

SUPPLEMENTAL DOCUMENT SD-8
FOR PART IIIA – Methods of Analysis/Sampling Seized Drugs for Qualitative
Analysis
Encountering Negative Results in a Statistical Sampling Plan

Introduction

Statistical sampling is utilized when an inference about the entire population is desired. This supplemental document provides guidance via various example scenarios in which negative results are encountered when using a hypergeometric sampling plan.

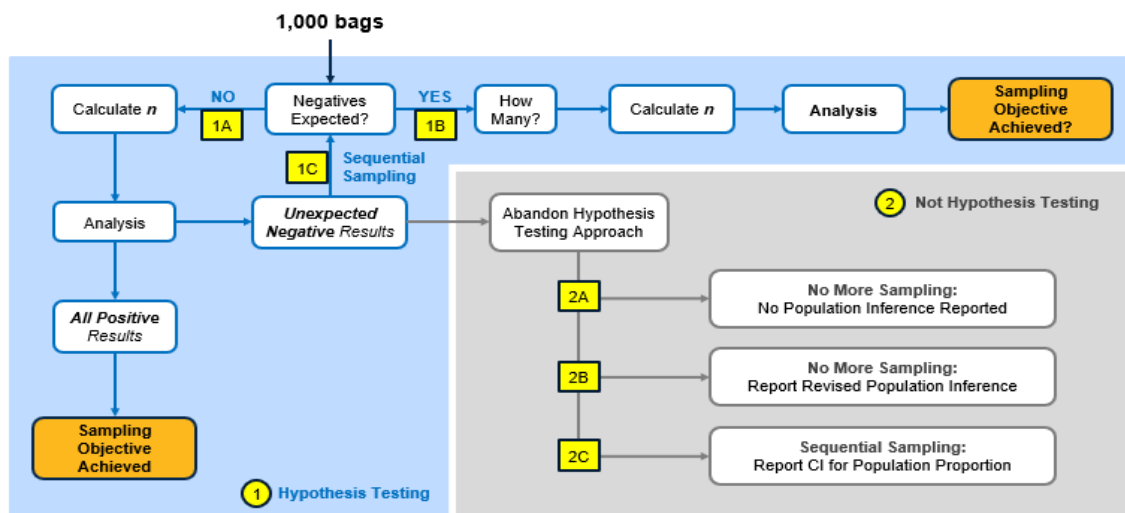
Scenario:

A laboratory receives an exhibit containing 1000 bags of white powder suspected to contain fentanyl.

Objective:

The analyst wants to use statistically based sampling without replacement to determine an appropriate number of bags to analyze and the appropriate conclusions to report based on the results obtained. A statistical sampling plan is devised with the objective of reporting an inference regarding 70% of the population with a minimum 95% level of confidence¹. The flowchart shown in Figure 1 provides a roadmap to follow.

Figure 1. Sampling Roadmap



Example 1 will discuss three options within a hypothesis testing framework (1A, 1B, and 1C in Figure 1). Example 2 will discuss three options available after abandoning the hypothesis testing approach (2A, 2B, and 2C in Figure 1).

¹ Significance level (α) = 0.05 corresponding to 5% probability of incorrectly rejecting a true null hypothesis (Type I error)

Example 1: Hypothesis Testing

A hypothesis testing approach is utilized wherein the analyst assesses the sampling objective and devises a set of complementary hypotheses. If enough evidence is gathered (via testing) to reject the null hypothesis (with a maximum tolerable error rate), then H_1 is reported (at the desired level of confidence) and the objective of the sampling plan is achieved.

In this instance, there are two possible hypotheses or outcomes:

Null Hypothesis (H_0): < 700 bags contain fentanyl

Alternative Hypothesis (H_1): $\geq$ 700 bags contain fentanyl

Procedure:

1. Assess how many sampled bags may produce a negative result.

Population determination considers the question being asked to minimize inconsistencies. Thus, it is typically assumed that all sampled units contain a drug of interest or are positive for a given characteristic (e.g. opiates).

One or more units in the sample set may not contain the drug of interest or demonstrate the given characteristic (e.g. contains methamphetamine instead of an opiate). These samples are considered “negatives” in the context of statistical sampling and based on the hypotheses proposed above.

2. Use a hypergeometric sampling calculator (See References 1 and 2) to determine the number of samples (n) that must be analyzed and found positive in order to reject the null hypothesis at a desired level of confidence, given an anticipated number of negative results.

Option 1A. No anticipated negative results

If no negative results are anticipated and the desired level of confidence is 95%, the hypergeometric sampling calculators indicate that 9 bags must be randomly selected and analyzed, as seen in Figure 2.

Figure 2. a) ENFSI Sampling calculator and b) NIST Sampling App showing how many bags to sample given no anticipated negative samples.

Calculation based on minimal PROPORTION (k) of positive samples in population N

At least requested proportion (k) of population (N) contain drugs at a confidence level of at least $(1-\alpha)$, if $(n-r)$ tested samples are positive.

