Inter-laboratory comparison of methodologies for the measurement of urinary 8-oxo-7,8-dihydro-2'-deoxyguanosine

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Abstract

Urinary 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodGuo) is widely used as a marker of oxidative stress. Here~~in~~ we report the comparison of two, distinct chromatographic assays, with ELISA. Chromatographic assays displayed good agreement ($r = 0.89$, $p < 0.0001$), whereas there was markedly worse, albeit still significant, agreement with ELISA (HPLC-GC/MS: r=0.43, HPLC-EC: r=0.56; p<0.0001). Mean values differed significantly between chromatographic assays and ELISA (HPLC-GC/MS: 3.86, HPLC-EC: 4.20, ELISA: 18.70 ng/mg creatinine, p<0.0001). Whilst it is reassuring to note good agreement between chromatographic assay, this study reveals significant short-comings in the ELISA, which bring into question its continued use in its present form.

Keywords: oxidative stress; 8-oxodGuo; ELISA; HPLC; GC/-MS; urine; biomarkers; molecular epidemiology.

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Introduction

Oxidative stress is purported to have an important role in a variety of pathological conditions, including cancer, cardiovascular disease and neurodegenerative disease (Olinski, Gackowski et al. 2002; Cooke, Evans et al. 2003). Oxidative damage to DNA perhaps receives the most attention, for not only can this lead to mutation (Cheng, Cahill et al. 1992), but it may have numerous other effects upon cell function, such as acceleration of telomere shortening, or modification of gene expression and cell signalling (Evans and Cooke 2004). Overall, this demonstrates that oxidative modification of DNA possesses clear mechanistic relevance to the pathogenesis of both malignant and non-malignant disease (Evans and Cooke 2006).

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Of all the identified oxidative modifications of DNA, the nucleobase 8-oxo-7,8-dihydro-guanine (8-oxoGua), or its 2'-deoxyribonucleoside equivalent 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodGuo), is the most studied, and is widely acknowledged as a ~~robust~~ biomarker of oxidative stress (Kasai 1997). Numerous approaches can determine *cellular* DNA levels of ~~8-oxodG~~ 8-oxodGuo (Guetens, De Boeck et al. 2002) but, in addition to being necessarily invasive, many of the procedures also risk the production of artefactual damage during sample workup (Gedik and Collins 2005), which rather limit its utility in molecular epidemiology studies.

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However, measurement of 8-oxodGuo in *urine*, has excellent stability (> 10 years, (Loft, Svoboda et al. 2006)), can circumvent issues of invasiveness and artefact (Lin, Jenner et al. 2004). The presence of 8-oxodG in urine is thought to derive from the activity of nudix hydrolases (also known as pyrophosphohydrolases), which hydrolyse the modified deoxyribonucleotide triphosphate (8-oxodGTP) to the corresponding monophosphate, which cannot be re-phosphorylated, removing 8-oxodGTP as a

substrate for polymerases (Cooke, Olinski et al. 2008). Potentially, measurement of urinary 8-oxodG may provide information concerning the prevention of mis-incorporation (Cooke, Evans et al. 2005). These properties are crucial to the development of assays which will improve our understanding of the role of oxidative stress in disease, and the development of biomarkers for this condition. A further pre-requisite is that such assays should be well-validated, in terms of being robust and reliable, with inter-laboratory consensus.

The principal chromatographic techniques applied to the analysis of ~~8-oxodG~~ 8-oxodGuo in urine are high pressure liquid chromatography pre-purification followed by gas chromatography (HPLC-GC/MS (Gackowski, Rozalski et al. 2001)), liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS (Weimann, Belling et al. 2002; Malayappan, Garrett et al. 2007)), and (column switching) liquid chromatography with electrochemical detection (HPLC-EC (Loft, Deng et al. 1998; Kasai 2003)); or immunoassay (enzyme linked immunosorbant assay, ELISA (Cooke, Evans et al. 1998; Rossner, Svecova et al. 2007)). The most frequently used ELISA applied to urinary analysis of ~~8-oxodG~~ 8-oxodGuo is that manufactured by the Japanese Institute for the Control of Aging ('8-OH-dG Check'; JaICA). However, there is significant precedent in the literature for this approach reporting greater levels of urinary ~~8-oxodG~~ 8-oxodGuo than chromatographic methods. Previous typical mean values for urinary 8-oxodG, in healthy volunteers, by the JaICA ELISA kit have ranged from 8.16 to 11.13 µg/g creatinine (Shimoi, Kasai et al. 2002; Yoshida, Ogawa et al. 2002), compared to 0.79 – 2.13 µg/g creatinine, for example, by chromatographic techniques (discussed in Lin et al. (Lin, Jenner et al. 2004)). It should also be noted that the reproducibility of ~~8-oxodG~~ 8-oxodGuo analysis by ELISA can also be very low. When the same urine samples were analysed

