Nature Remade: falsifying Polymerase Chain Reaction

Kevin P Corbett, MSc PhD

‘Biotechnology’

Hungarian scientist Karl Ereky coined the word "biotechnology" in 1919 in ***Biotechnology of Meat, Fat and Milk Production in an Agricultural Large-Scale Farm*** on a technique to convert natural materials into products.

1953
James Watson and Francis Crick first unravelled the structure of DNA at Cambridge University, they hypothesised that one day it would be possible to copy genetic material.

1956
Arthur Kornberg, based at Washington University St Louis, identified and isolated DNA polymerase, an enzyme that replicates a cell's DNA.

1960s
Gobind Kohrana, an Indian-American biochemist based at the Massachusetts Institute of Technology, found a means to synthesise DNA oligonucleotides, short bits of nucleic acids vital for artificial gene synthesis.

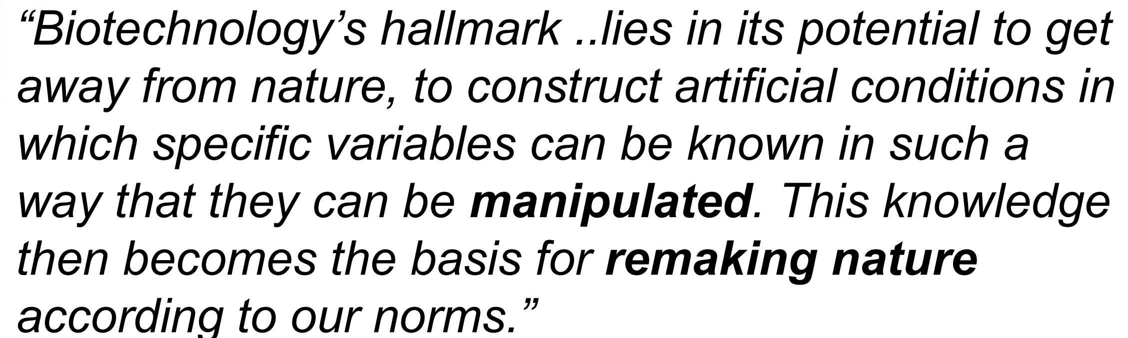
1971
Kjell Kleppe, a Norwegian researcher working with Kohrana, came up with the founding principle for PCR. He suggested bracketing a targeted DNA sequence using a pair of primers, strands of nucleic acids which serve as the starting point for DNA synthesis, and then copying the sequence using DNA polymerase.

1977
Use of primers was to become much easier to put into action with the development of techniques to sequence DNA by Fred Sanger at Cambridge University

PCR is a ***biotechnology***

“New biological techniques that found commercial applications during the 1970s and 1980s.”

(Kenney 1986 p,2)



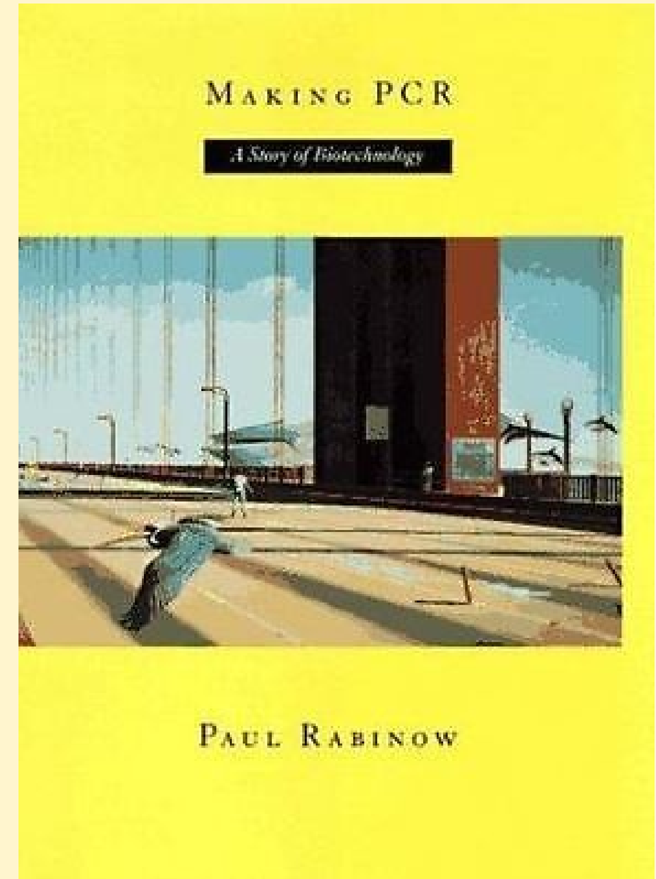
“Biotechnology’s hallmark ..lies in its potential to get away from nature, to construct artificial conditions in which specific variables can be known in such a way that they can be ***manipulated****. This knowledge then becomes the basis for* ***remaking nature*** *according to our norms.”*

