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Economic Comparison of Enzyme Immunoassay and Virus Isolation Procedures for Surveillance of Arboviruses in Mosquito Populations

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Cost-effectiveness analysis of an enzyme immunoassay (EIA) for the surveillance of arboviruses was conducted. The EIA was compared with conventional virus isolation and serologic identification procedures (virus isolation procedures; VIP). Under most circumstances, EIA was more cost-effective than VIP. Costs for processing mosquito pools by VIP increased with the number of viruses included in the surveillance program and with the prevalence rate of each virus. In contrast to VIP, the prevalence rate did not affect costs for processing pools by EIA. In general, EIA was the most cost-effective procedure, followed by cell culture and mouse bioassays. In a 5-year cost-effectiveness analysis of a model surveillance program in which EIA and cell culture bioassays were used, the EIA again proved to be the most cost-effective assay procedure under most circumstances.

Enzyme immunoassays (EIAs) have found wide applicability in virus diagnosis and surveillance (18, 21). The development of EIA procedures is typically justified on the basis of the reduced time and expense that are necessary to obtain diagnostic results in comparison with conventional virus isolation and identification procedures. The ability of EIAs to provide rapid, clinically relevant diagnostic results has been well documented (2, 4, 10). However, there is little information concerning the relative cost-effectiveness of EIA and conventional diagnostic procedures (S. W. Hildreth, Ph.D. dissertation, Yale University, New Haven, Conn., 1984).

Previous studies have documented the diagnostic efficacy of a capture antigen EIA for La Crosse encephalitis virus, eastern equine encephalitis virus, and Highlands J virus (6–9; Hildreth, Ph.D. dissertation). The EIA proved to be a sensitive and specific alternative for the surveillance of eastern equine encephalitis and Highlands J viruses (8). However, expense is also a major factor in judging the utility of a diagnostic procedure. Thus, the EIA procedure was compared with conventional virus isolation and subsequent identification procedures in a cost-effectiveness analysis (CEA).

MATERIALS AND METHODS

EIA. The EIA procedure and materials used for the cost analysis have been reported previously (7, 9; Hildreth, Ph.D. dissertation). The EIA provided diagnostic results in much less time than the virus isolation procedures (VIPs); however, this time that was saved was not included in cost calculations as money that was saved.

VIPs. Two general VIPs were considered: the suckling mouse bioassay and cell culture bioassays. For CEA each bioassay was partitioned into primary virus isolation with amplification and serologic identification of the isolated viruses. For CEA it was assumed that the suckling mouse bioassay was performed following standard procedures (14). For the cell culture procedures, the basic costs of virus isolation with different culture vessel types and sizes were examined. Two general methods for virus isolation were selected for the cost model (11, 14). In the first method, mosquito pools were inoculated onto cell cultures with a fluid culture medium. Cultures were observed daily for 5 to 7 days for cytopathic effects. In the second method, a semisolid, agar-based culture medium was used; this restricted the cytopathic effect to discrete plaques. The cost for preparing continuous cell cultures (Vero or BHK-21 cells) did not differ significantly from that for primary cell cultures (chick embryos); the average cost was used. All suspect infected mice or cell cultures were assumed to be passed for virus amplification and serologic identification by the complement fixation (CF) test (14).

CEA procedure. For the CEA of the EIA and bioassays, general procedures were followed (13, 15). The first phase of the analysis consisted of estimating the costs of testing mosquito pools by EIA or one of the two VIP bioassays. The influence of different variables (number of pools tested [*N*], number of different viruses [*V*], and prevalence rate of mosquito pools containing virus [*P*]) on these costs was also determined. The second phase of the CEA consisted of implementing these assays in a 5-year surveillance program model. The CEA addressed only those issues that were pertinent to obtaining diagnostic results; the costs associated with subsequent actions or inactions of health officials due to test results were not analyzed.

The following general assumptions were made. (i) All three diagnostic assays were begun with a fully processed mosquito pool. (ii) All assays were performed in a standard laboratory facility, and the cost of laboratory or building maintenance was not incorporated into any of the analyses. (iii) Common reusable glassware supplies and common major laboratory equipment were not included in cost estimates. Costs that were incurred during the basic research and development of the EIA were not included in the CEA.

