THE USE OF RICHARDSON'S MODIFICATION OF THE MINISTRY OF HEALTH'S STANDARD WASSERMANN REACTION (HARRISON-WYLER) AS A VERIFICATION TEST

BY

A. C. T. VAUGHAN

From the R.A.F. Institute of Pathology and Tropical Medicine, Halton

The "ideal" serological test for syphilis, giving maximal sensitivity with absolute specificity, has still to be evolved; indeed, owing to the widespread distribution of "Wassermann type substance" (Weil, 1941) it is doubtful whether the second requirement can ever be attained; the malarial patient, for instance, is long likely to give at least a short-lived reaction with the various serological tests for syphilis (Burney and others, 1942).

Normally there is an inverse relation between sensitivity and specificity, and in order to preserve the latter at a workable level sensitivity has been deliberately curtailed. The Ministry of Health's standard Wassermann Reaction (Wyer, 1929), which gave good results in the International Conferences held at Copenhagen in 1923 and 1928, is, according to present day standards, relatively insensitive; but its chief fault lies in the rather large number of doubtful reactions which are given with both syphilitic and non-syphilitic sera. Subsequently it was shown that by the use of larger amounts of patient's serum the sensitivity of the test could be increased some 20 per cent. without any reduction in specificity (Wyer, 1932; confirmed by Fairbrother, 1933). Recently Richardson (1940) introduced a further modification of the standard technique which he claimed not only enhanced specific reactions but at the same time weakened the non-specific, thereby increasing both the sensitivity and specificity of the test.

For every serological system there is a definite ratio, the optimal ratio, of antigen-antibody concentration at which aggregation occurs most rapidly (Marrack, 1938). Marked departure of either reactant from this ratio may lead to inhibition of the reaction, the so called zone phenomenon. This is well shown in the precipitin reaction in which visible particulation is suppressed by large excess of antigen.

The optimal ratio for precipitation does not coincide with that for maximal complement fixation. The former occurs in the zone of slight antigen excess, whereas the latter is favoured by the presence of slight antibody excess (Dean, 1912). Complement is fixed during the early stages of particulation; therefore fixation is most rapid at the optimal ratio for the system, and becomes slower the greater the distance from this point in either direction (Goldsworthy, 1928).

In the standard Wassermann, using alcoholic tissue extract diluted 1/15 for "antigen," with syphilitic sera of minimal reactivity the reaction takes place deep in the "post zone" of antigen excess, where particulation is slowed if not suppressed, and hence the fixation of complement is delayed or may not take place in detectable amounts during the period allowed for fixation to occur. This state of affairs leads to weak or false negative results being registered with such sera. On the other hand the comparable "pro-zone" due to antibody

excess is not appreciable even with highly reactive sera (Wadsworth and others, 1938).

For this reason many workers (Eagle, 1936; Boerner and Lukens, 1937; Kolmer, 1937) have recommended that the optimum dilution, that is, that which detects the smallest amount of "antibody," should be found by experiment for each batch of antigen as prepared. Richardson (1940) explored "the possibilities of using stepped combining proportions of serum and antigen with constant complement." He advocates what amounts to the setting up of an additional tube in the Ministry of Health's test, in which the conditions of the reaction are so adjusted, by doubling the volume of patient's serum (thus following Wyler's suggestions (Wyler, 1932) for increasing sensitivity) and by the use of a greater dilution of antigen, that something approaching maximal fixation of complement by sera of low reactivity takes place in or near the zone of equivalence; at the same time non-specific fixations due to "impurities" in the antigen are diluted out.

In this paper an account is given of the results obtained by the use of Richardson's modification in the examination of those sera which gave weak or doubtful reactions with the standard technique, out of 3,826 unselected (but not consecutive owing to gaps caused by leave etc.) sera sent for examination to this Institute during five months in the first half of 1944. For the majority of sera the result of the standard Kahn Test (Kahn, 1928) was also available for comparison. Richardson's claim that by his modification the Bordet-Wassermann, as presented by Harrison and Wyler (Wyler, 1929) has been rendered considerably more sensitive without significant loss in specificity, appears to be fully substantiated.

The modified test deserves widespread adoption in this country wherever the Harrison-Wyler technique is in use, for great advantage can be obtained at little extra cost or trouble. The results also furnish additional circumstantial evidence (if this be still needed) of the essentially antigen-antibody-like reaction which takes place between lipid tissue extract and the reacting substances in syphilitic sera (Eagle, 1937).

