Appendix A: Testosterone Testing and Measurements in BC

Testosterone exists in multiple compartments in the patient plasma. Owing to its non-polar chemical structure, only a very small fraction of testosterone is freely dissolved, and this is the biologically active fraction. Testosterone is also heavily bound to two plasma proteins: (1) albumin which is high-capacity (i.e., high in concentration) and low-affinity (i.e., loosely binding) and (2) sex hormone binding globulin (SHBG) which is low-capacity (low in concentration) and high-affinity (avidly binding). Pardridge[1] hypothesized that both the albumin-bound and the free testosterone were relatively biologically available compared to the SHBG-bound testosterone. He popularized the measurement of so–called "bioavailable testosterone" which is the sum of the free and albumin–bound fractions of testosterone. With these definitions in mind, testosterone can be measured as follows:

Total Testosterone: The sum of all testosterone fractions in the patient plasma, whether free or protein bound. In BC this is routinely measured by automated immunoassay at most laboratories and by tandem mass spectrometry at St. Paul's Hospital.

Bioavailable Testosterone: The sum of free testosterone and albumin-bound testosterone. This can be measured by selectively precipitating SHBG and performing a total testosterone assay on the remaining solution ("supernatant"). Due to poor repeatability, this method is no-longer employed in BC. Alternatively, bioavailable testosterone can be calculated from the total testosterone, SHBG and albumin concentrations using one of several formulas,[2] most often Vermeulen's equation.[3] Calculating bioavailable testosterone is probably a superior approach to the method of selective precipitation but is hampered by the fact that different kits for measuring total testosterone and SHBG produce different numerical results on the same patient sample. This leads to poor inter–laboratory comparability for this test. Consequently, each lab must generate reference intervals specific to their methods for total testosterone and SHBG. Given the fact that free and bioavailable testosterone demonstrate more age–dependence in older males than total testosterone,[4,5] many subjects are required to accomplish this.

Free Testosterone: This is the testosterone that is freely dissolved in the patient plasma. In BC, this is performed by measuring total testosterone, SHBG, and albumin and then calculating the free testosterone in the same manner as the bioavailable testosterone is determined.2 Calculated free testosterone is essentially a constant multiple of calculated bioavailable testosterone and neither offers any advantage over the other if both reference ranges are identically validated. In extenuating circumstances, free testosterone can be measured by a reference method using equilibrium–dialysis followed tandem mass spectrometry. This is not offered in Canada at the present time. Methods for free testosterone by analogue–based radioimmunoassay perform so poorly that they have been discontinued in BC and elsewhere.[6,7,8]

Salivary Testosterone: Saliva offers an ultrafiltrate of the plasma and therefore salivary testosterone correlates with free testosterone in both men and women.[9,10] However, tandem mass spectrometry is required to accurately quantify testosterone at the low levels seen in saliva (down to 0.005 nmol/L for women). This assay is not currently offered at any Canadian reference laboratory. However, results depend on the manner in which saliva is collected and are not clinically equivalent to free testosterone.[10] For this reason, salivary testosterone is, at present, a research–level tool we do not recommend incorporating it into routine clinical practice.

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