
Fall 1990

[Serologic Testing to Monitor for Viral and Mycoplasmal Infection](#)

Rodent viruses and mycoplasmas can cause overt disease and mortality. In addition, the scientific literature is replete with examples of how latent and unsuspected infections may complicate research. In fact, many indigenous viruses of rodents were first discovered as extraneous agents that contaminated biological materials and clouded the interpretation of experimental results.

In addition, viruses such as lymphocytic choriomeningitis virus (LCMV) and the hantaviruses, which cause asymptomatic infections in their natural rodent hosts, have been reported to cause mild to severe disease in humans. (*Herpesvirus simiae*, or monkey B virus, although not a rodent virus, is notable because infections with this agent, while asymptomatic in macaques, are frequently fatal in humans.)

In summary, research animals must be routinely monitored for viral and mycoplasmal infections not only to avoid wasting resources but to safeguard the health of laboratory workers.

Approaches for Diagnosing Infection: Why Serology is Employed for Routine Monitoring

Several approaches can be used for diagnosing viral and mycoplasmal infections: observation of animals for disease signs and pathologic changes; attempted isolation and identification of the pathogen itself by inoculation of a susceptible host, e.g., cell culture, embryonated eggs, or sucking mice; and testing for specific antibodies formed in response to infection.

During the active phase of viral infections, disease symptoms and pathologic changes may be observed. However, the observation of disease is an ineffective approach because natural viral infections of immunocompetent rodents are often asymptomatic. Furthermore, pathologic changes are frequently non-specific.

Virus isolation and identification are essential for studying infectious disease but not practical for routine health monitoring because they are complicated, time-consuming and expensive. Many different specimens are required for virus detection, and many host systems are needed for virus isolation. Field strains are often difficult to isolate, and some viruses will not grow at all in culture. Virus isolation and detection methods are also likely to yield false negative results in recovered animals.

By contrast, specific antibodies formed as part of the immune response have been shown to persist in the blood of rodents for many months. Serologic assays are rapid and specific when constructed and performed properly. Therefore, serologic testing for specific antibodies is the approach of choice for routine monitoring.

Figure 1, from a study by Parker and Reynolds (*Am. J. Epidem.* 88:112, 1968), shows data from an experiment in which mice were inoculated with Sendai virus. Saliva and lung were tested for the presence of infectious virus, and serum was collected and tested by the hemagglutination inhibition (HAI) method for virus specific antibodies. Sendai virus was isolated during the first two weeks after exposure, but virus-specific antibody could not be detected, as the mice had not yet mounted an antibody response. However, antibody titers became detectable at 12 days, and virus titers dropped precipitously. By 15 days post inoculation, virus was no longer detectable, while antibody titers continued to rise. Testing at this time for Sendai, using virus isolation, would yield false negative results. Serology testing during the active phase of infection would also yield false negative results.

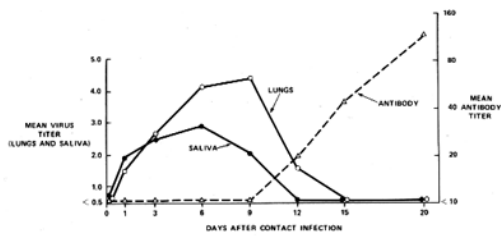


Figure 1. Sendai virus titers in the lungs and saliva of infected mice. Mean antibody titer is the reciprocal geometric mean HAI antibody titer of positive mice. Virus titers are mean titers of positive specimens expressed as negative log₁₀ TCID₅₀ per 0.05 ml. (From *Am. J. Epidemiol.*, 88:112, 1968, by permission.)

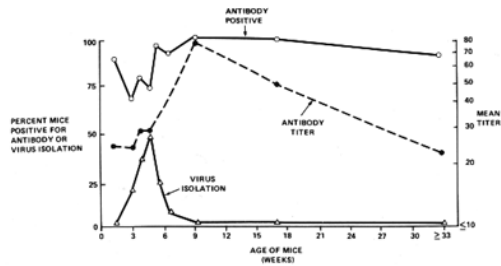


Figure 2. Sendai virus infection pattern in an enzootically infected mouse breeder colony. Mean titer is the reciprocal geometric mean HAI antibody titer of positive mice. (From *Am. J. Epidemiol.*, 88:112, 1968, by permission.)

