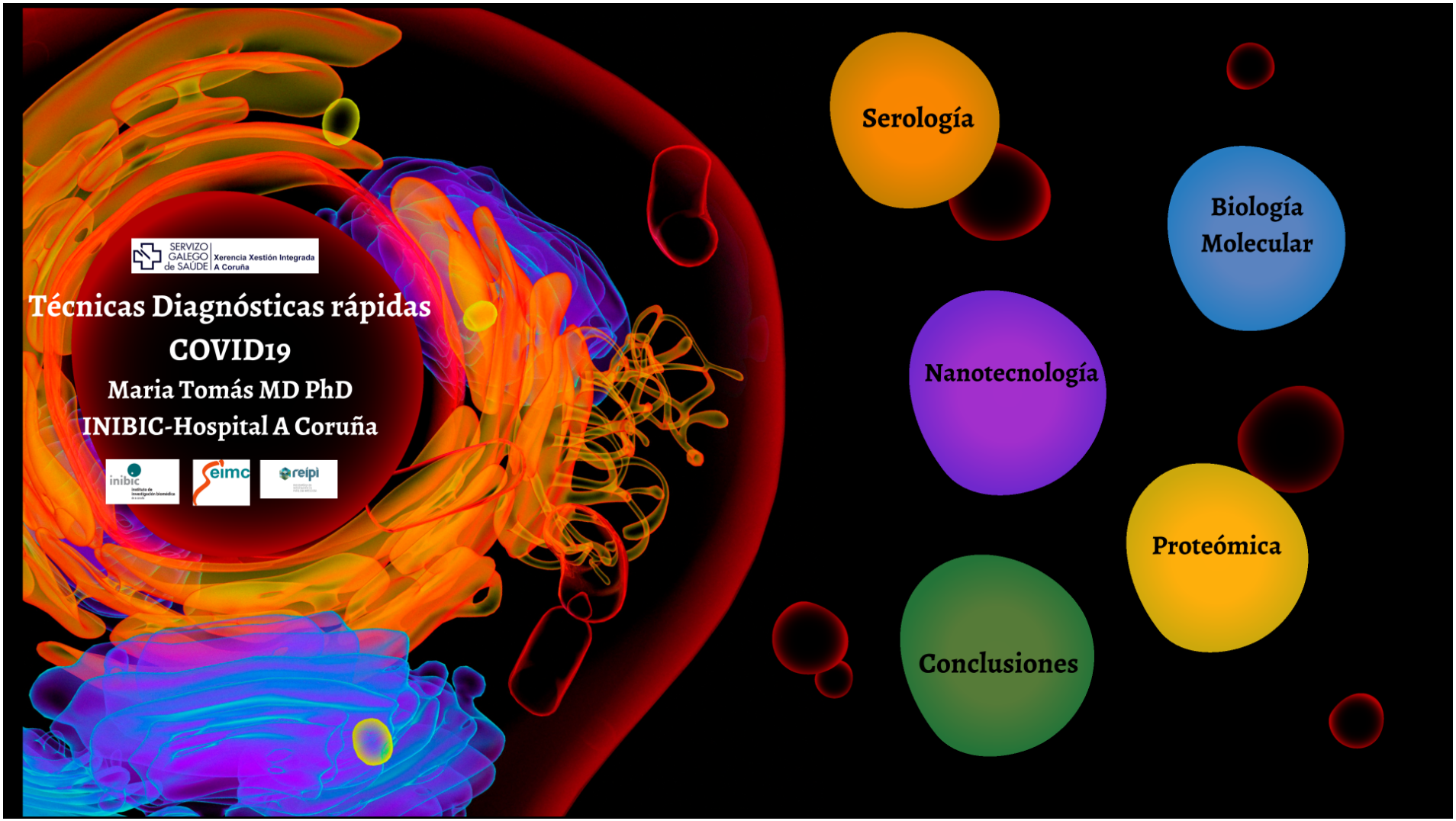
SERVIZO GALEGO de SAUDE
Xerencia Xestión Integrada A Coruña
Técnicas Diagnósticas rápidas
COVID19
Maria Tomás MD PhD
INIBIC-Hospital A Coruña
inibic
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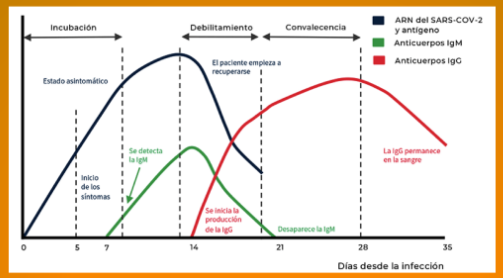
Serología
Biología Molecular
Nanotecnología
Proteómica
Conclusiones

Antígenos y Anticuerpos



Anticuerpos IgM

Son los primeros que se producen en respuesta a las proteínas virales **(antígenos)** y serán detectables principalmente durante el **inicio temprano** de la enfermedad.

Anticuerpos IgG

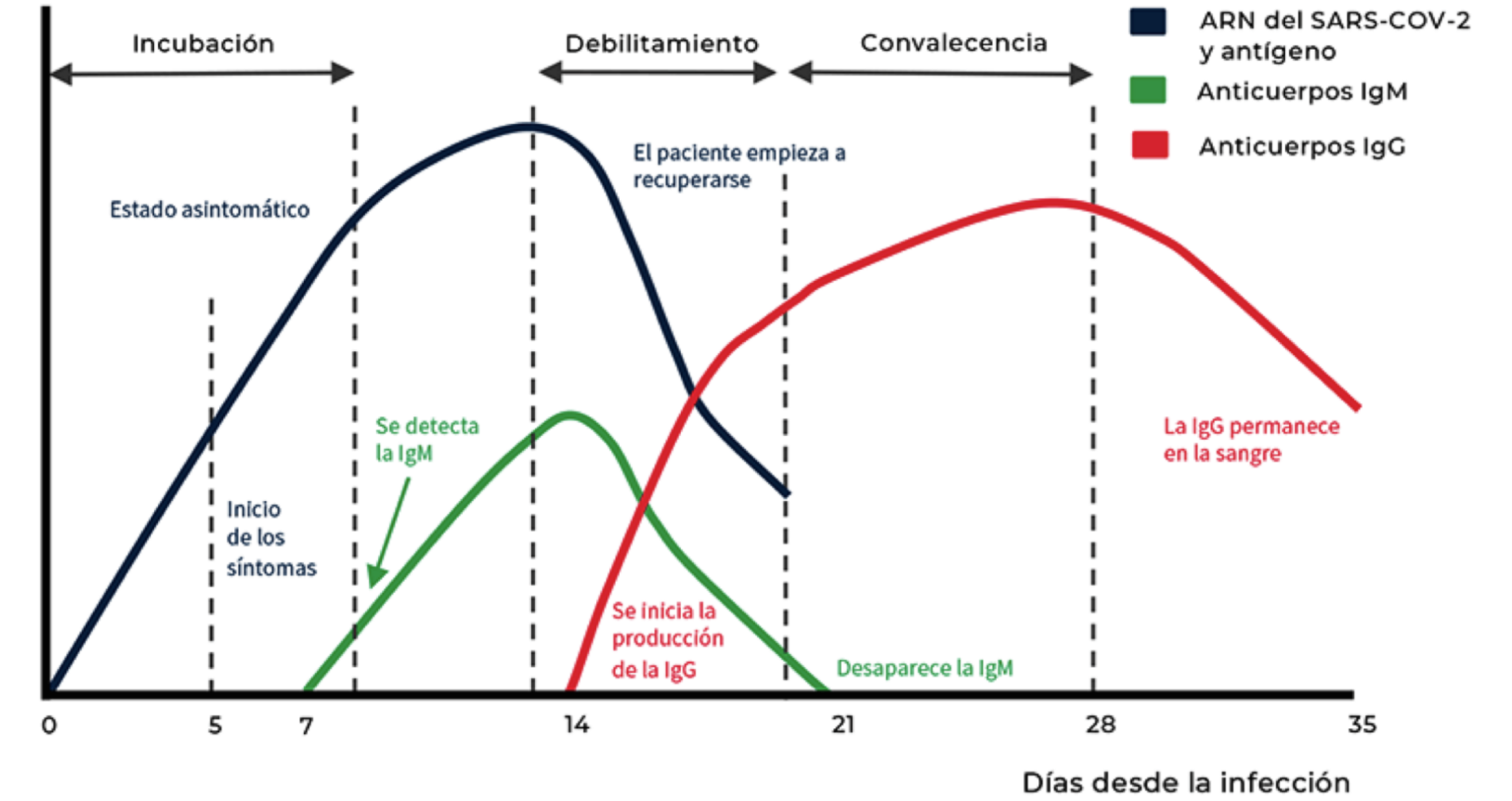
Se producen más tarde en respuesta a un antígeno y habitualmente **se mantienen durante un periodo más prolongado** en el cuerpo, para una respuesta a largo plazo.

Antígenos

Anticuerpos

Antígenos y Anticuerpos

Antígenos y Anticuerpos


Incubación
Debilitamiento
Convalecencia
ARN del SARS-COV-2 y antígeno
Anticuerpos IgM
Anticuerpos IgG
El paciente empieza a recuperarse
Estado asintomático
Se detecta la IgM
Inicio de los síntomas
Se inicia la producción de la IgG
Desaparece la IgM
La IgG permanece en la sangre
0
5
7
14
21
28
35
Días desde la infección

Anticuerpos IgM

Son los primeros que se producen en respuesta a las proteínas virales **(antígenos)** y serán detectables principalmente durante el **inicio temprano** de la enfermedad.

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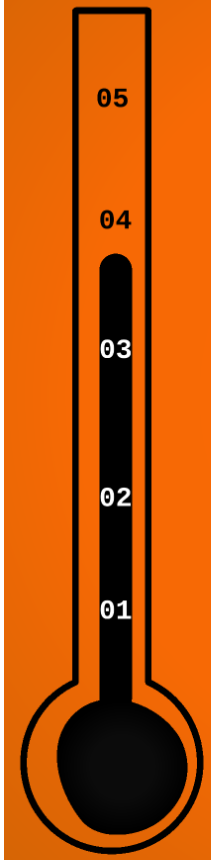
Pruebas rápidas de detección antígenos



Journal of Clinical Virology 129 (2020) 104472

Contents lists available at ScienceDirect

Journal of Clinical Virology

journal homepage: www.elsevier.com/locate/jcv

Short communication

Implementation of rapid SARS-CoV-2 antigenic testing in a laboratory without access to molecular methods: Experiences of a general hospital

Laurent Blairon[a], Alain Wilmet[b], Ingrid Beukinga[a], Marie Tré-Hardy[a,b,*,1]

[a] Department of Laboratory Medicine, Iris Hospitals South, Brussels, Belgium
[b] Department of Pharmacy, Namur Research Institute for Life Sciences, University of Namur, Belgium
[c] Faculty of Medicine, Université Libre de Bruxelles, Brussels, Belgium

ABSTRACT

Background: The COVID-19 Ag (Antigen) Respi-Strip assay is a new immunochromatographic diagnostic tool recently available for antigenic diagnosis of SARS-CoV-2. The proposed sensitivity is not higher than 60 %, but its high specificity allows both quick decisions for the management of patients and confirmation by molecular diagnosis for only negative tests. However, from the first tests performed, we suspected that the sensitivity observed with routine use was much lower than that announced by the manufacturer.
Materials and methods: Over a period of one month, we compared the negative results obtained with the COVID-19 Ag Respi-Strip kit with those obtained from qRT-PCR performed in a laboratory qualified for the molecular diagnosis of SARS-CoV-2. All samples tested were naso-pharyngeal smears from UTM-RT medium.
Results: Of 774 patients tested, 714 negative samples were sent for confirmation, and 159 were found to be positive by qRT-PCR. The median positive percentage agreement was 23.9 % (95 % CI: 14.2 %-38.2 %). The Cohen's kappa score was 0.35.
Conclusion: Using this immunochromatographic assay as a triage test did not significantly reduce the number of samples outsourced for COVID-19 confirmation by qRT-PCR. In addition, even if the turn-around time is short, the assay is completely manual, which is not suitable for large volumes of routine samples. The sensitivity of this rapid test is poor, and improvements are needed to enhance its performance.

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Contents lists available at ScienceDirect

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Low performance of rapid antigen detection test as frontline testing for COVID-19 diagnosis

Anaïs Scohy[*], Ahalieyah Anantharajah, Monique Bodéus, Benoît Kabamba-Mukadi, Alexia Verroken, Hector Rodriguez-Villalobos

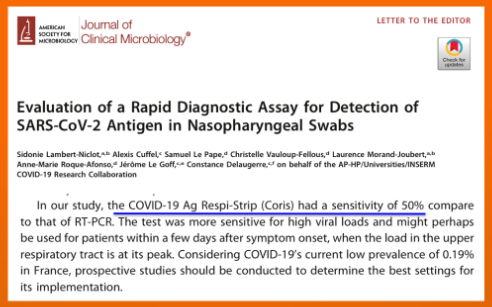
Department of Microbiology, Cliniques Universitaires Saint-Luc – Université Catholique de Louvain, Avenue Hippocrate 10, Brussels, Belgium

ARTICLE INFO

Keywords:
COVID-19
Rapid antigen detection test
SARS-CoV-2

ABSTRACT

Background: Ensuring accurate diagnosis is essential to limit the spread of SARS-CoV-2 and for the clinical management of COVID-19. Although real-time reverse transcription polymerase chain reaction (RT- qPCR) is the current recommended laboratory method to diagnose SARS-CoV-2 acute infection, several factors such as requirement of special equipment and skilled staff limit the use of these time-consuming molecular techniques. Recently, several easy to perform rapid antigen detection tests were developed and recommended in some countries as the first line of diagnostic.
Objectives: The aim of this study was to evaluate the performances of the Coris COVID 19 Ag Respi-Strip test, a rapid immunochromatographic test for the detection of SARS-CoV-2 antigen, in comparison to RT-qPCR.
Results: 148 nasopharyngeal swabs were tested. Amongst the 106 positive RT-qPCR samples, 32 were detected by the rapid antigen test, given an overall sensitivity of 30.2%. All the samples detected positive with the antigen rapid test were also positive with RT-qPCR.
Conclusions: Higher viral loads are associated with better antigen detection rates. Unfortunately, the overall poor sensitivity of the COVID-19 Ag Respi-Strip does not allow using it alone as the frontline testing for COVID-19 diagnosis.



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LETTER TO THE EDITOR

Evaluation of a Rapid Diagnostic Assay for Detection of SARS-CoV-2 Antigen in Nasopharyngeal Swabs

Sidonie Lambert-Niclot,[a,b] Alexis Cuffel,[c] Samuel Le Pape,[d] Christelle Vauloup-Fellous,[d] Laurence Morand-Joubert,[a,b] Anne-Marie Roque-Afonso,[d] Jérôme Le Goff,[c,e] Constance Delaugerre,[c,f] on behalf of the AP-HP/Universities/INSERM COVID-19 Research Collaboration

In our study, the COVID-19 Ag Respi-Strip (Coris) had a sensitivity of 50% compare to that of RT-PCR. The test was more sensitive for high viral loads and might perhaps be used for patients within a few days after symptom onset, when the load in the upper respiratory tract is at its peak. Considering COVID-19's current low prevalence of 0.19% in France, prospective studies should be conducted to determine the best settings for its implementation.

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ELSEVIER

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Anaïs Scohy*, Ah
Alexia Verroken,

Department of Microbiology, C

ARTICLE INFO

Keywords:
COVID-19
Rapid antigen detection test

Journal of Clinical Virology 129 (2020) 104455

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journal homepage: www.elsevier.com/locate/jcv

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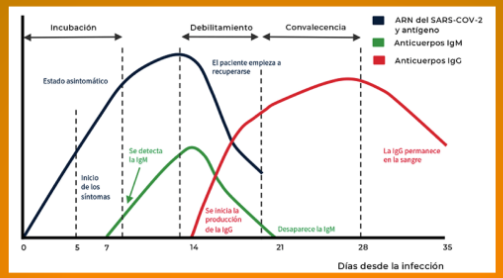
Antígenos y Anticuerpos



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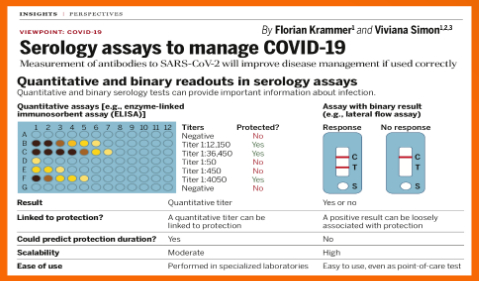
Antígenos

Anticuerpos

Pruebas rápidas de detección de IgM/IgG (Inmunocromatografía)



INSIGHTS | PERSPECTIVES

VIEWPOINT: COVID-19

By Florian Krammer[1] and Viviana Simon[1,2,3]

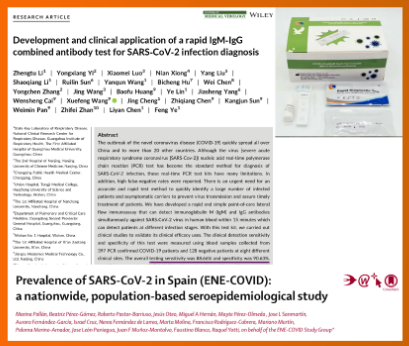
Serology assays to manage COVID-19

Measurement of antibodies to SARS-CoV-2 will improve disease management if used correctly

Quantitative and binary readouts in serology assays

Quantitative and binary serology tests can provide important information about infection.

