GUIDANCE DOCUMENT

VALIDATION OF RAPID DIAGNOSTICS FOR PATHOGEN IDENTIFICATION AND ANTIMICROBIAL SUSCEPTIBILITY TESTING (AST)

2025

Division of Descriptive Research,
Indian Council of Medical Research

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Division of Descriptive Research

In-Vitro Diagnostics Division

ICMR, New Delhi

GUIDANCE DOCUMENT: VALIDATION OF RAPID DIAGNOSTICS FOR PATHOGEN IDENTIFICATION AND ANTIMICROBIAL SUSCEPTIBILITY TESTING (AST), 2025.

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PREFACE

This document has been designed to provide assistance to the innovators and testing laboratories in validating diagnostics meant for pathogen identification and antimicrobial susceptibility testing. This validation protocol lays out a comprehensive framework to systematically evaluate and confirm the diagnostic performance of a given test, ensuring its reliability and its utility for clinical decision-making. This document describes evaluation criteria for methods to detect, identify and quantify pathogenic micro-organisms or the genetic materials i.e. DNA, RNA, toxins, antigens, or any other product of these organisms as well as methods for antimicrobial susceptibility and illustrates the steps to validate these diagnostic tests. This guidance document encompasses the assessment of precision, accuracy, reproducibility, and the ability to correctly identify the target pathogen and/or antimicrobial susceptibility. It will also help the innovators and developers understand the kind of evidence required to be generated before approaching the validating laboratory and prepare for the validation process. This document delineates the steps in the pathway of regulatory approval for a test. This is an evolving document that aims to align with emerging technologies and novel methodologies.

This document has been prepared by the AMR co-ordination unit of ICMR and is the third version of the draft protocol, having undergone two rounds of inputs from experts and Central Drugs Standard Control Organization (CDSCO) through e-mails and stakeholder consultations held at ICMR headquarters at New Delhi.

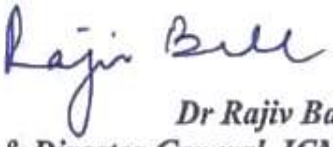
FOREWORD BY DIRECTOR GENERAL, ICMR

Antimicrobial resistance (AMR) poses a significant global threat to our efforts in combating infectious diseases. Inability to provide accurate and timely diagnosis is one of the major contributors to the misuse and overuse of antimicrobials. In India, there is a pressing need for high-quality, affordable diagnostic tools tailored to meet the specific requirements of the healthcare system.

Recent advances in molecular techniques have enabled the development of new and improved diagnostic tests capable of identifying microorganisms and detecting antimicrobial resistance genes. However, the lack of a standardized framework for validation process to be followed for new diagnostics has impeded their progress and uptake in the health care system.

To address this gap, the Indian Council of Medical Research (ICMR), in consultation with the Central Drugs Standard Control Organisation (CDSCO) and relevant experts, has developed a comprehensive protocol to guide the validation process for rapid diagnostic tests for pathogen identification and antimicrobial susceptibility testing (AST). This protocol provides guidance to innovators and developers for evaluating their test and demonstrating their effectiveness. By adhering to this standardized process, innovators can expedite validation, reducing the time and steps required for regulatory approval

We sincerely hope that this document will be instrumental in bridging the gap between developers and regulatory bodies thus minimizing delays and facilitating the timely introduction of validated tests in Indian health care system, thus supporting efforts to combat AMR.



Dr Rajiv Bahl
Secretary, DHR & Director General, ICMR

FOREWORD BY DRUGS CONTROLLER GENERAL OF INDIA

Antimicrobial resistance (AMR) is one of the most pressing public health challenges of our time, threatening to undermine the efficacy of many life-saving antibiotics and other antimicrobial agents. The rapid and accurate diagnosis of infectious diseases is a critical first step in ensuring the appropriate use of antimicrobials. Unfortunately, delays in diagnosis, combined with a lack of affordable and reliable diagnostic tools, contribute significantly to the inappropriate use of antibiotics, further driving the emergence and spread of AMR.

In India, where infectious diseases continue to pose a significant burden on public health, the need for high-quality, affordable, and easily accessible diagnostic tools is more urgent than ever. Recent advancements in molecular diagnostics and antimicrobial susceptibility testing (AST) hold great promise in addressing this gap. However, despite these advancements, the absence of a clear, standardized framework for validating these diagnostic tests has hindered their widespread adoption and integration into the healthcare system.

In recognition of this critical gap, the Indian Council of Medical Research (ICMR), in collaboration with the Central Drugs Standard Control Organisation (CDSCO) and other relevant stakeholders, has developed a comprehensive validation protocol for rapid diagnostic tests aimed at pathogen identification and antimicrobial susceptibility testing. This protocol provides a clear, systematic approach for innovators and developers to evaluate the performance and efficacy of their diagnostic tools in line with regulatory standards.

It is our hope that this initiative will foster greater collaboration between innovators, regulatory bodies, and the healthcare system, facilitating the introduction of effective diagnostic tools that will play a pivotal role in the fight against AMR in India.

Dr. Rajeev Singh Raghuvanshi
Drugs Controller General of India

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ABBREVIATIONS

AD	Agar Dilution
AMR	Antimicrobial resistance
AST	Antimicrobial Susceptibility Testing
ATCC	American Type Culture Collection
BMD	Broth Microdilution
CA	Categorical Agreement
CDSCO	Central Drugs Standard Control Organization
CLIA	Clinical Laboratory Improvement Amendments
CLSI	Clinical and Laboratory Standards Institute
CPE	Clinical Performance Evaluation
CRS	Composite Reference Standard
DD	Disk Diffusion
DNA	Deoxyribonucleic Acid
EA	Essential Agreement
ER	Error Rates
EUCAST	European Committee on Antimicrobial Susceptibility Testing
FDA	Food and Drug Administration
ID	Infectious Diseases
ISO	International Organization for Standardization
IVD	In Vitro Diagnostics
IVDMD	In Vitro Diagnostic Medical Device
LLOQ	Lower limit of quantification
LOD	Limit of Detection
MDR	Multiple Drug Resistance
MEs	Major Errors
mEs	Minor Errors

MIC	Minimum Inhibitory Concentration
MTCC	Microbial Type Culture Collection & Gene Bank
NABL	National Accreditation Board for Testing and Calibration Laboratories
NPV	Negative Predictive Value
PHC	Primary Health Care
PPV	Positive Predictive Value
QA	Quality Assurance
QC	Quality Control
RNA	Ribonucleic Acid
SDD	Susceptible Dose Dependent
S/I/R	Susceptible/Intermediate resistance/Resistance
SOP	Standard Operating Procedure
S/R	Susceptible/Resistance
UTI	Urinary Tract Infection
VMEs	Very Major Error

Introduction

Chapter 1

Introduction

Antimicrobial resistance (AMR) poses a critical global health concern, particularly in countries like India with dense population, a significant burden of infectious diseases and diverse healthcare practices which may lead to antibiotic consumption. Accurate diagnosis of the infection using a quality assured diagnostic test is essential for the timely initiation of treatment and for reducing the misuse of antimicrobials. A number of innovators are working towards the development of new indigenous diagnostics to address the healthcare needs of Indian population. These tests can be helpful in early identification of the pathogen and rapid testing of antimicrobial susceptibility phenotype or detection of antimicrobial resistance genes or markers. The impact of these tests on clinical decisions relies on their accuracy, which is established by comparing them to reference standard methods. However, systematic validation of these diagnostics remains challenging, impacting their adoption in healthcare (Sharma et al., 2021). Despite the availability of enormous literature on the validation of diagnostic tests, the steps that should be followed to undertake validation needs to be clearly defined for the diagnostic tests meant for AMR containment.

Validation of a diagnostic test is a systematic evaluation of the test which has been developed, standardized and optimized, to determine its fitness for the specific intended use (OIE, 2019). The World Health Organization (WHO) defines validation as 'the action (or process) of proving that a procedure, process, system equipment or method used works as expected and achieves the intended result' (WHO, 1995). The process of validation includes assessment of the analytical and diagnostic performance characteristics of a test. The parameters that should be tested are often considered to be based upon the type of test and are not uniform across all the tests or assays.

One of the major bottlenecks in undertaking a validation study is the lack of defined uniform protocols and absence of clarity on the parameters that need to be tested to establish the fitness-of-purpose of a new indigenous test. Guidelines for study design, interpretation of the results, execution in appropriate settings and performance evaluation remains ambiguous which decelerate the processes of development as well as regulatory approvals for indigenous AMR diagnostic tests. The developer derives information for test validation from various standard

documents that are available from Clinical and Laboratory Standards Institute (CLSI), European Committee on Antimicrobial Susceptibility Testing (EUCAST), Clinical Laboratory Improvement Amendments (CLIA), the International Organization for Standardization (ISO), Bureau of Indian Standards (BIS), United States' Food and Drug Administration (FDA) and WHO.

