AOAC SP 2025.001

Standard Protocol (SP) for Single and Independent Laboratory Validation of Methods for Detection of Benzodiazepines, Fentanyl, Medetomidine, Nitazenes, and Xylazine in Selected Drug Product Solids, Liquids, and Residues to Accompany AOAC SMPR 2025.002

Table of Contents

Approved by stakeholders associated with AOAC Working Group on Drug Test Strips. Effective date: October 5, 2025

Development of this standard was funded by the U.S. National Institute of Standards and Technology (NIST), Agreement No. 60NANB24D286.

1. Introduction

1.1 Candidate Methods

The candidate methods to be validated are rapid screening methods for known or suspected drug products intended to be used in nonlaboratory settings by law enforcement, rapid response personnel, public health staff, or other community members. These candidate methods will be validated in the AOAC *Performance Tested Methods*℠ (PTM) Program administered by the AOAC Research Institute (AOACRI) according to the PTM Policies and Procedures and will evaluate conformity to AOAC Standard Method Performance Requirements (SMPRs®) 2025.002 for Detection of Benzodiazepines, Fentanyl, Medetomidine, Nitazenes, and Xylazine in Selected Drug Product Solids, Liquids, and Residues.

Candidate methods include competitive antibody- or aptamer-based lateral flow assays (LFAs) with simple sample preparation steps that can be carried out in indoor or outdoor settings with minimal hands-on manipulation. Methods are to be specific for a drug or drug class. Multiplex methods, those with more than one reported result from a single LFA, must be evaluated for each compound or class of compounds according to this validation protocol, as well as cross-interference using co-spiking.

Potential target analytes of candidate methods include benzodiazepines, fentanyl, medetomidine, nitazenes, and xylazine. Candidate methods for other target analytes can be validated using the same general protocol, but with amendments as needed (e.g., appropriate selectivity tables) in consultation with AOAC volunteer experts.

The matrices to be analyzed may include bulk solids, liquids, and residues, but are exclusive of foods, beverages, plant materials, and biological fluids. The

target matrices are representative of substances or excipients that may contain drug products or residues from apparent drug use paraphernalia. Examples include tablets, powders, and residues on nonporous surfaces.

Each candidate method must include a detailed product insert consistent with the PTM Product Insert Requirements (*see* PTM Policies and Procedures). Due to the intended field use, candidate methods must be compatible with a visual read of results.

1.2 Validation Protocol Overview

The PTM validation has two main parts: single-laboratory validation (SLV) and independent laboratory validation (ILV) studies. For this validation protocol, SLV studies include selectivity, matrix study, determination of preliminary and final limit of detection (LOD), product consistency, robustness, environmental factors, and product stability.

The ILV study includes a subset of the SLV matrix study to verify method performance.

Note that the validation protocol includes the minimal ILV study, but the method developer (MD) may choose to transfer some or all of the SLV studies to the AOACRI independent laboratory.

This standard validation protocol document serves as a template to be tailored to the scope of the candidate method.

Candidate Method Scope:

Analyte or analyte class.—Benzodiazepines, fentanyl, medetomidine, nitazenes, xylazine or other

Matrix categories.—Bulk solids, liquids, and/or residues

The Candidate Method product insert is to clearly state the scope of analytes and matrix categories/items included in the PTM validation as well as any limitations of the method.

Validation guidelines for the design of experiments include AOAC OMA Appendix N and U.S. Food and Drug Administration's (FDA) Template for Developers of Molecular Diagnostic Tests.

2. Good Laboratory Practices

2.1 Specified reference standards and/or reference materials must either be obtained directly from the manufacturer or be traceable to direct procurement from the manufacturer.

2.1.1 All reference standards for this validation must be accompanied by a Certificate of Analysis showing a purity of ≥95%.

2.2 One or more analysts should prepare validation materials, and one or more independent analysts should perform the assays on randomized blind-coded samples.

2.2.1 All test portions must be fully randomized and labeled with a code so that the analyst performing the assays is unaware of the concentration or nature of the analyte present.

2.3 All experiments should include positive and negative controls with each batch of analyses. Use the test kit controls, or if there are no test kit controls, use a target analyte at 10x LOD as the positive control and water as the negative control.

