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An Africa Lateral Flow Assay-Based Early Recognition Test for Tenofovir-Induced Acute Kidney Injury (ALERT-AKI) Development †

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Abstract

The prevalence of (TDF)-induced Acute Kidney Injury (AKI) in Africa is a concern, given the widespread use of Tenofovir disoproxil fumarate (TDF) for Human Immunodeficiency Virus (HIV) therapy. Current tests used to detect AKI are based on increased urinary creatinine levels and are often not sensitive and specific enough for early detection. There is a need for more sensitive and specific rapid tests for the early detection of AKI, particularly in resource-limited countries for early detection and timely intervention. In the present study, a multiplex lateral flow assay, named An Africa Lateral Flow Assay-Based Early Recognition Test for Acute Kidney Injury (ALERT-AKI), detecting a set of three unique biomarkers of TDF-induced AKI was developed with a limit of detection of 2 ug/mL to 50 ug/mL, indicating the suitability of developed test for determining the selected AKI biomarkers in a clinically relevant range. This study shows proof of concept for a multiplex lateral assay tested on clinical samples. The ALERT-AKI multiplex platform will be the first of its kind once further clinical studies are conducted, offering multiple advantages, including early detection, simplicity, rapidity, high sensitivity, cost-effectiveness, and timely intervention, potentially reducing the severity and prevalence of AKI. Using a multiplexed protein biomarker rather than single protein molecules will allow for a much more accurate detection of kidney damage before it becomes significant or irreversible.

Keywords: Acute Kidney Injury (AKI); lateral flow assay; point of care (POC); tenofovir disoproxil fumarate (TDF)

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1. Introduction

Acute kidney injury (AKI) is a rapid decline in renal function, characterized by elevated serum creatinine levels and reduced urine output, which can result from various factors, including dehydration, infections, and the use of nephrotoxic drugs. Early detection and intervention are crucial, as AKI can lead to life-threatening complications such as end-stage renal failure, volume overload, electrolyte disturbance, and multi-organ dysfunction [1]. AKI induced by Tenofovir-based antiretroviral therapy (ART) represents a significant clinical concern, particularly in Africa where HIV prevalence remains high. Tenofovir disoproxil fumarate (TDF), often referred to as 'tenofovir', is the antiretroviral drug most frequently linked to kidney toxicity [2]. The prevalence of TDF-induced AKI in Africa is a

matter of concern, given the widespread use of Tenofovir disoproxil fumarate (TDF) for Human Immunodeficiency Virus (HIV) therapy and other risk factors for kidney diseases such as diabetes and hypertension, which are on the rise in the aging population of People Living with HIV (PLHIV) on ART. A study conducted in South Africa found that 19.4% of the patients taking TDF experience a significant decrease in renal function [3]. Another study in Uganda indicated that around 8% of the individuals on TDF-based antiretroviral treatment developed AKI [4]. The risk is amplified by factors such as co-existing infections, like tuberculosis, and pre-existing renal conditions [5]. In African populations, genetic factors such as polymorphisms in drug-metabolizing enzymes may also contribute to higher susceptibility to TDF-induced AKI [6]. AKI contributes to increased morbidity, mortality, and healthcare costs, presenting a significant public health challenge [7]. In Africa, where healthcare resources are limited, the impact is devastating, exacerbating already strained healthcare systems [7]. Delayed diagnosis not only places the individual at risk but also has public health implications by potentially limiting the effective management of HIV/AIDS within communities [8]. There is a growing consensus on the need for developing a rapid diagnostic test for the early detection of TDF-induced AKI, suitable for resource-limited settings [9], to enable timely intervention, potentially reducing the severity and prevalence of AKI [10].

2. Materials and Methods

2.1. Materials and Equipment

Antibodies and antigens were purchased from Biocom Africa (Johannesburg, South Africa). We obtained 40 nm gold nanoparticles and the lateral flow material from Diagnostic Consulting Network (Carlsbad, CA, USA). XYZ 3060 Batch Dispenser and Guillotine cutter were purchased from BioDot (Chichester, UK).

