A Systematic Approach for Generation and Qualification of New Lots of Negative Control for Supporting Immunogenicity Assays

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During execution of anti-drug antibody (ADA) testing it is common for laboratories to periodically exhaust their supply of negative control (NC) due to its multiple uses in an assay method. The NC is used for calculation of plate-specific cut points, as a base pool for preparation of positive controls (PC), and as a diluent in the evaluation antibody titers. Whenever the NC is depleted, a new lot is needed to replace it. However, without appropriate upfront qualification of the replacement NC lot, issues can arise that complicate its implementation and use as a key critical reagent.

Ideally a new NC lot is generated in such a way that its performance is comparable to the previous lot that it is intended to replace. Unfortunately, simply combining matrix from multiple subjects to create a new pool of presumed antibody-free NC matrix seldom results in a new lot that performs similarly to the original. To aid the process of generating a new NC lot, we have devised a systematic approach that we believe increases the likelihood that the new NC lot will perform comparably to the previous one. Below are recommendations for screening matrix for NC lot generation, followed by NC qualification and cut point adjustment, if needed. For implementation of this NC preparation strategy, it is important to execute the work prior to completely depleting the original lot of NC.

Key Points

- **Negative control (NC) lot replacement is an essential part of critical reagent management**
- **Proactive management of NC changeover reduces risk of lot-to-lot inconsistency**
- **A systematic approach provides a high prospect for successful NC lot qualification**

Step 1 – Matrix Screening

We recommend evaluating matrix lots from 50 to 100 drug-naïve individual subjects, representative of the target population[1], for suitability to include in the new NC lot. At least 1 L of NC pool should be generated to support the multiplicity of uses in ADA testing. Prospective individual subject matrix lots need a volume of at least 25 mL to be useful for generating the required volume.

The individual matrix lots are analyzed with and without supplementation with PC antibody, typically at or about the low positive control level, and in each of these states, with and without added therapeutic (confirmatory assay format). The information provided under these four sets of conditions is shown in *Table 1*.

Table 1: Conditions for Screening Individual Matrix Lots

Conditions		**Information Provided**
PC Antibody Supplementation	*Confirmatory Drug Supplementation*	
No	No	Signal response similar to negative samples
	Yes	Absence of positive or negative inhibition
Yes	No	Lack of a false negative response
	Yes	Acceptable level of inhibition

Samples are tested in a block design, such as the one shown in *Table 2*. An example plate map for this type of evaluation is shown in *Figure 1*. This design allows for testing 96 individual subject lots in the four conditions discussed above. If fewer lots are tested, the number of samples per plate should be decreased, and evenly divided across the 8 plates (e.g., 64 lots tested as 8 lots per plate).

Table 2: Experimental Design for Screening Individual Matrix Lots

Plate Order	**Analyst 1**		**Analyst 2**	
	Run 1	*Run 3*	*Run 2*	*Run 4*
1	Plate 1	Plate 3	Plate 5	Plate 7
2	Plate 2	Plate 4	Plate 6	Plate 8

Figure 1: Example Plate Map for Screening Individual Matrix Lots

ROW	1 2	3 4	5 6	7 8	9 10	11 12
A	S1*	S9	S5	S1*	S9	S5
B	S2	S10	S6	S2	S10	S6
C	S3	S11	S7	S3	S11	S7
D	S4	S12*	S8	S4	S12*	S8
E	S5	S1*	S9	S5	S1*	S9
F	S6	S2	S10	S6	S2	S10
G	S7	S3	S11	S7	S3	S11
H	S8	S4	S12*	S8	S4	S12*

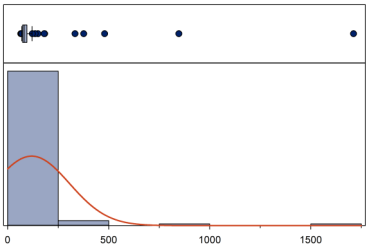
Neat	With added Drug	With added PC	With added Drug and PC

