



OxiDates

ZeptoMetrix Corporation

Oxidative Stress/Free Radical News

Issue 10, April 2008

Automated TBARS

The availability of the OXItek kit for measuring TBARS as a screening biomarker for oxidative stress makes it possible to use this kit in automated systems where service labs with high volume can assay multiple samples in an efficient and cost-effective manner. Many specialty laboratories currently offer fluorometric assays of TBARS which can easily be performed on most autoanalyzers. Normally, TBARS must be done by analyzing samples individually, and if large numbers of samples are being tested, it is labor intensive and requires a highly trained Medical Technologist. Automation speeds up sampling time, reduces variability and avoids reagent handling. Another advantage permits standardization and validation of large-scale research studies, as well as in clinical trials. Operational parameters can be stored in the autoanalyzer memory unit, thus simplifying TBARS measurements.

There are two ways TBARS can be automated. One is by using an autoanalyzer and the other is by using HPLC. In 1993, Svensson et al reported a continuous-flow technique for TBARS that they found was reliable, fast, and inexpensive (1). A comparison between the automated method and a traditional manual method gave an $r^2=0.988$. It was linear in the range from 50 -100 $\mu\text{mol/L}$ (nmol/mL) and the CV was 3 %. It was also reported by others that the determination of malondialdehyde adducts of TBA could be automated using HPLC techniques (2, 3). In one system, the detection limit was 0.8 pmol, where $r^2 = 0.999$, the standard curve was linear up to 10 pmol/L, and the CV was 9.1%. Separation was accomplished within 2 minutes. In another system, the detection was 0.15 μmol and the CV was between 8-13 %. By comparison, an earlier report indicated a detection limit of 1 ng and a CV of 7 % (4). In addition, an autoanalyzer has been used for determining lipid hydroperoxides, the precursor of TBARS (5). The CV was approximately 4 % and the calibration curve linear to 50 $\mu\text{mol/L}$. This publication also simultaneously automated the total antioxidant capacity. ZeptoMetrix has an automated TEAC assay.

This in-house TEAC assay can be run on request (contact jane_s@zeptometrix.com for details).

More recently, an automated system for determining reactive oxygen metabolites (d-ROMS) and antioxidant capacity (OXY Adsorbent Test) has been described (6-8). The r^2 value for their calibration curve was >0.99 and CVs for between-run and within-run were 3.9 and 2.7 respectively. An Oxidative-INDEX is calculated by subtracting the OXY Adsorbent Test value from the d-ROMS value to provide a global estimate of oxidative stress. Combining both measurements makes this approach more powerful because it reflects both oxidative and antioxidant components. TBARS and TEAC can easily be substituted and the autoanalyzer adapted for the these tests.

There are several clinical chemistry analyzers that have microprocessors with special programming features and can be adapted to measure TBARS. Many autoanalyzers use only kits or reagents offered by the manufacturer (closed system). However, it is necessary that the analyzer of choice be an open system to accommodate the TBARS and TEAC tests (9). Such analyzers can be used for both routine and clinical research and are ideal for mid-volume to high-volume laboratories and can run up to 400 tests per hour. ZeptoMetrix has established linearity and precision data for these tests.

There are at least 14 autoanalyzers on the market capable of running ZeptoMetrix kits. As equipment becomes more compact, "bed-side" autoanalyzers may become common (10). The economics of Point-of-Care (POCT) testing have been discussed including positive and negative cost factors, as well as overall benefits (11). TBARS and TEAC are logical tests to apply to automated testing strategies, especially for large volume studies.

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- LHP Profile
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- Oxidized DNA (8-Oxo-dG/8-Oxo-d/A)

Antioxidant Enzymes

- Erythrocyte Glutathione Peroxidase
- Erythrocyte Glutathione Reductase
- Erythrocyte Glutathione S-Transferase
- Plasma Glutathione Peroxidase
- Catalase
- Paraoxonase (PON, Arylesterase Activity)
- Paraoxonase (PON, Paraoxonase Activity)
- PON1 Gln-Arg192 Phenotyping Assay
- Superoxide Dismutase (SOD)
- Glucose-6-Phosphate Dehydrogenase

Antioxidant Micronutrients

- Fat Soluble Vitamin Profile:
 - Vitamin E
 - Beta-Carotene
 - Lycopene
 - Lutein/Zeaxanthin
 - Beta-Cryptoxanthin
 - Vitamin A
- Vitamin C
- TEAC
- Reduced Glutathione

Routine Analytes

- Total Cholesterol
- HDL Cholesterol
- LDL Cholesterol
- Triglycerides
- Total Protein
- Hemoglobin
- Uric Acid



Products

- TBARS Assay cat. # 0801192
- DNA Isolation Kit cat. # 0805001
- Total Glutathione Peroxidase Assay Kit cat. # 0805002
- Glutathione Reductase Assay Kit cat.# 0805004
- Arylesterase/Paraoxonase Assay Kit cat.# 0801199
- PON Standards cat.# 0801384
- Nucleoside Standards Inquire
- Dinucleotide Standards Inquire