2.4 Package insert instructions must be followed to correctly carry out and interpret the candidate method, including identification of invalid results and subsequent corrective actions. For all experiments, analysts must record and report the number of invalid results, reason for each if known (e.g., no capillary action), corrective action taken, and results of the corrective action.

3. Safety Precautions

3.1 Wear protective gloves and safety glasses while performing the validation studies as all materials, containers, and other items that come into contact with the sample may be contaminated with hazardous substances.

3.2 In addition to **3.1**, wear a particle mask and work in a fume hood when powders are produced or may become airborne. Avoid inhalation of powders.

3.3 Dispose of all used test strips, drug solutions, spiked matrices, and other waste according to local and federal regulations.

4. Definitions

4.1 *Antibody-based LFA*.—Test that uses antibodies to detect specific substances or specific class of substances using lateral flow technology.

4.2 *Aptamer-based LFA*.—Test that uses short single-stranded nucleic acid sequences to detect specific substances or specific class of substances using lateral flow technology.

4.3 *Naïve end user*.—Nonscientist with no prior experience using LFAs for detection of drugs.

4.4 *Probability of detection (POD)*.—Proportion of positive analytical outcomes for a qualitative method for a given matrix at a given analyte level or concentration. POD is concentration dependent.

4.5 *Reader*.—Person who visually interprets results of LFA test.

4.6 *Surrogate analyte*.—Nontoxic compound that is cross reactive in the LFA test.

5. Single-Laboratory Validation Studies

The SLV studies are the responsibility of the product manufacturer and may be carried out in the MD's laboratory or contracted to another laboratory. The MD may choose to transfer any or all of the SLV studies to a third-party laboratory that they have contracted or to the independent laboratory.

5.1 Selectivity

The selectivity study determines the range of compounds detected and not detected by the LFA.

5.1.1 Prepare a solution of each compound in water or in the aqueous buffer supplied in the LFA kit at 2 mg/mL or the maximum analyte concentration based on the manufacturer's sample preparation instructions, whichever is higher.

5.1.1.1 For analytes with a solubility below 2 mg/mL, prepare a solution at the maximum solubility.

5.1.1.2 Refer to Table 3 (a–f) in AOAC SMPR 2025.002 to determine which compounds to test.

5.1.1.3 Table 3 (a–f) of AOAC SMPR 2025.002 includes minimum requirements for selectivity testing. Additional drugs/compounds may be included in the study.

5.1.2 Randomize and blind code the solutions.

5.1.3 Test each solution once according to the product insert.

5.1.3.1 Use one lot of LFA test strips.

5.1.3.2 One analyst should read all LFA results.

5.1.3.3 If any target analyte yields a negative result, dilute the solution 10-fold and retest in case of negative results due to potential matrix effects or other high-concentration interferences that may affect binding efficiency.

5.1.3.4 If any nontarget compound produces a positive result, prepare and test serial dilutions to determine the concentration at which the compound no longer cross reacts. Start with 10-fold serial dilutions and identify the lowest concentration testing positive and the highest concentration testing negative. Then test 2-fold serial dilutions within that range and report the lowest concentration yielding a positive result.

5.1.4 Decode the data, tabulate, and report all test and retest results.

5.1.5 Determine the scope of compounds that yield a positive result.

5.1.5.1 Report the target drug and its analogues that yield positive reactions as the scope of the method.

5.1.5.2 Report as limitations of the method any compounds yielding positive reactions that are not analogues of the target analyte. Report as "[NAME OF COMPOUND] causes a positive test result at concentrations of [X] mg/mL or higher."

5.2 Preliminary Limit of Detection

This study is intended to determine a preliminary limit of detection (pLOD) in water or aqueous solution and serves to guide further experiments.

5.2.1 Prepare each target analyte in water or in the aqueous buffer supplied in the LFA kit at a starting concentration of 100 µg/mL.

5.2.2 Make dilutions in water or the aqueous buffer supplied in the LFA kit to prepare target analyte solutions at 50, 20, 10, 5, 2, 1, 0.5, 0.2, 0.1, and 0.05 µg/mL.