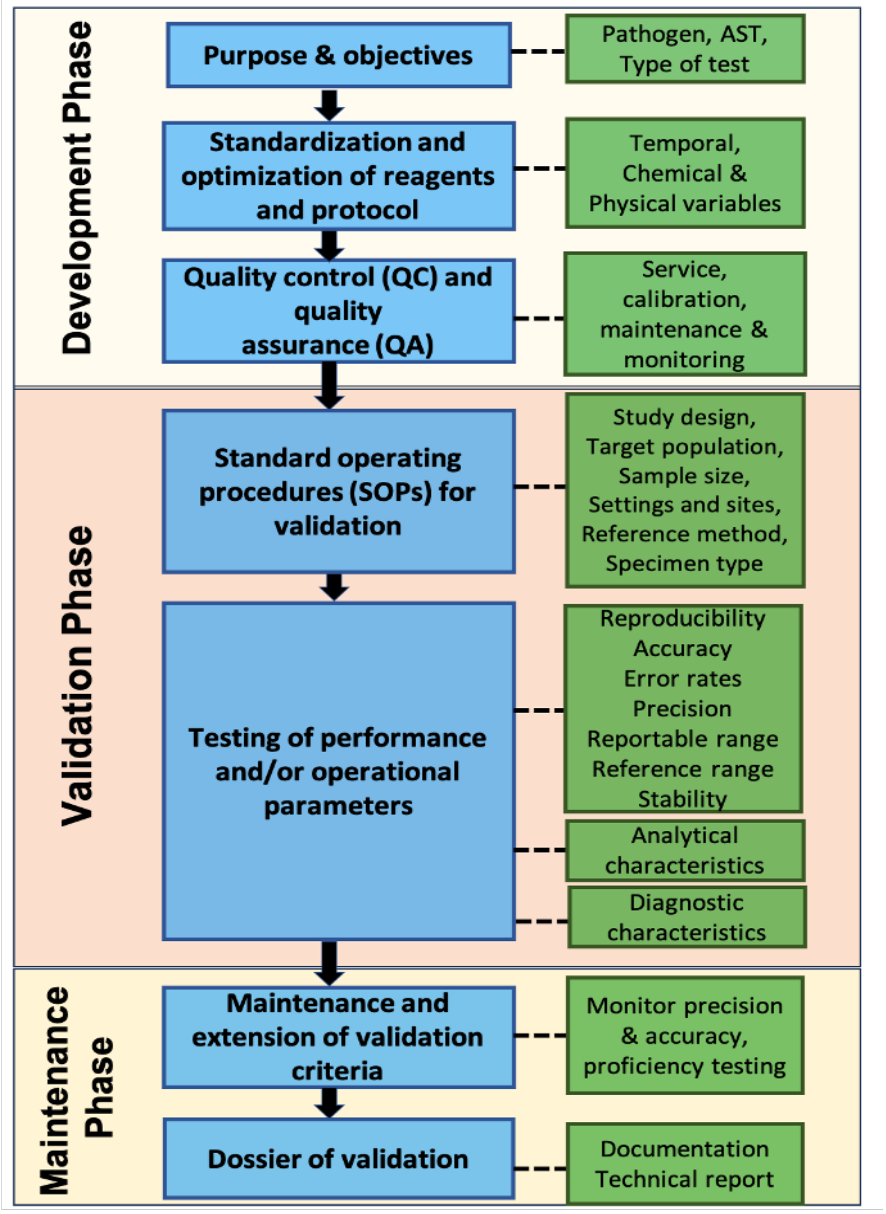

Figure 1: The process and the steps required in the validation of an indigenously developed diagnostic test

In India, various processes related to approvals on In Vitro Diagnostics (IVD) are regulated by CDSCO and a recently created online platform of MedTech Mitra (ICMR initiative in collaboration with the CDSCO and under the guidance of NITI Aayog) has been created by GoI to provide strategic support to MedTech innovators for clinical evaluation, regulatory facilitation

and the adoption of new products. Also, the Manthan digital platform, an initiative of the Office of the Principal Scientific Adviser, aims to bridge the gap between industry demand and the academic and startup ecosystem.

This document attempts to elaborate on the requisites, steps and process-flow for undertaking the validation of indigenous diagnostic tests for AMR. It supplements the existing CDSCO guidelines (CDSCO/IVD/GD/Stability/01/2022, CDSCO/IVD/GD/PER/01/2022) by bridging information gaps and providing guidance on test validation for innovators and developers in the country. This document establishes evaluation criteria for methods to detect, identify and quantify pathogenic micro-organisms or the genetic material *i.e.* DNA, RNA, toxins, antigens, or any other product of these organisms and testing method of antimicrobial susceptibility and illustrates the steps to validate these diagnostic tests. Figure1 illustrates the components and steps of validation study. This document provides predetermined acceptance criteria to ensure the safety and performance of IVD devices, in line with Medical Device Rules, 2017 and CDSCO requirements. Developers can evaluate their application using the guidance provided before submitting the application dossier to CDSCO (see Annexure 1-VI).

Regulatory process followed by CDSCO

The validation/performance evaluation of in-vitro diagnostics is crucial for verifying the performance and operational characteristics of IVDs during pre-qualification. Performance evaluation is a key part of pre-qualification assessments, conducted by specified CDSCO collaborating centres or designated laboratories. An IVD validation study consists of two important aspects: Analytical performance studies and Clinical performance studies. The clinical performance studies need to be conducted as per the standard ISO 20916:2019 in vitro diagnostic medical devices, in compliance with good study practice and with the national or regional requirements for ethics committee approval (ISO 20916, 2019). ISO 15189:2022 & ISO 20916:2019 emphasises on establishing the performance specifications as well as quality assessment measures as part of the validation (Álvarez & Andreu, 2011; ISO 15189, 2022; ISO 20916, 2019).

For a new In-vitro Diagnostic Medical Device (where no predicate device is available in the country), the applicant must first obtain a Test License in form MD-13 (for domestic manufacture) to produce test batches (Figure 2). These batches are necessary for generating in-house quality/validation data or for evaluation in an external laboratory, as applicable. Subsequently, to generate clinical performance data for the device, the applicant must secure permission to conduct CPE by obtaining MD-25 (for protocol approval by the IVD experts

committee constituted by CDSCO). Upon receiving MD-25, the applicant can proceed with the CPE. The study results and generated data, must be submitted for approval to manufacture the new IVD, using form MD-29 (for clinical data approval by the IVD experts committee constituted by CDSCO). Following this, the applicant must obtain the appropriate manufacturing license, either MD-5/MD-6 (approved by the State Licensing Authority) or MD-9/MD-10 (approved by the Central Licensing Authority, DCGI) for sale or distribution. The details for submitting applications are outlined in Annexures IV and V.

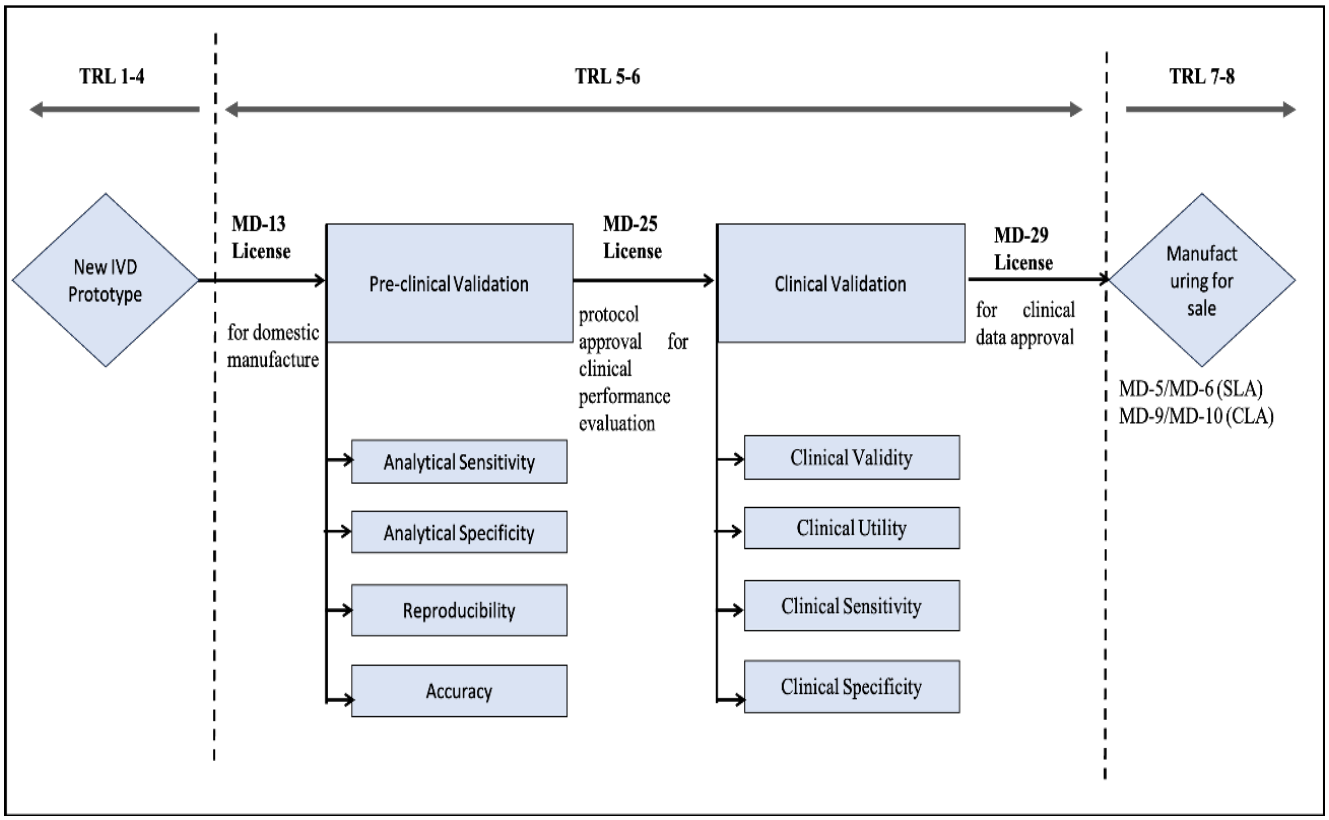

Fig 2: Regulatory licenses required for evaluation and approvals

For an In-vitro Diagnostic Medical Device with an existing predicate device in the country, the applicant must first obtain a Test License in form MD-13 (for domestic manufacture) to produce test batches. These batches are necessary for generating in-house quality/validation data or for evaluation in an external laboratory, as applicable. Following this, the applicant must secure the appropriate manufacturing license for sale or distribution. This involves obtaining MD-5/MD-6 (approved by the State Licensing Authority) or MD-9/MD-10 (approved by the Central Licensing Authority, DCGI).

The classification of in vitro diagnostic (IVD) medical devices is determined by the level of risk associated with their use, ranging from Class A (lowest risk) to Class D (highest risk). For any new IVD devices classified as Class B, C, or D, a CPE is mandatory. Table 1 provides a curated

list of selected examples of CDSCO-approved IVDs that are crucial for diagnosing pathogens and/or antimicrobial resistance, with further details available in Annexure VII (source: https://cdscomdonline.gov.in/NewMedDev/ListOfIvdMdApprovedDevices).

