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International Standard for Antistreptolysin-O

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A pool of highly potent freeze-dried sera from three patients with streptococcal infection has been examined by twelve laboratories in eleven countries and has now been established as the International Standard for Antistreptolysin-O by the WHO Expert Committee on Biological Standardization. The main part of this collaborative study consisted in a comparison of the proposed international standard serum with other standard preparations. Samples of serum from eight different patients were titrated by the different laboratories. The laboratories each carried out two series of tests, one using their own streptolysin-O preparations and the other using a particular streptolysin-O preparation supplied to all the participants. There was fairly good agreement between the results from the majority of the laboratories, particularly in the tests with the same preparation of streptolysin-O. Some of the variations could be traced to the use of unsuitable streptolysin reagents. On the basis of the results, the International Unit of Antistreptolysin-O has been defined as the activity contained in 0.0213 mg of the International Standard.

The antistreptolysin-O unit was originally defined by Todd (1932a, 1932b) as follows: One unit is contained in an amount of serum capable of neutralizing 2.5 minimal haemolytic doses of streptolysin-O, while one minimal haemolytic dose just suffices to haemolyse completely 0.5 ml of a 5% suspension of rabbit erythrocytes.

Hodge & Swift (1933) showed that this definition was not explicit, because of the instability of the haemolytic effect of streptolysin-O. In a personal communication to these authors, Todd then proposed that a serum containing a fixed number of antistreptolysin-O units should be universally employed as a standard. A horse globulin preparation containing 20 000 " Todd units " per ml was subsequently prepared by Todd. This preparation was later used by many laboratories as a basis for setting up their own antistreptolysin-O standards.

The National Danish Standard for Human Antistreptolysin-O was established in 1944. Kalbak (1942) and Ipsen (1944), respectively, described the use and the technical background of this standard. The agreement of the unit of the national standard with that of the standard established by Todd was specially studied by Ipsen.

The National Danish Standard has been widely distributed to laboratories in other countries—first, in the other Scandinavian countries and in Central Europe, and later in other parts of the world. The distribution at present amounts to about fifty samples a year, sent to some thirty different laboratories.

The unit of the National Danish Standard for Human Antistreptolysin-O established in 1944 was defined as the activity contained in 0.0809 mg of the Standard. This unit was equivalent to the unit defined by Todd (see Ipsen, 1944). The first Standard was replaced in 1952 by the second, one antistreptolysin-O unit of which was contained in 0.0643 mg of the preparation. In both cases the standard preparation was human serum of high titre. The Standard has been distributed to the users in the fluid state, in ampoules containing 10 antistreptolysin-O units per ml. It has proved to be most satisfactory for the receivers to have the Standard dispensed in this form.

In its second report, the WHO Expert Committee on Rheumatic Diseases (1957) recommended the establishment of an international standard for human antistreptolysin-O. The WHO Expert Committee on Biological Standardization (1957) accordingly requested the International Laboratory for Biological Standards, Statens Seruminstitut, Copenhagen, to obtain material suitable for such a standard and to arrange for an international collaborative assay.

A brief description of the material selected for the proposed international standard and of the collaborative assay will be presented here.

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MATERIAL FOR THE PROPOSED INTERNATIONAL STANDARD

During 1957, a pool of highly potent serum from three patients with streptococcal infection was collected, freeze-dried and sealed under vacuum in ampoules containing 0.5 ml each. A total of some 600 ampoules was obtained.

The stability of the product has been investigated (unpublished working document, WHO/BS/402). The antistreptolysin-O activity of a 1 : 10 dilution in buffered saline of the reconstituted serum was found to remain stable at −15°C, +4°C, +20°C and +37°C for at least four weeks.

THE COLLABORATIVE ASSAY

The names and addresses of the twelve laboratories that took part in the collaborative assay are listed in the Annex (see page 278). Ten of these laboratories participated in the main part of the study, six participated in an evaluation of the activity of the proposed international standard in comparison with that of the current Danish standard, and two made a direct comparison between the proposed international standard and the standard globulin preparation established by Todd and now held at the Medical Research Council Laboratories, Hampstead, London.

