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Enzyme-Linked Immunosorbent Assay for Detection and Identification of Coxsackieviruses A

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Coxsackieviruses A are known to cause a wide range of human disease processes. However, because many coxsackieviruses A present in clinical specimens do not produce a recognizable cytopathic effect in readily available tissue culture systems, infections with coxsackieviruses A are often difficult to diagnose. We have thus developed enzyme-linked immunosorbent assay (ELISA) systems for the detection and serotyping of coxsackievirus A antigens. The assays consist of a double-antibody ELISA which utilizes type-specific monkey and mouse coxsackievirus antisera. Although some cross-reactivity was noted, the ELISA systems correctly identified the serotypes of 22 of 23 coxsackievirus A complement fixation antigens available for testing. Testing of tissue culture fluids revealed that antigen could often be detected by ELISA before the appearance of a cytopathic effect. In addition, the infecting coxsackievirus A antigen could be unequivocally identified in 8 of 11 stool specimens obtained from patients with coxsackievirus A infections. The ELISA system might thus represent an important tool in the diagnosis and study of coxsackievirus A infections.

The coxsackievirus A group of human picornaviruses has been associated with a number of human diseases, including meningitis, myocarditis, pleurodynia, paralysis, and conjunctivitis (7, 10, 11, 13, 17). However, the diagnosis of infections caused by coxsackieviruses A has been hampered by the fact that many clinical isolates grow poorly, if at all, in readily available tissue culture cell lines (2, 14). Although the coxsackieviruses A can be cultivated in infant mice, the lack of the widespread availability of mouse systems has limited the usefulness of this diagnostic method (1, 5, 14). Similarly, the ability to establish the diagnosis of coxsackievirus A infection on serological grounds has been hampered by the existence of a large number of distinct serotypes and the fact that many patients exhibit heterotypic responses after infection (3, 9).

Enzyme immunoassays have been developed for the rapid, simple detection of a number of infecting antigens in tissue culture fluids and clinical specimens (15, 21, 22, 26). This report describes the development of solid-phase, enzyme-linked immunosorbent assay (ELISA) systems for the detection and identification of coxsackievirus A antigens.

MATERIALS AND METHODS

Antisera. Monkey antisera to coxsackieviruses were obtained from the Reference Reagents Branch, National Institute of Allergy and Infectious Diseases, Bethesda, Md. (16). Mouse antisera were obtained from Gabe Castellano, Microbiological Associates, Rockville, Md. The method of preparation of the mouse antisera has been previously described (19). Goat anti-mouse immunoglobulin G (IgG) was purchased from Antibodies Inc., Davis, Calif., and conjugated with alkaline phosphatase (type VII; Sigma Chemical Co., St. Louis, Mo.) by the method of Engvall and Perlman (6).

Coxsackievirus antigens. Coxsackievirus A complement fixation antigens, largely prepared in suckling mice, were obtained from Microbiological Associates. The preparation and potency of these antigens have been previously described (19). Seed virus preparations were obtained from the Reference Reagents Branch, National Institute of Allergy and Infectious Diseases. In both instances, types A1 through A22 and A24 were available for testing.

Control antigens. Antigens containing high concentrations of echoviruses, coxsackieviruses B, reoviruses, and adenoviruses were obtained from Microbiological Associates, the American Type Culture Collection, or the Reference Reagents Branch, National Institute of Allergy and Infectious Diseases (16).

Specimens consisting of uninoculated human embryonic fibroblast, human embryonic kidney, and African green monkey kidney, mouse brain, and mouse torso homogenates were utilized as negative controls for experiments in which complement fixation of tissue culture antigens was assayed.

Clinical specimens. Stool specimens were available from 11 patients from whom coxsackieviruses A were isolated by conventional means (some of the coxsackievirus A specimens were provided by N. Schmidt, Virology Laboratory, California State Department of Health). Four specimens were available containing coxsackievirus A1, two containing coxsackievirus A5, and one each containing coxsackieviruses A2, A9, A10, A16 and A24.

