MCHD Laboratory Developed Tests

Laboratory developed tests are defined as tests that have not been approved by FDA for commercial manufacture and use as medical devices. These tests have not gone through FDA premarket review and approval. The FDA has no regulatory oversight of the manufacturing process. By default, **these tests are regulated as high complexity tests under CLIA** and the laboratory must conduct internal verification and implement quality control systems including quality control of reagents and ongoing verification of accuracy. Unless noted otherwise, these tests may be used for clinical diagnosis when performed on validated specimens. The various types of LDT used by the Monterey County Health Department (MCHD) Laboratory are described below:

Verified with all Performance Characteristics Fully Established

These tests have been verified by the laboratory and the performance characteristics, including clinical utility have been established. These tests use analytic specific reagents that are not regulated by FDA. The laboratory has developed quality assurance systems to assure reagents have been tested prior to being used for production. Although these tests are suitable for clinical purposes the following disclaimer is required on the laboratory report by FDA:

> *The disclaimer for Analyte Specific Reagents (ASR) should state ["This test was developed and its performance characteristics determined by (Laboratory Name). It has not been cleared or approved by the U.S. Food and Drug Administration"]. The ASR disclaimer on the test report is required by the FDA under 21 CFR, Part 809.30 "Restrictions on the sale, distribution and use of analyte specific reagents."*

PCR (Target)	Validated specimens/samples	Sensitivity, Specificity, PPV, NPV
TB (ITS 2)	Respiratory (Sputum & Bronch Wash) and culture isolates	Sensitivity 85.5% Specificity 99.3% PPV 82.1% NPV 96.8%
Pertussis (IS485)	Nasopharyngeal	Sensitivity 97.1% Specificity 100% PPV 100% NPV 99.5%
Coccidioides immitis (ITS 2)	Respiratory (Sputum & Bronch Wash) and culture isolates	Sensitivity 90.3% Specificity 98.5% PPV 89.3% NPV 98.6%
Influenza typing (A, & B)	Upper respiratory (Nasopharyngeal swab, wash or aspirate, throat swab) Lower respiratory tract (tracheal aspirate, bronchoalveolar lavage)	Sensitivity 99.3% Specificity 92.3% PPV: Unavailable NPV: Unavailable

Verified with Performance Characteristics Partially Established

These tests have been developed by a government agency (CDC, FDA, USDA). They use analytic specific reagents that are not regulated by FDA. The laboratory has developed quality assurance systems to assure reagents have been tested prior to being used for production. These tests have been verified by the laboratory; however, the performance characteristics are limited oftentimes because prevalence of infection is so low, sensitivity cannot be established. Consequently, although these tests are suitable for clinical use, results are only reported in the context of other conventional methods. For example, in many instances, PCR is highly sensitive technique, but because DNA, can persist for weeks or months, follow-up testing is required for positive specimens to confirm presence of viable organisms.

PCR (Target)	Validated specimen/samples	Performance Characteristics[1]
Staph aureus (Sa442)	Culture isolates or swab enriched in TSB with 6.5%NaCl	Sensitivity: 10^4cfu/mL Specificity: non-MRSA=60% MRSA=100% Precision: 1.75-2.39 Linearity R^2=0.9975
Staph aureus (mecA)	Culture isolates	Sensitivity: 10^4 cfu/mL Specificity: 100% Precision: 1.51-4.03 Linearity: R^2=0.9982
Strep pneumonia (lytA)	Culture isolates or environmental samples	Sensitivity: 312 cfu/mL Specificity: 100% Precision: 0.65-2.4% Linearity: R^2=0.9901
Haemophilus influenza (hpd)	Culture isolates or environmental samples	Sensitivity: 981 cfu/mL Specificity: 100% Precision: 1.04-4.10% Linearity: R^2=0.9995
Neisseria meningitidis (ctrA)	Culture isolates or environmental samples	Sensitivity: 220 cfu/mL Specificity: 100% Precision: 0.47-1.89% Linearity: R^2=0.9976
Salmonella sp. (spaQ)	Culture isolates, stool, rectal swab, or environmental samples (food or water) enriched in Selenite or RV broth	Sensitivity: 100% Specificity: 98.7% Precision: 0.53%-1.55% Linearity: 0.9952
Shigella sp (ipaH)	Culture isolates, stool, rectal swab, or environmental samples (food or water) enriched in GN broth	Sensitivity: 190cfu/mL Specificity: 100% Precision: 1.26-5.66% Linearity: R^2=0.9989
Campylobacter (16S)	Culture isolates, stool, rectal swab, or environmental samples (food/water) enriched in Bolton	Sensitivity: 3600 cfu/mL Specificity: 100% Precision: 0.65-2.8%

	broth	Linearity: R^2= 0.9989
Listeria sp. (23S)	Culture isolates, environmental samples (food/water) enriched in BLEB	Sensitivity: 18 cfu/ml Specificity: 100% Precision: 0.90 – 2.53% Linearity: 0.9916
Listeria monocytogenes (iap)	Culture isolates, environmental samples (food/water) enriched in BLEB	Sensitivity: 3.6 x 10^2 cfu/ml Specificity: 100% Precision: 0.64-2.59% Linearity 0.9911

(1) Sensitivity of method without culture amplification. When used in tandem with culture enrichment, sensitivity approaches the theoretic detection limit of 1cfu.

