1 **Head-to head comparison of anterior nares and nasopharyngeal swabs for SARS-CoV-2**

2 **antigen detection in a community drive-through test centre in the UK**

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17 **Abstract:**

18 **Objective:** To conduct a head-to-head diagnostic accuracy evaluation of anterior nares (AN)

19 and nasopharyngeal (NP) swabs for SARS-CoV-2 antigen detection using two brands of rapid

20 diagnostic tests (Ag-RDT).

21 **Methods:** Two prospective diagnostic evaluations were carried out at different time points

22 and participant cohorts to evaluate the performance of paired AN and NP swabs in two Ag-

23 RDT brands: Sure-Status (PMC, India) and Biocredit (RapiGEN, South Korea). The sensitivity

24 and specificity of AN and NP swabs for each of the index tests cohorts was calculated against

25 the reverse transcription quantitative polymerase chain reaction (RT-qPCR) TaqPath COVID-

26 19 (ThermoFisher, UK) using NP swabs as reference standard.

27 **Results:** A total of 372 participants were recruited for the Sure-Status cohort and 232 for the

28 Biocredit of which 119 (32.1%) and 122 (53.7%) were SARS-CoV-2 positive by RT-qPCR,

29 respectively. Sensitivity and specificity of AN swabs was equivalent to the obtained with NP

30 swabs in both cohorts: 83.9 (76.0-90.0) and 98.8 (96.6-99.8) utilising NP swabs and 85.6 (77.1-

31 91.4) and 99.2 (97.1-99.9) with AN swabs for Sure-Status and; 81.2% (73.1-87.7%) and 99.0%

32 (94.7-86.5%) with NP swabs and 79.5% (71.3-86.3%) and 100% (96.5-100%) with AN swabs

33 for Biocredit. The agreement of the AN and NP swabs was high for both brands with an inter-

34 rater relatability (κ) of 0.918 and 0.833 for Sure-Status and Biocredit, respectively. The overall

35 50% LoD and 95% LoD was 0.9-2.4 × 10^4 and 3.0-3.2 × 10^8 RNA copies/mL for NP swabs and

36 0.3- 1.1 x 10^5 and 0.7-7.9 x 10^7 RNA copies/mL and for AN swabs with no significant difference

37 on LoD for any of the swabs types or test brands.

38 **Conclusions:** The diagnostic accuracy of the two SARS-CoV-2 Ag-RDTs brands evaluated in this

39 study was equivalent using AN swabs than NP swabs. However, test line intensity was lower

40 when using AN swabs which could negatively influence the interpretation of the Ag-RDT

41 results by lay users.

42 **Key messages**

43 The use of AN and NP swabs for Ag-RDT detection is equivalent but further research is needed

44 on reading test lines of lower intensity to ensure Ag-RDT results are interpreted correctly.

45 **What is already known on this topic**

46 Studies on SARS-CoV-2 RT-PCR testing found that AN swabs were 12%-18% less sensitive than

47 NP swabs. Studies on Ag-RDTs using paired AN and NP are still very limited although the data

48 suggest that these are comparable.

49 **What this study adds**

50 We investigated the diagnostic accuracy of two commercially available SARS-CoV-2 Ag-RDTs

51 using paired AN and NP swabs form symptomatic patients attending a drive through test

52 centre and the diagnostic accuracy and the limit of detection were comparable in for both

53 swab types of both Ag-RDT brands. However, the test line intensity was lower when using AN

54 swabs which could influence negatively the interpretation of the Ag-RDT results for lay users.

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56 **How this study might affect research, practice, or policy**

57 The equivalent diagnostic accuracy using both swab types is an advantage as AN sampling

58 could enable scaling up antigen testing strategies. Additional studies on Ag-RDTs using AN

59 swabs on self-interpretation by a lay person are needed to ensure that low intensity test lines

60 are not classified as false negatives.

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Introduction

To meet the immense diagnostic demand of the COVID-19 pandemic, the development of rapid diagnostic tests for the detection of SARS-CoV-2 antigens (Ag-RDTs) became a priority [1]. Nasopharyngeal (NP) swabs are considered the standard of care for SARS-CoV-2 detection [2] and thus the majority of Ag-RDT kits were developed for NP swabs exclusively [1]. However, the use of anterior nasal (AN) swabs has been increasing as a less invasive alternative to promote access to testing in the community and facilitate mass testing programmes particularly in the UK [3].

For Ag-RDTs, studies on Ag-RDTs comparing sensitivity on AN swabs and NP swabs are very limited, there are three reported studies, one meta-analysis reporting pooled data on AN swabs and NP swabs [4] from 12 commercially available Ag-RDT and two studies performed a head-to-head comparison on the same Ag-RDT brand, Standard-Q (SD Biosensor, Inc., Korea), one study on professional taken swabs [5] and another in self-taken [6].

