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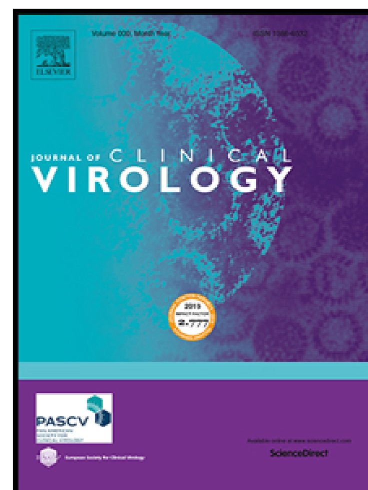

A multi-country phase 2 study to evaluate the suitcase lab for rapid detection of SARS-CoV-2 in seven Sub-Saharan African countries: Lessons from the field

Arianna Ceruti , Ndongo Dia , Adeleye Solomon Bakarey , Judah Ssekitoleko , Soa Fy Andriamandimby , Padra Malwengo-Kasongo , Rasheeda H.A. Ahmed , Rea Maja Kobialka , Jean Michel Heraud , Moussa Moise Diagne , Marie Henriette Dior Dione , Idrissa Dieng , Martin Faye , Ousmane Faye , Jean Théophile Rafisandratantsoa , Jean-Pierre Ravalohery , Claudio Raharinandrasana , Tsiry H. Randriambolamanantsoa , Norosoa Razanajatovo , Iony Razanatovo , Joelinotahina H. Rabarison , Phillipe Dussart , Louis Kyei-Tuffuor , Abigail Agbanyo , Olubusuyi Moses Adewumi , Adeola Fowotade , Muideen Kolawole Raifu , Patient Okitale-Talunda , Gracia Kashitu-Mujinga , Christelle Mbelu-Kabongo , Steve Ahuka-Mundeke , Anguy Makaka-Mutondo , Enas M. Abdalla , Sanaa M. Idris , Wisal A. Elmagzoub , Rahma H. Ali , Eman O.M. Nour , Rasha S.M. Ebraheem , Huda H.H. Ahmed , Hamadelniel E. Abdalla , Musab Elnegoumi , Izdihar Mukhtar , Muatsim A.M. Adam , Nuha Y.I. Mohamed , Shahinaz A. Bedri , Hamdan Mustafa Hamdan , Magid Kisekka , Monica Mpumwiire , Sharley Melissa Aloyo , Joanita Nabwire Wandera , Andrew Agaba , Rogers Kamulegeya , Hosea Kiprotich , David Patrick Kateete , Paul Kadetz , Uwe Truyen , Kamal H. Eltom , Anavaj Sakuntabhai , Julius Boniface Okuni , Sheila Makiala-Mandanda , Vincent Lacoste , George Olusegun Ademowo , Michael Frimpong , Amadou Alpha Sall , Manfred Weidmann , Ahmed Abd El Wahed

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1 Highlights

2 • The mobile Suitcase lab is a portable system for molecular diagnosis in the field, especially in
3 poor resource settings
4 • The recombinase aided amplification assay based on the RdRP gene is robust and accurate
5 for SARS-CoV-2 detection
6 • Diagnostic accuracy across different sites depends not only on the assay itself, but also on
7 other external factors such as implementation of standardized operation procedure,
8 enhanced quality control measures and importantly, in-person continuous staff training
9

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12 A multi-country phase 2 study to evaluate the suitcase lab for rapid detection of SARS-CoV-2 in seven
13 Sub-Saharan African countries: Lessons from the field

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95 Keywords: SARS-CoV-2; Diagnostics-in-a-Suitcase; Recombinase polymerase amplification
96 assay

