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Version: Accepted Version

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**Article:**

Di Martino, E, Kelly, G, Roulson, JA et al. (1 more author) (2015) Alteration of cell-cell and cell-matrix adhesion in urothelial cells: An oncogenic mechanism for mutant FGFR3. *Molecular Cancer Research*, 13 (1). 138 - 148. ISSN 1541-7786

<https://doi.org/10.1158/1541-7786.MCR-14-0022>

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## **ASSESSING THE QUALITY OF STUDIES ON THE DIAGNOSTIC ACCURACY OF TUMOR MARKERS §**

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Key words: diagnostic accuracy, study quality, IBCN classification, Oxford levels of evidence, QUADAS, NOS, STARD

word count: 4922 incl. references w/o tables

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§ This manuscript reflects and summarizes discussions held at the 10th Meeting of the International Bladder Cancer Network (IBCN e.V.), Nijmegen, The Netherlands, 20.–22.9.2012

**Disclosures:** The authors have no conflict of interest

## **Abstract**

**Objectives:** With rapidly increasing numbers of publications, assessments of study quality, reporting quality, and classification of studies according to their level of evidence or developmental stage have become key issues in weighing the relevance of new information reported. Diagnostic marker studies are often criticized for yielding highly discrepant and even controversial results. Much of this discrepancy has been attributed to differences in study quality. So far, numerous tools for measuring study quality have been developed, but few of them have been used for systematic reviews and metaanalysis. This is due to the fact that most tools are complicated and time consuming, suffer from poor reproducibility, and do not permit quantitative scoring.

**Methods:** The International Bladder Cancer Network (IBCN) has adopted this problem and has systematically identified the more commonly used tools developed since 2000. **Results:** In this review, those tools addressing study quality (QUADAS, NOS), reporting quality (STARD), and developmental stage (IBCN phases) of studies on diagnostic markers in bladder cancer are introduced and critically analyzed. Based upon this, the IBCN has launched an initiative to assess and validate existing tools with emphasis on diagnostic bladder cancer studies. **Conclusions:** The development of simple and reproducible tools for quality assessment of diagnostic marker studies permitting quantitative scoring is suggested.

Word count 208, letters 1462 (incl. blanks)

## Introduction

With rapidly increasing numbers of publications, assessments of study quality, reporting quality, and classification of studies according to their level of evidence or developmental stage have become key issues in weighing the relevance of new information reported. Diagnostic marker studies are often criticized for yielding highly discrepant and even controversial results [1, 2]. Thus, for an article on diagnostic accuracy of a molecular bladder cancer marker it is often nearly impossible to judge the methodological rigor of that study and to conclude whether the published results can be translated to clinical practice.

The International Bladder Cancer Network (IBCN) has adopted this problem for the area of diagnostic and prognostic biomarker research, focusing on studies related to bladder cancer. Recently, the **Phases Reporting and Assessment Optimization (PHARAO)** project has been proposed for developing a classification system to describe the developmental status of a given marker in analogy to the commonly accepted phases of clinical trials (phases I – IV) [3, 4]. In addition, the IBCN has initiated an analysis of published tools that are used to assess study quality and reporting quality of biomarker studies, exploiting the resources of the IBCN.

Although the use of such tools for the assessment of diagnostic marker trials is recommended, these have generally not been implemented by users, e.g. readers or reviewers. Some of them have been used in systematic reviews and metaanalyses or in education research [5]; however, in many tools sufficient external validation remains pending. One important reason for underutilization of these tools in the urology community is that urology training programs in general do not incorporate education on trial design, management, and analysis for their residents; further difficulties of these instruments reside in their deficiency to define what may be considered sufficient or adequate quality. This is in part due to the great variability in study settings and designs posing great challenges to a given tool with regard to its general applicability. As a consequence, application of most of the tools becomes rather complicated, further preventing their general use. These issues have fueled the development of numerous new instruments without finding a solution of existing problems.

In this context, it is the purpose of this review to introduce, classify, and analyze relevant available assessment tools designed to evaluate studies on the diagnostic accuracy of bladder cancer molecular markers. By this initiative, the use of assessment tools should be supported and, eventually, their practicability and applicability should be improved.

