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Multilaboratory Evaluation of Methods for Detecting Enteric Viruses in Soils

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Two candidate methods for the recovery and detection of viruses in soil were subjected to round robin comparative testing by members of the American Society for Testing and Materials D19:24:04:04 Subcommittee Task Group. Selection of the methods, designated "Berg" and "Goyal," was based on results of an initial screening which indicated that both met basic criteria considered essential by the task group. Both methods utilized beef extract solutions to achieve desorption and recovery of viruses from representative soils: a fine sand soil, an organic muck soil, a sandy loam soil, and a clay loam soil. One of the two methods, Goyal, also used a secondary concentration of resulting soil eluants via low-pH organic flocculation to achieve a smaller final assay volume. Evaluation of the two methods was simultaneously performed in replicate by nine different laboratories. Each of the produced samples was divided into portions, and these were respectively subjected to quantitative viral plaque assay by both the individual, termed independent, laboratory which had done the soil processing and a single common reference laboratory, using a single cell line and passage level. The Berg method seemed to produce slightly higher virus recovery values; however, the differences in virus assay titers for samples produced by the two methods were not statistically significant ($P \leq 0.05$) for any one of the four soils. Despite this lack of a method effect, there was a statistically significant laboratory effect exhibited by assay titers from the independent versus reference laboratories for two of the soils, sandy loam and clay loam.

Human enteric viruses are a common microbial contaminant of domestic drainage and sewerage systems. Effluents from septic tanks and related waste disposal facilities are generally discharged directly into soil. Also, municipal sewage is often discharged to land following various levels of formal treatment. One of the major public health concerns associated with human enteric viruses contained in domestic drainage and sewerage is that they may survive following introduction into the soil environment and subsequently cause illness in susceptible persons who may consume contaminated groundwaters or crops. Research has been done on several topics that are pertinent to this concern, including the extent of viral adsorption to soil (1, 5), viral persistence in soil (3, 4, 6), migration of viruses through soil in association with the movement of water or wastewater (4, 5, 7), viral persistence in groundwaters (10), and the survival of viruses on vegetables grown in contaminated soil (6) or vegetables that have been contaminated during wastewater irrigation (9).

The present study was designed to compare the sensitivity and reproducibility of two methods used for recovering and detecting enteric viruses contained in samples of different soil types. This study represents a voluntary participation effort performed under the auspices of the American Society for Testing and Materials task group D-19:24:04:04, responsible for developing standard methods for detecting viruses in solids. Evaluation of the test methods for recovering viruses from soil involved operators in nine different laboratories. The approach was generally similar to that of a previously published evaluation on methods for detecting viruses contained in wastewater sludges (2).

MATERIALS AND METHODS

Soil samples. Four different soil types were tested, and these are listed in Table 1 along with their physical characteristics. The soils were shipped to a single laboratory which assumed responsibility for inoculating them with viruses and subsequently distributed portions of the inoculated soils to individual testing laboratories. The viruses used as inoculum consisted of an indigenous population that had been isolated from raw sewage and passaged a limited number of times in a continuous African Green monkey kidney (BGM) cell culture line. Inoculation and distribution of the test soils to participants were done at intervals of 1 month, using a single different soil type each month, with virus addition performed within the 24-h period immediately prior to soil distribution. The amount of virus-laden fluid added during the virus-seeding operation differed according to soil type and was

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TABLE 1. Characteristics of the test soils

Parameter	Value for the following soil type			
	Fine sand (Eustis)	Organic muck (Ponzer)	Sandy loam (Flushing Meadows)	Clay loam
Sand, 2–1 mm (%)	0.0		2.5	0.6
Sand, 1–0.5 mm (%)	5.6		16.3	1.2
Sand, 0.5–0.25 mm (%)	38.8		19.0	5.0
Sand, 0.25–0.1 mm (%)	44.2		23.3	14.6
Sand, 0.1–0.05 mm (%)	6.4		18.1	25.4
Sand, total (%)	95.0		79.2	46.8
Silt (%)	3.4		17.6	36.0
Clay (%)	1.6		3.2	17.2
pH (standard units)	5.7	3.3	5.5	5.8
Organic carbon (%)	0.26	50.35	0.38	0.79
Individual extractable bases (meq/100 g)				
Calcium	0.12	1.38	4.90	29.00
Magnesium	0.04	0.63	2.30	2.80
Sodium	0.01	0.14	0.38	0.93
Potassium	0.00	0.19	0.55	0.95
Cation-exchange capacity, total (meq/100 g)	2.88	93.56	11.91	37.38
Base saturation (%)	6	3	68	90
Sodium adsorption ratio	0.025	0.099	0.142	0.165
Extractable acid (meq/100 g)	2.71	91.22	3.78	3.70

