CHAPTER 1
Role of the US Food and Drug Administration in the Regulation of Clinical Microbiology Devices

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1.1 Historical overview of in vitro diagnostics
The Medical Device Amendment (MDA) of 1976 [4] was the result of a long history of fraudulent medical devices beginning as early as the 1700s with Dr. Elisha Perkins’ patent tractors, which consisted of two rods of brass and iron about three inches long purported to eliminate disease from the body. Despite the surge of fraudulent medical devices throughout the years, no substantial regulation was enacted to ensure the safety and effectiveness of devices. The enactment of the Food Drug and Cosmetics (FD&C) Act of 1938 was the first step towards eventual implementation of device regulation.

1.1.1 Device classification via the MDA of 1976
The subject matter in this section as well as the remainder of the chapter will be confined to a discussion of the regulations that affect the classification of microbiological devices, as well as the various FDA processes which enable the commercial interstate sale and distribution of clinical microbiology in vitro diagnostic devices (IVDs) by the Center for Devices and Radiological Health (CDRH).

The following definition of an in vitro diagnostic device can be found in the FDA regulation 21CFR (Code of Federal Regulations) 809.3

(a) In vitro diagnostic products are those reagents, instruments, and systems intended for use in the diagnosis of disease or other conditions, including a determination of the state of health, in order to cure, mitigate, treat, or prevent disease or its sequelae. Such products are intended for use in the collection, preparation, and examination of specimens taken from the human body. These products are devices as defined in section 201(h) of the Federal Food, Drug, and Cosmetic Act (the act), and may also be biological products subject to section 351 of the Public Health Service Act.

(b) A product class is all those products intended for use for a particular determination or for a related group of determinations or products with common or related characteristics or those intended for common or related uses. A class may be further divided into subclasses when appropriate.

The MDA of 1976 required that all medical devices be divided into three product classes by virtue of the types of controls necessary to assure the safety and effectiveness of the device.

• Class I, general controls, includes devices which appear to pose relatively little risk to human health and for which a series of general controls are believed sufficient to ensure safety and effectiveness. The controls include regulations that: (i) prohibit the sale of adulterated or misbranded devices; (ii) require domestic device manufacturers and initial distributors to register their establishments with the FDA and provide the FDA with a list of all devices being sold before the passage of the law (pre-amendment devices); (iii) grant the FDA authority to ban certain devices; (iv) provide for notification to the FDA of risks and of repair, replacement, or refund (recall); (v) restrict the sale, distribution, or use of certain devices; and (vi) govern good manufacturing practices, records and reports, and inspections. These requirements also apply to Class II and Class III devices.

• Class II devices are those for which general controls alone are insufficient to assure safety and effectiveness, and existing methods are available to provide such assurances. In addition to complying with general controls, Class II devices are also subject to special controls. Special controls may include special labeling requirements, mandatory performance standards, and post-market surveillance.

• Class III devices are usually those that support or sustain human life, are of substantial importance in preventing impairment of human health, or which present a potential,
unreasonable risk of illness or injury. In many cases there is insufficient information to ensure that general controls and performance standards will provide assurance that the devices are safe and effective. New devices are assigned to Class III until they are found to be substantially equivalent to a pre-amendment device (Class I or II) or are reclassified as Class I or II devices through the de novo petition program.

As can be seen from the above descriptions, classification of an IVD is risk based. In addition, device classification is inherently tied to the intended use of a device. For example, when the intended use for a device is as an aid in the assessment of serological status for sexually active adults and expectant mothers the device is classified as Class II as risks may be mitigated with appropriate controls. However, when the intended use for a device is an aid in the assessment of response to therapy in immunocompromised patients, e.g., transplants recipients where there is a substantial increase in risk such as death from cytomegalovirus (CMV) infection, the device classification is Class III.

**Table 1.1** Types of FDA submissions with associated device classes

<table>
<thead>
<tr>
<th>Class</th>
<th>Premarket submission</th>
<th>Success metric</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>III</td>
<td>PMA</td>
<td>Safety and effectiveness</td>
<td>Approval</td>
</tr>
<tr>
<td>II</td>
<td>510(k)</td>
<td>Substantial equivalence</td>
<td>Clearance</td>
</tr>
<tr>
<td>I</td>
<td>None (if exempt)</td>
<td>Safety and effectiveness</td>
<td>Approval</td>
</tr>
<tr>
<td>II (de novo)</td>
<td>De novo request</td>
<td>Safety and effectiveness</td>
<td>Approval</td>
</tr>
</tbody>
</table>

Microbiology device regulations and their classifications can be found in 21 CFR, Microbiology Regulation, Sections 866.1 through 866.3980. The following are examples of Class I and Class II microbiology devices.

