

MAND Lab Handout #3: Glutathione Oxidative Stress Testing

IMPORTANT DISCLAIMER: These handouts are based on emerging research and mechanistic reasoning from animal models and cell studies — no MAND-specific clinical testing guidelines currently exist. The metabolic framework is hypothetical but grounded in published molecular data. Results need to be interpreted by providers familiar with both MAND and metabolic medicine.

Understanding Glutathione Oxidative Stress Testing for MAND

What is this testing for?

Glutathione is the body's most important antioxidant — it protects cells from damage caused by "oxidative stress" (a kind of rust that happens inside cells). Research on MBD5, the gene affected in MAND, has shown changes in glutathione metabolism at the genetic level. Combined with the mitochondrial energy problems in MAND, this means the body may be using up glutathione faster than it can make it.

These tests check whether the body's antioxidant defenses are working properly.

Tests that may be ordered:

- Reduced glutathione (GSH) and oxidized glutathione (GSSG) with GSH:GSSG ratio: This is the most direct test. GSH is the "active" protective form; GSSG is the "used up" form. A low GSH:GSSG ratio means the body is under oxidative stress.
- Glutathione peroxidase activity: This enzyme uses glutathione to neutralize harmful molecules. Low activity means less protection.
- 8-OHdG (8-hydroxy-2'-deoxyguanosine): This is a marker of DNA damage from oxidative stress. It can be measured in blood or urine. Elevated levels mean cells are being damaged.

- Pyroglutamic acid (5-oxoproline) in urine: When glutathione is severely depleted, this waste product builds up. It is measured as part of a urinary organic acids test.
- Plasma cysteine and homocysteine: Cysteine is the rate-limiting building block for making glutathione. Low cysteine may mean the body cannot make enough glutathione.
- Cystathionine: This is an intermediate in the pathway that makes cysteine. Elevated levels may mean the body is trying to push more material through this pathway to make glutathione.

What do the results mean?

If testing shows low GSH, high GSSG, low GSH:GSSG ratio, or elevated 8-OHdG, it confirms that the body is under oxidative stress. Possible interventions may include:

- Glutathione supplementation (liposomal or other bioavailable forms)
- Vitamins that support the glutathione pathway (B6, B12, folate)
- Antioxidant-rich foods

Important caution about N-Acetylcysteine (NAC):

NAC is commonly recommended as a glutathione precursor because it provides cysteine, the building block the body needs most to make glutathione. However, families and providers should be aware of an important consideration before using NAC:

NAC is a potent biofilm disruptor. Biofilms are protective "shields" that bacteria in the gut (and elsewhere) build around themselves. Many gut bacteria — both harmful and beneficial — live within biofilms. Research has shown that NAC can destabilize and destroy these biofilm structures, exposing bacteria that were previously hidden from the immune system.

When biofilms are disrupted, the immune system may suddenly "see" bacteria it was not previously reacting to. This can trigger increased immune activation and inflammation in the gut, which can paradoxically increase oxidative stress — the very problem NAC is being used to treat. In children with MAND who may already have a fragile balance between

their immune system and gut bacteria, this biofilm disruption could potentially:

- Cause GI symptoms such as bloating, diarrhea, cramping, or changes in stool
- Trigger increased systemic inflammation
- Temporarily worsen fatigue and behavioral symptoms
- Create a cycle where the immune response to newly exposed bacteria generates more oxidative stress, consuming the very glutathione that NAC was meant to support

For this reason, if NAC is being considered:

- Start at a very low dose and increase slowly
- Monitor for GI symptoms and behavioral changes
- Consider whether the gut microbiome should be assessed first (stool testing)
- Discuss with the provider whether alternative glutathione support strategies (such as liposomal glutathione, glycine supplementation, or whey protein) might be safer starting points
- Be aware that a temporary worsening of symptoms after starting NAC could be related to biofilm disruption, not a "detox reaction"

Important note: In MAND, the oxidative stress may be driven by the mitochondrial energy problems AND by iron that is not being stored properly. Addressing glutathione alone without considering the underlying causes may not be sufficient.

How to prepare:

- Blood tests for GSH/GSSG should ideally be processed quickly (the sample is time-sensitive)
- Ask the lab about special handling requirements
- 8-OHdG can be measured in urine (non-invasive)

- No special fasting is usually required, but check with the ordering provider

References:

- Bjørklund G, et al. "The Role of Glutathione Redox Imbalance in Autism Spectrum Disorder: A Review." *Free Radical Biology Medicine*. 2020;160:149-162.
- Meguro-Horike M, et al. "Haploinsufficiency of MBD5 and MBD6 Impairs Mitochondrial Respiration Through Chromatin-Mediated Gene Regulation." *Biochemical and Biophysical Research Communications*. 2026;800:153288.
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- Tieu S, et al. "N-Acetylcysteine and Its Immunomodulatory Properties in Humans and Domesticated Animals." *Antioxidants*. 2023;12(10): 1867.