

1 **Heterogeneity in susceptibility to hydroxychloroquine of SARS-CoV-2 isolates**

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21  
22 **Running title:** Hydroxychloroquine susceptibility of SARS-CoV-2.

23

24           **Abstract**

25           **Background**

26           Hydroxychloroquine has been demonstrated *in vitro* to control SARS-Cov2  
27 multiplication on Vero E6 cells. We herein tested the possibility that some patients with  
28 prolonged excretion of virus could be infected by less susceptible viral strains.

29           **Methods**

30           Using high-content screening method, we screened 30 different selected isolates of  
31 SARS-CoV-2 from different patients. All these patients received azithromycin and/or  
32 hydroxychloroquine. We focused our work on patients with viral persistence defined as  
33 patient having a positive detection of virus in nasopharyngeal sample for at least 10 days and  
34 tested during the two episodes of French epidemic, late winter-spring then summer. Dose-  
35 response curves in single-molecule assays with hydroxychloroquine were done for isolates  
36 with suspected reduced susceptibility. Genome clustering was done for all isolates.

37           **Results**

38           Among 30 tested strains, 3 were detected as replicating in presence of azithromycin  
39 and hydroxychloroquine at 5µM both. Dose-response model showed a decrease of  
40 susceptibility of these 3 strains to hydroxychloroquine. Whole genome sequencing showed  
41 that these three strains are all from the second epidemic episode and 2 clusters with isolates  
42 from Africa.

43           **Conclusions**

44           Reduced susceptibility to hydroxychloroquine was not associated with persistence of  
45 the virus in naso-pharyngeal sample. It was rather associated with occurrence during the  
46 second epidemic episode starting during summer and with strains clustering with those having  
47 a genotype which is common in Africa. This continent is the continent where  
48 hydroxychloroquine was the most used.

49           **Keywords:** SARS-CoV 2; Hydroxychloroquine; azithromycin; in vitro model; Vero  
50 E6; WGS; Africa.

51

52           **Abbreviations :** IHU Mediterranean infection, Hospitalo-Universitaire Institute  
53 Mediterranean infection ;  $\mu\text{M}$ , micromolar; NSB3 laboratory, Biosafety Level 3 laboratory;  
54 azithromycin, AZT; hydroxychloroquine, HCQ ; A5H5, azithromycin 5  $\mu\text{M}$  and  
55 hydroxychloroquine 5  $\mu\text{M}$ ; RT-PCR, real-time, Polymerase Chain Reaction; MOI,  
56 multiplicity of infection; HCS, High-content screening

57

58           **Introduction**

59           In December 2019, a novel coronavirus named SARS-CoV-2 emerged in Wuhan,  
60           China, in Hubei province [1–3]. Quickly, SARS-CoV-2 was spread around the world and the  
61           number of cases and deaths has increased rapidly ([https://www.nature.com/articles/d41586-](https://www.nature.com/articles/d41586-020-00758-2)  
62           [020-00758-2](https://www.nature.com/articles/d41586-020-00758-2)). Since then, finding effective treatments and vaccines remains a major issue in  
63           the world. Several molecules with an antiviral effect have been tested *in vitro* and *in vivo*,  
64           drug repurposing being one of the strategies applied. In this objective many drugs have shown  
65           an inhibition *in vitro* such as remdesivir, chloroquine, hydroxychloroquine, ivermectin,  
66           azithromycin, spiramycin, several protease inhibitors and some antimalarial drugs [4–16]. The  
67           combination of azithromycin and hydroxychloroquine showed synergistic effects *in vitro* at  
68           concentrations of 5 µM for each molecule and the combination was massively used in our  
69           institute to treat infected patients [17], as well as in several countries in particular in Africa  
70           (Zambia, Uganda, Egypt, Algeria, Morocco, Tunisia, Senegal, Cameroon) [18,19]. In  
71           literature, and in our experience, some patients, especially those immunocompromised or with  
72           other comorbidities (as hypertension, diabetes) have presented a positive viral load with a late  
73           clearance [20–23]. In our institute, we could isolate at least one SARS-CoV-2 strain for  
74           several patients defined as “persistent” with a positive RT-PCR test for more than 10 days  
75           after admission in spite of the fact that they received the combination of azithromycin and  
76           hydroxychloroquine [24]. In those cases the question of susceptibility to the antiviral drugs of  
77           the responsible strain was raised. Indeed, most *in vitro* studies evaluating susceptibility to  
78           antiviral drugs used a unique strain or clone of SARS-Cov 2, considering that this clone is  
79           representative of all, in spite of the fact that the variability of antiviral activities on an  
80           enlarged panel of strains is unknown. Herein, we decided to screen in an automated model of  
81           Vero E6 a single association of azithromycin-hydroxychloroquine on multiple strains issued  
82           of persistent and non-persistent patients randomly chosen to detect reduced susceptibility to

83 this combination [25]. After this preliminary screening, a dose-response study to  
84 hydroxychloroquine was done on suspect isolates and controls.