The same study showed that in enzootically infected colonies, virus can readily be isolated only from mice 3-6 weeks of age (Figure 2). Prior to 3 weeks, mice are protected by maternal antibody. After 6 weeks, they have developed active immunity and have recovered from infection. By contrast, antibody persists and is detectable essentially throughout the life of the mice. Clearly, as active infection is short-lived while specific antibodies persist, Sendai virus is more likely to be detected in a colony by serology than by attempted virus isolation.

As with Sendai, active infection by sialodacryoadenitis virus (SDAV) and most of its pathologic changes (except for rare chronic eye lesions) are ephemeral, whereas specific antibodies persist at high levels essentially throughout the life of the animal.

Not all agents cause acute infections. Some cause persistent infections, e.g., the parvoviruses, MCMV, LCMV, Hantaan, LDV, and *Mycoplasma pulmonis*, the cause of murine respiratory and genital mycoplasmosis. Nonetheless, except for lactate dehydrogenase-elevating virus (LDV), serology is most often used to screen for these agents.

In a Charles River study (Table 1), we infected mice and rats with *M. pulmonis* and collected sequential samples for mycoplasma isolation on cell-free medium and serologic testing by the indirect fluorescent antibody test (IFA), the enzyme-linked immunosorbent assay (ELISA), and the Murine ImmunoComb® modified ELISA (described later in this document). After inoculation, mycoplasma could be isolated at all sample times, demonstrating the persistent nature of *M. pulmonis* infections. By 14 and 16 days, antibody was detected by all test methods used. Though infection was detected sooner by cultural isolation, detection of specific antibodies is more often used because it is easier, less costly and more specific.

Table 1. Experimental Infection of Rodents with *M. pulmonis*

SPECIES (EXP)	DPI	ISOLATION	SCORE*		
			IMMUNOCOMB	ELISA	IFA
Mouse (V111)	0	-	0	0	198
	7	+	0	0	191
	11	+	+/-	1	239
	14	+	1	4	342
	20	+	2	NT	621

	28	+	3	11	1041
Rat (V106)	0	0	NT	0	133
	9	+	0	0	195
	16	+	1	4	482
	23	+	2	11	969
	43	+	2	15	1571

* ImmunoComb scores: 0 to +/- = negative; 1-3 = low to strong positive. ELISA scores: 0 to 1 = negative; 2 = equivocal; >= 3 = positive. IFA scores (Daryl Track X1 system): < 200 = negative; 200 to 299 = equivocal; >= 300 = positive.

However, serology is not always the method of choice. It is not appropriate for agents for which antigen of specific purity and potency cannot be prepared. It may not be appropriate when the antigen is very complex and likely to cross-react with other microorganisms, causing the assay to be non-specific. As this is the case for bacteria, diagnostic laboratories often use cultural isolation, rather than serology, to detect bacteria. (The complexity of bacterial antigens is underscored by comparing them to viruses. Viruses consist of 5-100 proteins. By comparison, *E. coli* has genetic material sufficient to code for 5000 proteins.) Finally, serology is of little use when an agent does not elicit detectable levels of antibodies.

LDV is a virus for which serology is not appropriate. Specific antibodies are formed in response to LDV infection, but they do not neutralize the virus. Consequently, an antigen excess exists and because LDV antibodies are complexed to virus, they are very difficult to demonstrate by serology. In bacterial infections, in addition to problems of antigen complexity and cross-reactions, serology is often ineffective because specific antibody levels are low. For example, when attempting to eliminate the *Bordetella bronchiseptica* carrier state in a Charles River colony of guinea pigs, we were able to isolate *B. bronchiseptica* from the trachea and nares of most guinea pigs prior to vaccination. On the other hand, microagglutination antibody titers were insignificant. Following vaccination, titers reached high levels but, unfortunately, the carrier state was not eliminated.

Serologic Test Methods with Emphasis on Non-Radioisotopic Solid Phase Immunoassays

Virus and mycoplasma specific antibodies are detected in rodents, as in other species, by various assay methods. These include conventional or traditional tests such as complement fixation (CF) and hemagglutination inhibition (HAI), as well as non-radioisotopic solid phase immunoassays, notably, the enzyme-linked immunosorbent assay (ELISA) and the indirect fluorescent antibody test (IFA). Understanding these methods is simplified by considering their common aspects:

All employ antigen of defined purity and potency to test for specific antibodies. All begin with a test serum-antigen incubation. (In the CF and HAI tests, the antigen is in solution. In the ELISA and IFA, antigen is attached to a surface called a "solid phase.") All utilize indicator systems to demonstrate whether antigen-antibody reactions have occurred, and controls to attempt to distinguish between specific and non-specific reactions. Finally, the CF, HAI and ELISA are all usually performed in plastic 96-well microplates.

