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<1223.1> VALIDATION OF ALTERNATIVE METHODS TO ANTIBIOTIC MICROBIAL ASSAYS

INTRODUCTION

Microbiological assay methods have traditionally been used to quantify the potency, or antimicrobial activity, of antibiotics. These microbiological procedures were historically used to certify antibiotics on a lot-by-lot basis to ensure sufficient activity. Before 1998, monographs for several approved antibiotics were published in FDA's Code of Federal Regulations, 21 CFR. These regulatory antibiotic assay procedures were later published in *USP–NF* as the official referee methods to determine the potency of antibiotics. The details of the microbial assay procedures for individual antibiotics, including the challenge organisms and test parameters, are described in [Antibiotics–Microbial Assays \(81\)](#). Microbial assays provide a direct measure of the effectiveness of the antibiotic against a reference microorganism. Although these microbiological methods have continued to serve as the official compendial referee methods since their publication in *USP–NF*, many manufacturers have replaced these microbiological analyses with high-performance liquid chromatography (HPLC) methods.

Although the microbiological methods provide direct proof of antimicrobial effectiveness and can integrate all moieties that contribute to antimicrobial effects in a formulation, these methods are less precise, more complex to perform, and slower to complete than alternative methods such as HPLC. Microbial assays also have limited selectivity and are not appropriate for evaluating organic impurities. The specific skill sets required to perform the microbiological antibiotic assays, their unique equipment requirements, and their comparative complexity deter many stakeholders from using these methods.

There are numerous reasons for replacing the microbiological antibiotic assays with chemical assays that use purity or content as surrogates for the measurement of biological activity. The advantages of chemical-based analytical methods have been described previously for simple, single-component antibiotics, as well as complex, multicomponent antibiotics (1). Physicochemical procedures, such as HPLC, allow for simpler preparation and rapid data acquisition with improved precision, accuracy, selectivity, and specificity. HPLC methods can be used effectively for both potency assignment and organic impurity testing. Additionally, because modern instruments and the expertise to use such equipment are widely available, the conversion to alternative methods may be economically advantageous.

This general chapter provides points to consider for manufacturers who want to use physicochemical alternatives instead of the microbial assay methods described in (81). Given the widespread use of HPLC as an alternative to microbial assay methods, this chapter focuses on HPLC methods. However, the principles set forth in this chapter are applicable to any alternative physicochemical procedure.

Change to read:

GENERAL CONSIDERATIONS FOR ALTERNATIVE METHOD DEVELOPMENT

Multiple important factors need to be considered when replacing microbial methods with HPLC or other chemical techniques. This is because of the specific characteristics of antibiotics.

1. It is essential to know whether the antimicrobial activity manifested by a preparation results from a single, active ingredient or arises from multiple, often related, moieties. Where technically possible, the analyst should determine the contribution of major moieties ▲ and the potential impact of their interaction ▲ (USP 1-Aug-2022) to antibiotic effectiveness. For the purposes of this chapter, major moieties are defined as those that contribute more than 1% of the antibiotic potency. Where multiple moieties contribute to antibiotic activity, the chemical assay should be able to resolve all major moiety peaks in the formulation.
2. ▲ To the extent possible, the individual activities of process impurities and degradation products that contribute more than 1% of the antimicrobial activity of an antibiotic preparation should be evaluated. This may not be necessary where evidence exists that each of the process impurities and degradation products contributes less than 1% of the total antimicrobial activity of a preparation.
3. As an alternative approach, testing a high number of lots of variable potency can be considered. A model taking into account the contribution of the process impurities and degradation products to the activity can be evaluated and validated. This approach may allow the demonstration of equivalency between the microbial assay and the alternative procedure without knowing the activity of individual moiety and impurity. ▲ (USP 1-Aug-2022)
4. General chapter (81) is the referee standard in any procedure comparison. Therefore, the currently official USP procedure should be performed by the manufacturer (or under that manufacturer's direction) to establish reference assay values within the test range. It may be necessary to evaluate data using the guidelines in [Analysis of Biological Assays \(1034\)](#). ▲ (USP 1-Aug-2022) Manufacturers should establish appropriate limits for precision and accuracy of the microbial assay procedure based upon their product knowledge.
5. The candidate alternative HPLC or other chemical method should be fully validated according to [Validation of Compendial Procedures \(1225\)](#).