Enter data (steps 1 to 4) and see results (steps 5 to 7)		Max. population size $N_{\max}$ allowed (current value):
		2000
Step 1: Enter population size (N) = number of samples in population:	1000	Step 6: Actual proportion (k^*) for sample size n ▼
Step 2: Enter proportion (k) of positives in population: (range: $0.0001 < k \leq 1$)	0.7	0.70000
Step 3: Enter expected number of negatives (r): (at max. 2)	0	Step 7: Actual CL for sample size n ▼
Step 4: Enter confidence level (CL) ($1-\alpha$) (range: $0.0001 \leq CL \leq 0.9999$)	0.95	0.96078
Step 5: Sample size (n)	9	

a.

Lower Confidence Bounds for Seized Material Sampling

Identification Counting Disclaimer

Population Size
1000

Significance Level (alpha)
0.05

Desired Minimum Proportion/Sample Size
Minimum Proportion ▼

Targeted Minimum Proportion of Population
0.70

Negatives to Expect while Maintaining Minimum Proportion
0

Recommended Sample Size: 9.

Observed.Positives	Lower.Confidence.Bounds	Inferred.Proportion
9	719	0.719
8	573	0.573
7	452	0.452
6	347	0.347
5	253	0.253
4	170	0.17
3	99	0.099
2	42	0.042
1	6	0.006
0	0	0

b.

See Appendix A1 for the equation used to calculate sample size, $n = 9$.

The probability of randomly selecting 9 bags and having them all test positive is too low to occur by chance alone (below established significance level of 0.05) if only 699 of the 1000 bags contain fentanyl (the null hypothesis scenario). Therefore, if fentanyl is identified in all 9 bags, we can reject H_0 and accept H_1 , that there are ≥ 700 bags that contain fentanyl in the population.

The results of the analysis can be reported in various ways. For example:

- A) A total of 1000 bags were received. By using statistical sampling and analysis of 9 bags, all of which contain fentanyl, it is concluded at a 95% level of confidence that at least 70% of the population contains fentanyl.
- B) A total of 1000 bags were received. Using statistical (hypergeometric) sampling and by testing 9 bags, it is concluded that fentanyl is present in at least 700 bags at a level of confidence of at least 95%.

Option 1B. Anticipated negative results

Using the hypergeometric calculators, sampling plans can be proposed when negative results are anticipated. Allowing for 1 negative sample, it is determined that 14 bags would need to be randomly selected and tested, as seen in Figure 3. If testing results in all positives or only one negative, then rejection of the null hypothesis is supported. Thus, it can be concluded that over 70% of the 1000 bags contain fentanyl.

Figure 3. a) ENFSI Sampling calculator and b) NIST Sampling App showing how many bags to sample given one anticipated negative result

Calculation based on minimal PROPORTION (k) of positive samples in population N		
At least requested proportion (k) of population (N) contain drugs at a confidence level of at least (1- α), if (n-r) tested samples are positive.		
Enter data (steps 1 to 4) and see results (steps 5 to 7)		Max. population size N_{max} allowed (current value):
		2000
Step 1: Enter population size (N) = number of samples in population:	1000	Step 6: Actual proportion (k') for sample size n ▼
Step 2: Enter proportion (k) of positives in population: (range: 0.0001<k<=1)	0.7	0.70000
Step 3: Enter expected number of negatives (r): (at max. 2)	1	Step 7: Actual CL for sample size n ▼
Step 4: Enter confidence level (CL) (1- α) (range: 0.0001<=CL<=0.9999)	0.95	0.95436
Step 5: Sample size (n)	14	

a.

Lower Confidence Bounds for Seized Material Sampling

b.

Identification Counting Disclaimer

Population Size

Significance Level (alpha)

Desired Minimum Proportion/Sample Size

Targeted Minimum Proportion of Population

Negatives to Expect while Maintaining Minimum Proportion

Recommended Sample Size: 14.

Observed.Positives	Lower.Confidence.Bounds	Inferred.Proportion
14	809	0.809
13	705	0.705
12	617	0.617
11	536	0.536
10	462	0.462
9	392	0.392
8	327	0.327
7	265	0.265
6	208	0.208
5	154	0.154
4	105	0.105

See Appendix A2 for the equation used to calculate sample size, $n = 14$.

The probability of randomly selecting 14 bags and having all, *or all but one*, test positive is too low to occur by chance alone (below established significance level) if only 699 of the 1000 bags contain fentanyl.

Therefore, H_0 is rejected and H_1 , that there are ≥ 700 bags that contain fentanyl in the population, is accepted.

The results of the analysis can be reported in various ways. For example:

- A) A total of 1000 bags were received. By using statistical sampling and analysis of 14 bags, 13 of which contained fentanyl, it is concluded at a 95% level of confidence that at least 70% of the population contains fentanyl.
- B) A total of 1000 bags were received. Using statistical (hypergeometric) sampling and by testing 14 bags, it is concluded that fentanyl is present in at least 700 bags at a level of confidence of at least 95%.

