# Assay of Antibodies to Caliciviruses by Radioimmune Precipitation Using Staphylococcal Protein A as IgG Adsorbent

By

M. E. Soergel, F. L. Schaffer, J. C. Sawyer[1], and C. M. Prato

Naval Biosciences Laboratory, University of California, School of Public Health, Berkeley, California, U.S.A.

With 3 Figures

## Summary

A radioimmune assay method designated St-RIP using a staphylococcal IgG adsorbent, which potentially has broad applications to viral (and nonviral) antigen-antibody systems, was applied to detection of calicivirus antibodies. Purified $^{125}$I-labeled virions of San Miguel sea lion virus serotypes 4 (SMSV-4) and 5 (SMSV-5) were incubated with sera; the immune complexes were reacted with an immunoadsorbent, formaldehyde-fixed staphylococci (Staphylococcus aureus protein A producer, strain Cowan I), and collected by centrifugation. Broad cross-reactivity was observed among serotypes of SMSV and vesicular exanthema of swine virus (VESV), but there was no reaction with antisera to six noncaliciviruses. Antibody production in a rabbit inoculated with SMSV-5 polypeptide was monitored by St-RIP assay; reactivity with intact SMSV-4 virion antigen was slightly less than, but closely paralleled, reactivity with SMSV-5 virion antigen. Applicability of the St-RIP test to serologic survey was demonstrated with pinniped, swine, and human (laboratory personnel) sera; numerous positive St-RIP reactions suggested the occurrence of widespread contacts with caliciviruses.

## Introduction

A number of radioimmunoassay (RIA) procedures have been developed which assay minute amounts of a variety of substances, including certain viral antigens and antibodies. Technical hurdles include preparation of antigen or antibody radiolabeled to high specific activity, and an optimum technique for separation of labeled antigen-antibody complexes from unbound labeled reagent. One approach to detection of viral antibodies is radioimmune precipitation (RIP),

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[1] Veterinary Services, APHIS, U.S. Department of Agriculture.

which employs radiolabeled antigen and, usually, a second antibody. In 1962, Gerloff et al. (2) found RIP to be a rapid and sensitive method for assay of poliovirus antibodies, and RIP has subsequently been used in various research applications. We have developed an advantageous modification of conventional RIP for assay of viral antibodies, using staphylococcal protein A as an immunoadsorbent.

Staphylococcal protein A binds to immunoglobulin molecules of the IgG class, but not to IgM or IgA; specificity is attributed to the Fc region of the H chain (6). This binding is exhibited by IgG of most mammalian species (6), though the proportion of immunoglobulins bound varies among species (5). Recently, protein A-bearing Staphylococcus aureus has been utilized to isolate antigens from cells (5), and in quantitation of α-fetoprotein in serum (4). Our RIP assay, designated St-RIP, also employs protein A-bearing Staphylococcus aureus as an IgG-binding reagent. The bacteria, substituting for the second antibody used in conventional RIP systems, acts as an immunoadsorbent, rapidly binding IgG; efficient separation of antibody-bound antigen from free antigen is then achieved by centrifugation. In this paper we substantiate the usefulness of St-RIP for sensitive and rapid detection of minute levels of viral antibodies.

Investigations in this laboratory have been concerned with isolation, characterization and epizootiology of several serotypes of a calicivirus of marine mammals, San Miguel sea lion virus (SMSV), and its relationship to a virus of a terrestrial mammal, vesicular exanthema of swine virus (VESV) (10, 11, 13—18). This report concerns establishment of the St-RIP procedure, its application to investigation of cross-reactivity among SMSV and VESV serotypes using specific antisera, and to serologic surveys.

## Materials and Methods

### Staphylococcal Immunoadsorbent

*Staphylococcus aureus* strain Cowan I was grown in Penassay broth, formalin-fixed, and heat-killed, essentially according to the method of Kessler (5). Quantitation of immunoadsorbing capacity of the staphylococcus preparations was measured by their ability to bind $^{125}I$-human gamma globulin; approximately 90 per cent of the radioactivity was precipitated. The immunoadsorbent was stored at 4° C. Prior to use, a portion was washed twice in Dulbecco's phosphate buffered saline (PBS), and resuspended to a 10 per cent (or 5 per cent) suspension in PBS. Tween-20, 0.5 per cent, was included to reduce nonspecific adsorption of antigen (4).