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repeatedly (30 times), the raw ~~8-oxodG~~ 8-oxodGuo values showed a very high coefficient of variation (CV= 28.4 - 47.3 (Yoshida, Ogawa et al. 2002)).

A more recent report indicates that strict temperature control during the incubation steps of the JaICA ELISA can decrease mean values (e.g. 1.67 pmol/μmol creatinine for ELISA vs. 0.41 pmol/μmol creatinine for LC-MS/MS (Cooke, Singh et al. 2006)), but this still has not resolved this problem. Further complicating this issue, there have been instances when the ELISA has been shown not to correlate with chromatographic techniques (Prieme, Loft et al. 1996; Cooke, Singh et al. 2006); whilst in other reports they do correlate, using HPLC-ECD (Shimoi, Kasai et al. 2002; Yoshida, Ogawa et al. 2002); and LC-GC/MS (Cooke, Rozalski et al. 2006). However, it appears that the good correlation noted by Yoshida et al. was obtained after certain erroneous data points were excluded. Specifically, data from an ELISA plate would be excluded if there was >25% variation in ~~8-oxodG~~ 8-oxodGuo values from any of the three different standard urine samples applied to the plate (Yoshida, Ogawa et al. 2002). This implies a problem with the uniformity of commercial ELISA plates.

To date, no ~~single~~ study has made comparisons between more than one chromatographic assay and ELISA, and there is only a single report of a comparison between two chromatographic assays (Harri, Kasai et al. 2007). Such experiments would be the first step towards establishing validated assays for application in future molecular epidemiology studies. ~~Like the~~As for European Standards Committee on Oxidative DNA Damage before it, a goal of the European Standards Committee on Urinary (DNA) Lesion Analysis (ESCULA, (Cooke, Olinski et al. 2008)) is to achieve consensus between the various methods for measuring urinary 8-oxodG. Here~~in~~ we report the comparison of three distinct methods for the analysis of urinary 8-oxodG:

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HPLC-GC/MS, HPLC-EC and ELISA, in order to begin the process of validating assays, which can ultimately be used to establish reference ranges for healthy individuals and patient groups with specific disease, stratified for parameters known to affect oxidative stress (e.g. age and smoking).

Methods

Subjects and sampling

Urine samples in the present study were collected from Swedish men (n = 140), aged 20-60 years (mean = 43 years), participating in the EU-funded, 'EMECAP' (European Mercury Emissions from Chloralkali Plants) project (Barregard, Horvat et al. 2006; Jarosinska, Barregard et al. 2006). Whilst the primary purpose of that project was to quantify the internal dose of mercury in chloralkali workers and general populations living close to mercury cell chloralkali plants, this population presented the opportunity to compare multiple methods of analysing urinary 8-oxodG. Of the 140 subjects, 57 were chloralkali workers exposed to inorganic mercury and 83 were age-matched control subjects living in the same region. Since there were only small differences between exposed workers and ~~referents~~controls, we pooled these 140 subjects into a single group for the method comparisons.

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Each subject provided a first void urine sample. The samples were subsequently aliquotted into multiple polyethylene tubes (cryotubes 4.5 mL, Nunc^{Tm}, Denmark), and stored frozen at –25 ^{o}C. The sample and background data collection was performed in 2002. Subsequently the samples were sent "blindly" to the following laboratories for ~~8-oxodG~~ 8-oxodGuo analysis: HPLC-~~/~~GC-~~/~~MS (Olinski laboratory, Bydgoszcz, Poland), HPLC-EC (Kasai laboratory, Kitakyushu, Japan) and ELISA (Cooke laboratory, Leicester, UK).

Urine sample preparation for HPLC purification with GC-~~/~~MS analysis (HPLC-GC/MS)

To two mL of human urine were added 0.05 nmol of [$^{15}N_5$] ~~8-oxodG~~ 8-oxodGuo (Gackowski, Rozalski et al. 2001). After centrifugation (2000 × g, 10 min), the

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supernatant was filtered through a Millipore GV13 0.22 μm syringe filter and 500 μL of the filtrate was injected onto the HPLC system.