Option 1C. Sequential sampling to support the original sampling objective after encountering unanticipated negative results

In accordance with SWGDRUG Recommendations Part IIIA.4.1, the original sampling objective, to infer that at least 70% of the 1000 bags contain fentanyl at a 95% level of confidence, would be invalidated upon encountering a negative result in the 9 bags sampled in Option 1A.

It is not statistically correct to simply replace the negative sample with an additional bag or to analyze additional samples to reach the number of bags that would have been sampled if the negative result was originally accounted for. In other words, it

would not be appropriate to analyze 5 additional bags to bridge the gap between a sampling plan devised when no negative results are expected (Option 1A - 9 samples) versus the allowance of 1 negative result (Option 1B - 14 samples).

If the goal is to reject H_0 (or accept H_1) and infer that there are ≥ 700 bags that contain fentanyl in the population, then the 8 established positives can be used to reduce the remaining number of bags needed to be inferred to be positive from 700 to 692 bags. Meaning that a population re-assessment must take place prior to subsequent sampling.

The new sampling question is how many bags out of the remaining 991 (1000-9) must be sampled to infer, with a 95% level of confidence, that ≥ 692 additional bags contain fentanyl. The new set of hypotheses being tested is:

Null Hypothesis (H_0): < 692 bags contain fentanyl

Alternative Hypothesis (H_1): ≥ 692 bags contain fentanyl

Using these new values, the hypergeometric sampling calculators can be used again to assess how many additional samples must be randomly selected and analyzed, as seen in Figures 4 and 5. As before, a number of anticipated negative results must be specified. Laboratories should exercise caution when assessing whether additional negative samples are anticipated. Since one or more negative results were observed during the first round of sampling, such information should be considered when forecasting subsequent sampling rounds.

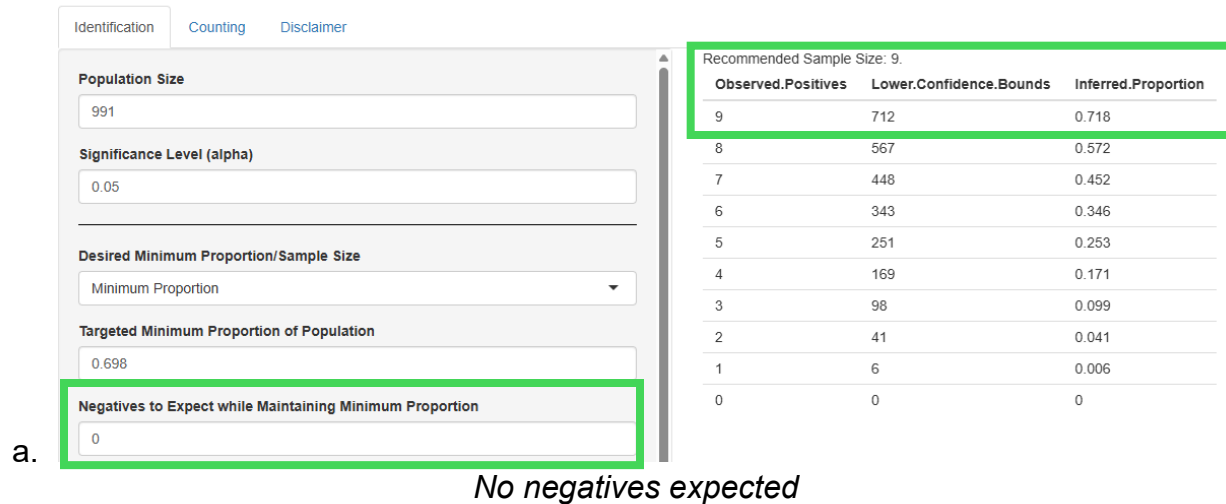
Figure 4. The ENFSI sampling calculator showing how many additional samples are required based on a) no anticipated additional negatives and b) one anticipated additional negative

Calculation based on NUMBER (K) of positive samples in population N			Calculation based on NUMBER (K) of positive samples in population N		
Exactly requested number (K) of population (N) contain drugs at minimum at requested confidence level (1- α), if (n-r) tested samples are positive!			Exactly requested number (K) of population (N) contain drugs at minimum at requested confidence level (1- α), if (n-r) tested samples are positive!		
Enter data (steps 1 to 4) and see results (Steps 5 to 7) :		Max. population size N_{max} allowed (current value):	Enter data (steps 1 to 4) and see results (Steps 5 to 7) :		Max. population size N_{max} allowed (current value):
		1000			1000
Step 1: Enter population size (N) = number of samples in population:	991	Step 6: Actual proportion (k') for sample size n	Step 1: Enter population size (N) = number of samples in population:	991	Step 6: Actual proportion (k') for sample size n
Step 2: Enter number of positives (K) in population: (range 1<K<=N)	692	0.69828	Step 2: Enter number of positives (K) in population: (range 1<K<=N)	692	0.69828
Step 3: Enter expected number of negatives (r): (at max. 2)	0	Step 7: Actual CL for sample size n	Step 3: Enter expected number of negatives (r): (at max. 2)	1	Step 7: Actual CL for sample size n
Step 4: Enter confidence level (CL) (1- α) (range:0.0001<=CL<=0.9999)	0.95	0.96165	Step 4: Enter confidence level (CL) (1- α) (range:0.0001<=CL<=0.9999)	0.95	0.95562
Step 5: Sample size (n)	9		Step 5: Sample size (n)	14	

