

Introduction to diagnostic test accuracy studies

Sitch, Alice; Dekkers, Olaf; Scholefield, Barney; Takwoingi, Yemisi

DOI:

[10.1530/EJE-20-1239](https://doi.org/10.1530/EJE-20-1239)

License:

None: All rights reserved

Document Version

Peer reviewed version

Citation for published version (Harvard):

Sitch, A, Dekkers, O, Scholefield, B & Takwoingi, Y 2021, 'Introduction to diagnostic test accuracy studies', *European Journal of Endocrinology*, vol. 184, no. 2, pp. E5–E9. <https://doi.org/10.1530/EJE-20-1239>

[Link to publication on Research at Birmingham portal](#)

Publisher Rights Statement:

The definitive version is now freely available at <https://doi.org/10.1530/EJE-20-1239>

© 2021 European Society of Endocrinology 2021

General rights

Unless a licence is specified above, all rights (including copyright and moral rights) in this document are retained by the authors and/or the copyright holders. The express permission of the copyright holder must be obtained for any use of this material other than for purposes permitted by law.

- Users may freely distribute the URL that is used to identify this publication.
- Users may download and/or print one copy of the publication from the University of Birmingham research portal for the purpose of private study or non-commercial research.
- User may use extracts from the document in line with the concept of 'fair dealing' under the Copyright, Designs and Patents Act 1988 (?)
- Users may not further distribute the material nor use it for the purposes of commercial gain.

Where a licence is displayed above, please note the terms and conditions of the licence govern your use of this document.

When citing, please reference the published version.

Take down policy

While the University of Birmingham exercises care and attention in making items available there are rare occasions when an item has been uploaded in error or has been deemed to be commercially or otherwise sensitive.

If you believe that this is the case for this document, please contact UBIRA@lists.bham.ac.uk providing details and we will remove access to the work immediately and investigate.



Introduction to diagnostic test accuracy studies

Journal:	<i>European Journal of Endocrinology</i>
Manuscript ID	Draft
mstype:	Methodology Editorial
Date Submitted by the Author:	n/a
Complete List of Authors:	Sitch, Alice; University of Birmingham, Institute of Applied Health Research ; University Hospitals Birmingham NHS Foundation Trust, NIHR Birmingham Biomedical Research Centre Dekkers, Olaf; Leids Universitair Medisch Centrum Centrum voor Humane en Klinische Genetica, Department of Clinical Epidemiology; Scholefield, Barnaby; University of Birmingham College of Medical and Dental Sciences, Birmingham Acute Care Research Group (BACR), Institute of Inflammation and Ageing; Birmingham Women and Children's Hospital NHS Foundation Trust, Paediatric Intensive Care Unit Takwoingi, Yemisi; University of Birmingham, Institute of Applied Health Research ; University Hospitals Birmingham NHS Foundation Trust, NIHR Birmingham Biomedical Research Centre
Keywords:	Biomarker, Biostatistics, Diagnostic tests

SCHOLARONE™
Manuscripts

1

2 **Introduction to diagnostic test accuracy studies**

3

4 Alice J Sitch^{1,2}, Olaf M Dekkers³, Barnaby R Scholefield^{4,5}, Yemisi Takwoingi^{1,2}

5

6 ¹ NIHR Birmingham Biomedical Research Centre, University Hospitals Birmingham NHS Foundation

7 Trust and University of Birmingham, UK

8 ² Test Evaluation Research Group (TERG), Institute of Applied Health Research, University of

9 Birmingham, UK

10 ³ Departments of Clinical Epidemiology, Leiden University Medical Center, Leiden, the Netherlands

11 and Endocrinology, Leiden University Medical Center, Leiden, the Netherlands

12 ⁴ Birmingham Acute Care Research Group (BACR), Institute of Inflammation and Ageing, University of

13 Birmingham, UK

14 ⁵ Paediatric Intensive Care Unit, Birmingham Women & Children's Hospital NHS Foundation Trust, UK

15

16

17 **Abstract**

18 Diagnostic accuracy studies are fundamental for the assessment of diagnostic tests. Researchers
19 need to understand the implications of their chosen design, opting for comparative designs where
20 possible. Researchers should analyse test accuracy studies using the appropriate methods,
21 acknowledging the uncertainty of results and avoiding overstating conclusions and ignoring the
22 clinical situation which should inform the trade-off between sensitivity and specificity. Test accuracy
23 studies should be reported with transparency using the STARD checklist (1).

