USING A MICROPLATE READER TO QUANTIFY DOT IMMUNOBINDING ASSAYS

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Dot immunobinding assays (dot-ELISA) are increasingly being applied to immunodiagnosis, mainly in seroepidemiology. Besides visual evaluation, instrumental readings can be made with the aid of commercially available reflectance densitometers (D. I. Stott, 1989, *J. Immunol. Methods, 119:* 153-187). Such instruments are complex and consequently expensive, which stimulates the search for alternative solutions for the quantification problem (C. Bordier & P. Ryter, 1986, *Analyt. Biochem., 152:* 113-118). In our laboratory it was decided to adapt an existing photometer (C. S. Ferreira, 1968, *Rev. Paul. Med., 73:* 394) to dot-ELISA readings. We first confirmed the viability of carrying out transmitted light measurements from dot-ELISA reactions, according to the technique described by P. H. Nibbering et al. (1985, p. 97-113, in G. R. Bullock & P. Petrusz (eds) *Techniques in immunocytochemistry*, vol. 3, Academic Press, London). Hence, transmitted light microplate reader and reflected light microphotometer determinations were compared. The latter were made according with the scanning technique (C. S. Ferreira & M. U. Ferreira, 1991, *Brazilian J. Med. Biol. Res., 24:* 495-498) which can be summarized as follows. The photometer is incorporated to a monocular microscope provided with a ten-power achromatic objective, a ten-power eyepiece and a mechanical stage. The nitrocellulose membrane filter is placed on a microscope slide. After focussing the microscope upon the membrane, the original eyepiece is replaced by a similar one provided with a CdS photoresistor. Readings are made by scanning each dot across its maximum diameter.
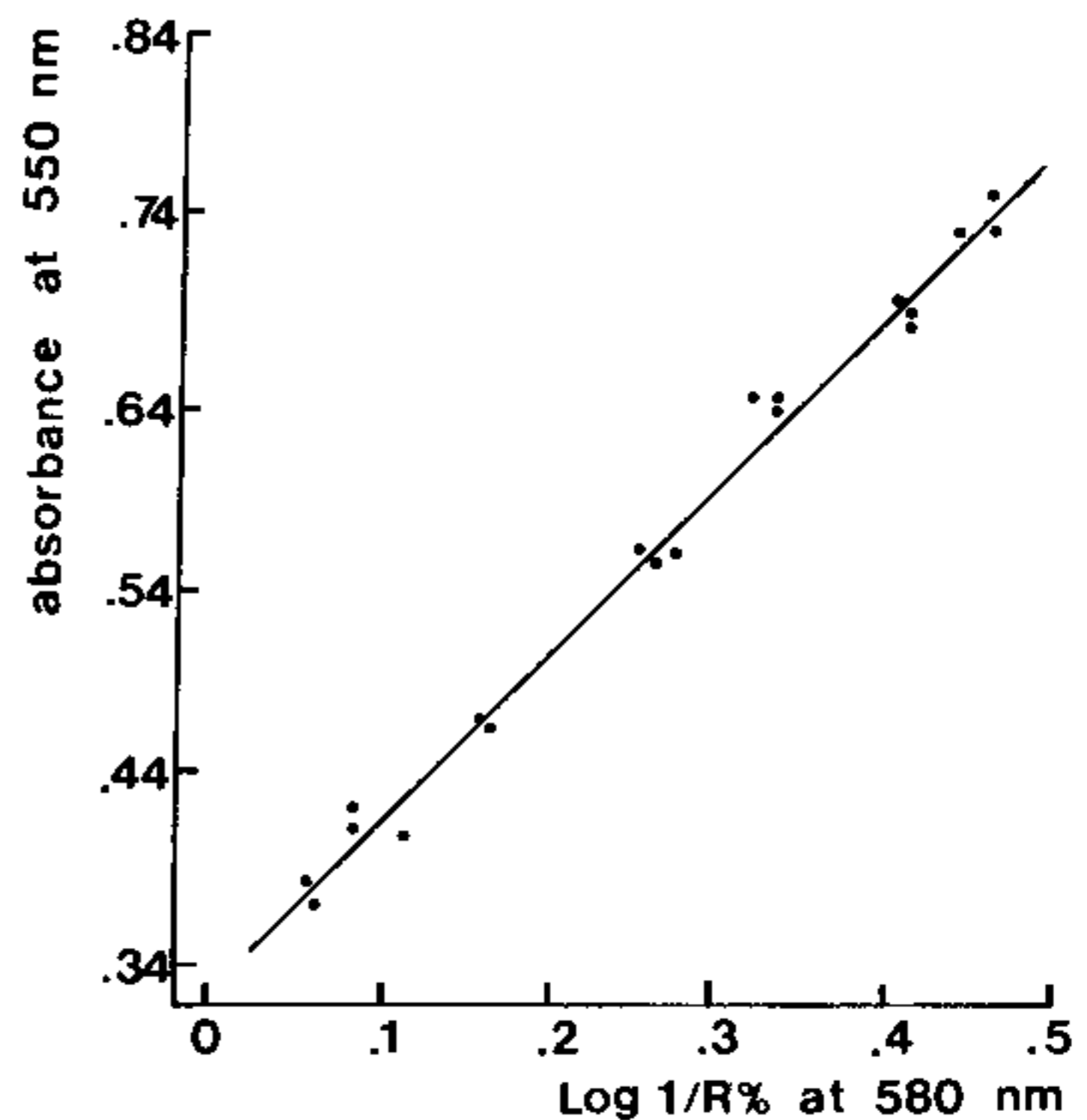
Correlation between reflectance microphotometer (X axis) and microplate reader (Y axis) quantifications of dot immunobinding assays on a glycerol-cleared nitrocellulose membrane filter.

A dot test according to P. H. Nibbering et al. (*loc. cit.*) was performed to test instrumental readings. Nine $40 \mu l$ aliquots of seven serial dilutions of peroxidase-conjugated goat anti-human IgG (Biolab Diagnóstica, Brazil) in distilled water were transferred to a nitrocellulose membrane filter, using a Bio-Dot Microfiltration Apparatus (Bio-Rad, USA). After drying for 10 min at 37 °C, the peroxidase activity on the filter was visualized by incubation with H_2O_2 and 4-chloro-1-naphtol for 10 min. The resulting circular dots were 3 mm in diameter. The developing substrate presents a broad maximum light absorption region about 590 nm (C. Bordier & P. Ryter, *loc. cit.*). The peroxidase reaction was stopped by transferring the filter to 2N HCl. The nitrocellulose filter was cut into three pieces, each one corresponding

to triplicate conjugate dilutions. They were immersed, respectively, in chemically pure glycerol, xylene and mineral immersion oil to compare their clearing properties. Microplate reader (MR 5000 — Dynatech Laboratories, USA) determinations at 550 nm were made with filter pieces placed, for optical geometry considerations, upon a clean microplate, coincidence between dots and microplate wells having been provided for. Each piece was then placed upon a dull white surface and subjected to a second series of determinations, this time using our reflectance microphotometer (580 nm).

Glycerol was the best of the essayed clearing media, as it did not modify the color of the dots. To test the precision of the method, a selected dot on a glycerol-cleared membrane filter was ten times placed upon, read and removed from the microplate. The arithmetic mean of these readings was 0.5525 with a standard deviation of 0.00469 (coefficient of variation = 0.85%). Figure shows the correlation between microphotometer and microplate reader quantification results of glycerol-cleared dots. A high degree of correlation was observed (r^2 = 0.9867, standard error of estimate = 0.0169), suggesting the use of microplate readers as an alternative to usual dot-ELISA densitometers.