2.2. Target Biomarker Selection

Based on the literature studies, the three most sensitive biomarkers for AKI were selected and used to develop the multiplex lateral flow test. All the antibody pairs for the selected biomarkers were sourced commercially from Biocom Africa (Johannesburg, South Africa), Johannesburg, South Africa and were validated using Enzyme-Linked Immunosorbent Assay (ELISA).

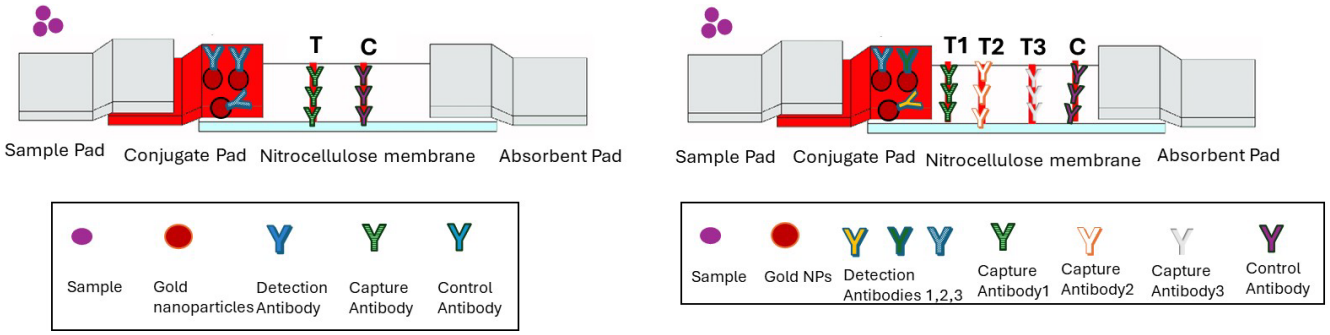
2.3. Conjugation of Antibodies to Gold Nanoparticles

Stable conjugation parameters were determined using the flocculation and salt test method [11,12]. The optimal antibody concentration was added directly to the required volume of gold nanoparticles and allowed to mix at room temperature for 30 min, with agitation. The conjugate mixture was blocked with equal volume of the starting conjugate with conjugate blocking buffer 10% Bovine Serum Albumin (BSA), pH 9 and further incubated for 30 min. This was followed by centrifugation at 15,000 rpm for 20 min at 4 °C. The supernatant was discarded and the nanoparticles were re-suspended in diluent buffer containing Borate buffer, with 5% sucrose added to an OD 10.

2.4. Singleplex and Multiplex Lateral Flow Test Developments

The lateral flow test was assembled as illustrated in Figure 1. The Lateral Flow Immuno Assay (LFIA) strips consisted of five parts, a sample pad, conjugate pad, stacking pad, Nitrocellulose (NC) membrane, and absorbent, with capture antibody and control antibody at 1 mg/mL and 0.3 mg/mL, respectively, at a rate of 1 µL/cm using the BioDot Airjet front line XYZ 3060 (BioDot, Chichester, UK). For the multiplex test strip, three test lines were dispensed on the NC membrane, positioned at a 2.5 mm interval. The gold

nanoparticle conjugates were sprayed onto a pre-treated conjugate pad using the BioDot Airjet front line XYZ 3060 (BioDot, Chichester, UK) at a rate of 10 µL/cm. The sample pad, conjugate pad, stacking pad, NC membrane, and absorbent pad were laminated onto a plastic backing with 2 mm overlaps. The fully laminated card was then cut into 4 mm wide strips using the BioDot Guillotine cutter (BioDot, Chichester, UK). The strips were either tested as dipsticks or assembled into cassette housing before testing.



(**a**) (**b**)

Figure 1. Schematic representation of sandwich lateral flow assay formats: (**a**) monoplex test for a single target and (**b**) multiplex test for three targets. In both designs, the sample passes through the conjugate pad containing antibody-conjugated gold nanoparticles (AuNPs) before reaching the test and control areas. "T" (monoplex) or "T1–T3" (multiplex) denote the test lines corresponding to specific target biomarkers, while "C" represents the control line confirming assay validity.