Complement Fixation (CF). *Figure 3* provides a schematic representation of the CF test. Broadly applicable, it can be used to test for antibodies to most infectious agents, but it is time consuming and not very sensitive.

As noted above, the CF test begins by incubating test serum with antigen of defined purity and potency. Typically, serial dilutions of the serum are tested. Test sera are heat-inactivated to destroy endogenous complement activity. Guinea pig serum is added as a source of exogenous complement. If a serum contains specific antibodies, antigen-antibody complexes form and fix complement. If the serum is non-immune, complement is not fixed. The amount of free (unfixed) complement is measured with an indicator system consisting of antibody-coated sheep red blood cells. If the serum is non-immune, complement remains free and hemolysis is observed. If serum contains specific antibodies and complement is fixed, hemolysis does not occur (*Table 6*).

In the CF test, serum samples are serially diluted in triplicate. The first dilution is tested against antigen; the second is tested against a tissue control. The tissue control does not contain virus. It is usually prepared from the host system in which the virus is propagated. For example, we prepare Sendai virus antigen from allantoic fluid collected from infected embryonated eggs. The tissue control for Sendai virus is allantoic fluid collected from uninfected eggs. In the case of *M. pulmonis*, however, the tissue control is another cross-reacting rodent mycoplasma, *M. arthritidis*. A third serial dilution, incubated without antigen or tissue control, detects anticomplement activity in the serum sample (*Table 2*).

A negative result is recorded when the serum does not fix complement in the presence of antigen or the controls. A titer is recorded when complement fixation, indicated by the absence of hemolysis, is observed in the serum-antigen wells but not in the controls. A titer is the reciprocal of the highest serum dilution to fix complement in the presence of antigen. For a serum to be considered antibody positive, the titer must be equal to or greater than the minimum significant titer for that test.

A specimen may fix complement because it reacts with the tissue in which the antigen is propagated. Such reactions are detected in the tissue control wells and recorded as "TC." A non-immune specimen should not fix complement. However, it may non-specifically activate complement because it contains immune complexes or is contaminated with bacteria. Activation of complement by serum alone is detected in the anticomplementary control and recorded as "AC." TC and AC results are considered non-specific and do not indicate whether a sample contains antibodies. TC or AC reactivity may be reduced by absorbing the serum with the tissue control or guinea pig serum, respectively. Alternatively, we prefer to retest samples by a different serologic method or to test new samples.

The concentration and activity of reagents in the CF test are crucial. Too much complement can reduce the sensitivity of the assay, whereas too little can increase the non-specific reactions.

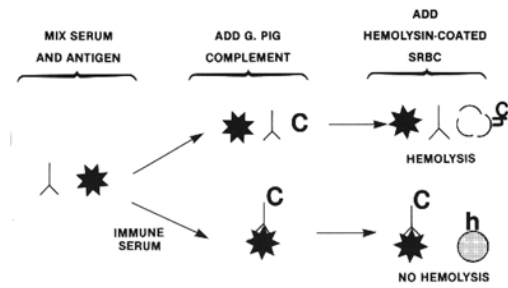


Figure 3. Complement Fixation Test (CF)

Table 2. CF Reaction Interpretation

REACTION			
ANTIGEN	TISSUE	ANTI-COMP	RESULT
NEGATIVE	NEGATIVE	NEGATIVE	-
POSITIVE	NEGATIVE	NEGATIVE	TITER*
POSITIVE	POSITIVE	NEGATIVE	TC
POSITIVE	POSITIVE	POSITIVE	AC

* A titer is the highest dilution of specimen incubated with antigen that shows less than 50% hemolysis. For a specimen to be considered antibody positive, the titer must be significant.

Hemagglutination Inhibition (HAI). The HAI test, represented in Figure 4, is predicated on the ability of specific antibodies to inhibit virus-mediated hemagglutination. Its principal limitation is that some viruses do not agglutinate red cells. Antibodies to these viruses cannot be measured by HAI.