intended to achieve a normal field moisture saturation level for each of the different soils. The moisture levels used were as follows: fine sand, 15.1%; organic muck, 74.3%; sandy loam, 14.3%; clay loam, 29.6%. The target level of virus added was intended to be 5 to 10 PFU/g of final wet soil weight for each soil type. This target level was missed in the case of the sandy loam soil.

For each soil type, a total of nine separate 50- to 70-g portions were sent on ice to each of the nine designated operators, using an overnight air delivery service (a total of 81 portions for each soil type). Each portion contained a designation as to which detection method should be used for its analysis. Every operator received three soil portions designated for each of the two required detection methods, "Berg" and "Goyal." The other three portions were available for processing by any alternative method of the operators' own choice. As a quality control check, for each soil type, two of the distributed portions were inoculated with distilled water rather than virus suspension. These two portions served as "virus blanks" and were sent to randomly selected individual operators as substitutes for virus-seeded soil portions intended to be processed by one of the two required techniques.

Soil testing protocol. Participating operators were required to adhere strictly to established protocols for performing the Berg and Goyal techniques and to initiate comparative testing within 24 h of the arrival of soil portions. All method comparisons were performed in triplicate, with a single performance (trial) of each method on days 1, 2, and 3 following receipt of the soil portions. A 50-g (wet weight) amount of soil was used for each trial, and this had to be taken from the soil portion designated for that particular detection method and trial number. After the soil portions were processed, each of the resulting samples was divided into three aliquots, and all aliquots were stored frozen. Virus titers for one of these aliquots were determined via plaque formation assay on BGM cells by that laboratory which processed the soil. A second aliquot was shipped to a common reference laboratory for virus titration. The third aliquot was to be used as a backup, for assay as needed by either the processing laboratory or the reference laboratory. A common data reporting form was used by all of the operators.

Berg method for virus recovery from soil. For processing by the Berg technique, 50 g (wet weight) of soil was suspended in 50 ml of a buffered 10% beef extract solution (containing, per liter, 100 g of commercial powdered beef extract, 13.4 g of Na_2HPO_4, and 1.2 g of citric acid). The beef extract used throughout this study was beef extract V (BBL Microbiology Systems, Cockeysville, Md.). The suspensions of soil and buffer were then agitated for 30 min with a magnetic stirrer, during which time the pH of the suspensions was maintained at 7.0 ± 0.1 by adding 5 M HCl or NaOH as needed. The suspensions were then centrifuged for 30 min at 2,500 × *g*, after which the supernatant material was collected and the pellet was discarded. The supernatants were subsequently passed through a stacked sandwich of 3.0-, 0.45-, and 0.25-μm-pore-size filters (Duo-Fine; Memtec America Corp., Timonium, Md.). For this processing technique, the filtered supernatant was considered to be the final sample, and it was stored at −70°C until assayed.

Goyal method for virus recovery from soil. For processing by the Goyal technique, 50 g (wet weight) of soil was combined in a 400-ml capacity screw-capped bottle with 4 equivalent volumes (200 ml) of pH 10.5 3% (wt/vol) beef extract solution. The bottle containing the soil and beef extract mixture was then vigorously shaken by hand for 5 min. The pH of the soil and beef extract mixture was checked and, if necessary, adjusted to 9.5 by addition of 1 M NaOH. This mixture was then centrifuged at 1,500 × *g* for 10 min at 4°C, after which the supernatant fluid was saved and the pellet was discarded.