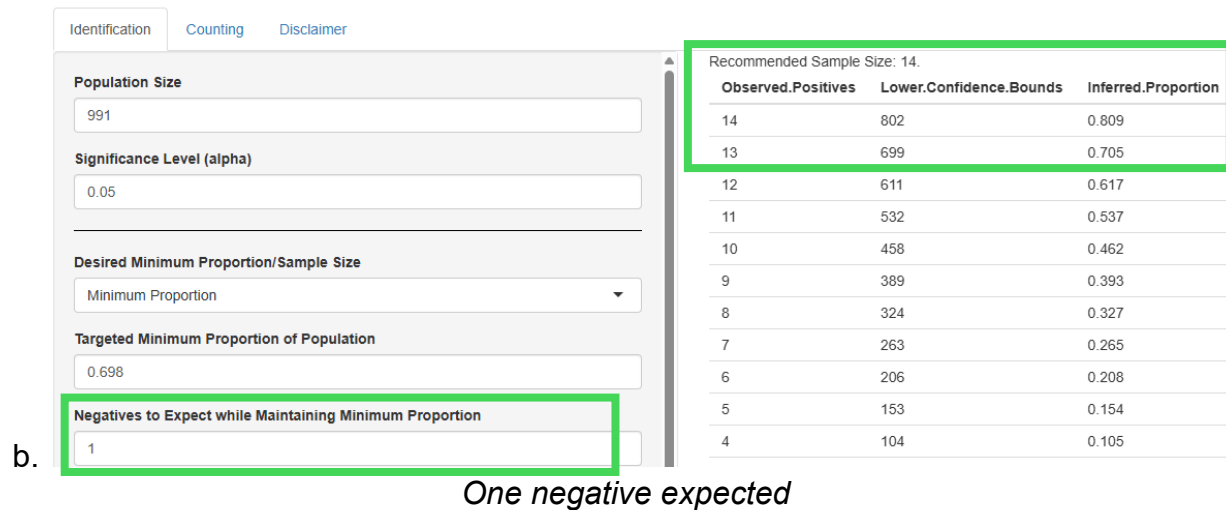
a. *No negatives expected* b. *One negative expected*

Figure 5. The NIST sampling app showing how many additional samples are required based on a) no anticipated additional negatives and b) one anticipated additional negative

Lower Confidence Bounds for Seized Material Sampling



Lower Confidence Bounds for Seized Material Sampling



Note: Not all sampling calculators have the option to specify a number versus a proportion of population to make an inference about, so the number may need to be manually converted into a proportion.

See Appendices A1 and A2 for the equations used to calculate sample size for no negatives expected and one negative expected, respectively.

When no additional negatives are considered, the calculators state that an additional 9 samples need to be randomly selected and analyzed. If an additional negative is anticipated, then 14 additional samples would be randomly selected and analyzed.

If the results are, once again, not consistent with the assumption made, then further sequential sampling may be performed, or one of the non-hypothesis testing approaches in Example 2 may be utilized.

The results can be reported in various ways. For example:

- A) A total of 1000 bags were received. By using sequential statistical sampling and analysis of 18 bags, 17 of which contained fentanyl, it is concluded at a 95% level of confidence that at least 70% of the population contains fentanyl.
- B) A total of 1000 bags were received. Using statistical (hypergeometric) sampling and by testing 23 bags, and finding fentanyl present in 21 of them, it is concluded that fentanyl is present in at least 700 bags at a level of confidence of at least 95%.

Appendix A1

To manually determine the number of bags, n , to be tested using the established significance level of 0.05 (95% level of confidence), we need to obtain enough evidence to reject the null hypothesis that there are only 699 bags containing fentanyl in the entire population (H_0).

This is done by using the hypergeometric distribution equation (Eq 1.) to calculate the probability (p -value) of obtaining a positive result at successive sample sizes, n , until it falls below the established significance level.

$$P_k = \frac{\binom{K}{k} \binom{N-K}{n-k}}{\binom{N}{n}} \text{ where } \binom{a}{b} = \frac{a!}{b!(a-b)!} \quad [\text{Eq. 1}]$$

N = Total number of bags in the population

K = Total number of positive bags in the population

n = Number of bags analyzed

k = Number of positive bags analyzed

When $k = n$, reflecting no anticipated negative samples, the equation simplifies to:

$$\begin{aligned} P_n &= P_{(bag\ 1)} * P_{(bag\ 2)} * P_{(bag\ 3)} * P_{(bag\ 4)} * \dots * P_{(bag\ n)} \\ &= \frac{700-1}{1000} * \frac{700-2}{1000-1} * \frac{700-3}{1000-2} * \frac{700-4}{1000-3} * \dots * \frac{700-n}{1000-(n-1)} \\ &= P(\text{all } n \text{ bags in the sample set contain fentanyl}) \end{aligned}$$