Quantitative assays [e.g., enzyme-linked immunosorbent assay (ELISA)]			Assay with binary result (e.g., lateral flow assay)	
	Titers	Protected?	Response	No response
	Negative	No		
	Titer 1:12,350	Yes		
	Titer 1:36,450	Yes		
	Titer 1:50	No		
	Titer 1:450	No		
	Titer 1:4050	Yes		
	Negative	No		
Result	Quantitative titer		Yes or no	
Linked to protection?	A quantitative titer can be linked to protection		A positive result can be loosely associated with protection	
Could predict protection duration?	Yes		No	
Scalability	Moderate		High	
Ease of use	Performed in specialized laboratories		Easy to use, even as point-of-care test	

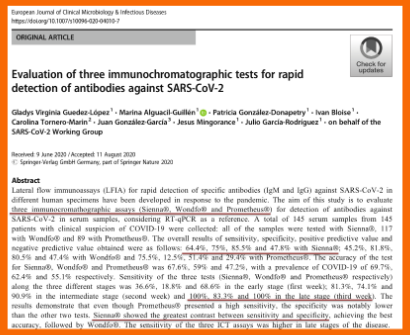


RESEARCH ARTICLE

WILEY

Development and clinical application of a rapid IgM-IgG combined antibody test for SARS-CoV-2 infection diagnosis

Zhengtu Li[1] | Yongxiang Yi[2] | Xiaomei Luo[3] | Nian Xiong[4] | Yang Liu[5] | Shaojiang Li[1] | Ruilin Sun[6] | Yanqun Wang[3] | Bicheng Hu[7] | Wei Chen[8] | Yongchen Zhang[1] | Jing Wang[9] | Baofu Huang[9] | Ye Lin[1] | Jiasheng Yang[4] | Wensheng Cai[10] | Xuefeng Wang[9] | Jing Cheng[1] | Zhiqiang Chen[9] | Kangjun Sun[9] | Weimin Pan[9] | Zhifei Zhang[10] | Liyan Chen[1] | Feng Ye[1]



European Journal of Clinical Microbiology & Infectious Diseases

https://doi.org/10.1007/s10096-020-04010-7

ORIGINAL ARTICLE

Check for updates

Evaluation of three immunochromatographic tests for rapid detection of antibodies against SARS-CoV-2

Gladys Virginia Guedez-López[1] · Marina Alguacil-Guillén[1] · Patricia González-Donapetry[1] · Ivan Bloise[1] · Carolina Tornero-Marín[1] · Juan González-García[1] · Jesus Mingorance[1] · Julio García-Rodríguez[1] · on behalf of the SARS-CoV-2 Working Group

Received: 9 June 2020 / Accepted: 11 August 2020

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Abstract

Lateral flow immunoassays (LFIA) for rapid detection of specific antibodies (IgM and IgG) against SARS-CoV-2 in different human specimens have been developed in response to the pandemic. The aim of this study is to evaluate three immunochromatographic assays (Sienna®, Wondfo® and Prometheus®) for detection of antibodies against SARS-CoV-2 in serum samples, considering RT-qPCR as a reference. A total of 145 serum samples from 145 patients with clinical suspicion of COVID-19 were collected: all of the samples were tested with Sienna®, 117 with Wondfo® and 89 with Prometheus®. The overall results of sensitivity, specificity, positive predictive value and negative predictive value obtained were as follows: 64.4%, 75%, 85.5% and 47.8% with Sienna®; 45.2%, 81.8%, 80.5% and 47.4% with Wondfo® and 75.5%, 12.5%, 51.4% and 29.4% with Prometheus®. The accuracy of the test for Sienna®, Wondfo® and Prometheus® was 67.6%, 59% and 47.2%, with a prevalence of COVID-19 of 69.7%, 62.4% and 55.1% respectively. Sensitivity of the three tests (Sienna®, Wondfo® and Prometheus® respectively) along the three different stages was 36.6%, 18.8% and 68.6% in the early stage (first week); 81.3%, 74.1% and 90.9% in the intermediate stage (second week) and 100%, 82.3% and 100% in the late stage (third week). The results demonstrate that even though Prometheus® presented a high sensitivity, the specificity was notably lower than the other two tests. Sienna® showed the greatest contrast between sensitivity and specificity, achieving the best accuracy, followed by Wondfo®. The sensitivity of the three ICT assays was higher in late stages of the disease.

Journal Pre-proof

Two serological approaches for detection of antibodies to SARS-CoV-2 in different scenarios: A screening tool and a point-of-care test

Alexis C.R. Hoste, Angel Venteo, Alba Fresco-Taboada, Istar Tapia, Alejandro Monedero, Lissette López, Maarten F. Jebbink, Elisa Pérez-Ramírez, Miguel Angel Jiménez-Clavero, Mercedes Almonacid, Patricia Muñoz, Jesus Guinea, Carmen Vela, Lia van der Hoek, Paloma Rueda, Patricia Sastre

T δ D Diagnostics Canada Pvt

2019-nCoV IgG/IgM Rapi

DR-ELISA

DR-LFA

Diagnostic

Sens: 100,0

Spec: 98,2

A total of 1065 serum samples, including positives for COVID-19 and negative samples from healthy donors or infected with other respiratory pathogens, were analyzed. The

VIEWPOINT: COVID-19

By Florian Krammer[1] and Viviana Simon[1,2,3]

Serology assays to manage COVID-19

Measurement of antibodies to SARS-CoV-2 will improve disease management if used correctly

Quantitative and binary readouts in serology assays

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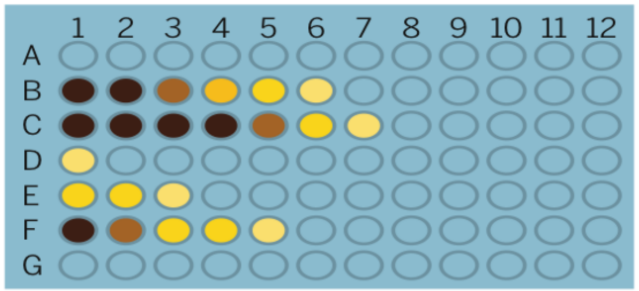
Quantitative assays [e.g., enzyme-linked immunosorbent assay (ELISA)]



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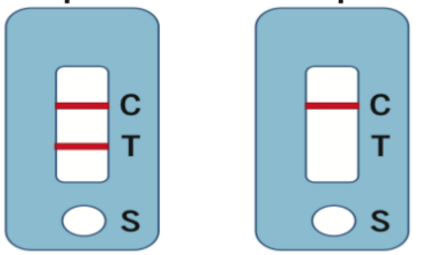
Negative	No

Assay with binary result (e.g., lateral flow assay)



Result	Quantitative titer	Yes or no
Linked to protection?	A quantitative titer can be linked to protection	A positive result can be loosely associated with protection
Could predict protection duration?	Yes	No
Scalability	Moderate	High
Ease of use	Performed in specialized laboratories	Easy to use, even as point-of-care test

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RESEARCH ARTICLE

JOURNAL OF MEDICAL VIROLOGY WILEY

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[1]State Key Laboratory of Respiratory Disease, National Clinical Research Center for Respiratory Disease, Guangzhou Institute of Respiratory Health, The First Affiliated Hospital of Guangzhou Medical University, Guangzhou, China

[2]The 2nd Hospital of Nanjing, Nanjing University of Chinese Medicine, Nanjing, China

[3]Chongqing Public Health Medical Center, Chongqing, China

[4]Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China

[5]The 1st Affiliated Hospital of Nanchang University, Nanchang, China

[6]Department of Pulmonary and Critical Care Medicine, Guangdong Second Provincial General Hospital, Guangzhou, Guangdong, China

[7]Wuhan No. 1 Hospital, Wuhan, China

[8]The 1st Affiliated Hospital of Xi'an Jiaotong University, Xi'an, China

[9]Jiangsu Medomics Medical Technology Co., Ltd, Nanjing, China

Abstract

The outbreak of the novel coronavirus disease (COVID-19) quickly spread all over China and to more than 20 other countries. Although the virus (severe acute respiratory syndrome coronavirus [SARS-Cov-2]) nucleic acid real-time polymerase chain reaction (PCR) test has become the standard method for diagnosis of SARS-CoV-2 infection, these real-time PCR test kits have many limitations. In addition, high false-negative rates were reported. There is an urgent need for an accurate and rapid test method to quickly identify a large number of infected patients and asymptomatic carriers to prevent virus transmission and assure timely treatment of patients. We have developed a rapid and simple point-of-care lateral flow immunoassay that can detect immunoglobulin M (IgM) and IgG antibodies simultaneously against SARS-CoV-2 virus in human blood within 15 minutes which can detect patients at different infection stages. With this test kit, we carried out clinical studies to validate its clinical efficacy uses. The clinical detection sensitivity and specificity of this test were measured using blood samples collected from 397 PCR confirmed COVID-19 patients and 128 negative patients at eight different clinical sites. The overall testing sensitivity was 88.66% and specificity was 90.63%.

Prevalence of SARS-CoV-2 in Spain (ENE-COVID): a nationwide, population-based seroepidemiological study

*Marina Pollán, Beatriz Pérez-Gómez, Roberto Pastor-Barriuso, Jesús Oteo, Miguel A Hernán, Mayte Pérez-Olmeda, Jose L Sanmartín, Aurora Fernández-García, Israel Cruz, Nerea Fernández de Larrea, Marta Molina, Francisco Rodríguez-Cabrera, Mariano Martín, Paloma Merino-Amador, Jose León Paniagua, Juan F Muñoz-Montalvo, Faustino Blanco, Raquel Yotti, on behalf of the ENE-COVID Study Group**

European Journal of Clinical Microbiology & Infectious Diseases
https://doi.org/10.1007/s10096-020-04010-7

ORIGINAL ARTICLE

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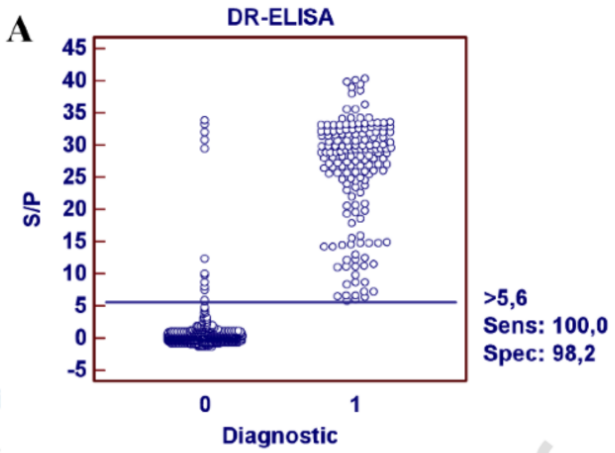
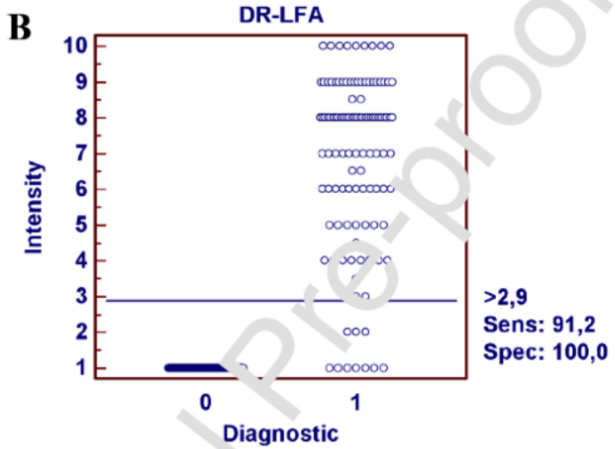
T δ D Diagnostics Canada Pvt

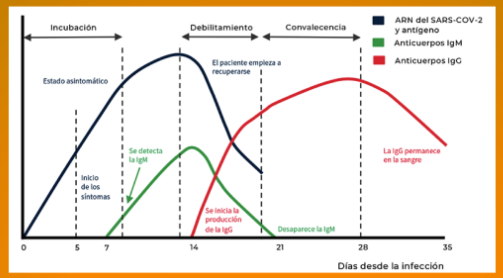
2019-nCoV IgG/IgM Rapi





A total of 1065 serum samples, including positive for COVID-19 and negative samples from healthy donors or infected with other respiratory pathogens, were analyzed. The results showed values of sensitivity between 91.2%-100%, and specificity of 100%-98.2%, for DR-LFA and DR-ELISA, respectively. No cross-reactivity against seasonal coronavirus (HCoV-NL63, HCoV-229E, HCoV-HKU1, HCoV-OC43) was found. These results demonstrate the importance of serology as a complementary tool to PCR, for follow up of recovered patients and identification of asymptomatic individuals.

Antígenos y Anticuerpos



Anticuerpos IgM

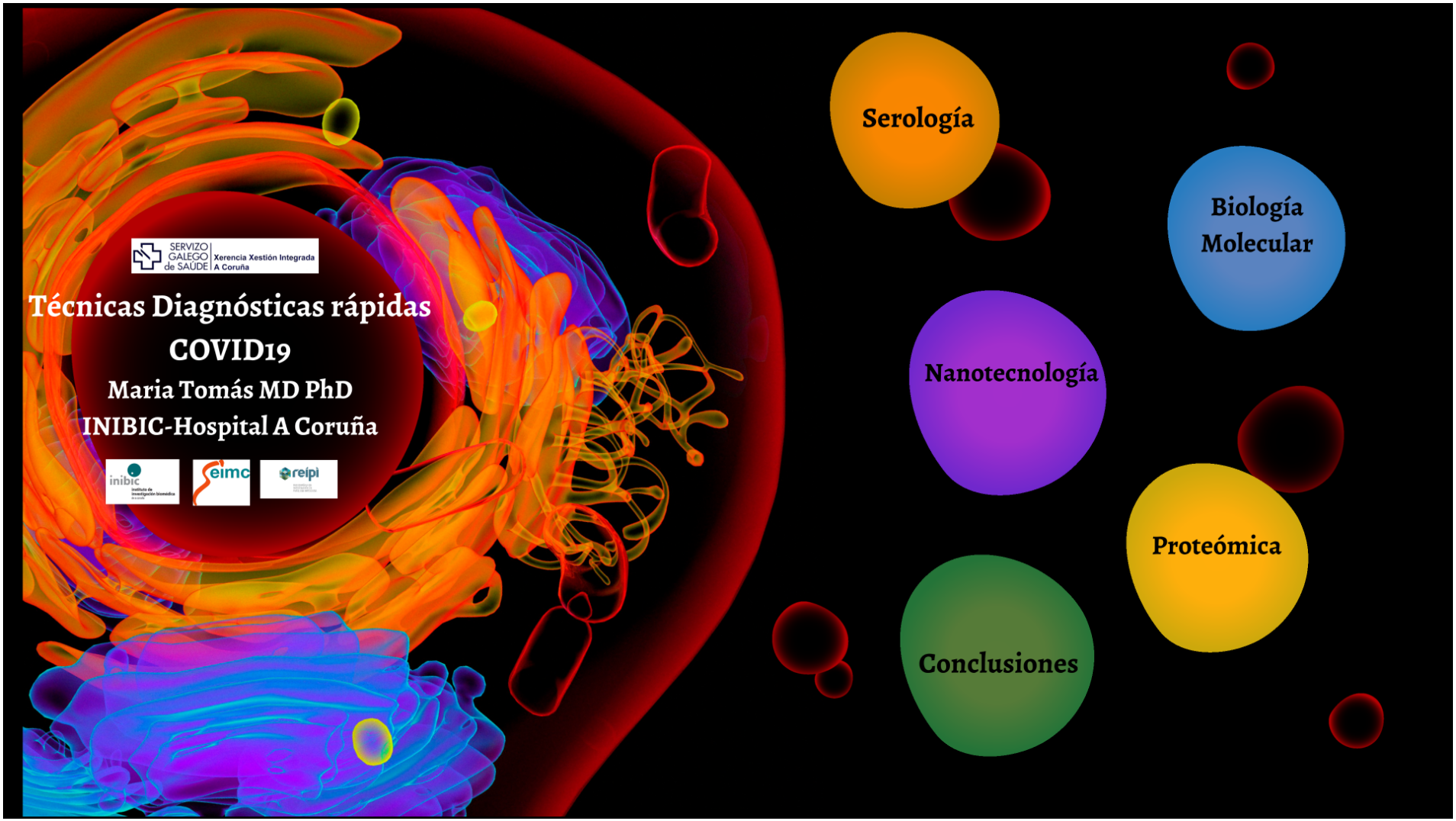
Son los primeros que se producen en respuesta a las proteínas virales **(antígenos)** y serán detectables principalmente durante el **inicio temprano** de la enfermedad.