Two stools containing Norwalk virus were provided

by Harry Greenberg, Laboratory of Infectious Diseases, National Institutes of Health, Bethesda, Md. Fourteen stool specimens containing human rotavirus were obtained from children with rotavirus gastroenteritis occurring during the summer months. In addition, eight stools containing echoviruses and four containing coxsackieviruses B of various serotypes, identified by standard tissue culture techniques (14), were available for testing.

Negative control stools were available from five normal adult volunteers during the winter months. These were utilized as negative controls for experiments in which clinical specimens were assayed.

Double-antibody ELISA. The double-antibody ELISA was adapted from the previously described ELISA for the detection and serotyping of human rotavirus (25, 26). The optical dilutions of all reagents were determined by checkerboard titration. Briefly, wells of round-bottomed polyvinyl microtiter plates (Dynatech 220-24) were coated with monkey anti-coxsackievirus serum raised against a single serotype and diluted in carbonate buffer. In addition, wells were coated with nonimmune monkey serum to serve as control wells. The plates were stored at 4°C until used.

Immediately before use, the plates were washed five times with phosphate-buffered saline (pH 7.4) containing 0.05% Tween 20, and a portion of antigen, tissue culture fluid, or clinical specimen was added to the wells in such a way that the test specimen was reacted in two wells coated with an individual monkey anti-coxsackievirus antiserum and in two wells coated with the normal monkey serum (24). Complement fixation antigens were tested at a concentration of 10^{-2} complement fixation units as defined by Gnesh et al. (8). Clinical specimens and tissue culture fluids were diluted at least 1:10 before testing. All dilutions were made in phosphate-buffered saline–0.05% Tween 20–1% fetal calf serum except for stool specimens, which were diluted in 20% buffered *N*-acetylcysteine (Mucomist). This reducing agent was added to reduce nonspecific reactivity due to antiglobulins which might be present in stool specimens (24).

After an incubation for 2 h at 37°C or overnight at 4°C, the plates were washed, and a dilution of mouse anti-coxsackievirus, directed against the same serotype as the monkey serum used to coat the plate, was added and incubated for 1 h at 37°C. (Nonimmune mouse serum was added to the wells coated with the nonimmune monkey serum.) The plates were again washed five times with alkaline phosphatase, and labeled goat anti-mouse IgG was added to each well. After another incubation for 1 h at 37°C, the plates were washed five times, and *p*-nitrophenyl phosphate substrate (Sigma 104-5) was added. The color resulting from the hydrolysis of the substrate by the alkaline phosphatase bound in the previous steps was measured in the microplate colorimeter (4).

The optical density readings derived from the above assays were interpreted as follows. For the wells coated with each coxsackievirus A serotype (or nonimmune serum), the mean absorbance in the wells containing phosphate-buffered saline–0.05% Tween 20–1% fetal calf serum was subtracted from the mean absorbance of the wells containing the specimens. This correction was necessary to adjust for variable amounts of nonspecific interaction between the mouse coxsackievirus antiserum and the solid phase. After this adjustment, a coxsackievirus A-specific absorbance was calculated for each specimen and each coxsackievirus A serotype. This was performed by subtracting the mean corrected absorbance of the specimen in the wells coated with the nonimmune serum from the mean corrected absorbance of the same specimen in the wells coated with the individual anti-coxsackievirus antisera.

At least three negative control specimens were assayed in each test run. These control antigens were either uninfected mouse brain homogenates, uninfected tissue culture cell lysates, or stool specimens known to be negative for coxsackievirus. A coxsackievirus A-specific absorbance was calculated for each negative control as described above, and a mean and standard error of the negative controls was determined. A clinical specimen or tissue culture antigen was considered to be positive for a coxsackievirus A serotype if it yielded a coxsackievirus-specific absorbance which was at least two standard deviations greater than the mean of the negative control specimens. When a specimen yielded a positive result for more than one serotype, a serotype was assigned if the specific absorbance for one serotype was at least 0.1 optical density units greater than the absorbance for any of the other serotypes. This arbitrary value was determined after initial experiments with complement fixation antigens (see Table 1).

