Rapid diagnostic tests versus RT–PCR for Ebola virus infections: systematic review and meta-analysis

Basilua Andre Muzembo, a Kei Kitahara, b Ayumu Ohno, b Ngangu Patrick Ntontolo, c Nlandu Roger Ngatu, d Keinosuke Okamoto a & Shin-Ichi Miyoshi a

a Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University, 1-1-1 Tsushimanaka, Kita Ward, Okayama, 700-8530, Japan.
b Collaborative Research Center of Okayama University for Infectious Diseases in India, Kolkata, India.
c Institut Médical Evangélique, Kimpese, Democratic Republic of the Congo.
d Department of Public Health, Kagawa University Faculty of Medicine, Miki, Japan.

Correspondence to Basilua Andre Muzembo (email: muzembo_andre@okayama.ac.jp).

(Submitted: 28 October 2021 – Revised version received: 8 May 2022 – Accepted: 16 May 2022 – Published online: 1 June 2022)

Abstract

Objective To evaluate the clinical accuracy of rapid diagnostic tests for the detection of Ebola virus.

Methods We searched MEDLINE®, Embase® and Web of Science for articles published between 1976 and October 2021 reporting on clinical studies assessing the performance of Ebola virus rapid diagnostic tests compared to reverse transcription polymerase chain reaction (RT–PCR). We assessed study quality using the QUADAS-2 criteria. To estimate the pooled sensitivity and specificity of these rapid diagnostic tests, we used a bivariate random-effects meta-analysis.

Findings Our search identified 113 unique studies, of which nine met the inclusion criteria. The studies were conducted in the Democratic Republic of the Congo, Guinea, Liberia and Sierra Leone and they evaluated twelve rapid diagnostic tests. We included eight studies in the meta-analysis. The pooled sensitivity and specificity of the rapid tests were 86% (95% confidence interval, CI: 80–91) and 95% (95% CI: 91–97), respectively. However, pooled sensitivity decreased to 83% (95% CI: 77–88) after removing outliers. Pooled sensitivity increased to 90% (95% CI: 82–94) when analysis was restricted to studies using the RT–PCR from altona Diagnostics as gold standard. Pooled sensitivity increased to 99% (95% CI: 67–100) when the analysis was restricted to studies using whole or capillary blood specimens.

Conclusion The included rapid diagnostic tests did not detect all the Ebola virus disease cases. While the sensitivity and specificity of these tests are moderate, they are still valuable tools, especially useful for triage and detecting Ebola virus in remote areas.
Introduction
Ebola virus disease was first discovered in 1976 in the Democratic Republic of the Congo and South Sudan. This highly pathogenic disease is often fatal in humans; in past outbreaks the case fatality rate ranged from 25% (37/149) to 90% (128/143). At the initial stages of the disease, symptoms include fever, vomiting, diarrhoea, anorexia and fatigue. Diagnosing the disease on these symptoms alone is challenging, because they are similar to common endemic diseases present in Africa, such as typhoid fever, malaria and yellow fever.

To confirm the diagnosis of Ebola disease, a positive result from a reverse transcription polymerase chain reaction (RT–PCR) test is required. However, lateral flow assays (that is, rapid diagnostic tests) are valuable tools to limit the spread of the disease since their fast turnaround time have the potential to trigger early outbreak alerts. For instance, researcher estimated that if a combination of rapid diagnostic tests and RT–PCR assays would have been available during the 2013–2016 Ebola virus disease outbreak, the number of infections would have been up to a third less in Sierra Leone.

Most Ebola outbreaks begin in remote or rural areas with limited hospital availability and trained clinicians. Laboratory equipment needed for diagnosis and trained equipment users are rarely available; it can take hours or days to get the RT–PCR results. If rapid diagnostic tests for the disease were readily available in high-risk outbreak areas, lives could be saved since the time between virus introduction into a community until implementation of countermeasures can be decreased.

According to the World Health Organization (WHO), rapid diagnostic tests for Ebola virus should have a desired clinical sensitivity of > 98% and an acceptable clinical sensitivity of more than 95%. Since 1976, many Ebola virus rapid diagnostic tests have been developed, but researchers have not yet thoroughly assessed the evidence of their performance in clinical samples. The few rapid diagnostic tests that have been assessed in field conditions demonstrated uncertainty and variability in performance. We therefore conducted a meta-analysis to increase the evidence base of current rapid diagnostic tests detecting Ebola virus in suspected cases.

