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REVIEW ARTICLE

Non-enzymatic antioxidant capacity assays: Limitations of use in biomedicine

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Abstract
The 'Total antioxidant capacity' (TAC) is a parameter frequently used for characterization of food products and of the antioxidant status of the body. This mini-review shows shortcomings of TAC assays and points of concern that should be considered when performing and interpreting results of such assays. The term TAC is not optimal since the assay measures only part of antioxidant capacity, usually excluding enzymatic activities. Antioxidant and oxidant-regenerating enzymes in blood cells and the blood vessel wall have a profound impact on the antioxidant properties of blood plasma, which is not reflected in the *in vitro* assays of isolated plasma. The term 'Non-enzymatic antioxidant capacity' (NEAC) is suggested as more relevant than TAC. NEAC is estimated by various methods, which yield different values and results obtained using different methods do not always show satisfactory correlation. One reason for the discrepancy of results is the use of different oxidants in NEAC assays. The use of hydroxyl radical as the oxidant is not recommended in view of the high and non-specific reactivity of this species.

Keywords: *Antioxidant, total antioxidant capacity, ABTS, DPPH˙, FRAP, ORAC, TRAP*

The idea

Antioxidant is defined, from a chemical point of view, as 'a substance that, when present at low concentrations compared to those of an oxidizable substrate, significantly delays or prevents oxidation of that substrate' (Page 1341) [1]. This operational definition provides also an instruction of how to assess the antioxidant activity of a substance. This instruction can be easily applied for a simple determination of a resultant antioxidant activity of all substances present in a sample. This quantity is often called *antioxidant capacity* or *total antioxidant capacity* (TAC) to differentiate it from the *antioxidant activity* of a simple compound characterized by the rate constant of its reaction with a specified oxidant [2]. As the legitimacy of the term TAC has been questioned [3], let us use the term 'non-enzymatic antioxidant capacity' (NEAC) at least throughout this review. Estimation of NEAC does not provide information on the nature of contributing compounds, but is simple and should allow for detection of unknown antioxidants and of synergistic interactions between antioxidants.

Direct assays of NEAC

Numerous protocols have been proposed for the assays of NEAC, some of them gaining broad popularity (Table I). From a mechanistic point of view, they are based on hydrogen atom transfer (HAT) or single electron transfer (SET), usually (but not always) coupled with proton transfer (Scheme 1). HAT reactions are generally quite rapid, typically completed in seconds-to-minutes, are weakly dependent on solvent and pH and represent a typical way of reaction of biologically relevant antioxidants. SET reactions are usually slow and require long times to reach completion, their rate generally augmenting with increasing pH since deprotonation enhances electron donating activity [4].

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Table I. Most popular direct assays of NEAC.

Method	Indicator	Oxidant	Principle of measurement	Mechanism	References
Antioxidant activity	Peroxides of methyl linoleate	Oxygen	Iodometric assay of peroxides	HAT	[58,59]
Total radical-trapping antioxidant parameter (TRAP)	Oxygen uptake	Peroxyl radicals (AAPH)	Oxygen electrode	HAT	[60,61]
Total oxidant scavenging capacity (TOSC)	α-keto-γ-methiolbutyric acid (KMBA)	Peroxyl radicals (AAPH), $^{\bullet}OH$, peroxynitrite (SIN-1)	Gas chromatography	HAT	[62,63]
Total antioxidant status (TAS)	2,2′-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS)	Ferrylmyoglobin + H_2O_2	Spectrophotometric fixed time assay	SET	[5,64]
Oxygen radical absorbance capacity ($ORAC_{PE}$)	R-Phycoerythrin	Peroxyl radicals (AAPH)	Fluorimetric	HAT	[65,66]
Oxygen radical absorbance capacity ($ORAC_{FL}$)	Fluorescein	Peroxyl radicals (AAPH)	Fluorimetric	HAT	[6]
Inhibition of crocin bleaching	Crocin	Peroxyl radicals (AAPH)	Spectrophotometric	HAT	[67]
Inhibition of LDL oxidation	LDL lipid dienes	Usually Cu^{2}+ ions	Spectrophotometric	HAT	[68]
Chemiluminescence	Luminol	AAPH, H_2O_2/peroxidase, UV	Luminometric	?	[39,69]

