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Brief communication (Original)

Sensitivity enhancement of nucleic acid detection by lateral flow strip test using UV crosslink method

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Background: Nucleic acid lateral flow (NALF) strip test is currently a promising method in biomedical applications for point-of-care DNA detection. However, sensitivity of NALF is a major limitation when tested without amplification of the DNA sample.
Objective: This study introduces UV crosslink as an additional step to enhance sensitivity of the test strip.
Methods: By applying UV exposure to the NALF platform with different irradiation energies and times, specificity test with target DNA and non-target DNA.
Results: the results revealed increasing signals of approximately 40% from all test strips compared to those without UV exposure. Furthermore, the sensitivity enhancement by UV crosslink of NALF dipstick has been shown to be independent from DNA sequences, hybridization specificity and target DNA length.
Conclusion: Data presents a new step to improve the sensitivity of NALF assay. It allows immediate visualization and quantification. There may be a potential application in other NALF platform products.

Keywords: Biosensor, gold nanoparticles, lateral flow, nanomedicine, strip test, UV crosslink

In biomedicine, nucleic acid detection systems are essential in many applications for diagnostics and monitoring of illnesses such as infectious diseases and genetic material detection [1-3]. Conventional Northern and Southern blot methods use the hybridization principle for nucleic acid detection with rather high specificity and sensitivity, but their time-consuming and complicated processes make the systems difficult to be used for point-of-care testing [4].

Lateral flow test strips are simple devices used for detecting the presence of a target analyte in a sample.€The lateral flow is usually produced in a dipstick format, using capillary force to control the flow of a liquid sample in a solid support membrane. Recently, nucleic acid lateral flow (NALF) microchromatographic assay has been developed with potential to be used as a new alternative screening test for wider applicability of a rapid strip test. The current NALF platform has been proposed to detect the presence of the target nucleic acid analyte. This provides a faster, cheaper, and easier procedure than those of Northern and Southern techniques [3-6]. Generally, the platform of the NALF strip has been described elsewhere [7, 8]. In brief, NALF is composed of a test line and a control line on a nitrocellulose membrane. The test line and the control line contain different biotin-modified nucleic acid probes grafted on to a streptavidin-functionalized nitrocellulose membrane support. A tested DNA sample can be dropped on to a sample pad and mixed with conjugate probes (complementary probes to DNA of interest attached on gold nanoparticles). Target DNAs of the same sequence that have been hybridized with the conjugate probes will be further sandwich-hybridized with specific biotin-modified probes at the test line while the excess conjugate probes will be trapped with another specific biotin-modified probes at the control line. Nonetheless, achieving a high sensitivity remains a major problem of this test strip. Previous studies reported the use of several reporters and enzymatic methods to improve the test's sensitivity [4, 9, 10], but the outcomes remain varying depending on protocols, and the standards of NALF procedures have not been clearly elucidated. Based

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on the DNA fixation step on nitrocellulose membrane of Southern blot technique [11, 12], we herewith report on the application of the rapid ultraviolet (UV) crosslink technique as a process to fix target DNA probes on nitrocellulose membrane of NALF test strip. The procedure consequently increases the signals of both test line and control line that are observable by naked eye.

Material and methods

Conjugation of thiolated probe on AuNPs

Thiolated probe that specific to the sequence of RT gene of HIV-1 virus (in the sequences below) have been conjugated on gold nanoparticles surface. This process has been previously described by Hill and Mirkin(13). Briefly, 50 ul of thiolated DNA probe and 7.7mg of DDT were mixed and incubated for 30 minutes. Activated thiolate DNA probes were collected by NAP-10 column and mixed with 1ml of AuNPs, then the solution were incubated for 24 hrs. Later, 2M NaCl buffer was added until the final concentration was 150 mM. The solution was centrifuged at 12,000 rpm for 12 minutes. The pellet was collected and dispersed in 1 ml eluent buffer (20 mM Na3PO4, 5% BSA, 0.25% Tween and 10 % sucrose)

Conjugate probe: 5' -Thiol-CTGTACCAGTAA CATTAAAGCCAGG -3'

Capture probe (test line): 5'- GTT AAA CAG TGG CCA TTG ACA GAA GA -Biotin -3'

Control probe (control line): 5'-Biotin-CCT GGC TTT AAT GTT ACT GGT ACA G -3'

