
A rapid, reproducible method for coating Rotorod Sampler collector rods with silicone grease

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Background: The Rotorod Sampler (Sampling Technologies, St. Louis Park, MN) is a rotating-arm impactor that recovers airborne particles on two rapidly moving plastic collector rods. For decades, the standard method for applying silicone grease to collector rods has been with one's finger. Although this method can yield excellent results when performed by practiced investigators, a relatively high skill level is required, and significant intrapreparer variability has been reported in the medical and technical literature.

Objective: The purpose of this investigation was to develop and evaluate a new method for coating Rotorod collector rods with silicone grease.

Methods: Collector rods were coated with silicone grease by dipping them into a solution consisting of silicone grease and hexane. Pollen recovery by these dipped collector rods was compared with pollen counts obtained with hand-greased collector rods.

Results: Twenty-three paired samples were obtained during five sampling periods. Pollen recovery by the hand-greased and dipped collector rods was similar ($P = 0.410$). Dipped collector rods generally offered a lower standard deviation than hand-greased collector rods, however, differences were not statistically significant ($F = 1.782$, $P = 0.087$). Dipped collector rods were also superior to hand-greased collector rods in several qualitative categories such as grease uniformity, time required for microscopic analysis, and visual quality.

Conclusions: Dipped collector rods offered a time-efficient means to obtain atmospheric samples with excellent visual quality. The resulting pollen counts were similar to data obtained via the standard, manual method. Allergists are encouraged to consider using this new method in their office practices and for drug studies.

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INTRODUCTION

The Rotorod Sampler (Sampling Technologies, St. Louis Park, MN) is a rotating-arm impactor that recovers airborne particles on two rapidly moving plastic collector rods.^{1–4} Silicone grease applied to these collector rods

serves as an adhesive to capture particles impinged on them.⁵

For decades the standard method for applying silicone grease to collector rods has been with one's finger. The manufacturer recommends dipping an index finger into the grease and then transferring it to the collector rod with several strokes down the length of the rod.⁶

Although this method seems a bit crude, it can yield excellent, reproducible results when performed by practiced investigators.⁷ A relatively high skill level is required, however, and significant intrapreparer variability has been reported in the medical and technical literature (Table

1).^{8,9} Reports concerning interpreparer variability are not available but one might anticipate substantial differences between investigators.

The purpose of this investigation was to develop and evaluate a new method for coating Rotorod collector rods with silicone grease. The experimental approach involved dipping collector rods into a solution composed of silicone grease and hexane, a highly volatile organic solvent. The performance of these dipped collector rods was compared with collector rods coated via the standard, manual method.

MATERIALS AND METHODS

Study Location and Sample Acquisition

A compact sampling array was installed on the flat rooftop (23 × 55 m) of a three-story (12 m above grade), suburban office building in Minnetonka, Minnesota.¹⁰ The array was placed in an unobstructed location in the southwest corner of the rooftop. There was no immediately adjacent vegetation.

The sampling array consisted of five modified Rotorod Samplers controlled by a modified Cycling Field Timer (Sampling Technologies).¹⁰ A standard 10% duty cycle that exposed collector rods for 1 minute out of every 10 minutes was used.

Twenty-four hour sampling periods were used. Collector rods were changed at approximately 3:00 PM daily.

A Rotorod Sampler holds two collector rods in its retracting head. A hand-greased collector rod and a dipped collector rod were therefore paired in each sampler. Ten collector rods (five hand-greased and five dipped) could thus be obtained during each sampling period.

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As demonstrated by Solomon et al,⁷ the two positions on a retracting head yield slightly different particle counts (even when random variability in the atmospheric pollen aerosol is considered). We controlled for this systematic source of error by alternating the type of collector rod placed in each position: a hand-greased collector rod was placed in a particular position during one sampling period; during the subsequent sampling period, a dipped collector rod was placed in that same position.

The performance of the Rotorod Samplers was monitored throughout the study to assure proper functioning.¹¹

Hand-Greased Collector Rods

One set of collector rods was coated with silicone grease by hand by D.A.F. in the manner recommended by the manufacturer.⁶ These collector rods were placed in storage vials (Sampling Technologies) before use.

Dipped Collector Rods

A second set of collector rods was coated with silicone grease by B.L.G. by dipping them into a hexane-silicone solution.

A special 55-mL glass flask was blown to facilitate dipping. The inner

diameter (26 mm) of the flask's round mouth was just large enough to admit the base of a Rotorod storage vial.

A stock solution consisting of silicone grease (Sampling Technologies) and hexane (Mallinckrodt Baker Inc., Paris, KY) was created by mixing the ingredients in the flask. Silicone grease is largely insoluble, so an emulsion was formed by agitating the solution with a magnetic stir bar.