(Rabinow 1996, p20 emphasis added)

PCR invented the 1980s

Bio-
technology
The
University–
Industrial
Complex
Martin Kenney

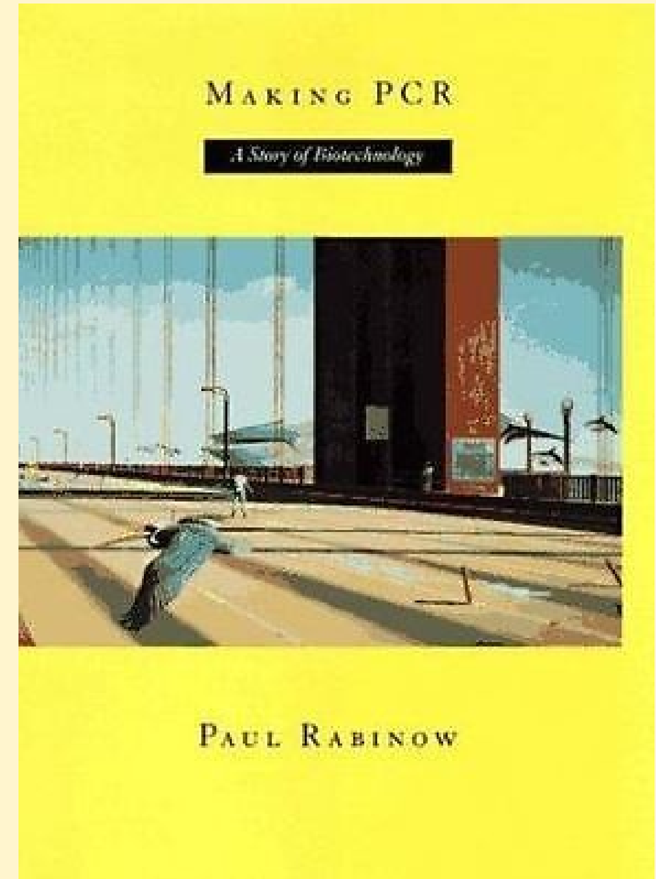
- Kary B. Mullis shared 1993 Nobel Prize for PCR *concept invention*
- Others helped refine the *technique*: Henry Erlich, Norman Arnheim, Randall Saiki, Glen Horn, Corey Levenson, Steven Sharf, Fred Faloona and Tom White (Rabinow 1996)

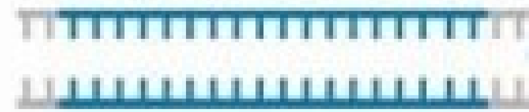


Cetus Corporation: first recombinant DNA start-up company founded in 1971

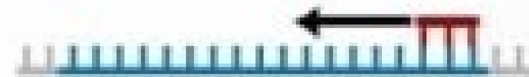
- PCR developed at the Cetus Corporation (Rabinow 1996)
- Develop and commercialise a new method of: ***"diagnosing disease at the nucleic acid level without the need for tissue culture"***