### Virus Antigen

The viruses used as precipitin antigens were SMSV-4 and SMSV-5 (16, 18). A preparation of SMSV-4 was purified, cross-linked with dimethyl suberimidate to preserve virion integrity, and labeled with $^{125}I$ essentially as described (13). A preparation of SMSV-5 was purified and labeled similarly, but without cross-linking. These viral antigens were stored at 4° C in glycerol, and were stable more than 7 months. Another SMSV-4 preparation used for some of the assays had been prepared for a long term stability study (13) and had been held at −70° C for nearly 1.5 years (approximately 8 half lives of $^{125}I$). Though the virions retained physical and antigenic integrity, specific activity was too low for practical use; relabeling with $^{125}I$ (to approximately the same initial specific activity) and rebanding by glycerol density gradient sedimentation, yielded a satisfactory preparation.

## Sera

Hyperimmune antisera to SMSV and VESV serotypes were prepared in rabbits by weekly (4—6 weeks) intravenous (i. v.) injections of 1 ml of cell culture-propagated virus. Additional antisera prepared in rabbits and laboratory swine were from earlier studies with VESV in this laboratory (8, 9, 19). One rabbit was immunized with denatured polypeptide (13) antigen prepared from purified SMSV-5 virions: virions in 1 per cent sodium dodecyl sulfate and 0.1 per cent 2-mercaptoethanol were heated in a boiling water bath for 2 minutes; equal amounts of antigen and $AlPO_4$, pH 5.0, were held at room temperature for 1 hour, and then inoculated intravenously (3); six 1 ml injections containing approximately 55 μg protein each were made over a 12-week period. Antisera to other viruses, from various sources, were available in our laboratory. Pinniped sera were from a study reported elsewhere (14). The field swine sera were from a more extensive survey for serum neutralizing antibodies to SMSV (J. C. Sawyer, manuscript in preparation). Human sera were from laboratory personnel. All sera were stored at $-20°$ C; some had been heated at 56° C for 30 minutes. Some sera had visible precipitates after extended frozen storage; it was necessary to centrifuge such sera prior to use.

## St-RIP Test

The procedure for the St-RIP assay was as follows: 10 μl of test serum at a dilution of 1:10 or greater was placed in a 1.5 ml microcentrifuge tube (12). Normal rabbit serum (1:10 in PBS) was used as diluent for dilutions of test serum greater than 1:10. Approximately 1000 cpm of $^{125}$I-SMSV in 250 μl PBS was added and mixed on a Vortex mixer. (Alternatively, for screening at the equivalent of a 1:10 dilution, 1 μl undiluted serum was added to 209 μl PBS followed by addition of 50 μl labeled antigen.) The quantity of antigen represented about 10 ng virus purified from 14 μl of original cell culture fluid. Samples were incubated at 37° C for approximately 1.5 hours, followed by the addition of 0.1 ml of 10 per cent staphylococcus preparation in 0.5 per cent Tween-20. (This quantity of immunoadsorbent was estimated to be approximately a 10-fold excess of that required to bind IgG in 10 μl of a 1:10 serum; subsequent experiments showed that smaller quantities gave identical results, and 5 per cent suspensions were then routinely used.) After mixing, and 5 minutes incubation at room temperature, samples were centrifuged in a Brinkman/Eppendorf centrifuge 3200 (Bio-Rad). Supernates were transferred to separate microcentrifuge tubes which were counted in a Beckman gamma-300 counter, as were the precipitates. Percent radioactivity for each precipitate was calculated; no correction was applied for the small volume of supernate remaining on the precipitate since controls with normal serum (included in each set of assays) had comparable minute amounts of residual supernate. Preliminary experiments with specific rabbit antiserum showed no difference between heated and unheated serum.

## Neutralization Test

Titrations for neutralizing antibodies were done as previously described (10, 14).

