Serological tests for syphilis in pregnancy False and missed positive reactions

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A leading article in the Lancet (1967) emphasized the continuing need to maintain serological supervision of pregnant women, and that advice still holds good. Wherever and whenever antenatal serological tests for syphilis are carried out routinely, the incidence of congenital syphilis falls and remains low. The incidence of congenital syphilis in the first year of life, as reported in Great Britain, has remained low for about 20 years, but some such cases are still diagnosed annually. Most of these are due to the fecklessness of the mothers concerned, who have not availed themselves of antenatal care, but others occur because of the low index of suspicion of the clinicians in charge of that care.

Since the introduction of specific serological tests, using *Treponema pallidum* as an antigen, biological false positive (BFP) reactions can be detected, and pregnant women in whom they are found can be spared an erroneous diagnosis, with all its social consequences, and unnecessary treatment. According to Wilkinson (1970), those classed as acute BFP reactions are not thought to have any adverse significance apart from the diagnostic problems they pose. Some of the serological tests used in the screening of pregnant women for syphilis have continued in use over many years, such as the various Wassermann reactions and Kahn tests, and this suggests that there is a need for a reappraisal of the tests in current use.

This paper reports on the sensitivity and specificity of the various serological tests for syphilis used in the screening of pregnant women in Glasgow in a 3-year period, 1969–1971.

Material and methods

Since 1964 all the laboratories which carry out screening tests for syphilis on pregnant women in Glasgow have reported their results to the Department of Venereology, so that they could be incorporated in the Annual Report of the Medical Officer of Health. Since 1965 every positive or doubtful result has been confirmed either by the Treponema Pallidum immobilization (TPI) test and/or by

the absorbed fluorescent treponemal antibody (FTA-ABS) test.

The results from five laboratories, which together carry out 93 per cent. of all the antenatal tests done in Glasgow, have been used in this report for the 3-year period 1969–1971. Three laboratories carried out a 'battery' of tests, the Venereal Diseases Research Laboratory (VDRL) slide test, the Reiter protein complement-fixation (RPCF) test, and a Wassermann reaction (WR) either standard or cardiolipin. (In this report, no account has been taken of the type of WR.) During 1969 one laboratory carried out a Kahn test in addition to the others. Two other laboratories used only a single flocculation test in the screening of pregnant women, during 1969 a Kahn test, thereafter the VDRL test.

No note has been taken of the type or stage of syphilis diagnosed in those cases in which the serological diagnosis was confirmed, as it is considered that screening tests should be reactive whether the syphilis is in an early or late stage, treated or untreated, as long as there is serological evidence available.

Results

During the 3-year period, sera from 64,404 pregnant women were examined, 285 (442.5 per 100,000) were reactive but syphilis was confirmed only in 39 (13.7 per cent.) or 60.6 per 100,000.

Table I sets out the results of this antenatal screening, by laboratory. Laboratories A, B, and C carried out the 'battery' of tests, while D and E did only the flocculation tests mentioned. Differences in techniques between the laboratories account for the various rates of reactive sera found, but differences in the population served by the laboratories account for the variations in the rates/100,000 of syphilis found. There are marked social and economic differences between the women attending the hospitals served by Laboratories B and C, and similarly between those served by Laboratories D and E. Laboratory A serves most of the area of the City of Glasgow.

The use of a 'battery' of tests allows comparison not only between the specificity of individual tests but also between their relative sensitivity. The results of each serological test are presented in Table II irrespective of laboratory. The VDRL was the mos sensitive test, missing only three out of 25 cases o

			Reactive sera		Reactive sera confirmed due to syphilis			
Lab	ooratory	No. of sera screened	No.	Rate/100,000	No.	Percentage of reactive sera	Rate/100,000	
A		17,146	67	390.8	9	13		
В		14,211	36	253.3	13	36	91.5	
С		6,095	9	147.7	3	33	49.2	
D	Kahn	6,479	11	169.8	3	27	46.3	
	VDRL	11,855	101	850∙0	8	8	67.5	
E	Kahn	2,962	18	607.7	1	6	33.8	
	VDRL	5,656	43	760.3	2	5	35.4	
Tot	al	64,404	285	442.5	39	13.7	60.6	

TABLE I Results of antenatal serological screening for syphilis, by laboratory

syphilis, but it lacked the specificity of the RPCF test, having 38 BFP reactions compared with only three by the latter. The RPCF tests and the WRs each missed eight cases of syphilis, but the WRs, with 66 BFPs, were the least specific of all. The Kahn test, dropped by the one laboratory which had been using it until 1970, while not providing any BFP reactions, lacked sensitivity and missed two out of five cases of syphilis.