Methods
We conducted a systematic review and meta-analysis of studies that assessed the performance of rapid diagnostic tests for Ebola virus compared to RT–PCR. We followed the Preferred Reporting Items for a Systematic Review and Meta-analysis of Diagnostic Test
Accuracy Studies.\textsuperscript{10} This review is registered with the International Prospective Register of Systematic Reviews (CRD42021278280).

We searched MEDLINE®, Embase® and Web of Science for articles published from 1976 to 7 October 2021. Search terms used are available in Box 1. We applied no language restrictions during the search. We also hand-searched the articles included in the reference lists of relevant studies, related key reviews and a book chapter on Ebola rapid diagnostic tests.\textsuperscript{8} The studies that we retrieved were exported to EndNote software X9 (Clarivate, Philadelphia, United States of America) and from there, we removed duplicated studies.

Two authors screened all the titles and abstracts identified through the search, and reviewed the full text of potentially relevant article against the inclusion and exclusion criteria (Box 2). Each researcher was blind to the selection of the other researcher. We recorded reasons for excluding articles; disagreements were discussed and arbitrated by consensus.

**Data extraction**

Two investigators independently extracted data from individual studies. We then compared extracted data and any disagreements were resolved through discussion.

Prior data extraction, we designed a standardized data-extraction form. Extracted data included: setting, study period, sample size, type of specimen, index test, reference standard and reported conflicts of interest. We also extracted reported sensitivity and specificity, raw data on true positives, false positives, false negatives and true negatives to recreate two-by-two tables.

**Methodological assessment**

Two authors independently evaluated the methodological quality of the included studies using the Quality Assessment of Diagnostic Accuracy Studies 2 (QUADAS-2) tool.\textsuperscript{11} We resolved any disagreements through consensus and further article reading.

**Data synthesis and analysis**

All statistical analyses were performed using Stata (version 16, StataCorp LP, College Station, USA). We applied a bivariate-effect model to calculate the pooled sensitivity, specificity, positive and negative likelihood ratios and the diagnostic odds ratio (OR). We generated forest plots of the sensitivity and specificity of each data point.
We conducted meta-analysis using the bivariate-effect models because of the heterogeneity expected between studies assessing diagnostic test accuracy. We also applied the bivariate-effect model to generate plot hierarchical summary receiver-operating characteristic curves.

Heterogeneity was assessed by visual inspection of forest plots of the sensitivity and specificity and the shape of the hierarchical summary receiver-operating characteristic curves.

In our subgroup analysis, we stratified studies into seven subgroups based on: specimen type (serum alone, plasma alone, serum and plasma combined, whole blood and capillary blood combined); reference standard (RealStar Filovirus Screen RT–PCR Kit 1.0 [altona diagnostics GmbH, Hamburg, Germany, hereafter altona] alone and other RT–PCR tests such as Trombley assay) and exclusion of outliers (studies reporting that the rapid diagnostic test is 100% sensitive or specific). We also performed a subgroup analysis using only the ReEBOV™ Antigen Rapid Test kit (Corgenix Inc., Broomfield, USA; hereafter ReEBOV™) because it had enough data points to be pooled separately.

**Results**

The searches yielded 113 studies for screening after we removed duplicates (Fig. 1). Of the 36 full-text studies that we evaluated for eligibility, nine studies met the inclusion criteria. Of these, eight were eligible for the meta-analysis. We excluded 10 studies because they were non-lateral flow assays, we were unable to reconstitute the 2 × 2 diagnostic table or had scarce evidence on clinical performance (e.g. studies showing only analytical performance of the rapid diagnostic test).

**Study characteristics**

The included studies were cross-sectional studies published in English and French between 2015 and 2020. Sample size ranged from 105 to 928 tests performed. The studies were conducted in four countries: the Democratic Republic of the Congo, Guinea, Liberia and Sierra Leone.

Specimens were from either Ebola-suspected patients, those hospitalized at an Ebola treatment centre or from deceased people. One study tested post-mortem oral swabs specimens; all the studies tested blood specimens: capillary blood, whole blood, plasma and serum. Haemolyzed specimens were also included. Four studies clearly stated that rapid tests were performed on stored serum or stored plasma.
Further details on study characteristics are shown in Table 1. Five studies indicated participants’ age (available in the data repository).  