# Results

### Evaluation of St-RIP Test With Homologous Hyperimmune SMSV Antisera

Results of two methods of radioimmune precipitation in microtubes using radioactive SMSV-4 virus with a homologous rabbit hyperimmune serum are presented in Figure 1. Precipitation using staphylococcal protein A as IgG adsorbent, was compared to the conventional RIP system (2) that uses a second antibody (goat anti-rabbit γ-globulin). Two different preparations of St-RIP immunoadsorbent yielded nearly identical results. At low dilutions of hyperimmune serum, the St-RIP method showed more complete precipitation of the

antigen than did the second antibody method, and it was approximately 3—5 fold more sensitive at the 50 per cent precipitation level. The difference between the two methods was less apparent at high dilutions of antibody. (The possibility that prolonged incubation with second antibody would increase the RIP titer was not tested.) The limit of detection of antibody was beyond the 1:312,500 dilution, if as suggested (2), positive detection of antibodies is defined as a 5 per cent increment of precipitation over that of the control serum base line.

A St-RIP curve similar to those in Figure 1 was obtained with SMSV-5 and homologous hyperimmune serum. This SMSV-5 serum had a lower St-RIP titer than did the SMSV-4 serum, in spite of similar homologous neutralization titers (Table 1). However, in both cases, St-RIP was considerably more sensitive than neutralization.
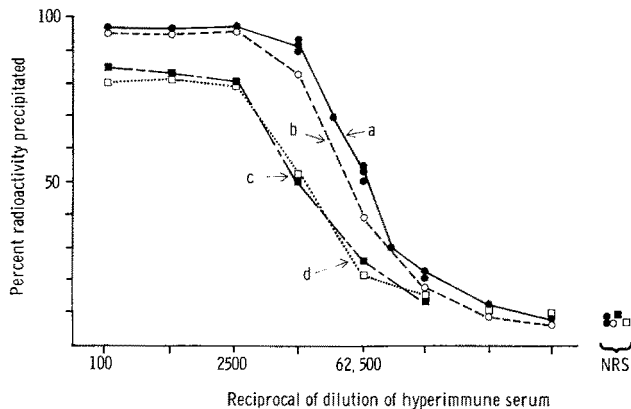


Fig. 1. Precipitation of ^{125}I-SMSV-4 virus with a homotypic rabbit hyperimmune serum. Quantitation of amount of antigen precipitated in St-RIP assay (a, b) was compared with RIP assays using second antibody precipitation (c, d). St-RIP was performed as described in text. Second antibody RIP was performed similarly except, in place of staphylococcal immunoadsorbent, 0.3 ml of 1:30 dilution of goat anti-rabbit γ-globulin (dilution selected from prior determinations to approximate conditions of equivalence) was added, followed by an additional two hours incubation. NRS indicates the baseline of nonspecific precipitation with the diluent, 1:10 normal rabbit serum (from different rabbits). Lines a and b represent two staphylococcal preparations in the St-RIP assays which employed a single SMSV-4 antigen preparation; the same antigen preparation was used in second antibody RIP, line d; a different SMSV-4 preparation (which gave a St-RIP curve virtually identical to line a) was used for c

## Specificity of SMSV St-RIP

St-RIP reactions of SMSV-4 and SMSV-5 intact virion antigens with hyper-immune rabbit sera prepared against the 4 previously characterized SMSV sero-types are shown in Table 1. There was considerable cross reactivity between SMSV-4 and SMSV-5. However, reciprocal cross-reactivities were not equivalent; the dilution giving 50 per cent St-RIP in the homologous reactions were 2600-fold and 79-fold greater than those for the heterologous reactions with these SMSV-4

and SMSV-5 antisera, respectively. There were considerably less cross-reactivities with SMSV-1 and SMSV-2 hyperimmune sera. The lack of cross-reactivity by neutralization was in accord with previous observations of specificity (16).