2.5. Limit of Detection (LOD)

The monoplex lateral flow test strips were tested in decreasing concentrations (1000, 100, 10, 5, 1, and 0 ug/mL) of recombinant protein, which were used as sample analytes. To perform the test, 5 µL of the sample was added onto the sample well, followed by addition of 100 µL of running buffer and allowed to run for 15 min and images of the test strips using a digital camera were taken.

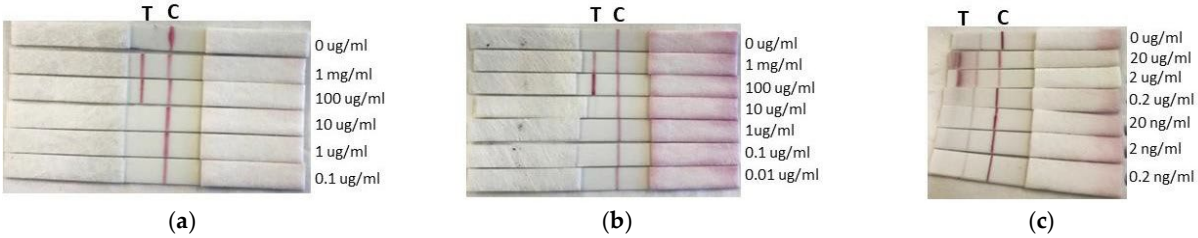
3. Results

3.1. Optimization of the Monoplex Lateral Flow Test

A sandwich lateral flow assay was designed as illustrated in Figure 1. The antibody pairs for each biomarker were optimized for use as either the capture antibody or as a detection antibody. The conditions for the stable detection of the chosen biomarkers in LFIA were determined separately in a monoplex format before a multiplex test was developed. A positive test was defined as the presence of a visible test line in the presence of a visible control line, while a negative test was defined as the presence of a visible control line and the absence of a visible test line.

3.2. Evaluation of Limit of Detection (LOD)

Each test line had a cutoff, which is the minimum concentration of a target biomarker in the sample necessary for the test line to become visible, and this cutoff was adjusted by varying the density of the capture antibody applied. The LOD was evaluated using recombinant proteins for the three biomarkers. The recombinant proteins were serially diluted and tested on the monoplex test strips (Figure 2). The LOD for the three biomarkers was determined to be 50 ug/mL for Biomarker 1, 10 ug/mL for Biomarker 2 and 2 ug/mL for Biomarker 3, indicating that the developed test is suitable for detecting AKI biomarkers in a clinically relevant range of 0.02 to 1.5 mg/mL [13–15].

