Title: Calcium

Presenter: Dr. Christopher McCudden, Ph.D., FACB, DABCC

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Slide 2:
The objectives of this presentation are to describe laboratory assessment of calcium, focusing on the differences between total and free calcium measurement and the use of various correction formulas to adjust for albumin or pH. Pre-analytical considerations involved in calcium testing will also be described.

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Calcium is an essential mineral required for a number of physiological processes, including muscle contraction, cell division, and signal transduction. The vast majority of calcium is found within the skeleton, where it serves a key structural role. Approximately 1% of calcium is found in soft tissues and only a small fraction is found in the extracellular fluid. It is from this small pool that blood samples are drawn to assess calcium balance clinically.

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The extracellular fluid calcium pool is divided into three different forms. These consist of protein-bound calcium, free or ionized calcium, and anion-bound calcium. The majority of protein-bound calcium is complexed with albumin, the most abundant serum protein, but binding also occurs with other globulins. Free calcium accounts for 45-50% of the total and is the biologically active form of the mineral. It is this free form that is directly regulated by hormones such as PTH and vitamin D. Extracellular free calcium exerts its action by binding to the calcium sensing receptor, which is widely distributed throughout the body. Anion-bound forms include complexes with phosphate, bicarbonate, citrate, and lactate. Together, protein-bound, free, and anion-bound calcium are measured as total calcium. While total calcium does not change as the mineral shifts between pools, it is important to consider that each individual pool is highly pH-dependent.

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The concentrations of free and bound calcium depend directly on pH. pH shifts occur both within the body and in vitro, such that pH changes after specimen collection can affect the measured concentration of free calcium. As shown in the Figure, calcium shifts between these pools as the concentration of hydrogen ions changes. For example, free calcium will decrease in cases of alkalemia but will increase in cases of acidemia. These shifts occur as hydrogen ions compete with calcium for charged sites on serum proteins such as albumin. It is estimated that for each 0.1 pH unit change there is approximately a 5% change in the free calcium concentration. Again, total calcium does not change with pH.

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Let's explore some of the differences between total and ionized calcium. Free calcium reflects the true calcium status of the body and is more accurate at indicating whether there is a calcium homeostasis disorder present. Free calcium is not affected by protein concentration, but it may not be available in all laboratory environments. Typically, ionized calcium is measured on a blood gas analyzer using an ion selective electrode with whole blood specimens. Free calcium measurement does have quite stringent collection and handling requirements, which is often cited as the reason it is not used universally.

In contrast, total calcium includes free, anion-bound calcium and protein-bound calcium and is thus dependent on the protein concentration. Samples with high protein concentrations will typically have higher calcium regardless of the free calcium status of the patient. Total calcium measurement is widely available on most chemistry analyzers and is part of the routine metabolic panel. Most chemistry analyzers rely on spectrophotometry to measure total calcium using indicator dyes, although atomic absorption spectrometry may be used in some labs and is considered a reference method. An advantage of total calcium is its stability allowing it to be measured in routine blood collections without any special handling or processing considerations.

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Because the total calcium concentration changes with protein concentration, several efforts have been made to develop formulae to correct for protein. The equation shown here demonstrates one of the most widely used examples. Correction formulas do work in some cases and regressions demonstrate good agreement between corrected and actual calcium concentrations. However, they often fail to accurately predict the calcium status of an individual patient.

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Another commonly encountered correction equation is for pH. pH correction is used to adjust the free calcium concentration of a sample for changes that may have occurred during transport or storage. These changes can result from metabolism or loss of CO2. Again, numerous formulae are available and again, there are limitations. One limitation is that equations have a small range of pH over which they are valid. They also make a number of major assumptions, such as the patient having a neutral pH, normal levels of serum albumin, and no additional calcium binding proteins or anions, such as citrate, present. When these assumptions cannot be made, it is preferable to avoid pH changes during transport and processing, such that correction is unnecessary.

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In order to avoid these pH changes during collection and transport and eliminate the need for correction, it is worth considering specimen requirements for free calcium measurement. Heparinized whole blood is most commonly used for free calcium measurement. Serum may be used, but is described elsewhere. Please see the reference below for more details.

Whole blood samples should be collected in devices containing balanced heparin to avoid artificially lowering calcium concentrations due to chelation. Vacutainer tubes or syringes should be filled adequately to avoid dilutional effects of liquid heparin or excess calcium from balanced heparin. Air bubbles should also be avoided to minimize loss of CO_2 and associated pH changes. Likewise, anaerobic conditions must be maintained to avoid pH changes which would affect the concentration of free calcium. Ice storage is appropriate for samples that will take longer than 30 minutes to analyze, where cooling the samples will minimize the effects of metabolic changes on pH.

Total calcium has less stringent processing and handling requirements than free calcium. Total calcium samples may include either serum or plasma, although when plasma samples are used it is important to avoid additives, such as EDTA, which chelate calcium. Handling and transport samples for total calcium should follow routine practice guidelines, but do not require the special considerations required for free calcium.

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The key points to remember from this short presentation are:

1. Free calcium is the most accurate laboratory test to assess the true calcium status of a patient. It is the biologically active pool which is hormonally regulated.
2. Total calcium concentrations can be misleading in patients with abnormal protein concentrations, acid-base disorders, or in patients receiving citrated blood products.
3. Correction formulas have significant limitations that need to be considered before being utilized.
4. If free calcium measurement is being used, it is essential to pay attention to the special collection and handling requirements.

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Resources