Chlamydial infection

Advances in the diagnostic isolation of Chlamydia, including TRIC agent, from the eye, genital tract, and rectum

S. DAROUGAR*†, B. R. JONES*, J. R. KINNISON†, J. D. VAUGHAN-JACKSON*‡,
AND E. M. C. DUNLOP*‡

From the Department of Clinical Ophthalmology and the Virus Laboratory†, Institute of Ophthalmology, London, and the Whitechapel Clinic, The London Hospital‡*

The inclusion bodies of TRIC (trachoma-inclusion conjunctivitis) agent were first described by Halberstaedter and Prowazek (1907) in smears taken from the conjunctiva of Orang-utans experimentally infected with trachomatous material and from the conjunctiva of patients suffering from trachoma. 50 years later the trachoma agent was cultured by T'ang, Huang, Chang, and Wong (1957) in the yolk sacs of fertile hens' eggs. The detection of inclusions in smears using Giemsa, iodine, or fluorescent antibody staining techniques, and the isolation of chlamydial agents by culture of clinical specimens in the yolk sac of the fertile egg, are relatively insensitive and leave much to be desired as diagnostic procedures for chlamydial infection of the eye and genital tract in developed countries or for studies of hyper-endemic trachoma in developing countries.

The technique of culture in irradiated McCoy cells (Gordon, Harper, Quan, Treharne, Dwyer, and Garland, 1969) provided the first alternative to culture in yolk sac for the primary isolation of *Chlamydia*. Studies by Gordon and others (1969) and by Darougar, Dwyer, Treharne, Harper Garland, and Jones (1971a) and Darougar, Treharne Dwyer, Kinnison, and Jones (1971d) have shown that this is three to four times more sensitive than detection of inclusions in smears or isolation of agent in yolk sac. Further studies have led to a simplified one-passage technique which is more sensitive and rapid than the original technique (Darougar and others, 1971a, d; Darougar, Kinnison, and Jones, 1971b, c). The purpose of this paper is to report the sensitivity of the simplified one-passage technique of culture in irradiated McCoy cells combined with new developments in collection and storage for transportation of clinical specimens for the isolation of *Chlamydia* including TRIC agent.

Material and methods

SOURCE OF SPECIMENS

Specimens were collected from patients who presented at the following clinics and who had not been treated with tetracyclines or sulphonamides during the current episode of infection:

(A) The External Diseases Clinic, Moorfields Eye Hospital, London

(B) The Diagnostic Clinic, and the External Diseases Clinic, Institute of Ophthalmology, London

(C) The Whitechapel Clinic, The London Hospital

METHODS OF COLLECTION

Specimens processed in this study were collected as follows:

(1) Of a total of 281 ocular specimens, 113 were collected by scraping and 168 were collected by swabbing the different areas of conjunctiva, *i.e.* upper tarsus, upper fornix, and lower lid (Darougar and Jones, 1971).

(2) Of a total of 155 urethral specimens collected from patients suffering from 'non-specific' urethritis (NSU), 95 were collected with endourethral scrapers and sixty with endourethral swabs (Dunlop, Vaughan-Jackson, Darougar, and Jones, 1972).

(3) Cervical, rectal, and urethral materials from 21 women with proven TRIC infection of the eye, nineteen female consorts of patients with TRIC infection of the eye, and fifteen mothers of babies with TRIC ophthalmia neonatorum (ON), were collected with cotton-wool swabs or curettes, or both (Dunlop, Vaughan-Jackson, and Darougar, 1972a; Vaughan-Jackson, Dunlop, Darougar, Dwyer, and Jones, 1972).

(4) Urethral material from ten men suffering from Reiter's disease, and from 22 men suffering from proven TRIC infection of the eye, and from eleven male consorts of women suffering from TRIC infection of the eye were collected with an endourethral curette, or an endourethral swab, or both (Dunlop and others, 1972a; Vaughan-Jackson and others, 1972).