Procedures

The Harrison-Wyler is the standard Wassermann technique in use at the R.A.F. Institute of Path-

ology and Tropical Medicine. The tests were carried out on two mornings a week. All sera which gave weak or otherwise doubtful reactions were retested in the afternoon by the modified technique. For the standard test the directions contained in the Medical Research Council's Special Report No. 129 were closely followed. Such minor differences as existed will be noted. Donald's nozzles for dropping were used for adding the reagents, and were found to be both speedy and accurate; graduated pipettes were, however, preferred for the complement titration.

Patient's serum.—The sera received for examination came from various R.A.F. Hospitals and sick quarters. Strongly lysed or heavily infected sera were discarded. Sera waiting to be tested or retested were kept at 4° C. Inactivation at 55° C. for half an hour was carried out the day before the test: the Kahn test, when performed, was done on the afternoon before the Wassermann test.

Complement.—Pooled lyophilized complement (from a commercial source) was used. A small quantity of salted complement (Richardson, 1941) was also tried. Both were found to be very satisfactory and kept well in sealed tubes in the cold. Haemolytic activity was always titrated on each morning of the test. While in use it was kept on the bench in a stoppered tube immersed in iced water.

Antigen.—The alcoholic extract of beef heart was obtained from the Ministry of Health Laboratory. The diluted antigen for the standard test was prepared by adding quickly 14 volumes of saline to 1 volume of the cholesterolized (0.4 per cent.) heart extract; after thorough mixing it was left to "ripen" for at least half an hour before use.

Haemolytic system.—A fresh supply of sheep cells was obtained from a commercial source each week. After washing, a 3 per cent. suspension by volume was sensitized with 6 M.H.D. of anti-sheep rabbit haemolysin.

Complement titration.—In order to obtain prompt lysis in the test proper, the minimal haemolytic dose was measured by observing the highest dilution of complement which showed complete haemolysis.

Standard Wassermann (Wyler, 1929).—Known positive and negative sera were included in each batch, as well as the usual reagent controls. The 2 M.H.D. serum control was omitted, as it was found that by using it many sera were classified as anticomplementary which gave readable results with a 3 M.H.D. serum control. A similar conclusion was reached by Webb (1936) when using the Kolmer 2-tube complement fixation test.

Reading.—As soon as the 3 M.H.D. serum controls had lysed, usually after 7 to 10 minutes' incubation in the 37° C. water bath, the degree of lysis (or its complete inhibition) was noted in the test rows. As the average batch of sera under test was too large to allow of their all being read at this precise moment, the practice was adopted of leaving them in the water bath for half an hour, when a final reading was made after they had all been removed. There is some theoretical justification for allowing the complement to act on the sensitized cells for the same length of time as used in its titration (Eagle, 1937).

Positive reactions after 10 and 30 minutes of incubation were graded as follows:

Strongly positive (+ +): complete inhibition of lysis in both 3 and 5 M.H.D. tubes.

Positive: some degree of lysis in the 5 M.H.D. tube but complete inhibition in the 3 M.H.D. tube.

Weakly positive: complete lysis in the 5 M.H.D. tube and some degree of lysis (up to approximately 50 per cent.) in the 3 M.H.D. tube.

In addition fleeting reactions ($\pm$) at 10 minutes were noted.

All sera which gave the following results in the standard test were re-examined the same afternoon by Richardson's modified test:

1. After 30 minutes' incubation: weak reaction.
2. After 10 minutes' incubation: (a) positive (these usually became "weak" or negative after 30 minutes); (b) weak reaction (usually negative at 30 minutes); (c) fleeting ($\pm$) reaction.
3. Any result which did not agree with the Kahn.
4. Any result which seems to differ from that expected by the clinical history.
5. Any serum which had given variable results in previous tests.

The Modified Test

The procedure of the modified test is summarized in Table I; for full details the original paper must be consulted (Richardson, 1940).

It will be seen that Row 1 constitutes the modified test. Row 2 is just the 3 M.H.D. tube of the standard test over again, and serves both as a reading comparator and as a check on any change that may have taken place in the reagents since their use in the morning. Row 3 is the serum control and therefore contains the increased volume of serum.

