European Court Rules PCR Tests Unreliable

11th Nov 2020 : A Portuguese Court of Appeal has made a judgement in relation to a detention case. In it the Court analysed how reliable the PCR Test is and concluded that if misused the PCR Test would have a reliability as low as 3%. for the detection of Coronavirus, and with a False Positive rate of 97%.

The judge references a Sept 2020 paper in the Clinical Infectious Diseases Journal which determined that the quality of a PCR Test depends on the amount of Amplification Cycles used in the test with the following Cycles vs Quality tradeoff:

Cycles	Reliability
25	70%
30	20%
35	3%
>35	0%

The Portugeuse judge concluded that at 35cycles a PCR Test produces only 3% reliability and 97% False Positives.

I attach herein the following documents:

1. A summary of the Portuguese case in the Court of Appeals Lisbon (Nov 2020)

2. A summary of the recent expert paper (Clinical Infectious Diseases Journals Sept 2020) upon which the court made its analysis of the PCR Test

3. The NHS guidance for the use of PCR for the diagnosis of Coronavirus, specifying the usage as 45 Cycles.

4. Comparison of Covid 2020 Deaths vs Total UK Deaths 2004-2019 (ONS Data)

5. Preliminary Results from the Liverpool Mass Testing programme, comparing two different Covid tests, the older PCR Test and a newer LFT (lateral flow test).
The results after 150,000 patients in Liverpool is that the LFT shows 1/5th of the cases of the PCR Test. However please note that these figures are not *directly* comparable as the selection of patients for each test is different.

Note:

False Positive = “a test result which wrongly indicates that a particular condition or attribute is present”

Cycles (Amplification Cycles) = A PCR Test first “amplifies” the material in the tube a specified number of times before then trying to match the material in the sample against a genetic signature of a virus.

Shortcuts:

1783 / 20.7T8PDL.L1-3
Rapporteur: MARGARIDA RAMOS DE ALMEIDA
Descriptors: HABEAS CORPUS
INTEREST IN ACTING SARS-COV-2 RT-PCR TESTS
DEPRIVATION OF FREEDOM
ILLEGAL DETENTION RL
Date of Agreement: 11/11/2020
Voting: UNANIMITY
Decision: Denied Providence

I. The ARS cannot appeal against a decision that ordered the immediate release of four people, due to illegal detention, in the context of a habeas corpus process (art. 220 als. C) and d) of CPPenal), asking for the validation of the compulsory confinement of the applicants, for being carriers of the SARS-CoV-2 virus (A....) and for being under active surveillance, due to high risk exposure, decreed by the health authorities (B..., C.... and D... ..) because it has no legitimacy or interest in acting.

II. The request made would also be manifestly unfounded because:

A. The prescription and diagnosis are medical acts, under the exclusive responsibility of a doctor, registered with the Ordem dos Médicos (Regulation No. 698/2019, of 5.9). Thus, the prescription of auxiliary diagnostic methods (as is the case of tests for the detection of viral infection), as well as the diagnosis of the existence of a disease, in relation to each and every person, is a matter that cannot be performed by law , Resolution, Decree, Regulation or any other normative way, as these are acts that our legal system reserves to the exclusive competence of a doctor, being sure that, in advising his patient, he should always try to obtain his informed consent (1 of article 6 of the Universal Declaration on Bioethics and Human Rights).

B. In the case that we are dealing with, there is no indication or proof that such diagnosis was actually carried out by a professional qualified under the terms of the Law and who had acted in accordance with good medical practices. In fact, what follows from the facts taken for granted, is that none of the applicants was even seen by a doctor, which is frankly inexplicable, given the alleged seriousness of the infection.

C. The only element that appears in the proven facts, in this respect, is the performance of RT-PCR tests, one of which presented a positive result in relation to one of the applicants.

D. In view of the current scientific evidence, this test is, in itself, unable to determine, beyond reasonable doubt, that such positivity corresponds, in fact, to the infection of a person by the SARS- CoV-2 virus, by several reasons, of which we highlight two (to which is added the issue of gold standard which, due to its specificity, we will not even address): For this reliability depend on the number of cycles that make up the test; For this reliability depend on the amount of viral load present.

