

Isolation of chlamydia in irradiated and non-irradiated McCoy cells

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SYNOPSIS Specimens from eye and genital tract were cultured in parallel in irradiated and non-irradiated McCoy cells and the frequency of isolation of chlamydia using these culture methods was compared. There was a significant difference between the frequencies of isolation; irradiated McCoy cells produced a greater number of positive results.

In 1969 Gordon and others showed that a method of culturing chlamydia in irradiated McCoy cells could be used to isolate this microorganism from clinical specimens. Since then such techniques have been simplified and altered (Darougar *et al*, 1971; Richmond, 1974) and recently a further modification, in which non-irradiated McCoy cells were used, has been described (Hobson *et al*, 1974). We have compared such a method with another method using irradiated McCoy cells.

Material and Methods

Conjunctival specimens were obtained from individuals who had clinical features of chlamydial ocular infection. Genital tract specimens were obtained from people attending a special clinic for sexually transmitted diseases and were mainly from cases of untreated non-specific urethritis (NSU) and consorts of such cases.

TISSUE CULTURE

McCoy cell cultures were maintained and passaged using methods similar to those of Darougar *et al* (1971) and Richmond (1974), the cells being grown and maintained in Falcon plastic flasks using Eagle's MEM (Hanks based) containing 10% previously tested fetal calf serum, 100 mg vancomycin, and 50 mg streptomycin per ml.

Where irradiated cells were used confluent monolayers in flasks were exposed to 6000 rad and two to four days later were trypsinized and seeded into flat-bottomed plastic tubes containing coverslips (150 000 cells per tube). These were incubated at 37°C; monolayers usually formed 24 hours after

seeding and at this stage the medium was replaced by 1 ml of fresh Eagle's MEM after which the tubes were inoculated with specimens.

Where non-irradiated McCoy cells were used, a similar procedure was carried out except that before specimen inoculation medium in the tubes was replaced by 1 ml of medium 199 containing fetal calf serum and antibiotics as above.

SPECIMEN PREPARATION

Specimens were collected in 1.5 ml of an appropriate transport medium (Richmond, 1974) and cultured as soon as possible. When suitable monolayers of cells were available for inoculation within 24 hours, specimens were stored at +4°C, otherwise specimens were kept at -70°C.

Each specimen was shaken with glass beads in a vortex mixer, and 0.2 ml was inoculated into each of three tubes containing coverslip monolayers, one of irradiated and two of non-irradiated McCoy cells. The tubes were centrifuged at 2000 RCF for 1 hour, after which the tube with irradiated cells was incubated at 35°C and the tubes with non-irradiated cells at 37°C, one of these being tightly capped and the other loosely capped in an atmosphere of 5-10% CO₂. After 48 hours' incubation, coverslips were stained using Giemsa's stain and were mounted in immersion oil; typical chlamydial inclusions were sought using a × 20 apochromat dry objective and a wide-field dark-ground oil immersion condenser.

Results and Conclusions

Results are shown in the table. The difference in numbers between specimens from cases of NSU and NSU consorts on one hand, and the totals in the respective sexes on the other hand, is composed of

	Conjunctiva	Male Genital Tract (Total)	Genital Tract NSU only	Female Genital Tract (Total)	Genital Tract Consorts of NSU only
No. cultured for chlamydia	76	44	28	26	12
No. +ve in irradiated McCoy cells	16	16	14	7	7
No. +ve in non-irradiated McCoy cells*	9	11	9	6	4

Table *Isolations of chlamydia in irradiated and non-irradiated McCoy cells*

*not in atmosphere of CO₂

specimens from individuals who had gonorrhoea, trichomonas infection, or monilial infection, or who were attending the special clinic but had no evidence of genital tract infection. Irradiated cell cultures were associated with an increased frequency of isolation of chlamydia from both eye and genital tract; the differences in numbers were small and when results from different sites were considered separately, $P > 0.05$, indicating that differences in frequency of isolation using the methods shown were not significant. However, when the *total* isolations obtained using the different methods were compared, applying Cochran's test, the isolation rate in irradiated cells was significantly higher ($P < 0.02$). Isolations in cultures incubated in CO₂ were fewer than in those not incubated in CO₂ and are not tabulated. The number of inclusions present was divided into three categories: (a) a few per coverslip; (b) at least one inclusion in many of the fields; and (c) one or more in most fields. There was little difference between the number of inclusions found using irradiated and non-irradiated cells, and about half of the positive results belonged to (a). The inclusions present in irradiated cells were larger, however, and more easily seen. It seems that a simplified culture method similar to that described by Hobson and others (1974) may be useful in the investigation of chlamydial infections, since it should be possible to employ such techniques more readily in microbiology laboratories, as they have already suggested; however, we did find that significantly more isolates were obtained using irradiated cells.

The number of specimens involved in our study is small, and it would be advisable to confirm these

findings in a larger series of culture attempts. Although these methods are technically simple, they are time-consuming, and we consider that one technician carrying out all the procedures necessary for cell culture, isolation, and microscopy could reasonably deal with about 35 specimens per week.

We wish to thank the consultant staff in the Special Department, The Royal Hospital, Wolverhampton and the Wolverhampton and Midland Counties Eye Infirmary for their invaluable help in supplying specimens, and the consultant staff of the Radiotherapy Department, Royal Hospital for arranging irradiation of tissue cultures. We are grateful to Professor R. E. O. Williams for general advice, and to Mrs. H. E. Tillett, Epidemiological Research Laboratory, Public Health Laboratory Service for advice concerning the statistics.