Sensitivity obtained with AN swabs was comparable (although 3% to 5% lower) than with NP swabs sensitivity but neither of the swab types fulfilled WHO target product profile (TPP) standards in either of the two published studies [7]. AN swabs are considered accurate and clinically acceptable alternatives to NP swabs in outpatient settings for SARS-CoV-2 reverse transcription polymerase chain reaction (RT-PCR) testing [8]. However, an in depth metanalysis on SARS-CoV-2 RT-PCR testing found that anterior nares specimens were 12%-18% less sensitive than NP swabs [9].

The aim of this study was to perform a head-to-head comparison of AN and NP swabs using two World Health Organisation (WHO) approved for Emergency Use Listing (WHO-EUL) SARS-CoV-2 Ag-RDT brands that are marketed for both sample types: Sure-Status COVID-19 Antigen

86   Card Test (Premier Medical Corporation, India) and Biocredit COVID-19 Antigen Test

87   (RapiGEN, South Korea) respectively.

88   This study is of particular interest in the UK as the use of home Ag-RDTs on AN swabs was

89   integral to combatting the spread of COVID-19 during the pandemic [3], as on the 1st of April

90   2022 free national RT-PCR COVID-19 testing was suspended, with the purchase of Ag-RDTs

91   using AN-swabs online or in pharmacies the only approach to access COVID-19 testing in a

92   non-clinical setting.

93   **Methods**

94   **Clinical evaluation**

95   The STARD (standard for reporting of diagnostic accuracy studies) statement was adopted as

96   a guideline for study design and reporting. This was a prospective evaluation of consecutive

97   participants enrolled at a community National Health Service (NHS) drive-through COVID-19

98   test centre located at the Liverpool John Lennon Airport. Two Ag-RDT brands were evaluated;

99   Sure-Status COVID-19 Antigen Card Test (Premier Medical Corporation India) and Biocredit

100   COVID-19 Antigen Test (RapiGEN, South Korea) referred as Sure-Status and Biocredit

101   thereafter. Swab samples used for the evaluation of Sure-Status were obtained from

102   participants recruited between August and October 2021 and for the evaluation of Biocredit

103   from participants recruited between December 2021 and March 2022. The study progressed

104   until at least 100 Ag-RDT positives using AN swabs in line with WHOs requirements for

105   evaluation of alternative sample type [10].

106   All adults over the age of 18 who attended the drive-through test centre with symptoms of

107   COVID-19 were asked to participate in the study. The symptoms included fever, cough,

108   shortness of breath, tight chest, chest pain, runny nose, sore throat, anosmia, ageusia,

109   headache, vomiting, abdominal pain, diarrhoea, confusion, rush, or tiredness. Participants

110   were recruited under the Facilitating Accelerated COVID-19 Diagnostics (FALCON) study using

111   verbal consent. Ethical approval was obtained from the National Research Ethics Service and

112   the Health Research Authority (IRAS ID:28422, clinical trial ID: NCT04408170).

113   Swabs were collected by trained health care workers following the same process with the NP

114   swab collected first in one nostril and placed in Universal Transport Media (UTM) (Copan

115   Diagnostics Inc, Italy) for the reference RT-qPCR test. This was followed by the collection of

116   two swabs to evaluate the Ag-RDTs, first an NP swab in the other nostril and finally a AN swab

117   in both nostrils following the manufacturer's instructions for use (IFU). Samples were given a

118   unique identification code and transported within cooler bags to the Liverpool School of

119   Tropical Medicine (LSTM) where samples were processed in category level 3 (CL3)

120   containment laboratory upon arrival by trained research technicians.

121   Sure-Status and Biocredit Ag-RDTs were carried out following their instructions for use (IFU).

122   The protocol for both Ag-RDT was the same when using AN and NP swabs. Results were read

123   by two operators, blinded to one another and if a discrepant result occurred, a third operator

124   acted as a tiebreaker. The visual read out of the Ag-RDT test band was scored on a quantitative

125   scale from 1 (weak positive) - 10 (strong positive). Ag-RDT results were classified as invalid

126   when the control line was absent. Photos were taken of all Ag-RDTs and results were QC

127   against the reported results by the operators performing and interpreting the Ag-RDT results.

