

A RAND NOTE

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Does Pooling Saliva for Cotinine Testing Save Money Without Losing Information?

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Although testing for cotinine in saliva offers an attractive means to verify survey responses about recent use of tobacco, its relatively high cost prevents its use in many studies of substance use. Pooling two or more samples can dramatically reduce the cost when prevalence rates are low, but many researchers fear that failures in detecting users will outweigh the monetary benefit. Results from pools of two saliva specimens collected from seventh-grade students provide the first empirical evidence that pooling saves money without compromising the test's accuracy to detect recent tobacco use. Pooling successfully identified all specimens near or above the 10-ng/ml threshold for evidence of active tobacco exposure. We conclude that analysts can realize substantial savings by pooling saliva samples from young populations without losing valuable information.

KEY WORDS: cotinine; smoking; adolescents; pooling.

INTRODUCTION

Although testing for cotinine in saliva offers an attractive method for measuring active exposure to tobacco and verifying self-reports of recent smoking, its relatively high cost prevents its use in many studies of substance

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use (Biglan and Ary, 1985; Pechacek *et al.*, 1984). Several researchers have performed the less expensive tests for thiocyanate or carbon monoxide; others have collected saliva but either not analyzed any specimens (Botvin *et al.*, 1984) or analyzed only a few (McAlister *et al.*, 1982). Pooling two or more samples can dramatically reduce the cost when prevalence rates are low, but many researchers fear that corresponding increases in information loss will outweigh the monetary benefit.

This study uses the results of saliva tests from young adolescents to assess the impact of pooling (two-stage testing) on both the cost of cotinine testing and the resulting quality of information. Specifically, we estimate the probability that saliva specimens at various concentration levels would fail to be detected by the procedure.

METHODS

In Stage 1 of the procedure, two or more specimens are combined to form a "pool," which is tested in the standard way. Specimens are tested individually in Stage 2 only if the test result for the Stage 1 pool exceeds a prespecified trigger value.

Saliva specimens from 1404 seventh-grade students were tested for cotinine, using radioimmunoassay procedures described by Haley *et al.* (1983). Saliva specimens were packed in dry ice within a few hours of being collected and remained frozen until they were assayed. Most students provided at least 1 ml of saliva. Each individual test required .025 ml of saliva, mixed with .250 ml of buffer. For tests of pools, .0125 ml of saliva from each test tube was added directly to the buffer.

The assay was designed to have the greatest accuracy at and above 10 ng/ml, the lowest concentration at which active exposure to tobacco can be reliably distinguished (Jarvis *et al.*, 1988; Carey and Abrams, 1988). Most nonsmokers attain a concentration level of less than 2 ng/ml, with values at or above 10 ng/ml being rare or nonexistent (Jarvis *et al.*, 1985; Hoffmann *et al.*, 1984); hence, the risk of incorrectly labeling self-reported nonusers as liars based on a 10-ng/ml cutoff point is very small.

To evaluate two-stage testing, we want to compare the benefit of reducing the number of tests required against the potential cost of losing information by not detecting specimens with positive cotinine levels. We define the benefit (cost *saving*) as the reduction in the expected number of cotinine tests required to process 100 specimens. The expected number of tests per 100 specimens is given by

$$\begin{aligned} \text{expected number of tests} &= (\text{Stage 1 test}) + (\text{Stage 2 tests}), \\ &= 100/k + 100 \times p(k), \end{aligned}$$

where k is the pool size and $p(k)$ is the probability that a pool of size k triggers Stage 2. The expected saving is

$$\text{saving} = 100 \times [(k-1)/k - p(k)].$$

As one increases k to reduce the number of Stage 1 tests, we would expect $p(k)$ to grow, requiring more Stage 2 tests. We set the pool size (k) at 2 for this analysis, because laboratory tests of larger pools yielded unsatisfactory reproducibility of test values.

To assess the information loss of pooling—how often the procedure misses specimens with substantial positive values—we define the *trigger rate*, $t(x)$, as the probability that a positive specimen will belong to a triggered pool. It is a function of the specimen's true value x . Ideally, $t(x)$ would equal 1.0 for all true values that interest the analyst ($x > 10$ in our case) but drop close to 0 for uninteresting values (e.g., $x < 5$).

Data on information loss came from two data sets, which were combined to estimate the trigger rate function. Specimens from each data set were tested both individually and as part of a two-member pool. One set consisted of 303 specimens that were initially tested as part of a pool, and later tested individually; these specimens came from students whom we judged likely to yield positive cotinine values because they had either admitted recent use or avoided answering the relevant questions. The second set included 56 specimens with individual cotinine values ranging from 2 to 16 ng/ml, most between 6 and 11 ng/ml. Each of these 56 samples was paired with a negative specimen to form a pool for cotinine testing. Using the resulting information, we then calculated the empirical trigger rate at different values of x (i.e., the proportion of positive pools out of all pools where the positive individual specimen scored x). We employed isotonic regression to make the function monotonic (Barlow *et al.*, 1972).

RESULTS

Two-stage testing detected all specimens near or above the 10 ng/ml threshold for evidence of active tobacco use. Indeed, the function approached 1.0 for values of x over 5.0 ng/ml and equaled 1.0 for values that exceeded 8.0 ng/ml (Table I). A one-sided 95% confidence interval for $t(10)$ extends from 85.0 to 100%. There is no reason to expect that these results would change for a different population.

The two-stage procedure also yielded dramatic improvement in the efficiency of cotinine testing. With a population of seventh-grade students, it reduced costs by 40%—requiring 844 tests of a possible 1404. From another

Table 1. Estimated Trigger Rate as a Function of the Individual Test Value of the Positive Specimen

Mean value on individual test (ng/ml) ^a	Estimated trigger rate ^b	Sample size
2.0-4.5	.15	27
5.0	.63	8
5.5-6.5	.89	9
7.0-8.0	.92	12
8.5-9.5	1.00	14
10-12	1.00	14

^aEach positive specimen was tested individually twice.

^bBased on isotonic regression.

perspective, we processed 1404 specimens for the cost of 844 tests. Thus, the 40% reduction in the cost per test translates into *66% more* tests for the same cost ($1404/844 = 1.66$). At costs of 20 dollars or more per test, the savings can be substantial.

DISCUSSION

These results demonstrate that pooling saliva samples can provide substantial cost reductions without entailing corresponding losses of important information. Analysts can rest assured that pooling will identify all individuals whose cotinine values indicate active exposure to tobacco (above 10 ng/ml). Because the cost reductions are greater when the prevalence rate is low, two-stage testing is particularly relevant for studies of active exposure among school-age children. It is less appropriate for studies of older populations with higher rates of use or for studies of the effects of passive smoking where values of 5 ng/ml or less are important. Applying the procedure to plasma samples might yield similar advantages but requires future evaluation.

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