Table III compares the results of the Kahn test and the VDRL test when used as the sole screening test by two laboratories. In 1969, using the Kahn test, the diagnostic rate of syphilis was 42.4 per 100,000. Because they used only one test it is impossible to know how many cases of syphilis they missed, but the

overall diagnostic rate that year was 54·4 per 100,000 so they may have missed 12 per 100,000 (one case among the 9,441 tested). The Kahn test used does not appear to bear any relation in selectivity or sensitivity to that used by another laboratory the same year (see Table II).

During 1970 and 1971 the VDRL test carried out by the two laboratories diagnosed syphilis at a rate of 57·1 per 100,000, a similar rate to the 58·7 per 100,000 when it was done by the three laboratories (see Table II), but the VDRL test of the latter missed 8·0 per 100,000 cases of syphilis and a similar incidence might well have been missed by these two laboratories. This would have amounted to at least one case of syphilis.

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TARLE II	Comparison o	t results of	t ındırıdı.al	tests used in	'hattery' or	t tests hw	three laboratories

		Reactive sera						Nonreactive sera (Missed cases of		
Serological test	No. of sera screened	Total		Confi syphil	rmed due to is	BFPs		syphili syphili		
		No.	Rate/100,000	No.	Rate/100,000	No.	Rate/100,000	No.	Rate/100,000	
VDRL	37,452a	60	159.8	22	58.6	38	101.2	3	8.1	
RPCFT	37,452	20	53.3	17	45.3	3	8.0	8	21.3	
WRs	37,452	83	221.1	17	45.3	66	175.8	8	21.3	
KAHN	4,808b	3	62.4	3	62.4	0	0	2	41.6	

^{*25} of the 37,452 sera were from patients with confirmed syphilis $(66\cdot7/100,000)$ b5 of the 4,808 sera were from patients with confirmed syphilis $(104\cdot0/100,000)$

TABLE III Comparison of results of Kahn and VDRL tests used alone

		Reactive	e sera				
Serological test	No. of sera screened	Total		Confirm	ned due to syphilis	BFPs	
		No.	Rate/100,000	No.	Rate/100,000	No.	Rate/100,000
Kahn VDRL	9,441 17,511	29 144	307·2 822·3	4 10	42·4 57·1	25 134	264·8 765·2
Total	26,952	173	641.8	14	51.9	159	589.9

The 'battery' of tests revealed reactive sera from 112 pregnant women (299 per 100,000). Table IV shows which of the tests, or combination of tests, were reactive in the 25 later confirmed to have syphilis and the 87 found to have BFP reactions. All three tests were reactive in 12 out of the 25 cases of syphilis, but were also positive in one further case where the results were confirmed to be a BFP reaction by repeated negative results of the TPI and FTA-ABS tests. Whenever the RPCF test was one of the two tests reported reactive, no BFP reaction was found, but these were common when the two reagin tests were reactive. When single tests only were reported reactive the VDRL was more sensitive, but less specific than the RPCF, although in two cases positive reactions of the latter were not confirmed. Whenever the WRs alone were reported reactive, it was always because of a BFP reaction. Lack of sensitivity of the combinations of tests, save that of the VDRL with the RPCF, and of each individual test is shown by the number of negative reactions in the presence of syphilis.

TABLE IV Results of 'battery' of tests on routine sera from 112 pregnant women

Serological tests	Reactions of tests							Total
VDRL RPCF WRs	+	+	+ + +	+		+		
Results confirmed due to syphilis	12	2	4	1	4	2	0	25
Biological false positive results	1	0	18	0	19	2	47	87

The rating of individual tests is set out in Table V, taking as the prime criterion of a screening test its sensitivity. Thus, the VDRL, with a sensitivity of 88 per cent., was the best test. The difference between the RPCF test and the WRs, equal in regard to sensitivity, lay in the better specificity of the former which was rated second. The WRs, which were even less specific than the VDRL, were rated third.