Table 1: Examples of CDSCO approved antimicrobial resistant IVDs (August 2024)*

Class	Risk Level	Example
A	Low Risk	WASPLab® System, Microplate ELISA Reader
B	Low Moderate Risk	ETEST Azithromycin, ETEST Cefixime, Antimicrobial Susceptibility System(ASS)-HiMic Plate Kit, Mueller Hinton 2 agar + 5% sheep blood
C	Moderate High Risk	AMR Direct Flow Chip Kit (Manual and Auto), NG-Test CARBA 5, UTI Antimicrobial Susceptibility Testing PCR Kit
D	High Risk	-

**Table 1 and Annexure VII provide examples of CDSCO approved IVDs purely for reference purposes only. None of the examples indicate any endorsement through this document.*

General considerations for validation of diagnostics

Chapter 2

General considerations for validation of diagnostics

I. **Sites**: In an ideal situation, the validation studies must preferably be carried out at multiple sites. However, in cases where this is not feasible, validation should be carried out in a minimum of two different sites representing the country's geographic variations to test the validity and usefulness of the diagnostic. The performance evaluation sites should be chosen from the CDSCO approved list and must comply with ISO15189 standards. (CDSCO, 2023).

II. **Sample**: The type of specimen to be used for testing must be mentioned, such as invasive (CSF, blood) or non -invasive (sputum, urine) etc. Isolates from sterile sites like blood or CSF are more clinically significant than those from non-sterile sites like urine or sputum, which may indicate colonization. The sample size must be determined with the help of statistical expert to ensure that sufficient number of specimens are tested to provide statistically reliable results taking into consideration the prevalence of the condition/infection. These are required to justify any claim and to provide reasonable estimates of uncertainty. Sample collection and handling must be performed by trained personnel. Also the sample storage must be done at optimum conditions to preserve integrity of the samples.

III. **Test methods:** Specify the type of diagnostic, whether it is a method, assay, kit or a device and detail its necessary components. Indicate the method involved e.g. biochemical assay, molecular assay or mass-spectrometry based assay. While most test methods yield numeric, quantitative results, some assays only provide qualitative outcomes.

IV. **Standards**: Reference material (standard strains, toxins, analytes, antimicrobials etc) for validating the test must be of certified quality; if not available, then the molecule with highest possible purity must be used to ensure the test results are reliable (GUIDELINE 2.1., 2012). Strains used to measure test performance should be obtained from reference centre collections (e.g., ATCC, etc), academic government reference laboratories, or other collections that are available to the scientific community. The characteristics of the reference material conferring

it a particular trait must be ascertained while procuring the material. For validation of the test performance characteristics, the target strain should be associated historically with the specimen, or an outbreak. These should be the first strains of choice for conducting the validation study (Caliendo et al., 2013).

Standards for interpretation of results and susceptibilities:

Breakpoints: These are the values used by clinical microbiology laboratories to interpret the results of antimicrobial susceptibility testing (AST) and classify isolates as susceptible or resistant. Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) set clinical breakpoints. Indian laboratories majorly use CLSI breakpoints. For a given antibiotic, the breakpoint may differ for different sites (e.g., urine versus tissues or skin) and for different infecting organisms.

Minimum Inhibitory Concentration (MIC): It is defined as the lowest concentration of an antibiotic which prevents visible growth of a bacterium. MICs are determined and then interpreted according to the breakpoint – if the MIC is less than the susceptible breakpoint, the organism is considered as susceptible and can be successfully treated with that particular antibiotic, whilst if the MIC is higher than the susceptible breakpoint, it is considered as non-susceptible (intermediate resistant or resistant).

V. **Reference method**: The reference method is defined as a method/test by which the performance of an alternate method is measured or evaluated. The reference method is usually the gold standard for the organism under study. Validation studies must include comparison to a recognized reference method to demonstrate equivalence of performance, the significance of which must be determined statistically. The performance of the test under question must be comparable to the gold standard. In case there is no gold standard for an organism then the next best test available or composite reference standard (CRS) is used. A composite reference standard is a fixed rule used to make a final diagnosis based on the results of two or more tests, referred to as component tests. For each possible pattern of component test results (test profiles), a decision is made about whether it reflects presence or absence of the target disease. Though it is simple and easy to interpret, it can lead to seriously biased estimates of accuracy indices (sensitivity and specificity) and should be avoided whenever possible.

VI. **Media**: Bacteriological media that ISO standards should be utilised for antimicrobial susceptibility testing. All the media must be tested for physical and chemical parameters

and growth promotion parameters. The composition of the culture media used and the name and catalogue number of the manufacturer needs to be mentioned. This is important as the accuracy of the test results is dependent on the quality of media used for susceptibility testing.

VII. **Supplies**: The test kits, reagents, chemicals, equipment etc required to conduct validation of the test will be provided by the developer of the test. The test kits and reagents must be supplied at temperature that is suitable to preserve the test efficacy. Also, the storage conditions must be clearly stated by the developer and must be followed as indicated in the instructions for use / labels. Some products may not need refrigeration. If refrigerated storage space is inadequate to store the entire test kit, they may be divided such that labile reagents can be refrigerated separately from the non-labile supplies. Calibrated thermometers or other environmental monitoring devices must be placed at each location where reagents and specimens are stored, i.e. ambient, refrigerator and freezer; and temperatures must be recorded daily.

Criteria for development and validation of assays for new rapid diagnostics in pathogen identification and antimicrobial susceptibility testing (AST)

Chapter 3

Criteria for development and validation of assays for new rapid diagnostics in pathogen identification and antimicrobial susceptibility testing (AST)

Phase 1: Assay Development

Step 1: Define the purpose of validation for diagnostics

The intended goals and applications of the diagnostic, such as the identification of pathogens, testing of antimicrobial susceptibility, or detection of antimicrobial resistance markers, should be outlined. The type of diagnostic, whether it is a method, assay, kit, or device, must be specified, along with a detailed description of its necessary components. It should be indicated whether the test detects the whole organism, toxin, analyte, DNA/RNA, etc. The following considerations should be taken into account when planning the validation:

1.1. Pathogen identification

The disease or condition to be diagnosed.

Whether the test can provide a qualitative or quantitative result for optimal clinical utility.

Whether a screening or confirmatory test is required. Screening tests determine the status of a disease, disorder or other physiological state in an asymptomatic individual whereas confirmatory test establish the presence (or absence) of infection for treatment decisions in symptomatic or screen positive individuals.

The test purpose will directly influence the subject sample size (N) and selection criteria (including inclusion and exclusion) when planning and designing the study. For example, if the prevalence is low and test is meant to screen asymptomatic individuals, specimens from a large number of subjects may be required to provide sufficient evidence of optimal performance. However, if the test is to be used for diagnosis in symptomatic individuals, specimens from a smaller number of subjects may be adequate.

1.2. Antimicrobial susceptibility testing (AST)

Whether genotypic or phenotypic based AST.

Whether test can differentiate between susceptible(S)/resistance (R) or susceptible/intermediate resistance/resistance (S/I/R) for a particular antibiotic.

Whether a single test or a diagnostic algorithm is required.

Whether test can provide a qualitative or quantitative result for optimal clinical utility.

Whether a screening or confirmatory test is required.

Step 2: Optimization of the reagents and protocol

The reagents and protocol must be optimized to ensure that consistency of the assay is maintained, considering factors such as temporal, chemical and physical variables. These include test operating conditions, storage conditions during transportation, temperature, pH, dilutions, buffers and other relevant factors during laboratory usage (Jennings et al., 2009). A kit should be self-sufficient to allow testing and analysis e.g., brochure/software to facilitate training of the laboratory personnel, instructions for storage, processing of samples and analysis and reporting of the data (Crowther et al., 2006). Shelf life (expiry date) and the lot number of the reagents and kit should be clearly labelled. A certificate of analysis for reagents in the kit can be provided. 'Single test' device or consumables not to be reused should be preferred. Optimal quality of materials and media should be included for the study i.e. as certificate of analysis. The sample profile should be clearly detailed for precise validation (CLSI/NCCLS, 2003).

Step 3. Quality control (QC) and Quality assurance (QA)

Before validation is initiated, the test and reference method should have passed defined quality control criteria. QC is necessary to ensure the performance of the test method under evaluation and the reproducibility of the results. All QC parameters should be in range before validation. If any result is obscured, then additional replicates should be used until 95% of results are in the expected range.QC measures and strains to be used for the test method should be defined by the manufacturer and at least three replicates should be recommended. For the reference method, QC protocols and strains should follow CLSI guidelines (CLSI, 2018c) and up to nine replicates should be tested, including at least one QC organism (Humphries et al., 2018). Microbial reference stains should be obtained from standard sources (e.g. ATCC, MTCC (IMTECH), MCC (NCCS)) and should be well characterized, including stable defined antimicrobial susceptibility phenotypes. Isolates should be stored under optimal conditions to

ensure that their phenotypic and genotypic characteristics are retained, for e.g., resistance mechanisms especially plasmid-borne resistance can be lost under suboptimal conditions. The common storage conditions recommended for the isolates are -70°C to -80°C, in 20% glycerol or any another suitable medium such as dimethyl sulfoxide (Humphries et al., 2018).

Quality assurance implements monitoring and evaluating the records, calibration and maintenance of equipment, training and QC and helps to ensure that materials and processes consistently give quality results (OIE, 2019). If a diagnostic is a machine or uses auxiliary equipment, it must be serviced, calibrated, maintained and monitored appropriately to ensure the reproducibility of the test conditions in assay performance.

Phase 2: Assay Validation

Step 4: Analytical validation: Standard Operating Procedures (SOPs) for validation

Analytical performance describes a device's capacity to precisely detect or measure a specific analyte. These studies are generally designed following CLSI guidelines, with the study approach and plan recorded in an analytical performance study. Standard Operating Procedures (SOPs) should be prepared as ready-to-use working drafts and updated as needed throughout the validation process. Upon completion of the validation study, these SOPs will serve as controlled documents.

4.1. Study design and site selection

The design of a study for diagnostic validation requires meticulous planning, sample size calculation, an appropriate study design, specimen type, considerations for multiple matrix specimen and establishment of a clear reference standard. Additional considerations includes standardization of data collection, planning statistical analyses, obtaining ethical approval and adherence to reporting guidelines.

4.2. Sample size

A defined number of adequate samples are required to ensure that precise inferences can be made from the statistical analysis and level of confidence can be achieved for accurate results (Hajian-Tilaki, 2014). Sample size and data points for different parameters are mentioned in Table 2.

4.2.1. Pathogen identification- A representative number of samples including positives and negatives should be tested in parallel to check the performance parameters of diagnostic test (Rabenau et al., 2007).

