A Cleaning Technique for Cuvettes Used in Enzyme-Linked Immunosorbent Assay

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ABSTRACT

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Cuvettes (Gilford) used for enzyme-linked immunosorbent assay of potato viruses S, X, and Y and of oat blue dwarf virus could be used repeatedly when they were cleaned with concentrated sodium hydroxide in water and ethanol (1:1, v/v) or with 1% Haemo-Sol in water. Cuvettes could be used interchangeably following use with different viruses. Concentrated sulfuric acid:potassium dichromate was another potentially useful cleaning agent, but it was not tested as extensively.

Cuvettes used once for enzyme-linked immunosorbent assay (ELISA) and discarded represent a major expendable-item expense for this assay. We have found it necessary to use five cuvette packs (Gilford) for 100 samples. This includes two wells per sample and 40 wells per cuvette pack. The first and 10th well in each strip have frequently given higher than expected absorbancy values and cannot be used. Our experiences with the dissociation technique reported by Bar-Joseph et al (2) for recycling cuvettes were not satisfactory for potato virus S (PVS), potato virus Y (PVY), or oat blue dwarf virus (OBDV) (1). Therefore, we sought a method that might simply remove coating γ-globulin and other components bound to plastic walls of the cuvette wells.

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MATERIALS AND METHODS

Viruses. Potato viruses X (PVX), PVS, and PVY were maintained in Norland, Kennebec, and Russet Burbank potatoes; healthy plants of each cultivar were used for controls. OBDV was maintained in Lodi oats and propagated by periodically infesting oat seedlings with viruliferous aster leafhoppers. Healthy oat plants were used as controls.

ELISA. The double-antibody sandwich method of ELISA was used in all experiments and γ-globulins and γ-globulin enzyme conjugates were prepared according to techniques described by Clark and Adams (3) except that γ-globulins were not filtered through DE cellulose. Coating γ-globulins, at a total protein concentration of 1.0 μg/ml for each antiserum, were deposited in wells and incubated 4–5 hr at 36 C. Crude healthy or virus-infected plant saps were added 1:1 (v/v) into wells in which phosphate-buffered saline + 0.5% Tween 20 (polyoxyethylene sorbitan monolaurate) (PBS + T) + 0.01 M sodium diethyldithiocarbamate (NaDIECA) had been deposited, and cuvettes were incubated overnight at 4–5 C. Enzyme γ-globulin conjugates for each antiserum were used at a 1/400 dilution in PBS + T, and cuvettes were incubated at 20–24 C for 3–5 hr. Substrate, *p*-nitrophenyl phosphate (Sigma 104, Sigma Chemical Co., P.O. Box 14508, St. Louis, MO 63178) was used at a concentration of 0.6

mg/ml in diethanolamine, sodium carbonate buffer, pH 9.8. A Gilford PR-50 EIA washer, dispenser, and absorbance monitor (Gilford Instrument Laboratories, Inc., Oberlin, OH 44074) was used in all experiments. The cuvettes were machine-washed seven times with PBS + T following incubation of coating γ-globulins, nine times following incubation of antigens, and seven times following incubation of γ-globulin enzyme conjugates. Absorbance (A_{405}) of the hydrolyzed substrate was recorded immediately and 15 min, 30 min, and 1 hr following addition of substrate so that all comparisons were made at equivalent times and within the linear range of absorbance increase. The maximum A_{405} for the Gilford absorbance monitor is 2.702, and absorbance values were calculated for infected samples as follows: (mean [$\bar{X}$] of infected samples) − ($\bar{X}$ of healthy samples + two standard deviations of $\bar{X}$ of healthy samples).

Cleaning solutions. The following cleaning solutions were prepared in deionized water: a) Sulfuric acid: potassium dichromate (H_2SO_4:$K_2Cr_2O_7$) cleaning solution (4). b) Concentrated sodium hydroxide (NaOH) (500–600 g/L of water): absolute ethanol (EtOH) cleaning solution, prepared by mixing one volume of concentrated NaOH with one volume of absolute ethanol. When the concentrated NaOH solution was added to the ethanol, the mixture separated into layers. This was stirred for 1–2 hr, and sufficient deionized water was added with stirring until the layers became miscible. The NaOH:EtOH solution had a yellow color and was discarded when it turned brown. Occasionally a precipitate formed after several cuvette packs had been cleaned. The precipitate was removed by filtration through Whatman No. 1 filter paper, and the filtrate was effective for additional cleaning. c) Alcotab (Alconox, Inc., New York, NY 10003), one tablet per liter of water. d) 1.0% Haemo-Sol in water (Scientific Products, 13505 Industrial Park Blvd., Minneapolis, MN 55427).

After use, cuvettes were first rinsed several times with tap water to remove hydrolyzed substrate and then immersed in the cleaning solution and agitated to remove any trapped air in wells. Unless stated otherwise, the cuvettes were soaked in each cleaning solution for 48 hr, rinsed five or six times with tap water, four or five times in deionized water, two or three times in distilled water, and then inverted on paper towels to dry. After drying, cuvette packs were stored in polyethylene bags.

