POOLING TECHNIQUES FOR BIOASSAY SCREENING[1]

L.C. Sun, J.W. Baum, E. Kaplan, and A.R. Moorthy

Brookhaven National Laboratory, Upton NY 11973-5000

ABSTRACT

Pooling techniques commonly are used to increase the throughput of samples used for screening purposes. While advantages of such techniques are increased analytical efficiency and cost savings, the sensitivity of measurements decreases because it is inversely proportional to the number of samples in the pools. Consequently, uncertainties in estimates of dose and risk which are based on the results of pooled samples increase as the number of samples in the pools increases in all applications. However, sensitivities may not be seriously degraded, for example, in urinanalysis, if the samples in the pools are of known time duration, or if the fraction of some attribute of the grab urine samples to that in a 24-hour composite is known (e.g., mass, specific gravity, creatinine, or volume, per 24-h interval). This paper presents square and cube pooling schemes that greatly increase throughput and can considerably reduce analytical costs (on a sample basis). The benefit-cost ratios for 5x5 square and 5x5x5 cube pooling schemes are 2.5 and 8.3, respectively. Three-dimensional and higher arrayed pooling schemes would result in even greater economies; however, significant improvements in analytical sensitivity are required to achieve these advantages. These are various other considerations for designing a pooling scheme, where the number of dimensions and of samples in the optimum array are influenced by: 1) the minimal detectable amount (MDA) of the analytical processes, 2) the screening dose-rate requirements, 3) the maximum masses or volumes of the composite samples that can be analyzed, 4) the information already available from results of composite analysis, and 5) the ability of an analytical system to guard against both false negative and false positive results. Many of these are beyond the scope of this paper but are being evaluated.

INTRODUCTION

Often a large number of samples must be analyzed to screen them for any possible high values. If the costs of analysis are high, it may be cost-effective to combine (pool) several samples and analyze the pool, or a fraction of it, to decide if specific analyses of the individual samples in the pool are justified. In 1987, a fission track analysis (FTA) method was developed at Brookhaven National Laboratory (BNL) (1) and used for plutonium urinalysis for the Marshallese (2,3). Although sample analysis using the FTA method is expensive, because of the FTA's ultra-low level of detection sensitivity, pooling methods given in this paper can be used to increase the throughput of samples, saving both time and money.

POOLING SCHEMES AND TECHNIQUES

In the same available time, square and cube pooling schemes allow a significantly larger throughput of samples than making individual analyses, and hence can considerably reduce analytical costs (on a per sample basis). For a square matrix composite method, a total of N^2 (N is an integer) individual samples can be pooled in batches of 2N single composite samples; the composite samples are pooled from the corresponding rows and columns, respectively. Once all composite samples have been analyzed, no further testing is needed for determining individual dose levels. In a square composite pooling scheme, all rows' and columns' test samples can check and verify for each other. This thesis applies also to a multidimensional pooling scheme. Therefore, the additional advantage of using a square matrix or multidimensional pooling scheme over a simple composite pooling method is reducing the probability for false positive and false negative results.

In a square pooling matrix, the probability of two composite samples, from an intersecting row and an intersecting column, being false positive is p^2. There is a 10% probability of false positives in the existing BNL FTA system established for plutonium dose assessments; therefore, the chance of generating positive results in both a column and row composite urine is reduced to 1% (0.1^2). Similarly, if spike (e.g., calibration) samples are used in a pooling scheme, then the probability of false negative results also can be improved because positive results in each intersecting composite must be identified and be traceable for all spiked samples. Overall, the final

[1]This work was supported by the U.S. Department of Energy under contract No. DE-AC02-76CH00016.

positive results are only accepted as positive, for example, using a square matrix method, when both the row and column composite results are positive simultaneously, and both results also are within a factor of two of each other. The criterion of 2 can be changed according to the precision required of the measurements and the mass balance of plutonium activity in the samples. Therefore, all "non-coincident" row and column or array composite results are to be treated as false positive outcomes and should be eliminated for dose calculations. These include the following three cases: 1) only one out of the ten test samples is positive, 2) only multiple row (or columns) composite test samples are positive, 3) all imbalanced (greater than outside preset limits) plutonium activity in all positive coincident rows and column results.

