The standardisation of tuberculin / by A.J. Eagleton.

THE STANDARDISATION OF TUBERCULIN

BY

A. J. EAGLETON, M.D.Lond.

(Reprinted from "The Lancet," 1921, I., 429)

From

THE WELLCOME PHYSIOLOGICAL RESEARCH LABORATORIES

Henry S. Wellcome—British, formerly USA

BROCKWELL HALL

HERNE HILL

LONDON, S.E. 24

THE STANDARDISATION OF TUBERCULIN

BY

A. J. EAGLETON, M.D. LOND.

(From the Wellcome Physiological Research Laboratories)

Reprinted from THE LANCET, 1921, I., 429

THE STANDARDISATION OF TUBERCULIN.

TUBERCULIN has at present three chief uses: (1) the testing of cattle for tuberculosis; (2) the diagnosis of tuberculosis in man; and (3) the treatment of tuberculosis in man. Tuberculin prepared in the usual way has often been found inefficient for (1) and (2), and inactive preparations have doubtless been used in treatment. The standardisation of such a drug is then of great importance; the object of this communication is to show the degree of accuracy with which it may be done.

The various methods of standardising tuberculins are based on Koch's original discovery that tuberculous animals show a characteristic reaction when injected with doses of tuberculin which are quite innocuous to the normal animal. The official Frankfort[1 2] test is essentially identical with that of Koch; a similar test is used in Vienna. The Bureau of Animal Industry, Washington, U.S.A.,[3] employs a slight modification of the original method. There is at present no official test in Great Britain.

In this paper is described the test as applied in this laboratory, with the difficulties presented and the degree of accuracy permitted by its use. The subcutaneous test, which is a modification of the Frankfort test, will first be considered. The improvements introduced in the intradermic and von Pirquet tests will then be reviewed. Finally, a résumé will be given of all tests carried out with the tuberculin which we employ as our standard.

Preliminary sensitisation.—A batch of guinea-pigs of approximately equal weight, and from 50 to 70 in number, is injected intramuscularly with a saline suspension of tubercle bacilli (1 mg. per c.cm.). Half a cubic centimetre is injected into the anterior and inner aspect of the right thigh, deep to the adductor muscles. The right inguinal region of the injected guinea-pigs is examined periodically for enlargement of the lymphatic glands; any animals that may die from extraneous causes are carefully searched for signs of infection. After

about 21–30 days glandular enlargement will be appreciable; we can then proceed to the preliminary test, which is as follows.

1. *Subcutaneous Test.*

Into one animal of the batch 0·5 c.cm. of a tuberculin which has remained constant in value for a long period and is used as a "standard" is injected under the skin of the abdomen. If death occurs within 24 hours with typical lesions, then the main test can be undertaken. Should the animal survive, or should the post-mortem findings be atypical, then a further preliminary test must be made at a later date, and so on, until a typical death is obtained.

The main test consists in the comparison of a sample with the standard tuberculin. Doses of 0·5 c.cm., 0·25 c.cm., 0·1 c.cm., 0·05 c.cm. of each tuberculin are injected, and the lowest dose found that will produce a fatal result in 24 hours with typical post-mortem signs. At least two animals must be injected with the "minimal lethal dose." The following scheme will explain the procedure:—

Batch X., injected 0·5 mg. standard strain tubercle bacilli.

Preliminary Test.

25 days later	...	0·5 c.cm.	Standard tuberculin	...	S.
7 ,, ,,	...	0·5 c.cm.	,,	,,	...	D. 2 days.
7 ,, ,,	...	0·5 c.cm.	,,	,,	...	D.o.n.

Main Test.

C.cm.	Standard.	Sample A.	Sample B.	C.cm.	Standard.	Sample A.	Sample B.
0·5	D.o.n.	D.o.n.	S.	0·05	S.	S.	—
0·25	D.o.n.	D.o.n.	S.	0·05	S.	S.	—
0·1	D.o.n.	D. 48 hs.	S.	2·0	—	—	S.
0·1	D.o.n.	S.	—	1·0	—	—	S.
0·1	D.o.n.	S.*	—				

S. = survived; D. = death; o.n. = overnight. *Seedy.

Sample A is just under standard strength, while sample B is useless. The minimal lethal dose of the standard varies with the batch of animals, as will be shown later; the samples can only be appraised by comparison with the standard on the same batch of animals. In other words, no absolute value can be given to any tuberculin as the reagent employed to test it is a variable. As much as 5 c.cm. of the standard has been injected into non-tuberculous guinea-pigs without giving rise to any result except the immediate irritation, due to the glycerine content of the inoculum.

