

1 **Title:** Assessment of Inactivation Procedures for SARS-CoV-2

2

3 **Authors:** Heidi AUERSWALD¹, Sokhoun YANN¹, Sokha DUL², Saraden IN¹, Philippe
4 DUSSART¹, Nicholas J. MARTIN³, Erik A. KARLSSON^{1*}, Jose A. GARCIA-RIVERA^{2#}.

5

6 **Affiliations:**

7 ¹ Virology Unit, Institut Pasteur du Cambodge, Institut Pasteur International Network, Phnom
8 Penh, Cambodia

9 ² Naval Medical Research Unit TWO, Phnom Penh, Cambodia

10 ³ Naval Medical Research Unit TWO, Singapore

11

12 *For correspondence on COVID-19 and other emerging infectious disease diagnostic testing and
13 response in Southeast Asia, please contact Dr. Erik Karlsson, ekarlsson@pasteur-kh.org

14 #For correspondence on diagnostic testing for infectious disease on naval vessels and other
15 austere sites, please contact Dr. Jose A. Garcia-Rivera, jose.a.garciarivera.mil@mail.mil

16

17 **Keywords:** SARS-CoV-2, heat inactivation, chemical inactivation, COVID-19, biosafety

18

19

20

21

22

23

24 **Abstract**

25 Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), the causative agent of
26 Coronavirus disease 2019 (COVID-19), presents a challenge to laboratorians and healthcare
27 workers around the world. Handling of biological samples from individuals infected with the
28 SARS-CoV-2 virus requires strict biosafety and biosecurity measures. Within the laboratory, non-
29 propagative work with samples containing the virus requires, at minimum, Biosafety Level-2
30 (BSL-2) techniques and facilities. Therefore, handling of SARS-CoV-2 samples remains a major
31 concern in areas and conditions where biosafety and biosecurity for specimen handling is difficult
32 to maintain, such as in rural laboratories or austere field testing sites. Inactivation through physical
33 or chemical means can reduce the risk of handling live virus and increase testing ability worldwide.
34 Herein we assess several chemical and physical inactivation techniques employed against SARS-
35 CoV-2 isolates from Cambodian COVID-19 patients. This data demonstrates that all chemical
36 (AVL, inactivating sample buffer and formaldehyde) and heat treatment (56°C and 98°C) methods
37 tested completely inactivated viral loads of up to 5 log₁₀.

38

39

40

41

42

43

44

45

46

47 **Introduction**

48 Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), the causative agent of
49 Coronavirus disease 2019 (COVID-19) has rapidly spread across the world. On January 30th, 2020,
50 the World Health Organization (WHO) declared the outbreak a Public Health Emergency of
51 International Concern and upgraded it to a pandemic on March 11, 2020 [1]. As of May 28th,
52 2020, there have been over 5.6 million laboratory-confirmed cases and greater than 350,000 deaths
53 reported globally [2]. Extensive testing is necessary to ensure accurate diagnosis, contact tracing,
54 and mitigate SARS-CoV-2 spread through isolation and quarantine procedures. Additionally,
55 extensive testing facilitates the global public health response against COVID-19, providing critical
56 information regarding the effectiveness of mitigation efforts.

57 Given the lack of approved drugs and a vaccine, SARS-CoV-2 isolates should be handled
58 according to strict biosafety and biosecurity measures [3, 4]. Extreme care in handling live samples
59 prevents occupational exposure and requires extensive technical training and appropriate primary
60 and secondary containment devices wearing recommended personal protective equipment.
61 Therefore, with the need for extensive testing in areas and conditions where biosafety and
62 biosecurity for specimen handling is difficult to maintain remains a major concern. Such scenarios
63 include the need to sample outside of designated testing centers, conducting field investigations in
64 difficult locales, non-secure sample transportation, and even testing in underequipped or under-
65 maintained laboratories. Inactivation through physical or chemical means reduces the risk from
66 handling live samples and increase testing ability worldwide.

