COMPARISON OF SIX IMMUNOASSAY SCREENING METHODS FOR PCBs IN TRANSFORMER OIL.

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Abstract
One hundred and three transformer oil samples with various levels of PCBs were sent to six immunoassay manufacturers as blind samples. Measurements from the six assay methods were correlated with measurements from both HRMS and LRMS. Screening performance of each assay was evaluated by computing a receiver operating characteristic (ROC) curve and estimating the area under the ROC curve. All six assays were found to have utility for PCB screening at the concentration of 0.5 ppm(mg-PCB/kg-oil).

Introduction
Prior to 1977 polychlorinated biphenyls (PCBs) were widely used industrial chemicals. Their high stability and excellent electrical and thermal characteristics made them especially desirable in electrical equipment and they were often introduced into the insulating oil of capacitors and transformers.[1] An unknown number of PCB containing transformers remain in service around the world. In Japan, production, import and usage of PCBs was restricted by law on 1973, and used PCBs have been securely kept in storage. Although PCB was not used for a long time, unexpected PCB contamination of insulating oil was recognized on 2002. The contaminated concentration of PCB is low level as ppm, but the contamination may extend to approximately six million transformers in Japan.
PCBs in transformer oil have been measured by several procedures after purification of PCBs from oil matrix by pretreatment. Currently available detection systems are mainly based on Gas Chromatography followed by either electron capture detection(ECD) or mass spectroscopy (Low or high resolution). These systems are well established but faster and less expensive methods are highly desirable. Recently, immunoassay has been identified as a useful method to sense environmental chemicals. Immunoassays for the detection of PCBs in transformer oil with an associated extraction procedure of PCBs from oil matrix.[3] This extraction was improved using a column based method and a battery operated handheld immunoassay instrument was developed.[3] This immunoassay system consisting of simple column extraction and rapid measurement using a portable instrument was proposed as a screening method and tested on more than 1000 real transformer oil samples.[4] The performance reported was adequate for screening purposes, with zero false negatives and 7% false positives for 500 samples confirmed by GPC/GC/ECD.
The screening system described is now commercially available in Japan but the magnitude of the screening task underway remains enormous. Several other companies have come forward with extraction and test kits of their own to help meet the demand. In order to evaluate and compare the sensitivity and specificity of the various tests, we sent 103 oil samples to each lab and asked each to report back to us with their estimated concentration. Using Receiver Operating Characteristic curves in conjunction with known values for the total PCB in the samples (measured by LRGC/LRMS or the spiked concentration) we estimated the area under the ROC curve for each immunoassay and compared them using a permutation test.

Materials and Methods
Sixty seven used transformer oil samples and thirty six new transformer oil samples spiked with known quantities of various Kanechlors were aliquoted and supplied to six immunoassay providers. Used Transformer oil samples were measured using LRMS and spiked samples were evaluated based on their nominal value estimated from the known quantity of pure Kanechlor added. In addition, nine of the samples were also tested by HRMS for comparison. The combined distribution of all samples is as shown in Figure 1.

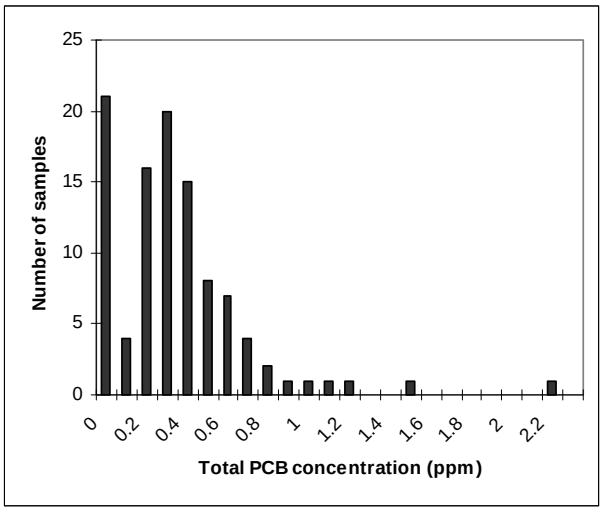

Figure 1. Histogram showing the distribution of oil samples supplied to the immunoassay providers.

The immunoassay providers participating in the study along with a brief summary of their technical approach are shown in Table 1.

Table 1. Companies and Technology evaluated

A	Sumika Chemical Analysis Service. A inhibition assay in which binding between a colloidal gold labeled anti-PCB antibody and a solid phase is inhibited by PCB in the sample.
B	Japan EnviroChemicals. An ELISA method using an enzyme labeled anti-PCB antibody.
C	EnBioTec Laboratories. Immunochromatography method with absorbance detection.
D	Kyoto Electronic Manufacturing. Automated, particle based immunoassay using fluorescently labeled antibody.
E	Sekisui Chemical. Immunochromatography method using magnetic particles for detection.
F	Aisin Seiki. Immunochromatography method with absorbance detection.

Results and Discussion

The results from each immunoassay supplier were regressed against the HRMS results (nine points only) and against combined LRMS and spiked results (103 points) to obtain the best fit straight line. Tables 2 and 3 show the slope, intercept, and correlation coefficient for each immunoassay.