4.2.2. Antimicrobial susceptibility testing- A representative number of samples positive for clinically relevant pathogens indicative of susceptibility, intermediate resistance and resistance (S/I/R) for antimicrobials must be tested in parallel in the study. A minimum of 100 isolates recovered from clinical samples are recommended for each group of micro-organisms (CLSI, 2018c) to determine the relevance of antimicrobials for infectious disease management. Susceptibility testing will be performed for a panel of antibiotics based on the drug-bug profile combination, including the recently approved novel antibiotics using the reference broth microdilution method. However, a greater number of samples will always help in improving the confidence in test results and minimize the error or bias in process.

4.3. Reference or gold standard method

The reference or gold standard should be used as per the type of diagnostic test for the validation. Standard and updated guidelines should be followed for the testing protocol. In case there is no gold standard for an organism then the next best test available or composite reference standard (CRS) is used. Another approach to analytical validation entails comparing the performance and specifications of the IVD with an existing predicate device. Demonstrating that the new diagnostic is equivalent or superior to the predicate in all aspects supports a claim of substantial equivalence.

4.3.1. Pathogen identification- Standard reference materials/isolates recommended by CLSI in their recent guidelines should be used. These standards perfectly discriminate between participants with or without the disease and provide unbiased results for the diagnostic accuracy measure of index test. Ideally, a gold standard with 100% sensitivity and specificity should be used as reference. The storage conditions of the reference materials/ isolates should be defined in accordance with standards such as those defined by CLSI, ATCC etc., to preserve the genotypic and phenotypic stability.

4.3.2. Antimicrobial susceptibility testing- The standard methods and guidelines on S/I/R as recommended by CLSI should be used for diagnostic evaluation. For AST, ideally a gold standard with 100% category agreement and essential agreement should be used as reference method. The reference method for AST is MIC determination by the broth microdilution (BMD) method (CLSI, 2018a). Whereas other reference methods such as agar dilution (AD) and disk diffusion (DD) are acceptable substitutes in case of non-availability of BMD (CLSI, 2018b). In such cases, exceptions or modifications as suggested by CLSI must be considered as per requirement while evaluating the diagnostic test. For example, inducible clindamycin resistance is determined by the D-zone test, a reference DD method. Another exception is for fosfomycin resistance or

testing for *Neisseria gonorrhoeae,* because of complications with BMD, the AD is used as a reference method (CLSI, 2018c). Other exception include the case of methicillin resistant *Staphylococci* (detection of *mecA* or *mecC*) and vancomycin resistant Enterococci and Staphylococci, where molecular detection of a resistance gene is used as the gold standard method. However, the resistance in other species is multifactorial and not defined by single molecular target, especially in gram-negative bacteria. Problems such as bias, errors and inadequate results can occur in absence of reference/gold standard or in using imperfect reference standards. Therefore, multiple imperfect reference standards should be considered, but only with expert informed opinion.

4.4. Specimen type

The capability of the diagnostic to analyse one sample-type or multiple sample-types (e.g. urine, blood, cerebrospinal fluid, etc.) should be clearly outlined. If diagnostic is capable of utilizing more than one sample type (e.g. urine, serum, spinal fluid, etc.) for microbial detection, all the analytical and performance studies should be independently repeated. A linearity study should be carried out for each specimen type to omit the effect of matrix background on results.

4.5. Shelf life (Stability) of the diagnostic

The shelf-life of a diagnostic, whether an assay or device, must be assessed to ensure its viability in maintaining acceptable performance characteristics over a defined time interval under specified storage conditions. The shelf-life cannot be measured directly and it is assessed from the accuracy and performance characteristics. Stability of diagnostic includes reagents, media, controls, reconstituted lyophilized materials, working solutions, matrix and calibrators etc. when used, stored and transported under specific conditions (CDSCO/IVD/GD/Stability/01/2022, 2022). EN 13640 and CLSI EP25-A describe the need for different types of product stability testing(CLSI EP25, 2024).

The shelf life of a diagnostic can be assessed by performing real-time or accelerated stabilities studies, component stability studies, reconstituted stability testing, transport simulated stability assessment, open or in-use stability evaluation. The selection of lot/batches and the number of samples used affect the stability assessment. A minimum of three different batches to verify shelf life in real-time are recommended (Marimuthu et al., 2019; *Medical Devices Rules, 2017*, n.d.).

4.6. Statistical evaluation of results

A thorough statistical analysis of the assay's test results should be conducted to ensure its accuracy and all findings and inferences should be documented with precision. Additionally, the method's

characteristics should be compared with those of a reference standard or a previously validated method to assess its reliability. Establish cut-off values or assign numerical parameters to further evaluate the method's effectiveness. Cut-off values should be established, or numerical parameters should be assigned, for further evaluation of the method's effectiveness.

Step 5: Analytical validation: Testing the performance and/or operational parameters

The performance and/or operational parameters that need to be validated for a particular test/method should be identified and the formulas and worksheets and should be defined. Each analytical parameter should be considered on a case by case basis and if any are not applicable a justification should be provided (e.g. linearity is not applicable to qualitative devices). The number of parameters that should be tested can vary slightly based on the type of diagnostic i.e. whether test is biochemical, molecular or microbiological etc., or its format i.e. whether it is a method, device and/or a test kit.

A diagnostic test can be qualitative (reported as positive or negative or indeterminate) or quantitative. Assessment of minimum three determinants of validity i.e. accuracy, reproducibility and precision have been recommended for both types of diagnostic test outputs (Cleophas & Zwinderman, 2009).

While CLIA lists the performance specifications that must be established, it does not specify the scientific methodology or data analysis tools to be used (Burd, 2010). Guidelines to assist in establishing these performance specifications have been published by the CLSI and ISO in several documents (CLSI, 2018b).

5.1. Pathogen identification

For diagnostic aimed at rapid identification of pathogen, performance parameters should be assessed as mentioned in Table 2.

5.1.1. Accuracy: The test should accurately identify individuals with the disease and provide insight into its severity.

5.1.2. Analytical Sensitivity: The test should be able to distinguish between two close concentrations of the analyte.

5.1.3. Analytical Specificity: The test's ability to accurately quantify the analyte in the presence of potentially interfering substances must be thoroughly evaluated. This evaluation should take

into account potential interference from both endogenous and exogenous sources, with appropriate testing conducted to ensure reliable performance.

5.1.4. Reproducibility: Consistency is ensured when the second test yields the same result as the first when a subject is tested twice.

5.1.5. Precision: A test method is said to be precise when repeated determinations (analyses) on the same sample give similar results. When a test method is precise, the amount of random variation is small. The test method can be trusted because results are reliably reproduced time after time.

5.1.6. Reportable range: The range of analyte concentrations for which the test system can accurately report results.

5.1.7. Reference range: The range of IVD output values that correspond to normal or healthy populations.

Table 2. Evaluation of performance parameters for diagnostic for rapid pathogen identification

S. No	Performance characteristics/parameters			Data point and minimum sample testing requirements
	Determinant of validity	Statistical test for		
		Qualitative diagnostic	Quantitative diagnostic	
1	Accuracy Sensitivity Specificity Overall Accuracy	Receiver operated curves (ROC)	Barnett's test; Intraclass correlation vs gold test, Bland-Altman test	**# Analytical Sensitivity (limit-of-detection study):** 60 data points (e.g. 8-12 replicates from 4-5 samples in the range of the expected detection limit); conduct the study over 5 days; probit regression analysis (or standard deviation with confidence limits if limit of blank studies are used) **# Analytical Specificity (interference studies):** No minimum no. of samples recommended; test sample-related interfering substances (hemolysis etc.) and genetically similar organisms or organisms found in same sample sites with same clinical presentation; spike with low concentration of analyte; paired-difference (t test) statistics

				# Accuracy (comparison-of-methods study): Test in duplicate by both the comparative and test procedures over at least 5 operating days; typically 40 or more specimens; xy scatter plot with regression statistics; Bland-Altman difference plot with determination of bias; % agreement with kappa statistics and/or Lin's concordance correlation coefficient # Calculate the Diagnostic sensitivity (TP/TP+FN) and diagnostic specificity (TN/ TN+FP). # Calculate the Positive Predictive Value (PPV), Negative predictive value (NPV), Likelihood Ratio (LR+ and LR-) of a positive and negative test.
2	Reproducibility	Cohen's kappas;	Duplicate standard errors, Repeatability coefficients, Intraclass correlations vs duplicate test	At least 10 and preferably 20 runs of the assay to give estimates of these parameters
3	Precision (replication study)	Confidence intervals	Confidence intervals	**# For qualitative test:** minimum of 3 concentrations (LOD, 20% above LOD, 20% below LOD) and obtain 40 data points; test in duplicate over 15 days (include data from analytical sensitivity runs to provide data over 20 days) **# For quantitative test:** minimum of 3 concentrations (high, low, LOD) and test in duplicate 1-2 times/day over 20 days (include data from reportable range study as day 1 to provide data over 20 days); calculate SD and/or CV within run, between run, day to day, total variation
4	Reportable Range or Analytical Measurement Range (linearity study)	NA (Qualitative tests do not require linearity, Analytical Measurement Range	a linearity experiment to determine reportable range and lower limit of quantification (LLOQ)	**# For quantitative assays:** 7-11 concentrations across anticipated measuring range (or 20- 30% beyond to ascertain widest possible range); (CLSI/NCCLS, 2003)

		reference range studies)		2-4 replicates at each concentration on same day; polynomial regression analysis
5	Reference Interval (reference value study)	NA (reference range is typically negative or not-detected and reference interval studies do not need to be performed if target is always absent in a healthy individual)		# **For quantitative assays:** reference interval will be reported as below the LOD or LLOQ; for some analytes, the reference interval may be a clinical decision limit; if the intended use of the test is limited to patients known to be positive for the analyte being assayed, a reference interval may not be applicable it can be verified by testing 20 known normal samples; if no more than 2 results fall outside the manufacturer/published range then that reference range can be considered verified.

Source: (Burd, 2010; CLSI, 2008; CLSI and IFCC, 2008; CLSI/NCCLS, 2003; Humphries et al., 2018, 2023; Patel et al., 2013; Rabenau et al., 2007; van Belkum et al., 2019)

5.2. Antimicrobial susceptibility testing

For detection of the S/I/R criteria, assessment of accuracy, error rates, precision and reproducibility of diagnostics for AST has been recommended for qualitative and quantitative types of diagnostics (Humphries et al., 2018; Patel et al., 2013). Data points and interpretive criteria for DD and MICs methods as described by CLSI guidelines M02 and M23 documents (CLSI, 2008, 2018b) should be used for testing so as to minimize the number of category errors during testing.