5.2.3 Randomize and blind code triplicate aliquots of each solution.

5.2.4 Test each blinded aliquot on the LFA according to the product insert.

5.2.4.1 Use one lot of LFA test strips.

5.2.4.2 One analyst should read all LFA results.

5.2.4.3 The range of concentrations tested can be narrowed as long as data are collected for at least two successive concentrations yielding 3/3 positive results and two successive concentrations yielding 0/3 positive results.

5.2.4.4 LFAs applicable to testing of bulk solids and liquids may have a higher LOD than LFAs claiming applicability for analysis of residues.

5.2.5 Decode the data, tabulate, and report the number of positive results from the triplicate

analyses for each concentration of each analyte tested.

5.2.6 The lowest concentration yielding 3/3 positive results is the pLOD.

5.3 Final Limit of Detection

Based on the pLOD estimated from three replicate analyses on one lot of test strips with one reader, a final LOD is to be determined with 20 replicate analyses and is to account for variability due to test strip lot and reader. Carry out the experiment under indoor daylight lighting conditions (5000K, 5000–10000 lumens).

5.3.1 For each target analyte, prepare test solutions at 2x pLOD, 1x pLOD, and 0.5x pLOD.

5.3.2 Prepare a randomized testing plan and coding scheme such that 20 replicates of each solution are tested using three lots of LFA test strips, and carry out the testing according to the product insert.

5.3.2.1 The 20 replicate analyses should be approximately evenly divided among the three lots.

5.3.2.2 The three lots of test strips must be different manufacturing batches utilizing at least two lots of antibody or aptamer.

5.3.2.3 Readers should not be aware of the test solution or test strip lot for any given strip.

5.3.2.4 Randomly include two positive and two negative controls in the experiment.

5.3.2.4.1 Use kit controls if provided by the manufacturer.

5.3.2.4.2 If no kit controls, use drug solution at 10x pLOD and blank water or test kit buffer.

5.3.2.5 Use three readers to interpret the results of each test strip within a short time span (e.g., <1 min). For example, have one analyst initiate the LFA tests at regular intervals using a timer. When a test strip has developed according to the time required by the product insert, the strip is passed to Reader 1, then to Reader 2, then to Reader 3. Each reader visually interprets and records their own results (positive or negative) independently.

5.3.3 Decode the data and ensure that all readers correctly interpreted the positive and negative control strips.

5.3.4 Tabulate results by reader, test solution, and lot of test strips. Look for aberrant data by reader or test strip lot.

5.3.4.1 If any reader or test strip lot appears to be an outlier, perform root-cause analysis to determine the cause of the outlying data, then repeat the experiment.

5.3.4.2 An outlier is defined as one reader or one test strip lot differing from the others by >4/20 (>20%) replicates at any given concentration.

5.3.5 If there are no obvious outlier data sets, then determine the lowest concentration that yields an observed ≥95% POD across LFA lots and readers (≥57/60).

5.3.6 Additional concentrations can be tested if all concentrations are below 95% POD or if all concentrations are at 100% POD.

5.3.6.1 Additional concentrations may be, for example, 4x or 0.25x pLOD. Include positive and negative controls in the retesting.

5.3.7 Report the final LOD as the lowest concentration that yields an observed ≥95% POD across LFA lots and readers (≥57/60).

5.4 Matrix Study

Matrix studies determine the method performance in each matrix item. *See* Table 1 for preparation of validation materials. Carry out the experiment under indoor daylight lighting conditions (5000K, 5000–10000 lumens).

5.4.1 Include at least three matrix items per claimed category. *See* Table 6 of AOAC SMPR 2025.002 for example matrix compounds. Do not use any matrix compounds that were found to cross react in the selectivity study. If any matrix compound results in a faint, but visible, test line, another matrix item from that category may be chosen for the validation study.

5.4.2 For each matrix item, prepare validation materials according to Table 6 of AOAC SMPR 2025.002. There is a blank, low, and high material for each matrix. The blank material is matrix alone, the high material is target drug alone, and the low material is a low level of target drug in matrix. All materials are prepared in aqueous solution (e.g., water or a buffer supplied with the test kit). Matrix testing conditions are standardized at 2.0 mg/mL for bulk solids, 0.1 mL/1.0 mL (10%) for liquids, and 0.1 mg/mL for residues.

