ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA
CAMPUS DI FORLÌ

IL FUTURO DEGLI IVD:NOVITÀ E CRITICITÀ

Il punto di vista del clinico

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Dichiaro che negli ultimi due anni NON ho avuto rapporti, anche di finanziamento, con soggetti portatori di interessi commerciali in campo sanitario.

Proceedings of the Clinical Microbiology Open 2018 and 2019 - a Discussion about Emerging Trends, Challenges, and the Future of Clinical Microbiology

Christopher D. Doern,[a] Melissa B. Miller,[b] Kevin Alby,[b] Michael A. Bachman,[c] Stephen M. Brecher,[d] Aida Casiano-Colon,[e] Marc Roger Couturier,[f] J. Kristie Johnson,[g] James E. Kirby,[h,i] Erin McElvania,[j] Duane W. Newton,[k] Frederick S. Nolte,[l] Preeti Pancholi,[m] Peggy McNult,[n] Vaishali Dharmarha,[n] Sherry Dunbar,[o] on behalf of the American Society for Microbiology (ASM) Clinical and Public Health Microbiology Committee and the ASM Corporate Council

July 2022 Volume 60 Issue 7 Journal of Clinical Microbiology 10.1128/jcm.00092-22

TABLE 1 Executive summary: major topics of discussion

Topic	Key opportunities	Key challenges	Next steps
Widespread use of NGS in clinical microbiology	Rapid strain typing Direct detection from clinical specimens Detection of resistance genes and virulence factors	Defining the specific clinical need Lack of standardized databases, interpretation, and quality control protocols Cost relative to conventional methods	Create guidelines to standardize best laboratory practices
Big data in clinical microbiology	Antimicrobial resistance profiling based on patient risk factors Analysis of protein profiles generated by MALDI-TOF MS AST variance detection Improved image and NGS dataset analyses	Computing power required Microbiologists trained in bioinformatics Artificial intelligence programs needed to perform analyses Variability in microbiology specimens	Performing collaborative outcome studies which include laboratories, information technology, and data scientists
Laboratory diagnostic stewardship	Optimized use of multiplex PCR testing Reduction in unnecessary test utilization Cost control	Integration of LIS with EMR to facilitate data analyses Provider buy-in Ensuring interventions Lack of data demonstrating clinical outcomes of new technology	Generate outcomes data Publish guidelines to establish a laboratory stewardship program Create order templates to assist in test ordering Develop best practices for uniformity in community laboratory stewardship practices Improve laboratory test catalogs
Staffing shortage	Capitalize on technology skills of younger generation microbiologists	Decreasing no. of training programs Pandemic-induced fatigue Extended training required for new hires Limited opportunities for advancement within the laboratory	Promote alternative training mechanisms Advocate for improved pay
Promoting investment in the clinical laboratory	Capitalize on innovations that can be used to improve patient care	Laboratories are often considered a cost-center Siloed thinking in finance departments Indirect benefit of laboratory testing on patient care and cost savings	Perform outcome studies to demonstrate the benefit of investment in laboratory technology Include business analysts in laboratory stewardship programs Engage Chief Financial Officers in ASM-sponsored sessions Participate in pathology management groups and meetings
Rapid susceptibility testing	Improved patient outcomes through faster therapeutic decision making Improved rapid phenotypic testing methods Enhanced predictive value for genotypic susceptibility methods	Cost compared to conventional methods Biological and technical challenges must be overcome Polymicrobial specimens	Microbiology laboratories and industry collaborate to demonstrate improved patient care with rapid AST methods
Point of care testing for infectious diseases	Improved access to testing Near-patient diagnosis facilitating treatment decisions	Easy access promotes overutilization Inaccurate results when performed outside the laboratory Difficult to capture data from POCT Inferior performance to laboratory-based testing	Study the impact of COVID-19 at-home testing
Molecular diagnostics for fungal infections	Improved diagnosis of fungal infections in vulnerable patients	Difficult to validate due to low frequency Cost prohibitive clinical trials False-positive results due to environmental contamination	Develop collaborative groups to create a more efficient test development and evaluation process

A Recent Update on Advanced Molecular Diagnostic Techniques for COVID-19 Pandemic: An Overview