128   RNA was extracted using the QIAamp® 96 Virus QIAcube® HT kit (Qiagen, Germany) on the

129   QIAcube® (Qiagen, Germany) and screened using TaqPath COVID-19 (ThermoFisher, UK) on

130   the QuantStudio 5™ thermocycler (ThermoFisher, UK), an internal extraction control was

131   incorporated before the lysis stage, as recommended by the manufacturer. SARS-CoV-2 RT-

132   qPCR result was considered (1) positive if any two of the three SARS-CoV-2 target genes (N

133   gene, ORF1ab and S gene) amplified with cycle threshold (Ct) ≤ 40, (2) indeterminate if only

134   one SARS-CoV-2 gene amplified and (3) negative if the internal extraction control amplified

135   and the SARS-CoV-2 target genes did not. Samples with invalid RT-qPCR results (no

136   amplification of the internal extraction control) were re-extracted and re-run once. Viral loads

137   in UTM swabs were measured with a ten-fold serial dilution standard curve of quantified

138   specific in vitro-transcribed RNA using five replicates for each standard curve point [11].

139   **Statistical Analysis**

140   Sensitivity, specificity, positive predicted value (PPV) and negative predictive values (NPV)

141   were calculated with 95% confidence intervals (CIs) by comparing the Ag-RDT results by swab

142   type to the RT-qPCR, as the reference standard. Sub-analyses of diagnostic performance were

143   performed by swab type (AN and NP), Ct-value ranges, onset of symptoms and vaccination

144   status using nonparametric statistics. The level of agreement between AN and NP swabs was

145   determined using Cohen's kappa (κ) [10]. The correlation between test line intensity and viral

146   loads were measured by Person correlation, coefficient (r_P) [12] and to further analyse Ag-RDT

147   sensitivities, we used logistic regression, with RNA copy numbers of the RT-qPCR NP swab and

148   swab type (AN and NP) as independent variables and test outcomes as the dependent

149   variable, yielding detection probabilities for each viral load level. Statistical analyses were

150   performed using SPSS V.28.0, Epi Info V3.01 and R scripts. Statistical significance was set at *P*

151   < 0.05.

152   **Patient and public involvement**

153 Participants were not involved in setting the research question, the outcome measures or the

154 design and implementation of the study. Patients did not receive the results of the RDTs

155 evaluated in the study.

156 **Results**

157 Participant demographics

158 A total of 604 participants were recruited for this study, 372 recruited between August and

159 October 2021 were enrolled for the Sure-Status Ag-RDT evaluation and 232 recruited

160 between December 2021 and March 2022 were enrolled for the Biocredit Ag-RDT evaluation

161 (supplementary material 1). Details of the demographics of the population of study are found

162 in Table 1. Our study population had a mean age of 43 years (range 18-81, interquartile range

163 [IQR] 33.0-50.0), 348 (58%) were female and 566 were British (94%), with the remaining 36

164 participants being of other nationalities. Three hundred and fourteen participants of the 372

165 enrolled for the Sure-Status evaluation (84.4%) and 217 participants of the 232 recruited for

166 Biocredit (93.5%) received complete SARS-CoV-2 vaccination (2 doses). Additionally, 143 of

167 the participants enrolled from December 2021 (61.6%) for the Biocredit evaluation received

168 a third dose as part of the UK booster roll out [13]. All participants were symptomatic with a

169 median onset of symptoms of 2 days (IQR 1-3). The most common symptoms were cough

170 (387, 64.3%), sore throat (232, 38.5%), headache (203, 33.7%), fever (160, 26.6%), body aches

171 (80, 13.3%) and runny nose (80, 13.3%) (Table 1).

172 Overall, 240 participants (40.1%, CI95% 36.1-44.1%) were SARS-CoV-2 positive by RT-qPCR, 5

173 had indeterminate RT-qPCR results and the remaining were negative. Participants with

174 indeterminate RT-qPCR results were excluded from further analysis.

175 RT-qPCR positivity was significantly higher (p<0.05) among the participants enrolled for the

176 Biocredit evaluation cohort (53.7%, CI95% 47-60.4%) during December 2021 and March 2022

177 which coincided with the Omicron wave in the UK [14] than among the participants enrolled

178 between August and October 2021 (31.7%, CI95% 27.0-36.7%) when Delta was the dominant

179 SARS-CoV-2 variant.

180 Diagnostic evaluations

181 *Sure Status*

182 The sensitivity and specificity for the Sure-Status Ag-RDT compared to RT-qPCR was 83.9

183 (76.0-90.0) and 98.8 (96.6-99.8) utilising NP swabs and 85.6 (77.1-91.4) and 99.2% (CI95%

184 97.1-99.9%) with AN swabs. For individuals with Cts < 25, the sensitivity was 92.8% (CI95%

185 85.7-97.1%) and 94.9% (CI95% 88.4-98.3%) for NP and AN-swabs respectively. Seven Ag-RDTs

186 gave invalid results, one NP swab (0.03%) sample and six AN swab samples (1.6%). The

187 difference of invalid results by sample type was not statistically significant by Fisher test (*P* =

188 0.06381). Invalid Ag-RDTs results were excluded from further analysis. Four SARS-CoV-2

189 positive cases were detected by NP only (3.4%) and six cases were detected by AN only (5.0%)

190 but this discrepancy on sensitivity between swab types was not significant (*P* = 0.43). The

191 percentage of agreement of NP and AN swab using Sure-Status was 96.7% (95% CI 94.7-

192 98.5%) and inter-rater reliability was almost perfect (κ = 0.918). Inter-rater reliability was

193 strong for both NP (κ = 0.871) and AN (κ = 0.852) swabs when compared to RT-qPCR.