The following calculations show the p -values (and resulting levels of confidence, LoC) obtained for each successive sample tested (with no negatives found) until a value below 0.05 is obtained (which is sample 9).

$$P_1 = \frac{699}{1000} = 0.6990 \text{ (30.10\% LoC)}$$

$$P_2 = \frac{699}{1000} * \frac{698}{999} = 0.4884 \text{ (51.16\% LoC)}$$

$$P_3 = \frac{699}{1000} * \frac{698}{999} * \frac{697}{998} = 0.3411 \text{ (65.89\% LoC)}$$

$$P_4 = \frac{699}{1000} * \frac{698}{999} * \frac{697}{998} * \frac{696}{997} = 0.2381 \text{ (76.19\% LoC)}$$

$$P_5 = \frac{699}{1000} * \frac{698}{999} * \frac{697}{998} * \frac{696}{997} * \frac{695}{996} = 0.1662 \text{ (83.38\% LoC)}$$

$$P_6 = \frac{699}{1000} * \frac{698}{999} * \frac{697}{998} * \frac{696}{997} * \frac{695}{996} * \frac{694}{995} = 0.1159 \text{ (88.41\% LoC)}$$

$$P_7 = \frac{699}{1000} * \frac{698}{999} * \frac{697}{998} * \frac{696}{997} * \frac{695}{996} * \frac{694}{995} * \frac{693}{994} = 0.0808 \text{ (91.92\% LoC)}$$

$$P_8 = \frac{699}{1000} * \frac{698}{999} * \frac{697}{998} * \frac{696}{997} * \frac{695}{996} * \frac{694}{995} * \frac{693}{994} * \frac{692}{993} = 0.0563 \text{ (94.37\% LoC)}$$

$$P_9 = \frac{699}{1000} * \frac{698}{999} * \frac{697}{998} * \frac{696}{997} * \frac{695}{996} * \frac{694}{995} * \frac{693}{994} * \frac{692}{993} * \frac{691}{992} = 0.0392 \text{ (96.08\% LoC)}$$

Appendix A2

The hypergeometric probability formula (Eq. 1) allows for the calculation of the probability of encountering a particular number of positive samples (k).

To account for the possibility of up to 1 negative result, the individual probabilities of encountering either zero negatives ($k = n$) or one negative ($k = n - 1$) must be calculated. These probabilities are then added together for an overall probability of either situation occurring.

If only one sample is analyzed, the only possible outcomes are zero or one negative result. No inferences about untested bags are supported since it is guaranteed that one of those two results will occur (the total probability $P = 1.0$).

If two samples are analyzed, the following p -values are calculated:

$$\text{2 sampled (0 negatives): } P_{k=2} = \frac{\binom{699}{2} \binom{1000-699}{2-2}}{\binom{1000}{2}} = \frac{\frac{699!}{2!(699-2)!} * \frac{301!}{0!(301-0)!}}{\frac{1000!}{2!(1000-2)!}} = \frac{243951}{499500} = 0.4884$$

$$\text{2 sampled (1 negative): } P_{k=1} = \frac{\binom{699}{1} \binom{1000-699}{2-1}}{\binom{1000}{2}} = \frac{\frac{699!}{1!(699-1)!} * \frac{301!}{1!(301-1)!}}{\frac{1000!}{2!(1000-2)!}} = \frac{210399}{499500} = 0.4212$$

When p -values are combined, the total probability of encountering one or fewer negative results in 2 randomly selected samples, given 699 bags in the population are positive, is $0.4884 + 0.4212 = 0.9096$ (LoC = 9.039%).

Table A.1 shows the p -values (and resulting LoC) obtained for each successive sample tested (with zero or one negatives encountered) until a total p -value below 0.05 is obtained (which is sample 14).

Table A.1: Calculations for varying sample size, n

N	K	n	# of negatives	k	P _k	Total P	LoC
1000	699	2	0	2	0.4884	0.9096	9.04%
			1	1	0.4212		
		3	0	3	0.3411	0.7830	21.70%
			1	2	0.4419		
		...					
		9	0	9	0.03922	0.1930	80.70%
			1	8	0.15376		
		10	0	10	0.02731	0.1464	85.36%
			1	9	0.11913		
		11	0	11	0.01901	0.1103	88.97%
			1	10	0.09133		
		12	0	12	0.01322	0.0826	91.74%
			1	11	0.06941		
		13	0	13	0.00919	0.0616	93.84%
			1	12	0.05236		
		14	0	14	0.00639	0.0456	95.44%
			1	13	0.03925		

Example 2. Abandoning Hypothesis Testing

In accordance with SWGDRUG Recommendations Part IIIA.4.1, the original sampling objective, to infer that at least 70% of the 1000 bags contain fentanyl at a 95% level of confidence, would be invalidated upon encountering unexpected negative results within either the initial sample set or a sequential sample set. In this scenario, it may be desirable to abandon the hypothesis testing approach.

