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DEVELOPMENT OF LATERAL FLOW FLUORESCENCE ASSAY FOR THE DETECTION OF *TRYPANOSOMA*

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Trypanosoma such as *Trypanosoma brucei* and *Trypanosoma cruzi*, the causative agents for African sleeping sickness and Chagas disease, respectively, have important influence on human health. The methods such as microscopic examination, immunological methods, and molecular methods are used for the identification and detection of *Trypanosoma*, but none of these methods are ideal to mass screening of samples such as onset of outbreak, epidemiological surveys, and blood unit screening. Therefore, there is a need for an assay which can rapidly, sensitively and specifically detect *Trypanosoma*.

In this study, Ru(bpy)₃²⁺-doped silica nanoparticles (RuSNP) were used to target nucleic acid sequences in a lateral flow fluorescent assay. This assay was developed to improve the sensitivity and lower the limit of detection as compared to the traditional lateral flow assay. The assay targeted both the spliced leader sequence as well as the polyA tail of the mRNA. The surface of spherical RuSNP was modified by glycidoxypropyl trimethoxysilane (GOPTMS). Amine-terminated oligonucleotides as a bioreceptor were immobilized onto the RuSNP via the interaction between the NH₂ and the epoxy group of the GOPTMS. The conjugate complexes formed were immobilized on the conjugate pad, and the capture oligonucleotides used for test and control lines were immobilized on the nitrocellulose membrane. The effects of the amount of RuSNP, GOPTMS, amine-capped oligonucleotides, and capture oligonucleotides on the test line on the performance of the test strips were investigated and optimized. The fluorescence intensity was evaluated by using a fluorescent microplate reader.

The experimental results showed that the nucleic acid sequence-based and RuSNP-labeled lateral flow assay was very sensitive compared with the gold-labeled test strips and the chemiluminescent test strips we developed previously, and that the limit of detection (LOD) of the test strips developed is 0.4 fmol. The LOD can further be reduced about one order of magnitude when dipstick format was used.