Full title

Reference materials for SARS-CoV-2 molecular diagnostic: validation of encapsulated synthetic RNAs for room temperature storage and shipping

Short title

Room temperature storage and shipping of RNA reference materials for covid-19 detection

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Abstract

The Coronavirus pandemic unveiled the unprecedented need for diagnostic tests to rapidly detect the presence of pathogens in the population. Real-time RT-PCR and other nucleic acid amplification techniques are accurate and sensitive molecular techniques that necessitate positive controls. To meet this need, Twist Bioscience has developed and released synthetic RNA controls. However, RNA is an inherently unstable molecule needing cold storage and costly shipping and resource-intensive logistics. Imagene provides a solution to this problem by encapsulating dehydrated RNA inside metallic capsules filled with anhydrous argon, allowing room temperature and eco-friendly storage and shipping. Here, RNA controls produced by Twist were encapsulated (RNAshells) and distributed to several laboratories that used them for COVID-19 detection tests by amplification. One RT-LAMP procedure, four different RT-PCR devices and 6 different PCR kits were used. The amplification targets were genes E, N; RdRp, Sarbeco-E and Orf1a/b. RNA retrieval was satisfactory, and the detection was reproducible. RNA stability was checked by accelerated aging. The results for a 10-year equivalent storage time at 25 °C were not significantly different from those for unaged samples. This room temperature RNA stability is a great advantage because it allows the preparation and the distribution of large strategic batches which can be kept for a long time and used for standardization processes between detection sites. Moreover, it makes it possible also to use these controls as single use and in the field where large differences in temperature can occur. In conclusion, these encapsulated RNA controls processed at room temperature can be used as reference materials for SARS-Cov-2 virus and other pathogen detection.

44 # Introduction

45 The Coronavirus pandemic has led to an unprecedented need for diagnostic tests to rapidly
46 detect the presence of pathogens in the population. Real-time RT-PCR and RT-LAMP
47 amplification techniques proved to be accurate and sensitive molecular tools for virological
48 diagnosis [1,2,3,4,5].

49 A key aspect of any ongoing pathogen detection effort is the use of quality control
50 measures for a wide range of applications from diagnostic assay development to day-to-
51 day testing. This includes verifying and validating diagnostic tests of both Next-Generation
52 Sequencing (NGS) and reverse transcription-amplification (RT-PCR or RT-LAMP) assays
53 [6,7,8,9]. Controls based on viral nucleic acids extracted from infected patients or live
54 viruses propagated in cell culture have safety and security concerns. Synthetic RNA
55 controls, like those generated through gene synthesis by Twist Bioscience, mitigate the
56 biohazard risk and broaden access across diverse strains [10]. Additionally, as DNA
57 synthesis is a readily available technology, new RNA controls can be rapidly developed in
58 response to the emergence of new strains or pathogens.

59 However, RNA is an inherently unstable molecule. In ambient conditions, RNA rapidly
60 degrades, which presents a storage and cost challenge for laboratories that require large
61 stocks of control RNA (for a review see [2]). Imagene's technology consists in confining
62 dehydrated RNA, in the presence of proprietary stabilizing solutions, inside metallic
63 capsules filled with anhydrous argon (RNAshells®), keeping away the samples from
64 deleterious factors. Thus, it offers a solution to the stability problem by allowing extensive
65 and eco-friendly storage and shipping a room temperature [2].

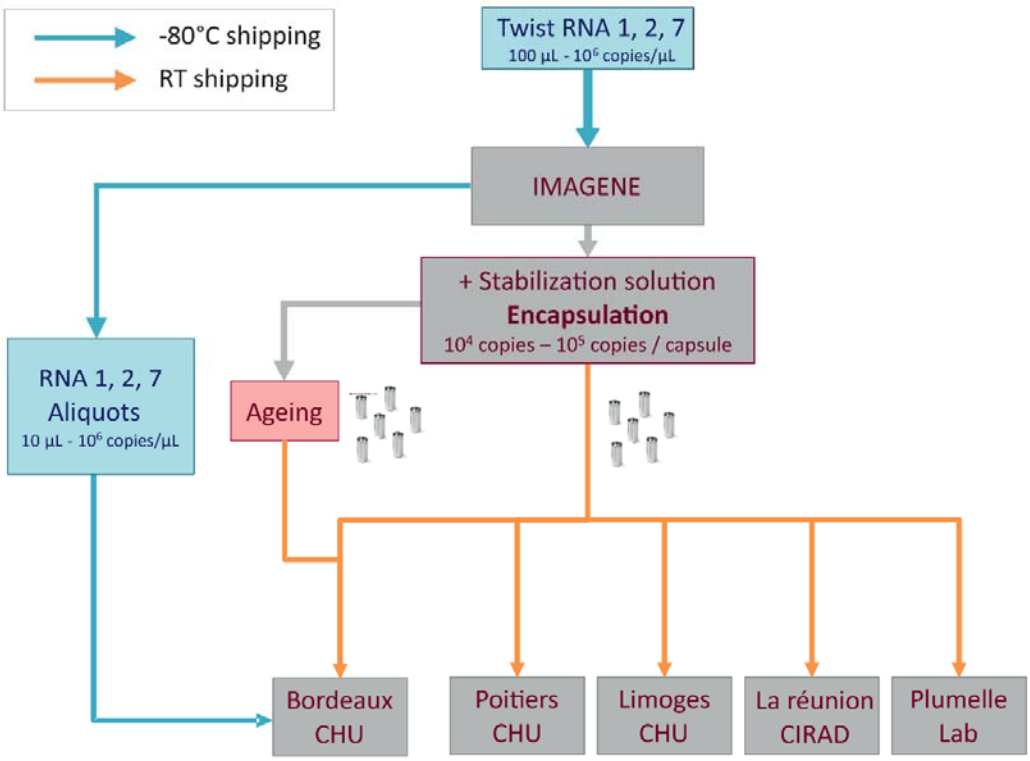
66 Here, Imagene encapsulated synthetic RNA controls produced by Twist Bioscience
67 (RNAshells). These capsules of controls were then distributed to five laboratories for
68 fitness-for-purpose analysis. The objective was to verify, first that the detection of the virus
69 was reliable using multiple PCR procedures and one LAMP procedure on 3 RNA variants
70 and 5 gene targets, using 4 PCR thermocyclers and 6 PCR kits, and second that the RNA
71 stability was insured over the equivalent of a 10-year storage period at room temperature.