6. The value of any method comparison is dependent upon the precision and accuracy of the assay test results obtained from both methods. No statistical comparison should be undertaken if the assay data from either method does not meet predetermined acceptance criteria for method validation.
7. The guidelines in this chapter do not apply to already marketed products for which the manufacturer has already received regulatory approval to use an alternative method. In all cases, the applicability of an alternative method can only be determined through submission and review by the relevant regulatory authority.
8. Any alternative to a compendial procedure must be validated and ▲ must produce comparable results to the compendial method or procedure within allowable limits established on a case-by-case basis ▲ (USP 1-Aug-2022) (2). A *Stimuli* article published in *PF* 35(3) [May–June 2009] discusses the “equivalent or better” approach to evaluating alternatives to compendial procedures (3). It is expected that such a comparison will use appropriate statistical analysis. Examples of the recommended types of statistical analyses are outlined in this chapter.

Change to read:

TECHNICAL CONSIDERATIONS

▲ The microbial assay and the candidate alternative method should be performed as described in the appropriate general chapter (e.g., (81) for microbial assays and *Chromatography* (621) for HPLC procedures); other physicochemical methods may be used (e.g., capillary electrophoresis) and should be conducted according to the specific general chapter. Stability-indicating alternative procedures are recommended.

Complex antibiotics, which have more than one active moiety, generally require additional data compared to simple antibiotics, where all or almost all antimicrobial activity is contributed by a single moiety. ▲ (USP 1-Aug-2022)

Change to read:

BRIDGING STUDIES

Bridging studies are used to compare the data obtained from candidate alternative procedures to the microbial assay data to determine whether the alternative procedure is an acceptable substitute. ▲ Two approaches for developing the alternative procedure are described in detail below.

Approach I

1. Validate the candidate alternative procedure according to (1225). The procedure should be specific, linear, precise, accurate, sensitive, robust, and stability indicating.
2. Where process impurities or degradation products contribute more than 1% of the antimicrobial activity of an antibiotic preparation, the process impurities or degradation products should be evaluated whenever possible. Each antimicrobial moiety that has the potential to contribute more than 1% of the antimicrobial activity of the antibiotic preparation should be isolated, and values for the relative microbial activity factor (*F*) against the USP Reference Standard should be established. Note that purification of moieties present at low levels could be a difficult and time-consuming work. Also, evaluation of the activity of minor moieties can be difficult due to limited quantities available and precision associated with the purity of the samples. Active moieties including process impurities and degradation products at levels not more than 1% of antimicrobial activity may be disregarded. For simple antibiotics, this step may be skipped.
3. Evaluate a suitable number of production lots of the antibiotic to determine the variation range of process impurities and degradation products of the antibiotic. When available, lots representing the specification range should be evaluated. This step is done before testing the samples using the two methods to evaluate the variation of the production and help the selection of representative batches for the bridging study. ▲ (USP 1-Aug-2022)
4. ▲▲ (USP 1-Aug-2022) Test a minimum of three separate lots of the ▲ sample against ▲ (USP 1-Aug-2022) the USP Reference Standard using the microbial assay method and the candidate alternative procedure. ▲ It is important to select lots that cover the activity and purity specification range. Testing lots with similar activity and purity will not demonstrate method comparability. As a consequence, more than three lots will usually be tested. Successful method comparability necessitates testing lots with variation in activity and purity. Stability samples with varying amounts of active moieties should have a change in potency that directly correlates to the alternative method. Forced degradation products or out-of-specification (OOS) lots may be introduced in the study to strengthen its robustness.
5. A minimum of three replicate samples per lot using both the candidate alternative method and the microbial assay procedures should be tested. One replicate is defined as the test measurement of one sample preparation (i.e., one sample stock solution). When a limited number of similar lots is used, it ▲ (USP 1-Aug-2022) may be necessary to increase the number of replicates based on the maximum allowed percentage difference (see *Data Evaluation*) and the standard deviation of the method (4). ▲ When a complex antibiotic displays significant composition variability, more lots may be used in the study with reduction in the number of replicates. The number of lots and replicates should be balanced according to the precision of each method and the complex antibiotic composition variability to ensure the maximum potential causes of variability are taken into account. If the HPLC assay has better precision than the microbial assay, fewer replicates may be needed. Manufacturers may work with the relevant regulatory authority to decide whether the replicates and lots are sufficient to demonstrate the method equivalency. *Appendix 4* presents one methodology for determining sample size for bridging studies. ▲ (USP 1-Aug-2022) If possible, prepare standard stock and sample solutions and