Throughout this report the participating laboratories are referred to by arbitrary numbers, which have no connexion with the order in which the laboratories are listed in the Annex.

Each participant in the main part of the study was asked to investigate a collection of ten serum samples, comprising eight single serum samples, designated I-VIII, from different patients and two samples of the proposed international standard serum, one of which was given the code number IX. Samples I and VIII were high-titre sera, samples II, IV, VI and VII were borderline sera in the clinical sense, and samples III and V were low-titre sera.

The laboratories were asked to test all the ten sera simultaneously, by the technique and with the streptolysin-O usually employed by them. The tests were preferably to be repeated on four different days, using erythrocytes from different rabbits and differents lots of the streptolysin-O preparation in normal use. In addition, a series of tests was to be performed with a particular preparation of streptolysin-O [1] supplied to all the participants. The latter series of tests was intended to demonstrate the influence of using blood corpuscles from different rabbits, while the former series was designed to indicate the significance of using different preparations of streptolysin-O.

The participants received the test materials in April 1958, and returned their protocols to Copenhagen at the end of that year.

[1] This preparation of streptolysin-O was kindly made available by Dr A. L. Lane of Difco Laboratories, Detroit, Mich., USA.

RESULTS AND DISCUSSION

Agreement between the laboratories

To make the results from the ten different laboratories comparable, the statistical evaluation was initiated on each single test for each serum from each laboratory by estimating the serum dose (titre) yielding, or better preventing, 50% haemolysis. This interpolation could be done only in the cases where observations around the 50% end-point were available. The laboratories have an upper as well as a lower limit for their titration rows, varying from laboratory to laboratory. The observation that a given serum has a titre lower or higher than the lower or upper limits, respectively, for the titration is of value, but is not the precise information required for the straightforward statistical treatment used here. Such observations, therefore, have not been included in the calculations. In consequence, the standard deviations are, in all such cases, somewhat underestimated and the mean titres are usually overestimated, since in most cases observations below the lower limit have been excluded.

The results of all the tests are summarized in Tables 1 and 2. For each serum tested in each laboratory an average titre is given; in addition, an unweighted average of the titres for each serum has been calculated. Table 1 deals with the tests performed with streptolysin-O preparations of widely different origin, mostly made up by the laboratories themselves. Table 2 deals with the tests performed with the particular preparation of streptolysin-O supplied to all the laboratories.

For the tests performed in each laboratory an analysis of variance was carried out, the main results of which have been presented in the last two columns of Tables 1 and 2. This analysis of variance has furnished us with estimates of the titration error and the variation between experimental series, including, in most cases, information regarding the influence of using in each laboratory different lots of streptolysin-O on different days.