24

25

For Review Only

26 **Introduction**

27 Diagnosing diseases is crucial in medicine, and for this purpose many diagnostic tests and
28 procedures are applied. For the diagnosis of a suspected adrenal carcinoma a CT scan is performed,
29 and an insulin tolerance test (ITT) for adrenal insufficiency. The performance of these tests can be
30 investigated in diagnostic accuracy studies.

31 Medical diagnostic tests are evaluated in different ways, depending on the stage of evaluation and
32 the purpose of the test. A fundamental aspect of the evaluation of diagnostic tests is *test accuracy*,
33 that is, the ability of a test to differentiate between those who have and those who do not have the
34 condition or disease of interest. In this article we define key terminology (see box 1) used in the
35 context of test accuracy, and describe basic aspects of study design and analysis.

36 Guidelines for reporting of test accuracy studies, The STAndards for the Reporting of Diagnostic
37 accuracy studies (STARD) checklist (1), have been published and we recommend their use to
38 increase quality and transparency of reporting.

39

40 **Measures of test accuracy**

41 Test accuracy is determined by cross classifying the results (positive and negative) of an index test
42 against those of the reference standard. This produces a two-by-two table giving the number of true
43 positives, false positives, false negatives and true negatives, see box 2. Standard methods for
44 estimating test accuracy require binary classification of the results of the index test and the
45 reference standard. As such when test results are non-binary, criteria (referred to as thresholds, cut-
46 offs or cut-points) are needed to define test negatives and test positives. For example, when
47 assessing the test accuracy for the CRH-test for adrenal insufficiency, a cut-off needs to be defined.

48 Measures of test accuracy should always be accompanied by a 95% confidence interval (CI), which is
49 a measure of uncertainty for the point estimate. In example given in box 2, the 95% CI for the
50 sensitivity ranges from 0.96 to 0.99; the 95% CI for specificity is wider, ranging from 0.67 to 0.78.

51

52 **Study population and design**

53 There are different phases in the evaluation of a diagnostic test. Firstly, test performance is
54 determined in a population of clearly established cases and non-cases (2, 3), this referred to as
55 proof-of-concept or exploratory study. Secondly, assessment in a representative population in an
56 appropriate clinical setting (prospective consecutive recruitment of suspected cases) can be
57 performed (4). The spectrum of disease will vary between these designs; researchers should be
58 aware of this difference when planning studies and generalising results of studies to clinical settings
59 (5, 6). When researchers perform an exploratory study involving known cases and non-cases
60 (referred to as a diagnostic case-control or two-gate design (2)), (positive and negative) predictive
61 values should not be directly calculated using two-by-two data from such studies. This is because
62 predictive values are directly related to prevalence and the proportion of participants with the target
63 condition in case-control studies is artificial, i.e. determined by the study investigators. For example,
64 doubling the number cases would directly affect the calculated NPV and PPV. This is not the case
65 when a representative population is sampled, for example all pituitary adenoma patients with
66 suspected ACTH deficiency.

67 Test accuracy studies often evaluate a single index test but where alternative tests exist that can be
68 used at the same point in the diagnostic pathway (providing the tests do not interfere with each
69 other and the patient burden is not too great), these test can be evaluated in one study population
70 (7). The ideal comparative study design is to perform all tests and the reference standard on all
71 participants (paired or within-subject design) or to randomise participants to receive one of the
72 index tests (8). The randomised design is preferred when it is not possible to perform multiple index

73 tests on each individual for ethical or logistical reasons. Additionally, the role of the test in the
74 diagnostic pathway—replacement, triage or add-on—should be considered when designing a study
75 (8).

76

77 **Sample size**

78 Sample size calculations for test accuracy studies should be determined prior to recruitment; see (9,
79 10) for details. When evaluating a single test, a common approach is based on the precision around
80 an estimate of sensitivity and/or specificity (i.e. width of the confidence intervals). The precision of
81 the sensitivity estimate will increase with the number of participants with the target condition
82 (reference standard positive) and the precision of the specificity estimate will increase with the
83 number of participants without the target condition (reference standard negative). Hence, it is vital
84 to have an estimate of the prevalence of the target condition to plan the sample size.

85

86 **Statistical analysis of accuracy studies**

87 Measures of test accuracy (see box 1) can be calculated along with 95% confidence intervals (11, 12).
88 For test accuracy studies comparing two tests, additionally the difference in sensitivity and
89 specificity between the index tests can be computed. With the paired comparative design,
90 McNemar's test can be used to test differences in sensitivity and specificity. Alternatively, regression
91 modelling taking into account the paired nature of the data can be performed. The effect of
92 important clinical characteristics on test accuracy can also be explored using such models; for
93 example it can be assessed whether age determines differences of two index tests. For the
94 randomised comparative design, a test of independent proportions can be used to compare
95 sensitivity and specificity between groups.

