Techniques

Construction of an Inexpensive ELISA Plate Reader

L. W. Stobbs

Research scientist, Agriculture Canada, Research Station, Vineland Station, Ontario L0R 2E0.
Research supported under a Natural Sciences and Engineering Research Council Operating Grant A7321.
The technical assistance of D. Reider, Science Electronics, University of Waterloo, Waterloo, Ontario, is appreciated.
Accepted for publication 26 May 1986 (submitted for electronic processing).

ABSTRACT

Stobbs, L. W. 1986. Construction of an inexpensive ELISA plate reader. Phytopathology 76:1217-1221.

An inexpensive enzyme-linked immunosorbent assay (ELISA) plate reader was constructed by interfacing a solid-state photometer with a light microscope. By using the light path system of the microscope, only a light-sensing circuit and narrow bandpass filter were necessary to obtain reliable ELISA substrate readings. Laboratory tests have shown that this unit provides accuracy, reproducibility, and linearity comparable to those of a state-of-the-art commercial unit.

Enzyme-linked immunosorbent assay (ELISA) is a highly sensitive serological procedure widely used in research and diagnostic laboratories to detect antigens. Routine diagnoses of many diseases are being made with this technique in medical, veterinary, and agricultural fields. Besides providing qualitative assessment, quantitative data can be obtained because the amount of immunospecific antigen in the sample is directly related to the degree of substrate hydrolysis, which is measured colorimetrically.

Several ELISA readers are commercially available that will provide absorbance readings of the substrate in the test plates, although such instruments are generally costly. Examining the design of these readers, we can break each into several operational subunits. A light source of fixed intensity with a 405-nm narrow bandpass filter provides a beam of monochromatic light. This light beam is transmitted through the substrate, contained within the ELISA plate, which is supported on either a fixed or moveable stage, to a photosensor mounted in a telescoping tube supported over the plate. Finally, a photometer provides sample absorbance readings, which may be recorded on a peripheral printer.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

Many of these features are incorporated in the laboratory light microscope. By using the light path of the microscope interfaced with a compact solid-state photometer, light absorbance readings of substrate in microtiter wells can be easily obtained. In this way, only a photosensor and a 405-nm bandpass filter are necessary to convert a microscope to an ELISA plate reader, thus providing an inexpensive means of determining absorbance values. This paper describes the construction and test results with such an instrument.