Many times assay developers will look at a classification and find it exempt from section 510(k) of the act. It is important to note here that 21 CFR 866.9 Limitations of Exemptions from Section 510(k) of the Federal Food, Drug, and Cosmetic Act (the act) contains several important points that are specifically related to microbiology devices. They are:

1. (6) For identifying or inferring the identity of a microorganism directly from clinical material;
2. (7) For detection of antibodies to microorganisms other than immunoglobulin G (IgG) or IgG assays when the results are not qualitative, or are used to determine immunity, or the assay is intended for use in matrices other than serum or plasma;
3. (8) For noninvasive testing as defined in 812.3(k) of this chapter; and
4. (9) For near patient testing (point of care)

Table 1.1 illustrates the types of submissions sent to the FDA for review for each device class, the metric for the successful completion of that submission, and the final action taken to enable that device to enter interstate commerce.

### 1.2 Current microbiology device review regulatory pathways: practical considerations

#### 1.2.1 Premarket notification 510(k)

After having established the regulatory class of a device and prior to introduction into the market of a device intended for human use, each assay developer must submit a 510(k) to the FDA unless the device is exempt from 510(k) requirements of the Federal Food, Drug, and Cosmetic Act (the act) and does not exceed the limitations of exemptions in the device classification regulation chapters (e.g., 21 CFR 862.9, 21 CFR 864.9 and 21 CFR 866.9).

The purpose of the 510(k) submission is to demonstrate to the FDA that the new device is at least as safe and effective, that is, substantially equivalent to, as a legally...
marketed device or predicate device (21 CFR 807.92(a)(3)). While the basic content of a 510(k) has been established under 21 CFR 807.87, it is important to note that the types of information and data required may likely vary according to the analyte being detected as well as the differences between the new device and the predicate device. The FDA has 90 days for review of the submitted 510(k) in which to make a decision on whether a device is substantially equivalent.

Primary in the determination of substantial equivalence is that the new device has the same intended use as the predicate device. In addition, while the new device generally has the same technological characteristics, this is not a requirement. New devices with the same intended use yet different technological characteristics will be expected to supply appropriate clinical or scientific data if deemed necessary by the Secretary of Health and Human Services or a person accredited under Section 523, that demonstrates that the device is as safe and effective as a legally marketed device, and does not raise different questions of safety and effectiveness than the predicate device.

Following review of a 510(k) submission which the FDA finds substantially equivalent, each submitter will receive an order, in the form of a letter, from the FDA which finds the device to be substantially equivalent and states that the device can be marketed in the United States. This “clearance order” permits commercial distribution of the device.

Alternatively, following the review of a 510(k) submission, the FDA may find the device not to be substantially equivalent (NSE) to the predicate device. In general, an NSE decision is based on one of the following reasons.

1. No predicate device exists for the device, or the intended use is different than the predicate. In each case the device is deemed to be a Class III device and cannot be reviewed via a 510(k) submission.

2. The submitted information and data do not support a decision of substantial equivalence.

In the first case, the FDA will issue a letter stating that the device has automatically been classified as Class III and requires a premarket amendment (PMA), or that the device may be eligible for the de novo petition program (see below).

In cases where the information submitted is not sufficient to make a determination of substantial equivalence, the FDA will issue a letter identifying the deficiencies, which are usually performance related, and request additional information to resolve these deficiencies. The FDA will interactively work with developers to resolve the deficiencies within the time frame specified by regulation and guidance. If the information or justifications supplied are insufficient, the FDA will issue a NSE letter. If a sponsor does not respond at all to the request for additional information within the time frame specified by regulation and guidance, the FDA will consider the submission withdrawn. In either case above, the sponsor does have the opportunity to make a new 510(k) submission that addresses the outstanding items from the original review. The new 510(k) submission should cite the previous 510(k) number and specify how the outstanding items have been addressed and whether the new submission contains only the original information or additional new information.

1.2.2 De novo petitions

In response to devices that had been found NSE due to the lack of a predicate device, the Food and Drug Administration Modernization Act of 1997 (FDAMA) added the “de novo” classification option as an alternate pathway to classify such devices. The formation of this pathway enables FDA to make a risk-based classification determination for the device under Section 513(a)(1) of the FD&C Act.

Devices which may be eligible for this pathway must be low to moderate risk devices and the assignment of substantial benefits to patients is not as high as that for Class III devices. Such devices should be sufficiently understood to explain all the risks and benefits of the device such that all risks can be appropriately mitigated through the application of general and/or special controls to provide reasonable assurance of safety and effectiveness.