96 For tests with non-binary results, receiver operating characteristic (ROC) curve analysis is typically
97 performed. There is a negative relationship between sensitivity and specificity as the cut-point
98 changes (threshold effect); if we for example lower the cortisol threshold for the diagnosis of
99 adrenal insufficiency, this will increase sensitivity (less false negatives), as a consequence however,
100 the specificity will be lower (more false positives) A ROC curve displays this trade-off between
101 sensitivity and specificity at different cut-points for a test (see figure 2), and curves for different tests
102 in a comparative study can be compared on a single ROC plot. A simplistic cut-point would maximise
103 sensitivity and specificity. However, an appropriate cut-point for use in clinical practice should be
104 driven by the consequences for false positive and false negative results. If a study is used to derive a
105 cutpoint for a test the performance, external validation is required, as a single study will likely
106 overestimate the test's performance. This is especially the case for small studies.

107

108 **Concluding remarks**

109 Diagnostic test accuracy studies are required to understand the potential for new diagnostic
110 technologies. It is vital that researchers understand the implications of the design of their studies
111 and the impact on the study conclusions. Researchers need to understand the clinical situation and
112 weigh the consequences of misidentifying positive and negative participants. There is a need for
113 clear and transparent reporting allowing the limitations of studies to be identified. We encourage
114 researchers to seek specialist support when embarking on these studies.

115

116 **Acknowledgements**

117 B.S. is funded by a National Institute for Health Research (NIHR) Clinician Scientist Fellowship (NIHR-
118 CS-2015-15-016) for this research project. Y.T. is funded by an NIHR Postdoctoral Fellowship. Y.T. is a

119 co-convenor of the Cochrane Screening and Diagnostic Tests Methods Group. This work is supported
120 by the NIHR Statistics Group (<https://statistics-group.nihr.ac.uk/>). A.S. and Y.T. are supported by the
121 NIHR Birmingham Biomedical Research Centre at the University Hospitals Birmingham NHS
122 Foundation Trust and the University of Birmingham. The views expressed are those of the author(s)
123 and not necessarily those of the NHS, the NIHR or the Department of Health and Social Care.

124

125 AJS is an Advisory Editor and O M D is a Deputy Editor for European Journal of Endocrinology.

126 Neither were involved in the review or editorial process for this paper, on which they are listed as
127 authors.

128

129

For Review Only

130 **References**

131

- 132 1. Bossuyt PM, Reitsma JB, Bruns DE, Gatsonis CA, Glasziou PP, Irwig L, et al. STARD 2015: an
133 updated list of essential items for reporting diagnostic accuracy studies. *BMJ : British Medical*
134 *Journal*. 2015;351:h5527.
- 135 2. Rutjes AW, Reitsma JB, Vandenbroucke JP, Glas AS, Bossuyt PM. Case-control and two-gate
136 designs in diagnostic accuracy studies. *Clinical chemistry*. 2005;51(8):1335-41.
- 137 3. Pepe MS, Etzioni R, Feng Z, Potter JD, Thompson ML, Thornquist M, et al. Phases of
138 biomarker development for early detection of cancer. *Journal of the National Cancer Institute*.
139 2001;93(14):1054-61.
- 140 4. Sackett DL, Haynes RB. The architecture of diagnostic research. *Bmj*. 2002;324(7336):539-41.
- 141 5. Whiting P, Rutjes AW, Reitsma JB, Glas AS, Bossuyt PM, Kleijnen J. Sources of variation and
142 bias in studies of diagnostic accuracy: a systematic review. *Ann Intern Med*. 2004;140(3):189-202.
- 143 6. Irwig L, Bossuyt P, Glasziou P, Gatsonis C, Lijmer J. Designing studies to ensure that estimates
144 of test accuracy are transferable. *Bmj*. 2002;324(7338):669-71.
- 145 7. Takwoingi Y, Leeflang MM, Deeks JJ. Empirical evidence of the importance of comparative
146 studies of diagnostic test accuracy. *Ann Intern Med*. 2013;158(7):544-54.
- 147 8. Bossuyt PM, Irwig L, Craig J, Glasziou P. Comparative accuracy: assessing new tests against
148 existing diagnostic pathways 2006 2006-05-04 21:59:05. 1089-92 p.
- 149 9. Obuchowski NA. Sample size calculations in studies of test accuracy. *Stat Methods Med Res*.
150 1998;7(4):371-92.
- 151 10. Pepe MS, Pepe PBMS. *The Statistical Evaluation of Medical Tests for Classification and*
152 *Prediction*: Oxford University Press; 2003.
- 153 11. Wilson EB. Probable Inference, the Law of Succession, and Statistical Inference. *Journal of*
154 *the American Statistical Association*. 1927;22(158):209-12.