67 Cambodia is a tropical, resource poor, least developed country (LDC) in Southeast Asia
68 with a large socio-economic dependence on tourism [5]. Cambodia is also a major hotspot of
69 endemic and emerging infectious disease [6]. One particular, but not unique, issue faced in LDCs

70 is the expansion of testing capacity due to a scarcity of testing laboratories, especially in remote
71 provincial health centers. Therefore, SARS-CoV-2 samples from these rural health centers requires
72 safe but rapid transportation to designated testing sites. Aside from active training and great care
73 when handling live specimens from suspected cases, transportation of potentially infectious
74 material requires increased protective equipment and packaging, often in reduced supply or of poor
75 quality, even in the best of scenarios. Therefore, simple and effective inactivation of suspected
76 samples that can be conducted onsite can greatly decrease risk of exposure during transportation,
77 handling, and testing, as well as reduce demand for protective equipment and supplies at a current
78 global scarcity.

79 Herein, we evaluated the efficiency of various thermal and chemical inactivation methods
80 on SARS-CoV-2 utilizing three separate SARS-CoV-2 isolates cultured from patient samples
81 collected in Cambodia to determine their effect on viral infectivity and RNA integrity tested via
82 real-time RT-PCR.

83

84

85

86

87

88

89

90

91

92

93 **Materials and Methods**

94

95 **Cell lines**

96 African green monkey kidney cells (Vero; ATCC# CCL-81) were used for the isolation and culture
97 of SARS-CoV-2 isolates. Vero E6 cells were used for the titration of infectious virus via TCID₅₀.
98 Both cell lines were cultivated in Dulbecco's modified Eagle medium (DMEM; Sigma-Aldrich,
99 Steinheim, Germany) supplemented with 10% fetal bovine serum (FBS; Gibco, Gaithersburg, MD,
100 USA) and 100 U/ml penicillin-streptomycin (Gibco) at 37°C and 5% CO₂ atmosphere. Upon
101 infection with SARS-CoV-2 the culture medium was replaced by infection medium containing
102 DMEM, 5% FBS, antibiotics, 2.5 µg/mL Amphotericin B (Gibco) and 16 µg/mL TPCK-trypsin
103 (Gibco).

104

105 **Virus culture and titration**

106 Three SARS-CoV-2 isolates (designated hCoV-19/Cambodia/1775/2020, 1775; hCoV-
107 19/Cambodia/2018/2020, 2018; and, hCoV-19/Cambodia/2310/2020, 2310) were obtained from
108 patient's swabs (combination of one nasopharyngeal and one oral swab in one tube of viral
109 transport medium) and cultured in Vero cells. Virus-containing supernatants, as determined by the
110 presence of cytopathic effect (CPE), were harvested six days after infection by centrifugation at
111 1,500 rpm for 10 min. The concentration of viable virus was measured by TCID₅₀ assay on Vero
112 E6 cells in 96-well plates (TPP, Trasadingen, Switzerland) [7]. Briefly, serial dilutions of viral
113 culture supernatant were inoculated onto cells using infection medium. After 4 days of incubation,
114 plates were inactivated with 4% formaldehyde for 20 minutes then stained with 1% crystal violet

115 solution in phosphate buffered saline (PBS) for 20 min. Titer of viable virus was calculated
116 applying the Spearman-Karber formula [8].

117

118 **Inactivation of SARS-CoV-2 isolates**

119 Inactivation was performed in triplicate using 140 μ L aliquots of SARS-CoV-2 isolates (1775,
120 2018, and 2310; passage 3 from Vero cells). Chemical inactivation included: (i) adding 560 μ L
121 viral lysis buffer (AVL buffer including carrier RNA; AVL buffer) from the QIAamp Viral RNA
122 Mini Kit (Qiagen, Hilden, Germany) for 10 min at room temperature according the manufacturer's
123 recommendations; (ii) 200 μ L inactivating sample buffer (GeneReach, Taichung City, Taiwan)
124 containing 50% guanidinium thiocyanate (GITC) and 6% t-Octylphenoxypolyethoxyethanol
125 (Triton X-100) for 15 min at room temperature; or, (iii) 140 μ L 4% Formaldehyde in PBS (General
126 Drugs House Co. Ltd., Bangkok, Thailand) for 15 min at room temperature. Thermal inactivation
127 similarly performed on 140 μ L aliquots of fresh virus culture: (iv) 56°C for 30 min; (v) 56°C for
128 60 min; and, (vi) 98°C for 2 min in a thermo-block (Eppendorf, Hamburg, Germany). Sterile
129 DMEM treated in the similar methods served as negative controls, and untreated SARS-CoV-2
130 isolates as positive controls.

