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Laboratory diagnosis of trachoma: a collaborative study

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A collaborative study on the laboratory diagnosis of trachoma was carried out in three laboratories. A standardized complement fixation (CF) test with chlamydial (bedsonial) group antigen was found to be highly reproducible. The results obtained by different laboratories using the method and reagents suggested by the WHO International Reference Centre for Trachoma and other Chlamydial Infections agreed in more than 95% of the tests. Similar agreement was observed between the results obtained with these reagents and those routinely used in one of these laboratories. In confirmation of previous studies, the CF test was found to give positive results in only a limited proportion of trachoma cases. However, in an area where the disease is hyperendemic the rates showed good correlation with the intensity of clinical signs. A comparison was also made between Giemsa staining and a fluorescent antibody (FA) technique for the cytological examination of conjunctival scrapings. The results obtained with the former method correlated well with clinical activity but the positivity rate was lower than that obtained by the FA technique. The FA results, however, were not an accurate indicator of clinical intensity. These results suggest that the Giemsa method may detect only the most heavily infected individuals.

The complement fixation test with chlamydial group antigen is commonly used in the diagnosis of psittacosis and lymphogranuloma venereum, but its application to the diagnosis of trachoma or inclusion conjunctivitis has been more limited. Recent studies indicate that approximately 50% of patients with acute ocular or oculogenital TRIC (trachoma–inclusion conjunctivitis) infections have significant levels of complement-fixing (CF) antibodies (Schachter et al., 1970). However, in previous studies of chronic trachoma the CF response has been found to be erratic and to vary considerably from area to area. Nevertheless, such a test might possibly provide useful information as an adjunct to clinical diagnosis in large-scale population surveys, the rates of positivity reflecting the degree of endemicity or activity of trachoma in a given area (Tarizzo et al., 1968). It is recognized, of course, that the CF test is group-specific; psittacosis and lymphogranuloma venereum among other infections can cause significant CF antibody levels, but they are usually rare enough in large populations not to have a significant effect on the results.

In this study sera were collected from children in Morocco and in Western Samoa and were tested in three laboratories—in Copenhagen, Adelaide, and San Francisco. The same CF test and antigens were used in each laboratory; the Copenhagen laboratory also used its own routine testing procedures. An attempt was also made to compare the Giemsa and the fluorescent antibody (FA) staining techniques for the detection of inclusions. The Giemsa method is still generally considered to be the standard test, while FA methods have proved the most sensitive when quantification of inclusions is sought (Nichols et al., 1967) or in cases of mild chronic trachoma where other cytological methods have failed (Hanna, 1968).

MATERIALS AND METHODS

Serological studies

Sera collected in Morocco were sent to the Statens Seruminstitut (SSI) in Copenhagen and those from

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Western Samoa to the Institute of Medical and Veterinary Science (IMVS) in Adelaide. The clinical diagnoses were made and recorded according to the criteria recommended by WHO. The record cards were sent to Geneva, where the data were collated. The diagnoses were not available to the laboratories performing the tests.

The WHO International Reference Centre for Trachoma and other Chlamydial Infections (IRC) in San Francisco supplied antigens, complement, and haemolysin for the tests. The CF method was that of Meyer & Eddie (1964), boiled phenolized antigens prepared from the 6BC psittacosis strain and the Ap2 trachoma strain (TRIC//USA-Ariz/Cal-4/OT) being used. All materials were pretitrated at the IRC and protocols, together with the observed titres, were sent to the collaborating laboratories.

The SSI performed the tests according to IRC-recommended methods, as well as by their own standard procedures. For comparative purposes they also used their standard antigen, an ether-soluble, acetone-precipitated, phospholipid-type group antigen (Volkert & Christensen, 1955) prepared from the DK/SS-1/ON inclusion conjunctivitis isolate (Mordhorst, 1965). The IMVS tested the sera collected in Western Samoa, using the IRC method.

Once the collaborating laboratories had finished their tests, they sent aliquots of representative serum samples without results to the IRC for retesting.