Most of the methods to assay NEAC stemmed out from the antioxidant definition [1] and are based on a kinetic assessment of inhibition of oxidation of an indicator substance, these assays are often referred to as *direct assays*. An alternative approach to assess the total content of antioxidants in a sample is based on determination of their reductive properties, i.e. their ability to reduce an indicator substance, or to evoke a reduction peak in a voltammetric measurement; these assays are sometimes called *indirect assays*.

Kinetic assays of NEAC are based on determination of lag (induction) time of the reaction induced by antioxidants, on the comparison of areas under curve of absorbance or fluorescence of an indicator vs time or on a fixed-time measurement of absorbance (fluorescence) of an oxidized (reduced) form of an indicator (Figure 1, Table I). The popular Total Antioxidant Status (TAS) assay commercialized by the Randox company is based on the measurement of reaction product after a fixed time under standard reaction conditions [5]. Not all antioxidants induce a lag time. A clear-cut lag time is expected when the rate constant of the antioxidant k_A for the reaction with the oxidizing agent is much higher than that of the indicator k_I ($k_A >> k_I$), so the oxidation of the indicator becomes visible when the antioxidant is exhausted (sample 1 in Figure 1C). When both rate constants are comparable ($k_A \approx k_I$) and concentrations of both substance are similar, both will be oxidized simultaneously and the rate of oxidation of the indicator will be lower and lag time in the oxidation of the indicator will not be apparent (sample 1 in Figure 1C). A fixed-time assay or the area-under-curve approach is applicable for both these cases (Figure 1C) so they are more universal than lag-time assays.

The obvious drawback of the most popular kinetic assays involving 2,2′-azobis(2-amidinopropane) (AAPH) as a source of free radicals is the rather long time of analysis, which may be compensated by parallel analysis of multiple samples in absorbance or fluorescence microplate readers.

HAT reactions

$$X^{\bullet} + AH_2 \rightarrow XH + AH^{\bullet}$$

SET reactions

$$X^{\bullet} + AH \rightarrow X^{-} + AH^{\bullet+}$$
$$AH^{\bullet+} + H_2O \rightarrow A^{\bullet} + H_3O^{+}$$
$$X^{-} + H_3O^{+} \rightleftharpoons XH + H_2O$$

$$M^{n+} + AH \rightarrow M^{(n-1)+} + AH^{+}$$

Scheme 1. Hydrogen atom transfer (HAT) and single electron transfer (SET) reactions.

Which oxidant should be employed?

The oxidant, or source of oxidant to be used in NEAC assay should be easy to obtain/handle and biologically relevant. Superoxide and hydrogen peroxide are of obvious biological relevance, but they are disposed of mainly be enzymatic means. It is therefore of doubtful value to introduce new NEAC assays based on the use of these reactive oxygen species (ROS) as there are well established essays of activities of superoxide dismutase and catalase, although sometimes it might be of interest to control the share of non-enzymatic scavenging in the assayed activities ascribed to those enzymes.

NEAC assays based on the scavenging of the hydroxyl radical have been proposed [6,7] and employed. Hydroxyl radical is thought to be crucial

