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THE PRACTICAL APPLICATION TESTS OF ECO-ASSAY® DIOXIN ELISA KIT FOR THE MEASUREMENT OF DIOXINS IN ASH SAMPLES

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Introduction

Dioxins (polychlorinated dibenzo- *p*- dioxins, polychlorinated dibenzofurans and coplanar-polychlorinated biphenyl) have severe toxicity and the actual level of dioxins in the environment may cause harmful effect on the reproductive ability and the immune response. Dioxins exist a quite small amount in the environment, and the measurement of dioxins is required a high-sensitive and high-accuracy analytical method. Because of this reason, High-Resolution Gas Chromatograph / High-Resolution Mass Spectrometer (HRGC/HRMS) is adopted in the official analytical method of dioxins. In the official method, however, there are some problems such as the necessity of an expensive and appropriate instrument, an educated technical expert and a long turnaround time. So, in case of the necessity of acute feedback of results and the total evaluation of many dioxins pollutant points, it seems to be very inconvenient to measure the dioxins using the official analytical method. Consequently, a simple, economical and rapid dioxins assay method (alternative assay method) is required and the performance of the alternative assay method is also requested the almost same ability to GC/MS. One of the candidate of alternative method is an Enzyme-Linked Immunosorbent Assay (ELISA). Recently, the ecoassay® dioxin ELISA kit was already developed and can very conveniently determine dioxins[1)]. As there are only a few data that was utilized the ELISA kit, in this paper, we reported the results of the reliability and practical applications on this ELISA kit.

Methods and Materials

We tested the performance of the ELISA kit in five laboratories and also tested the variance among laboratories.

Sample preparation: Ash samples were extracted with toluene after treatment with 2N HCl. After concentration, dryness and dissolution with n-hexane, the extracts were clean up by a sulfuric acid treatment and silica gel column chromatography, or by a multi-layer silica gel column chromatography. The cleaned-up samples were perfectly dried and then dissolved with DMSO. After addition of the citrate buffer, the DMSO solution was assayed for dioxins using ELISA kit.

ELISA kit procedure: Procedure of ELISA kit method is shown in Figure 1.

Results and Discussion

A correlation between the toxic equivalent (TEQ) in ash samples by GC/MS and Dioxin Equivalent (DEQ) by ELISA kit is summarized in Figure 2. This figure illustrates a significant positive correlation expressed by gradient of 1.56 with a correlation coefficient of 0.757. Each data (n = 52) was independently measured in five laboratories, showing a ratio of DEQ/TEQ to be a range of 0.42 to 14.0 with an average of 3.4. When compared the two analytical data, there was a tendency that the DEQ was higher than the TEQ in 90% of the samples. On the other hand, the samples, in which the DEQ was lower than the TEQ, showed the high TEQ value. These results induced that further investigations concerning extraction and clean-up methods were required.

Therefore, we tested a variance among five laboratories when used the same sample (3.0 ngTEQ/mL) and the ELISA kits. The results of the intra and inter assay variation are shown in Table 1. The DEQ values in each laboratory showed almost the same and were quite similar to the TEQ values. In the next step, in order to confirm an effect of low chlorinated dioxins on the high ratio of DEQ/TEQ, we measured the DEQ value of samples which were cleaned up on a multi-layer silica gel column and an alumina column. The results of the intra and inter assay variation using this cleaned up sample are shown in Table 2. A decrease of the DEQ value was observed in the laboratory A, D and E, but the reverse result was obtained in the laboratory B and C. This result showed that there was no change of the DEQ value, in spite of an incorporation with the further alumina column clean up. Consequently, we concluded that the low chlorinated dioxins gave no effect on the DEQ value, but there are still some possibility that impurities that can not remove in the clean-up processes may cause non-specific reactions.

Conclusion

These results clearly show the validity for the measurement of dioxins using the ecoassay® dioxin ELISA kit. In addition, this presents a new, simple and economical method for measuring dioxins in ash samples.

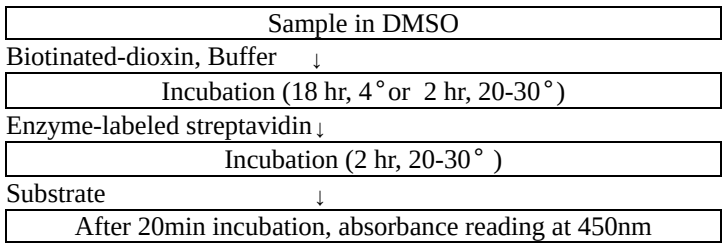

Figure 1 Procedure of the Eco Assay® Dioxin ELISA kit

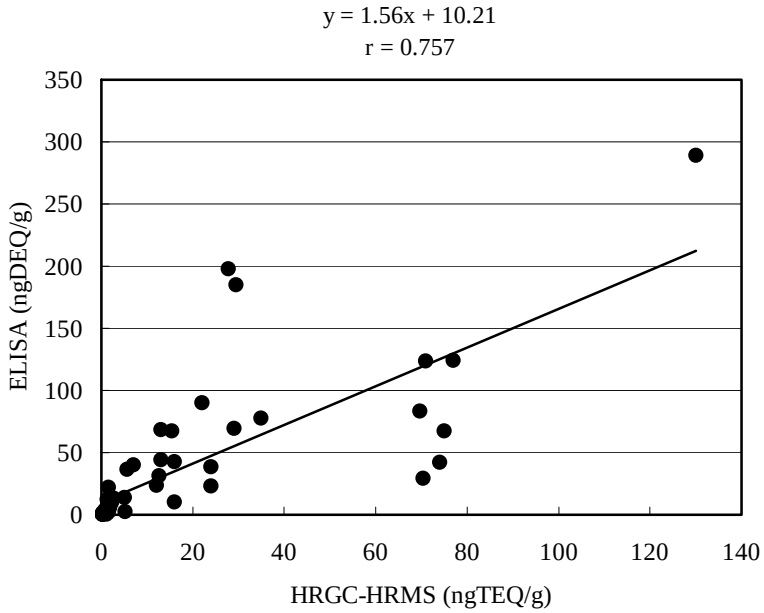

Figure 2 Correlation between the TEQ value by GC/MS and the DEQ value by the ELISA kit (Fly ash sample: n = 52)

	1st	2nd	3rd	Mean±S.D.
A	4.6	4.0	2.3	3.7±1.2
B	1.5	3.1	2.2	2.3±0.83
C	4.2	4.2	4.1	4.2±0.076
D	-	2.7	1.5	2.1±0.86
E	2.2	2.0	2.2	2.1±0.088

Table 1 Inner and inter assay variations among 5 laboratories using 5 samples (A – E) cleaned up on a multi layer column

	1st	2nd	3rd	Mean±S.D.
A	3.5	1.9	2.8	2.7±0.81
B	2.8	3.5	-	3.1±0.43
C	5.6	5.5	5.5	5.6±0.049
D	2.2	1.5	1.4	1.7±0.45
E	2.0	2.2	1.6	1.9±0.34

Table 2 Inner and inter assay variations among 5 laboratories using 5 samples (A – E) cleaned up on a multi layer column and an alumina column

References

1. Tsukasa Kodaira, Yasuteru Usuki, Biomedical Research, under pressing