Cytological studies

Conjunctival scrapings were also collected from the same children in Morocco and Western Samoa and sent to the same laboratories. The smears were stained by the standard Giemsa method and by an indirect FA technique; reagents and criteria for the latter were supplied by the IRC (Schachter et al., 1970). In addition to a direct comparison between FA and Giemsa methods this study was expected to show whether a laboratory otherwise competent and experienced in work on the trachoma agent could easily use this method, as the other two laboratories did not use FA methods routinely.

SEROLOGICAL RESULTS

Morocco series

Testing of all the 154 sera from Morocco by the IRC method and with 6BC and Ap2 antigens gave results in 100% agreement within one tube dilution (Table 1). Negative (<1:5) results were obtained with 126 of 154 specimens with both antigens; 22 specimens gave positive reactions with both, while 6 that gave titres of 1:5 with one antigen reacted at 1:10 with the other. A series of 13 coded serum specimens tested at IRC gave results agreeing within one dilution. The reciprocal of the average titre for all specimens was approximately 3.6.

Table 1. Number of cases, by CF titre, obtained with two antigens (Morocco series, tested in Copenhagen)

Titre with 6BC antigen	Titre to Ap.2 antigen						Total
	<5	5	10	20	40	80	
<5	103	4					107
5	1	18	3				22
10		3	13				16
20			3	2	1		6
40					2		2
80						1	1
total	104	25	19	2	3	1	154

Comparison of methods. A total of 145 sera were tested against the 3 antigens by both CF methods. There were thus 435 direct comparisons of the methods. Agreement within one twofold dilution was seen in 413 (95%). In 22 cases of disagreement of at least 2 dilutions, a higher titre was obtained by the IRC method in 18 instances and by the SSI method in 4; 14 of these specimens were positive at 1:10 by the IRC method and negative by the SSI method. For practical purposes, therefore, the two methods are comparable although the IRC method may be slightly more sensitive in detecting low-grade reactions. For example, with the Ap2 antigen the SSI method gave 36 positive reactions (24.8%) at 1:5 or higher, while the IRC method gave 46 (31.7%). Most of these disparate positive reactions had complete fixation at 1:5 compared with a negative result. The difference between methods is not important in the diagnosis of psittacosis or lymphogranuloma venereum but may be significant in surveys where high titres are the exception.

Comparison of antigens. The three antigens (6BC and Ap2 boiled phenolized and ether–acetone DK/SS-1) were tested by both methods against 145 sera and 290 direct comparisons were thus available.

Table 2. Number of cases, by CF titre, obtained with two antigens (Western Samoa series, tested in Adelaide)

Titre with 6BC antigen	Titre to Ap.2 antigen					Total
	<4	4	8	16	32	
<4	145	6				151
4	7	13	1			21
8	1	4	11	2		18
16			3	2	1	6
32				1	1	2
total	153	23	15	5	2	198

Agreement within one dilution was observed in 282 of 290 (97.3%). In the other 8 the highest titre was obtained in 6 cases with 6BC, while in 2 instances the titres with both 6BC and Ap2 were higher than with DK/SS-1. These group antigens may therefore be considered practically equivalent for CF tests.

Western Samoa series

The Western Samoan specimens were collected from children 5–10 years old. Clinical trachoma was not diagnosed in any of these children. A titre of 1:8 or higher was considered to be positive, in view of the different dilution series used by the Adelaide laboratory and of the low titres often observed in such surveys. Of the 200 sera tested, 2 were anti-complementary, leaving 198 valid tests. Of this group, 171 (86.3%) were negative (≤1:4) with both antigens. One that was negative with 6BC was positive with Ap2, while 5 that were negative with the Ap2 antigen were positive at 1:8 with 6BC. Titres for 197 of 198 specimens (99.5%) agreed within one dilution (Table 2). The overall positivity rates of 3.5–4% at 1:16 or higher do not differ significantly from the results obtained in screening normal adult populations in the USA. The reciprocal of the average titre for all specimens was 1.96 with the 6BC antigen and 1.80 with the Ap2 antigen. Sixteen serum samples from the Samoa series were tested at the IRC, and 15 agreed within one dilution with the IMVS results.