131

132 **Analysis for viable virus post inactivation**

133 To determine if any viable virus remained post inactivation, 50% Polyethylene glycol 8000
134 (Sigma-Aldrich, St. Louis, USA) in PBS was added (1/5 of total sample volume) to an aliquot
135 from each sample condition and incubated overnight at 4°C. Following incubation, virus was
136 recovered by centrifugation at 1,500 rpm for 1h. Precipitates were washed twice with sterile PBS,
137 re-constituted with infection medium, and used for infecting the TCID₅₀ on Vero E6 cells and

138 recovery cultures on Vero cells. Negative controls were treated the same way to examine
139 cytotoxicity of possible remaining traces of inactivation solutions.

140

141 **SARS-CoV-2 real-time RT-PCR**

142 Following inactivation, RNA from one aliquot per condition per virus isolate and negative control
143 was immediately extracted with the QIAamp Viral RNA Mini Kit (Qiagen) and stored at -80°C
144 until further processing. Real-time RT-PCR assays for SARS-CoV-2 RNA detection were
145 performed in duplicate using the Charité Virologie algorithm (Berlin, Germany) to detect both E
146 and RdRp genes [9]. In brief, real-time RT-PCR was performed using the SuperScript™ III One-
147 Step RT-PCR System with Platinum™ Taq DNA Polymerase (Invitrogen) on the CFX96 Touch
148 Real-Time PCR Detection System (BioRad, Hercules, CA, USA)ABI. Each 25 µl reaction mixture
149 contained 5 µl of RNA, 3.1 µl of RNase-free water, 12.5 µl of 2X PCR buffer, 1 µl of
150 SuperScript™ III RT/Platinum Taq Mix, 0.5ul of each 10 µM forward and reverse primer, and
151 0.25 µl of probe (E_Sarbeco_P1 or RdRP_SARSr-P2) using the following thermal cycling
152 conditions; 10 min at 55°C for reverse transcription, 3 min at 94°C for PCR initial activation, and
153 45 cycles of 15 s at 94°C and 30 s at 58°C.

154

155 **Statistical Analysis**

156 Statistics were performed using GraphPad Prism for Windows, version 7.02 (GraphPad Software,
157 Inc., La Jolla, CA,, USA). Analysis of variance was performed comparing mean Ct values for each
158 inactivation method. Difference between standard (AVL) and each specific inactivation method
159 was determined using Dunnett's test for many-to-one comparison. A p-value of less than 0.05 was
160 considered to indicate statistical significance. Agreement, including bias and 95% confidence

161 interval, between Ct values following inactivation by AVL and other methods was assessed using
162 a method described by Bland and Altman [10]. The mean Ct value of AVL and the other
163 inactivation method assessed was plotted on the X-axis. The difference between the two values
164 was plotted on the Y-axis. Cut-off values of 2 and -2 are plotted.

165

166

167

168

169

170

171

172

173

174

175

176

177

178

179

180

181

182

183

184 **Results**

185 **Inactivation efficiency**

186 All chemical and thermal inactivation methods resulted in the reduction of viable SARS-CoV-2 to
187 undetectable levels. Untreated virus isolates had a concentration of viable virus up to 6.67×10^5
188 (isolate 2310) before treatment (Table 1). Therefore, the reduction of viable virus across
189 inactivation levels was at least 5 log₁₀. Precipitation of virus and complete removal of inactivation
190 solution before infecting Vero E6 cells for TCID₅₀ titration ensured that no CPE was induced by
191 chemical products used in the inactivation procedure. Successful recovery of virus post-PEG
192 precipitation was determined by RT-PCR on the same samples used for TCID₅₀. All attempts to
193 recover viable virus post inactivation on Vero cells were unsuccessful up to day 6 post-inoculation.