TRANSPORT MEDIUM

Of 281 ocular specimens, 213 were collected in plastic-capped glass vials containing sucrose-phosphate solution

with antibiotics (2SP) (Gordon and others, 1969). The other 68 specimens were collected in plastic capsules with soft silicone washers or stoppers (made by Sterilin Ltd., 12-14 Hill Rise, Richmond, Surrey) containing 2SP and 3 per cent. foetal bovine serum. Of 95 specimens of urethral scrapings, 41 were collected in glass vials containing 2SP and 54 in plastic capsules containing 2SP with additional foetal bovine serum.

Other specimens processed in this study were collected in plastic capsules containing 2SP with additional foetal bovine serum.

TRANSPORTATION OF SPECIMENS TO THE LABORATORY

113 of 281 ocular specimens and 41 of 95 urethral specimens were kept in the clinic in a $+4^{\circ}\text{C}$. refrigerator for 2 to 6 hours until they were transferred to the laboratory in a thermos flask packed with ice. In the laboratory these specimens were stored in a -70°C . refrigerator until they were inoculated into the cell culture tubes. The other specimens were collected in plastic capsules and were snap frozen in the clinic in a portable liquid nitrogen refrigerator (-170°C .). These specimens were transferred to the laboratory and stored in a -70°C . refrigerator until they were inoculated into the cell culture tubes at a convenient time.

CELL CULTURE TECHNIQUE

The McCoy cell line was grown and irradiated and mono-layers of these cells were prepared in flat-bottomed tubes containing coverslips, following the method used by Darougar and others (1971b). The clinical specimens were inoculated on to the cell culture, using either the modified two-pass technique (Darougar and others, 1971c) or the simplified one-pass technique of culture in irradiated McCoy cells (Darougar and others, 1971b).

The principles involved in each technique are shown in Table I. Details of the earlier three-pass technique of culture in irradiated McCoy cells are included in this Table for comparison.

Results

OCULAR SPECIMENS

Of 281 clinical specimens collected from 281 patients, eighty were inoculated by the modified two-pass technique and 201 by the simplified one-pass technique of culture in irradiated McCoy cells (Table II, overleaf).

Modified two-pass technique

Specimens were collected in 2SP by scraping, transported on ice to the laboratory, and stored in a -70°C . refrigerator. Of eighty specimens inoculated by this technique, 46 (57.5 per cent.) gave positive TRIC agent cultures (Table II).

Simplified one-pass technique

Of 201 ocular specimens inoculated by this method, 33 were collected in 2SP by conjunctival swabbing and stored in a -70°C . refrigerator before inoculation. Of these 33 specimens, nineteen (57.5 per cent.) gave isolates of TRIC agent (Table II). Of 100 specimens collected in 2SP by conjunctival swabbing and snap frozen in a portable liquid nitrogen refrigerator, 49 (49 per cent.) gave isolates of TRIC agent (Table II). Of the 68 remaining specimens, which were collected in 2SP with additional 3 per cent. foetal bovine

TABLE I *Modification in methods of collection, storage, and inoculation of conjunctival specimens onto irradiated McCoy cell culture for isolation of Chlamydia*

<i>Techniques of culture</i>	<i>Standard 3-pass</i>	<i>Modified 2-pass</i>	<i>Simplified 1-pass</i>
Collection of specimens	Scraping	Scraping	Swabbing
Transport medium	2SP	2SP	2SP + serum
Method of transportation	In thermos-flask packed with ice delivered same day	In thermos-flask packed with ice delivered same day	In liquid nitrogen delivered at a convenient time
Disintegration of specimens for inoculation	Indirect sonication (7 min.)	Indirect sonication (7 min.)	Shaking with glass beads on a whirli-mixer (20 sec.)
No. of tubes	12	4	2
Force of centrifugation (G.)	1,800	2,700	2,700
Temperature during centrifugation	18–20°C. refrigerated centrifuge	35–38°C. bench centrifuge	35–38°C. bench centrifuge
Time required for inoculation (min.)	40	20	10
Maximum incubation period (days)	9–10	6–7	2–3
Total time for screening mono-layers for inclusions (min.)	60	20	10

serum by conjunctival swabbing and snap frozen in a liquid nitrogen refrigerator, 54 (79.4 per cent.) gave isolates of TRIC agent. This increase in the sensitivity of the technique, from the 49 per cent. rate of isolation obtained from specimens collected in 2SP alone by swabbing, is statistically significant ($\chi^2 = 15.95$; $P < 0.01$).