## 85 **Materials and Methods**

### 86 **Ethic concerns and sample collection**

87 Nasopharyngeal samples were done at the IHU Mediterranean infection as part of  
88 Covid-19 diagnosis and follow-up of patients. The study was approved by the ethical  
89 committee of the University Hospital Institute Méditerranée Infection (N°: 2020-029).  
90 Regarding both the French and the local situations, we defined 2 periods of time in this  
91 pandemic situation: a first one consisting in the arrival of the virus then the lockdown from  
92 February to May 2020, and a second one since June 2020, still ongoing. Information on the  
93 sample collection, name of the strains, and treatments are summarized in Table 1. All patients  
94 received treatment by Azithromycin that was in most cases associated to hydroxychloroquine  
95 [24]. Persistent patients are defined as presenting 2 viral swabs positive by RT-PCR during a  
96 period up to 10 days [25]. For these patients, when it was possible, we evaluated the  
97 susceptibility of 2 strains, the first isolated at admission and the second isolated during  
98 evolution under treatment. IHUMI-3 isolate was among the first strains isolated in the  
99 laboratory and used as control, as in all our previous experiments [17]. Viral isolation was  
100 done following the procedure described [26]. After isolation, the viruses were harvested and  
101 frozen at -80°C. TCID<sub>50</sub> were performed for each strain and MOI for inoculation was  
102 adjusted in function of the RT-PCR values in order to inoculate the same virus concentration  
103 for each virus. Before inoculations for antiviral assays the viral stock was diluted into the M4  
104 medium.

### 105 **Screening for reduced susceptibility and dose-response to hydroxychloroquine of** 106 **selected isolates**

107 All 30 strains from 20 patients (Table 1) and IHUMI-3 strain control were screened  
108 using the high-content screening procedure to the combination of hydroxychloroquine and  
109 azithromycin (Sigma-Aldrich) at 5  $\mu$ M each to evaluate the possible reduction of  
110 susceptibility. First, 200  $\mu$ L of  $5 \cdot 10^5$  cells /mL of Vero-E6 were incubated overnight at 37°C  
111 with 5% CO<sub>2</sub> in 96 well plates. Supernatant was removed four hours before the infection by  
112 SARS-CoV 2 and drug dilutions were incubated in the M4 medium 4 hours before. Viral  
113 infection of each strain was achieved with a MOI 0,001 (50uL per well) except in negative  
114 controls. Imaging and cell analyses were performed by high-content-screening using the CX7  
115 automated cell-insight optical microscope (Thermofisher Scientific, USA). The proof of  
116 concept was used by Francis *et al.* and developed to automatically detect infections in cells  
117 [25]. Briefly at time points (H0 and 72 hours post-infection) wells were stained by NucBlue™  
118 Live ReadyProbes™ reagent (Molecular Probes, Life Technologies, USA) at a final  
119 concentration of 2 ng/mL (5  $\mu$ L per well directly from stock solution). Image acquisition and  
120 analyses were performed using the automated CellInsight™ CX7 High-Content Analysis  
121 Platform coupled with an automation system including an Orbitor™ RS Microplate mover  
122 and a Cytomat™ 2C-LIN (Thermo Scientific) incubator. We evaluated the protective effect of  
123 A5H5 by comparison to the positive control without addition of drugs and measured the  
124 difference in total cell count and % on infected cells according to the following formula [total  
125 cell counts (A5H5 -positive control )] \* [% injured cells (A5H5 – positive control) / 10 ]. As  
126 a consequence of this initial screening, 3 strains suspected to have possible reduced  
127 susceptibility to the combination (IHUMI-2123, IHUMI-2137 and IHUMI-2178) were first  
128 tested against hydroxychloroquine and azithromycin at 5  $\mu$ M each then in a serial dilution  
129 range from 25  $\mu$ M to 0,39  $\mu$ M of hydroxychloroquine to determine dose-response assays. In  
130 order to confirm that the effect was not a genotype-selection effect, we tested IHUMI-2122,  
131 IHUMI-2177 and IHUMI-3 as control. Dose-effect curve was determined using a range of