Table 2. Correlation parameters for Immunoassay vs. HRMS measurements (9 points)

	A	B	C	D	E	F
Slope	0.92	1.04	1.08	1.07	1.15	0.96
Intercept	0.04	0.04	-0.07	0.1	0	0.26
R^2	0.973	0.983	0.97	0.9915	0.9538	0.8773

Table3. Correlation parameters for Immunoassay vs. LRMS measurements or the spiked concentration (103 points)

	A	B	C	D	E	F
Slope	0.96	1.09	1.00	1.20	1.13	1.12
Intercept	0.03	0.02	-0.03	0.04	0.00	0.02
R^2	0.85	0.87	0.86	0.88	0.86	0.83

Total PCB concentrations of nine samples for the HRMS analysis were determined at 0.22mg/kg, 0.23mg/kg, 0.28mg/kg, 0.51mg/kg, 0.65,g/kg, 0.79mg/kg, 1.3mg/kg, 1.5mg/kg and 2.4mg/kg. In the range of concentrations of the samples, all six assays were correlated with the slope from 0.92 to 1.15 and more than 0.87 coefficients. In the same manner, a correlation between immunoassay and LRMS measurements for 103 samples ranging until 2.3 mg/kg in each assay indicated the slope from 0.96 to 1.20 and more than 0.83 coefficients.
Receiver operating characteristic (ROC) curves, which plot the true positive rate vs. the false positive rate for all possible cutoff values, have been widely used in medical diagnostics for evaluating screening tests [5,6] and have begun to be more widely applied in evaluation of environmental screening tests.[7, 8] In particular, the area under the curve (AUC) which has been shown to be equal to the probability of correctly ranking a positive sample as higher than a negative sample[6], is often used as a summary figure of merit for the screening test. The AUC ranges between 0.5 (a test no better than random chance) to 1.0 (a perfect test). Figure 2 below shows the ROC curves for the six immunoassays based on the assumed definition of positives as samples that are 0.5 ppm total PCB or higher. Presently there is no official guideline to identify low level PCB contamination in transformer, but there is statutory concentration for breaking PCBs down to harmless constituents in oil is 0.5 mg/kg in Japan. This study thus assumed 0.5 mg/kg as definition of positives

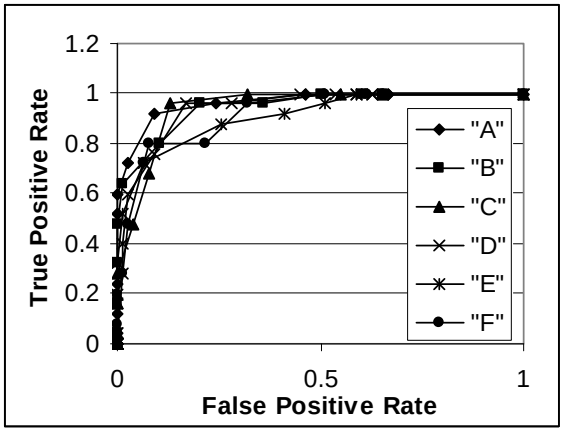

Figure 2. ROC curves for all six immunoassays.

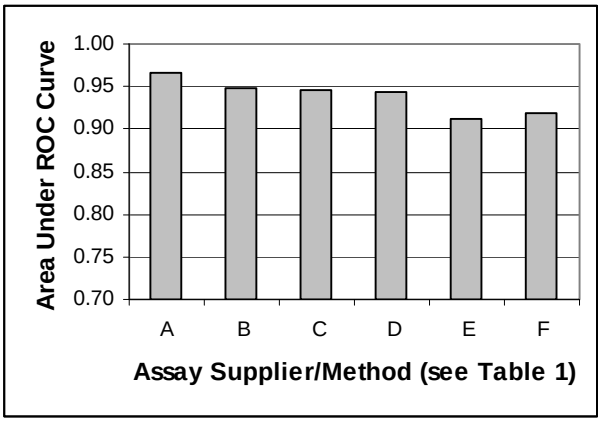

Figure 3. AUC value for each ROC curve of Figure 2.

Figure 3 summarizes the AUC for the six assays. The relatively high areas under the ROC curves (all are greater than 0.9) suggest that all of these tests can be useful in screening transformer oil for total PCBs. Bandos[9] suggests a permutation method for comparing the significance of observed differences in AUCs in cases where the assays being evaluated are tested on the same samples. Following his approach, and using a p-value of 0.05 (95% confidence) only small differences between assay "A" and assays "E" and "F" are found, all other differences are within the range expected from random error. In summary, all six assays were found to have utility for PCB screening at 0.5 ppm(mg-PCB/kg-oil).

References

(1) Erickson MD. *Analytical Chemistry of PCBs Second Edition,* CRC Press, Boca Raton FL, 1997.
(2) Glass TR, Ohmura N, Taemi Y, Joh T. *Environ Sci Technol* 2005; 45: 5005.
(3)Ohmura N, Glass TR, Sasaki K, Joh T, Taemi Y, Yokobori N, Terakado S. *Bunseki Kagaku* 2006;55:341.
(4)Ohmura N, Glass TR, Sasaki K, Joh T, Taemi Y, Imanishi K, Terakado S. *Bunseki Kagaku* 2006;55:519.
(5)Zwieg MH, Cambel G. *Clinical Chemistry* 1993;39:561.
(6)Hanley JA, McNeil BJ. *Radiology* 1982;143:29.
(7)Martin OV, Lai KM, Scrimshaw MD, Lester JN. *Environ. Sci. Technol.* 2005;39:5349.
(8)Syage JA, Cai, SS, Li, J, Evans MD. *Anal. Chem.* 2006;78:2967.
(9)Bandos AI, Rockette HE, Gur D. *Stat Med* 2005; 24:2873.