The supernatant fluid was next adjusted to pH 3.5 ± 0.1 by dropwise addition of 5 M HCl and agitated with a magnetic stirrer until either a flocculant precipitate formed or 30 min had passed. The pH of the solution was periodically checked during the course of stirring and readjusted as necessary to 3.5 by adding either 1 M HCl or NaOH. The solution was then centrifuged at 1,000 × *g* for 5 min at 4°C, following which the resulting supernatant fluid was carefully removed by pipetting and discarded. A magnetic stirring bar was then added to the centrifuge bottle containing the precipitate, and the precipitate was suspended in 8 to 10 ml (total volume) of pH 11.0 0.05 M glycine. The pH of the precipitate and glycine buffer mixture was periodically checked during this stirring and, if necessary, increased to ≥9.5 by addition of 1 M NaOH. After dissolution, the suspension was centrifuged at 1,000 × *g* for 10 min at 4°C for collection of the resulting supernatant. This supernatant was adjusted to pH 7.5 by adding 1 M HCl (with a caution not to lower the pH below 7.4 to avoid formation of a troublesome precipitate), supplemented with antibiotics (final concentrations, 200 U of penicillin G, 200 μg of streptomycin sulfate, and 2.5 μg of amphotericin B per ml), made isotonic by adding 0.5 ml of 3.0 M NaCl per 10 ml of sample, and frozen at −70°C until assayed.

Virus enumeration. Each of the soil processing laboratories was responsible for titrating one aliquot (or two, if they experienced difficulties with assay performance) from each of the samples that they had produced. In doing so, the individual laboratories were permitted to exercise their choice in selecting any single cell line and quantitative viral plaque assay method. All laboratories chose the BGM cell line. Viral assays performed in the reference laboratory were

TABLE 2. Recovery of viruses from four soil types utilizing the different processing techniques[a]

Soil processing laboratory	Soil processing technique	Assay titer (PFU/g [dry wt] of soil)							
		Fine sand (Eustis)		Organic muck (Ponzer)		Sandy loam (Flushing Meadows)		Clay loam	
		Independent laboratory	Reference laboratory	Independent laboratory	Reference laboratory	Independent laboratory	Reference laboratory	Independent laboratory	Reference laboratory
1	Berg	3.9 ± 1.9	7.3 ± 2.3	4.1 ± 1.1	1.2 ± 0.4	157.8 ± 68.0	104.6 ± 19.4	0.2 ± 0.3	2.1 ± 2.2
	Goyal	3.2 ± 1.2	9.0 ± 1.1	0.3 ± 0.5	5.7 ± 4.4	81.8 ± 31.3	117.4 ± 21.8	0.2 ± 0.3	0.3 ± 0.2
2	Berg	4.1 ± 1.0	7.4 ± 1.2	6.8 ± 1.1	4.9 ± 0.1	6.7 ± 1.0	166.4 ± 1.9	2.7 ± 1.7	2.8 ± 3.4
	Goyal	5.4 ± 0.5	8.4 ± 1.5	6.8 ± 3.7	4.1 ± 3.7	8.6 ± 3.1	200.7 ± 72.3	Toxicity	Toxicity
3	Berg	0.1 ± <0.1	7.2 ± 0.3	3.5 ± 0.4[b]	3.3 ± 2.3	0.6 ± 1.0	157.3 ± 29.6	<0.1 ± 0.1	2.7 ± 4.7
	Goyal	<0.1 ± <0.1	8.4 ± 4.3	1.0 ± <0.1[b]	1.2 ± <0.1[b]	0.4 ± 0.2	100.9 ± 45.2	0.1 ± <0.1[b]	Toxicity
4	Berg	9.4 ± 2.8	8.2 ± 1.2	3.3 ± 0.1[b]	1.6 ± 1.6	43.1 ± 8.7	228.6 ± 28.4	0.7 ± 1.3	8.0 ± 5.5
	Goyal	9.4 ± 0.1	9.2 ± 1.7	4.3 ± 0.6[b]	0.7 ± 1.2	11.5 ± 2.0	120.5 ± 66.5	0.3 ± <0.1[b]	Toxicity
5	Berg	8.4 ± 0.7	8.7 ± 0.4	Toxicity	6.8 ± 3.5	163.2 ± 4.8	204.1 ± 25.4	5.9 ± 1.1	1.7 ± 0.4
	Goyal	6.9 ± 0.5	8.5 ± 1.1	Toxicity	9.2 ± 4.4	113.9 ± 98.8	151.4 ± 30.8	Toxicity	Toxicity
6	Berg	8.0 ± 0.8	8.8 ± 1.3	3.6 ± 0.7	2.6 ± 0.8	143.5 ± 11.0	153.5 ± 13.3	3.3 ± 1.2	11.8 ± 2.3
	Goyal	8.8 ± 1.0	8.3 ± 0.7	0.1 ± 0.2	0.0[c]	168.0 ± 14.2	118.7 ± 5.2	1.5 ± 0.3	5.2 ± 1.7
7	Berg	5.9 ± 1.0	7.2 ± 0.7	6.2 ± 4.0	1.8 ± 2.1	10.6 ± 7.6	70.0 ± 83.9	6.5 ± 1.4	10.0 ± 3.4
	Goyal	5.8 ± 1.5	6.9 ± 0.6	4.2 ± 3.0	5.2 ± 3.0	169.0 ± 69.3	120.5 ± 26.7	2.0 ± 0.7	5.0 ± 2.8
8	Berg	17.8 ± 2.1	10.6 ± 1.6	6.6 ± 3.3	3.5 ± 4.7	189.2 ± 110.2	112.2 ± 31.1	4.8 ± 0.3	9.0 ± 2.6
	Goyal	13.0 ± 0.2	3.6 ± 0.8	7.4 ± 3.9	2.4 ± 2.6	211.8 ± 68.4	136.2 ± 53.8	5.0 ± 0.7	13.2 ± 1.4
9	Berg	7.7 ± 3.1	8.1 ± 1.8	Toxicity	No values	148.3 ± 49.7	169.1 ± 60.5	Toxicity	7.8 ± 1.7
	Goyal	6.4 ± 2.6	4.7 ± 1.2	Toxicity	No values	107.2[d]	87.0[d]	Toxicity	Toxicity

