

## RADIAL HAEMOLYSIS IN GEL FOR DETECTION OF ANTIBODIES TO BUNYAVIRUS LEDNICE (TURLOCK GROUP)

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*Summary.* — The method of radial haemolysis in gel (RHG) was used for detection of antibodies to Lednice virus. Repeated and comparative experiments proved that the method gave reliable and sufficiently specific standard results. No cross-reactions were detected with sera to other arboviruses. Comparison of antibody titres achieved in RHG with those of haemagglutination inhibition (HIT) and indirect immunofluorescence (IF) tests showed a satisfactory sensitivity, as the antibody titres in RHG and HIT were practically of the same range.

*Key words:* *Lednice virus (Turlock group); radial haemolysis in gel; antibody detection*

Radial haemolysis in gel (RHG) originally described by Schild et al. (1975) is based on haemolytic action of antiserum on erythrocytes sensitized by antigen in the presence of complement in agarose gel. This method proved to be successful in detection of antibodies to various viruses including togaviruses (Gaidamovich and Melnikova, 1979, 1980; Odelola, 1979; Duca *et al.*, 1979). Simplicity, specificity and economy of the method was the reason for its verification also with the Lednice 6118 virus belonging to the Turlock group family *Bunyaviridae* (Málková *et al.*, 1972).

The RHG reaction was performed with plates produced by Medtehnika, U.S.S.R. according to Gaidamovich and Melnikova (1979, 1980). Sheep erythrocytes washed and stored as a 10% suspension in dextrose-gelatine-veronal buffer were used in the amount of 0.3 ml/plate. Before each experiment, they were washed in the phosphate buffered saline (PBS) of pH 7.2, sedimented and then diluted to original volume (0.3 ml) in borate-phosphate buffer with optimum pH for the tested virus (Table 1). To 0.3 ml of erythrocytes 0.1 ml of antigen was added and the mixture was kept for 10 min at room temperature. Thereafter, the erythrocytes were washed again in PBS and diluted with the optimum pH buffer to original volume. The erythrocytes together with 0.1 ml of complement were added to 2.5 ml of heated agarose (45 °C) and the mixture was poured on the plate. Wells ( $\varnothing$  2 mm) were punched into the hardened agarose containing the antiserum. The plate was placed into the humid chamber (37 °C) and the reaction was read after 18–20 hr. It was considered positive if the diameter of the haemolytic zone was at least 4 mm. Haemagglutination inhibition test (HIT) was performed according to Kolman and Meergansová (1973). Indirect immunofluorescence (IF) method was made as described Holubová, 1980.

Sheep erythrocytes have proved suitable either defibrinized or in Alsever solution. They

Table 1. Antibody titres against some arboviruses in RHG, HI and indirect IF tests

Antigen	Optimum pH for HA	HA-titre of antigen	Antibodies detected by		
			RHG	HIT	IF
Lednice (6118)	<b>6.0-6.2*</b>	320	512-1024	160-1280	64-128
Sindbis	<b>6.2-6.4</b>	2560	128-256	320-640	256
TBE	<b>6.4-6.6</b>	1280	128-256	160-320	512
WN	<b>6.6-6.8</b>	1280	256-512	160-640	1024
Ťahyňa	6.0- <b>6.2</b>	640	512-1024	160-320	128-512
Čalovo	6.2- <b>6.4</b>	160	1024-2048	1280-2560	1024**

HA = haemagglutination.

\* Thick printed values were used.

\*\* Single assay.

were used mostly in Alsever solution with regard to non-standard quality of defibrinized erythrocytes.

The antigen was prepared from suckling mouse brains by ultrasonic sucrose-acetone extraction (Clarke and Casals, 1958). The highest sensitizing effect on erythrocytes was obtained when using concentrated or 1:2 diluted antigen. Higher dilution of antigen weakened the haemolytic effect and thereby the contrast of the haemolysis zone. No strict correlation between the sensitizing ability of the antigen and the haemagglutination titre was found. Tissue medium from infected chick embryo fibroblasts (virus titre 5.5-6.5 log TCID<sub>50</sub>/0.1 ml), though five times concentrated (using lyophilized virus), proved to be a very weak antigen.

Standard mouse hyperimmune serum inactivated for 30 min at 56 °C was used. Concentration of antibodies influenced the size of the haemolysis zone in dependence on the time. The haemolysis zone began to appear after 3-4 hr. The optimal time for reading was less than 24 hr with regard to size and contrast effect of zones. In experiments, standard time 18-20 hr was used. Several commercial agaroses prepared in PBS were tested: U.S.S.R. (Oljanskij zavod chimpreparatov), French (L'industrie biologique française SA) and Fluka AG (F.R.G.) agaroses. All gave good results, the U.S.S.R. and the French ones in concentration 1.2% (30 mg/2.5 ml) and Fluka in concentration 0.6% (15 mg/2.5 ml). The Fluka agarose was advantageous because of lower consumption and clearer colour of erythrocytes, however, it required speedy manipulation.

The specificity of the reaction was tested in comparison with arboviruses of various taxonomic groups (showing haemagglutination properties) which occur in Czechoslovakia. Neither the 6118 antigen reacted in RHG with any of the compared antisera (Sindbis, WN, Ťahyňa, Čalovo and tick-borne encephalitis viruses), nor the antiserum 6118 reacted with any of the antigens compared.

As for sensitivity of the reaction, antibody titres of hyperimmune sera in RHG, HIT and indirect IF were compared. As seen from Table 1, antibody titres in RHG and HIT were practically of the same range except the Ťahyňa virus, where lower titres in HIT were repeatedly found. More differentiated picture was obtained by indirect IF when comparing the results of

RHG and indirect IF tests. IF antibody titres of 6118 virus were repeatedly lower, whereas those of TBE and WN were repeatedly higher. These differences can be explained by full diversity of both reactions.

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