5.2.1. Accuracy: It shows the closeness of the result under evaluation to the true value (i.e., agreement with the reference standard or predicate test) that can be measured by two ways, categorical agreement (CA) and essential agreement (EA).

Categorical agreement: Percentage of isolates tested producing the same category result i.e. susceptible, intermediate, susceptible dose dependent, resistant, or non-susceptible as compared to the reference standard method. Susceptible-dose dependent (SDD) category implies that susceptibility of an isolate is dependent on the dosing regimen that is used in the patient. The dosing regimens (i.e., higher doses, more frequent doses, or both) used to set the

SDD interpretive criterions are provided in Appendix E in M100 (CLSI, 2018b). Non-susceptible implies that only a susceptible interpretive criterion has been designated in these isolates because of the absence or rare occurrence of resistant strains. An isolate that is interpreted as non-susceptible does not necessarily mean that the isolate has a resistance mechanism.

Essential agreement: Percentage of isolates tested producing MICs that are within 1 $\log_2$ dilution (±1 doubling dilution) of the reference BMD MIC value. EA is applicable only to those AST methods that determine MIC values.

5.2.2. Error rates (ER): ER shows the CA discrepancies of the test from the reference method. These are divided into following three types of errors:

Minor errors (mEs): the test shows minor discrepancies/errors between susceptible vs. intermediate and intermediate vs. resistant when compared to reference method. It can have the least detrimental influence on therapeutic decision.

Major errors (MEs): the test displays 'resistant' results while the result is 'susceptible' by the reference method. These errors limits the therapeutic options and tends to overuse last resort of antibiotics.

Very major error (VMEs): the test expresses 'susceptible' result while the result is 'resistant' by the reference method. This is a critical error that leads to use of an ineffective therapeutic agent for treatment against an infection and are associated with high mortality rate.

5.2.3. Precision: It is the closeness of agreement of the test to give same value when the same isolate is tested repeatedly under specified conditions. It can be determined within a run (repeatability); across several runs in one day; or across multiple runs across multiple days (reproducibility).

5.2.4. Reproducibility: It can be determined by testing the QC organisms using different personnel/operators, different test batches, different lots, different laboratories and after different time intervals.

For diagnostic aimed at AST of pathogen, performance parameters should be assessed as mentioned in Table 3.

Table 3. Evaluation of performance parameters for diagnostic for rapid AST

S. No.	Performance parameters	Calculations	Calculation terms	Acceptance criteria*
1	**Accuracy** Categorical Agreement (CA)	N_{CA}/NT *100	N_{CA}= number of isolates with an AST result with the same categorical interpretation as reference method ; NT= number of isolates tested	≥90% CA
	Essential Agreement (EA)	N_{EA}/NT * 100	N_{EA}= number of isolates with thesame or within one doubling dilution MIC value as the reference method; NT= number of isolates tested;	≥90% EA **(comparison-of-methods study)**Test in duplicate at least 5 operating days; n=≥40; xy scatter plot with regressionstatistics; Bland-Altman difference plot with determination of bias; % agreement with kappa statistics
	Sensitivity Specificity	TP/TP+FN TN/TN+FP	TP=True positive TN=True negative FP=False positive FN=False negative	
2	**Error rates (ER)** Minor Errors (mEs)	N_{mE}/NT * 100	N_{mE}= number of isolates havingminor errors NT= number of isolates tested;	≤10% mE
	Major Errors (MEs)	N_{ME}/N_{RefS} *100	N_{ME}= number of isolates that yielded false-resistant results;N_{RefS}= number of isolates susceptible by the reference method	<3% ME
	Very Major Errors (VMEs)	NVME/NRef R * 100	N_{VME}= number of isolates that tested false-susceptible results N_{RefR}= number of isolates resistant by the reference method	<3% VME (FDA uses <1.5% VME)

3	Precision	% CV= SD/mean *100	Calculate in terms of Imprecision (random error): standard deviation(SD) & coefficient of variation (CV)	Sample testing in duplicate over 20days
4	Reproducibility	NA	More number of variables can strengthen the results	≥95%
5	Reportable Range	NA (for quantitative analysis)	A linearity experiment to determine reportable range andlower limit of quantification (LLOQ)	At least 10 and preferably 20 runsof the assay; 7-11 concentrations across anticipated measuring range (or 20-30% beyond to ascertain widest possible range); 2-4 replicates at each conc. on sameday; polynomial regression analysis
6	Reference range	NA		n=20 representative of the population [if the population is different, n=60 (minimum, 40)]; Out of the 20 samples, if no more than 2 results fall outside the published range then the reference range can be considered to be verified

*Wherever applicable, percent (%) values are as recommended by ISO 20776-2:2007 and FDA.

Source: (CLSI, 2008; CLSI and IFCC, 2008; CLSI/NCCLS, 2003; Humphries et al., 2018, 2023; Patel et al., 2013; Rabenau et al., 2007; van Belkum et al., 2019)

5.2.5. Address errors and bias

Errors and variables can be assessed using various methods. Due to the inherent variation in MIC end points, the error rate will be directly proportional to the percentage of isolates with antimicrobial agent MICs in the range of one two-fold concentration above the intermediate MIC (I + 1) and two-fold concentration below the MIC (I-1)(Humphries et al., 2018). Thus, when an entire population is used as the denominator for calculating error rates, the rate will be

determined largely by the population of MICs in the I + 1 to I − 1 range. For example, when 90% of the isolates have highly susceptible drug MICs (as it is common with newer antimicrobial agents), the error rate will be considerably less than that of a population in which 40% of the MICs fall in the I + 1 to I − 1 range. Using the total I + 1 to I − 1 subpopulation as the denominator for calculating discrepancies provides a more accurate assessment of the discrepancy by accounting for normal technical variability in the testing method. For antimicrobial agents for which clinical use will primarily be for organisms with specific types of resistance mechanisms, scatterplots and error rates are evaluated and presented separately for these types of organisms.

Table 3 : Acceptance performance rates for ASTs by error-rate bound method for antimicrobials with an intermediate category

Reference MIC range for isolates to include in denominator of error calculations		**Acceptable Error Rates**		
1-dilution intermediate range	**2-dilution intermediaterange**	**mE**	**ME**	**VME**
≥ I + 2	≥ I_{high} + 2	< 2%	ND	< 5%
I+1 to I-1	I_{high} +1 to I_{low} -1	< 40%	< 10%	< 10%
≤ I-2	≤ I_{low} -2	ND	< 2%	< 5%

MIC=minimal inhibitory concentration; mE=minor error; ME=major error; VME=very major error; I=Intermediate MIC value; I_{high}=high end of the MIC range for intermediate category; I_{low}=low end of the MIC range for intermediate category; ND=not determined

Error-rate-bound method (Brunden et al., 1992; FDA, 2009): It can be used for following conditions:

To evaluate MIC distribution in the isolates that differs significantly from the normal distribution of MICs.
To calculates the breakpoints, if >20% of the isolates tested are within 1log2 dilution.
To analyse linear regression when the population of bacteria tested is enriched with a non-wild-type population
Traditionally used to evaluate disk breakpoints when the bacterial population is not binomial

Model based approach (DePalma et al., 2017): Determine Disk diffusion breakpoints interpretive criteria, whereby a fitted model is used to take into account the proportion of isolates at each

Table 4: Acceptance performance rates for ASTs by error-rate bound method for antimicrobials when no intermediate category exists

Reference MIC range for isolates to include in denominator of error calculations	Acceptable Error Rates		
	mE	ME	VME
≥ R + 1	< 2%	ND	5%
R + S	< 40%	< 10%	< 10%
≤ S - 1	ND	< 2%	< 5%

MIC=minimal inhibitory concentration; mE=minor error; ME=major error; VME=very major error; R=resistant MIC value; S=Susceptible MIC value; ND=not determined

5.2.6. Address void testing bias: Define possible bias that can be introduced in testing e.g., constant bias, proportional bias etc. Avoid discrepant analysis techniques (McAdam, 2000) as it has tendency to overestimate the sensitivity and specificity and PPV of a test. It has been emphasized that these should be avoided especially for diagnostic tests (like therapeutics). Re-testing of both concordant and discrepant samples should be done to avoid test bias.

Step 6: Clinical validation: Testing of clinical characteristics

Clinical performance evaluation (CPE) is the systematic evaluation of a new *in vitro* diagnostic device using the specimens collected from human participants to assess its performance. It evaluates the accuracy and reliability of a diagnostic test in a clinical setting. These studies often involve testing the diagnostic test on patients with known disease or condition and comparing the results to a gold standard or reference standard (Baumfeld Andre et al., 2022).

In India, clinical performance evaluation is required for all the class B, C and D (classification as per Chapter II, Rule 4, Sub-rule (2) of MDR 2017) (*Medical Devices Rules, 2017*, n.d.) in vitro diagnostic devices that are new. The IVD may be exempted from clinical performance evaluation if it is being marketed for at most two years in one of the countries like United States of America, Australia, Canada and Japan. The manufacturer or importer is required to take permission to conduct clinical performance evaluation from the Central licensing authority (CLA). Before beginning the enrolment process, the clinical performance evaluation must be recorded in the Clinical Trial Registry of India. The annual status report for every clinical

performance evaluation concerning whether evaluation is ongoing, complete, or ended should be sent to CDSCO.

6.1. Study design

CPE studies should be designed to maximize the value of the data while minimizing bias. Evaluating both diagnostic accuracy and clinical utility ensures robust validation studies, producing reliable results for diagnostic tests. IVD medical device performance evaluation can be designed as observation or intervention. An observational study is the one in which the results obtained during the study are not used in the treatment of the patient and do not affect treatment decisions. An interventional study is a study in which the results obtained from the study can influence patients' decisions and be used to guide treatment. The design of the study must be decided by taking opinion from experts in the field.

6.1.1. Selection of target population: The condition or disease targeted by the diagnostic determines selection of appropriate population (for e.g. whether diagnostic aimed at neonatal sepsis or sepsis etc.), the inclusion and exclusion criteria for enrolling participants and selecting appropriate study settings. The letter of consent should be signed by the participant (or their legal guardian) for enrolment in the study.