HPLC purification of urinary ~~8-oxodG~~ 8-oxodGuo was performed according to the method described by Ravanat *et al*. (Ravanat, Guicherd et al. 1999) with some modifications. Briefly, urine samples spiked with labelled compounds were injected onto a Supelcosil LC 18 column (250 × 10 mm) equipped with Supelguard LC18 guard column (20 × 4.6 mm), both from Supelco. A 30 min linear gradient elution was performed (0.5 % acetic acid at 0 min to 0.5 % acetic acid with an addition of 5 % of acetonitrile 30 min), at a flow rate of 3 mL/min. After this time the column was washed with 70 % of acetonitrile for 20 min and equilibrated with 0.5 % acetic acid for 10 min prior to a further injection.

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The collected fractions were dried by evaporation under reduced pressure in a Speed–Vac system. The ~~8-oxodG~~ 8-oxodGuo fraction was treated with 400 μL of 60 % formic acid (Sigma) for 30 min. at 130 ^{o}C. Subsequently, samples were prepared for GC-MS analysis which was performed according to the method described by Dizdaroglu (Dizdaroglu 1994), adapted for additional [$^{15}N_5$] 8-oxoGua analyses (m/z 445 and 460 ions were monitored).

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Urine sample preparation and HPLC-EC analysis

Urine samples were defrosted and mixed with an equal volume of a 4 % (v/v) acetonitrile in double-distilled water, containing the ribonucleoside marker 8-oxoguanosine (8-oxoGuo; 120 ug/mL), 130 mM NaOAc and 0.6 mM H_2SO_4. The treated urines were then stored at 5 °C overnight, before being centrifuged at 15,700 x ~~g~~ for 5 min~~s~~. Samples were transferred to plastic HPLC injector vials and 20 μL was injected, using an automated HPLC system similar to that described in detail

previously (Kasai 2003). In essence, the system comprised a sampling injector (Gilson 231XL), a pump (Gilson 307) for the anion exchange guard (MCI GEL CA08F, 7 µm, 1.5 x 50 mm) and main column (MCI GEL CA08F, 7 µm, 1.5 x 150 mm) in HPLC-1 (flow rate was 50 µL/min and column oven was set at 65 ^{o}C), a UV detector (Gilson UV/VIS-151 with micro cell), a second pump (Gilson 307) for the analysis of the ~~8-oxodG~~ 8-oxodGuo fraction with a reverse-phase column (Shiseido, Capcell Pak C18, 5 µm, 4.6 x 250 mm) in HPLC-2 (flowrate was 0.9 mL/min and column oven set at 40 ^{o}C), connected with an EC detector (ESA Coulochem II) and two switch valves. A third pump (Gilson 307) was used to back wash the guard column (flowrate 70 µL/min) for 32 min~~utes~~ after valve switching at around 13 minutes after each sample injection. For HPLC-1 the solvent used was ~~composed of~~ 2 % acetonitrile in 0.3 mM sulfuric acid, in HPLC-2 the solvent was composed of 10 mM phosphate buffer (pH 6.7), 5 % methanol, plus an antiseptic, Reagent MB (KCG reagent, MC Medical Inc. Japan; 100 µL/L), and was recycled for a ~~time~~ period of one week. The guard column was back washed with 0.5 M ammonium sulphate:acetonitrile (7:3 v/v). Both the 8-oxoGuo marker peak, used for automatic peak detection, and ~~–~~ the ~~8-oxodG~~ 8-oxodGuo fraction, were detected at 305 nm (Svoboda and Kasai 2004). After automatic peak detection the ~~8-oxodG~~ 8-oxodGuo fraction was collected by valve switching and then injected on HPLC-2 to be detected by a Coulochem II EC detector (ESA) with a guard cell (5020) and an analytical cell (5011), applied potentials: guard cell = 400 mV, E1 = 150 mV and E2 = 350 mV. The total time between analysis of consecutive samples was 52 min~~s~~. The automatic peak detection was controlled by software from Gilson and chromatograms were recorded and integrated with the computer software (Unipoint 3.30).

