RECOMMENDED "BEST PRACTICES" FOR TCO2 TESTING AND SAMPLE COLLECTION

DEFINITIONS

Bicarbonate ion – Bicarbonate ion (HCO_3^-) is a normal constituent of the blood.

Bicarbonate is naturally produced by the reaction of carbon dioxide (CO_2) with water (H_2O) to produce carbonic acid (H_2CO_3), which dissociates to a bicarbonate ion and a proton (H^+). Acid/base metabolism in the body is regulated by this chemical equation:

$$\mathbf{CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow H^+ + HCO_3^-}$$

TCO2 – The total concentration of all forms of carbon dioxide (Total Carbon Dioxide) in the sample including bicarbonate and carbonate as well as dissolved CO2. The TCO2 of the plasma or serum is used for regulatory purposes. The dissolved carbon dioxide is only a small fraction (about 3%) of the total carbon dioxide.

Total CO2 analyzer – A TCO2 analyzer adds acid to the plasma or serum sample thereby converting all the carbonate, bicarbonate and carbonic acid to water and CO2. The concentration of CO2 is measured represents the total concentration of available CO2 in the sample or TCO2.

Measurement Uncertainty (MU) – The measurement uncertainty is a value (with units of concentration) that is determined experimentally and characterizes the variability of the analytical process. The measurement uncertainty is used to eliminate all reasonable variability originating from the measurement process. The measurement uncertainty is a property of the method and is therefore unique to each laboratory unless measures have been taken to standardize methods between laboratories.

Regulatory Threshold- The threshold is the maximum permitted concentration of TCO2 that has been established by the regulatory body.

Decision Level- The regulatory threshold including the measurement uncertainty is the decision level. For TCO2 the decision level is the regulatory threshold level plus the measurement uncertainty for the laboratory providing the testing.

REGULATORY THRESHOLD

The 37 millimoles/L regulatory threshold for TCO2 is a statistically based threshold designed to identify a state of metabolic alkalosis in horses. Most racing regulators in the U. S. apply a threshold of 37 millimoles of total carbon dioxide per liter of plasma/serum. The decision level for the regulation of TCO2 is 37 millimoles/L plus the measurement uncertainty.

COLLECTION OF SAMPLES

Blood samples should be collected 45 minutes (+ or – 15min) pre-race and approximately 3 hours after furosemide administration. The samples must be handled in a consistent manner and cannot be frozen. If samples are obtained pre-furosemide a lower regulatory threshold is necessary and the horses must be kept in a secure detention barn until race time.

Furosemide (Lasix/Salix) produces an elevation in Total Carbon Dioxide averaging approximately 1.7mml/l for a standard 250mg IV dose administered four (4) hours pre-race. The furosemide effect has been considered in the 37mml/l regulatory threshold.

In harness racing, it is necessary to collect samples from horses about 30-45 minutes pre-race. The horse needs to be reasonably well cooled off before being sampled. A horse that is hyperventilating from exercise may produce an artificially low TCO2.

Horses selected for post race TCO2 testing should be required to remain in the test barn for a minimum of one and one-half hours before being sampled for TCO2 testing. This is to permit the TCO2 value to approach accurate values. Post-race sampling of horses for TCO2 testing should be discouraged.

INSTRUMENTATION

Most laboratories doing TCO2 testing laboratories are utilizing Beckman instruments. Beckman EL-ISE analyzers were used to establish the international threshold for TCO2 testing. Other TCO2 instruments may be used for TCO2 analysis but must be demonstrated to be equivalent and consistent with Beckman equipment.

Blood samples must be processed and tested using standardized, reproducible, validated procedures. Furthermore, test samples submitted for TCO2 testing should be tested within 120 hours in order to limit sample degradation that results in decreased TCO2 values.

COMMENTARY

Regulators should be advised that an elevated TCO2 concentration is indicative only of metabolic alkalosis and is not proof of the administration of sodium bicarbonate or other alkalinizing substances. For example, the feeding of certain feeds with highly positive dietary cation-anion balances (the DCAB is a measure of the difference between the concentrations of the major cations (primarily sodium and potassium) and the major anions (primarily chloride and sulfate) in the feed) may cause metabolic alkalosis that is characterized by increased TCO2 values. Furthermore, the administration of furosemide produces a mild metabolic alkalosis that results in an average increase in the TCO2 of 1.7 millimoles/L.

Regulators should conduct an investigation to determine the cause of an elevated TCO2 value, which should include bur not necessarily be limited to, the quarantining of the horse and the taking of periodic blood samples.

Trainers should be advised that elevated TCO2 values, regardless of causation, are violations of the rules and that the penalties for excessive TCO2 values are severe. Therefore, trainers and their veterinarians should work closely to identify any procedures or practices that may elevate the TCO2 value.

Split sample analyses can be done with TCO2 testing but the split sample must be run at the split sample laboratory in parallel with the official sample at the official laboratory in order to avoid delays in testing that result in lower TCO2 values as a result of sample degradation.