

ORAL PRESENTATIONS

NEW ANTIGEN-OLIGONUCLEOTIDE BIOCONJUGATES FOR MULTIPLEX ASSAY OF PATHOGENIC BACTERIA

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Our research involved the development and evaluation of new oligonucleotide bioconjugates with low molecular weight antigens, intended for use in multiplex assays of pathogenic bacteria. These antigens function as essential markers within a diagnostic platform that integrates isothermal amplification and immunoassay. Specifically, the recombinase polymerase amplification (RPA) method is employed for its high sensitivity and specificity; it is carried out using an enzyme complex at a single temperature of 40°C for 15 minutes, which means that expensive equipment is not needed. The resulting amplicons are subsequently analyzed via lateral flow assay, where detection is facilitated by specific antibodies immobilized on the test strip and streptavidin-conjugated gold nanoparticles that target the biotinylated markers. The interaction leads to the formation of a ternary complex that becomes immobilized within the analytical zones of the test strip. The assay outcome can be visually evaluated after 7 minutes based on coloration. In the study, two antibiotics, streptomycin and chloramphenicol, as well as two hormones, cortisol and testosterone, were used as labels (antigens). Specific, highly sensitive antibodies were generated and validated against these labels. Derivatives of the investigated antigens, prepared as esters and carboxymethyl oximes, were attached to the amino group of oligonucleotides through activated ester formation and amidation reactions, with HBTU used as the activating reagent. The optimal conditions for conjugate synthesis, including reagent concentration, reaction time, and purification parameters, were investigated and established. The enzyme immunoassay showed that the immunochemical properties of the modified antigens were retained within the oligonucleotides. Double-stranded oligonucleotide probes, 50 bp in length, bearing one of the antigen labels and a biotin label at opposite ends, were characterized by their interaction with the corresponding immobilized specific antibodies and streptavidin conjugated to peroxidase. Isothermal amplification using primer pairs bearing the new antigen labels and biotin demonstrated higher amplification activity than the standard fluorescein label, and this activity was sufficient to detect amplicons in an immunoassay performed in a microplate or on a test strip. These new labels were shown to be suitable for multiplex assay of two different bacteria using two primer pairs with distinct antigen labels on the forward primers and biotin on the reverse primers, respectively. Detection was carried out using test strips. Specific antibodies were immobilized in the two analytical zones of the test strips, and a streptavidin-gold nanoparticle conjugate was employed. Therefore, the proposed antigen labels and the developed conjugation methods represent promising reagents and tools for multiplex assay technologies.