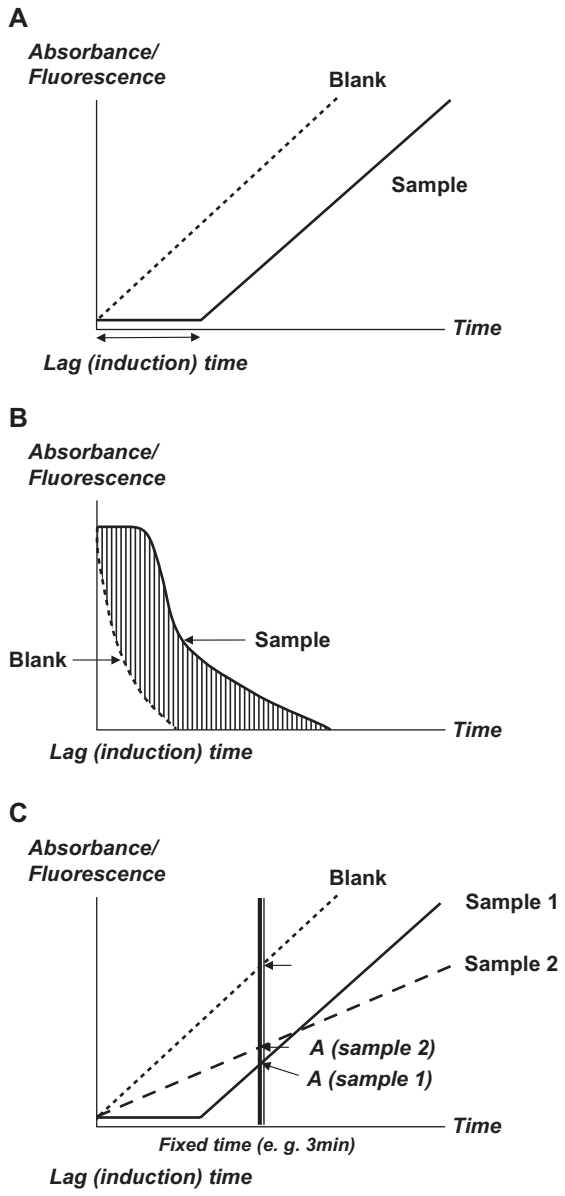

Figure 1. Various principles of direct NEAC assays: (A) determination of lag (induction) time of a reaction of oxidation of an indicator (duration of the lag time is a measure of NEAC); (B) determination of area-under-curve of decay of absorbance of fluorescence (area-under-curve is a measure of NEAC); (C) fixed point assay (inhibition of the increase in absorbance or fluorescence during a fixed time of reaction is a measure of NEAC).

for the biological damage inflicted by ROS, but $^{\bullet}OH$ is a species reacting avidly with most molecules so NEAC against this radical is closer to measure the total content of organic molecules rather than that of antioxidants.

There are biologically relevant antioxidants, for which no specific enzymatic detoxification systems exist, like peroxyl radicals, peroxynitrite, hypochlorite and ozone, although peroxiredoxins, selenoproteins and hemoproteins react with peroxynitrite at a high rate [8]. Measurement of the scavenging capacity of these species by biological material may be of interest and of possible physiopathological significance [9,10].

Indirect NEAC assays

As the ability of a compound to act as an antioxidant depends in the vast majority of cases on its ability to reduce oxidants, their reactive intermediates or primary oxidation products, measurement of reducing properties of a sample may be an indirect measure of the content of antioxidants of this sample. However, some synthetic antioxidants having no reducing properties, as nitroxides will not be measured by this approach. The most popular NEAC assays based on reduction of indicators are listed in Table II. They are based on reduction of stable free radicals (of which $DPPH^{\bullet}$ can be purchased and $ABTS^{\bullet+}$ is prepared by oxidation of ABTS [11]) or metal ions. An obvious drawback of the FRAP assay is the low pH (3.6), at which especially thiols show low reactivity [12,13].

The indicators used in these assays differ in reactivities, so numerical values obtained in various assays are different. Another problem of these assays is the kinetics of reduction. Although generally these assays are less time-consuming than direct ones, in many cases the reaction is far from complete after the recommended fixed reaction time. Reaction rates of various antioxidants differ significantly [14,15] and biological samples containing a variety of antioxidants show usually long reaction times. Therefore, results of measurements of $ABTS^{\bullet+}$ reduction cannot be directly compared if measurements were done after different reaction times (or the reaction time is not specified). The measurements are usually done at ambient temperature, which can be different in various laboratories and between seasons. Sometimes measurements at two time points are employed to measure 'slow' and 'fast' antioxidants, but complete separation of both contributions is not possible since 'slow' antioxidants contribute also to the 'fast' reactivity [16–18].