**Index tests**

The included studies evaluated twelve index tests: ReEBOV™, QuickNavi™-Ebola (Denka Seiken, Tokyo, Japan); DEDIATEST EBOLA (Senova, Weimar, Germany); One step Ebola test (Intec, Xiamen, China); SD Ebola Zaire Ag (SD Biosensor, Suwon-si, Republic of Korea); EBOLA Ag K-SET (Coris BioConcept, Gembloux, Belgium); eZYSCREEN® (Vedalab, Alençon, France); NMRC Ebola virus lateral flow immunoassay (Naval Medical Research Center, Bethesda, USA); Defence Science and Technology Laboratory Ebola virus disease rapid diagnostic test (Defence Science and Technology Laboratory, Salisbury, United Kingdom of Great Britain and Northern Ireland); and three tests using the dual path platform from Chembio Diagnostics (Medford, USA): Fever Panel Antigen System, Ebola Antigen System and Ebola–Malaria Antigen duplex system.

Further details on the tests are available from the data repository. The tests using the dual path platform are different from classic lateral flow assays as they contain a cartridge with a battery. ReEBOV™ should be stored at 2–8 °C, hence a cold chain is needed which could be a concern for use in remote field conditions. In addition, ReEBOV™, DEDIATEST EBOLA and SD Ebola Zaire Ag have been reported to have operational biosafety concern when using the test. The NMRC Ebola virus lateral flow immunoassay could not yield readable results in samples containing red blood cells.

All studies compared the rapid diagnostic test against RT–PCR, notably altona,  

**Methodological assessment**

The results of the quality assessments are available in the data repository. In the patient selection domain, 78% (7/9) of the studies had an unclear or high risk of bias, because these studies did not clearly specify random or consecutive recruitment of participants. Furthermore, some studies had suboptimal study design (two studies), missing information on patients’ exclusion criteria (seven studies) and the use of stored blood samples collected for other purposes (three studies). Regarding the reference standard domain, one study was judged as having a high risk of bias for incorrect use of
Altona’s RT–PCR kit, by modifying the manufacturer’s instructions. We judged applicability concerns to be low in the patient selection, index test and reference standard domains.

Five (56%) studies explicitly stated no conflicts of interest. In two (22%) studies, authors acknowledged having potential conflicts of interest. Two (22%) studies reported receiving test kits from manufacturers but did not consider it as a conflict of interest.

**Meta-analysis**

Nineteen data points covering 5332 tests performed were available to summarize the performance of rapid diagnostic tests for Ebola virus.

Table 2 shows the bivariate-effect model estimates for the pooled sensitivity, specificity, positive and negative likelihood ratios and the diagnostic OR. The pooled sensitivity and specificity were 86% (95% confidence interval, CI: 80–91) and 95% (95% CI: 91–97), respectively (Fig. 2). While sensitivity estimates varied widely from 62% to 100% across studies, the range for specificity estimates were narrower (80–100%; Fig. 3).

**Subgroup analysis**

In the subgroup analyses, the pooled specificity estimates were more consistent across subgroups compared to pooled subgroup sensitivity estimates (Table 2).

**Sample type**

**Serum and plasma**

We included five studies, representing 3754 samples from 14 data points. Nine tests were assessed: ReEBOV™, DEDIATEST EBOLA, SD Ebola Zaire Ag, eZYSSCREEN®, Ebola Antigen System, Ebola–Malaria Antigen duplex system, EBOLA Ag K-SET, One step Ebola test and Fever Panel Antigen System. The pooled sensitivity and specificity were 84% (95% CI: 77–89) and 94% (95% CI: 89–97), respectively.

**Plasma**

Studies assessing performance of rapid diagnostic tests on plasma samples used either ReEBOV™, DEDIATEST EBOLA, SD Ebola Zaire Ag, EBOLA Ag K-SET, One step Ebola test or Fever Panel Antigen System. We included three studies in this subgroup with 2995 specimens and 10 data points. The pooled sensitivity and specificity were 85% (95% CI: 76–91) and 93% (95% CI: 85–97), respectively.
Whole and capillary blood

Index test used in this subgroup were eZYSSCREEN®, QuickNavi-Ebola, ReEBOV™ and Defence Science and Technology Laboratory Ebola virus disease rapid diagnostic test. Among five data points and 1578 specimens, the pooled sensitivity and specificity were 99% (95% CI: 67–100) and 98% (95% CI: 91–99), respectively.

Reference standard

altona

We restricted the analysis to 13 data points (2925 specimens tested) where the altona RT–PCR kit had been used as the gold standard. Index tests used in this subgroup were eZYSCREEN®, Ebola Antigen System, Ebola–Malaria Antigen duplex system, DEDIATEST EBOLA, SD Ebola Zaire Ag, EBOLA Ag K-SET, Fever Panel Antigen System, ReEBOV™, One step Ebola test and Defence Science and Technology Laboratory Ebola virus disease rapid diagnostic test. The pooled sensitivity increased to 90% (95% CI: 82–94) compared to the overall estimates (86%; 95% CI: 80–91), while the pooled specificity (94%; 95% CI: 90–97) was similar (95%; 95% CI: 91–97).