Tissue culture experiments. Tissue culture tubes containing African green monkey kidney and human embryonic cells (WI-38; Flow Laboratories, McLean, Va.) were inoculated with seed virus preparations of coxsackieviruses A in a concentration of 100 50% tissue culture infective doses per ml by following established procedures (14). Briefly, 0.2 ml of virus diluted in minimum essential medium was added to duplicate tubes and allowed to absorb for 4 h at 37°C. The tubes were washed three times with minimal essential medium and reincubated at 37°C. The tubes were observed for cytopathic effect every 24 h. In addition, 0.1 ml of tissue culture fluid was aseptically removed every 24 or 48 h and tested for antigen by the ELISA system with wells coated with the homologous coxsackievirus A antiserum. Positivity in these assays was defined as an ELISA value of 2 standard deviations greater than the value of fluid removed from uninoculated control tubes. Tissue culture fluids that were positive in this assay were serotyped as described above.

RESULTS

Complement fixation antigens. Antigens prepared from 23 coxsackieviruses A (A1 to A24, except A23) were available for ELISA testing. All of the antigens except A19 could be detected at concentrations containing 10^{-2} complement fixation units, i.e., 100-fold-less antigen than could be detected by complement fixation. The ELISA specific activity of these antigens tested with homologous antisera ranged from 0.22 optical density units for coxsackievirus A20 to 0.62 optical density units for coxsackievirus A16 (Table 1).

Testing of these antigens in wells coated with

TABLE 1. *Cross-reactivity of coxsackievirus A antigens*

Antigen[b]	Optical density units for following antiserum[a]:																						
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	24
1	**0.31**[c]			0.14								0.13			0.11							0.17	
2		**0.34**	0.14	0.10				0.10	0.17			0.15			0.12							0.18	
3			**0.33**					0.13															
4				**0.42**													0.16						
5					**0.40**																		
6			0.10			**0.29**									0.16								
7							**0.33**																
8			0.19					**0.44**									0.13						
9									**0.35**														
10		0.10								**0.57**				0.13	0.13	0.12						0.11	
11											**0.28**	0.14											0.12
12								0.17		0.10		**0.41**			0.10							0.13	0.12
13								0.11	0.13		0.13	0.12	**0.27**				0.12	0.13					0.13
14			0.14						0.10		0.10	0.12		**0.30**	0.10			0.14				0.10	
15															**0.26**								
16							0.13									**0.62**						0.11	
17			0.20						0.19			0.20			0.17	0.10	**0.33**	0.13					0.16
18																	0.16	**0.45**					
19																							
20																				**0.22**			
21			0.16									0.11									**0.58**		
22									0.10			0.13										**0.46**	
24									0.13			0.12			0.10								**0.44**

[a] Values represent the means of duplicate determinations. Values of less than 0.10 are not shown. Negative control antigens consisting of uninoculated human embryonic fibroblast, mouse brain, and mouse torso cells gave values of ≤0.10 optical density units for all antigens tested.

[b] All antigens were tested at a concentration of 10^{-2} complement fixation units.

[c] Homologous reactions are shown in boldface type.

each of the coxsackievirus A antisera revealed extensive cross-reactivity. Antisera to some types, such as A3, A8, A9, A12, A15, and A22, reacted with a number of different coxsackievirus A antigens. However, quantitative determination revealed that each antiserum reacted with the homologous antigen to a statistically significantly greater extent than with the heterologous coxsackievirus A antigens. Similarly, the serotype of each antigen could be identified by the fact that the antigen reacted with the homologous antiserum to a greater extent than it did with heterologous antisera. For example, coxsackievirus A17 antigen reacted with eight different coxsackievirus A antisera. However, the reactivity with the homologous antiserum was 0.33. This reactivity was 0.13 optical density units greater than the reactivity with any of the other coxsackievirus A antisera. Similarly, coxsackievirus A10 antigen reacted with six coxsackievirus A antisera. However, the homologous reaction (0.57 optical density units) was at least 0.44 optical density units greater than any of the heterologous reactions. Utilizing a similar form of analysis, the correct antigenic type of all the antigens except for A19 could be successfully assigned.

