COLA Clinical Laboratory Accreditation

COLA Primers Accreditation

COLA PRIMER 75

Verification of Performance: For Microbiology Identification and Susceptibility Procedures

● INTRODUCTION ●

Verification of performance specifications serves several purposes:

1) It is a regulatory requirement
2) Verification ensures that the test system is operating according to expected performance standards and that it is capable of producing accurate and reliable results.
3) It will demonstrate that laboratory personnel have been trained to operate the system correctly by producing consistently accurate results.

Verification is a one-time process performed when a new system is introduced into a laboratory or when a current system is going to be updated (such as adding a new antibiotic to a panel). These studies are time consuming and expensive, therefore, each laboratory must make their own decisions regarding the scope of testing (i.e. how many organisms to test, what pool of organisms will be tested, etc.)

The key points to remember are that:

1) The Laboratory Director, in consultation with the Clinical Consultant, the Technical Supervisor, and the manufacturer, should establish the study protocols.
2) Study protocols and acceptability criteria must be developed prior to the initiation of any verification testing.
3) The studies must be performed in ***your*** laboratory by ***your*** staff.
4) The data must be reviewed and analyzed to determine acceptability by the Laboratory Director, ***prior*** to the initiation of patient testing.
5) You must document data collection and validation and retain these materials for the life of the analyzer or test method plus two (2) years.
6) Loaner instruments require verification; relocation of an existing analyzer requires re-verification (to what extent is dependent upon the type of move - a bench to bench move would require less than a move on a truck to an entirely new location)
7) Each instrument's performance must be verified – even if you have multiple instruments of the same make and model.

● WHAT TYPE OF VERIFICATION STUDY DO I NEED? ●

There are two types of verification studies: ***comprehensive*** studies and ***limited*** studies. You must first determine which type of study you need.

Perform a ***comprehensive*** study if:

1) You have never performed identification and/or susceptibility testing in your laboratory previously
2) You change from one manufacturer's system to another manufacturer's system (e.g. Vitek to Microscan)
3) You change from one model to another model (e.g. Microscan AutoScan to Microscan Walkaway
4) You change your test method (e.g. manual to automated, API strips to Vitek cards)
5) You add a new panel for testing (e.g. MicroStrep panels to add *Strep. pneumoniae* susceptibility testing to your laboratory

Perform a ***limited*** study when you *add* new features to a previously verified system, such as when:

1) You add a new antibiotic to a previously verified system (you would only verify the new antibiotic)
2) You change to a different panel on a previously verified system (you would only verify the new panel)
3) You want to report a previously unverified antibiotic on a previously verified system (you would only verify the previously unverified antibiotic)
4) The manufacturer updates the formulation to an antibiotic on a previously verified system
5) When you add another analyzer or replace an analyzer with the same make/model which has been previously verified (you verify the new analyzer against the current analyzer)
6) When new dilutions are added to a panel to address new breakpoints to a previously verified system. If the interpretive criteria have not changed, no study is required in this circumstance. **IMPORTANT:** if the system is not FDA-cleared for new breakpoints, this is considered a "modification" of the test system requiring the *establishment* of performance specifications.
7) When there are certain software updates to a previously verified system (e.g. expert software updates or organism database updates). In these cases, your verification efforts should be concentrated on the specific change. Not all software updates would require verification; however, you may need to consider verifying your LIS in some cases.

NOTE: In an effort to provide as much information as possible, this Lab Guide will detail the process of performing a ***comprehensive*** verification study.

• WHEN ARE VERIFICATION STUDIES NOT REQUIRED? •

Verification studies are NOT required following:

1) Routine maintenance
2) Repairs
3) Certain updates to software (e.g. taxonomic software updates) or instrument updates that do not affect the test panels.

• SELECTING ORGANISMS FOR TESTING •

Although CLIA does not provide specifics for the quantity and selection of organisms to test, these are very important considerations.

The laboratory should choose a variety of organisms for testing appropriate for the scope and extent of testing to be performed in your laboratory. If the plan is to report both gram positive and gram-negative organisms in your laboratory, you will need to verify both the gram positive and gram-negative types of panels. Choose several different species and strains of organisms you expect to see in your laboratory. Several species and strains of Staphylococcus, Streptococcus and Enterococcus should be used for gram-positive panels. For gram-negative organisms, choose several strains of fermenters and non-fermenters that you will expect to see, as well as some unique organisms (*Pasteurella*, *Acinetobacter*, and *Stenotrophomonas maltophilia*).