Other RT–PCR tests

We included three studies using the following reference standards: Ebola-specific qRT–PCR, Trombley and GeneXpert® Ebola assays. Index tests were ReEBOV™, DEDIATEST EBOLA, SD Zaire Ag, QuickNavi-Ebola and One step Ebola test.

Among six data points and 2407 specimens tested, the pooled sensitivity of decreased to 78% (95% CI: 69–86). However, the pooled specificity (96%; 95% CI: 86–99) was comparable to the overall estimates.

Removing outliers

We excluded four data points that had sensitivity or specificity of 100%. We found that the pooled sensitivity slightly decreased to 83% (95% CI: 77–88) compared with the overall sensitivity estimates of 86% (95% CI: 80–91). However, the pooled specificity was the same as the overall specificity estimates; 95% (95% CI: 90–98) versus 95% (95% CI: 91–97).

ReEBOV™

When we considered only studies that used ReEBOV™ as index test (five data points with 1215 tests performed), the pooled sensitivity and specificity were 95% (95% CI: 70–99) and 89% (95% CI: 83–94), respectively (Fig. 4).
Discussion

In this study, we conducted meta-analyses on clinical accuracy studies to assess the performance of rapid diagnostic tests to detect Ebola virus in individuals suspected of having the disease. Compared to the gold standard RT–PCR, the overall pooled sensitivity for rapid tests was 86%, falling short of desired sensitivity of > 98% and an acceptable sensitivity of > 95% listed in a WHO target product profile document released during the 2013–2016 Ebola virus disease outbreak. Furthermore, we show that the overall pooled specificity was 95%, also lower than WHO’s recommended level of > 99%.

While the clinical value for Ebola virus screening to contain the outbreak is indisputable, our findings suggest that better performing rapid diagnostic tests in field conditions is needed. Our results indicate that current tests miss 14% of cases, which is considerable proportion because of the contagiousness and high mortality of Ebola disease. False-negative results should be minimized to the lowest level possible, since false-negative individuals might infect other people. In hospitals, false-negative patients might be treated with less precaution than positive patients and hence the likelihood of infecting health-care workers and other patients is greater. Letting false-negative patients waiting for a confirmatory RT–PCR test at home increase the risk of infecting people in the community. In addition, false-negative patients will not be included in contact tracing, which might lead to that the transmission chain is sustained. Noteworthy is that a low viral load in the specimen could also lead to false-negative result even with a rapid test with high sensitivity.

False positivity can have severe implications for false-positive individuals and their families since they would be subject to unnecessary quarantines. These individuals can also be exposed to the potential Ebola patients when waiting for the confirmation of the diagnosis. When assessing in which type of sample the rapid test performed best, we found that tests made on whole or capillary blood had the highest sensitivity, specificity, likelihood ratios and diagnostic OR. Using whole or capillary blood has the advantage that blood centrifugation is not required which would reduce the turn-around time and make the test more accessible in remote field settings. However, this subgroup analysis included only five data points, from four studies, and two of the data points were from a study that have been a subject of debate. Hence, further field studies are required to confirm results.

Using different gold standard also affected the results. The six data points with higher sensitivity all used altona as the gold standard. We also noted that pooled sensitivity
was higher in studies using altona (90%) compared with other gold standards (78%). However, only six data points were available for other gold standards versus 13 for altona. Why using altona yielded a sensitivity superior to that of other gold standards is unclear. Reduced sensitivity of altona to specimens with cycle threshold values above 30 (i.e. low viral loads) have been observed. This reduced sensitivity of altona may affect the interpretation of our pooled estimates. Our pooled sensitivity might have been underestimated, since altona might miss detecting positive samples with low viral loads. However, we cannot rule out the possibility that studies using altona might also overestimate the sensitivity of rapid tests, since one study that used altona reported an unusual sensitivity of 100%.

The review identified sufficient data points for assessing ReEBOV™ performance. This test received an emergency use authorization from the Food and Drug Administration (FDA) and WHO during the 2013–2016 Ebola outbreak. But, in 2018 FDA revoked this authorization when the new manufacturer (Zalgen Laboratories) that had acquired the company failed to reproduce the claimed test accuracy of ReEBOV™. The claimed sensitivity and specificity of the test were 91.8% and 84.6%, respectively. Our results are in line with the suboptimal performance: we showed a pooled sensitivity of 95% with a wide confidence interval (95% CI: 70–99), suggesting that the result is overestimated due to included outlier studies. The pooled specificity was also below WHO criteria of 99% analytical specificity.