Laboratory procedures were divided into those conducted by a senior technician (salary, $24,000 per annum) or a junior technician (salary, $16,800 per annum). The costs of animals and daily care were estimated from those at the Yale University Animal Care Facility. The cost of a pregnant Swiss albino mouse was estimated to be $6.41, and the cost of a New Zealand rabbit (2 kg) was estimated to be $17.60.

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Daily housing and maintenance charges were estimated to be $0.75 per mouse and $0.90 per rabbit.

Cost assumed for the EIA procedure. The cost of the EIA procedure included the preparation of immunoglobulin reagents and the performance of the assay. Mouse hyperimmune ascitic fluids (MIAFs) were produced by obtaining 20 pregnant mice and producing a suckling mouse brain virus stock with their litters (14). The procedure yielded about 300 ml of ascitic fluid, at a total cost of $255.43. For the subsequent production of MIAFs the remaining virus stock and nonpregnant mice were used at an anticipated cost of $168.01 per 300 ml of MIAF.

Rabbit antibodies were produced by using purified viruses. Standard procedures (7, 9) were followed for virus purification, and for the cost model four infected roller bottle cell cultures were required. The cost of labor (senior technician) and supplies was calculated to be $106.80 for 5.0 ml of virus stock. Rabbits were inoculated subcutaneously twice with purified virus-Freund adjuvant; all inoculations and bleedings were assigned to a junior technician. By assuming a yield of approximately 85 ml, the cost of this serum sample was $258.51.

Immunoglobulins were precipitated from MIAFs and serum samples by using saturated ammonium sulfate (7, 9). The cost of buffers, reagents, dialysis tubing, and labor (junior technician) was $27.87 per sample. Immunoglobulin G (IgG) was separated from the saturated ammonium sulfate product by either protein A affinity chromatography or DEAE Affi-Gel Blue (Bio-Rad Laboratories, Richmond, Calif.) affinity chromatography (7, 9). The total cost of labor (junior technician) plus reagents and supplies was estimated to be $75.57 for a final product of 10 mg of IgG. For the analyses, all enzyme conjugates were obtained from commercial sources. The EIA used in phase I analysis was a qualitative EIA; thereefore, the cost of an automated plate reader was not included.

Cost assumed for the suckling mouse bioassay procedure. A 10% nonfertilized rate or nonspecific death rate was assumed for the pregnant mice. It was assumed that approximately 40 pools per hour could be processed by a junior technician. The cost of inoculation of a mosquito pool into a litter of mice and observation for 10 days was estimated to be $9.07 per pool.

Assumptions for serologic identification. Virus isolates were identified by the CF test. The following assumptions were made about the procedure. (i) Every virus was tested against three different MIAFs for each arbovirus that the surveillance program attempted to monitor, (ii) positive control antigens were included for each of the controls, (iii) each virus isolate was tested at fourfold dilutions against fourfold dilutions of the MIAF, and (iv) the CF test was performed by a senior technician. Costs of reagents and disposable plastic microplates were estimated at $0.15 and $0.14 per 16 wells, respectively. The labor time for the CF test was calculated for two different testing schedules: once a week for 12 weeks and once at the end of 12 weeks. These values were then averaged.

Assumptions for the 5-year surveillance program analysis. By using the cost estimates from phase I, the 5-year model was constructed with the present values of the net costs for the EIA. These net costs were estimated by discounting all monies spent throughout the 5-year model to their theoretical worth in year 1 of the program. The formula used to discount yearly expenditures was as follows:

$$\sum_{i=0}^{n=4} (\text{yearly net cost for testing mosquito pools})_i/(1 + d)^i,$$

where i is the year (0, 1, 2, 3, or 4) of the program and d is the discount rate (expressed as a proportion). Year 1 of the actual program was considered year 0 in this formula, year 2 was considered year 1, and so forth, to ensure that only monies spent beyond the first year of operation were discounted. The discount rate varied between 5 and 15%. For each program, the cost-effectiveness ratio was calculated; the 5-year total net cost was divided by the total number of mosquito pools that were tested, resulting in the cost per mosquito pool. The procedure that produced the lowest cost per mosquito pool was considered the most cost-effective method.