The main difficulty in connexion with this test lies in the fact that different batches of animals vary in the time which they take to become sensitive to the tuberculin. It will be seen later that the individual members of a batch vary so little in sensitivity that it is rare to find any aberrant deaths or survivals when testing serial dilutions of tuberculins. The different batches, on the other hand, show variation,

both in the length of time taken to become sensitive and in the degree of sensitivity attained, as judged by the minimal lethal dose of standard tuberculin.

The earliest period at which any batch is sensitive can only be found by a preliminary test as described above, and in some batches as many as five such tests have been negative. Again, if 0·5 c.cm. of standard kills one or two animals of a batch we have to inject lower doses to find the degree of sensitivity present; in this way many animals are wasted. In order to obviate this difficulty we tried both the von Pirquet and intracutaneous tests parallel with the subcutaneous test. As a result of many experiments we found a correlation between the three methods, as shown in the table (vide infra).

2. *The von Pirquet Test.*

Von Pirquet himself studied the cutaneous reaction of tuberculous guinea-pigs to tuberculin; he states that these animals are not very sensitive to the drug, and that a deeper scarification is necessary than that used for the test in human beings. Our experiments confirm this, but we found that when deep scarification was used the resulting trauma sometimes made the reactions difficult to read. We changed our method of scarification from two intersecting parallel lines to two and finally three parallel scratches ///, and the "three-scratch" test is the one we now employ. With this method the trauma is not sufficient to obscure the reaction, and well-graduated readings can be obtained with different dilutions of tuberculin on the same animal. Before the tests, a white area of the animal is deprived of hair by clipping and the application of a calcium sulphide paste, after which the area is washed with warm water and the animal thoroughly dried. The groups of three scratches are spaced out as far as possible. Different dilutions of the tuberculins are applied to the areas, and glycerine broth, ten times concentrated as in the preparation of the tuberculin, is used as a control. The reactions can be read in 24 hours, and have generally diminished by 48 hours.

3. *Intracutaneous Reaction.*

The intracutaneous reaction was first described in 1905 by Mantoux, Moussu, and Römer, and adopted later by Loewenstein, but is not used in any official laboratory.

The test consists in the injection of 0·1 c.cm. of diluted tuberculin into the skin of the shaven area. As in the von Pirquet test, we have always used a control of broth, but in this case diluted 1/10. The reactions cannot be read

in 24 hours, taking three days to reach their maximum development. The control injection gives a transitory reaction, but this fades by the second or third day, while the different dilutions of tuberculin give rise to lesions of varying intensity. In the most marked, a central area of necrosis is surrounded by a bright red halo, the whole mounted on an œdematous disc with a sharply defined border. The slighter degrees are marked by diminishing necrosis until the end-point consists merely of a small raised disc; often, however, there is a sudden transition from a lesion showing necrosis to a completely negative reaction.

The von Pirquet and intracutaneous tests can be used for the standardisation of tuberculins, but hitherto we have always checked the results so obtained by the subcutaneous method. The skin reactions are, however, indispensable in experimental work, where we have a large number of samples made in different ways, where the available material is small, or where we must test for small traces of active principle. It should be pointed out that in all cases where the skin tests are used alone, a control and the standard must be included on the same animal as the samples under experiment. Slight individual variations occur in the members of a batch. These are due partly to the differences in the skin trauma, and partly to the fact that the skin reactions, being more delicate, detect variations which do not affect the subcutaneous test.

The value of the von Pirquet test as a guide to the sensitivity of a batch, and the comparison of the skin reactions with the subcutaneous test, are shown in the accompanying table.

4. *Constancy of Test.*

Since August, 1919, when our present standard was adopted, 13 different batches of animals have been infected with a constant dose of the same culture. Of these animals 91 have received doses of standard ranging from 1·0 c.cm. to 0·025 c.cm. In only two cases were irregular results produced with animals of the same batch—that is, a low dose killed, while a higher dose was ineffective. The individual variation of members of the same batch gives an error then of just over 2 per cent.

The constancy of the test on all batches is shown in the diagram. It represents animals injected with tuberculin at least 30 days after infection. The tops of the black columns show the percentage of deaths at each dose. The tops of the shaded columns show the increased percentage of deaths since we have employed the von Pirquet test to indicate the dosage for subcutaneous inoculation.

The following factors must be controlled if accurate results are to be obtained: (1) the same strain of tubercle bacillus must be used for injecting the animals; (2) the guinea-pigs should be about the same size; (3) all animals to be given injections of tuberculin

Batch XV.

19/6/20.—0·5 mg. *B. tuberculosis* H12, injected intramuscularly into batch of animals.