Complement containing 3 M.H.D. was used throughout. After the addition of the sensitized cells a careful watch was kept on the behaviour of the sera under test. This was now possible because in the morning the standard test had eliminated all sera giving unequivocal results: it was rare that more than a dozen sera needed re-examination.

Reading.—If the fixation recorded in the standard test had been caused by "impurities" in the antigen, then the tube in the first row showed more rapid lysis than the middle tube and the result was read as negative.

In specific reactions the reverse sequence of events occurred; compared with the middle tube, lysis in the front tube (in Row 1) occurred more slowly, if at all. The classification of difficult sera with a low degree of reactivity was greatly facilitated by this direct comparison of the modified test

TABLE I
THE MODIFIED TEST

Row	Serum	Saline	Complement containing 3 M.H.D. per unit vol.	Lipoid emulsion diluted		Sensitized R.B.C. ²
				1/15	1/120 ¹	
	vol.	vol.	vol.	vol.	vol.	vol.
3	2/5	1+3/5	1	—	—	1
2	1/5	1	1	1	—	1
1	2/5	3/5	1	—	1	1

1 vol. = 0.11 ml. = 2 drops from No. 30 Starrett gauge dropper { saline complement sensitized cells 1/120 antigen emulsion

= 2 drops from No. 18 Starrett gauge dropper { 1/15 antigen emulsion

1/5 vol. = 0.022 ml. = 1 drop from No. 56 Starrett gauge dropper { Saline serum

¹ The Ministry of Health antigen at this dilution (1/120) gave "complete absence of lysis with strongly reacting syphilitic sera and something near the optimum fixation with weakly reacting syphilitic sera" and at the same time weakened non-specific fixation. Antigens with a constant (e.g. 0.4 per cent.) cholesterol content optimally sensitized with lipid are of comparable "strength" (Kent, 1942). The 1/120 dilution was prepared by taking the 1/15 antigen used in the morning and further diluting it eight times with saline. According to Richardson (1940) "since the dilution is always carried out on a pre-formed lipoidal emulsion (*itself constant*) the exact change of haptene strength is reproducible from day to day."

² Magnesium ion was not added to the sensitized cells as it did not seem to have any appreciable effect on the rate of hæmolysis.

with the standard (both tubes containing complement of identical hæmolytic activity and subject to the same conditions of incubation).

The results were recorded as positive (i.e. as "diagnostic" of syphilis) if at the end of half an hour of incubation the front tube showed complete inhibition (or only slight occurrence) of hæmolysis. The middle tube was usually more hæmolysed, sometimes completely so.

In a weak, but nevertheless significant reaction (see later), lysis in the front tube was partial or nearly complete at 30 minutes but always occurred much more slowly than in the middle tube.

Results.—During 40 sessions 3,826 sera were examined, an average of 95.6 sera per session. This is rather more than the optimal number, but a very competent technician carried out the tests with assistance, and the only difficulty experienced was in reading the weaker reactions as their serum controls cleared. The standard test was always completed by midday. Ten sera were found to be anticomplementary and are excluded from further discussion.

The evaluation of serological tests based on a comparison of the results obtained by the examination of a series of unselected sera is rendered difficult by the absence of any practicable objective standards, save in the early primary stage, on which to base a diagnosis of syphilis (Wadsworth and Brown, 1936; Gilbert and Maltaner, 1945). An attempt, however, was made to classify on clinical grounds all the sera examined as either

syphilitic or non-syphilitic. The results, being thus based on data open to subjective error, have no absolute value and cannot legitimately (except as an indication of broad trends) be compared with those of other workers (see Table II).

The results obtained by the examination of these sera by the various ways described above are summarized in Table III.

It must be emphasized that neither sera which after 30 mins. secondary incubation in the standard technique were positive or strongly positive, nor those which lysed at the same rate as their serum controls (that is, clear-cut negatives) were retested by the modified method if the results were compatible with the clinical history. But it is not considered that even if the composite test had been carried out on all the sera, any marked change in the above figures would have resulted. The reason for not making the full comparison was to prevent overburdening an already hard-worked staff.

Table III shows (as far as the small numbers will allow) that the increased sensitivity of Richardson's modification test is not obtained at the price of specificity. In order to bring out this enhancement of sensitivity more sharply, the results with lowly reactive sera from known syphilitics are set out in Table IV. The majority of the patients had received varying amounts of treatment.

From these results (Tables III and IV) the following deductions may be made.