Preparing and maintaining the hexane-silicone solution within tight analytical limits proved untenable because silicone grease is exceedingly difficult to quantitatively transfer between vessels, and hexane is extremely volatile (boiling point, 38.3° C)¹². The tolerances for the solution were therefore 7.5 to 9.5% wt/vol.

The composition of the solution was monitored throughout the period of time that the collector rods were dipped. Briefly, a plastic weigh boat and micropipet tip were weighed. The tip was then used to withdraw 1 mL of hexane-silicone solution from the flask. This aliquot was expelled into the boat; the tip was also placed into the boat. After the hexane had completely evaporated (at least 10 minutes was allowed to elapse), the boat and its

contents were weighed. The mass of silicone grease (grams) per milliliter of solution was then calculated.

The hexane-silicone solution tended to become richer over time despite precautions to minimize solvent loss. The solution was maintained within the stated tolerances by adding appropriate volumes of hexane.

Rotorod collector rods were loaded into the bases of storage vials (each vial holds 12 collector rods). These collector rods were then dipped en masse into the hexane-silicone solution for several seconds and withdrawn. Collector rods were dipped three times with at least 10 minutes between each dip to allow the hexane to evaporate.

The vials' clear plastic covers were press-fitted onto the bases after the hexane had completely evaporated.

Sample Analysis

Collector rods were placed in stage adapters, stained with Calberla solution and covered with cover glass (all items, Sampling Technologies). Pollen grains were counted by evaluating the entire surface of each collector rod with a light microscope by B.L.G. at 400×.¹³ All data were then converted to volumetric equivalents (pollen grains per cubic meter of air, henceforth abbreviated p/m³).

Statistical Methods

A target sample size was calculated based on the standard deviation observed during previous work with paired samples.¹⁴ The desired sample size was calculated such that a difference of 15 p/m³ or more in the means would be identified as statistically significant at the 0.05 level with a power of 0.80. Based on preliminary studies, the standard deviation was predicted to be 10 p/m³. It was thus concluded that a sample size of 20 collector rod pairs would be sufficient to achieve the desired result.

The mean and standard deviation were computed for each sampling period for both the hand-greased and dipped collector rods (Table 2).

Differences in pollen recovery between the hand-greased and dipped col-

Table 1. Pollen Recovery by Rotating-Arm Impactors Used in Previous Investigations

R*	Device†	n‡	Minimum	Maximum	Mean
7	RR	70	—	—	0.56 p/m ³
	RR	70	—	—	2.80 p/m ³
	RR	70	—	—	4.47 p/m ³
	RR	70	—	—	2.87 p/m ³
	RS	70	—	—	0.55 p/m ³
	RS	70	—	—	4.33 p/m ³
	RS	70	—	—	0.33 p/m ³
	RS	70	—	—	0.33 p/m ³
8	RS	11	0%	21%	6%
	RS	33	—	—	10%
	RS	38	0%	74%	15%
	RS	41	2%	244%	29%
	RS	60	0%	196%	23%
	RS	43	0%	89%	10%
	RS	243	0%	209%	32%
	RR	18	—	61%	15%

Data represent the difference between paired samples obtained with the same device during the same sampling period (percent or pollen grains per cubic meter of air [p/m³]); data for multiple devices were presented in References 7 and 8.

* Reference number.

† RR = Rotorod Sampler; RS = Rotoslide Sampler.

‡ Number of paired samples.

Table 2. Pollen Recovery (Pollen Grains per Cubic Meter of Air) by the Hand-Greased and Dipped Collector Rods

SP*	n†	Hand-greased collector rods		Dipped collector rods	
		Mean $\pm$ SD	Range	Mean $\pm$ SD	Range
1	5	58.8 $\pm$ 6.5	51.3–67.6	60.1 $\pm$ 3.3	54.8–63.1
2	4	52.4 $\pm$ 11.4	36.2–62.2	56.5 $\pm$ 7.6	51.3–67.6
3	5	105.2 $\pm$ 11.5	89.1–116.7	96.9 $\pm$ 10.4	87.8–114.7
4	5	68.8 $\pm$ 6.2	59.6–74.7	72.0 $\pm$ 8.9	60.3–83.7
5	4	101.0 $\pm$ 14.0	87.2–116.0	91.4 $\pm$ 3.7	85.9–93.6

* Sampling period.

† Number of paired samples per sampling period.

lector rods were evaluated using a paired comparisons *t* test. Our null hypothesis was that differences in recovery would not be statistically significant.

The standard deviations of the two test populations were compared using an *F* statistic. We hypothesized that the variance would be greater in the hand-greased population, thus the *P* value associated with this test was for a one-tailed test.