Table 1. *St-RIP assays for antibodies reacting with SMSV-4 and SMSV-5 in hyperimmune sera of four SMSV serotypes, and comparisons with neutralizing antibodies*

| | St-RIP[a] | | | | | | | | | |
| | SMSV-4 | | | SMSV-5 | | | | | | |
| Rabbit antisera[c] | %St-RIP at 1:10 dilution | Reciprocal of dilution at | | %St-RIP at 1:10 dilution | Reciprocal of dilution at | | Neutralization[b] SMSV Serotype | | | |
| | | 50% St-RIP | Detectable St-RIP | | 50% St-RIP | Detectable St-RIP | 4 | 5 | 1 | 2 |
| SMSV-1 | 25 | | | 21 | | | — | — | 640 | <32 |
| SMSV-2[d] | 18 | | | 26 | | | — | — | — | 5,120 |
| SMSV-4 | 97 | 62,500 | 312,500 | 65 | 24 | 1,000 | 226 | — | — | — |
| SMSV-5 | 88 | 51 | 100+ | 97 | 4,000 | 62,500 | — | 320 | <16 | <16 |

a Per cent St-RIP is the percentage of $^{125}$I-SMSV precipitated. 50 per cent St-RIP was estimated from plots of per cent St-RIP vs. log serum dilution. Detectable St-RIP is defined as the greatest serum dilution tested at which precipitation was >5 per cent above normal rabbit serum diluent control (controls were 4—9 per cent with SMSV-4 and 2—4 per cent with SMSV-5, depending upon individual rabbit serum used); + indicates precipitation >15 per cent above control. Blank spaces indicate test not done

b Neutralization of SMSV serotypes. Homotypic neutralization expressed as reciprocal of dilution; heterotypic neutralization assays were all negative at 1:10 dilution, indicated by —, or at dilutions representing 20 antibody units to homologous virus, 1:32 for SMSV-1 antisera or 1:16 for SMSV-5 antisera

c Serum from a single rabbit was used for each serotype

d St-RIP of pre-immune serum was 2 and 4 per cent with SMSV-4 and SMSV-5 antigen, respectively. Pre-immune samples were not available for other sera

To examine St-RIP specificity further, SMSV-4 and SMSV-5 labeled antigens were tested with various other calicivirus and noncalicivirus antisera (Table 2). No St-RIP reaction was found with noncalicivirus antisera, whereas the calicivirus antisera showed varying degress of reaction. Antiserum to a new SMSV isolate (temporarily designated 436), which by neutralization showed no relationship to 4 previous SMSV serotypes, cross-reacted with both SMSV-4 and SMSV-5 in St-RIP test. St-RIP assay of antisera to 9 VESV serotypes also showed cross-reactivities with SMSV-4 and SMSV-5, substantiated by negative St-RIP in matching pre-immunization sera (where available). Variations in St-RIP reactions among animals immunized with the same serotype may reflect differing immunization procedures, as well as differences among individuals. A dramatic increase in heterologous St-RIP titers was seen in the VESV-E pig from which multiple bleedings were available.

Table 2. *St-RIP assays for antibodies reacting with SMSV-4 and SMSV-5 in hyper-immune sera of caliciviruses and noncaliciviruses*

| Antisera | Species | St-RIP[a] | | | |
| | | SMSV-4 | | SMSV-5 | |
| | | Immune serum | Pre-immune serum | Immune serum | Pre-immune serum |
|---|---|---|---|---|---|
| **SMSV** | | | | | |
| New isolate | Rabbit | 43 | | 57 | |
| **VESV** | | | | | |
| Type A | Rabbit | 67 | | 11 | |
| | Rabbit[b] | 38 | | 65 | |
| | Swine[c] | 71 | | 73 | |
| Type C | Rabbit | 29 | | 43 | |
| Type D | Rabbit | 20 | | 14 | |
| | Swine | 38 | 9 | 22 | 5 |
| | Swine | 39 | 9 | 24 | 5 |
| Type E | Rabbit | 24 | | 25 | |
| | Swine[c] | | | | |
| | 1st bleed | 13 ⎫ | 4 | 17 ⎫ | 4 |
| | Final bleed | 79 ⎭ | | 78 ⎭ | |
| | Swine | 18 | | 22 | |
| Type F | Rabbit | 82 | | 80 | |
| | Swine | 40 | 8 | 18 | 6 |
| | Swine | 13 | 7 | 17 | 6 |
| Type G | Rabbit | 59 | | 63 | |
| | Swine | 21 | 6 | 17 | 3 |
| | Swine | 27 | 7 | 28 | 6 |
| Type I | Rabbit | 35 | | 29 | |
| Type J | Rabbit | 14 | | 13 | |
| Type K | Rabbit | 13 | | 18 | |
| **Noncalicivirus** | | | | | |
| Vaccinia | Rabbit | 10 | | 6 | |
| Blue Tongue | Sheep | 6 | | 3 | |
| Poliovirus type 2 | Rabbit | 10 | | 5 | |
| Sindbis | Rabbit | 7 | | 5 | |
| Influenza | Rabbit | 6 | 5 | 3 | 4 |
| VSV — Indiana | Rabbit | 7 | | 6 | |
| Ogden | Rabbit | 5 | | 5 | |
| Coccal | Rabbit | 6 | | 5 | |