The $DPPH^{\bullet}$ reduction assay is often used, especially when studying plant material. It is cumbersome when applied to blood plasma since the reaction is performed in a medium containing methanol or ethanol, which precipitates proteins [19]. Due to the low rate of the reaction, many assays are not run until completion of the reaction, but are based on a fixed time of incubation or measurement of the reduction rate during a short time. Often, the results are given not in equivalents of a standard antioxidant but in percentage reduction of absorbance.

Different assays yield different values

The same antioxidants show different reactivities in various assays and activity of the same antioxidants usually differs between assays (Table III). Reactivity of antioxidants vary with pH, e.g. in the crocin bleaching assay, the reactivity ratio at pH of 8.0 to that at pH of 6.0 is 1.11, 0.78, 1.67 and 12.1 for Trolox, ascorbate, urate and BSA, respectively [20]. The phenoxyl

Table II. Most popular direct assays of NEAC.

Method	Indicator	Measurement	References
$DPPH^{\bullet}$ decolourization	Diphenylpicrylhydrazyl stable radical ($DPPH^{\bullet}$)	Spectrophotometric or EPR	[25,70]
$ABTS^{\bullet+}$ decolourization	Preformed $ABTS^{\bullet+}$	Spectrophotometric	[11,24]
Ferric-reducing activity of plasma (FRAP)	Ferrous tripyridyltriazine	Spectrophotometric	[12,13]
Cupric ion reducing antioxidant capacity (CUPRAC)	Bathocuproine (BC), bathocuproinedisulfonic acid disodium salt (BCS) or neocuproine (NC)	Spectrophotometric	[71,72]
Cyclic voltammetry	—	Voltammetric	[73]

radicals of vitamin E and of its synthetic, less hydrophobic analogue, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), formed upon one-electron reactions of these molecules with oxidants, is relatively stable and prone to a reaction with a next oxidant molecule [21]. The pH sensitivity of their reactions is also relatively low. As a result, a tocopherol or Trolox molecule undergoes two one-electron reactions under most situations of NEAC assays. Therefore, Trolox is the most often used antioxidant standard and NEAC is usually expressed in Trolox equivalents. If another substance is used as a standard (e. g. ascorbic acid or uric acid), the conversion into Trolox equivalents is not 1:1 and will differ between various assays.

Since NEAC of a biological material is a function of the content of individual antioxidants and their contribution will vary from one method to another, NEAC of a given sample is different in various assays [19]. As a result, correlations between results obtained by various methods are sometimes not impressive, e.g. correlation coefficients between results of $ORAC_{FL}$ and FRAP assays for various vegetables of 0.36–0.93 were reported [22]; the correlation coefficient between results of NEAC measurement of human serum by $ORAC_{PE}$ and FRAP was 0.35 ($p < 0.02$) and no correlation was found between NEAC values of the serum obtained by $ORAC_{PE}$ and TAS and by FRAP and TAS [23].

Which antioxidants are measured?

Most NEAC assays are performed in aqueous solutions, which creates problems for measuring antioxidants that are hydrophobic and barely soluble or completely insoluble in such systems. The contribution of these antioxidants to NEAC of a tested sample will be under-estimated. Some assays can or even must be performed in non-aqueous media, which gives a better opportunity for measuring activities of hydrophobic antioxidants. $ABTS^{\bullet+}$ reduction can be measured in ethanol [11,24], while $DPPH^{\bullet}$ reduction must be measured in methanol, ethanol or methanol(ethanol)/water mixtures [14,25]. Other solutions of this problem have been proposed, e.g. the use of cyclodextrins to enhance the solubility of hydrophobic antioxidants [26] or measurement of inhibition of oxidation of a hydrophobic fluorescent probe C11-BODIPY581/591 induced by a hydrophobic azo initiator 2,2′-azobis(4-methoxy-2,4-dimethylvaleronitrile) (MeO-AMVN) [27]. In turn, the contribution of hydrophilic antioxidants should be expected to be minute in assays of this type. Interestingly, however, the MeO-AMVN/C11-BODIPY581/591 assay [27] was reported to be sensitive to hydrophilic antioxidants, especially uric acid [28].