### **Current tools**

A systematic review of medical data bases by Dreier et al [6] identified 17 tools designed to assess studies investigating the diagnostic accuracy of molecular markers. Only the instruments generated after 2000 and those more frequently cited in the literature were considered for this review. For this review, the tools were divided into four categories, based upon their objective:

- **Study Quality:**  
(e.g. Newcastle-Ottawa scale [7], QUADAS [8] and the QUADAS-2 tool [9])
- **Quality of Reporting:**  
(e.g. STARD criteria [10, 11])
- **Study Phases**  
(e.g. IBCN criteria [, 3, 4, 12])
- **Level of Evidence**  
(e.g. Oxford criteria 2001/2009 [13])

### **Study quality**

*Newcastle-Ottawa quality assessment Scale (NOS):*

The NOS was designed to evaluate the quality of non-randomized studies, discriminating between case control trials and cohort studies [7]. Both scales include three categories with a total of 8 items (Tab. 1). When analyzing case control trials, NOS addresses three areas including selection, comparability, and exposure, whereas in cohort studies it includes selection, comparability, and outcome.

This scale was originally developed for application in systematic reviews and metaanalyses. A study can be awarded a maximum of one star for each numbered item within the selection and exposure categories in case control studies, or the selection and outcome categories in cohort studies. A maximum of two stars can be given for comparability, in either type of study, resulting in a maximum of 9 points. No cut-off for good or poor quality is provided. The questions are clear and apparently easy to answer; however, the options provided are difficult to apply to some study concepts. Furthermore, the NOS has been criticized for having a high inter-rater variability [14-17].

The discrimination between case-control studies and cohort trials, as well as its easy applicability, are important factors that explain why the NOS has been frequently used in the past, mainly for systematic reviews and metaanalyses [18, 19].

*Quality Assessment of studies of Diagnostic Accuracy (QUADAS):*

The QUADAS instrument is presumably the most widely accepted tool for quality assessment. It is considered a retrospective instrument for evaluation of the methodological rigor of a study investigating the diagnostic accuracy of a given test. The QUADAS tool was developed through a Delphi procedure eventually reducing an initial list of 28 items down to 14 questions [8]. The items include patient spectrum, reference standard, disease progression bias, verification bias, review bias, clinical review bias, incorporation bias, test execution, study withdrawals, and indeterminate results (Tab. 2). The QUADAS tool is presented together with recommendations for scoring each of the items included. The QUADAS tool provides a matrix in which readers can examine the internal and external validity of a study.

The majority of items included in QUADAS relate to bias (items 3, 4, 5, 6, 7, 10, 11, 12 and 14); only two items relate to variability (items 1 and 2) while three relate to reporting (items 8, 9 and 13). The questions posed are focused and clear; their accompanying guidelines appear helpful. However, there is much room for subjective interpretation since several items may be answered differently by reviewers based upon their individual perception. Any item may be answered with either “yes”, “no”, or

“unclear”; however, no advice is provided on scoring, cut-off and, as a result, on classifying a study as having good or poor quality.

The QUADAS tool has experienced fairly frequent use predominantly within systematic reviews and metaanalyses. For bladder cancer markers Xia et al. [20] reported its use in a metaanalysis on the accuracy of survivin in the diagnosis of bladder cancer.

Several reports have been published regarding the external validation of QUADAS [21, 22]. Oliveira et al. [21] applied the QUADAS score alone and in combination with the STARD tool to assess a malaria test in a semi-quantitative way. A combination of QUADAS criteria and STARD criteria was compared (see discussion of STARD below) with the QUADAS criteria alone. Articles fulfilling at least 50% of QUADAS criteria were considered as having regular to good quality without providing a definition for this allocation. Of the 13 articles retrieved, 12 fulfilled at least 50% of QUADAS criteria; only two fulfilled the combined STARD/QUADAS criteria. The authors concluded that the STARD/QUADAS combination might have the potential to provide greater rigor when evaluating the quality of studies, given that it incorporates relevant information not contemplated in the QUADAS criteria alone.

Hollingworth et al. [22] used data from a systematic review of magnetic resonance spectroscopy (MRS) in the characterization of suspected brain tumors to provide a preliminary evaluation of the inter-rater reliability of QUADAS. Nineteen publications were distributed randomly to primary and secondary reviewers for dual independent assessment. Most studies in this review were judged to have used an accurate reference standard. There was good correlation ( $\rho = 0.78$ ) between reviewers in assessment of the overall number of quality criteria met. However, mean agreement for individual QUADAS questions was only fair ( $\kappa = 0.22$ ) and ranged from no agreement ( $\kappa < 0$ ) to moderate agreement ( $\kappa = 0.58$ ). These findings suggest that different reviewers will reach different conclusions when using QUADAS. These findings are similar to those observed by Whiting et al. [23], reporting an adequate inter-rater reliability for individual items in the QUADAS checklist (range 50–100%, median 90%).

Recently, the QUADAS2 tool has been presented [9]. It basically follows the original QUADAS tool; however, items were now reduced down to 11 questions in four new domains (patient selection, index test(s), reference standard, and flow and timing) (tab. 3). In contrast to the original scale, the QUADAS2 tool provides advice on the rating of study quality. To date, experience concerning the use of this instrument is limited [24, 25] and external validation is underway.

