Research Testing

**Determination of residual superoxide function** is important in management: patients with little to no superoxide production are at the greatest risk for mortality [Kuhns et al 2010] and, thus, are the most likely candidates for HSCT. The following three tests are performed in research laboratories only:

- **Cytochrome c reduction assay** quantitates indirectly the actual amount of superoxide produced by measuring spectrophotometrically the inhibitable reduction of ferricytochrome c by superoxide dismutase to ferrocytochrome c [Elloumi & Holland 2014]. Results of this test correlate well with results of the DHR test.

- **Chemiluminescence.** Because superoxide can cause a variety of chemical agents to luminesce, measurement of luminescence (typically using dichlorofluorescein [DCF]) can quantitate the amount of superoxide produced [Elloumi & Holland 2014]. While this assay can rapidly detect superoxide activity and identify hypomorphic forms of CGD, it lacks cellular resolution and thus cannot identify female carriers of X-linked CGD.

- **Neutrophil superoxide production of reactive oxygen intermediates (ROI).** The quantitation of superoxide produced can be obtained directly from the cytochrome c reduction assay (a research laboratory test) or indirectly from the DHR test (a routine clinical test). In general, a DHR test value in the lower range (i.e., <225 arbitrary units) correlates with poor superoxide production, which can be predicted from the specific NADPH oxidase pathogenic variant (see Genotype-Phenotype Correlations).

**Immunoblot test for the NADPH complex proteins.** Failure to detect the following cytoplasmic subunits of the phagocyte NADPH oxidase (phox) proteins suggests autosomal recessive inheritance: p47^{phox} (encoded by NCF1), p67^{phox} (NCF2), or p40^{phox} (NCF4) (Table 1 and Table A). Immunoblotting is currently performed only in research laboratories.

Note: This technique cannot distinguish between pathogenic variants in **CYBB** (encoding gp91^{phox}) and **CYBA** (encoding p22^{phox}). Because the protein products of these two genes stabilize each other within the phagocyte membrane, absence of one protein results in the absence of the other [Segal et al 2000] (see Molecular Genetics). Pathogenic variants in **CYBB** or **CYBA** that cause a failure to bind heme (leading to a loss of the cytochrome b558) have been referred to as cytochrome negative. In contrast, pathogenic variants in **NCF1, NCF2,** and **NCF4** leave cytochrome b558 intact and have been referred to as cytochrome positive. Because pathogenic missense variants in either **CYBB** or **CYBA** can also support cytochrome b558 persistence without function, the terminology ‘cytochrome negative’ and ‘cytochrome positive’ is not preferred.
References
