Oxidants and Antioxidants in Medical Science

Oxidative stress research: a framework to relate basic science to clinical practice

Ezekiel Uba Nwose[1], Phillip Taderera Bwititi[2], Melissa Judith Chalada[1]

[1]*School of Psychological and Clinical Sciences, Charles Darwin University, Darwin, Northern Territory;*
[2]*School of Biomedical Sciences, Charles Sturt University, Wagga Wagga, New South Wales; **Australia***

Abstract

Oxidative stress research has been going on for a long time, but a review of recent free radicals research literature presents little or no framework for translation into clinical practice. Instead, there seems to be a paradigm shift whereby the current trend in oxidative stress research has moved from the yet-to-be translated basic concepts to new hypotheses and theories that can hardly be separated from the basics. This commentary focuses on the issue of 'translating basic science to clinical practice'; with a view to draw attention to untranslated basic concepts, some new theories that are inseparable from the basic concepts, the gap between negative *vs* positive research outcomes, and the gap between diagnostic pathology *vs* research in assessment of oxidative stress. Attempt is made to explain how oxidative stress research can be adopted in clinical practice and pathology, in particular. Importantly, this article identifies that the confounding effects of various default metabolites with anti- or pro-oxidant properties require to be acknowledged; and also hypothesize a framework to relate any new theory to the basics as well as to clinical practice.

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INTRODUCTION

Untranslated basic concepts

Oxidative stress research has been going on for over sixty years. One of the major challenges facing adoption in clinical practice is the choice of ideal biomarkers that are informative for diagnosis and management of diseases. The dilemma for choice for ideal markers in part stems from the fact that there are complex endogenous mechanisms for correction and repair of oxidative damage [1] and that oxidative stress can be due to reduced antioxidant protection and/or increase in free radical production [2]. This is compounded by the fact that there are numerous antioxidant and pro-oxidant species whose contribution to oxidative stress is still not clear and some of these compounds have oxidant and antioxidant properties depending on the micro-environment [3].

Several indices of oxidative stress are measured in various disease and these include total oxidant capacity [4] as well as H_2O_2, a product of partial reduction of molecular oxygen [5], thiobarbituric acid reactive substances (TBARS) for lipid peroxidation, malondialdehyde (MDA) for lipid peroxidation [4, 6], 8-isoprostane for lipid peroxidation, and 8-hydroxy-2-deoxyguanosine for DNA oxidation [7]. Peroxynitrite ($ONOO^-$) nitrates proteins [8]; 3-nitrotyrosine (3-NT) is therefore a biomarker for the generation of reactive nitrogen intermediates [9]. Several endogenous antioxidant enzymes including superoxide dismutase (SOD), glutathione peroxidise (GSH-Px), glutathione reductase and catalase (CAT) [6] counter the damaging effects of free radicals [10].

Beside the requirements of substantiation and validity, there is also a need for rational application of biomarkers of oxidative stress to observational studies and clinical trials [11]. It has been identified that appropriate participant selection is a panacea for consistent outcomes in antioxidant trials; for instance, the need to select patients who show evidence of pro-oxidant [12]. On association of oxidative stress with various diseases of interest, increased SOD and CAT red blood cells'

activities in farmers as a result of pesticide exposure were observed. The report attributed the increased SOD activity to activation of compensatory mechanism through the effects of pesticides on progenitor cells, while the high CAT activities were possibly adaptive responses to the produced free radicals [13]. In Alzheimer's disease there is a decrease in antioxidant capacity and beta amyloids also possibly act as pro-oxidants and combine with redox active metals to form reactive oxygen and nitrogen species (ROS and RNS) [14]. Such observations link directly or indirectly pro-oxidant production to pesticide exposure. Conducting animal experimentation in a manner which is not compatible to human volunteer experimentation is not necessary as the experimental outcome would be unverifiable and untranslatable to clinical practice. In other words, research methodology should be such that it can be duplicated in a clinical setting, which would also include validated diagnostic biomarkers. The point being brought to the fore is that it is important to have a basic oxidative stress concept that is experimented by a laboratory method and can be duplicated in a diagnostic pathology.

