A universal challenge in quantifying the accuracy of diagnostic tests is establishing whether each participant has the disease of interest (1). This classification is necessary to calculate various measures of diagnostic accuracy for the test being studied, such as sensitivity and specificity, predictive values, likelihood ratios, or receiver-operating characteristic curves (2). It is also a prerequisite for the derivation and validation of multivariate diagnostic prediction models. When making the classifications, researchers aim to use the best available method (the preferred reference standard) to verify the diagnosis in all participants.

Because of practical or ethical constraints, it is not always possible to ascertain disease status in all participants by using the preferred reference standard. Often an alternative, less accurate method (inferior reference standard) is used in patients who do not receive the preferred reference standard. The use of different reference standards in different groups of participants in a diagnostic study is known as differential verification (3). Differential verification is common; it may occur in up to one quarter of all diagnostic accuracy studies (4, 5).

As shown in Table 1, differential verification occurs for various reasons and in a wide range of clinical fields. A distinctive example comes from studies on the accuracy of mammography in detecting breast cancer (6, 11). The preferred reference standard, biopsy, is performed only when a lesion is found during mammography, indicating where to perform biopsy. In the ideal scenario, patients without lesions would also undergo this preferred reference standard, which would mean that they should have random biopsies. However, this option is not ethical and is not equivalent to a targeted biopsy. Instead, patients without lesions are followed to see whether breast cancer develops before the next screening.

Relying on disease classification that is based on an alternative reference standard may seem logical, but problems arise if one mistakenly treats the alternative and preferred reference standards as interchangeable when analyzing, reporting, or making inferences. When reference standards do not correspond well with the underlying “true” disease status (as is often the case with an inferior reference standard), the final disease classification will be wrong in some patients. The misclassification can cause biased estimates of diagnostic accuracy and regression parameters (12). When a mix of reference standards is used, index test or model accuracy estimates will systematically differ from the ideal study in which all patients have the preferred reference standard. This systematic deviation is called differential verification bias (4, 13, 14). Figure 1 depicts a clinically relevant example of it.

This article elaborates on problems that can be confused with differential verification, proposes a classification system for types of differential verification, and explores the mechanisms by which each type leads to bias. It provides guidance on how to clearly report results when differential verification is present and how to assess and correct for the risk of bias. We believe that such guidance extends and improves STARD (Standards for the Reporting of Diagnostic Accuracy Studies) and QUADAS-2 (Quality Assessment of Diagnostic Accuracy Studies) (13, 16).

**Classification of Verification Patterns**

Figure 2 gives an overview of the main verification patterns. Complete verification is the ideal situation in which all participants are verified by the same reference standard. A reference standard is often a single test, but when no single highly accurate test is available, multiple component tests may be used as a single reference method instead. Component tests can be used to make a final diagnosis in several ways. These include composite reference standards (a fixed rule for combining individual test results into a final diagnosis), panel diagnosis (consensus diagnosis by a group of experts), and latent class models (a statistical
Clear reporting of differential verification requires addressing the accuracy of the alternative reference standard, providing information about why patients received the reference standards they did (the verification pattern), and analyzing and presenting results for each reference standard.

Judging the risk of bias due to differential verification requires considering the accuracy of the inferior reference standard and investigating the verification pattern.

The situation in which disease status is not ascertained at all in some patients, and is thus missing, is termed partial verification. When the missingness of the reference standard is somehow related to the index test results, index test accuracy estimates based on only the complete cases is probably biased but the bias can be corrected for if these missingness is completely at random, estimates will be unbiased but inefficient, leading to CIs that will be wider than those obtained with complete verification. If missingness is dependent on patient characteristics, estimates are probably biased but the bias can be corrected for if these patient characteristics are measured.

In pattern A depicted in Figure 3, reference standard assignment depends entirely on the index test results. The earliest definition of differential verification was limited to pattern A and was described as the situation in which “negative [index] test results are verified by a different, often less thorough, [reference] standard, for example follow-up” (4). This particular situation, in which all positive index test results are verified by the preferred reference standard whereas all negative index test results are verified by an inferior standard, was later termed complete differential verification (11), but we propose referring to it as complete index test–dependent differential verification for clarity. Pattern A either is a product of the clinical situation or is determined a priori by the researcher.