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Urine sample preparation and ELISA

Following thawing and centrifugation (300 x g for 10 mins) of the urine samples, the supernatants (50 μL/well) were applied to the competitive ELISA plate according to the protocol (Japanese Institute for the Control of Aging, Fukuroi, Japan). On the basis of our previous observations (Cooke, Singh et al. 2006) we adhered strictly to the incubation temperature of 37 °C for the primary antibody, as described by the manufacturer.

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Urinary creatinine

Urinary creatinine levels were determined by the photometric 'Jaffe' method at the University Hospital in Gothenburg, Sweden.

Statistics

Associations between assays were assessed by plots and the Pearson correlation coefficient (r_p), as well as the Spearman rank correlation coefficient (r_s). Differences between assays were tested with paired t-tests of untransformed and log-transformed results. Results were also expressed per mg creatinine in order to normalise for urine concentration, and allow comparison with previous literature values. The possible impact of smoking and age was tested in multiple linear regression models including these covariates and the creatinine concentration of the samples. Statistical analyses were performed using the SAS package, version 9.1. P-values < 0.05 were considered to be statistically significant.

Results

Descriptive statistics are shown in Table 1. The mean level of urinary ~~8-oxodG~~ 8-oxodGuo was similar to those previously reported in the literature, for samples collected from the general population, when comparing like-for-like analytical methods (chromatography (Harri, Kasai et al. 2007); ELISA(Leinonen, Lehtimaki et al. 1997; Thompson, Heimendinger et al. 1999)). As shown in the Table 1, the mean ~~8-oxodG~~ 8-oxodGuo levels, determined by the HPLC-EC assay, were on average ~10% higher than the HPLC/GC-MS method (paired analysis, $P<0.01$). Mean levels by the ELISA assay were 5-7 times higher than those found for the other two methods ($P<0.0001$). [Insert Table 1 here.]

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Figure 1A shows the strong, linear association between the concentrations of urinary 8-oxodG, analysed by HPLC-GC/MS and HPLC-EC ($n = 115$, $r_p=0.89$, $P<0.0001$; Table 1). Expressed in another way, had the result been duplicates from the same assay, the coefficient of variation would have been only 25%. The strength of the association was identical based on all 140 samples ($r_p=0.89$). The Bland-Altman plot (Figure 1B) shows that the association between results from the two assays was similar over the whole range of concentrations although, as would be expected, the absolute deviations between results increased with concentration. [Insert Figure 1 here.]

In contrast, the linear associations between urinary ~~8-oxodG~~ 8-oxodGuo concentrations measured by ELISA and (i) HPLC-GC/MS ($r_p=0.17$, $P=0.08$), and (ii) HPLC-EC methods ($r_p=0.28$, $P=0.003$), were much weaker. As evident from Table 1 and Figures 2A and 3A~~B~~, the results using ELISA provide, on average, much higher concentrations of urinary 8-oxodG. Consequently the associations with the ELISA assay were stronger using the Spearman rank correlation coefficient, which puts less

weight on the extremes, and reflects the rank order of the sample concentrations, irrespective of the fact that ELISA results in paired samples are higher than the results for the HPLC-based methods (r_s=0.43, P<0.0001 when comparing to HPLC-GC/MS and r_s=0.56; P<0.0001 when comparing to HPLC-EC; Figure 2A and ~~B~~3A). Bland-Altman plots, of the data represented in Figures 2A and 3A, show that the association between results from the two assays was similar (within 95 % limits of agreement) at relatively low concentration of urinary 8-oxodG (< 40 ng/mL) although, as would be expected, the absolute deviations between results increased significantly with increasing concentration. The plots for ELISA vs. HPLC-GC/MS and ELISA vs. HPLC-EC were almost identical in terms of mean bias and 95 % limits of agreement. [Insert Figures 2 and 3 here.]

The levels of urinary 8-oxodGuo in self-reported smokers were slightly higher, as measured by both HPLC-GC/MS and HPLC-EC (point estimates, 24 % and 15 % higher, respectively, n=115) than in non-smokers. Surprisingly, the results using ELISA show, on average, 17 % lower urinary ~~8 oxodG~~ 8-oxodGuo in smokers. In linear multiple regression models with self-reported smoking (yes/no), age and creatinine concentration as independent variables, the increase with creatinine concentration was by far the strongest effect, significant (P<0.0001) for all assays. The effect of smoking on urinary ~~8 oxodG~~ 8-oxodGuo was statistically significant in the results from the HPLC-EC assay (P=0.03) and borderline for the HPLC-GC/MS assay (P=0.05).