To demonstrate HAI antibodies, serial dilutions of a specimen are incubated with antigen in V-bottom microplate wells. A suspension of red blood cells is then added. The species of blood cells and the incubation temperature vary according to the virus. If the specimen contains antibodies to the viral hemagglutinin, these will coat the virus and prevent it from agglutinating the red blood cells. Unagglutinated red blood cells appear in the well bottom as a button that streams when the plate is tilted. Conversely, if the sample is HAI antibody-negative, red cells do not stream but instead blanket the well bottom, indicating that hemagglutination has occurred (Table 6). In the HAI test, each serum sample is titrated in duplicate. One dilution is incubated with virus, the other without (Table 3). If hemagglutination is detected in the virus wells but not in control wells, the sample is considered to be HAI antibody-negative. If hemagglutination is inhibited in the virus wells, and the control wells show no evidence of hemagglutination, the result is recorded as a titer.

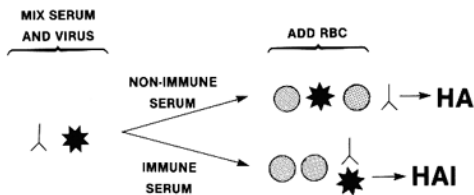


Figure 4. Hemagglutination Inhibition Test (HAI)

Table 3. HAI Reaction Interpretation

AGGLUTINATION		
ANTIGEN	CONTROL	RESULT
POSITIVE	NEGATIVE	-
NEGATIVE	NEGATIVE	TITER
POSITIVE	POSITIVE	AGG

Significant titers can also be caused by non-specific inhibitors of hemagglutination. These are usually removed from sera by treatments including heat inactivation to destroy heat-labile inhibitors; digestion of mucoprotein inhibitors with *Vibrio cholerae* neuraminidase (receptor-destroying enzyme) and kaolin absorption of lipoproteins.

If hemagglutination is found in the control wells, the sample contains serum agglutinins that mask the presence of specific antibodies. We record such a reaction as AGG. Samples that contain non-specific hemagglutinins can be absorbed with red blood cells to attempt to remove this activity.

Precise titration of antigen prior to use is crucial. Too many hemagglutination units decrease HAI antibody titers; too few units can increase the incidence of positive results due to nonspecific inhibitors of hemagglutination.

Enzyme-Linked Immunosorbent Assay (ELISA). The indirect ELISA is the ELISA method most often used for detection of antibodies (Figure 5). Typically, antigen is immobilized on the surface of wells in microplates made of specially prepared polystyrene or polyvinyl. Test specimens are incubated in the well to which antibodies may bind. Unbound antibodies are removed by washing. Those that attach are demonstrated by incubating first with an enzyme-conjugated anti-immunoglobulin, and then (following a wash to remove unbound conjugate) with a chromogenic enzyme substrate. A colored product develops at a rate proportional to the amount of antibodies from the specimen that have attached to a well. (See Table 6 for positive and negative reaction.) Color intensity can be assessed visually or spectrophotometrically (in absorbance units) with an ELISA reader.

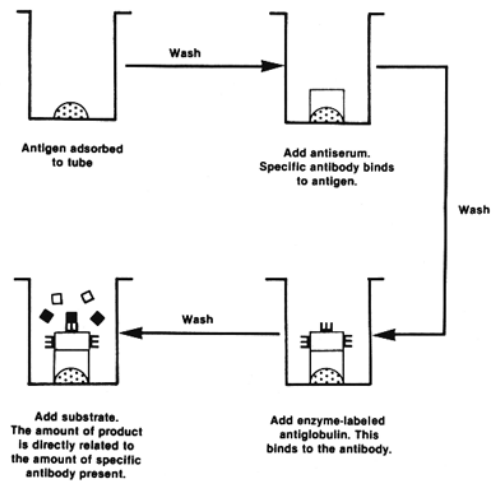


Figure 5. Indirect Enzyme-Linked Immunosorbent Assay (ELISA)

Table 4. ELISA and IFA Reaction Interpretation

REACTION		
ANTIGEN	TISSUE	RESULT
NEGATIVE	NEGATIVE	-
POSITIVE	NEGATIVE	+
POSITIVE	POSITIVE	TC

Ideally, attached antibodies are antigen-specific. In practice, however, they may bind non-specifically. We incubate each sample in a tissue control well to detect non-specific binding (*Table 4*). When a color reaction occurs in the tissue control well in addition to the antigen well, the result is recorded as TC. As in the CF test, a TC result in the ELISA is considered non-specific and does not indicate whether a sample is antibody positive or negative. In our experience, tissue control reactivity can be reduced by absorption with kaolin or by ultracentrifugation. However, rather than using these treatments, we usually retest the sample by an alternate method. If necessary, we test additional samples.