97 **Abstract**

98 Background: The COVID-19 pandemic led to severe health systems collapse, as well as
99 logistics and supply delivery shortages across sectors. Delivery of PCR related healthcare
100 supplies continue to be hindered. There is the need for a rapid and accessible SARS-CoV-2
101 molecular detection method in low resource settings.
102 Objectives: To validate a novel isothermal amplification method for rapid detection of SARS-
103 CoV-2 across seven sub-Sharan African countries.
104 Study design: In this multi-country phase 2 diagnostic study, 3,231 clinical samples in seven
105 African sites were tested with two reverse transcription Recombinase-Aided Amplification
106 (RT-RAA) assays (based on SARS-CoV-2 Nucleocapsid (N) gene and RNA-dependent RNA
107 polymerase (RdRP) gene). The test was performed in a mobile suitcase laboratory within 15
108 minutes. All results were compared to a real-time RT-PCR assay. Extraction kits based on
109 silica gel or magnetic beads were applied.
110 Results: Four sites demonstrated good to excellent agreement, while three sites showed fair
111 to moderate results. The RdRP gene assay exhibited an overall PPV of 0.92 and a NPV of
112 0.88. The N gene assay exhibited an overall PPV of 0.93 and a NPV 0.88. The sensitivity of
113 both RT-RAA assays varied depending on the sample Ct values. When comparing sensitivity
114 between sites, values differed considerably. For high viral load samples, the RT-RAA assay
115 sensitivity ranges were between 60.5 and 100% (RdRP assay) and 25 and 98.6 (N assay).
116 Conclusion: Overall, the RdRP based RT-RAA test showed the best assay accuracy. This
117 study highlights the challenges of implementing rapid molecular assays in field conditions.
118 Factors that are important for successful deployment across countries include the
119 implementation of standardized operation procedures, in-person continuous training for staff,
120 and enhanced quality control measures.

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122 **1. Background**

123 In March 2020, the World Health Organization (WHO) declared COVID-19 caused by severe
124 acute respiratory syndrome coronavirus type 2 (SARS-CoV-2) as a global pandemic [1].
125 Early detection of infected cases is still regarded as essential to reduce the disease burden
126 [2].

127 Real-time reverse transcription polymerase chain reaction (RT-PCR) is the standard
128 approach in terms of sensitivity and accuracy. However, this technique requires well-
129 established laboratory. Additionally, supply and delivery shortages have been reported
130 across various sectors [3,4]. Antigen lateral flow tests were quickly developed and deployed,
131 allowing carriers with high viral load to be diagnosed more easily. However, these tests often
132 do not reach the WHO recommended minimum of >80% sensitivity and >97% specificity for
133 new diagnostics tests [5]. Deployment of a simple, rapid molecular test method could offer
134 marked advantages.

135 Recombinase-Polymerase/aided Amplification (RPA-RAA) assays have been described as a
136 rapid and effective nucleic acid amplification technique, due to its simplicity and fast sample-
137 to-result test time [6]. RPA/RAA is an isothermal probe-based nucleic acid detection method
138 that neither requires template denaturation nor primer annealing steps [7]. The proper
139 selection of a polymerase working at low temperature (39-42° C), the robustness of the
140 assay, and compactness of the fluorescence detection device make RPA/RAA an optimal
141 technique for molecular diagnosis at the point of need. To enable the widespread use of the
142 technology, mobile suitcase laboratories were deployed to many sub-Saharan African
143 countries [8].

144 **2. Objectives**

145 To further clinically evaluate the system in real-life settings, a multi-country single blinded
146 phase 2 study was conducted in seven sub-Saharan African countries. The aim was not only

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147 to determine the accuracy of RAA assay for detection of SARS-CoV-2 in local African
148 settings, but also to assess performance differences between research institutions.

149

150 **3. Material and Methods**

151 **3.1 Sample size calculation**

152 The sample size was calculated using the formula for comparing two independent
153 proportions, which is used to estimate sample size for studies comparing sensitivity and/or
154 specificity of two tests of unpaired design [9]. A minimum of 300 samples per site were
155 required to achieve 95% confidence.

156

157 **3.2 Study site and population**

158 A multi-country, single blinded, phase 2 diagnostic evaluation study was conducted in seven
159 sites: Institut Pasteur de Dakar (IPD), Senegal; Institut Pasteur de Madagascar (IPM),
160 Madagascar; Kumasi Centre for Collaborative Research (KCCR), Ghana; University of
161 Ibadan (UI), Nigeria; Institut National de Recherche Biomédicale (INRB), Democratic
162 Republic of the Congo; University of Khartoum (UofK), Sudan; and Makerere University
163 (MAK), Uganda. For the purpose of this study, a total of 3,231 archived samples were used
164 to evaluate the assay. Archived samples included in this study were from patients who tested
165 positive for SARS-CoV-2 by the reference laboratory and patients who were suspected
166 COVID-19 and tested negative by the reference laboratory. All samples were tested
167 irrespective of age, sex, and race. During the study, all samples were handled anonymously.
168 Samples included nasal, mid-turbinate swab or saliva in viral transport media (VTM), PBS or
169 stored dry and maintained in –80°C. Additional information regarding the samples was
170 included in supplementary file #1.