III. Any diagnosis or any act of health surveillance (as is the case of determining the

detect the RNA of the virus, commonly used in Portugal to test and list the number of infected (after nasopharyngeal collection), are performed by amplifying samples , through repetitive cycles.

The number of cycles of such amplification results in the greater or lesser reliability of such tests.

iii. And the problem is that this reliability is shown, in terms of scientific evidence (and in this field, the judge will have to rely on the knowledge of experts in the field), more than debatable.

This is the result, among others, of the very recent and comprehensive Correlation study between 3790 qPCR positives samples and positive cell cultures including 1941 SARS-CoV-2 isolates, by Rita Jaafar, Sarah Aherfi, Nathalie Wurtz, Clio Grimaldier, Van Thuan Hoang, Philippe Colson, Didier Raoult, Bernard La Scola, Clinical Infectious Diseases, ciaa1491, https:

//doi.org/10.1093/cid/ciaa1491,em https://academic.oup.com/cid/advance-article/doi/10.1093 / cid / ciaa1491 / 5912603, published at the end of September this year, by Oxford Academic, carried out by a group that brings together some of the greatest European and world experts in the field.

This study concludes [2], in free translation: "At a cycle threshold (ct) of 25, about 70% of the samples remained positive in the cell culture (i.e. they were infected): in a ct of 30, 20% of the samples remained positive; in a ct of 35, 3% of the samples remained positive; and at a ct above 35, no sample remained positive (infectious) in cell culture (see diagram).

This means that if a person has a positive PCR test at a cycle threshold of 35 or higher (as in most laboratories in the USA and Europe), the chances of a person being infected are less than 3%. The probability of a person receiving a false positive is 97% or higher ".

iv. What follows from these studies is simple - the possible reliability of the PCR tests performed depends, from the outset, on the threshold of amplification cycles that they support, in such a way that, up to the limit of 25 cycles, the reliability of the test will be about 70%; if 30 cycles are carried out, the degree of reliability drops to 20%; if 35 cycles are reached, the degree of reliability will be 3%.

v. However, in the present case, the number of amplification cycles with which PCR tests are carried out in Portugal, including the Azores and Madeira, is unknown, since we were unable to find any recommendation or limit in this regard. saw. For its part, in a very recent study by Elena Surkova, Vladyslav Nikolayevskyy and Francis Drobniewski, accessible at https://www.thelancet.com/journals/lanres/article/PIIS2213-2600(20)30453-7/fulltext, published in the equally prestigious The Lancet, Respiratory Medicine, it is mentioned (in addition to the multiple questions that the precision of the test itself raises, regarding the specific detection of the sars-cov virus 2, due to strong doubts about the fulfillment of the so-called gold standard) that (free translation):

"Any diagnostic test must be interpreted in the context of the actual possibility of the disease, which existed before its realization. For Covid-19, this decision to perform the test depends on the previous assessment of the existence of symptoms, previous medical

