Testosterone (T) has multiple significant physiological effects in women. To date no rapid, simple assay of total T has been shown to produce reliable results in women at the low end of the normal female range. The aim of this study was to evaluate the accuracy of a direct radioimmunoassay (dRIA) for total T by comparing values for total T measured by this assay with values determined by a conventional RIA (cRIA) method that utilizes extraction and chromatographic steps prior to quantification. Methods: Fasting serum samples were obtained from a sub-group of 259 healthy women, aged 18-75 years, randomly recruited from the community and stored at -80°C. Total T was measured by the dRIA method using antibody coated tubes and iodine-labeled T tracer. For comparison, total T levels were also measured using the cRIA after organic solvent (ethylacetate : hexane (3 : 2)) extraction and Celite column partition chromatography prior to RIA. Results: The mean T level by dRIA was 0.76 nmol/L (median 0.70, SD 0.54, min 0.10, max 3.2). The mean difference between the two measurements (dRIA-cRIA) was –0.28 (SD 0.3). The limits of agreement using the Bland-Altman approach on log transformed data showed that, on average, the dRIA value was 63% of the cRIA value and that 95% of the time the dRIA estimate lay between 26% and 155% of the cRIA estimate. However, with respect to clinical application, for classification of values in the lowest 10th centile, agreement between assays was seen in 245/259 women (Kappa = 0.68) Conclusion: The dRIA is a clinically useful assay that provides precise measurements of total T in women, particularly when values are low, and is appropriate for the study of the issue of ‘low’ T within the female population.