### **Quality of reporting**

Although the general quality of the study and the reporting are difficult to separate from each other, the QUADAS tool has already been supplemented in 2003 by another tool, specifically addressing the issue of quality of reporting.

#### *STAndards for the Reporting of Diagnostic accuracy studies (STARD)*

The STARD tool was developed to improve the quality of reporting in diagnostic accuracy studies [e.g. 10, 11]. It comprises 25 items, mirroring the classical sections of a manuscript including title, keywords, abstract (1 item), introduction (1 item), methods (11 items), results (11 items), and discussion (1 item). The reader may rate each item as either “present” or “absent” (Tab. 4).

Smidt and coworkers reported on external validation by applying the STARD tool to 32 diagnostic accuracy studies published in medical journals with an impact factor of at least 4 in 2000 [26]. All manuscripts were independently reviewed by two experts at the beginning of the study and again almost two years later.

The overall inter-assessment agreement for all items of the STARD statement was 85% (Cohen's kappa 0.70) varying from 63% to 100% for individual items. The inter-assessment reliability of the STARD checklist was satisfactory (ICC = 0.79 [95% CI: 0.62 to 0.89]). The authors concluded that although the overall reproducibility of the quality of reporting using the STARD statement was good, substantial differences were found for specific items. These disagreements were not likely caused by



differences in the interpretation by the reviewers but rather by difficulties in assessing the reporting of these items due to lack of clarity within the articles.

In summary, despite some deficiencies concerning reproducibility, the STARD tool is a validated tool for the assessment of reporting quality. However, several issues have emerged with this tool. The underlying questions are not always easy to apply to a given manuscript. Further, no recommendations for scoring are provided that may allow classifying a manuscript as having sufficiently good reporting or not.

### **Study phases**

The definition of study phases addresses the need to identify the current status of development of a given procedure (treatment, diagnostic procedure). This should support an adequate and systematic development of new diagnostic or therapeutic concepts. Due to a lack of recommendations for the development of diagnostic marker trials, the IBCN Phases classification was developed in 2003 (and revised in 2007) in analogy to the 4 phases of clinical trials [3, 4, 12].

#### *Phase I: Assay Development and Evaluation of Clinical Prevalence (Feasibility Studies)*

This phase involves the identification of a target potentially suited for diagnostic use. Identification of the target may occur in many ways, classically by identifying the target in tumor cells. However, with the advent of molecular technology other ways or definitions of a variety of targets are conceivable. The key issue is whether a difference between tumor cells and normal urothelial cells can be demonstrated. It has to be noted that field effects are an integral part of the development of bladder cancer. This warrants not only inclusion of “normal” adjacent tissue but also tissue and samples from healthy individuals as important controls in evaluating a markers definition.

#### *Phase II: Evaluation Studies for Clinical Utility*

This phase involves optimization of the assay technique (e.g. standardization, automatization) and/or interpretation of the assay results. The ultimate goal of this

phase is to develop hypotheses and to define standards that can be used to perform Phase III studies.

Phase II trials are mostly single-institutional studies. However, adequately sized and representative samples of patients may be easier to achieve in a large collaborative network with sufficient numbers of specimens to define and select the most appropriate set of samples. In addition, identifying the sources of variability during this phase of biomarker development is required for designing a Phase III study.

Based upon the results in such studies, adequate cut-off values will be defined for quantitative assays. It is essential that the outcome from Phase II studies is translated into hypotheses that form the basis for Phase III analyses.

#### *Phase III: Confirmation Studies*

In Phase III, hypotheses emerging from previous phase II studies are tested with sufficient power in a defined clinical setting using an independent, prospective and controlled cohort of patients. The clinical utility of a given marker assay, its performance, and interpretation are established in this phase, the aim of which is the generation of (evidence-based) information that may eventually be included into clinical guidelines.

#### *Phase IV: Validation and Technology Transfer as Application Studies*

The aims of Phase IV studies are (a) to transfer the techniques and established methods of the assays and other aspects of the technology into clinical practice; and (b) to evaluate the ability of investigators and clinicians at other institutions to apply these methods and interpret the results in a similar and comparable way.

The IBCN classification is easy to use; nevertheless, it has only been rarely applied in the past in systematic reviews [1].

