Separating pure sperm cells from sexual assault specimens is a key to solving various sex offenses. The methods to separate sperm and epithelial cells from sexual assault specimens have been researched diversely, but controlling sexual assault specimens not only need skilled techniques, but also require a considerable amount of time and effort in laboratory works. Differential DNA extraction methods have been assessed using various tests to get intact male DNA from mixed vaginal secretion. In this study, the multiplex separation method assessed with the single-tube preferential lysis frequently used method to the present has been verified applicability of sexual assault specimens (virginal swabs, underpants, and sanitary pad in twenty-five specimens) to separate efficiently sperms from sexual assault specimens. The results show multiplex separation method was able to separate intact sperm cells, but not significantly different from preferential lysis method in terms of purity, and no distinct differences were found on extraction efficiency as a result of quantifying DNA concentration using Quantifiler® Duo DNA Quantification Kit. Moreover, multiplex separation method may have many advantages, most notably rapidity and processing capacity to deal with twenty or more sexual assault specimens at the same time. And to conclude, we suggest that select each method depending on the amount of specimens.