- subdivide them for use with the microbial assay and candidate alternative assay procedures. This provides paired data that can be analyzed (see [Appendix 2](#)).
6. Use the relative microbial activity factor (F) to convert the percentage purity values for each moiety, and then sum them to determine a combined potency value. \blacktriangle For example, $\text{potency} = \sum x_i F_i$, where x_i is the content of moiety i and F_i is its relative microbial activity factor.
 7. Apply the appropriate analysis on the results obtained by the two methods to determine whether the candidate alternative and the microbial assay procedures yield comparable results. If necessary, \blacktriangle (USP 1-Aug-2022) apply appropriate outlier (see [Analytical Data— Interpretation and Treatment \(1010\)](#) and [\(81\)](#)) and comparison tests (see [Data Evaluation](#)). \blacktriangle The number of outliers should be limited and justification for their rejection should be provided in the bridging study report. \blacktriangle (USP 1-Aug-2022)
 8. \blacktriangle (USP 1-Aug-2022) If the bridging study fails, it may be necessary to include potency contributions from \blacktriangle process \blacktriangle (USP 1-Aug-2022) impurities \blacktriangle and degradation products \blacktriangle (USP 1-Aug-2022) that make a contribution below 1% \blacktriangle or take into account synergistic activity. Additional analysis may also be required to evaluate the accuracy of measured purity of isolated impurity samples.

Approach II

In the case of complex antibiotics, it can be difficult, time-consuming, or even technically infeasible to separate and purify all active antimicrobial moieties and test their activity. An alternative approach that tests a significant number of lots of samples may be considered. An example of this approach implemented for proteins can be found in a *Stimuli* article published in *PF* 40(4) [July–Aug. 2014] [\(5\)](#).

1. Validate the candidate alternative procedure according to [\(1225\)](#). The procedure must be specific, linear, precise, accurate, sensitive, robust, and stability indicating.
2. Identify moieties that may contribute to antimicrobial activity. Active moieties including process impurities and degradation products at levels below 1% of antimicrobial activity may be disregarded.
3. Test an appropriate number of lots, with varying amounts of active moieties covering the specification range, by both methods. Forced degradation products and/or OOS lots may be used in the study to demonstrate robustness. These are called "training lots". The number of replicates and lots should be based on the maximum allowed percentage difference (see [Data Evaluation](#)) and the standard deviation of the method [\(4\)](#). [Appendix 4](#) presents one methodology for determining sample size for bridging studies. Manufacturers may work with the relevant regulatory authority to decide whether the replicates and lots are sufficient to demonstrate the method equivalency and whether the model is appropriate. When possible, prepare standard stock and sample solutions and subdivide them for use with the microbial assay and candidate alternative assay procedures. This provides paired data that can be analyzed (see [Appendix 2](#)).
4. Identify an appropriate model to convert the sum of percentage purity values for each moiety to a combined potency value by applying conversion factors (F). For example, $\text{potency} = \sum x_i F_i$, where potency is the microbial activity from the microbial test; x is the content of moiety from the physicochemical method; F is the conversion factor. F is determined in step 5. More complex models may be used.
5. Apply appropriate statistical analysis on the results obtained by the two methods to determine the conversion factors (F) so that the candidate alternative procedure and the microbial assay procedure yield equivalent results to find out the correlation between the two methods. Confirm the validity of the model obtained by comparing the predicted and obtained potency on another set of lots called "validation lots". The involvement of a statistician to help design the study, determine the relevant variables, analyze the results, and define the model is highly recommended. Outlier analysis in step 7 of *Approach I* can be applied.
6. Stability samples with varying amounts of active moieties should have a change in potency that directly correlates to the alternative method.
7. If the bridging study fails, it may be necessary to test the relative microbial activity for each moiety as stated in *Approach I*. \blacktriangle (USP 1-Aug-2022)

Change to read:

DATA EVALUATION

Described below are USP's recommendations for data evaluation. Alternative approaches such as Deming regression [\(6\)](#) \blacktriangle (USP 1-Aug-2022) may also be used. For an antibiotic with a comparatively narrow assay range (80%–125% or narrower), follow step 1. For an antibiotic with a wide assay range (80%–125% or wider), follow steps 1 and 2.