Table 1. Validation materials and acceptance criteria for SLV matrix studies

Matrix category	Material	Target drug concentration	Matrix amount	Aqueous diluent, mL[a]	*N* (number of replicates)[b]	Acceptance criterion, POD[c]
Bulk solid	Blank	0	10.0 mg	5.0	5	0.0
	Low	2 µg/mL[d]	10.0 mg		33	≥0.9
	High	2.0 mg/mL[e]	0		5	1.0
Liquid	Blank	0	0.10 mL	1.0	5	0.0
	Low	2 µg/mL[d]	0.10 mL		33	≥0.9
	High	2.0 mg/mL[e]	0		5	1.0
Residue	Blank	0	0.10 mg	1.0	5	0.0
	Low	0.10 µg/mL[d]	0.10 mg		33	≥0.9
	High	0.10 mg/mL[e]	0		5	1.0

[a] Diluent to be defined (e.g., tap water, bottled water, or buffer provided in kit, etc.).

[b] Replicates are aliquots of the same solution tested using individual test strips.

[c] POD = Probability of detection. Results at the low level must meet POD ≥0.9 with 95% confidence.

[d] Or 2x LOD, whichever is greater.

[e] Or maximum solubility, whichever is less.

5.4.2.1 If a method is designed to detect a class of drugs, then use a representative drug compound to prepare these solutions (*see* Table 1 of AOAC SMPR 2025.002).

5.4.3 Prepare enough of each material to accommodate testing of five replicate aliquots of blank and high materials and 33 replicate aliquots of the low material, depending on the test aliquot size of the candidate method.

5.4.4 Prepare a randomized testing plan and coding scheme such that the 33 replicates of low material, five replicates of high material, and five replicates of blank are tested across three lots of LFA test strips, and carry out the testing according to the product insert.

5.4.4.1 Each set of replicate analyses should be approximately evenly divided among the three lots. Therefore, each lot should receive 1–2 blank and high material aliquots and 11 low-level material aliquots.

5.4.4.2 Readers should not be aware of the test solution or test strip lot for any given strip.

5.4.4.3 Randomly include three positive and three negative controls in the experiment.

5.4.4.3.1 Use kit controls if provided by the manufacturer.

5.4.4.3.2 If no kit controls are provided, use drug solution at 10x LOD and blank water or test kit buffer.

5.4.4.4 Use three readers to interpret the results of each test strip within a short time span (e.g., <1 min). For example, have one analyst initiate the LFA tests at regular intervals using a timer. When a test strip has developed according to the time required by the product insert, the strip is passed to Reader 1, then to Reader 2, then to Reader 3. Each reader visually determines and records their own results (positive or negative) independently in quick succession. A data sheet pre-printed with blind codes is helpful.

5.4.5 Decode the data and ensure that all readers correctly interpreted the positive and negative control strips.

5.4.6 Tabulate results by reader, test solution, and lot of test strips. Look for aberrant data by reader or by test strip lot.

5.4.6.1 If any reader or test strip lot appears to be an outlier, perform root-cause analysis to determine the cause of the outlying data, then repeat the experiment.

5.4.6.2 An outlier is defined as one reader or one test strip lot differing from the others by >3/11 or >7/33 (>20%) replicates at the low level.

5.4.7 If there are no obvious outlier data sets, then determine the POD for each material using a consensus positive read for each test strip. A consensus positive read is one in which all three readers interpret the result as positive. If at least one reader interprets a strip as negative for the presence of analyte, the strip is deemed negative.

5.4.8 If consensus reads do not meet the acceptance criteria, perform root-cause analysis and test additional concentrations in matrix if needed. Report any matrix effects observed. Concentrations required to meet the low-level acceptance criteria may vary with matrix and drug analog.

5.4.9 The low-level acceptance criterion (POD ≥0.9) must be met with 95% confidence.

5.5 Product Consistency

5.5.1 Tabulate data from the low-level materials in the matrix studies by test strip lot.

5.5.2 For each drug/matrix combination, determine whether POD differs by product lot.

5.6 Robustness

Robustness evaluates the effects of making small changes in method steps (not environmental factors) on the results of the analysis. As these methods are expected to require few steps to perform, this study may be limited in scope.

5.6.1 Choose up to three method parameters to vary. These should be parameters that the user could vary in the field when performing the test, for example, timing, volume, or mass measurements.