A result is recorded as positive if there is a positive reaction in the antigen well but little or no color development in the tissue control well. Little or no color development in either the antigen or control well is recorded as a negative result.

We routinely evaluate the specificity of our ELISAs by assaying standard immune and non-immune sera. The results of such a check for the Sendai and MHV ELISAs are shown in *Table 5*. In the Sendai ELISA, only mouse and rat Sendai antisera give positive reactions. Reactions with heterologous immune and non-immune sera are negative. In the MHV ELISA, mouse MHV antiserum reacts with MHV, but so does rat SDAV antiserum. This cross-reaction is expected and acceptable since both MHV and SDAV are rodent coronaviruses that share antigens.

Table 5. Quality Control of CRL ELISA Antigen Purity Assay

SPECIES	STANDARD SERUM SPECIF. (LOT)	AG (LOT):	NET SCORE*	
			SENDAI (1)	MHV (ORGMH3)
Mouse	SEND (S219)		11	0
	MHV (S222)		0	9
	REO-3 (S210)		0	0
	GD-7 (S227)		0	0
	MVM (S231)		0	0
	MAD (S233)		0	0
	LCMV (S262)		0	1
	NON-IMMUNE (S241)		0	0
Rat	SEND (S220)		10	0
	SDAV (S232)		0	6
	REO-3 (S211)		0	0
	PVM (S213)		0	0
	KRV (S223)		0	0
	MPUL (S174)		0	0
	NON-IMMUNE (S239)		0	0

* NET SCORE = (Abs 405nm ANTIGEN-TC) / 0.13

NET SCORE 0,1 = negative; 2 = equivocal; >= 3 = positive

SENDAI ELISA 03/25/87; MHV ELISA 05/01/87

Indirect Fluorescent Antibody Test (IFA). The steps of the IFA are very similar to those of the ELISA (*Figure 6*). Virus-infected cells and uninfected cells are fixed to wells in a glass slide. The fixative is usually cold acetone, which permeabilizes the cell membrane, making the intracellular viral antigens more accessible to antibodies. As with the ELISA, unbound antibodies are removed by washing. Instead of the enzyme conjugate and substrate indicator system used in the ELISA, the binding of primary antibodies to the solid phase is demonstrated in the IFA with a fluorescent dye-conjugated anti-immunoglobulin. After washing to remove unbound conjugate, slides are covered with buffered glycerol and examined with a fluorescence microscope.

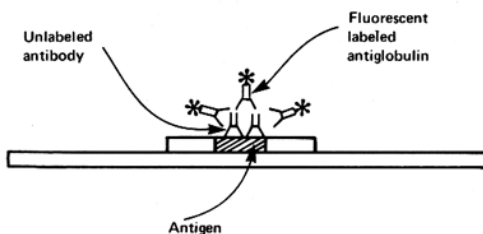
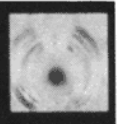
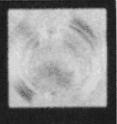

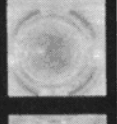
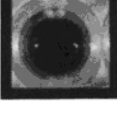
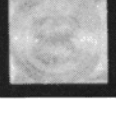


Figure 6. Indirect Fluorescent Antibody Test (IFA)

The expense of these microscopes can be a significant barrier to doing IFA. On the other hand, they can be found in most microbiology laboratories. Fluorescence microscopes have a light source with an exciter filter to exclude all but the appropriate wavelengths and a reflector/barrier filter combination to reflect the light onto the slide, so fluorescence may be observed.

Bright, granular fluorescence is typical of an antibody-virus reaction, whereas diffuse fluorescence indicates non-specific reactions. The location of fluorescence is also an important factor. In the case of certain DNA viruses, such as the rodent parvoviruses (MVM, KRV, and H-I), strong nuclear fluorescence is characteristic. The ability of IFA to include evaluation of fluorescence morphology and location in the interpretation of reactions is its major advantage over other serologic methods.

Table 6. Appearance of Reactions in Various Serologic Tests

TEST ^a	REACTION ^b	
	POSITIVE	NEGATIVE
CF	 NO LYSIS	 HEMOLYSIS
HAI	 NO HA	 HA
ELISA	 COLOR	 NO COLOR

^aCF = complement fixation; HAI = hemagglutination inhibition;
ELISA = enzyme-linked immunosorbent assay.