TABLE V Rating by sensitivity and specificity of individual screening tests

Rating	Test	Missed cases of syphilis	Sensitivity (per cent.)	BFPs	Specificity (per cent.)
1	VDRL	3	88	38	37
2	RPCF	8	68	3	85
3	WRs	8	68	66	20

Ideally, screening tests should not miss any case of syphilis, no matter how weak the reagin or antibody

production, and irrespective of whether the patient has received treatment or not. When the tests were paired (Table VI) and either or both tests were reactive, then the requirement of 100 per cent.

TABLE VI Rating by sensitivity and specificity of pairs of screening tests

Rating	Pairs of tests	Missed cases of syphilis	Sensitivity (per cent.)	BFPs	Specificity (per cent.)
1	VDRL+RPCF	0	100	40	38·5
2	VDRL+WRs	2	92	85	21·3
3	RPCF+WRs	4	84	68	23·6

sensitivity (judged on the basis of the total number of cases of syphilis picked up with the battery of three tests) was met by the combination of VDRL and RPCF tests. Between them they were reactive in all 25 cases of syphilis (100 per cent. sensitivity) and, at the same time, they were associated with only forty BFP reactions, a specificity of 38.5 per cent. Combinations of the VDRL test with a WR and of the RPCF test with a WR had sensitivities of 92 per cent. and 84 per cent. respectively, but both pairs lacked specificity, 21.8 per cent. for the former and 23.6 per cent. for the latter.

The addition of a WR to the combination of VDRL and RPCF tests, as has been the practice at the three laboratories to date, has added nothing to the pick-up rate of syphilis among pregnant women, but has added a further 47 BFP reactions to the forty already mentioned. This has increased the demand for FTA-ABS and/or TPI tests by 117.5 per cent. (125.5 per 100,000 specimens) from pregnant women.

Discussion

Several reports have been published on the results of serological screening for syphilis on unselected pregnant women. Morris (1968) reported on the use of the VDRL, RPCF, and CWR in Bristol from 1963 to 1967; Salo, Aho, Neiminen and Hormila (1969) from the State Serum Institute at Helsinki on the VDRL test used between 1963 and 1967; Quaife and Gostling (1971) on BFP reactions to the WR in Portsmouth early in 1969; and Adeoba (1967) on the results of a WR and Kahn test on Rh-positive women in Dublin during 1965. The last author also quoted a series from Nigeria where a proportion of BFP reactions were due to malaria, but these results have been omitted from Table VII in which those from the other European centres are compared with those from Glasgow.

6 1 1 1		Results by centre							
Serological te	sts	Glasgow	Bristol	Helsinki	Portsmouth	Dublin			
VDRL	Reactive sera Confirmed due to syphilis BFP reactions	159·8 58·6 101·2	147·6 94·7 52·9	164 118 46					
RPCF	Reactive sera Confirmed due to syphilis BFP reactions	53·3 45·3 8·0	228·3 125·3 103·0						
WRs	Reactive sera Confirmed due to syphilis BFP reactions	221·1 45·3 175·8	172·7 75·2 95·5		190				
Battery	Reactive sera Confirmed due to syphilis BFP reactions	299·0 66·7 232·3	353·6 133·7 220·0			400 145·5 254·5			

TABLE VII Comparison between the results of antenatal serological screening for syphilis in several centres, expressed in rates/100,000

All three centres using the VDRL test (Glasgow, Bristol, and Helsinki) had similar rates for reactive sera, but, because of a BFP reaction rate double that of the others, the tests carried out in Glasgow appeared to be much more sensitive. The rates per 100,000 of those confirmed to be due to syphilis were lower in Glasgow than elsewhere not only for the VDRL but for all the tests. From this it would appear that the incidence of syphilis among pregnant women in Glasgow was lower than in the other centres.