This study has some limitations. First, our meta-analysis included some studies with some methodological limitations and studies where tests’ accuracy could have been overestimated. However, this concern was addressed by conducting subgroup analyses. For instance, we assessed whether the pooled estimates differed by removing outliers in the meta-analysis. This approach did indeed change the pooled sensitivity but not its specificity. Second, synthesizing the included studies in one pooled estimate of sensitivity and specificity could be inaccurate as there was substantial variation in the used cut-off for RT–PCR cycle threshold (data repository), which limits comparability of rapid tests. These issues are illustrated by pooling only data from ReEBOV™ studies. The results showed a suboptimum pooled sensitivity with a great uncertainty.

Third, some studies used stored blood samples. Using frozen blood samples that were processed through freeze–thaw cycles may have compromised the tests’ sensitivity. Nevertheless, this explanation is unlikely since a newly published study conducted on
patients also demonstrated that the sensitivity of three rapid tests (QuickNavi-Ebola, OraQuick Ebola Rapid Antigen Test (OraSure Technologies, Bethlehem, USA) and EBOLA Ag K-SET rapid test) varied, from 40% to 87%. Thus, for Ebola rapid tests with potential acceptable sensitivity, well designed clinical studies with larger sample sizes are necessary for an adequate assessment of their current performance.

Finally, we could not perform subgroup analysis comparing test performance by symptoms duration because of lack of data stratified by timing of the tests. Therefore, future field studies need to evaluate the performance of these test in relation to symptoms duration. As an example, rapid diagnostic tests for severe acute respiratory syndrome coronavirus 2 are more sensitive (83.8%) when used within 7 days of symptoms onsets than when used at later than 7 days (sensitivity of 61.5%). These limitations, however, would not modify the usefulness of Ebola rapid diagnostic tests as tool for separating suspected Ebola patients while they are waiting for their RT–PCR results.

The strength of our study is that our literature was extensive, without any language restrictions, although only studies published in English and French were included. However, some studies were excluded because of low evidence on clinical performance and studies could have been missed by our search strategy.

In conclusion, the results from this meta-analysis suggest that currently there is no commercial rapid diagnostic test for Ebola virus disease that has sufficient sensitivity and specificity to meet WHO standards. Despite the suboptimal performance, these tests still have clinical value because of their rapid turnaround time. Clinicians, especially those in settings where RT–PCR tests are not immediately available, should be aware of the existence, availability and limitations of the rapid tests. A negative result is unreliable in a subject highly suspected of having Ebola virus disease and the result must be confirmed using RT–PCR. Current commercially available tests include ReEBOV™, SD Zaire Ag, OraQuick, Ebola Antigen System and QuickNavi-Ebola and health-care workers can procure rapid tests, such as OraQuick, from the US Centers for Disease Control and Prevention or WHO. Our findings stress a great need for more accurate (if possible) rapid diagnostic test for Ebola virus. Tests with improved performance should undergo additional field testing, if blood samples are available or conducted during future outbreaks.
Acknowledgements

We thank Matthew James Mclaughlin, Martin Barnabas, and Mansongi Biyela Carine. Ngangu Patrick Ntontolo is also affiliated to the Department of Family Medicine and Primary Health, Protestant University of Congo, Kinshasa, Democratic Republic of the Congo.

Funding:

This work was supported by JSPS KAKENHI (Grants-in-Aid for Scientific Research; Grant Number 19K19467) and the Program of the Japan Initiative for Global Research Network on Infectious Diseases, JP22wm0125004, from Ministry of Education, Culture, Sports, Science and Technology in Japan, and Japan Agency for Medical Research and Development.

Competing interests:

None declared.

References


Box 1 Search terms and keywords used to identify studies on diagnostic accuracy of rapid tests for Ebola virus disease

For Ebola virus disease we used the keywords:


We combined these keywords with:

“rapid diagnostic test” [MeSH Terms] OR “Rapid test” [All fields] OR “rapid assay” [All fields] OR “EBOV lateral flow assay” [All fields].

We further narrowed searches by including the following names for index test: “ReEBOV,” “QuickNaviTM-Ebola,” “eZYSSCREEN,” “DSTL EVD lateral flow assay,” “OraQuick Ebola rapid antigen test kit,” “SD Q Line Ebola Zaire Ag” and “NMRC EBOV LFI.”