If you are only performing urine cultures, use organisms that will be frequently isolated from this site. You should also have a good mix of sensitive and resistant organisms. You need to know the identification and/or susceptibility results for the organisms being used for the verification, if you are not currently performing this testing in your laboratory. If you already perform identification and susceptibility testing, you can use your current system as the comparison method. Testing only quality control strains of organisms is not sufficient; you must include organisms isolated from patient samples. Regarding the number of isolates to use in the verification study, again CLIA does not specify a minimum number.

The number of organisms you test plays a role in determining the outcome of the verification study. At least 30 isolates (each for gram positive and gram negative testing) should be tested at a minimum for a comprehensive verification study; however, you can test more. Testing at least 30 isolates is statistically significant, as it will provide you with a good chance of meeting the acceptability criteria for the study. You will need to try to obtain 30 results for all of the antibiotics you will be reporting, so you may need to use more isolates. Consider how few antibiotics are reported for *Enterococcus* species, for example. For limited verification studies, testing ten isolates is usually sufficient. Consider the reasons for implementing a limited study, usually only a few antibiotics, at most, are at issue with a limited study. All isolates should be fresh (< 24 hours old) when you are ready to begin testing.

Where can I obtain isolates for testing?

You will need to know the identification and susceptibility information of the test isolates in order to compare that data to the information you obtain on the system being verified.

1. You can use *some* ATCC Quality Control (QC) strains for verification testing. The identification and sensitivity patterns of these organisms are already known. **CAUTION**: do not use a majority of QC strains in the study (with the exception of precision studies).
2. You can also use previously tested patient isolates that you have already identified on the current, verified system. If you are new to microbiology testing, ask another laboratory* to send you some isolates with the identification and sensitivity data they obtained. (*These isolates should come from a CLIA-certified laboratory.)
3. If you have isolates in which the results (ID or sensitivity or both) are not known, you can send these out to a CLIA-certified reference laboratory for testing and compare these results.
4. You can use previous Proficiency Testing samples (these usually have limited susceptibility data but are great for identification verification).

● VERIFICATION PREPARATION ●

Important steps need to be taken *prior* to beginning the verification study. CLIA does not provide specific recommendations on how to perform verification studies. It is important that the Laboratory Director approve all laboratory verification protocols prior to the initiation of the study. It is recommended that the laboratory:

1) Have staff thoroughly trained on the procedure prior to initiating the study.
2) Have staff run Quality Control (QC) on the system (to ensure the system is installed correctly, is functioning properly, and staff have been trained correctly). **QC must pass prior to *initiating the* study.**
3) Write the study protocol to include:
    a. the name of the system being verified, software version number, panel type, comprehensive or limited study, the method and the procedure that will be used for testing
    b. What testing will be performed (including QC, how many isolates will be used, names of the isolates you will use in the study, what will you be verifying, the specific antibiotics that your laboratory will be reporting). Verification should be performed on all uses the instrument will have (Are you going to test yeast on the system? If so, you will need to verify for yeast, etc.)
    c. What are the acceptability criteria (90% Accuracy? 95% Precision? What constitutes a match for identification and susceptibility?)

d. How will the results be analyzed (e.g. categorical or essential agreement, or both?)
e. What is the comparison method that will be used (a previously verified system? A third party laboratory?)
f. How will discrepancies be resolved? (Will you repeat them? Will you send out to a third party if necessary? Is genus level identification acceptable? What confidence percentage will be accepted for identifications: 85%? 90 %?)

4) It is highly recommended that you create a spreadsheet for all of the data. You will be generating and comparing a large amount of data.

REMEMBER – the Laboratory Directory must approve the verification protocols before the study begins.

● IDENTIFICATION VERIFICATION PROCESS ●

Regarding organism identification, you only need to verify the **Accuracy** and **Precision** of the system.

For both of these studies, ensure that QC has been run on the panels (and it was acceptable) prior to beginning the study.