TABLE 1

AVERAGE LOG TITRES OF ANTISTREPTOLYSIN-O: TESTS WITH DIFFERENT STREPTOLYSIN-O PREPARATIONS

Lab. No.	Serum sample [a]										Standard deviation due to variation between			
	I	II	III	IV	V	VI	VII	VIII	IX	PIS [b]	titrations [e]	df	experiments [f]	df
1	3.19 (2)	2.78 (2)	2.14 [c] (1)	2.44 (2)	A	2.39 (2)	2.44 (2)	3.67 (2)	3.89 (2)	3.96 (2)	0.08	7	0.08 *	1
2	3.36 (4)	3.09 [c] (2)	B	B	B	2.85 [c] (1)	2.91 [c] (1)	3.57 (4)	3.87 (4)	3.80 (4)	0.10	9	0.31 ***	3
3	2.85 (12)	B	B	B	B	B	B	3.15 (12)	3.47 (12)	3.90 (12)	0.08	33	0.05 *	11
4	2.93 (4)	2.45 (4)	1.56 (4)	2.02 (2)	1.66 (4)	2.36 (4)	2.17 (3)	3.39 (3)	3.73 (3)	3.71 (2)	0.06	12	0.05 *	3
5	3.26 (4)	2.77 (4)	1.62 (4)	2.43 (4)	1.80 (4)	2.56 (4)	2.44 (4)	3.62 [d] (2)	3.92 [d] (3)	4.00 [d] (1)	0.04	18	0.05 ***	3
6	2.99 (5)	2.73 (5)	1.83 [c] (4)	2.43 (5)	1.89 [c] (4)	2.49 (5)	2.46 (5)	— —	— —	3.68 (5)	0.14	21	0.05	3
7	3.30 (4)	2.78 [c] (2)	B	B	B	2.70 [c] (2)	2.70 [c] (1)	3.73 (4)	4.04 (4)	3.94 (4)	0.09	9	0.06	3
8	3.45 (12)	3.01 [c] (11)	B	B	B	B	B	3.67 (12)	3.97 (12)	—	0.13	40	0.09 **	10
9	3.32 (8)	2.98 (8)	2.03 (8)	2.60 (8)	2.27 (8)	2.70 (8)	2.72 (8)	3.66 (8)	3.95 (8)	3.96 (8)	0.03	70	0.01 **	
10	2.80 (3)	2.58 (3)	2.00 (3)	2.70 (3)	2.25 (3)	2.53 (3)	2.85 (3)	3.85 (3)	4.15 (3)	—				
Average	3.15	2.80	1.87	2.44	1.97	2.57	2.59	3.59	3.89	3.88				
Range	0.65	0.64	0.58	0.68	0.61	0.49	0.74	0.70	0.68	0.32				

[a] The figures in parentheses indicate the number of titrations included in the averages.

[b] Proposed international standard.

[c] The average titre has been computed without taking into account one or more titrations yielding complete, or almost complete, haemolysis even in the lowest dilution tested.

[d] The average titre has been computed without taking into account one or more titrations yielding no haemolysis, or practically no haemolysis, even in the highest dilution tested.

[e] The standard deviation due to variation between titrations = $\sqrt{s^2_R}$, s^2_R being the residual variance; df = degrees of freedom.

[f] The standard deviation due to variation between experiments = $\sqrt{\frac{s^2_E - s^2_R}{n}}$, n being the number of sera and s^2_E the variance between experiments; df = degrees of freedom for s^2_E.

* The variance ratio s^2_E/s^2_R is significant at the 5% level.

** The variance ratio s^2_E/s^2_R is significant at the 1% level.

*** The variance ratio s^2_E/s^2_R is significant at the 0.1% level.

A = all titration results < 2.079; B = all titration results < 2.699; C = all titration results > 3.398; D = all titration results > 3.699.

All sera with complete titrations in the experimental series used have been included in the analyses of variance. The National Danish Standard is thus included for some laboratories.

TABLE 2

AVERAGE LOG TITRES OF ANTISTREPTOLYSIN-O: TESTS WITH THE SAME STREPTOLYSIN-O PREPARATION

Lab. No.	Serum sample [a]										Standard deviation due to variation between			
	I	II	III	IV	V	VI	VII	VIII	IX	PIS [b]	titrations [e]	df	experiments [f]	df
1	3.29 (2)	2.73 (2)	A	2.46 (2)	A	2.44 (2)	2.46 (2)	3.67 (2)	4.00 (2)	3.91 (2)	0.06	7	0.03	1
2	3.19 (4)	2.66 (4)	A	2.26 (4)	A	2.48 (4)	2.39 (4)	3.61 (4)	3.90 (4)	3.91 (4)	0.10	21	0.04	3
3	—	—	—	—	—	—	—	—	—	—				
4	3.06 (4)	2.61 (3)	1.56 (4)	2.25 (2)	1.68 (3)	2.46 (3)	2.42 (4)	C	D	D	0.09	8	0.01	2
5	3.23 (4)	2.75 (4)	1.55 (4)	2.34 (4)	1.79 (4)	2.46 (4)	2.40 (4)	3.64 [d] (1)	3.69 [d] (1)	3.93 [d] (3)	0.08	18	0.01	3
6	3.03 (5)	2.56 (5)	1.45 (5)	2.18 (5)	1.67 (5)	2.45 (5)	2.24 (5)	—	3.85 (5)	3.85 (5)	0.07	36	0.02	4
7	3.08 (4)	2.50 (4)	A	2.37 (4)	A	2.39 (4)	2.39 (4)	3.56 (4)	3.88 (4)	—	0.05	18	0.01	3
8	3.03 (4)	2.56 (4)	A	2.43 (4)	A	2.65 (4)	2.65 (4)	3.58 (4)	3.81 (4)	—	0.16	18	0.00	3
9	3.13 (4)	2.68 (4)	1.70 (4)	2.38 (4)	1.87 (4)	2.51 (4)	2.44 (4)	3.47 [d] (1)	3.78 [d] (2)	3.78 [d] (1)	0.03	21	0.03 ***	3
10	—	—	—	—	—	—	—	—	—	—				
Average	3.13	2.63	1.57	2.33	1.75	2.48	2.42	3.59	3.88	3.88				
Range	0.26	0.25	0.25	0.28	0.20	0.26	0.41	0.20	0.22	0.15				