[a] Per cent St-RIP is the percentage of $^{125}$I-SMSV precipitated; 1 μl undiluted serum was used in each instance.
Blank spaces indicate not tested

[b] Immunized by subcutaneous injections of purified virus in Freunds adjuvant (19). All other calicivirus rabbits were given crude virus intravenously

[c] Immunized by two intramuscular injections of purified virus in Freunds adjuvant, two weeks apart, and one intravenous injection of purified virus at 10 weeks; the 1st bleeding was obtained just prior to, and the final bleeding 2 months after the intravenous injection (9). Other swine were immunized by intradermal injections of crude virus

*Immunization With Virion Polypeptide*

To ascertain if antibody produced against denatured viral polypeptide would be reactive in St-RIP tests with homologous and heterologous virions, a rabbit was immunized by repeated i.v. injections of SMSV-5 polypeptide with AlPO$_4$ as adjuvant. The immune response was followed by St-RIP reaction to homologous intact SMSV-5 and heterologous SMSV-4 intact virion antigens (Fig. 2). The homologous St-RIP titer rose following the second inoculation and further increases (and decreases) correlated with additional booster inoculations. The heterologous St-RIP titers paralleled the homologous pattern. Serum dilutions giving 50 per cent St-RIP in the heterologous reaction were only 2- to 4-fold less than the dilutions giving 50 per cent St-RIP in the homologous. This cross-reactivity was much greater than that observed in serum from the rabbit immunized with intact virions without adjuvant (79-fold difference, mentioned above).
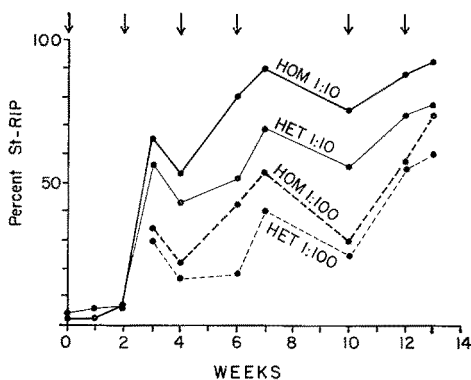


Fig. 2. Immune response in a rabbit injected with SMSV-5 polypeptide, as measured by St-RIP assays with homologous and heterologous intact virion antigens. Polypeptide was injected intravenously at each time indicated by arrows. Solid lines and broken lines indicate results of St-RIP assays at 1:10 and 1:100 dilutions of serum, respectively, with SMSV-5 (HOM) and SMSV-4 (HET) antigens

*Serologic Survey With St-RIP*

To demonstrate the suitability of St-RIP for serologic surveys for calicivirus antibodies, a small number of sera from pinnipeds (Table 3) and humans (Table 4) and a larger number of swine (Fig. 3) were tested with SMSV-4 and -5 antigens. High St-RIP titers with both SMSV-4 and SMSV-5 were observed in selected sera from 2 sea lions known to have neutralization antibodies to 4 SMSV serotypes. Varying St-RIP titers were found in sera from the 7 fur seals tested, none of which had detectable neutralizing antibodies to SMSV-4 or SMSV-5 (Table 3). Nineteen sera from 23 laboratory personnel, many of whom had worked with SMSV and/or VESV, were clearly negative (Table 4). However, 3 individuals who had collected samples from pinnipeds in the field, as well as having worked with viruses in the laboratory, showed high St-RIP titers which correlated serotypically with neutralization titers. Noteworthy was the increase of SMSV-5 antibodies shown by individual 1001, over the negative pre-employment serum. Paired sera from individual 1002 showed a small increase in SMSV-4 St-RIP over a 15 year period.