As illustrated in Figure 1, there are several options available:

- 2A – Sampling and analysis are stopped, and no inference is made about the contents of the population.
- 2B – Sampling and analysis are stopped, and the final population inference is revised.
- 2C – Additional bags are sampled and tested to generate a confidence interval for the population proportion.

Option 2A. No inference

A conservative approach to take when encountering unanticipated negative samples is to abandon making any statistical inferences. In this instance, only results for the tested items are reported and no inferences are made (reported) about the untested items.

It must be considered how the absence of a statistical inference will affect the end-user of the results. If 8 of 9 analyzed bags are reported to contain fentanyl, and the net weight of the entire population was extrapolated to exceed a statutory weight threshold, will the court draw its own, statistically unsupported, inference regarding the contents of the unanalyzed bags?

While this scenario leads to the reporting of unambiguous results, efforts should be made to ensure the needs of the end-user are met.

Option 2B. Revising the inference

In accordance with SWGDRUG Recommendations Part IIIA.3.2.2.3, hypothesis testing can be abandoned after encountering unexpected negative results. This may still allow a revised inference to be made, if the samples have been randomly drawn.

Appropriate inferences include decreasing either the number of inferred positives in the population, K , or the reported level of confidence (LoC). [ASTM E2548-26]

Decreasing K :

Oftentimes it is not desirable to reduce the level of confidence given the presentation of seized drug evidence in court. As seen in Appendix B1, the hypergeometric equation (Eq. 1) can be used with decreasing K values to determine the lowest K value at which the probability of encountering the observed number of negatives or fewer is higher than the desired significance level. That reflects the inference that can be supported by the observed results.

This calculation is automatically performed by the NIST sampling app when initially determining the number of units to sample, as shown in Figure 6.

Figure 6. The NIST sampling app automatically provides alternative values of K that can be reported given differing numbers of observed positive results within a sample set. Note that this screenshot is the same as in Figure 2b.

Lower Confidence Bounds for Seized Material Sampling

The screenshot shows the NIST sampling app interface. On the left, there are input fields for Population Size (1000), Significance Level (alpha) (0.05), Desired Minimum Proportion/Sample Size (Minimum Proportion), Targeted Minimum Proportion of Population (0.70), and Negatives to Expect while Maintaining Minimum Proportion (0). On the right, there is a table titled 'Recommended Sample Size: 9.' with three columns: Observed.Positives, Lower.Confidence.Bounds, and Inferred.Proportion. The table lists values for observed positives from 9 down to 0. The row for 8 observed positives is highlighted with a green border.

Observed.Positives	Lower.Confidence.Bounds	Inferred.Proportion
9	719	0.719
8	573	0.573
7	452	0.452
6	347	0.347
5	253	0.253
4	170	0.17
3	99	0.099
2	42	0.042
1	6	0.006
0	0	0

The ENFSI sampling calculator can also be used, as seen in Figure 7, by keeping the significance level (α) constant and determining the revised K associated with the given values of N and n , and the observed positives, k . It should be noted that the ENFSI calculator was primarily designed for the purpose of hypothesis testing rather than retrofitting a statistical model after analysis.

Figure 7. The ENFSI sampling calculator showing that a K value of 574 (a) would require a sample size of 10, given the presence of 1 negative result, but a K value of 573 (b) only requires a sample size of 9, given the presence of 1 negative result.

ε Calculation based on NUMBER (K) of positive samples in population N Exactly requested number (K) of population (N) contain drugs at minimum at requested confidence level (1-α), if (n-r) tested samples are positive!			ι Calculation based on NUMBER (K) of positive samples in population N Exactly requested number (K) of population (N) contain drugs at minimum at requested confidence level (1-α), if (n-r) tested samples are positive!		
Enter data (steps 1 to 4) and see results (Steps 5 to 7) :		Max. population size N_{max} allowed (current value):	Enter data (steps 1 to 4) and see results (Steps 5 to 7) :		Max. population size N_{max} allowed (current value):
		1000			1000
Step 1: Enter population size (N) = number of samples in population:	1000	Step 6: Actual proportion (k') for sample size n ▼	Step 1: Enter population size (N) = number of samples in population:	1000	Step 6: Actual proportion (k') for sample size n ▼
Step 2: Enter number of positives (K) in population: (range 1<K<=N)	574	0.57400	Step 2: Enter number of positives (K) in population: (range 1<K<=N)	573	0.57300
Step 3: Enter expected number of negatives (r): (at max. 2)	1	Step 7: Actual CL for sample size n ▼	Step 3: Enter expected number of negatives (r): (at max. 2)	1	Step 7: Actual CL for sample size n ▼
Step 4: Enter confidence level (CL) (1-α) (range: 0.0001<=CL<=0.9999)	0.95	0.96839	Step 4: Enter confidence level (CL) (1-α) (range: 0.0001<=CL<=0.9999)	0.95	0.95005
Step 5: Sample size (n)	10		Step 5: Sample size (n)	9	

This indicates that if the number of inferred positive bags in the population, K , is set at 573 and one negative was allowed, the number of required samples would be 9, which is exactly the scenario at hand.