Anticuerpos IgG

Se producen más tarde en respuesta a un antígeno y habitualmente **se mantienen durante un periodo más prolongado** en el cuerpo, para una respuesta a largo plazo.

Antígenos

Anticuerpos


SERVIZO GALEGO de SAÚDE
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COVID19
Maria Tomás MD PhD
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Biología Molecular
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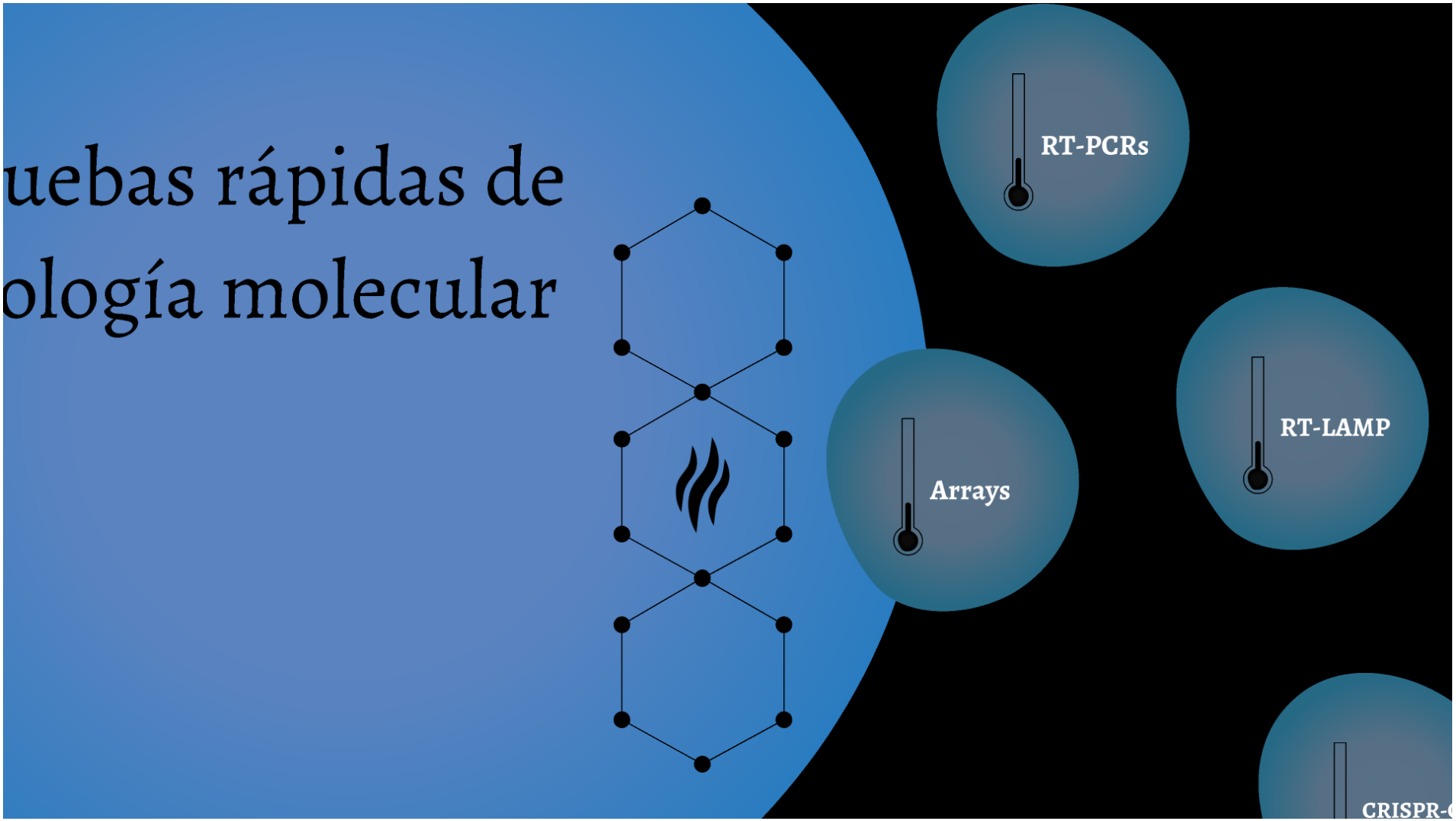
Conclusiones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Arrays
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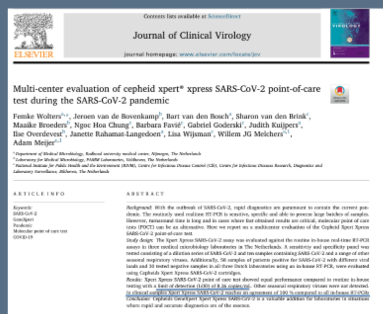
RT-PCRs (reacción en cadena de la polimerasa con transcriptasa reversa)



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Journal of Clinical Virology

journal homepage: www.elsevier.com/locate/jcv

Multi-center evaluation of cepheid xpert® xpress SARS-CoV-2 point-of-care test during the SARS-CoV-2 pandemic

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ARTICLE INFO

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SARS-CoV-2
GeneXpert
Pandemic
Molecular point of care test
COVID-19

ABSTRACT

Background: With the outbreak of SARS-CoV-2, rapid diagnostics are paramount to contain the current pandemic. The routinely used realtime RT-PCR is sensitive, specific and able to process large batches of samples. However, turnaround time is long and in cases where fast obtained results are critical, molecular point of care tests (POCT) can be an alternative. Here we report on a multicenter evaluation of the Cepheid Xpert Xpress SARS-CoV-2 point-of-care test.

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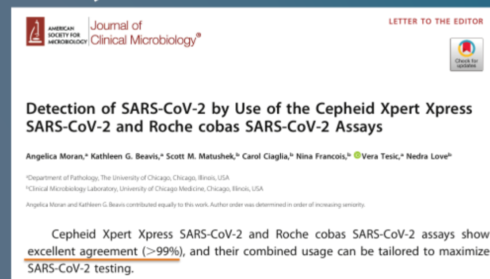

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LETTER TO THE EDITOR

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Angelica Moran, Kathleen G. Beavis, Scott M. Matushek, Carol Ciaglia, Nina Francois, Vera Tesic, Nedra Love

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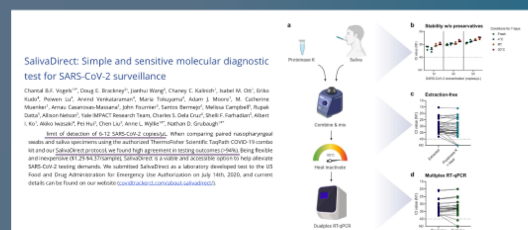

SalivaDirect: Simple and sensitive molecular diagnostic test for SARS-CoV-2 surveillance

limit of detection of 6-12 SARS-CoV-2 copies/μL. When comparing paired nasopharyngeal swabs and saliva specimens using the authorized ThermoFisher Scientific TaqPath COVID-19 combo kit and our SalivaDirect protocol, we found high agreement in testing outcomes (>94%). Being flexible and inexpensive ($1.29-$4.37/sample), SalivaDirect is a viable and accessible option to help alleviate SARS-CoV-2 testing demands. We submitted SalivaDirect as a laboratory developed test to the US Food and Drug Administration for Emergency Use Authorization on July 14th, 2020, and current details can be found on our website (covidtrackerct.com/about-salivadirect/).

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VIROLOGY

Comparison of Commercially Available and Laboratory-Developed Assays for *In Vitro* Detection of SARS-CoV-2 in Clinical Laboratories

Joshua A. Lieberman, Gregory Pepper, Samia N. Naccache, Meei-Li Huang, Keith R. Jerome, Alexander L. Greninger

ABSTRACT Multiple laboratory-developed tests (LDTs) and commercially available assays have emerged to meet diagnostic needs related to the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic. To date, there is limited comparison data for these different testing platforms. We compared the analytical performance of a LDT developed in our clinical laboratory based on CDC primer sets and four commercially available, FDA emergency use authorized assays for SARS-CoV-2 (Cepheid, DiaSorin, Hologic Panther, and Roche Cobas) on a total of 169 nasopharyngeal swabs. The LDT and Cepheid Xpert Xpress SARS-CoV-2 assays were the most sensitive assays for SARS-CoV-2 with 100% agreement across specimens. The Hologic Panther Fusion, DiaSorin Simplexa, and Roche Cobas 6800 failed to detect positive specimens only near the limit of detection of our CDC-based LDT assay. All assays were 100% specific, using our CDC-based LDT as the

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[b] Laboratory for Medical Microbiology, PAMM Laboratories, Veldhoven, The Netherlands
[c] National Institute for Public Health and the Environment (RIVM), Centre for Infectious Disease Control (CIb), Centre for Infectious Diseases Research, Diagnostics and Laboratory Surveillance, Bilthoven, The Netherlands

ARTICLE INFO

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LETTER TO THE EDITOR

Check for updates

Detection of SARS-CoV-2 by Use of the Cepheid Xpert Xpress SARS-CoV-2 and Roche cobas SARS-CoV-2 Assays

Angelica Moran,[a] Kathleen G. Beavis,[a] Scott M. Matushek,[b] Carol Ciaglia,[b] Nina Francois,[b] Vera Tesic,[a] Nedra Love[b]

[a]Department of Pathology, The University of Chicago, Chicago, Illinois, USA

[b]Clinical Microbiology Laboratory, University of Chicago Medicine, Chicago, Illinois, USA

Angelica Moran and Kathleen G. Beavis contributed equally to this work. Author order was determined in order of increasing seniority.

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VIROLOGY

Comparison of Commercially Available and Laboratory-Developed Assays for *In Vitro* Detection of SARS-CoV-2 in Clinical Laboratories

Joshua A. Lieberman,[a] Gregory Pepper,[a] Samia N. Naccache,[b] Meei-Li Huang,[a,c] Keith R. Jerome,[a,c] Alexander L. Greninger[a,c]

[a]Department of Laboratory Medicine, University of Washington Medical Center, Seattle, Washington, USA
[b]LabCorp Seattle, Department of Microbiology, Seattle, Washington, USA
[c]Vaccine and Infectious Disease Division, Fred Hutchinson Cancer Research Center, Seattle, Washington, USA

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SalivaDirect: Simple and sensitive molecular diagnostic test for SARS-CoV-2 surveillance

Chantal B.F. Vogels[1†*], Doug E. Brackney[2†], Jianhui Wang[3], Chaney C. Kalinich[1], Isabel M. Ott[1], Eriko Kudo[4], Peiwen Lu[4], Arvind Venkataraman[4], Maria Tokuyama[4], Adam J. Moore[1], M. Catherine Muenker[1], Arnau Casanovas-Massana[1], John Fournier[5], Santos Bermejo[6], Melissa Campbell[5], Rupak Datta[5], Allison Nelson[5], Yale IMPACT Research Team, Charles S. Dela Cruz[6], Shelli F. Farhadian[5], Albert I. Ko[1], Akiko Iwasaki[4], Pei Hui[3], Chen Liu[3], Anne L. Wyllie[1#*], Nathan D. Grubaugh[1#*]

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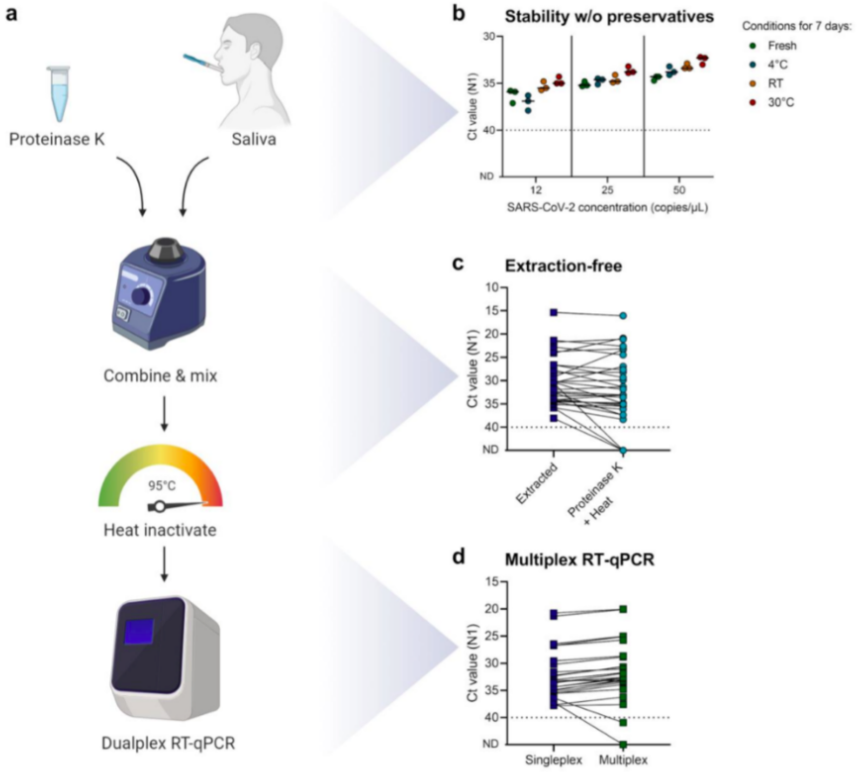

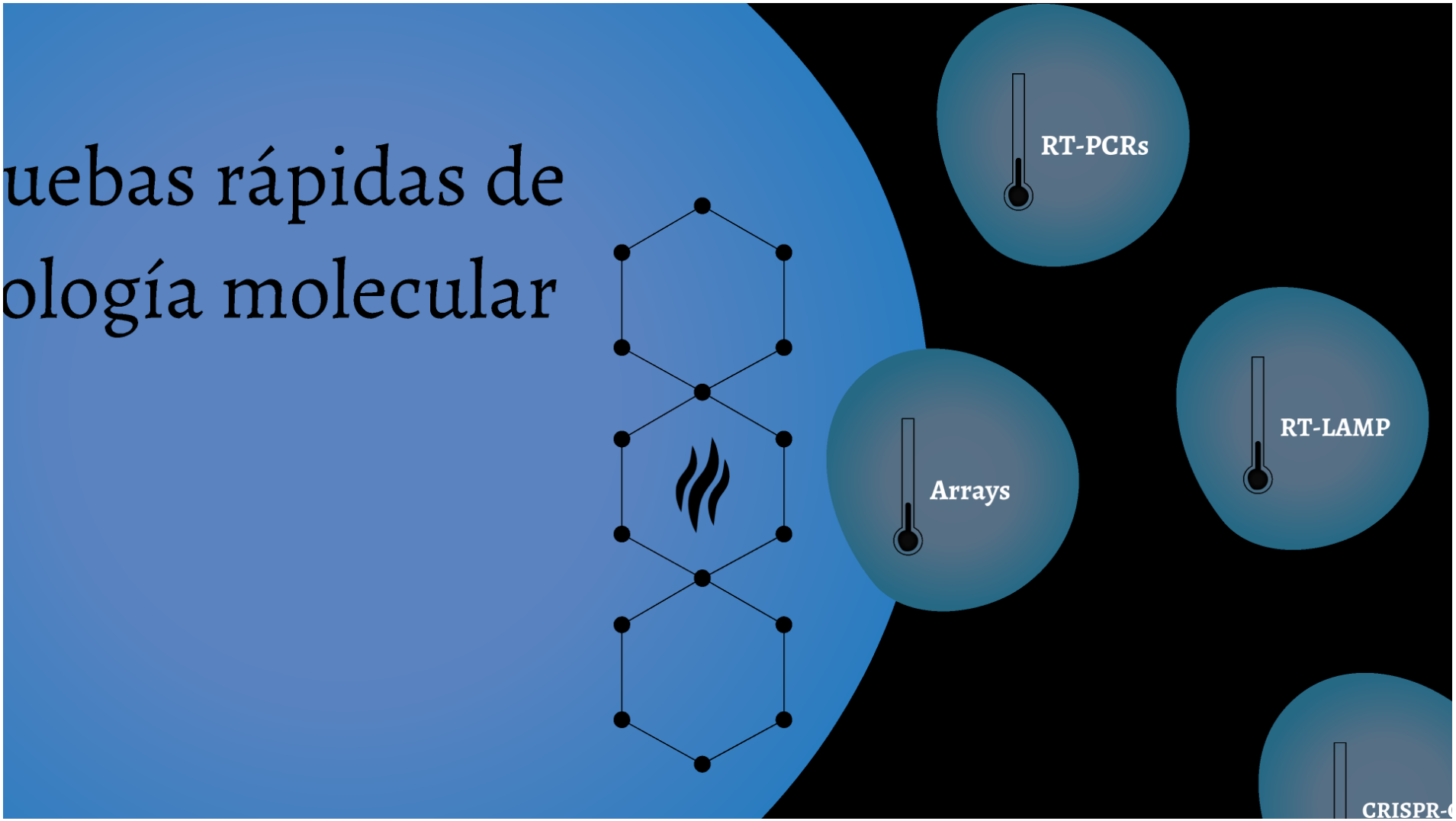
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RT-PCRs
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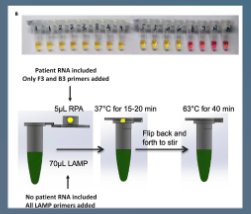
RT-LAMP(Transcripción reversa de la amplificación isotérmica