194

195 **Effect of inactivation procedure on RT-PCR**

196 There were significant differences between the Ct values for the RdRp (ANOVA; $p < 0.0001$) and
197 E (ANOVA; $p < 0.0001$) genes. Following many-to-one comparison of AVL to all other forms of
198 inactivation used in this study (Figure 1), only formaldehyde inactivation was significantly
199 different for the RdRp (Dunnet's Test; $p = 0.0016$) and E (Dunnet's Test; $p = 0.0007$) genes. In order
200 to demonstrate the agreement in Ct values for the inactivation methods compared to the standard
201 AVL, Bland-Altman plots are graphically presented in Figure 2 with cut-off values marked at two
202 Ct differences (dashed lines). Samples inactivated by formaldehyde were the only ones where the
203 absolute bias Ct value for all samples was greater than two compared to AVL for RdRp ($-20.32 \pm$
204 1.75) and E (-19.80 ± 1.17) genes. All other inactivation methods resulted in absolute bias Ct
205 values of less than one except for the RdRp gene following inactivation at 56°C for 30 min (-1.15
206 ± 1.08), though this was still within the limits of agreement.

207 **Discussion**

208 Following the rapid global spread of SARS-CoV-2 and the need for universal testing, more
209 and more individuals are exposed to live virus samples, thereby increasing the chances of
210 occupational infection. The WHO and United States Centers for Disease Control (USCDC) have
211 released laboratory guidelines to mitigate risk of exposure during diagnostic and research
212 procedures [3, 4]. However, despite initial recommendations for handling within contained
213 biosafety cabinets, individuals working with these samples are still required to handle potentially
214 live virus at the initial steps of acquiring and preparing the suspected samples prior testing, thereby
215 increasing the risk of exposure. Potential exposure greatly increases in situations requiring large
216 numbers of samples to be processed under harsh conditions, in underequipped or poorly
217 maintained laboratories, and even within the sample transportation system, such as found in
218 developing or rural areas of the world. Therefore, the continued need for COVID-19 testing
219 worldwide requires utilization of simple and effective inactivation techniques.

220 Previous studies have been conducted on the effectiveness of chemical inactivation
221 techniques on SARS-CoV-2 [11, 12], the majority of these based on infectious agents of concern
222 such as Ebola [13] and SARS and MERS coronaviruses [14]. As with other viruses, the primary
223 step in the molecular detection of SARS-CoV-2 is viral lysis to begin the extraction of nucleic
224 acids. The buffers used in this lysis step yield varying results [11, 13, 15, 16]; however, unlike
225 previous studies [11], this study found that AVL buffer alone was successfully able to fully
226 inactivate up to 5 log₁₀ of virus from three different primary isolates of SARS-CoV-2. Apart from
227 differences in isolates utilized and a slight reduction in titer, it is unclear as to the reasons why
228 AVL buffer fully inactivated in this study versus others, but further work is warranted to determine
229 the exact effectiveness of this step alone.

230 Inactivating sample transport media, either made in-house or commercially available, also
231 presents an attractive way to inactivate samples at the point of sampling to ensure safe handling
232 along the transport chain and within the laboratory. These inactivating transport media include the
233 key components of many viral lysis buffers including chaotropic agents (GITC), detergents (Triton
234 X-100) and buffering agents (EDTA, Tris-HCL) to inactivate a preserve viral RNA. Previous
235 studies have shown that GITC-lysis buffers are able to inactivate SARS-CoV-2 samples [11, 12];
236 however, the addition of Triton-X may be necessary for complete inactivation [11]. In line with
237 these studies, commercial sample transport media containing both GITC and Triton-X was
238 successfully able to inactivate up to 5 log₁₀ of virus with no loss of molecular diagnostic sensitivity.