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172 **3.3 Study design**

173 This study was conducted according to the guidelines for diagnostic kit evaluation (figure 1)
174 [9].
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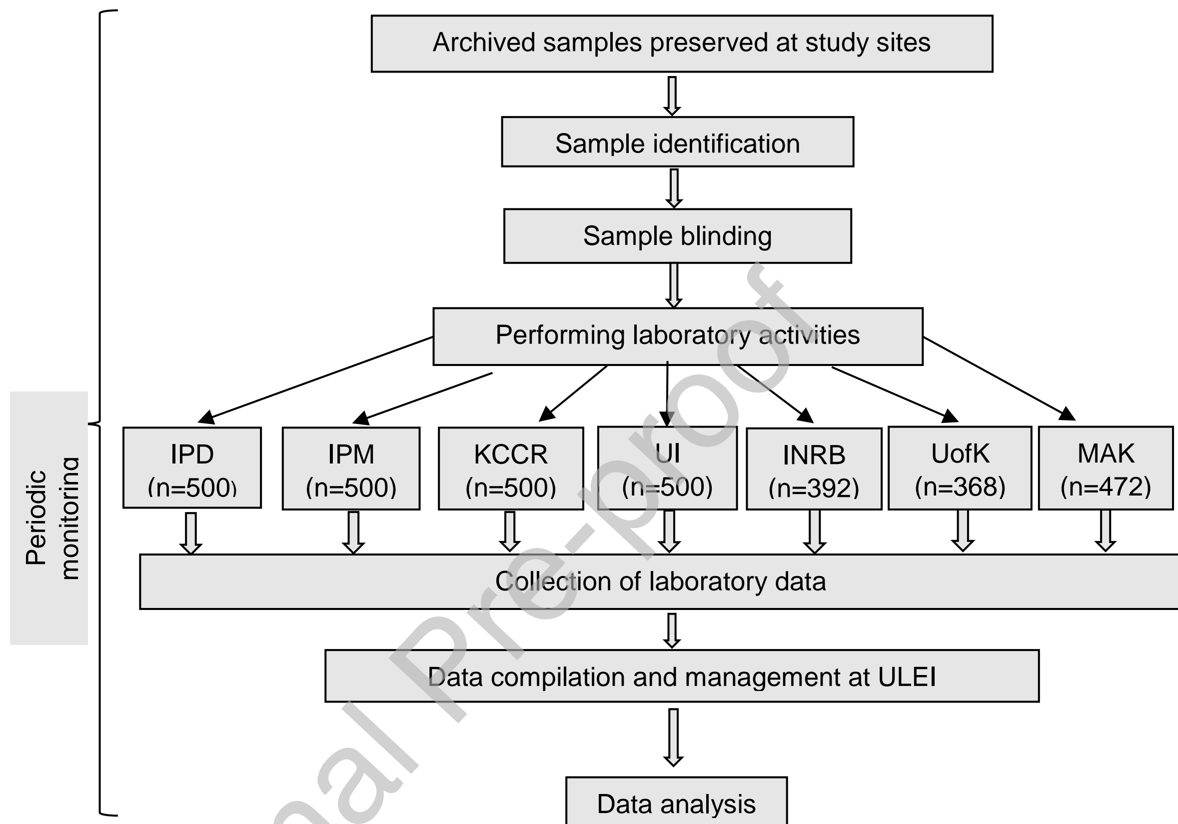
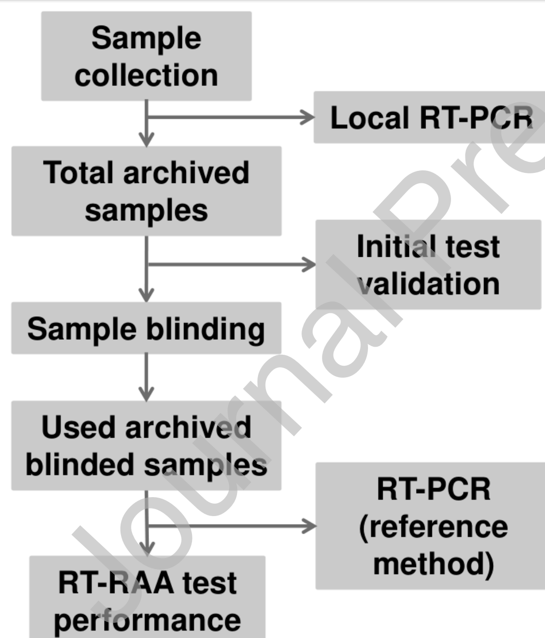