Table III. Relative reactivities of antioxidants in various assays, with respect to Trolox. Reactivity of Trolox is assumed as 1.

Assay	TOSC	TRAP (oxygen electrode)	$ORAC_{PE}$/ $ORAC_{FL}$	FRAP
Uric acid	0.70	0.65	Not measured	0.84
Ascorbic acid	0.46	0.85	0.22/0.48	1.04
Glutathione	0.19	0.18	0.16/0.31	0.11
Reference	[62]	[61]	[6]	[19]

Main fields of application of NEAC assays

Two main fields of applications of NEAC assays in biomedical sciences are: (i) studies of foods, beverages and medicinal plants to estimate their total content of antioxidants, (ii) studies of blood plasma/serum, other body fluids and tissue homogenates aimed at understanding the overall defense status against oxidative stress in diseases.

Food, spices, herbs and beverages

NEAC of food products has been a field of extensive research, which produced thousands of papers. Not surprisingly, however, results of comparisons of NEAC of the same fruits and vegetables bring divergent results. NEAC of tea leaves, medicinal herbs and herbs which are sources of spices is also frequently studied. It should be remembered that the NEAC of the plant material is affected by the stage of growth, light intensity, temperature and other environmental factors [29,30] so once obtained data cannot be treated as universally applicable.

Building a database of NEAC activity of foods has been proposed [31]. Such a database contains data

collected using one method, $ORAC_{FL}$ (http://www.ars.usda.gov/Services/docs.htm?docid=15866). Even if methodological differences have been eliminated by the use of one method, the utility of such data is compromised by the dependence of the NEAC of fruits and vegetables on the conditions of growth, degree of ripeness, time and conditions of storage and way of preparation [32]. Often fruit peels have a high content of antioxidants and peeling may decrease NEAC activity of fruits [33–35]. Various cooking methods affect NEAC of food products in different ways, usually decreasing but in some cases augmenting it [36]. A more substantial reservation concerning the practical use of food antioxidant databases concerns the question of equivalence of NEAC contributed by various compounds. Plant metabolites have various biological activities, apart from antioxidant properties. Ingestion of 5 g of ascorbate is not equivalent to consumption of an equivalent amount of 1 g of the phytoestrogen genistein, which shows approximately equivalent antioxidant activity. Diverse bioavailability of various antioxidants is another question; consumption of two portions of different foods of the same NEAC may lead to absorption of disparate amounts of Trolox equivalents from the intestine if the content of available antioxidants differs from one food to another. Still, food NEAC data may be valuable to consider appropriate antioxidant milieu in the intestine [37].

What determines NEAC of blood plasma?

As antioxidants react stoichiometrically with oxidants, the main contribution to NEAC belongs to those antioxidants that are present at the highest concentrations. For human blood plasma, the main contributors to NEAC are: uric acid and protein thiol groups. The contribution of various antioxidants to NEAC of blood varies depending on the assay used, e.g. the share of thiol groups is low in FRAP (Table IV).

The presence of unidentified component of NEAC of blood plasma has been postulated by a Finnish group on the basis of comparison of measured NEAC with a value predicted on the basis of concentrations and activities of known antioxidants [38,39]. This fraction is decreased in immobilized patients [40] but increased in pneumonia [39]. The contribution of uric acid to NEAC of human blood plasma is prevalent and changes of the concentration of this metabolite may affect NEAC significantly. Some authors suggest the determination of calculation of uric-acid independent NEAC either by uricase treatment of plasma [41] or by subtraction of the contribution of uric acid calculated on the basis of its plasma concentration and reactivity [42].