239 Apart from sample media and buffers utilized for diagnostic testing, various disinfectant
240 and inactivating chemicals are available for sample treatment. Formaldehyde has a long history of
241 use for inactivation against a number of viruses and in a number of fixation techniques, including
242 vaccine preparations [17, 18]. Formaldehyde has been shown to successfully inactivate both SARS
243 and MERS [14, 19, 20] and has been suggested to be a viable alternative for disinfection and
244 inactivation of SARS-CoV-2 [19, 20]. Formaldehyde treatment did successfully inactivate up to 5
245 log₁₀ of virus; however, this treatment severely impacted viral detection in subsequent molecular
246 testing. This decreased detection is not unexpected as formaldehyde treatment results in RNA
247 degradation and modification [21]. Therefore, formaldehyde treatment does not appear to be a
248 solution for increased molecular SARS-CoV-2 testing; however, it does remain a viable alternative
249 for sample inactivation or disinfection.

250 Perhaps the most studied technique thus far regarding SARS-CoV-2 has been thermal
251 inactivation at various times and temperatures [11, 22-24]. Several previous studies have shown
252 heat to be an effective inactivation technique against other coronaviruses, including SARS, MERS,

253 and human seasonal strains [14, 23, 25]. Similar to previous studies, 56°C heat treatment for 30 or
254 60 minutes was fully able to inactivate up to 5 log₁₀ of SARS-CoV-2 from three different isolates
255 [11, 22]. Interestingly, while other studies utilized 95°C for 5 to 10 minutes for inactivation, heat
256 treatment at 98°C for only 2 minutes was also able to completely inactivate up to 5 log₁₀ of virus.
257 These results are very promising as high heat treatment is extremely rapid and may be a vital
258 addition to the testing arsenal, as RT-PCR can possibly be performed directly from these samples
259 without the need for nucleic acid extraction [26, 27]. Interestingly, the shortened time period of
260 high heat treatment may mitigate some of the reduction in detection seen in previous studies and
261 make this technique more employable [11].

262 Overall, the agreement and retained sensitivity amongst RT-PCR results, combined with
263 the fact that all methods resulted in 100% virus inactivation up to a viral load of 5 log₁₀, suggests
264 that any of the tested methods, except formaldehyde, are useful to inactivate SARS-CoV-2
265 samples. Given the WHO recommendation to “test, test, test,” these data can help to optimize
266 sample inactivation for austere or remote areas. Indeed, it may be possible to use basic tools such
267 as a stopwatch and boiling water to achieve 100% virus inactivation without compromising sample
268 integrity, significantly decreasing possible exposure during sample transportation and handling,
269 allowing for dissemination of testing to labs with decreased biosafety and biosecurity capacity,
270 and possibly reducing the global demand for a dwindling supply of PPE.

271

272

273

274

275

276 **Funding**

277 This work, including that of Dr. Auerswald, was supported, in part, by internal funding through
278 Institut Pasteur in Cambodia. Dr. Karlsson is supported, in part, by the U.S. Department of Health
279 and Human Services, Office of the Assistant Secretary for Preparedness and Response (Grant No.
280 1 IDSEP190051-01-00) and through internal funding at Institut Pasteur in Cambodia. The findings
281 and conclusions in this report are those of the authors and do not necessarily represent the official
282 views of the US Department of Health and Human Services. This work was also partially funded
283 and supported by the Armed Forces Health Surveillance, Branch Global Emerging Infections
284 Surveillance Section.

285

286 **Acknowledgements**

287 We would like to acknowledge the patients, Cambodian Ministry of Health and Cambodian Center
288 for Disease Control, Rapid Response Team members, and the doctors, nurses, and staff of the
289 reference hospitals in Cambodia for their response to the COVID-19 pandemic. We thank all of
290 the technicians and staff at Institut Pasteur du Cambodge in the Virology Unit and at NAMRU-2
291 in the Molecular Unit involved in this work.