132 hydroxychloroquine doses (from 25  $\mu$ M to 0,39  $\mu$ M) at MOI of 0,001. Hydroxychloroquine  
133 dilutions were done from a stock solution in M4 and then concentrations were adjusted. Each  
134 test was done at least in sixplicates and repeated twice independently and the potential effect  
135 was monitored by RT-PCR after 48H of incubation under previously described conditions  
136 [27], except concerning the polymerase replaced by the SuperScript™ III Platinum™ SYBR  
137 with ROX (Sigma-Aldrich, catalog number: 11736051). Relative viral quantification was  
138 done compared to the positive control (viruses without drugs) by the  $2^{-\Delta\Delta Ct}$  (-delta delta CT)  
139 method [28]. Statistical analyses were performed using GraphPad Prism v8.0.0 (GraphPad  
140 Software, La Jolla California USA).

#### 141 **Viral preparation and genomic sequencing, genomic assembly and bioinformatic** 142 **analyses**

143 In parallel of the antiviral assays, 500  $\mu$ L of the viral supernatant obtained from co-  
144 culture were centrifuged through UFC-filter (see previous section). Then viral RNA was  
145 extracted from 200  $\mu$ L of the filtrate supernatant using the QIAcube kit. Then, it was reverse  
146 transcribed using SuperScript IV (ThermoFisher Scientific, Waltham, MA, USA) prior to  
147 cDNA second strand synthesis with Klenow Fragment DNA polymerase (New England  
148 Biolabs, Beverly, MA, USA). The next step concerned the DNA purification done by using  
149 Agencourt AMPure XP beads (Beckman Coulter, Villepinte, France) and finally sequenced on  
150 Illumina technology with the Illumina Nextera XT Paired-end strategy on a MiSeq instrument  
151 (Illumina Inc., San Diego, CA, USA). The Wuhan-Hu-1 isolate genome served as reference  
152 (consensus sequences GenBank Accession no. MN908947) and mapping was done by CLC  
153 Genomics workbench v.7. Sequences were compared to the GISAID database and a  
154 phylogenetic tree was done by using next train ncov (<https://github.com/nextstrain/ncov>).

#### 155 **Results**

156           **High-content screening for reduced susceptibility detection**

157           Among the 30 strains (plus IHUMI-3 strain control) screened 72 hours after the viral  
158 infection by SARS-COV-2 on the high-content screening with or without treatment by the  
159 combination of hydroxychloroquine and azithromycin both at 5  $\mu$ M, IHUMI-2123, IHUMI-  
160 2137 and IHUMI-2178 had a low threshold obtained on the HCS software (1099, -1021 and -  
161 257 respectively), suggesting possible reduced susceptibility to A5H5 (Figure 1a and  
162 supplementary Table 1). This result was confirmed by SARS-CoV-2 replication analysis  
163 (Figure 1b).

164           **Dose-effect curves of hydroxychloroquine assays**

165           Concerning the IHUMI-3, IHUMI-2122 and IHUMI-2177 strains used as controls, we  
166 observed a consistent viral inhibition compatible with the results observed previously in  
167 SARS-CoV-2 isolates. On the contrary, concerning IHUMI-2123, IHUMI-2137 and IHUMI-  
168 2178 we observed a displacement of susceptibility to hydroxychloroquine (Figure 2)  
169 confirming a specific pattern of reduced susceptibility for these isolates.

170           **Genome analysis**

171           We first conducted a global genome-to-genome comparison on the couple of strains  
172 isolated in persistent patients and could not detect any modification (Table 1). We also  
173 analyzed 20 genomes to place them in a phylogenetic tree. Regarding the quality score on the  
174 next clade, all strains received a good quality score (Supplementary file S1). We could detect  
175 that all the strains of the second period have 10 or more amino-acid changes in their genome  
176 compared to strains of the first period. On the contrary all the strains of the first period have  
177 less than 10 amino-acid mutations except one (patient 12). All viruses in those studies have  
178 the D614G mutation in the spike, described elsewhere as potentially increasing the infectious  
179 effects [29]. The phylogenetic tree was reconstructed by integrating all IHUMI strains and  
180 evolutionary relationships were evidenced (Figure 3). All 5 strains from the second period