For the initial model, the surveillance program was assumed to require the testing of $N = 1{,}000$ mosquito pools for $V = 1$ with $P = 1\%$ of the pools tested. All costs for supplies, reagents, and labor were assumed to increase yearly by a 7% inflation increment. The discount rate was chosen to be 10%. All equipment costs for the 5-year surveillance program were budgeted for the first year. Additional analyses (15; Hildreth, Ph.D. dissertation) were conducted by varying N (1,000 to 4,000 pools), P (1 to 30%), V (1 to 4 viruses), and the discount rate (5 to 15%); however, only representative results are presented.

The general cost-effectiveness ratios were adjusted to account for the diagnostic value of each assay by using the probability of false-negative $[P_r(F^-)]$ or false-positive $[P_r(F^+)]$ test results (8; Hildreth, Ph.D. dissertation). The method was as follows:

$$\frac{\text{cost-effectiveness ratio per mosquito pool}}{[1 - P_r(F^-)][1 - P_r(F^+)]}$$

where

$$P_r(F^-) = \frac{\beta P}{(1 - \alpha)(1 - P) + \beta P} \text{ and}$$

$$P_r(F^+) = \frac{\alpha(1 - P)}{(1 - P) + (1 - \beta)P},$$

where α is the test false-positive rate, and β is the test false-negative rate. If the assay were 100% accurate, the cost estimate would not be inflated. The method was adjusted for the probability that the assay results were correct for a given prevalence rate of virus.

RESULTS

Costs of performing the EIA were estimated by totaling the costs of reagents, labor, and supplies that were needed to test mosquito pools that originated from surveillance programs that varied by N, P, and V. To simplify these estimates, N was assumed to be 1,000, 2,000, 4,000, or 8,000 mosquito pools. Labor costs were calculated by assuming that N was tested over 12 weeks. P was assumed to be 1, 5, or 10%. V was assumed to be 1, 2, 3, or 4 different viruses. An example of the cost calculations was as follows: N = 1,000 mosquito pools tested over a 12-week period (84 pools per week); 84 pools required 6 plates at $7.92 per plate ($6.12 for reagents plus $1.80 for the plate). Labor costs were $98.48 (senior technician, 8 h), with a total cost of $1.74 per mosquito pool.

The cost estimated to process a mosquito pool by EIA ranged from $1.27 to $5.94, depending on the variables N and V (Table 1). The P value did not affect the cost of performing the EIA, because the assay simultaneously de-

TABLE 1. The qualitative EIA: cost of antigen detection and identification

No. of mosquito pools tested/yr	Total cost ($)/pool to screen the following no. of viruses[a]:			
	1	2	3	4
1,000	1.74	3.38	4.65	5.94
2,000	1.55	2.70	4.09	5.26
4,000	1.33	2.58	3.61	4.15
8,000	1.27	2.03	2.75	3.26

[a] Total cost per pool for detection and identification of viruses within mosquito pools by EIA.

tected and identified specific viral antigens in each pool. However, this same attractive feature of the EIA increased costs when each pool was tested for more than one virus (Table 1). The magnitude of the increase was greatest for the low-volume surveillance program (N = 1,000). This was not unexpected because reagents costs were the greatest for such a program.

The cost for the suckling mouse bioassay included the cost for primary isolation, passage, and serologic identification of the virus isolate. Frequently, primary virus isolations had to be repassaged to ensure a characteristic survival pattern of inoculated mice and to produce sufficient viral antigen for serologic identification. The number of pools requiring passage was directly related to the prevalence rate of viruses in the mosquito population and to nonspecific death rates in mouse litters. Costs for passage of the virus isolates were calculated for P values of 1 to 30% (Table 2). For P = 1%, the increased cost was estimated to be $0.09 per mosquito pool. However, with P = 10 and 30%, the additional costs per mosquito pool were estimated to be $0.91 and $2.72, respectively. For a surveillance program characterized by N = 1,000 and P = 1 to 30%, the total cost of performing the suckling mouse bioassay was estimated to be between $9.36 and $15.52 per mosquito pool, depending on V (Table 2). Similarly, with surveillance programs with N = 4,000, the cost per mosquito pool was calculated to be $9.24 to $15.58. In contrast to the EIA, the cost associated with the suckling mouse bioassay was affected by P as well as by N and V. However, the major expense (e.g., 59 to 98%) was attributable to the initial VIP.