292

293 **Competing Interests**

294

295 The authors declare that they have no competing interests.

296

297

298

299 **References**

- 300 1. World Health Organization. *Coronavirus disease (COVID-19) outbreak*. 2020 [cited
301 2020 May 13th]; Available from:
302 <https://www.who.int/westernpacific/emergencies/covid-19>.
- 303 2. Dong, E., H. Du, and L. Gardner, *An interactive web-based dashboard to track COVID-*
304 *19 in real time*. *The Lancet Infectious Diseases*, 2020. **20**(5): p. 533-534.
- 305 3. United States Centers for Disease Control and Prevention. *Interim Laboratory Biosafety*
306 *Guidelines for Handling and Processing Specimens Associated with Coronavirus Disease*
307 *2019 (COVID-19)*. 2020; Available from: [https://www.cdc.gov/coronavirus/2019-](https://www.cdc.gov/coronavirus/2019-ncov/lab/lab-biosafety-guidelines.html)
308 [ncov/lab/lab-biosafety-guidelines.html](https://www.cdc.gov/coronavirus/2019-ncov/lab/lab-biosafety-guidelines.html).
- 309 4. World Health Organization. *Laboratory testing for coronavirus disease 2019 (COVID-*
310 *19) in suspected human cases: interim guidance, 2 March 2020*. 2020; Available from:
311 <https://apps.who.int/iris/handle/10665/331329>.
- 312 5. United Nations. *UN list of Least Developed Countries*. 2019 [cited 2020 May 28th];
313 Available from:
314 [https://unctad.org/en/Pages/ALDC/Least%20Developed%20Countries/UN-list-of-Least-](https://unctad.org/en/Pages/ALDC/Least%20Developed%20Countries/UN-list-of-Least-Developed-Countries.aspx)
315 [Developed-Countries.aspx](https://unctad.org/en/Pages/ALDC/Least%20Developed%20Countries/UN-list-of-Least-Developed-Countries.aspx).
- 316 6. Burgos, S. and S. Ear, *Emerging infectious diseases and public health policy: insights*
317 *from Cambodia, Hong Kong and Indonesia*. *Transbound Emerg Dis*, 2015. **62**(1): p. 96-
318 101.
- 319 7. Flint, S.J.E., W.; Racaniello, V.R.; Skalka, A.M., , "*Virological Methods*". *Principles of*
320 *Virology*. . 2009: ASM Press.

- 321 8. Ramakrishnan, M.A., *Determination of 50% endpoint titer using a simple formula*. World
322 journal of virology, 2016. **5**(2): p. 85-86.
- 323 9. Corman, V.M., et al., *Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-*
324 *PCR*. Eurosurveillance, 2020. **25**(3): p. 2000045.
- 325 10. Martin Bland, J. and D. Altman, *STATISTICAL METHODS FOR ASSESSING*
326 *AGREEMENT BETWEEN TWO METHODS OF CLINICAL MEASUREMENT*. The
327 Lancet, 1986. **327**(8476): p. 307-310.
- 328 11. Pastorino, B., et al., *Evaluation of heating and chemical protocols for inactivating SARS-*
329 *CoV-2*. bioRxiv, 2020: p. 2020.04.11.036855.
- 330 12. Scallan, M.F., et al., *Validation of a Lysis Buffer Containing 4 M Guanidinium*
331 *Thiocyanate (GITC)/ Triton X-100 for Extraction of SARS-CoV-2 RNA for COVID-19*
332 *Testing: Comparison of Formulated Lysis Buffers Containing 4 to 6 M GITC, Roche*
333 *External Lysis Buffer and Qiagen RTL Lysis Buffer*. bioRxiv, 2020: p.
334 2020.04.05.026435.
- 335 13. Smither, S.J., et al., *Buffer AVL Alone Does Not Inactivate Ebola Virus in a*
336 *Representative Clinical Sample Type*. Journal of Clinical Microbiology, 2015. **53**(10): p.
337 3148-3154.
- 338 14. Darnell, M.E.R., et al., *Inactivation of the coronavirus that induces severe acute*
339 *respiratory syndrome, SARS-CoV*. Journal of virological methods, 2004. **121**(1): p. 85-91.
- 340 15. Blow, J.A., et al., *Virus inactivation by nucleic acid extraction reagents*. Journal of
341 Virological Methods, 2004. **119**(2): p. 195-198.
- 342 16. Ngo, K.A., et al., *Unreliable Inactivation of Viruses by Commonly Used Lysis Buffers*.
343 Applied Biosafety, 2017. **22**(2): p. 56-59.