Control antigens. Complement fixation antigens or seed virus preparations of the following viruses were tested in the coxsackievirus A ELISA: coxsackievirus B (types 1 through 6), echoviruses (types 1 through 8), adenovirus (types 1, 14, and 21), reovirus (types 1 through 3), and rotavirus (types NCDV and SA-11). None of the antigens gave an ELISA-specific value greater than 0.10 to any of the coxsackievirus A antisera. In addition, the following negative control antigens were tested: uninoculated African green monkey kidney, human embryonic fibroblast, mouse brain, and mouse torso cells. None of these antigens showed specific reactivity in the ELISA system for coxsackievirus A antigen.

Tissue culture studies. The coxsackievirus A ELISA was compared with the observation of cytopathic effect for the detection of the growth of coxsackievirus A seed virus preparations in African green monkey kidney cells and human embryonic fibroblast (WI-38) cells. The results are shown in Tables 2 and 3. In the case of the

TABLE 2. *Comparison of cytopathic effect (CPE) and ELISA for detection of coxsackievirus A antigen in human embryonic fibroblast (WI-38) cells*

Coxsackievirus A serotype	Test results on following day:											
	1		2		3		4		5		6[a]	
	CPE[b]	ELISA[c]	CPE	ELISA	CPE	ELISA	CPE	ELISA	CPE	ELISA	CPE	ELISA
1	–[d]	–	–	–	–	NT[e]	–	–	–	74	–	72
2	–	–	–	–	–	NT	1	199	1	112	1	42
3	–	–	–	–	–	NT	–	73	–	24	–	–
4	–	–	–	–	–	NT	–	63	–	–	–	22
5	–	–	–	–	–	NT	–	78	–	74	–	51
6	–	–	–	–	–	NT	2	90	3	44	4	93
7	–	18	3	190	3	NT	4	999	4	406	4	318
8	–	–	–	–	–	NT	–	–	–	70	–	23
9	–	–	1	95	3	NT	4	1,999	4	1,194	4	810
10	–	–	–	–	–	NT	1	606	1	351	1	168
11	–	–	–	–	–	NT	–	–	–	55	–	76
12	–	–	–	–	–	NT	–	62	–	36	–	44
13	–	–	–	–	–	NT	2	202	3	88	4	130
14	–	–	–	–	–	NT	2	–	3	–	3	–
15	–	–	–	–	1	NT	2	–	3	–	4	122
16	–	–	–	–	–	NT	–	–	–	–	–	34
17	–	–	–	–	–	NT	1	–	1	–	3	34
18	–	–	–	–	1	NT	3	222	3	154	4	230
19	–	–	–	–	–	NT	–	50	–	–	–	40
20	–	–	–	–	–	NT	1	111	1	55	3	102
21	–	–	–	–	–	NT	–	52	2	624	2	799
22	–	–	–	–	–	NT	–	–	–	57	–	54
24	–	–	–	–	–	NT	–	–	–	22	–	29
Control	–	–	–	–	–	–	–	–	–	–	–	–

[a] Cells could not be read for CPE after day 6 due to nonspecific degeneration of the cells.
[b] Number of positive seed virus preparations.
[c] Absorbance units (optical density × 1,000). Values shown are the means of duplicate determinations.
[d] –, Negative for CPE, or an ELISA value of less than 2 standard deviations above control antigen.
[e] NT, Not tested.

