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THE PROVISIONAL INTERNATIONAL STANDARD ANTITYPHOID SERUM

A. FELIX, D.Sc., F.R.S.

Lister Institute of Preventive Medicine, London
formerly Director, Central Enteric Reference Laboratory and Bureau, Public Health Laboratory Service (Medical Research Council), London

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SYNOPSIS

The Provisional International Standard Antityphoid Serum, prepared in 1935, provided standards of the O and the Vi antibodies, both contained in a single serum preparation, and was used for standardizing the potency of therapeutic antityphoid serum.

The "Provisional Standard Serum for Vi Agglutination", which is one of the series of proposed Standard Agglutinating Sera for Typhoid and Paratyphoid A and B Fevers, is based on the Provisional International Standard Antityphoid Serum.

It is recommended that the Provisional International Standard Antityphoid Serum, in conjunction with a standard typhoid vaccine, be used in the test for the determination of the "functional efficacy" of the Vi antibody. This is one of the several tests recommended for the official control of the immunizing potency of typhoid vaccines.

The Provisional Standard Antityphoid Serum was described by the present author in 1938, and has been formally included in the list of international standards and reference preparations.[23] The object of this article, written at the request of the World Health Organization, is to describe briefly the purposes for which the preparation has been employed in the past and for which it is intended now.

1. Standardization of Therapeutic Antityphoid Serum

The Provisional Standard Antityphoid Serum was introduced in connexion with the somewhat complicated process of preparation of a potent serum from the horse for therapeutic use in man. It was prepared in 1935 by Felix & Petrie[11] at the Lister Institute of Preventive Medicine, London, and ampouled (about 250 ampoules, each containing the dried material

from 5-ml volumes of the serum) by Sir Percival Hartley at the National Institute for Medical Research, London.

The serum was adopted to supply two standards, both contained in a single serum preparation, in order to estimate the two essential protective substances present in antityphoid serum, namely, the O and the Vi antibodies. It was suggested that the following provisional unitages be assigned to 1 ml of the reconstituted Provisional Standard Serum :

(1) 5 units of Vi antibody, as defined by mouse-protection tests against 3 minimum lethal doses (MLD) of the living virulent strain Ty 2;

(2) 2.5 units of O antibody, as defined by mouse-tests for the neutralization of 1 MLD of killed typhoid bacilli (strain O 901).

The corresponding Standard O-Agglutinin Titre and Standard Vi-Agglutinin Titre were also defined in 1938 (Felix [5]).

At the last session of the Permanent Commission on Biological Standardisation of the Health Organisation of the League of Nations, held in Paris in October 1938, the Commission accepted the Provisional Standard Antityphoid Serum as the basis for an international comparative investigation, and designated institutes in ten countries to which the serum and the recommended test strains were to be distributed.[17] However, the outbreak of the second World War in 1939 prevented the comparative investigation from being carried out.

Serum therapy of typhoid fever with O + Vi antityphoid serum has been favourably reported in the literature [1, 3, 4, 14, 15, 18-21] and it held its own in spite of rival claims in favour first of the sulfonamides and later of penicillin, both of which proved to be ineffective. Antityphoid serum continued to be manufactured until about 1949, and the Provisional Standard Antityphoid Serum, though its potency had never been officially defined in units, continued to be employed by serum institutes in China, Czechoslovakia, Italy, Poland, and South Africa, and at the Lister Institute.

Because of the success of chloramphenicol, serum therapy of typhoid fever is no longer practised routinely. Demands for antityphoid serum are still made occasionally by clinicians in charge of patients who fail to respond to chloramphenicol. In view of the extreme degree of toxaemia commonly seen in severe typhoid cases, a combination of chloramphenicol and a potent Vi + O antityphoid serum might prove more effective. The report of the study-group on typhoid which met in May 1953 during the Sixth World Health Assembly,[2] also mentioned the beneficial results obtained with antityphoid serum. It is, however, unlikely that the Provisional International Standard will be in any great demand in the future for use in the standardization of therapeutic antityphoid serum.

2. Standardization of the Typhoid Vi-Agglutination Test

The Provisional International Standard Antityphoid Serum served as the basis for the " Provisional Standard Serum for Vi Agglutination ". This has been distributed, in the form of liquid serum, to workers in various parts of the world since January 1939, first by the present author working at the Lister Institute, London, and later in co-operation with the Standards Laboratory, Oxford, England, and the Standards Laboratory for Serological Reagents, Public Health Laboratory Service (Medical Research Council), London.