Several examples of cell culture bioassays were included in the cost model. The culture systems varied by the source of cells, the culture vessels, and the type of culture medium

TABLE 2. The suckling mouse bioassay: cost of virus isolation and identification

No. of mosquito pools tested/yr	P (%)	Cost ($)/mosquito pool for virus isolation for:		Total cost ($)/mosquito pool to detect the following no. of viruses[a]:	
		Primary isolation	Additional passage	1	4
1,000	1	9.07	0.09	9.36	9.53
	10	9.07	0.91	10.48	11.25
	30	9.07	2.72	13.11	15.52
4,000	1	9.07	0.09	9.24	9.32
	10	9.07	0.91	10.64	11.17
	30	9.07	2.72	13.26	15.58

[a] Total cost per mosquito pool for virus isolation and serologic identification.

TABLE 3. Cell culture bioassay: cost of virus isolation and identification

Cell culture bioassay type	No. of mosquito pools tested/yr	P (%)	Cost ($)/mosquito pool for virus isolation for:		Total cost ($)/mosquito pool to detect the following no. of viruses[a]:	
			Primary isolation	Additional passage	1	4
Single cell culture test tube	1,000	1	1.95	0.02	2.16	2.34
		10	1.95	0.19	2.64	3.42
		30	1.95	0.58	3.66	6.06
	4,000	1	1.95	0.02	2.05	2.12
		10	1.95	0.19	2.63	3.33
		30	1.95	0.58	3.81	6.13
Agar overlay on a six-well culture plate	1,000	1	2.40	0.02	2.61	2.79
		10	2.40	0.24	3.13	3.91
		30	2.40	0.71	4.44	6.84
	4,000	1	2.40	0.02	2.50	2.58
		10	2.40	0.24	3.13	3.82
		30	2.40	0.71	4.59	6.91

[a] Total cost per mosquito pool for virus isolation and serologic identification.

used. Five different types of culture vessel systems were used in the cost model: culture test tube (16 by 1.5 cm), 96-well microculture plate, 24-well microculture plate, 6-well culture plate, and culture petri dish (60 by 15 mm). The cell culture medium was either totally fluid or a semisolid overlay containing agar.

Costs were estimated for the preparation of cell cultures in each of the five types of culture vessels. However, for the analysis these costs were expressed per unit of vessel used by each mosquito pool. A single pool was assumed to use the following vessel units: one culture dish, one well of a 6-well culture plate, two wells of a 24-well microculture plate, eight wells of a 96-well microculture plate, and one culture test tube. These vessel unit assignments ensured a minimum of 600 mm^2 of surface area of cell culture per mosquito pool.

Preparation costs were greatest for culture petri dishes, followed by those for the six-well culture plate and finally those for the culture test tube (data not shown). The costs associated with the use of the 24- or 96-well microculture plates were similar. Labor costs for the preparation of cell cultures by either the senior- or junior-level technicians were also similar enough to justify the use of the average labor cost.

The overall cost of primary virus isolation included the costs of cell culture production and maintenance, mosquito pool inoculation, and daily monitoring of cells for the detection of the cytopathic effect. As with the EIA and the suckling mouse bioassay, the size of N did not affect the cost per mosquito pool of primary virus isolation (Table 3). The cost of primary virus isolation varied with the culture method. The single cell culture test tube method was the least expensive and was estimated to be $1.95 per mosquito pool.