Once a device is granted marketing authority under the de novo petition program it is also eligible to serve as a predicate device for new devices that can be regulated via a 510(k) submission, thus enhancing the need for an appropriate risk-benefit profile when considering the safety and effectiveness of the device.

1.2.2.1 Scientific evidence supporting a de novo petition

The majority of microbiology devices using the de novo classification option will have both analytical and clinical data in support of the petition. Nonclinical testing may include, but is not limited to, testing such as the limit of detection (LoD) of the analyte, precision and/or reproducibility of the assay, interference testing, and cross-reactivity with other microbiological or nonmicrobiological analytes. A variety of clinical testing methods are also used in support of the petition. The clinical testing may involve anything from a randomized clinical trial, comparison of the new device to a putative gold standard using clinical specimens, and comparison of the new device to a patient infected status (PIS).

1.2.2.2 Benefit-risk determinations

Since the factors that the FDA considers as part of the benefit-risk determination (as well as examples) when reviewing a de novo petition are detailed in the guidance document “Factors to Consider When Making Benefit-Risk Determinations in Medical Device Premarket Approval and De Novo Classifications” only a brief mention of them will be made here.
1.2.2.3 Benefits of a new device
For microbiological devices the types of benefits may include the impact on clinical management of a patient, for example, the use of viral load assays to predict a patient’s response to therapy; the diagnosis of a specific pathogen which may have an impact on public health such as devices that identify influenza virus or *Chlamydia trachomatis*; and the ability to identify a specific pathogen to enable correct immediate treatment, such as those devices that identify *Clostridium difficile*.

Other benefit considerations may be the magnitude of the benefit, the probability of the patient experiencing a benefit and the duration of the effect(s) of the benefit.

1.2.2.4 Potential risks associated with a new device
The risks associated with microbiological devices are most often considered in terms of the risk of a false positive or a false negative result. A false positive result for a diagnostic assay may cause a patient to receive unnecessary therapy such as receiving a course of antibiotics for a viral infection, or may have severe consequences if a pregnant woman is misdiagnosed with a positive result for herpes simplex virus or rubella. A false negative result may also have either mild or severe consequences. For example, a false negative may lead to the incorrect treatment for bacterial meningitis, with severe consequences.

In addition to the individual effects, the aggregate effects of a misdiagnosis are also considered. Misdiagnosis of an individual with *C. difficile* infection may cause an infected patient to be housed with uninfected patients thus causing spread of the disease to the other residents of a hospital ward. Similarly, misdiagnosis of a sexually transmitted infection (STI) may also have significant ramifications in the spread of the pathogen in a population.

Possible mitigation of potential risks is also extremely important when determining the risk-benefit of a device. Mitigations such as restrictions in the labeling of a diagnostic device and restrictions in the use of a device to a segment of a population are two important safeguards that are often employed.

1.2.3 Premarket application
The PMA process is a scientific and regulatory review to assess the safety and effectiveness of Class III devices. Any microbiological device that involves measuring, detecting, or identifying a Class III analyte, is subject to a PMA review (see discussion above on classifying analytes). The applicant must receive FDA approval of its PMA application prior to marketing the device. Such an approval is based on a determination by the FDA that the PMA contains sufficient valid scientific evidence to assure that the device is safe and effective for its intended use(s). An approved PMA is, in effect, a private license granting the applicant (or owner) permission to market the device. While the PMA is considered to be the most stringent type of device marketing application required by the FDA, this is primarily due to the PMA Manufacturing Quality System section, which must be included with the application. It is expected that all medical devices be manufactured under a quality system that is defined by FDA Regulation 21 CFR. 820, however, the details of the manufacturing of a specific device need only to be submitted to the FDA for Class III products. In addition a PMA may also be the subject of a review by an FDA designated microbiology panel of independent outside experts and is also subject to a quality audit by FDA’s Bioresearch Monitoring Program (BIMO) of a clinical study site’s compliance with a study protocol and the data generated during the study. The FDA has 180 days to review a submitted PMA and to make a decision of approval, approvable, or not approved. An approved PMA gives the applicant permission to market the device. A finding of approvable means that there are certain items outstanding, usually related to manufacturing documents that need to be corrected prior to approval, and not approved means that the application is denied and the applicant may not market the device.