Target sequence: 5'- TCT TCT GTC AAT GGC CAC TGT TTA ACC TTT GGT CCA TCC ATT CCT GGC TTT AAT GTT ACT GGT ACA G -3'

Non target sequence: 5'- GAC ATG GTC TTA GTA ATT TCG GTC CTT ACC TAC CTG GTT TCC AAT TTG TCA CCG GTA ACT GTC TTC TT -3'

Lateral flow strip test construction

The lateral flow test strip composes of four main parts (**Figure 1A** and **B**); 1) sample pad; location

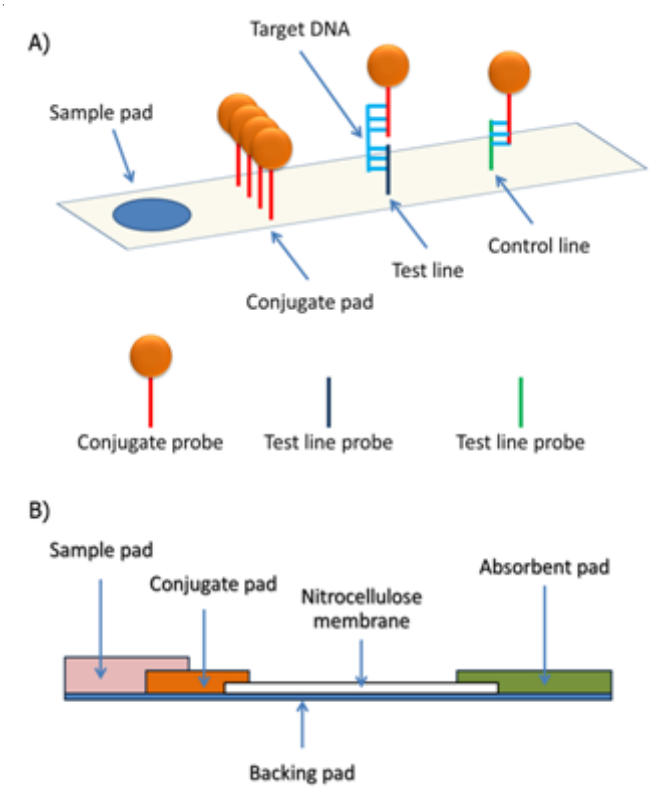

Figure 1. Diagram representing the lateral flow assay. This composes of 1) sample pad; the location that the sample is applied, 2) conjugate pad; site of nanogold combined with conjugate probes, 3) Reaction membrane; the main area containing test line- and control line-probes, available for sandwich hybridization of target DNA-nanogold-test line probe, and 4) absorbent pad; waste reservoir to draw the sample and collect it.

where sample is applied, 2) conjugate pad; site of nanogold combined with conjugate probes, 3) reaction membrane; the main area containing test line- and control line-probes, available for sandwich hybridization of target DNA-nanogold-test line probe, and 4) absorbent pad; waste reservoir to draw the sample and collect it. The method used to construct the lateral flow test strips, was a slight modification to the method previously described by Mao et al [4]. Briefly, 60 uL of 1 mM of DNA probe (control or test line probe) mix with 140 uL PBS and 1.67 mg/mL of streptavidin solution (300 ul) incubated for 1 hour. Then the excess streptavidin is removed by centrifugal filter at 6,000 rpm for 20 minutes washed 3 times with 1ml of PBS solution and eluted in 500 uL of PBS buffer. Then it is drop on nitrocellulose membrane (Millipore HF240) as test and control line. Finally, nitrocellulose membranes, are exposed to UV light in the UV crosslink machine, followed by assembly of the nitrocellulose membrane, sample pad, conjugate pad, and absorption pad on a backing card.

Results and discussion

Select UV exposure time

UV exposure has been performed in two groups of NALF dipsticks: 50mJ/cm^2 for 30, 60, 120 seconds, and 125mJ/cm^2 for 30, 60, and 120 seconds. The result showed that both UV-exposed groups had clear observable signals at test lines, whereas the control group (no UV treatment) displayed no signal detectable by naked eyes (**Figure 2a** and **b**). Image analyzer also confirmed that the signal increases when more exposure time was increased in both groups (**Figure 2c**). In particular, these figures suggest that the distinct advantage of UV application on NALF is to increase naked-eye detection of the low-signal test strip.

