

**SONA NANOTECH'S MULTICOLOURED
GOLD NANORODS: THE GOLD STANDARD
FOR COMPETITIVE LATERAL FLOW ASSAYS**



SONA NANOTECH INC | HALIFAX, NOVA SCOTIA, CANADA



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Based in Halifax, Nova Scotia, Canada, Sona Nanotech Inc. has developed a unique proprietary method of manufacturing gold nanorods (GNRs) without the toxic surfactant CTAB (cetyltrimethylammonium bromide).

Sona's GNRs are an improvement on gold and other nanoparticle products already on the market, exhibiting characteristics including increased sensitivity, varied colour results, tunable aspect ratios, long-term stability, facile bioconjugation and improved shelf life.

In this white paper we will show how Sona Nanotech's GNRs are perfectly suited for competitive lateral flow assays.

They offer clear visual readings, distinct colour patterns and greater sensitivity, and can be read quantitatively by a reader, making them ideal for use in multiplexed devices.

A lateral flow test using non-CTAB synthesized gold nanorods for dual color detection of delta-9-tetrahydrocannabinol

Abstract:

Lateral flow tests exist for the detection of $\Delta 9$ -Tetrahydrocannabinol (THC) in saliva. These tests generally employ a gold nanosphere to generate the detection and control zone signals. In this study Sona Nanotech's gold nanorods (AuNRs) with two different colors (blue and red) were successfully conjugated to anti-THC and IgY antibodies and used as detection and control particles in a THC lateral flow test. The developed lateral flow strip could detect 25 ng/mL THC within 10 minutes. A dose-dependent displacement of signal was seen from 0 to 500ng/mL. The test was able to be read using a low-cost, portable reader system with coefficient of variations ranging from 2.0 to 12.2% being seen across the range of concentrations tested. It was further shown that AuNRs can be combined with traditional gold nanospheres in a lateral flow format, increasing the possible range of application of the AuNR particles in the field of lateral flow testing.

1. Introduction

THC lateral flow tests are designed to measure the level of $\Delta 9$ -Tetrahydrocannabinol, the principal psychoactive cannabinoid of the cannabis plant, in saliva. The test is carried out by adding the saliva sample onto the sample addition port of the lateral flow test cassette. The liquid moves by capillary action through the sample pad and conjugate pad hydrating the dried AuNR labelled anti THC antibody conjugate. The whole mixture continues its flow through the nitrocellulose membrane towards the absorbent pad at the end of the strip. As the mixture flows across the membrane, the AuNR labelled anti-THC antibody will be captured by a BSA-THC hapten conjugate test line, resulting in the appearance of a visible capture line. If THC is present in the sample, this will bind to the AuNR labelled anti-THC antibody inhibiting the binding of the AuNR conjugated antibody to the THC test line. Capture line intensity is therefore inversely proportional to the THC concentration in the sample. An anti-IgY control line binds to AuNR labelled IgY antibody also dried onto the conjugate pad. The intensity of this control line should remain stable independent of THC concentration in the sample.

2. Materials and Methods

2.1 Reagents and instruments

AuNR, Blue Gemini Nanorods ($\lambda=650\text{nm}$) and Red Gemini Nanorods ($\lambda=850\text{nm}$), were provided by Sona nanotech. Artificial THC saliva samples were from Synergent Bio (California,USA). Anti-THC, IgY, anti-IgY, 20nm IgY gold nanosphere conjugate, THC-BSA hapten and general lateral flow pad materials were supplied by Lateral Dx Ltd (Alloa, Scotland). Other reagents were of analytical grade or higher. The Optricon Cube reader was from Chembio (Berlin, Germany).

2.2 Conjugation of anti-THC and IgY antibodies to AuNR

Blue Gemini nanorods were conjugated to the anti-THC antibody using the provided protocol supplied by Sona Nanotech. Red Gemini nanorods were conjugated to IgY using the provided protocol supplied by Sona Nanotech. The resulting conjugates were stored at 2-8 °C until ready for use.