Reporting language shall reflect the decrease in the number of bags in the population inferred to contain fentanyl. Examples are as follows:

- A) A total of 1000 bags were received. By using statistical sampling and analysis of 9 bags, 8 of which contained fentanyl, it is concluded at a 95% level of confidence that at least 57.3% of the population contains fentanyl.
- B) A total of 1000 bags were received. Using statistical (hypergeometric) sampling and by testing 9 bags, it is concluded that fentanyl is present in at least 573 bags at a level of confidence of at least 95%.

Analogous scenarios involving more than one negative can be addressed.

For example, as seen in the NIST sampling app in Figure 6, if only 5 of the 9 randomly selected bags had been found to contain fentanyl, it could be concluded at a 95% level of confidence that at least 253 bags contained fentanyl.

Notably, the ENFSI calculator is limited to a maximum of 2 negative samples.

Decreasing the LoC:

If priority is given to a jurisdictional weight threshold or to a proportion of the population on which to report a conclusion, the values for N , n , k , and the desired K can be used in conjunction with the hypergeometric equation (Eq. 1) or a sampling calculator to determine the level of confidence at which the final inference can be made.

For the example of a population made up of 1000 bags, 9 of which were randomly selected and 8 were found to contain fentanyl, the LoC can be decreased in the ENFSI sampling calculator until the sample size matches the number of analyzed samples, as seen in Figure 8. As with above, this modifies the original intended application of the ENFSI calculator from hypothesis testing (sampling plan established prior to analysis) to retrospectively determining the resulting decreased LoC.

Figure 8. The ENFSI sampling calculator showing that a confidence level $(1-\alpha)$ value of 0.8071 (a) would require a sample size of 10, given the presence of 1 negative result, but a confidence level $(1-\alpha)$ value of 0.8070 (b) only requires a sample size of 9, given the presence of 1 negative result.

Calculation based on minimal PROPORTION (k) of positive samples in population N			Calculation based on minimal PROPORTION (k) of positive samples in population N		
At least requested proportion (k) of population (N) contain drugs at a confidence level of at least $(1-\alpha)$, if $(n-r)$ tested samples are positive.			At least requested proportion (k) of population (N) contain drugs at a confidence level of at least $(1-\alpha)$, if $(n-r)$ tested samples are positive.		
Enter data (steps 1 to 4) and see results (steps 5 to 7)		Max. population size N_{max} allowed (current value):	Enter data (steps 1 to 4) and see results (steps 5 to 7)		Max. population size N_{max} allowed (current value):
		1000			1000
Step 1: Enter population size (N) = number of samples in population:	1000	Step 6: Actual proportion (k') for sample size n	Step 1: Enter population size (N) = number of samples in population:	1000	Step 6: Actual proportion (k') for sample size n
Step 2: Enter proportion (k) of positives in population: (range: 0.0001<k<=1)	0.7	0.70000	Step 2: Enter proportion (k) of positives in population: (range: 0.0001<k<=1)	0.7	0.70000
Step 3: Enter expected number of negatives (r): (at max 2)	1	Step 7: Actual CL for sample size n	Step 3: Enter expected number of negatives (r): (at max 2)	1	Step 7: Actual CL for sample size n
Step 4: Enter confidence level (CL) $(1-\alpha)$ (range: 0.0001<=CL<=0.9999)	0.8071	0.85357	Step 4: Enter confidence level (CL) $(1-\alpha)$ (range: 0.0001<=CL<=0.9999)	0.807	0.80702
Step 5: Sample size (n)	10		Step 5: Sample size (n)	9	

The same outcome can be obtained with the NIST sampling app, as seen in Figure 9, by increasing the α value until the inferred positive proportion (that can be made based on 8 observed positives among a sample set of 9 bags) surpasses the desired threshold of 70%.

Figure 8. The NIST sampling app showing that an α value of 0.1929 (a) would allow for an inference regarding 699 of 1000 bags, given the presence of 1 negative result, but an α value of 0.1930 (b) allows for the inference to be made about 700 of 1000 bags, given the presence of 1 negative result.

Lower Confidence Bounds for Seized Material Sampling

Identification
Counting
Disclaimer

Population Size

Significance Level (alpha)

Desired Minimum Proportion/Sample Size

Sample Size

Chosen Sample Size: 9.

Observed.Positives	Lower.Confidence.Bounds	Inferred.Proportion
9	834	0.834
8	699	0.699
7	579	0.579
6	468	0.468
5	363	0.363
4	265	0.265
3	174	0.174
2	91	0.091
1	24	0.024
0	0	0

Lower Confidence Bounds for Seized Material Sampling

Identification
Counting
Disclaimer

Population Size

Significance Level (alpha)

Desired Minimum Proportion/Sample Size

Sample Size

Chosen Sample Size: 9.

