This paper proposes a novel regularisation method for the estimation of large covariance matrices, which makes use of insights from the multiple testing literature. The method tests the statistical significance of individual pair-wise correlations and sets to zero those elements that are not statistically significant, taking account of the multiple testing nature of the problem. The procedure is straightforward to implement, and does not require cross validation. By using the inverse of the normal distribution at a predetermined significance level, it circumvents the challenge of evaluating the theoretical constant arising in the rate of convergence of existing thresholding estimators. We compare the performance of our multiple testing (MT) estimator to a number of thresholding and shrinkage approaches in the literature in a detailed Monte Carlo simulation study. Results show that the estimates of the covariance matrix based on MT procedure perform well in a number of different settings and tend to outperform other estimators proposed in the literature, particularly when the cross-sectional dimension, N, is larger than the time series dimension, T. Finally, we investigate the relative performance of the proposed estimators in the context of two important applications in empirical finance when N≯T, namely testing the CAPM hypothesis and optimising the asset allocation of a risky portfolio. For this purpose the inverse covariance matrix is of interest and we recommend a shrinkage version of the MT estimator that ensures positive definiteness.