6.1.2. Inclusion and Exclusion criteria: The inclusion and exclusion criteria for enrolment of the suitable target population should be outlined to ensure reliable results. Inclusion criteria might include specific age ranges, clinical setting (in-patient or out-patient), severity of illness and ability to provide informed consent. Exclusion criteria could encompass those outside the age range, individuals with co-morbid conditions that could confound results, previous participants in similar studies, known allergies or contraindications, pregnant or breastfeeding women, history of non-compliance, acute or chronic diseases and recent surgery or trauma. These criteria ensure a homogeneous population, enhancing the study's validity and reliability (Clodi-Seitz et al., 2024).

6.2. Study settings and number of sites

Site settings for diagnostic validation should be clearly defined, preferably using accredited laboratories (e.g., NABL). For testing at PHCs or in the field, training requirements for operating protocols and proficiency testing must be established to ensure competency. A documented criterion for the training and retraining of personnel, along with competency assessment records,

should be maintained. Personnel performing tests should have access to diagnosis and treatment in case of exposure and the anticipated number of operators should be specified without affecting test results. Requirements for the installation and maintenance of equipment, including electrical needs, should be outlined, with minimal needs for sites like PHCs or bedside locations. Validation should involve multiple sites to avoid bias and ensure accuracy and the validating site must be accredited by a competent authority.

6.3. Test purpose

IVD medical devices can be designed for a variety of intended uses like diagnosis, screening, monitoring. The purpose of the test will directly influence the study's sample size (N) and sample selection (including inclusion and exclusion) when planning and designing the evaluation plan. For example, if the prevalence of disease/infection is low and the purpose of the test is to examine the asymptomatic population, a sample of larger subjects would be required to provide sufficient evidence. However, if the test is used for diagnostic purposes in patients, a small sample of subjects will be sufficient. An expert's opinion must be taken while deciding the sample size.

6.4. Specimen Collection and Handling

Samples used in clinical performance studies can be derived from specimens which might have been obtained from different sources, including purposefully-collected specimens, leftover specimens, or archived specimens. The samples must be collected, transported and stored appropriately to preserve the integrity. In case where leftover, or archived specimens are used, there must be sufficient information available necessary to perform data analysis.

6.5. Ethical Considerations for Clinical Performance Studies

As a general principle, the rights, safety and well-being of subjects participating in IVD medical device clinical performance studies shall be protected. Approval of ethical committees, informed consents wherever applicable must be taken.

6.6. Clinical Performance Evaluation Criteria

Clinical validation evaluates the clinical validity and utility of a test based on the disease or marker being tested. Data can be sourced from laboratory studies, peer-reviewed literature, or other reliable sources. CLIA mandates that laboratories have a qualified director responsible for ensuring the clinical utility of the tests performed.

6.6.1. ***Clinical Validity:*** The ability of a test to detect or predict the associated disorder (phenotype)

6.6.2. ***Clinical Utility:*** The usefulness of the test in the diagnosis or treatment of patients. The purpose of test (screening, diagnostic, predictive, etc.) must be clearly defined. Documented via literature review and/or independent evaluation by the laboratory

6.6.3. ***Clinical Sensitivity:*** The proportion of patients with the mutation/disease who have a positive test result, or the likelihood that a positive result correctly determines that the patient has the condition being tested. Positive Predictive value = True positive results ÷ (True positive + False positive). Predictive values take into account the prevalence of the disease in the population being tested [e.g., the higher the prevalence, the higher the likelihood that a positive result is a true positive

6.6.4. ***Clinical Specificity:*** The proportion of patients who lack the mutation/disease who have a negative test result, or the likelihood that a negative result correctly determines that the patient does not have the condition being tested. Negative Predictive Value = True negative results ÷ (True negative + False negative)

Phase 3: Assay Maintenance

Step 7. Maintenance and extension of validation criteria

Validation ensures an understanding of the reproducibility, strengths, accuracy and limitations of the study. A validated assay needs constant monitoring and maintenance to retain its designation. Once the assay is used into routine, internal quality control is accomplished by consistently monitoring the assay for assessment of repeatability and accuracy (Cembrowski & Sullivan, n.d.). assay. Reproducibility is assessed through external quality control programmes such as proficiency testing. Continuous monitoring and timely review of assay performance is needed to:

monitor accuracy post validation e.g. the test's performance in routine usage and to ensure that the expected performance is maintained throughout the life of the test;

monitor reproducibility and limitations;

determine need of calibration and control procedures

perform risk assessments which need to reviewed or written

Step 8: Dossier of validation (technical report)

Documentation of all validation and verification experiments must be kept by the laboratory for as long as the test is in use but for no less than 2 years (US Federal code; Indian law/BIS/NABL requirement). It should contain following:

Record of each sample testing and method modification, result interpretation and analysis;

Deficiencies if any which could not be resolved in assay validation or in method;

Justification for inadequate data and for each possible scenario.

Annexure VI provides the format for the validation report summary. Interpretation of results should be detailed, easy and simple for the personnel performing the test. For instance, whether interpretation of results will be based on visual color change, (or) it will be generated automatically with the inbuilt database (or) results can be printable etc. Instructions for use (brochure)/software should be provided which include details like whether test results will be qualitative (positive/negative or present/absent) or quantitative. Uncertain test results must be reported and a protocol for repeat testing should be provided.

Results of validation from third-party (independent) evaluation of the test may be considered for objective assessment and ensuring diagnostic conformity to the stated purpose and claimed outcome.

Step 9: Outcome and Decision

Decision on the outcome of validation process of diagnostic will depend on the practicability of the method, assay usability, performance parameters data and user feedback. Approximate cost per test may have decisive impact on the usability of diagnostic which could be determined using cost-effectiveness studies. The barriers to implementation and final recommendations should be made based upon the validation exercise.

Annexures

Chapter 5

Annexures

S.NO.	TITLE
Annexure I.	INSTRUCTIONS
Annexure II.	CHECKLIST
Annexure III.	DEFINITIONS
Annexure IV.	PROCESS FOR SUBMISSION OF HARD COPY OF APPLICATION
Annexure V.	PROCESS FOR APPLICATION ON ONLINE SUGAM PORTAL
Annexure VI.	VALIDATION REPORT SUMMARY FORMAT
Annexure VII.	CDSCO APPROVED AMR PREDICATE IVDS

Annexure I

INSTRUCTIONS

Perform risk assessments and identify if any risk [(e.g. operational (chemical/physical), biohazard (biological infectious material) etc.] for which methods need to reviewed or written (ISO 14971:2009)

- To minimise the hazards to users of the assay/test

  - A risk assessment should be performed prior to using any reagent in a diagnostic test;

  - Infection risk of biological test materials or analyte that may pose a health threat (such as Mycobacterium tuberculosis).

  - Risk assessment of reporting a false-positive or false-negative result that would result in significant health problem/risk to the patient or general public.

Work place health and safety

- The developer must provide adequate data for reasonable assurance of safety and effectiveness of the test/assay/device;

- There must be documented policies and procedures relating to workplace health & safety that are consistent with relevant national & jurisdictional workplace health & safety requirements

- Waste disposal – to ensure safe disposal of biological waste

Ethical approval for the use of specimens should be taken care of the laboratory undertaking diagnostic testing for validation.

Quality control procedures should be followed strictly by the laboratory.

During validation, quality of raw reagents for testing, storage condition for kits and reagents be defined or recommended.

The storage conditions of reference materials used in testing should be followed as per the instructions given by standard-provider to preserve the quality and stability of the reference material. These should be documented in the dossier.

Annexure II

CHECKLIST

- Product information including specifications and Instructions for use must be provided by the developer. The lot number/batch number of the product should also be mentioned.
- Test requirements: Information regarding the reagents/chemicals required for the validation study should be provided. Data sheets for all the reagents required including safety data and details of the strains to be used must also be included.
- Equipment required to conduct tests must be mentioned by the developer of the test and the same may be supplied if required
- Details of the specimen to be used for the validation including the details of the cold chain and storage conditions must be provided. Specimen panel details should be mentioned
- SOPs for conducting tests must also be provided
- Interpretation criterion must also be provided by the test developer to the validating centre
- Statistical advice/analyses

Annexure III.

DEFINITIONS

Analytical sensitivity	Smallest amount of substance in a sample that can accurately be measured by an assay
Analytical specificity	Ability of an assay to measure on particular organism or substance, rather than others, in a sample
Diagnostic sensitivity	Percentage of persons who have a given disorder who are identified by the assay as positive for the disorder
Diagnostic specificity	Percentage of persons who do not have a given condition who are identified by the assay as negative for the condition
Accuracy	Closeness of agreement between the test results and an accepted reference value
Precision	Closeness of agreement between results of replicate measurements. It is also definedas level of concordance of the individual test results within a single run (intra - assayprecision) and from one run to another (inter - assay precision). It is usually characterised in terms of the standard deviation of the measurements and relative standard variation (coefficient of variation or %CV)
Reproducibility	Ability to produce essentially the same diagnostic result/consistent results, under different conditions (different operators, test batch, different apparatus - laboratory orvalidated ancillary equipment, different laboratories and/or after different intervals of time). Repeatability is used to indicate within-run reproducibility.
Linearity	Determination of the linear range of quantification for a test or test system. It is achieved when measured results are directly proportional to the concentration of the analytes (microorganisms or nucleic acid) in the test sample, within a given range.
Reportable range	Highest and lowest test values that can be analysed while maintaining accuracy without dilution or concentration.
Reference range	Range of test values expected for a designated population of individuals.

Positive predictive value (PPV)	PPV is the probability that when a test is positive, the specimen does contain the designated pathogen.
Negative predictive value (NPV)	NPV is the probability that when a test is negative, the specimen does not have the designated pathogen.
False Positive	False positive is a result that indicates a given condition exists when it does not.
False Negative	False negative is a result which wrongly indicates that a condition does not hold.
Cut off value	For diagnostic or screening tests, the value used to divide continuous results into categories; typically positive and negative

Annexure IV.