181 belonged to separate clades provisionally named Marseille 1 and Marseille 5 [30].  
182 Specifically, the strains with reduced susceptibility to hydroxychloroquine were from  
183 Marseille clades 1 (IHUMI-2123 and IHUMI-2178) and 5 (IHUMI-2137). All strains  
184 belonging to the Marseille 1 clade were positioned in proximity of genomes originating from  
185 Africa i.e; Senegal and Gambia. The phenotype with reduced hydroxychloroquine in  
186 Marseille 1 genotype was shared by IHUMI-2123 and IHUMI-2178 isolates but not by  
187 IHUMI-2122 and IHUMI-2177 isolates. IHUMI-2137 grouped within Marseille 5 clade.  
188 Meanwhile, regarding IHUMI-2123 and IHUMI-2178 isolates with a reduced susceptibility to  
189 hydroxychloroquine, they did not present mutations compared to close to IHUMI-2122 and  
190 IHUMI-2177 isolates without reduced susceptibility.

#### 191 **4. Discussion**

192 To the best of our knowledge, it was the first time that three SARS-CoV2 strains  
193 showed a profile of reduced susceptibility to hydroxychloroquine *in vitro*. Susceptibility to  
194 azythromycin was not tested independently as its effect alone *in vitro* is limited. The high-  
195 content screening technology, first applied to the high-throughput culture of giant viruses of  
196 amoeba then to SARS-CoV-2 [25], was used for the first time herein to quickly screen  
197 susceptibility to drugs of a large panel of viruses. If other work will be needed to confirm  
198 clearly that the procedure can be standardized enough to provide efficient large screening of  
199 strains, it was proven as efficient to detect isolates with reduced susceptibility. However, even  
200 if highly time-consuming and susceptible to many confounding factors as presented below,  
201 for fine determination of susceptibility, dose-effect determination by molecular biology  
202 remains necessary. Indeed, *in vitro* sensitivity assays carried out on the same virus can  
203 provide divergent results according to the great discrepancies due to several essential  
204 determinants in the experiments. First, cell lines used, although all the ones used in these  
205 assays need to be permissive, may harbor different permissivity levels resulting in differences

206 in viral titers. For SARS-CoV2, the entry step involves the ACE2 receptor and two  
207 independent host protease pathways TMPRSS2 or the cathepsins B/L that activate the Spike  
208 viral protein. The virus may not use these two ways similarly, and the expression level of  
209 these receptors mediating virus entry are differentially expressed according to the cell lines  
210 [31]. For example, VeroE6 engineered for expressing greater amounts of TMPRSS2 were  
211 used elsewhere, resulting in 100-fold higher titers of SARS-CoV-2 [32]. Inversely, viral titers  
212 provided by SARS-CoV2 infected calu-3 cells (Continuous human lung epithelial cell line)  
213 are lower than in Vero cells. [32]. It could make sense for the sensitivity assays to use the cells  
214 physiologically closest from those of the replication site in vivo. In this view, primary cells  
215 derived from organ explants were used for sensitivity assays and it seemed to be a relevant  
216 approach. However, variable effects which are donor-dependent on the sensitivity for some  
217 tested drugs should be expected due to differences in viral replication and gene expression  
218 [33]. Thus this approach could be a false good idea and testing molecules in a coarse model  
219 such as in Vero E6 that have genetic defect in interferon production could help in evidencing  
220 an effect. Secondly, the multiplicity of infection reported the drug concentration is not  
221 standardized. It seems obvious that the higher the MOI, the lower is the relative drug  
222 concentration, and the more likely the virus can replicate. This MOI is not even mentioned in  
223 some studies. Finally, time for end point evaluation and the method used for assessment of  
224 viral replication can also vary according to the studies from 1 hour to 120 hours [34,35].  
225 Besides, assessment of viral replication by PCR or fluorescent assay or visual inspection to  
226 monitor cell viability may also not have the same sensitivity. In example for the latter, some  
227 permissive cells such as human intestinal epithelial Caco-2 do not produce cytopathic effects  
228 after SARS-CoV2 infection and thus cannot be evaluated with such method [36]. As a result,  
229 it is risky to draw conclusions on a single sensitivity test, especially when testing a virus with  
230 a high genomic variability.