[a] Processing the different soils for recovery of seeded viruses was performed in triplicate by each of the nine independent laboratories. The resulting samples (one from each of three replicates per soil, per processing technique, per processing laboratory) were divided into portions. One portion of each sample was assayed by the independent laboratory that had processed the soil. A duplicate portion of each sample was then assayed by a single, common reference laboratory. The titers are listed as mean ± 1 standard deviation for the replicates of each soil type. "Toxicity" indicates that sample cytotoxicity prevented successful viral assay. "No values" indicates that no assay titers were available, presumably for reasons other than sample cytotoxicity. The individual techniques are described in Materials and Methods.

[b] If a sample was reported to contain no detectable viruses, then a maximum estimate for its virus titer was calculated which represented the mathematical limit of detection. The superscript *b* next to a number indicates that at least one of the values used in determining the listed average value for virus recovery was a maximum estimate.

[c] All values were 0.0.

[d] Only a single value was available.

done by using a soft-agar overlay technique and the BGM cell line. All virus titers were reported in terms of PFU per gram (dry weight) of soil. The type of cell culture medium to be used by the independent laboratories was not predesignated. The reference laboratory used an equal-parts mixture of Eagle minimum essential medium (made with Hanks balanced salt solution) and Leibovitz L-15 medium supplemented with fetal bovine serum to a final level of 10% (vol/vol) as the cell growth medium. The reference laboratory used Earle balanced salt solution supplemented with fetal bovine serum at a final level of 5% (vol/vol) for maintenance of confluent cell culture monolayers prior to performing the plaque assay procedure. The agar overlay medium used by the reference laboratory when performing plaque assays consisted of Eagle minimum essential medium prepared with Hanks balanced salt solution, lacking phenol red, and supplemented to final levels of 1% (vol/vol) fetal bovine serum and 1.5% (wt/vol) agar.

Each laboratory was requested to confirm the positive status of at least 10 plaques per sample for all cases in which assaying an aliquot of sample yielded at least that minimum number. Confirmation was accomplished by individually collecting material from each of the 10 plaques and then inoculating the collected material into separate fresh cell cultures. These fresh cultures were then overlaid with liquid medium and observed for the development of virus-characteristic cytopathogenic effects for 14 days or as long as the cells remained viable and healthy. The percentage of confirmable plaques was utilized to establish a corrected virus recovery level for each sample. It was those corrected values which were used for the statistical analyses we present.

Statistical analysis. Values of mean and standard deviation were calculated for viral assay titers representing the replicate trials from each combination of operator, soil type, and soil processing technique. Separate values were derived for titers from those assays performed by individual, termed independent, soil processing laboratories versus the reference laboratory. Further analyses consisted of two-way analysis of variance (ANOVA), one-way ANOVA, and *t* tests.