1.2.3.1 When is a PMA required?
The initial step in determining whether a device requires a PMA is to search the product classification database: [http://www.fda.gov/medicaldevices/deviceregulationandguidance/overview/classifyyourdevice/ucm051637.htm](http://www.fda.gov/medicaldevices/deviceregulationandguidance/overview/classifyyourdevice/ucm051637.htm). The resulting search will yield information on device names, classifications, and any existing links to the CFR. Please note that for many devices that are novel or for which there were no existing devices prior to 1976, there will be no classification number, only the name of the device, a product code, and in some cases it will state if a PMA is to search the product classification database: [http://www.fda.gov/medicaldevices/deviceregulationandguidance/overview/classifyyourdevice/ucm051637.htm](http://www.fda.gov/medicaldevices/deviceregulationandguidance/overview/classifyyourdevice/ucm051637.htm). The initial step in determining whether a device requires a PMA is to search the product classification database: [http://www.fda.gov/medicaldevices/deviceregulationandguidance/overview/classifyyourdevice/ucm051637.htm](http://www.fda.gov/medicaldevices/deviceregulationandguidance/overview/classifyyourdevice/ucm051637.htm). The resulting search will yield information on device names, classifications, and any existing links to the CFR. Please note that for many devices that are novel or for which there were no existing devices prior to 1976, there will be no classification number, only the name of the device, a product code, and in some cases it will state if a PMA is required for that device.

If it is still unclear whether a PMA is necessary, the next step is to use the product code to search both the 510(k) and PMA databases, which can be found as links on the product classification database. Should a 510(k) be found in the database, and the new device has an equivalent intended use, then it is generally safe to assume that a 510(k) submission will be applicable to the device.

Finally, a device may not be found at all in the product classification database. This may occur if the analyte detected by the device is “novel,” for example having only recently been discovered and isolated. For those devices not found in the database, consideration is given to whether the device is high risk, i.e., supports or sustains human life, is of substantial importance in preventing impairment of human health, or presents a potential, unreasonable risk of illness or injury. In addition, a device may have been foundNSE to a Class I or Class II device. In both of these cases a PMA submission is required.
1.2.4 Major elements of an IVD submission to the FDA

For a new IVD, both “safety” and “effectiveness” of a device must be demonstrated. The broad criteria for evaluating the safety and effectiveness of a new IVD are also defined by regulation:

- Safety: are there reasonable assurances based on valid scientific evidence that probable benefits to health from use of the device outweigh any probable risks? [21 CFR 860.7(d)(1)].
- Effectiveness: is there reasonable assurance based on valid scientific evidence that the use of the device in the target population will provide clinically significant results? [21 CFR 860.7(e)(1)].

Requirements for marketing also include the need for labeling, which includes, among other requirements, product performance characteristics “as appropriate... describing such things as accuracy, precision, specificity, and sensitivity” [21 CFR 809.10]. The following discussion provides an overview of how the FDA approaches these requirements during the development and review of a new microbiology diagnostic device.

There is significant overlap in the elements of a submission for an in vitro diagnostic device approval or clearance and these are presented in Table 1.2. Several of these elements will be discussed in detail below and references to guidance documents for specific elements and/or analytes are included at the end of this chapter.

1.2.4.1 Intended use

The intended use of an in vitro diagnostic device is possibly the most critical element of an IVD. It not only establishes the risk associated with that device but dictates what studies should be performed (both analytical and clinical) in support of the submission and, finally, drives the FDA review of that submission. In devising an intended use for a new IVD the sponsor should have a clear understanding of the conditions/disease associated with the target organism and how the results from the IVD will integrate into patient management, as well as the patient populations for whom the IVD is intended.

The intended use of a device describes the organism/analyte being detected or measured in relation to the disease or condition associated with that organism/analyte. It also describes whether the assay is a qualitative assay associated with the direct detection of an organism, e.g., Chlamydia trachomatis, or if the assay quantitatively detects an organism, e.g., viral load molecular assays for hepatitis B. The patient specimen matrix being tested by the device is also an important part of the intended use: whether the matrix is serum or plasma (including anticoagulation types), urine or CSF, the swab type (nasal, genital), whether it is a clinician-collected or self-collected sample, and where the specimen collection may take place, such as a professional clinical high-complexity laboratory; a point of care (POC) location in a hospital (such as an emergency room or patient bedside); a facility with untrained users (e.g., a physician’s office); or an “over the counter” home test.

Limitations may also be part of the intended use. For example, “This assay has not been licensed for testing of blood donors” or in the case of many quantitative viral load assays such as for hepatitis C virus (HCV), “This assay has not been approved for diagnosis of hepatitis C.”

1.2.4.2 Device description

This section should contain all the items associated with the IVD, whether they are contained within the IVD kit or need to be obtained separately. Instrumentation and software applications may be a substantial portion of this section.