TABLE II *Comparison of isolation of TRIC agent from conjunctiva, using different techniques of irradiated McCoy cell culture and different techniques of transportation*

Technique	No. of patients tested	Isolates obtained	
		No.	Per cent.
Modified 2-passage: Specimens collected in 2SP by scraping and stored in -70°C refrigerator	80	46	(57.5)
Simplified 1-passage: Specimens collected in 2SP by swabbing and stored in -70°C refrigerator	33	19	(57.5)
Specimens collected in 2SP by swabbing and stored in liquid nitrogen refrigerator (-170°C .)	100	49	(49) ^a
Specimens collected in 2SP with 3 per cent. foetal bovine serum by swabbing and stored in liquid nitrogen refrigerator (-170°C .)	68	54	(79.4) ^a

^a $\chi^2 = 15.95$; $P < 0.01$

URETHRAL SPECIMENS FROM MEN

Of 155 specimens collected from 95 patients suffering from NSU, 95 were collected by scraping. Of these 95 specimens, 37 (39 per cent.) gave chlamydial isolates (Table III). Of the sixty specimens collected by endourethral swabbing from this group of patients, 22 (37 per cent.) gave chlamydial isolates.

TABLE III *Results of different methods of collection of urethral material from men suffering from NSU*

Method of collection	No. of patients	Isolates obtained	
		No.	Per cent.
Endourethral swab	60	22	37
Endourethral scrape	95	37	39

Of 41 specimens which were collected in 2SP by scraping and were stored in a -70°C . refrigerator,

eighteen (44 per cent.) gave chlamydial isolates (Table IV). Of the remaining 54 specimens, which were collected in 2SP with 3 per cent. foetal bovine serum by scraping and snap frozen in a portable liquid nitrogen refrigerator, 26 (48.1 per cent.) gave positive chlamydial cultures. These slight differences are not statistically significant.

TABLE IV *Results of different methods of storage of clinical specimens collected from urethra of men suffering from NSU*

Method of storage	Transport medium	No. of patients	Isolates obtained	
			No.	Per cent.
Mechanical refrigerator (-70°C .)	2SP	41	18	44.0
Liquid nitrogen refrigerator (-170°C .)	2SP and serum	54	26	48.1

The results of isolation from urethral material from patients suffering from Reiter's disease and TRIC infection of the eye, and from consorts of women with TRIC infection of the eye, are presented in Table V.

TABLE V *Isolation of Chlamydia from urethra of men suffering from Reiter's disease, of men with TRIC infection of eye, or of male consorts of women with TRIC infection of eye*

Group	No. of patients	No. of isolates obtained from urethra
Reiter's disease	10	3
TRIC infection of eye	22	10
Male consorts of patients with TRIC infection	11	6

CERVICAL, URETHRAL, AND RECTAL SPECIMENS FROM WOMEN

Specimens from the cervix, urethra, and rectum were collected in 2SP with and without additional foetal bovine serum and stored in a -70°C . refrigerator.

Of 21 cervical specimens collected from women with TRIC infection of the eye, nineteen (more than nine out of ten) gave chlamydial isolates (Table VI). Of twelve urethral specimens and 21 rectal specimens collected from this group of patients, seven and eleven respectively (more than half) gave chlamydial isolates.

Of nineteen cervical specimens collected from female sexual consorts of men with TRIC infection of the eye, eight specimens (or nearly half) gave chlamydial isolates.

TABLE VI *Isolation of Chlamydia from cervical material from women suffering from TRIC infection of eye, or from female consorts of men with TRIC infection of eye, or from mothers of babies with TRIC ophthalmia neonatorum*

Group	No. of patients	No. of isolates obtained from cervix
TRIC infection of eye	21	19
Female consorts of patients with TRIC infection of the eye	19	8
Mothers of babies with TRIC ophthalmia neonatorum	15	11

Of cervical specimens collected from fifteen mothers of babies with TRIC ophthalmia neonatorum, eleven (over seven out of ten) gave chlamydial isolates.