CURRENT EVIDENCE

Some theories inseparable from the basic concepts

Reactive 'nitrogen' vs 'oxygen' species: one of the theories in oxidative stress research is that RNS are more potent oxidants than ROS. It is argued that oxidative stress is not sufficient to explain cellular damage while conventional antioxidants have been less useful than expected, and that nitro-oxidative stress is now considered as the fundamental harmful mechanism instead of oxidative stress. Therefore, a paradigm shift is now from oxidative stress management to nitrosative stress management [1, 15-17]. Yet, it is known that RNS arise as product(s) of reaction(s) of ROS, or even regarded as a subset of ROS [18].

The question is of a framework to monitor oxidative stress by laboratory methods. Though, superoxide anion ($O_2^{-\bullet}$) reacts with the nitrogen-centered radical nitric oxide (NO•) to form the $ONOO^-$; the development of $ONOO^-$ decomposition catalysts as a potential therapeutic agent is now being suggested [17].

$$O_2^- + NO \rightarrow ONOO^-$$
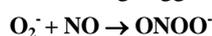
It may be difficult to separate RNS from ROS in several aspects. The reactive species are formed as part of normal physiology, and are associated with oxidative damage of the mitochondria, which underlies the concept that oxidative stress is synonymous with mitochondrial dysfunction or cytotoxicity. Further, they are both capable of altering cellular responses [19], and the tendency of reduced glutathione (GSH) to attenuate both RNS and ROS is apparent. Thus, we re-echo a recent

hypothesis that specific interactions between RNS and ROS are involved in causing the vulnerability to oxidative damage [20]. We also hypothesize that normal levels of GSH and its co-antioxidant network, including coenzyme Q, vitamins C and E [21], as well as bilirubin and uric acid [22], would confound RNS effects.

Despite the availability of a number of ROS and RNS and associated enzymes to measure and assess oxidative stress, few studies have been done to compare the two different reactive species. There is indication that cells can repair damage after nitrosative stress but not after oxidative stress [23], and there is also the opinion that RNS may be more potent than ROS [1]. Both NO• and $O_2^{-\bullet}$ are at the center of the complex interactions, whereby, in a simplified illustration (Fig.1), they have alternative pathways. Nitric oxide reacts with superoxide to form $ONOO^-$, which, under physiological conditions, exists in a dynamic equilibrium with peroxynitrous acid (ONOOH). What needs to be appreciated is that shifts in this equilibrium may show little no difference in damage potential. At equilibrium, up to 30% ONOOH could convert to nitrogen dioxide ($NO_2•$) and hydroxyl (•OH) radicals. If there is a shift in the equilibrium that limits the '**NO• + $O_2^{-\bullet}$**' reaction, NO• would go on to produce $NO_2•$, while $O_2^{-\bullet}$ would form hydrogen peroxide (H_2O_2) that could feed forward to $NO_2•$ and/or •OH formation [24]. Comparison or differentiation may be difficult, especially as both ROS and RNS can interact, integrate and synergize to cause oxidative damage. Therefore, the growing preference for RNS to ROS is subject to debate.

Metabolic waste products vs antioxidant activity: another set of theories in oxidative stress research is whether known metabolic waste products including uric acid and bilirubin are antioxidants in addition to their toxic properties. Uric acid is still being investigated and speculated for its antioxidant benefit. For instance, it was
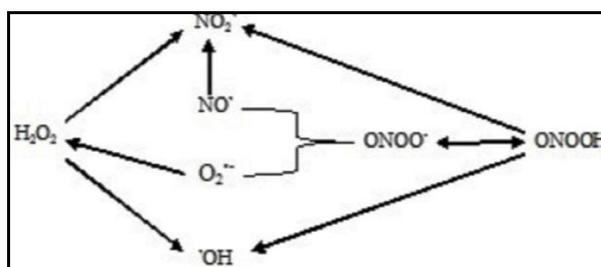

Fig. 1. Simplified interactions between reactive oxygen and nitrogen species. Both nitric oxide (NO•) and superoxide ($O_2^{-\bullet}$) are at the center of the complex interactions, whereby they have alternative pathways. NO• reacts with O^- to form peroxynitrite ($ONOO^-$), which under physiological conditions, exists in a dynamic equilibrium with peroxynitrous acid (ONOOH). At equilibrium, up to 30% ONOOH could convert to nitrogen dioxide ($NO_2•$) and hydroxyl (•OH) radicals. If there is a shift in the equilibrium that limits the '$NO• + O_2^{-\bullet}$' reaction, NO• would go on to produce $NO_2•$ while $O_2^{-\bullet}$ would go on to form hydrogen peroxide (H_2O_2), and could feed forward to $NO_2•$ and/or •OH formation. Thus, with or without equilibrium, NO• and $O_2^{-\bullet}$ would produce deleterious $NO_2•$ and •OH [24].