** If adequate volume of the old NC lot is available, samples may be included on the screening plates for comparison*

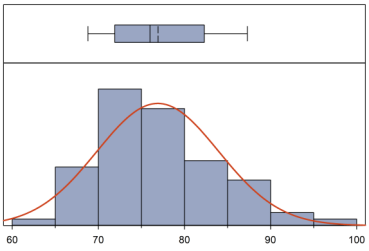
The distributions of results from each assay format are examined to choose lots that show a negative response in the samples without added PC and a positive response when supplemented with PC. The overarching aim is to create a pool of matrix that will perform comparably to the NC lot that is being replenished. Evaluation of the distribution includes a test for normality, such as the Sharpio-Wilk test[2], and calculation of the skewness coefficient[3]. If the distribution is non-normal, then individual matrix lots identified as outliers using Tukey's boxplot criteria[4] are removed iteratively until the remaining set of values assume a normal distribution and no outliers remain. An example of the response distribution before and after outlier removal is seen in *Figure 2*. Individual matrix lots identified as outliers are excluded from the new NC lot.

After selection and pooling of the candidate matrix lots, the second step in the process is to evaluate the similarity in signal responses and performance between the old and new NC lots.

Figure 2: Distribution of Individual Matrix Lot Responses Before and After Outlier Removal



Before Outlier Removal, significant non-normality ($p < 0.001$), skewness = 7.11



After Outlier Removal, good normality ($p > 0.05$), skewness = 0.63

Step 2 - New Negative Control Lot Qualification

Once a new NC lot has been created, the old and new lots are compared systematically to determine if they perform comparably, and they are investigated for potential differences that might impact the cut point factor. To accomplish this task in an efficient and practical manner, we recommend using a simple statistical design, such as the one shown in *Table 3*. Unspiked samples of the old and new NC lots are tested multiple times on the same plate, in two different arrangements (Plate Maps A and B) as seen in *Figure 3*.

Table 3: Experimental Design for Comparison of Old and New NC Lots

Plate Order	Analyst 1		Analyst 2	
	Run 1	*Run 3*	*Run 2*	*Run 4*
1	A	B	B	A
2	B	A	A	B

Figure 3: Plate Maps for Comparison of Old and New NC Lots

Plate Map A

ROW	1 2	3 4	5 6	7 8	9 10	11 12
A	Old NC	New NC	Old NC	New NC	Old NC	New NC
B	Old NC	New NC	Old NC	New NC	Old NC	New NC
C	Old NC	New NC	Old NC	New NC	Old NC	New NC
D	Old NC	New NC	Old NC	New NC	Old NC	New NC
E	Old NC	New NC	Old LPC	New LPC	Old NC	New NC
F	Old NC	New NC	Old LPC	New LPC	Old NC	New NC
G	Old NC	New NC	Old HPC	New HPC	Old NC	New NC
H	Old NC	New NC	Old HPC	New HPC	Old NC	New NC

