

Correlation between 3790 qPCR positives samples and positive cell cultures including 1941 SARS-CoV-2 isolates

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Dear Editor -

Since the beginning of the outbreak of the emerging epidemic (Covid-19) due to SARS-CoV-2, declared as a pandemic on March 12th 2020 by the WHO [1], a major issue has been to correlate viral RNA load obtained after RT-PCR and expressed in cycle threshold (Ct) with contagiousness and therefore duration of eviction from contacts and discharge from specialized infectious disease wards. Several works published recently and based on more than 100 studies attempt to propose such cut off for Ct value and duration of eviction with a consensus at approximately $Ct > 30$ and at least 10 days, respectively [2–5]. However, in an article published in this journal, a group reported that patients could be not be contagious above 25 Ct as the virus was not detected in culture above this Ct [6]. This limit was then evoked in the French media during the interview with the member of the French Scientific Council Covid-19 as a possible value above which patients are no longer contagious [7]. At the beginning of the outbreak, we correlated the Ct values obtained by our PCR technique based on the amplification of the E gene and the results of the culture [8]. Since the beginning of the epidemic, we have performed in our institute 250,566 SARS-CoV-2 RT-PCR for 179,151 patients, of which 13,161 (7.3%) tested positive. Up to the end of May, 3 790 of these samples reported positives on naso-pharyngeal samples were inoculated and managed for culture as previously described [8]. Of these 3 790 inoculated samples, 1941 SARS-Cov-2 isolates could be obtained after the first inoculation or up to 2 blind subcultures. The correlation between the scanner values and the positivity of the culture allows us to observe that the image obtained with ten times more isolates than our preliminary work (1941 versus 129) does not change significantly (Figure 1). It can be observed that at $Ct=25$, up to 70% of patients remain positive in culture and that at $Ct=30$ this value drops to 20%. At $Ct=35$, the value we used to report positive result for PCR, less than 3% of culture are negative. Our Ct value of 35 initially based on the results obtained by RT-PCR on control negative samples in

our laboratory and initial results of cultures [8] is validated by the present work and is in correlation with what was proposed i.e. in Korea [9] or Taiwan [10]. We could observe that subcultures, especially the first one, allow increasing percentage of viral isolation on high Ct samples, confirming that these high Ct are mostly correlated with low viral loads. From our cohort, we now need to try to understand and define the duration and frequency of live virus shedding in patients on a case-by-case basis, in the rare cases where the PCR is positive beyond 10 days, often at a Ct above 30. In any cases, these rare cases should not impact public health decisions.

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Ethical approval : The protocol was approved by the ethical committee of the University Hospital Institute Méditerranée Infection (N°: 2020-01). All subjects provided informed consent in accordance with the Declaration of Helsinki.

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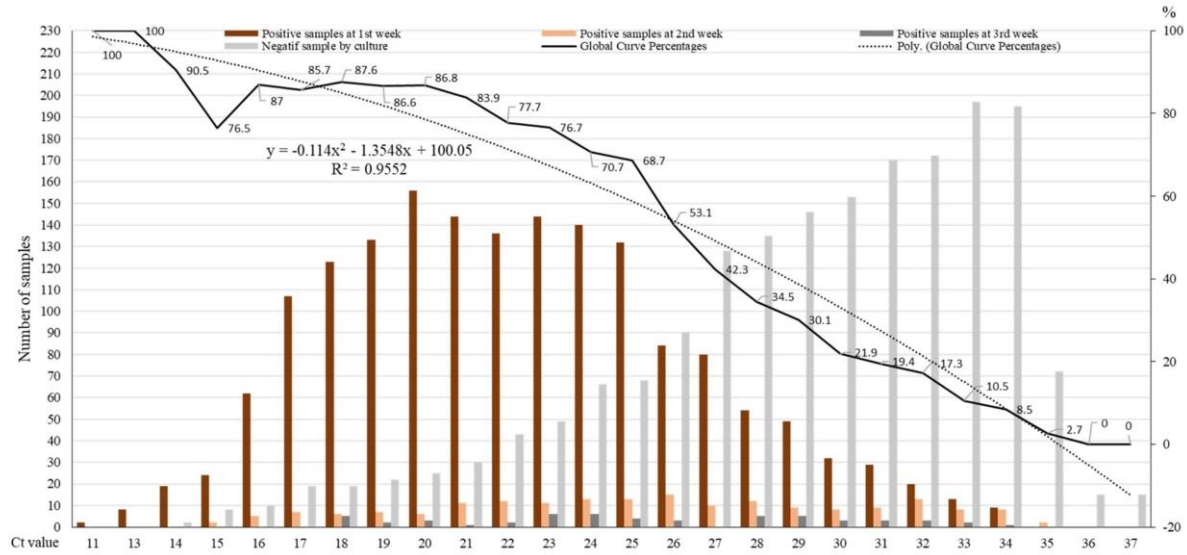
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Figure 1. Percentage of positive viral culture of SARS-CoV-2 PCR-positive nasopharyngeal samples from Covid-19 patients, according to Ct value (plain line). The dashed curve indicates the polynomial regression curve.

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Figure 1



Number of samples (N = 3790)	2	8	21	34	77	133	153	164	190	186	193	210	225	217	192	218	206	209	196	211	208	220	213	74	15	15
Total positive (n = 1941)	2	8	19	26	67	114	134	142	165	156	150	161	159	149	102	90	71	63	43	41	36	23	18	2	0	0
Week 1: number of positive (n = 1700)	2	8	19	24	62	107	123	133	156	144	136	144	140	132	84	80	54	49	32	29	20	13	9	0	0	0
(% by total positive)	(100)	(100)	(100)	(92.3)	(92.5)	(93.9)	(91.8)	(93.7)	(94.5)	(92.3)	(90.7)	(89.4)	(88.1)	(88.6)	(82.4)	(88.9)	(76.1)	(77.8)	(74.4)	(70.7)	(55.6)	(56.5)	(50.0)	(0)	(-)	(-)
Week 2: number of positive (n = 187)	0	0	0	2	5	7	6	7	6	11	12	11	13	13	15	10	12	9	8	9	13	8	8	2	0	0
(% by total positive)	(0)	(0)	(0)	(7.7)	(7.5)	(6.1)	(4.5)	(4.9)	(3.6)	(7.1)	(8.0)	(6.8)	(8.2)	(8.7)	(14.7)	(11.1)	(16.9)	(14.3)	(18.6)	(22.0)	(36.1)	(34.8)	(44.0)	(100)	(-)	(-)
Week 3: number of positive (n = 54)	0	0	0	0	0	0	5	2	3	1	2	6	6	4	3	0	5	5	3	3	3	2	1	0	0	0
(% by total positive)	(0)	(0)	(0)	(0)	(0)	(0)	(3.7)	(1.4)	(1.8)	(0.6)	(1.3)	(3.7)	(3.8)	(2.7)	(2.9)	(0)	(7.0)	(7.9)	(7.0)	(7.3)	(8.3)	(8.7)	(5.6)	(0)	(-)	(-)
Negative cultured (n = 1849)	0	0	2	8	10	19	19	22	25	30	43	49	66	68	90	128	135	146	153	170	172	197	195	72	15	15