72

73 # Material and methods

74 ## Workflow

75 The experimental strategy we followed is summarized in Fig 1.



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77

78 Fig 1. **Experimental strategy for the preparation, distribution and validation of**
79 **RNAshell® encapsulated RNA controls for SARS-CoV-2 virus detection**. The
80 frozen RNA samples were sent on dry ice to Imagene. One part of these was
81 aliquoted and sent on dry ice to Bordeaux CHU. The other part was aliquoted
82 and encapsulated. A set of these aliquots was heated to simulate a 10 years
83 room temperature storage and sent to Bordeaux CHU. The other aliquots were
84 sent to Bordeaux, Limoges, Poitiers CHUs, the CIRAD Réunion and a private
85 clinical laboratory (Plumelle, Salon de Provence).

86

87 Twist RNA controls

88 Synthetic SARS-CoV-2 RNA control molecules for three SARS-CoV-2 variants were graciously
89 supplied by Twist Bioscience. Details are given Table 1.

90

91 **Table 1. Synthetic SARS-CoV-2 RNA controls used in this study**

Control	GenBank ID	GISAID name	Name used
Control 1	MT007544.1	Australia/VIC01/2020	RNA 1
Control 2	MN908947.3	Wuhan-Hu-1	RNA 2
Control 7	EPI_ISL_418227	France/HF2393/2020	RNA 7

92 The controls are ssRNA *in vitro* transcribed from synthetic DNA. Each one consists of
93 six non-overlapping 5 kb fragments, providing greater than 99% coverage of the viral
94 genome.

95 RNA encapsulation and accelerated ageing

96 RNA controls were sent to Imagene over dry ice as 100 µL solutions at a concentration of
97 approximately 10^6 copies per microliter [10]. They were distributed in 10 µL aliquots and
98 stored at -80°C. Aliquots were thawed, diluted with an Imagene proprietary stabilization
99 solution and distributed into glass inserts fitted in capsules to obtain either 10^4 copies (low)
100 or 10^5 copies (high) per capsule.

101 RNA samples were vacuum-dried and encapsulated under anhydrous and anoxic
102 argon/helium according to the process developed by Imagene [11,12] to give RNAshells®. A
103 set of 3 capsules of each variant RNA and each copy number (a total of 18 capsules) were
104 heated at 90 °C for 16 h to simulate 10 years of storage at 25 °C according to our previously
105 published Arrhenius equation for encapsulated RNA [13]. RNAshells® were then shipped at
106 room temperature to the participating laboratories.

107 RNAshell® opening and RNA rehydration

108 The RNAshells® were opened with a portable ShellOpener™ supplied by Imagene following
109 the given instructions. After opening, a given volume of RNAse-free ultrapure water was
110 added in the capsule and the RNA was left 10-20 minutes on the bench to rehydrate. At
111 that point, the RNA was ready for analysis (RT-qPCR or RT-LAMP). The different volumes
112 used to rehydrate and analyze the RNA by RT-qPCR in the different laboratories are given
113 Table 2.

114 **Table 2. Summary of the material and methods for the different RT-qPCR analysis.**