RESULTS

Assay results representing replicate evaluations of the two principle test methods (Berg and Goyal) for the recovery of viruses from all four soil types are summarized in Table 2. The mean values listed in Table 2 representing virus recovery by the Berg versus Goyal methods are graphed in Fig. 1. Points lying above the 45° line indicate instances when the

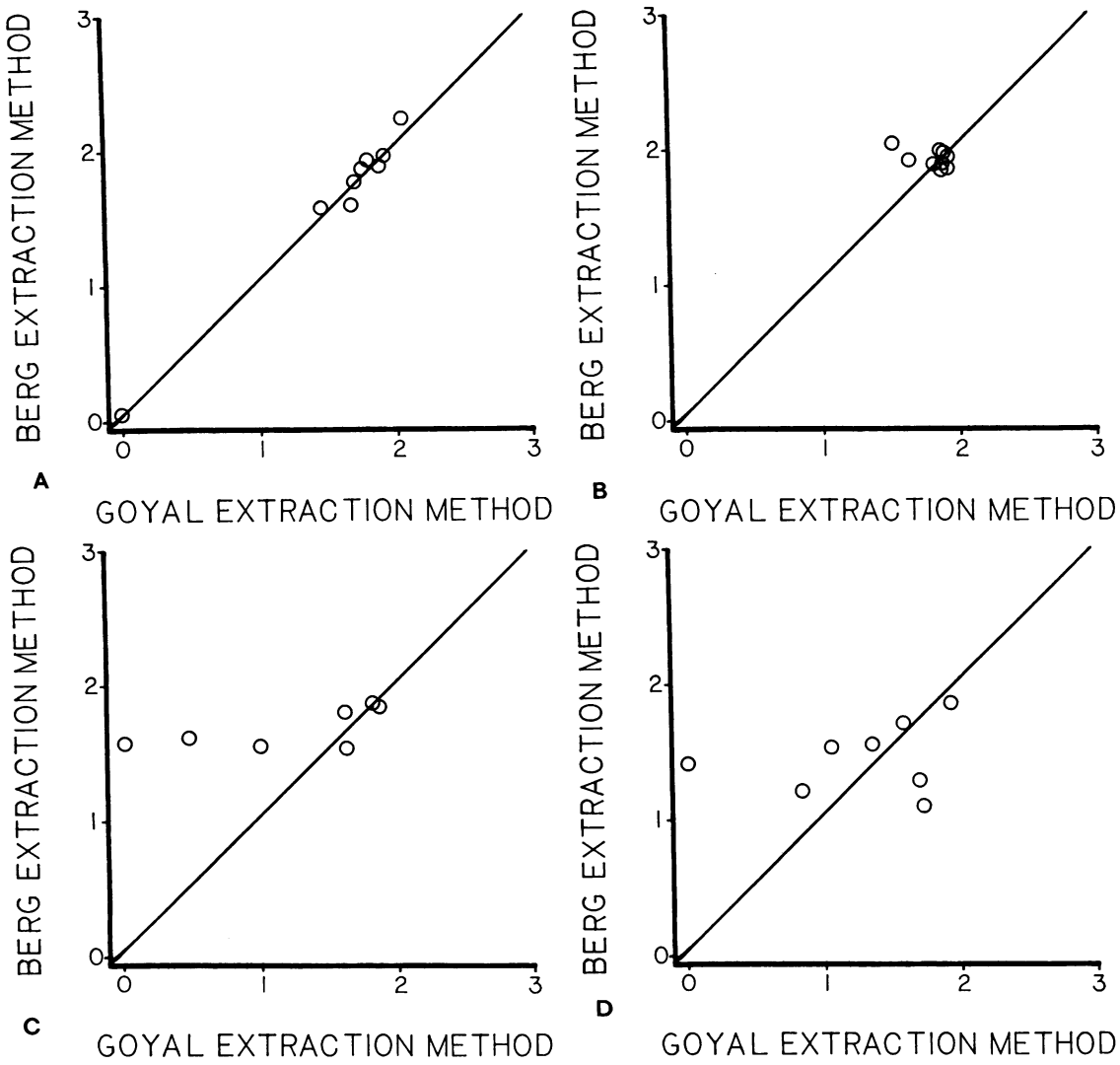

FIG. 1. Plot of $\log_{10}$ transformed virus assay titers for samples prepared with the Berg versus Goyal soil processing techniques. (A) Fine sand soil, assays performed by independent laboratories; (B) fine sand soil, assays performed by reference laboratory; (C) organic muck soil, assays performed by independent laboratories; (D) organic muck soil, assays performed by reference laboratory; (E) sandy loam soil, assays performed by independent laboratories; (F) sandy loam soil, assays performed by reference laboratory; (G) clay loam soil, assays performed by independent laboratories; (H) clay loam soil, assays performed by reference laboratory.

