A lyophilized formulation to extend the shelf-life of tuberculin PPD

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Abstract
This study was aimed to develop a dry purified protein derivative (PPD) preparation to extend the shelf-life of tuberculin PPD. Five percent sucrose (S), 6.5% mannitol (M), 2.5% trehalose (T) or 0.3% Hemaccel (H) was added to each formulation. In vivo and in vitro analyses were carried out to determine the efficacy of the lyophilized products. In the in vivo test, the delayed type hypersensitivity (DTH) responses of the lyophilized preparations were compared to the liquid preparation (CL) after injection into BCG vaccinated guinea pigs. The preparations of H, M, T, and S generated DTH responses of 100, 90, 89, and 60%, as compared to the response of CL, respectively. There was no loss of tuberculin activity in the H formula. A statistically significant difference in activity was found between S and CL (p<0.05). The cellular test for IFN-\(\gamma\) secretions was performed using the whole blood of human subjects screened for DTH response to tuberculin PPD Mantoux tests. The detection of IFN-\(\gamma\) secretions was done using ELISA and the efficacy was expressed in terms of percentage of IFN-\(\gamma\) responses to the tuberculin antigens. The results of CL, H, M, T and S were 3.28, 10.40, 0.84, 1.52 and 1.29%, compared to mitogen stimulation, respectively. The lyophilized H, M and T formulations and the liquid CL were studied for their shelf-life stability. Accelerated degradation was done by storing the samples at higher temperatures of 37°C and 56°C for 3, 6, 9 and 12 months. All the tuberculin PPD solutions were injected into BCG vaccinated guinea pigs at the end of each storage period and the activity of each solution was evaluated. The formulation with the Hemaccel as excipient gave a superior response than the others at the normal storage temperature of 4°C for 12 months. Therefore, Hemaccel provides protection for PPD activity. This supports the potential for the development of lyophilized tuberculin PPD with the addition of 0.3% Hemaccel to extend shelf life.