Costs for the combined virus isolation and serologic identification procedures were calculated by using two primary isolation systems and the CF test (Table 3). Costs of the other primary isolation systems have been presented elsewhere (Hildreth; Ph.D. dissertation). The culture test tube system was the least expensive for virus isolation and

TABLE 4. Cost-effectiveness ratios of the EIA for a 5-year surveillance program

No. of mosquito pools tested/yr	No. of viruses attempting to detect	Cost ($) ratios by[a]:	
		Qualitative EIA	Quantitative EIA
1,000	1	2.26	4.86
	3	5.02	7.62
4,000	1	1.41	2.06
	3	3.52	4.22

[a] Cost ratios of a 5-year program per mosquito tested by different EIAs (discount rate, 10%).

identification. The six-well culture plate system was also relatively inexpensive (Table 3). Costs for these bioassays (virus isolation and serologic identification) ranged between $2.05 and $6.13, and $2.50 and $6.91 per mosquito pool, respectively. However, the use of two different primary isolation cell culture assays per mosquito pool increased the estimated total cost for virus isolation and identification to $4.48 to $9.48 per mosquito pool for the test tube system and $6.32 to $11.92 per mosquito pool for the six-well culture plate system (data not shown). In general, costs for cell culture bioassays were found to be influenced predominately by P, followed by N and then V.

For the 5-year CEA model, surveillance programs were required to purchase equipment, depending on the method that was used for evaluating the EIA results. Qualitative evaluation of the EIA was performed by visual scoring (− to +4) of substrate degradation products and did not require instrumentation. Quantitative evaluation of the EIA required a spectrophotometric plate reader (cost, $13,000). Each surveillance program in which the EIA was used was also assigned the purchase of a semiautomatic plate washer (cost, $2,250) and two multichannel pipets (total cost, $800). The total equipment costs for the quantitative and qualitative EIAs were $16,060 and $3,060, respectively. The surveillance program in which cell culture bioassays were used required the following eqipment: large incubator, microscope, isolation hood, and a roller drum (total cost, $12,300).

The results of the CEA were expressed as the ratio of the total cost of the 5-year program (in currently valued monies) per total number of mosquito pools tested, yielding the cost per mosquito pool tested. For the initial parameters of the surveillance program (N = 1,000, P = 1%, V = 1, and discount rate of 10%), the cost of the use of the EIA was $2.26 per mosquito pool when it was tested with the qualitative system and $4.86 per mosquito pool when it was tested with the quantitative system (Table 4). The cost of the use of the suckling mouse bioassay or cell culture was $8.86 and $4.51 per mosquito pool tested, respectively (Table 5). The qualitative EIA was the most cost-effective, followed by the single cell culture test tube procedure and then the quantitative EIA.

Sensitivity analyses revealed several salient characteristics of the three assays. First, when 1,000 mosquito pools were tested for a single virus, the qualitative EIA was always the most cost-effective diagnostic procedure, regardless of the P value of the infected pools and the theorized discount rate. The quantitative EIA was always more cost-effective than the suckling mouse bioassay. However, the P value was required to be 10% before the quantitative EIA became more cost-effective than the single cell culture test tube assay. Second, when V = 3, the single cell culture test tube assay was the most cost-effective, except when the prevalence rate was 10%. However, it is unlikely that a single cell culture test tube assay would be used for detecting three different viruses; at least two different cell lines would be more realistic. Under these alternate conditions and by using the same test protocols, the costs of the cell culture assays were expected to be equal to or greater than $6.54 to $8.47 per mosquito pool (data not shown), which were substantially more than the qualitative EIA ($4.65 to $5.52 per mosquito pool). The suckling mouse bioassay was the least cost-effective assay, regardless of the P value and the discount rate.

The total costs for all assays declined when N was increased to 4,000. Furthermore, the difference in cost between the quantitative and the qualitative EIAs was found to decrease. With V = 1 and N = 4,000, the EIA was again found to be the most cost-effective method ($1.34 to $2.18 per pool), regardless of the value of P and the discount rate. Even with V = 3, the EIA was the most cost-effective assay, particularly when cell culture viral susceptibility was considered. The suckling mouse bioassay was again the least cost-effective.