References

- Darougar, S., Kinnison, J. R., and Jones, B. R. (1971). Simplified irradiated McCoy cell culture for isolation of *Chlamydiae*. In *Trachoma and Related Disorders*, edited by R. L. Nichols, pp. 63-70. Excerpta Medica, Amsterdam.
- Gordon, F. B., Harper, I. A., Quan, A. L., Treharne, J. D., Dwyer, R. St.C., and Garland, J. A. (1969). Detection of *Chlamydia* (*Bedsonia*) in certain infections of man. I. Laboratory procedures, comparison of yolk sac and cell culture for detection and isolation. *J. infect. Dis.*, **120**, 451-462.
- Hobson, D., Johnson, F. W. A., Rees, E., and Tait, I. A. (1974). Simplified method for diagnosis of genital and ocular infections with *Chlamydia*. *Lancet*, **2**, 555-556.
- Richmond, S. J. (1974). The isolation of *Chlamydia* subgroup A (*Chlamydia trachomatis*) in irradiated McCoy cells. *Med. Lab. Technol.*, **31**, 7-9.

usually be recognized without reference to the legend.

Long lists of useful references, including the full titles of the articles, are given after every chapter.

A. S. HILL

Ocular Pathology. A Text and Atlas. By Myron Yanoff and Ben S. Fine. (Pp. xiv + 762; illustrated; \$60.00.) Hagerstown, Maryland: Harper and Row. 1975.

Good textbooks on ocular pathology are very rare: indeed, there still exists a need for a major scholarly authoritative work, with an accurate historical bibliography, covering all aspects of this speciality; in short, a modern counterpart to the four volumes of Parsons' *Pathology of the Eye* (1904). Until this is forthcoming—and it would seem with the great advances in this field and in medical science in general that only multiple authorship could provide it—the subject is well served by the valuable Atlas and Textbook by Hogan and Zimmerman and now by this most impressive and beautifully produced volume by two well-known ocular pathologists. It is written in the form of tabulated notes which, although not attractive to read as a book, is of particular value for quick reference and for teaching. It is remarkable how ably the authors have condensed the most up-to-date knowledge of so many aspects of ocular pathology, including the complications of immunobiology, into such succinct and accurate summaries, all lavishly illustrated by excellent examples of the conditions described.

This fine book invites comparison with the French work *Anatomie Pathologique de l'Oeil et de ses Annexes* by G. Offret, P. Dhermy, A. Brini, and P. Bec, which provides a similarly up-to-date account of a comparably high standard. The French book, however, is written in essay form, concerns itself more with the pathogenesis of the conditions it describes, and pays more attention to the provision of original source references, whereas the book under review is more richly and better illustrated, often with electron micrographs, and is rather more comprehensive. It is undoubtedly the best textbook of descriptive pathology now available in the English language and can confidently be recommended to all those seeking knowledge in this branch of pathology whether for study, teaching or reference.

NORMAN ASHTON

1975 Review of the Year Book of Pathology and Clinical Pathology. Pathology. Edited by Frank A. Carone, **Clinical Pathology.** Edited by Rex B. Conn. (Pp. 558; illustrated; £13.75.) Chicago: Year Book Medical Publishers. 1975. Distributed in the United Kingdom by Lloyd-Luke (Medical Books) Ltd, London.

The Year Book series is as old as the century, and its 75th birthday, celebrated by changing to a rich burgundy-coloured cover, is an occasion to review this essentially unreviewable anthology.

Just over 300 articles from the pathology journals of the English-writing world are presented as digests of 200-500 words, often with an illustration and always with a brief editorial comment. The latter usually mentions some other relevant articles on the subject. It is not a bad way to make a quick dip into any special branch of pathology and to find out what has been happening; not really a substitute for authoritative reviews, or for reading the primary journals, even if one seldom goes further than the summaries and titles, but a good 'long stop'. Personally, I enjoy the comments which add perspective and aid digestion. I also applaud the essay by one editor, Dr. R. B. Conn, on the discarding of useless tests, with particular reference to the NBT test.

The delay in publication is minimal; the 1975 edition published in July deals with the literature to September 1974; or so it is claimed, but, in fact, no paper from the *J. clin. Path.* is included after May 1974, thus omitting important papers, on gentamicin assay, for example. The production is good; the cost is no less than one has learned to expect.

H. E. M. KAY

Quality Control in Microbiology. Edited by James E. Prier, Josephine Bantola, and Herman Friedman. (Pp. x + 188; illustrated; £7.25.) London: University Park Press, Baltimore. 1975.

This is a collection of contributions to a symposium held in Philadelphia in November 1973. There are 14 contributors, three of them medical microbiologists.

Six of the contributors come from Government supported laboratories including CDC and ATCC; four of them have university or research appointments, two of them are from hospital departments, and two from commercial firms:

all are known for their work in the field of diagnostic medical microbiology.

There is an excellent contribution by Dr. R. C. Bartlett on 'Can we afford the price of quality?' and he also contributes useful appendices illustrating the method of monitoring in his own hospital department.

Contributions are inevitably uneven but all are well worth reading. That of Dr. Wright from the US Food and Drug Administration on antibiotic susceptibility discs tells how the Bauer-Kirby method was accepted as the standard test in the USA. This is not a popular method in Europe but the problems they encountered are of general interest.

The publishers claim that this book should be essential reading for all clinical microbiology personnel. In Britain, where the organization of laboratories is somewhat different, it will be of interest to directors of diagnostic laboratories and should be read by all those concerned with distribution of quality control material.

E. JOAN STOKES

Correction

In the paper 'Isolation of chlamydia in irradiated and non-irradiated McCoy cells' by L. Johnson and I. A. Harper (*J. clin. Path.*, 1975, 28, 1003) there is an error in the third paragraph of the paper under the subheading 'tissue culture'; the last two lines should read:

'100 µg vancomycin, and 50 µg streptomycin per ml'.