just recently investigated whether uric acid serves as risk factor for cardiovascular diseases or as antioxidant defence. The report suggested that hyperuricemia improves physical activity after cardiac rehabilitation and might reflect the decline in antioxidant defences that occurs in old age [25]. However, an earlier report, which investigated whether regular physical activity results in hyperuricemia, indicated that intense physical function is associated with increased uric acid and a risk to immobility [26]; that is, physical activity is a cause, not an effect, of hyperuricemia. Further, the association of hyperuricemia with diseases are still being reported [27-31]. On bilirubin, suffice to say that while further toxic effect such as nephrotoxicity are still being reported [32-34], it is also speculated to show antioxidant protection effects on cardiovascular disease albeit at physiological level [35, 36].

We reviewed archive laboratory data, firstly to determine association and correlation of serum uric acid, albumin and estimated glomerular filtration rate (eGFR) in order to identify the potential of renal function testing in evaluation of oxidative stress. We observed that serum albumin levels were reduced as eGFR decreased and there was also an inverse relationship between eGFR and serum uric acid levels [37]. Secondly, on the basis that erythrocyte oxidative stress can cause both hyperviscosity and intravascular hemolysis with the latter able to cause hyperbilirubinemia, we evaluated the association and correlation of blood viscosity and bilirubin levels in a general population and observed a weak positive correlation between blood viscosity and bilirubinemia [38].

The results from our evaluations uphold the ancient concepts that hyperbilirubinemia and hyperuricemia are epiphenomenal to pathophysiology. If our interpretation is wrong, it is pertinent to know how to interpret and optimize the tests. For instance, it is important to know how bilirubin or uric acid would be useful as antioxidant therapeutic agents. It will be of interest for proponents of antioxidant activities of serum bilirubin and uric acid to know that interpretation of the levels of these metabolites can be taken into account in therapeutic management of patients.

The gap between negative and positive research outcomes is no real conflict

The objective of this section is to re-address the conflict between experimental positive (presence of antioxidant effect) and observational negative (no antioxidant effect) reports from antioxidant studies. Using three reports: observational negative, observational neutral and an experimental positive cardiovascular outcomes, critical points are brought to the fore that experimental and observational results are not conflicting, but a factor of different research protocols on the effectiveness of antioxidant in cardiovascular disease [39].

In this paper, reference is made to the seemingly conflicting reports on effectiveness of antioxidants in Down's syndrome:

Negative report: a study evaluated the efficacy and toxicity of antioxidants in people with Down's syndrome and the methodology involved semi-independent individuals who were on psychotropic and Parkinson disease medications. Both the control and treated groups had participants with existing moderate to severe levels of cognitive impairment. Further, vitamin E supplement administered to the treated group was 900 IU, in addition to 200 mg of ascorbic acid and 600 mg of alpha-lipoic acid. The results showed that compared to the placebo group the group administered antioxidant supplements neither improved cognitive functioning nor stabilization of cognitive decline possibly implying there was existing damage, which vitamin E supplementation alone could not reverse [40].

Positive report: in a mouse model of Down's syndrome that evaluated oxidative stress and assessed the efficacy of long-term antioxidant administration, it was reported that vitamin E delayed the onset of cognitive and morphological abnormalities [41]. The study protocol involved administration of vitamin E corresponding to 3000 IU for adult human.

Clearly, the two study protocols presented are not replicas of each other. Thus, it is incorrect to say they are conflicting. An error in oxidative stress studies that try to link research to clinical practice and involves reporting negative or no antioxidant effect is that at least one of the following three facts is often overlooked:

- Antioxidant imbalance means disturbance in antioxidant homeostasis. Antioxidant therapies can only improve existing imbalance in co-antioxidant network, thereby alleviate oxidative stress and prevent further oxidative damage

- Antioxidant therapy does not necessarily reverse any damage that has already occurred, or treats the cellular injury that disrupted its balance or homeostasis. Down's syndrome is caused by the presence of an extra copy of or all of chromosome 21 [42]. Thus, where cognitive impairment is associated with Down's syndrome, it is our view that antioxidant would not delete the extra copy of chromosome 21.