1. Demonstrate the equivalence of results at the targeted potency of 100% using a two one-sided test (TOST) to test for equivalence. TOST offers several advantages over the t -test, which looks for differences [\(7–10\)](#). This requires the laboratory to set a maximum allowed percentage difference (such as 3%, 4%, or 5%), denoted by k in the appendices. Use the formulas in [Appendix 1](#) for independent samples (different samples used for the two procedures) or [Appendix 2](#) for paired samples (a set of samples each of which is assayed by both procedures).
2. Compare the chapter microbial assay and candidate alternative assay procedures using \blacktriangle (USP 1-Aug-2022) samples that cover the full monograph range of activity values. Prepare a Bland–Altman (BA) plot [\(Appendix 3\)](#). There should be no evidence of an important trend and the BA 95% agreement limits should not extend outside a predetermined maximum difference established by the laboratory.

Change to read:

APPENDIX 1: TOST FORMULAS FOR INDEPENDENT SAMPLES

On the basis of the knowledge of the product, the laboratory must establish the maximum allowed percentage difference between the average result for the candidate alternative assay and the average result (USP 1-Aug-2022) for the microbial assay. A difference that meets the requirements indicates that the alternative method provides acceptable results in the specified compendial range as compared with the microbial assay. In statistical notation, what must be demonstrated is:

$$100 \left| \frac{\mu_{HPLC}}{\mu_{Microbial}} - 1 \right| < 100k \quad \text{Equation 1}$$

μ_{HPLC} = mean value for alternative method (HPLC)

$\mu_{Microbial}$ = mean value for the microbial assay (USP 1-Aug-2022)

k = a small positive number such as 0.03 (for an allowed 3% difference; when determining k , factors such as the variability of the microbial assay, product specification, and intended use of the product can be considered) (USP 1-Aug-2022)

These are the two values to be compared.

Rearranging, this becomes:

$$-k\mu_{Microbial} < \mu_{HPLC} - \mu_{Microbial} < k\mu_{Microbial} \quad \text{Equation 2}$$

The idea of TOST is to consider the two inequalities of Equation 2 separately. That is, to demonstrate the hypothesis in Equation 2 at the 5% level, one must demonstrate both of the inequalities in Equation 3 at the 5% level:

$$\mu_{HPLC} - (1 + k)\mu_{Microbial} < 0 \text{ and } \mu_{HPLC} - (1 - k)\mu_{Microbial} > 0 \quad \text{Equation 3}$$

For a comparison of means, the TOST is equivalent to considering two-sided 90% confidence bounds. If the bounds satisfy the inequalities in Equation 3, then equivalence has been demonstrated. When the samples for the two assays under comparison are different ("independent samples"), determine the following upper (U) and lower (L) confidence bounds:

$$U = \bar{X}_{HPLC} - (1 + k)\bar{X}_{Microbial} + t_{0.05,df} \sqrt{\frac{S_{HPLC}^2}{N_{HPLC}} + (1 + k)^2 \frac{S_{Microbial}^2}{N_{Microbial}}} \quad \text{Equation 4}$$

$$L = \bar{X}_{HPLC} - (1 - k)\bar{X}_{Microbial} - t_{0.05,df} \sqrt{\frac{S_{HPLC}^2}{N_{HPLC}} + (1 - k)^2 \frac{S_{Microbial}^2}{N_{Microbial}}} \quad \text{Equation 5}$$

\bar{X}_{HPLC} = sample average for the alternative method (HPLC)

k = a small positive number such as 0.03 (for an allowed 3% difference; when determining k , factors such as the variability of the microbial assay, product specification, and intended use of the product can be considered)

$\bar{X}_{Microbial}$ = sample average for the microbial assay

t = one-sided 5% value for a t -distribution

S_{HPLC} = sample standard deviation for the alternative method (HPLC)

N_{HPLC} = sample size for the alternative method (HPLC)

$S_{Microbial}$ = sample standard deviation for the microbial assay

$N_{Microbial}$ = sample size for the microbial assay (USP 1-Aug-2022)

For the number of degrees of freedom (df), use:

$$\frac{\left(\frac{S_{Microbial}^2}{N_{Microbial}} + \frac{S_{HPLC}^2}{N_{HPLC}} \right)^2}{\left[\left(\frac{1}{N_{Microbial} - 1} \right) \left(\frac{S_{Microbial}^2}{N_{Microbial}} \right)^2 \right] + \left[\left(\frac{1}{N_{HPLC} - 1} \right) \left(\frac{S_{HPLC}^2}{N_{HPLC}} \right)^2 \right]} \quad \text{Equation 6}$$