Exemplary results of studies of the effects of various treatments on the blood plasma NEAC and alterations of blood plasma NEAC in various diseases are shown in Table V. While in many diseases a decrease in blood plasma NEAC is a biomarker of systemic oxidative stress, not always diseases known to involve oxidative stress are characterized by lower NEAC of blood plasma. A classical example is chronic renal failure, in which NEAC of blood plasma shows inverse correlation with the glomerular filtration rate and higher NEAC values, due to increased concentration of uric acid, reflecting impaired renal function [43,44]. Being dependent on numerous variables and showing limited changes under pathologic conditions, blood plasma NEAC does not seem to be a potential diagnostic parameter, although scarce reports have suggested its suitability in some cases, e.g. for prediction of thrombosis-prone status in type 2 diabetes [45] or of plasma β-carotene level, especially in moderate alcohol drinkers [46].

Other body fluids

NEAC of other body fluids, especially those easy to obtain (milk, tears, saliva, seminal plasma, urine, but also cerebrospinal and amniotic fluid), apart from blood plasma, has been a subject of studies (Table VI). NEAC of urine is contributed mainly by uric acid so the uric-acid independent fraction of urine NEAC may be more useful than total NEAC of this fluid, reflecting mainly the rate of purine metabolism.

Post-prandial changes in plasma NEAC

The post-prandial changes in plasma NEAC seem convincing in showing a direct effect of consumption of

Table IV. Percentage contributions of antioxidants to the NEAC of human blood plasma estimated by various methods.

Method	AAPH/Luminol	TRAP AAPH/ Luminol	TRAP AAPH/ oxygen consumption	Crocin bleaching	$ABTS^{\bullet+}$ decolourization	FRAP	ORAC/ Phycoerythrin
Uric acid	53.1	42.9	47.4	50.9	33.1	60	36.9
Protein–SH	6.0	16.3	12.7	20.8*	52.9	10	31.5
Bilirubin	1.1	—	—	—	2.4	—	
Vitamin C	3.0	2.6	13.4	26.7	4.7	15	3.7
Vitamin E	3.0	3.1	5.4	1.6	1.7	5	4.8
Unidentified	33.8	35.1	21.1	0	5.2	10	23.1
Reference	[74]	[73]	[75]	[20]	[7]	[12]	[65]

*Calculated for BSA.

Table V. Examples of results of studies of NEAC of blood plasma. Results expressed in Trolox equivalents.