For explanation of symbols and conventions, see Table 1.

A glance at the last two serum columns in Tables 1 and 2 (serum IX and the proposed international standard (PIS) serum are identical) will show that in each laboratory the average titres in both series of tests are practically the same. In the assays with the same preparation of streptolysin-O (Table 2), the log titres arrived at in the different laboratories for the PIS serum and serum IX have ranges of 0.15 and 0.22, respectively. In the assays with different preparations of streptolysin-O (Table 1), the between-laboratories range amounts to a twofold dilution step for the PIS serum (0.32) and to approximately double this value for serum IX (0.68). For sera I-VIII the results from the different laboratories show a similar range of difference.

In Table 3 a survey is presented of the over-all averages from Table 2 and of the corresponding figures from Table 1 made comparable to the former by including only the results from laboratories represented in both tables. The over-all average titres for each serum are consistently slightly lower in the tests with the same preparation of streptolysin-O than in those with different preparations of streptolysin-O. It can be seen that the lower the serum titres, the greater the difference between the over-all averages in the two series of tests. This trend has

TABLE 3
SURVEY OF COMPARABLE RESULTS FROM TABLES 1 AND 2

	Serum sample									
	I	II	III	IV	V	VI	VII	VIII	IX	PIS[a]
Table 1: Over-all average titres calculated for the same laboratories as Table 2	3.23	2.82	1.84	2.38	1.91	2.58	2.55	3.62	3.91	3.86
Table 2: Over-all average titres	3.13	2.63	1.57	2.33	1.75	2.48	2.42	3.59	3.88	3.88
Table 1: Range of titres	0.52	0.64	0.58	0.58	0.61	0.49	0.74	0.34	0.31	0.32
Table 1: Potency range	0.34	0.49	0.45	0.32	0.45	0.33	0.48	0.13		
Table 2: Range of titres	0.26	0.25	0.25	0.28	0.20	0.26	0.41	0.20	0.22	0.15
Table 2: Potency range	0.16	0.25	0.28	0.29	0.24	0.35	0.45	0.08		

[a] Proposed international standard.

appeared mainly owing to the exclusion of incomplete observations—an exclusion more often affecting the low-titre sera in Table 1 than in Table 2.

The range of the titres for sera I-VIII obtained in the different laboratories is about one twofold dilution step in the tests with the same preparation of streptolysin-O and amounts to approximately double this value in the tests with different preparations of streptolysin-O.

It was thought that by using relative potencies—i.e., estimates of the potency of sera I-VIII in relation to the proposed international standard serum—as a basis for comparison instead of average titres, it might be possible to bring the laboratories into closer agreement with regard to their individual statements as to the activity of the sera under test. It can be seen from Table 3 that the relative potencies estimated in the different laboratories had a smaller range (by about half a twofold dilution step) than the average titres in the case of the tests with different preparations of streptolysin-O. In the case of the tests with the same preparation of streptolysin-O, however, the ranges of the relative potencies are approximately the same as those of the average titres.

Accuracy of the assays

It is appropriate here to discuss the accuracy of the results obtained in the different laboratories.

The variations arising from errors in titration are fairly uniform for all laboratories, the values of the standard deviation clustering around 0.08 in logarithmic value—that is, about one-fourth of a twofold dilution step. It can be seen from Tables 1 and 2 that the titration errors are of equal magnitude in the two assay systems, which is as expected. This means that an error of about $\pm 20\%$ has been introduced solely through the process of titration.

