Determination of Antibodies to Diphtheria and Tetanus Toxoid by Latex Agglutination Technique

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ABSTRACT

A micromethod of latex agglutination was worked out for determination of antibodies to diphtheria and tetanus toxoids. It is suitable for pediatrics since a minimum amount of serum (0.05 ml.) is required, which can be used without being inactivated or absorbed. Particles of polystyrene latex of Czechoslovak production were used as antigen carriers thus enabling better standardization of the technique. The particles are well defined both in physical and chemical terms and replace red blood cells in passive hemagglutination. The method was compared with passive hemagglutination in a dynamic study of children inoculated at monthly intervals with the trivalent vaccine Alditepera.

The technique of latex agglutination for determining antibodies to diphtheria and tetanus toxoids was worked out on the basis of experience using red blood cells treated with tannic acid, bis-diazo-lized or denatured with formaldehyde (Boyden, 1951; Stavitsky & Arquilla, 1954, Stavitsky, 1955; Gordon, Rose & Sehon, 1958; Lumenfeld, Isersky & Shelesnyak, 1962), which did not always give standard results, and required much serum, for this reason as so often in pediatrics' some data of the dynamic study were omitted. Because of the small amount of serum needed, the microtitrator of Takatsy (Takatsy, 1950) was used, making possible parallel tests (2 × 0.025 ml.) from 0.05 ml. of serum.

The latex particles are synthetized in Czechoslovakia (Institute for Production of Sera and Vaccines, Šár. Michalany). The particles are of polystyrene with a specific gravity close to water, of size 7,000 Å, and their advantages are due to the following properties: (1) monodispersity with respect to size, homogeneity of particles size rather than their absolute size being important; (2) coating of the free surface by emulsifying agents which help to keep the particles in suspension without clumping as well as binding the antigen. Sixty per cent of the surface of particles are occupied by emulsifying agents and forty per cent are left free for binding the antigen.

Advantages of latex particles for application in serology were summarized by Houba (1962).

MATERIALS AND METHODS

Material. One per cent solution of normal rabbit serum in saline, pH 7—7.2, was prepared for tests always fresh from the frozen rabbit serum stored in test tubes in 0.5 ml. lots at —18° C.

Diphtheria antigen of a known amount of Lf and total nitrogen was supplied by the Institute for Production of Sera and Vaccines, Praha, as purified ultrafiltrated toxoid.

Tetanus antigen was supplied by the same Institute, the parameters being identical as with the diphtheria toxoid.
The sera examined were stored either frozen at \(-18^\circ\text{C}\) or freeze-dried; all blood samples from a child were treated in one test. Standard antisera (anti-diphtheria and anti-tetanus) were used.

Suspension of purified polystyrene latex particles was supplied by the Institute for production of sera and vaccines, Šarišské Michalany. It was regarded as 100 per cent and diluted to 1 per cent suspension for binding the antigen.

**Method.** Diphtheria and tetanus antigen are diluted so that approximately 80 Lf units were present in the final volume of latex suspension. A relatively reliable parameter of the amount of antigen required for binding the antigen is the total nitrogen value, ranging from 0.15 to 0.30 mg. per ml. in the final dilution of latex particles. On keeping these values it is not necessary to make the suspension free of non-bound antigen residue by washing. The sera are tested without previous inactivation or absorption; they are diluted on microtitrator plates with 1 per cent solution of normal rabbit serum in saline using loops of 0.025 ml. volume and one drop of diluent per each well. The suspension of latex particles was incubated with antigen for one and a half hours at room temperature and occasional stirring and then one drop of the suspension (0.025 ml.) was placed into each well.

**Reading.** Agglutination was read after 24 hours incubation at room temperature (protected from direct source of heat and shocks) and a further reading was taken after 48 hours, when changes in titers usually cease. The reading is made against a black sheet in a horizontal position only. Positive results are represented by a diffuse sediment, negative results by a sharply bordered small white target on the bottom of the well — as illustrated on Fig. 1 and 2. Each titration is accompanied by parallel control tests with standard antiserum, with 1 per cent solution of normal rabbit serum in saline and with a similarly diluted suspension of latex particles, but without antigen. Only the control with standard antiserum is positive and of a known titer whereas other controls are negative. The control with latex suspension without antigen does not show tendency to agglutination, the latex particles remain in suspension and do not sediment at all.

**RESULTS**

Formation of anti-diphtheria and anti-tetanus toxoid (Lodinová & Jouja, 1964) was studied in 49 children aged from birth up to 1 year, injected with trivalent vaccine Alditepera at monthly intervals, the first administration being at the age of three months. Venous blood was taken at two week intervals in the period from the third to five and a half months; before that period blood was withdrawn more frequently and afterwards at monthly intervals. In the table, however, only those blood samplings are given which demonstrate formation of anti-diphtheria and anti-tetanus toxoid after three immunizations. Most children examined were in hospital from birth, in the physiological department of the Institute for the Care of Mother and Child (45 children) and later in the Nursery Institute at Krč (4 children).

In 26 children out of the total of 49, the method of passive hemagglutination by Stavitsky and Arquilla (1954) and Stavitsky (1955) in micromodification by Takatsy (1950) was used; for a better standardization, sheep red blood cells were denatured with formaldehyde according to Lunenfeld et al. (1962). In 23 children the modified method was used, with latex particles as antigen carrier. Some sera from the children were parallely examined in the Institute for production of sera vaccines, for the titer of anti-diphtheria toxoid by the intradermal toxin neutralization test in rabbits according to Jensen (1933). The results are summarized in Tab. 1. The table shows average values of antibody titer in children after vaccination estimated by the three methods: passive