Akanksha Roberts[1], Raghuraj Singh Chouhan[2], Deepshikha Shahdeo[1], Narlawar Sagar Shrikrishna[1], Veerbhan Kesarwani[1], Milena Horvat[2] and Sonu Gandhi[1]**

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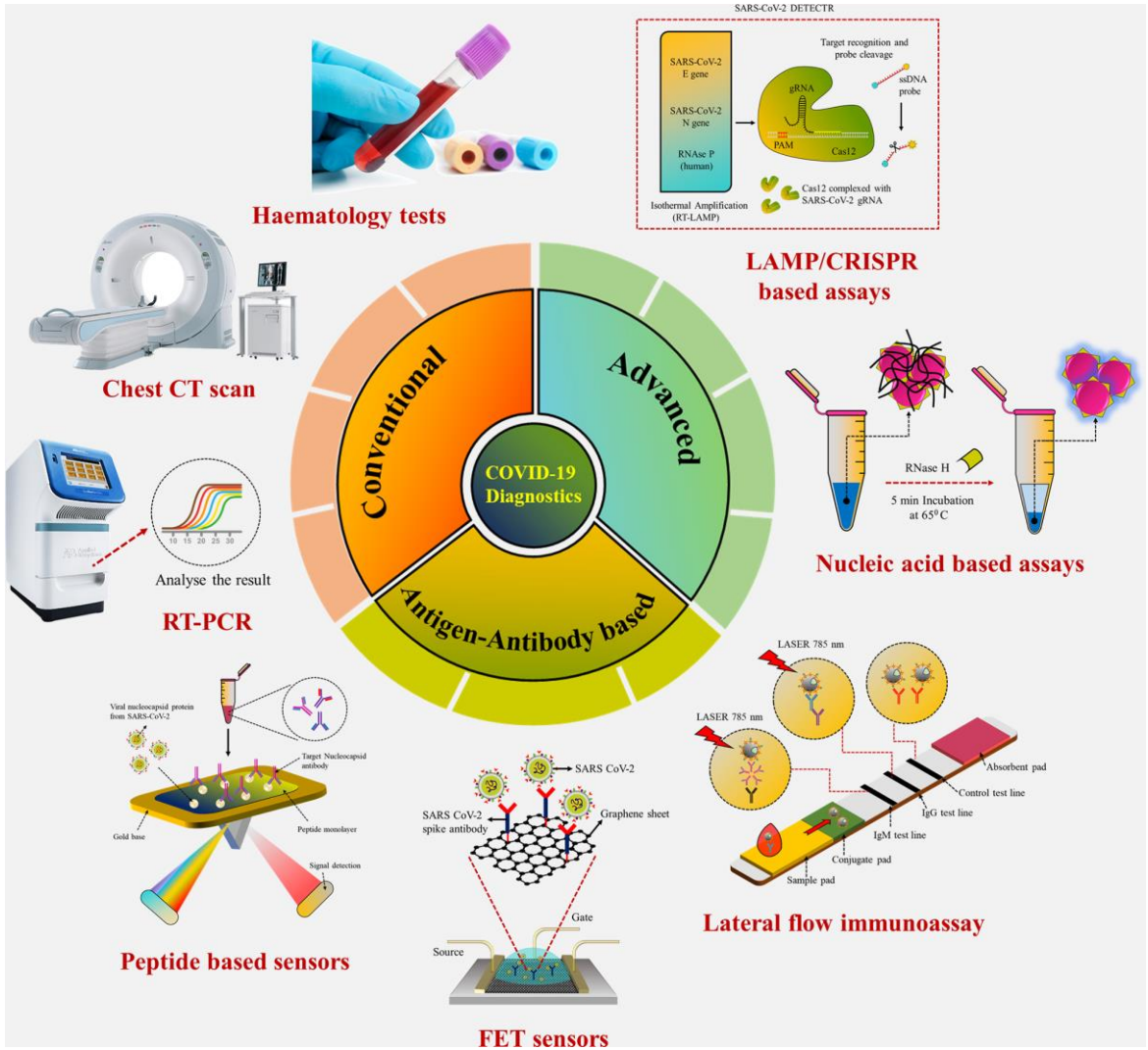

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TABLE 4 | Various antigen- and antibody-based diagnostic kits available for the detection of SARS-CoV-2.