Site	**Bordeaux CHU**	**Bordeaux CHU**	**Limoges CHU**	**Poitiers CHU**	**Poitiers CHU**	**Private clinical Laboratory**
Instrument	Light Cycler 480 (Roche)	Light Cycler 480 (Roche)	CFX96 (BioRad)	CFX96 (BioRad)	CFX96 (BioRad)	SLAN®-96P Real-Time PCR System (Sansure)
Kit	GeneFinder Covid-19 Plus, Osang Healthcare (distributed by Elitech)[2] [14]	SARS CoV-2 R Gene, Argene Biomérieux [14,15]	SuperScript™ III One-Step RT-PCR (Thermofisher)	TaqPath COVID-19 Multiplex Diagnostic Solution (Thermofisher)	Allplex™ 2019-nCoV Assay (Seegene)	Kit Novel coronavirus 2019-ncov (Sansure)
Nb cycles	45	45	45	40	45	45
Cycles	20 min @ 50°C 5 min @ 95°C 45 cycles : - 15 s @ 95°C - 50 s @ 58°C	5 min @ 50°C 15 min @ 95°C 45 cycles : - 10 s @ 95°C - 40 s @ 60°C - 25 s @ 72°C	ND	2 min @ 25 °C 10 min @ 53°C 2 min @ 95°C 40 cycles : - 3 s @ 95°C - 30 s @ 60°C	20 min @ 50 °C 15 min @ 95°C 45 cycles : - 15 s @ 94°C - 30 s @ 58°C	30 min @ 50°C 1 min @ 95°C 45 cycles : - 15 s @ 95°C - 30 s @ 60°C
Positivity	Ct < 43	ND	fluo signal > 6 000 for IP2 > 13 000 for IP4	Ct < 37	Ct < 40	ND
Target genes	E, N, RdRp	E, N, RdRp	IP2-RdRp, IP4-RdRp Sarbeco- E	S, orf1, N	E, N, RdRp	ORF1ab, N

RNA Controls	RNAshells #1, 2, 7 10^4 copies 10^5 copies 18 aged RNAshells (3 of each) Solution #1, 2, 7 10 µL @ 10^6 copies / µL	RNAshells #1, 2, 7 10^4 copies 10^5 copies 18 aged RNAshells (3 of each) Solution #1, 2, 7 10 µL @ 10^6 copies / µL	RNAshells #1, 2, 7 10^4 copies 10^5 copies	RNAshells #1, 2, 7 10^4 copies 10^5 copies	RNAshells #1, 2, 7 10^4 copies 10^5 copies	RNAshells #2 6x 10^4 copies 6x 10^5 copies
Rehydration volume	10 µL	10 µL	20 µL	25 µL	25 µL	ND
RNA volume in reaction	5 µL	5 µL	5 µL	10 µL	8 µL	ND
Theoretical number of copies / reaction	5,000 or 50,000	5,000 or 50,000	2,500 or 25,000	4,000 or 40,000	3,200 or 32,000	ND

ND: non disclosed

Analysis of RNA samples in solution

The non-diluted and non-encapsulated aliquots of RNA 1, 2 and 7, at approximately 10^6 copies per µL, were prepared and sent by Imagene on dry ice to Bordeaux CHU. The

119 samples were thawed and diluted to match the concentrations of the rehydrated capsules
120 (here, 10^3 and 10^4 copies per µL). They were analyzed in parallel by RT-qPCR (see Table 2).

121 RT-qPCR analysis

122 The reverse transcription polymerase chain reaction (RT-PCR) is an amplification technique
123 widely used to detect a specific RNA target such as viral RNA. The reaction mix includes
124 dNTPs, enzymes, a buffer solution, a fluorescent probe and one pair of primers to amplify a
125 specific region of the fragment. The reaction is carried out by a series of alternating
126 temperature steps in a thermal cycler. In a first stage, RNA conversion to cDNA is
127 performed. A second stage begins with an initial heating step to denature the RNA/cDNA
128 hybrid, activate the *Taq* DNA polymerase enzyme and degrade the target RNA. It is
129 followed by the amplification reaction which includes 3 steps: denaturation, annealing of
130 the primers and elongation, reproduced 40-45 times. At each elongation step a fluorescent
131 signal is generated proportionally to the amount of amplified product. A threshold cycle
132 (Ct) is obtained by measuring the number of amplification cycles necessary to obtain a
133 given minimum fluorescent signal.

134 A positive control for RT-qPCR should contain an amount of target sufficient to give a Ct
135 easily distinguishable from the negative control but not too high to avoid the risks of cross-
136 contamination from a well to the nearby wells in the analysis plate.

137 The different RT-qPCR materials and methods used in the study, when available, are
138 summed up in Table 2.

139 ## RT-LAMP analysis

140 The Reverse Transcription Loop mediated isothermal AMPlification (RT-LAMP) is a single-
141 tube technique for the detection of a target RNA. After an initial reverse transcription step,
142 the cDNA target sequence is amplified at a constant temperature by a DNA polymerase
143 with a high displacement activity using two pairs of primers. The amplified products are
144 detected by a colorimetric or a fluorescent probe. The parameter related to the
145 concentration of the target is the time necessary to detect a positive signal.

146 Using several pairs of primers makes LAMP more specific but more complex to design than
147 PCR. Another advantage is that LAMP being an isothermal amplification technique does not
148 require a thermal cycler allowing the procedure to be used in the field. In this context, a
149 positive control should give a signal in the first minutes of the reaction (as early as
150 possible).

151 Here, RT-LAMP was first tested on a dilution series of control RNA 2 Low or High,
152 rehydrated with 50 µL of RNAse-free pure water. For Control 2 Low: a single analysis was
153 done for 1000 copies/reaction, 2 replicates were done for 500 and 250 copies/reaction and
154 3 replicates for 100, 50 and 25 copies/reaction. For Control 2 High: 3 replicates were done
155 for 1000 copies/reaction and 4 replicates for 200, 40 and 8 copies/reaction.

156 To test the encapsulated RNA 2 as a positive control in the field, 60 independent RT-LAMP
157 runs were performed for the presence of SARS-CoV-2. Each run included 14 samples, a
158 negative control and a positive control. The positive control consisted of RNA 2 rehydrated
159 with water and further diluted in *Amies* medium to have a final concentration of 1000
160 copies/reaction.