^bHA = hemagglutination

As noted previously, all serologic test methods employ antigen of defined purity and potency. The steps for preparing antigen at Charles River include propagating the agents used to make the antigens, which are for the most part viruses; preparing viral seeds and stocks of the cells in which the viruses are grown; scaling up cell culture for production of ELISA antigen. Scale-up is followed by antigen purification and inactivation and, finally, quality control testing.

Viruses are obligate intracellular parasites and hence must be grown in a susceptible host, such as mice, embryonated eggs, or cell culture. MTLV and K are examples of rodent viruses that do not grow well in culture and must be grown in mice. Although Sendai virus will grow in the monkey kidney cell line LLC-MK2, we produce ELISA and HAI antigen in eggs where this virus grows to much higher titers.

We grow most viruses in cell culture, usually in immortal cell lines from a variety of species. However, some viruses (for example, the FL strain of mouse adenovirus, as well as SDAV, MCMV and polyoma) will grow only in primary embryo fibroblast or kidney cell cultures.

Cultures are handled under a stream of HEPA-filtered air in biological safety cabinets. This prevents their contamination by extraneous agents and also protects the technician and surrounding environment from viruses that may be in the cultures. Zoonotic agents including LCMV, Hantaan virus and *Herpesvirus simiae* (monkey B virus) are propagated in a Biosafety Level 3 laboratory. BL 3 practices apply to work done with "indigenous or exotic agents where the potential for infection by aerosols is real and the disease may have serious or lethal consequences" (*Biosafety in Microbiology and Biomedical Laboratories, CDC-NIH, 1984*).

Most cells that we use are attachment-dependent. That is, they grow attached to the culture vessel surface in sheets called monolayers. These cells can be removed from the surface by treatment with a saline solution containing trypsin and EDTA. Virus infection of cells can cause morphologic changes referred to as cytopathic effects (CPE). Examples of CPE are cell rounding and sloughing and syncytia (i.e., giant cell formation). Infected cultures are examined for CPE with an inverted microscope.

In general, cultures are incubated at 35-37 degrees Celsius in an atmosphere of 5 percent CO₂ in humidified air in an incubator that controls temperature, humidity and CO₂. The CO₂ acts as a buffer to control the pH of the cell culture medium, especially to keep the pH from getting too basic when the cell concentration is low.

Comparison of Solid Phase to Conventional Serologic Methods

Non-radioisotopic solid phase immunoassays are enjoying widespread use because, compared to conventional tests, they are simpler to perform and more sensitive. Both performance of the assays and reading of results are amenable to automation.

Unique solid phases simplify performance of assays. The simplicity of performance is derived, in part, from some unique solid phases and assay formats that are available. Formats that have been available in kit form for rodent serology include the Daryl Track XI quantitative IFA system and the Organon Teknika Enzabead System.

Several years ago, Charles River began collaboration with Orgenics of Yavne, Israel, toward the development of a modified ELISA kit: the Murine ImmunoComb® for detection of mouse or rat antibodies to *Mycoplasma pulmonis*, rodent coronavirus (including MHV, SDAV, and RCV) and Sendai virus. The agents included in this test kit were chosen because they are widespread and commonly associated with adventitious infections. They seriously impair the usefulness of infected mice and rats. Finally, these agents elicit strong, persistent, and specific antibody responses that can be demonstrated serologically.

The ImmunoComb derives its trade name from the unique, comb-shaped solid phase developed by Orgenics (Figure 7). The comb has 12 tines or teeth. Each tine is coated with three different antigens, allowing each sample to be simultaneously assayed for antibodies to the three different agents. Performance of the assay is facilitated by a developing plate divided into compartments pre-filled with all the solutions needed for the test. The assay results are developed by incubating the comb in consecutive compartments of the developing plate, following the steps of the indirect ELISA. Washing is done with tap water. A positive result is indicated by the development of a colored dot (equal to or greater than the corresponding positive control) at the site of antigen application. The color intensity can be scored on a scale of 0-3 by comparison with the CombKey, a color scale supplied with the kit.

