To obtain high selectivity and sensitivity, as well as accurate relative quantification of each protein, a mass spectrometry-based platform was used to analyze these samples. An MRM-based approach using a linear ion trap MS was taken to specifically measure PSA, hCG, and total Phos A/B proteins. Label-free quantification was used to determined relative abundance of each peptide/ protein.

The samples were suspended in 300mM NaCl and 10µL of human serum-equivalent was processed per sample per preparation method. Each sample was depleted of the top 12 highest abundant proteins in human serum using Genway Seppro immunoaffinity columns (IgY12). The proteins were then denatured with 8M urea, ammonium carbonate (pH 10.5) was added to increase pH, and then proteins were reduced and alkylated using a volatile reaction (acetonitrile, iodoethanol and triethylphosphine) at 37°C for one hour and then dried completely using a speed vacuum. Samples were resuspended in modified trypsin and incubated overnight at 37°C. The sample was acidified with formic acid and the entire sample was injected on a reverse-phase C18 column using a Thermo Surveyor autosampler and MS pump. The HPLC was coupled to a microspray ESI-MS/MS (Thermo LTQ).

Each protein was measured using two peptides in an SRM/ MRM method. The precursor ion of each peptide was scanned and then the peptides were fragmented. Three fragment ions per peptide were also collected. The Area-Under-the-Curve (AUC) was calculated for each peptide using XCalibur Processing Method software and relative quantification calculations were made using Microsoft Excel. This procedure was used to measure PSA, hCG, and total Phos A/B. The phosphopeptide from Phos A was not detected using this procedure due to the pH conditions of the volatile reduction and alkylation reaction. Due to time limitations we did not participate in quantification of the phosphorylated peptide for Phos A, and instead measured total Phos A/B using the LTQ.

The methods described here are published and well-accepted in the field of mass spectrometry. Label-free quantification using mass spectrometry is frequently used in our laboratory, and therefore, we did not incur any novel or unexpected results to report using this methodology.