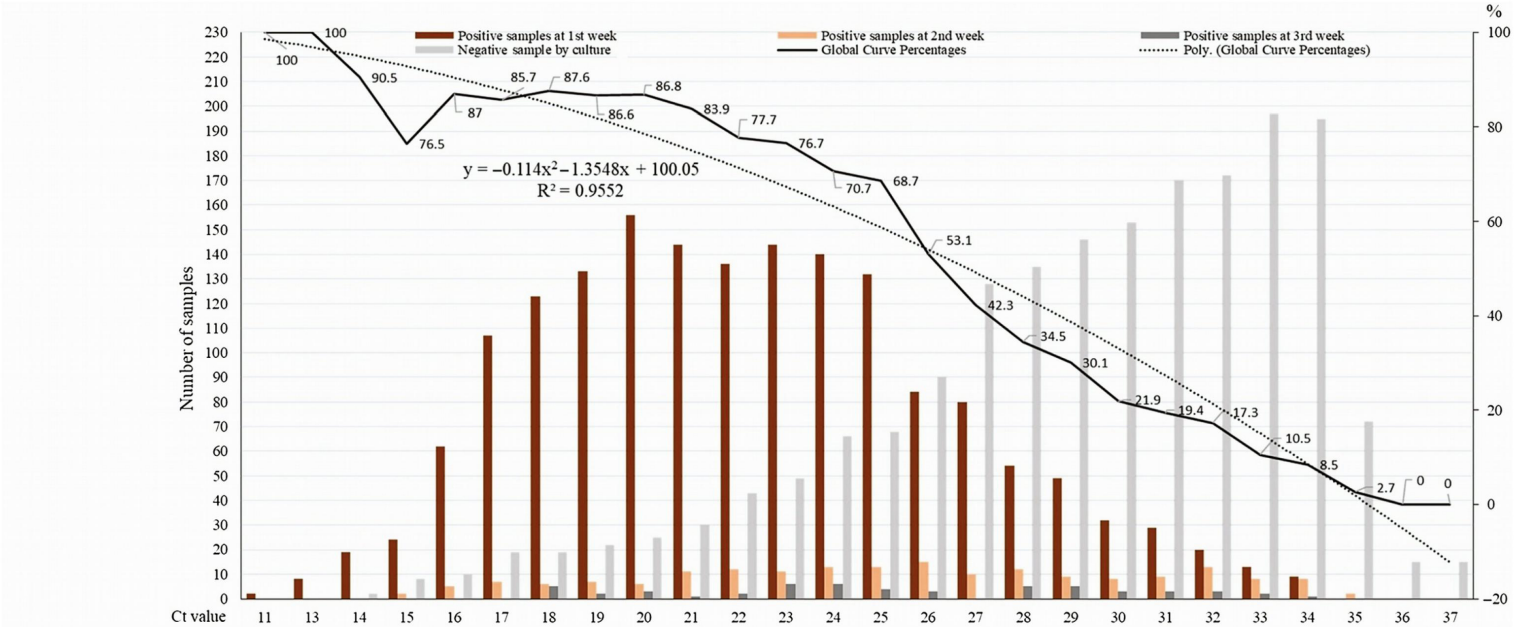
The Scientific Paper on which this judge's conclusions are based is:

Correlation Between 3790 Quantitative Polymerase Chain Reaction–Positives Samples and Positive Cell Cultures, Including 1941 Severe Acute Respiratory Syndrome Coronavirus 2 Isolates
by Rita Jaafar, Sarah Aherfi, Nathalie Wurtz, Clio Grimaldier, Thuan Van Hoang, Philippe Colson, Didier Raoult, Bernard La Scola

Clinical Infectious Diseases, ciaa1491, https://doi.org/10.1093/cid/ciaa1491
Published: 28 September 2020

In the following chart these scientists mark the reliability of the PCR test against the number of cycles (ct value) ... and the result is the thick black line which is at its maximum at about 20 cycles being 86.8%, but at 35 cycles is at 2.7% reliability.

Remember again the NHS is using 45 cycles.



Ct value	11	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37
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 positives (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 positives (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 positives (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

Clinical Infectious Diseases

IDSA Infectious Diseases Society of America

Clinical Infectious Diseases, ciaa1491, https://doi.org/10.1093/cid/ciaa1491
Published: 28 September 2020 **Article history** ▾

Guidance and Standard Operating Procedure
COVID-19 Virus Testing in NHS Laboratories

NHS England and NHS Improvement

Appendix 5: PHE COVID -19 Testing Protocol – <u>If not using Commercial Assay</u>

Public Health England

2019-nCoV real-time RT-PCR RdRp gene assay

A. Background

This protocol describes a uniplex real-time RT-PCR assay for the detection of the 2019 novel coronavirus (2019-nCoV). A 100 bp long fragment from a conserved region of the RNA-dependent RNA polymerase (RdRP) gene is detected with FAM labelled hydrolysis probes. The assay will detect 2019-nCoV and SARS virus, as well as other bat-associated SARS-related viruses (Sarbecovirus). In the validated and published format, the assay employs the use of two probes; one will detect 2019-nCoV, SARS-CoV and bat-SARS-related CoVs, and the other 2019-nCoV only.[1]
The RdRp gene assay has been evaluated in the Respiratory Virus Unit, PHE, on the ABI 7500 Fast real-time PCR system.