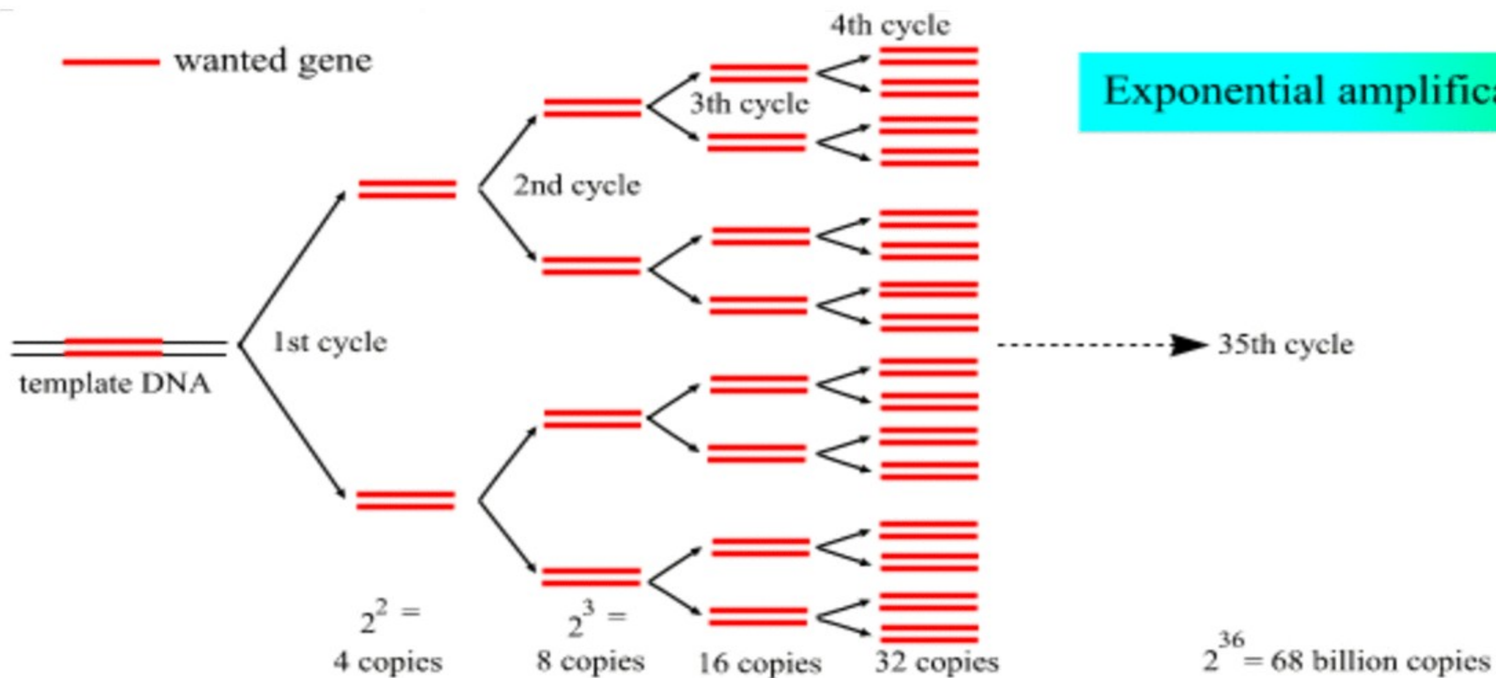
(Cetus Corporation Annual Report 1982, p.2)