- While experimental animals are regimented equally in their activities, clients in human observational studies are still exposed to varying levels of stress of daily life, which depends on the individuals' circumstances that are not within the control of the researcher.

The argument is that there is no real conflict in findings between negative observational and positive experimental studies. On one hand, observational studies *vis-a-vis* clinical trials are yet to exactly duplicate the

conditions and protocols of experimental studies with a view to limit confounding factors. On the other, experimental studies need to adopt research methodologies that can be duplicated in human studies. This view is supported by the report that inappropriate patient selection is one of the reasons for inconsistent outcomes in antioxidant trials [12]. The hypothesis hereby propagated is that observational study that exactly duplicates an experimental study could obtain near, if not exact, result. Hence, the framework being suggested here is that every experimental study should give recourse to clinical confounding factors in order to enable validation for practice.

THE IMPORTANCE OF THE HYPOTHESES

Application of antioxidant and oxidative stress knowledge to patient care

A cursory comparison of current *vs* past trend in oxidative stress research will reveal little or no framework for translation oxidative stress research methods and/or theories into clinical practice. Instead, there is continuing shift of emphasis in theories. The current trend in oxidative stress research has moved from the yet-to-be translated basic concepts to new hypotheses and theories that can hardly be separated from the basics. This commentary re-visits the topic of 'translating basic science to clinical practice' or 'from research to bedside' in oxidative stress research. If it is essential to relate knowledge to what it talks about [43], relating knowledge of antioxidant and oxidative stress to patient-care in conventional medical practice is important.

Implications for research methods and reagents

Perhaps another salient point of note is that analytical methods for oxidative stress research are not validated for use in clinical practice. Several indices of oxidative stress including biomarkers of RNS and ROS as well as indices of oxidative damage have been identified for a long time. For instance, GSH method has been standardized and reference value determined [44], but available reagents in the market are designated for 'research use only'. Such reagents have to be upgraded and validated for '*in vitro* diagnostic use', so that diagnostic pathology can duplicate experimental methodology with valid standard operational procedure. Otherwise, the issue remains how to compare apple with apple, or 'translating basic science to clinical practice'. The same importance applies to emerging computer-based diagnostic technologies, which is highlighted in the third article of this series [45].

Further, it is known that medical knowledge consists of norms, hypotheses and theories. Whereas some hypotheses are verifiable, most theories are empirically not testable at all due to non-existence of statements of facts [43]. Good examples that have been brought to the

fore in this article include the concept that bilirubin and uric acid are antioxidants. It is essential that proponents of these theories come up with a hypothesis on how bilirubin and/or uric acid would be useful as antioxidant therapeutic agents, *i.e.* considering their toxicities as waste products of metabolism.

CONCLUSION

The overreaching conclusion is that new biomarkers or theories should be rationalized and related with basic paradigm. Experimental oxidative stress assessment methods, especially in animals, need to use protocols that should be applicable and replicable in diagnostic pathology. This is necessary for comparison of results from experimental *vs* observational studies to be comparing apple with apple.

REFERENCES

1. Korkmaz A, Manchester LC. Reactive nitrogen species; devastating intracellular players and melatonin as a defender. J Exp Integr Med 2011; 1:63-5.

2. Sies H. Oxidative stress: from basic research to clinical application. Am J Med 1991; 91:S31-8.

3. Young IS. Measurement of total antioxidant capacity. J Clin Pathol 2001; 54:339.

4. Ramos D, Martins EG, Viana-Gomes D, Casimiro-Lopes G, Salerno VP. Biomarkers of oxidative stress and tissue damage released by muscle and liver after a single bout of swimming exercise. Appl Physiol Nutr Metab 2013; 38:507-11.

5. Pourova J, Kottova M, Voprsalova M, Pour M. Reactive oxygen and nitrogen species in normal physiological processes. Acta Physiologica 2010; 198:15-35.

6. Pandey KB, Rizvi SI. Markers of oxidative stress in erythrocytes and plasma during aging in humans. Oxid Med Cell Longev 2010; 3:2-12.

7. Muzembo BA, Narongpon D, Ngatu NR, Eitoku M, Hirota R, Suganuma N. Assessment of lifestyle effect on oxidative stress biomarkers in free-living elderly in rural Japan. Geriatr Gerontol Int 2012; 12:547-54.