Observed.Positives	Lower.Confidence.Bounds	Inferred.Proportion
9	834	0.834
8	700	0.7
7	579	0.579
6	468	0.468
5	363	0.363
4	265	0.265
3	174	0.174
2	91	0.091
1	24	0.024
0	0	0

See Appendix B1 for the calculation of levels of confidence ($1 - \alpha$) given the desired number of inferred positives in the population, K .

Both calculators show that the highest level of confidence at which it can be reported that at least 70% of the 1000 bags contain fentanyl, based on randomly selecting 9 bags and finding fentanyl in 8 of them, is 80.70% ($1 - 0.1930$).

Reporting language shall reflect the decreased LoC. Examples are as follows:

- A) A total of 1000 bags were received. By using statistical sampling and analysis of 9 bags, 8 of which contained fentanyl, it is concluded at a 80.70% level of confidence that at least 70% of the population contains fentanyl.
- B) A total of 1000 bags were received. Using statistical (hypergeometric) sampling and by testing 9 bags, it is concluded that fentanyl is present in at least 700 bags at a level of confidence of at least 80%.

Option 2C. Additional sampling to generate a confidence interval for the population proportion

If additional bags are randomly sampled and tested, the proportion of the population containing the drug of interest can be estimated with an associated margin of error.

The more samples that are analyzed, the smaller the margin of error. The laboratory will need to compromise between the additional time and resources required to perform additional testing and the size of the resulting margin of error.

For this option, the laboratory has elected to randomly sample 21 additional specimens, leading to a final sample count of 30. Of the 21 additional samples, 18 were found to contain fentanyl and 3 were found to contain only acetaminophen.

Using the proportion of bags in the sample which contained the drug of interest ($\hat{p}$), a confidence interval (P) representing the proportion of the entire population which can be reported to contain the drug of interest at the chosen level of confidence (95%) can be calculated.²

$$P = \hat{p} \pm MOE \quad MOE = SE * z_{95\%} \quad SE = \sqrt{\frac{\hat{p}(1-\hat{p})}{n}} \quad [\text{Eq. 2}]$$

The margin of error (MOE) is calculated in Eq. 2 by multiplying the standard error (SE) by a critical z score (1.96 for 95% LoC).

For the given example, $8 + 18 = 26$ of the 30 total analyzed samples were found to contain fentanyl. Thus, $\hat{p} = 26/30 = 0.8667$.

$$\text{With } n = 30, \text{ the SE is } \sqrt{\frac{0.8667(1-0.8667)}{30}} = \sqrt{0.00385} = 0.0621$$

$$\text{Using } z = 1.96, \text{ the margin of error is } 0.0621 * 1.96 = 0.1216$$

² This method assumes a normal distribution to approximate binomial probabilities which becomes more appropriate as sample size increases. To ensure enough samples have been analyzed to justify this approximation it should be assessed whether $n * p \geq 5$ and $n * (1-p) \geq 5$. See References 5 and 6.

The proportion of the total population that can be concluded to contain fentanyl with a 95% level of confidence is $86.67 \pm 12.16\%$.

The results can be reported in various ways. For example:

- A) A total of 1000 bags were received. Based on the sampling and analysis of 30 bags, and finding fentanyl present in 26 of them, it is concluded that fentanyl is present in 866 ± 122 bags at a 95% level of confidence.
- B) A total of 1000 bags were received. Using statistical sampling and by testing 30 bags, it is concluded that fentanyl is present in at least 744 bags at a level of confidence of at least 95%.

Appendix B1

When inferences are drawn (and revised) retrospectively after analysis, hypothesis testing is no longer utilized. The known values for N , n , and k , can be used to determine the probabilities (P_k) and resulting LoC ($1-\alpha$) for a varying number of positives in the population, K . The P_k values are combined (Total P) and subsequently subtracted from 1 to determine the associated level of confidence that can be reported. Table B.1 summarizes the probabilities for decreasing K values using Eq. 1.

Table B.1 Adjusted LoC for varying K values

N	n	k	K	P_k	Total P	LoC ($1 - \alpha$)
1000	9	9	700	0.0397	0.1948	80.52%
		8		0.1550		
		9	699	0.0392	0.1930	80.70%
		8		0.1538		
		9	698	0.0387	0.1912	80.88%
		8		0.1525		
		9	650	0.0203	0.1200	88.00%
		8		0.0997		
		9	600	0.0098	0.0697	93.03%
		8		0.0598		
		9	580	0.0072	0.0550	94.50%
		8		0.0478		
		9	575	0.0067	0.0518	94.82%
		8		0.0451		
		9	574	0.0066	0.0512	94.88%
		8		0.0446		
		9	573	0.0065	0.0506	94.94%
		8		0.0441		
		9	572	0.0064	0.0499	95.01%
		8		0.0436		

The table shows that an inference of $K \geq 700$ (rejecting $K < 700$) would be supported with a decreased LoC of 80.70%, given 9 randomly selected samples and 0 or 1 negatives observed.

On the other hand, if results must be reported with a 95% LoC (Total $P < 0.05$) and 0 or 1 negative results were observed, at least 573 of the bags in the population can be inferred to contain fentanyl.

References

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