Frequently in diagnostic studies, the decision about which reference standard each patient receives is less straightforward and is determined by various factors besides, or in addition to, index test results. In pattern B depicted in Figure 3, the choice of reference standard is influenced by other factors, such as signs and symptoms, other test results, or patient or physician preference. Pattern B may be broken down into 3 subtypes based on the method that uses associations among tests to define the unobserved disease status) (1). Although these methods use several pieces of information, the same information is available for all study participants and the same method is used to reach a final diagnosis.

The situation in which disease status is not ascertained at all in some patients, and is thus missing, is termed partial verification. When the missingness of the reference standard is somehow related to the index test results, index test accuracy estimates based on only the complete cases will be biased (17). Depending on the pattern of missingness, it may be possible to mathematically correct for partial verification bias (18, 19).

The situation in which different reference standards are used in different groups of patients is termed differential verification. The word differential means “varying according to circumstances or relevant factors” (20). A salient question in the case of differential verification is, “What factors determine which reference standard is used?” Figure 3 shows 2 basic differential verification patterns. The key distinction between the patterns is the reason that patients received one reference standard over another.

Differential verification can be thought of as a missing data problem in which the preferred reference standard is missing in some patients and replaced by an alternative, inferior reference standard (21). Data can be missing completely at random, or the missingness can be related to measured or unmeasured patient characteristics (17, 22). If missingness is completely at random, estimates will be unbiased but inefficient, leading to CIs that will be wider than those obtained with complete verification. If missingness is dependent on patient characteristics, estimates are probably biased but the bias can be corrected for if these patient characteristics are measured.

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### Table 1. Examples of Differential Verification

<table>
<thead>
<tr>
<th>Target Condition</th>
<th>Index Test</th>
<th>Preferred Reference Standard</th>
<th>Inferior Reference Standard</th>
<th>Reason for Not Using Preferred Reference Standard for All Patients</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast cancer</td>
<td>Mammography</td>
<td>Biopsy</td>
<td>Follow-up</td>
<td>Impossible to perform a biopsy when no lesion is detected during mammography</td>
<td>6</td>
</tr>
<tr>
<td>Deep venous thrombosis</td>
<td>Diagnostic rule for ruling out deep venous thrombosis</td>
<td>Ultrasonography together with follow-up</td>
<td>Follow-up</td>
<td>To test the safety of the rule in clinical practice, patients with a normal score on the diagnostic rule were sent home without extensive testing</td>
<td>7</td>
</tr>
<tr>
<td>Congenital heart defect</td>
<td>Pulse oximetry (blood oxygen levels)</td>
<td>Clinical work-up</td>
<td>Congenital anomalies registry</td>
<td>Too expensive to perform a clinical work-up of all infants to detect a rare disease</td>
<td>8</td>
</tr>
<tr>
<td>Depression</td>
<td>Diagnostic prediction rule for depression</td>
<td>In-person interview</td>
<td>Telephone interview</td>
<td>Not practically feasible to interview all participants in person</td>
<td>9</td>
</tr>
<tr>
<td>Tuberculosis</td>
<td>Screening test</td>
<td>Sputum culture</td>
<td>Chest radiography and/or follow-up</td>
<td>Clinical records were used instead of setting up a diagnostic study</td>
<td>10</td>
</tr>
</tbody>
</table>
pattern of “missingness” of the preferred reference standard (17). First, the choice of reference standard sometimes depends solely on known factors that are measured and recorded in the study. This pattern may occur, for example, when different study centers use slightly different imaging techniques as the reference standard because of availability of the technology. Second, unknown factors (those not recorded) could also influence this choice. An example of such a factor is patient preference: When the preferred reference standard is burdensome, some participants, particularly those with less severe symptoms, may opt out and be followed instead. Third, studies can be designed in

\[\text{Estimated accuracy of VIA:} \]
\[\text{Sensitivity} = \frac{150}{150 + 70} = 0.68 \]
\[\text{Specificity} = \frac{630}{630 + 150} = 0.81 \]

\[\text{Estimated accuracy of VIA:} \]
\[\text{Sensitivity} = \frac{150}{150 + 100} = 0.60 \]
\[\text{Specificity} = \frac{600}{600 + 150} = 0.80 \]

The example is loosely inspired by a study on the accuracy of VIA in screening for cervical cancer (15). The preferred reference standard is colposcopy plus biopsy when a lesion is detected. Because the preferred standard is invasive, one might use an alternative, less invasive reference standard, the Pap smear, for participants with a normal VIA result. If one assumed that the Pap smear had perfect accuracy, the naive (biased) estimates of sensitivity and specificity for the VIA would be 0.68 and 0.81, respectively. If one recognized the sensitivity of the Pap smear as only 0.70, the true estimate of the sensitivity for the VIA would be 0.60. Pap = Papanicolaou; VIA = visual inspection using acetic acid.
which the reference standard is assigned completely at random.