TABLE 3. *Comparison of cytopathic effect (CPE) and ELISA for detection of coxsackievirus A antigen in African green monkey kidney cells*

Coxsackievirus A serotype	Test result on following day:									
	1		2		3		4		5[a]	
	CPE[b]	ELISA[c]	CPE	ELISA	CPE	ELISA	CPE	ELISA	CPE	ELISA
1	–[d]	–	–	–	–	NT[e]	–	–	–	16
2	–	–	–	–	–	NT	–	55	–	42
3	–	–	–	–	–	NT	–	–	–	–
4	–	–	–	–	–	NT	–	26	–	24
5	–	–	–	–	–	NT	1	26	1	41
6	–	–	–	–	–	NT	1	122	1	27
7	–	–	–	14	1	NT	2	650	4	539
8	–	–	–	–	–	NT	–	40	–	–
9	1	88	3	508	3	NT	4	1,577	4	1,524
10	–	–	–	–	–	NT	–	–	–	12
11	–	–	–	–	–	NT	–	44	–	21
12	–	–	–	–	–	NT	–	21	–	–
13	–	–	–	–	–	NT	–	–	–	–
14	–	–	–	–	–	NT	–	–	–	44
15	–	–	–	–	–	NT	–	–	–	–
16	–	–	–	–	–	NT	–	–	–	–
17	–	–	–	–	–	NT	–	58	–	–
18	–	–	–	–	–	NT	–	–	–	–
19	–	–	–	–	–	NT	–	17	–	–
20	–	–	–	–	–	NT	–	–	–	–
21	–	–	–	–	–	NT	–	–	–	105
22	–	–	–	–	–	NT	–	–	–	–
24	–	–	–	–	–	NT	–	–	–	–
Control	–	–	–	–	–	–	–	–	–	–

[a] Cells could not be read for CPE after day 5 due to nonspecific degeneration of the cells.
[b] Number of positive seed virus preparations.
[c] Absorbance units (optical density × 1,000). Values shown are the means of duplicate determinations.
[d] –, Negative for CPE, or an ELISA value of less than 2 standard deviations above the control antigen.
[e] NT, Not tested.

human embryonic fibroblasts, ELISA activity was detected before cytopathic effect in the case of 13 of the seed virus preparations, whereas cytopathic effect was observed before ELISA positivity in 4. In three of these four instances, the ELISA became positive within 24 h of the first observed cytopathic effect. For six of the seed virus preparations, ELISA positivity and cytopathic effect were attained simultaneously. In the case of the African green monkey kidney cell lines, ELISA activity was noted before cytopathic effect in the case of 12 of the seed virus preparations and simultaneous with cytopathic effect in 3. For eight serotypes, neither cytopathic effect nor ELISA activity was noted in this cell line during the study period. The tissue culture experiments could not be conducted for more than 7 days due to nonspecific degeneration of the cell lines. During the study period, 11 of the 23 seed virus preparations failed to exhibit visible cytopathic effect in human embryonic fibroblast cells, and 19 failed to exhibit visible cytopathic effect in African green monkey kidney cells. On the other hand, only one seed virus specimen was negative by ELISA in human embryonic fibroblast cells, and eight seed virus specimens were negative after cultivation in African green monkey kidney cells (Tables 4 and 5).

Clinical specimens. A total of 11 stool specimens known to contain coxsackieviruses A were available for testing (Table 6). Eight of these specimens could be successfully identified by the ELISA system. In one instance, a specimen was positive for the correct serotype but was also positive for an additional serotype. In two cases, specimens from which coxsackievirus A was cultivated did not have significant reactivity in the ELISA system. Control stools containing Norwalk virus ($n = 2$), rotavirus ($n = 14$), echovirus ($n = 8$), coxsackievirus B ($n = 4$), or no identifiable virus ($n = 1$) failed to give significant ELISA-specific reactivities for any of the coxsackievirus A antisera.