176 **Figure 1.** Schematic representation of the multi-country, single blinded, Phase 2 study to test
177 archived samples with the RT-RAA assay for SARS-CoV-2. Institut Pasteur de Dakar (IPD);
178 Institut Pasteur de Madagascar (IPM); Kumasi Centre for Collaborative Research (KCCR);
179 University of Ibadan (UI); Institut National de Recherche Biomédicale (INRB); University of
180 Khartoum (UofK); and Makerere University (MAK).
181
182
183 Each country initially tested an inactivated test panel of 20 samples using RT-RAA and real-
184 time RT-PCR reagents to assure their preparedness to perform the tests (Figure 2). Once
185 successful (at least 90% accurate detection rate), each country started clinical sample testing
186 as follows: all samples were labelled with random numbers by the site principal investigator,

187 and the laboratory personnel were divided into two teams: one team performed the real-time
188 RT-PCR and the other performed the RT-RAA. Both teams were blinded and did not know
189 whether the sample was from a positive or negative subject. Simultaneously, the three parts
190 carbonless copy paper-based laboratory report forms (LRFs) were made available in two
191 modes, one by the unblinded staff and the other by the blinded staff (Supplementary file #2).
192 All laboratory data were reported in LRFs by both teams separately. The data was then
193 decoded by the data management team, where it was merged and statistically analysed.
194 Patient information was not shared between study sites, only positivity and negativity rates
195 were recorded. To ensure the quality of the study activities, periodic monitoring was
196 performed.

197



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199 **Figure 2.** Workflow of the performed tests at the study sites. The new RT-PCR performed
200 was used as the reference method to validate the RT-RAA assays.

201

202 **3.4 Mobile Suitcase Laboratory Set Up**

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203 The total set up consisted of a Glove Box (Bodo Koennecke, Berlin, Germany) and a mobile
204 suitcase laboratory (figure 3). The Glove Box protects the technician while handling and
205 inactivating the sample before nucleic acid extraction. The isothermal amplification test was
206 performed in the Mobile Suitcase Lab (figure 4).
207

208
209 **Figure 3.** Example of the suitcase lab, which is fully equipped to perform molecular tests in
210 the field.
211
212

213

214 **Figure 4.** The suitcase lab at various study sites.

215

216 **3.5 Laboratory Analysis**

217 **3.5.1 Real-time RT-PCR assay for Detection of SARS-CoV-2 RNA**

218

219 RNA was isolated from clinical samples of subjects who were recently suspected of
220 contracting COVID-19. At KCCR, UofK, UI and INRB, the QIAamp Viral RNA Mini Kit
221 (Qiagen, Hilden, Germany) was used. At MAK, Liferiver Viral DNA/RNA isolation kit
222 (Shanghai ZJ Biotech co. let, Shanghai, China) was used. At IPD the Veri-Q PREP M16
223 Automatic Nucleic Acid Extraction System (MiCo BioMed, South Korea) was used. IPM used
224 the NucleoSpin Dx Virus, Mini kit (Macherey-Nagel GmbH, Düren, Germany).

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225 All samples were originally tested as part of routine diagnostics and divided into positive and
226 negative according to the locally used real-time RT-PCR results (Supplementary file #2). To
227 assure integrity of RNA on the day of testing, an additional real-time RT-PCR was performed.
228 This result was considered as reference method (Figure 2). The archived patient material
229 was tested at each site with a commercially available real-time RT-PCR assay combining
230 oligonucleotide Lightmix Modular SARS-CoV-2 RNA-dependent RNA polymerase (RdRP)
231 and the lyophilized one-step RT-PCR Polymerase Mix kit from TIB MOLBIOL (Berlin,
232 Germany) according to the manufacturer's instructions. The real-time RT-PCR reaction
233 comprised 15 µL, including 0.5 µL oligonucleotide mix, 4 µL PCR-grade water, 10 µL qPCR
234 Master mix and 5 µL template or control. The following real-time PCR cycler was used:
235 BioRad CFX96 Touch (Bio-Rad Laboratories, Hercules, United States) at IPD, KCCR and UI,
236 Rotorgene Q (Qiagen, Hilden, Germany) at IPM, Applied Biosystems 7500 Fast (Thermo
237 Fisher Scientific, Waltham, United States) at INRB, qTower (Biometra, Analytik Jena, Jena,
238 Germany) at UofK and Applied Biosystems Quantstudio 7flex (Thermo Fisher Scientific,
239 Waltham, United States) at MAK.

