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## Preface

In our first protocols book, *Free Radical and Antioxidant Protocols (1)*, reference to *in vivo*, *ex vivo*, or *in situ* techniques were few compared to classical biochemical assays and only 6 of the 40 chapters were concerned with these applications. In our second book, *Oxidative Stress Biomarkers and Antioxidant Protocols (2)*, which is being published concurrently with this third volume, *Oxidants and Antioxidants: Ultrastructure and Molecular Biology Protocols*, the number of such chapters has increased. The literature dealing with histochemical/cytochemical and immunohistochemical techniques and staining to identify cellular/subcellular sites of oxidative stress has expanded rapidly, as has the molecular biology methodology used to analyze free radical and antioxidant (AOX) reactions, as well as the monitoring of living tissue.

A two-way search was performed for each technique listed in **Table 1**, coupled with “oxidative stress” using the PUBMED search engine from the National Library of Medicine at NIH. Most of the techniques involved in measuring oxidative stress employ molecular biology or ultrastructural approaches. Of these techniques, histology, polymerase chain reaction, and Western blotting are the most widely used. Several forms of therapy are now available for patients with increased oxidative stress. In addition to standard antioxidant therapy supplementation *in vivo* and *in vitro*, photodynamic therapy (PDT) employs excitation of a photon-emitting compound delivered systemically for free radical-mediated necrosis of affected tissues, and stem cells are also being used to induce signaling events or replace antioxidant enzymes.

From **Table 1**, one can appreciate how these various techniques have currently become valuable options for pathophysiological studies, especially in ultrastructure. Treatment is another important category: in most instances these are non-invasive. Consequently, this third volume in the series contains additional chapters written by authors who collectively provide 109 methods of oxidative stress measurement across the three books.

Some of the procedures listed within this volume require sophisticated instrumentation not generally found in the routine laboratory. However, some devices are usually available somewhere in a given institution and collaboration is often possible. Techniques introduced here alert the reader to the high level that oxidative stress measurements have reached. Imaging techniques employing photon biotechnology, confocal microscopy, and photodynamic therapy have been included in the present book chapters, a development that illustrates the degree of sophistication available for study at the molecular level.

**Table 1**  
**Recent Citations of Oxidative Stress Biomarkers**

	1997	1998	1999	2000	
<b>Molecular Biology</b>	Western blot	17	31	26	47
	Northern blot	21	23	29	21
	Southern blot	5	4	4	4
	PCR	33	22	55	61
	Differential display	5	9	7	6
	Mobility shift assay	8	14	16	14
	<i>In situ</i> hybridization	21	12	18	26
<b>Ultrastructure</b>	Histology	87	91	150	160
	Intracellular	94	122	155	204
	Cytology	431	523	641	820
	Immunohistochemistry	56	91	119	118
<b>Treatment</b>	AOX therapy	121	158	209	288
	PDT	4	3	7	12
	Stem cells	24	44	50	66

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## References

1. Armstrong, D. (ed.) (1998) *Free Radical and Antioxidant Protocols, Methods in Molecular Biology, vol. 108*, Humana Press Inc., Totowa, NJ.
2. Armstrong, D. (2002) *Oxidative Stress Biomarkers and Antioxidant Protocols, Methods in Molecular Biology, vol. 186*, Humana Press, Totowa, NJ.



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