

THE USE OF MICROTITER PLATES AND THE APPARATUS DYNATECH FOR AUTOMATION OF THE ROUTINE DETERMINATION OF ABO-GROUP FROM BODY FLUIDS (OR STAINS) AND HAIR

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Introduction

The routine analysis of ABO-groups continues to be one of the basic method of forensic serology examination but there exists a variety of used modifications. We describe a method with modified DYNATECH apparatus for time and material saving examination of large series of samples by absorption-elution and absorption-inhibition methods.

Absorption-elution method

The important part of the method is perfect washing of superfluous diagnostic antiserum from cotton segments soaked by the examined biological material. This time consuming part was solved by means apparatus ULTRAWASH II with interchangeable head with 96 pairs of aspirating and dispensing jets. For washing of segments it is necessary to cover aspirating jets by suitable net (100 µm). There is possible to program number of washing cycles, the intensity of aspiration and infusion and the amount of washing fluid.

The pressured segments of hair (apr. 5 - 8 mm) are collected (2 - 3 pieces together) by celluloid partially dissolved by acetone on apex.

One of the advantages of this modification is the fact that the samples remain from the very beginning (ensuring of traces) to the end of operation (evaluation of agglutination) in the same wells of microtiter plate and the danger of mistaking of samples is reduced.

After releasing of the thermolabile binding of absorbed antibodies in the elution part of the method (20 minutes - 56 °C) the cotton segments are removed and the suspension of testing erythrocytes is dropped to eluate. The agglutination is read after centrifugation.

In regard to the individual character of traces it is not possible to use the shaker but to resuspend agglutinates individually in each well of microplate under direct control by stereomicroscope. The agglutinates are resuspended by air flow directed to the individual wells.

Absorption-inhibition method

The use of connected apparatus AUTODILUTER II and dispenser SRD II (modern version AUTODILUTER III and SRD III)

allows a fast and exact dilution of tested sera to desired degree of dilution. The transport and mixing of sera in dilution rows of microplate is provided by means of Takatsy crowns (25 and 50 μ l). It is possible to choose the volume of dispensed buffered saline so that agglutination in dilution row of tested serum continually decreased from +++ to -.

Unlike the absorption-elution method shaker can be used to resuspension of agglutinates.

The evaluation of agglutination by apparatus MR 7000

The former reader MR 700 valued agglutination measuring the optical density in the centre of well after slopping whole apparatus.

Modern type MR 7000 with agglutination program cartridge can evaluate as many as 32 values of optical density measured in diameter of each well on microplate. The obtained data are processed in four parameters A, B, C and D, which can form any equation or rule. The producer recommends for evaluation of agglutinates the equation $A \times B$ or $A \times B / C$. We prefer the evaluation of parameter B after determination of suitable limits.

The results are printed in symbols +, ? and -. The apparatus MR 7000 is equipped by time-programmed shaker (0 - 59 sec.).

The microtiter plate is moved on on unclosed line and this allows the addition other apparatus. DYNATECH offers the temperate store and washing units. This set can be used as completely automatized equipment, where microplate with samples is moved on among apparatus, that provide the examination and printing documentation. The recommended connection of MR 7000 to PC enlarge the possibility of intervention to the programmes and documentation of results.