MATERIALS AND METHODS

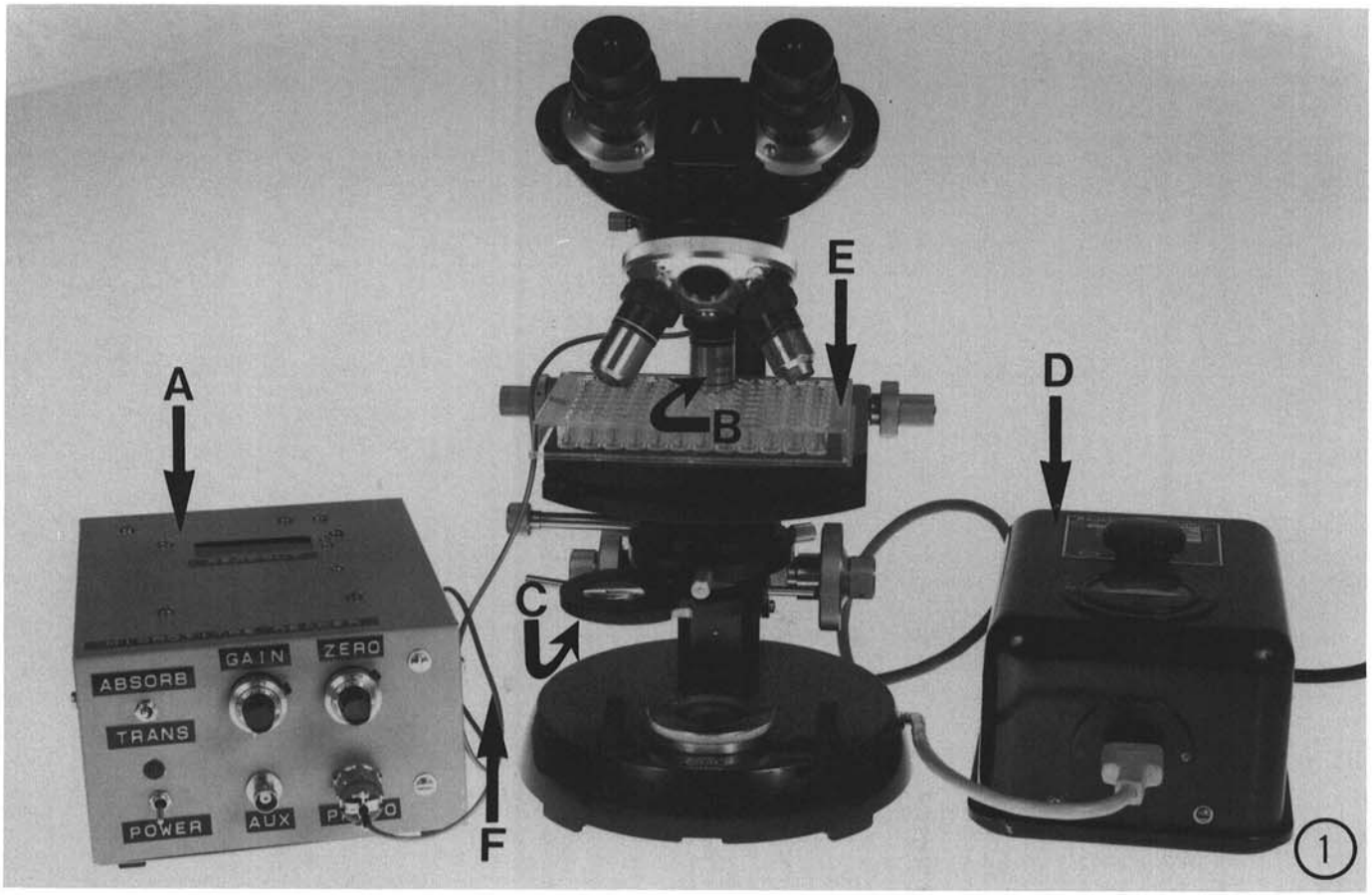
Basic design and operation. The ELISA microplate reader consists of three components: 1) a solid state photometer and photosensor interfaced with 2) a light microscope equipped with 3) a 405-nm narrow bandpass filter assembly (Fig. 1). A halogen light source connected to a variable power supply provided uniform light intensity for reliable photometer operation.

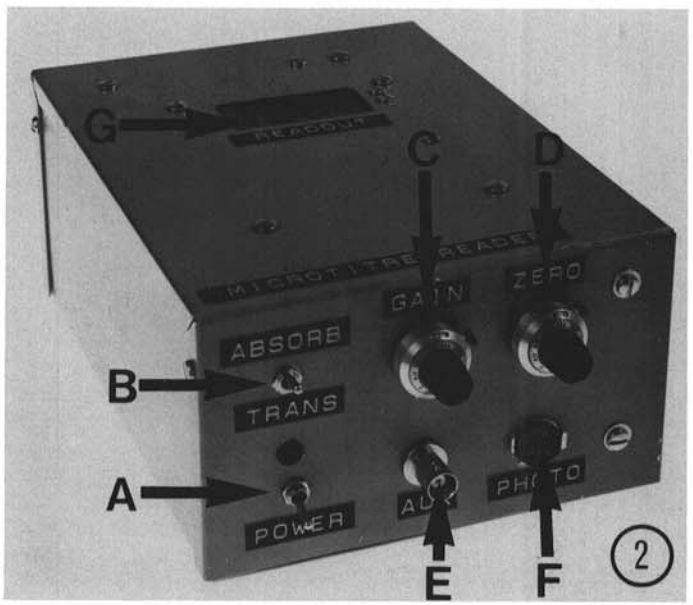
The photometer (Fig. 2) was designed to provide a digital readout of absorbance or percentage of light transmittance. Zero and gain controls allowed baseline and 100% transmittance adjustments, respectively. An auxiliary output connector was provided for interfacing with a microprocessor or compatible printout system.