Type of POCT Kit	Target Analyte	Sensitivity (%)	Specificity (%)	Detection Time	Reference
Graphene field effect transistor (Gra-FET)	Antibodies	N/A	N/A	N/A	(74)
LFIA	IgM and IgG	88.8	90.5	15 min	(22)
LFIA	IgM and IgG	97.7	N/A	N/A	(74)
Colloidal gold-based immune-chromatographic (ICG) strip	IgM or IgG	96.7	N/A	15 min	(67)
Chemi-luminescence immunoassay	IgM and IgG (recombinant nucleocapsid)	82.3	97.4	23 min	(75)
Immune-precipitation and parallel DNA sequencing	Antibody	90–97	~98	N/A	(76)
Colloidal Gold Immuno chromatographic assay (GICA)	Nucleoprotein	57.8	99.6	15 min	(77)
GICA	Nucleoprotein	30.3	100	15 min	(78)
Fluorescence immuno chromatographic assay (FICA)	SARS-CoV-2 antigen	93.8	100	<15 min	(79)
GICA	Nucleoprotein	50.5	100	N/A	(80)
Microfluidic FICA	SARS-CoV-2 antigen/IgG/IgM	N/A	N/A	<15min	(81)
Chemiluminescence Immunoassay (CLIA)	Nucleocapsid protein	55.3	99.7	30 min	(82)
FICA	Nucleocapsid protein	11.6	N/A	30 min	(83)
FICA	Nucleocapsid protein	67.8	100	10 min	(84)
GICA	IgG and IgM	86.88	99.40	5–10 min	(85)
GICA	IgG and IgM	95.10	91.3	15 min	(86)
CLIA	IgG and IgM	100	N/A	N/A	(87)
CLIA	IgG and IgM for nucleocapsid protein	81.54	96.62	N/A	(88)
ELISA	IgG and IgM	81.33	N/A	N/A	(89)
ELISA	IgG and IgM for nucleocapsid and spike protein	82.3	N/A	N/A	(90)
ELISA	IgA, IgM, and IgG	85.5	N/A	N/A	(91)
ELISA	IgG, IgA against spike protein	N/A	N/A	N/A	(92)

N/A, not available.

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TABLE 3 | Commercially available PoCT kits for SARS-CoV-2 detection with nucleic acid target analytes.

Detection Method	Target Analyte	Sensitivity (%)	Specificity (%)	Detection Time	Reference
RCA with magnetic nanoparticles	Synthetic complementary DNA (RdRp)	N/A	N/A	<2 h	(52)
CRISPR-based LAMP with lateral flow assay	RNA (E, N genes)	95	100	<1 h	(53)
Real-time qRT-PCR	RNA (RdRp, E, N genes)	100	100	>4 h	(40)
Reverse transcription-LAMP	RNA	N/A	N/A	<1 h	(49)
LAMP with colorimetric readout	RNA	N/A	N/A	<1 h	(54)
Digital PCR	RNA	N/A	N/A	<1 h	(55)
Reverse transcription-LAMP	RNA (ORF1ab, S genes)	100	100	<1 h	(56)
Reverse transcription-LAMP	RNA	N/A	N/A	<1 h	(57)

N/A, not available.

A deletion in the N gene may cause diagnostic escape in SARS-CoV-2 samples

Silvia Zannoli[a,*], Giorgio Dirani[a], Francesca Taddei[a], Giulia Gatti[a], Ilaria Poggianti[a], Agnese Denicolò[a], Valentina Arfilli[a], Martina Manera[a], Andrea Mancini[a], Arianna Battisti[a], Vittorio Sambri[a,b]

S. Zannoli et al. / Diagnostic Microbiology and Infectious Disease 102 (2022) 115540

Table 1
Ct values for the samples tested with both the Allplex SARS-CoV-2 assay and Xpert Xpress SARS-CoV-2 assay.