231 One of the most interesting perspectives should be also to test multiple viral strains to  
232 check concordance of the results. Currently, *in vitro* assays use essentially 1 or 2 SARS-CoV2  
233 strains. Our work suggests it is risky to draw conclusions on a single sensitivity test when  
234 testing a virus with a high genomic variability. Indeed we observe an heterogeneity in the  
235 hydroxychloroquine antiviral activity screening of 30 strains and could detect three strains  
236 with a lower susceptibility profile. For isolates from patients of the first period of the  
237 epidemic, persistence was clearly not associated to a lowered susceptibility profile to  
238 hydroxychloroquine *in vitro*. This confirmed the observation that persistence and severity are  
239 rather associated with host factors as suggested by recent genetic research on Covid 19  
240 severity-associated factors [37,38] or immunocompromised status [39,40]. Moreover,  
241 genomic analyses did not reveal any modification in these isolates that could explain  
242 persistence, neither in the sequence of the strain isolated at admission nor in that of the strain  
243 isolated during the course of the disease. The less susceptible hydroxychloroquine strain,  
244 IHUMI-2123, that belongs to Marseille 1 genotype was isolated in early summer at the  
245 beginning of the second episode from a patient returning from Tunisia [30], which is a  
246 country where hydroxychloroquine was massively used [19]. We evidenced a close  
247 phylogenetic proximity between all strains of the Marseille 1 clade (IHUMI-2123, IHUMI-  
248 2122, IHUMI-2178, and IHUMI-2177) with strains isolated in Senegal and Gambia, both  
249 countries using hydroxychloroquine to treat patients with COVID-19 [41,42]. We believe that  
250 it is possible that a large use of hydroxychloroquine in these countries selected strains with  
251 reduced susceptibility that were latter transmitted to Marseille population. Paradoxically, the  
252 patients tested in Marseille hospitals during the period of early summer and infected by  
253 isolates of this genotype presented milder infections and lower mortality than observed during  
254 the first part of the epidemic although the viral loads in their respiratory secretions were  
255 higher [43]. This observation raises several questions that will be difficult to resolve soon

256 such as does the lowered susceptibility to hydroxychloroquine reduces severity of infection or  
257 is it useful to use hydroxychloroquine in such case or only in patients with severity markers or  
258 risk factors such as anticoagulant lupus for which hydroxychloroquine is the treatment and  
259 thus, efficiency likely, not due to an antiviral effect [44–46]. But finally, the  
260 hydroxychloroquine concentration to achieve 50% of viral inhibition was around 3.125  $\mu\text{M}$   
261 for the three strains with high hydroxychloroquine susceptibility and  $> 12.5 \mu\text{M}$  for the three  
262 strains with reduced susceptibility, which require at least four times more hydroxychloroquine  
263 for the same effect (Figure 2). Moreover, a 90% viral inhibition required around 12.5  $\mu\text{M}$  for  
264 susceptible strains and  $> 25 \mu\text{M}$  for less susceptible strains. However, these concentrations  
265 remain consistent with concentrations observed in human plasma and lungs. An oral uptake of  
266 400 mg of hydroxychloroquine led to a maximum blood concentration ( $C_{\text{max}}$ ) of 1.22  $\mu\text{M}$   
267 [47]. But, hydroxychloroquine accumulated 30 times more in lungs than in blood [48],  
268 allowing a potential efficiency of hydroxychloroquine even against strains with reduced  
269 susceptibility. An oral uptake of 400 mg of hydroxychloroquine would still be effective *in*  
270 *vivo* in humans infected with the current strains with *in vitro* reduced susceptibility to  
271 hydroxychloroquine.

272         However, these genotypic and phenotypic variations could be frequent in the viral  
273 populations in the future and could apply to more drugs and need to be considered in the  
274 global repurposing strategy. By the description of Korber et al we know that the spike  
275 population evolved during February - April 2020 and constituted a fast replacing situation by  
276 the G614 [29]. Recently a major situation was noticed by Denmark where minks were  
277 infected with a strain presenting a few mutations notably in the spike protein and associated  
278 with a selection pressure in a potential zoonotic transfer. Those aspects need to be carefully  
279 considered, for testing and using antiviral compounds but also for epidemiology and  
280 vaccination strategy.

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284

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286           This manuscript has been edited by a native English speaker

287 **Conflicts of Interest:** The authors declare no conflict of interest.