**Factors Leading to Differential Verification Bias**

The differential verification pattern—and, notably, the reasons that patients receive the reference standards they do—play a major role in whether bias is introduced as well as whether this bias can be (partially) adjusted for. Current guidelines for assessing the risk of bias in a diagnostic study simply warn that there is a risk of bias when multiple reference standards are used (13). This guidance is supported by the results of 2 meta-analyses on factors influencing diagnostic accuracy estimates, which found that studies in which differential verification was present had a diagnostic odds ratio that was, on average, 1.6 (95% CI, 0.9 to 2.9) to 2.2 (CI, 1.5 to 3.3) times higher than that in studies of the same test that used a single reference standard (4, 5). The example in Figure 1 illustrates how differential verification can lead to overestimates of accuracy. Although differential verification seems to generally result in a substantial overestimate of index test accuracy, it does not lead to clinically relevant bias in some situations.

Given that differential verification often causes bias, the pertinent question is whether the bias is large enough to be clinically relevant. The magnitude and direction of differential verification bias are influenced by various factors: the verification pattern, the accuracy of the reference standards, the proportion verified by each reference standard, and disease prevalence (11). Formal correction methods may address these factors, but it is difficult for the reader to consider multiple factors simultaneously when performing an explicit assessment of the risk of clinically relevant bias.

We recommend that readers break down the problem into a few questions that can independently be used to rule out the risk of differential verification bias (Table 2). Information about the disease prevalence and the proportion verified by each reference standard may be the most readily available information, but knowledge about either of these factors alone does not allow one to rule out the risk of clinically relevant bias. In many situations, the preferred reference standard is assumed—correctly or incorrectly—to have near-perfect accuracy. We recommend, therefore, that readers focus on the accuracy of the inferior ref-

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**Figure 3. Classification of differential verification patterns.**

In pattern A, the test a participant receives depends solely on the index test result, whereas in pattern B, other variables may influence reference standard assignment.
ference standard and the verification pattern when ruling out the risk of clinically relevant differential verification bias.

**Accuracy of the Inferior Reference Standard**

As a general rule, the risk of clinically relevant differential verification bias decreases as the accuracy of the inferior reference standard increases. To estimate the accuracy of the inferior reference standard, its performance needs to be compared with that of the preferred reference standard. Although this may not be possible or ethical to do within the study, accuracy estimates may be available in the literature.

A commonly used alternative reference standard for making a final disease classification is follow-up (following patients over time to see whether symptoms worsen or improve). The follow-up information is used to decide retrospectively whether patients did indeed have the disease at the time the index test was done. Assessing the accuracy of follow-up is difficult, even when the preferred reference standard and follow-up are both done in a random subset of patients, because the condition of the patients can improve or worsen between when the preferred reference standard is performed and the end of follow-up.

Because estimating the accuracy of follow-up is difficult, it is particularly important to consider the quality and length of follow-up from a biological perspective, taking into account the natural course of existing cases as well as the incidence of new cases. We provide a cursory example of how this can be done using a study that investigated the accuracy of blood oxygen concentration measured at birth in detecting congenital heart defects (see Table 1) (23). In this study, newborns with low oxygen levels were treated by a cardiologist, whereas the rest were followed up after 1 year through a congenital anomalies register.

Follow-up should be long enough to allow as many hidden cases of disease to progress to a detectable stage as possible. However, if follow-up is too long, new cases developing after the index test was performed will also be detected. In the example, follow-up might be considered too short because some types of congenital heart defects are detected later in life. On the other hand, it was not too long because congenital heart disease is, by definition, already present at birth. The second point to consider is whether follow-up allows detection of the same type and severity of disease as the preferred reference standard. Researchers should focus on whether the test being studied detects cases that will benefit from clinical intervention rather than simply the presence of any disease (24). In the example, more serious types of defects are probably detected at birth, whereas less serious ones are detected during follow-up. Although this may not be a problem, if follow-up instead detects the more pronounced cases, the estimated sensitivity of the index test will be an overestimate of its sensitivity in detecting serious cases.