1.2.4.3 Assay interpretation

The interpretation of results from a new investigational device should be determined prior to conducting “pivotal” clinical studies. For a qualitative assay, this includes the criteria for scoring device results (e.g., as either “analyte detected,” “analyte not detected,” “equivocal,” or “invalid”). Invalid results may occur when one or more test parameters fail to meet the expected criteria. For equivocal results, rules for retesting specimens should be specified.

1.2.4.4 Analytical performance characteristics

The analytical performance characteristics are some of the initial characteristics studied on a new device. The results of these studies can provide an insight into the possibility of false negative and false positive results with the new IVD. An insight into the likelihood of false negative results can often be gleaned from the results of LoD, the effect of different matrices on the ability of the device to detect an organism, and the potential of interfering substances, either naturally occurring or introduced into the system in the case of drug interactions, to give a false result.

Table 1.2 Major elements in a submission for a new in vitro diagnostic device

<table>
<thead>
<tr>
<th>Intended use</th>
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<tbody>
<tr>
<td>Device description, internal/external controls</td>
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<tr>
<td>Assay interpretation</td>
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<tr>
<td>Pre-analytical (e.g., sample preparation) and analytical performance</td>
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<tr>
<td>Clinical performance</td>
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<tr>
<td>Instrument and software, if applicable</td>
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<tr>
<td>• If multiple platforms, assay performance on each</td>
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<tr>
<td>Labeling (package insert)</td>
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<tr>
<td>PMA-specific elements</td>
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<tr>
<td>• Manufacturing</td>
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<tr>
<td>• Quality systems inspections</td>
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<td>• BIMO inspections of clinical studies</td>
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</table>
Analytical studies such as cross-contamination or carry-over introduced by system components may provide evidence of the potential of the IVD to generate false positive results. Similarly, cross-reactivity with organisms other than the target analyte should also be studied to ensure positive results are not obtained in their presence.

The analytical studies also need to consider the intended use of the new device. The studies for a quantitative assay, e.g., viral load assays, will need to be more extensive than those for a qualitative assay and include limit of blank (LoB), LoD, lower limit of quantitation (LLoQ), upper limit of quantitation (ULoQ), and linearity. All assays, regardless of the quantitative or qualitative output, should have their performance near the LoD or assay cutoff thoroughly validated.

In general, analytical studies should also use real clinical matrices, e.g., negative serum, or a negative vaginal swab matrix. If the new device is for pathogen detection, actual organisms should also be used, as opposed to plasmids or nucleic acid transcripts.

1.2.4.4.1 Data reporting
All raw data listings for the analytical performance section should be included in the 510(k) or PMA submission. Statistical analyses of the data are discussed in a separate section below.

There are many analyte-/device-specific FDA guidance documents which contain more details on analytical performance studies and they may be found at: http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/PerformanceStudies.htm

1.2.4.5 Clinical performance
Clinical studies in support of a 510(k) submission or a PMA application may differ somewhat but the basic concepts are the same. In each case it is essential to design a clinical study that will support the claims of the proposed intended use. Some of the more important aspects of a clinical trial are discussed below.

1.2.4.5.1 Study population
The study population in the clinical study should directly reflect the intended use population. For example, if an IVD is to be used in a population with signs and symptoms of a disease or a population which is at risk for a disease, these are the patients who should be enrolled in the clinical study. The blood donor population for example, would not be representative of the intended use population for an infectious disease diagnostic device and therefore should not be eligible for inclusion in the study.

1.2.4.5.2 Clinical samples
Any clinical study should always, where possible, use real clinical samples. In some cases for rare analytes and for biological threat (biothreat) organisms there may be a provision for allowing the use of spiked or surrogate specimens. If multiple types of patient specimens are claimed for use in the device, all types of specimen matrices must be tested in the clinical study. The samples tested in the clinical study should be prospectively collected during the study and if not assayed immediately should be stored at the proper, validated, temperature range. For certain IVDs it may not be feasible to perform a prospective study. In that case, a set of retrospective banked samples that were at the time collected prospectively may be suitable for inclusion in the study. For example, studies found in certain PMA applications for viral load assays for hepatitis B or C may contain retrospective samples that were collected prospectively while patients were on antiviral therapy.

1.2.4.5.3 Determination of assay performance
In some 510(k) submissions the performance of the device in the clinical study may be compared to that of a predicate device that has FDA clearance for the same study population. More often, the performance of a new device is compared to a reference method such as bi-directional sequencing, viral culture, or a composite reference standard which may include both of those methods.