Medical Hypotheses

Loop mediated isothermal amplification (LAMP) assays as a rapid diagnostic for COVID-19




Medical Hypotheses

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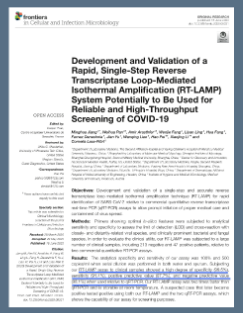
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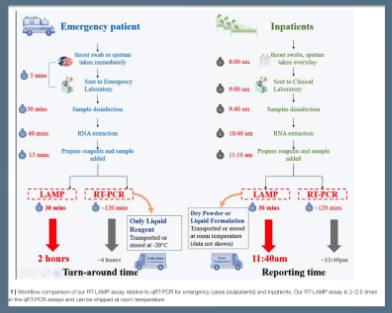

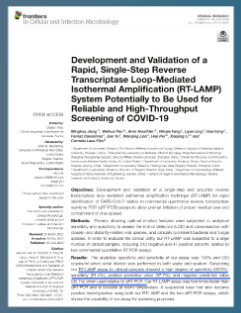
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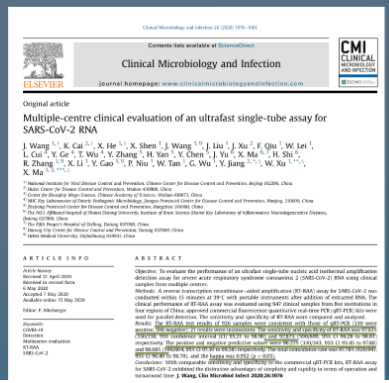
Clinical Microbiology and Infection

Original article

Multiple-centre clinical evaluation of an ultrafast single-tube assay for SARS-CoV-2 RNA

J. Wang [1,†], K. Cai [2,†], X. He [3,†], X. Shen [1], J. Wang [3,†], J. Liu [1], J. Xu [2], F. Qiu [1], W. Lei [1], L. Cui [4], Y. Ge [4], T. Wu [3], Y. Zhang [2], H. Yan [3], Y. Chen [5], J. Yu [6], X. Ma [6,7], H. Shi [8], R. Zhang [3,9], X. Li [1], Y. Gao [3,9], P. Niu [1], W. Tan [1], G. Wu [1], Y. Jiang [2,*,1], W. Xu [3,*,9], X. Ma [3,*,9]

ARTICLE INFO

ABSTRACT

Contents lists available at ScienceDirect

Medical Hypotheses

journal homepage: www.elsevier.com/locate/mehy

Loop mediated isothermal amplification (LAMP) assays as a rapid diagnostic for COVID-19

Junaid Kashir[a,b,*], Ahmed Yaqinuddin[a]

[a] College of Medicine, Alfaisal University, Riyadh, Saudi Arabia
[b] Department of Comparative Medicine, King Faisal Specialist Hospital and Research Center, Riyadh, Saudi Arabia

ARTICLE INFO

Keywords:
COVID-19
Polymerase chain reaction (PCR)
Loop mediated isothermal amplification (LAMP)
Diagnostic
Coronavirus

ABSTRACT

Recently, a novel coronavirus (SARS-CoV-2; coronavirus disease 2019, COVID-19) has emerged, rapidly spreading and severely straining the capacity of the global health community. Many nations are employing combinations of containment and mitigation strategies, where early diagnosis of COVID-19 is vital in controlling illness progression and limiting viral spread within the population. Thus, rapid and accurate methods of early detection are vital to contain COVID-19 and prevent further spread and predicted subsequent infectious waves of viral recurrence in future. Immediately after its initial characterization, Chinese and American Centers for Disease Control and Prevention (CDCs) rapidly employed molecular assays for detection of COVID-19, mostly employing real-time polymerase chain reaction (RT-PCR) methods. However, such methods require specific expensive items of equipment and highly trained analysts, requiring upwards of 4–8 h to process. These requirements coupled with associated financial pressures may prevent effective deployment of such diagnostic tests. Loop mediated isothermal amplification (LAMP) is method of nucleic acid amplification which exhibits increased sensitivity and specificity are significantly rapid, and do not require expensive reagents or instruments, which aids in cost reduction for coronavirus detection. Studies have shown the successful application of LAMP assays in various forms to detect coronavirus RNA in patient samples, demonstrating that 1–10 copies of viral RNA template per reaction are sufficient for successful detection, ~100-fold more sensitive than conventional RT-PCR methods. Importantly, studies have also now demonstrated the effectiveness of LAMP methodology in the detection of SARS-CoV-2 RNA at significantly low levels, particularly following numerous improvements to LAMP assay protocols. We hypothesise that recent advancements in enhanced LAMP protocols assay perhaps represent the best chance for a rapid and robust assay for field diagnosis of COVID-19, without the requirement of specialized equipment and highly trained professionals to interpret results. Herein, we present our arguments with a view to disseminate such findings, to assist the combat of this virus that is proving so devastating. We hope that this strategy could be applied rapidly, and confirmed for viability with clinical samples, before being rolled out for mass-diagnostic testing in these current times.

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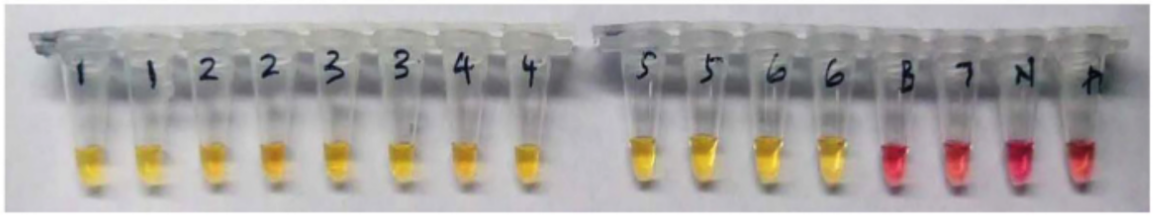

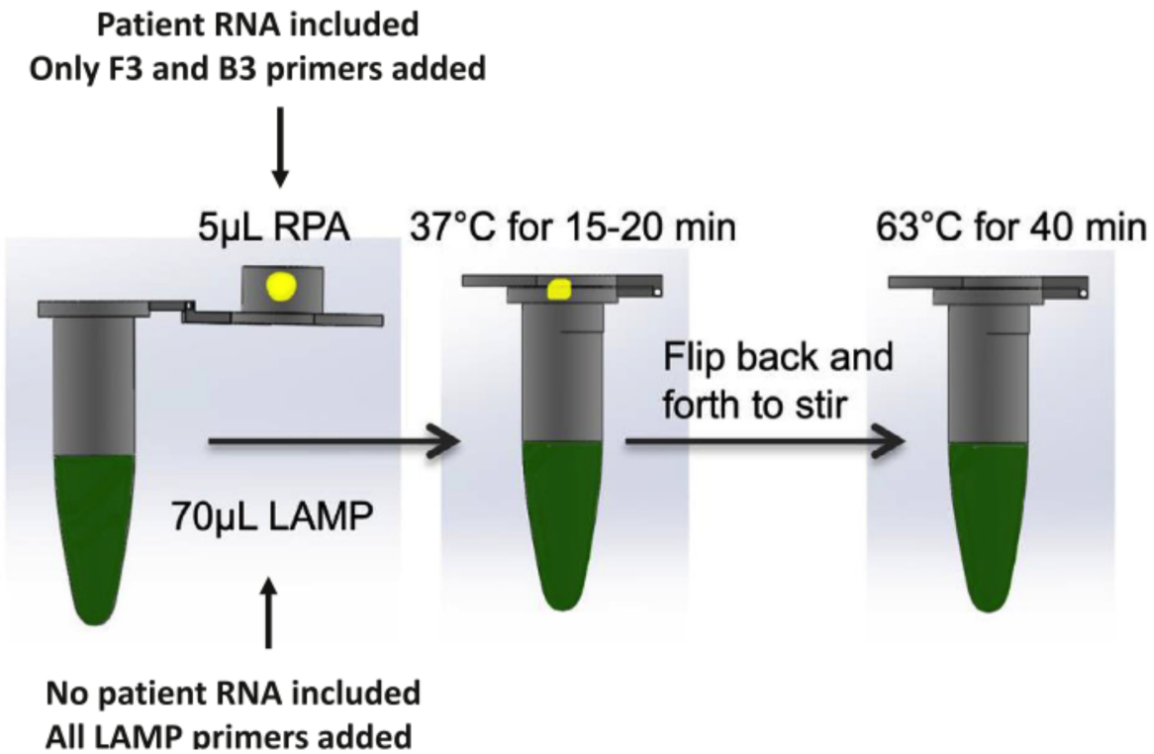

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[a] College of
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frontiers in Cellular and Infection Microbiology

ORIGINAL RESEARCH
published: 16 June 2020
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Development and Validation of a Rapid, Single-Step Reverse Transcriptase Loop-Mediated Isothermal Amplification (RT-LAMP) System Potentially to Be Used for Reliable and High-Throughput Screening of COVID-19

Minghua Jiang[†], *Weihua Pan*[2†], *Amir Arasthfer*[3†], *Wenjie Fang*[2], *Liyan Ling*[4], *Hua Fang*[5], *Farnaz Daneshnia*[3], *Jian Yu*[1], *Wanqing Liao*[2], *Hao Pei*[6*], *Xiaojing Li*[7*] and *Cornelia Lass-Flörl*[8]

[1] Department of Laboratory Medicine, The Second Affiliated Hospital and Yuying Children's Hospital of Wenzhou Medical University, Wenzhou, China, [2] Shanghai Key Laboratory of Molecular Medical Mycology, Shanghai Institute of Mycology, Shanghai Changzheng Hospital, Second Military Medical University, Shanghai, China, [3] Center for Discovery and Innovation, Hackensack Meridian Health, Nutley, NJ, United States, [4] Department of Laboratory Medicine, Pinghu Second People's Hospital, Jiaxing, China, [5] Department of Laboratory Medicine, Pudong New Area People's Hospital, Shanghai, China, [6] Department of Laboratory Medicine, Wuxi No. 5 People's Hospital, Wuxi, China, [7] Department of Dermatology, Affiliated Hospital of Hebei University of Engineering, Handan, China, [8] Institute of Hygiene and Medical Microbiology, Medical University of Innsbruck, Innsbruck, Austria

Objectives: Development and validation of a single-step and accurate reverse transcriptase loop-mediated isothermal amplification technique (RT-LAMP) for rapid identification of SARS-CoV-2 relative to commercial quantitative reverse transcriptase real-time PCR (qRT-PCR) assays to allow prompt initiation of proper medical care and containment of virus spread.

Methods: Primers showing optimal *in-silico* features were subjected to analytical sensitivity and specificity to assess the limit of detection (LOD) and cross-reaction with closely- and distantly-related viral species, and clinically prominent bacterial and fungal species. In order to evaluate the clinical utility, our RT-LAMP was subjected to a large number of clinical samples, including 213 negative and 47 positive patients, relative to two commercial quantitative RT-PCR assays.

Results: The analytical specificity and sensitivity of our assay was 100% and 500 copies/ml when serial dilution was performed in both water and sputum. Subjecting our RT-LAMP assay to clinical samples showed a high degree of specificity (99.5%), sensitivity (91.4%), positive predictive value (97.7%), and negative predictive value (98.1%) when used relative to qRT-PCR. Our RT-LAMP assay was two times faster than qRT-PCR and is storable at room temperature. A suspected case that later became positive tested positive using both our RT-LAMP and the two qRT-PCR assays, which shows the capability of our assay for screening purposes.

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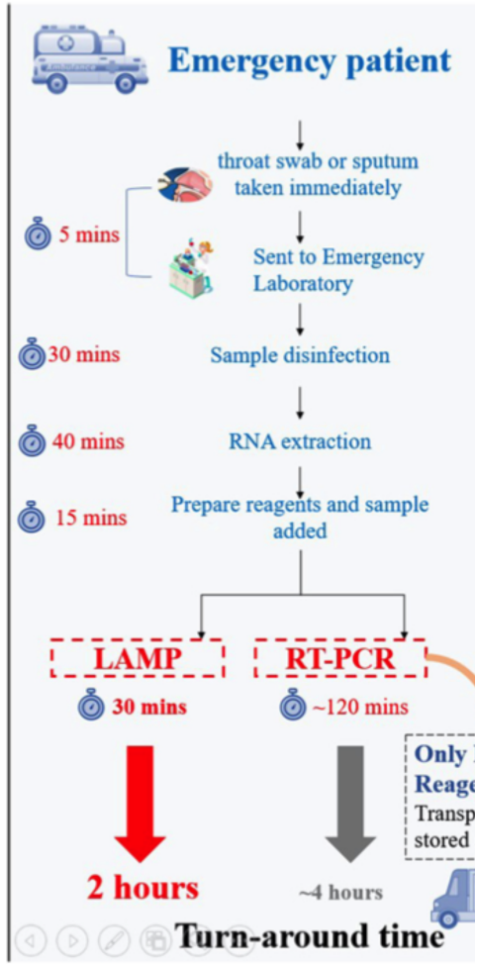

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ORIGINAL RESEARCH
published: 16 June 2020
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Fang[3], Liyan Ling[4], Hua Fang[5], Xiaojing Li[7]* and

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China, [3] Center for Discovery and Innovation,
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e and Medical Microbiology, Medical

gle-step and accurate reverse technique (RT-LAMP) for rapid uantitative reverse transcriptase tion of proper medical care and

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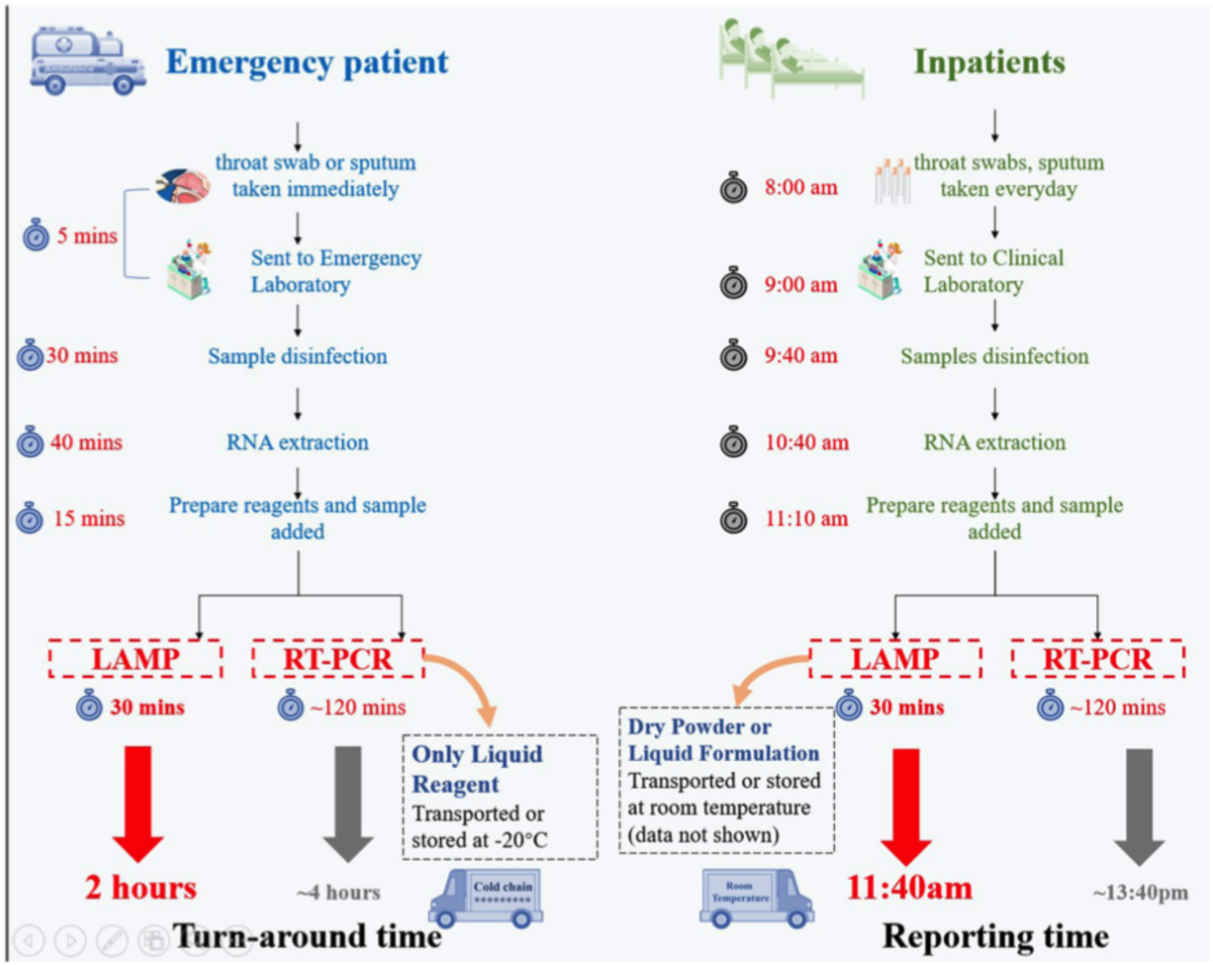

1 | Workflow comparison of our RT-LAMP assay relative to qRT-PCR for emergency cases (outpatients) and inpatients. Our RT-LAMP assay is 2–2.5 times n the qRT-PCR assays and can be shipped at room temperature.

