VALIDATION PLAN

Office of Microbiology

Validation Plan Title	
Qualitative or Quantitative Assay	
Date	
Lab Unit	
Written by	
Proposed Test Implementation Date	

This validation plan describes how the laboratory units in the Office of Microbiology at the Washington State Public Health Laboratories intends to establish the performance characteristics of lab developed tests (LDTs) or modified FDA cleared tests. Performance characteristics established for each test include: accuracy, precision, sensitivity, specificity, analytical sensitivity (limit of detection), and analytical specificity (cross reactivity/interfering substances), reportable range, and reference ranges/Intervals (Normal Values), analytical measurement range (AMR) as applicable. **This plan must be approved before performing the actual validation**.

VALIDATION PLAN

Office of Microbiology

PURPOSE: Please describe **below** the reason for implementing this test and its intended use. Include all acceptable specimen types. **Note:** Each acceptable specimen type to be tested at the WA PHL must be evaluated.

TEST PRINCIPLE: Please describe **below** the scientific basis of how this tests works.

INSTRUMENTATION & REAGENTS: Please describe **below** the reagents/ kit and instrumentation that will be used in the validation. Add rows below as needed.

Reagent	Lot Number	Expiration Date

VALIDATION PLAN

Office of Microbiology

Instrument	Serial Number	Manufacturer

SAMPLES: At the WA PHL a minimum of 60 samples/isolates per target and/or sample type is required during the validation process. This must include samples representing a combination of negative and positive samples at varying levels. **Note:** Less than 60 samples require approval **before** submitting the validation plan. Please list your samples below including data in appropriate fields. Add rows below as needed.

Sample #	Source	Specimen #	Target	Expected Result	Other

ACCURACY: Are your test results correct? This can be determined by testing commercially available calibrators/calibration and quality control materials with known values, proficiency testing materials that have established values, and previously tested patient specimens with established values. Total accuracy should be **at least 90%** relative to the reference method. Please describe your plan to determine accuracy **below** this will additionally satisfy calculations for PPV & NPV, clinical sensitivity & clinical specificity as well.

$$\text{Accuracy (Agreement)} = \frac{\text{No. of correct results}}{\text{Total no. of results}} \times 100$$

PREDICTIVE VALUE PPV& NPV: The predictive value of a test is the probability that a positive result (PPV) accurately indicates the presence of an analyte or a specific disease or that a negative result (NPV) accurately indicates the absence of an analyte or a specific disease. Calculations for these should come from your accuracy sample pool numbers (TP, FP, TN, FN).

Accuracy PPV= [TP/(TP+FP)] X 100 **Accuracy NPV = [TN/(TN+FN)] X 100**

SENSITVITY & SPECIFICITY: Clinical sensitivity refers to the ability of a test to detect a disease or clinical condition, while clinical specificity refers to the degree to which a test is negative when disease is absent. Determinations of sensitivity and specificity should be done in a **"blinded"** fashion (i.e. without prior knowledge of the patient's disease status). Calculations for these should come from your accuracy sample pool numbers (TP, FP, TN, FN). Both sensitivity and specificity should be **at least 95%** compared to the reference method.

Sensitivity = [TP/(TP+FN)] X 100 **Specificity= [TN/(TN+FP)] X 100.**

PRECISION/REPRODUCIBILITY: Can you obtain the same test result time after time? Confirming this parameter can be done repeatedly testing the same samples on the same day, and on different days and getting the same or comparable results (reproducible),regardless of which member of the laboratory's testing personnel performs the test (operator variance). Precision/reproducibility should be **at least 95%** compared to the reference method. **Exception:** Fully automated test systems that are not operator dependent, operator variance should not affect the test's precision and may not need to

be evaluated by more than one person. Please describe **below** how you plan to establish the intra-run and inter-run precision/reproducibility.

Qualitative Test **Reproducibility = [No. of repeated results in agreement/Total no. results] x 100**

Quantitative Test **Precision= (Coefficient of Variation (CV)) = [Standard Deviation/Average] x 100**

ANALYTICAL SENSITIVITY/LIMIT OF DETECTION: This refers to the ability of a test to detect a target analyte (e.g., an antibody or antigen), which is usually expressed as the minimum detectable concentration of the analyte also known as the limit of detection. LOD is expressed as a concentration (usually copies/ml; copies/µgDNA for molecular assays) such that the lower the detectable concentration of analyte, the greater the analytical sensitivity of the assay. Please describe **below** your plan to determine the analytical sensitivity of the assay.

ANALYTICAL SPECIFICITY/CROSS REACTIVITY/INTERFERING SUBSTANCES: Analytical specificity refers to the ability of an assay to detect only the intended target and that quantification of the target is not affected by cross-reactivity from related or potentially interfering nucleic acids or specimen-related conditions. The two aspects of analytical specificity are cross-reactivity and interference. For molecular assays this would include primers/probe sequences identified during blast searches. Please describe **below** your plan to establish the analytical specificity of the assay.

REPORTABLE RANGE: How high and how low can test result values be and still be accurate? If applicable this can be done by choosing samples with known values at the highest and lowest levels the manufacturer claims accurate results can be produced by the test system. Please describe **below** how you plan to establish the reportable range if applicable. **Note:** At the WA PHL we list positive, negative, and inconclusive other similar results for not applicable qualitative tests that do not have a reportable range.

REFERENCE RANGE/NORMAL VALUES: Do the reference ranges provided by the test system's manufacturer fit your patient population? You may begin patient testing using the manufacturer's suggested reference range(s) or you may use other published reference ranges from a textbook or a journal publication. When you test known normal patients, the results should be within your reference range and with abnormal patients, you should expect results outside the reference range. Note: At the WA PHL we list negative or not detected for many of the infectious disease conditions we use qualitative tests for. Please describe **below** what and how you plan to verify regarding the reference range.

ANALYTICAL MEASUREMENT RANGE/ AMR: **NOTE: QUANTITATIVE ONLY**. AMR is the range of analyte values that a method can directly measure on the specimen without any dilution, concentration, or other pretreatment not part of the usual assay process. AMR validation is the process of confirming that the assay system will correctly recover the concentration or activity of the analyte over the AMR. Materials used for validation must be known to have matrix characteristics appropriate for the method. The test specimens must have analyte values that, at a minimum, are near the low, midpoint, and high values of the AMR. For quantitative tests please describe below how you plan to establish the AMR.

LITERATURE REFERENCES/PACKAGE INSERTS: List **below** any technical and/or literature used in the verification planning process.

OTHER PERFORMANCE CHARACTERISITCS: List **below** any known limitations or unusual characteristics of the test that people performing the test should be aware of.

NEED FOR CLIENT EDUCATION: Please describe **below** how this will be provided **if needed**.

VALIDATION PLAN

Office of Microbiology

APPROVAL

Statement	This validation plan has been reviewed and has been considered acceptable for initiation of establishing performance characteristics of the proposed test.
Lead Signature & Date	
Supervisor Signature & Date	
Office Director Signature & Date	
QA Officer Signature & Date	
CLIA Director Signature & Date	