Berg extraction method yielded higher virus recoveries than the Goyal method. Points lying below the 45° line indicate instances when the Goyal method yielded higher virus recovery. These graphs demonstrate that the Berg method generally was somewhat better numerically in terms of recovering viruses from the soils. They also show that assay titers generated by the reference laboratory generally exhibited less scatter, or variability, when compared with titers generated by the independent laboratories.

The mean values listed in Table 2 for virus recovery by the Berg and Goyal techniques were then averaged. The resulting overall virus recovery averages are presented in Table 3 along with their corresponding standard deviations. These overall values were derived by using only those individual values from Table 2 which represent complete sets of data, i.e., those with corresponding numbers available for all four possible combinations of soil processing technique (Berg versus Goyal) and location where the assay was performed (independent laboratories versus reference laboratory). The number of data sets used in performing these calculations were as follows: fine sand, 9; organic muck, 7; sandy loam, 9; clay loam, 4. Inclusion of the unmatched values from Table 2 would change three of the overall virus recovery averages for clay loam soil (change to 3.0 ± 2.6 for samples representing the Berg technique with assay performed by the independent laboratories, 6.2 ± 3.9 for samples representing the Berg technique but assayed by the reference laboratory, and 1.5 ± 1.9 for samples representing the Goyal technique with assay by the independent laboratories). Inclusion of unmatched values from Table 2 would change two of the overall virus recovery averages for organic muck soil (change to 3.2 ± 1.9 for samples representing the Berg technique with assay performed by the reference laboratory and to 3.6 ± 3.1 for samples representing the Goyal technique with assay by the reference laboratory). There were no unmatched values in any of the other categories for either