Correlation of CF results with clinical diagnoses

The proportions of positive reactors in the Morocco series and the mean titres observed by clinical diagnoses are given in Tables 3 and 4. The proportions of positive results were in general fairly low; however, they and the titres appear to be higher in cases of active trachoma. When results are tabulated by the individual scores given to the presence of follicles, it appears clearly that there is a correlation between the results and the intensity of clinical activity.

Table 3. Results of CF tests by clinical diagnosis (specimens from Morocco, tested in Copenhagen)

	Clinical diagnosis					Active trachoma	Total trachoma	Total
	No Tr	Tr I	Tr II	Tr III	Tr IV			
no. tested	17	31	23	54	29	108	137	154
6BC antigen								
no. of positive sera [a]	1	2	6	13	3	21	24	25
% positive	5.9	6.4	26.1	24.1	10.3	19.4	17.5	16.3
titre [b]	0.88	1.61	6.96	3.70	2.93	4.17	3.91	3.57
Ap2 antigen								
no. of positive sera [a]	1	2	5	14	3	21	24	25
% positive	5.9	6.4	21.7	25.9	10.3	19.4	16.8	16.3
titre [b]	0.88	1.93	7.61	4.16	2.76	4.44	3.94	3.60

[a] Titre ≥ 1:10.
[b] Reciprocal of average titre of all specimens.

Table 4. Results of CF tests by intensity of clinical activity (specimens from Morocco, tested in Copenhagen)

	Intensity of clinical activity [a]				Total
	F_0	F_1	F_2	F_3	
no. tested	46	70	36	2	154
6BC antigen					
no. of positive sera [b]	4	8	11	2	25
% positive	8.7	11.4	30.5	(100.0)	16.3
titre [c]	2.17	2.36	7.08	15.00	3.57
Ap2 antigen					
no. of positive sera [b]	5	8	11	1	25
% positive	10.9	11.4	30.5	(50.0)	16.3
titre [c]	2.50	2.21	6.67	22.50	3.60

[a] F = conjunctival follicles.
[b] Titre $\geqslant 1:10$.
[c] Reciprocal of average titre of all specimens.

CYTOLOGICAL RESULTS

Morocco series

An attempt was made to compare FA and Giemsa staining methods on scrapings taken from upper and lower conjunctivae but, because of breakage and loss of specimens, a complete comparison was not possible. The quality of the specimens varied but in most instances appeared satisfactory for the FA method. The Giemsa preparations, however, were difficult to read, apparently because of deleterious conditions of storage or improper fixation. In general, the cells appeared hazy and lacked optimum definition. While the slides were readable, the process was much more arduous than usual. Because of this variation in quality, specimens collected from individual patients were not compared.

The overall results are shown in Tables 5 and 6. Positive results were obtained more often with the FA method than with Giemsa stain. The specimens collected from the upper conjunctiva tended to be more positive when stained by the Giemsa method than those from the lower conjunctiva, while those collected from the lower conjunctiva were more often positive when stained by the FA method than those from the upper conjunctiva.

The results obtained with the Giemsa stain correlate well with active disease, with a maximum of 27% inclusion-positive in Tr II. The FA method, on the other hand, gave relatively high positive results through the entire sample, although some association with clinical activity could be shown in Tr II, where 60% of the samples were inclusion-positive compared with approximately one-third for inactive cases.

Table 6 shows a correlation between the results of microscopic examination and the intensity of clinical activity. Essentially the same relationship between findings obtained with the two techniques and with material of different origin is maintained —i.e., higher positivity rates with the FA than with the Giemsa technique, the former giving more frequent positive results with material from the lower conjunctiva than with that from the upper, while Giemsa staining gave more frequent positive results with material from the upper conjunctiva than with that from the lower.

