Organohalogen Compounds, Volume 60, Pages 279-282 (2003)

DEVELOPMENT OF IMMUNOCHROMATOGRAPHIC TEST FOR SCREENING OF POLYCHLORINATED BYPHENYLS

Kazuto Nishi[1], Akira Okuyama[1], Hiroshi Takenaka[1], Haruki Mizukami[1], Hidetaka Takigami[2], Eiichi Tamiya[3], Shin-ichi Sakai[2], Masatoshi Morita[2]

EnBioTec Laboratories Co., Ltd., Time 24 Bldg., 4th Floor, 2-45 Aomi, Koto-ku, Tokyo 135-8073, Japan[1], National Institute for Environmental Studies, Onogawa 16-2, Tsukuba, Ibaraki 305-8506, Japan[2], Japan Advanced Institute of Science and Technology, 1-1, Asahidai, Tatsunokuchi, Ishikawa 923-1292, Japan[3]

Introduction

Polychlorinated biphenyls (PCBs) are one of the major concerns in environmental contaminants because of their toxicity to human and wildlife, and low rate of degradation in the environment. Several analytical methods of PCBs are currently available, such as gas chromatography/mass spectrometry (GC/MS) and enzyme-linked immunosorbent assay (ELISA). GC/MS method is very precise but expensive, time consuming and labor-intensive. ELISA system has been developed as an alternative means for the estimation of PCB concentration. We reported the development of ELISA system using monoclonal antibody which showed the highest reactivity against 2,3', 4,4', 5-pentachlorobiphenyl (PCB 118) for the quantification of PCBs[1, 2]. Although being a good tool for screening purpose, ELISA system needs some special equipments and skill for obtaining reliable data, and is not suitable for on-site measurement of PCBs.

Immunochromatographic test has been developed as more convenient assay than ELISA. The test, which is very simple and does not need special equipment, has been applied to many fields, especially to a diagnostic medicine[3, 4]. In these applications, its targets are mostly large molecule substances such as proteins. But the test concept, which is that the positive results are judged by the increase of color intensity, can not be applied to small molecule target like PCBs. Ordinal immunochromatographic tests use a sandwich type reaction with two different antibodies to obtain positive result line visibly. In case of a small molecule antigen, the sandwich-reaction does not occur due to an obstacle to the reaction between an antigen and the antibodies.

In this study, we report the development of a new immunochromatographic test for PCB using the PCB 118 monoclonal antibody and the performance of the test with PCB 118 and Japanese commercial PCB products; Kanechlor (KC), which have been considered to be a main source of PCBs contamination in Japan.

Materials and Methods

Test Principle

The test principle of the newly developed immunochromatographic test (Figure 1) is based on the gold labeled immunochromatography technology. Both PCB hapten as free gold-antibody conjugate trap line and anti-mouse immunoglobulin G as test line are immobilized on a nitrocellulose membrane. After gold-antibody conjugate and sample solution are mixed, the mixture is applied on the sample pad and moves forward on the membrane by capillary action.

When PCB negative samples are tested, all free gold conjugate is trapped by the PCB hapten on the trapper line and no color appears on the test line. On the other hand, for PCB positive samples, complex of PCB and gold-antibody conjugate passes through the trapper line and reaches to the test line. Since the complex is captured by the anti-mouse immunoglobulin, visible red color appears on the test line. Thus, the color intensity of the test line is proportional to the concentration of PCB in the sample. Detection of the reaction color can be performed by naked eyes or by an immunoreader (Otsuka electronics, Japan, DiaScan 10) which detects the reflectance of the red color.

