

## STUDY OF THE APPLICATION OF A MICROMETHOD OF IMMUNOELECTROSMOPHORESIS FOR THE DIAGNOSIS OF SOME BACTERIAL INFECTIONS

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Immunoelectrosmophoresis (IEOPh) is a technique of investigation of antigens and antibodies. It is based on two of their main properties: 1) Precipitating ability (or immunodiffusion-ability) in agar-gel with a corresponding specificity of both antigen and antibody, 2) Characteristic mobility in electric field (electrophoresis). This technique is very suitable and applicative in: detection and investigation (identification) of individual components in a multiple-component system (P. Grabar et al. — 1958); determination of the purity of a possible single-component system (C. A. Williams et al. — 1955); determination of the effectiveness of the fractionating method of isolation of certain materials (A-J-Crowle — 1960); identification of untypical proteins, especially pathological (P. Grabar — 1970). The latter advantage of IEOPh makes it a very useful and precise method in the microbiological diagnosis. Presuming all that we try to apply in the Department of Microbiology and Virology the rationalized method of IEOPh (Todorov T. — 1978).

### Material and methods

The method is based on the fact that the electrophoretic mobility of antigens in agar-gel with pH 8.2—8.6 is directed from cathode to anode, whereas gamma-globulins, being electroneutral, are transferred towards cathode. In the course of its motion the antigen interacts with the against moving specific antibodies and precipitating stripes are formed as a result. They confirm the diagnosis. The micromethod of IEOPh is performed on a glass-plate 26/76 mm. It is covered with 1% agarose (Koch-light) dissolved in veronal buffer with pH 8.6. 60 holes (2 mm in diameter) are cut in the gel-surface: 12 horizontal and 5 vertical. The distance between the holes is 3 mm in horizontal and 2 mm in vertical respectively. The vertical holes are filled with the investigated sera (first column) and antigens (second one). The plate is set in electrophoretical tank with veronal buffer (pH 8.6) in a position that holes with sera are directed towards anode, while those with antigen-diagnostics — towards cathode. The current flows through filter-paper bands. The voltage is 150 V and the duration of the electrophoretical motion is 30 min. Then the glass-plates are dipped in saline solution (24 hours), dried (37° C) and stained with Amidoschwarz 10B. The results can be registered after that. The established 1—2 or more precipitating stripes on the field between the holes with antigens and antibodies (sera) prove the positive diagnosis of the infection with the corresponding antigen-diagnostics. The micromethod is rationalized by T. Todorov (1978) and ap-

plied in the diagnosis of echynococcosis but our previous investigations (Metodiev Kr. — 1979) confirm the applicability of the method in the routine bacteriology.

The object of the present study is to investigate the role of some conditional pathogenic bacteria in the etiology of urinary tract infections of children hospitalized in the Clinic of Paediatry, Varna city.

The study covers 84 children with various urinary infections: cystopyelitis, cystitis, urethritis. The role of the following bacterial strains was investigated as etiological agent: *Escherichia coli* 017, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Pseudomonas aeruginosa*. The antigens were prepared ex tempore from standard strains (Institute of infectious and parasitic diseases, Sofia).

### Results and discussion

The results of the investigation show that in 76 of the patients (90.48%) the etiological agent is a bacterial one, while the rest 8 cases are probably a result of a viral, autoimmune or allergic influence. Out of all 76 bacterial infections 34 (44.74%) are due to conditional pathogenic bacteria. By using the micromethod of IEOPh it is established that the etiological role of the investigated strains for the infectious process in these 34 cases is the following: *E. coli* — 12 (35.28%), *Kl. pneumoniae* — 4 (11.76%), *Pr. vulgaris* — 10 (29.41%) and *Ps. aeruginosa* — 8 (23.55%). It is obvious that these bacteria play an important role in the etiology of the uro-genital tract infections. The precise diagnosis of bacterial infections (conditional pathogenic especially) is a serious problem for both microbiologists and clinicists. Therefore, it is obligatory to suggest new methods and approaches to support the uncertain routine bacteriological diagnosis. The preciseness of IEOPh is undoubtful and the fact that the micromethod allows the simultaneous investigation of 30 sera and 30 different antigen-diagnostics confirms the applicability of this method in the bacteriological practice. It is a quick and easy method and the results of its application in our study are definitely reliable statistically.

In conclusion it must be pointed that the micromethod of IEOPh could be applied successfully in every bacteriological laboratory but it is advised that all necessary bacterial strains (for antigen-diagnostics) must be standard and exclusively purified. Our experience shows that cross-reactions due to antigen shift are possible, therefore, it is recommended to investigate several times one single serum if it is suspected to be positive for any of the applied antigens.

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**ИССЛЕДОВАНИЕ ПРИМЕНЕНИЯ МИКРОМЕТОДА  
ИММУНОЭЛЕКТРООСМОФЕРЕЗА В ЦЕЛЯХ ДИАГНОСТИКИ  
НЕКОТОРЫХ БАКТЕРИАЛЬНЫХ ИНФЕКЦИЙ**

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**Р Е З Ю М Е**

Предлагается микрометод иммуноэлектроосмофореза (ИЭОФ), посредством которого исследовано 84 детей в клинике детских болезней в Варне. Изучается роль условно-патогенных штаммов (Е. коли, Кл. пнеумоние, Пр. вулгарис, Пс. аеругиноза) в этиологии некоторых урогенитальных инфекций (цистит, уретрит, цистопиелит). Доказывается их участие в инфекционном процессе у 34 их всех исследованных детей: Е. коли (12), Кл. пнеумоние (4), Пр. вулгарис (10), Пс. аеругиноза (8). Проведен анализ применимости микрометода ИЭОФ в бактериологической практике.

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