The most sensitive test used in Glasgow was the VDRL, but in Bristol it was the RPCF test. There were marked differences in the specificity of the RPCF test as carried out in the two cities, the BFP rate in Glasgow (8.0 per 100,000) being the lowest of the three tests, while in Bristol (103.0 per 100,000) it was the highest. This latter result might seem surprising for a test which uses a treponemal group antigen, but Sequeira (1962) reported that the incidence of BFP reactions was 1 in 500 for the RPCF test compared with 1 in 1,000 for the standard WR. From these results he calculated that, as the antigens were independent, a BFP reaction to both tests would coincide only once in every 500,000 tests.

The CWR as used on Bristol was less sensitive than the WRs used in Glasgow, but in both series they picked up fewer confirmed cases than did either of the other tests. In Glasgow, the WRs were also the least specific of the tests, the BFP rate being 175.8 per 100,000, similar to that of 190 per 100,000 reported by Quaife and Gostling (1971) from Portsmouth during the first 15 weeks of 1969, before an epidemic of enterovirus infection in the locality which raised the BFP rate to a maximum of 3,660 per 100,000 during the period from June 6 to June 17.

The 'battery' of tests used in Glasgow and Bristol gave results which are remarkably similar despite the variations in sensitivity and specificity noted between individual tests. Thus the different rates of reactive sera found in Glasgow and Bristol, 299 and 353·8 per 100,000 respectively, can be accounted for by the differences in the rates of those later confirmed as being due to treponemal infection, 66·7 and 133·7 per 100,000 respectively. There was little difference between the rates of BFP reactions, 232·3 per 100,000 in Glasgow and 220·0 per 100,000 in Bristol. The rate of BFP reactions reported by Adeoba (1967) was higher (254·5 per 100,000), but he used only the Kahn test and WR as screening tests.

The series from Glasgow and Bristol in which the 'battery' of tests were used both showed that individually the VDRL test, RPCF test, and WRs were not sufficiently sensitive and specific to be acceptable screening tests for syphilis in pregnant women. For this reason those laboratories currently using a flocculation test alone for such purposes would be well advised to reconsider their policy.

The results of the present investigation have indicated that, of the 'battery' of tests used in Glasgow, the WRs were of no value, in that none of the sera reactive in those tests alone was confirmed as positive. In fact, the use of the WRs merely increased by 47 (117.5 per cent.) the number of reactive sera requiring investigation by the FTA-ABS and/or TPI tests to diagnose them as BIP reactions. The same conclusions can be drawn from the results reported by Morris (1968). The CWR he used never, on its own, produced a reaction which was later confirmed as due to treponemal infection, but it did add a further 26 (41 per cent.) reactive sera from individual pregnant women which required further investigation to diagnose them as BFP reactions. In Morris's series, the VDRL and RPCF tests together had a sensitivity of 100 per cent. (they did not miss any case later

confirmed as a case of treponemal disease), and a specificity of 39.7 per cent. These results are almost identical to those obtained in Glasgow, where the same two tests were 100 per cent. sensitive and 38.5 per cent. specific, even when the results of three laboratories were combined.

If the VDRL and RPCF tests together are sufficient for the serological screening for syphilis in pregnant women, then they might well be the optimum combination of tests for screening of other groups within the community.

At present, too many laboratories are testing too few sera and using a wide variety of serological (usually 'reagin') tests. A number of them lack the backing of a major clinic for the sexually transmitted diseases to provide a constant source of sera from patients with syphilis, so that, all in all, the results tend to lack consistency and reliability.

Although found worthless in the 'battery' of tests carried out in Glasgow and Bristol, WRs (or 'complement-fixation tests using lipid antigens'), were the most common serological tests in use among the 68 laboratories from throughout Great Britain which provided information to the British Cooperative Clinical Group (1972). Sixty (88.2 per cent.) of these laboratories did WRs in 1971 while only 46 (67.6 per cent.) carried out the VDRL test and 44 (64.7 per cent.) the RPCF test.

So much for the serological tests in current use. However, the time is upon us when we must accept that the more recently developed specific tests, using T. pallidum as an antigen, have superseded, in sensitivity and specificity, the traditional 'reagin' tests and the RPCF test, and these newer tests should be used in all screening procedures, as soon as a nationwide specialist laboratory service can be set up.