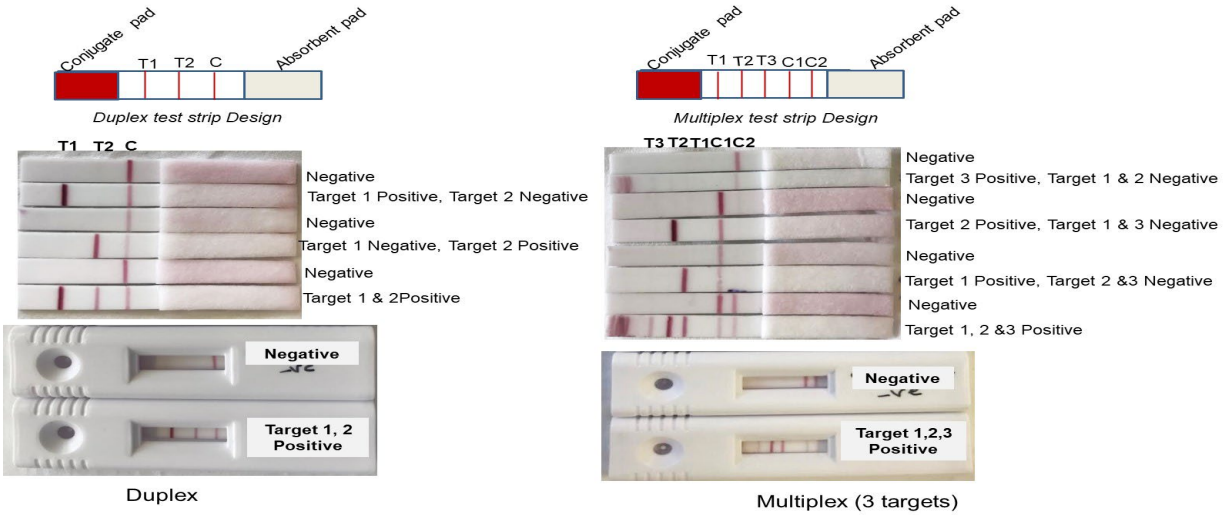


(**a**) (**b**) (**c**)

Figure 2. Limit of detection (LOD) of lateral flow antigen tests for (**a**) Biomarker 1, (**b**) Biomarker 2, and (**c**) Biomarker 3. Serial dilutions of recombinant proteins were tested, and assay results were recorded after 15 min. "T" denotes the test line indicating target detection, and "C" denotes the control line confirming assay validity.

3.3. Development of a Multiplex Lateral Flow Test Strip

A multiplex test was developed, incorporating all three capture antibodies in a single test strip and the conjugates sprayed in a single conjugate pad. The multiplex test was tested using the respective recombinant proteins representing each of the three biomarkers (Figure 3). The multiplex test could selectively detect the specific biomarker targets without cross-reactivity.



(**a**) (**b**)

Figure 3. Development of multiplex lateral flow tests: (**a**) dual-analyte strip for the detection of Biomarkers 1 and 2, and (**b**) triple-analyte strip for the detection of Biomarkers 1, 2, and 3, using recombinant proteins. Assays were allowed to develop for 15 min prior to result interpretation. "T" indicates the test line(s) corresponding to specific biomarkers, and "C" represents the control line confirming assay validity.

4. Discussion

In the present study, we have developed a lateral flow assay detecting three AKI biomarkers in a monoplex format, with a limit of detection of 2 ug/mL to 50 ug/mL, indicating the suitability of the developed test for determining the selected AKI biomarkers in a clinically relevant range. A multiplex lateral flow test was also designed to detect all three biomarker targets in one test. The multiplex test shows no cross-reactivity of the biomarker target conjugates and can detect each biomarker target with high specificity. This study shows proof of concept for a multiplex lateral assay that will be validated in clinical samples. The ALERT-AKI multiplex platform will be the first of its kind once further clinical studies are conducted, offering multiple advantages, including early detection, simplicity, rapidity, high sensitivity, cost-effectiveness, and timely intervention, potentially

reducing the severity and prevalence of AKI. Using a multiplexed protein biomarker rather than single protein molecules will allow for a much more accurate detection of kidney damage before it becomes significant or irreversible. Emerging biomarkers (such as the ones used in our study) can indicate kidney damage, while newer markers may evaluate kidney stress. In the future, a combination of functional, damage, and stress indicators may be utilized to define AKI more comprehensively.

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Institutional Review Board Statement: This study was conducted in accordance with the Declaration of Helsinki and approved by the CSIR Research Ethics Committee of Council for Scientific and Industrial Research (Reference 432/2023, 4 March 2024).

Data Availability Statement: Data is contained within the article.

