Acrosome reaction is a prerequisite for successful fertilization. Information on the inter and intraobserver variations in acrosome reaction results is not readily available. Our study examined the inter- and intraobserver variation in assessing the acrosome reaction in cryopreserved sperm. Semen specimens were obtained from 15 healthy volunteers with proven fertility according to the World Health Organization criteria. The ejaculates were diluted after liquefaction (1:1, vol./vol.) with TEST-yolk buffer containing glycerol and then frozen in liquid nitrogen. The spontaneous acrosome reaction was assessed by fluorescein isothiocyanate conjugated peanut lectin. Sperm viability was determined by Hoechst-33258. Seminal smears were prepared, and to assess interobserver variation, two andrologists (SCE and RKS) masked to the identity of the smears determined whether the acrosome reaction had occurred. A total of 100 spermatozoa were counted twice in each smear. Similarly, a rater (SCE) was masked to the identity of the slides and then scored each slide (n = 15) twice to determine the intraobserver variability. The differences in reaction frequency between observers were analyzed with the average coefficient of variation (CV), and the intraobserver variation was determined with the intraclass correlation coefficient (ICC). Differences were considered significant at P < 0.05. The interobserver difference was 1.31% ± 9.53% (P < 0.60; CV = 6.5%; and ICC = 0.81 at 95% confidence interval). The intraobserver difference was -0.29% ± 2.4% (P < 0.65; CV = 1.6%; and ICC = 0.98 at 95% confidence interval). We conclude that trained observers can assess the occurrence of the acrosome reaction with high accuracy and reproducibility.