161 RT-LAMP was performed according to the protocol RunCov developed by the CIRAD-UMR
162 PVBMT (3P) and validated by the National Reference Center [16]. The reactions were
163 performed in a 25 µL total reaction volume containing 15 µL of ISO-DR004-RT300
164 Isothermal Mastermix (OptiGene Ltd, Horsham, United Kingdom), 2.475 µL of each primer
165 pair (N and S), 0.05 µL (0.5 units) of AMV transcriptase (Promega, Charbonnières-les-Bains,
166 France) and 5 µL of template RNA. LAMP reactions were run on a Genie II instrument
167 (OptiGene) at 65°C for 25 min followed by an annealing step during which the temperature
168 was decreased from 92°C to 78°C at a speed of 0.05°C/s to determine the annealing
169 temperature (Ta) of the produced amplicon.

170 Statistical analysis

171 All statistical analyses including ANOVA, Fisher Test and previous verification of data
172 normality (histogram and normal: Q-Q plot), were performed using the R statistical
173 software, version 4.1.1 (2021-08-10; R Development Core Team, Vienna, Austria) with the
174 different packages: stats, multcomp, emmeans and ggplot2).

175 Results and discussion

176 Objectives and strategy

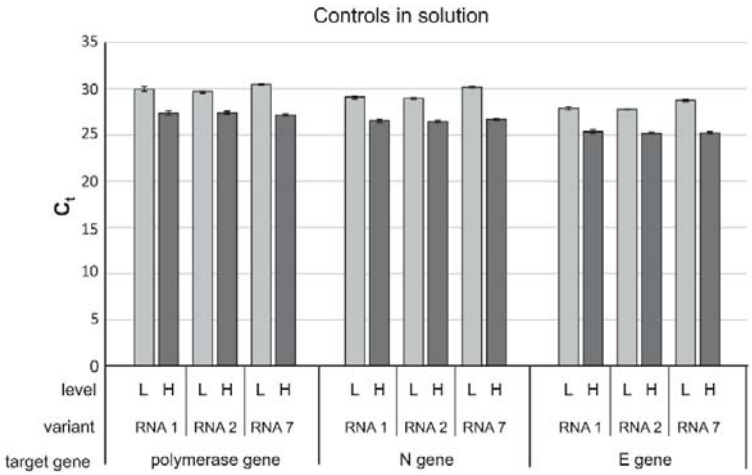
177 The aim of this work was to validate whether encapsulated RNA controls stored and
178 shipped at room temperature were fit for purpose as reference materials for internal
179 quality control of SARS-CoV-2 detection.

180 Bordeaux CHU received non-diluted, non-encapsulated, frozen samples in solution, to
181 compare the controls before and after encapsulation (i.e. to check the potential effect of

182 the process and stabilization solution). They also received the aged RNAshells® to validate
183 that 10 years of storage at room temperature did not alter the performance of the
184 encapsulated controls. Two other laboratories (Poitiers CHU and Limoges CHU) received
185 RNAshells® to verify that the controls gave the same performances when run with other
186 RT-qPCR techniques. The CIRAD-UMR PVBMT (3P) laboratory tested the controls in
187 multiple experiments while developing a RT-LAMP test for field use. Finally, RNAshells®
188 were sent to a private clinical laboratory to check that the capsules can be routinely used
189 by any operator.

190 Controls in solution

191 First, we checked the performance of the controls on non-encapsulated samples. The
192 results of these experiments are presented Fig 2.



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194 **Fig 2. RT-qPCR for controls in solution**. The controls were thawed and diluted at
195 reception. Measures were done at Bordeaux CHU on 3 variants RNA (RNA 1, 2, 7), 2
196 copy numbers: 5,000 (low, L) and 50,000 (high, H) copies/reaction, 3 target genes
197 (polymerase gene, N gene and E gene).

198 All the controls gave positive results. For a given low or high concentration and a given
199 target gene, the Ct were comparable between the different variants ($p = 0.647$). The
200 highest Ct differences came from the gene targets ($p=7.79 \times 10^{-4}$), probably due to
201 differences in PCR efficiency.

202 The chosen copy numbers gave Ct values in a suitable range (around 30 and 27 respectively
203 for the low and high controls), clearly different from the negative control (above 40-45).
204 The Ct were also not too low. This is advantageous because a positive control with too
205 many more copies than in patient samples could result in carry-over contamination in the
206 nearby wells of the PCR plate leading to false positive samples.

207 ## RNA controls encapsulation and accelerated aging

208 Then, we needed to verify, that encapsulation does not induce alterations in the controls.
209 Another important point is to test whether RNA controls could be stored at room
210 temperature for an extended period of time in RNAshell®, so a set of capsules were
211 submitted to an accelerated aging by heating to simulate a storage period of 10 years at
212 25°C according to our previously published Arrhenius study [13]. The results are shown Fig
213 3.