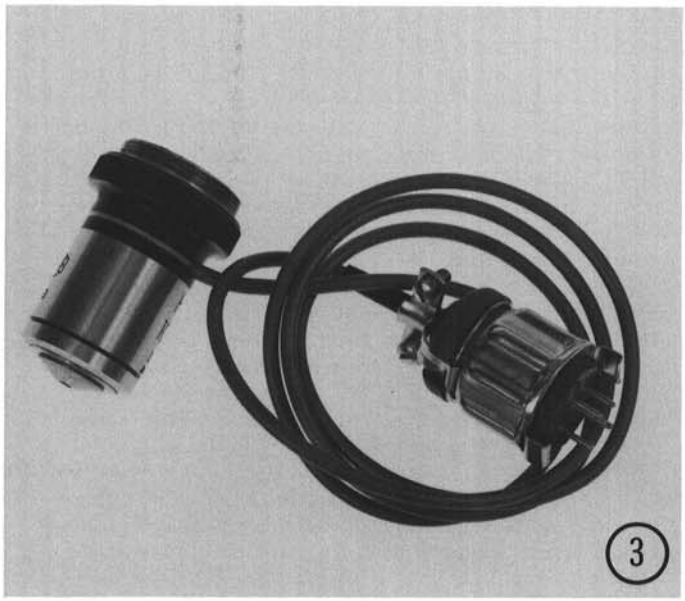
The photosensor (Fig. 3) was positioned in a discarded oil-immersion objective and located on the microscope objective

turret. Such nonuseable lenses can usually be obtained from the microscope manufacturers. The lens elements were removed, the aperture machined to 2 mm, and the photosensor centered 5 mm above the aperture, and secured in position with epoxy resin. This method was found to produce the most reproducible light measurement. Because only the outer sleeve of the photosensor assembly moves vertically, the distance between the photocell and the ELISA well remains constant. The spring tensioning mechanism in the objective permits the objective assembly to ride over the plate surface and to center uniformly as it partially drops into the next well. The tight fit minimizes the influence of extraneous incident light on transmitted light measurements. A coaxial cable inserted through the side of the objective lens housing provided electrical continuity between the photosensor and the photometer. The coaxial shield was grounded to both the microscope and photometer chassis.

A Corning #5113 narrow band blue filter and #5970 ultraviolet-transmitting visible absorption filter were cut to fit the microscope







Figs. 1–3. Light microscope ELISA plate reader. **1,** Photometer interfaced with light microscope (A) photometer, (B) photosensor mounted on objective turret, (C) filter holder for narrow-band wavelength filters, (D) variable power supply, (E) ELISA plate on microscope stage, (F) coaxial cable connecting photosensor to photometer. **2,** Photometer: (A) on/off power switch, (B) absorbance/transmittance selection switch, (C) gain adjustment, (D) zero adjustment, (E) auxillary output, (F) photosensor input connector, (G) digital display of absorbance or % transmittance. **3,** photosensor mounted in discarded objective lense housing.

filter holder. Technical specifications are available from the manufacturer (Corning Glass Works, Corning, NY). The combination of these filters provided a narrow bandpass transmission peak at 405 nm, which corresponds to the absorbance optimum of the ELISA substrate, *p*-nitrophenol. A neutral-density filter may be used to reduce light intensity depending on the light source used. Light-beam width was adjusted using the microscope aperture and condenser lens assembly to provide a beam slightly wider than the photosensor aperture. The beam was centered over the photosensor aperture with the condenser alignment aperture screws.

