

# Practical Guidelines for Chlamydia Test Verification

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## National Chlamydia Laboratory Committee National Infertility Prevention Project

### **A. Introduction and purpose of these guidelines.**

CLIA regulations require that clinical laboratories “verify” tests that are new to a laboratory. However, the regulations are mute with regard to how this verification needs to be done and how many specimens need to be tested. For this reason, the National Chlamydia Laboratory Committee undertook the writing of these guidelines. They are meant to assist the ordinary, perhaps small clinical laboratory, to perform reasonable, but meaningful procedures, in order to (1) satisfy the regulation, (2) gain experience in the performance of the new procedure, and (3) gain experience with the comparison of results obtained with the new test versus those found with the old, established test, particularly when changing from a non-amplification to an amplification procedure.

### **B. The testing described in this statement is limited to specific situations common to current chlamydia testing practices.** The situations addressed are as follows:

1. The introduction of nucleic acid amplification testing of endocervical specimens into a clinical laboratory already employing a non-amplification procedure for that specimen source.
2. The use of an amplification test that has been established in a laboratory for use with a given specimen source, for example, endocervix, for use with a specimen from a different source, for example, urine. In this situation, the test must have been FDA cleared or pre-market approved for use with both specimen types.
3. The use of endocervical swabs taken in a transport medium specific to a non-amplification test that is being used, for testing in an amplification test system. This would be an “off-label” use as it relates to the FDA. It would be employed in a testing algorithm designed to increase the sensitivity of testing, perhaps at lower cost than testing all specimens by amplification. Specimens giving high negative values on a non-amplification test would be retested using the amplification procedure. Without satisfactory findings on the testing of this algorithm described in Section D.3., below, an additional, second, specimen, one in the amplification test transport medium, would need to be obtained at the time of each patient visit. Note: As described in Section D.3., below, because this would be an “off-label” use of a specimen transport medium, a larger number of specimens will need to be tested than that required for the other two situations, 1 and 2, described in this section, above.

**C. The regulation and its application.** In accordance with Section 493.1213 of the Clinical Laboratory Improvement Amendments (CLIA) of 1988, final rule, February 28, 1992, “*verification*” (or establishment) of performance specifications is required for each new method, prior to reporting patient test results based on the method.

1. For FDA-cleared or FDA-pre-market-approved tests (B.1 and 2, above), the purpose of verification testing is to show that prior to reporting patient test results, the laboratory has demonstrated that it can obtain the performance specifications for accuracy, precision, and reportable range of patient results that are comparable to those established by the manufacturer. A description of these terms as they apply to the chlamydia testing involved is as follows:

a. The accuracy of a chlamydia test is a reflection of how reliable a negative or positive result may be as an indication of the absence or presence of the organism in the specimen. Accuracy may be measured in terms of sensitivity and specificity.

Regarding sensitivity, in accordance with the manufacturer’s claims and the published literature, it is expected that the sensitivity of the amplification test will exceed that of the non-amplification procedure. The laboratory’s data should show this increase in positivity when comparing the two (2) procedures, using endocervical specimens.

Regarding specificity, the current gold standard depends on a combination of culture and amplification test results, and can be a complex determination. For the purpose of verification in the ordinary clinical laboratory, it is suggested that laboratories do not attempt to measure this factor. Instead, it is recommended that laboratories assume that the specificity data in the package inserts and published results for both non-amplification and amplification tests are true for your population. As a rule, reported specificities are over 99%.

b. Precision is a measure of the reproducibility of the testing procedure. When repeat tests on the same specimens are performed, it is expected that the results will be close in all cases.

c. Regarding the reportable range of patient results, for chlamydia testing, no range of results such as would be appropriate with a chemical, physiologic, or cellular analyte is reported. *Any* amount of Chlamydia trachomatis that is

detected is a “ positive” result. Therefore, determination of a “reportable range” is not appropriate to this testing.

2. For non-FDA-cleared or non-FDA-pre-market approved tests, the CLIA regulation requires that before reporting results of such tests, the laboratory must verify or establish the performance characteristics of the method as applicable: Accuracy, precision, analytical sensitivity, specificity to include interfering substances, reportable range of patient test results, reference range(s), and any other performance characteristic required for test performance. Calibration and control procedures for patient testing must be established.

It is recommended that the application of this rule to the “off-label” use described in section B.3., above, require, only, that the result obtained in the amplification test using the swab in the non-amplification test transport medium must be the same as that obtained with the swab in the amplification test transport medium. As seen below, a relatively larger number of specimen pairs must be tested in order that any sporadic but systemic interfering substances, if present, might be detected.

**D. Recommended procedures for situations listed in section B., above.**

1. The establishment of a nucleic acid amplification test for endocervical specimens to replace a non-amplification procedure for that specimen source. (Note: Laboratorians must be trained by the manufacturer in all aspects of equipment maintenance and test performance, and run practice testing in the home laboratory to obtain experience and familiarity with the system before proceeding.)

a. Specimens.

Obtain 2 endocervical swabs from each patient, one for each of the 2 tests, in its appropriate transport medium as prescribed by the manufacturer. Randomize the order in which the specimens are taken.

b. Parameters to be examined.

- (1) To determine sensitivity, run the 2 tests in parallel. Record the results and calculate the sensitivity as described in section D.1.d., below.