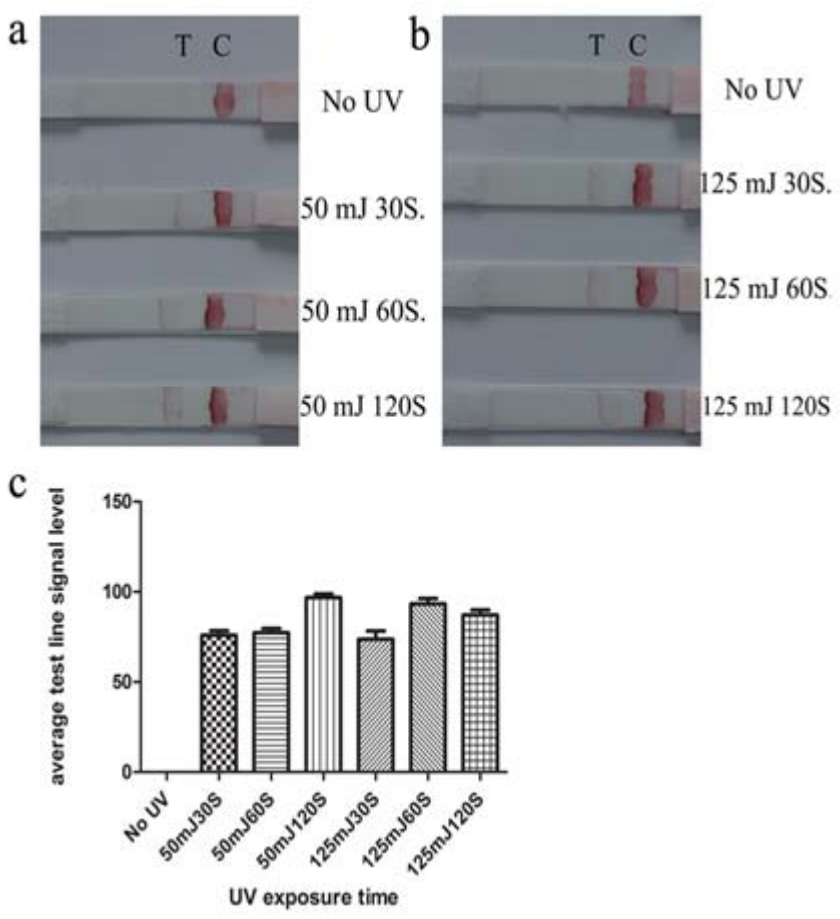

Figure 2. NALF signal comparison among different UV intensity and times. **a:** the test and control line's signals from 50 mJ/cm^2 UV-treated NALF at 30, 60, 120 second exposure times comparing with no UV-treated NALF. **b:** NALF signals from 125 mJ/cm^2 UV intensity at 30, 60, and 120 second. **c:** Quantitate signals of image analyzer from each platform.

Selectivity test

In order to prove whether the UV treatment can be used with other DNA sequences on NALF strip ubiquitously, different DNA probes sequences and synthetic target DNA samples have been tested. The NALF test strips were established and given a 50 mJ/cm^2 UV exposure for 120 seconds, compared with no UV exposure in the same platform of NALF strips. 100nM synthetic target DNA sample was run for 20 minutes on a conventional platform strip. The result confirmed that the UV-exposed group apparently increased the signal seen on both the test line and control line (**Figure 3a**).

We further evaluated whether the signal outcome after UV treatment is affected by hybridization specificity of the probe-target sequences. Three groups of extracted DNA were set as samples for UV-treated NALF platforms, namely: i) well-matched synthetic target DNA; ii) non-complementary synthetic DNA; iii) mixture of the target and non-target DNA. After 20 min of reaction, the NALF photos were taken. The results revealed that the first and the third groups displayed red bands from the control line and test line but the second group have only one band at the control line (**Figure 3b**). Image analyzer also indicated that the first and the third groups contained comparable signal intensity in test line but unobservable any signal from the test line in the second group (**Figure 3c**). This experiment strongly proposes that specificity of NALF assay has not been changed by UV crosslink, and DNA mixture with non-target domains does not interfere with the signal visualization.