### **Levels of evidence**

A very important dimension in the assessment of manuscripts is the consideration of the level of evidence that a given study provides. The Oxford Centre for Evidence-based Medicine (OCEBM) Levels of Evidence May 2001/2009 classification has been designed to classify the relevance of scientific contributions based mainly upon the study design [13]. Initially aiming at the classification of clinical trials, the 2009 modification also included adaptations for prognostic and diagnostic marker studies as well as for economic and decision analyses. A 5-scale classification was developed, starting from expert opinion (Level of evidence (LoE) 5) and extending to validating cohort studies for diagnostic markers (LoE 1b) (Tab. 5). Levels 1-3 received subclassifications with grade “a” representing systematic reviews or metaanalyses of respective trials and grade “b” representing results from a single study. It is of interest that absolute SpPins (case series reporting on highly specific tests, in which a positive result will confirm presence of a disorder), and absolute SnNouts (case series reporting on highly sensitive tests, in which a negative result will exclude a disorder) were defined as LoE 1c.

Although the 2009 classification was simple and easy to use, early hierarchies that placed randomized trials categorically above observational studies were criticized for being simplistic. This criticism was met by introducing the 2011 classification providing more flexibility insofar that upgrading and downgrading of studies is possible [Tab. 6]. Furthermore, different clinical settings, e.g. screening, diagnosis, prognosis and therapy are discriminated. Sub-classifications “a-c” were eliminated facilitating allocation to the different levels.

Somewhat surprisingly, randomized controlled trials (RCTs) are not listed as a separate level of evidence for diagnostic and prognostic studies, presumably due to the fact that RCTs in this field are extremely rare.

While the LoE classifications 2001/2009 have been well accepted by the scientific community, experience with the 2011 version is still limited.

## **Discussion**

The problem of defining the quality of a given study is as old as scientific communication. An extensive literature search recently performed by Dreier et al. [6] yielded a total of 147 different tools developed to assess study quality. While there has been a focus on therapeutic trials in the past, more recently instruments for assessment of diagnostic studies have also been developed. The large number of different assessment tools suggests that none of them is accepted as a “perfect” solution for the problem.

Doubtless, the challenge to develop a single tool which can be applied to all diagnostic studies is considerable. In contrast to clinical trials with similar designs comparing standard care vs. a new strategy using criteria defined by good clinical practice (GCP) guidelines, diagnostic trials may differ with regard to a variety of parameters. Furthermore, the quality of studies is heterogeneous and numerous methodological shortcomings are apparent in the design of diagnostic accuracy studies (Tab. 7). Finally, definition of study quality is difficult since expectations are different and viewpoints may vary.

One of the most widely used tools to assess study quality is the QUADAS instrument. However, it has been designed – and thus far is exclusively used - for systematic reviews or metaanalyses [8]. One problem in using the QUADAS tool lies in the distinction between general study quality and reporting quality. Inevitably, the assessment of quality relates strongly to the reporting of results; a well-conducted study will score poorly in a quality assessment tool if the methods and results are not reported in sufficient detail. The intention of the STARD document was to complement quality assessment of diagnostic accuracy studies by providing a tool focusing on quality of reporting [10, 11]. However, this requires the use of a second instrument and, in consequence, additional time.

Studies failing to report on aspects of quality may be considered as having inferior quality since faulty reporting generally reflects faulty methods. When using QUADAS, another important factor to consider is the difference between bias and variability. Study bias will limit the validity of the study results whereas variability may complicate the translation of study results into clinical practice.

It may be questioned whether a separate tool for assessment of reporting quality like STARD is necessary and reasonable, or if the study and the reporting quality are so closely linked that analysis along the STARD criteria is not likely to generate an added value with regard to study assessment [16]. In general, it would be considered preferable to assess overall quality using just a single tool.

A classification of the development phases concerning the status of a new test may well be necessary. The IBCN classification using a four phase scale (in analogy to the four phases of clinical trials) may constitute a first step in this direction [12]. Thus far, this classification is not generally accepted, despite its simplicity and similarity to clinical study phases. This may be partly due to a lack of precision in some of the definitions; however, the instrument is currently under review for further improvement.

The Oxford level of evidence (LoE) 2001/2009 scale has been widely accepted for classifying the scientific impact of a new study [13]. This may be attributed to the facts that (1) it can be more or less universally applied to different study designs, (2) it is clearly structured and (3) it can be easily used. Furthermore, the LoE classification is a rapid procedure and feasible even for inexperienced scientists. While the revised 2011 version provides more flexibility this feature makes the classification much more demanding since former LoE 1b studies may be downgraded to level 2, while convincing former level 3 studies might even be considered level 1 in the current system. However, this gain in flexibility may be traded in for a loss in reproducibility and discriminative power.