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Discussion

There is a need for well validated, preferably non-invasive, biomarkers of oxidative stress, that can be applied to the rapid analysis of samples from a large scale study. Immunoassay approaches have the potential to fulfil this criterion, as well as being easily established in even the most poorly equipped of laboratories, and with little need for specialist experience or equipment. However, the on-going lack of agreement between immunoassay and chromatographic techniques, widely considered to be the 'gold standard', limits the usefulness of this approach.

Cursory examination of the literature would indicate that there appears to be reasonable within-technique agreement in urinary ~~8 oxodG~~ 8-oxodGuo levels, in healthy adults (discussed in Cooke et al. (Cooke, Evans et al. 2000)). As mentioned above, a number of studies have compared chromatographic approaches with ELISA, with markedly different findings. However, there has been little formal comparison of chromatographic techniques, with only one report in the literature, which also showed a good correlation between HPLC-MS/MS and HPLC-EC (r = 0.93, n = 246 samples (Harri, Kasai et al. 2007)). Herein, we demonstrate the strong agreement between HPLC-EC and HPLC-GC/MS, although mean ~~8 oxodG~~ 8-oxodGuo values by HPLC-EC were significantly higher than by HPLC-GC/MS. It is of interest that Harri et al. (Harri, Kasai et al. 2007) also noted that, on the whole, the HPLC-EC method gave higher levels, although this was not examined for statistical significance. A possible explanation for this finding might derive from the use of mass labelled, internal standards in the HPLC-MS/MS assay. Nevertheless, both chromatographic methods were sensitive enough to show the expected slight increase in urinary ~~8-oxodG~~ 8-oxodGuo amongst smokers.

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Consistent with previous studies, we demonstrate that ELISA generates significantly higher values for urinary 8-oxodG. Possible reasons for this over-estimation are a source of much discussion within the literature~~;~~ for example, some authors claim that the monoclonal antibody used in the JaICA ELISA kit, and indeed other commercially available kits, is not sufficiently specific for the detection of 8-oxodG. Whilst it is possible that the antibody used in the ELISA kit may be cross-reactive towards 8-oxoGua or/and the ribonucleoside or even the unmodified nucleoside/base, there is no experimental evidence for this (Cooke, Olinski et al. 2008). In contrast, a recent publication demonstrated that performing the primary antibody incubation at 4 ºC overnight, increases the selectivity of the ELISA (Evans, Singh et al. 2008). This modification to the protocol brings mean urinary 8-oxodG levels into agreement with chromatographic methods.

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We also show ~~that ELISA can demonstrate~~ a significant correlation between ELISA and ~~with~~ two, distinct, chromatographic techniques. ~~However,~~ ~~s~~Since there is a reasonable association between the ranks of the values for ELISA and chromatographic techniques, it is perhaps not surprising that the ELISA can identify associations with ~~8-oxodG~~ 8-oxodGuo in pathological conditions (Rossner, Svecova et al. 2007; Chung, Huang et al. 2008), although this technique should be expected to be less precise than HPLC-based techniques. It is also of concern that the relationship between ELISA and chromatographic techniques can be so variable – sometimes correlating with chromatographic techniques, as reported here, and sometimes not (for example, Prieme et al. (Prieme, Loft et al. 1996)). It might be speculated that the basis for this variability derives from some intrinsic variability in the urine samples used in one study, compared to those used in another, e.g. a higher concentration of interfering compounds. Certainly, isolation of ~~8-oxodG~~ 8-oxodGuo

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from the urine, and hence removal of any interfering constituents, with subsequent analysis by ELISA, vastly improves the agreement between ELISA and HPLC-EC (r=0.550 prior to purification, and r=0.833, post-purification (Shimoi, Kasai et al. 2002)). In itself, this implies that this is either an issue of specificity, or some other kind of assay interference. Of course, this detracts from any benefit offered by ELISA, and is not really a practical solution.

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The source of the ELISA should also be considered. As we have highlighted previously, certain manufacturer's kits have severe shortcomings over and above those noted here (Cooke, Singh et al. 2006). Taken together, and in particular given the ability for absolute identification of target compound by mass spectrometry, it is reasonable to assume that the chromatographic techniques reflect the 'true' concentrations of the modified 2'-deoxyribonucleoside, 8-oxodG.