240 **3.5.2 RT-RAA Assay for Detection of SARS-COV-2 RNA**

241 Two RT-RAA assays were evaluated: one based on the RdRP gene and one on the
242 Nucleocapsid (N) gene. The primers, probe and reaction conditions for SARS-CoV-2 genes
243 N and RdRP RT-RPA assays were based on a previous study [8] Primer and probe were
244 synthesized by TIB MOLBIOL (Berlin, Germany). The RT-RAA nucleic acid amplification kit
245 (Fluorescent RT-RAA) from Jiangsu Qitian Gene Biotechnology Co. (Wuxi, China) was used.
246 The kit comprises lyophilized enzymes, including the reverse transcriptase necessary for
247 RNA amplification. The RT-RAA reaction total volume was 50 µL including 21.5 µL of the
248 oligonucleotide mix (21 pMol for forward primer, 42 pMol for reverse primer and 6 pMol for
249 exo-probe), 25 µL rehydration buffer, 2.5 µL Magnesium Acetate and 1 µL template or
250 control. The mix was added into the lid of the reaction tube containing the freeze-dried pellet.
251 The tube was closed, centrifuged, mixed, centrifuged, and placed immediately into the

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252 isothermal device: UofK and IPM used the TwistDx TS1 device (Cambridge, UK). UI used
253 the Qiagen ESEquant TS2 model (Hilden, Germany), while all other countries used the Axxin
254 T8 Isothermal instrument (Fairfield, Australia). The reaction was incubated at 42 °C for 15
255 minutes. A mixing step was conducted after 320 s for the N gene assay and after 230 s for
256 the RdRP gene assay. For signal interpretation, a combined threshold time (TT) and first
257 derivative analysis was used with the corresponding software of each device.

258

259 **3.6 Data Analysis**

260 Standard formulas were used with MedCalc [10] to determine the sensitivity, specificity,
261 positive predictive value (PPV), and negative predictive value (NPV) [11]. Cohen's kappa
262 coefficient (k) and McNemar's test were performed to determine the concordance and
263 discordance between the RT-RAA assays and RT-PCR-based method. The values of
264 Cohen's kappa coefficients were interpreted according to Landis and Koch [12] and
265 calculated using the online Graphpad version. The McNemar test was calculated via
266 https://epitools.ausvet.com.au/mcnemar. A P-value <0.05 was considered to be statistically
267 significant.

268 **3.7 Mutational analysis of the RdRP amplicon**

269 Over 10,000 SARS-CoV-2 RNA sequences from local strains in the involved countries were
270 screened for potential mutations in the targeted RdRP region. A previously published pipeline
271 was used [13].

272

273 **4. Results**

274 **4.1 Performance of the deployed isothermal amplification assays**

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In total, 3,231 samples were identified at study sites (Figure 1) to validate the assays. After data curation, the N gene assay results were evaluated with a total of 1890 negative samples and 580 positive samples and the RdRP gene assay with a total of 2326 negative samples and 868 positive samples (Supplementary file #3 displaying sample flowchart and raw data). The RdRP gene assay showed an overall PPV of 0.92 and a NPV of 0.88. The N gene assay showed an overall PPV of 0.93 and a NPV 0.88.

The sensitivity of both RT-RAA assays varied depending on the sample Ct values (Table 1). RT-PCR positive samples with high viral load (Ct <30) showed the best results with 90.8% and 81.8% overall sensitivities for the RdRP and N gene RT-RAA assays, respectively. When comparing sensitivity between sites, values differed considerably. For high viral load samples, the RdRP assay sensitivity ranged between 60.5 and 100%. The N gene did not perform as well as the RdRP gene, with a sensitivity between 25 and 98.6 %. The specificity among all study sites ranged from 91.1% to 100% for the RdRP gene RT-RAA assay and from 94.3% to 100% for the N gene RT-RAA assay (Table 2). The overall sensitivity and specificity with both targets combined was 58.2% and 99.2%, respectively. The agreement between test methods varied across sites (Table 3). Sites IPD, KCCR, UI and INRB showed good to excellent agreement while sites IPM, UofK and MAK showed fair to moderate results.