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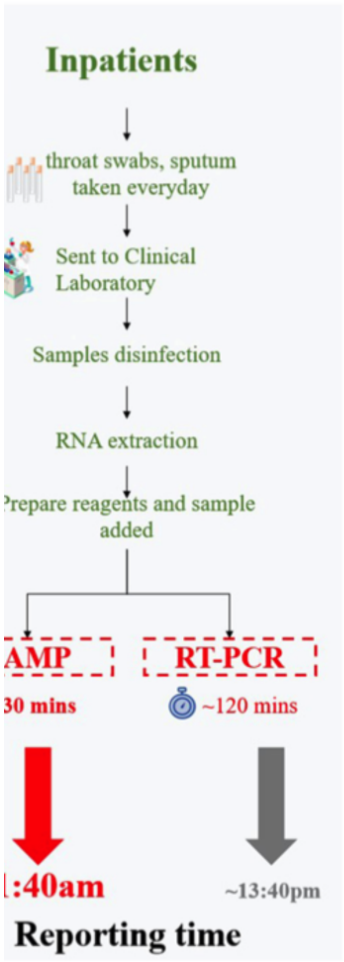

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ORIGINAL RESEARCH
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Development and Validation of a Rapid, Single-Step Reverse Transcriptase Loop-Mediated Isothermal Amplification (RT-LAMP) System Potentially to Be Used for Reliable and High-Throughput Screening of COVID-19

Minghua Jiang[†], *Weihua Pan*[2†], *Amir Arasthfer*[3†], *Wenjie Fang*[2], *Liyan Ling*[4], *Hua Fang*[5], *Farnaz Daneshnia*[3], *Jian Yu*[1], *Wanqing Liao*[2], *Hao Pei*[6*], *Xiaojing Li*[7*] and *Cornelia Lass-Flörl*[8]

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Objectives: Development and validation of a single-step and accurate reverse transcriptase loop-mediated isothermal amplification technique (RT-LAMP) for rapid identification of SARS-CoV-2 relative to commercial quantitative reverse transcriptase real-time PCR (qRT-PCR) assays to allow prompt initiation of proper medical care and containment of virus spread.

Methods: Primers showing optimal *in-silico* features were subjected to analytical sensitivity and specificity to assess the limit of detection (LOD) and cross-reaction with closely- and distantly-related viral species, and clinically prominent bacterial and fungal species. In order to evaluate the clinical utility, our RT-LAMP was subjected to a large number of clinical samples, including 213 negative and 47 positive patients, relative to two commercial quantitative RT-PCR assays.

Results: The analytical specificity and sensitivity of our assay was 100% and 500 copies/ml when serial dilution was performed in both water and sputum. Subjecting our RT-LAMP assay to clinical samples showed a high degree of specificity (99.5%), sensitivity (91.4%), positive predictive value (97.7%), and negative predictive value (98.1%) when used relative to qRT-PCR. Our RT-LAMP assay was two times faster than qRT-PCR and is storable at room temperature. A suspected case that later became positive tested positive using both our RT-LAMP and the two qRT-PCR assays, which shows the capability of our assay for screening purposes.

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Clinical Microbiology and Infection 26 (2020) 1076–1081

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Original article

Multiple-centre clinical evaluation of an ultrafast single-tube assay for SARS-CoV-2 RNA

J. Wang [1, †], K. Cai [2, †], X. He [1, †], X. Shen [1], J. Wang [1, 9], J. Liu [1], J. Xu [2], F. Qiu [1], W. Lei [1], L. Cui [4], Y. Ge [4], T. Wu [4], Y. Zhang [5], H. Yan [5], Y. Chen [5], J. Yu [6], X. Ma [6, 7], H. Shi [8], R. Zhang [1, 9], X. Li [1], Y. Gao [1, 9], P. Niu [1], W. Tan [1], G. Wu [1], Y. Jiang [2, *, §], W. Xu [1, **, §], X. Ma [1, 3, ***, §]

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SARS-CoV-2

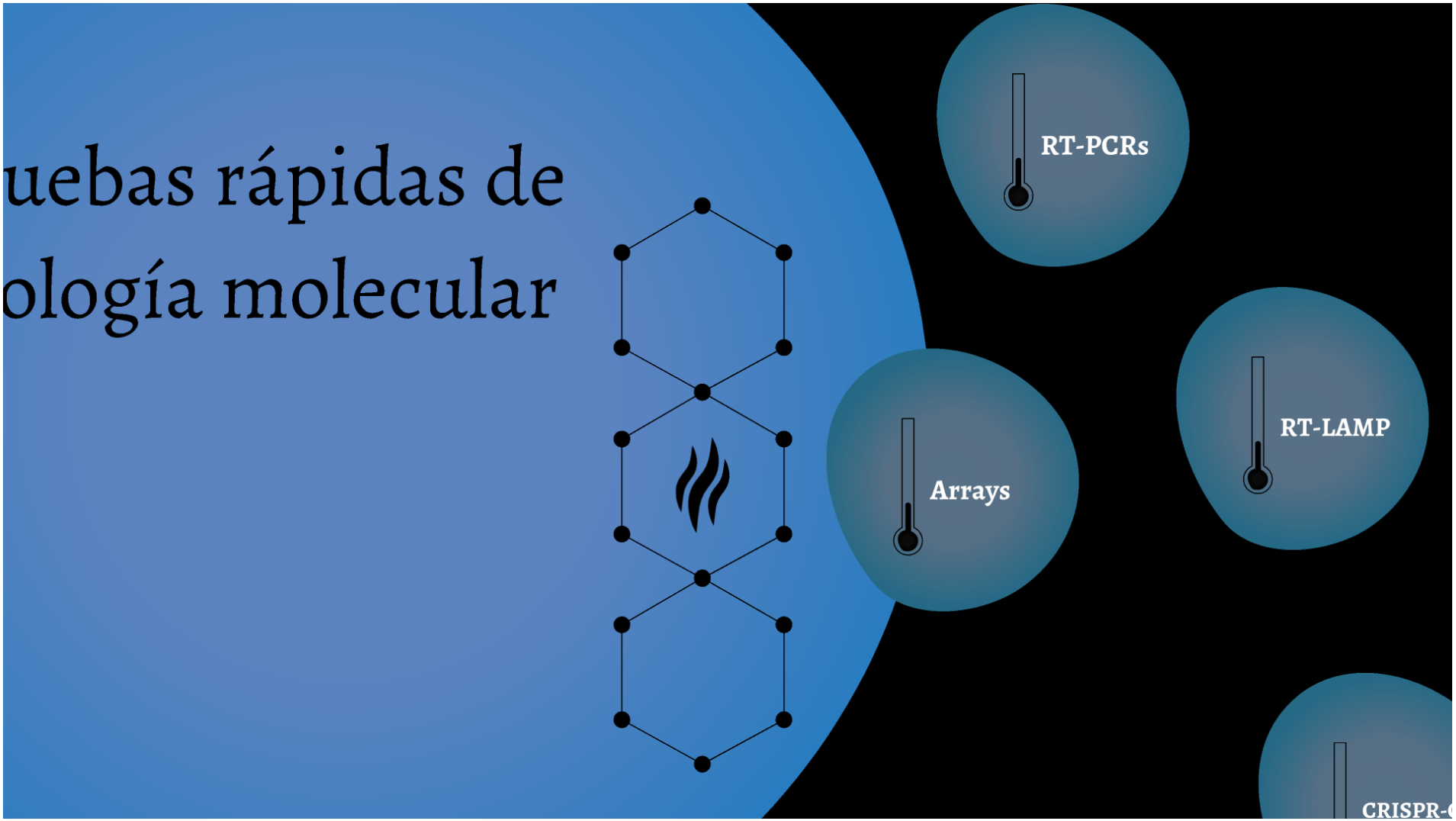
ABSTRACT

Objective: To evaluate the performance of an ultrafast single-tube nucleic acid isothermal amplification detection assay for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) RNA using clinical samples from multiple centres.

Methods: A reverse transcription recombinase–aided amplification (RT-RAA) assay for SARS-CoV-2 was conducted within 15 minutes at 39°C with portable instruments after addition of extracted RNA. The clinical performance of RT-RAA assay was evaluated using 947 clinical samples from five institutions in four regions of China; approved commercial fluorescence quantitative real-time PCR (qRT-PCR) kits were used for parallel detection. The sensitivity and specificity of RT-RAA were compared and analysed.

Results: The RT-RAA test results of 926 samples were consistent with those of qRT-PCR (330 were positive, 596 negative); 21 results were inconsistent. The sensitivity and specificity of RT-RAA was 97.63% (330/338, 95% confidence interval (CI) 95.21 to 98.90) and 97.87% (596/609, 95% CI 96.28 to 98.81) respectively. The positive and negative predictive values were 96.21% (330/343, 95% CI 93.45 to 97.88) and 98.68% (596/604, 95% CI 97.30 to 99.38) respectively. The total coincidence rate was 97.78% (926/947, 95% CI 96.80 to 98.70), and the kappa was 0.952 ($p < 0.05$).

Conclusions: With comparable sensitivity and specificity to the commercial qRT-PCR kits, RT-RAA assay for SARS-CoV-2 exhibited the distinctive advantages of simplicity and rapidity in terms of operation and turnaround time. **J. Wang, Clin Microbiol Infect 2020;26:1076**


uebas rápidas de
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Journal of Clinical Virology

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Clinical evaluation of the BioFire® Respiratory Panel 2.1 and detection of SARS-CoV-2

Hannah M. Creager[a], Barbara Cabrera[a], Andy Schnaubelt[a], Jesse L. Cox[a], Allison M. Cushman-Vokoun[a], Salika M. Shakir[b], Keith D. Tardif[b], Meei-Li Huang[c], Keith R. Jerome[c,d], Alexander L. Greninger[c], Daria Drobysheva[e], Usha Spaulding[e], Margarita Rogatcheva[e], Kevin M. Bourzac[e], S.H. Hinrichs[a], M.J. Broadhurst[a,1], P.D. Fey[a,*,1]

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Keywords:
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ABSTRACT

We evaluated the performance of the BioFire® Respiratory Panel 2.1 (RP2.1) in the detection of SARS CoV-2 in comparison against three other SARS CoV-2 EUA assays. In these studies, the RP2.1 panel had 98 % positive percent agreement (48/49) and 100 % negative percent agreement (49/49). Since 30 % of nasopharyngeal swab specimens have a SARS CoV-2 Ct > 30 and thus detection of virus in low titers is clinically relevant, a sample with a high titer was diluted and each 10 fold dilution was tested in triplicate and compared against 6 other EUA approved SARS CoV-2 assays. These data suggested that the BioFire® RP2.1 panel, along with four other SARS CoV-2 assays (Roche cobas, Cepheid Xpert Xpress, BioFire® Defense COVID19, and NECoV19), consistently detected viral RNA at the 10−7 dilution. Overall, these studies suggest that the BioFire® RP2.1 assay can be used to detect acute cases of SARS CoV2 in addition to patients with low viral titer later in disease presentation.

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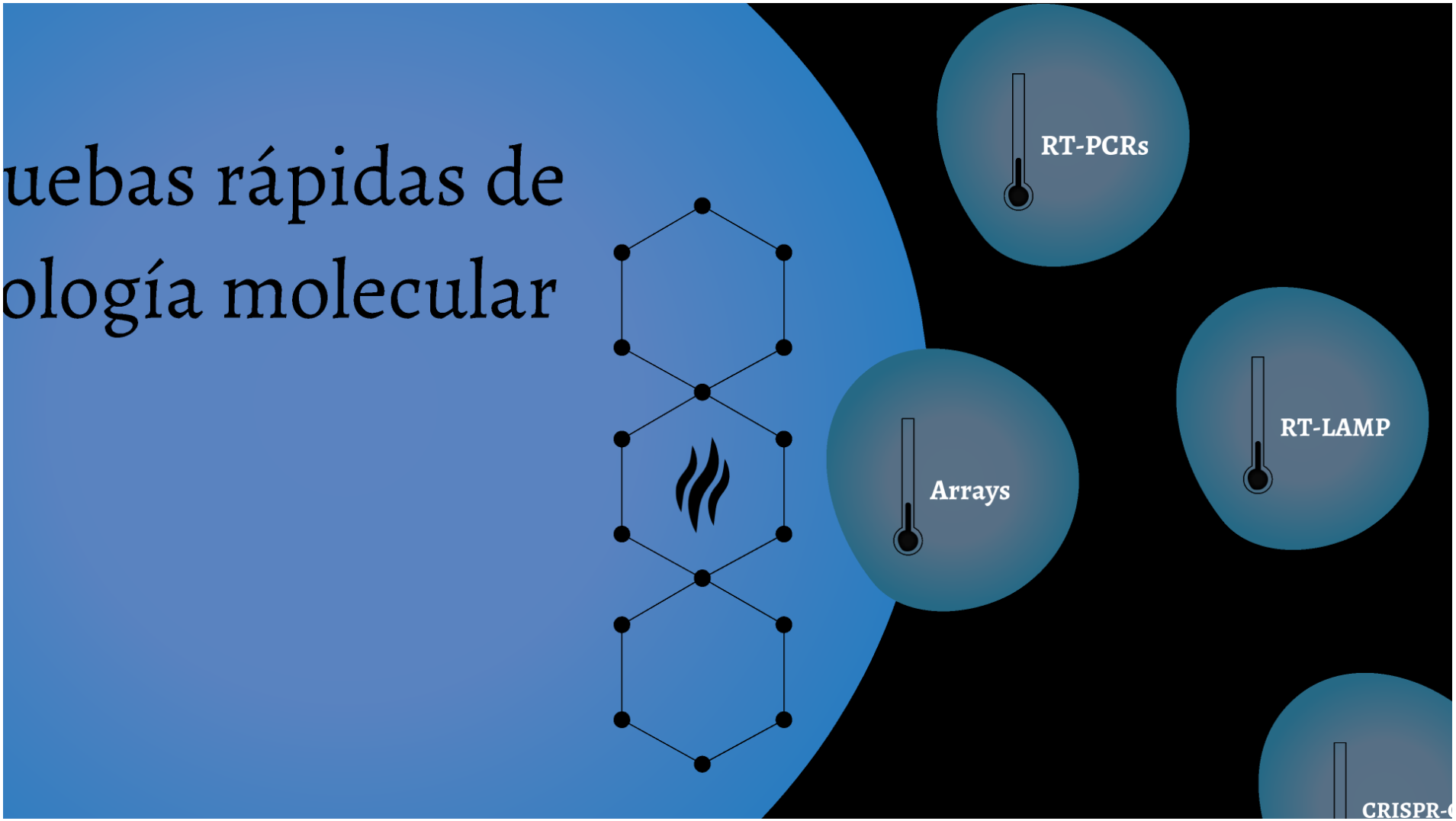
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CRISPR-Cas