5.6.2 If more than one parameter is varied, set up a factorial design of experiments using the high- and low-parameter settings. Compare to the nominal parameter settings. *See* Table 2.

5.6.3 For each trial, test three replicate aliquots of pure analyte at 2x LOD and three replicate aliquots of aqueous diluent in a randomized blind-coded manner.

5.6.4 Decode the data and report the number of positive results for the drug at 2x LOD and for the aqueous diluent for each trial. Report any parameter variation or interaction that causes a change in results from the nominal setting results.

5.7 Environmental Factors

5.7.1 *See* AOAC SMPR 2025.002 Table 7 for the environmental factors to test depending on the test kit configuration.

5.7.1.1 Elevated temperature storage = 55°C for 2 weeks

5.7.1.2 Decreased temperature storage = 0°C for 2 weeks

Table 2. Factorial design of experiment for robustness with 3-parameter variations

Trial	Parameter 1	Parameter 2	Parameter 3
1	High	High	High
2	High	High	Low
3	High	Low	High
4	High	Low	Low
5	Low	High	High
6	Low	High	Low
7	Low	Low	High
8	Low	Low	Low
9	Nominal	Nominal	Nominal

5.7.1.3 Low pH = pH 3

5.7.1.4 High pH = pH 11

5.7.1.5 Mechanical manipulation = manually twist test strip 90° in both directions three times

5.7.1.6 Elevated humidity storage = 30°C with 90% relative humidity for 2 weeks

5.7.1.7 Water hardness = water with $CaCO_3$ at 100, 200, 300, 400, and 500 mg/kg

5.7.1.8 Lighting = outdoor: direct sunlight and low sunlight (<300 lumens), indoor: daylight (5000K, 5000–10000 lumens) and warm white (2700K, 5000–10000 lumens).

5.7.2 Test each environmental factor individually.

5.7.3 For each environmental factor setting, test three replicate aliquots of pure analyte at 2x LOD and 10 replicate aliquots of aqueous diluent in a randomized blind-coded manner. Use one reader.

5.7.4 Decode the data and report the number of positive results for the drug at 2x LOD and for the aqueous diluent for each environmental factor setting. Report any factor setting that results in <3/3 positive results for the drug solution or any positive results for the blank. Also report any invalid results (control line invalid).

5.7.5 Add a limitation to the product insert if needed.

5.8 Product Stability

This study is intended to provide data to support the shelf-life of the test kit under normal storage conditions. There are three options for product stability testing. Five time points are required. [Examples are provided for a 12-month shelf life at 25 ± 5°C storage. Adjustments will be made for other storage and shelf-

life claims.] Existing product stability data from the MD's quality assurance program may be considered in lieu of the proposed studies below.

Prospective study.—Place three lots of newly manufactured test kits at 25 ± 5°C. Test at 0, 3, 6, 9, and 12 months. The three lots must be different manufacturing batches utilizing at least two lots of antibody or aptamer.

Retrospective study.—Choose test kits from five retention lots representing 0, 3-, 6-, 9-, and 12-months storage since manufacture.

Accelerated study.—If test kits of sufficient age are not available for testing prior to certification, accelerated stability can be conducted. For accelerated stability, store one lot of newly manufactured test kits at 45 ± 1°C. Test at 0, 5, 10, 30, and 50 days. Real-time stability data (via prospective study or retrospective study) are required at the first PTM annual renewal to verify the results of an accelerated study.

5.8.1 Prepare an aqueous solution of target analyte at 2x LOD in water or the aqueous buffer supplied in the LFA kit.

5.8.2 Randomize and blind code 10 replicate volumes of the analyte solution and 10 replicate volumes of water or aqueous buffer supplied in the LFA kit.

5.8.2.1 Volumes should be sufficient to accommodate all time points (i.e., >5x the test volume of the LFA).

5.8.2.2 If conducting a prospective study and the analyte solution will be stored frozen, prepare and freeze single-use volumes to avoid repeated freeze-thaw cycles. Solutions can also be prepared fresh for each time point if stability of the analyte is unknown.

5.8.3 For each stability time point or lot of test strips, test one aliquot of each blind-coded solution according to the product insert.