The ELISA plate to be read was placed on the microscope stage. The photosensor tube was positioned over a control well on an ELISA plate and lowered until the spring tensioning mechanism was engaged. Subsequent reading of adjacent wells was easily facilitated by sliding the plate so that the objective moved from well to well. Replacing the microscope stage with a sheet of clear Plexiglas larger than the microscope stage permitted easier manipulation of the plates. A 1-cm-diameter hole in the Plexiglas in line with the light path limited extraneous light absorption and scattering. Although the zero control permitted ample adjustment for zeroing incident background light, readings were taken under low ambient room lighting.

Circuit description. In designing the electronic circuitry for the photometer, a photocell was selected from specifications with maximum linearity and speed at the desired wavelength of 405 nm (Fig. 4). A cadmium sulphite photoconductive cell (CLAIREX CL705HL, Electro Sonic, Toronto, Ontario, Canada) was used with a maximum dark resistance of 18.7 MΩ (5 sec at 21.5 lx) and an on resistance of 28 KΩ (21.5 lx). An FET input LF356 operational amplifier (op amp) with a high-input impedance was used because of the high resistance of the photocell (18 MΩ for 0% transmittance). The op amp functioned as a current amplifier, and because the resistance of the photocell (R_{pc}) was a linear function of the light it receives, the output voltage (V_{OUT}) was inversely proportional to the transmittance by the following:

(1) $I_{IN} = 15\ V / R_{pc}$ where I_{IN} is the photocell current;

(2) $V_{OUT} = I_{IN} \times R_f$ where R_f is the feedback resistor. Experimentally it was determined that 100% T resulted in a photocell resistance of about 1.5 MΩ for optimum optical dispersion. V_{OUT} was, therefore, selectable by choosing R_f. V_{OUT} will not exceed the +/− 15 supply voltages to the op amp; saturation will occur and the output voltage will no longer be able to change with a varying input. Also, to ensure a good safety margin, and considering possible increases in light input of 100% T due to optical variations, V_{OUT} was set at −5 V for 100% T. R_f was then calculated as follows:

$$\text{from (2) } R_f = -5\ V / I_{IN}$$

substituting (1) in (2) $R_f = -5V / 15V \times 1.5\ M\Omega = 500\ K\Omega$ A 741 op amp inverted this voltage from −5V to +5V and allowed for adjustable gain at 100% T. For optimal operation of the absorbance log convertor, and for a simple translation to transmittance, V_{OUT} of the 741 was chosen to be +10 V. The 741 gain was determined by R_f / R_L and was set for a range of 1–3 (or +5 V to +15 V at 100% T). It was better to set the gain here rather than on the first op amp for two reasons. First, the speed of response was not affected by the gain setting, and second, when 100% T was adjusted to 10 V, the optical safety margin for saturation on the first op amp was maintained. The voltage was directly visible on the output meter as 10 V at 100% T, and provided a means of checking saturation. The zero control for nulling any dark currents and/or offset currents from the amplifiers was designed around the 741 op amp.

Circuit calibration was set by adjusting the zero control for 0 V at 0% T and by using the gain control for 10 V at 100% T. Transmittance could be obtained by simply reading the voltage

