Abstract:

A method for purifying macroschizonts of Theileria parva from bovine lymphoblastoid cells, propagated in vitro, was developed. This method involved three steps. First, the macroschizonts were liberated by disrupting host cells suspended in growth medium at 4 × 106 cells/ml at 300–400 psi, using the Stansted cell disrupter. This yielded 80–90% disrupted cells while causing minimum damage to the macroschizonts. Second, the host cell nuclei were separated by (a) centrifuging the lysate at 300g for 60 min, (b) resuspending the pellet in 0.02 times the volume of initial host cell suspension in Leibovitz's L15 growth medium, and (c) lysing the host cell nuclei by adding nucleus-lysing buffer (NLB, containing 0.14 M Tris, 0.1 M HCl, 0.12 M glucose, and 0.5 M NaCl adjusted with NaOH to pH 7) to 0.2 times the volume of initial host cell suspension. The resulting chromatin precipitate was removed by adding DE-52 cellulose equilibrated with NLB and allowing the precipitate to sediment. Lastly, the final suspension obtained in the second step was applied on a DE-52 cellulose column which was equilibrated with the elution buffer (NLB with 10% fetal, or newborn, bovine serum, pH 7). Macroschizonts free of intact host cells and naked host cell nuclei were collected in the eluate. The protein yield was 2.7 mg per 109 starting undisrupted host cells, which was 1.7% of the total starting protein