Another metric of assay performance is comparison to a patient infected status (PIS) which may or may not involve clinical outcome. For example, assays for C. trachomatis are evaluated against a PIS of infected or not infected status using an algorithm based on multiple cleared nucleic acid amplification tests (NAAT) using multiple matrices. Likewise, the performance of serological assays for hepatitis B may be judged in relation to the clinical status of a patient (e.g., acute, chronic, recovered) which has been determined by an algorithm using multiple FDA approved hepatitis B assays. In contrast, viral load assay performance characteristics are primarily based on clinical outcome, such as if a patient achieves a sustained viral load in response to drug therapy.

1.2.4.5.4 Additional studies required for assays that are run in a moderate complexity location
In order for an assay to be used in a setting such as a physician's office which is not a Clinical Laboratory Improvement Amendments of 1988 (CLIA) certified, high complexity laboratory, by operators that are untrained personnel, the assay must obtain (in addition to an FDA clearance or approval) a certificate of CLIA waiver. The basic principles that should be demonstrated are that the assay procedure is simple, has easy to understand instructions for use, and the performance when run by an untrained operator is the same as when performed by a trained laboratory technician. The additional studies that are required to attain CLIA waiver certification are outlined in the FDA guidance document entitled
and are positive via the reference assay divided by the percentage of patients that both test positive by the new device. For example, in a study of novel H1N1 influenza, it is the percentage of patients that have a positive result given the clinical reference method. Higher specificity yields greater confidence in a positive test result, and thus can be a reason for low sensitivity. Specificity is the converse, i.e., the probability that the new device will have a negative result given the clinical reference method is negative. Specificity is estimated in the pivotal study by the fraction of subjects that are negative by the new test among those that are negative by the clinical reference method. Higher specificity yields greater confidence in a positive result. It is important to recognize that although patients included in specificity calculations do not have the disease of interest, in most cases they are still symptomatic (depending on the specific intended use for the test); asymptomatic subjects would not be considered part of the intended use population for such a device and therefore would not be appropriate for use in a specificity calculation. The formulae for calculating sensitivity and specificity are illustrated in Table 1.3.

### Table 1.3 Sensitivity, specificity, and predictive values

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Clinical reference standard</th>
<th>Total</th>
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<tbody>
<tr>
<td></td>
<td>Condition present</td>
<td>Condition absent</td>
</tr>
<tr>
<td>Test positive</td>
<td>True positive (TP)</td>
<td>False positive (FP)</td>
</tr>
<tr>
<td>Test negative</td>
<td>False negative (FN)</td>
<td>True negative (TN)</td>
</tr>
<tr>
<td>Total</td>
<td>N+</td>
<td>N−</td>
</tr>
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</table>

For a prospective study: prevalence is estimated as 100% × (TP + FN)/N; sensitivity is estimated as 100% × TP/(TP + FN); specificity as 100% × (TN)/N; PPV is estimated as 100% × TP/(TP + FP); NPV is estimated as 100% × TN/(TN + FN).

There may be significant challenges with estimating the sensitivity of a new assay if the prevalence of the disease being tested is low. Estimates of sensitivity are dependent both on the true underlying sensitivity and sample size. In a clinical study with only 5 specimens positive for a specific pathogen (via the reference method) and an observed sensitivity of 5/5 or 100% results in a lower confidence bound of only 55.6%, meaning that diagnostic performance is very uncertain. Even for a test with perfect sensitivity, at least 35 patients with the condition of interest would be needed to yield a 95% lower bound greater than 90%. In a pivotal study for devices to detect more common conditions, it is not uncommon to see a sample size of 50 or more patients that are positive via the reference method. The FDA considers the observed sensitivity and specificity and their respective 95% lower confidence bounds as part of device performance.
The formulas for NPV and PPV in Table 1.3 are inappropriate when prevalence is unknown (e.g., when representative cases and controls are selected for study). Instead, formulas in Table 1.4 provide a means of estimating NPV and PPV under various scenarios for prevalence. More details on the design and analysis of diagnostic device studies can be found in FDA guidance [2] and Pepe [3].

In Table 1.4, prevalence is assumed known, i.e., measured without error. So if we had a device with a sensitivity of 90% and a specificity of 90% as in Table 1.5, but the prevalence of the target condition of interest was now 10% rather than the 1% in Table 1.5, the PPV would be 0.50 or 50% using the equation in Table 1.4 rather than 8.33% as in Table 1.5 and NPV would be 0.99 or 99%. The NPV is negligibly lower than in Table 1.5.

When a clinical study only assesses agreement to another previously cleared device rather than to a reference method, reporting NPV and PPV would be inappropriate.