- 344 17. Sabbaghi, A., et al., *Inactivation methods for whole influenza vaccine production*.
345 *Reviews in Medical Virology*, 2019. **29**(6): p. e2074.
- 346 18. Salk, J.E., et al., *Formaldehyde treatment and safety testing of experimental poliomyelitis*
347 *vaccines*. *American journal of public health and the nation's health*, 1954. **44**(5): p. 563-
348 570.
- 349 19. Henwood, A.F., *Coronavirus disinfection in histopathology*. *Journal of Histotechnology*,
350 2020: p. 1-3.
- 351 20. Kampf, G., et al., *Persistence of coronaviruses on inanimate surfaces and their*
352 *inactivation with biocidal agents*. *Journal of Hospital Infection*, 2020. **104**(3): p. 246-251.
- 353 21. Evers, D.L., et al., *The effect of formaldehyde fixation on RNA: optimization of*
354 *formaldehyde adduct removal*. *The Journal of molecular diagnostics : JMD*, 2011. **13**(3):
355 p. 282-288.
- 356 22. Batéjat, C., et al., *Heat inactivation of the Severe Acute Respiratory Syndrome*
357 *Coronavirus 2*. *bioRxiv*, 2020: p. 2020.05.01.067769.
- 358 23. Kampf, G., A. Voss, and S. Scheithauer, *Inactivation of coronaviruses by heat*. *Journal of*
359 *Hospital Infection*, 2020.
- 360 24. Lista, M., et al., *Resilient SARS-CoV-2 diagnostics workflows including viral heat*
361 *inactivation*. 2020.
- 362 25. Leclercq, I., et al., *Heat inactivation of the Middle East respiratory syndrome*
363 *coronavirus*. *Influenza and other respiratory viruses*, 2014. **8**(5): p. 585-586.
- 364 26. Grant, P.R., et al., *Extraction-free COVID-19 (SARS-CoV-2) diagnosis by RT-PCR to*
365 *increase capacity for national testing programmes during a pandemic*. *bioRxiv*, 2020: p.
366 2020.04.06.028316.

- 367 27. Fomsgaard, A.S. and M.W. Rosenstjerne, *An alternative workflow for molecular*
368 *detection of SARS-CoV-2 - escape from the NA extraction kit-shortage, Copenhagen,*
369 *Denmark, March 2020. Euro Surveill, 2020. 25(14).*

370

371

372

373

374

375

376

377

378

379

380

381

382

383

384

385

386

387

388

389

390 **Figure Legends**

391

392 **Figure 1:** Comparison of Ct values of SARS-CoV-2 (A) E gene, and (B) RdRp gene for three
393 isolates (1775; circles, 2018; squares, 2310; triangles) inactivated by different methods.
394 Inactivation with 2% formaldehyde for 15 min at RT results in significantly elevated Ct values for
395 both genes (**p=0.0001, one-way ANOVA comparison to AVL inactivation). Bland Altman
396 Plots comparing Ct values for (C) E gene, and (D) RdRp gene following inactivation by sample
397 transport buffer (circles), formaldehyde (squares), 30 min at 56°C (triangle), 60 min at 56°C
398 (inverted triangle) and 2 min at 98°C (diamond) compared to AVL. Ct value difference between
399 the two values is plotted on the Y-axis. Cut-off values of 2 and -2 Ct are plotted as dashed lines.