RESULTS

Twenty-three paired samples (46 collector rods total) were obtained during five sampling periods (Table 2).

Pollen recovery by the hand-greased and dipped collector rods was similar (*P* = 0.410).

Dipped collector rods offered a lower standard deviation than hand-greased collector rods during 4 of the 5 sampling periods. A formal analysis demonstrated, however, that differences in the standard deviations between groups were not statistically significant (*F* = 1.782, *P* = 0.087).

Dipped collector rods required a shorter period of time to coat with silicone grease than hand-greased collector rods when prepared in large numbers. Several thousand collector rods could be dipped per hour; at best, several hundred collector rods could be prepared by hand during the same time period.

Hand-greased collector rods did not accept Calberla solution as well as dipped collector rods. Pollen grains on the former collector rods were often poorly stained or not stained; this made counting these grains more difficult, time-consuming, and subjective.

Dipped collector rods were easier to analyze than hand-greased collector rods. The silicone grease applied to the former collector rods was apparently thinner and/or more uniform in depth than the latter. Less microscope refocusing was thus required because the pollen grains occupied a relatively narrow optical plane. Although a formal time trial was not conducted, hand-greased collector rods required approximately 10 to 20 minutes more time to process compared with their dipped counterparts.

Dipped collector rods seemed to have a "cleaner" appearance than hand-greased collector rods. The latter collector rods contained microscopic air bubbles within the silicone grease that complicated visual analysis. It was not established whether these bubbles existed in the native silicone grease or were introduced when the grease was applied to the collector rods.

DISCUSSION

This investigation presented a new method for coating Rotorod collector rods with silicone grease. Collector rods were dipped into a solution composed of silicone grease and hexane, a highly volatile organic solvent. Pollen recovery by these dipped collector rods was then compared under field conditions to collector rods prepared via the standard, manual method. Four principal conclusions emerged.

First, there were no statistically significant differences in pollen recovery between the dipped and hand-greased collector rods. This finding was reassuring because collector rods have been prepared by hand for several de-

cades. It thus seems that pollen counts obtained with dipped collector rods can be directly compared with data obtained via the standard manual method.

Second, dipped collector rods appeared to offer a more reproducible sample than hand-greased collector rods. The standard deviation was less in the former population in 4 of the 5 sampling periods in this investigation. Although differences were not statistically significant, these data suggested that dipped collector rods offered less rod-to-rod variability.

Third, dipped collector rods took less time to prepare and analyze than hand-greased collector rods. Time savings were accrued because dipped collector rods were prepared en masse and required less microscope refocusing during sample analysis. The financial benefits of these time savings have been obvious to the authors in several drug studies where thousands of collectors rods have been required.

Last, the visual appearance of the dipped collector rods was superior to hand-greased collector rods. Pollen grains were often unstained on the latter collector rods. A likely explanation for this phenomenon was that these pollen grains penetrated into areas with relatively thick silicone grease.¹⁵ The silicone grease, a very hydrophobic compound, made these grains less accessible to Calberla solution. The silicone grease on the hand-greased collector rods was also found to contain small air bubbles that degraded overall image quality. These problems were not encountered with the dipped collector rods.

When taken together, these conclusions suggested that dipped collector rods offered a time-efficient means to obtain atmospheric samples with excellent visual quality. The resulting pollen counts were similar to data obtained via the standard, manual method. Allergists are encouraged to consider using this new method in their office practices and for drug studies.

APPENDIX

This investigation, a feasibility study, was conducted during the 1999 pollen season. The key objective finding—that dipped collector rods yielded similar estimates of the atmospheric pollen concentration as hand-greased collector rods—permitted a larger field study during the 2000 pollen season. The purpose of this field study was to test the subjective conclusions presented in the body of this paper.

Dipped collector rods were supplied to 20 experienced Rotorod users in cities across the United States. These individuals were asked to compare the “readability,” or visual quality, of dipped collector to hand-greased collector rods. A five-point scale was used: 1 indicated that the dipped collector rods compared poorly to hand-greased collector rods and 5 indicated that they compared favorably. The resulting scores were distributed as follows: 1, 1 user; 2, 1 user; 3, 2 users; 4, 4 users; and 5, 12 users.

Representative positive user comments were thus: “consistency of grease [on dipped collector rods] is wonderful from rod to rod;” “[dipped collector rods have a] uniform coat of grease;” “[dipping collector rods at the factory] prevented globbing/areas too thin;” “[dipped collector rods]

stained more evenly, quicker;” “[dipped collector rods were] easier to count—there were no streaks on the rods;” and “counts [obtained with dipped collector rods] looked good—less mess for me.”

Representative negative user comments were thus: “grease on the [dipped collector] rods seemed thicker;” and “[grease on dipped collector rods contains] too many air bubbles.”

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