Western Samoa series

Slides from Western Samoa were generally unsatisfactory with too few cells for evaluation. Where sufficient cells could be discerned, neither inclusions nor the cytology associated with trachomatous infection could be found.

DISCUSSION

This study confirmed the findings of previous investigators that CF tests show a relative lack of sensitivity in the diagnosis of trachoma. The highest

Table 5. Results of microscopic examination by clinical diagnosis (specimens from Morocco, examined in Copenhagen)

	Clinical diagnosis					Active trachoma	Total trachoma	Total
	No Tr	Tr I	Tr II	Tr III	Tr IV			
upper conjunctiva								
Giemsa								
ratio [a]	1/7	0/17	5/18	6/29	0/14	11/64	11/78	12/85
% positive	(14.3)	0	27.8	20.7	0	17.2	14.1	14.2
FA								
ratio [a]	2/7	6/16	8/15	16/33	8/23	30/64	38/87	40/94
% positive	(28.6)	37.5	53.5	48.5	34.8	46.9	43.7	42.6
lower conjunctiva								
Giemsa								
ratio [a]	0/16	3/29	5/19	2/44	2/25	10/92	12/117	12/133
% positive	0	10.3	26.3	4.5	8.0	10.9	10.3	9.0
FA								
ratio [a]	6/17	17/30	13/20	31/54	11/28	61/104	72/132	78/149
% positive	35.3	56.7	65.0	57.4	39.3	58.7	54.5	52.3

[a] No. positive/no. examined.

Table 6. Results of microscopic examination of conjunctival scrapings by intensity of clinical activity (specimens from Morocco, examined in Copenhagen)

	Intensity of clinical activity [a]				Total
	F_0	F_1	F_2	F_3	
upper conjunctiva					
Giemsa					
ratio [b]	1/23	4/34	6/25	1/3	12/85
% positive	4.4	11.8	24.0	(33.3)	14.2
FA					
ratio [b]	10/30	16/39	11/22	3/3	40/94
% positive	33.3	41.0	50.0	(100.0)	42.6
lower conjunctiva					
Giemsa					
ratio [b]	2/42	4/57	4/31	2/3	12/133
% positive	4.8	7.3	12.9	(66.7)	9.0
FA					
ratio [b]	17/46	33/64	25/36	3/3	78/149
% positive	37.0	51.6	69.4	(100.0)	52.3

[a] F = conjunctival follicles.
[b] No. positive/no. examined.

rate of positive reactions obtained was approximately 25% in cases with active trachoma. There was, however, a definite correlation with the degree of activity of the disease. These findings suggest that, if the antigenic stimulus associated with trachoma infection is inadequate, both from the point of view of positivity rates and from that of long-lasting CF response, to permit the use of this method for the diagnosis of individual cases, the test might still be a useful adjunct to clinical diagnosis as an indicator of the degree of active trachoma in a particular population. Further work will be necessary to determine whether the positivity rates also correlate with the severity of the disease and whether they are precise and sensitive enough to change as a consequence of changes occurring in the degree of endemicity of the disease.

The CF test itself proved to be highly reproducible in competent microbiological laboratories that were accustomed to performing serological tests. The reagents are stable and the methods are standard. The degree of reliability of the tests was outstanding, more than 95% of all the results agreeing within one dilution. It would seem reasonable to suggest that such antigens should be routinely used not only in studies of trachomatous populations but also in surveys of respiratory or venereal diseases because of the possible role of chlamydiae as etiological agents of these conditions.

Other serological methods, such as the recently described micro-immunofluorescence test (Wang & Grayston, 1970), may possibly be more useful in the study of trachoma and of its epidemiology, but they are more complex and time-consuming. The CF test may be a helpful screening procedure and may provide some laboratory support for comparative studies of large population groups.

Unpublished work at the IRC and SSI indicates that micro-methods for CF tests can also be adapted to trachoma. Macro-methods remain necessary for standardizing the reagents and often give higher titres, but micro-methods can be used for testing large numbers of sera.

