0224 SENSITIVE AND QUANTITATIVE ANALYSIS OF WESTERN BLOTS BY USING TIME-RESOLVED IMMUNOFLUOROMETRY. Eleftherios P. Diamandis, Theodore K. Christopoulos, Courtney C. Bean (Dept. Clin. Biochem., The Toronto Hospital and Univ. of Toronto, Toronto, Ontario, Canada)

A new method is described for detection and quantification of proteins blotted or spotted on nitrocellulose (NC).Proteins are reacted with specific antibodies followed by reaction with biotinylated secondary antibodies.The immunocomplex is then detected with a streptavidin-based macromolecular complex (SBMC) labeled with the fluorescent europium chelate 4,7-bis (chlorosulfophenyl) 1,10-phenanthroline-2,9-dicarboxylic acid. Alternatively, proteins are labeled with digoxigenin and then electrophoresed, transferred to NC, reacted with biotinylated anti-digoxigenin antibody and stained with SBMC.The fluorescent spots or bands are detected by visual inspection under UV illumination or by instant photography or quantified by scanning with time-resolved fluorometer. A front surface time-resolved fluorometer suitable for microtiter plates, was converted to a scanner and evaluated. Spotted biotinylated goat anti-mouse antibodies (GAMIg) were detected down to 25 pg. CVs were 10-15% in the range of 400-10,000 pg/1 μL spot.Spotted unlabeled mouse IgG was reacted with biotinylated GAMIg and visualized with SBMC.The detection limit was 10 pg. Spotted α-fetoprotein (AFP), ferritin and carcinoembryonic antigen were reacted with specific antibody followed by the addition of biotinylated GAMIg and then visualized by SBMC. Detection limits were 5-10 pg. Human serum albumin, somatotropin and AFP were electrophoresed, transferred to NC and reacted with primary antibody. Then a biotinylated secondary antibody was added followed by SBMC.We analyzed, by Western blot, proteins covalently linked to digoxigenin. The detection was accomplished with biotinylated anti-digoxigenin antibody followed by SBMC. Proteins tested were bovine IgG, ovalbumin, bovine serum albumin and lysozyme. The proposed method doesn't involve enzyme detection, is simple, sensitive and gives sharp bands which remain fluorescent for months to years. Furthermore, it is the only time-resolved fluorometric method suitable for analysis of Western blots. Methods which use enhancement solution to extract $Eu^{3+}$ after the formation of immunocomplexes, are'nt suitable forWestern blot analysis because after $Eu^{3+}$-extraction, the spatial resolution is lost.