B. Reagents

1. Primers and probes – order from TIB Molbiol, Germany.

Assay	Oligonucleotide ID	Sequence (5' - 3')	Concentration*
RdRp gene	RdRp_SARSr-F2	GTGARATGGTCATGTGTGGCGG	use 600 nM per reaction
	RdRp_SARSr-R1	CARATGTTAAASACACTATTAGCATA	use 800 nM per reaction
	RdRp_SARSr-P2	FAM-CAGGTGGAACCTCATCAGGAGATGC-BBQ	Specific for 2019-nCoV, will not detect SARS-CoV use 100 nM per reaction and mix with P1
	RdRp_SARSr-P1	FAM-CCAGGTGGWACRTCATCMGGTGATGC-BBQ	Pan Sarbeco-Probe, will detect 2019-nCoV virus, SARS-CoV and bat-SARS-related CoVs use 100 nM per reaction and mix with P2

FAM, 6-carboxyfluorescein; BBQ, blackberry quencher

*Optimized concentrations are mol per liter of final reaction mix.
(e.g., 1.5 microliters of a 10 micromolar (uM) primer stock solution per 25 microliter (ul) total reaction volume yields a final concentration of 600 nanomol per liter (nM) as indicated in the table)

[1]Drosten et al. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Eurosurveillance 2020; 25 (3).

Version 1.0 28.01.2020

2. Invitrogen SuperScript III Platinum one-step qRT-PCR kit. Cat nos. 11732-020 and 11732-088. Order from ThermoFisher Scientific, UK.

C. Preparation of RT-PCR mix and cycling conditions

RdRp-assay

MasterMix:	**Single rxn (µl)**
H_2O (RNAse free)	2.1
2x Reaction mix	12.5
$MgSO_4$(50mM)	0.4
RdRp_SARSr-F2 primer (10 µM)	1.5
RdRp_SARSr-R1 primer (10 µM)	2
RdRp_SARSr-P1 probe (10 µM)	0.25
RdRp_SARSr-P2 probe (10 µM)	0.25
SSIII/Taq Enzyme Mix	1
MasterMix per well / total	20
Template RNA	5
	25µl

NHS Specifies Usage of 45 Cycles in PCR Tests.