1. The modified test is much more sensitive than the standard test and with little loss of specificity. If the number of patients, rather than specimens of sera, giving false positive reactions be considered, the specificity of the Richardson modification is considerably improved: 0.09 per cent. (3 patients) for positive results and 0.12 per cent. (4 patients) if weakly positive results are included (see Table III).

2. Partial fixations in the modified test thus have a specificity denied to those obtained with the parent technique.

3. Results obtained are comparable with the standard Kahn test. The Kahn appears to be rather more sensitive in early untreated primary syphilis, but this is not an important advantage because the diagnosis may usually be more satisfactorily made in these cases by the demonstration of *Treponema pallidum* in the primary lesion. In older untreated

TABLE II
CLINICAL CLASSIFICATION OF SERA

Number of sera giving positive (that is, "weakly positive" and stronger) reactions with either complement fixation or Kahn flocculation test	503
Number of sera giving negative reactions ..	3,313
Total	3,816
False positive sera (from history and clinical examination)	18
False negative sera (<i>Treponema pallidum</i> seen in primary lesion)	8
Number of presumed syphilitic sera (503-18+8)	493
Number of presumed non-syphilitic sera (3313+18-8)	3,323
Total	3,816

¹ 470 of these were also examined by the standard Kahn test in addition to the complement fixation reaction.

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TABLE III

COMPARISON OF THE SENSITIVITY AND SPECIFICITY OBTAINED FOR THE WASSERMANN (STANDARD AND MODIFIED) AND THE STANDARD KAHN

Test	Standard Wassermann Reaction				Standard + modified Wassermann		Kahn
Duration of secondary incubation	30 mins.		10 mins.		see text		
Strength of reaction	++, +	++, +, ±	++, +	+++, +, ±	+	+, ±	+++ ++ +
Sensitivity	55% (271)	58.4% (288)	62.7% (309)	73% (360)	83.6% (412)	91% (451)	93.4% (439)
Specificity	0.12% (4)	0.18% (6)	0.18% (6)	0.54% (18)	0.15% (5)	0.21% (7)	0.21% (7)

Figures in parenthesis = number of sera out of the total examined (493 for complement fixation and 470 for Kahn) giving the reactions indicated.

Strength of reaction = as defined above p. 79.

Sensitivity = percentage of the 493 syphilitic sera which gave positive reactions with the technique indicated (470 for the Kahn).

Specificity = percentage of the 3,323 non-syphilitic sera which gave (false) positive reactions with the technique indicated. Not all the 3,323 Wassermann-negative sera were tested by the Kahn method, so that the percentage for the specificity given for the Kahn is a minimum value.

TABLE IV

SYPHILITIC SERA GIVING NEGATIVE OR WEAK REACTIONS IN THE STANDARD TEST BUT WEAKLY POSITIVE OR STRONGER WITH THE MODIFIED TEST

	HW 30	HW 10	HR	Kahn
A. <i>Congenital syphilis</i> Young adults; treated ..	1/17 (5.9%)	9/17 (47%)	17/17 (100%)	13/16 (81%)
B. <i>Acquired syphilis</i> (i) Untreated primary (a) <i>Treponema pallidum</i> seen	1/23 (4.3%)	5/23 (21.7%)	7/21 (33.3%)	12/23 (52.2%)
(b) Sero positive but <i>Treponema pallidum</i> not seen	2/12 (16.6%)	8/12 (66.6%)	11/12 (91.6%)	11/12 (91.6%)
Total; untreated primary syphilis (a and b)	3/35 (8.6%)	13/35 (37%)	18/33 (54.5%)	23/35 (65.7%)
(ii) Treated primary and secondary syphilis	15/188 (8.0%)	153/187 (81.8%)	153/187 (81.8%)	139/166 (83.7%)

HW 30 = Harrison-Wyler (i.e. Ministry of Health's standard technique) read after 30 minutes' secondary incubation.

HW 10 = Harrison-Wyler read when 3 M.H.D. serum control had completely lysed (this was usually after about 10 minutes' secondary incubation).

H.R. = Richardson's modification of the Harrison-Wyler.

Kahn = Results of Kahn test on (most of the) same sera for comparison.

The results are expressed as a fraction; the numerator represents the number of sera giving a serologically diagnostic result (weakly positive or stronger in a known syphilitic) and the denominator the total number of sera examined by the particular technique; the percentage positives are enclosed in brackets.

primary syphilis both tests are apparently of equal sensitivity, but the numbers concerned are too small for definite conclusions to be made.