8/7/20.—Von Pirquet test: standard tuberculin undiluted +, control −; 1/10 (+).

12/7/20.—Same animal as above, standard 0·5 c.cm., subcut. D.o.n.

15/7/20.—Von Pirquet:

	Tuberculin.		Broth.
	Standard.	Sample A.	Control.
Undiluted	++	+	−
1/5	(+)	−	
1/10	−	−	

23/7/20.—Von Pirquet:

	Standard.	Sample A.	Control.
Neat	++	+	−
1/5	++	(+)	
1/10	+	−	
1/20	?	−	

Intracutaneous (72 hours reading).

	Standard.	Sample A.	Control.
1/5	.....	++	−
1/10	.....	++	
1/15	.....	++	
1/20	.....	−	
1/50	++	.....	

24/7/20.—Subcutaneous:

Standard 0·25 c.cm. D.o.n. Sample A 0·5 c.cm. D.o.n.
0·25 c.cm. S.

3/8/20.—Subcutaneous:

Standard 0·1 c.cm. D.o.n. Sample A 0·5 c.cm. D.o.n.

4/8/20.—Standard 0·05 c.cm. D.o.n. Sample A 0·5 c.cm. D.o.n.
0·05 c.cm. S.; seedy. 0·25 c.cm. S.

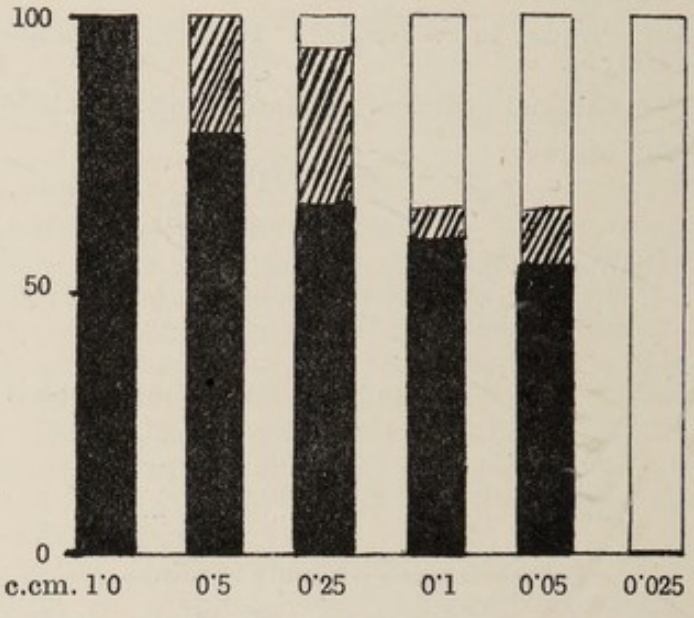
++ = Very strong. + = Strong. (+) = Slight. − = Negative. } Von Pirquet reaction.

should be examined and any sick ones discarded—no animal should be used unless the inguinal glands are palpable; (4) the von Pirquet test should be used to guide the dosage for subcutaneous injection.

The results show that tuberculins can be tested with a reasonable degree of constancy. They also show the value of the von Pirquet test as an adjunct to the sub-

cutaneous test. It may, I think, also be claimed that the standard in use in this laboratory has been thoroughly tested and is suitable for this reaction. We have tested tuberculins made in 1889 and since that date, and many of the earliest samples are still potent. We are, however, at work on the exact

Diagram showing Dose in Cubic Centimetres.



Dark columns show percentage of deaths at each dosage. Shaded columns show percentage improvement after adoption of von Pirquet reaction.

standardisation of a purified, dried tuberculin, which may, if satisfactory, be used as a standard in future.

In conclusion, I have to thank Dr. Stanley Griffiths for his kindness in sending us our standard culture, and all my colleagues for criticism, suggestions, and help. Above all, I am indebted to Dr. R. A. O'Brien, who initiated this work and placed at my disposal his knowledge of the test derived from long experience.

References.—1. Loewenstein, E.: "Anwendung des Tuberkulins beim Menschen," Handbuch der Pathogenen Micro-organismen, Kolle and Wassermann. 2. Otto, R.: "Prüfungstechnische Erfahrung bei der Wertbestimmung des Tuberkulins," Klin. Jarhb., Bd. xiii., H. 1, 1904. 3. Schroeder and Brett: Journal of the American Veterinary Medical Assoc., 1919, vol. liv., p. 357. "The Methods of the Bureau of Animal Industry for Testing the Potency of Tuberculins."

Printed at The Lancet Office,
423 Strand, London, W.C. 2.