194 *Biocredit*

195 For the Biocredit Ag-RDT the sensitivity and specificity were 81.2% (CI95%73.1-87.7%) and

196 99.0% (CI95%94.7-86.5%) with NP swabs and 79.5% (CI95%71.3-86.3%) and 100%

197   (CI95%96.5-100%) with AN sampling compared to RT-qPCR. Sensitivity was 92.2% (CI95%84.6-

198   96.8%) and 95.5% (CI95%89.0-98.8%) using NP and AN swabs among participants with Ct <

199   25. Ten SARS-CoV-2 positive cases were detected solely by NP (8.2%) and eight cases were

200   detected only by AN (6.6%) but no significance on sensitivity was observed between NP and

201   AN swabs for this brand of Ag-RDTs either (P = 0.43). No invalid results were observed for this

202   Ag-RDT. The percentage of agreement of NP and AN swab for Biocredit was 91.6% (95% CI

203   87.2-94.9%) and inter-rater reliability was strong (κ = 0.833). Inter-rater reliability was

204   moderate for both NP (κ = 0.790) and AN (κ = 0.782) sampling compared to RT-qPCR.

205   Diagnostic accuracy for both Sure-Status and Biocredit is displayed in Table 2.

206   *Head-to-head comparison of Sure Status and Biocredit*

207   We report non-significant difference in the diagnostic accuracy among participants with

208   symptoms irrespective of days since onset, or vaccination status for all Ag-RDTs and swabbing

209   combination (all P values > 0.05). Both Biocredit and Sure-Status Ag-RDTs using both swab

210   types had better sensitivities on detecting SARS-CoV-2 antigens on individuals with Ct values

211   < 25 than >30 (P = 0.029 in NP and P = 0.032 in AN for Sure-Status and P = 0.018 and P = 0.0002

212   for Biocredit).

213   The RNA copy numbers per mL (RNA copies/mL) of RT-PCR NP swabs was calculated and

214   statistically higher viral loads were obtained for the Sure-Status cohort than Biocredit (Figure

215   1) measured by Kruskal–Wallis (P= 0.006). We determined the 50% and 95% limits of

216   detection (LoD) for both Ag-RDT and swab types based on a logistic regression model (Figure

217   2). For Sure-Status, the RNA copies/mL for 50% LoD and 95% LoD were 2.4×10^4 and 3.2×10^8

218   for NP specimen and 3.4×10^4 and 7.94×10^7 for AN swabs. All participants that had a negative

219   Ag-RDT result using AN swab and a positive result using NP swab had a viral load below 95%

220   LoD of both swab types (3.0×10^5 - 4.4×10^6 copies/mL). Five out of six participants that had a

221   negative Ag-RDT result using NP swab and a positive result using AN swab had a viral load

222   below 95% LoD of both swab types (3.3×10^5 - 1.8×10^7 copies/mL) and one above (1.9×10^9).

223   For Biocredit, the RNA copies/mL for LoD50 and LoD95 were 9.12×10^3 and 3.02×10^8 for NP

224   specimen and 1.12×10^5 and 6.76×10^6 for AN swabs. Although the LoD95 was better for AN

225   swabs for both Ag-RDT brands (3.98 for Sure-Status and 44.67 for Biocredit), there was no

226   statistical difference on LODs neither by swab type and Ag-RDT brand (all P values > 0.05).

227   All participants that had a negative Ag-RDT result using AN swab and a positive result using

228   NP swab had a viral load below 95% LoD of both swab types (3.6×10^1 – 3.7×10^6 copies/mL).

229   Seven out of eight participants that had a negative Ag-RDT result using NP swab and a positive

230   result using AN swab had a viral load below 95% LoD for NP swab (4.4×10^4 - 1.6×10^8

231   copies/mL) and one above (2.0×10^9 copies/mL).