RESULTS

In two experiments, cuvettes cleaned with several cleaning agents were tested with several dilutions of sap from PVS-infected leaves and with healthy potato leaf sap. Cuvettes used previously for ELISA of PVS-infected or healthy potato sap were cleaned with H_2SO_4:$K_2Cr_2O_7$, NaOH:EtOH, Alcotab, or Haemo-Sol and then reused with undiluted sap from PVS-infected foliage, PVS-infected sap diluted 1:10–1:500 with healthy potato sap, and with sap from healthy foliage. Previously unused cuvettes were also charged for check purposes. The absorbance values (A_{405}) for PVS-infected samples were in most cases slightly higher when PVS followed PVS than when PVS followed healthy (Table 1). Absorbance values (A_{405})

Table 1. Assay of effectiveness of several chemicals for cleaning cuvettes after enzyme-linked immunosorbent assay of potato virus S (PVS)-infected and healthy potato sap

Cleaning solution	Prior use	Mean absorbance (A_{405})[a]: PVS crude sap	PVS, 1:10	PVS, 1:100	PVS, 1:200	PVS, 1:500	Healthy crude sap
H_2SO_4:$K_2Cr_2O_7$[b]	PVS	1.870	1.410	0.638	0.462	0.300	0.168
	Healthy	1.773	1.395	0.589	0.324	0.239	0.159
Concentrated	PVS	2.096	1.645	0.687	0.652	0.306	0.085
NaOH:EtOH[c]	Healthy	2.208	1.605	0.680	0.631	0.289	0.073
Alcotabs	PVS	2.083	1.596	0.732	0.557	0.402	0.326
	Healthy	1.874	1.436	0.662	0.557	0.391	0.334
Haemo-Sol	PVS	1.983	1.503	0.758	0.567	0.306	0.146
	Healthy	1.877	1.372	0.691	0.514	0.258	0.123
Fresh cuvette (check)		1.749	1.351	0.623	0.461	0.242	0.098

[a] Crude sap from PVS-infected potato diluted 1:1 with phosphate-buffered saline + 0.5% Tween 20 + 0.01 M sodium diethyldithiocarbamate; PVS dilutions made with healthy potato sap. PVS values = ($\bar{X}$ of infected samples) − ($\bar{X}$ of healthy + 2 standard deviations of $\bar{X}$ of healthy). Values = $\bar{X}$ of two experiments.
[b] Sulfuric acid:potassium dichromate.
[c] Sodium hydroxide:absolute ethanol.

Table 2. Repeated use and cleaning of cuvettes used for enzyme-linked immunosorbent assay of potato virus S (PVS), potato virus X (PVX), and potato virus Y (PVY)

Cleaning solution	Repeat uses (no.)	Immediate previous use[a]	Absorbance (A_{405})[b]: Virus	Healthy
NaOH:EtOH[c]	PVX (3)	PVX	1.20	0.16
		Healthy	1.25	0.08
1.0% Haemo-Sol	PVX (3)	PVX	1.19	0.07
		Healthy	1.20	0.09
New cuvettes (check)	...	...	1.63	0.07
NaOH:EtOH	PVY (4)	PVY	1.33	0.11
		Healthy	1.19	0.15
1.0% Haemo-Sol	PVY (4)	PVY	1.90	0.23
		Healthy	1.95	0.23
New cuvettes (check)	...	...	1.07	0.10
NaOH:EtOH	PVS (5)	PVS	2.09	0.22
		Healthy	2.16	0.31
1.0% Haemo-Sol	PVS (5)	PVS	2.02	0.16
		Healthy	1.91	0.16
New cuvettes (check)	...	...	1.84	0.13

[a] Sap from virus-infected or healthy potato.
[b] Absorbance for virus values = ($\bar{X}$ of infected samples) − ($\bar{X}$ of healthy + 2 standard deviations of X of healthy).
[c] Sodium hydroxide:absolute ethanol.

Table 3. Use of cuvettes for enzyme-linked immunosorbent assay following use with potato virus S (PVS), potato virus X (PVX), potato virus Y (PVY), oat blue dwarf virus (OBDV), and cleaning with NaOH:EtOH[a] solution

Virus (2nd use) / Virus (1st use)	Absorbance (A_{405}) value (2nd use): Virus[b]	Healthy
PVX / PVS	1.34	0.29
PVX / PVY	1.56	0.26
PVX / OBDV	1.54	0.18
PVS / OBDV	1.96	0.19
PVX check[c]	1.60	0.29
PVS check[c]	1.79	0.12

[a] Sodium hydroxide:absolute ethanol.
[b] Absorbance of virus infected = ($\bar{X}$ of infected samples) − ($\bar{X}$ of healthy + 2 standard deviations of $\bar{X}$ of healthy).
[c] Cuvettes previously unused.

