Critical comparison of three 8-hydroxy 2'-deoxyguanosine monoclonal antibodies

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ABSTRACT

Oxidative damage to nucleic acids is associated with a variety of diseases including cancer, aging, and obesity. Guanine is the base that is most prone to oxidation, and 8-hydroxy-2'-deoxyguanosine from DNA is the form of oxidized guanine that is most commonly studied. In recent years, it has become increasingly clear that both DNA and RNA are damaged by oxidation in disease states, and that the repair processes that are initiated to correct this damage release multiple oxidized guanine species into the urine, including the ribose-free base (8-hydroxyguanine), the nucleoside from RNA (8-hydroxyguanosine), and the deoxynucleoside from DNA (8-hydroxy-2'-deoxyguanosine). While 8-hydroxy-2'-deoxyguanosine is the form most researchers study, it has been also reported that 8-hydroxyguanosine is a better marker of age-related oxidant damage, and that 8-hydroxyguanine is a better marker in some cancer patients.

There are several commercially available 8-hydroxy-2'-deoxyguanosine monoclonal antibodies that have been used to develop immunoassays. For the average consumer, it is unclear whether these antibodies are specific for 8-hydroxy-2'-deoxyguanosine or also recognize other closely-related guanine species. In addition, it is often unclear how closely the ELISA results using these antibodies to assess biological samples match LC/MS values for the same biological samples. Finally, it remains unclear whether quantification of any single guanine species or multiple species is the better assessment of oxidant stress *in vivo*.

In this report, 7E6.9 a novel monoclonal antibody (CCC kit # 501130) and two commercially available monoclonal antibodies (N45.1 and 15A3), are compared for binding specificity and LC/MS correlation of 8-hydroxy-2'-deoxyguanosine levels in human urine.

ELISA

Method 1 – The antigen (8-hydroxy-2'-deoxyguanosine) is coated on the plate. The specific monoclonal antibody and unknown sample containing 8-hydroxy-2'-deoxyguanosine are added to the plate. Antibody that is not bound by 8-hydroxy-2'-deoxyguanosine in solution is free to bind the plate-coated 8-hydroxy-2'-deoxyguanosine . The plate-bound antibody is detected using anti-mouse IgG-HRP and TMB substrate.

Method 2 – The antigen (8-hydroxy-2'-deoxyguanosine) is directly labeled with an enzyme (tracer). The specific antibody is bound to the plate. The tracer competes for binding to the antibody with the 8-hydroxy-2'-deoxyguanosine in the sample. The plate-captured tracer is detected with Ellman's reagent.

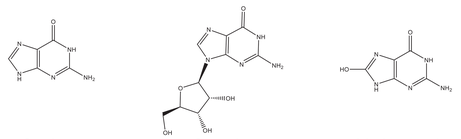
Both ELISA methods were used for each of the monoclonal antibodies. The data obtained with the two methods were comparable but the second method used less antibody, and required fewer steps, and was therefore preferred.

LC/MS

Quantitative determination of 8-hydroxy-2'-deoxyguanosine and 8-hydroxyguanosine in human urine was performed by liquid chromatography-tandem mass spectrometry utilizing an Agilent 1200 HPLC coupled to an Applied Biosystems API 4000 triple quadrupole mass spectrometer.

Each urine sample was diluted 1:1 with 1M ammonium acetate containing 5ng/mL of internal standard. A calibration curve was prepared in the dilution solvent. A 10 µL injection of each sample and standard was made onto the LC/MS/MS.

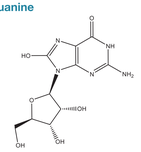
The method precision proved 3.74% RSD (n=6) at 1 ng/mL with a limit of quantitation equal to 100 pg/mL and a detection limit of 30 pg/mL.



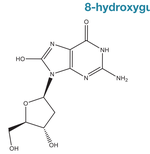
Guanine

Guanosine

8-hydroxyguanine

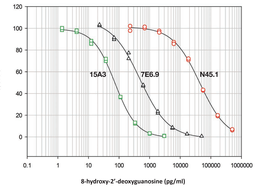


8-hydroxyguanosine



8-hydroxy-2'-deoxyguanosine

Problem: Monoclonal antibodies produced against 8-hydroxy-2'-deoxyguanosine may cross-react with 8-hydroxyguanine and 8-hydroxyguanosine (from RNA).

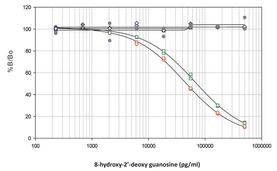


Graph 1. Sensitivity Comparison. Standard Curves were performed for each antibody to compare the ELISA's working range.