288

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**Table 1. Strains and patients information**

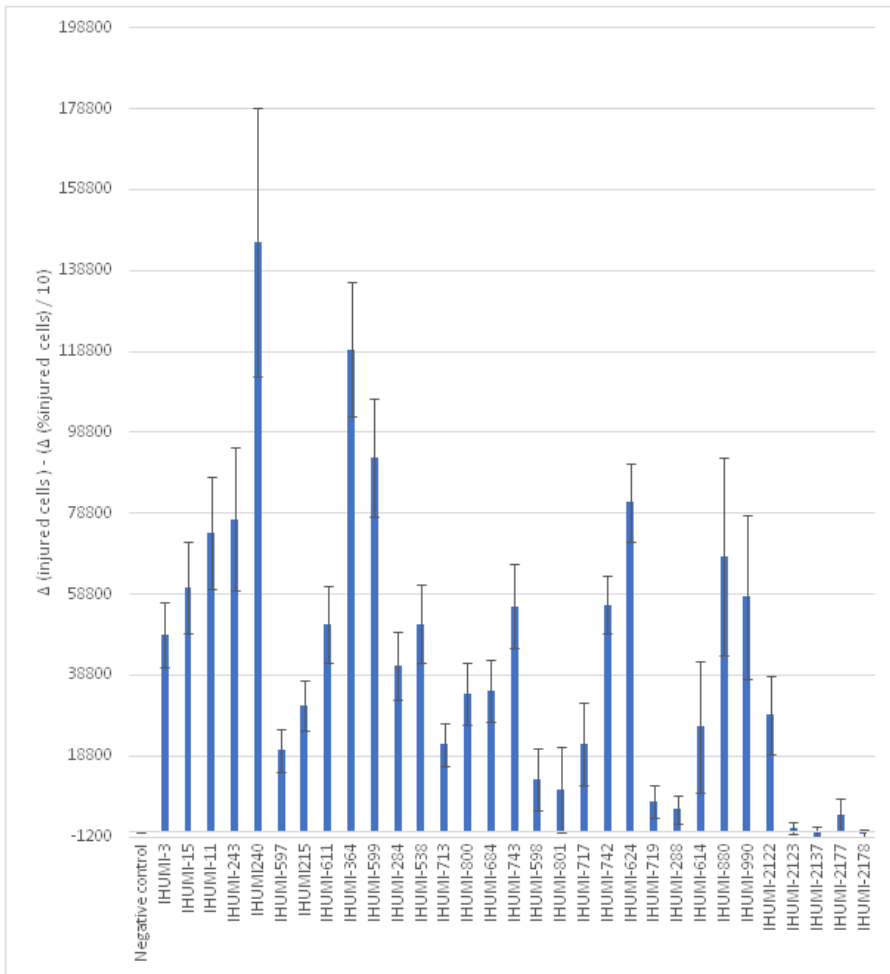
Patients number	Strains	Month of sampling	Persistence	Days of the lastest isolate after onset	RT-PCR Ct values	Treatment	Clade	GISAID access number
Patient 1	IHUMI-11	March 2020	No	NA	29	AZT HCQ	20A/15324T	IHUCOVID-0760
Patient 2	IHUMI-15	March 2020	No	NA	23	HCQ	20B	IHUCOVID-0649
Patient 3	IHUMI-240	March 2020	No	NA	22	AZT HCQ	20C-5	IHUCOVID-0152
Patient 4	IHUMI-243	March 2020	No	NA	29	AZT HCQ	20A/15324T	IHUCOVID-0762
Patient 5	IHUMI-597	March 2020	No	NA	20	/	20A/25563T	IHUCOVID-0142
Patient 6	IHUMI-215	March 2020	Yes	8	23	AZT HCQ	20A/25563T-1B	IHUCOVID-756
	32,2				20A/25563T-1B			
Patient 7	IHUMI-364	March 2020	Yes	4	21	AZT HCQ	20A/15324T	IHUCOVID-143
	IHUMI-599				29,1			
Patient 8	IHUMI-284	March 2020	Yes	4	30		20A/A0268G-2	IHU-0144
	IHUMI-538				20,5	AZT	20A/A0268G-2	
Patient 9	IHUMI-713	March 2020	Yes	3	31	AZT HCQ	20A/25563T-1	IHUCOVID-0272
	IHUMI-800				23,1		20A/25563T-1	