PROCESS FOR SUBMISSION OF HARD COPY OF APPLICATION

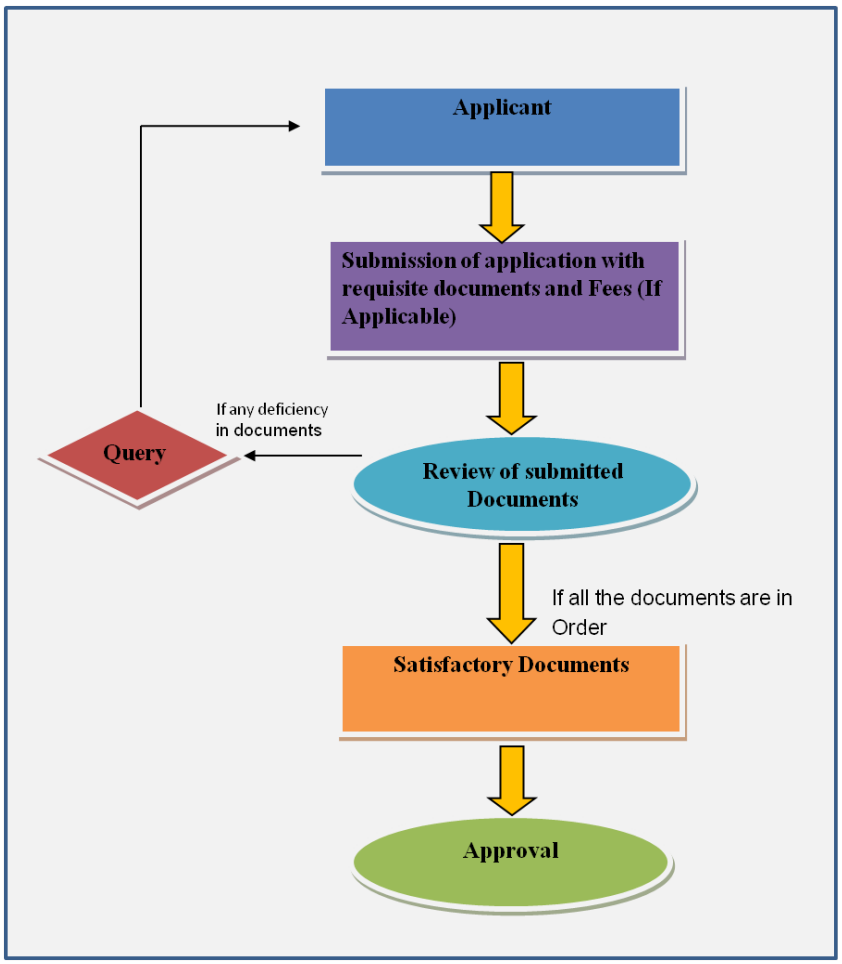

Figure 1: Approval process for Application received in Hard copy with respect to In Vitro Diagnostic Division. (Retrieved from CDSCO website https://cdsco.gov.in/opencms/export/sites/CDSCO_WEB/Pdf-documents/medical-device/Approval_process_flowchart_MD_hardcopy2.pdf)

Annexure V.

PROCESS FOR APPLICATION THROUGH ONLINE SUGAM PORTAL

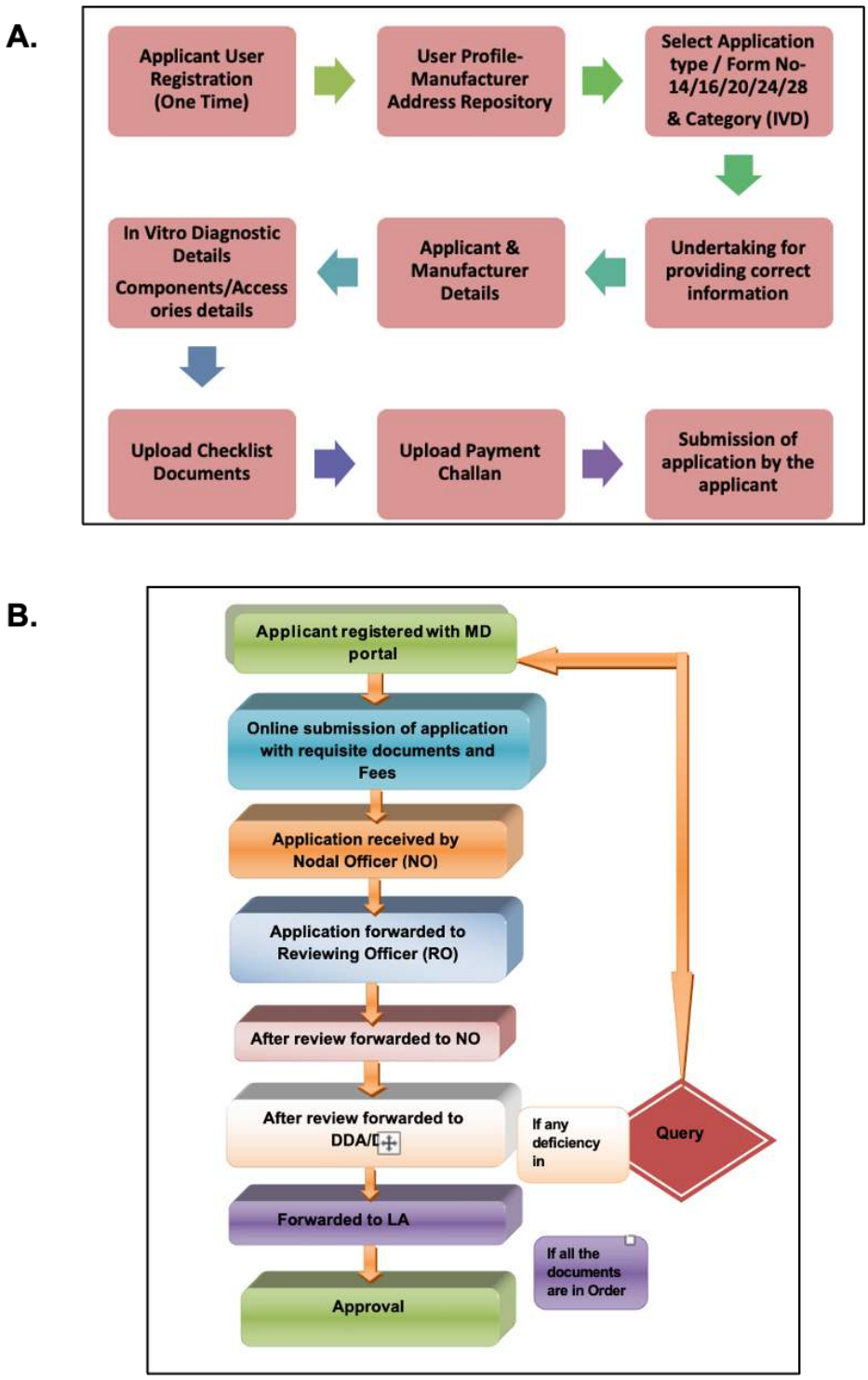

Figure 2: Approval process for Application received through Online Sugam Portal for grant of permissions with respect to In Vitro Diagnostics. (A) Step-1:Registration of applicant with MD portal (B) Step-2:Submission and processing of application. (Retrieved from CDSCO website: https://cdsco.gov.in/opencms/export/sites/CDSCO_WEB/Pdf-documents/medical-device/Approval_process_flowchart_MD_Online1.pdf)

ANNEXURE VI.

VALIDATION REPORT SUMMARY FORMAT

Name of the product (Brand /generic):	
Name and address of the legal manufacturer:	
Name and address of the actual manufacturing site:	
Type of test:	
Lot No./Batch No.	
Manufacturing date	
Expiry date	
Number of tests received:	
Intended use	
Regulatory Approval: Test License/Manufacturing License License No: Issue date: Valid upto:	
Brief details of the test	
Reference standard/Product	
Samples used	
Controls used	
Reference method used	
Brief details of the method validation plan	
Relevant SOPs (Provide SOP Nos. and titles)	
Calculation: Clinical sensitivity: Sensitivity (%) = $\frac{\text{True Positives}}{\text{True Positives + False Negatives}}$ X 100	

Clinical specificity: Specificity (%) = True Negatives X 100 True Negatives + False Positives Positive predictive value (PPV) : PPV = (prevalence) (sensitivity) (prevalence) (sensitivity) + (prevalence) (1- sensitiv Negative predictive value (NPV): NPV = (1 - prevalence) (specificity) (1 - prevalence) (specificity) + (prevalence) (1-sensitivity) Accuracy: Accuracy = (True Negatives + True Positives) (True Negatives + True Positives + False Negatives + False Positives)	

Results:

S.No	Testing Parameter	Criteria / specification	Result obtained	Remark

Conclusion:

Signature of the Analyst
Name:
Designation:
Date:

Signature of the Lab. Head
Name:
Designation:
Date:

ANNEXURE VII.

Examples of CDSCO approved antimicrobial resistant IVDs (August 2024)*

(source: https://cdscomdonline.gov.in/NewMedDev/ListOfIvdMdApprovedDevices)

S. No.	Manufacturer/ Importer Name	Name of Device	Device Class	Intended Use	Issuing Authority
1	Tulip Diagnostics Private Limited	Microplate ELISA Reader	Class A	Antibiotic Susceptibility Testing machine designed for clinical ELISA test analysis using water-soluble samples and reagent.	SLA - Goa
2	Copan India Private Limited	WASPLab® System	Class A	Used for the incubation and the digital imaging of agar culture plates.	CLA - CDSCO
3	BioMerieux India Pvt. Ltd.	ETEST Azithromycin	Class B	For determining the MIC of antimicrobial agents	CLA - CDSCO
4		ETEST Cefixime	Class B	For determining the MIC of antimicrobial agents	CLA - CDSCO
5		ETEST Clarithromycin	Class B	For determining the MIC of antimicrobial agents	CLA - CDSCO
6		ETEST Daptomycin	Class B	For determining the MIC of antimicrobial agents	CLA - CDSCO
7		ETEST Erythromycin	Class B	For determining the MIC of antimicrobial agents	CLA - CDSCO
8		ETEST Fluconazole	Class B	For determining the MIC of antimicrobial agents	CLA - CDSCO
9		ETEST Flucytosine	Class B	For determining the MIC of antimicrobial agents	CLA - CDSCO
10		ETEST Itraconazole	Class B	For determining the MIC of antimicrobial agents	CLA - CDSCO
11		Etest Levofloxacin	Class B	For determining the MIC of antimicrobial agents	CLA - CDSCO
12		ETEST Linezolid	Class B	For determining the MIC of antimicrobial agents	CLA - CDSCO
13		ETEST Moxifloxacin	Class B	For determining the MIC of antimicrobial agents	CLA - CDSCO
14		ETEST Spectinomycin	Class B	For determining the MIC of antimicrobial agents	CLA - CDSCO
15		ETEST Teicoplanin	Class B	For determining the MIC of antimicrobial agents	CLA - CDSCO
16		ETEST Tetracycline	Class B	For determining the MIC of antimicrobial agents	CLA - CDSCO
17		ETEST Trimethoprim Sulfamethoxazole	Class B	For determining the MIC of antimicrobial agents	CLA - CDSCO
18		ETEST Voriconazole	Class B	For determining the MIC of antimicrobial agents	CLA - CDSCO
19		CEFTAZIDIME / AVIBACTAM CZA	Class B	For determining the MIC of antimicrobial agents	CLA - CDSCO
20		ETEST AZITHROMYCIN	Class B	For determining the MIC of antimicrobial agents	CLA - CDSCO

