

ORT_20 - Comparison of chaotropic agents and incubation temperatures for Avidity-ELISA

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Introduction: The avidity index (AI) measures the binding strength between the antibody and the antigen, being an important tool to evaluate the affinity maturation of the immune response in vaccine studies. It can be assessed by a modified ELISA, using chaotropic agents; however, standardization differences affect the final results.

Objective: This study compared the effect of three chaotropic agents and their incubation temperatures in avidity-ELISA.

Methodology: Immune sera from Swiss mice were collected after immunization with three doses of outer membrane vesicles of meningococci alone (OMV) or complexed with Aluminium hydroxide (OMV+AH) or dimethyl dioctadecyl ammonium bromide (OMV+DDA). Avidity-ELISA was conducted using potassium thiocyanate (KSCN) 1.5 M, Urea 6 M or Diethanolamine (DEA) 0.1 M as chaotropic agent, which was incubated for 20 minutes after sera incubation, at 4°C, room temperature (RT) (20-25°C) or 37°C. AI was considered as the ratio between the OD without treatment / the OD with treatment and classified as low if ≤ 30 , intermediate if between 30-50% and high if $\geq 70\%$. The AI was compared using Friedman and Dunn's post-test. $P \leq 0.05$ was considered significant.

Results: The AI presented little variability, without a statistical difference, when Urea and DEA were used, even at different temperatures. KSCN incubated at 4°C provided statistically higher AI than at 37°C ($P \leq 0.05$) for OMV+AH and OMV samples. 2/5 samples of OMV+AH, 3/4 samples of OMV+DDA and 2/4 samples of OMV changed the AI range when KSCN was used at different temperatures. Thus, the mean AI of each group, obtained by KSCN, were lower than Urea and DEA.

Conclusion: Urea and DEA provided more similar results regardless of incubation temperature, while KSCN seemed to be more temperature-sensitive. The lower AI provided by KSCN might reflect its chaotropic activity, which impairs not only hydrogen bonds and van der Waals forces but also electrostatic interactions. When using ELISA-Avidity, it is important to standardize the chaotropic agent and incubation temperature, which can also fluctuate inside a laboratory.

Keywords: Avidity index; ELISA; Chaotropic agent