### 1.4 Common issues with new FDA submissions

Having discussed analytical, clinical, and statistical considerations that should be present in both 510(k) and PMA submissions, it is a useful undertaking to examine some of the common deficiencies that are encountered during review of these submissions. The Safe Medical Devices Act (SMDA), the final report of the Temple Committee issued in 1993 [5], was the first such study to independently examine these issues. Many of their conclusions are still accurate today. The following examples represent the most common deficiencies that must be rectified before an application can be cleared or approved. In addition, the deficiencies are also responsible for more than one review cycle prior to clearance or approval of a device, leading to delays in ability to commercialize the device.

- Poorly designed studies that result in either intentional or unintentional biased datasets, particularly when investigational (or similar approved) test outcomes influence patient enrollment and/or disease verification procedures in a manner that is not consistent with indications for use.
- Instead of clinical accuracy (sensitivity, specificity, and predictive values), agreement of results with some other test is estimated. If these agreement measures are not high enough, it can make it difficult to conclude whether the device is safe and effective.
- Insufficient number of study samples resulting in performance estimates that are not powered statistically and clinically to justify safety and effectiveness claims made in the intended use.
- Insufficient or a complete lack of any study site monitoring during the clinical trial which often results in protocol deviations, or incorrectly recorded data.
- All workflow processes are not fully validated (e.g., IVD test-system processes multiple sample tube types, but only one type is included in the clinical trial).
- The addition of bridging studies when a clinical study for safety and effectiveness fails to include all the necessary intended-use subpopulations or types of specimens.

### Table 1.4 Positive predictive value and negative predictive value formulas

<table>
<thead>
<tr>
<th>Prevalence</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>100%</td>
<td>90%</td>
<td>90%</td>
<td>91%</td>
<td>99%</td>
</tr>
<tr>
<td>10%</td>
<td>90%</td>
<td>90%</td>
<td>91%</td>
<td>99%</td>
</tr>
</tbody>
</table>

### Table 1.5 Hypothetical example for diagnostic: low prevalence of condition clinical reference standard

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Clinical reference standard</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Condition present</td>
<td>Condition absent</td>
</tr>
<tr>
<td>Test</td>
<td>9</td>
<td>99</td>
</tr>
<tr>
<td>positive</td>
<td>1</td>
<td>891</td>
</tr>
<tr>
<td>Test</td>
<td>10</td>
<td>990</td>
</tr>
</tbody>
</table>

Sensitivity = 90%; specificity = 90%; PPV = 9/108 or 8.33%; NPV = 891/892 = 99.9%.

When sensitivity, specificity, and prevalence are known, one can calculate positive and negative predictive values (PPV and NPV, respectively). The PPV and NPV ask an alternative question, i.e., given a positive or negative test result, what is the probability that the patient has (or does not have) the condition being tested for. In a prospective study, this would be estimated as the percentage of patients with the condition of interest from all patients that test positive (see Table 1.3); similarly, the negative predictive value is estimated as the percentage of patients without the condition of interest among those that test negative.

The relationship between sensitivity, specificity, prevalence, PPV, and NPV is illustrated in Table 1.3 for prospective studies and in Table 1.4 more generally. As can be seen, the PPV and NPV are expressed as percentages and, ideally, both will be close to 100%. The importance of test prevalence is illustrated with hypothetical data in Table 1.5, where, despite high sensitivity and specificity, test utility is extremely limited in the context of ruling in a diagnosis as the PPV of the test result is still only 8%, underscoring the value of tests as an adjunct to diagnosis rather than the sole means for diagnosis.

However, tests may still have substantial clinical value even when only the NPV is very high. A high NPV indicates that if the patient tests negative it is very unlikely that the disease is present and the physician may want to pursue further workup to obtain a diagnosis.

The formulas for NPV and PPV in Table 1.3 are inappropriate when prevalence is unknown (e.g., when representative cases and controls are selected for study). Instead, formulas in Table 1.4 provide a means of estimating NPV and PPV under various scenarios for prevalence. More
• The often long time lag between a preliminary contact of a device developer with the FDA for preclinical advice and the submission of the 510(k) or PMA can result in the omission of studies that may then be necessary due to a change in clinical practice.
• Device used to validate the performance during the clinical evaluation is still a prototype and not the final design.
• Organizational problems:
  ° nonintuitive grouping of information;
  ° lack of a single detailed table of contents (TOC) at the front of a submission, lack of sequential pagination;
  ° organizational structure differs between submitted electronic and hard copies, and no TOC that aligns the two;
  ° studies not described clearly or completely (especially, analytical studies);
  ° important study details scattered throughout a submission;
  ° electronic data are provided but not in Excel (it requires a lot of time to convert non-E Excel files), and a description of variables in the data sets are not provided.