Table 1. Overall clinical sensitivity of all samples across sites categorized according to the Ct values of real-time RT-PCR.

RT-PCR Ct range	Target gene for RT-RAA	Overall Sensitivity (%)	RT-PCR positive	RT-RAA positive
0-30	RdRP	90.8 (82.6 - 99.7)	493	448
	N	81.8 (72.5 - 92)	341	279

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≥30-35	RdRP	45.6 (36.3 - 56.5)	182	83
	N	41.8 (30.98 - 55.4)	117	49
≥35-40	RdRP	13.5 (9 -19.7)	193	26
	N	12.3 (6.9 - 20.2)	122	15
Total	RdRP	64.2 (58.9 - 69.7)	868	557
	N	59.1 (53 - 65.7)	580	343

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297 **Table 2.** Clinical sensitivity and specificity at each study site. NA is not applicable as the site
298 did not perform the assay. The 95% confidence interval is showed in parenthesis.

Study site	RdRP Sensitivity (%)				RdRP Specificity (%)	N Sensitivity (%)				N Specificity (%)
	<30 Ct	30-35 Ct	>35 Ct	Overall		<30 Ct	30-35 Ct	>35 Ct	Overall	
IPD	100 (95 - 99.9)	75 (47.6 - 92.7)	21.4 (4.7 - 50.8)	85.3 (76.9 - 91.5)	99 (97.4 - 99.7)	98.6 (92.5 - 99.9)	81.2 (54.3 - 95.9)	14.3 (1.8 - 42.8)	84.3 (75.8 - 90.8)	99 (97.8 - 99.8)
IPM	94.4 (89.3 - 97.5)	23.9 (12.6 - 38.7)	4.2 (0.5 - 14.2)	62.4 (55.9 - 68.6)	93.5 (89.8 - 96.2)	82.5 (75.3 - 88.3)	10.8 (3.6 - 23.6)	12.5 (4.7 - 25.2)	54,4 (47.9 - 60.9)	94.3 (90.8 - 96.8)
KCCR	95.6 (78 - 99.9)	84 (63.9 - 95.5)	47.6 (25.7 - 70.2)	76.8 (65 - 86.1)	99.7 (98.7 - 100)	91.3 (72 - 98.9)	68 (46.5 - 85)	19 (5.4 - 41.9)	60.8 (48.4 - 72.4)	99 (97.6 - 99.7)
UI	95.5 (84.8 - 99.5)	90 (55.5 - 99.7)	42.9 (9.9 - 81.6)	88.7 (78.1 - 95.3)	100 (99.1- 100)	95.5 (84.8 - 99.5)	90 (55.5 - 99.7)	42.9 (9.9 - 81.6)	88.7 (78.1 - 95.3)	100 (99.1 - 100)
INRB	89.2 (79.8 - 95.2)	50 (28.2 - 71.8)	3.6 (0.1 - 18.3)	62.9 (53.8 - 71.4)	99.6 (97.9 - 100)	25 (8.7 - 49.1)	16.6 (0.4 - 64.1)	0 (0 - 70.7)	20.4 (8 - 39.7)	100 (96 - 100)
UofK	60.5 (40.4 - 76)	23 (5 - 53.8)	14.3 (4 - 32.7)	38 (27.3 - 49.6)	91.1 (87.2 - 94.3)	55.3 (38.3 - 71.4)	28.6 (8.4 - 58.1)	0 (0 - 30.9)	30.8 (21.1 - 42.1)	98.5 (96.4 - 99.6)
MAK	87.9 (79.8 - 95.6)	32 (19.5 - 46.7)	6.5 (1.4 - 18)	54.3 (47.09 - 61.5)	99.3 (97.4 - 99.9)	NA	NA	NA	NA	NA

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300

301 **Table 3.** Agreement between RT-PCR and RT-RPA assays at different sites. NA is not
302 applicable as the site did not perform the assay. Fair= 0.21 - 0.40; moderate= 0.41 - 0.60;
303 good= 0.61 - 0.80; excellent: 0.81 - 1.