When the inferior reference standard is believed to have high accuracy, clinically relevant differential verification bias is unlikely and there is no need to look into the other factors influencing bias. This was believed to be the case in an example of differential verification from a study involving a clinical prediction rule for screening for depression in primary care (see Table 1) (9). In this study, in-person or telephonic questionnaires were used as the reference standard, but because the authors had reason to assume that these methods for assessing depression had similar accuracy, they argued that clinically relevant differential verification bias was unlikely. When the inferior reference standard’s accuracy is questionable, however, the next step is to consider the verification pattern.

**Verification Pattern**

The pattern of verification plays an important role in whether bias is introduced. The most straightforward verification pattern is when the choice of reference standard is fully dependent on the index test results (Figure 3, pattern A). Studies with this pattern are likely to have biased estimates of sensitivity, specificity, diagnostic odds ratios, and likelihood ratios because these estimates rely partly on disease status classification by the inferior reference standard. An exception is positive predictive value estimates (the probability that a person has the disease given that the index test results are abnormal). The positive predictive value estimate is not affected by differential verification bias in pattern A because all patients with positive index test results receive the preferred reference standard (3). Negative predictive value estimates can also still be interpreted in a meaningful way in the sense that they provide information on the proportion of missed cases, as defined by the inferior reference standard (see Figure 4 for an example).

When the choice of reference standard is not fully dependent on the index test results (Figure 3, pattern B), differential verification is likely to bias all accuracy estimates because they rely to some degree on disease classification by the inferior reference standard. In the rare case

### Table 2. Questions to Ask When Assessing Risk of Differential Verification Bias

<table>
<thead>
<tr>
<th>Question</th>
<th>Answer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Was the choice of reference standard completely dependent on the results of the index test? (If so, the predictive values are clinically interpretable.)</td>
<td>No</td>
</tr>
<tr>
<td>If the answer to the first question is no, how accurate is the inferior reference standard? (The higher the accuracy of the inferior reference standard, the lower the risk of bias.)</td>
<td>High</td>
</tr>
<tr>
<td>What percentage of the participants were diagnosed by use of the inferior reference standard? (If a negligible percentage of participants received an inferior standard, the risk of bias is low. Several factors must be taken into account when determining whether the percentage is negligible.)</td>
<td>Low</td>
</tr>
<tr>
<td>If follow-up is used as the inferior reference standard, does it identify almost all hidden cases present at the time of the index test but very few new cases that develop afterward? Does follow-up detect the same type of cases as the preferred reference standard? (If the answer to both questions is yes, the risk of bias is low.)</td>
<td>No</td>
</tr>
</tbody>
</table>

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that reference standard assignment is completely random, index test accuracy estimates calculated using only data from patients verified by the preferred reference standard will be unbiased, although they will generally lack precision because of the smaller sample size.

When the pattern of differential verification is not reported clearly, it is difficult for the reader to assess whether differential verification introduces clinically relevant bias. As a last resort, it is possible to take a pragmatic approach. This approach is to consider studies to have a low risk of bias if only a negligible percentage of participants receive the inferior reference standard. However, determining this percentage requires consideration of other factors that may influence this bias, particularly the degree of imperfection of the inferior reference standard and the correlation of errors between the inferior reference standard and the test under evaluation.

Because differential verification often biases diagnostic index test or model accuracy estimates, all efforts should be made to avoid it. When it is unavoidable, the fully index test–dependent differential verification pattern (Figure 3, pattern A) is preferable because it still produces clinically interpretable predictive values, as discussed earlier. Pattern B in Figure 3 probably biases all index test accuracy estimates if analyzed naively.

**Methods for Dealing With Differential Verification Bias**

Adjusting for differential verification bias is possible when the indication to perform a particular reference standard can be explained by the data at hand. These methods can improve accuracy estimates, but the gains in accuracy are correlated with a loss in precision.

