RAD 2022 Conferences

Evaluation of radiosensitivity and in vitro immunoreactivity of *Streptococcus agalactiae* strains, γ- irradiated in different conditions

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Introduction

Group B Streptococcus (GBS) is an opportunistic pathogen that asymptomatically can colonize the genitourinary tract of adults, including pregnant women, being the main cause of bacterial infection associated with birth. Rapid tests are usually performed to reduce the risk of transmission from the infected mother to the newborns or take appropriate measures to control the infection. The majority of these rapid tests lack associated positive control.

In order to develop a safe, reactive, and stable positive control, we applied gamma irradiation as inactivation method on bacterial suspensions of *Streptococcus agalactiae* – the group representative. For this we have investigated also the influence of storage conditions after gamma irradiation on the viability of the bacterial cells and, the *in vitro* antibody binding affinity (ELISA).

The *aim* of this study is to investigate the influence of a series of factors on the stability of *Streptococcus agalactiae* strains suspensions, exposed to gamma irradiation treatments, for future applications like developing external positive controls for rapid identification kits or as the main component of gamma inactivated vaccines.

Materials and Methods

- Bacterial strain: *Streptococcus agalactiae* (ATCC 13813)

S. agalactiae was cultivated overnight in Brain Heart Infusion Broth at 37^0 C with stirring (200 rpm).

For establishing the D_{10} value, *S. agalactiae* cells were resuspended in Phosphate Buffer (PBS) (pH=7.4) and adjusted at ~10^7 CFU / ml (CFU – Colony Forming Units)

For assessing the immunologic reactivity (ELISA), the suspension was made in PBS with Sodium Azide (0.09%) (PBS with 0.09%NaN3) and adjusted at ~10^{13} CFU/ml.

- Irradiation treatment

Exposures to gamma radiation were performed within a Gamma Chamber GC-5000 (Co-60 Research Irradiator). The dosimetry was conducted with ECB dosimetry system (oscillometric reading) (ISO/ASTM 51538:2009) with an average uncertainty of 3 %. All doses are expressed as absorbed dose in water.

- Establishing D_{10} value for *S. agalactiae* (ATCC 13813)

D_{10} value for *S. agalactiae* was established by irradiating equal portions of suspensions at incremental doses ranging from 25 Gy to 3 kGy, in triplicates. Unirradiated controls were associated with the test.

- *In-house* indirect ELISA protocol was developed for assessing the immunologic reactivity of suspensions, at each irradiation dose, after different storage conditions and time periods. The test was performed on a time-base to check the stability of suspensions.
- Irradiation dose applied for evaluation the immunologic reactivity: 3 kGy, 5 kGy, 10 kGy, 25 kGy. Unirradiated controls were associated with the tests.
- Storage conditions: room temperature (22-25^{0}C) and refrigerator (2-8^0)
- Storage time: freshly prepared (24 h) suspensions and 6 months old suspensions.

ELISA protocols were performed in high binding 96 wells plates (Nunc) using primary mouse monoclonal antibody against *Streptococcus agalactiae* (Santa Cruz Biotechnology, Inc) and secondary monoclonal rabbit - mouse IgGκ light chain binding protein (m-IgGκ BP) conjugated to horseradish peroxidase (HRP). Every condition was tested in four replicates. Positive and negative controls were associated to the tests.

- The plate read and data acquisitions: after stopping the reaction, the plates were read with SpectraMax i3x Multi-Mode Plate reader (Molecular Devices) using a predefined reading protocol of the device "ELISA with HRP and TMB" in the end point mode. The dates were acquired using SoftMax Pro 7.1 and analyzed in Excell.

Results and discussions

- Establishing D_{10} value for *S. agalactiae* (ATCC 13813)

D_{10} of ~50 Gy, was calculated for bacterial suspensions, through linear regression analysis.

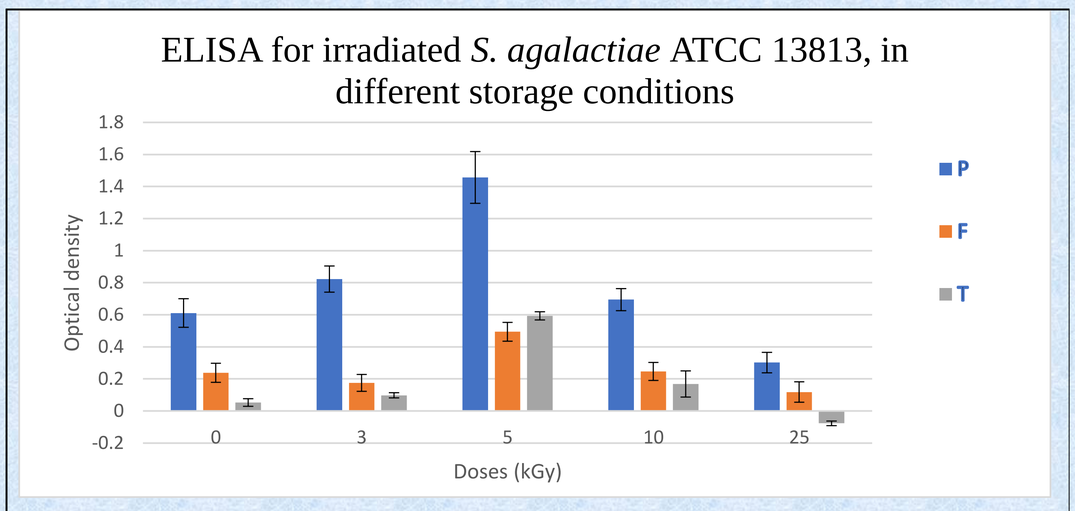

Fig. 1. Irradiated *S. agalactiae* ATCC 13813 - ELISA tests results, in different storage conditions: P- freshly prepared suspensions; F – 6 months old irradiated suspensions, stored at refrigerator; T - 6 months old irradiated suspensions, stored at room temperature

ELISA results for *S. agalactiae* ATCC 13813 show that irradiated samples at 5 kGy have the highest immunoreactivity values for all three storage conditions. Most probably, at this dose, the epitopes (antigens) are better exposed, being favorable to the specific interaction between Ac-Ag. The chemical interaction resulting from the combination of irradiation dose and sodium azide is not excluded to have a preservative effect on the structure of the protein of interest.

The immunogenic reactivity of the 6 months old suspensions significant decrease for the irradiated suspensions at 25 kGy and kept at room temperature. A similar decrease was recorded for non-irradiated suspensions and room temperature.

The low calculated D_{10} value (50 Gy) allowed us to make very concentrated suspensions, that irradiated at the chosen dose of 5 kGy , are safe to handle by the medical staff - the target group for possible future use of the suspensions. The high concentration of the suspensions covers possible reactivity decay over time and since, prolongs the shelf life of the product.

Conclusions

Gamma irradiation treatment of the final product can be applied for stabilizing a ready-to-use, external positive control for rapid GBS tests. Results of *in-vitro* immunoreactivity also have the potential of being further exploited for developing irradiated vaccines.

References

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