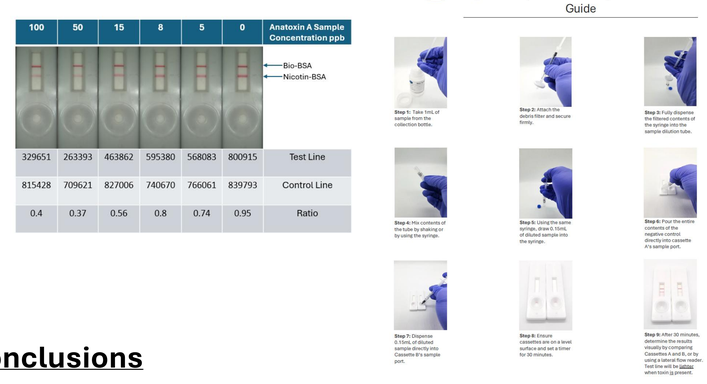


Figure 7. Confirmation of UTEX2497 containing AnaC gene cluster by PCR and positive in our assay. Data demonstrating the use of the AchR-based lateral flow assay with an algae strain containing the gene region needed to express anatoxin-a family of toxins based on real-time PCR analysis. Strain photo included for reference.



Conclusions

1. We have developed a novel receptor binding assay that permits rapid detection of extremely low amounts of anatoxin-a and dihydroanatoxin-a
2. The test is isomer specific – able to differentiate between cis, trans, +/-
3. Created a test kit that is field deployable, stable, convenient, rugged, robust and reasonably priced

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