This is an approximation that assumes k is small. If using software that only allows for integer degrees of freedom (e.g., Excel), use linear interpolation to obtain the t -value. Conclude that the two procedures are equivalent (i.e., any difference on average is acceptably small) for the given lot (L) if:

$$L > 0 \text{ and } U < 0 \quad \text{Equation 7}$$

Example 1 ▲(Results for one sample lot):▲ (USP 1-Aug-2022)

Microbial Assay Data	HPLC Assay Data
72.02	▲72.38▲ (USP 1-Aug-2022)
67.3	▲71.92▲ (USP 1-Aug-2022)
71.79	▲72.25▲ (USP 1-Aug-2022)
71.16	—
69.06	—
75.56	—
74.7	—
74.16	—
76.48	—

Following the above formulas with $k = 0.03$:

$$N_{\text{HPLC}} = 3; \bar{X}_{\text{HPLC}} = \text{▲}72.18\text{▲} \text{ (USP 1-Aug-2022)} \quad S_{\text{HPLC}} = \text{▲}0.237\text{▲} \text{ (USP 1-Aug-2022)}$$

$$N_{\text{Microbial}} = 9; \bar{X}_{\text{Microbial}} = \text{▲}72.47\text{▲} \text{ (USP 1-Aug-2022)} \quad S_{\text{Microbial}} = 3.045$$

Degrees of freedom (df) = ▲8.283▲ (USP 1-Aug-2022) interpolated t -value = ▲1.860▲ (USP 1-Aug-2022)

$L = \text{▲}0.039\text{▲}$ (USP 1-Aug-2022) and $U = \text{▲}-0.500\text{▲}$ (USP 1-Aug-2022) so the 3% equivalence criterion is satisfied.

Change to read:

APPENDIX 2: TOST FORMULAS FOR PAIRED SAMPLES

When the samples for the two assays are the same, the data are considered “paired”. The hypotheses are the same as those in [Appendix 1](#). Because of the pairing of samples, the standard deviation calculations differ.

Determine the confidence bounds, U and L , as follows:

$$U = \bar{X}_{\text{HPLC}} - (1 + k)\bar{X}_{\text{Microbial}} + t_{0.05,df} \sqrt{S_U^2 / N} \quad \text{Equation 8}$$

$$L = \bar{X}_{\text{HPLC}} - (1 - k)\bar{X}_{\text{Microbial}} - t_{0.05,df} \sqrt{S_L^2 / N} \quad \text{Equation 9}$$

N = number of samples for each procedure

$df = N - 1$

S_L and S_U are calculated as follows:

$$\text{▲}S_U^2 = \frac{1}{N-1} \sum_{j=1}^N [X_{\text{HPLC},j} - (1 + k)X_{\text{Microbial},j} - \Delta\bar{X}_U]^2 \quad \text{Equation 10}$$

in which, $\Delta\bar{X}_U = \frac{1}{N} \sum_{j=1}^N [X_{\text{HPLC},j} - (1 + k)X_{\text{Microbial},j}] \text{▲}$ (USP 1-Aug-2022)

$$\text{▲}S_L^2 = \frac{1}{N-1} \sum_{j=1}^N [X_{\text{HPLC},j} - (1 - k)X_{\text{Microbial},j} - \Delta\bar{X}_L]^2 \quad \text{Equation 11}$$

in which, $\Delta\bar{X}_L = \frac{1}{N} \sum_{j=1}^N [X_{\text{HPLC},j} - (1 - k)X_{\text{Microbial},j}] \text{▲}$ (USP 1-Aug-2022)

Conclude that the two procedures are equivalent (i.e., any difference on average is acceptably small) for the given lot if:

$$L > 0 \text{ and } U < 0 \quad \text{Equation 12}$$

Example 2 ▲(Results for multiple sample lots):▲ (USP 1-Aug-2022)

Microbial Assay Data	HPLC Assay Data
1011	980.9
990	981.4
960	978.3
1000	974.3
970	966.7

Following the above formulas with $k = 0.03$:

$$N = 5; \bar{X}_{\text{HPLC}} = 976.3; \bar{X}_{\text{Microbial}} = 986.2$$

$$S_L = 18.749; S_U = 19.958$$

$$df = 4; t\text{-value} = 2.132$$

$L = 1.830$ and $U = -20.438$, so the 3% equivalence criterion is satisfied.