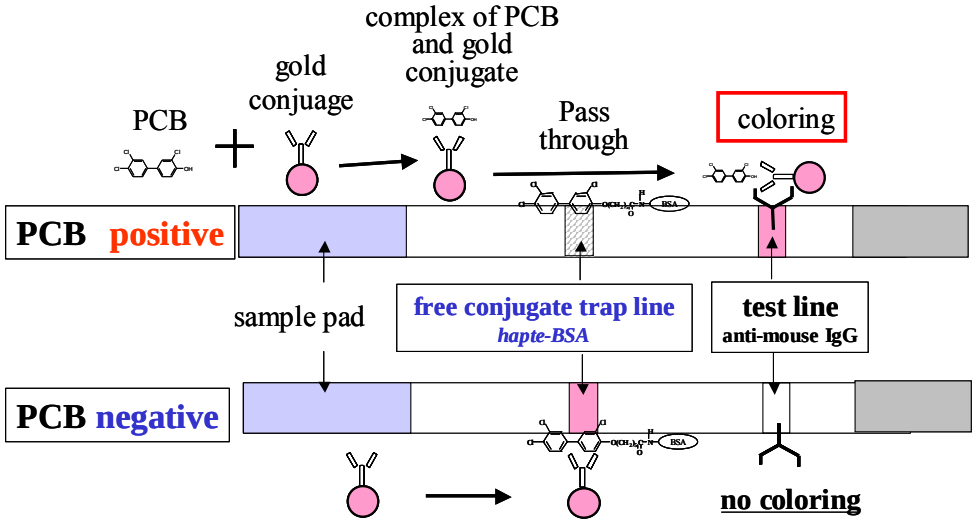

Figure 1, Test principle of new immunochromatograhic

Construction of immunochromatographic test

The PCB 118 monoclonal antibody was coupled to gold colloid particles by the procedures described previously[5] and the gold colloid conjugate was dried at 50℃ for 2 h in 1.5 mL glass vials. The dried conjugates were kept in a desiccator at room temperature until use.

PCB hapten and anti-mouse Immnoglobulin G antibody were immobilized on a nitrocellulose membrane with the width of 1 mm as a free gold-conjugate trap line and a test line, respectively. A glass fiber sheet as sample application pad and a cotton sheet as absorbent pad were assembled with the two-line-immobilized membrane on a laminate card. The assembled sheet was cut into strips with the width of 5 mm and the strips were placed into plastic cassettes.

Assay procedure

After the dried gold conjugate in the glass vial was suspended with 125 µL of tris buffered saline (TBS), 25 µL of PCB 118 or KC samples dissolved in dimethylsulfoxide (DMSO) was added and mixed thoroughly. 100 µL of the mixture was applied to the sample pad of the strip and kept for 20 minutes at room temperature. The color intensity on the test line or both lines was measured by the observation with naked eyes or by the immunoreader, respectively.

Data analysis

In case of judgment by naked eyes, the appearance of the red color on the test line was regarded as sample-positive. No color appearance was regarded as sample-negative. On the other hand, in case of judgment by the immunoreader, the ratio of the color intensity on the trap line to that on the test line was calculated as judgment value by the following formula;
Judgment value (%) = test line color intensity / trap line color intensity x 100

Results and Discussion

The qualitative results for PCB 118 by the observation of naked eyes showed that the detection limit was 0.1ppm and samples of more than 1ppm, from 1ppm to 10ppm, were judged to be positive (data not shown).

Figure 2 and Figure 3 showed the results of PCB 118 analysis using the immunoreader. The color intensity of the test line increased in proportion to the concentration of PCB 118, while the color intensity of the trapper line decreased (Figure 2). Figure 3 showed the results of the judgment value calculated by the color intensity of both lines. Since the values were amplified in proportion to the concentration of PCB 118 concentration, the proposed judgment value was seemed to be a suitable index for the quantification of PCB.

The test results of KC series analysis using the immuoreader were shown in Figure 4. KC 500 showed the highest sensitivity and sensitivity of each KC was as follows, KC 300; 3 ppm, KC 400; 0.5 ppm, KC 500; 0.1 ppm, KC 600; 3 ppm. These results were reasonable because the monoclonal antibody used for the assay was highly specific to PCB 118 congener and KC500 had the