4. The standard test, if read after 30 minutes' secondary incubation, is only moderately sensitive but has a satisfactory degree of specificity.

5. The standard test, read (as directed by Wyler, 1929) as soon as the serum controls clear, has a better sensitivity, but the percentage of false positives among the partial fixations is considerably increased so that only weak results in known syphilitics may be considered significant (see Table IV).

Discussion

The modern tendency in routine work is to supplant the Wassermann reaction by a more easily performed flocculation test, and it is claimed that the advantages gained from doing two tests do not compensate for the extra work involved, particularly when some of the methods for performing the Wassermann in current use give inferior results (Kahn, 1940). This is probably true for small remote laboratories, but in large central reference laboratories it is desirable that results should be cross checked by the use of two tests. And if, as Weil (1941) points out, a complement fixation test be combined with a flocculation test, an increased number of positive results are obtained owing to the somewhat different ranges of sensitivity possessed by the two different types of reaction. Thus, of the syphilitic sera with low reactivity which are considered in Table IV, 12 per cent. were Richardson-positive but Kahn-negative; in 16 per cent. the results were reversed (see Table V).

These divergent results are probably significant, since they were obtained in known cases of syphilis undergoing treatment. Webb (1936) came to the same conclusion. Berger and Sutherland (1944) obtained higher percentages for discordant results when testing a larger number of sera with the Standard Kahn and the Harrison-Wyler Wassermann method modified by the use of an optimum antigen dose. The relatively fewer discordant results in the present series may follow the use of pooled complement, thereby eliminating

day-by-day variation in the deviability of complement—an important cause of such discrepancies.

The Wassermann reaction has the great advantage of an easily visible indicator system. The Kahn, probably the most reliable of the various flocculation techniques, suffers from the difficulty of reading the weaker reactions: there is an almost insensible gradation in particle size from the positive through the doubtful to the negative reaction, so that magnification except by low power (as Kahn himself insists) is of no help.

In order to obtain consistent results reading of the Kahn should always be done under standard conditions (such as the use of concealed artificial light against a dark background) which, having been adjusted to suit, should always be kept constant for any one observer. Even then, the Richardson test was found to be of more help in checking weak Kahn reactions than the reverse. The deliberate use by Richardson of the zone phenomenon to differentiate specific from non-specific reactions makes his modification a valuable "verification test," easy to read, and more readily carried out than Kahn's own verification test (Kahn, 1940).

TABLE V

COMPARISON OF PARALLEL WASSERMAN (RICHARDSON MODIFICATION) AND KAHN TESTS ON SYPHILITIC SERA

HR	Kahn	Sera of low reactivity (see Table IV)	Syphilitic sera subjected to both complement fixation and Kahn (see footnote in Table II)
		No.	No.
+, ±	++, +	132 (66.7%)	404 ¹ (86%)
+, ±	—	25 (12.6%)	25 (5.3%)
—	++, +	33 (16.7%)	33 (7%)
—	—	8 ² (4%)	8 ² (1.7%)
Totals ..		198 (100%)	470 (100%)

HR +, Kahn ++ = positive reaction.

HR ±, Kahn + = weakly positive reaction.

¹ includes 272 Kahn positive sera which gave positive or strongly positive readings with the Standard Wassermann.

² These sera although negative to both techniques were obtained from patients in whose primary lesions *Treponema pallidum* was demonstrated.

It must not be forgotten that the antigen is the most important reagent in serological tests for syphilis, and any general improvement in specificity must now await its further fractionation. For instance cephalin, which possesses strong anticomplementary qualities (Wadsworth and others, 1934), may be removed by ethereal extraction with apparent improvement. But the preliminary elimination of ether-soluble substances prior to the alcoholic extraction of normal bovine heart muscle is not carried out in the preparation of the McIntosh and Fildes' antigen used in the Standard Harrison-Wyler Wassermann technique. Richardson (1940) believes that "impurities" play a part in producing a stabilized haptene emulsion, and relies on the effect of dilution to cause weakening or disappearance of any non-specific reactions caused by them. This seems to be justified, since it was found both in the present investigation and by Berger and Sutherland (1944) that the majority of partial fixations are specific when diluted antigens are used. It is interesting to note in this connexion that Hecht (1945) found that previous ethereal extraction of tissue produces an antigen of inferior value. The most effective fraction of dried beef heart appears to be one soluble in acetone and petrol ether. Moreover, he suggests (like Richardson) that use may be made of the "impurities," that is, the petrol ether and acetone insoluble fraction, in verifying non-specific "positives," since they give a stronger reaction with non-syphilitic sera (but weaker with syphilitic) than do the "specific" antigens. It is possible that cultures of non-pathogenic spirochaetes closely related to *Treponema pallidum* may be used to provide an antigen, but experiments in this direction have so far been only partially successful. Non-specific fixations are common; in experimental human *P. vivax* infection Eagle and others (1941) found that spirochaetal antigen derived from the Reiter strain gave more biological (false positive) reactions than the use of lipoid mammalian tissue extract in his own Wassermann and micro-flocculation techniques.