Rationalization of the serological services with centralization of effort is required, now it has been established that automation of serological tests for syphilis is a practical proposition. A few specialized laboratories, each to serve a large area, should be recognized and suitably funded to set up automated serological techniques, the results of which would be consistently sensitive and specific. This would be of benefit to the community in general and to patients and staff of the clinics for sexually transmitted diseases in particular.

Of the specific tests the TPI cannot be automated and the FTA-ABS only to the point of reading the results by darkground fluorescence microscopy. This puts a severe strain on the eyes of the microscopist who has to take rests away from the microscope every so often throughout the day. The reading of the results of the automated microhaemagglutination (Treponema pallidum) (AMHA (TP)) test is not a

strain, and this test, or a development of it, would appear to be the most suitable for use in the screening of large numbers of sera to eliminate syphilis. BFP reactions would no longer be a problem and all 'positive' sera could be subjected to a quantitative 'reagin' test, the result of which would act as a baseline if treatment were required.

The screening of sera for chronic BFP reactors, in an attempt to diagnose collagen vascular diseases at an early or presymptomatic stage, will still require the use of 'reagin' tests. Further investigation is needed to find out which test is the most suitable for this purpose.

Summary and conclusions

Over a 3-year period, 1969 to 1971, five laboratories in Glasgow carried out serological screening tests for syphilis on the sera from 64,404 pregnant women. 285 tests (442.5 per 100,000) were reported reactive, of which 39 (13.7 per cent.) were confirmed as specific by means of the FTA-ABS and/or TPI test, a rate of 60.6 per 100,000.

Three laboratories used a 'battery' of tests, VDRL, RPCF, and either a standard or cardiolipin WR. During 1969, one of these laboratories carried out a Kahn test in addition. Of 37,452 sera tested by these laboratories, 112 (299 per 100,000) were reactive, of which 25 (22.3 per cent.) were confirmed as due to syphilis, a rate of 66.7 per 100,000. The two other laboratories carried out a flocculation test alone; during 1969 a Kahn test, and thereafter the VDRL test. Of 26,952 sera tested, 173 (641.8 per 100,000) were reactive, fourteen (8.1 per cent.) being confirmed as due to syphilis (51.9 per 100,000).

The overall incidence of BFP reactions was 86.3 per cent., 91.9 per cent. for the flocculation tests when used alone and 77.7 per cent. when the 'battery' of tests was used. However, when the 'battery' of tests was carried out, each individual test was seen to have missed a proportion of cases of syphilis. This amounted to 40 per cent. (2 out of 5) for the Kahn test; 32 per cent. (8 out of 25) for the RPCFT and the WRs, and 12 per cent. (3 out of 25) for the VDRL test. None of these tests alone could therefore be considered a satisfactory one for screening purposes.

Screening tests should not miss any positive reactions and this result was obtained in each laboratory by the combination of the VDRL and RPCF tests. Together they had a sensitivity of 100 per cent., not missing any of the 25 confirmed cases, and provided only forty BFP reactions (a specificity of 38.5 per cent.). The VDRL test with a

WR missed two positives (a sensitivity of 92 per cent.), but between them they had 85 BFP reactions (a specificity of 21·3 per cent.). The RPCF test with a WR missed four positives (a sensitivity of 84 per cent.) and gave 68 BFP reactions (a specificity of 23·6 per cent.).

From these findings, it is apparent that all the various Wassermann reactions have become redundant and should certainly be dropped from the 'battery' of tests used in the serological screening of pregnant women and probably from all other serological screening programmes as well. Provided the VDRL and RPCF tests are used, the WRs add nothing except to the cost, not only in materials but also in precious laboratory bench space and man hours.

It is to be hoped that in the near future serological screening to eliminate or diagnose syphilis will be carried out in only a few specialized laboratories throughout the country, all of which will use an automated test with T. pallidum as an antigen, such as the automated microhaemagglutination (T. pallidum) (AMHA(TP)) test, so eliminating the problem of BFP reactions as well as discovering cases missed by the reagin and RPCF tests. Funds, from Government sources, will have to be provided to the selected specialist laboratories to set up and maintain the centralized service, but the improvement in reliability, as far as sensitivity and specificity are concerned, will be of great benefit.

