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Zeitschrift für Immunitätsforschung

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Volume 170

Gustav Fischer Verlag · Stuttgart · New York · 1985

Universitäts-
Bibliothek
München

ISSN Immunobiology · Zeitschrift für Immunitätsforschung · 0171-2985
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Printed by Druckerei Ungeheuer + Ulmer KG GmbH + Co, Ludwigsburg
Printed in Germany

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155. Detection of autoantibodies against thyroglobulin, thyroid microsomes, and other soluble antigens with a rational and economical immunoblotting microtechnique

W. STÖCKER, H. FINKBEINER, R. GUTEKUNST, G. GEUSENDAM, H. BERNDT, and P. C. SCRIBA

Nitrocellulose (nc) was introduced as antigen support to detect antibodies in enzyme-immunoassays (1, 2). Only nanograms of antigens are required, and a great number of antibodies can be screened with the same test protocol. Nc-pieces are incubated in the wells of microtiter trays (1) or in sealed plastic bags (2). This is cumbersome and requires large volumes of samples and reagents. The *«titerplane-technique»* (3) was applied to facilitate the assay procedure and to minimize volumes of samples and reagents.

Methods: a) Two plane glass-plates were furnished with 96 hydrophilic reaction areas surrounded by hydrophobic zones. The plates could be arranged face to face in such a way that the reaction-areas of one plate exactly covered those of the opposite plate. Each pair of reaction areas formed a separate chamber in which a liquid sample or reagent could be contained from both sides and evaporation was drastically retarded. b) A surgical specimen of a nontoxic nodular goiter was frozen and homogenized by sectioning in a cryotome. Thyroglobulin and thyroid microsomal antigen were separated by fast protein liquid chromatography (Superose 6; Pharmacia Fine Chemicals) and applied to nc-pieces (0,2 µl antigen solution per piece of 2 mm × 2 mm). Antigen-nc-pieces were prepared in advance and stored in liquid nitrogen until use. c) They were adhered to the reaction areas of one of the plates *(antigens'-support).* A mylar-backbone prevented impregnation of the nc with the adhesive. d) Serum-dilutions and, in the further steps, reagents (peroxydase-labeled antihuman serum, diaminobencidine) were applied to the reaction areas of the other plate *(reagents' support).* Volumes as small as 0,5 µl were sufficient (3), but usually 10 µl were applied per reaction area. Reactions were started by superimposing the two plates.

Preliminary *results* suggest that the immunoblotting test with the «titerplane-technique» for autoantibodies against thyroglobulin and thyroid microsomes is highly sensitive and specific: Each of 50 sera with an unequivocal reaction in the indirect fluorescent antibody test was markedly positive with the corresponding antigens in the nc-test. Of 20 healthy control persons only one exhibited a positive reaction with both antigens. Both antibodies were exactly discriminated by immunoblotting. Reactions with sera exhibiting antibodies against cell nuclei and mitochondria, but not against thyroid, in the immunofluorescent test, were negative. A study with a greater number of patients is under way. Compared to conventional techniques, the amount of work spent on bulk examinations was drastically reduced, the volumes of samples and reagents could be cut down to 10 %. The simultaneous performance of numerous single tests permitted a better standardisation. In *conclusion,* the «titerplane-technique» considerably improves the immunoblotting with nc. This technique is especially suitable for screening supernatants of hybridomas on monoclonal antibodies. A number of nc-pieces can be fixed side by side to simultaneously detected autoantibodies against thyroglobulin, thyroid microsomes, and other antigens.

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2. P. HERBRINK, F. J. VAN BUSSEL, and S. O. WARNAAR. 1982. J. immunol. meth. **48:** 293.
3. W. STÖCKER. 1985. Acta histochem. Suppl. **31:** 269.

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