Neither QUADAS nor STARD can be used to provide a reproducible quantitative value or score for study or reporting quality. At best, the QUADAS instrument provides a qualitative assessment of study design, permitting the conclusion that weaknesses in certain parameters may alter some test findings more than others. However, there are several reasons for not incorporating a quality score into QUADAS. Scores are necessary if the investigator intends to use a quantitative indicator of quality to provide weight in a meta-analysis, or if a continuous variable in a meta-regression is required. Since quality scores are very rarely used in these

ways, the authors of QUADAS felt no need to introduce such a score. They stated that definitions on how to weigh and calculate quality scores might be in fact arbitrary, thus preventing development of an objective quality score [8, 23]. In consequence, the application of scores without consideration of the individual items may dilute or entirely miss potential associations.

The authors of this review would challenge this line of reasoning, believing instead that it is necessary to add a semi-quantitative estimation of the quality of a given study. In particular, we believe that journal editors and reviewers should be highly interested in tools permitting quantifying quality in a score, thereby permitting a more transparent review process. Furthermore, existing quality assessment tools still include arbitrary and debatable items notwithstanding the care invested in the development process. Finally, it should be the intention of an assessment tool to permit estimates of study quality or reporting whether or not formal scoring is included.

Similar to the QUADAS instrument, the NOS has been developed for quality assessment in reviews and metaanalyses [7, 27]. In contrast to QUADAS, it permits a semi-quantitative scoring although no cut-off for good/poor quality studies is provided. In a recent validation trial the inter-rater reliability of the NOS varied from substantial for the length of follow-up to poor for both the selection of a non-exposed cohort and the fact that the outcome was not present at study outset [15, 16]. Investigators reported no association between individual NOS items or overall NOS score and effect on estimates. Variable agreement for the NOS and the lack of evidence showing that it is able to discriminate studies with biased results underscores the need for more detailed guidance to apply this tool in systematic reviews [15, 16].

In general, it may be hypothesized that reliability and reproducibility are better achieved by simpler instruments. Final conclusions cannot be drawn since systematic validation of intra- and inter-rater reproducibility has rarely been reported for assessment tools in general. In particular, information concerning the use of the instruments by reviewers/investigators with limited experience is lacking.

Based upon this analysis the authors feel that most instruments available are too complicated and time consuming for an application beyond systematic reviews and metaanalysis (e.g. in peer review or identifying the relevance of a study after reading the manuscript). The reason underlying is the desire to generate comprehensive (ideal) assessment tools covering all possible aspects of quality/reporting quality. In order to improve the current situation we see a need for two measures: first of all a simple and robust assessment tool should be developed and validated. In a second step journal editors and publishers must be encouraged to request reviewing on the basis of such a tool. Acceptance by the reviewers can be obtained if the alternate review process will not require additional time.

As a starting point the IBCN is planning to support assessment of marker studies through investigation of existing tools for analysis of studies on diagnostic accuracy, delineating limitations, proposing modifications, or, if considered necessary, developing new tools targeting the needs of potential users. Embedded in the PHARAO initiative, the IBCN is preparing a validation trial for assessment tools focusing on studies on diagnostic accuracy. Instruments directed towards the assessment of study quality and reporting quality will be studied. In addition, further classification instruments for study phases and LoE will be included in this project.