Of the factors tending to depress the greater inherent sensitivity of the complement fixation test, to which Berger and Sutherland refer in their paper (Berger and Sutherland, 1944), that due to variations in the deviability of the complement used (and which may, therefore,

affect whole batches of results) may be eliminated by the use of pooled complement from which unsatisfactory guinea-pig sera have been excluded. The large-scale issue of this pooled complement, either lyophilized or salted, would do much to make the performance of the Wassermann easier and more reliable. For factors present in certain individual sera which tend to lower the sensitivity of the Wassermann, central laboratories could try to eliminate the "complementoid" effect (Ehrlich and Sachs, 1902), which sometimes "antagonizes" the fixation of complement by weakly sensitized antigen, that is, with a lowly reactive sera (Bordet, 1939), by precipitating it out in the carbon dioxide insoluble fraction of heat-inactivated serum (Berger and Sutherland, 1944). The occasional excess of natural antishoop hæmolyisin may be removed by absorption with unsensitized sheep red cells; the use, however, of a well-sensitized (6 M.H.D.) hæmolytic system renders it less liable to be affected by the presence of natural hæmolyisins in the patient's serum.

For the standardization of the cells it is more logical to use a method based on volumes, such as the measurement of a known volume following centrifugation of the thrice-washed cells to a constant volume, than to rely on the hæmoglobin estimation recommended in the Ministry of Health method (Wyler, 1929). Hæmolyisin possibly acts on the monolayer lipoprotein membrane investing the red blood cell (Schulman and Rideal, 1937) and the liberation of hæmoglobin is a passive accompaniment of its destruction.

Finally a word may be said about the method of reporting results. Most of the so-called quantitative methods of performing the Wassermann test give only an approximate estimate of the "antibody" titre; and the same applies, only to a higher degree, to flocculation tests: the amount of precipitate is not a true index of reactivity (Gilbert and Maltaner, 1945), so that, while the modern tendency to ignore roughly quantitative findings (expressed in the plusses beloved of old by serologists) and to report a result as simply "positive" (i.e. diagnostic), "doubtful" (significance depending on clinical assessment), and "negative" must in many ways be considered an improvement, inconsistencies still remain. The performance of a

strictly quantitative test, expressing the result as a figure directly related to the true "antibody" content would be even better. The serologist is concerned with the accurate measurement of antibody titres, (Maltaner, 1940): the clinician with its interpretation (Wadsworth and others, 1938). No positive serological result of the approximately quantitative type can be considered diagnostic on its own merits. Many causes of what are sometimes called "biological" positives are known. Malaria, leprosy, yaws, relapsing fever, vaccinia, infectious mononucleosis, and pregnancy may be cited. But Maltaner (1940, 1941), using a strictly quantitative complement fixation test, was able to establish a close serological relationship between syphilis and yaws, whereas with the same technique sera from lepers was readily distinguished from the sera of syphilitics by its uniformly low titre with a cholesterolized tissue extract as antigen.

Summary

A brief account is given of the employment of Richardson's modification of the standard Harrison-Wyler Wassermann technique as a verification test for those sera which gave doubtful results with the standard test. By this means the sensitivity of the complement fixation reaction was considerably increased without any loss in specificity. This was particularly noticeable in sera from patients undergoing treatment, and is felt to be an important improvement (where serological tests are included among the various "tests of cure") in a disease such as syphilis, where it is doubtful whether clinical cure is ever accompanied by the complete eradication of spirochaetes from the body.

Compared with the standard Kahn, the tests were very similar in specificity and sensitivity, but in the case of lowly reactive sera the Richardson modification was much the easier to read.

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