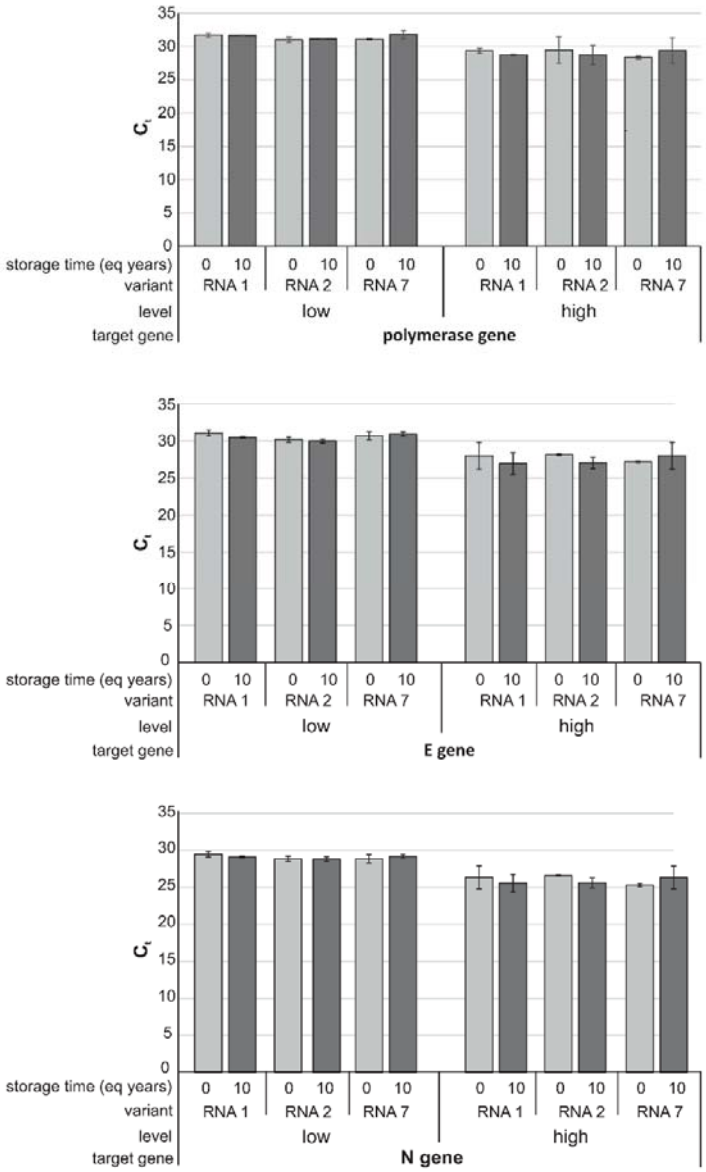


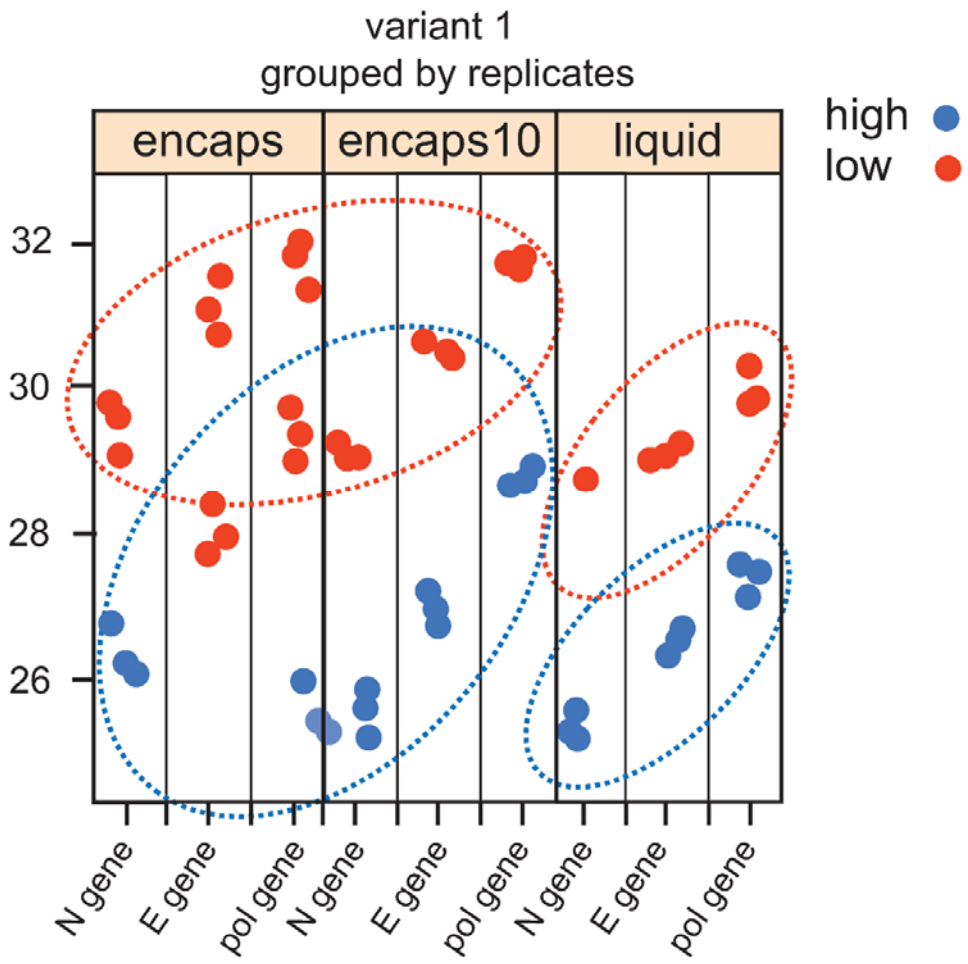
Fig 3. RT-qPCR on RNAshell®-encapsulated and aged controls. RT-qPCR measures were done by Bordeaux CHU on 3 variants RNA (RNA 1, 2, 7), 2 copy numbers: 5,000 (low, L) and 50,000 (high, H) copies/reaction, 3 target genes (polymerase gene, N gene and E gene) and after 2 storage times: time 0 post-encapsulation and after heating for a period equivalent to 10 years at room temperature (0 and 10).