232   *Quantitative read-out analysis*

233   Quantitative read-out in paired positive AN and NP was more often higher for the NP (40

234   instances higher on NP and four higher on AN in Sure-Status; and 35 instances higher on NP

235   and 12 higher on AN in Biocredit) and gave significantly higher scores for both Ag-RDT, Sure-

236   Status (P = 0.007) and Biocredit (P = 0.013) (Figure 3) measured by Kruskal–Wallis.

237   Additionally, test lines scores were analysed by RNA copies/mL and these had a positive

238   correlation. For Biocredit, strong correlation using AN swabs (r_P = 0.727) but moderate using

239   NP swabs (r_P = 0.591). For Sure-Status, both swab types had a moderate correlation to viral

240   loads (NP swab r_P = 0.614 and AN swab r_P = 0.661).

241   **DISCUSSION**

242   To our knowledge, this is the first diagnostic clinical evaluation of Sure-Status Ag-RDT at the

243   time of this publication and the point estimates for sensitivity (≥80%) and specificity (≥97%)

244   have shown a satisfactory performance for both AN and NP swabs fulfilling the target product

245   profile (TPP) WHO standards [7] although the lower bound of the 95%CI were below the TPP

246   threshold. Further evaluations should be performed with larger sample size for a more precise

247   estimate.

248   For Biocredit Ag-RDT, there are five studies to date that have evaluated the performance of

249   NP swabs reporting varied sensitivities from 52% to 85% [4]. In this study we reported a

250   sensitivity and specificity of 81.2%, CI95%73.1-87.7% and 99.0%, CI95%94.7-86.5% fulfilling

251   the WHO standards using NP swab when using the point estimates for sensitivity and

252   specificity. Biocredit Ag-RDT point estimate for sensitivity was below the threshold (79.5%,

253   CI95% 71.3-86.3%) of the WHO TPP when using AN. Differences in sensitivity between sample

254   type and Ag-RDT brands was not statistically significant.

255   It was observed during Ag-RDT testing that AN swabs had higher viscosity when compared to

256   NP swabs. Although no significant, this viscosity caused inappropriate sample flow in Sure-

257   Status RDT giving the higher invalid rate compared to NP swabs.

258   Even through the Ag-RDT were evaluation in different cohorts (different recruitment times,

259   SARS-CoV-2 variant, etc), results presented here demonstrate that AN swabs are equivalent

260   to NP swabs for SARS-CoV-2 Ag-RDT testing giving comparable sensitivities, 50% LoD and 95%

261   LoD for both Ag-RDTs brands evaluated here. Our results supports previous findings where

262   AN and NP swabs were compared for the Ag-RDT Standard-Q (SD Biosensor, Inc., Korea) in

263   Lesotho [5], and also obtained lower sensitivities in AN (67.3%) than NP (70.2%) swabs [5].

264   Studies on RT-qPCR have found lower sensitivity using AN swabs compared to NP swabs

265 consistently [9]. However, the difference in sensitivity was only significant for patients with

266 viral loads < 10^3 copies/mL [15] and this threshold is not relevant to Ag-RDTs of which the limit

267 of detection ranges between 10^4- 10^8 RNA copies/mL in swabs [11].

268 Quantitative assessment of the test line scores showed that test line intensity was

269 significantly higher on NP swabs than AN swabs. The line intensity is an important component

270 of home testing as studies have shown fainter lines are more difficult to interpret for a lay

271 person, likely due to lower signal intensity [16]. In an user experience home based study,

272 77.1% of the cases that the participants interpreted wrongly as negative being positive, were

273 weak and moderate positives while only 22.9% were strong positives [16]. The lower intensity

274 of the AN swab compared to NP swab is likely attributed to the differences of SARS-CoV-2

275 viral loads in the respiratory tract. Studies have found lower viral loads on AN swabs

276 compared to NP swabs [15]. Statistical analysis supported this hypothesis where a positive

277 correlation between viral loads and Ag-RDT test line scores was shown. Further

278 implementation studies on Ag-RDT test results interpretation by patients or within a home

279 testing setting are urgently needed to drive self-testing to scale.

280 This study has several strengths, the use of standardised sampling methods, independent

281 blinded readers, robust statistical analysis, quantitative assessment of Ag-RDT test line results

282 and the evaluation of two approved WHO-EUL Ag-RDT test brands. Qualifying it to have high

283 global public health relevance [17].

284 The main limitation of this study is that, although the operators were blinded to each other

285 and to the SARS-CoV-2 RT-qPCR result, they were not blinded to the swab type as the shape

286 and size of these is different and the tests were done in parallel. We do not expect that this

287 could have caused a bias in reading the Ag-RDT results as the reference standard result was

288   unknown for the operators and data was QC but this is a consideration for future studies.