Table 1. Percent cross-reactivity of antibodies against molecules with structural similarities

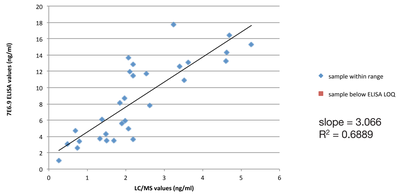
	Monoclonal antibody		
	15A3	**N45.1**	**7E6.9**
8-hydroxy-2-deoxyguanosine*	100	100	100
8-hydroxyguanosine	38	66	100
8-hydroxyguanine	23	<1	0.15
guanosine	<0.1	<1	<0.1
guanine	<0.1	<1	<0.1

*recognition of 8-hydroxy-2'-deoxyguanosine is 100% benchmark



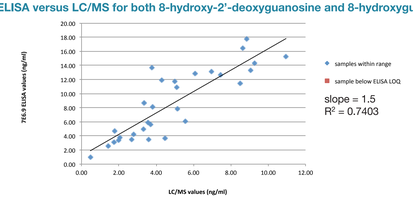
Graph 2. N45.1 specificity. Competitive ELISA was used to determine specificity of N45.1 with 8-hydroxy-2'-deoxyguanosine (○), 8-hydroxyguanosine (□), 8-hydroxyguanine (△), guanosine (◊), and guanine (●).

7E6.9 ELISA versus LC/MS for 8–hydroxy-2'-deoxyguanosine



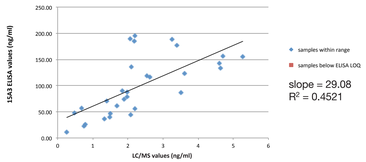
Graph 3. 30 human urine samples were quantified by 7E6.9 based ELISA and LC/MS for 8-hydroxy 2'-deoxyguanosine

7E6.9 ELISA versus LC/MS for both 8-hydroxy-2'-deoxyguanosine and 8-hydroxyguanosine



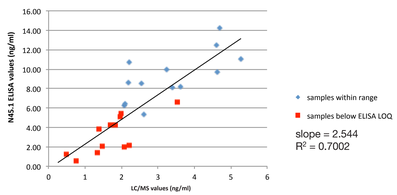
Graph 4. 30 human urine samples were quantified by 7E6.9-based ELISA and the sum of 8-hydroxy-2'-deoxyguanosine and 8-hydroxy guanosine by LC/MS

15A3 ELISA versus LC/MS for 8-hydroxy-2'-deoxyguanosine



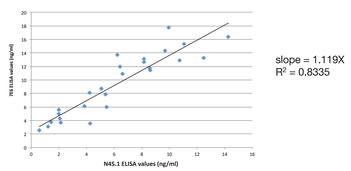
Graph 5. 30 human urine samples were quantified by 15A3-based ELISA and LC/MS for 8-hydroxy-2'-deoxyguanosine

N45.1 ELISA versus LC/MS for 8-hydroxy-2'-deoxyguanosine



Graph 6. 30 human urine samples were quantified by N45.1-based ELISA and LC/MS for 8-hydroxy-2'-deoxyguanosine

Direct comparison of ELISAs values from N45.1 and 7E6.9



Graph 7. 30 human urine samples were quantified by N45.1-based and 7E6.9-based ELISA.

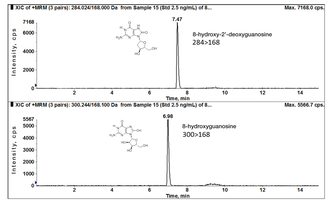


Figure 1. LC/MS/MS Chromatogram.

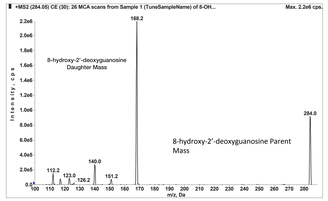


Figure 2. 8-hydroxy-2'-deoxyguanosine Product Ion Scan (284>168)

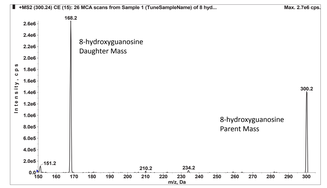


Figure 3. 8-hydroxyguanosine Product Ion Scan (300>168)

Discussion:

- ELISAs using all three monoclonal antibodies (7E6.9, N45.1 and 15A3) correlate with LC/MS values for detection of 8-hydroxy-2'-deoxyguanosine, with slopes ranging from 2.5 to 30.
- A 7E6.9-based ELISA correlates better to LC/MS measurements of a combination of 8-hydroxy-2-deoxyguanosine and 8-hydroxyguanine (slope =1.5) than 8-OH-2-dG alone.
- It is unknown what other metabolites 15A3 may be measuring but they appear to be related to 8-hydroxy-2'-deoxyguanosine.
- Future work should include validation against biological samples from an *in vivo* oxidative stress model in order to better assess these antibodies.

Conclusions:

- The 7E6.9 antibody shows a high degree of correlation with N45.1, which exhibits cross-reactivity with the RNA metabolite 8-hydroxyguanosine (not previously reported).
- The ELISA using the 15A3 antibody also correlates with LC/MS but exhibits a high slope. This may indicate detection of other oxidized guanosine metabolites, which could be advantageous in some models of oxidative stress.