21		ETEST BENZYLPENICILLIN	Class B	For determining the MIC of antimicrobial agents	CLA - CDSCO
22		ETEST CHLORAMPHENICOL	Class B	For determining the MIC of antimicrobial agents	CLA - CDSCO
23		ETEST CIPROFLOXACIN	Class B	For determining the MIC of antimicrobial agents	CLA - CDSCO
24		ETEST COLISTIN	Class B	For determining the MIC of antimicrobial agents	CLA - CDSCO
25		ETEST DAPTOMYCIN	Class B	For determining the MIC of antimicrobial agents	CLA - CDSCO
26		ETEST DORIPENEM	Class B	For determining the MIC of antimicrobial agents	CLA - CDSCO
27		ETEST ERTAPENEM	Class B	For determining the MIC of antimicrobial agents	CLA - CDSCO
28		ETEST IMIPENEM	Class B	For determining the MIC of antimicrobial agents	CLA - CDSCO
29		ETEST LINEZOLID	Class B	For determining the MIC of antimicrobial agents	CLA - CDSCO
30		ETEST MEROPENEM	Class B	For determining the MIC of antimicrobial agents	CLA - CDSCO
31		ETEST MOXIFLOXACIN	Class B	For determining the MIC of antimicrobial agents	CLA - CDSCO
32		ETEST POLYMYXIN B	Class B	For determining the MIC of antimicrobial agents	CLA - CDSCO
33		ETEST TEICOPLANIN	Class B	For determining the MIC of antimicrobial agents	CLA - CDSCO
34		ETEST VANCOMYCIN	Class B	For determining the MIC of antimicrobial agents	CLA - CDSCO
35		Ceftazidime/ceftazidime + clavulanic acid	Class B	For determining the MIC of antimicrobial agents	CLA - CDSCO
36		Etest Cefotaxime/ Cefotaxime+ clavulanic acid,	Class B	For determining the MIC of antimicrobial agents	CLA - CDSCO
37		Etest Imipenem/ Imipenem +EDTA,	Class B	For determining the MIC of antimicrobial agents	CLA - CDSCO
38		Etest Meropenem/ Meropenem +EDTA	Class B	For determining the MIC of antimicrobial agents	CLA - CDSCO
39		Etest Piperacillin/ Tazobactam	Class B	For determining the MIC of antimicrobial agents	CLA - CDSCO
40	Becton Dickinson India Private Limited	BD BACTEC Myco/F Lytic Culture Vials	Class B	Nonselective culture medium to be used as an adjunct to aerobic blood culture media for the recovery of	CLA - CDSCO

				mycobacteria, yeast and fungi from blood.	
41		BD BACTEC Plus Aerobic/F Culture Vials	Class B	Used in a qualitative procedure for the aerobic culture and recovery of microorganisms (bacteria and yeast) from blood.	CLA - CDSCO
42		BD BACTEC Plus Anaerobic/F Culture Vials	Class B	BD BACTEC Plus Anaerobic/F medium is used in a qualitative procedure for the anaerobic culture and recovery of microorganisms (bacteria and yeast) from blood. The principal use of these media is with the BD BACTEC fluorescent series instruments.	CLA - CDSCO
43		BD BBL Sensi-Disc Ethionamide - 25 µg	Class B	These discs are used in qualitative susceptibility testing procedures in culture media. They serve as a convenient method for addition of antimicrobial agents to culture media, especially for qualitative studies of mycobacteria and related organisms	CLA - CDSCO
44		BD BBL Sensi-Disc Rifampin, RA25, 25ug	Class B	These discs are used in qualitative susceptibility testing procedures in culture media. They serve as a convenient method for addition of antimicrobial agents to culture media, especially for qualitative studies of mycobacteria and related organisms	CLA - CDSCO
45	HiMedia Laboratories Pvt. Ltd.	Positive Blood Cultures Pretreatment Reagent	Class B	This is a pretreatment reagent which used for the identification of positive blood culture microorganisms using the AUTOF MS. It is used in conjunction with other clinical and diagnosis procedures as an aid in the early diagnosis of, for example, bloodstream infection.	CLA - CDSCO
46	Suyog Diagnostic Private Ltd	URO QUICK SCREENING KIT	Class B	Semi-quantitative single use in vitro diagnostic kit, general microbiological culture liquid media, intended to be used by professional users only for the detection of the microbial growth in human urine.	CLA - CDSCO
47	HiMedia Laboratories Pvt Ltd.	Dehydrated Culture Media(DCM), HiMedia, HiCrome	Class B	Devices intended t to grow meant to grow/ isolate/identify and handle microorganisms /infectious agent.	SLA - Nashik Division
48		Dehydrated Culture Media (DCM), HiMedia, HiVeg, Granulated	Class B	Devices intended t to grow meant to grow/ isolate/identify and handle microorganisms /infectious agent.	SLA - Nashik Division

49	CML BIOTECH LIMITED	Candida Agar Plate	Class B	A selective differential media used for rapid isolation and identification of Candida species from mixed cultures in clinical and non-clinical samples.	SLA - Kerala
50	HiMedia Laboratories Pvt Ltd.	Anaerobic Blood Agar Plate w/ Neomycin	Class B	Anaerobic Blood Agar Plate w/ Neomycin is recommended for isolation and cultivation of Group A and Group B Streptococci from throat cultures and other clinical samples.	SLA - Kokan Division
51		Antimicrobial Susceptibility System(ASS)-HiMic Plate Kit (MPK071 - Amphotericin B)	Class B	To determine Minimum Inhibitory Concentration (MIC) of antibiotic.	SLA - Kokan Division
52		Antimicrobial Susceptibility System(ASS)-HiMic Plate Kit (MPK012 - Ceftazidime)	Class B	To determine Minimum Inhibitory Concentration (MIC) of antibiotic.	SLA - Kokan Division
53		Antimicrobial Susceptibility System(ASS)-HiMic Plate Kit (MPK709 - Levonadifloxacin)	Class B	To determine Minimum Inhibitory Concentration (MIC) of antibiotic.	SLA - Kokan Division
54		Antimicrobial Susceptibility System(ASS)-HiMic Plate Kit (MPK001 - Amikacin)	Class B	To determine Minimum Inhibitory Concentration (MIC) of antibiotic.	SLA - Kokan Division
55	Bio-Rad Laboratories (India) Pvt Ltd	Ceftolozane + Tazobactam 30/10 ug	Class B	Antibiotic disks are used to perform a semi-quantitative antimicrobial susceptibility testing using disk diffusion method.	CLA - CDSCO
56	M/s.Transasia Bio-Medicals Ltd.	Suspension Medium MIC	Class B	Supplementary preparation for MIC kits (MIC G-I, G-II, URINE, NEFERM, STAPHY), which are designed for antibiotic susceptibility testing.	CLA - CDSCO
57	BioMerieux India Pvt. Ltd.	Mueller Hinton 2 agar + 5% sheep blood	Class B	Susceptibility of pneumococci and other streptococci to antibiotics.	CLA - CDSCO
58	Rivaara Labs Private Limited	AMR Direct Flow Chip Kit (Manual and Auto)	Class C	Allows a quick detection of twenty AMR gene families, which are associated with multidrug-resistant organisms (MRO)	CLA - CDSCO

59	Abbott Diagnostics Medical Private Limited	SD Bioline TB Ag MPT64 Rapid	Class C	For the detection of antigen MPT64 MTB complex in samples from liquid or solid culture media.	CLA - CDSCO
60	HiMedia Laboratories Pvt. Ltd.	NG-Test CARBA 5	Class C	For the detection of the KPC, OXA, VIM, IMP, NDM carbapenemases in a bacterial colony obtained from culture.	CLA - CDSCO
61	KILPEST INDIA Ltd	UTI Antimicrobial Susceptibility Testing PCR Kit	Class C	For the detection & differentiation of AMR genes in uropathogens	CLA – CDSCO
62	Q-Line Biotech Private Ltd	MTB RT-PCR Kit	Class C	Used for the detection of MTB DNA from suspected samples	CLA - CDSCO
63	EMPE Diagnostic Pvt Ltd	mfloDx MDR-TB AMP Kit	Class C	Used for amplification and detection of MTB and its resistance to Rifampicin and Isoniazid genes.	CLA – CDSCO
64	Roche Diagnostics India Pvt. Ltd.	cobas® 4800 MRSA/SA Amplification/Detection Kit	Class C	For the rapid in vitro qualitative detection of MRSA and SA DNA from nasal swabs	CLA - CDSCO
65	Instrumentation Laboratory India Pvt Ltd	HemosIL Liquid Anti-Xa	Class C	For the qualitative detection of MTB complex DNA in smear positive or smear negative specimens	CLA - CDSCO
66	Abbott Healthcare Pvt. Ltd.	Abbott RealTime MTB Amplification Reagent Kit	Class C	For the qualitative detection of MTB complex DNA in smear positive or smear negative specimens	CLA - CDSCO
67		Abbott RealTime MTB Control Kit	Class C	To establish run validity of the Abbott RealTime MTB assay	CLA - CDSCO

**Table 1 and Annexure VII provide examples of CDSCO approved IVDs purely for reference purposes only. None of the examples indicate any endorsement through this document.*

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Chapter 5

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