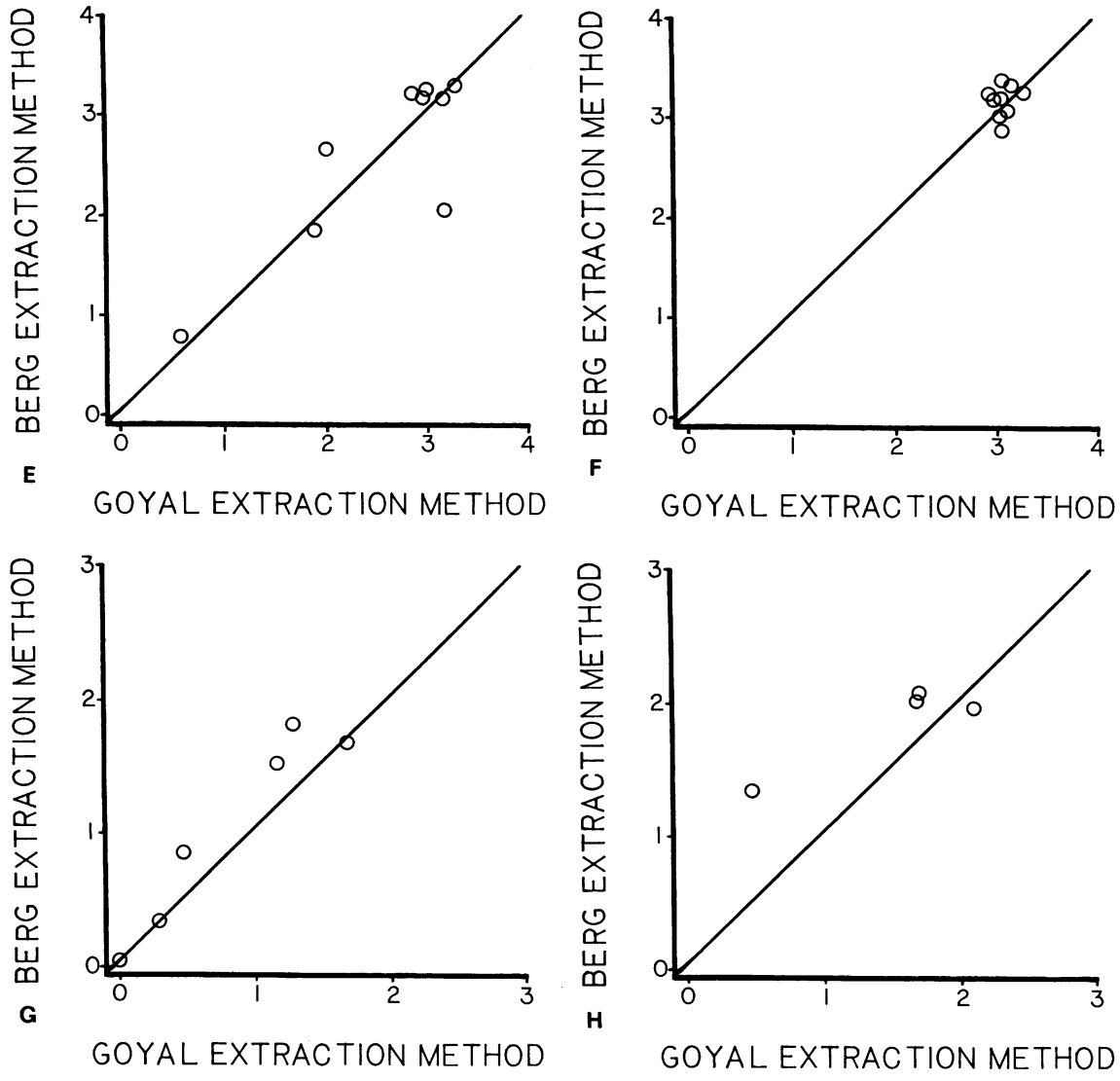

FIG. 1—*Continued.*

the clay loam or organic muck soils and no unmatched values for processing either the fine sand or sandy loam soils by the Berg or Goyal technique.

Table 4 presents the results of using two-way ANOVA analyses on the mean virus recovery values listed in Table 2. The ANOVA testing was used to determine the possible existence of either method effects within the data attributable to whether soil was processed by the Berg technique or

TABLE 3. Overall averages for virus recovery[a]

Soil processing technique	Location (laboratory) of assay	Assay titers (PFU/g [dry wt] of soil)			
		Fine sand	Organic muck	Sandy loam	Clay loam
Berg	Independent	7.3 ± 4.9	4.9 ± 1.6	95.9 ± 78.4	3.7 ± 2.7
	Reference	8.2 ± 1.1	2.7 ± 1.3	151.8 ± 49.6	8.2 ± 4.2
Goyal	Independent	6.6 ± 3.7	3.4 ± 3.0	96.9 ± 77.8	2.2 ± 2.0
	Reference	7.4 ± 2.0	2.8 ± 2.3	128.1 ± 32.8	5.9 ± 5.4

[a] Values are means ± standard deviations calculated from the values given in Table 2, but using only those values from Table 2 which represented complete sets of data, i.e., having numbers available for all four possible combinations of soil processing technique (Berg versus Goyal) and location of assay (independent versus reference laboratory). The number of data sets used in preparing the calculations shown here were as follows: fine sand, 9; organic muck, 7; sandy loam, 9; clay loam, 4. Assuming a 10-PFU/g (wet weight) input level of virus for each of the different soil types, and with proper allowance made for the target soil moisture levels, estimated values for 100% virus recovery would be as follows: 8.5 PFU/g (dry weight) for the fine sand soil, 2.6 PFU/g (dry weight) for the organic muck soil, 8.6 PFU/g (dry weight) for the sandy loam soil, and 7.0 PFU/g (dry weight) for the clay loam. The target level for virus addition was obviously missed in the case of the sandy loam soil.

TABLE 4. Examination of virus recovery values for effects attributable to soil processing technique or location of assay[a]

Effect examined	Virus recovery with given soil type			
	Fine sand	Organic muck	Sandy loam	Clay loam
Processing technique	0.516	0.408	0.593	0.335
Assay location	0.413	0.092	0.045	0.050
Interaction	0.988	0.370	0.560	0.842

[a] Values are the one-tailed P values derived by two-way ANOVA, using those values from Table 2 which represented complete sets of data, i.e., having numbers available for all four possible combinations of soil processing technique (Berg versus Goyal) and location of assay (independent versus reference laboratory). The number of data sets used in preparing the calculations shown here were as follows: fine sand, 9; organic muck, 7; sandy loam, 9; clay loam, 4. Incorporation of the unmatched values from Table 2 into these calculations was also evaluated but did not improve any of the levels of significance.