The variations between experimental series are clearly of different magnitude in Tables 1 and 2, the values of the standard deviation being higher in Table 1. Here we may recall that in Table 1 these variations include, in nearly all the laboratories, the effect of using different preparations of streptolysin-O on different days, whereas in Table 2 this effect has been eliminated since the same preparation of streptolysin-O was used for all titrations in all laboratories. In both tables, however, the experimental variations include the effect of using erythrocytes from different rabbits.

In Table 2 the values of the standard deviation due to the variation between experiments are estimated as being around 0.02 in logarithmic value—that is, about one-sixteenth of a twofold dilution step. These variations seem to be without any practical significance in view of the fact that, in most cases, they include the effect of using erythrocytes from different rabbits.

The variation between experiments is considerably greater in Table 1 than in Table 2. Most laboratories have standard-deviation values of about 0.05. Laboratory 2 has a somewhat higher value (0.31), probably because one of the streptolysin-O preparations used had been contaminated during storage. Except for laboratories 6 and 7, the variation between experiments has been estimated, by a variance ratio test, as statistically significant, in most cases at the 5% level but in some at the 1% or even the 0.1% level.

It is an interesting question whether the variation between experiments shown in Table 1 would be smaller if estimates of the potency of sera I-VIII in relation to the proposed international standard serum were used as a basis for comparison instead of the average titres. With the exception of laboratory 2, all the laboratories arrived at values for the standard deviation due to variation between experiments that were lower than or almost equal to the values for the standard deviation due to variation between titrations. An estimate of the total variance on a titre in a laboratory can be obtained by adding the squares of the values for variation between experiments and variation between titrations. In Table 1 the total variance will thus be about $0.05^2 + 0.08^2$, and the corresponding standard deviation will be $\sqrt{0.05^2 + 0.08^2} = 0.09$. A relative potency, however, will include titration errors originating from the titration of the standard serum as well as from the titration of the serum under test. The titration error on a potency estimate will therefore, for most laboratories, be in the neighbourhood of $\sqrt{0.08^2 + 0.08^2} = 0.11$. Hence it follows that the use of relative potencies does not diminish the total error involved in titrating the same serum on different days.[1]

From the above it can be calculated that the total error, including the titration error as well as the error arising from variation between experiments, amounts to about one-third of a twofold dilution step.

Advantages of using a standard serum

It may be questioned whether the laboratories benefit at all by using a standard. Hodge & Swift (1933) proposed adjusting the streptolysin-O dose to be used by a preliminary titration against a known serum. Since then, this procedure has become common practice in many laboratories. All the laboratories participating in this collaborative study carried out such a preliminary adjustment of their streptolysin preparations—i.e., converted the antistreptolysin-O unit into a corresponding " binding unit " of the streptolysin-O preparations used. It would seem that the use of the standard serum for this adjustment procedure would result in a reasonable limitation of the experimental error.

In only a few cases were the records from the laboratories suitable for comparison of the dose-response curves obtained by the titration of the test sera and the standard serum. In a graphical analysis of the results from laboratories 5, 6 and 9, only the last-mentioned, which had the smallest experimental error, indicated differences in the slopes of the reaction curves. The differences between the slopes of the test sera and the slope of the standard serum were most marked in the case of the low-titre sera; the high-titre sera gave slopes that were very similar to the slope of the standard serum. The slope of the Danish standard serum did not differ from that of the proposed international standard.

The question of differing slopes of reaction curves was dealt with some years ago by Ipsen (1944) in his comparison of the National Danish Standard for Human Antistreptolysin-O with Todd's standard (a purified globulin preparation from hyperimmune horse serum). The slope problem encountered with sera from patients has recently been considered thoroughly by Kusama et al. (1958). These workers have found a practical way of overcoming the difficulties—namely, by increasing the test dose of the streptolysin-O used for the assay—and in their paper have discussed in detail the theoretical problems involved.

