**How is oxidative stress defined in laboratory medicine?**

A measurable imbalance between oxidant production and antioxidant defense inferred through biochemical, enzymatic, and molecular surrogate markers in biological specimens.

**What is the core laboratory challenge in assessing oxidative stress?**

There is no single definitive analyte; interpretation depends on multi-marker panels reflecting different redox compartments and reaction products.

**Which specimen types are used in oxidative stress testing?**

Serum, plasma, urine, whole blood, erythrocytes, and tissue homogenates depending on analyte stability and analytical target.

**What are the major analytical approaches used in oxidative stress measurement?**

Spectrophotometric assays

Fluorometric assays

Enzyme activity assays

Immunoassays (ELISA)

Chromatography-based methods (HPLC, GC)

Mass spectrometry (LC-MS/MS, GC-MS)

Electrochemical assays (redox potential measurements)

**What is the TBARS assay and what does it measure?**

A spectrophotometric method estimating malondialdehyde (MDA) as a marker of lipid peroxidation.

**What are limitations of TBARS methodology?**

Low specificity, interference from aldehydes and carbohydrates, and susceptibility to sample oxidation artifacts.

**How is MDA measured more accurately?**

By HPLC or LC-MS/MS following derivatization, improving specificity over TBARS.

**What is 4-HNE and how is it measured?**

A reactive lipid peroxidation aldehyde measured using ELISA, immunohistochemistry, or LC-MS/MS.

**What is 8-OHdG and what methods detect it?**

A DNA oxidation product measured in urine, plasma, or DNA hydrolysates using ELISA, HPLC-ECD, or LC-MS/MS.

**Why is LC-MS/MS considered a gold standard in oxidative stress testing?**

It provides high specificity, structural confirmation, and accurate quantification of multiple oxidative biomarkers simultaneously.

**What are isoprostanes and how are they measured?**

Stable lipid peroxidation products quantified primarily by GC-MS or LC-MS/MS, considered highly reliable oxidative stress markers.

**What does the GSH/GSSG assay measure?**

Cellular redox balance by quantifying reduced and oxidized glutathione levels.

**What analytical methods are used for glutathione measurement?**

Enzymatic recycling assays, HPLC with fluorescence detection, and LC-MS/MS.

**What are enzymatic antioxidant assays used in labs?**

Activity measurements of SOD, catalase, and glutathione peroxidase using spectrophotometric or colorimetric methods.

**How is superoxide dismutase (SOD) activity measured?**

By inhibition of superoxide-dependent reactions using spectrophotometric or colorimetric assays.

**How is catalase activity measured?**

By monitoring decomposition rate of hydrogen peroxide.

**What is total antioxidant capacity (TAC)?**

An assay estimating cumulative antioxidant effect using colorimetric or chemiluminescent methods.

**What are limitations of TAC assays?**

Non-specific, influenced by diet, and unable to distinguish individual antioxidant contributions.

**What is chemiluminescence in oxidative stress testing?**

A method detecting ROS generation through light emission during redox reactions.

**What is electron paramagnetic resonance (EPR) used for?**

Direct detection of free radicals using spin trapping techniques.

**Why is EPR considered highly specific?**

It directly measures unpaired electrons rather than downstream oxidation products.

**What is redox potential measurement in clinical labs?**

Electrochemical assessment of oxidation-reduction balance in biological fluids.

**What pre-analytical factors affect oxidative stress assays?**

Temperature, oxygen exposure, delay before processing, hemolysis, and freeze–thaw cycles.

**Why is sample handling critical in ROS-related assays?**

Reactive species and oxidation products can form or degrade rapidly ex vivo, altering results.

**What is the role of derivatization in oxidative stress analytics?**

It stabilizes reactive aldehydes (e.g., MDA, 4-HNE) for accurate chromatographic detection.

**What is multiplex oxidative stress profiling?**

Simultaneous measurement of multiple biomarkers using combined LC-MS/MS or bead-based immunoassays.

**What is oxidative metabolomics?**

Global profiling of redox-related metabolites to map systemic oxidative state.

**How are oxidative stress results clinically interpreted?**

As integrated patterns rather than single thresholds, often correlated with inflammation and metabolic markers.

**What is the main limitation of oxidative stress diagnostics in routine labs?**

Lack of standardized reference ranges and inter-laboratory harmonization.

**What is the future direction of oxidative stress laboratory science?**

Integration of LC-MS/MS, metabolomics, and systems biology-based redox network modeling.