2.3 Assembly of the ICA strip

The nitrocellulose membranes were coated with 0.5 mg/mL BSA-THC hapten conjugate (Capture line) and goat anti-IgY antibody (0.5 mg/mL, control line). Membranes were dried for 18 hours at 37 °C then stored desiccated at room temperature. The conjugate pad was impregnated with AuNR THC and IgY conjugates (both AuNR and 20nm gold nanosphere) diluted 1:10 with conjugate dilution buffer. Conjugate pad was dried for 18 hours at 37 °C then stored desiccated at room temperature. Cards were assembled with treated sample pad, AuNR treated conjugate pad, coated nitrocellulose and absorbent pad. The assembled cards were cut into 4.0 mm width and stored at room temperature under desiccated condition. Strips were then assembled into lateral flow cassette housings.

2.4 Testing of performance of THC test strips

The prepared test cassettes were tested with artificial saliva at the following levels 0,25,50,100,150 and 500 ng/mL. 100ul of each standard was applied to the cassette sample port. Each standard concentration was run in triplicate. After 10 min of running time, the results were analyzed quantitatively by the lateral flow strip reader. Kinetic measurements of the test strips were also made using the reader by running the 0, 50, 100 and 500ng/mL standards. Measurements were made every 30 seconds for 15 minutes.

3. Results and Discussions

Performance of AuNR THC Test

Figure 1 shows the capture line signals achieved for the standard concentrations tested. The T/C ratio was also determined by dividing the capture line signal (T) by the control line signal (C), Figure 2. As shown in Figure 3, the colour of blue capture lines gradually became lighter on the strips with the increase concentrations of THC. There was a determinable difference in signal seen between 0 and 25 ng/mL. The T lines disappeared at 500 ng/mL of THC.

Figure 1: Capture Line THC Displacement Curve

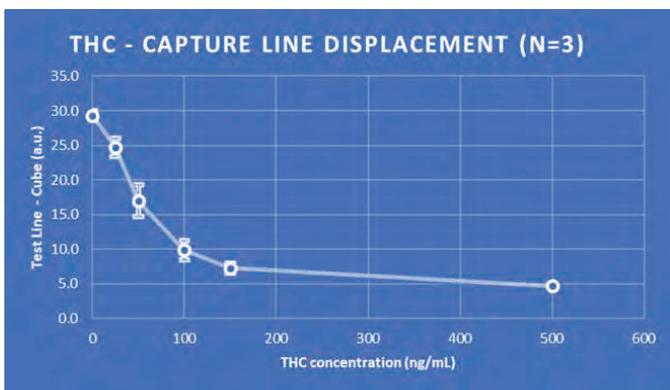


Figure 2: T/C Ratio THC Displacement Curve

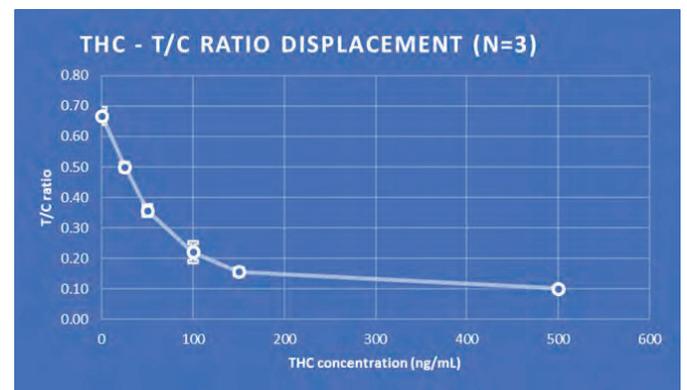


Figure 3: Photograph of AuNRs Capture and Control Test strips. Replicates 1,2 and 3 at 10 minutes