289   Another limitation of this study is that the AN swabs were always taken last. The order of

290   sample collection could have negatively biased the results obtained for AN swabs caused by

291   a possible sample depletion. However, in the two studies that compared Ag-RDT using AN

292   swabs, the AN swab was collected first and in both studies the sensitivity obtained with AN

293   swabs was lower than when using NP swabs [5,6]. Further, studies on RT-qPCR found also

294   lower sensitivity using AN swabs compared to NP swabs [9], even when AN swabs were

295   collected first [15,18,19]. Thereby it is unlikely that the order of the swabs impacted sample

296   availability for AN and NP sampling.

297   An interesting point to discuss is the circulation of different SARS-CoV-2 variant of concern

298   during the study period. Sure-Status was evaluated during Delta wave and Biocredit during

299   the Omicron wave. A later study to this one evaluated the analytical sensitivity of Biocredit

300   and Sure-Status using clinical samples positive to Omicron and Delta [20]. Interestingly, both

301   Ag-RDT brands had higher sensitivity point estimates among Omicron positive samples than

302   Delta, however this was not significant suggesting that it is unlikely that the SARS-CoV-2 strain

303   present at the time of evaluation had impact on the sensitivity estimates of the ag-RDTs.

304   In conclusion, this study demonstrates the sensitivity of two SARS-CoV-2 Ag-RDTs using AN-

305   sampling are comparable to that of NP-sampling. AN-sampling can be performed with less

306   training, reduces patient discomfort, and enables scaling up of antigen testing strategies. Test

307   line intensity however is lower when using AN swabs which could influence negatively the

308   interpretation of the Ag-RDT results. Additional studies on Ag-RDTs using AN swabs on self-

309   interpretation by a lay person are needed and further education around how to interpret a

310   positive Ag-RDT to the wider community.

CONTRIBUTION STATEMENT

ACA and ERA secured the funding for this research in collaboration with RB, MdV and CE. ACA

designed the study. KC, JW collected the samples and RLB, KK, KC, MM and CTW performed all

laboratory procedures and analysis. GA led all data analysis.

RLB and ACA prepared the first draft of this manuscript and all other authors (GA, KK, KC, MM, JW,

CTW, RB, ERA, MdV, CE) edited, reviewed and approved the final version of this manuscript. ACA is

the guarantor of this study.**COMPETING INTERESTS**

The authors report no competing interests.

336   support during the study and Daisy Bengey, Rachel Watkins, and Lorna Finch for their help on

337   sample collection and recruitment.

338   **ETHICS APPROVAL**

339   Ethical approval was obtained from the National Research Ethics Service and the Health

340   Research Authority (IRAS ID:28422, clinical trial ID: NCT04408170).

341   **REFERENCES**

342   1   FIND. SARS-CoV-2 diagnostic pipeline. https://www.finddx.org/covid-19/pipeline/

343   2   Center of Disease Control and Prevention. Interim Guidelines for Collecting, Handling, and
344   Testing Clinical Specimens for COVID-19. May 22. 2020.

345   3   GOV.UK. New campaign urges public to get tested twice a week. 2021.

346   4   Brümmer LE, Katzenschlager S, Gaeddert M, *et al.* Accuracy of novel antigen rapid diagnostics
347   for SARS-CoV-2: A living systematic review and meta-analysis. PLoS Medicine. 2021.

348   5   Labhardt, Niklaus D González Fernández L, Katende, Bulemba Muhairwe J, Bresser M, *et al.*
349   Head-to-head comparison of nasal and nasopharyngeal sampling using SARS-CoV-2 rapid
350   antigen testing in Lesotho. *medRxiv*. Published Online First: 2022. doi:
351   10.1101/2021.12.29.21268505

352   6   Lindner AK, Nikolai O, Kausch F, *et al.* Head-to-head comparison of SARS-CoV-2 antigen-
353   detecting rapid test with self-collected anterior nasal swab versus professional-collected
354   nasopharyngeal swab. *European Respiratory Journal*. 2020;57:2003961.

355   7   (WHO) WHO, R&D Blue Print WH (HQ). Target product profiles for priority diagnostics to
356   support response to the COVID-19 pandemic v.1.0. 2020.

357   8   Péré H, Péré H, Péré H, *et al.* Nasal swab sampling for SARS-CoV-2: A convenient alternative
358   in times of nasopharyngeal swab shortage. Journal of Clinical Microbiology. 2020.

359   9   Zhou Y, OLeary TJ. Relative sensitivity of anterior nares and nasopharyngeal swabs for initial
360   detection of SARS-CoV-2 in ambulatory patients: Rapid review and meta-Analysis. PLoS ONE.
361   2021.

362   10   World Health Organization (WHO). Prequalification Teams Diagnostics. Instructions and
363   requirements for Emergency Use Listing (EUL) Submission: In vitro diagnostics detecting
364   SARS-CoV-2 nucleic acid or antigen.