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**Tables:**

CASE CONTROL STUDIES	COHORT STUDIES
<p>A study can be awarded a maximum of one star for each numbered item within the Selection and Exposure categories. A maximum of two stars can be given for Comparability.</p>	<p>A study can be awarded a maximum of one star for each numbered item within the Selection and Outcome categories. A maximum of two stars can be given for Comparability</p>
<p><b>Selection</b></p> <ol style="list-style-type: none"> <li>1) <u>Is the case definition adequate?</u> <ol style="list-style-type: none"> <li>a) yes, with independent validation *</li> <li>b) yes, eg record linkage or based on self reports</li> <li>c) no description</li> </ol> </li> <li>2) <u>Representativeness of the cases</u> <ol style="list-style-type: none"> <li>a) consecutive or obviously representative series of cases *</li> <li>b) potential for selection biases or not stated</li> </ol> </li> <li>3) <u>Selection of Controls</u> <ol style="list-style-type: none"> <li>a) community controls *</li> <li>b) hospital controls</li> <li>c) no description</li> </ol> </li> <li>4) <u>Definition of Controls</u> <ol style="list-style-type: none"> <li>a) no history of disease (endpoint) *</li> <li>b) no description of source</li> </ol> </li> </ol> <p><b>Comparability</b></p> <ol style="list-style-type: none"> <li>1) <u>Comparability of cases and controls on the basis of the design or analysis</u> <ol style="list-style-type: none"> <li>a) study controls for _____ (Select the most important factor.) *</li> <li>b) study controls for any additional factor * (This criteria could be modified to indicate specific control for a second important factor.)</li> </ol> </li> </ol> <p><b>Exposure</b></p> <ol style="list-style-type: none"> <li>1) <u>Ascertainment of exposure</u> <ol style="list-style-type: none"> <li>a) secure record (eg surgical records) *</li> <li>b) structured interview where blind to case/control status *</li> <li>c) interview not blinded to case/control status</li> <li>d) written self report or medical record only</li> <li>e) no description</li> </ol> </li> <li>2) <u>Same method of ascertainment for cases and controls</u></li> </ol>	<p><b>Selection</b></p> <ol style="list-style-type: none"> <li>1) <u>Representativeness of the exposed cohort</u> <ol style="list-style-type: none"> <li>a) truly representative of the average _____ (describe) in the community *</li> <li>b) somewhat representative of the average _____ in the community *</li> <li>c) selected group of users eg nurses, volunteers</li> <li>d) no description of the derivation of the cohort</li> </ol> </li> <li>2) <u>Selection of the non exposed cohort</u> <ol style="list-style-type: none"> <li>a) drawn from the same community as the exposed cohort *</li> <li>b) drawn from a different source</li> <li>c) no description of the derivation of the non exposed cohort</li> </ol> </li> <li>3) <u>Ascertainment of exposure</u> <ol style="list-style-type: none"> <li>a) secure record (eg surgical records) *</li> <li>b) structured interview *</li> <li>c) written self report</li> <li>d) no description</li> </ol> </li> <li>4) <u>Demonstration that outcome of interest was not present at start of study</u> <ol style="list-style-type: none"> <li>a) yes *</li> <li>b) no</li> </ol> </li> </ol> <p><b>Comparability</b></p> <ol style="list-style-type: none"> <li>1) <u>Comparability of cohorts on the basis of the design or analysis</u> <ol style="list-style-type: none"> <li>a) study controls for _____ (select the most important factor) *</li> <li>b) study controls for any additional factor * (This criteria could be modified to indicate specific control for a second important factor.)</li> </ol> </li> </ol> <p><b>Outcome</b></p> <ol style="list-style-type: none"> <li>1) <u>Assessment of outcome</u> <ol style="list-style-type: none"> <li>a) independent blind assessment *</li> <li>b) record linkage *</li> <li>c) self report</li> </ol> </li> </ol>

<p>a) yes ✱ b) no</p> <p>3) <u>Non-Response rate</u> a) same rate for both groups ✱ b) non respondents described c) rate different and no designation</p>	<p>d) no description</p> <p>2) <u>Was follow-up long enough for outcomes to occur</u> a) yes (select an adequate follow up period for outcome of interest) ✱ b) no</p> <p>3) <u>Adequacy of follow up of cohorts</u> a) complete follow up - all subjects accounted for ✱ b) subjects lost to follow up unlikely to introduce bias - small number lost - &gt; ____ % (select an adequate %) follow up, or description provided of those lost) ✱ c) follow up rate &lt; ____% (select an adequate %) and no description of those lost d) no statement</p>
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Table 1: NOS items for assessment of study quality of diagnostic studies [5].

Item	Yes	No	Unclear
1. Was the spectrum of patients representative of the patients who will receive the test in practice?	( )	( )	( )
2. Were selection criteria clearly described?	( )	( )	( )
3. Is the reference standard likely to correctly classify the target condition?	( )	( )	( )
4. Is the time period between reference standard and index test short enough to be reasonably sure that the target condition did not change between the two tests?	( )	( )	( )
5. Did the whole sample or a random selection of the sample, receive verification using a reference standard of diagnosis?	( )	( )	( )
6. Did patients receive the same reference standard regardless of the index test result?	( )	( )	( )
7. Was the reference standard independent of the index test (i.e. the index test did not form part of the reference standard)?	( )	( )	( )
8. Was the execution of the index test described in sufficient detail to permit replication of the test?	( )	( )	( )
9. Was the execution of the reference standard described in sufficient detail to permit its replication?	( )	( )	( )
10. Were the index test results interpreted without knowledge of the results of the reference standard?	( )	( )	( )
11. Were the reference standard results interpreted without knowledge of the results of the index test?	( )	( )	( )
12. Were the same clinical data available when test results were interpreted as would be available when the test is used in practice?	( )	( )	( )
13. Were uninterpretable/ intermediate test results reported?	( )	( )	( )
14. Were withdrawals from the study explained?	( )	( )	( )

Table 2: QUADAS tool for assessment of study quality of diagnostic studies [6].