Table 3. *St-RIP assays for antibodies reacting with SMSV-4 and SMSV-5 in pinniped sera, and comparisons with neutralizing antibodies*

| Animal | Serum code | St-RIP[a] | | | | | | Neutralization[b] | | | |
| | | SMSV-4 | | | SMSV-5 | | | SMSV serotype | | | |
| | | %St-RIP at 1:10 dilution | Reciprocal of dilution at | | %St-RIP at 1:10 dilution | Reciprocal of dilution at | | | | | |
| | | | 50% St-RIP | Detectable St-RIP | | 50% St-RIP | Detectable St-RIP | 4 | 5 | 1 | 2 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Sea lion[c] | 72—M9 | 94 | 316 | 1250 | 98 | 790 | 1250+ | 10 | 40 | 20 | 80 |
| | 0—8 | 95 | 170 | 1250 | 91 | 126 | 250+ | 10 | 10 | 20 | 20 |
| Fur seal[d] | P730110 | 68 | 27 | 100+ | 27 | | | — | — | — | 13 |
| | P730111 | 20 | | | 2 | | | — | — | — | 10 |
| | P730040 | 12 | | | 97 | | | — | — | — | — |
| | P730038 | 6 | | | 17 | | | — | — | — | — |
| | P730009 | 4 | | | 49 | | | — | — | — | 5 |
| | P730005 | 6 | | | 97 | | | — | — | — | 5 |
| | P730044 | 18 | | | 98 | | | — | — | — | — |

[a] See footnote, Table 1
[b] See footnote, Table 1
[c] Geographical source of sea lion sera was Channel Islands, California
[d] Geographical source of fur seal sera was St. Paul Island (Pribilof Islands), Alaska

Table 4. *St-RIP assays for antibodies reacting with SMSV-4 and SMSV-5 in human sera, and comparisons with neutralizing antibodies*

| Human sera (lab personnel) | St-RIP[a] | | | | | | Neutralization[b] | | | |
| | SMSV-4 | | | SMSV-5 | | | SMSV serotype | | | |
| | %St-RIP at 1:10 dilution | Reciprocal of dilution at | | %St-RIP at 1:10 dilution | Reciprocal of dilution at | | | | | |
| | | 50% St-RIP | Detectable St-RIP | | 50% St-RIP | Detectable St-RIP | 4 | 5 | 1 | 2 |
|---|---|---|---|---|---|---|---|---|---|---|
| 1001 | 13 | | | 99 | 525 | 6250 | — | 40 | — | — |
| 1001 Pre[c] | | | | 3 | <10 | <10 | | | | |
| 1002 | 15 | | | 3 | | | — | — | — | — |
| 1002 Pre[c] | 7 | | | 3 | | | | | | |
| 1003 | 88 | 36 | 250 | 9 | | | 20 | — | — | — |
| 1004 | 96 | 63 | 250+ | 15 | | | 40 | — | — | — |
| 19 others[d] | <8 | | | <6 | | | | | | |

[a] See footnote, Table 1
[b] See footnote, Table 1
[c] Pre: indicates sera from individuals prior to employment. No assays for neutralizing antibodies were done
[d] No neutralizing antibodies were detected in selected individuals, others not done