365   11   Cubas-Atienzar, Ana I., Kontogianni K, Edwards T, *et al.* Limit of detection in different
366   matrices of 19 commercially available rapid antigen tests for the detection of SARS-CoV-2.
367   *Scientific Reports*. 2021;11:1–8. doi: https://doi.org/10.1038/s41598-021-97489-9

368   12   Schober P, Schwarte LA. Correlation coefficients: Appropriate use and interpretation.
369   *Anesthesia and Analgesia*. Published Online First: 2018. doi:
370   10.1213/ANE.0000000000002864

371 13 GOV.UK. Vaccinations in the UK. Coronavirus (Covid-19) in the UK. 2022.
372   https://coronavirus.data.gov.uk/details/vaccinations

373 14 Mahase E. Covid-19: Is the UK heading for another omicron wave? *The BMJ*. 2022;376. doi:
374   https://doi.org/10.1136/bmj.o738

375 15 Callahan C, Lee R, Lee G, *et al.* Nasal-Swab Testing Misses Patients with Low SARS-CoV-2 Viral
376   Loads. *medRxiv*. 2020.

377 16 Jing M, Bond R, Robertson LJ, *et al.* User experience of home-based AbC-19 SARS-CoV-2
378   antibody rapid lateral flow immunoassay test. *Scientific Reports*. 2022;12:1173. doi:
379   10.1038/s41598-022-05097-y

380 17 World Health Organization (WHO). Coronavirus disease (COVID-19) Pandemic — Emergency
381   Use Listing Procedure (EUL) open for IVDs. Prequalification of Medical Products (IVDs,
382   Medicines, Vaccines and Immunization Devices, Vector Control).
383   https://extranet.who.int/pqweb/vitro-diagnostics/coronavirus-disease-covid-19-pandemic-—
384   -emergency-use-listing-procedure-eul-open

385 18 Hanson KE, Barker AP, Hillyard DR, *et al.* Self-collected anterior nasal and saliva specimens
386   versus health care worker-collected nasopharyngeal swabs for the molecular detection of
387   SARS-CoV-2. *Journal of Clinical Microbiology*. Published Online First: 2020. doi:
388   10.1128/JCM.01824-20

389 19 Tu Y-P, Jennings R, Hart B, *et al.* Swabs Collected by Patients or Health Care Workers for
390   SARS-CoV-2 Testing. *New England Journal of Medicine*. Published Online First: 2020. doi:
391   10.1056/nejmc2016321

392 20 Byrne RL, Aljayyoussi G, Greenland-Bews C, *et al.* Comparison of the analytical and clinical
393   sensitivity of thirty-four rapid antigen tests with the most prevalent SARS-CoV-2 variants of
394   concern during the COVID-19 pandemic in the UK. *medRxiv*. 2023;2023.07.24.23293072. doi:
395   10.1101/2023.07.24.23293072

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397   **Supplementary material 1. Diagram of participant flow in the study.**

398   **Figure 1. Boxplot of the SARS-CoV-2 viral load distribution of the RT-qPCR NP swabs used**
399   **as reference standard for the participants enrolled for Sure-Status and Biocredit Ag-RDT**
400   **evaluation.** The whiskers show the maximum and minimum values and the vertical line the
401   median. Asterisks indicate statistical significance between AN and NP swab types.

402   **Figure 2. Limit of detection analyses of upper-respiratory samples positive by RT-qPCR for**
403   **Sure-Status and Biocredit using AN and NP swabs.** The log10 RNA copies on the x axis were
404   plotted against a positive (1.0) or negative (0.0) Ag-RDT result on the y axis. Green (Sure-
405   Status) and purple (Biocredit) curves show logistic regressions of the viral load on the Ag-
406   RDT result; vertical dashed lines indicate log10 RNA copies subjected to the test at which
407   50% and 95% LoD of the samples are expected positive based on the regression results. No
408   significant differences were observed.

409   **Figure 3. Boxplot of the scores of the test lines for both Ag-RDT Sure-Status and Biocredit**
410   **using AN and NP swabs.** The whiskers show the maximum and minimum values and the
411   vertical line the median. Asterisks indicate statistical significance between AN and NP swab
412   types.