Two large batches of the " Provisional Standard Serum for Vi Agglutination " were prepared in horses at the Lister Institute, the first in 1938 and, when this was exhausted, the second in 1946. These two Substandards were both based on the (dried) Provisional Standard Antityphoid Serum held by the Department of Biological Standards, National Institute for Medical Research, London (see Felix [7]).

Following the decision taken by the WHO Expert Committee on Biological Standardization at its third session, held in 1949,[22] a third batch of " Provisional Standard Serum for Vi Agglutination " has now been prepared and standardized in a similar manner. This new batch has been dried and represents one of the series of seven proposed Standard Agglutinating Sera for Typhoid and Paratyphoid A and B Fevers described in another article in this number of the *Bulletin* (see page 919).

In the future, therefore, it will no longer be necessary to use the Provisional International Standard Antityphoid Serum for standardizing further Substandards of agglutinating typhoid Vi serum.

3. Standardization of Typhoid Vaccine

Tests essentially identical with those employed in the standardization of therapeutic antityphoid serum form part of the several tests required for the evaluation of the immunizing potency of typhoid vaccines. The Provisional Standard Antityphoid Serum played a definite role in the experimental work that led to advances in the preparation and testing of typhoid vaccine.

Earlier attempts at standardizing typhoid vaccine failed because no vaccine existed at that time that would meet the requirements of a standard vaccine. Recent work has shown that the alcohol-preserved vaccine, which shows no loss of potency in 10 years at 1°C-2°C, or one of the dried vaccines now available, could serve as a standard vaccine.[9, 12, 13, 16] Standardization of typhoid vaccine has thus become feasible.

A complete assay of differently prepared typhoid vaccines should comprise :

(1) active immunization of mice;

(2) immunization of rabbits with a view to establishing :

(*a*) whether circulating Vi and O antibodies are readily elaborated;

(*b*) whether the Vi antibody produced possesses full " functional efficacy " in passive-protection tests in mice;

(3) tests of the Vi- and O-antibody responses in man.

The tests under (1) can be used to reject unsatisfactory vaccines, but the sensitivity of the active-protection test in the mouse is not as high as that of the tests listed under (2) and (3).[6, 8] The latter tests are standardized by means of the Provisional International Standard Antityphoid Serum, which contains Vi antibody elaborated in response to immunization with the natural Vi antigen as present in the living bacterial cell.

In any scheme for the standardization of typhoid vaccine, whether on a national or an international level, two different aspects of the problem need to be considered :

1. The routine examination in the course of preparation of typhoid vaccine.

2. The official control of the potency of typhoid vaccine.

Fortunately, it is not necessary to employ all the tests listed under (1), (2), and (3) in the routine examination in the course of preparation of typhoid vaccine. For this purpose, estimation of the Vi- and O-agglutinin responses in groups of rabbits is sufficient, controlled by parallel tests of the standard vaccine, provided that it is known that the method used for making the vaccine does not lead to " functional deficiency " of the Vi antibody.[10] It is thus unnecessary routinely to carry out passive-protection tests in mice.

On the other hand, the position is quite different when the protective value of vaccines is estimated for purposes of official control. In this case it is clearly indispensable to employ all the available tests, irrespective of whether the method by which any given vaccine has been prepared is known or not. This is the purpose for which the Provisional International Standard Antityphoid Serum is now recommended.

The question may be asked whether this standard preparation will in fact still retain a unique potential value after the separate TO-Agglutination and TVi-Agglutination Standards have been established. This question should be answered in the affirmative, for the following reasons :

1. The Provisional International Standard Antityphoid Serum was produced by immunization with living bacilli and contains the " ideal " Vi antibody endowed with the property of full " functional efficacy ", that is to say, the power of promoting phagocytosis of, and protection against infection with, virulent O + Vi typhoid bacilli. For this reason it is the ideal standard for use in passive-protection tests in mice.

2. The " Provisional Standard Serum for Vi Agglutination " has been prepared by immunization with alcohol-treated Vi antigen, and the Vi antibody it contains is believed to possess full " functional efficacy ". Nevertheless, it appears to be appropriate that the serum containing the " ideal " antibody should remain the standard for measuring " functional efficacy ".