5.8.3.1 If the LFA kit includes positive and negative controls, test one replicate of each at each time point or with each lot of matched test strips.

5.8.3.2 If no kit controls are provided, use drug solution at 10x LOD and blank water or test kit buffer as experimental controls.

5.8.3.3 The report should specify what kind of study was performed, and how the test solutions were prepared and stored.

5.8.3.4 Use one reader to interpret all test strips.

5.8.4 Decode results and tabulate the number of positive responses for the target drug at each time point or for each lot. Report any cases of positive results for the blanks. If the LFA kit includes kit controls, report those results at each time point or for each lot.

5.8.5 Plot the data and report any loss of detection over time.

6. Independent Laboratory Validation Study

The MD in consultation with the AOAC technical consultant is to choose an approved AOACRI independent laboratory with no conflict of interest and enter into a financial agreement. The ILV study is supervised by the AOAC technical consultant. The ILV study includes a subset of the SLV matrix study at a minimum. The MD may choose to transfer any or all of the SLV studies to a third-party laboratory that they have contracted or to the independent laboratory.

6.1 Matrix Study

The ILV matrix study verifies the method performance in one matrix item for each claimed category. *See* Table 3 for preparation of validation materials. Carry out the experiment under indoor daylight lighting conditions (5000K, 5000–10000 lumens).

6.1.1 The independent laboratory is to test one matrix item per claimed category. Matrices are to be chosen by the volunteer expert and specified in the candidate method validation protocol.

6.1.2 For each matrix item, prepare validation materials according to Table 6 of AOAC SMPR 2025.002. There is a blank, low, and high material for each matrix. The blank material is matrix alone, the high material is target drug alone, and the low material is a low level of target drug in matrix. All materials are prepared in aqueous solution (e.g., water or a buffer supplied with the test kit). Matrix testing conditions are standardized at 2.0 mg/mL for bulk solids, 0.1 mL/1.0 mL (10%) for liquids, and 0.1 mg/mL for residues.

6.1.2.1 If a method is designed to detect a class of drugs, then use a representative drug compound to prepare these solutions (*see* Table 1 of AOAC SMPR 2025.002).

6.1.3 Prepare enough of each material to accommodate testing of five replicate aliquots of blank and high materials and 33 replicate

Table 3. Validation materials and acceptance criteria for ILV matrix studies

Matrix category	Material	Target drug concentration	Matrix amount	Aqueous diluent, mL[a]	*N* (number of replicates)[b]	Acceptance criterion, POD[c]
Bulk solid	Blank	0	10.0 mg	5.0	5	0.0
	Low	2 µg/mL[d]	10.0 mg		33	≥0.9
	High	2.0 mg/mL[e]	0		5	1.0
Liquid	Blank	0	0.10 mL	1.0	5	0.0
	Low	2 µg/mL[d]	0.10 mL		33	≥0.9
	High	2.0 mg/mL[e]	0		5	1.0
Residue	Blank	0	0.10 mg	1.0	5	0.0
	Low	0.10 µg/mL[d]	0.10 mg		33	≥0.9
	High	0.10 mg/mL[e]	0		5	1.0

[a] Diluent to be defined (e.g., tap water, bottled water, or buffer provided in kit, etc.).

[b] Replicates are aliquots of the same solution tested using individual test strips.

[c] POD = Probability of detection. Results at the low level must meet POD ≥0.9 with 95% confidence.

[d] Or 2x LOD, whichever is greater.

[e] Or maximum solubility, whichever is less.

aliquots of the low material, depending on the test aliquot size of the candidate method.

6.1.4 Prepare a randomized testing plan and coding scheme such that the 33 replicates of low material, five replicates of high material, and five replicates of blank are tested across three lots of LFA test strips, and carry out the testing according to the product insert.

6.1.4.1 Each set of replicate analyses should be approximately evenly divided among the three lots. Therefore, each lot should receive 1–2 blank and high material aliquots and 11 low-level material aliquots.