DISCUSSION

Coxsackieviruses A are known to cause a number of different clinical syndromes (13). How-

TABLE 4. *Time needed for detection of coxsackievirus A antigen in tissue cultures by the appearance of cytopathic effect (CPE) and ELISA activity*

Coxsackievirus A antigen	Day of onset			
	CPE		ELISA	
	AGMK[a]	WI-38[b]	AGMK	WI-38
1	–[c]	–	5	5
2	–	4	4	4
3	–	–	–	4
4	–	–	4	4
5	4	–	4	4
6	4	4	4	4
7	3	2	2	1
8	–	–	4	5
9	1	2	1	2
10	–	4	5	4
11	–	–	4	5
12	–	–	4	4
13	–	4	–	4
14	–	4	5	–
15	–	3	–	6
16	–	–	–	6
17	–	4	4	6
18	–	3	–	4
19	–	–	4	4
20	–	4	–	4
21	–	5	5	4
22	–	–	–	5
24	–	–	–	5

[a] AGMK, African green monkey kidney cells.
[b] WI-38, Human embryo fibroblast cells.
[c] –, No CPE or ELISA reactivity.

TABLE 5. *Comparison of time of onset of cytopathic effect (CPE) and ELISA reactivity for coxsackieviruses A*

Order of appearance	No. of serotypes	
	WI-38[a]	AGMK[b]
ELISA before CPE	13	12
CPE before ELISA	4	0
Simultaneous ELISA and CPE	6	3
No ELISA or CPE	0	8

[a] WI-38, Human embryo fibroblast cells.
[b] AGMK, African green monkey kidney cells.

ever, diagnosis of infection due to coxsackievirus A is difficult in clinical situations due to the fact that many clinical isolates grow poorly in tissue culture systems (13, 14). Although most of the coxsackieviruses A have been adapted to growth in tissue culture systems such as human embryonic fibroblasts, this adaptation requires techniques not usually performed in the clinical laboratory (2). In addition, although the coxsackieviruses A are cultivable in infant mice, many laboratories do not have the facilities to routinely inoculate specimens in infant mice (1, 5). There is thus the need for a simple, rapid system for the detection of coxsackievirus A antigen.

ELISA systems have been developed for the detection and serotypic identification of a number of medically important viruses such as rotavirus (25, 26), hepatitis B virus (21), and herpes simplex virus (15). We thus devised a double-antibody ELISA system for the detection and identification of coxsackievirus A antigen utilizing readily available reagents. In all instances except for coxsackievirus A19, such ELISA systems were capable of detecting at least 0.01 complement fixation units of the homologous coxsackievirus A antigen and were thus at least 100-fold-more sensitive than the corresponding complement fixation assay. When heterologous and homologous antigens were tested, extensive cross-reactivity among the coxsackievirus A antigens was noted. However, quantitative analysis revealed that homologous reactions were all at least 0.10 optical density units greater than heterologous reactions. Thus, all of the antigens with the exception of A19 could be successfully serotyped by means of the ELISA system. The cross-reactivity of these coxsackievirus reagents has been noted previously and has led to the suggestion that these reagents are not ideal for viral identification systems other than viral neutralization assays (12, 14; M. G. Katze and R. L. Crowell, J. Gen. Virol., in press). In fact, variable amounts of cross-reactivity necessitated the use of a system for correcting for such cross-reactivity. This system consisted of determining the nonspecific reactivity between the monkey antisera used to coat the solid-phase and the mouse anti-mouse liquid-phase antibodies. In addition, the values obtained for clinical specimens had to be corrected to account for nonspecific interaction between the specimens and the immunoglobulins tested. These interactions, which have been described for other solid-phase systems (23, 24), are probably due to rheumatoid factor-like antiglobulins present in some stool specimens. However, the quantitative nature of the ELISA system allowed for the performance of these corrections, and the system could then be used to identify coxsackievirus A antigens. Similar quantitative analyses have been utilized for the successful identification of other viruses by the ELISA systems (15, 25).