Conflicts of Interest: The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

References

1. Levey, A.S.; James, M.T. Acute kidney injury. *Ann. Intern. Med.* **2017**, *167*, ITC66–ITC80. [CrossRef] [PubMed]
2. Venter, W.D.F.; Fabian, J.; Feldman, C. An overview of tenofovir and renal disease for the HIV-treating clinician. *S. Afr. J. HIV Med.* **2018**, *19*, a817. [CrossRef] [PubMed]
3. Mwafongo, A.; Nkanaunena, K.; Zheng, Y.; Hogg, E.; Samaneka, W.; Mulenga, L.; Siika, A.; Currier, J.; Lockman, S.; Hughes, M.D.; et al. Renal events among women treated with tenofovir/emtricitabine in combination with either lopinavir/ritonavir or nevirapine. *AIDS* **2014**, *28*, 1135–1142. [CrossRef] [PubMed]
4. Bygrave, H.; Kranzer, K.; Hilderbrand, K.; Jouquet, G.; Goemaere, E.; Vlahakis, N.; Triviño, L.; Makakole, L.; Ford, N.; Gray, C. Renal safety of a tenofovir-containing first-line regimen: Experience from an antiretroviral cohort in rural Lesotho. *PLoS ONE* **2011**, *6*, e17609. [CrossRef] [PubMed]
5. Olowu, W.A.; Niang, A.; Osafo, C.; Ashuntantang, G.; Arogundade, F.A.; Porter, J.; Naicker, S.; Luyckx, V.A. Outcomes of acute kidney injury in children and adults in sub-Saharan Africa: A systematic review. *Lancet Glob. Health* **2016**, *4*, e242–e250. [CrossRef] [PubMed]
6. Perazella, M.A. Drug-induced nephropathy: An update. *Expert. Opin. Drug Saf.* **2010**, *9*, 865–878. [CrossRef] [PubMed]
7. Lameire, N.; Van Biesen, W.; Vanholder, R. The changing epidemiology of acute renal failure. *Nat. Clin. Pract. Nephrol.* **2013**, *2*, 364–377. [CrossRef] [PubMed]
8. Wyatt, C.M.; Schwartz, G.J.; Owino Ong'or, W.; Abuya, J.; Abraham, A.G.; Mboku, C.; M'mene, L.B.; Koima, W.J.; Hotta, M.; Maier, P.; et al. Estimating kidney function in HIV-infected adults in Kenya: Comparison to a direct measure of glomerular filtration rate by iohexol clearance. *PLoS ONE* **2016**, *11*, e69601. [CrossRef] [PubMed]
9. Glassock, R.J.; Christopher, W. An epidemic of chronic kidney disease: Fact or fiction? *Nephrology Dialysis Transplantat.* **2008**, *23*, 1117–1121. [CrossRef] [PubMed]
10. Kalim, S.; Szczech, L.A.; Wyatt, C.M. Acute kidney injury in HIV-infected patients. *Semin Nephrol.* **2008**, *28*, 556–562. [CrossRef] [PubMed]
11. Chang, C.C.; Chen, C.P.; Wu, T.H.; Yang, C.H.; Lin, C.W.; Chen, C.Y. Gold Nanoparticle-Based Colorimetric Strategies for Chemical and Biological Sensing Applications. *Nanomaterials* **2019**, *9*, 861. [CrossRef] [PubMed]
12. Oliveira, J.P.; Prado, A.R.; Keijok, W.J.; Antunes, P.W.P.; Yapuchura, E.R.; Guimarães, M.C.C. Impact of Conjugation Strategies for Targeting of Antibodies in Gold Nanoparticles for Ultrasensitive Detection of 17β-Estradiol. *Sci. Rep.* **2019**, *M9*, 13859. [CrossRef] [PubMed]
13. Murty, M.S.; Sharma, U.K.; Pandey, V.B.; Kankare, S.B. Serum cystatin C as a marker of renal function in detection of early acute kidney injury. *Indian J. Nephrol.* **2013**, *23*, 180–183. [CrossRef] [PubMed]

14. Al-Taee, I.K.; Al-Safar, J.J.; Al-Falahi, Y.S.; Al-Shamma, I.A. The Clinical Significance of beta2-microglobulin in End-Stage Renal Disease. *Saudi J. Kidney Dis. Transplant.* **2003**, *14*, 492–496. [PubMed]
15. Haase-Fielitz, A.; Haase, M.; Devarajan, P. Neutrophil gelatinase-associated lipocalin as a biomarker of acute kidney injury: A critical evaluation of current status. *Ann. Clin. Biochem.* **2014**, *51 Pt 3*, 335–351. [CrossRef] [PubMed]