GOBIERNO DE ESPAÑA · MINISTERIO DE CIENCIA E INNOVACIÓN · Instituto de Salud Carlos III

INFORME DEL GRUPO DE ANÁLISIS CIENTÍFICO DE CORONAVIRUS DEL ISCIII (GACC-ISCIII)

LA TECNOLOGÍA CRISPR EN LA INFECCIÓN POR SARS-CoV-2

** Este informe está realizado con la evidencia científica disponible en la fecha de su elaboración y podrá ser actualizado si surgen nuevas evidencias.*

Emerging Microbes & Infections 2020, VOL. 9 https://doi.org/10.1080/22221751.2020.1793689

EMI · Taylor & Francis

REVIEW · OPEN ACCESS

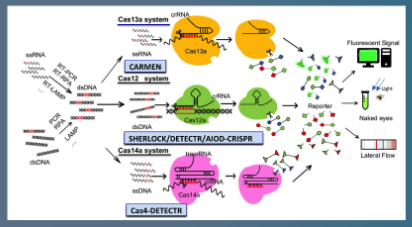
Next-generation pathogen diagnosis with CRISPR/Cas-based detection methods

Xinjie Wang, Xiaoyun Shang and Xingxu Huang

[a]Shanghai Institute for Advanced Immunochemical Studies, ShanghaiTech University, Shanghai, People's Republic of China; [b]Suzhou Maximum Bio-tech Co., Ltd., Suzhou, People's Republic of China; [c]School of Life Science and Technology, ShanghaiTech University, Shanghai, People's Republic of China

ABSTRACT

Ideal methods for detecting pathogens should be sensitive, specific, rapid, cost-effective and instrument-free. Conventional nucleic acid pathogen detection strategies, mostly PCR-based techniques, have various limitations, such as expensive equipment, reagents and skilled performance. Recently, CRISPR/Cas-based methods have burst onto the scene, with the potential to power the pathogen detection field. Here we introduce these unique methods and discuss its hurdles and promises.



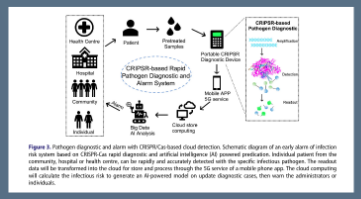


Figure 3. Pathogen diagnostic and alarm with CRISPR/Cas-based cloud detection. Schematic diagram of an early alarm of infection risk system based on CRISPR-Cas rapid diagnostic and artificial intelligence (AI) powered predication. Individual patient from the community, hospital or health centre, can be rapidly and accurately detected with the specific infectious pathogen. The readout data will be transformed into the cloud for store and process through the 5G service of a mobile phone app. The cloud computing will calculate the infectious risk to generate an AI-powered model on update diagnostic cases, then warn the administrators or individuals.

	COVID-19 RT-PCR	STOP-Covid (SHERLOCK)	CARMEN	DETECTR	AIOD-CRISPR	CONAN
Diana molecular	Nucleocápside	ORFLab (Espícula)	ORFLab (Espícula)	Nucleocápside	Nucleocápside	Nucleocápside
CRISPR-Cas		Cas12b	Cas13	RT-LAMP/Cas12a	Cas12a	Cas3
Tipo muestra	ARN	ARN	ARN	ADN	ADN	ARN
Duración	120 min	40-70 min	+ 30 min	30-40 min	40 min	40 min
Número de muestras	1	1	1000	1	1	1
Resultados	Cuantitativa	Semi-cuantitativa	Cualitativa	Cualitativa	Cualitativa	Cualitativa
Límite detección	> 10 viral copias	100 viral copias	10 viral copias	10 viral copias	1.3 viral copias	100 copias
Aprobación FDA	Si	Si	En proceso	En proceso	-	-
Publicaciones	-	Joung et al. Medrxiv 2020 (PMID: 32511521)	Ackerman CM et al. Nature 2020 (PMID: 32349121)	Broughton et al. Nat Biotech 2020 (PMID: 32300245)	Ding et al. bioRxiv 2020- (PMID: 32511323)	Yoshimi K et al. medRxiv 2020-

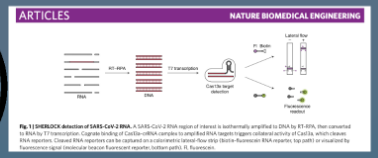
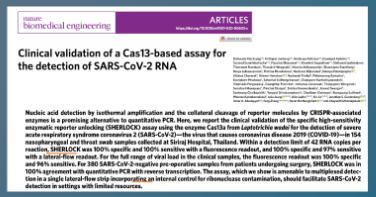


Fig. 1 | SHERLOCK detection of SARS-CoV-2 RNA. A SARS-CoV-2 RNA region of interest is isothermally amplified to DNA by RT-RPA, then converted to RNA by T7 transcription. Cognate binding of Cas13a–crRNA complex to amplified RNA targets triggers collateral activity of Cas13a, which cleaves RNA reporters. Cleaved RNA reporters can be captured on a colorimetric lateral-flow strip (biotin–fluorescein RNA reporter, top path) or visualized by fluorescence signal (molecular beacon fluorescent reporter, bottom path). FI, fluorescein.



Nucleic acid detection by isothermal amplification and the collateral cleavage of reporter molecules by CRISPR-associated enzymes is a promising alternative to quantitative PCR. Here, we report the clinical validation of the specific high-sensitivity enzymatic reporter unlocking (SHERLOCK) assay using the enzyme Cas13a from *Leptotrichia wadei* for the detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)—the virus that causes coronavirus disease 2019 (COVID-19)—in 154 nasopharyngeal and throat swab samples collected at Siriraj Hospital, Thailand. Within a detection limit of 42 RNA copies per reaction, SHERLOCK was 100% specific and 100% sensitive with a fluorescence readout, and 100% specific and 97% sensitive with a lateral-flow readout. For the full range of viral load in the clinical samples, the fluorescence readout was 100% specific and 96% sensitive. For 380 SARS-CoV-2-negative pre-operative samples from patients undergoing surgery, SHERLOCK was in 100% agreement with quantitative PCR with reverse transcription. The assay, which we show is amenable to multiplexed detection in a single lateral-flow strip incorporating an internal control for ribonuclease contamination, should facilitate SARS-CoV-2 detection in settings with limited resources.

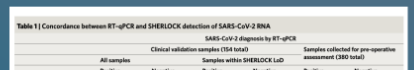


Table 1 | Concordance between RT–qPCR and SHERLOCK detection of SARS-CoV-2 RNA

	SARS-CoV-2 diagnosis by RT–qPCR			
	Clinical validation samples (154 total)		Samples collected for pre-operative assessment (380 total)	
	All samples	Samples within SHERLOCK LoD		

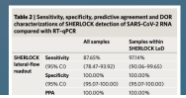
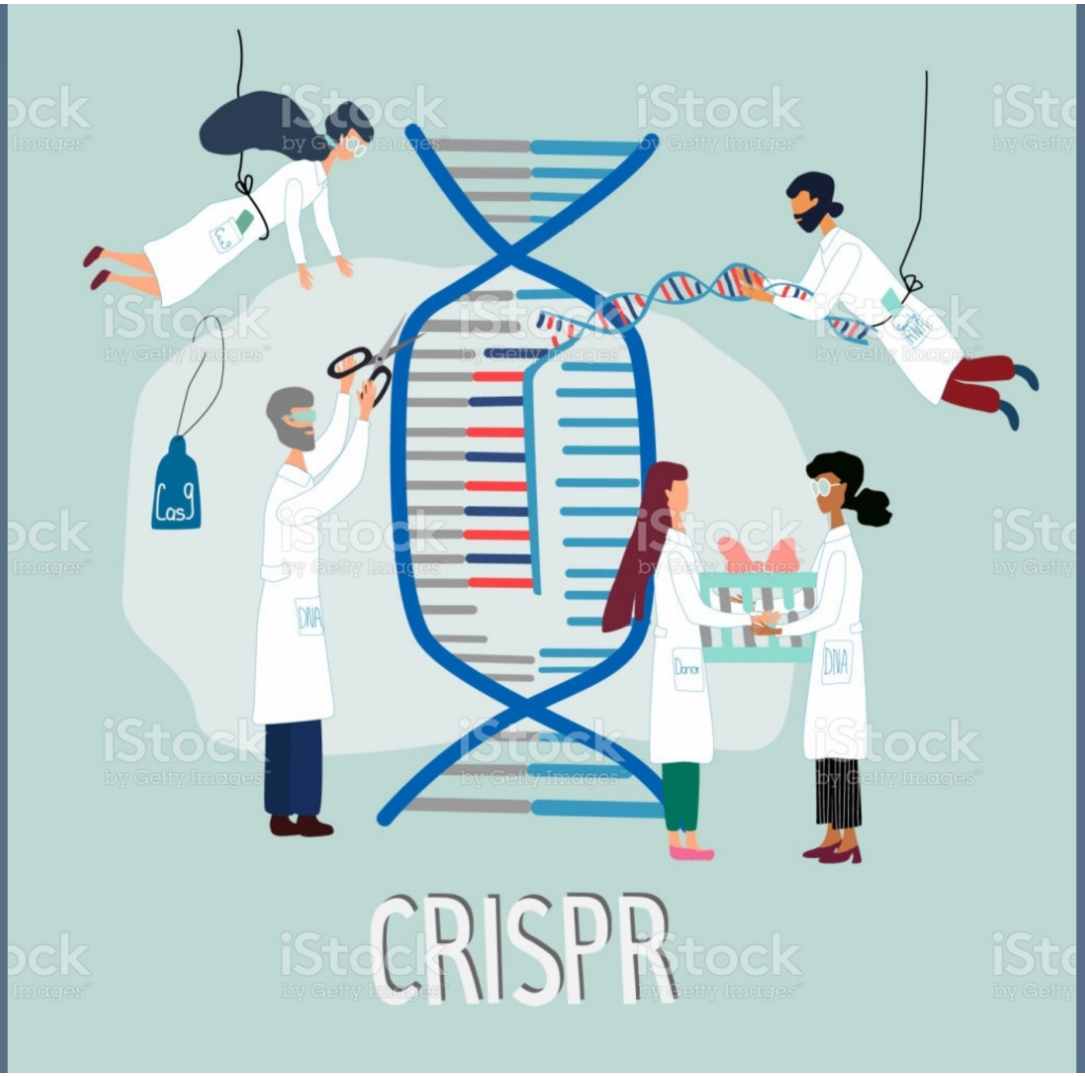


Table 2 | Sensitivity, specificity, predictive agreement and DOR characterizations of SHERLOCK detection of SARS-CoV-2 RNA compared with RT–qPCR

		All samples	Samples within SHERLOCK LoD
SHERLOCK lateral-flow readout	Sensitivity	87.66%	97.94%
	(95% CI)	(78.67–93.92)	(90.06–99.65)
	Specificity	100.00%	100.00%
	(95% CI)	(95.07–100.00)	(95.07–100.00)
	PPA	100.00%	100.00%



INFORME D

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crRNA

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REVIEW

Next-generation pathogen diagnosis with CRISPR/Cas-based detection methods

Xinjie Wang [a], Xiaoyun Shang[b] and Xingxu Huang [c]

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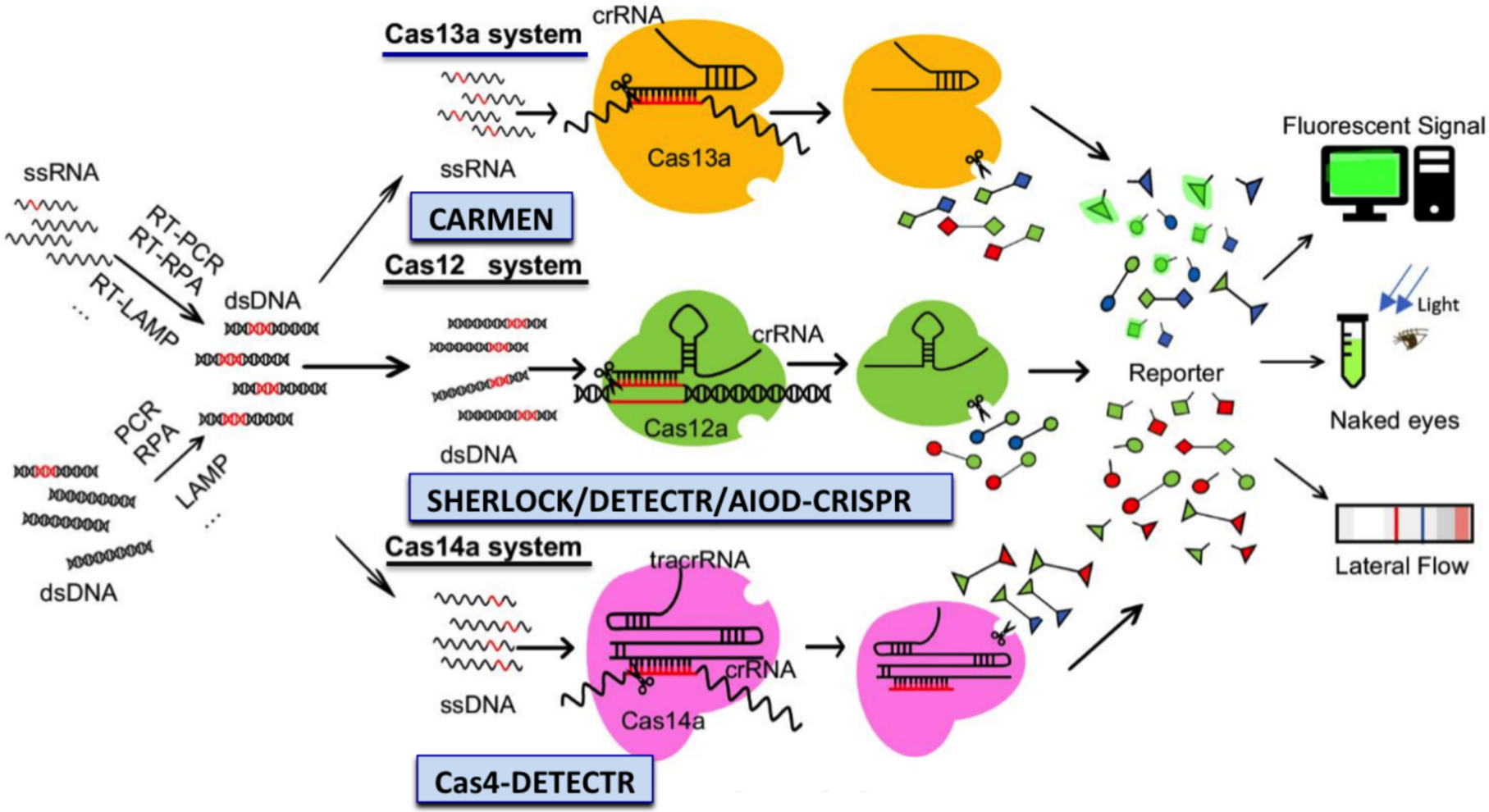
ABSTRACT

Ideal methods for detecting pathogens should be sensitive, specific, rapid, cost-effective and instrument-free. Conventional nucleic acid pathogen detection strategies, mostly PCR-based techniques, have various limitations, such as expensive equipment, reagents and skilled performance. Recently, CRISPR/Cas-based methods have burst onto the scene, with the potential to power the pathogen detection field. Here we introduce these unique methods and discuss its hurdles and promises.