Table 4. Comparison of dissociation of antigen-antibody complexes vs. sodium hydroxide:ethanol cleaning for recycling Gilford cuvettes for enzyme-linked immunosorbent assay of healthy potato foliage and foliage infected with potato virus X (PVX), potato virus Y (PVY), and potato virus S (PVS)

		Mean absorbance (A_{405}) value for second use[a]					
		Virus (2nd)[b] / Virus (1st)	Virus (2nd)[c] / Healthy (1st)	Healthy (2nd)[d] / Virus (1st)	Healthy (2nd)[e] / Healthy (1st)		
Recycling method	**Virus**					**Virus**	**Healthy**
NaOH:EtOH[f]	PVX	2.47	2.53	0.16	0.13		
Dissociation	PVX	2.21	2.50	0.37	0.13		
Check (new cuvette)	PVX	...	...	...	...	2.41	0.22
NaOH:EtOH	PVY	0.72	0.71	0.15	0.16		
Dissociation	PVY	0.28	0.33	0.15	0.11		
Check (new cuvette)	PVY	...	...	...	...	0.62	0.20
NaOH:EtOH	PVS	1.66	1.58	0.08	0.08		
Dissociation	PVS	0.94	0.64	0.72	0.07		
Check (new cuvette)	PVS	...	...	...	...	1.92	0.11

[a] Absorbance of infected = ($\overline{X}$ of infected samples) − ($\overline{X}$ of healthy + 2 standard deviations of $\overline{X}$ of healthy). PVX and PVY experiments repeated three times, PVS repeated once.
[b] $\overline{X}$ of absorbancy of virus-infected samples in wells used for virus in the previous use.
[c] $\overline{X}$ of absorbancy of virus-infected samples in wells used previously for healthy samples.
[d] $\overline{X}$ of absorbancy of healthy samples in wells used previously for virus-infected samples.
[e] $\overline{X}$ of absorbancy of healthy samples in wells used previously for healthy samples.
[f] Sodium hydroxide:absolute ethanol.

for infected samples at all dilutions and with all cleaning agents with the exception of PVS following healthy in H_2SO_4:$K_2Cr_2O_7$-cleaned cuvettes were higher than those obtained in previously unused cuvettes. Absorbance values (A_{405}) for healthy samples were lower in NaOH:EtOH-cleaned cuvettes and higher in cuvettes cleaned with other chemicals than in previously unused cuvettes. The readings for healthy sap were unacceptably high in Alcotab-cleaned cuvettes.

NaOH:EtOH and 1% Haemo-Sol were used for repeated cleaning of cuvettes that had been used for ELISA of PVX, PVS, and PVY. Cuvettes used five times for PVS and cleaned with either cleaning solution were as effective for ELISA as previously unused cuvettes (Table 2). Cuvettes used four times for PVY and cuvettes used three times for PVX and cleaned with NaOH:EtOH were also consistently effective for ELISA.

Tests were made to determine whether the virus involved in previous use affected subsequent assays with different viruses. Cuvettes were first used for assay of PVS, PVY, or OBDV cleaned with NaOH:EtOH solution and were then used for a different virus. Assays with such cuvettes were comparable to assays using new cuvettes (Table 3).

Double-antibody sandwich dissociation vs. simple cleaning. Bar-Joseph et al (2) used low pH glycine buffer to cause double-antibody sandwiches of citrus tristeza virus (CTV) to dissociate from antibody in γ-globulin-coated plates. Such plates could be used without recoating and were reusable for five consecutive assays.

In three experiments, cuvettes were used for ELISA of PVS, PVX, PVY, and healthy controls; one cuvette pack for each virus assay was then treated by the double-antibody dissociation technique, and the other cuvette pack for each virus was cleaned with NaOH:EtOH. The cuvette packs were then reused for ELISA of PVS, PVX, and PVY. Previously unused cuvette packs were charged for check purposes. The NaOH:EtOH-cleaned cuvettes performed as effectively as previously unused cuvettes for each of the three viruses (Table 4). The glycine-HCl buffer treatment caused dissociation of the double-antibody sandwich of PVX, but not of PVX or PVY, in γ-globulin-coated cuvettes.

DISCUSSION

NaOH:EtOH, H_2SO_4:$K_2Cr_2O_7$, and Haemo-Sol were effective for removal of all components in Gilford cuvettes used in the double-antibody sandwich method for ELISA of PVS, PVX, PVY, and OBDV. This cleaning technique is simple, convenient, and more effective than attempting to dissociate antigens of PVS or PVY from coating γ-globulins. Occasionally, an absorbance value (A_{405}) deviated markedly from other replicated wells. Possibly, air trapped in corners of the square wells prevented cleaning solution from contacting all well surfaces. This was overcome by ensuring that cuvette packs were sufficiently agitated in the cleaning solutions to remove all trapped air.

It appears that recycled cuvettes can be repeatedly used and cleaned, that they can be used interchangeably for ELISA of different viruses, and that they can be stored indefinitely after cleaning. Because we have not tested these cleaning procedures with any other plates or cuvettes used with ELISA, we cannot comment on their efficacy with microplates available from other manufacturers.

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