8. Coppola L, Pastore A, Adamo G, Coppola A, Manzella D, Gombos I, Luongo M, Mastrolorenzo L. Circulating free nitrotyrosine and cognitive decline. Acta Neurol Scand 2010; 122:175-81.

9. Baraldi E, Giordano G, Pasquale MF, Carraro S, Mardegan A, Bonetto G, Bastardo C, Zacchello F, Zanconato S. 3-Nitrotyrosine, a marker of nitrosative stress, is increased in breath condensate of allergic asthmatic children. Allergy 2006; 61:90-6.

10. Naito Y, Lee MC, Kato Y, Nagai R, Yonei Y. Oxidative stress markers. Anti-Aging Medicine 2010; 7:36-44.

11. Blumberg J. Use of biomarkers of oxidative stress in research studies. J Nutr 2004; 134:3188-9S.

12. Levy Y, Blum S, Levy AP. Antioxidants in the prevention of atherosclerosis: the importance of proper patient selection. Clin Nutr 2009; 28:581-2.

13. Wafa T, Nadia K, Amel N, Ikbal C, Insaf T, Asma K, Hedi MA, Mohamed H. Oxidative stress, hematological and biochemical alterations in farmers exposed to pesticides. J Environ Sci Health B 2013; 48:1058-69.

14. Sutherland GT, Chami B, Youssef P, Witting PK. Oxidative stress in Alzheimer's disease: primary villain or physiological by-product? Redox Rep 2013; 18:134-41.

15. Pacher P, Beckman JS, Liaudet L. Nitric oxide and peroxynitrite in health and disease. Physiol Rev 2007; 87:315-424.

16. Valez V, Cassina A, Batinic-Haberle I, Kalyanaraman B, Ferrer-Sueta G, Radi R. Peroxynitrite formation in nitric oxide-exposed submitochondrial particles: detection, oxidative damage and catalytic removal by Mn-porphyrins. Arch Biochem Biophys 2013; 529:45-54.

17. Liaudet L, Rosenblatt-Velin N, Pacher P. Role of peroxynitrite in the cardiovascular dysfunction of septic shock. Curr Vasc Pharmacol 2013; 11:196-207.

18. Doshi S, Khullar K, Sharma R, Agarwal A. Role of reactive nitrogen species in male infertility. Reprod Biol Endocrinol 2012; 10:109.

19. Lee J, Giordano S, Zhang J. Autophagy, mitochondria and oxidative stress: cross-talk and redox signalling. Biochem J 2012; 441:523-40.

20. Newsholme P, Rebelato E, Abdulkader F, Krause M, Carpinelli A, Curi R. Reactive oxygen and nitrogen species generation, antioxidant defenses, and β-cell function: a critical role for amino acids. J Endocrinol 2012; 214:11-20.

21. Nwose EU, Jelinek HF, Richards RS, Kerr PG. The 'vitamin E regeneration system' (VERS) and an algorithm to justify antioxidant supplementation in diabetes - a hypothesis. Med Hypotheses 2008; 70:1002-8.

22. Mekki K, Taleb W, Bouzidi N, Kaddous A, Bouchenak M. Effect of hemodialysis and peritoneal dialysis on redox status in chronic renal failure patients: a comparative study. Lipids Health Dis 2010; 9:93.

23. Kroncke KD, Klotz LO, Suschek CV, Sies H. Comparing nitrosative versus oxidative stress toward zinc finger-dependent transcription. Unique role for NO. J Biol Chem 2002; 277:13294-301.

24. Mikkelsen RB, Wardman P. Biological chemistry of reactive oxygen and nitrogen and radiation-induced signal transduction mechanisms. Oncogene 2003; 22:5734-54.

25. Molino-Lova R, Prisco D, Pasquini G, Vannetti F, Paperini A, Zipoli R, Luisi ML, Cecchi F, Macchi C. Higher uric acid levels are associated with better functional recovery in elderly patients receiving cardiac rehabilitation. Nutr Metab Cardiovasc Dis 2013; doi:10.1016/j.numecd.2013.04.009.

26. Beavers KM, Hsu FC, Serra MC, Yank V, Pahor M, Nicklas BJ. The effects of a long-term physical activity intervention on serum uric acid in older adults at risk for physical disability. J Aging Phys Act 2013: in press.