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There are surprisingly few studies focussing upon urinary 8-oxodGuo and smoking. Of these, the general finding is that levels of 8-oxodGuo are elevated in the urine of smokers, compared to non-smokers (Prieme, Loft et al. 1998; Hu, Wang et al. 2006), our findings, using chromatographic techniques, agree with this. However, the reverse was true, based upon ELISA estimates, which is difficult to reconcile, although a previous study of smoking status and urinary 8-oxodGuo, determined by ELISA, failed to establish a significant difference between the two groups (Besaratinia, Van Schooten et al. 2001).

This study is the first step ~~reveals itself to the first step~~ towards obtaining validated methods for measuring urinary 8-oxodGuo, ~~and~~ highlighting the limitations of immunoassay, ~~which requires further attention~~ and bringing into question its continued use in its present form. ~~As such, it highlights the need for a~~A larger study is needed, incorporating multiple methods, and analysing pure standards,

as well as multiple urine samples~~.~~, and ~~T~~this is a goal of ESCULA. It should be noted that, whilst presently the most studied product of oxdatively-generated damage to DNA, 8-oxodGuo is not the sole product (Cooke, Evans et al. 2003). Other lesions exist which, under various cellular conditions, may be more abundant than 8-oxodGuo (Pang, Zhou et al. 2007; Dedon 2008). This presents the possibility of other lesions being studied in urine, and other biological matrices, as potential biomarkers of oxidative stress and disease.

Acknowledgements

MSC, MDE, RR, DG, AS, MF and RO are partners of ECNIS (Environmental Cancer Risk, Nutrition and Individual Susceptibility), a network of excellence operating within the European Union 6th Framework Program, Priority 5: "Food Quality and Safety" (Contract No 513943).

The research related to the analysis of ~~8-oxodG~~ 8-oxodGuo using the HPLC-EC method was supported by the Japan Society for the Promotion of Science.

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Table 1. Comparison of urinary ~~8-oxodG~~ 8-oxodGuo (ng/mL) by three different methods, in 115 Swedish men aged 20-60 years.

Method	Mean 8-oxodG (ng/mL) [1]	Median [2]	SD	Min	Max
HPLC-GC/MS	6.01	4.99	5.22	0.66	47.3
HPLC-EC	6.52	5.62	4.59	1.07	38.8
ELISA	29.8	18.0	31.3	2.89	172

[1]Mean ~~8-oxodG~~ 8-oxodGuo in ng/mg creatinine was 3.86 for HPLC-GC/MS, 4.20 for HPLC-EC, and 18.70 for ELISA; mean creatinine concentration 1.6 g/L.

[2]For HPLC-GC/MS and HPLC-EC results were available for 140 men. Mean ~~8-oxodG~~ 8-oxodGuo values were very similar to those shown above: 6.29 ng/mL (HPLC-GC/MS) and 6.74 ng/mL (HPLC-EC).

Figure legends

Fig. 1. (A) Comparison of urinary ~~8-oxodG~~ 8-oxodGuo concentrations as determined by HPLC-EC and HPLC-GC/MS. Lines of linear regression (——) and perfect correlation (·····) are both shown. (B) Bland-Altman plot displaying the comparison of HPLC-EC and HPLC-GC/MS techniques. The plot shows the differences between paired measurements plotted against their respective means. The 95% limits of agreement (mean bias +/- 2 SD) and mean bias are shown.

Fig. 2. (A) Comparison of urinary ~~8-oxodG~~ 8-oxodGuo concentrations as determined by ELISA and HPLC-GC/MS. (B) Bland-Altman plot displaying the comparison of ELISA and HPLC-GC/MS techniques. The plot shows the differences between paired measurements plotted against their respective means. The 95% limits of agreement (mean bias +/- 2 SD) and mean bias are shown.

Fig. 3. (A) Comparison of urinary 8-oxodGuo concentrations as determined by ELISA and HPLC-EC. (B) Bland-Altman plot displaying the comparison of ELISA and HPLC-EC techniques. The plot shows the differences between paired measurements plotted against their respective means. The 95% limits of agreement (mean bias +/- 2 SD) and mean bias are shown.

Fig. 1A.