Discussion

Using a higher temperature during the period of centrifugation (35–38°C.) and the higher force of centrifugation (2,700 G) in the three-passage system, the rate of isolation of TRIC agent in the first pass of the standard technique of culture in irradiated McCoy cells was increased from 55 to 87 per cent. when applied to conjunctival material from patients suffering from TRIC infection of the eye (Darougar and others, 1971b). After this improvement the modified two-passage technique was developed which proved to be as sensitive as the modified three-passage technique. A further simplification of this technique resulted in the development of the simplified one-passage technique which required only two tubes of cells for each clinical specimen and 2 to 3 days for completion of tests. Studies of the sensitivity of the simplified technique for primary isolation of *Chlamydia* have shown it to be as sensitive as the two-passage technique of culture in irradiated McCoy cells (Table II).

Swabbing has provided a simple, fast, sensitive and non-traumatic technique for the collection of specimens (Darougar and Jones, 1971; Dunlop and others, 1972a). The simplicity and speed of collection by swabbing has facilitated the use of culture in

irradiated McCoy cells in the diagnosis and study of chlamydial infection of the eye and genital tract.

Lack of a convenient and efficient method of storage for transportation of clinical specimens has been a major handicap in the use of this new technique for the diagnosis of TRIC infection, especially in studies of chlamydial infection in areas where well-equipped laboratories with skilled personnel were not available. The liquid nitrogen refrigerator used in previous studies (Darougar and others, 1971d) has offered a practical method of preserving and transporting specimens. The use of plastic capsules to contain the transport medium has obviated the hazards of explosion associated with the use of glass ampoules or glass vials for the storage and transportation of clinical specimens in liquid nitrogen. The rate of isolation of TRIC agent was slightly lower after clinical specimens were snap frozen and stored in liquid nitrogen when compared with the result of isolation after storage in a –70°C. refrigerator. Although this difference is not statistically significant, it suggests that snap freezing may possibly damage the infectivity and viability of TRIC agent. Studies in our laboratory have shown that the addition of serum to the transport medium will significantly protect the viability and infectivity of *Chlamydia* against the effects of snap freezing in liquid nitrogen (Table II). In the present study 3 per cent. foetal bovine serum has been added to the transport medium because the McCoy cells used were adapted to this serum. The use of this technique of storage for transportation allows specimens to be collected at any time in clinics or in the field for transfer to the laboratory and culture when convenient.

Using these developments of methods for the collection, transportation, and culture of specimens by the simplified one-passage technique of culture in irradiated McCoy cells, a very high level of sensitivity for the isolation of TRIC agent has been achieved, reaching 79.4 per cent. for the infected eye. Using the different variations in technique, *Chlamydia* has been isolated in cell culture from genital material from over nine in ten female patients suffering from TRIC infection of the eye and from over seven in ten of mothers of babies with TRIC ophthalmia neonatorum. The simplicity of the one-passage technique, which makes it three times faster and five times cheaper than the earlier method, will facilitate the application of culture in irradiated McCoy cells to routine diagnostic services for chlamydial infection in developed areas. Furthermore, it makes the culture method practicable for epidemiological studies and for assessment of the results of treatment of hyperendemic trachoma in developing areas.

Summary

The simplified one-passage technique of culture in irradiated McCoy cells was used for the isolation of *Chlamydia*, including TRIC agent, in conjunction with certain developments in the collection and storage of specimens.

Using swabbing for the collection of material from the conjunctiva, the addition of animal serum to the transport medium, and a liquid nitrogen refrigerator for storage and transportation of clinical specimens to the laboratory, a very high level of sensitivity has been reached for the isolation of TRIC agent from the infected eye (79.4 per cent.). Using the different variations in technique, *Chlamydia* has been isolated in cell culture from genital material from men and women suffering from TRIC infection of the eye, from nearly half and from over nine of ten respectively, from 44.8 per cent. of men suffering from NSU, and from genital material from over seven in ten mothers of babies with TRIC ophthalmia neonatorum.

The simplicity and speed of these techniques will facilitate the application of culture in irradiated McCoy cells in routine diagnostic services for chlamydial infection of the eye or genital tract in developed areas. Furthermore, it makes it practicable to apply these techniques in epidemiological studies, and in the assessment of the results of treatment of hyperendemic trachoma in developing countries.