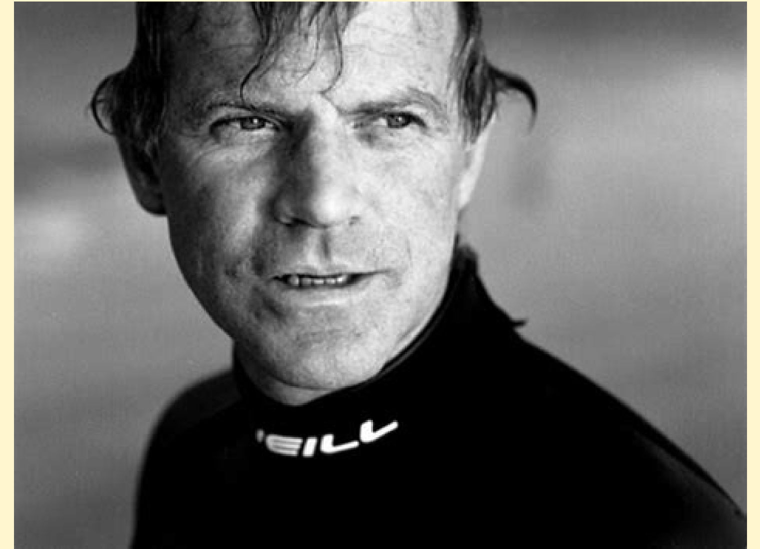
POLYMERASE
CHAIN REACTION




wanted gene
Exponential amplification
4th cycle
3th cycle
2nd cycle
1st cycle
template DNA
35th cycle
2^2 =
4 copies
2^3 =
8 copies
16 copies
32 copies
2^{36} = 68 billion copies

Amplifying power

"Beginning with a single molecule PCR can generate 100 billion similar molecules in an afternoon".



Mullis KB (1990) The unusual origin of the polymerase chain reaction. Scientific American 262, 36-43.

The Perth Group

The HIV-AIDS debate

- Home
- What the Perth Group has argued
- Papadopulos redox theory of cellular function papers
- Papers and letters published in scientific journals
- Monograph on mother-to-child transmission
- Papers published in Continuum magazine
- Papers published in the popular press
- Papers/letters rejected by the scientific press
- Presentations
- Interviews
- Selected email correspondence
- A virus like no other
- Oxidation, Montagnier and the Perth Group
- Montagnier Nobel Prize 2008
- The Parenzee Case
- The House of Numbers
- Latest files

The view of The Perth Group is that the HIV/AIDS experts have not proven:

1. The existence of a unique, exogenously acquired retrovirus, HIV.
2. The "HIV" antibody tests are specific for "HIV" infection.
3. The HIV theory of AIDS, that is, that HIV causes acquired immune deficiency (destruction of T4 lymphocytes=AID) or that AID leads to the development of the clinical syndrome AIDS.
4. The "HIV genome", (RNA or DNA) originates in a unique, exogenously acquired infectious retroviral particle.
5. HIV/AIDS is infectious, either by blood, blood products or sexual intercourse.
6. Mother to child transmission of a retrovirus HIV or its inhibition with AZT or nevirapine.

The Perth Group has argued:

1. The impossibility of haemophiliacs acquiring HIV following factor VIII infusions.
2. That AIDS and all the phenomena inferred as "HIV" are induced by changes in cellular redox brought about by the oxidative nature of substances and exposures common to all the AIDS risk groups and to the cells used in the "culture" and "isolation" of "HIV".
3. That AIDS will not spread outside the original risk groups.
4. That the cessation of exposure to oxidants and/or use of anti-oxidants will improve the outcome of AIDS patients.
5. That the pharmacological data prove AZT cannot kill "HIV" and AZT is toxic to all cells and may cause some cases of AIDS.

Identification ≠ Purification

"Sequence-based approaches to microbial identification and disease causation share some problems with more traditional approaches but also generate some additional problems. Perhaps the **most obvious and perplexing issue** raised by sequence-based approaches is the **absence of a viable or even intact microorganism** with which to reproduce disease. Strict adherence to the principle behind Koch's third postulate poses a major difficulty for the evaluation of microorganisms that have **not yet been purified** or propagated in the laboratory." (emphasis added, p29)

CLINICAL MICROBIOLOGY REVIEWS, Jan. 1996, p. 18–33 Vol. 9, No. 1
0893-8512/96/$04.00+0
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Sequence-Based Identification of Microbial Pathogens: a Reconsideration of Koch's Postulates

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“PCR is a technique that amplifies small amounts of DNA or RNA but the same fundamental problem exists..Unless you can isolate the virus free of cellular contamination, you cannot be certain that the DNA RNA fragments are viral and not cellular” (p.25)

1999

THE PRACTISING MIDWIFE

Harrison, Rosalind and Corbett, Kevin (1999) *Screening pregnant women for HIV: the case against.*
The Practising Midwife 2 (7), pp. 24-29.

