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A comparative study of Radiation Sterilization of cell culture media (RPMI) and filtration sterilization method in cell culture laboratory

ALKHANSA, Mahmoud, ABDALLA.OMER, ALMAHI. Waleed, ELTEIB. Nahla, YOUSIF. Amna, SALIH. Ashraf
Sudan Atomic Energy Commission
Poster Number: ID: 401
alkhansa.salih@gmail.com

International Conference on Applications of Radiation Science and Technology
(ICARST 2017)
24 to 28 April 2017, Vienna, Austria

Abstract:

Introduction: Filtration is a safe method uses for media sterilization in cell culture laboratory. Aim of this study is using gamma radiation to sterile RPMI 1640 media in cell culture laboratory compared to filtration sterilization method. **Methods**: RPMI media was sterilized by gamma radiation and compared with filtered media; microbiology and Cell viability testing were done. **Results:** 20 kGy was destroyed bacteria rather than 5.0 kGy, while poor cell growth was observed in irradiated media compared to filtered media. **Conclusion:** Gamma radiation could be sterile media with doses larger than 5 KGy but not suitable for sterile media cell culture in laboratory because increasing the probability of poor cell growth and phenol red was degraded by gamma radiation.

1. Introduction:

Introduction: Cell culture media is uses in biopharmaceutical to stimulate the natural environment of the cell (1). RPMI-640 was developed to culture human lymphocytes cells (2).

2. Methods:

2.1. Media preparation:

RPMI-1640 media in powder form, it was added to NaHCO3 and dissolved in distilled water, pH meter used for pH determination

2.2. Irradiation

Cobalt 60 gamma irradiator at 1.26 kGy/h was used for radiation purpose.

2.3. Microbiology screening :

Testing of bacteria, fungi and moulds

2.4. Cell viability test:

0.5 ml of lymphocytes added to 4.5 ml from RPMI media, 1.2% penicillin/streptomycin, 1% Fetal Bovine Serum, 30µml Phytohemagglutinin and then incubated in CO_2 incubator. Cells were counted using chamber.

3. Results :

Table1: Microbiology test

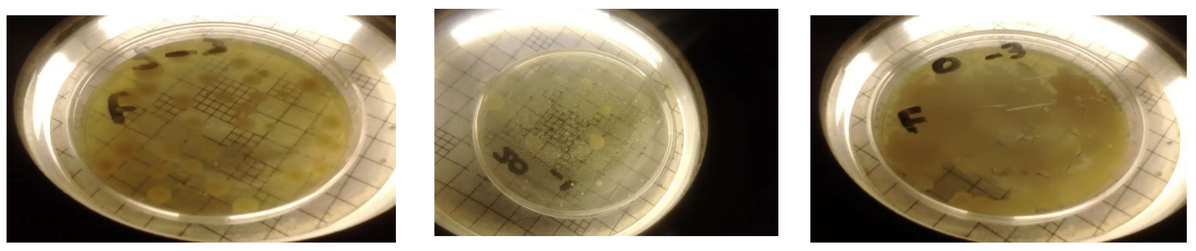
Testing	5 KGy	20 KGy	filtration
Total viable count	7.1×10^6	6.65×10^2	1.55×10^3
Total coli form	Negative	4.0	>18
E. Coli	Negative	Negative	Negative
Fungi	Negative	Negative	Negative
Yeast	Negative	Negative	Negative
Moulds	Negative	Negative	Negative

Table2: pH for doses of gamma radiation and filtration

pH pre irradiation	pH post irradiation	Media color
7.2	7.2	change
7.2	8.0	colorless
7.2	7.3	Not change

Table3: Cell viability test

Sterile media	Cell Death after 24 hours	Cell growth after 48 hours
05 kGy	50%	50%
20 kGy	70%	10%
Filtration	10%	> 60%



Figur1: Fungi testing for media irradiated of 5 KGy ,20 KGy and filtered media

Figure 2: Bacteria testing for media irradiated of 5 KGy and 20 KGy

Figure 3: Non irradiated RPMI media - pH 7.2

4. Conclusion :

Gamma radiation could be sterile RPMI 1640 media with doses larger than 5 KGy but media integrity was effected. Radiation sterilization among RPMI media may useful for liquid RPMI media without red phenol. Further research on phenol red degradation by gamma radiation, and amino acids concentrations post gamma irradiation strongly recommended, that might help in modifications of media for various cell culture applications using gamma radiation.

5. References:

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DOI http:// dx.doi.org /10.13070 /mm.en.3.17 5

Bibliography :

Alkhansa Salih Mahmoud, BSc, MSc in biochemistry
Nuclear Application in Biological Sciences Institute