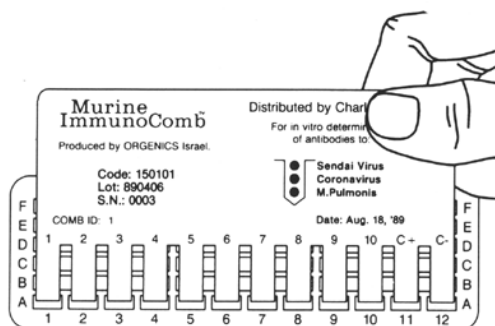
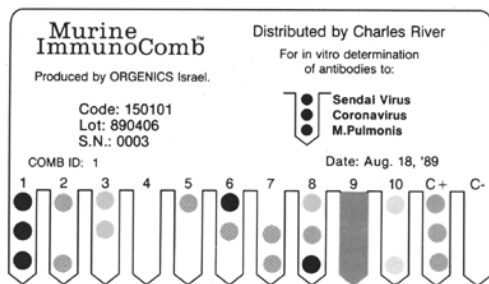


Figure 7. Above, insertion of ImmunoComb tines into wells of compartment A on developing plate. Below, example of a developed comb.



Solid phase assays are exquisitely sensitive. Solid-phase assays are generally more sensitive than conventional tests. Sensitivity can be defined as the smallest amount of antibody that can be detected (i.e., titer) or the number of animals found to be antibody

positive over the number affected.

Data from a study by Parker et al. (*J. Clin. Micro.* 9:444, 1979) on the antibody response of mice to Sendai virus show ELISA titers at various times after virus inoculation to be consistently and substantially higher than those obtained by HAI and CF (*Figure 8*). In the same study, the ELISA was shown to be somewhat more sensitive than the HAI and CF tests for identifying antibody-positive individuals in a Sendai-infected colonies. In particular, of the 20 animals tested in one colony, none were positive by HAI and only four were positive by CF, while all were antibody-positive by ELISA.

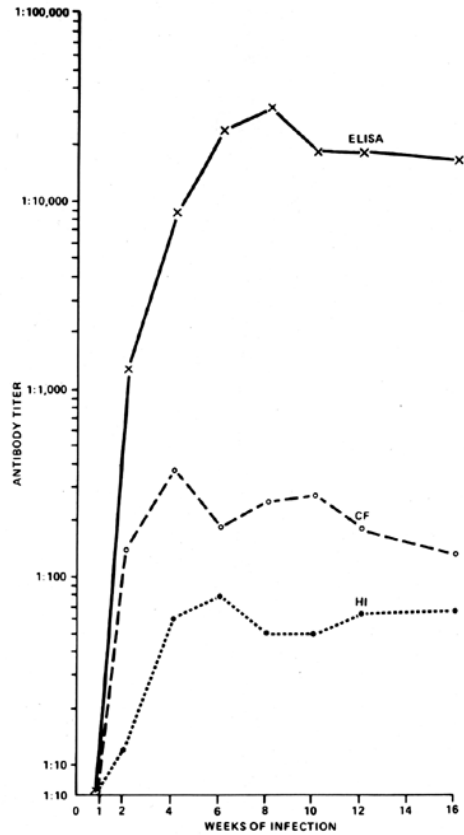


Figure 8. ELISA, complement fixation (CF), and hemagglutination inhibition (HAI) antibody responses in Swiss mice inoculated with ten 50% tissue culture-infective doses of Sendai virus. (From Parker et al., *J. Clin. Micro.* 9:444, 1979, by permission.)

In our unsuccessful attempt to eliminate the *B. bronchiseptica* carrier state in guinea pigs by vaccination, we were unable to demonstrate *B. bronchiseptica* antibodies by microagglutination in guinea pigs found to be infected with *Bordetella* by cultural isolation. By contrast, in a recent study, Manning and coworkers (*Lab. Anim. Sci.* 37:615, 1987) demonstrated that serology could be used to identify rabbits infected with *Bordetella* and also *Pasteurella multocida*. Their results show how the extreme sensitivity of ELISA and other non-radioisotopic immunoassays has broadened the applicability of serology for diagnosing infection.

The CRL ELISA is automated. Our ELISA protocol utilizes programmable equipment for more efficient, accurate, and reproducible sample processing. In addition, our data processing department has developed custom computer programs for accessioning and tracking test specimens, recording and processing results, and generating reports.

Sample transfer and dilution are a key part of any serologic test, irrespective of the method. Precision and accuracy are reduced when the amount of sample is transferred or when the sample is transferred to the wrong location. We have reduced the tedium involved in sample transfer, and the likelihood of errors, by using programmable pipettes to do preliminary sample transfer and dilutions. We have also simplified the transfer of diluted samples to antigen-coated test plates by using 96-well transfer devices.