Patient 10	IHUMI-684	March 2020	Yes	4	21,6	AZT HCQ	20A/25563T	IHUCOVID-0147
	IHUMI-743				20,4		20A/25563T	
Patient 11	IHUMI-598	March 2020	Yes	4	20,5	AZT HCQ	20C-5	IHUCOVID-0151
	IHUMI-801				20,7		20C-5	
Patient 12	IHUMI-717	March 2020	Yes	2	21,2	AZT HCQ	20B-1a	IHUCOVID-0312
	IHUMI-742				19,1		20B-1a	
Patient 13	IHUMI-624	March 2020	Yes	2	16,1	AZT HCQ	20A/25563T-1b	IHUCOVID-0749
	IHUMI-719				17,7			
Patient 14	IHUMI-288	March 2020	Yes	5	23	AZT HCQ	20C-4	IHUCOVID-0752
	IHUMI-614				26			
Patient 15	IHUMI-880	April 2020	Yes	3	19	HCQ	20B	IHUCOVID-°0641
	IHUMI-990				21,4		20B	
Patient 16	IHUMI-2122	July 2020	Unknown	NA	17,8	AZT	Marseille 1	IHUCOVID0976
Patient 17	IHUMI-2123	July 2020	Yes	NA	17,7	AZT HCQ	Marseille 1	IHUCOVID0982
Patient 18	IHUMI-2137	August 2020	Yes	NA	14,7	AZT HCQ	Marseille 5b	IHUCOVID1329
Patient 19	IHUMI-2177	August 2020	No	NA	25,1	AZT	Marseille 1A	

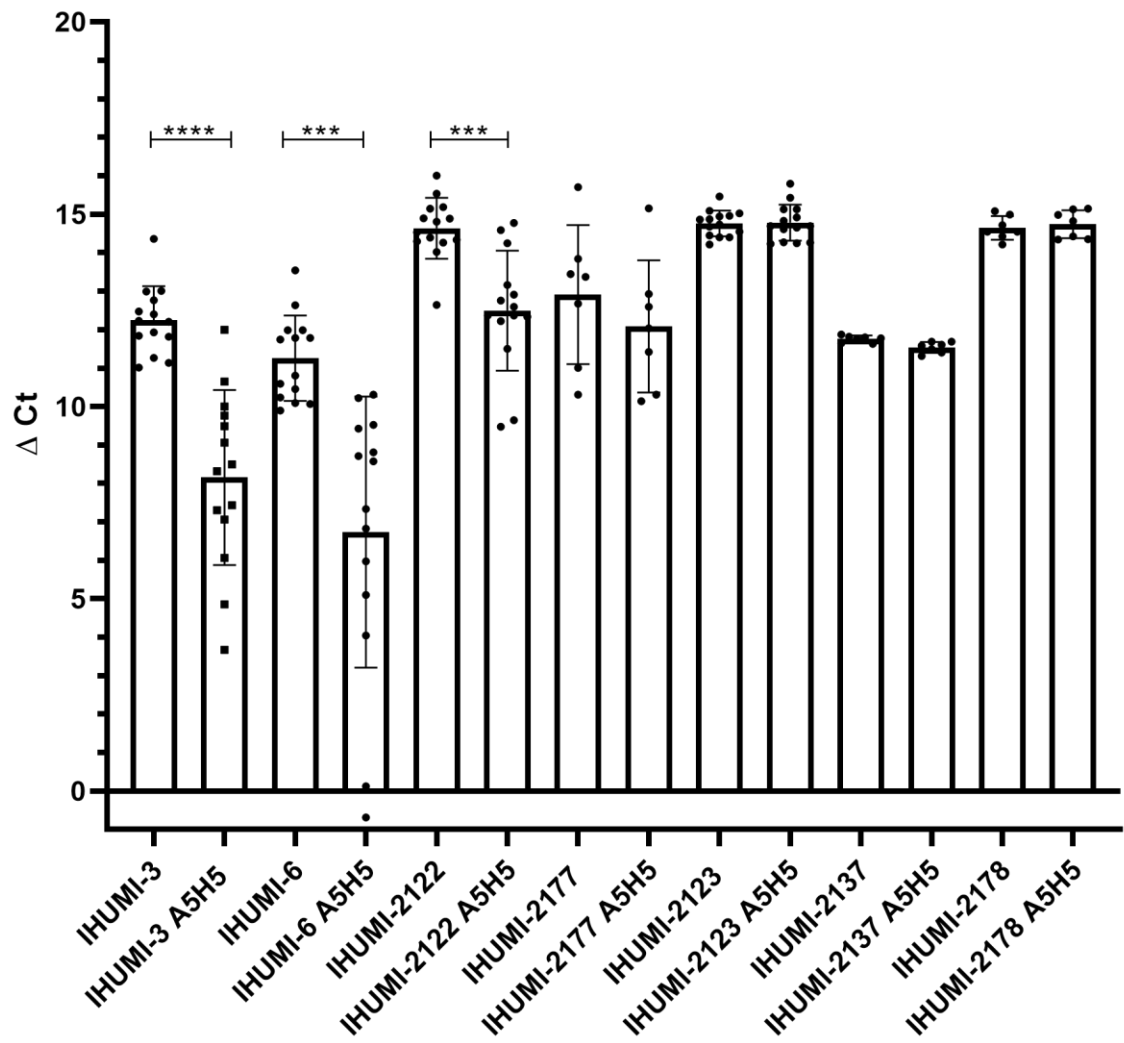
Patient 20	IHUMI-2178	August 2020	Unknown	NA	21,6	AZT HCQ	Marseille 1A	IHUCOVID1212
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NA : not applicable because only one strain was obtained