Plate Map B

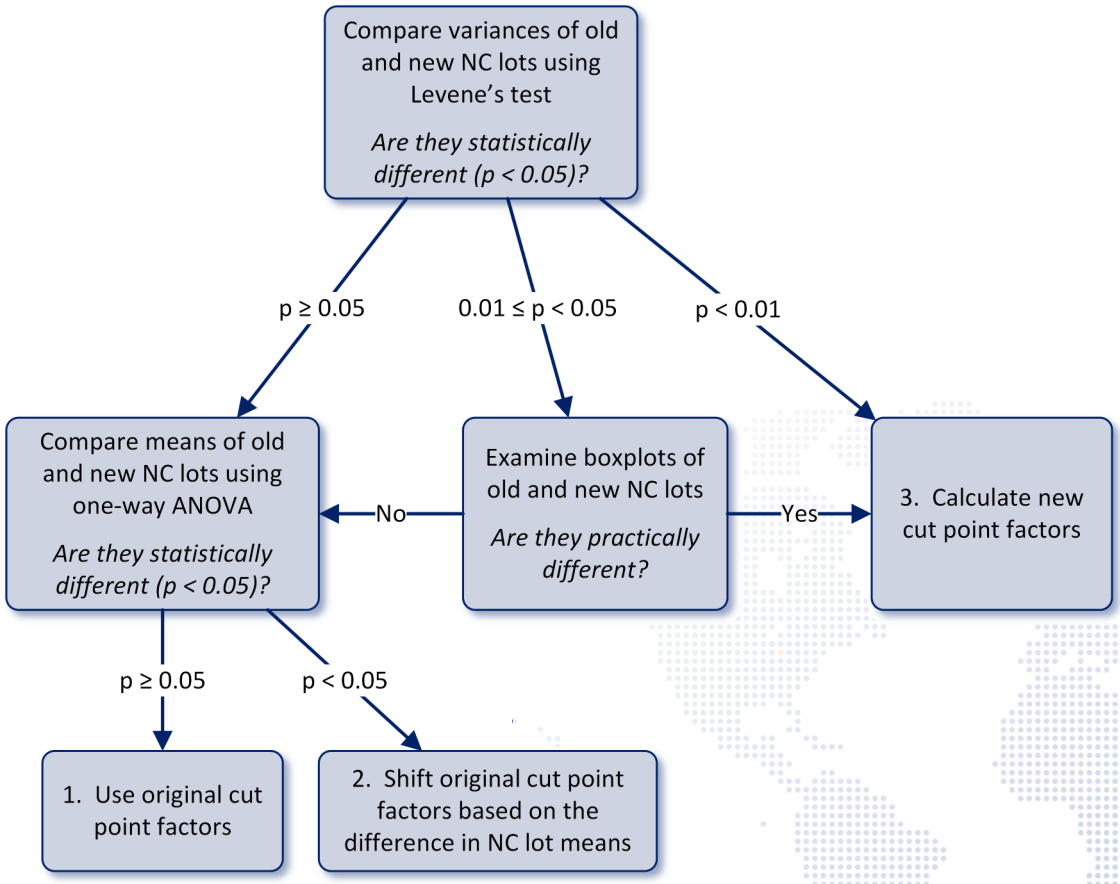
ROW	1 2	3 4	5 6	7 8	9 10	11 12
A	New NC	Old NC	New NC	Old NC	New NC	Old NC
B	New NC	Old NC	New NC	Old NC	New NC	Old NC
C	New NC	Old NC	New NC	Old NC	New NC	Old NC
D	New NC	Old NC	New NC	Old NC	New NC	Old NC
E	New NC	Old NC	New LPC	Old LPC	New NC	Old NC
F	New NC	Old NC	New LPC	Old LPC	New NC	Old NC
G	New NC	Old NC	New HPC	Old HPC	New NC	Old NC
H	New NC	Old NC	New HPC	Old HPC	New NC	Old NC

The data from the above experiment are analyzed to determine if there are statistical differences between the means or variances of the old and new NC lots. A one-way analysis of variance (ANOVA) is used to determine if there is a difference in the mean response between the old and new NC lots, and Levene's test[5] is used to evaluate differences in the variability of the responses between the two NC lots.

The flow chart in *Figure 4* is used to assess the impact of the new NC lot on the assay cut point factor and aid decision-making. There are three possible outcomes based on the comparative analysis:

1. Continue using the original cut point factor
2. Adjust the cut point factor based on the new NC pool performance
3. Determine a new cut point factor

Figure 4: Flow Chart for Assessment of Cut Point Impact



Summary

Generation of a new NC lot is an important component of critical reagent management to ensure consistency in the performance of anti-drug antibody assays. Bridging to a new NC lot during sample analysis should be planned well in advance in order to have a controlled changeover to the new NC lot. By using a systematic approach for the NC lot generation and comparison to the previous lot, the effect on the cut point factors can be evaluated and managed to provide analytical consistency. This will lessen the risk of timeline delays by avoiding generation of an unsatisfactory lot of NC, a key critical reagent in ADA assays.

References

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