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Title: The effect of temperature during sputum processing on inflammatory markers

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Body: Objective: To evaluate the effect of temperature in sputum processing on biochemical markers in induced sputum. Method: Sputum specimens from 28 asthmatics were divided into two or four equal parts. 0.1% dithiothreitol (DTT) were added and placed in a shaking water bath at 37°C or rocker at 4°C for 15 minutes and 30 minutes. The collected supernatants stored at -80°C. Cells were stained with hematoxylin eosin stain. IL-5, IL-10, IL-17, IFN gamma, and TNF alpha were measured using the Bio-Plex® suspension array system in the supernatants. Results: Processing sputum at 4°C for 15 minutes and 30 minutes, processing sputum at 37°C for 15 minutes and 30 minutes resulted in similar total cell counts and differential cell counts. The concentration of all cytokines processed at 4°C showed no change when the processing time increased. However, the concentration of IL-5, IL-17 and TNF alpha processed at 37°C appeared to increase when the processing time increased. There were no differences between the concentration of IL-5, IL-10, IL-17, IFN gamma of the samples treated at 4°C and 37°C for 15 minutes. Further, there were no differences between the concentration of IL-5, IL-10, IL-17, IFN gamma of the samples treated at 4°C and 37°C for 30 minutes. The concentration of TNF alpha in the samples treated at 37°C for 30 minutes was significantly higher than those treated at 4°C for 30 minutes [22.90(52.01) vs 13.86(10.47)pg/ml, p<0.05]. Conclusion: Processing sputum at 4°C and at 37°C resulted in similar cell total counts and cell differential counts. Processing sputum at 4°C provides a valid characterization of cytokine expression profile and appears to be superior to processing sputum at 37°C.