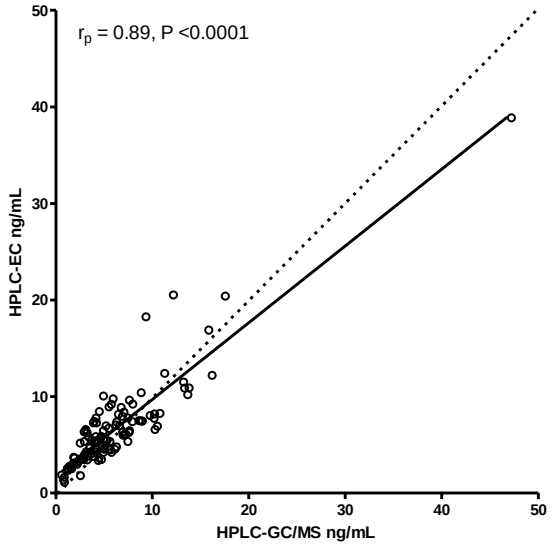

r_p = 0.89, P <0.0001
HPLC-EC ng/mL
HPLC-GC/MS ng/mL
0
10
20
30
40
50

Fig. 1B.

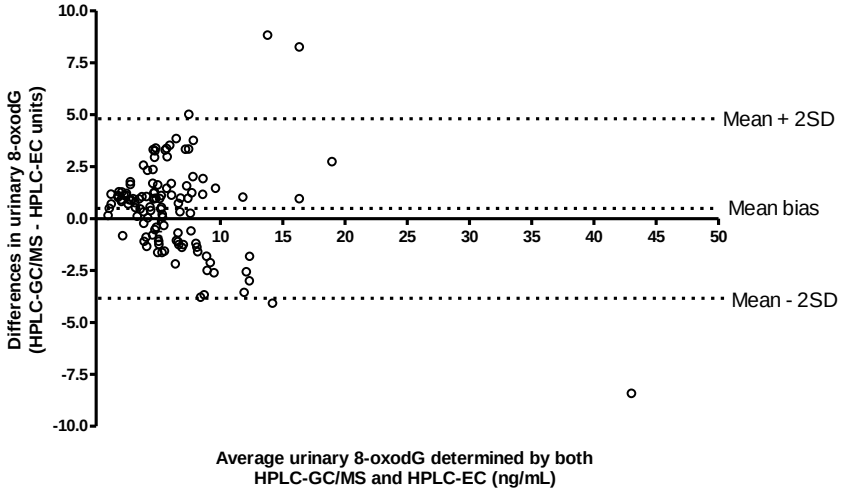

Differences in urinary 8-oxodG (HPLC-GC/MS - HPLC-EC units)
Average urinary 8-oxodG determined by both HPLC-GC/MS and HPLC-EC (ng/mL)
Mean + 2SD
Mean bias
Mean - 2SD
10.0
7.5
5.0
2.5
0.0
-2.5
-5.0
-7.5
-10.0
10
15
20
25
30
35
40
45
50

Fig. 2A.

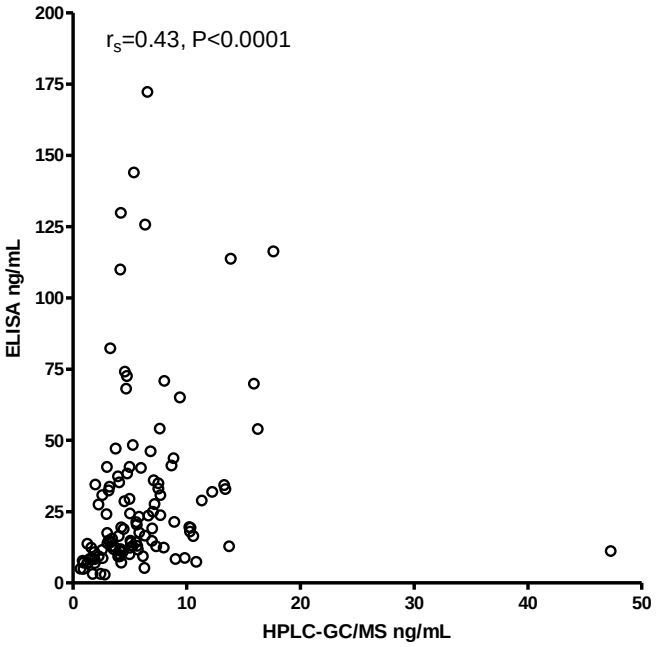

r_s=0.43, P<0.0001
ELISA ng/mL
HPLC-GC/MS ng/mL
0
25
50
75
100
125
150
175
200
0
10
20
30
40
50

Fig. 2B.