Cas13a system
crRNA
Cas13a
ssRNA
ssRNA
CARMEN
Cas12 system
crRNA
Cas12a
dsDNA
dsDNA
SHERLOCK/DETECTR/AIOD-CRISPR
Cas14a system
tracrRNA
crRNA
Cas14a
ssDNA
Cas4-DETECTR
RT-PCR
RT-RPA
RT-LAMP
PCR
RPA
LAMP
dsDNA
Reporter
Fluorescent Signal
Light
Naked eyes
Lateral Flow

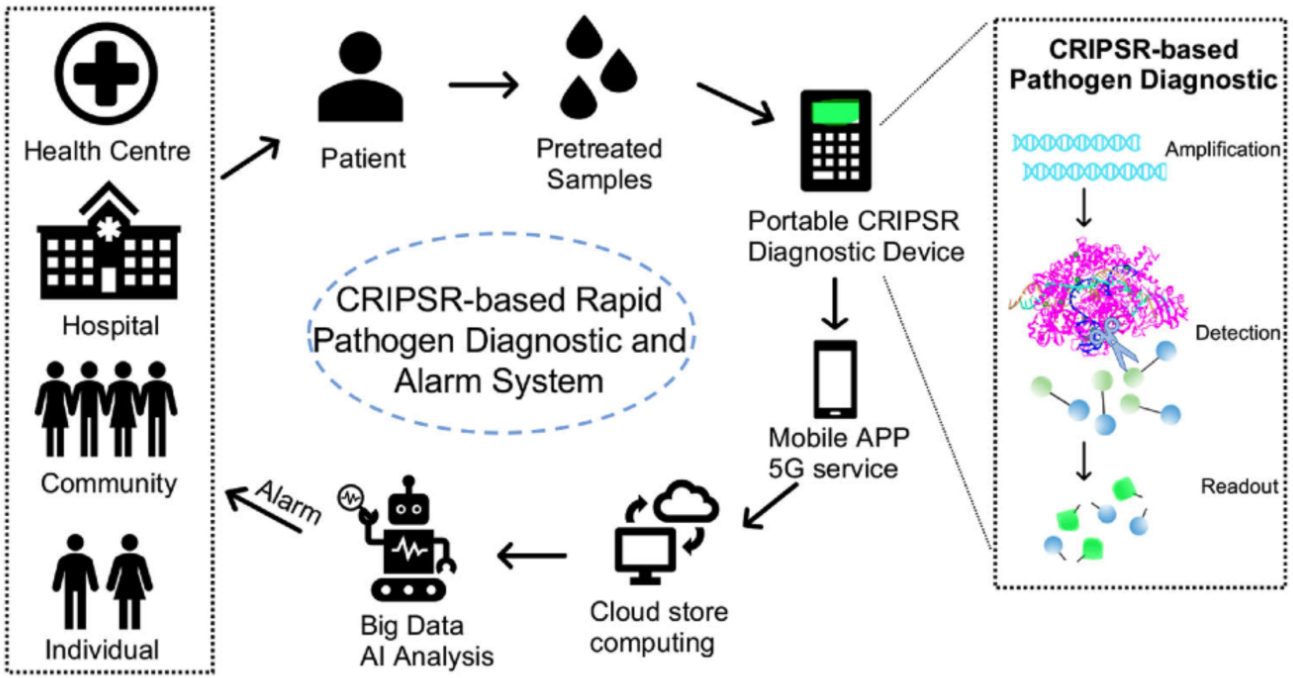


Figure 3. Pathogen diagnostic and alarm with CRISPR/Cas-based cloud detection. Schematic diagram of an early alarm of infection risk system based on CRISPR-Cas rapid diagnostic and artificial intelligence (AI) powered predication. Individual patient from the community, hospital or health centre, can be rapidly and accurately detected with the specific infectious pathogen. The readout data will be transformed into the cloud for store and process through the 5G service of a mobile phone app. The cloud computing will calculate the infectious risk to generate an AI-powered model on update diagnostic cases, then warn the administrators or individuals.

Cas4-DETECTR

1

	COVID-19 RT-PCR	STOP-Covid (SHERLOCK)	CARMEN	DETECTR	AIOD-CRISPR	CONAN
Diana molecular	Nucleocápside	ORF1ab (Espícula)	ORF1ab (Espícula)	Nucleocápside	Nucleocápside	Nucleocápside
CRISPR-Cas	-	Cas12b	Cas13	RT-LAMP/Cas12a	Cas12a	Cas3
Tipo muestra	ARN	ARN	ARN	ADN	ADN	ARN
Duración	120 min	40-70 min	≈ 30 min	30-40 min	40 min	40 min
Número de muestras	1	1	1000	1	1	1
Resultados	Cuantitativa	Semi-cuantitativa	Cualitativa	Cualitativa	Cualitativa	Cualitativa
Límite detección	> 10 viral copias	100 viral copias	10 viral copias	10 viral copias	1.3 viral copias	100 copias
Aprobación FDA	Si	Si	En proceso	En proceso	-	-
Publicaciones	-	Joung et al. MedRxiv 2020 (PMID: 32511521)	Ackerman CM et al. Nature 2020 (PMID: 32349121)	Broughton et al. Nat Biotech 2020 (PMID: 32300245)	Ding et al. bioRxiv 2020- (PMID: 32511323)	Yoshini K et al. medRxiv 2020-

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ARTICLES

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Clinical validation of a Cas13-based assay for the detection of SARS-CoV-2 RNA

Maturada Patchsung[1,15], Krittapas Jantarug[1,15], Archiraya Pattama[2,15], Kanokpol Aphicho[1,15], Surased Suraritdechachai[1,15], Piyachat Meesawat[1,15], Khomkrit Sappakhaw[1,15], Nattawat Leelahakorn[1], Theerawat Ruenkam[1], Thanakrit Wongsatit[1], Niracha Athipanyasilp[2], Bhumrapee Eiamthong[1], Benya Lakkanasirorat[1], Thitima Phoodokmai[1], Nootaree Niljianskul[3], Danaya Pakotiprapha[4], Sittinan Chanarat[4], Aimorn Homchan[4], Ruchanok Tinikul[4], Philaiwarong Kamutira[4], Kochakorn Phiwkaow[1], Sahachat Soithongcharoen[1], Chadaporn Kantiwiriyawanitch[1], Vinutsada Pongsupasa[1], Duangthip Trisrivirat[1], Juthamas Jaroensuk[1], Thanyaporn Wongnate[1], Somchart Maenpuen[5], Pimchai Chaiyen[1], Sirichai Kamnerdnakta[6], Jirawat Swangsri[6], Suebwong Chuthapisith[6], Yongyut Sirivatanauksorn[6], Chutikarn Chaimayo[2], Ruengpung Sutthent[2], Wannee Kantakamalakul[2], Julia Joung[7,8,9,10], Alim Ladha[7,8,9,10], Xin Jin[8,9,11,12], Jonathan S. Gootenberg[9,13], Omar O. Abudayyeh[9,13], Feng Zhang[7,8,9,10,13,14], Navin Horthongkham[2]✉ and Chayasith Uttamapinant[1]✉

Nucleic acid detection by isothermal amplification and the collateral cleavage of reporter molecules by CRISPR-associated enzymes is a promising alternative to quantitative PCR. Here, we report the clinical validation of the specific high-sensitivity enzymatic reporter unlocking (SHERLOCK) assay using the enzyme Cas13a from *Leptotrichia wadei* for the detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)—the virus that causes coronavirus disease 2019 (COVID-19)—in 154 nasopharyngeal and throat swab samples collected at Siriraj Hospital, Thailand. Within a detection limit of 42 RNA copies per reaction, SHERLOCK was 100% specific and 100% sensitive with a fluorescence readout, and 100% specific and 97% sensitive with a lateral-flow readout. For the full range of viral load in the clinical samples, the fluorescence readout was 100% specific and 96% sensitive. For 380 SARS-CoV-2-negative pre-operative samples from patients undergoing surgery, SHERLOCK was in 100% agreement with quantitative PCR with reverse transcription. The assay, which we show is amenable to multiplexed detection in a single lateral-flow strip incorporating an internal control for ribonuclease contamination, should facilitate SARS-CoV-2 detection in settings with limited resources.

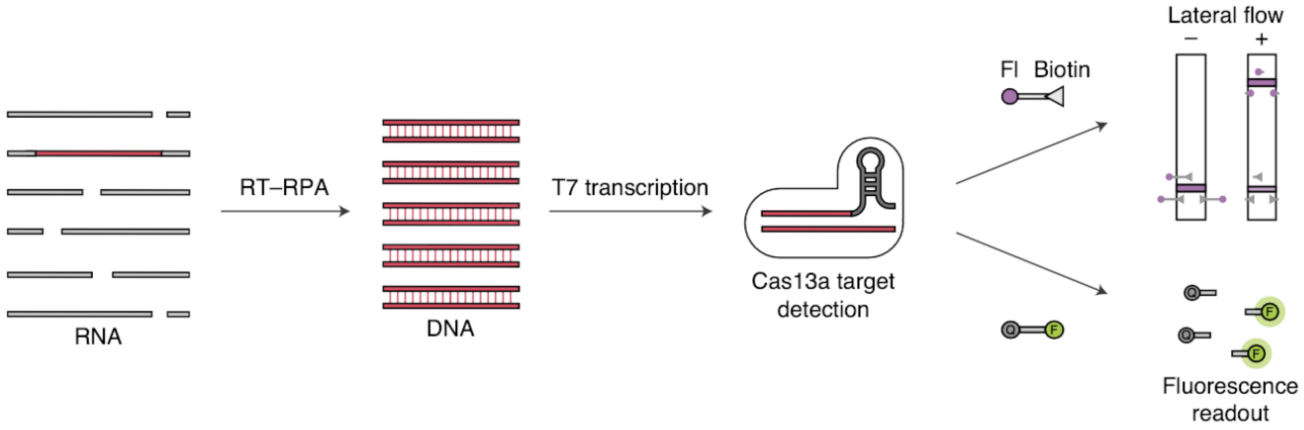


Fig. 1 | SHERLOCK detection of SARS-CoV-2 RNA. A SARS-CoV-2 RNA region of interest is isothermally amplified to DNA by RT–RPA, then converted to RNA by T7 transcription. Cognate binding of Cas13a–crRNA complex to amplified RNA targets triggers collateral activity of Cas13a, which cleaves RNA reporters. Cleaved RNA reporters can be captured on a colorimetric lateral-flow strip (biotin–fluorescein RNA reporter, top path) or visualized by fluorescence signal (molecular beacon fluorescent reporter, bottom path). Fl, fluorescein.

Table 1 | Concordance between RT–qPCR and SHERLOCK detection of SARS-CoV-2 RNA

		SARS-CoV-2 diagnosis by RT–qPCR					
		Clinical validation samples (154 total)				Samples collected for pre-operative assessment (380 total)	
		All samples		Samples within SHERLOCK LoD			
		Positive	Negative	Positive	Negative	Positive	Negative
SHERLOCK lateral-flow readout	Positive	71 (true positive)	0 (false positive)	68 (true positive)	0 (false positive)	N/A	N/A
	Negative	10 (false negative)	73 (true negative)	2 (false negative)	73 (true negative)	N/A	N/A
	Total	81	73	70	73	N/A	N/A
SHERLOCK fluorescence readout	Positive	78 (true positive)	0 (false positive)	70 (true positive)	0 (false positive)	0 (true positive)	0 (false positive)
	Negative	3 (false negative)	73 (true negative)	0 (false negative)	73 (true negative)	0 (false negative)	380 (true negative)
	Total	81	73	70	73	0	380

In total, 154 clinical samples (81 positive and 73 negative) were analysed for both lateral-flow and fluorescence readouts for clinical validation of SHERLOCK. An additional 380 clinical samples collected as part of pre-operative assessment of surgical patients at Siriraj Hospital, Bangkok during May 2020 were further analysed using the fluorescence readout of SHERLOCK. The LoD of SARS-CoV-2 *S* gene by SHERLOCK was at $C_t < 33.5$ (Fig. 2). N/A, not applicable.

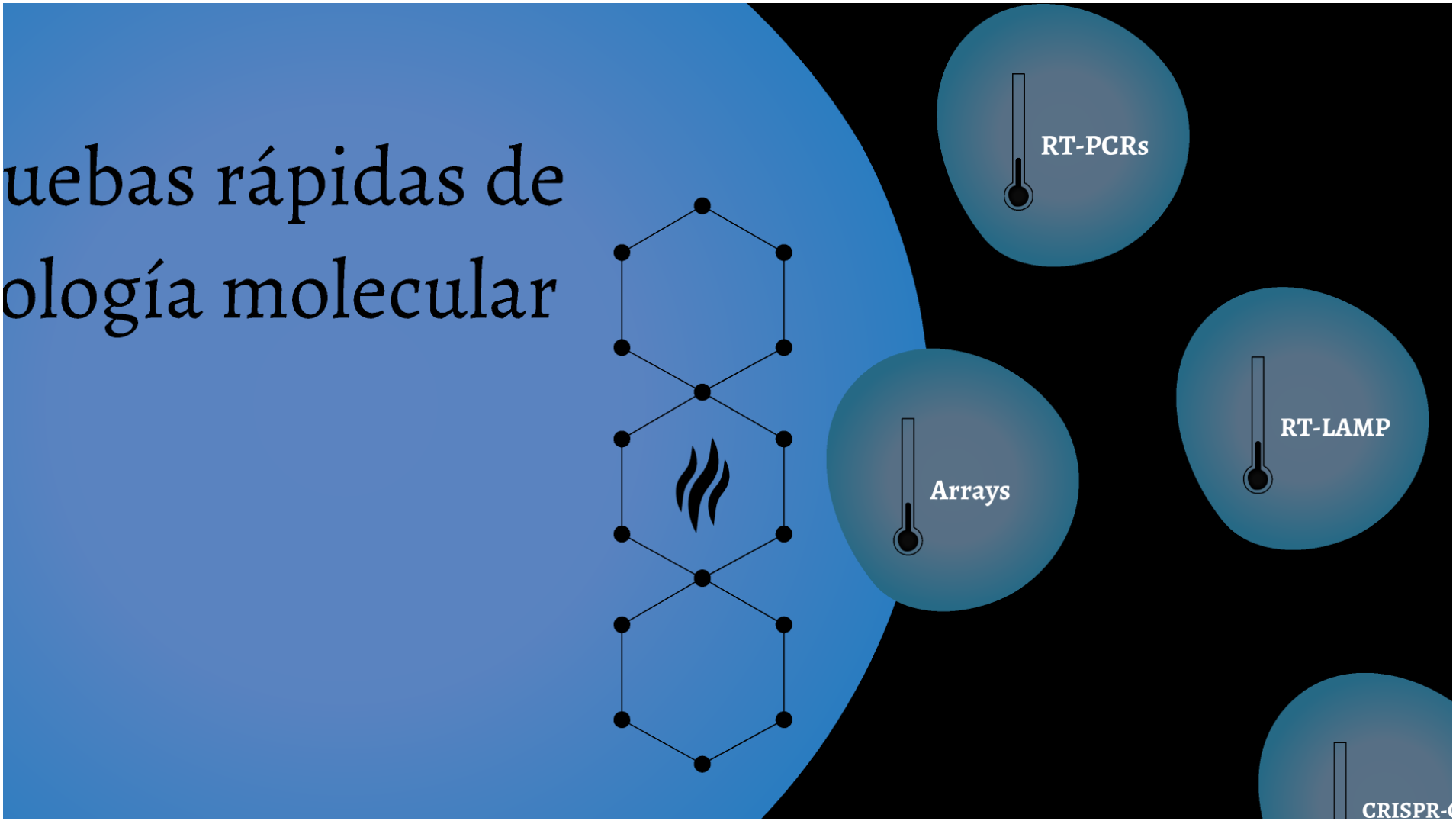
es collected for pre-operative ment (380 total)	
e	**Negative**
	N/A
	N/A
	N/A
positive)	0 (false positive)
e negative)	380 (true negative)
	380

dditional 380 clinical samples collected as .OCK. The LoD of SARS-CoV-2 *S* gene by

Table 2 | Sensitivity, specificity, predictive agreement and DOR characterizations of SHERLOCK detection of SARS-CoV-2 RNA compared with RT–qPCR

		All samples	Samples within SHERLOCK LoD
SHERLOCK lateral-flow readout	**Sensitivity**	87.65%	97.14%
	(95% CI)	(78.47–93.92)	(90.06–99.65)
	Specificity	100.00%	100.00%
	(95% CI)	(95.07–100.00)	(95.07–100.00)
	PPA	100.00%	100.00%
	(95% CI)	(94.94–100.00)	(94.72–100.00)
	NPA	87.95%	97.33%
	(95% CI)	(78.96–94.04)	(90.70–99.68)
	ln(DOR)	6.91	8.30
	(95% CI)	(4.05–9.76)	(5.25–11.40)
SHERLOCK fluorescence readout	**Sensitivity**	96.30%	100.00%
	(95% CI)	(89.56–99.23)	(94.87–100.00)
	Specificity	100.00%	100.00%
	(95% CI)	(95.07–100.00)	(95.07–100.00)
	PPA	100.00%	100.00%
	(95% CI)	(95.38–100.00)	(94.87–100.00)
	NPA	96.05%	100.00%
	(95% CI)	(88.89–99.18)	(95.07–100.00)
	ln(DOR)	8.10	9.94
	(95% CI)	(5.12–11.08)	(6.01–13.87)