Box 2 Inclusion and exclusion criteria used to identify studies on diagnostic accuracy of rapid tests for Ebola virus disease

Inclusion criteria:

• Patients: Individuals with suspected Ebola virus disease;
• Index test: classic and non-classic lateral flow assays carried out in any specimen to diagnose Ebola disease;
• Reference standard: the study evaluates rapid diagnostic tests for Ebola virus against RT–PCR.
• Outcomes: the study report sensitivity and specificity of rapid diagnostic tests for Ebola virus or contains sufficient data to calculate sensitivity and specificity, by recreating the 2 × 2 diagnostic table.

Exclusion criteria:

• Rapid PCR-based Ebola virus tests
• Review articles, editorials and non-clinical studies.

RT–PCR: reverse transcription polymerase chain reaction.
<table>
<thead>
<tr>
<th>Study</th>
<th>Country</th>
<th>Sample size, no.</th>
<th>Study design</th>
<th>Reference standard</th>
<th>Specimen</th>
<th>Industry funded</th>
<th>Index test</th>
<th>No. of samples</th>
<th>% (95% CI)</th>
<th>Prevalence, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Makiala, 2019&lt;sup&gt;15&lt;/sup&gt;</td>
<td>Democratic Republic of the Congo Sierra Leone</td>
<td>928</td>
<td>Cross-sectional</td>
<td>GeneXpert&lt;sup&gt;®&lt;/sup&gt; Ebola</td>
<td>Whole blood</td>
<td>2018</td>
<td>No</td>
<td>DEDIATEST Ebola</td>
<td>68</td>
<td>2</td>
</tr>
<tr>
<td>Wonderly, 2019&lt;sup&gt;17&lt;/sup&gt;</td>
<td>Sierra Leone</td>
<td>428</td>
<td>Cross-sectional</td>
<td>RT–PCR (altona)</td>
<td>Whole blood</td>
<td>2015</td>
<td>No, but received rapid diagnostic tests from manufacturers</td>
<td>RT–PCR (Trombley)</td>
<td>109</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>RT–PCR (altona)</td>
<td>Stored plasma Ebola Zaire Ag</td>
<td>110</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>RT–PCR (Trombley)</td>
<td>Stored plasma Ebola Zaire Ag</td>
<td>123</td>
<td>39</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>RT–PCR (altona)</td>
<td>Stored plasma ReEBOV™ Antigen Rapid Test kit</td>
<td>133</td>
<td>29</td>
</tr>
<tr>
<td>Study Reference</td>
<td>Location</td>
<td>Study Type</td>
<td>Sample Type</td>
<td>Sample Year</td>
<td>Sample Method</td>
<td>Test Method</td>
<td>Test</td>
<td>Sensitivity</td>
<td>Specificity</td>
<td>95% CI Low</td>
</tr>
<tr>
<td>-----------------</td>
<td>----------</td>
<td>------------</td>
<td>-------------</td>
<td>-------------</td>
<td>---------------</td>
<td>-------------</td>
<td>------</td>
<td>-------------</td>
<td>------------</td>
<td>-----------</td>
</tr>
<tr>
<td>Colavita, 2018</td>
<td>Sierra Leone</td>
<td>Cross-sectional</td>
<td>Stored plasma</td>
<td>2015</td>