“Detection of viral nucleic acid is not equivalent to isolating a virus.”

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EMERGING INFECTIOUS DISEASES®

Emerg Infect Dis. 2001 Jul-Aug; 7(4): 756–758.

PMCID: PMC2631763
PMID: 11585546

Identification of arboviruses and certain rodent-borne viruses: reevaluation of the paradigm.

American Committee on Arthropod-borne Viruses, Subcommittee on InterRelationships among Catalogued Arboviruses

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Abstract

Diagnostic and epidemiologic virology laboratories have in large part traded conventional techniques of virus detection and identification for more rapid, novel, and sensitive molecular methods. By doing so, useful phenotypic characteristics are not being determined. We feel that the impact of this shift in emphasis has impaired studies of the biology of viruses. This position paper is a plea to the scientific and administrative communities to reconsider the importance of such information. We also suggest a revised paradigm for virus isolation and characterization and provide a rationale for accumulating biologic (phenotypic) information.

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Calisher et al (2001) Identification of Arboviruses and Certain Rodent-Borne Viruses: Reevaluation of the Paradigm. Emerging Infectious Diseases (2001) 7 (4), July–August, 756-758

2001

Science, Technology, &
Human Values
Volume 34 Number 1
January 2009 102-125
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10.1177/0162243907310376
http://sth.sagepub.com
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http://online.sagepub.com

2009

"You've Got It, You May Have It, You Haven't Got It"

Multiplicity, Heterogeneity, and the Unintended Consequences of HIV-related Tests

Kevin P. Corbett
Independent Research Consultant

Viral Load
"..goals of undetectability and virological success were seen as unobtainable and as devaluing bodily responses."
(p.118)

https://www.eurosurveillance.org/content/10.2807/1560-7917.ES.2020.25.3.2000045

Other sites: ECDC European Antibiotic Awareness Day ESCAIDE - Scientific conference Eurosurveillance journal EVIP - Vaccina

Eurosurveillance Europe's journal on infectious disease surveillance, epidemiology, prevention and

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Research

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Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR

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Victor M Corman[1], Olfert Landt[2], Marco Kaiser[3], Richard Molenkamp[4], Adam Meijer[5], Daniel KW Chu[6], Tobias Bleicker[1], Sebastian Brünink[1], Julia Schneider[1], Marie Luisa Schmidt[1], Daphne GJC Mulders[4], Bart L Haagmans[4], Bas van der Veer[5], Sharon van den Brink[5], Lisa Wijsman[5], Gabriel Goderski[5], Jean-Louis Romette[7], Joanna Ellis[8], Maria Zambon[8], Malik Peiris[6], Herman Goossens[9], Chantal Reusken[5], Marion PG Koopmans[4], Christian Drosten[1]

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Corman Victor M, Landt Olfert, Kaiser Marco, Molenkamp Richard, Meijer Adam, Chu Daniel KW, Bleicker Tobias, Brünink Sebastian, Schneider Julia, Schmidt Marie Luisa, Mulders Daphne GJC, Haagmans Bart L, van der Veer Bas, van den Brink Sharon, Wijsman Lisa, Goderski Gabriel, Romette Jean-Louis, Ellis Joanna, **Zambon Maria**, Peiris Malik, Goossens Herman, Reusken Chantal, Koopmans Marion PG, **Drosten Christian**. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Euro Surveill. **2020**;25(3):pii=2000045. https://doi.org/10.2807/1560-7917.ES.2020.25.3.2000045

"A viral genome sequence was released for immediate public health support via the community online resource virological.org on 10 January (Wuhan-Hu-1, GenBank accession number MN908947..***followed by four other genomes....virus isolates or samples from infected patients have so far not become available..*** ..[the PCR was] ***designed in absence of available virus isolates or original patient specimens***..***Design and validation were enabled..*** [by] ***the use of synthetic nucleic acid technology."***