Sample ID	Allplex SARS-CoV-2 result (Ct)			Xpert Xpress SARS-CoV-2 result (Ct)	
	E gene	RdRP/S gene	N gene	E gene	N gene
1	21	23	N/A	20	22
2	19	21	N/A	19	20
3	22	24	N/A	21	22
4	21	22	N/A	21	23
5	19	19	N/A	18	19

Five SARS-CoV-2-positive samples showed N-gene drop-out with a RT-PCR multiplex test. WGS found all samples to harbor a deletion in the same region of the N gene, which is likely to impair the efficiency of amplification. This highlights the need for a continued surveillance of viral evolution and diagnostic test performance.

Importance of external quality assessment for SARS-CoV-2 antigen detection during the COVID-19 pandemic

Oliver Donoso Mantke [a,*], Victor M. Corman [b,c], Francesca Taddei [d], Elaine McCulloch [a,*], Daniela Niemeyer [b], Laura Grumiro [d], Giorgio Dirani [d], Paul S. Wallace [a], Christian Drosten [b], Vittorio Sambri [d], Hubert G.M. Niesters [e]

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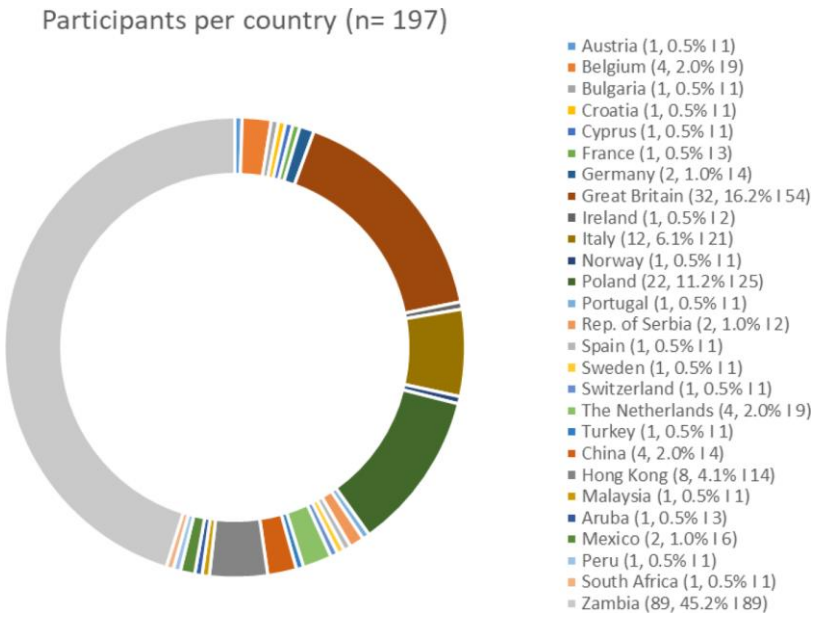

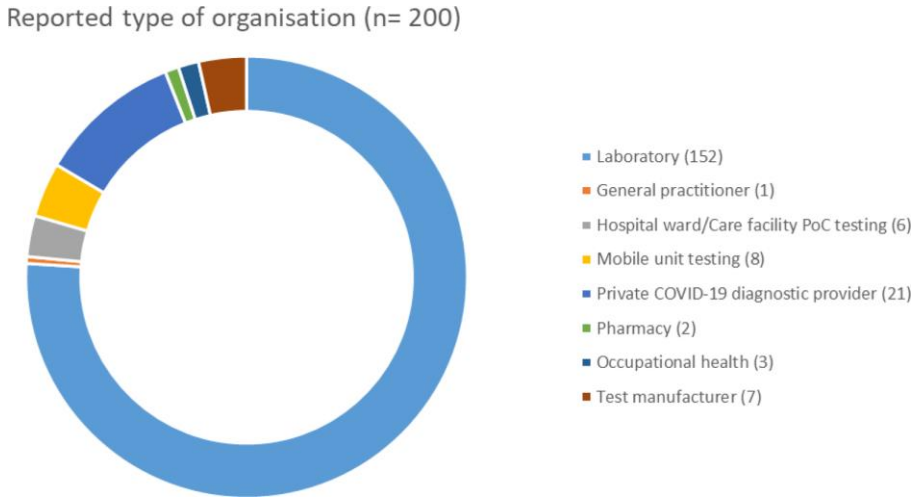

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Journal of Clinical Virology 154 (2022) 105222

Table 1
QCMD 2021 SARS-CoV-2 Antigen Testing EQA Study - Composition of panels and overall performance per sample.