155 12. Brown LD, Cai TT, DasGupta A. Interval Estimation for a Binomial Proportion. Statist Sci.
156 2001;16(2):101-33.

157

For Review Only

<p>Target condition</p> <p>The target condition is the disease or condition the test(s) are aiming to diagnose e.g. adrenocortical carcinoma (ACC) or adrenal insufficiency.</p>
<p>Target population</p> <p>The target population is the population of interest e.g. patients with an incidentally discovered adrenal mass, or patients with an adrenal mass found on imaging for staging purposes of extra-adrenal malignancy; patients with a pituitary adenoma in whom ACTH deficiency is assessed.</p>
<p>Index test(s)</p> <p>An index test is a test the researchers aim to evaluate. A study may evaluate more than one index test, e.g. non-contrast computerised tomography (CT) and MRI for an adrenal mass.</p>
<p>Reference standard</p> <p>The reference standard, sometimes referred to as the “gold” standard, is the best way of verifying the presence or absence of the target condition. This may be a test that is not normally used or available in practice, such as a period of follow up to confirm or exclude the presence of the target condition (for example ACC) at the time the index test was done, or a combination of several pieces of information (known as a composite reference standard) e.g. histologically proven diagnosis (obtained through adrenalectomy or adrenal biopsy) or imaging-based follow-up (for example, twice yearly CT). Be aware that the even the reference standard is not always perfect (ITT for adrenal insufficiency).</p>
<p>Sensitivity and specificity</p> <p>The <i>sensitivity</i> of a test is the proportion of participants with the target condition (positive reference standard) that have a positive index test result, <i>specificity</i> is the proportion of participants without the target condition (negative reference standard) that have a negative index test result.</p>
<p>Positive and negative predictive values</p> <p>The <i>positive predictive value</i> (PPV) is the proportion of participants with a positive index test result who truly have the target condition. The <i>negative predictive value</i> (NPV) is the proportion of participants with a negative index test result who truly do not have the target condition.</p>

Box 1: Key terminology for test accuracy studies

146x157mm (120 x 120 DPI)

2x2 table			Calculations	
Index test	Reference test			
	+	-		
+	279	61	• Sensitivity=TP/(TP+FN)=279/(279+5)	
-	5	163	• Specificity=TN/(TN+FP)=163/(163+61)	
			• PPV=TP/(TP+FP)=279/(279+61)	
			• NPV=TN/(TN+FN)=163/(163+5)	
Results			Interpretation	
	Estimate	95% CI		
Sensitivity	0.98	0.96, 0.99	• Sensitivity: 98% of the participants with disease have a positive index test.	
Specificity	0.73	0.67, 0.78	• Specificity: 73% of the participants without disease have a negative index test.	
PPV	0.82	0.78, 0.86	• PPV: 82% of the participants with a positive index test have the disease.	
NPV	0.97	0.93, 0.99	• NPV: 97% of the participants with a negative index test do not have the disease.	

Box 2: Example calculations, results and interpretation. TP is the number of true positive results; TN is the number of true positive results; FP is the number of false positive results; FN is the number of false negative results; PPV is the positive predictive value; NPV is the negative predictive value.

149x97mm (120 x 120 DPI)