The diagnostic value of each assay was considered in the final CEA of the EIA. The diagnostic value was the ability of each assay to correctly diagnose both virus-containing pools and normal noninfected mosquito pools (i.e., sensitivity and specificity rates). The sensitivity rates of the EIA and the cell culture assay were estimated previously (6, 7, 9; Hildreth, Ph.D. dissertation) for assaying mosquitoes during the early and late phases of infection. During the early phase of infection, the EIA was assigned sensitivity and specificity rates of 0.418 and 1.0, respectively. During the late phase of infection, the sensitivity and specificity rates of both assays were expected to be 1.0 (or very close to 1.0). For this CEA model, when error rates were corrected for mosquitoes that were assayed in the early phase of infection, the EIA was predicted to be more cost-effective than the cell culture methods when V = 1 (Table 6). When V = 3 the EIA was only superior to the cell culture method when two cell culture assays were used per mosquito pool and was only

TABLE 5. A 5-year surveillance program: cost-effectiveness ratios of suckling mouse and cell culture bioassays

Bioassay	No. of pools tested/yr	No. of viruses attempting to detect	Cost ($) ratios for the following P values[a]:		
			1%	5%	10%
Suckling mouse	1,000	1	8.86	9.36	9.92
		3	8.97	9.66	10.48
	4,000	1	8.75	9.23	9.92
		3	8.80	9.47	10.35
Single cell culture[b]	1,000	1	4.51	4.74	4.96
		3	4.61	5.04	5.48
	4,000	1	2.55	2.76	3.11
		3	2.60	3.00	3.33
Two cell cultures[c]	1,000	1	6.80	7.12	7.46
		3	6.90	7.42	7.94
	4,000	1	4.84	5.44	5.84
		3	5.13	5.61	6.26

[a] Cost ratios of a 5-year program per mosquito pool tested as influenced by the prevalence rates of viruses. Costs were estimated by using a discount rate of 10%.

[b] For the single cell culture, each pool was assayed by using a single test tube.

[c] For the two cell culture assay, each pool was assayed with two cell lines by using a single test tube plus one well of a six-well culture plate.

minimally less cost-effective than the single cell culture test tube assay. The same analysis on late-phase-infected mosquitoes showed no inflation of the cost-effectiveness ratios of either assay.

DISCUSSION

New diagnostic assays must not only be evaluated by their sensitivity and specificity characteristics but also by the economics of performing them. Recently, several medical diagnostic procedures have been evaluated economically, and the most cost-efficient use of the procedures have been defined (17). Virus diagnostic methods have not been formally evaluated in this manner. Economic analyses have been applied to arboviruses, but only to the evaluation of epidemics and control of vectors (1, 5, 12, 19), revealing that routine surveillance and vector control can be less expensive than the cost of a major epidemic. A substantial proportion of the expense of vector surveillance or of a vector control evaluation program is the cost of monitoring the virus within mosquito populations.

The economic analyses demonstrate that the EIA is a cost-effective surveillance diagnostic tool. The cost of the EIA is directly correlated with the number of viruses that are monitored in the surveillance program. Cost is not correlated with the prevalence rate of the virus in the sampled mosquito population. These two characteristics are to be expected from an assay that is designed to measure only a single factor within a specimen. In contrast, bioassays, such as suckling mice and cell culture bioassays, use biologic amplification procedures to separate specimens containing infectious agents from specimens lacking such agents. Subsequently, serologic assays are used to identify the agents. Due to the two-level screening process, the cost of performing such bioassays is influenced by the prevalence rate. Furthermore, the cost is also influenced by the susceptibility range of these bioassays to different arboviruses. For example, two different cell lines may be required when attempting to isolate two or more different viruses from a mosquito pool. Therefore, the number of viruses that are monitored may influence both the cost of virus isolation and the serologic identification procedure.

TABLE 6. Error-adjusted cost-effectiveness ratios of the EIA and cell culture bioassay

Assay type	No. of mosquito pools tested/yr	No. of viruses attempting to detect	Cost ($) ratios/ mosquito pool[a]	
			Unadjusted	Adjusted
EIA	1,000	1	2.26	2.33
		3	5.44	5.61
	4,000	1	1.41	1.46
		3	3.52	3.63
Single cell culture[b]	1,000	1	4.74	4.77
		3	5.04	5.07
	4,000	1	2.76	2.77
		3	3.00	3.02
Two cell cultures[c]	1,000	1	7.12	7.16
		3	7.42	7.46
	4,000	1	5.44	5.47
		3	5.61	5.64

[a] Prevalence rate of pools containing infected mosquitoes was assumed to be 5%.