Challenge C1A (May to Jul 2021)						
Sample code	**Sample content**	**Matrix**	**Viral RNA concentration (ddPCR log_{10}copies/mL)**	**Sample status**	**Percentage correct qualitative results (all)**	
					%	**Total datasets**
SCV2Ag21C1A-01	SARS-CoV-2 Lineage B.1	Stabilisation buffer	6.4	Educational	66.4	143
SCV2Ag21C1A-02	SARS-CoV-2 Lineage B.1	Stabilisation buffer	7.4	Core	89.5	143
SCV2Ag21C1A-03	True Negative	Stabilisation buffer	N/A	Core	90.2	143
SCV2Ag21C1A-04	SARS-CoV-2 Lineage B.1	Transport medium	6.4	Core	96.5	143
SCV2Ag21C1A-05	SARS-CoV-2 Lineage B.1	Transport medium	5.4	Educational	32.9	143
Challenge C1B (Aug to Oct 2021)						
Sample code	**Sample content**	**Matrix**	**Viral RNA concentration (ddPCR log_{10} copies/mL)**	**Sample status**	**Percentage correct qualitative results (all)**	
					%	**Total datasets**
SCV2Ag21C1B-01	SARS-CoV-2 Delta variant	Transport medium	6.4	Core	98.1	216
SCV2Ag21C1B-02	SARS-CoV-2 Lineage B.1	Stabilisation buffer	6.4	Educational	43.5	216
SCV2Ag21C1B-03	SARS-CoV-2 Delta variant	Transport medium	5.4	Educational	53.2	216
SCV2Ag21C1B-04	SARS-CoV-2 Alpha variant	Transport medium	6.4	Core	98.6	216
SCV2Ag21C1B-05	SARS-CoV-2 Alpha N	Transport medium	6.4	Core	85.6	216
SCV2Ag21C1B-06	SARS-CoV-2 Lineage B.1	Transport medium	6.4	Core	94.4	216
Challenge C1C (Nov to Dec 2021)						
Sample code	**Sample content**	**Matrix**	**Viral RNA concentration (ddPCR log_{10} copies/mL)**	**Sample status**	**Percentage correct qualitative results (all)**	
					%	**Total datasets**
SCV2Ag21C1C-01	SARS-CoV-2 Alpha variant	Transport medium	6.4	Core	96.5	113
SCV2Ag21C1C-02	True Negative	Transport medium	N/A	Core	95.6	113
SCV2Ag21C1C-03	SARS-CoV-2 Delta variant	Transport medium	6.4	Core	100	113
SCV2Ag21C1C-04	SARS-CoV-2 Lineage B.1	Transport medium	6.4	Core	98.2	113
SCV2Ag21C1C-05	SARS-CoV-2 Alpha N	Transport medium	6.4	Core	77.9	113

Viral RNA concentrations quantified with ddPCR E gene reference assay (modified from [18]).
N/A: not applicable.

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Results: Participants registered for each individual challenge in any combination. In total, 258 respondents from 27 countries worldwide were counted submitting 472 datasets. All core samples were correctly reported by 76.7 to 83.1% at participant level and by 73.5 to 83.8% at dataset level. Sensitivity differences could be shown in viral loads and SARS-CoV-2 strains/variants including the impact on performance by a B.1.1.7-like mutant strain with a deletion in the nucleoprotein gene. Lateral flow rapid antigen tests showed a higher rate of false negatives in general compared with automated point-of-care tests and laboratory ELISA/immunoassays.