We are grateful to our colleagues who referred patients for investigation, to colleagues at the External Diseases Clinic, Moorfields Eye Hospital, for the collection of specimens, to Mrs. Bryony Phillips, B.Sc., of the Computer Section, Institute of Ophthalmology, for statistical advice, to Mrs. J. Hook, Miss M. Vance, Miss S. Argent, and Mr. R. Hejazi for technical help, and to Miss C. M. Carlyle and Mrs. R. E. Cohen for secretarial assistance.

We thank the Research and Development Division of the Department of Health and Social Security for a grant in support of the laboratory work.

References

- DAROUGAR, S., DWYER, R. St. C., TREHARNE, J. D., HARPER, I. A., GARLAND, J. A., and JONES, B. R. (1971a) 'Trachoma and Related Disorders', ed. R. L. Nichols, p. 445. Excerpta Medica, Amsterdam and New York.
- and JONES, B. R. (1971) *Brit. J. Ophthalm.*, **55**, 585.
- , KINNISON, J. R., and JONES, B. R. (1971b) 'Trachoma and Related Disorders', ed. R. L. Nichols, p. 63. Excerpta Medica, Amsterdam and New York.
- , —, — (1971c) *Idem.*, p. 501.
- , TREHARNE, J. D., DWYER, R. St. C., KINNISON, J. R., and JONES, B. R. (1971d) *Brit. J. Ophthalm.*, **55**, 591.
- DUNLOP, E. M. C., VAUGHAN-JACKSON, J. D., and DAROUGAR, S. (1972a) *Brit. J. vener. Dis.*, **48**, 421.
- , —, —, and JONES, B. R. (1972b) *Ibid.*, **48**, 425.
- GORDON, F. B., HARPER, I. A., QUAN, A. L., TREHARNE, J. D., DWYER, R. St. C., and GARLAND, J. A. (1969) *J. infect. Dis.*, **120**, 451.
- HALBERSTAEDTER, L., and PROWAZEK, S. VON (1907) *Arb. Gesundh.-Amte (Berl.)*, **28**, 44.
- T'ANG, F. F., HUANG, Y. T., CHANG, H. L., and WONG, K. C. (1957) *J. Hyg. Epidem. (Praha)*, **1**, 109.
- VAUGHAN-JACKSON, J. D., DUNLOP, E. M. C., DAROUGAR, S., DWYER, R. St. C., and JONES, B. R. (1972) *Brit. J. vener. Dis.*, **48**, 445.

Infection chlamydiale

Les progrès dans l'isolement dans un but diagnostique des *Chlamydia*, agents TRIC inclus, à partir de l'oeil, des voies génitales et du rectum

SOMMAIRE

La technique simplifiée d'un seul passage en culture sur cellules McCoy irradiées, en association avec certains perfectionnements pour la récolte et la conservation des spécimens, fut utilisée pour l'isolement de *Chlamydia*, dont l'agent TRIC.

L'emploi d'un écouvillon pour la récolte du matériel sur la conjonctive, l'addition de sérum animal au milieu de transport et l'utilisation d'un réfrigérateur à azote liquide pour la conservation et le transport des échantillons de la clinique au laboratoire ont permis d'atteindre un très haut niveau de sensibilité pour l'isolement de l'agent TRIC à partir d'yeux infectés (79,4 pour cent). Faisant intervenir différentes variantes de technique et à partir de prélèvements génitaux chez des malades atteints d'affections oculaires à l'agent TRIC, des *Chlamydia* ont été isolées en culture cellulaire chez presque la moitié des hommes et chez plus de 9 femmes sur 10. Des *Chlamydia* ont été isolés chez 44,8 pour cent des hommes présentant une urétrite non spécifique et, à partir de prélèvements génitaux, chez plus de 7 sur 10 des mères d'enfants atteints d'ophtalmie du nouveau-né due à l'agent TRIC.

La simplicité et la rapidité de ces techniques faciliteront, dans les pays développés, l'application en routine de la culture en cellules McCoy irradiées dans les services de diagnostic des infections de l'oeil à *Chlamydia* ou dans celles des voies génitales. En outre, dans les pays en voie de développement, il devient possible d'appliquer ces techniques dans des études épidémiologiques et dans l'évaluation de la valeur du traitement du trachome hyperendémique.