One application of the ELISA system is the detection and identification of coxsackievirus A antigens in tissue culture systems. In the case of both human embryonic fibroblast cells and African green monkey kidney cells, the presence of coxsackievirus A antigen could be detected before the occurrence of visible cytopathic effect for a majority of the antigenic types. The fact

TABLE 6. *Reactivity of stool specimens in coxsackievirus A ELISA*

Coxsackievirus A serotype[a]	Optical density units for following antiserum[b]:																						ELISA identification[c]
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	24	
1	**0.44**[d]			0.16																				1
1	**0.38**			0.12																		0.14		1
1	**0.19**																							1
1	**0.31**																					0.11		1
2		**0.18**							0.14															2, 9
5					**0.10**																			5
5					**0.27**																			5
9																								Negative
10										**0.21**														10
16																**0.37**		0.11						16
24																								Negative

[a] Specimens were serotyped by mouse or tissue culture neutralization assays.

[b] Values are expressed as ELISA specific activities and are the means of duplicate determinations. Values of less than 0.10 (2 standard deviations) are not shown. Stools from patients with rotavirus (n = 14), Norwalk virus (n = 2), echovirus (n = 8), and coxsackievirus B (n = 4) gave values of less than 0.10 with all antisera.

[c] The ELISA serotype was assayed as described in the text.

[d] Homologous reactions are shown in boldface type.

that most of the ELISA evaluations were negative 24 h after the inoculation and washing of the tissue culture cells suggests that the positive ELISA reactions represent viral growth rather than residual inoculated antigen. It is of interest that coxsackievirus A19 could be detected in the tissue culture system in spite of the fact that the complement fixation antigen could not be detected. This discrepancy is probably due to the relative quantities of reactive antigen in the specimens tested. Concentrated antigens containing echoviruses, adenoviruses, reoviruses, or coxsackieviruses B were negative in the coxsackievirus A ELISA.

A number of immunological methods, including complement fixation (8), immunofluorescence (20), and hemagglutination inhibition (18) have been proposed to identify coxsackieviruses in tissue culture fluids. However, complement fixation lacks sensitivity, immunofluorescence requires the subjective microscopic reading of stained material, and hemagglutination inhibition is not applicable to the coxsackievirus serotypes which do not hemagglutinate (14). The ELISA system might thus provide a useful alternative to these systems for the detection and identification of coxsackieviruses A growing in tissue culture systems.

An additional application of the coxsackievirus A ELISA might be the direct detection of coxsackievirus A antigens in clinical specimens such as stools. The serotype of the infecting coxsackievirus A antigen could be identified in 8 of 11 specimens tested. In one additional instance, coxsackievirus A antigen was detectable but the serotype could not be unequivocally assigned. Stools containing other viruses such as echovirus, coxsackievirus B, rotavirus, and Norwalk virus were negative in the coxsackievirus A system. The number of specimens available for testing was small, and, thus, a larger number of specimens containing a wider variety of serotypes need to be tested before the true efficiency of the coxsackievirus ELISA can be determined. However, these experiments reveal that, under some circumstances, the ELISA system is capable of rapidly detecting coxsackievirus A antigens in clinical specimens.

Although the coxsackieviruses A have been reported to cause a number of different clinical syndromes, the relative unavailability of practical means of diagnosing these infections has limited research into the pathophysiology of coxsackievirus A infections. The ELISA system for the detection of coxsackievirus A antigen utilizes readily available reagents and does not require the use of sophisticated technology. However, the system could be improved by the availability of more specific reagents which would obviate the need for the quantitative correction system required with the use of the available antisera. The availability of a simple coxsackievirus A diagnostic system might increase the capability of the diagnostic laboratory to diagnose coxsackievirus A infections and might thus lead to an increase in the state of knowledge of the role of coxsackieviruses A in human diseases.

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