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MYCOPLASMA INFECTION OF TISSUE CULTURE

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SUMMARY

Thirty Vero (green monkey kidney cell) cultures were examined for detection of mycoplasma infection. Nine samples were found to be positive. The incidence of contamination caused by *Mycoplasma orale* (4 isolates) followed by *M. arginin* (3 isolates). *Mycoplasma salivarium* and *Acholeplasma laidlawii* were the least (1 isolate for each).

INTRODUCTION

Mollicutes; mycoplasmas and acholeplasmas, are samll, free-living unwalled prokaryotes that often contaminate cell cultures. The mycoplasma contamination causes serious problem in vaccine production, since tissue culture contaminated with mycoplasma grows rather well without apparent morphological changes and moreover, they may exert immunological activities that otherwise cannot be observed (Biberfeld and Gronowicz, 1976; Naot et al., 1979; Stanbridge et al., 1981; Proust et al., 1985).

Between 5 and 35 per cent of cell cultures in current use are infected with mycoplasmas, with the species *Mycoplasma orale, M. hyorhinis, M. arginini* and *Acholeplasma laidlawii* representing the majority of contaminating isolates (McGarrity and Carson, 1982).

Many species of mycoplasma exert no cytopathogenic effects on host cells, making infection difficult to detect, in cell culture the concentration of mycoplasma can easily reach 10^7-10^8 colony forming unit per millilitre without over turbidity, coupled with the fact that most mycoplasmas are resistant to commonly used antibiotics. Numerous methods for detecting mycoplasma infection have been developed but the most sensitive and specific is the standard microbiological cultivation procedure (DelGuidice and Gardella, 1984).

The cell cultures, serum, yeast extract have become indispensable substrates for the vaccine production, viral cultivation, titration and analysis of virus activity, therefore great precautions should be taken before using these materials.

The aim of the present work was the detection and exclusion of contaminated materials with mycoplasma before using in the vaccine production.

MATERIAL AND METHODS

1- Cell lines: Thirty Vero (green monkey kidney cell) from different departments at Serum & Vaccine Institute, were examined for mycoplasma.

2- Media:

Fluid media consisted of 70 ml PPLO broth (Difco), 20 ml heated-inactivated horse serum, 10ml fresh yeast extract, glucose or arginine (1%), phenol red (0.002%), and penicillin G (1000 u/ml) (McGarrity and Barile, 1983).

The samples were cultured for detection of mycoplasma contamination as described by Hay et al., 1989. The isolates were checked for biochemical behaviour (Ern and Stipkovits, 1973). Serological identification of recovered strains by growth inhibition test was carried out as described by Clyde (1964).

3- Reference antisera:

Specific antisera against *M. arginini, M. orale, M. salivarium* and *A. laidlawii* were obtained from Dr. S. Geary, Department of Pathobiology, Unviersity of Connecticut, USA.

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RESULTS

Thirty Vero (green monkey kidney cell) were checked for detection of mycoplasma contamination. Nine isolates were recovered, tested for digitonin sensitiviy and biochemical characterization. Eight isolates were found to be mycoplasma and only one acholeplasma. Serological identification of the strains gave the results shown in Table (1).

Table (I): Biochemical characterization and Serological identification of mycoplasmas contamianting cell cultures.

No. of isolates	Biochem. Glucose	Characterization Arginine	Digitonin	Serological identification
4	+	-	+	*M. orale*
3	-	+	+	*M. arginini*
1	-	+	+	*M. salivarium*
1	+	-	-	*A. laidlawii*

The highest incidence of contamination with *M. orale* (4 isolates), followed by *M. arginini* (3 isolates. *Mycoplasma salivarium* and *Acholeplasma laidlawii* were found to be the least (1 isolate for each).

DISCUSSION

Numerous methods for detecting mycoplasma infection have been developed, but the most sensitive and specific is the standard microbiological cultivation procedure (DelGuidice and Gardella, 1984).

In the present study, 30 Vero cell cultures from different departments of Serum & Vaccine Institute were examined for detection of mycoplasma contamination. Nine samples were found to be contaminated. Biochemical characterization and serological identification of the isolates showed that the highest incidence of contamination (may be transferred through the technicians or contaminated reagents) caused by *M. orale* (4 isolates) followed by *M. arginini* (3 isolates), while *M. salivarium* and *Acholeplasma laidlawii* were the the least (1 isolate for each).

McGarrity and Carson (1982) proved that *M. orale, M. hyorhinis, M. arginini* and *A. laidlawii* representing the majority of contaminating isoaltes of cell cultures in current use.

In the present study, we confirm the advantage of microbiological cultivation procedures for detection of mycoplasma contaminating cell cultures and we recommend that new lots of horse serum, yeast extract and other ingredients should be tested for mycoplasma before use in cell cultures.

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