Table 1

***. Primers and probes, real-time RT-PCR for 2019 novel coronavirus

Assay/use	Oligonucleotide	Sequence[a]	Concentration[b]
RdRP gene	RdRp_SARSr-F	GTGARATGGTCATGTGTGGCGG	Use 600 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, SARS-CoV and bat-SARS-related CoVs. Use 100 nM per reaction and mix with P2
	RdRp_SARSr-R	CARATGTTAAASACACTATTAGCATA	Use 800 nM per reaction
E gene	E_Sarbeco_F	ACAGGTACGTTAATAGTTAATAGCGT	Use 400 nM per reaction
	E_Sarbeco_P1	FAM-ACACTAGCCATCCTTACTGCGCTTCG-BBQ	Use 200 nM per reaction
	E_Sarbeco_R	ATATTGCAGCAGTACGCACACA	Use 400 nM per reaction
N gene	N_Sarbeco_F	CACATTGGCACCCGCAATC	Use 600 nM per reaction
	N_Sarbeco_P	FAM-ACTTCCTCAAGGAACAACATTGCCA-BBQ	Use 200 nM per reaction
	N_Sarbeco_R	GAGGAACGAGAAGAGGCTTG	Use 800 nM per reaction

[a] W is A/T; R is G/A; M is A/C; S is G/C. FAM: 6-carboxyfluorescein; BBQ: blackberry quencher.

[b] Optimised concentrations are given in nanomol per litre (nM) based on the final reaction mix, e.g. 1.5 µL of a 10 µM primer stock solution per 25 µL total reaction volume yields a final concentration of 600 nM as indicated in the table.

Centers for Disease Control and Prevention, 2020

"CDC 2019-Novel Coronavirus (2019-nCoV)
Real-Time RT-PCR Diagnostic Panel, For Emergency Use Only, Instructions For Use"

"Since no *quantified* virus isolates of the 2019-nCoV are currently available, assays designed for detection of the 2019-nCoV RNA were tested with characterized stocks of in vitro transcribed full length RNA **(N gene; GenBank accession: MN908947.2)** of known titer (RNA copies/µL) spiked into a diluent consisting of a suspension of human A549 cells and viral transport medium (VTM) to **mimic clinical specimen**".

https://www.fda.gov/media/134922/download

PUBLIC HEALTH ENGLAND*, 2020

"Professor Zambon asked that we respond to your request for data, as below.

RT-PCR tests –

the gold standard for PCR tests is not virus isolation

PCR tests are developed using synthetic transcripts.."

"Professor Maria Caterina Zambon FMedSci FRCPath, is a British virologist, Director of Imoerial College NIHR Research Unity for Respiratory Infections and lead for UK Health Security Agency (formerly Public Health England)" https://www.imperial.ac.uk/medicine/nihr-hpru-respiratory-infections/people/

*United Kingdom Health Security Agency

CORMAN-DROSTEN REVIEW REPORT

CURATED BY AN INTERNATIONAL CONSORTIUM OF SCIENTISTS IN LIFE SCIENCES (ICSLS)

Review report Corman-Drosten et al. Eurosurveillance 2020

November 27, 2020

This extensive review report has been officially submitted to Eurosurveillance editorial board on 27th November 2020 via their submission-portal, enclosed to this review report is a retraction request letter, signed by all the main & co-authors. First and last listed names are the first and second main authors. All names in between are co-authors.

External peer review of the RTPCR test to detect SARS-CoV-2 reveals 10 major scientific flaws at the molecular and methodological level: consequences for false positive results.