3. Whenever typhoid vaccines are tested in passive-protection tests, both the O and Vi antibodies are present in the sera under test. It is, therefore, an advantage to employ a standard serum which contains the two antibodies in relative amounts similar to those present in the average serum under test.

4. The quantities of standard serum required for performing passive-protection tests in mice are very much greater than those needed for agglutination tests. The available quantities of the two separate O-Agglutination and Vi-Agglutination Standards would soon be exhausted if they were employed for mouse-protection tests.

This is not the place to make detailed recommendations for the standardization of typhoid vaccines. Only the test for the " functional efficacy " of the Vi antibody and the special part in this test assigned to the existing Provisional International Standard Antityphoid Serum have been discussed.

RÉSUMÉ

Le sérum antityphoïdique devant représenter l'Etalon International a été préparé par l'auteur de cet article en 1935 et accepté par la Commission permanente de Standardisation biologique de l'Organisation d'Hygiène de la Société des Nations en 1938, pour être soumis à un essai comparatif. La guerre empêcha l'exécution de cet essai.

Les sérums antityphoïdiques O+Vi ont gardé une grande valeur dans le traitement de la fièvre typhoïde chez l'homme, jusqu'à l'introduction du chloramphénicol. Cet antibiotique a fait passer la sérothérapie à l'arrière-plan. Cependant, dans les cas de toxémie aiguë observés généralement chez les sujets gravement atteints, l'adjonction au chloramphénicol de sérum antityphoïdique O+Vi actif pourrait assurer au traitement une plus grande efficacité. Quoi qu'il en soit, il est probable que l'Etalon Provisoire de Sérum Antityphoïdique ne sera désormais que peu demandé pour la standardisation des sérums thérapeutiques. D'autre part, cet étalon, qui a servi de base lors de l'établissement du Sérum-étalon Provisoire pour l'agglutination Vi, ne sera guère utilisé non plus à l'avenir pour la standardisation des étalons secondaires de sérums agglutinants Vi, puisque l'on dispose maintenant d'un étalon spécifique pour ces sérums.

Mais l'Etalon Provisoire de Sérum Antityphoïdique a joué un grand rôle dans les recherches expérimentales qui ont abouti à la préparation et à l'essai du vaccin typhoïdique et il a encore un rôle à jouer dans la standardisation de ces vaccins et les essais d'activité.

Ces essais comprennent : 1) l'immunisation active des souris; 2) l'immunisation des lapins, en vue d'établir *a*) si les anticorps circulants Vi et O sont rapidement élaborés et *b*) si l'anticorps Vi ainsi produit possède sa pleine « efficacité fonctionnelle » dans les tests de protection passive de la souris; 3) les essais sur la réponse immunologique, chez l'homme, par production d'anticorps Vi et O.

Les tests 2) et 3) sont standardisés par l'intermédiaire de l'Etalon Provisoire de Sérum Antityphoïdique, contenant l'anticorps Vi qui a pris naissance sous l'action de l'antigène Vi naturel de la cellule bactérienne vivante.

Comme il est indispensable d'effectuer tous les essais précités pour contrôler officiellement le vaccin typhoïdique, l'Etalon Provisoire est alors d'une grande utilité.

On s'est demandé si cette préparation garderait toute sa valeur, après la création des Etalons d'agglutination TO et TVi. On peut répondre par l'affirmative pour les raisons suivantes : L'Etalon Provisoire de Sérum Antityphoïdique, résultat de l'immunisation par des bacilles vivants, contient l'anticorps Vi idéal, doué de la pleine « efficacité fonctionnelle », c'est-à-dire ayant le pouvoir de susciter la phagocytose du bacille typhique O+Vi et de protéger l'organisme contre l'infection par ce bacille. Ce sérum constitue l'étalon idéal pour les tests de protection passive de la souris. En outre, du fait que les sérums soumis à l'essai contiennent les anticorps Vi et O, il y a avantage à utiliser, pour les tests de protection, un sérum étalon qui contienne ces anticorps dans des proportions analogues à celles que l'on trouve dans les sérums moyens. Enfin, les quantités de sérum étalon nécessaires pour le test de protection passive de la souris sont beaucoup plus élevées que celles que nécessite le test d'agglutination. Les stocks des deux étalons d'agglutination séparés, Vi et O, seraient bientôt épuisés, s'ils étaient employés pour le test de protection de la souris.

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