1.4.1 IVD labeling
Federal IVD regulations apply to all legally marketed commercial IVDs [21 CFR 809.10(a) and (b)], whether class I exempt or nonexempt, Class II, or Class III; devices for investigative use, IUO, [21 CFR 809.10(–)(2)(ii)] and research use, RUO, [21 CFR 809.10(c)(2)(I)].

1.4.1.1 510(k) and PMA labeling
The proposed labeling is an important part of the 510(k) notification or PMA and is reviewed by the FDA prior to issuing clearance or approval of an IVD. As noted above labeling requirements for inserts and outer packaging can be found in 21 CFR 809.10(b).

When reviewing labeling, particular attention is paid to the intended use, device description, specimen collection procedures, transport and storage recommendations, quality control recommendations, warnings and limitations, expected validation of cutoff, results and their interpretation, expected values, and specific performance characteristics. It is of particular importance that the clinical studies are clearly described and the associated performance characteristics match what was determined during review of the data submitted. The FDA is committed to “truth in labeling” in order to provide the end user with a product that performs as expected.

1.4.2 New technologies
1.4.2.1 How have FDA review policies and regulations kept up with the technological revolution in the microbiology laboratory?
Recent changes in the methodologies applied to rapid pathogen identification have been “game-changers” in microbiology laboratory practice, beginning with the move towards nonculture methods when singleplex polymerase chain reaction and other nucleic acid amplification tests were developed and leading towards the development and use of multiplex nucleic-acid-based tests, mass spectrometry, and high-throughput sequencing technologies. New FDA regulatory science concepts on how to determine the performance of these new and more complex technologies have been open for comment and discussion by way of a series of public workshops and guidance documents.

The FDA held such a workshop in 2011 to discuss how best to establish the performance of “highly multiplexed microbiology assays” and to discuss the clinical and public health applications of such devices.

As a follow up, the FDA drafted a guidance document published in 2014 describing how to validate the performance of these types of technologies as they apply to the diagnosis of infectious disease: http://www.fda.gov/medicaldevices/deviceregulationandguidance/guidancedocuments/ucm373750.htm.

To date several multiplex devices with up to 30 plus microbial analytes and resistance markers have been cleared using the principles outlined in the guidance. Similarly in April 2014, the FDA held a public workshop entitled “Advancing Regulatory Science for High Throughput Sequencing Devices for Microbial Identification and Detection of Antimicrobial Resistance Markers.” The purpose of this workshop was to discuss the clinical and public health applications and performance validation of new high-throughput sequencing devices, the quality criteria for establishing the accuracy of reference databases for regulatory use, and ways to streamline clinical trials of such devices for microbial identification. This discussion was essential to establish the safety and effectiveness of high-throughput sequencing devices when used to test human specimens or clinical isolates for the diagnosis of infectious diseases and detection of antimicrobial resistance markers. A guidance document outlining the types of studies the FDA is proposing be undertaken to validate the performance of such devices is currently under development.

1.4.3 How have FDA regulations and policies fostered preparedness for new and emerging pathogens and other public health emergencies?
During public health emergencies, medical countermeasures (MCMs) including diagnostic assays may be needed to prevent or treat diseases or conditions caused by biothreat agents or emerging infectious disease threats, like pandemic influenza or MERS-CoV.

The Pandemic and All-Hazards Preparedness Reauthorization Act of 2013 (PAHPRA) provides key legal authorities’ guidelines to sustain and strengthen national preparedness for public health emergencies; and the Emergency Use Authorization (EUA) authority allows
the FDA to help strengthen the nation’s public health pro-
tections against biothreats by facilitating the availability of
MCMs needed during public health emergencies. Under
Section 564 of the Federal Food, Drug, and Cosmetic Act,
the FDA Commissioner may allow unapproved medical
products or unapproved uses of approved medical prod-
ucts to be used in an emergency to diagnose, treat, or
prevent serious or life-threatening diseases or conditions
caused by biothreat agents when there are no adequate,
approved, and available alternatives.

The FDA published *Guidance – Emergency Use
Authorization (EUA) of Medical Products* in 2007, explaining
the FDA’s policies for authorizing the emergency use of
medical products under Section 564 of the FD&C Act.
The guidance is intended to inform industry, government
agencies, and FDA staff of FDA’s general recommendation
and procedures for issuance of EUAs.

During the 2009 H1N1 influenza pandemic, over 18
EUAs were granted to microbiology assays that could
detect the H1N1 virus from appropriate respiratory speci-
cmens, and similarly, EUAs have been issued for assays that
could detect the H7N9 influenza and MERS-CoV viruses
when the potential for a pandemic scenario was declared
in 2013 for both of these organisms.

### Appendix: web sites

<table>
<thead>
<tr>
<th>Statutory document</th>
<th>Web site</th>
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### References