6.1.4.2 Readers should not be aware of the test solution or test strip lot for any given strip.

6.1.4.3 Randomly include three positive and three negative controls in the experiment.

6.1.4.3.1 Use kit controls if provided by the manufacturer.

6.1.4.3.2 If no kit controls are provided, use drug solution at 10x LOD and blank water or test kit buffer.

6.1.4.4 Use three readers to interpret the results of each test strip within a short time span (e.g., <1 min). For example, have one analyst initiate the LFA tests at regular intervals using a timer. When a test strip has developed according to the time required by the product insert, the strip is passed to Reader 1, then to Reader 2, then to Reader 3. Each reader visually interprets and records their own results (positive or negative) independently in quick succession. A data sheet pre-printed with blind codes is helpful.

6.1.5 Decode the data and ensure that all readers correctly interpreted the positive and negative control strips.

6.1.6 Tabulate results by reader, test solution, and lot of test strips. Look for aberrant data by reader or test strip lot.

6.1.6.1 If any reader or test strip lot appears to be an outlier, perform root-cause analysis to determine the cause of the outlying data, then repeat the experiment.

6.1.6.2 An outlier is defined as one reader or one test strip lot differing from the others by >3/11 or >7/33 (>20%) replicates at the low level.

6.1.7 If there are no obvious outlier data sets, then determine the POD for each material using a consensus positive read for each test strip. A consensus positive read is one in which all three readers interpret the result as positive. If at least one reader interprets a strip as negative for the presence of analyte, the strip is deemed negative.

6.1.8 If consensus reads do not meet the acceptance criteria, perform root-cause analysis and test additional concentrations in matrix if needed. Report any matrix effects observed. Concentrations required to meet the low-level acceptance criteria may vary with matrix and drug analog.

6.1.9 The low-level acceptance criterion (POD ≥0.9) must be met with 95% confidence.

6.2 Product Consistency

6.2.1 Tabulate data from low-level materials in the matrix studies by test strip lot.

6.2.2 For each drug/matrix combination, determine whether detection differs by product lot.

7. Field Use Study

This study is intended to demonstrate acceptable performance of the candidate method in the hands of naïve end users. Field use studies may identify test kit limitations due to difficult manipulations or results interpretation or poorly written instructions for use. Carry out the experiment under indoor daylight lighting conditions (5000K, 5000–10000 lumens).

7.1 Assemble at least 15 individuals with no experience using LFAs for drug testing.

7.1.1 Include a variety of ages (≥21 years), levels of education, and socioeconomic backgrounds.

7.2 For each user, provide three replicate aliquots of a surrogate analyte at 2x LOD and three replicate aliquots of aqueous diluent in a randomized blind-coded manner. Also provide negative bulk matrix.

7.2.1 If no surrogate analyte is available, provide only the diluent aliquots and the bulk matrix.

7.3 Ask each intended user to read the instructions for use and familiarize themselves with the kit contents.

7.4 Provide appropriate personal protective equipment for each user, such as latex gloves, eye protection, and lab coat.

7.5 Ask each user to perform the sample preparation with the bulk matrix. Observe closely for proper sample handling and manipulation. Record any errors observed.

7.6 Ask each user to perform the assay with the prepared bulk matrix and supplied blinded liquid aliquots, and record positive, negative, or invalid results.

7.6.1 If no surrogate analyte is available, provide additional "pre-run" test strips for the user to interpret. These should include at least three negative (aqueous diluent) and three positive (drug at 2x LOD) tests in random order.

7.7 Decode the data and report the number of positive results for the surrogate drug at 2x LOD, the aqueous diluent, and the negative matrix for each participant. Report any user experiencing difficulty in carrying out the method or interpreting the results.

7.7.1 If no surrogate analyte is available, report the number of positive results for the "pre-run" test strips by sample type (drug vs diluent).

7.8 Provide corrective action once, if needed, for each participant and retest.

7.9 Report all test and retest results.

7.10 Record any comments from the user, including clarity of the method instructions.

7.11 Revise product insert to clarify where needed based on study results.

8. Report

The validation study report is to be submitted by the MD at the completion of data collection. This report is to incorporate the results generated by the SLV and ILV studies as well as results from the field use study.

The assigned expert reviewers and volunteer expert will review the study report and the product insert. Once reviewed and any revisions are made, the reviewers will determine if the method has met the AOAC SMPRs and approves it.

The AOACRI will issue certification for PTM status. The PTM certificate and certification mark will also be issued.

The MD is encouraged to submit the approved report for publication in the *Journal of AOAC INTERNATIONAL*.