221 All analyzed RNA, whatever the variant, target gene or storage time were successfully

222 amplified.

223 To analyze the extent and sources of variations on these experiments we used variant 1.

224 The results are shown Fig 4.



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226 **Fig 4. Group analysis for Ct on variant 1 controls.** Ct (3 replicates) are shown

227 according to: a) the state: encapsulated (encaps), encapsulated and aged (encaps

228 10) or in solution (liquid) and b) the number of copies (5,000 (low) and 50,000

229 (high) of each target gene. Groups of Ct values from the same state (dry vs liquid)

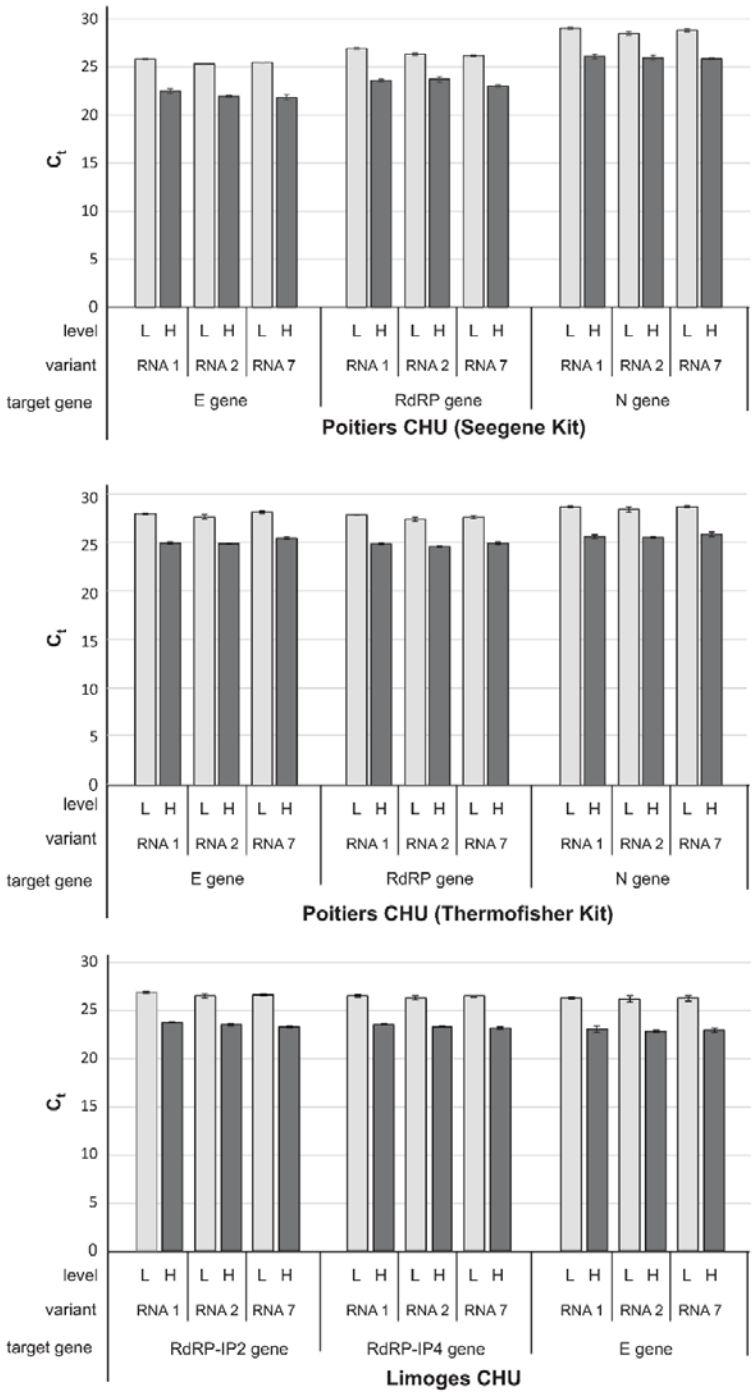
230 and copy number are circled.

231 The encapsulated controls and the controls in solution show significant Ct differences
232 (p=0.001999), due partly to the encapsulation step, including the addition of a stabilization
233 solution, but more probably to the fact that the controls in solution did not come from the
234 same diluted samples. However the Ct values of encapsulated RNA are maintained in the
235 same suitable range, meaning that the encapsulation process did not lead to RNA loss,
236 degradation or PCR inhibition to an extent that would make RNAshell®-encapsulated RNA
237 unfit for purpose.