The square matrix composite pooling approach can be generalized further to three and higher dimensions. Significant improvements in analytical sensitivity would be required for four-dimensional and higher arrays. Table 1 illustrates several options including square, cube, and four (quad) dimension arrays. For example, a 3x3x3 array would allow 27 individual urine samples to be linked using 9 composites, with portions of 9 samples in each composite. Each sample is uniquely identified at the intersection of three composites. Further, the benefit-cost ratios in a 5x5 square matrix and a 5x5x5 cube schemes are 25/10=2.5 and 125/15=8.3, respectively. Three-dimensional arrayed pooling schemes and higher ones would result in even greater economies, as the last column of Table 1 shows. However, significant improvements in analytical sensitivity are required to achieve these advantages. The probability of accepting false positives is reduced as p^n, where n is the dimension of the pooling array. Although we do not expect improvements in the probabilities of avoiding false negatives for screening the maximum individual outcomes, this is unimportant for the FTA system.

Table 1. Multidimensional Pooling Options

Array		No. of Samples	Samples per Composite	Volume of Each Urine Sample	Composites Analyzed	Benefit-Cost Ratio
Dimensions	**Size**					
Square	5x5	25	5	1/5	10	25/10=2.5
Square	10x10	100	10	1/10	20	100/20=5.0
Cube	3x3x3	27	9	1/9	9	27/9=3.0
Cube	4x4x4	64	16	1/16	12	64/12=5.3
Cube	5x5x5	125	25	1/25	15	125/15=8.3
Quad	4x4x4x4	256	64	1/64	16	256/16=16

The number of dimensions and samples in the optimum array is influenced by: 1) the MDA of analytical processes, 2) the maximum masses or volumes of the composite sample that can be managed in the analytical process, 3) the information available from results of previous composite analyses, and 4) the criteria that the analytical system must meet to guard against both false negative and false positive results. Many of these, and other, considerations are beyond the scope of this paper, and are being evaluated for application in future pooling methodologies.

CONCLUSIONS

The main advantage for pooling urine samples (grab or 24-h) for bioassays is increased analytical efficiency and cost savings. The sample's screening power is inversely proportional to the duration of collection of a grab sample, and may be derived from a recommended guideline or a specific protection standard. Using a larger fraction of the collected individual sample lowers the screening goal. In cases where it is necessary to ensure that the dose rate of the maximally exposed individual in the pool is below some fixed value, pooling samples can be a viable option. It must be recognized that interpretations of the results of short-term samples are based on assumptions which require further verification (e.g., a constant rate of diurnal excretion; the ability of approximating the duration of grab samples using measurements of specific gravity or creatinine). There also may be no opportunity for analytical verification because small grab-sample volumes can be quickly depleted in the preliminary stages of pooling.

In general, for designing a pooling scheme, the optimum screening plan will depend on the following parameters: 1) the total number of samples to be analyzed, 2) the sensitivity (MDA) of the analytical method to be used, 3) the cost per sample to be analyzed, and 4) desired screening goals or acceptable sensitivity limit. We are making further studies to optimize this multidimensional approach.

ACKNOWLEDGMENT

The authors wish to thank Maria Beckman for her help in preparing this manuscript and Avril Woodhead for her editorial assistance.

REFERENCES

1. A.R. Moorthy, C. J. Schopfer, and S. Banerjee, *Anal. Chem.* 60: 857A (1988).
2. L.C. Sun, A.R. Moorthy, E. Kaplan, et al. *Appl. Rad. and Isotop.* 46(11): 1259-1269 (1995).
3. L.C. Sun, C.B. Meinhold, A.R. Moorthy, et al., *Proceedings of IRPA8,* Vol. 2, 1320-1323 (1992).