To verify the **ACCURACY** of the identification system, perform the following:

1. Be certain that you have at least 30 isolates to test (each for gram positive and gram negative testing) and that the growth is fresh (<24 hours old). Refer to the *"Selecting Organisms for Testing"* section above.
2. If you test over the course of a several days, daily QC is not required for identification verification. Identification panels require QC per new lot and shipment. **NOTE**: once the identification verification study is completed and deemed acceptable, you can opt to follow CLSI M50 protocol for Streamlined QC as part of a written and implemented IQCP. Qualifying studies are not required to move to streamlined QC for identifications.
3. Test the isolates on the system being verified.
4. To verify accuracy for organism identification, you will be comparing the *actual identification* of each organism, **not** the individual biochemical reactions.
5. Compare the identification of the isolate, from the system being verified, to the known identification for that isolate (comparison method). Follow the pre-determined rules (see Verification Preparation section) for what constitutes a "match". The identifications should match.

Analyze the data as follows:

$$\frac{\text{\# of identifications that matched comparison method}}{\text{total \# of identifications tested}} \times 100 = \%\ \text{Accuracy}$$

If you test a total of 30 organisms and 27 out of 30 match the comparison method, this equals 90% agreement.

$$\frac{\text{27 identifications matched comparison method}}{\text{30 identifications tested}} \times 100 = 90\%\ \text{Accuracy}$$

The Laboratory Director should set the acceptable limit for accuracy prior to the initiation of the study. Accuracy should be ≥ 90% at a minimum for organism identifications.

Discrepancies should always be investigated by repeat testing on the method being verified and the comparison method (if possible). The predetermined acceptability criteria should help reduce the number of discrepancies. For example, if it was predetermined that you would accept an identification to the genus level, this would resolve a discrepancy between an identification of *Citrobacter freundii* from the comparison method vs. *Citrobacter* species on the method being verified (i.e. this would be considered a match).

To verify the **PRECISION** of a new identification system in a comprehensive study, perform the following:

1. Choose at least five isolates (each for gram positive and gram negative) to test. You can use the key indicator strains from the manufacturer's listing of required quality control organisms for the system. You can use a couple of patient strains also if you need more organisms. Be certain that the growth is fresh (<24 hours old).
2. If you test over the course of a several days, daily QC is not required for identification systems. Identification systems require QC per new lot and shipment. **NOTE**: you can also opt to follow CLSI M50 protocol for Streamlined QC as part of a written and implemented IQCP once this portion of the study (identifications) is completed and deemed acceptable. Qualifying studies are not required to move to streamlined QC for identifications.
3. Since we are evaluating the *reproducibility* of results, you will not need a comparison method to evaluate precision.
4. Test the five isolates each in triplicate (as a minimum), using a *new inoculum* for each identification panel. You can always test more organisms and perform the replicate testing for a few days. The more tests you perform, the better chance of achieving the acceptability goals. These can be run on the same day or over a period of a few days. It is also a good idea to have several members of your trained staff set up the panels, as this will be a good assessment of their skills with the new system.

5. Each isolate should produce the same identification for each replicate. If you test an *E. coli* in triplicate and for each replicate, you obtain an identification of *E.coli*, this would equate to 100% Precision.

Analyze the data using the same formula used for the accuracy verification:

$$\frac{\text{\# of identifications that matched}}{\text{total \# of identifications tested}} \times 100 = \text{\% Precision}$$

In the chart below, we tested five different organisms, each run in triplicate, for a total of 15 identifications tested. Two isolates did not give the correct identification (highlighted).

Known Organism ID	Replicate #1	Replicate #2	Replicate #3
E.coli	*E.coli*	*E.coli*	*E.coli*
E. cloacae	*E. cloacae*	*E. cloacae*	*E. cloacae*
Staph aureus	*Staph aureus*	*Staph capitis*	*Staph capitis*
S. bovis	*S. bovis*	*S. bovis*	*S. bovis*
K. oxytoca	*K. oxytoca*	*K. oxytoca*	*K. oxytoca*

Using the formula, we can now calculate precision:

$$\frac{\text{13 identifications matched}}{\text{Total of 15 identifications tested}} \times 100 = 86.6\% \text{ Precision}$$

If the predetermined acceptability rate for our study for identification precision is ≥ 90 %, then the results attained above are **not** acceptable (86.6%). If we had tested one more replicate for each organism and each of them matched, the precision would have passed at 90%.

$$\frac{\text{18 identifications matched}}{\text{Total of 20 identifications tested}} \times 100 = 90\% \text{ Precision}$$

It is up to the Laboratory Director to determine the acceptability criteria, however, the acceptable limit for precision be ≥ 90% at a minimum for identification testing.