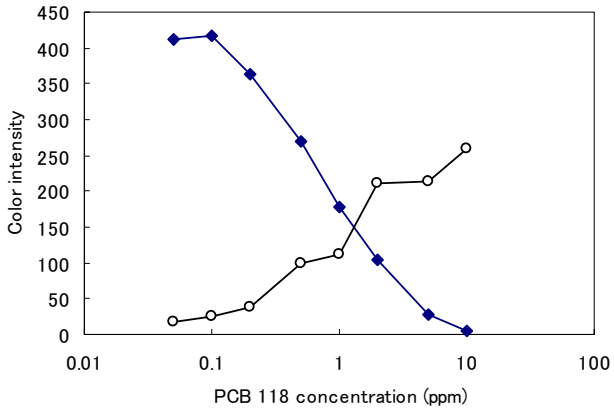

Figure 2, The color intensity for PCB 118 (in DMSO) assayed by using the immunoreader. ♦ trap line, O test line.

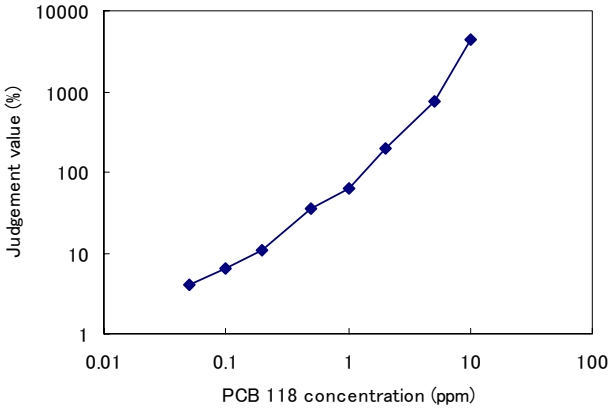

Figure 3. The judgment values for PCB 118 (in DMSO) using the immunoreader. The judgment value was calculated as described in Materials and Methods.

highest content of PCB 118 among the KC series.

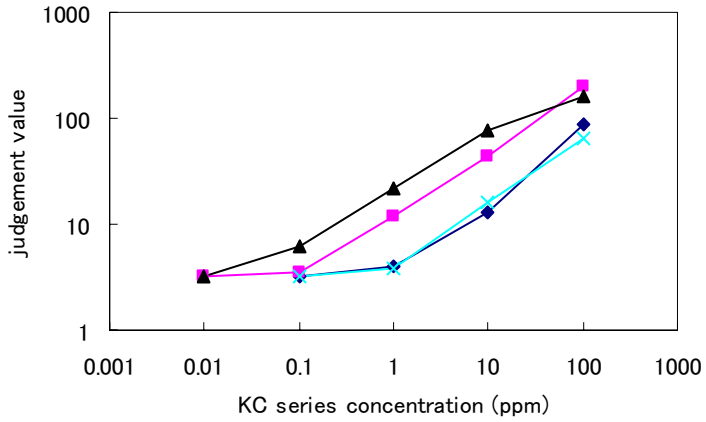

Figure 4, Dose response curve for KC series. + KC300 ■ KC400, ▲ KC500, ♦ KC600. Judgment value was calculated as described in Materials and Methods.

Conclusions

The newly developed immunochromatographic test can be used not only for qualitative analysis of PCB but also quantitative analysis. Since the new assay is simpler in handling than ELISA, it could be very useful tool for on-site PCB screening.

Acknowledgments

This work was supported by Kanto Bureau of Economy, Trade and Industry, local new birth consortium program.

Reference

1. Okuyama, A., Takenaka, H., Nishi, K., Mizukami, H., Kozaki, S., Kirihata, M., and Miyatake, K. (2001) Organohalogen Compounds 54, 44
2. Okuyama, A., Takenaka, H., Nishi, K., Mizukami, H., Kozaki, S., Kirihata, M., Miyatake, K., Takigami, H., Sakai, S., and Morita, M. (2002) Organohalogen Compounds 58, 333
3. May, K. (1991) J. Obstet. Gynecol. 165, 2000
4. Wenning R., Moeller M.R., Haguenoer J.M., Marocchi A., Zoppi B.L., de la Torre R., Carstensen C.A., Goerlach-Graw A., Schaeffler J., and Leinberger R. (1998) J. Anal. Toxicol. 22, 148
5. Peak S.H., Lee S.H., Cho J.H., and Kim Y.S. (2000) Methods 22, 53