Pieter Borger[1], Bobby Rajesh Malhotra[2], Michael Yeadon[3], Clare Craig[4], Kevin McKernan[5], Klaus Steger[6], Paul McSheehy[7], Lidiya Angelova[8], Fabio Franchi[9], Thomas Binder[10], Henrik Ullrich[11], Makoto Ohashi[12], Stefano Scoglio[13], Marjolein Doesburg-van Kleffens[14], Dorothea Gilbert[15], Rainer Klement[16], Ruth Schruefer[17], Berber W. Pieksma[18], Jan Bonte[19], Bruno H. Dalle Carbonare[20], Kevin P. Corbett[21], Ulrike Kämmerer[22]

ABSTRACT

In the publication entitled "Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR" (Eurosurveillance 25(8) 2020) the authors present a diagnostic workflow and RT-qPCR protocol for detection and diagnostics of 2019-nCoV (now known as SARS-CoV-2), which they claim to be

2020

"The first and major issue is that the novel Coronavirus SARS-CoV-2..is based on ***in silico (theoretical) sequences***, supplied by a laboratory in China [1], because at the time neither **control material** of infectious ("live") or **inactivated** SARS-CoV-2 nor **isolated genomic RNA** of the virus was available to the authors. **To date no validation has been performed by the authorship based on isolated SARS-CoV-2 viruses or full length RNA thereof."**

1 of 29 100%

International Journal of Vaccine Theory, Practice, and Research

IJVTPR 2023

RT-PCR Test Targeting the Conserved 5′-UTR of SARS-CoV-2 Overcomes Shortcomings of the First WHO-Recommended RT-PCR Test

Ulrike Kämmerer, PhD [1], Sona Pekova, PhD [2], Rainer J. Klement, PhD [3], Rogier Louwen, PhD [4], Pieter Borger, PhD [5], Klaus Steger, PhD [6]

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[4] Erasmus University Medical Center Rotterdam, Department of Medical Microbiology and Infectious Diseases, Netherlands; Current address: CCassured (CRISPR Commons assured), Breda, Netherlands; rlouwen@hotmail.com, https://orcid.org/0000-0003-2537-4941

[5] The Independent Research Institute on Information and Origins, Lörrach, Germany; pieter.borger@hotmail.com

[6] Corresponding Author: Professor Emeritus, Justus Liebig University Giessen, Germany; Klaus.Steger@chiru.med.uni-giessen.de; https://orcid.org/0000-0002-2104-0840

Abstract

For the first time in medical history, a laboratory assay (RT-PCR) was used as the sole criterion to diagnose a disease

No diagnostic value of RT-PCR for the proof of an infectious virus -

Kammerer et al, 2023

“Of utmost importance and irrespective of any protocol design, RT-PCR **solely detects** the reverse_x005F_x0002_transcribed and amplified RNA target(s) selected by applied primers and, therefore, **can by no means prove that a replication-competent, infectious virus is actually present** in a given sample…

However, neither a certain RNA copy number, nor a specific Ct-value used as a threshold can enable any secure conclusion even concerning whether the viral load is increasing or decreasing.”

Reaffirming RNA is ‘virus’ -

Kammerer et al, 2023

“Of note, due to the high sensitivity of RT-PCR, residual, **non-infectious <u>viral</u>** RNA remains detectable even in the absence of infectious viruses. When applying external standards with defined **viral** RNA copy numbers, RNA **viral** loads can be correlated with Ct-values obtained by RT-qPCR.”

Problematic claim -

Kammerer et al, 2023

"..we selected a ***unique region*** *['consensus region'] located within the conserved and specific 5'-UTR of SARS-CoV-2 to serve as a specific and sensitive target for real-time RT-qPCR detection of the viral RNA resulting in a 207 base pairs (bp) amplicon"*

Question: How do Kammerer et al know the 'consensus region' within 5'-UTR is 'viral' in origin?

Conclusion

1) PCR **cannot** be used to detect a ‘virus’ unless what is detected is first **proven** to be viral in origin;

[*“detection of viral nucleic acid is not equivalent to isolating”* Calisher et al 2001];

2) PCR can only be used to **amplify** specific known nucleotide sequences;

3) PCR **cannot** determine the origin (‘provenance’) or significance of any nucleotide sequence, thus what is detected / amplified cannot be ‘viral’ (unless 1) above is proven);

4) PCR analytical sensitivity and specificity is **not equivalent to diagnostic specificity** for a clinical condition.