Le nuove regole di classificazione: le 4 classi di rischio

IVDD
List-based approach

List A	List B	Unlisted
Detection of HIV infection	Blood sugar self-testing	Companion Diagnostics Detection of SARS-CoV-2

IVDR
Risk-based approach

Class D	Class C	Class B	Class A
Detection of HIV infection Detection of SARS-CoV-2	Companion Diagnostics Blood sugar self-testing	Detection of glucose in urine	Specimen receptacles

Near-patient testing (POCT) and MDSW are classified in their own right

MDCG 2020-16 Guidance on Classification Rules for in vitro Diagnostic Medical Devices under EU) 2017/745

2. PERFORMANCE ANALITICA (*section 9.1 of Annex I*)

Le prestazioni analitiche sono la capacità di un dispositivo di rilevare o misurare correttamente un particolare analita

- Generally verified by means of analytical performance studies
- For novel markers or markers without available reference material/measurement:
  - Comparison to other well-documented methods or the composite reference standard
  - Clinical performance study may be required comparing the performance of the novel device to the current standard in clinical practice
- Results are documented in the analytical performance report

Examples of characteristics to be verified

- Analytical sensitivity (e.g. limit of detection)
- Analytical specificity (e.g. interference, cross-reactivity)
- Cut-off values
- Absence of inacceptable cybersecurity vulnerabilities

- LOD: limit of detection corrispondente a valore di cT PCR (cycle threshold - polymerase chain reaction) pari a 20 o inferiore misurato sul gene N (valore misurato tramite PCR real time previa estrazione e purificazione dell'RNA).

Tamponi orofaringeo: lo studio clinico confronta con RT-PCR

Metodo		RT-PCR		Risultati totali
SARS-CoV-2 Antigen Rapid Test Cassette	Risultati	Positivi	Negativi	
	Positivi	113	2	115
	Negativi	3	212	215
Risultati totali		116	214	330

Sensibilità relativa:97.4%(95%CI*:90.39%-98.61%)
Specificità relativa:99.1%(95%CI*:98.20%-99.87%)
* Intervalli di confidenza

Tamponi nasofaringei: studio clinico confronta con RT-PCR

Metodo		RT-PCR		Risultati totali
SARS-CoV-2 Antigen Rapid Test Cassette	Risultati	Positivi	Negativi	
	Positivi	113	3	116
	Negativi	5	480	485
Risultati totali		118	483	601

Sensibilità relativa: 95.76%(95%CI*:90.39%-98.61%)
Specificità relativa: 99.38%(95%CI*:98.20%-99.87%)
* Intervalli di confidenza
I tamponi orofaringei, nasofaringei o nasalis ono stati ottenuti dai pazienti. La RT-PCR è stata usata come metodo di riferimento per il SARS-CoV-2 Antigen Rapid Test Cassette. I campioni sono stati considerati positivi se così indicato dalla PCR.