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Épreuves sérologiques pour la syphilis dans la grossesse; positivités non découvertes et fausses positivités

SOMMAIRE

Pendant une période de trois ans, de 1969 à 1971, cinq laboratoires à Glasgow ont pratiqué des tests de dépistage sérologique pour la syphilis sur les sérums de 64.404 femmes enceintes. 285 tests (442,5/100.000) furent déclarés positifs, parmi lesquels 39 (13,7 pour cent) eurent leur spécificité confirmée par les tests FTA-ABS et/ou TPI, ce qui donne un taux de 60,6 pour 100.000.

Trois laboratoires employaient une batterie de tests: le VDRL sur lame, le test RPCF et un BW, soit standard, soit à l'antigène cardio-lipidique.

Pendant 1969, un de ces laboratoires pratiqua en plus le test de Kahn. Sur 37.452 sérums examinés par ces laboratoires, 112 (299/100.000) furent positifs parmi lesquels, chez 25 (22,3 pour cent), le diagnostic de syphilis fut confirmé, soit un taux de 66,7/100.000. Les deux autre laboratoires pratiquèrent seulement un test de floculation: le test de Kahn en 1969, le VDRL ensuite. Sur 26.952 sérums examinés, 173 (641,8/100.000) furent positifs, la syphilis étant confirmée dans 14 cas (8,1 pour cent) soit un taux de 51,9/100.000.

Pour l'ensemble, l'incidence de réactions biologiques faussement positives fut de 86,3 pour cent: 91,9 pour cent lorsque les tests de floculation furent employés seuls et 77,7 pour cent lorsqu'une batterie de tests fut utilisée. Cependant, lorsqu'on utilisa la batterie de tests, chaque test pris individuellement se montra avoir laissé échapper un certain nombre de cas de syphilis. Ceci atteint 40 pour cent (2 sur 5) pour le test de Kahn, 32 pour cent (8 sur 25) pour le RPCF et le BW, et 12 pour cent (3 sur 25) pour l'épreuve du VDRL. Aucun de ces test isolés ne peut donc être considéré comme satisfaisant dans les opérations de dépistage.

Les tests de dépistage ne doivent laisser passer aucune réaction positive et ceci fut obtenu dans chaque laboratoire par l'emploi combiné des tests VDRL et RPCF. Leur utilisation simultanée donne une sensibilité de 100 pour cent, n'ayant laissé passer aucun des 25 cas confirmés et donnant seulement 40 réactions faussement positives (38,5 pour cent de spécificité). Le VDRL couplé au BW a laissé passer 2 cas positifs (92 pour cent de sensibilité) mais avec ce complexe il y eut 85 réactions faussement positives (21,3 pour cent de spécificité). Le RPCF couplé au BW a laissé passer 4 positifs (84 pour cent de sensibilité) et a fourni 68 réactions faussement positives (23,6 pour cent de spécificité).

D'après ces résultats, il apparaît que toutes les diverses réactions de Wassermann sont devenues superflues et qu'elles doivent certainement disparaître des batteries de tests utilisés dans le dépistage sérologique chez les femmes enceintes et probablement aussi de tous les autres programmes sérologiques de dépistage. A condition que l'on emploie le VDRL associé au RPCF, le BW n'ajoute rien qu'une dépense, non seulement en matériel mais aussi en précieuse place sur la paillasse et en heures précieuses de technicien.

On espère que dans un proche futur le dépistage sérologique, pour éliminer ou trouver une syphilis, sera pratiqué seulement dans un petit nombre de laboratoires spécialisés pour tout le pays qui, tous, utiliseront un test automatisé avec le *T. pallidum* comme antigène, tel que la micro-hémagglutination du TP automatisée (AMHA (TP)) pour éliminer le problème des réactions biologiques faussement positives aussi bien que pour découvrir les cas

non découverts par les tests de réagine ou le RPCF. Un budget gouvernemental devra être accordé à des labora-toires spécialisés choisis, pour organiser et faire fonc-tionner un service centralisé. Le gain de confiance obtenu tant pour la sensibilité que pour la spécificité bénéficiera à la communauté toute entière mais, plus spécialement, aux malades et au personnel des cliniques pour les maladies sexuellement transmises.