Supplementary data: Testosterone suppression in the treatment of recurrent or metastatic prostate cancer – A Canadian consensus statement

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Accurate measurement of testosterone levels during androgen-deprivation therapy (ADT) is critical to providing optimal care for men with prostate cancer. The following tables provide guidance for sample collection and handling, direction on data reporting (Supplementary Table 1) and specifications for the use of mass spectrometry for assay of testosterone in serum samples in this context (Supplementary Table 2). This document is intended to facilitate dialogue between clinicians and clinical laboratory staff by: 1) providing the details needed to assess the capacity for a selected assay to accurately measure serum testosterone levels ≤0.7 nmol/l; 2) indicating steps to ensure analyte (i.e., testosterone) preservation in collected samples; and 3) providing detailed settings to use in performing the liquid chromatography tandem mass spectrometry (LC-MS/MS)-based assay.

General notes on testosterone assay for men receiving ADT for prostate cancer

- **Frequency of testing:** Repeat testing on a regular basis in the context of ADT for prostate cancer (every 3–6 months) is recommended.
- **Assay consistency:** Consistent use of the same methodology is preferable for monitoring a patient's testosterone levels throughout ADT. A change in testosterone assay during treatment may result in less comparable results and may impact clinical decisions and treatment strategy (whether or not castrate level is attained).

Steps clinicians may take to ensure accurate testosterone measurement in the context of ADT for prostate cancer

- Communicate with directors of laboratories to which patients receiving ADT will be referred and provide specifications for sample collection, processing and analysis (Supplementary Tables 1 and 2) to ensure they can provide accurate testing either in-house or through outsourcing. Elicit feedback on sample collection/handling to ensure analyte preservation.
- Clearly indicate the need for measurement accuracy at low testosterone levels on requisition form (e.g., select testosterone assay for women or children, add notation indicating purpose for assessing castrate level, i.e., patient on ADT, etc.).
- Direct patient to deliver sample only to specific labs vetted for providing accurate assay of testosterone at low levels by:
  - Providing a list of pre-approved labs.
  - Sending an assay specifications sheet (Supplementary Table 2) to accompany the requisition for the patient to present to their lab of choice to ensure the lab can provide the appropriate assay.

References

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Supplementary Table 1. Details of sample processing for testosterone analysis

Sample collection, handling, analysis, and reporting
Sample collection and handling
• Specimen: Serum (or plasma according to lab preference) • Collection medium: Type of tube and volume according to lab preference • Volume: Preferred minimum to permit repeat analysis
Forms and shipping
• Requisition form: Look for any additional specifications regarding type of test or assay, including notes that indicate need for accuracy at low testosterone levels • Transportation (if outsourcing): Send specimen frozen (or according to lab preference)
Testing method and timing
• Validated LC-MS/MS is preferred. Use IA only if MS unavailable or analysis at a reference lab not possible; ensure the IA method is externally validated against MS • Turn-around time: Goal is to provide test results to clinician within 7–10 days after receipt of sample
Report to clinicians
• Test result: Total testosterone assay result and comparison to castrate level goal of ≤0.7 nmol/l in males undergoing ADT for prostate cancer • Assay type: Inform clinician of method used (preferably MS; alternative is IA externally validated against MS). If externally validated IA is used, assay and method details should be communicated to the clinician with the assay result

ADT: androgen-deprivation therapy; IA: immunoassay; LC-MS/MS: liquid chromatography tandem mass spectrometry; MS: mass spectrometry.

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Supplementary Table 2. Mass spectrometry (MS) specifications for testosterone analysis

Mass spectrometry assay specifications, equipment, method development, and validation
Sensitivity of MS methods
• A sensitivity of <0.3 nmol/l (corresponding to approximately half of the target value of 0.7 nmol/l), with a CV ≤20% and a bias <15% is required[1] - The sensitivity of <0.3 nmol/l by LC-MS/MS is achievable - van der Sluis et al reports a sensitivity of 0.1 nmol/l[2]
Equipment and settings
• The LC-MS/MS laboratory equipment needs to function in selected reaction monitoring (SRM) mode (enhanced specificity) - Total testosterone is detected by SRM in the positive ion mode, quantified by the ion transition m/z 289 → 97 for testosterone and 292 → 100 for the internal analytical isotopic standard [$^{13}C_3$-testosterone][3,4] - For confirmation of the analyte, the transitions m/z 289 → 109 for testosterone and 292 → 112 for the isotopic standard are used[3,4] - Assay testing intervals: <0.3 nmol/l to 100 nmol/l[2]
Method selection, development, and validation
• LC-MS/MS methods optimized for higher throughput analysis are available • Development and validation of LC-MS/MS methods[1-3,5-10] includes - Use of an internal standard [$^{13}C_3$-testosterone] - Use of a certified source of testosterone standard (e.g., Australian National Measurement Institute reference material [NMI M914]) or NIST (National Institute of Standards and Technology) - Use of commercially available calibrators (e.g., ChromSystems) • Best practices according to clinical laboratory standard of practices: Clinical and Laboratory Standards Institute Guidance documents for analytical assays (C62-A and C57; www.CLSI.org)[1,6] • External quality assessment programs: UK NEQAS, CDC program[5,7]

ADT: androgen-deprivation therapy; CV: coefficient of variation; IA: immunoassay; LC-MS/MS: liquid chromatography tandem mass spectrometry; MS: mass spectrometry; SRM: selected reaction monitoring.