Tamponi nasale: lo studio clinico si confronta con RT-PCR

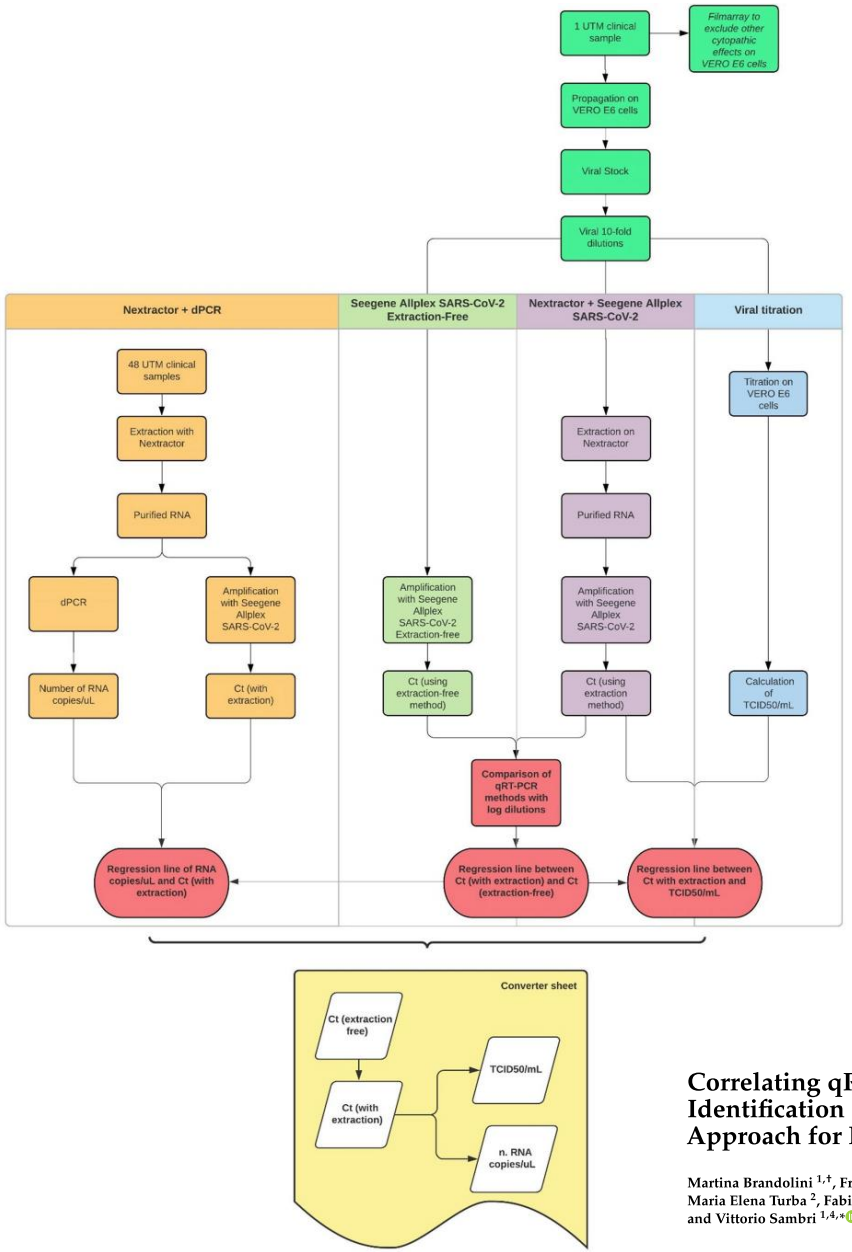
Metodo		RT-PCR		Risultati totali
SARS-CoV-2 Antigen Rapid Test Cassette	Risultati	Positivi	Negativi	
	Positivi	113	2	115
	Negativi	3	212	215
Risultati totali		116	214	330

Sensibilità relativa:97.4%(95%CI*:90.39%-98.61%)
Specificità :99.1%(95%CI*:98.20%-99.87%)
* Intervalli di confidenza

DETECTION LIMIT

Se la concentrazione di virus è superiore a 400$TCID_{50}$ /ml, I risultati positivi sono oltre il 95%. Se la concentrazione di virus è inferiore a 200$TCID_{50}$ /ml, gli esiti positivi sono inferiori a 95%, quindi il limite di rilevazione (LoD) del prodotto è 400TCID50 /ml.

PRECISIONE



MODULO RISULTATI GENEXPERT

DATA: 24/10/2022	E gene (Ct)	N gene (Ct)
10^{-1}	14.3	16.4
$10^{-1.5}$	15.3	17.5
10^{-2}	17	19.2
$10^{-2.5}$	17.3	19.5
10^{-3}	18.2	20.5
$10^{-3.5}$	18.9	21.2
10^{-4}	20.3	21.4

MODULO RISULTATI PROVE LOTTO 1

DATA: 24/10/2022
DITTA: PICKDARE
REF KIT: COVG-602/02010399043250
LOTTO KIT: COVG202202

DATA: 24/10/2022	E gene (Ct)	N gene (Ct)
10^{-1}	POS	POS
$10^{-1.5}$	POS	POS
10^{-2}	POS	POS
$10^{-2.5}$	POS	POS
10^{-3}	POS	POS
$10^{-3.5}$	POS	POS
10^{-4}	NEG	NEG

Correlating qRT-PCR, dPCR and Viral Titration for the Identification and Quantification of SARS-CoV-2: A New Approach for Infection Management

Martina Brandolini [1,†], Francesca Taddei [1,†], Maria Michela Marino [1], Laura Grumiro [1], Agata Scalcione [1], Maria Elena Turba [2], Fabio Gentilini [3], Michela Fantini [1], Silvia Zannoli [1], Giorgio Dirani [1] and Vittorio Sambri [1,4,*]

Thank you for your attention

When I say "we"..... I mean THEM

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