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**Figure 4. An example of how to report complete index test–dependent differential verification.**

![Diagram showing differential verification process]

- **Patients with suspected DVT** (n = 1100)
- **Patients included** (n = 1010)
  - **Diagnostic decision rule score ≥4** (n = 505)
    - **No ultrasonography** (n = 5)
      - DVT+ (n = 5)
    - **Referred to ultrasonography**
      - DVT+ (n = 125)
      - DVT− (n = 375)
  - **Diagnostic decision rule score ≤3** (n = 505)
    - **3-mo follow-up**
      - DVT+ (n = 5)
      - DVT− (n = 495)
    - **Lost to follow-up** (n = 5)
      - **DVT detected by ultrasonography**
        - Wells rule + 125
        - Wells rule − 0
      - **DVT detected during follow-up**
        - Wells rule + 0
        - Wells rule − 5

**PPV** = 125/(125 + 375) = 0.25

**NPV** = 495/(5 + 495) = 0.99

The example is inspired by AMUSE-1 (Amsterdam Maastricht Utrecht Study on Thromboembolism) (7), which looked at the safety of a diagnostic rule (the Wells rule) for excluding DVT. When the rule predicted a high risk for DVT, the patients were referred for further testing (ultrasonography). Those with a low predicted risk were sent home and followed instead. In this example, the probability that a participant truly has DVT given that they had a diagnostic score ≥4 (that is, the PPV) is 25%. Likewise, the probability that a person who was sent home because they had a diagnostic score ≤3 would soon thereafter be diagnosed with DVT (and was therefore erroneously sent home) was 1%. DVT = deep venous thrombosis; NPV = negative predictive value; PPV = positive predictive value.
Verification pattern A in Figure 3 is obvious to the investigator, can be confirmed by the data, and is probably well-explained to the reader. However, in pattern B of Figure 3, the indication to perform a particular reference standard may require assessment of the associations between observed variables in the data set and the choice of reference standard, similar to the way that the pattern of missingness is investigated in methods for handling missing data (17). The more the important patient characteristics differ between reference standard groups, the more likely differential verification introduces bias. However, if all key patient characteristics are found to be similar between the groups, it may, for the sake of practicality, be reasoned that reference standard assignment was a near-random process (17).

When the indication to perform a particular reference standard is known (or can be assumed to be completely random) and well-reported, the investigator or a reader can use a recently proposed Bayesian correction method (14). This model takes into account the verification pattern as well as bias due to one or both imperfect reference standards in a single model. The model requires the investigator to specify the verification pattern and give a best guess of the accuracy of both reference standards in the form of a prior distribution. The data (that is, the cross-tabulations of the index test results by the results of the reference standards) are also required.

When the preferred reference standard is not too burdensome for the patient, investigators might consider performing both reference standards in a random subset of participants (for pattern A in Figure 3, this could be done in a random subset of patients with negative index test results) to estimate the concordance, and thus the accuracy, of the inferior reference standard compared with the preferred reference standard. This information is helpful in the Bayesian method discussed earlier because it provides a better estimate of the accuracy of the imperfect reference standard (that is, a narrower prior distribution that is closer to the truth), which will result in more precise and less biased estimates. Another option is to use the information on the patients who received both reference standards to predict what the result of the preferred reference standard will be in those missing this result by using a method called multiple imputation (19).

**CONCLUDING REMARKS**

Differential verification refers to a common situation in diagnostic studies in which different reference standards are used for different groups of patients. The use of different methods to determine the final disease status often results in biased estimates of diagnostic test or model accuracy and should therefore be avoided, if possible. Because of insufficient reporting, readers may struggle with identifying differential verification and assessing the risk of clinically relevant bias. When differential verification occurs, we recommend that authors not only assess the risk of bias themselves and try to correct for it when appropriate but also allow the reader to do the same by following the suggested reporting guidelines. When readers assess whether differential verification caused clinically relevant bias in accuracy estimates, we recommend that they consider both the accuracy of the inferior reference standard and the verification pattern.
In situations where differential verification is unavoidable, it may be impossible to obtain sufficiently unbiased and precise accuracy estimates even after making adjustments in the analysis for the imperfectness of the inferior reference standard. This is the case when the reason that patients receive one reference standard over another is unclear (and also cannot be assumed to be completely at random) or when the accuracy of the inferior reference test is poor or highly uncertain. When traditional diagnostic accuracy studies are unable to produce precise and unbiased results, comparative accuracy studies or randomized trials that explore the relationship between testing and improved health outcomes may be preferable (27, 28). However, these studies also have limitations and are not always feasible. Our guidance for studies with differential verification can be used with STARD to improve the reporting of diagnostic accuracy studies and with QUADAS to improve the assessment of risk of bias due to differential verification in reviews of diagnostic accuracy studies.

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