**Figure 1. Initial screening of the 31 selected SARS-CoV-2 isolates to a combination of hydroxychloroquine and azithromycin at 5  $\mu$ M each. (1a)** Difference observed between cells treated or not treated calculated by high-content screening for each strain. **(1b)** Effect of hydroxychloroquine and azithromycin association on SARS-CoV 2 replication on selected isolates. Delta Ct between 0 and 48 h post infection. Ordered axis represents the variation of delta cycle-thresholds obtained by RT-PCR between H0 and H48 for each condition. Each point represents data obtained for one well. Median and interquartile range were indicated for each condition. \*\*\* represent significant results under  $p < 0,0005$ . Others are not significant compared to the control

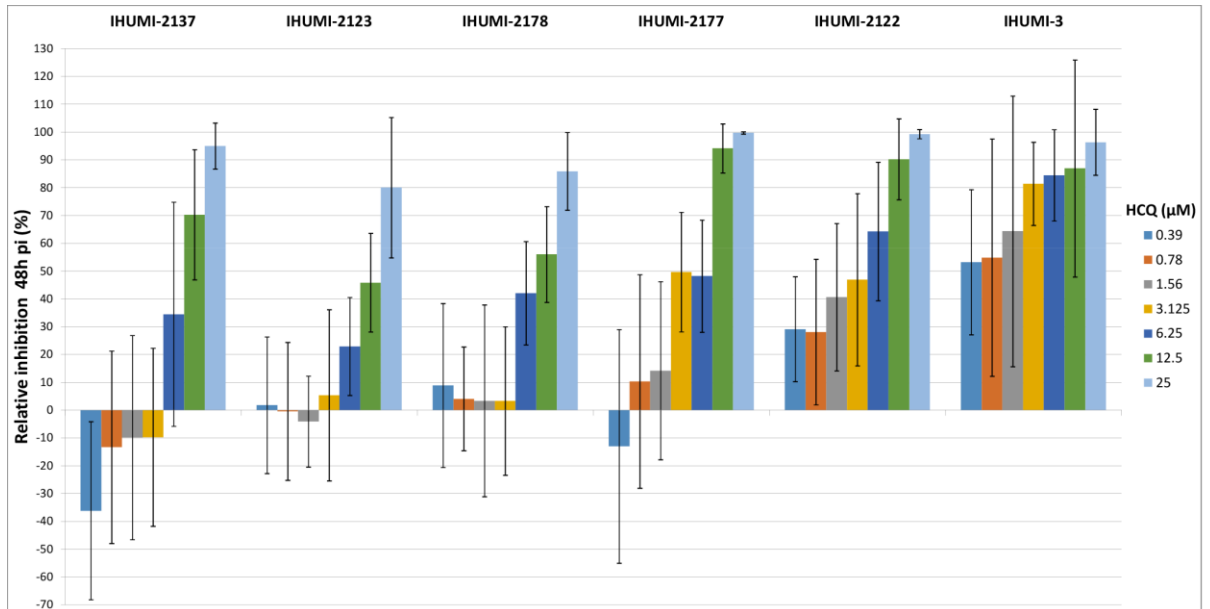






**Figure 2:** Exploration of effect-dose curves of hydroxychloroquine. The range used from 25  $\mu\text{M}$  to 0,39  $\mu\text{M}$  tested on IHUMI-3, IHUMI-2122, IHUMI-2123, IHUMI-2137, IHUMI-2177 and IHUMI-2178 strains.

Abbreviations : p.i, post-infection; HCQ, hydroxychloroquine ;  $\mu\text{M}$ , micromolar



**Figure 3:** Phylogenetic tree of whole genomes from IHUMI strains including closely related genomes available from GISAID. Mutation scales are compared to the Wuhan reference genome.

