IVD_05 - Development and Optimization of Immunological Assay for Evaluation of FITC Immunoconjugates

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Introduction: The use of fluorescein isothiocyanate (FITC) immunoconjugates is fundamental in various biomedical applications, especially in diagnostic techniques such as indirect immunofluorescence (IFI) and flow cytometry (FC). These conjugates are crucial for the precision of the results, directly influencing the quality of the kits provided by institutions like Bio-Manguinhos, which is a reference in the development and production of diagnostic kits, including those for Human Leishmaniasis and Chagas Disease.

Objectives: This study aims to develop and validate a methodology for evaluating the quality and functionality of an anti-HumanIgG-FITC conjugate, ensuring the efficacy and reliability of diagnostic kits.

Methodology: We used beads coupled with Human Anti-IgG, followed by the addition of Human IgG and the anti-HumanIgG-FITC conjugate, in serial dilutions from 1/500 to 1/32000. The evaluation was performed in comparison with a commercial conjugate, following the internal standards and procedures of SEFEN/DERED/Bio-Manguinhos. Fluorescence was quantified using the GloMax® Discover (Promega), with excitation and emission lengths of 475nm and 550nm, respectively, aiming to establish a standardized protocol for routine quality assessments.Statistical analysis included tests for adherence to the normal curve (Anderson- Darling) and T-tests for differences between means, both with a significance level of 5%.

Results: The results showed adherence to the normal curve for all dilutions and the white control ($p > 0.05$). Furthermore, there were statistically significant differences in fluorescence intensities between each dilution and the blank control, with significance observed up to the dilution of 1/8000 ($p < 0.05$).

Conclusion: The Bead-Based Immunological Methodology (BBIM) proved effective in evaluating the quality of the FITC immunoconjugate, demonstrating the ability to distinguish significant differences in fluorescence intensity among the tested dilutions. This protocol offers a reliable tool for the internal control of FITC conjugates, ensuring the quality of diagnostic kits provided to the Ministry of Health.

Keywords: FITC Immunoconjugates; Immunological Assay Optimization; Diagnostic Kit Quality