400

401

402

403

404

405

406

407

408

409

410

411

412

413 **Table 1: SARS-CoV-2 isolates used for inactivation**

414

SARS-CoV-2 isolate	Before treatment TCID₅₀/mL	After PEG precipitation (positive control) TCID₅₀/mL	Post inactivation (all methods) TCID₅₀/mL
1775	2.11E+05	4.10E+04	Not detected
2018	1.19E+04	6.09E+03	Not detected
2310	6.67E+05	1.22E+05	Not detected

415

416

417

418

419

420

421

422

423

424

425

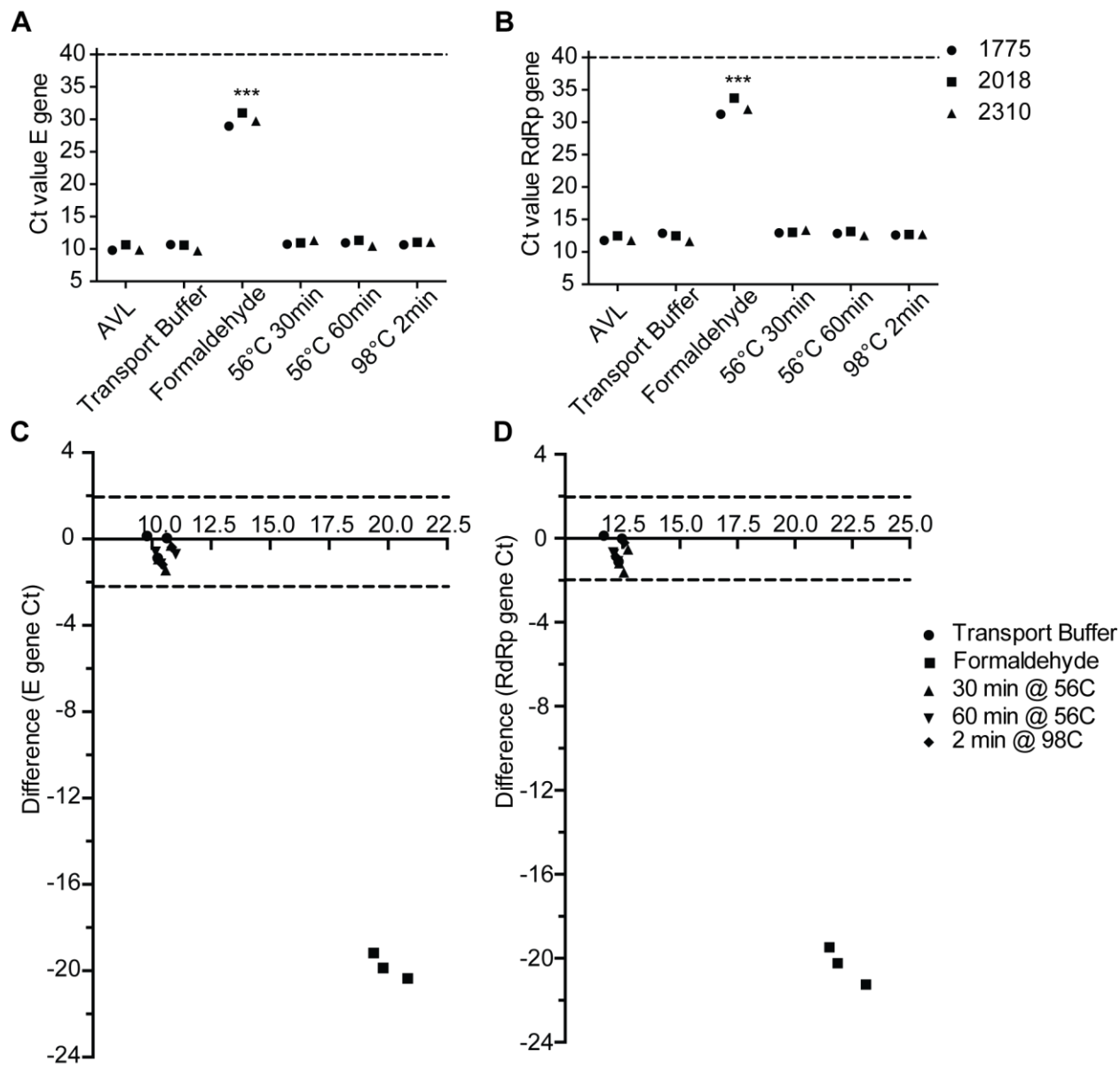
426

427

428

429

430 **Figure 1**



431