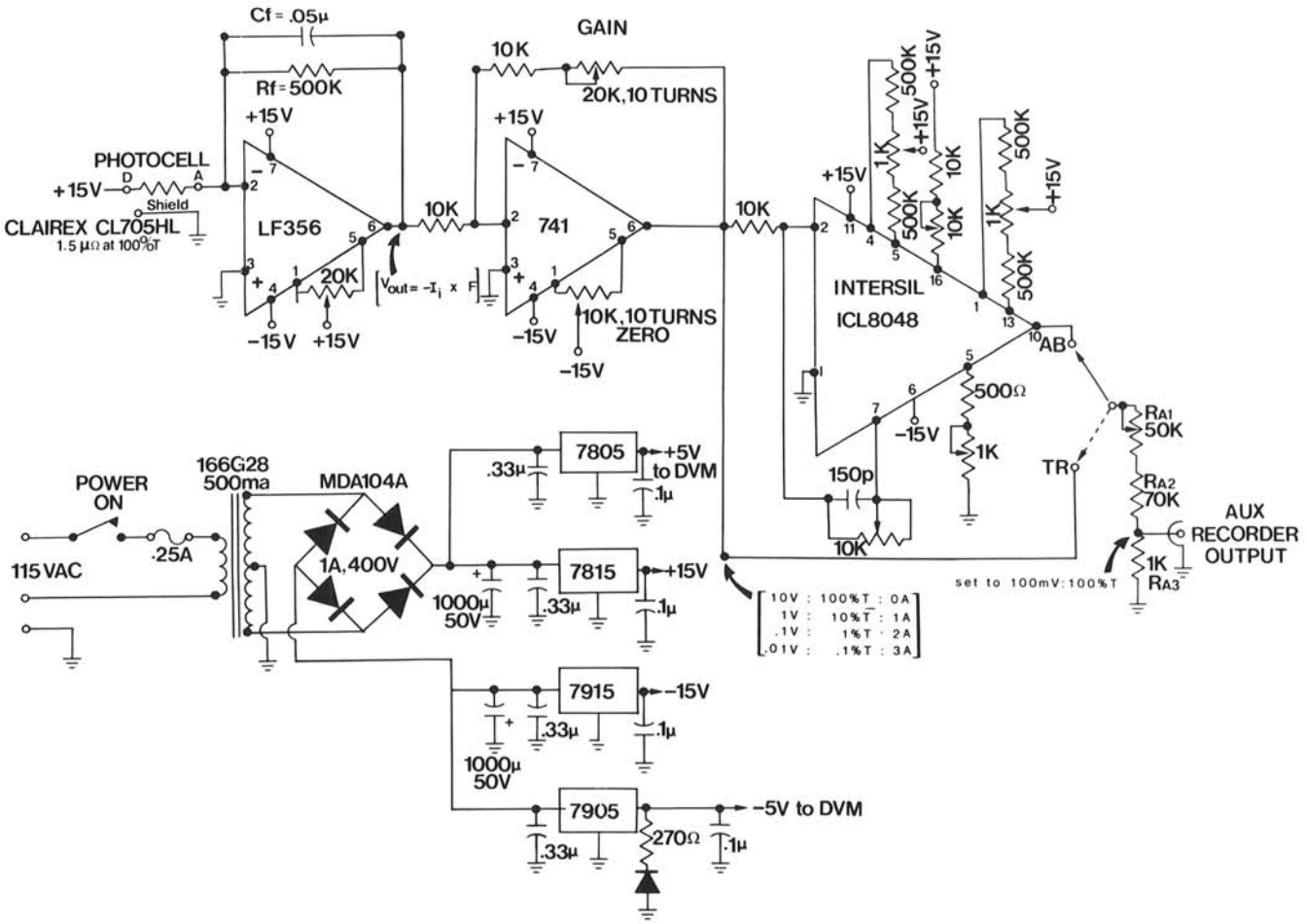


Fig. 4. Electronic schematic of photometer circuit.

with either a recorder or a digital voltmeter. The output was attenuated 100:1 by variable resistors (R1, R2, R3) for 100 mV full scale for interfacing with most recorders. The 10-V output also interfaced with an inexpensive digital voltmeter kit (Intersil ICL-7107 EV/Kit, Electro Sonic, Toronto, Canada); however, any digital voltmeter could be connected to the recorder output.