Change to read:

APPENDIX 3: BLAND-ALTMAN PLOTS

[Figure 1](#) shows the data plotted as HPLC assay (x-axis) versus microbial assay (y-axis). In [Figure 2](#), the x-axis represents the average response obtained using the alternative and microbial assay methods. The y-axis represents the difference in responses measured with the alternative and microbial assay methods.

The bias for each data set is represented by the average difference.

95% limits of agreement are represented by $\text{bias} \pm 2S$.

Data points outside the limits of agreement are considered outliers.

The BA plot ([11-12](#)) should show data points scattered within the limits of agreement with no obvious pattern. If a pattern exists (e.g., proportional pattern or funneling outwards with increasing average), perform a log transformation. If performing a log transformation, take the antilog of results for analysis using the original scale of measurement.

Example 3:

▲Lot	Microbial Assay Data	HPLC Assay Data	Microbial Assay-HPLC Assay
1	1213	1226	-13
	1253	1284	-31
	1276	1285	-9
	1293	1354	-61
	1222	1240	-18
2	1155	1181	-26
	1108	1131	-23
	1118	1169	-51
	1101	1082	19
	1136	1134	2
3	893	881	12
	805	790	15
	827	811	16
	821	812	9

▲Lot	Microbial Assay Data	HPLC Assay Data	Microbial Assay-HPLC Assay
	867	839	28
4	1085	1096	-11
	1032	1050	-18
	1046	1052	-6
	1019	1024	-5
	1048	1035	13
5	1000	977	23
	920	918	2
	978	964	14
	906	924	-18
	903	898	5