[b] Single cell culture assumes that each pool was assayed with a single test tube format.

[c] Two cell culture assay assumes that each pool was assayed with a single test tube plus one well of a six-well culture plate.

The cost analysis (phase I) ranked the EIA, cell culture assays, and the suckling mouse bioassay in order of cost-effectiveness. These differences between the first two assays may be underestimated. First, the cost of performing the cell culture bioassay would increase if other serologic assays, such as hemagglutination inhibition or neutralization, were substituted for the CF test or were used to further identify the virus. Second, each mosquito pool may need to be tested by using multiple cell lines.

The use of the 5-year surveillance program model (phase II cost analysis) allows for a more realistic comparison of different diagnostic methods, regardless of how and when the costs are accrued. In general, CEA is a useful tool in aiding program planners (13, 15, 20), and it is best applied and evaluated for each unique surveillance program. For example, surveillance programs designed for eastern equine encephalitis virus in Massachusetts and La Crosse virus in Wisconsin differ markedly due to the effects of cell culture viral susceptibility and serologic cross-reactions that are encountered with the viruses under surveillance (3, 16).

The 5-year model analyzed in this study considers the cost of equipment as well as the error rates of the assays. For some laboratories the costs of establishing a cell culture capability may be less than that estimated in the 5-year model. However, for new surveillance programs or temporary field stations, the establishment of cell culture capability will probably exceed the costs estimated in the model. The qualitative EIA was the most cost-effective assay in this study. Furthermore, many laboratories now have EIA plate readers. Removal of this item of equipment from the CEA would significantly lower the estimated cost for the quantitative EIA processing of pools. Under these circumstances, the EIA (qualitative or quantitative) is the most cost-effective assay.

The cost per assay must be adjusted for the quality of the results. Errors associated with the EIA occur with mosquitoes containing low titers of virus, which is characteristic of either the early phase of infection or incompetent vectors. However, the probability of sampling infected mosquitoes during this early phase of infection is low (6,7; Hildreth, Ph.D. dissertation). As such, the effect of the error rates of the EIA on the diagnostic quality of the assay results is minimal when both the overall low prevalence rate of all infected pools and the sensitivity and specificity rates of the EIA are considered.

For these economic analyses, the CEA method was chosen instead of the benefit-cost analysis method. The major difference between the two methods is that a monetary value is assigned to the benefit, which is done in the benefit-cost analysis (15, 20). In the analysis reported here a major benefit of the EIA was not measured: the time saved due to a rapid assay. Even when a large number of samples were tested, the EIA yielded final results in 48 h. The two bioassays (cell culture and suckling mice) yielded final results at different time intervals, depending on the following: (i) virulence of the virus, (ii) sensitivity of the bioassay, and (iii) quantity of infectious units within the sample. We choose not to estimate this time interval because of the number of arboviruses but acknowledged a general time interval for completion of results of 4 to 14 days. The 2 to 12 days that are saved by the EIA is important to public health and clinical laboratories and would easily justify any minor differences in costs per test.

Two major limitations of the EIA need to be noted. First,

only the virus(es) previously decided on would be detected by the chosen immunologic reagents. Alternate viruses that would be detected in the more expensive VIP systems would not be detected. Although this attribute may be advantageous in situations in which many medically unimportant viruses are circulating, it is disadvantageous when a new or unexpected virus is encountered. Second, the use of the EIA alone would not result in the development of a repository of virus isolates. Such repositories of isolates collected from different locations or circumstances over time are invaluable for epidemiologic and virologic studies. However, in this regard, the EIA could be used as a screening device, and only positive pools could be processed by VIPs.

In conclusion, the results of this study indicate that the EIA is a potentially powerful public health surveillance tool. The EIA procedure should be applicable for monitoring any pathogen in a vector population. The EIA provides results rapidly, reliably, and inexpensively; all are features of a diagnostic assay that will be useful not only as a population surveillance tool but also as a standard research tool for many laboratories.

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