Disease/Effect studied	Control group	Compared group	Method of assay	*p*	Reference
Repeated red wine consumption (300 ml daily for a week)	324 ± 125 μM	510 ± 391 μM	FRAP	<0.05	[76]
Omnivores vs vegetarians	Omnivores: 1.160 ± 0.163 mM	Vegetarians: 1.135 ± 0.177 mM	FRAP	NS*	[77]
Maximal aerobic exercise at high altitude in highlanders	0.49 ± 0.04 mM	1.23 ± 0.05 mM	Inhibition of ABTS oxidation	<0.001	[78]
Supplementation with ascorbic acid and Coenzyme Q_{10}	1.15 ± 0.14 mM	1.16 ± 0.07 mM	AAPH/Luminol	NS	[79]
Major depression	1.75 ± 0.08 mM	1.69 ± 0.11 mM	Crocin bleaching	<0.05	[80]
Smokers vs non-smokers	126 ± 54 μM	68 ± 35 μM	Cu^2+ reduction	<0.02	[81]
Old (65–96 y) vs young (19–26 y)	Young 1.31 ± 0.06 mM	Old 1.02 ± 0.06 mM	Inhibition of ABTS oxidation	<0.01	[82]
Essential hypertension	426 ± 14 μM	311 ± 8 μM	FRAP	<0.01	[83]
Type 2 diabetes	146 ± 8 μM	111 ± 4 μM	FRAP	NS	[84]
Schizophrenia	1.128 ± 0.145 mM	1.058 ± 0.101 mM	Inhibition of ABTS oxidation	<0.05	[85]
Systemic inflammatory response syndrome	803 ± 31 μM	606 ± 20 μM	AAPH/Luminol	<0.001	[74]
Amyotrophic lateral sclerosis	1.96 ±1.01 mM	9.59 ± 11.7** mM	Inhibition of ABTS oxidation	<0.00002	[86]
Epilepsy	1.64 ± 0.14 mM	0.89 ± 0.23 mM	Inhibition of ABTS oxidation	<0.001	[87]
Alzheimer's disease	1.040 ± 0.280 mM 4.861 ± 0.479 mM	1.094 ± 0.154 mM 4.942 ± 0.415 mM	FRAPABTS$^{\bullet+}$ decolourization	NS NS	[88]
Parkinson's disease	410 ± 19 μM	295 ± 26 μM	AAPH/Luminol	<0.01	[89]
Cystic fibrosis	1.40 ± 0. 20 mM	1.35 ± 0.11 mM	Inhibition of ABTS oxidation	NS	[90]
Cardiovascular disease	789 ± 159 μM	538 ± 119 μM	FRAP	<0.0001	[91]
Acute lymphocytic leukaemia, children	739 ± 48 μM 156 ± 4 μM	514 ± 46 μM 162 ± 6 μM	FRAP DPPH$^{\bullet}$ decolourization	<0.01 NS	[92]
End-stage renal disease, before and after haemodialysis	Before: 1.4 ± 0.2 mM	After: 1.1 ± 0.2 mM	Inhibition of ABTS oxidation	<0.001	[93]

*NS, not significant at P = 0.05.
**High variation due to the heterogeneity of the group studied.

food and beverages on the redox status of the body. It has been found, e.g. that consumption of tea [47] or certain berries and fruits such as blueberries, mixed grape and kiwifruit, is associated with increased plasma NEAC [48] and that plasma NEAC decreases after a test meal ('Milanese' meat, i.e. beef, egg and breadcrumbs, fried in corn oil, and fried potatoes) and this increase is prevented by simultaneous consumption of red wine [49]. However, these changes are small and sometimes cannot be ascribed to the consumed antioxidants, e.g. increase of plasma NEAC after consumption of apples is due to the effect of fructose, which augments the level of uric acid; this effect can be mimicked by ingestion of an equivalent amount of fructose [50,51].

Interaction between antioxidants

It can be argued that one of the advantages of NEAC measurements over determination of content of single antioxidants is the possibility of detection of synergistic interactions between antioxidants present in the sample. However, there is little room for such interactions if all antioxidants in the sample react stoichiometrically in the assay and, in contrast to the situation *in vivo*, cannot be regenerated. Such synergistic interactions between hydrophobic and hydrophilic antioxidants have been reported [52], but evidence for such interactions is scarce. On the other hand, sub-additive effects were observed in NEAC measurements. NEAC of mixtures of proteins (albumin or casein) and flavonoids is lower than the sum of antioxidant activities of pure components. This effect is due to binding of flavonoids to the proteins [53]. However, sub-additive effects may occur also between antioxidants in the absence of binding, apparently due to interactions between free radicals of antioxidants formed during the assay [54]. These effects may cast doubt on the validity of the simple assumption that NEAC measured is an algebraic sum of contributions of individual antioxidants present in the sample [20,55] and of calculations based on this assumption.

Conclusions: Handle with care

NEAC of biological materials is an easily measurable parameter, which may be useful in some cases due to its simplicity, rapidity and low cost. Its lack of specificity may be an advantage, e.g. when screening chromatographic fractions for the presence of antioxidants

Table VI. Examples of results of studies of NEAC of other body fluids.