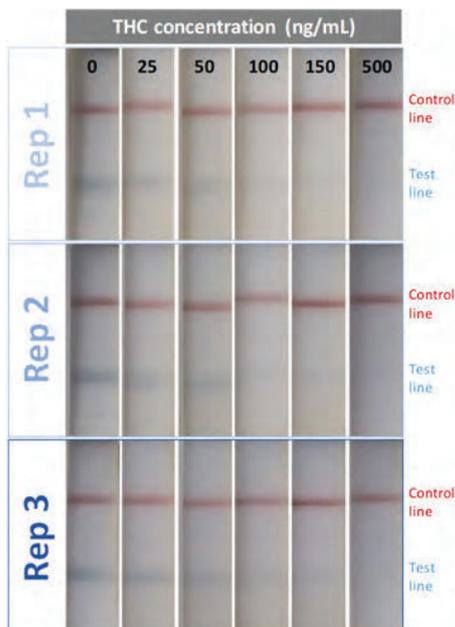
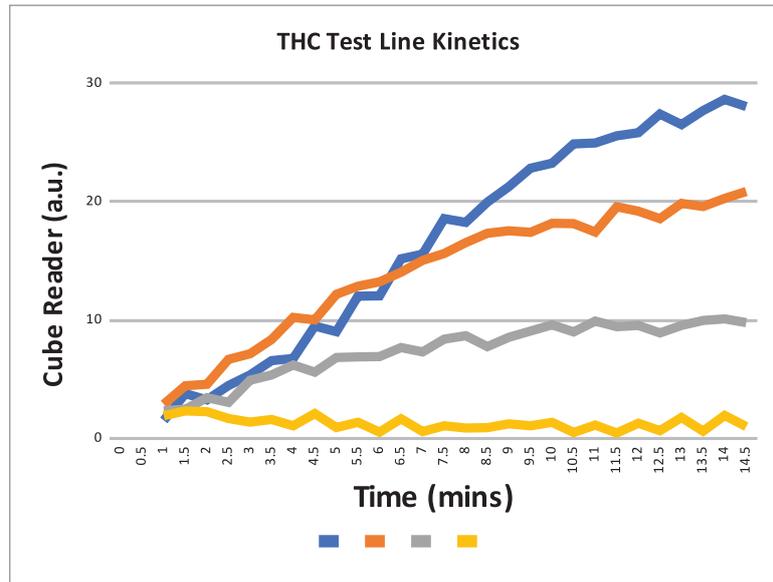


Figure 4: Photograph of AuNRs Capture and 20nm Gold Nanosphere Control Test strips. Replicates 1,2 and 3 at 5 minutes

The ability of the AuNR THC conjugate to work with a 20nm IgY gold nanosphere conjugate was tested by running standards of 0, 50,100 and 500 ng/mL. Tests were read visually at 5 minutes with no reader data being captured. The AuNR and 20nm IgY conjugates formed clear capture and control line signals and were able to be used together on the one test strip, Figure 4.

Kinetic measurement of capture line signal development is shown in Figure 5. Capture line signal increased from 0 to 15 minutes for the 0 and 50ng/mL samples. Signals for the 100 ng/mL and 500 ng/mL standards plateaued at ~10 and 5 minutes respectively. Maximal differential between standards was estimated to be between 10 and 15 minutes.

Figure 5: Kinetic Measurement of Capture line signal AuNRs, 0-15 minutes. 0, 50, 100, 500 ng/mL



4. Conclusion

Non-CTAB synthesized AuNRs (blue and red) were successfully conjugated to anti-THC and IgY antibodies. The resulting conjugates were able to be used in a lateral flow test format to provide a test with different capture and control line colors. Test displacement increased with increasing THC concentration. A limit of detection of 25ng/mL was determined under the conditions tested. With further development is expected that this LOD could be improved further. The AuNRs were able to be used in conjunction with 20nm gold nanospheres with no interaction of the two different particle types being seen. This could allow the AuNRs be incorporated into existing on-market lateral flow tests where the existing capture and control conjugates are using gold nanospheres. The AuNR conjugates were able to be detected using the Cube reader system, allowing for the potential of low cost, on-site testing.

From this experiment we have demonstrated that:

- Sona's GNRs can be successfully used in a displacement assay.
- Traditional spherical gold colloid and Sona's GNRs can be combined in the same assay system.
- Two different colored GNRs can be combined in one assay system.

Sona Nanotech's gold nanorods are perfect for:

- Multiplexing
- Qualitative and quantitative tests
- Sandwich and competitive lateral flow assays

For more information on all of Sona's products and services:

Visit: www.sonanano.com

Email: darren@sonanano.com or anu@sonanano.com

Call: +1 (902) 880 - 9925 or +1 (902) 989 - 2693