<td>No, but received rapid diagnostic tests from manufacturers</td>
<td>One step Ebola test</td>
<td>125</td>
<td>39</td>
<td>2</td>
<td>158</td>
</tr>
<tr>
<td>Gallais, 2017</td>
<td>Guinea</td>
<td>Cross-sectional</td>
<td>Store residual plasma samples</td>
<td>2014–2015</td>
<td>Yes</td>
<td>EBOLA Ag K-SET</td>
<td>93</td>
<td>2</td>
<td>12</td>
<td>103</td>
</tr>
<tr>
<td>Phan, 2016</td>
<td>Liberia</td>
<td>Cross-sectional</td>
<td>Plasma</td>
<td>2014–2015</td>
<td>No</td>
<td>NMRC Ebola virus lateral flow immunoassay</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Boisen, 2016</td>
<td>Sierra Leone</td>
<td>Cross-sectional</td>
<td>Ebola virus-specific qRT-PCR</td>
<td>2014</td>
<td>Yes</td>
<td>ReEBOV™ Antigen Rapid Test kit</td>
<td>72</td>
<td>2</td>
<td>44</td>
<td>58</td>
</tr>
<tr>
<td>Broadhurst, 2015</td>
<td>Sierra Leone</td>
<td>Cross-sectional</td>
<td>Plasma (for the reference test) and RT–PCR (altona)</td>
<td>2015</td>
<td>No, but received rapid diagnostic tests from manufacturers</td>
<td>ReEBOV™ Antigen Rapid Test kit</td>
<td>28</td>
<td>6</td>
<td>0</td>
<td>71</td>
</tr>
<tr>
<td></td>
<td>Country</td>
<td>Sample Size</td>
<td>Study Type</td>
<td>Sample Type</td>
<td>Study Year</td>
<td>Tests Received</td>
<td>Test Used</td>
<td>Sensitivity</td>
<td>Specificity</td>
<td>Total Cases</td>
</tr>
<tr>
<td>----------------</td>
<td>------------------</td>
<td>-------------</td>
<td>------------------</td>
<td>-----------------------------</td>
<td>------------</td>
<td>----------------</td>
<td>----------------------------------</td>
<td>-------------</td>
<td>-------------</td>
<td>-------------</td>
</tr>
<tr>
<td>Walker, 2015²³</td>
<td>Sierra Leone</td>
<td>131</td>
<td>Cross-sectional</td>
<td>Capillary blood</td>
<td>2015</td>
<td>No</td>
<td>Defence Science and Technology Laboratory Ebola virus disease rapid diagnostic test</td>
<td>15</td>
<td>9</td>
<td>107</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Capillary blood</td>
<td>2015</td>
<td>No</td>
<td>ReEBOV™ Antigen Rapid Test kit</td>
<td>45</td>
<td>18</td>
<td>214</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Whole blood</td>
<td>2015</td>
<td>No, but received rapid diagnostic tests from manufacturers</td>
<td>RealStar Filovirus Screen RT–PCR Kit 1.0 (altona diagnostics GmbH, Hamburg, Germany), Trombley refer to the Trombley assay, GENEJET® Ebola (Cepheid, Sunnyvale, United States of America), Weidmann refer to the Weidmann technique (that is, a quantitative one-step RT–PCR).¹⁹</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CI: confidence interval; PCR: polymerase chain reaction; RT–PCR: reverse transcription PCR; rRT–PCR: real-time RT–PCR; qRT–PCR: quantitative RT–PCR.