The St-RIP test was applied to swine sera from apparently healthy animals, 51 collected in California (Fig. 3A) and 75 collected in Utah (Fig. 3B). Little or no reactivity with either SMSV-4 or SMSV-5 (<20 per cent St-RIP) was found in 24 per cent of the California and 37 per cent of the Utah swine. Three (6 per cent) of the California swine but none of the Utah swine showed high reactivities (> 40 per cent St-RIP) with both antigens. Although a few individual sera had high St-RIP titers with SMSV-5, reactions with SMSV-4 predominated in both groups. There was only partial correlation between St-RIP and neutralization titers (not shown). Some sera showed direct correlation, but many that were negative (<1:10) by neutralization showed moderate to high St-RIP titers; conversely, a few sera with <15 per cent St-RIP had neutralizing antibodies to one or both the SMSV serotypes.
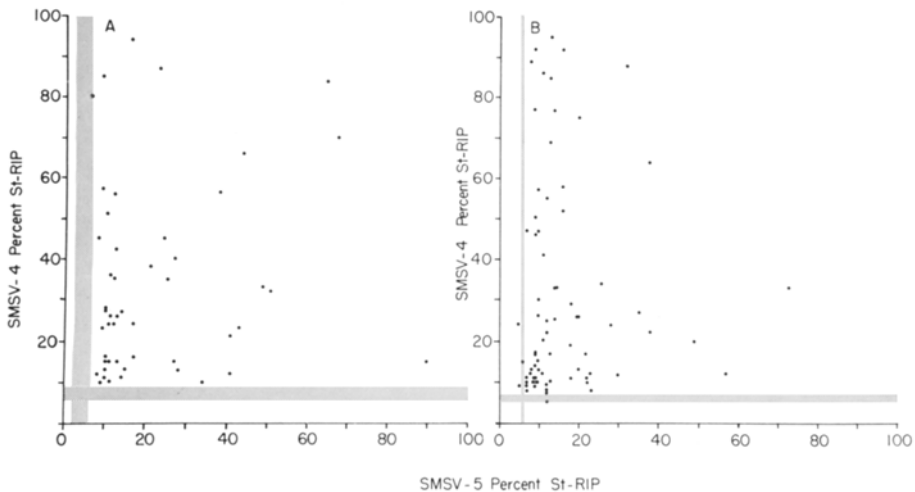


Fig. 3. St-RIP reactivity of swine sera from California (A) and Utah (B) with SMSV-4 and SMSV-5 antigens. Each point represents an individual animal; 1 μl of serum (or 10 μl of a 1:10 dilution) was used for each assay. Stippled bands represent control baseline St-RIP levels with normal rabbit sera assayed at the same time; a single control serum was used in B, the same serum was used for some of the assays in A, and another rabbit serum for others

## Discussion

Conventional RIP with second antibody is a rapid and sensitive method for detection of minute levels of viral antibodies. However, the procedure requires incubation with a specific antiimmunoglobulin which may not be readily available, especially for wild animal species. Furthermore, the antiimmunoglobulin concentration must be adjusted to the equivalence zone to achieve optimal precipitation (1). St-RIP, using a "universal" IgG adsorbent, obviates such limitations of second antibody RIP. Other advantages of St-RIP including low nonspecific reactivity, rapidity (by virtual elimination of a second incubation period), and use of a large batch of staphylococcal adsorbent (stable for months at 4° C)

contribute to usefulness of the procedure. A disadvantage is that certain immuno-globulin molecules, such as IgM, IgA, and some IgG, may not react with protein A (4, 5, 6).

In the present study in which reaction of antigen with antibody, precipitation of immune complexes, and radioassay of precipitate were all performed in the same microcentrifuge tube, both supernates and precipitates were assayed to achieve more complete evaluation of the technique. Further expediency (especially helpful when screening large numbers of sera) could be achieved at some sacrifice of quantitative precision, by assaying precipitates or supernates only. Alternatively, total and bound $^{125}$I could be assayed by counting the precipitation tube before and after removal of the supernate.

The significance of low (10—20 per cent) St-RIP values for unpaired sera at a 1:10 dilution is difficult to assess. Such values are above minimal St-RIP levels of 2—6 per cent found for some individuals among each of the various species (except for the two sea lions, both of which were known to be positive for SMSV antibodies), as well as those for normal rabbit serum controls. At dilutions beyond 1:10, however, low St-RIP levels are much more meaningful since they are compared with a baseline of a normal serum used as diluent, similar to second antibody RIP (1, 2). With paired sera from the same individual, changes in St-RIP levels are more readily interpreted as an antibody response to an antigen, as exemplified by the experimental animals inoculated with caliciviruses (Table 2) or polypeptide (Fig. 2). It is impractical to collect paired sera from wild animals such as pinnipeds, however, to monitor changes in individual animals it would be feasible to collect paired sera from domestic animals. Based on current information (Fig. 3; J. C. Sawyer, unpublished) such a study in selected populations of swine appears desirable.