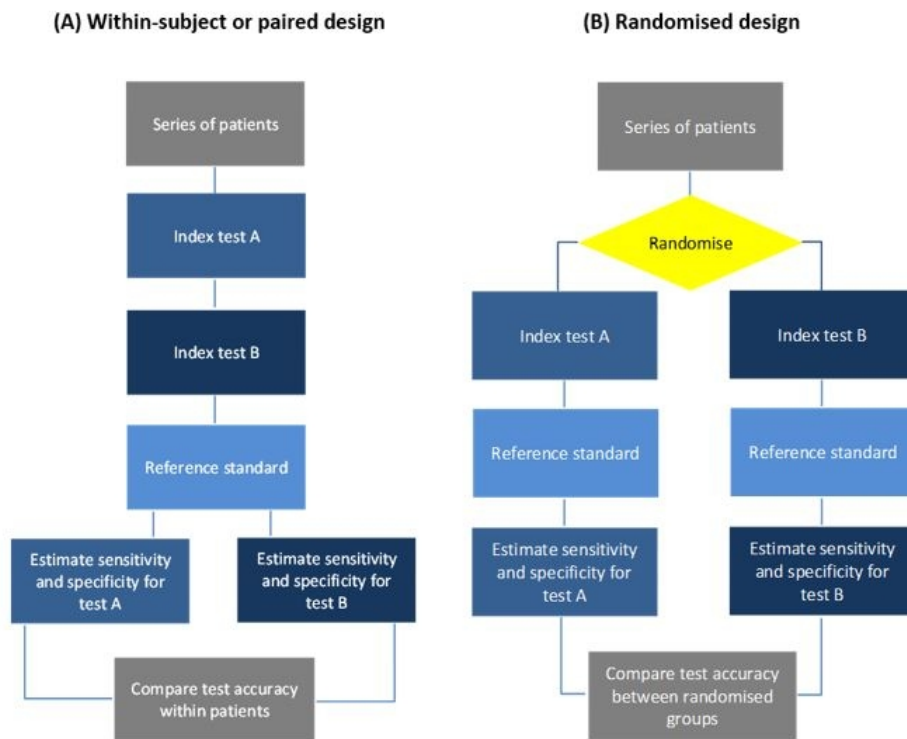


Figure 1: Robust study designs for comparing test accuracy by Y. Takwoingi (9). In (A) all patients undergo all index tests while in (B) patients are randomly assigned to only one of the index tests. In both (A) and (B), all patients receive the reference standard. Both designs are valid, although the paired design requires a smaller study sample.

150x117mm (120 x 120 DPI)

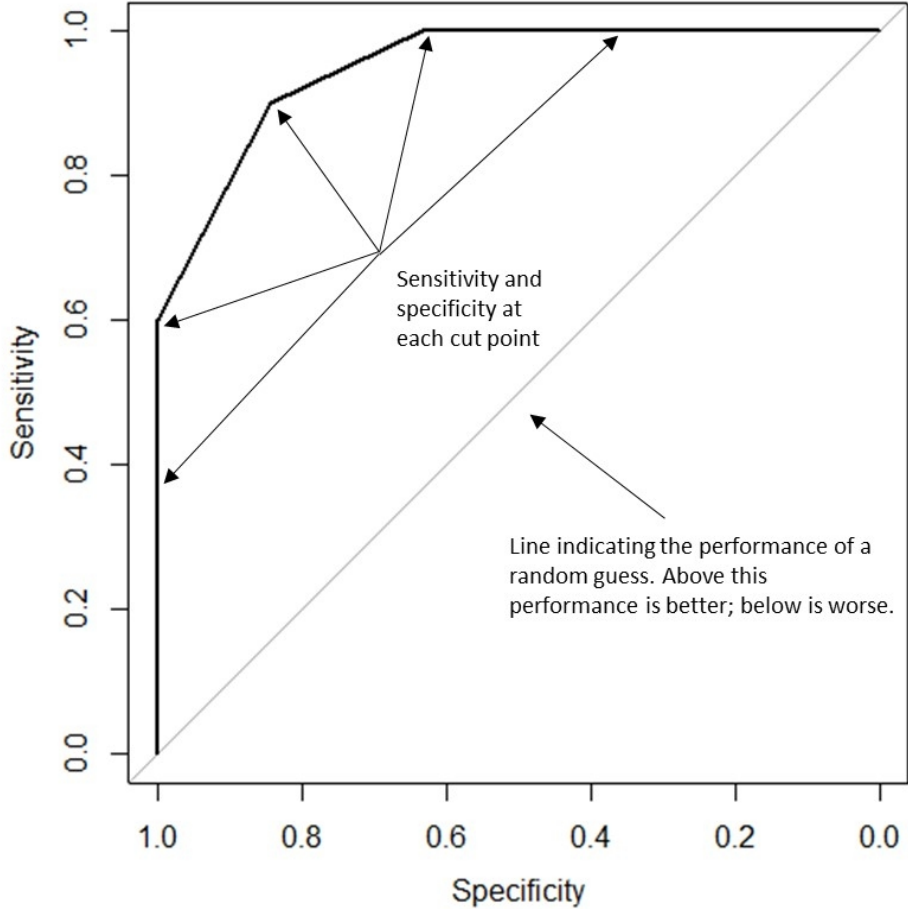


Figure 2: ROC curve example

142x142mm (150 x 150 DPI)