* altona refer to RealStar Filovirus Screen RT–PCR Kit 1.0 (altona diagnostics GmbH, Hamburg, Germany), Trombley refer to the Trombley assay, GeneXpert® Ebola (Cepheid, Sunnyvale, United States of America), Weidmann refer to the Weidmann technique (that is, a quantitative one-step RT–PCR).

* We were unable to accurately reconstitute the 2x2 table used to calculate specificity and sensitivity.

* While not specifically mentioned in article, the authors cite the Trombley assay in their methods.
Table 2. Meta-analysis of rapid diagnostic tests for Ebola virus disease

<table>
<thead>
<tr>
<th>Group and study</th>
<th>Data points (no. of studies)</th>
<th>Sample size</th>
<th>Pooled sensitivity</th>
<th>Pooled specificity</th>
<th>Positive likelihood ratio</th>
<th>Negative likelihood ratio</th>
<th>Diagnostic OR</th>
</tr>
</thead>
<tbody>
<tr>
<td>All studies15–16,19,23</td>
<td>19 (8)</td>
<td>5332</td>
<td>86 (80–91)</td>
<td>95 (91–97)</td>
<td>18.0 (9.9–32.9)</td>
<td>0.14 (0.10–0.21)</td>
<td>126 (66–240)</td>
</tr>
<tr>
<td>Sample type</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum and plasma15,17–19,21</td>
<td>14 (5)</td>
<td>3754</td>
<td>84 (77–89)</td>
<td>94 (89–97)</td>
<td>13.7 (7.4–25.5)</td>
<td>0.17 (0.12–0.24)</td>
<td>79 (42–148)</td>
</tr>
<tr>
<td>Plasma17,18,21</td>
<td>10 (3)</td>
<td>2995</td>
<td>85 (76–91)</td>
<td>93 (85–97)</td>
<td>11.9 (5.7–24.8)</td>
<td>0.16 (0.10–0.25)</td>
<td>74 (34–160)</td>
</tr>
<tr>
<td>Serum15,19</td>
<td>4 (2)</td>
<td>759</td>
<td>80 (73–86)</td>
<td>95 (89–98)</td>
<td>17.6 (7.5–41.0)</td>
<td>0.21 (0.15–0.28)</td>
<td>85 (38–190)</td>
</tr>
<tr>
<td>Whole blood and capillary blood16,19,22,23</td>
<td>5 (4)</td>
<td>1578</td>
<td>99 (67–100)</td>
<td>98 (91–99)</td>
<td>45.5 (11.3–182.7)</td>
<td>0.02 (0.00–0.45)</td>
<td>3011 (184–49177)</td>
</tr>
<tr>
<td>Reference standard</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>altona1,15,17,18,19,22,23</td>
<td>13 (6)</td>
<td>2925</td>
<td>90 (82–94)</td>
<td>94 (90–97)</td>
<td>15.9 (8.9–28.4)</td>
<td>0.11 (0.06–0.19)</td>
<td>146 (73–293)</td>
</tr>
<tr>
<td>Other standardsb,16,17,21</td>
<td>6 (3)</td>
<td>2407</td>
<td>78 (69–86)</td>
<td>96 (86–99)</td>
<td>21.5 (5.3–87.2)</td>
<td>0.22 (0.15–0.32)</td>
<td>96 (23–408)</td>
</tr>
<tr>
<td>Other</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Removing outlier studiesb,15,16,17,18,19,21</td>
<td>15 (6)</td>
<td>4675</td>
<td>83 (77–88)</td>
<td>95 (90–98)</td>
<td>16.9 (8.4–33.9)</td>
<td>0.17 (0.13–0.24)</td>
<td>97 (48–196)</td>
</tr>
<tr>
<td>ReEBOV™ Antigen Rapid Test kit17,21,22</td>
<td>5 (3)</td>
<td>1215</td>
<td>95 (70–99)</td>
<td>89 (83–94)</td>
<td>8.9 (5.3–15.1)</td>
<td>0.06 (0.01–0.42)</td>
<td>157 (18–1403)</td>
</tr>
</tbody>
</table>

CI: confidence interval; OR: odds ratio.
a RealStar Filovirus Screen reverse transcription polymerase chain reaction Kit 1.0 (altona diagnostics GmbH, Hamburg, Germany).
b Gold standard tests were Trombley assay,14 GeneXpert® Ebola (Cepheid, Sunnyvale, United States of America) and RT–PCR specific for Ebola (while not specifically mentioned in article, the authors cite the Trombley assay in their methods).21

We excluded studies reporting that rapid diagnostic tests were 100% sensitive or specific.
Fig. 1. Flowchart of the selection of articles included in study on the diagnostic accuracy of rapid tests for Ebola virus disease

3985 records identified through database search:
- 310 through PUBMED®
- 3358 through Embase®
- 317 through Web of science™

Record removed before screening:
- 1129 duplicate records removed
- 2743 records ineligible

113 records screened

76 records excluded

37 articles sought for retrieval

One report no retrieved

36 articles assessed for eligibility

27 articles excluded:
- Three were reviews
- 10 provided insufficient clinical data or no lateral flow assay
- 14 reports did not study Ebola virus disease rapid test.

Nine articles included in qualitative synthesis
And
Eight articles included in meta-analysis
Fig. 2. Forest plots of the sensitivities and specificities of Ebola virus rapid diagnostic tests compared to RT–PCR

CI: confidence interval; RT–PCR: reverse transcription polymerase chain reaction.

*While not specifically mentioned in article, the authors cite the Trombley assay in their methods.21

Note: altona refers to RealStar Filovirus Screen reverse transcription polymerase chain reaction Kit 1.0 (altona diagnostics GmbH, Hamburg, Germany).
Fig. 3. Hierarchical summary receiver-operating characteristic curves of the sensitivity and specificity of Ebola virus rapid diagnostic tests compared to RT–PCR.

Notes: Each open circle represents the sensitivity and specificity of each included data point (n = 19). The size of each circle is proportional to the sample size of the study. A curve close to the upper left-hand corner of the hierarchical summary receiver-operating characteristic curve indicates better overall performance.35

RT–PCR: reverse transcription polymerase chain reaction.
Fig. 4. Forest plots of the sensitivities and specificities of ReEBOV™ Antigen Rapid Test kit for the detection of Ebola virus

Cl: confidence interval; RT–PCR: reverse transcription polymerase chain reaction.

Note: altona refers to RealStar Filovirus Screen reverse transcription polymerase chain reaction Kit 1.0 (altona diagnostics GmbH, Hamburg, Germany).

While not specifically mentioned in article, the authors cite the Trombley assay in their methods.21