Study site	Kappa Value RdRp	Agreement	p-value RdRP	Kappa Value N	Agreement	p-value N
IPD	0.878	Excellent	0.022	0.854	Excellent	<0.001
IPM	0.568	Moderate	<0.001	0.497	Moderate	<0.001
KCCR	0.843	Excellent	<0.001	0.697	Good	<0.001
UI	0.901	Excellent	0.03	0.901	Excellent	0.03
INRB	0.696	Good	<0.001	0.283	Fair	<0.001
UofK	0.328	Fair	0.05	0.382	Fair	<0.001
MAK	0.570	Moderate	<0.001	NA	NA	NA

304

305

306 **4.2 Mutational analysis of the amplicon**

307 No significant mutations were found in the RdRP amplicon across countries (supplementary
308 files #4 and #5).

309

310 **5. Discussion**

311 The worldwide spread of SARS-CoV-2 has taken viral diagnostics to a new level of
312 importance and publicity. Alternative methods to real-time RT-PCR with equal sensitivity and

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313 specificity are urgently needed to overcome shortage in supply chains [14]. The WHO
314 recommended the ASSURED criteria (affordable, sensitive, specific, user-friendly, robust,
315 deliverable to end-users) for future diagnostics [15]. Isothermal amplification assays address
316 most of these criteria as shown in many outbreak situations [16-18]. Although clinical
317 research is rapidly progressing in the field of new diagnostic tests, multi-country approaches
318 for SARS-CoV-2 are lacking, especially in sub-Saharan Africa [40]. Only one multicenter
319 study was reported using RT-loop mediated amplification in four African countries in both
320 east and west Africa with very promising sensitivity of 87% [19].

321 Using the RT-RAA technology helped to circumvent the worldwide supply shortages of real-
322 time RT-PCR test kits [20,21]. The specificity for the two SARS-CoV-2 genome targets across
323 sites ranged from 91.1 - 100% (RdRP gene RT-RAA) and 94.3 - 100% (N gene RT-RAA). In
324 contrast the SARS-CoV-2 an E gene target RT-RAA, showed a high number of false positive
325 results in a previous study and was not included in the current screening [22]. Detecting true
326 negative samples accurately at high specificity avoids unnecessary clinical implications and
327 social upset [23-25]. SARS-CoV-2 Rapid antigen tests have shown a higher false positive
328 rate (96-99.7%) [26], and especially in low prevalence settings a molecular confirmatory test
329 is needed [27].
330 When deploying both RT-RAA assays in this study, sensitivity showed a large range of
331 intercountry variations. For high viral load samples, promising overall 90.8% (RdRP RT-RAA)
332 and 81.8% (N RT-RAA) sensitivities were determined. For samples with Ct 31 - 40, values
333 were inconsistent between sites. While higher assay accuracy was identified at three sites,
334 two sites did not produce the expected outcomes despite the success during the preparatory
335 phase. Compared to the RdRP gene, the N-gene RT-RAA assay demonstrated lower
336 sensitivity. The insufficient performance of N gene RT-RAA assay led to its exclusion for
337 further testing to maximize usage of laboratory materials and resources, further underlining
338 the importance of adaptation to unanticipated events during a large diagnostic study. Overall,
339 the performance of the RT-RAA assays is much better than the commercially available rapid
340 antigen tests, whose sensitivity values differed considerably with sensitivities ranging from