Troubleshooting Unacceptable Results

If the accuracy and/or precision results do not meet the predetermined acceptability criteria established prior to the start of the study, ***troubleshoot*** to try to raise the level of agreement.

Here are some things you can do:

- Ensure isolate subcultures are pure and fresh.
- Ensure the correct organism is used at the correct concentration.
- Repeat the testing that failed.

- If repeating the test does not provide the correct identification, you may choose to have an independent, third party laboratory test it, as the referee. The organism could have been misidentified in the first place.
- If the results are still not acceptable, you may need to contact the system manufacturer for assistance to help determine the cause of the problem.

●SUSCEPTIBILITY VERIFICATION PROCESS ●

Regarding susceptibility testing, you only need to verify the **Accuracy** and **Precision** of the system.

Quality control should be performed ***each day*** of the study for susceptibility testing. If it is your intent to follow CLSI M-02 and M-07 to reduce susceptibility quality control frequency from daily to weekly, you will need a written and implemented IQCP once the verification studies are completed and deemed acceptable. You can use the data from the quality control organisms you test here in the susceptibility verification toward the 20-day study, 30-day study or 15 replicate plan study that is also required to reduce quality control frequency for susceptibility testing from daily to weekly.

To verify the **ACCURACY** of the susceptibility system in a comprehensive study, perform the following:

1. Be certain that you have at least 30 isolates to test for each panel type (e.g. Gram Positive, Gram Negative, etc.) and for the scope of testing (e.g. urine panel) that will be reported. Ensure the organisms are fresh (<24 hours old). You need to test enough isolates to yield 30 results for **each** antibiotic you will be reporting. You may need to test more isolates since not all of the antibiotics on the panel will be reported for some organisms (e.g. *Enterococcus* species, *Stenotrophomonas maltophilia*). **IMPORTANT:** Antibiotics are formulated differently on gram negative and gram-positive panels. If the panels have the same antibiotics on both the gram negative and gram-positive panels (e.g. SXT), you will need to verify those antibiotics on both panels.

   Refer to the *"Selecting Organisms for Testing"* on page 4 of this document. The use of clinical patient isolates is *preferred* for accuracy in susceptibility testing. Incorporate some quality control organisms only if you need to.

2. You must perform QC each day of the study using the QC strains required by the manufacturer for the susceptibility testing.
3. As the comparison method, you *can* use a previously verified system present in your laboratory. If verifying a new MIC system and you used the Kirby-Bauer method previously, the Kirby-Bauer method can be the comparison method (you will be limited to calculating the Categorical Agreement only). Likewise, if you have a previously verified

Vitek system in the laboratory and you are switching to Microscan, you can use the Vitek as the comparison method. If microbiology testing is new for your laboratory, you can send the isolates out to a reference laboratory for testing and compare those results.

4. Test the isolates on the system being verified.
5. When verifying the accuracy of the new susceptibility system, you will need to calculate the Categorical Agreement **(CA)** between the method being verified and the comparison method to see if the two systems match **S**ensitive, **I**ntermediate and **R**esistant results.

Categorical Agreement (CA) is the minimum analysis required for susceptibility testing verification.

EXAMPLE: if you test an *E.coli* strain on the new method and it tests Sensitive (S) for ampicillin and the comparison method tests Sensitive (S) for that same *E. coli* strain vs. ampicillin then that is a match (agreement). You will calculate the **CA** for each drug tested.