238 On the other side, no significant difference was observed between 0- and 10-years
239 RNAshells® (Fig 3, p=0.7606) meaning that the simulated long-term storage of RNA did not
240 lead to significant degradation. One can therefore consider that the encapsulated RNA
241 controls are stable over at least 10 years at room temperature and are suitable reference
242 materials.

243 Compatibility with other RT-qPCR kits and instruments

244 To broaden the validation, capsules were distributed to laboratories that performed tests
245 with three other RT-amplification techniques, including the method used by the National
246 Reference Center for SARS-CoV 2 (on RdRp IP2 and IP4 regions). The results are shown
247 Fig 5.



248

249 **Fig5. RT-qPCR results from Limoges and Poitiers CHU.** For Limoges CHU, the RT-
250 qPCR measures were done on 3 variants RNA (RNA 1, 2 and 7), 2 copy numbers:
251 2,500 (Low, L) and 25,000 (High, H) copies/reaction), and 3 target genes (RdRp-IP2,
252 RdRp-IP4 and E genes). For Poitiers CHU, RNAshells® were rehydrated with 25 µL of

253 water. Eight µL were used for the Seegene kit and 10 µL for the Thermofisher kit. Ct
254 values were obtained by RT-qPCR measures done on 3 variants RNA (RNA 1, 2 and
255 7), 3 target genes (E, N and RdRp genes), with 2 copy numbers: 10,000 (Low, L) and
256 100,000 copies/capsule (high, H) giving 3,200 and 32,000 copies per reaction for the
257 Seegene kit and 4,000 and 40,000 copies per reaction for the Thermofisher kit. For
258 each technique, the control capsules were run in 3 distinct experiments (Poitiers
259 CHU) and the mean and SE of the 3 Ct were calculated.

260

261 Although the experiments were run over 3 days (at Poitiers CHU), the sampling from a
262 given capsule gave constant results. In all the experiments, techniques and targets, the
263 results obtained in the different laboratories were reproducible among all the capsules,
264 showing that the encapsulated control RNA are suitable for their use as internal quality
265 controls. The Ct for variants were strictly equivalent. The highest variability came from
266 the analysis kits and target genes as demonstrated above with the controls in solution.

267 Compatibility with a RT-LAMP procedure

268 SARS-Cov-2 genome was successfully amplified using the RT-LAMP assay from both RNA 2
269 low and RNA 2 high capsules with a high sensitivity (100% positive samples at 25 copies per
270 reaction for RNA2 low, 40 copies for RNA2 high and 50% positive samples at 8 copies for
271 RNA 2 high). Annealing temperature peaks were obtained for both S and N regions for 90%
272 of the positive samples with annealing temperatures ranging between 88.4-88.7 °C and
273 83.4-83.8 °C, for N and S regions, respectively. For 10% positive samples only one annealing
274 temperature peak (S or N) was obtained. This last result was principally obtained for low
275 copy numbers. This was already observed in other RT-LAMP experiments involving other

276 types of samples and is probably due to differences in primers binding probabilities to their

277 respective targets.

278 In the field, 1000 copies per reaction were taken so that the signal came out early,

279 validating the run before or at the same time as positive samples came out.

280 The results are given Table 3.

281 **Table 3. RT-LAMP results from CIRAD.**

			Annealing temperature	
RNAshell RNA 2	Copies / reaction	Amplification time (min)	N gene	S gene
Low	1000	10.00	88.5	83.50
Low	500	11.75	88.5	83.7
Low	500	11.00	88.5	83.7
Low	250	12.75	88.5	83.6
Low	250	13.50	88.6	83.7
Low	200	14.50	88.6	83.8
Low	200	14.50	88.4	83.8
Low	200	16.75	88.7	83.7
Low	100	13.00	88.6	83.8
Low	100	12.50	88.8	83.7
Low	100	13.00	88.5	83.7
Low	50	13.50	88.7	83.8
Low	50	17.00	88.7	83.8
Low	50	14.00	88.7	83.7
Low	25	16.75	88.3	83.8
Low	25	14.00	88.6	83.8
Low	25	13.50	88.4	83.6
High	1000	10.30	88.7	83.7
High	1000	10.15	88.7	83.7
High	1000	10.15	88.6	83.7
High	200	12.00	88.6	83.7
High	200	11.30	88.6	83.6
High	200	11.30	88.6	83.6
High	200	10.45	88.7	83.7
High	40	12.30	88.7	83.7
High	40	13.45	-	83.7
High	40	13.15	-	83.7
High	40	13.45	88.6	88.5
High	8	19.45	-	83.4