The poor quality of the material collected for cytological studies shows the need for improving and standardizing methods for the collection of specimens before any attempt can be made to compare results obtained with different methods or under different conditions. It was apparent that the FA technique is sufficiently standardized for it to be introduced into laboratories that are willing to invest the time needed to familiarize their personnel with the method. It is not possible to compare the results of the serological and microscopic methods obtained in this study.

With these limitations in mind, it is believed that the results obtained with the two staining techniques on the material from Morocco permit some tentative interpretation. The higher rates of positivity, together with their relatively poor correlation with clinical findings, might indicate that the greater sensitivity of the FA technique is associated with less specificity. An alternative explanation would be that only the most actively infected individuals are detected by the Giemsa method, while the more sensitive FA technique gives higher infection rates. This latter explanation is particularly attractive for an area of high endemicity. It suggests that persistence of the agent need not be correlated with the clinical activity. Clarification of these points would have to be made by further comparative studies performed with uniform methods in areas with different degrees of trachoma endemicity. Similar observations—i.e., a high rate of FA-positive results in the absence of Giemsa-staining inclusions—have been noted in comparative studies in American Indians (Schachter et al., 1971). In the study reported here the rates of positivity were approximately 30–40% higher for the FA method than for Giemsa staining in each clinical category. This difference may be interpreted as representing the clinically inapparent " infectious load " of the community.

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RÉSUMÉ

DIAGNOSTIC DE LABORATOIRE DU TRACHOME: UNE ÉTUDE COLLECTIVE

Le diagnostic de laboratoire des infections à chlamydiae, couramment utilisé dans le cas de l'ornithose et de la lymphogranulomatose vénérienne, a des applications plus limitées dans le cas du trachome du fait de l'irrégularité de la réponse immunitaire.

Une étude a été faite en collaboration par trois laboratoires, à San Francisco, Copenhague et Adelaïde, sur des échantillons de sérums et des grattages de conjonctive prélevés au Maroc et au Samoa-Occidental, dans le but de vérifier la reproductibilité des résultats et d'évaluer leurs rapports avec les données cliniques.

Les méthodes de laboratoire utilisées ont été la réaction de fixation du complément et l'examen microscopique de frottis conjonctivaux après coloration par le Giemsa ou selon la méthode de l'immunofluorescence.

Les études sérologiques ont montré que dans les deux séries de sérums étudiées, 154 du Maroc, et 198 du Samoa-Occidental, les résultats obtenus dans les trois laboratoires étaient en accord dans plus de 95% des cas. Le même accord a été observé en comparant les résultats obtenus dans un même laboratoire avec des techniques ou des réactifs différents. Les taux de positivité ont été relativement bas dans l'ensemble, se situant légèrement au-dessous de 20% pour les cas de trachome actif. Toutefois, une bonne corrélation a été observée entre les variations des taux de positivité et plus encore celles des titres moyens, et le stade de la maladie. Cette corrélation était aussi très nette entre résultats sérologiques et intensité des signes cliniques.

En ce qui concerne les résultats des examens microscopiques, la première constatation a été que les techniques de récolte et de préparation des frottis exigent d'être standardisées avant qu'on puisse procéder à des études comparatives ou même interpréter les résultats obtenus dans des conditions différentes. Une corrélation a été observée, dans ce cas aussi, entre résultat de l'examen microscopique d'une part et d'autre part le stade de la maladie ainsi que l'intensité des signes cliniques. La méthode d'immunofluorescence s'est avérée plus sensible et apparemment moins spécifique que la coloration par le Giemsa. Cette différence paraît indiquer qu'une positivité à l'immunofluorescence serait en rapport avec l'infection indépendamment des manifestations cliniques alors que, dans le cas du Giemsa, la positivité dépendrait de l'intensité de ces manifestations.

En conclusion, les résultats obtenus confirment que les méthodes de laboratoire pour le diagnostic du trachome, et plus particulièrement la réaction de fixation du complément, pourraient trouver une utilisation pour mesurer le degré d'endémie de la maladie malgré leur valeur limitée pour le diagnostic de cas individuels.

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