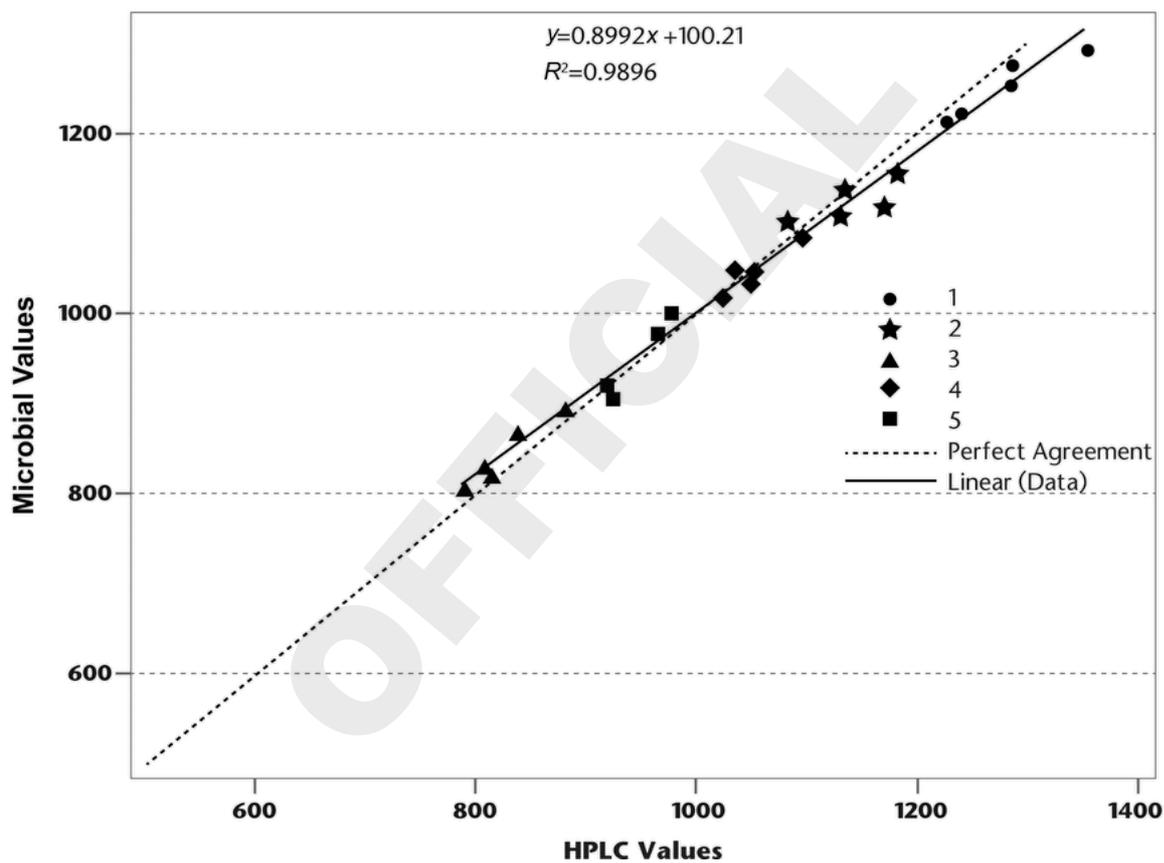


Figure 1. Example data plotted as microbial assay data versus HPLC assay data. (R^2 = coefficient of determination)

▲ (USP 1-Aug-2022)

[Figure 1](#) shows the data plotted as microbial (y-axis) versus HPLC (x-axis). This appears to be in good agreement. [Figure 2](#) shows what happens when the same data are instead plotted as recommended by Bland-Altman.

▲

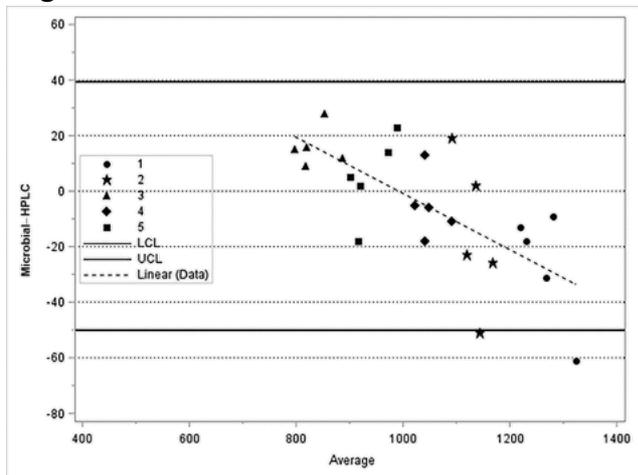


Figure 2. Example data in a BA plot. (LCL = lower control limit; UCL = upper control limit.)

▲ (USP 1-Aug-2022)

Calculate the bias, estimated by the mean difference (d) and standard deviation (SD) of the difference:

$$d = -5.28$$

$$\text{SD of difference} = 22.36$$

Most of the differences are expected to lie between $d - 2SD$ and $d + 2SD$ (if normally distributed, 95% will lie in these limits). If differences within $d \pm 2SD$ (limits of agreement) are not important, the two measurement methods can be used interchangeably:

$$\text{LCL } (d - 2SD) = -49.95 \quad \text{Equation 13}$$

$$\text{UCL } (d + 2SD) = 39.39 \quad \text{Equation 14}$$

The difference between results from the two procedures has a strong trend. This trend can be seen in [Figure 1](#), but the BA plot in [Figure 2](#) makes it clearer.

Add the following:

▲APPENDIX 4: SAMPLE SIZE JUSTIFICATION FOR ANTIBIOTIC BRIDGING STUDIES

The methodology for determining sample size for antibiotic bridging studies presented in [\(1010\)](#) for the estimate to reflect the equivocal zone can be used. [Table 1](#) lists the sample sizes corresponding to different choices for three parameters in [Equation 15](#).

$$N \geq \frac{2\sigma^2}{\delta^2} (Z_\alpha + Z_{\beta/2})^2 \quad \text{Equation 15}$$

N = sample size

σ = standard deviation of the microbial assay [assuming 1000 $\mu\text{g}/\text{mg}$, $\sigma = 10 \times \text{CV}$ (coefficient of variation)]

δ = difference (\pm) from the true value that is considered equivalent

Z_α = Type I errors (Z_α is usually set at 95%)

$Z_{\beta/2}$ = Type II errors

Table 1

Power	$Z_{\beta/2}$	δ	σ	N
80%	0.842	30	50	35
		40	50	20
		50	50	13
		60	50	9
		70	50	7
		80	50	5
90%	1.282	30	50	48

Power	$Z_{\beta/2}$	δ	σ	N
		40	50	27
		50	50	18
		60	50	12
		70	50	9
		80	50	7 [▲] (USP 1-Aug-2022)

Change to read:

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