Gold Standard Verified by Performance Panel

These tests have been developed by a government agency (CDC, FDA, USDA). They use analytic specific reagents that are not regulated by FDA. The laboratory has developed quality assurance systems to assure reagents have been tested prior to being used for production. Our laboratory has not determined performance characteristics because a gold standard is not readily available. These tests have been verified by comparing our performance with the State Laboratory or the CDC. These tests are for clinical use, since the method is the current the gold standard.

PCR (Target)	Validated specimens/samples	Source of performance panel
Influenza subtyping (PdmA H1, H3, H5& PdmH1)	Upper respiratory (Nasopharyngeal swab, wash or aspirate, throat swab) Lower respiratory tract (tracheal aspirate, bronchoalveolar lavage)	VRDL Performance Panel
stx 1 & 2	Stool enriched in GN broth.	MDL Performance Panel
Measles	Nasopharyngeal swab and aspirates, Urine	VRDL & CDC Performance Panel
Norovirus G1 & G2	Stool	VRDL Performance Panel
Enterovirus	Respiratory (e.g.Nasophayngeal swab and aspirates, nasal swab, throat swab, BAL & tracheal) CSF	VRDL Performance Panel

Investigational Use or Research use Only (RUO)

These tests have been developed by a government agency (CDC, FDA, USDA). They use analytic specific reagents that are not regulated by FDA. The laboratory has developed quality assurance systems to assure reagents have been tested prior to being used for production. While these tests are derived from reliable sources, their verification has been limited to performance

parameters measured under laboratory conditions (i.e. not involving clinical specimens). Clinical sensitivity and specificity have not been established.

> *Interpretive Guidelines §493.1291(c)(4):*
> *Laboratories using manufacturer's instruments, kits or test systems labeled for "investigational use only" or "research use only" must clearly state that the test results are not to be used for treatment or diagnostic purposes. If results of such tests are being reported without a disclaimer statement, or are being used by the provider for patient care, they are in the same category as in-house developed tests and the laboratory must establish performance specifications in accordance with §493.1253 as described above*

PCR (Target)	**Validated specimens/samples**	**Sensitivity, Specificity, PPV, NPV**
Cyclospora	Stool, Food, Water	Sensitivity: 0.02 pg/ml Specificity: TBD Precision: 0.53-9.20 Linearity: 0.9963
Strep pneumonia (lytA)	Blood, CSF	Sensitivity: 312 cfu/mL Specificity: 100% Precision: 0.65-2.4% Linearity: R^2=0.9901
Haemophilus influenza (hpd)	Blood, CSF	Sensitivity: 981 cfu/mL Specificity: 100% Precision: 1.04-4.10% Linearity: R^2=0.9995
Neisseria meningitidis (ctrA)	Blood, CSF	Sensitivity: 220 cfu/mL Specificity: 100% Precision: 0.47-1.89% Linearity: R^2=0.9976
Bacillus anthracis (capA)	Culture isolates or environmental samples	Sensitivity: 0.0002 pg/ml Specificity: TBD Precision: 0.97-1.75% Linearity: R^2=0.993

Comment: TB, pertussis, Cocci, Influenza
This PCR is a lab developed test and its performance characteristics determined by the Monterey County Public Health Laboratory. It has not been cleared or approved by the U.S Food and Drug Administration.

Comment: All other PCR's
This PCR is a lab developed test and its performance characteristics determined by the Monterey County Public Health Laboratory. It has not been cleared or approved by the U.S Food and Drug Administration. This test has been verified under laboratory conditions and is suitable for clinical purposes when used as an adjunct ot other conventional methods; it is not limited to investigations or research.

Comment: Influenza subtyping, Norovirus, Measles, Stx 1&2 and Enterovirus
This PCR is a lab developed test obtained from CDC. It is the current gold standard for clinical purposes. Its performance parameters cannot be established since there is no other readily available lab method for clinical comparison.

Comment for Cyclospora, H. influenza, Neisseria meningitidis, and Strep. pneumonia PCR on source material
This PCR is a lab developed test obtained from CDC. Performance parameters are limited to parameters measured under laboratory conditions (detection limit, linearity, accuracy and precision). Clinical sensitivity and specificity have not been established.

Abbreviations

ASR Analyte Specific Reagents
BAL Bronchoalveolar lavage
BLEB Buffered Listeria Enrichment Broth
CDC Centers for Disease Control
cfu Colony forming units
CLIA Clinical Laboratory Improvement Amendments
CSF Cerebral spinal fluid
FDA Food and Drug Administration
GN Gram-Negative
LTD Laboratory Developed Tests
MDL Microbial Diseases Laboratory (Richmond, CA)
ml milliliter
NPR Negative Predictive Value
PCR Polymerase Chain Reaction
pg picogram
PPV Positive Predictive Value
R^2 R squared (coefficient of determination)
TBD To Be Determined
USDA United States Department of Agriculture
VRDL Viral & Rickettsial Disease Laboratory (Richmond, CA)