Table 7. Charles River Serology Assays

AGENT	(ABBREV)	METHOD		
		PRIMARY	ALTERNATE	SPECIES
Sendai virus	(SEN)	ELISA	HAI, IFA	M, R, GP, H
Simian virus 5	(SV5)	ELISA	HAI, IFA	GP, H
Pneumonia virus of mice	(PVM)	ELISA	HAI, IFA	M, R, GP, H
Mouse hepatitis virus	(MHV)	ELISA	IFA	M
Rat coronavirus/Sialodacryodenitis virus	(RCV/SDA)	ELISA	IFA	R
Minute virus of mice	(MVM)	ELISA	HAI, IFA	M
Kilham rat virus	(KRV)	ELISA	HAI, IFA	R
Toolan's H-1 virus	(H-1)	ELISA	HAI, IFA	R
Mouse polio virus	(GD-7)	ELISA	IFA	M, R
Reovirus type 3	(REO-3)	ELISA	HAI, IFA	M, R, GP, H
Lymphocytic choriomeningitis virus	(LCMV)	ELISA	IFA	M, R, GP, H
Hantaan virus	(HANT)	IFA	NONE	M, R
Ectromelia virus	(ECTRO)	ELISA	HAI, IFA	M
Mouse adenovirus FL/K87	(MAD)	ELISA	IFA	M, R
Mouse pneumonitis virus	(K)	ELISA	HAI	M
Polyoma virus	(POLY)	ELISA	HAI	M
Epizootic diarrhea of infant mice virus	(EDIM)	ELISA	IFA	M
Mouse cytomegalovirus	(MCMV)	ELISA	IFA	M
Mouse thymic virus	(MTLV)	ELISA	IFA	M
Herpesvirus simiae	(B VIRUS)	IFA	NONE	MK
Lactate dehydrogenase-elevating virus	(LDV)	ENZYME	NONE	M
<i>Mycoplasma pulmonis</i>	(MPUL)	ELISA	IFA	M, R
<i>Encephalitozoon cuniculi</i>	(ECUN)	ELISA	IFA	M, R, GP, H, RB

METHODS: ELISA = Enzyme-linked immunosorbent assay; IFA = Indirect fluorescent antibody test; HAI = Hemagglutination inhibition test; and ENZYME = Assay of serum for lactate dehydrogenase activity.

SPECIES: M=Mouse, R=Rat, GP=Guinea Pig, H=Hamster, RB=Rabbit, MK=Monkey.

Washing and dispensing steps are crucial in the ELISA, as they are in any solid phase immunoassay, so we use a programmable washer/dispenser. It has a feeder and stacker so that technicians need not add and remove plates individually. Similarly, to eliminate the tedium of feeding plates one at a time, we use an ELISA plate reader with a feeder and stacker.

Serum specimens are received accompanied by an Accession Form that includes the name and address of the person to be given the results and information on each specimen, including a unique identification code, species of origin, dilution and treatment(s) such as heat inactivation, and the test or profile of tests to be performed. Specimens are arranged in groups by customer and species. Each group is assigned by computer an 8-digit accession number in the Order Entry Maintenance screen of the Laboratory Accession Management System (LAMS). The first two digits of the number indicate the year, while remaining numbers identify the group (e.g., 90-001234). The customer address, service(s) requested and other pertinent information (listed above) are entered in the Data Maintenance screen. The Data Maintenance information is used by LAMS to generate a Serology Test Results Maintenance screen for entering results. The results may be manually entered or automatically uploaded from the Charles River ELISA program described in the next paragraph.

As manual processing of results is laborious and filled with potential for mistakes, we have minimized the likelihood of errors by computerizing the process. ELISA absorbance values are sent from the ELISA reader to a personal computer. The data is collated by a program that converts the absorbance values to integers by dividing them by 0.13. Based on experience, we consider a net score (i.e., the antigen score minus the tissue control score) of 3 or higher to be a positive result. A net score of 2 is equivocal, and 0-1 are considered negative. If there is reactivity in the tissue control well with a score of 2 or more, the result is TC.

The assays we perform are shown in Table 7. Because of the advantages just described, we try to use the ELISA as often as possible for primary assays. However, since no assay method always gives accurate results, we maintain the capability of testing for antibodies to most agents by alternate methods.

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