<p><b>DOMAIN 1: PATIENT SELECTION</b></p> <p><b>A. Risk of Bias</b></p> <p>Describe methods of patient selection:</p> <p> <input type="checkbox"/> Was a consecutive or random sample of patients enrolled? Yes/No/Unclear  <input type="checkbox"/> Was a case-control design avoided? Yes/No/Unclear  <input type="checkbox"/> Did the study avoid inappropriate exclusions? Yes/No/Unclear            Could the selection of patients have introduced bias? <b>RISK: LOW/HIGH/UNCLEAR</b> </p> <p><b>B. Concerns regarding applicability</b></p> <p>Describe included patients (prior testing, presentation, intended use of index test and setting):</p> <p>Is there concern that the included patients do not match the review question? <b>CONCERN: LOW/HIGH/UNCLEAR</b></p>	<p><b>DOMAIN 3: REFERENCE STANDARD</b></p> <p><b>A. Risk of Bias</b></p> <p>Describe the reference standard and how it was conducted and interpreted:</p> <p> <input type="checkbox"/> Is the reference standard likely to correctly classify the target condition? Yes/No/Unclear  <input type="checkbox"/> Were the reference standard results interpreted without knowledge of the results of the index test? Yes/No/Unclear            Could the reference standard, its conduct, or its interpretation have introduced bias? <b>RISK: LOW/HIGH/UNCLEAR</b> </p> <p><b>B. Concerns regarding applicability</b></p> <p>Is there concern that the target condition as defined by the reference standard does not match the review question? <b>CONCERN: LOW/HIGH/UNCLEAR</b></p>
<p><b>DOMAIN 2: INDEX TEST(S)</b></p> <p>If more than one index test was used, please complete for each test.</p> <p><b>A. Risk of Bias</b></p> <p>Describe the index test and how it was conducted and interpreted:</p> <p> <input type="checkbox"/> Were the index test results interpreted without knowledge of the results of the reference standard? Yes/No/Unclear  <input type="checkbox"/> If a threshold was used, was it pre-specified? Yes/No/Unclear            Could the conduct or interpretation of the index test have introduced bias? <b>RISK: LOW/HIGH/UNCLEAR</b> </p> <p><b>B. Concerns regarding applicability</b></p> <p>Is there concern that the index test, its conduct, or interpretation differ from the review question? <b>CONCERN: LOW/HIGH/UNCLEAR</b></p>	<p><b>DOMAIN 4: FLOW AND TIMING</b></p> <p><b>A. Risk of Bias</b></p> <p>Describe any patients who did not receive the index test(s) and/or reference standard or who were excluded from the 2x2 table (refer to flow diagram):</p> <p>Describe the time interval and any interventions between index test(s) and reference standard:</p> <p> <input type="checkbox"/> Was there an appropriate interval between index test(s) and reference standard? Yes/No/Unclear  <input type="checkbox"/> Did all patients receive a reference standard? Yes/No/Unclear  <input type="checkbox"/> Did patients receive the same reference standard? Yes/No/Unclear  <input type="checkbox"/> Were all patients included in the analysis? Yes/No/Unclear            Could the patient flow have introduced bias? <b>RISK: LOW/HIGH/UNCLEAR</b> </p>

Table 3: QUADAS-2 tool for assessment of study quality of diagnostic studies [9].

Section and Topic	Item #		On page #
TITLE/ABSTRACT/ KEYWORDS	1	Identify the article as a study of diagnostic accuracy (recommend MeSH heading: 'sensitivity and specificity').	
INTRODUCTION	2	State the research questions or study aims, such as estimating diagnostic accuracy or comparing accuracy between tests or across participant groups.	
METHODS		Describe	
Participants	3	The study population: The inclusion and exclusion criteria, setting and locations where the data were collected.	
	4	Participant recruitment: Was recruitment based on presenting symptoms, results from previous tests, or the fact that the participants had received the index tests or the reference standard?	
	5	Participant sampling: Was the study population a consecutive series of participants defined by the selection criteria in items 3 and 4? If not, specify how participants were further selected.	
	6	Data collection: Was data collection planned before the index test and reference standard were performed (prospective study) or after (retrospective study)?	
Test methods	7	The reference standard and its rationale.	
	8	Technical specifications of material and methods involved including how and when measurements were taken, and/or cite references for index tests and reference standard.	
	9	Definition of and rationale for the units, cutoffs and/or categories of the results of the index tests and the reference standard.	
	10	The number, training and expertise of the persons executing and reading the index tests and the reference standard.	
	11	Whether or not the readers of the index tests and reference standard were blind (masked) to the results of the other test and describe any other clinical information available to the readers.	
Statistical methods	12	Methods for calculating or comparing measures of diagnostic accuracy, and the statistical methods used to quantify uncertainty (e.g. 95% confidence intervals).	
	13	Methods for calculating test reproducibility, if done.	
RESULTS		Report	
Participants	14	When study was done, including beginning and ending dates of recruitment.	
	15	Clinical and demographic characteristics of the study population (e.g. age, sex, spectrum of presenting symptoms, comorbidity, current treatments, recruitment centers).	
	16	The number of participants satisfying the criteria for inclusion that did or did not undergo the index tests and/or the reference standard; describe why participants failed to receive either test (a flow diagram is strongly recommended).	
Test results	17	Time interval from the index tests to the reference standard, and any treatment administered between.	
	18	Distribution of severity of disease (define criteria) in those with the target condition; other diagnoses in participants without the target condition.	
	19	A cross tabulation of the results of the index tests (including indeterminate and missing results) by the results of the reference standard; for continuous results, the distribution of the test results by the results of the reference standard.	
	20	Any adverse events from performing the index tests or the reference standard.	
Estimates	21	Estimates of diagnostic accuracy and measures of statistical uncertainty (e.g. 95% confidence intervals).	
	22	How indeterminate results, missing responses and outliers of the index tests were handled.	
	23	Estimates of variability of diagnostic accuracy between subgroups of participants, readers or centers, if done.	
	24	Estimates of test reproducibility, if done.	
DISCUSSION	25	Discuss the clinical applicability of the study findings.	