The present collaborative assay seems to indicate (*a*) that the use of a standard serum has afforded a reasonable comparability between the results obtained by different laboratories, and (*b*) that adjustment of the various streptolysin-O preparations used in each laboratory by means of a standard serum has equalized the results obtained in the same laboratory at different times. Differences in the slopes of the dose-response curves for the various sera have not caused difficulties in this comparative assay, the main purpose of which was to compare practical determinations of levels of antistreptolysin-O activity for a series of sera, rather than to provide a detailed description of the sera—an undertaking which would require a much more elaborate titration system than is usually employed.

Unitage assigned to the proposed international standard

It has always been the custom to try to define the unit of an international standard at a level of activity such that laboratories which previously used a national or a provisional laboratory standard do not have to depart from their usual practice with regard to unitage. It seems appropriate, therefore, to examine here the information on unitage provided by the results obtained in the collaborative study.

In addition to the results presented in Tables 1, 2 and 3, results are available from a total of 41 experi-

[1] If only a single titration of the standard serum and the test serum is carried out.

ments, carried out in six different laboratories, in which the activity of the proposed international standard was compared with that of the current Danish standard, the unitage of which corresponds to the unit originally defined by Todd. The results of the tests, which were performed simultaneously in pairs in each laboratory, are presented in Table 4. It can be seen that the average potency of the proposed international standard is estimated at 4305 Danish units per millilitre, with a standard error amounting to approximately 13% of that value (standard error on the average log potency, 0.05).

TABLE 4

AVERAGE LOG TITRES FOR PROPOSED INTERNATIONAL STANDARD AND DANISH NATIONAL STANDARD AND POTENCY OF INTERNATIONAL STANDARD RELATIVE TO DANISH STANDARD

Lab. No.	Number of tests	Average log titre		Log potency of PIS relative to DS	PIS in Danish units [c]
		PIS [a]	DS [b]		
Tests with different streptolysin preparations					
2	2	4.099	1.440	2.659	4560
6	5	3.683	1.314	2.369	2339
7	4	3.987	1.313	2.674	4721
8	12	3.972	1.363	2.609	4064
9	8	3.953	1.338	2.615	4121
10	3	4.154	1.333	2.821	6622
Tests with the same streptolysin preparation					
6	5	3.856	1.169	2.687	4864
9	2	3.783	1.140	2.643	4395
Geometric mean				2.635	4305
Standard deviation				0.13	
Standard error				0.05	

[a] Proposed international standard.
[b] Danish standard.
[c] The Danish standard contains 10 Danish units per ml.

Table 5 gives the results obtained by weighing the contents of ten ampoules of the proposed international standard. As no figures are available for the weights of the contents of the ampoules used for the tests in Table 4, the average figure in Table 5 represents our best estimate of the amount of material in the ampoules actually used in the assays. In both cases the ampoules were selected at random from the whole batch of standard ampoules. The average contents of one ampoule (corresponding to half a millilitre) were 45.95 mg, with a standard error of 0.20 mg, representing about 0.4% of the mean weight. The difference between the highest and the lowest weight was 1.9 mg, which is equal to 4.1% of the mean.

TABLE 5

CONTENTS BY WEIGHT OF TEN AMPOULES [a] OF THE PROPOSED INTERNATIONAL STANDARD FOR ANTISTREPTOLYSIN-O

Ampoule No.	Weight of contents (mg)
1	46.3
2	46.4
3	46.5
4	46.1
5	45.5
6	45.3
7	46.4
8	46.3
9	46.1
10	44.6
Average	45.95
Standard deviation	0.62
Standard error	0.20

[a] Each ampoule represents 0.5 ml of standard serum.

By taking the mean weight of the contents per ampoule from Table 5 and the mean value for the potency in Danish units from Table 4, the unit of the proposed international standard may be defined on a weight basis as follows: $\frac{1}{2} \times 4305$ units = 45.95 mg or 1 unit = 0.0213 mg. Defined in this way, the unit represents a level of activity that is in agreement with the results obtained by the majority of the laboratories participating in the collaborative assay. The unit has, by comparison with the second Danish standard, retained the activity of the unit originally defined by Todd.