An Intersil ICL 8048 log converter was used to provide the absorbance output. It had an optimum operational range of 0.01–10 V, corresponding with the 10-V output from the 741 op amp. Several trimpots were used to calibrate the log converter, as specified in the manufacturers handbook. Regulated +/− 5-V supplies for the voltmeter and +/− 15-V supplies for the op amps were built using the standard regulators LCS7805, 7905, 7815, and 7915.

Operation. 1. A Zeiss model M-20 binocular microscope was placed on a flat, stable surface at a convenient working height. Best results are obtained under low ambient light, perferably in a darkened room. Direct sunlight or bright light may cause inaccurate readings.

2. Remove one of the objective lenses and replace with the light sensor tube. Position the sensor in the light path by rotating the objective turret. Connect the interfacing cable to the photometer.

3. Turn on the microscope light source. Close the microscope aperture to minimum setting and adjust the centering controls until a narrow beam of light is centered on the under surface of the light sensor tube. Lowering of the condenser lens will narrow the light beam further. Once this operation is complete, further adjustments are unnecessary.

4. Place the 405-nm blue filters into the microscope filter holder and insert into the light path. The filters should be clean and free of fingerprints and other debris. If cleaning is necessary, wipe gently with a dry cloth or lens tissue.

5. Turn on the photometer and allow it to warm up for approximately 5 min to allow components to stabilize.

6. Set the photometer function switch to the transmission position.

7. Switch off the light source and adjust the ZERO control to give a reading of 0.

8. With the light source on, place the microplate on the microscope stage and position the control well, which will be used for blanking under the sensor objective. Gently lower the objective until it is centered over the well and tension is applied to the spring-loaded mechanism. Adjust the gain to give a transmittance value of 100%. If sufficient adjustment is not possible using the gain control, increase the diameter of the microscope iris diaphragm and make fine adjustments using the gain control.

9. Move the microplate until the next well to be evaluated is positioned under the light sensor. The spring-loaded objective will center on each well. No vertical adjustment of the objective is necessary when wells are changed.

10. Change the photometer function switch to the absorbance position.

11. Read the absorbance value from the display.

Performance evaluation. A. Effect of well volume on light transmittance. To examine the influence of slight variances in substrate volumes on light transmittance, six wells in each of five ELISA plates were loaded with increasing incremental volumes (Table 1) of hydrolyzed substrate. Substrate used in these tests was prepared by adding 50 μl of alkaline phosphatase (Type 7, Sigma Chemical Co., St. Louis, MO) to 30 ml of *p*-nitrophenol substrate (Sigma Chemical Co.) used in the ELISA procedure (Clark and Adams, 1977). The reaction was stopped after 5 min by the addition of 3 ml of 3 M NaOH and the absorbance (405 nm) was adjusted to 1.95 using distilled water as diluent. The percentage of light transmission was recorded for each well using both the microscope photometer and a Beckman DU-8 spectrophotometer equipped with an ELISA microplate reader accessory (Beckman Instruments Inc., Irvine, CA).

B. Reproducibility between ELISA wells. Three ELISA plates were loaded with 200 μl of hydrolyzed substrate as indicated in Table 2. Percentage of light transmission (405 nm) was recorded for horizontal, vertical, and diagonal rows using the microscope reader and Beckman spectrophotometer.

C. Linearity and sensitivity test. With water as the diluent, a linear dilution series was prepared (Table 3), using hydrolyzed substrate with an absorbance of 1.95. Ten wells in each of three plates were loaded with 200 μl of each substrate dilution and the absorbance determined on both readers. The absorbance was compared graphically with the expected absorbance obtained by multiplying the dilution decimal equivalent by the undiluted substrate solution absorbance.