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341 only 28-86 % (Ct 17- 36) [28,29]. Rapid antigen tests are suited best for detection of
342 symptomatic carriers with high viral load [30,31]. Low viral load samples are often undetected
343 as well as certain SARS-CoV-2 mutations [32,33].
344 The RdRP gene RT-RAA assay showed promising but very variable sensitivity values across
345 sites. Some sites showed more than 90% sensitivity and others under 50% even with high
346 viral load samples. A potential mutation in the target region of RdRP primers, was excluded
347 by screening over 10,000 SARS-CoV-2 RNA sequences from local strains in the involved
348 countries (Supplementary file #4 and #5) using a recently published screening method [13],
349 identifying no significant changes. Reagents deterioration during transportation was unlikely,
350 since RAA reagents are lyophilized, cold-chain independent, robust, and stable over long
351 periods of time [6]. In addition, a quality control check was conducted upon delivery of the kits
352 to exclude this possibility. Clinical sample integrity is one of the factors for decreasing assay
353 sensitivity especially since RNA is unstable [8]. Degraded RNA or samples contaminated with
354 RNases can lead to poor assay performance [20]. In our study, all samples were tested with
355 real-time RT-PCR and RT-RAA in a very short time window to assure sample integrity. Thus,
356 it can be assumed that each sample had a similar viral load when tested with both methods.
357 A limitation represents the use of different extraction kits and amplification devices across
358 sites. This adds on the variability of the clinical settings of the study. It is difficult to
359 standardize protocols and equipment across healthcare laboratories. Nonetheless, all
360 devices and equipment used were approved to be used for *in vitro* diagnostics. Nucleic acid
361 extraction remains the bottleneck of molecular diagnostics. In this study, standardized kits
362 were used to ensure RNA quality to validate the isothermal amplification assays. However, to
363 implement point-of-need molecular tests, extraction protocols need to be simplified and user-
364 friendly. Different rapid methods have been described to extract SARS-CoV-2 RNA.
365 Combinations of detergent, heat and magnetic beads can be used for quick extraction
366 [34,35]. RPA/RAA has been shown to be more tolerant against inhibitors in clinical samples
367 [36-38]. Thus, rapid extraction methods are feasible with this technology, as shown in various
368 studies [39,40]. However, further refinement is needed to enhance RNA purity and yield. In

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369 contrast, the RT-PCR is more intolerant to inhibitors from different matrices and requires
370 highly purified RNA [41-43]. Further large clinical studies are needed to combine both rapid
371 extraction and SARS-CoV-2 molecular assays on site.
372 Surprisingly, one test site discovered an unusual cluster of discordance on certain days. After
373 retesting those samples, better results were achieved. Deviations in performance of
374 diagnostic tests have been attributed to sample quality/quantity, settings, and operators [22].
375 The latter could hinder the homogeneity of sensitivity values between sites. Thus, correct
376 sample handling is an essential factor to be considered. Although the influence of individual
377 operators on the results of a diagnostic test cannot be fully avoided, certain actions could
378 help to reduce such events. In this study, continuous and in-person training was not possible
379 due to the COVID-19 pandemic and travel restrictions. Thus, the variability of the assay's
380 results could be partially explained due to this difference in quality of training. Furthermore,
381 attention to details regarding workflow should not be underestimated. For example, after
382 careful troubleshooting, inaccuracies while transcribing records and test results, or the
383 influence of reduced concentration while working after a certain hour of the day were
384 reported. As a consequence, standardized operations and in-person training for staff are of
385 utmost importance before operating diagnostic samples, in addition to quality control checks
386 [44].
387
388 The findings of this study provide evidence for the importance of the suitcase lab as a
389 deployable and feasible setup for accurate, sensitive and specific pathogen detection,
390 particularly in low-resource settings. Furthermore, the RT-RAA method, especially based on
391 the RdRP gene, is a promising on-site detection method for SARS-CoV-2 infection, overall
392 showing higher accuracy than commercially available rapid antigen test and bypassing
393 supply shortages. However, variations in assay sensitivity between sites revealed the
394 importance of quality control and face to face training for the staff. The influence of global
395 and regional disruptions should not be underestimated in large multi-country diagnostic trials.
396 Additionally, sample handling by staff was regarded as a bottleneck for test performance.

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397 Continuous in-person training is an essential tool for successful diagnostic testing, in case of
398 excellent quality and the quantity of kits and devices. These lessons learned should be
399 considered when planning and performing large multi-country diagnostic clinical trials in poor
400 resource settings.
401

402 Ethical approval

403 Ethical approval was granted from each study site before performing the testing:
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412

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416

417 Supplementary material

418 Supplementary file #1: additional information of the samples collected at each study site;
419 Supplementary file #2: laboratory report forms; Supplementary file #3: sample flowcharts,
420 results of each study site; Supplementary file #4: raw data of the mutational analysis;
421 Supplementary file #5: summary of the mutational analysis.
422

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430

431 **Declaration of interests**

432 All authors declare no conflicts of interest.

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585 **Declaration of interests**

586

587 ☒ The authors declare that they have no known competing financial interests or personal
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590 ☐The authors declare the following financial interests/personal relationships which may be
591 considered as potential competing interests:
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