BIO_06 - Anti-PBP2a monoclonal antibody Fab-like fragment radiolabeling with Technetium-99m for *in situ* diagnosis of methicillin-resistant Staphylococcus aureus (MRSA)

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Introduction: Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the main pathogens associated with serious nosocomial and community infections and has high mortality rates. The existing diagnostic methods are not efficient for the exact detection of infectious foci, which would facilitate the best treatment choice. In this scenario, the MRSA anti-PBP2a Fab-like fragment (Fab) can be used as an imaging agent for a precise *in situ* diagnosis of infectious foci.

Objectives: This study aims to perform and evaluate the Fab radiolabeling with Technetium-99m (99mTc) for *in situ* diagnosis of MRSA studies.

Methodology: The Fab was radiolabelled with 99mTc (99mTc-Fab) by the indirect method using HYNIC as bifunctional chelating agent and the stability of the complex was analyzed by paper chromatography (ITLC-SG) in a gamma counter after different incubations time in mouse serum at 37ºC. After, radioimmunoconjugate structural integrity was analyzed by SDS-page and 99mTc-Fab binding ability to recombinant protein PBP2a was assessed by western blot (WB). 99mTc-Fab concentration was estimated by ELISA, using a non-labeled Fab on a standard curve. Finally, to analyze bacteria detection limit *in vitro* with 99mTc-Fab, serial dilution of MRSA Brazilian epidemic clone inoculum were added with 99mTc-Fab at an average activity of 300 microcuries (μCi) into different tubes, incubated at 37ºC for 30 minutes, and washed. After that, CT (computed tomography) and SPECT (single photon emission computed tomography) images were performed (in duplicates) to obtain estimated values of bacteria concentration detectable through imaging.

Results: The 99mTc-Fab maintained radiochemical purity close to 100%, demonstrating stable radiolabeling up to 8h after inoculation in mouse serum, but maintained high purity rates until the last point analyzed (24h). Radiolabelling did not affect the structural integrity of Fab, by SDS-page analysis, and WB demonstrated that Fab maintains the ability of target epitope binding, but apparently weaker. Before radiolabeling, Fab concentration was 0.6mg/ml, and ELISA showed that 99mTc-Fab was 0.3mg/ml. The bacteria limit of detection *in vitro* by SPECT was $8x10^7$ UFC.

Conclusion: The antibody radiolabeling process was successful, despite the impact on its yield, as it did not interfere with its structure and binding ability to its target, both the recombinant and the native protein, the latter present on the bacterial surface. From perspective, we will realize the optimization of radiolabeling to increase its yield and perform imaging tests in a murine animal model.

Keywords: Radioimmunoconjugate, Technetium-99m, Methicillin-resistant *Staphylococcus aureus*