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414 **Table 1.** Demographics of the population of study for Sure-Status and Biocredit cohorts

	Sure-Status	Biocredit	All
Total	372	232	604
Age [mean (min-max), IQR]	43 (18-81), 33-53	43 (18-78), 33-51	43 (18-81), 33-52
Gender [%F, (n/N)] IQR]	57%, (211/372)	59%, (137/232)	58%, (348/602)
Triple vaccinated (n, %)	NA*	143 (61.6%)	143 (23.8%)
Double vaccinated (n, %)	314 (84.4%)	74 (40%)	388 (64.4%)
Partially vaccinated (n, %)	29 (7.8%)	4 (1.7%)	33 (5.5%)
Not vaccinated (n, %)	27 (7.3%)	10 (4.3%)	37 (6.2%)
Vaccination not disclosed (n, %)	2 (0.5)	1 (0.3%)	3 (0.5%)
Days symptoms onset [median (IQR); N]	2 (1-3), 371	2 (1-3), 232	2 (1-3), 601
Days 0-3 (n, %)	304, 81.7%	186, 80.2%	490, 81.1%
Days 4-7 (n, %)	56, 15.1%	41, 17.7%	97, 16.1%
Days 8+ (n, %)	10, 2.7%	5, 2.2%	15, 2.5%
RT-qPCR SARS-CoV-2 Positivity [%, (n/N)]	31.7%, (118/372)	53.7%, (122/227)	40.1% (240/599)
Symptom [total n (%), in RT-qPCR positive n (%)]			
Cough	248 (66.7%), 73 (61.3%)	139 (60.0%), 71 (58.2%)	387 (64.3%), 144 (60.0%)
Sore throat	129 (34.7%), 34 (28.6%)	103 (44.4%), 56 (45.9%)	232 (38.5%), 90 (37.4%)
Headache	123 (33.1%), 57 (47.9%)	80 (34.5%), 45 (36.9%)	203 (33.7%), 102 (42.3%)
Fever	106 (28.5%), 30 (25.2%)	54 (23.3%), 28 (22.9%)	160 (26.6%), 58 (24.1%)
Body aches	41 (11.0%), 21 (17.7%)	39 (16.8%), 29 (23.8%)	80 (13.3%), 51 (21.2%)
Runny nose	39 (13.2%), 20 (16.8%)	41 (17.7%), 31 (25.4%)	80 (13.3%), 51 (21.2%)
Loss taste	48 (12.9%), 19 (16.0%)	19 (8.2%), 10 (8.2%)	67 (11.1%), 29 (12.0%)
Loss smell	29 (7.8%), 9 (7.6%)	14 (6.0%), 7 (5.7%)	43 (7.1%), 16 (6.6%)
Chest pain	18 (4.8%),7 (5.9%)	12 (5.2%), 8 (6.6%)	30 (5.0%), 15 (6.2%)
Fatigue	13 (3.5%), 4 (3.4)	17 (7.3%), 10 (8.2%)	30, (5.0%), 14 (5.8%)
Shortness of breath/tight chest	13 (3.5%), 3 (2.5%)	9 (3.9%), 5 (4.1%)	22 (3.6%), 15 (6.2%)
Vomiting	11 (3%), 5 (4.2%)	2 (8.6%), 2 (1.6%)	13 (2.2%), 7 (2.9%)
Diarrhoea	9 (2.4%), 3 (2.5%)	3 (13%), 3 (2.5%)	12 (2.0%), 6 (2.5%)
Abdominal pain	6 (1.6%), 3 (2.5%)	1 (0.4%), 1 (0.8%)	7 (1.2%), 4 (1.7%)
Rash	3 (0.8%), 0 (0.0%)	1 (0.4%), 1 (0.8%)	4 (0.6%), 1 (0.4%)
Confusion	1 (0.3%), 0 (0.0%)	0 (0%)	1 (0.2%), 0 (0%)
Other	159 (42.7%), 68 (57.4%)	134 (57.8%), 85 (69.7%)	293 (48.7%), 153 (63.5%)

415 *****Participants were enrolled before booster rolled out in the UK

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420   **Table 2**. Clinical sensitivity and specificity of Sure-Status and Biocredit using NP and Nasal Swab

All Ct values	TP	FP	TN	FN	Sensitivity	Specificity	NPV	PPV
Sure-Status								
NP swab	99	3	250	19	83.9 (76.0-90.0)	98.8 (96.6-99.8)	92.9 (89.7-95.2)	97.1 (91.44-99.03)
AN swab	101	2	246	17	85.6 (77.1-91.4)	99.2 (97.1-99.9)	93.5 (90.3-95.1)	98.1 (92.7-99.5)
Biocredit								
NP swab	99	1	104	23	81.2 (73.1-87.7)	99.0 (94.7-86.5)	81.9 (73.99-85.2)	99.0 (93.4-99.9)
AN swab	97	0	105	25	79.5 (71.3-86.3)	100 (96.5-100)	80.8 (74.8-85.6)	100 (100-100)
All NP	198	4	354	42	82.5 (77.1-87.1)	98.9 (97.2-99.7)	89.4 (86.5-91.7)	98.0 (94.9-99.2)
All AN	198	2	351	42	82.5 (77.1-87.1)	99.4 (97.9-99.9)	89.3 (86.4-91.7)	99.0 (96.1-99.8)

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