Calculate susceptibility accuracy as follows:

$$\frac{\text{\# of sensitivities that matched comparison method for S, I, R}}{\text{Total \# of sensitivities tested}} \times 100 = \text{Categorical Agreement}$$

ORGANISM	Ampicillin New Method	Ampicillin Current Method	Agreement?
E.coli	S	I	NO
K. pneumonia	R	R	YES
C. freundii	R	R	YES
K. aerogenes	S	I	NO
S. marcescens	S	S	YES
P. mirabilis	S	S	YES

Example: if you tested 33 organisms vs. ampicillin and all of them matched the comparison method for the S, I, R categories *except two of them*, our Categorical Agreement would be:

$$\frac{\text{31 sensitivities matched comparison method for S, I, R for Ampicillin}}{\text{33 total sensitivities tested for Ampicillin}} \times 100 = 93.9\% \text{ Categorical Agreement for Ampicillin}$$

Again, it is up to the Laboratory Director to determine the acceptability criteria; however, the acceptable limit of agreement for CA should be ≥ 90% at a minimum.

1. If you are verifying MIC's on the new system and comparing the results to comparison MIC results (not Kirby Bauer method) and you have greater than four (4) dilutions for the individual drugs on the panels, you should also calculate the Essential Agreement (**EA**). If you do not report MIC values in the laboratory, you do not need to analyze the data for

EA. For Essential Agreement, all MIC results for each organism/antibiotic combination on the new system should fall within +/- 1 doubling dilution of the comparison method MIC. Calculating the EA holds no value if the antibiotics on the panels have four (4) or less dilutions.

Example: The ampicillin dilutions on the panel are 2, 4, 8, 16 and 32 (there are >4 dilutions for ampicillin). You test an *E. coli* strain against ampicillin on the new system and the resulting MIC is 2. The MIC for the same organism vs. ampicillin from the comparison method is 4. These two MICs are within one doubling dilution of each other and are therefore "in Essential Agreement".

If you tested 33 organisms for MIC vs. ampicillin and all except two of them matched the comparator method within +/- 1 doubling dilution for each corresponding organism/antibiotic combination, the equation would look like this:

$$\frac{\text{31 MICs for ampicillin matched +/- 1 doubling dilution to comparison method}}{\text{33 total sensitivities tested for ampicillin}} \times 100 = 93.9\%\ \text{EA (Essential Agreement) for ampicillin}$$

2. It is up to the Laboratory Director to determine what the limits of acceptability are for the study. Essential Agreement **(EA)** should be ≥90% at a minimum for MIC accuracy.

To verify **PRECISION** of the susceptibility system, perform the following:

1. Select at least five isolates from the original pool of organisms. Ensure that the growth is fresh (≤24 hours old). You can use the QC strains required by the manufacturer as well as some patient isolates if you need more organisms. Refer to the *"Selecting Organisms for Testing"* information above.
2. You must perform QC with the QC strains required by the manufacturer each day you perform susceptibility testing for the verification. **NOTE:** You can use the data from the QC strains toward the 20 or 30 day or 15 replicate qualifying study to reduce quality control frequency.
3. You will not need a comparison method for precision, as you will be analyzing the reproducibility of results from the system being verified.
4. Test the five isolates in triplicate (as a minimum) on the panels using a *new inoculum* for each susceptibility panel. It is also a good idea to have several members of your trained staff set up the panels, as this will be a good assessment of their skills with the new system.
5. Evaluate the results for precision using Essential Agreement **(EA).** In this instance, a good match is MIC results within +/- 1 doubling dilution for each organism tested. If comparing results of Kirby-Bauer testing, you will need to evaluate the results for Categorical Agreement **(CA)** only.

The data should look something like this:

E. coli	Ampicillin	AMP/Sulbactam	Cefoxitin	Ciprofloxacin	Gentamycin	Levofloxacin	Piper/Tazo	Tetracycline	SXT	DAY	Comments
Repeat #1	4	4	2	2	0.5	2	1	8	8	**1**	Repeat #2 for Ampicillin is more than 1 doubling dilution from the MIC of 4, therefore, it is not an "equivalent" result. If this same result were an MIC of 2 or 8, it would be a match
Repeat #2	16	4	2	4	1	4	1	4	8		
Repeat #3	4	2	4	4	0.5	4	1	8	4		
Repeat #4	2	2	2	2	2	2	2	8	4	**2**	All of the results through Day 2 are all within +/- 1 doubling dilution
Repeat #5	4	2	2	2	0.5	2	1	8	8		
Repeat #6	4	1	2	2	1	2	1	4	2		
Repeat #7	2	2	2	1	0.5	4	2	4	8	**3**	All of the results through Day 3 are all within +/- 1 doubling dilution
Repeat #8	4	4	2	4	0.5	4	2	8	8		
Repeat #9	4	4	4	2	1	4	2	8	4		
MATCH	8/9	9/9	9/9	9/9	9/9	9/9	9/9	9/9	9/9	80/81	80 ÷ 81= 0.987 x 100 = 98.7% Precision for *E.coli*