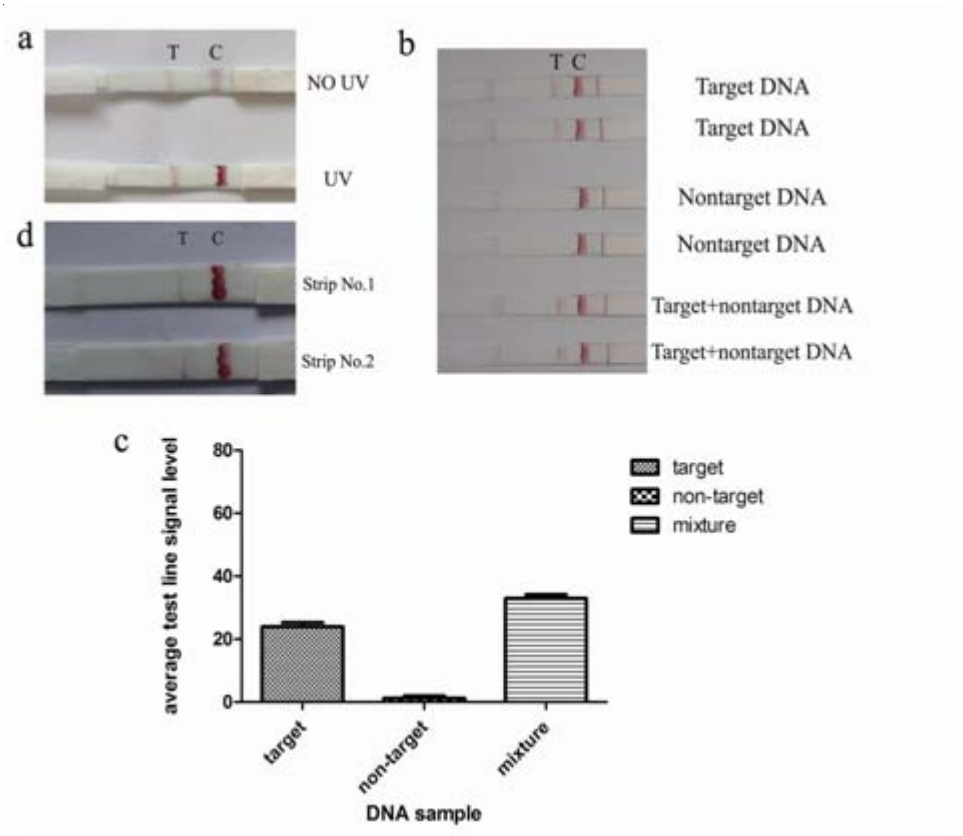

Figure 3. Validation of UV treatment and influencing parameters. **a:** Test with different sequences: Signal of NALF platform that uses other probe's sequences, UV-exposed group showed stronger band of test line and control line than those of no UV-treated group. **b:** NALF signal visualization and hybridization specificity: three groups of dipsticks are shown for comparison by using target DNA, non-target DNA and mixture of target and non-target DNA as tested samples on each dipstick. Photos of all groups are taken in duplicate strips. **c:** test line signal level from dipstick showed no signal in non-target groups and the same level in target and target+non-target groups. **d:** Test with long length DNA samples: the signal from dipstick running with 1.3 kb PCR product. Strip No.1 and No.2 were performed by the similar DNA sample at the same condition.

Test with PCR product

For further application of UV treatment in the real usage with long-length target double-stranded DNA, another experiment is performed: a NALF platform test in optimized condition with 1.3kb PCR product as a DNA sample. The test strips were exposed with 50mJ/cm^2 UV for 120 second. Wherein, 300nM PCR product was denatured by boiling in water for 5 minutes and chilled on ice for 15 minutes. Then the PCR product was dropped on the sample pad and run with 5x SSC buffer. After running for 20 minutes, both strips gave well-defined bands in test lines and control lines (**Figure 3d**). This implies the practicability of this method to long-length DNA target.

Conclusion

To conclude, here we introduce UV treatment on NALF assay as a new step to improve sensitivity and visualization detectable by naked eyes. The image analyzer verified that UV-crosslink enhances signals approximately 40% of the strip test. By using this novel technique, UV allows signal amplification regardless of DNA sequences, hybridization specificity, and target DNA length.

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