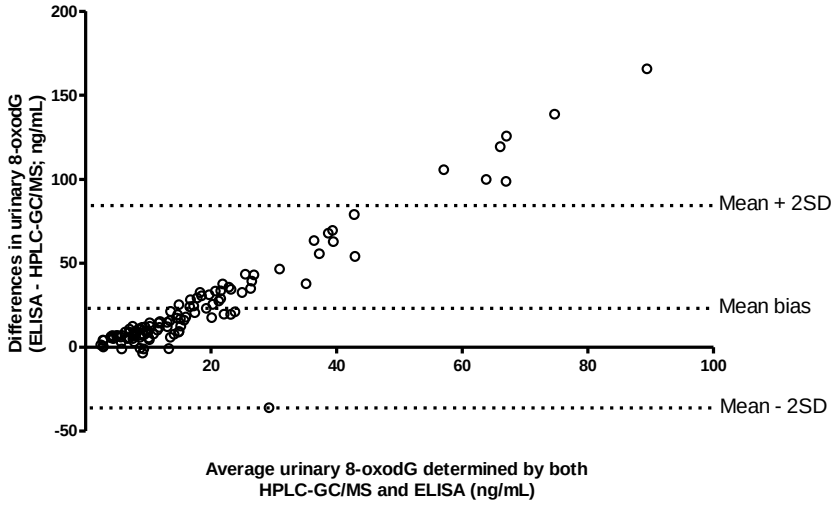

Differences in urinary 8-oxodG (ELISA - HPLC-GC/MS; ng/mL)
Mean + 2SD
Mean bias
Mean - 2SD
200
150
100
50
0
-50
20
40
60
80
100
Average urinary 8-oxodG determined by both HPLC-GC/MS and ELISA (ng/mL)

Fig. 3A.

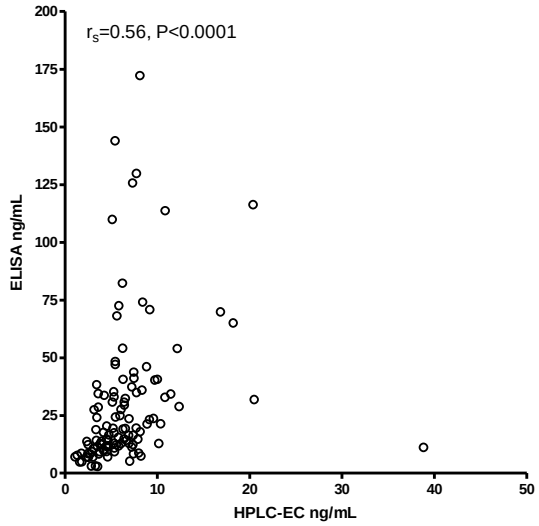
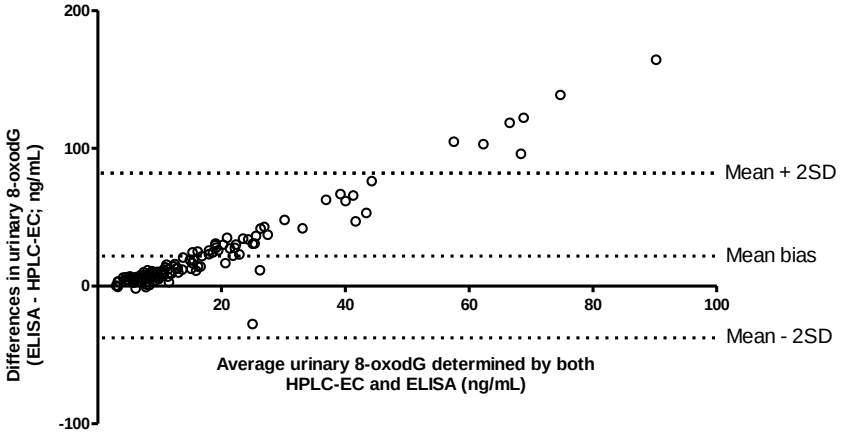

r_S=0.56, P<0.0001
ELISA ng/mL
HPLC-EC ng/mL
0
25
50
75
100
125
150
175
200
0
10
20
30
40
50

Fig. 3B.


Differences in urinary 8-oxodG (ELISA - HPLC-EC; ng/mL)
Average urinary 8-oxodG determined by both HPLC-EC and ELISA (ng/mL)
Mean + 2SD
Mean bias
Mean - 2SD
200
100
0
-100
20
40
60
80
100