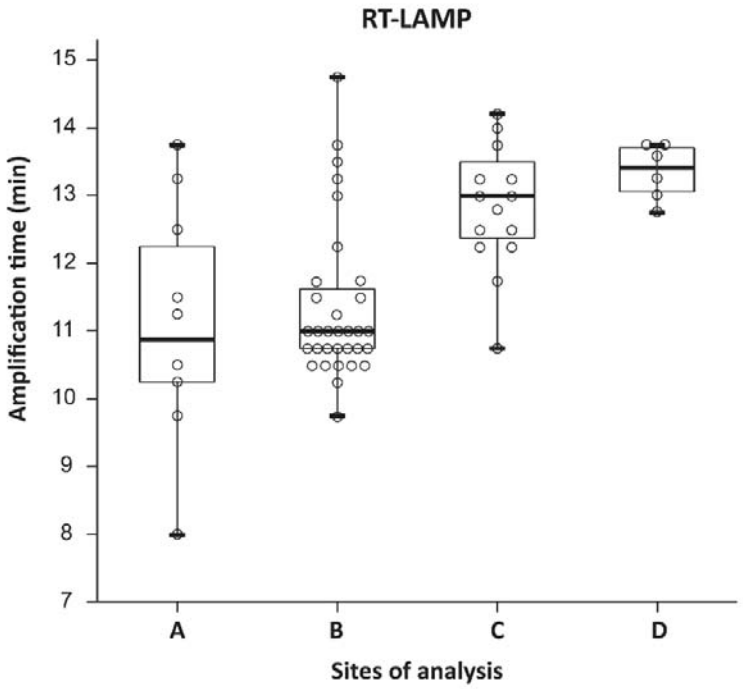
High	8	23.30	88.7	-
High	8	-	-	-
High	8	-	-	-

282 RNA 2 samples High and Low were rehydrated with 50 µL of water and diluted to
283 have between 1000 and 8 copies per reaction and amplified using the Runcov RT-
284 LAMP protocol of the CIRAD La Réunion. The amplification time and annealing
285 temperatures are given.

286

287 ” Real life” use of the capsules as internal quality controls

288 For the monitoring of SARS-CoV 2 detection in the field by RT-LAMP, the RNA 2 variant
289 (1000 copies) was used as a positive control for 60 RT-LAMP tests run on samples taken
290 and analyzed in 4 different sites (A, B, C, D). This control was successfully amplified in each
291 run (Fig 6).



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293 **Fig 6. LAMP tests** The RNA 2 variant (1000 copies) was used as a positive control
294 for 60 (real life) tests run on samples analyzed at 4 different sites (A, B, C, D). For
295 each test, both N and S regions were amplified.

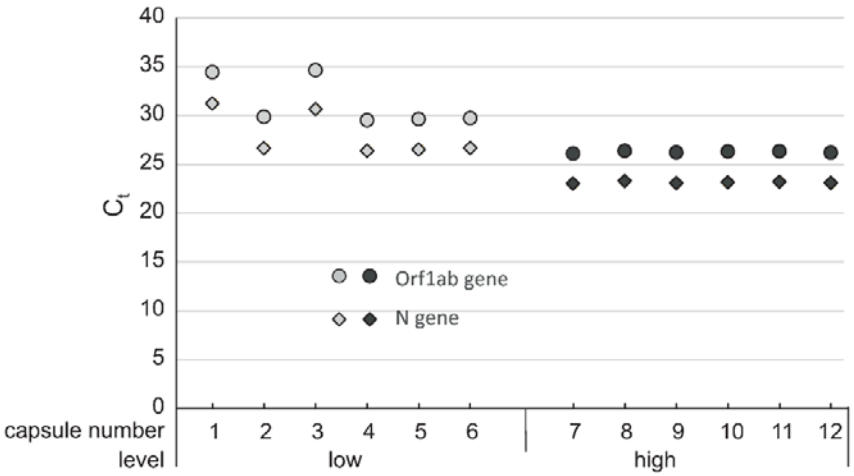
296 For each test, both N and S regions were amplified, generating annealing peaks within the
297 expected annealing temperature ranges (not shown).

298 This field study shows that the capsules can be used as positive controls at the point of care
299 or on site with simplified logistics due to their stability at room temperature.

300

301 Validation of RNA controls for private clinical laboratories

302 To test the robustness of the controls, a series of experiments we conducted in a private
303 clinical laboratory. The laboratory received the capsules and the instructions for use and
304 included the rehydrated RNA in their routine diagnostic test. The results are shown Fig 7.



305

306 **Fig 7. Private clinical laboratory-.** Results on 2 target genes and 2 levels on 6 capsules
307 for each.

308 The results showed a good reproducibility among the capsules except for 2 capsules with a
309 low RNA copy number where the operator probably had rehydrated the RNA with a higher
310 volume of water, decreasing the number of copies in the test.

311 So, the capsules can easily be used as reference materials for internal quality control of the
312 monitoring of the diagnostics procedures, without the need for extensive training of the
313 operators.

314

315 Conclusions

316 The RNAs synthetized by Twist Bioscience and encapsulated in RNAshells® by Imagene
317 have been validated as positive controls for SARS-CoV-2 detection. Both 10,000 and
318 100,000 copy numbers per capsule are suitable for their use in RT-qPCR and RT-LAMP
319 analysis. The signal is reproducible among the variants, the targets and the analysis
320 methods. Moreover, the RNAshell®-encapsulated RNA showed an excellent stability, with
321 an unprecedented shelf life of more than 10 years at room temperature. This stability is a
322 great advantage because, first, it facilitates the use of these controls in the field, in
323 particular when large variations of temperature can be expected, and second, importantly,
324 it allows the preparation and the distribution of large strategic batches which can be kept
325 for a long time and used for standardization between processes or detection sites.

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373 Acknowlegdements

374 We thank Twist bioscience for the gift of the RNA controls and suggestions for the writing
375 of the paper.