ABSTRACT

The theoretical part describes the basic characteristics of antigens and antibodies, including their structure and functions. The different classes of antibodies are also presented along with the process of antibody production in the human body. Additionally, various methods of antibody preparation or production are explained, such as monoclonal, polyclonal, and recombinant antibodies for diagnostic purposes, with their advantages and disadvantages evaluated. The use of antibodies in diagnostics and therapy is summarized, and the significance of the affinity interaction between antigen and antibody is explained, along with the use of magnetic particles in diagnostics. The following chapters mention selected methods used for the characterization of affinity pairs, particularly immunoblotting techniques such as Dot blot, Affiblot, and Western blot, as well as immunoenzymatic methods like ELISA.

The experimental part contains the results obtained from Affiblot version 3.5.2 to verify the functionality of the model antigen-antibody system (human IgG and its detection antibodies or conjugates) and to optimize the detection method. Two detection methods, colorimetric and chemiluminescent were tested and compared. In the next phase, two different membranes were tested to determine their impact on the accuracy and sensitivity of the measurements. An important part of the experiments was the verification of the model antigen-antibody system for the purpose of antigen isolation using magnetic particles with immobilized antibodies and the quantification of the protein obtained in this way. The measurements on Affiblot were compared with those on a classic dot blot. It was also tested whether the magnetic particles exhibit nonspecific adsorption or not.

Keywords: affiblot, dot blot, western blot, ELISA, antibody, antigen, magnetic particles