Table 4: The STARD tool for assessment of reporting quality of in diagnostic studies [8, 9]

Level	Prognosis	Diagnosis
1a	Systematic review (SR) (with homogeneity) of inception cohort studies; CDR" validated in different populations	SR (with homogeneity) of Level 1 diagnostic studies; CDR" with 1b studies from different clinical centres
1b	Individual inception cohort study with > 80% follow-up; CDR" validated in a single population	Validating cohort study with good reference standards; or CDR" tested within one clinical centre
1c	All or none case-series	Absolute SpPins and SnNouts" "
2a	SR (with homogeneity) of either retrospective cohort studies or untreated control groups in RCTs	SR (with homogeneity) of Level >2 diagnostic studies
2b	Retrospective cohort study or follow-up of untreated control patients in an RCT; Derivation of CDR" or validated on split-sample§§§ only	Exploratory cohort study with good reference standards; CDR" after derivation, or validated only on split-sample§§§ or databases
2c	"Outcomes" Research	-
3a	-	SR (with homogeneity) of 3b and better studies
3b	-	Non-consecutive study; or without consistently applied reference standards
4	Case-series (and poor quality prognostic cohort studies)	Case-control study, poor or non-independent reference standard
5	Expert opinion without explicit critical appraisal, or based on physiology, bench research or "first principles"	Expert opinion without explicit critical appraisal, or based on physiology, bench research or "first principles"

" Clinical Decision Rule. (These are algorithms or scoring systems that lead to a prognostic estimation or a diagnostic category.)

§§§ Split-sample validation is achieved by collecting all the information in a single tranche, then artificially dividing this into "derivation" and "validation" samples.

" " An "Absolute SpPin" is a diagnostic finding whose Specificity is so high that a Positive result rules-in the diagnosis. An "Absolute SnNout" is a diagnostic finding whose Sensitivity is so high that a Negative result rules-out the diagnosis.

Table 5: Oxford Center of Evidence Based Medicine (OCEBM) 2009 criteria for diagnostic and prognostic marker trials [13]

Question	Step 1 (Level 1*)	Step 2 (Level 2*)	Step 3 (Level 3*)	Step 4 (Level 4*)	Step 5 (Level 5)
<b>Is this diagnostic or monitoring test accurate?</b> (Diagnosis)	Systematic review of cross sectional studies with consistently applied reference standard and blinding	Individual cross sectional studies with consistently applied reference standard and blinding	Non-consecutive studies, or studies without consistently applied reference standards**	Case-control studies, or "poor or non-independent reference standard**"	Mechanism-based reasoning

\* Level may be graded down on the basis of study quality, imprecision, indirectness (study PICO does not match questions PICO), because of inconsistency between studies, or because the absolute effect size is very small; Level may be graded up if there is a large or very large effect size.

\*\* As always, a systematic review is generally better than an individual study.

Table 6: Oxford Center of Evidence Based Medicine (OCEBM) 2011 criteria for diagnostic and prognostic marker trials [13]

Study design  
Sample size  
Patient selection  
Selection of adequate control population  
Prevalence of target condition  
Technique/standardization  
Test experience  
Insufficient operational definition of positive and negative test findings  
Cut-off definition (e.g. post-hoc definition)  
Absence of a third category of indeterminate test findings  
Use of an inappropriate gold standard or reference test  
Lack of rater blinding

Tab. 7: Frequent methodological shortcomings and parameters varying between diagnostic accuracy trials.