Analyze the data *for each organism* as follows, using the data in the table above:

$$\frac{\text{80 MICs matched within +/- 1 doubling dilution for } \textit{E. coli}}{\text{81 total MICs tested for } \textit{E.coli}} \times 100 = \text{98.7\% Essential Agreement (EA) for this } \textit{E.coli} \text{ strain}$$

If you choose to, you can calculate the MIC precision via Essential Agreement using all of the organism and antibiotic MIC data combined.

It is up to the Laboratory Director to determine what the limits of acceptability are for the study. Essential Agreement **(EA)** should be ≥90% at a minimum for precision with susceptibility studies.

Types of Errors

It is important to remember that when the MIC results do not match, you should investigate further, especially if you have any Very Major Errors **(VME)**. A Very Major Error occurs when the system being verified reports an organism/antibiotic combination as Susceptible (S) and the comparison method for that same organism/antibiotic combination is Resistant (R). You will need to review the data thoroughly to locate any VMEs. **VMEs are the most serious of errors.** If these errors go unchecked, it could mean that an ineffective antibiotic could be used in the treatment of a patient. These errors should always be investigated thoroughly. Performing repeat testing on the results in question on the system being verified (and the comparison method, if possible) using the same inoculum is the logical first step to take when investigating errors and discrepancies.

The VME rate should be < 3%. This can be calculated as follows:

$$\frac{\text{\# of Very Major Errors}}{\text{\# of resistant isolates per comparison method}} \times 100 = \text{VME Rate}$$

Other errors may also require investigation:

Major Error: this error occurs when the new system being verified reports an organism as Resistant (R) when the reference method is Susceptible (S). In clinical practice, this can cause a serious problem for the patient if the antibiotic choices are limited (i.e. an antibiotic that would otherwise be effective has been wrongly discounted). Review the data carefully to locate any Major Errors and attempt to resolve them if the Major Error rate is above the threshold.

The Major Error rate should be <3%. This can be calculated as follows:

$$\frac{\text{\# of Major Errors}}{\text{\# of susceptible isolates by comparison method}} \times 100 = \text{Major Error Rate}$$

Minor Error: this error has occurred when one system reports an antibiotic as Intermediate (I) while the other method reports Susceptible (S) or Resistant (R). These types of errors are the least harmful to the patient. A Minor Error Rate of ≥3% needs to be investigated.

The Minor Error Rate should be <3%. This can be calculated as follows:

$$\frac{\text{\# of Minor Errors}}{\text{Total \# of isolates tested}} \times 100 = \text{Minor Error Rate}$$

• VERIFICATION SUMMARY •

It is important that you create a summarized, written report of the verification study. Summarize the accuracy and precision results for both identification and susceptibility testing. Be sure to indicate the acceptability criteria set forth prior to testing and compare them to your test results. Explain whether acceptability criteria were met. Remember to document all raw data, calculations, any repeat testing and error resolution and keep all the materials created during the study with the summary. Lastly, the Laboratory Director must review all of this information, determine whether it is acceptable, and sign off on the study prior to the implementation of the new system.

Save the verification study for as long as the test system is in use PLUS two years.

Resources:

COLA Core Criteria Interpretations. (March 2018). Baltimore, MD: COLA, Inc.

Patel, J. B., Sharp, S., Novak-Weekley, S. (2013). Verification of Antimicrobial Susceptibility Testing Methods: a Practical Approach. *Clinical Microbiology Newsletter*, 35(13), 103-109.

Mann, L., et al. (2015). M-52: *Verification of Commercial Microbial Identification and Antimicrobial Susceptibility Testing Systems*. Wayne, PA: Clinical and Laboratory Standards Institute.