27. Jin YL, Zhu T, Xu L, Zhang WS, Liu B, Jiang CQ, Yu H, Huang LM, Cheng KK, Thomas GN, Lam TH. Uric acid levels, even in the normal range, are associated with increased cardiovascular risk: The Guangzhou Biobank Cohort Study. Int J Cardiol 2013; 168:2238-41.

28. Eisen A, Benderly M, Goldbourt U, Haim M. Is serum uric acid level an independent predictor of heart failure among patients with coronary artery disease? Clin Cardiol 2013; 36:110-6.

29. Turak O, Ozcan F, Tok D, Işleyen A, Sokmen E, Tasoglu I, Aydogdu S, Sen N, McFann K, Johnson RJ, Kanbay M. Serum uric acid, inflammation, and nondipping circadian pattern in essential hypertension. J Clin Hypertens (Greenwich) 2013; 15:7-13.

30. Kohagura K, Kochi M, Miyagi T, Kinjyo T, Maehara Y, Nagahama K, Sakima A, Iseki K, Ohya Y. An association between uric acid levels and renal arteriolopathy in chronic kidney disease: a biopsy-based study. Hypertens Res 2013; 36:43-9.

31. Moon SS. Relationship between serum uric acid level and nonalcoholic fatty liver disease in pre- and postmenopausal women. Ann Nutr Metab 2013; 62:158-63.

32. Rafat C, Burbach M, Brocheriou I, Zafrani L, Callard P, Rondeau E, Hertig A. Bilirubin-associated acute tubular necrosis in a kidney transplant recipient. Am J Kidney Dis 2013; 61:782-5.

33. Horsfall LJ, Nazareth I, Petersen I. Cardiovascular events as a function of serum bilirubin levels in a large, statin-treated cohort. Circulation 2012; 126:2556-64.

34. McArdle P, Whitcomb B, Tanner K, Mitchell BD, Shuldiner AR, Parsa A. Association between bilirubin and cardiovascular disease risk factors: using Mendelian randomization to assess causal inference. BMC Cardiovasc Disord 2012; 12:16.

35. Kamisako T, Masuda S, Tanaka Y. Relationship between serum bilirubin and remnant lipoprotein cholesterol level. Clin Lab 2013; 59:435-8.

36. Oda E, Kawai R. A possible cross-sectional association of serum total bilirubin with coronary heart disease and stroke in a Japanese health screening population. Heart Vessels 2012; 27:29-36.

37. Bwititi P, Nwose U, Nielsen S, Ruven Henk, Kalle W, Richards Ross, Butkowski E. Serum uric acid and albumin levels and estimated glomerular filtration rate: oxidative stress considerations. Australian J Med Sci 2012; 33:82-7.

38. Nwose EU, Richards RS, Bwititi PT, Butkowski E. Serum bilirubin and lipoprotein-a: How are these associated with whole blood viscosity? Redox Rep 2012; 17:8-13.

39. Nwose EU. Oxidative Stress Concepts in Clinical Practice - Towards optimizing medical nutrition therapy with laboratory methods. Lambert Academic Publishing, **CITY ???,** 2010.

40. Lott IT, Doran E, Nguyen VQ, Tournay A, Head E, Gillen DL. Down syndrome and dementia: a randomized, controlled trial of antioxidant supplementation. Am J Med Genet A 2011; 155:1939-48.

41. Lockrow J, Prakasam A, Huang P, Bimonte-Nelson H, Sambamurti K, Granholm AC. Cholinergic degeneration and memory loss delayed by vitamin E in a Down syndrome mouse model. Exp Neurol 2009; 216:278-89.

42. Miller RJ. The ups and downs of Down's syndrome. Lancet 2002; 359:275-6.

43. Reid BL, Bourke C. Control of metabolic energy by space systems. Part 1: Theoretical reports of space flow structure and function. Med Hypotheses 2003; 61:351-65.

44. Lehmann HP, Henry JB. SI units. In: Henry JB (ed) Clinical Diagnosis and Management by Laboratory Methods. 20th edition, WB Saunders, Philadelphia, PA, p 1433, 2001.

45. Nwose EU, Bwititi PT. Can Computer Meridian Diagnostic be useful in diagnosis and management of diseases? Med Hypotheses 2013; 81:564-7.