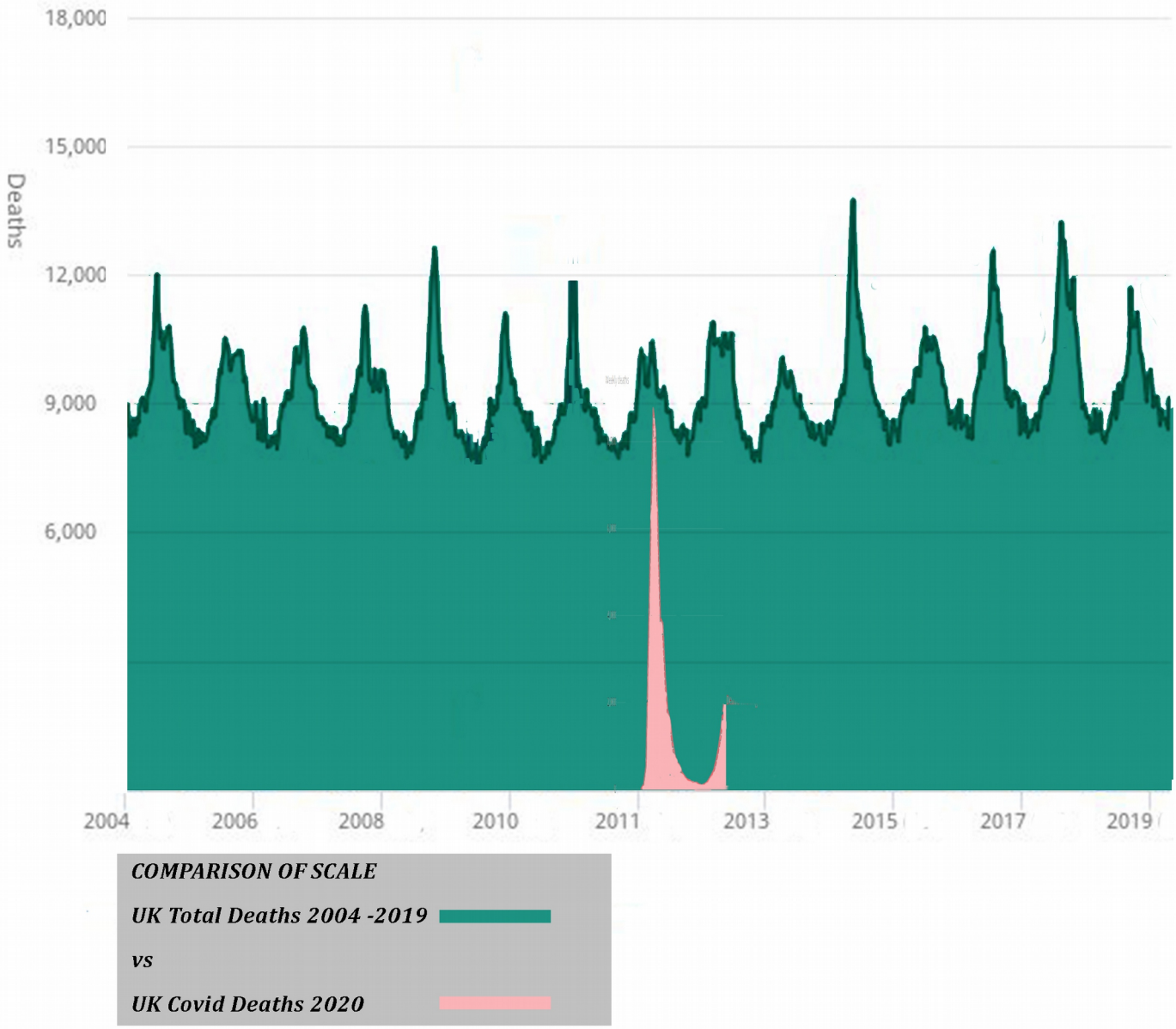
Cycler:

Each Cycle takes 13mins 45secs and has 4 steps

55°C	10 min	
94°C	3 min	45x cycles
94°C	15 sec	
58°C	30 sec	

Passive reference: none
Standard mode

COMPARISON OF SCALE
UK Total Deaths 2004-2019
vs
UK Covid Deaths 2020



Office for National Statistics

Covid Deaths 2020
https://www.ons.gov.uk/peoplepopulationandcommunity/birthsdeathsandmarriages/deaths/bulletins/deathsregisteredweeklyinenglandandwalesprovisional/latest

Total Deaths 2004 2019
https://www.ons.gov.uk/peoplepopulationandcommunity/birthsdeathsandmarriages/deaths/bulletins/excesswintermortalityinenglandandwales/latest

Liverpool mass testing programme

Provisional figures as at 18 November 2020 13:02

Source: Combined Intelligence for Population Health Action (CIPHA)
Pillar 2 (swab testing for the wider community population) testing data only

Tests - Liverpool Residents	06 - 18 November 2020
No of people tested: PCR	55,827
No of positive tests: PCR	1,708

= 0.031% positive

Tests - Liverpool Residents	06 - 18 November 2020
No of people tested: LFT	82,106
No of positive tests: LFT	518

= 0.0063% positive

The new LFT Covid Test finds far fewer cases than the older PCR Test

Further information on how to get tested is available from here:

Symptom-free mass testing - Liverpool City Council

Note : this is not a *direct* comparison as there is a different selection of patients for each test.

Liverpool City Council : Mass Testing Data
https://tinyurl.com/y5svgpa5

The LFT Test : Developed by Oxford University and Porton Down
https://tinyurl.com/yx9ftyhh