the Goyal technique, laboratory effects attributable to whether viral assay of the prepared samples was done by the independent laboratories or the reference laboratory, and possible interaction effects between the processing technique and location where the assay was performed. The ANOVA findings demonstrate an apparent lack of method effect or interaction effect. A statistically significant ($P \leq 0.05$) laboratory effect was observed for samples representing the sandy loam and clay loam soils. Comparable laboratory effects were not found for the assay values representing either the fine sand soil or the organic muck soil. As with the information presented in Table 3, the numbers shown in Table 4 were produced with only those values from Table 2 which represented complete sets of data, i.e., having numbers available for all four possible combinations of soil processing technique (Berg versus Goyal) and location of assay (independent laboratories versus reference laboratory). Incorporation of the unmatched values from Table 2 into these ANOVA calculations was also evaluated but did not improve any of the levels of significance for laboratory, method, or interaction effects (results not shown). Paired two-tailed t tests (in which equal numbers of values were being compared) and one-way ANOVA (in which the numbers of values being compared were unequal) were used in a further effort to examine the reference laboratory assay values from Table 2 for presence of possible method effects exhibited by samples from any of the four different soils. No statistically significant ($P \leq 0.05$) method effects were revealed by this evaluation (results not shown). Three of the values listed in Table 2 were judged outlyers because they were >2 standard deviations away from the appropriate mean. Changing these three outlying values to bring them within the second deviation did not improve any of the levels of significance as determined by either ANOVA or t test.

DISCUSSION

The findings from this study suggest that the Berg method may have yielded slightly higher values for recovery of viruses from soil than the Goyal method. These differences were not statistically significant ($P \leq 0.05$) for any of the four soil types examined. In making this comparison, it should be recognized that, while both the Berg and Goyal procedures used beef extract as a proteinaceous fluid for desorbing viruses from the soil particles, the Goyal method carried this procedure much further by using organic flocculation to reduce the volume of the produced samples (eluates). This is a very important difference in that organic flocculation greatly decreases the volume of sample material which must then be assayed for presence of viruses. However, secondary concentration processes such as organic flocculation typically involve some loss of virus, and this particular technique has been estimated to be 50 to 60% efficient in terms of virus recovery. This loss of virus could more than account for any observed differences in titer between samples processed by the two methods. It is important to note that toxicity was observed when assaying some of the virus concentrates prepared from the organic muck and clay loam soils. There were five occasions when identically paired samples were determined to be cytotoxic by an independent laboratory but not by the reference laboratory, and vice versa on two occasions. This is assumed to reflect the inexact nature of performing viral assays by means of cell culture techniques. Nevertheless, cell culture techniques still offer the only means available for determining viral infectivity short of directly inoculating laboratory animals. While all of the laboratories voluntarily used the same cell line (BGM) for performing the viral plaque assay procedure, the individual laboratories were allowed to select their own choice of cell culture media and to use their own protocols for both cell cultivation and viral assay. These allowances may have influenced the relative sensitivity of the cultured BGM cells to cytotoxins present in the processed samples. The media used by the reference laboratory are defined in Materials and Methods.

The method used for adding viruses to soil was believed to be comparable to what occurs under natural conditions in sewage effluent infiltration basins. It is thought that virus recovery efficiencies similar to those reported for this study could be expected if the same virus recovery procedures were used on natural samples of virally contaminated soil. It should, of course, be noted that virus recovery can vary according to virus type. This is why a natural virus population was used for seeding the soil samples examined in this study. The target virus level we used for seeding the soil samples was chosen to provide numerical recovery values that would be sufficiently high to permit reliable statistical analyses. In comparison, this virus level exceeded that previously observed in a field study which examined naturally contaminated soil from the Flushing Meadows site in Phoenix, Ariz. (4). The Flushing Meadows site consists of sandy loam soil identical to that used in our study.

Virus recovery values provided by the individual laboratories often varied over a broader range than did those reported by the reference laboratory for the identical samples. This was anticipated in advance and was the reason for having portions of all samples assayed by both the laboratory which produced the sample and the reference laboratory. For the purpose of this study, virus recovery was examined with regard to the comparative sensitivities of the Berg and Goyal techniques. The data generated by the study were also examined for precision and bias. The differences between virus titers reported by the independent laboratories and the reference laboratory were statistically significant ($P \leq 0.05$) in the case of the sandy loam and clay loam soils, but were not significant in the case of either the fine sand or organic muck soil.

There were no significant interaction effects observed between the choice of method used for processing soil samples (Berg versus Goyal) and the laboratory location of assays (independent laboratories versus reference laboratory). In summary, then, the two methods compared in this

study can be considered roughly equivalent in terms of their capability for recovering viruses from contaminated soil.

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