Direct Measurement of Microplates and Its Application to Enzyme-Linked Immunosorbent Assay

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A commercially available system for enzyme analysis was adapted for high-speed reading of microplates in which the enzyme-linked immunosorbent assay was performed.

The enzyme-linked immunosorbent assay (ELISA) is an interesting alternative for other serological methods hitherto described (1, 3). The test is sensitive, and, since practically all steps of the assay can be mechanized, it has a potential for large-scale screening of body fluids in general and sera in particular. In most ELISA applications, polystyrene tubes are used as antigen carriers (macro-ELISA). However, performance of ELISA in a polystyrene microplate containing 96 wells (micro-ELISA) was reported as well (2). Advantages of the latter procedure are that quantities of necessary reagents can be considerably reduced and handling of the samples in microplates is much easier.

For some field applications, visual reading of the color of the reaction product in the wells of the microplates is acceptable. For other applications, however, measurement of the reaction product is felt necessary, particularly since the data thus obtained are objective and are expressed in figures. So far, the only way to measure micro-ELISA results has been by transferring the contents from the individual wells to a spectrophotometer. Direct reading of the plate would shorten the assay time considerably. This paper describes an adaptation of semi-automated equipment for enzyme analysis for the measurement of micro-ELISA results.

Available equipment. For the macro-ELISA a system Olli 3000 (Olli Medical Electronics, Kivenlahti, Finland) was used. This system consists of an Olli 234 photometer, which measures 24 discrete samples in one sequence. The measuring speed is 24 samples/25 s. The light source is a Tungsten lamp (12 V, 50 W) with stabilized power supply. As light detectors 24 silicon photodiodes are used. The light from the Tungsten lamp, divided in 24 branches of a fiber optic assembly, passes through the cuvettes and is then received by the detectors.

Alterations of equipment. In our adaptation the light path has been changed from horizon-

tal to vertical, enabling measurement through the flat-bottomed wells of a microplate.

Since most commercially available microplates contain 96 wells, an additional device was developed to measure the whole microplate

FIG. 1. Photometer (1) with adaptations (2, 3, and 4) for reading of a microplate (96 wells with flat bottoms). (1) Input for holder with 24 polystyrene cuvettes; (2) carrier tray for microplate; (3) input for carrier with microplate; (4) device to shift microplate in four positions enabling the measurement of 4×24 wells.

FIG. 2. Photometer with adaptations for reading a microplate. (1) Handle for changing the light path: horizontal for measurement of cuvettes; vertical for measurement of a microplate.

in four steps without losing the original speed of operation. In each position 24 samples can be read sequentially (Fig. 1). Since an additional set of fiber optics and detectors was used, the photometer could be used for measuring both cuvettes (macro-ELISA) and microplates (micro-ELISA) by changing a handle (Fig. 2).

With the adapted system it was possible to measure microplates with 96 wells. The measuring procedure of the whole plate takes 150 s. The accuracy of the method depends on the optical quality of the microplates. Microplates are not developed for measuring purposes. Reading is generally done by visual interpretation of the color of the contents. From the preliminary results obtained with the adapted spectrophotometer, it can be concluded, however, that most batches of polystyrene microplates yielded acceptable and reproducible

readings. However, it was essential to use wells with flat bottoms.

It is felt that the development of systems enabling assessment of ELISA results in microplates directly presents an important further step in the application of ELISA for large-scale screening purposes.

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