Fluid/disease or treatment studied	Control group	Compared group	Method of assay	*p*	Reference
Cerebrospinal fluid/ amyotrophic lateral sclerosis	61.3 ± 3.2 μM	48.8 6 2.2 μM	Ferric reducing ability (FRA)	<0.003	[94]
Cerebrospinal fluid/ meningismus vs meningitis	Meningismus 0.38 ± 0.11 mM	Meningitis 0.4 ± 0.07 mM	$ABTS^{\bullet+}$ decolourization	NS*	[95]
Seminal plasma/exposure to radiofrequency magnetic waves	1.68 ± 0.48 mM	1.55 ± 0.38 mM	Inhibition of ABTS oxidation	NS	[96]
Seminal plasma/fertile donors vs infertile patients	1.83 ± 0.54 mM	1.38 ± 0. 43 mM	Inhibition of ABTS oxidation	<0.001	[97]
Follicular fluid/*in vitro* fertilization, intracytoplasmic sperm injection	Pregnant: 1.054 ± 0.189 mM	Non-pregnant: 0.685 ± 0.203 mM	AAPH/Luminol	<0.02	[98]
Follicular fluid/smoking	440 (340–545) μM	362 (254–533) μM	AAPH/Luminol	<0.004	[99]
Amniotic fluid/2nd trimester vs 3rd trimester	2nd trimester: 347 (210–565) μM	3rd trimester: 776 (636–1116) μM	Inhibition of ABTS oxidation	<0.001	[100]
Blister fluid		2.82 ± 0.24 mM	Inhibition of ABTS oxidation	<0.02	[101]
Saliva/Crohn's disease	0.342 ± 0.110 mM	0.248 ± 0.145 mM	FRA	NS	[102]
Saliva/smoking	Non-smokers: 0.14 ± 0.15 mM	Smokers: 0.24 ± 0.31 mM	Inhibition of ABTS oxidation	<0.01	[103]
Tears, old (> 59 y) vs young (≤ 59 y, = C)	Young: 179.4 ± 95.4 μM	Old: 237.2 ± 151.7 μM	FRA		[104]
Urine	3.2 ± 0.1 mM		AAPH/Pyrogallol Red		[105]
Urine/Down's syndrome < 14 y Urate-independent NEAC	445 ± 119 mmol/mol creatinine 111 ± 81 mmol/mol creatinine	692 ± 343 mmol/mol creatinine 174 ± 118 mmol/mol creatinine	CUPRAC-BCS,Uricase	<0.02 NS	[106]
Faeces	26.6 ± 10.2 mmol/kg wet faeces		Inhibition of ABTS oxidation		[107]

*NS, not significant at P = 0.05.

[38,39,56,57]. However, in many cases the value of the sum of antioxidant activities of individual components does not provide sufficient information. Compounds which act as antioxidants have also other more specific functions and are not biologically equivalent. In a clinical laboratory, NEAC may be useful as a simple initial test for diagnosis of oxidative stress and monitoring therapy for first orientation, to be followed by more specific analyses of individual antioxidants, like erythrocyte sedimentation rate employed as a screening test. Restricting analysis to NEAC should be discouraged, especially in situations where it is readily assayable what component is responsible for changes of antioxidant properties of a sample.

Suggestion: Nomenclature

NEAC is referred to using different names, Total Antioxidant Capacity (TAC) being used most frequently. The critique of this term [3] seems justified: this parameter does not reflect the full capacity of the organism to deal with oxidative stress. NEAC of isolated blood plasma does not reflect the antioxidant properties of whole blood, which is contributed to significantly by antioxidant enzymes (first of all superoxide dismutase, catalase, glutathione peroxidase and peroxiredoxins) present in blood cells and cells of the vessel walls and by enzymatic systems present in these cells which regenerate oxidized forms of antioxidants. Sometimes the results of NEAC assays are labelled only with the name of a specific procedures (ORAC, FRAP) or name of the test (TAS), which contributes to the confusion. In my opinion, NEAC used in this paper would be the most appropriate term to replace TAC. Since the results of the assays strongly depend on the oxidant used, the oxidant should be mentioned in direct assays, which results could be referred to as e.g. 'NEAC for hypochlorite' or 'NEAC for peroxynitrite'.

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