RESULTS

Differences in well volume significantly influenced the percentage of light transmittance readings (Table 1). Accurate

TABLE 1. Effect of well volume on transmittance readings

Well volume (ml)	Transmittance (% at 405 μm)	
	Microscope reader	Beckman DU8
0.00	83.7 ± 0.8[a]	81.3 ± 0.09
0.03	73.3 ± 1.4	71.0 ± 1.0
0.06	68.9 ± 1.6	66.9 ± 1.8
0.09	63.4 ± 2.0	61.0 ± 2.2
0.13	59.6 ± 2.2	57.6 ± 3.0
0.16	54.6 ± 2.6	52.6 ± 2.1
0.19	51.6 ± 2.9	49.2 ± 2.0
0.22	49.2 ± 3.1	46.8 ± 2.8
0.25	45.1 ± 3.4	43.0 ± 3.8
0.28	40.9 ± 3.9	38.1 ± 3.8
0.32	43.1 ± 3.7	42.6 ± 4.2
0.35	not reproducible	not reproducible

[a] Values represent mean ± standard deviation of 30 replicate wells.

TABLE 2. Percentage of light transmittance of substrate loaded wells

Sample position	Absorbance reader	Transmittance (% at 405 nm)		
		Plate 1	Plate 2	Plate 3
Row D, wells 1 to 12[a]	Microscope	40.8 ± 1.9	40.5 ± 2.1	40.4 ± 2.5
	DU8	39.6 ± 1.8	38.8 ± 2.6	40.1 ± 2.8
Well 6, row A to H[b]	Microscope	43.4 ± 2.1	43.5 ± 2.2	43.7 ± 3.0
	DU8	42.8 ± 2.0	42.6 ± 2.6	42.2 ± 2.9
Diagonal[c]	Microscope	43.4 ± 2.2	43.4 ± 2.6	43.3 ± 2.5
	DU8	42.8 ± 2.4	43.2 ± 2.1	43.1 ± 2.2

[abc] Values represent mean ± standard deviation of [a] 12, and [b],[c] 8 replicates of three ELISA plates.

TABLE 3. Absorbance (405 nm) linearity test of substrate dilutions

	Tube number						
	1	2	3	4	5	6	7
Volume of distilled water added (ml)	-	1.87	3.75	5.00	6.25	7.50	8.75
Volume of solution from Tube #1 added (ml)	-	8.13	6.25	5.00	3.75	2.50	1.25
Decimal dilution	1.0	0.81	0.63	0.50	0.38	0.25	0.13
Expected absorbance	1.95	1.58	1.22	0.98	0.73	0.49	0.24
Actual absorbance[a]	1.95 ± .06	1.55 ± .05	1.22 ± .07	0.99 ± .08	0.78 ± .06	0.50 ± .03	0.26 ± .04

[a] Values represent mean ± standard deviation of 30 replicate wells.

dispensing of substrate solution is essential for reproducible sample well readings. Addition of substrate volumes greater than 320 μl to the wells created a convex meniscus, which caused excessive light scatter and irregular readings on both the microscope and Beckman readers. The percentage of light transmittance through wells loaded with equal volumes of substrate did not differ significantly with respect to plate position or the type of reader used (Table 2). When serial dilutions of substrate were read under the microscope reader, absorbance readings were linear and compared favorably with the calculated absorbance values and readings on the Beckman DU-8 microplate reader (Table 3).

DISCUSSION

Construction of the ELISA photometer and photosensor element can be easily handled at reasonable cost by most facilities with electronic workshops. Attachment of the light sensor to a compatible light microscope and subsequent alignments can be easily accomplished by laboratory personnel. Comparative substrate absorbance readings taken on both the microscope reader and the Beckman DU-8 microplate reader were not different, with corresponding variability. This illustrates similar photometric sensitivity and reproducibility over an analogically linear absorbance range. Although lacking several of the more automated features found on commercial units, the reader is relatively inexpensive and particularly useful in teaching laboratories and smaller research or diagnostic facilities where budgeting constraints may limit access to otherwise expensive units.

LITERATURE CITED

1. Clark, M. F., and Adams, A. N. 1977. Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. J. Gen. Virol. 34:475-483.