*Title:* **Update of Isoluminol-labelled monoclonal antibodies for human IgG/IgM detection**

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Biokit S.A., through its AcuStar®/BIO-FLASH® analyser platform, distributes chemiluminescence immunoassays for the detection of coagulopathies, thrombotic disorders and infectious diseases. AcuStar®/BIO-FLASH® reagents are mainly based on the antigen-antibody (Ag/Ab) recognition principle.

Among the different assays schemes for these immunoassays, one of the most used is the detection of antibodies in the patient sample through a two-step reaction. In the first step of the reaction, magnetic beads coated with an antigen (Ag) capture the analyte (Ab) if present in the sample. In a second step of the reaction, a chemiluminescent labelled specific antibody reacts with the antibody captured by the microparticles. The label produces light in presence of an oxidiser and a catalyst and this amount of light emitted is used to detect analyte presence/concentration in the sample.

AcuStar®/BIO-FLASH® reagents use N-(4-Aminobutyl)-N-ethylisoluminol (ABEI) as chemiluminescent label that is chemically attached to antibodies with different recognition epitopes. When isoluminol is covalently bound to a macromolecule, the intensity of light decreases compared to free isoluminol due to quenching effects. Recently, derivatives of Isoluminol have been introduced to improve light emission and stability of the Isoluminol-labelled components.

Two different monoclonal antibodies have been used in this study: monoclonal antibodies against human IgM and IgG (different immunoglobulin classes related to first and long term adaptative immune response respectively) that have been labelled using the new Isoluminol derivative. The optimum conditions to label each monoclonal antibody have been studied, standard physicochemical characteristics of the resulting conjugates have been evaluated, and luminosity of each new labelled-antibody have been compared with reference materials in order to be considered an efficient alternative of the standard ABEI-labelled conjugates.

Additionally, the macromolecule could also suffer changes that may affect its functionality. Functionality of newly developed conjugates should be assayed at least in one model system. Different AcuStar®/BIO-FLASH® model assays have been selected and prepared using the optimised conjugates, and the performance and stability of each replaced component have been checked.

**Keywords:** Isoluminol, chemiluminescence, conjugation, immunoassay