The relationship between SMSV and vesicular exanthema of swine has been summarized recently (11). Although neutralization assay shows a high degree of serotypic specificity, complement fixation, immunodiffusion and immuno-electron microscopy reveal antigenic relationships among the serotypes of SMSV and VESV (8; 11; 17; J. C. Sawyer, unpublished data; F. Brown, personal communication). St-RIP assay using SMSV-4 and SMSV-5 antigens also revealed broad cross-reactivities with SMSV and VESV caliciviruses. St-RIP, which is more readily quantitated than immunodiffusion or immunoelectron microscopy, should lend itself to more extensive studies of cross-reactivity among SMSV and VESV serotypes and provide further insight into antigenic and, possibly, evolutionary relationships. Antiserum to virion polypeptide, and/or labeled polypeptide as a St-RIP antigen, may prove very useful in such studies.

The St-RIP test appears to have potential utility for serologic surveys of antibodies to caliciviruses. Because of its high sensitivity and broad cross-reactivity, St-RIP may uncover the existence of previously unrecognized sero-types. Our findings of St-RIP reactivity in a high proportion of healthy swine is consistent with a hypothesis that inapparent infections with one or more calici-virus serotypes have occurred in these populations. It should be noted that clinical vesicular exanthema of swine has not been reported in the United States since 1956, but prior to that time inapparent infections were also recognized (8, 11). Lack of correlation between St-RIP and neutralization remains unexplained, but

may relate to differences in sensitivity, specificity, and the particular populations of immunoglobulins involved in these reactions.

Whether or not caliciviruses infect man is of considerable interest. SMITH *et al.* (15) have suggested infection of humans with SMSV may occur, and MADELEY and COSGROVE (7) have presented electron microscopic evidence of calicivirus infection associated with gastroenteritis in human infants. Our finding of St-RIP reactions (strictly correlating with neutralization reactivity) in sera of laboratory personnel indicates an antibody response does result from exposure (perhaps as an inapparent infection) to SMSV. Whether or not the SMSV-VESV caliciviruses show any relationship to those caliciviruses found in human infants, or to feline calicivirus, remains to be determined.

In preliminary experiments we explored other potential uses of St-RIP. Rapid detection of virus or antigen (rather than antibody) was demonstrated in competition assay, wherein binding of known antibody by unlabeled antigen competes with labeled antigen (2, 12); SMSV-4 virus in 10 µl of culture fluid from infected cells inhibited precipitation of labeled SMSV-4 with an appropriate dilution of hyperimmune serum. Other viruses, blue tongue, Sindbis and encephalomyocarditis extrinsically labeled with $^{125}$I, and poliovirus propagated in $^{3}$H-uridine-containing medium, were reactive in St-RIP tests with homotypic hyperimmune sera prepared in sheep, rabbits or hamsters. St-RIP was also applicable to a purified bacterial antigen; *Yersinia pestis* fraction IB, labeled with $^{125}$I, showed St-RIP reactivity with antibodies in sera of rabbits immunized with purified antigen and with a commercial plague vaccine. St-RIP also detected antibodies in sera of experimental mice and human recipients of plague vaccine. As an alternative to the use of serum from clotted blood, antibodies in heparinized plasma from freshly drawn mouse blood reacted satisfactorily in St-RIP. This suggests feasibility of very rapid detection of antibodies in single drop (e.g., finger or ear lobe) blood samples. Provided that suitable labeled antigens can be prepared, application of St-RIP to additional viral and nonviral systems can be envisioned.

## Acknowledgments

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Authors' address: Ms. M. E. Soergel, Naval Biosciences Laboratory, Naval Supply Center, Oakland, CA 94625, U.S.A.