For any patient specimen that is positive on the non-amplification test but negative on the amplified procedure, steps should be taken to resolve the

difference in the two results. Possibly attempt to confirm the non-amplification result with DFA testing on the sediment or by using the prescribed blocking agent, etc; possibly investigate the negative amplification test result by examining for the presence of amplification inhibitors in the specimen. The home laboratory or the manufacturer might do the latter, by testing the specimen at dilution or testing a DNA extract of the sample.

- (2) To determine precision, aliquots of specimens tested in the amplification procedure should be tested a second time using the same procedure.
  - (3) The laboratory must share 10 or more specimens with the manufacturer or a laboratory they recommend to assure that there is reasonable agreement between the other laboratory's numerical results and interpretation and theirs.
- c. The number of specimen pairs that needs to be tested depends on a number of factors that may be subjective. For example, if the contemplated new test has been used widely and is generally accepted by the public health and microbiology communities, and there is a decent number of published evaluations and a high level of agreement among the evaluations and with the manufacturer's claims, a comparatively low number of trials would be appropriate.

The ASM Cumitech #31, p.8, col. 2 (see ref. 1), suggests that the minimum number of paired specimens that should be run is that number which would give at least 20 positives. This would mean a total of about 200 specimen pairs where the frequency of amplification positives is about 10%. However, for example, for Roche PCR and Abbott LCR, a quantity of data has been published, and about all of it is in agreement regarding sensitivity and specificity. It is the consensus of the committee that half that number of specimens, or a number sufficient to obtain 10 positive amplification tests would serve. On the other hand, for newer products, with less time on the market and fewer publications, etc., more pairs should be tested, perhaps enough to obtain 20 positives.

- d. For testing reproducibility, it is suggested that at a minimum, 10 specimens for LCR/PCR and 20 for the newer tests, be repeated. The specimens selected should represent the entire spectrum (high to low) of results, but should concentrate on those near the pos./neg. cut-off point, that is, low positives and high negatives.

- e. For quantitative expression of the performance of the non-amplified test as compared with that of the amplified procedure, assume that the amplification test is the gold standard and create a “2 x 2” table, accordingly. An example of such a table, using numbers derived from simulated results, and calculations of parameters follows:

		Amplification test	
		Pos.	Neg.
Non-amplification test	Pos.	6 A	0 B
	Neg.	4 C	90 D

Sensitivity of the non-amplified test as compared with the amplification procedure =  $\frac{A}{A+C} = \frac{6}{6+4} = 60\%$

Positive Predictive Value (PPV) (of the non-amplified test) =  $\frac{A}{A+B} = \frac{6}{6+0} = 100\%$

Negative Predictive Value (NPV) (of the non-amplified test) =  $\frac{D}{D+C} = \frac{90}{90+4} = 96\%$

2. The establishment of an amplification procedure for testing urine samples. Note: If the laboratory is currently testing endocervical specimens with a non-amplification procedure, it is recommended that the amplification procedure be first verified for endocervical specimens, as above.

- a. Regarding the number of tests that should be run, if this is the first use of the amplification test in the laboratory, obtain enough appropriate urine samples from patients of each gender to be tested, to obtain a total of at least 10 positives from each gender. If this amplification procedure has previously been verified in the laboratory for endocervical specimens, it would be appropriate to test fewer specimens, perhaps enough to obtain at least a total of five (5) positives from each gender for which the test is to be used.
- b. After running the tests in your laboratory, submit the remainder or an aliquot of ten selected specimens for testing by the manufacturer or a laboratory they recommend. The samples selected for retesting should represent an array of results obtained, from high to low readings, with particular

emphasis on specimens with results that fall near the cut-off point, *i.e.*, low positives and high negatives. Ninety five percent of the qualitative results, *i.e.*, negative vs. positive, must agree with those of the reference laboratory. All differing results should be investigated for reconciliation.

3. The establishment of the use of swabs taken in a non-amplification transport medium for testing in an amplification system. Note: For any other off- label use, use the outline, below, as a guide, but note that the number of specimens/trials and parameters may need to be increased and expanded, respectively.
  - a. Verify the use of the amplification system in the laboratory employing the corresponding specimen, as described in section D.1., above.
  - b. Obtain two specimens from each patient involved, one in each of the appropriate transport media, in random order, on each of approximately 300 or more patients. Inasmuch as the new use is neither FDA-cleared nor FDA-pre-market-approved, a higher number of test specimens than used in the methods described above, is required in its establishment.
  - c. Test both specimens in the amplification system. (Note: When the non-amplification specimen used in this protocol is tested, it must be in the same condition that it would have been, had it already been employed in the non-amplification procedure and stored until the time for high negative specimens to be retested by amplification.)
  - d. Compare the numerical readings and “positive” vs. “negative” result interpretations for each of the specimen pairs. Retest an array of 20 non-amplification specimens, selected to represent the whole range of numerical results, in the amplification procedure for reproducibility.

If paired numerical readings throughout the range agree and the non-amplification transport specimen results are reproducible, the non-amplification specimen may be used in the amplification system.

- f. Once the procedure is established, validation (on-going QC and QA) must be done. For every new lot of non-amplification collection kits in use, several pairs of control specimens, one in each type of transport, must be run. Results must be consistent with the findings on the initial

verification procedure if patient results are to be considered valid.

- g. If the formulation of the non-amplification collection kit is changed, the procedure must be verified as if for the first time.

**References:**

1. Clinical Laboratory Improvement Amendments of 1988, final rule, February 28, 1992, Section 493.1213.
2. Verification and validation of procedures in the clinical microbiology laboratory, Cumitech 31, B.L. Elder, et. al., Am. Soc. for Microbiology, Wash., D.C. February, 1997.

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