The results from the two laboratories that made a direct comparison between the proposed international standard and the standard globulin preparation established by Todd are given overleaf.

Laboratory 11

Experiment	*Ratio of International Unit to Todd unit*	*Contents of ampoule of dried PIS in Todd units*
1	0.985	2119
2	0.909	1954
3	0.971	2087
4	0.917	1972
Mean		2033

Laboratory 12

Experiment	*Contents of ampoule of dried PIS in Todd units*
1	2450
2	2500
3	2550
4	2400
5	2350
Mean	2450
Standard deviation	70

The mean of the results from these two laboratories (2242 Todd units) is within 5% of the value (2150 International Units) assigned to the proposed international standard.

In order not to depart from present practice, the International Standard for Antistreptolysin-O will be dispensed in the fluid state, in ampoules containing 10 International Units per ml. For special purposes, however, ampoules of the freeze-dried standard containing 2150 International Units ± 65 International Units (95% limits) will be available.

Annex

LABORATORIES PARTICIPATING IN THE COLLABORATIVE ASSAY OF THE PROPOSED INTERNATIONAL STANDARD FOR ANTISTREPTOLYSIN-O

ARGENTINA

Professor Dr Ernesto Grichener
Facultad de Ciencias Médicas de la Universidad Nacional del Litoral
Rosario

DENMARK

Streptococcus Department
Statens Seruminstitut
Copenhagen

FRANCE

Dr R. Caravano
Rheumatic Fever Research Laboratory
International Children's Centre
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GERMAN DEMOCRATIC REPUBLIC

Dr W. Köhler
Hygienisches Institut der Universität Rostock
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HUNGARY

Dr V. Balazs
I. Belgyógyászati Klinika
Szeged

ITALY

Professor Domenico d'Antona
Istituto Sieroterapico e Vaccinogeno Toscano " Sclavo "
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JAPAN

Dr Hideo Kusama
Department of Bacteriology
National Institute of Health
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NETHERLANDS

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RÉSUMÉ

A la suite de la recommandation formulée en 1957 par le Comité OMS des Maladies rhumatismales, qui avait souligné l'importance de la détermination du taux des antistreptolysines dans le diagnostic du rhumatisme articulaire aigu et la nécessité d'établir un étalon international, le Laboratoire international pour les Etalons biologiques, au Statens Seruminstitut de Copenhague a été chargé de prendre les mesures nécessaires.

Les auteurs décrivent les conditions techniques et l'analyse statistique des résultats de l'essai collectif auquel ont procédé 12 laboratoires de 11 pays, portant sur un mélange de sérums lyophilisés très actifs, qui provenaient de trois malades atteints d'infections à streptocoques. D'après les résultats, dont la concordance a été très satisfaisante, l'Unité Internationale d'Antistreptolysine-O a été définie comme correspondant à l'activité de 0,0213 mg de l'Etalon International. Cet étalon est distribué aux laboratoires qui en font la demande sous forme liquide, en ampoules de 10 UI/ml. Dans des cas particuliers, il peut être obtenu sous forme lyophilisée, en ampoules contenant 2150 $\pm$ 65 UI.

REFERENCES

Hodge, B. E. & Swift, H. F. (1933) *J. exp. Med.*, **58**, 277

Ipsen, J. (1944) *Acta path. microbiol. scand.*, **21**, 203

Kalbak, K. (1942) [*Experimental and clinical investigations on O-streptolysin and the occurrence of O-antistreptolysin in serum*], Copenhagen, Ejnar Munksgaard

Kusama, H., Ohashi, M., Shimazaki, H. & Fukumi, H. (1958), *Jap. J. med. Sci. Biol.*, **11**, 359

Todd, E. W. (1932a) *Brit. J. exp. Path.*, **13**, 248

Todd, E. W. (1932b) *J. exp. Med.*, **55**, 267

World Health Organization, Expert Committee on Biological Standardization (1957) *Wld Hlth Org. techn. Rep. Ser.*, **127**, 9

World Health Organization, Expert Committee on Rheumatic Diseases (1957) *Wld Hlth Org. techn. Rep. Ser.*, **126**, 20