J. clin. Path., 1975, **28**, 1003-1004

Isolation of chlamydia in irradiated and non-irradiated McCoy cells

L. JOHNSON AND I. A. HARPER

From the Public Health Laboratory, New Cross Hospital, Wolverhampton WV10 0QP

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% CO_2. 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

Received for publication 10 June 1975.

	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 CO_2

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, $P > 0{\cdot}05$, 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 ($P < 0{\cdot}02$). Isolations in cultures incubated in CO_2 were fewer than in those not incubated in CO_2 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.