Values calculated from 154 samples used for clinical validation. CI, confidence interval; PPA, positive predictive agreement; NPA, negative predictive agreement; ln(DOR), the natural logarithm of diagnostic odds ratio.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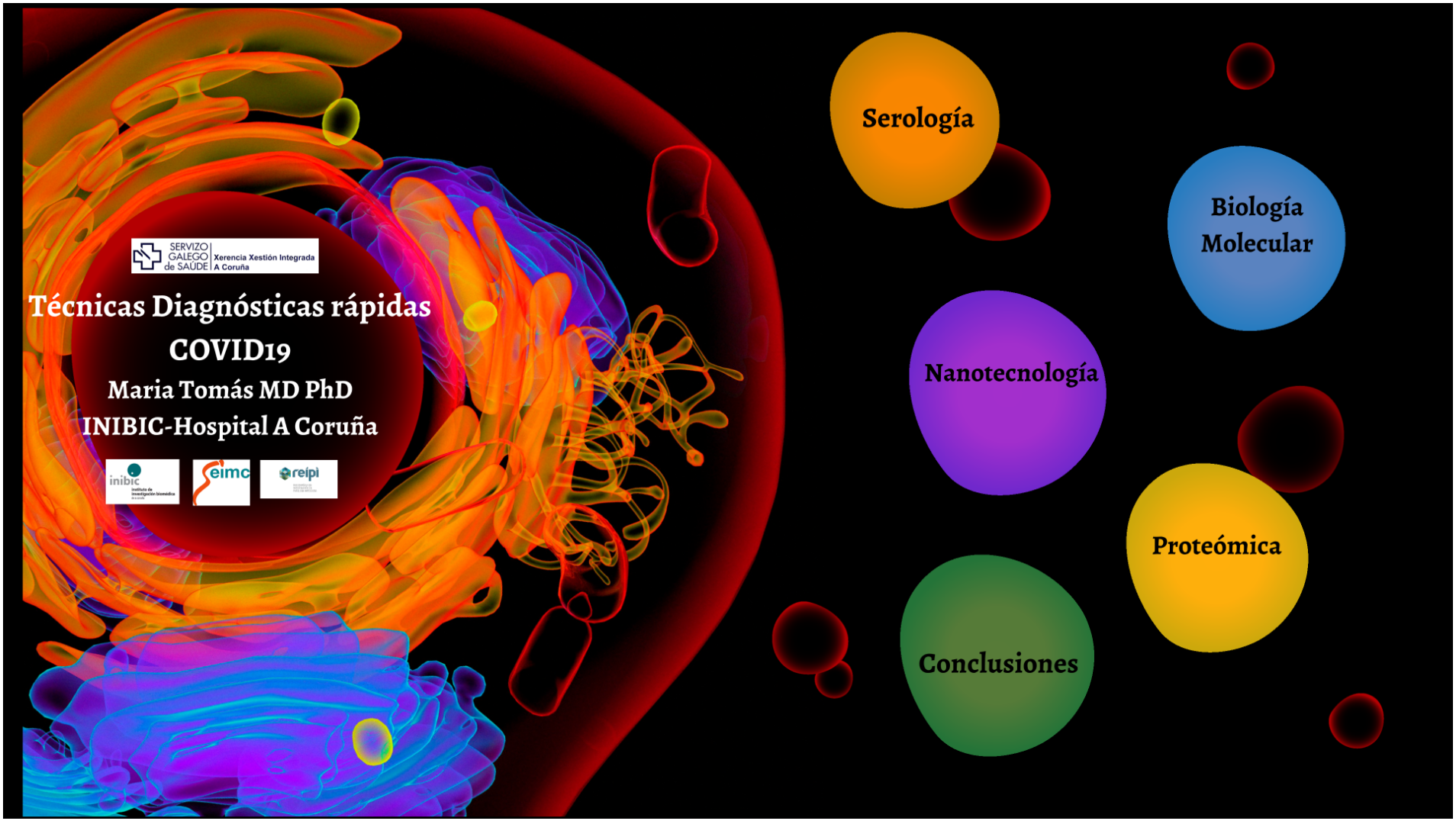
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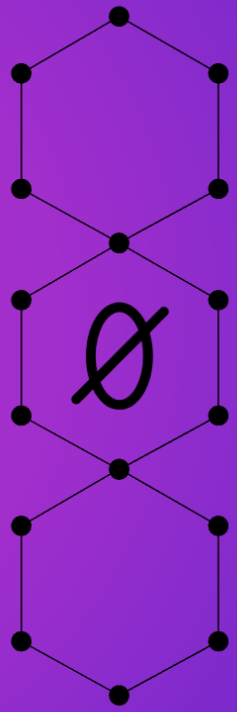

Anticuerpos

mRT-LAMP-LFB

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SARS-CoV-2 IGM and IGG rapid serologic test for the diagnosis of COVID-19 in the emergency department

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Emergency Department, Hôpital Pitié-Salpêtrière, 7-83 Boulevard de l'Hôpital, 75013 Paris, France

Table 2
Comparison of SARS-CoV-2 RT-PCR and LFI's results .

		RT-PCR		Rapid IgM/IgG	
		Positive	Negative	Sensitivity (95% CI)	75% (69.5–80.5)
LFI IgM/IgG	Positive	15	27	Specificity (95% CI)	80.3% (75.2–85.4)
	Negative	5	110	Positive predictive value (95% CI)	35.7% (29.6–41.8)
	Total	20	137	Negative predictive value (95% CI)	95.7% (93.1–98.3)

Table 2 Sample serum classification table using three-antigen Boolean classifier

	Serum (+)	Serum (−)	Total	Serum (+) percentage
RNA(−)	7	16	23	30% (95% CI 11–48)
RNA(+) all intervals	76	20	96	79% (95% CI 71–97)
Total	83	36	119	
Interval samples				
RNA(+) < 10 days	9	16	25	36% (95% CI 17–54)
RNA(+) 10–20 days	53	16	69	77% (95% CI 67–87)
RNA(+) > 21 days	21	2	23	95% (95% CI 86–100)
Total	83	34	117[a]	

[a] Time interval not available for two patients.

ROYAL SOCIETY OF CHEMISTRY

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Real-world evaluation of a novel technology for quantitative simultaneous antibody detection against multiple SARS-CoV-2 antigens in a cohort of patients presenting with COVID-19 syndrome†

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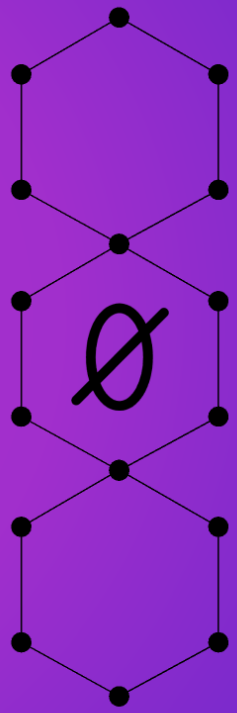

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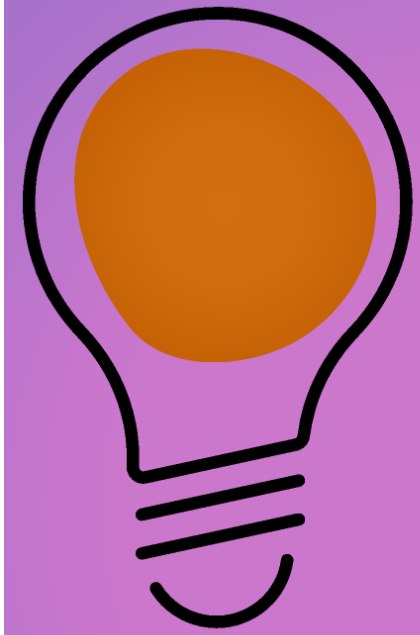

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Multiplex reverse transcription loop-mediated isothermal amplification combined with nanoparticle-based lateral flow biosensor for the diagnosis of COVID-19

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[d] Key Laboratory of Major Diseases in Children, Ministry of Education, Beijing Key Laboratory of Pediatric Respiratory Infection Disease, National Clinical Research Center for Respiratory Diseases, Beijing Children's Hospital, Capital Medical University, National Center for Children's Health, Beijing, 10045, PR China

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Keywords:
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COVID-19
Multiplex reverse transcription loop-mediated isothermal amplification
Lateral flow biosensor
Rapid diagnosis

ABSTRACT

The ongoing global pandemic (COVID-19), caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has become a huge public health issue. Hence, we devised a multiplex reverse transcription loop-mediated isothermal amplification (mRT-LAMP) coupled with a nanoparticle-based lateral flow biosensor (LFB) assay (mRT-LAMP-LFB) for diagnosing COVID-19. Using two LAMP primer sets, the ORF1ab (opening reading frame 1a/b) and N (nucleoprotein) genes of SARS-CoV-2 were simultaneously amplified in a single-tube reaction, and detected with the diagnosis results easily interpreted by LFB. In presence of FITC (fluorescein)-/digoxin- and biotin-labeled primers, mRT-LAMP produced numerous $FITC_n$/digoxin- and biotin-attached duplex amplicons, which were determined by LFB through immunoreactions ($FITC_n$/digoxin on the duplex and anti-FITC/digoxin on the test line of LFB) and biotin/treptavidin interaction (biotin on the duplex and strptavidin on the polymerase nanoparticle). The accumulation of nanoparticles leaded a characteristic crimson band, enabling multiplex analysis of ORF1ab and N gene without instrumentation. The limit of detection (LoD) of COVID-19 mRT-LAMP-LFB was 12 copies (for each detection target) per reaction, and no cross-reactivity was generated from non-SARS-CoV-2 templates. The analytical sensitivity of SARS-CoV-2 was 100% (33/33 oropharynx swab samples collected from COVID-19 patients), and the assay's specificity was also 100% (96/96 oropharynx swab samples collected from non-COVID-19 patients). The total diagnostic test can be completed within 1 h from sample collection to result interpretation. In sum, the COVID-19 mRT-LAMP-LFB assay is a promising tool for diagnosing SARS-CoV-2 infections in frontline public health field and clinical laboratories, especially from resource-poor regions.

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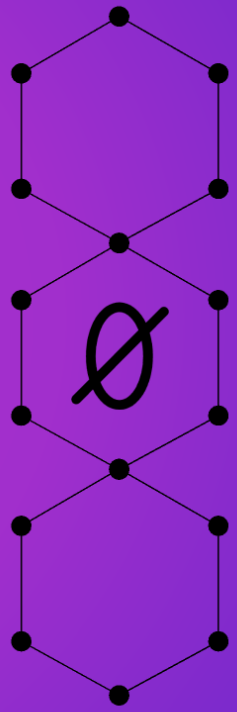
Rapid diagnosis

ABSTRACT

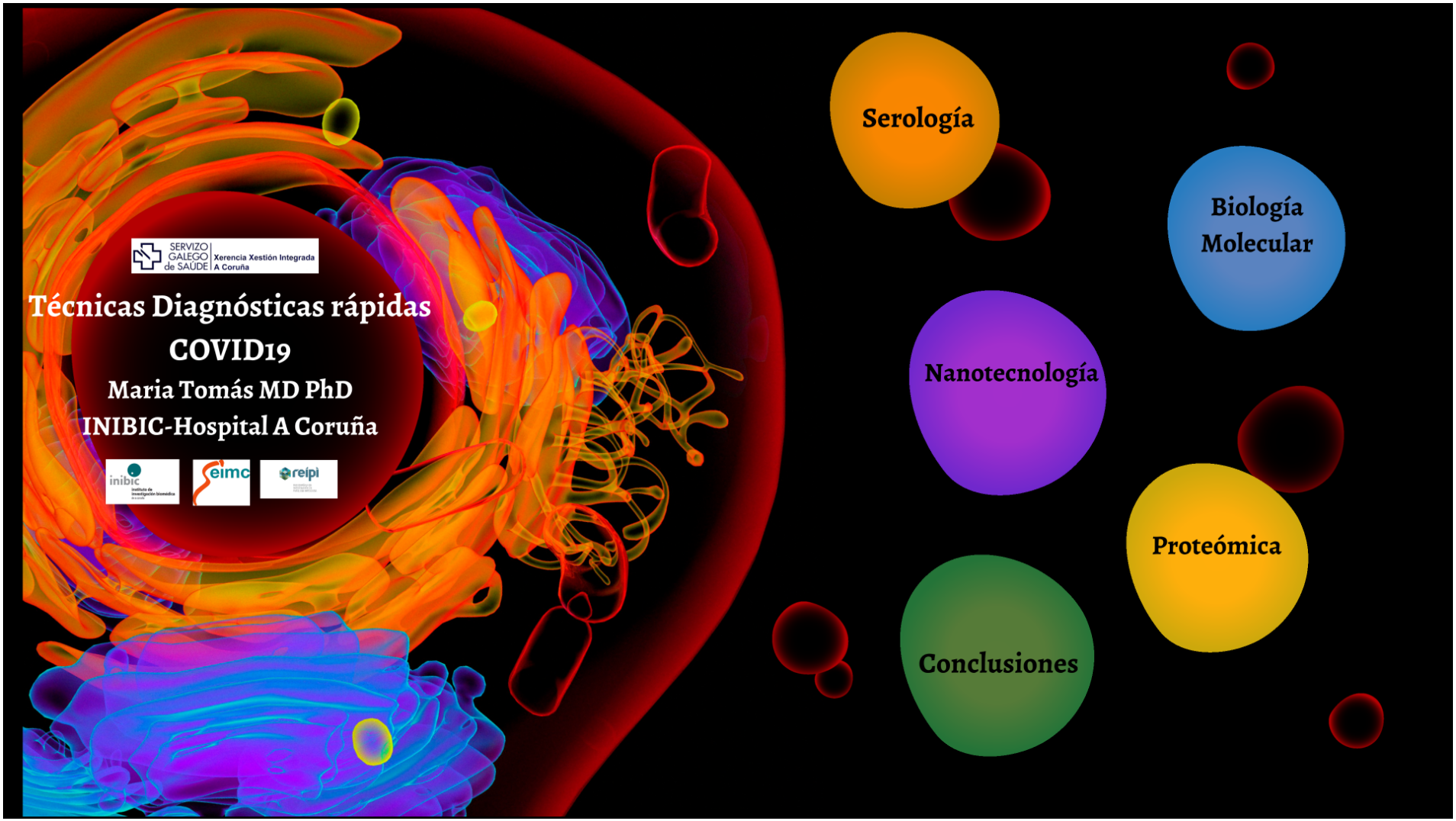
The ongoing global pandemic (COVID-19), caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has become a huge public health issue. Hence, we devised a multiplex reverse transcription loop-mediated isothermal amplification (mRT-LAMP) coupled with a nanoparticle-based lateral flow biosensor (LFB) assay (mRT-LAMP-LFB) for diagnosing COVID-19. Using two LAMP primer sets, the ORF1ab (opening reading frame 1a/b) and N (nucleoprotein) genes of SARS-CoV-2 were simultaneously amplified in a single-tube reaction, and detected with the diagnosis results easily interpreted by LFB. In presence of FITC (fluorescein)-/digoxin- and biotin-labeled primers, mRT-LAMP produced numerous FITC-/digoxin- and biotin-attached duplex amplicons, which were determined by LFB through immunoreactions (FITC/digoxin on the duplex and anti-FITC/digoxin on the test line of LFB) and biotin/treptavidin interaction (biotin on the duplex and strptavidin on the polymerase nanoparticle). The accumulation of nanoparticles leaded a characteristic crimson band, enabling multiplex analysis of ORF1ab and N gene without instrumentation. The limit of detection (LoD) of COVID-19 mRT-LAMP-LFB was 12 copies (for each detection target) per reaction, and no cross-reactivity was generated from non-SARS-CoV-2 templates. The analytical sensitivity of SARS-CoV-2 was 100% (33/33 oropharynx swab samples collected from COVID-19 patients), and the assay's specificity was also 100% (96/96 oropharynx swab samples collected from non-COVID-19 patients). The total diagnostic test can be completed within 1 h from sample collection to result interpretation. In sum, the COVID-19 mRT-LAMP-LFB assay is a promising tool for diagnosing SARS-CoV-2 infections in frontline public health field and clinical laboratories, especially from resource-poor regions.

Nanotecnología



Anticuerpos

mRT-LAMP-LFB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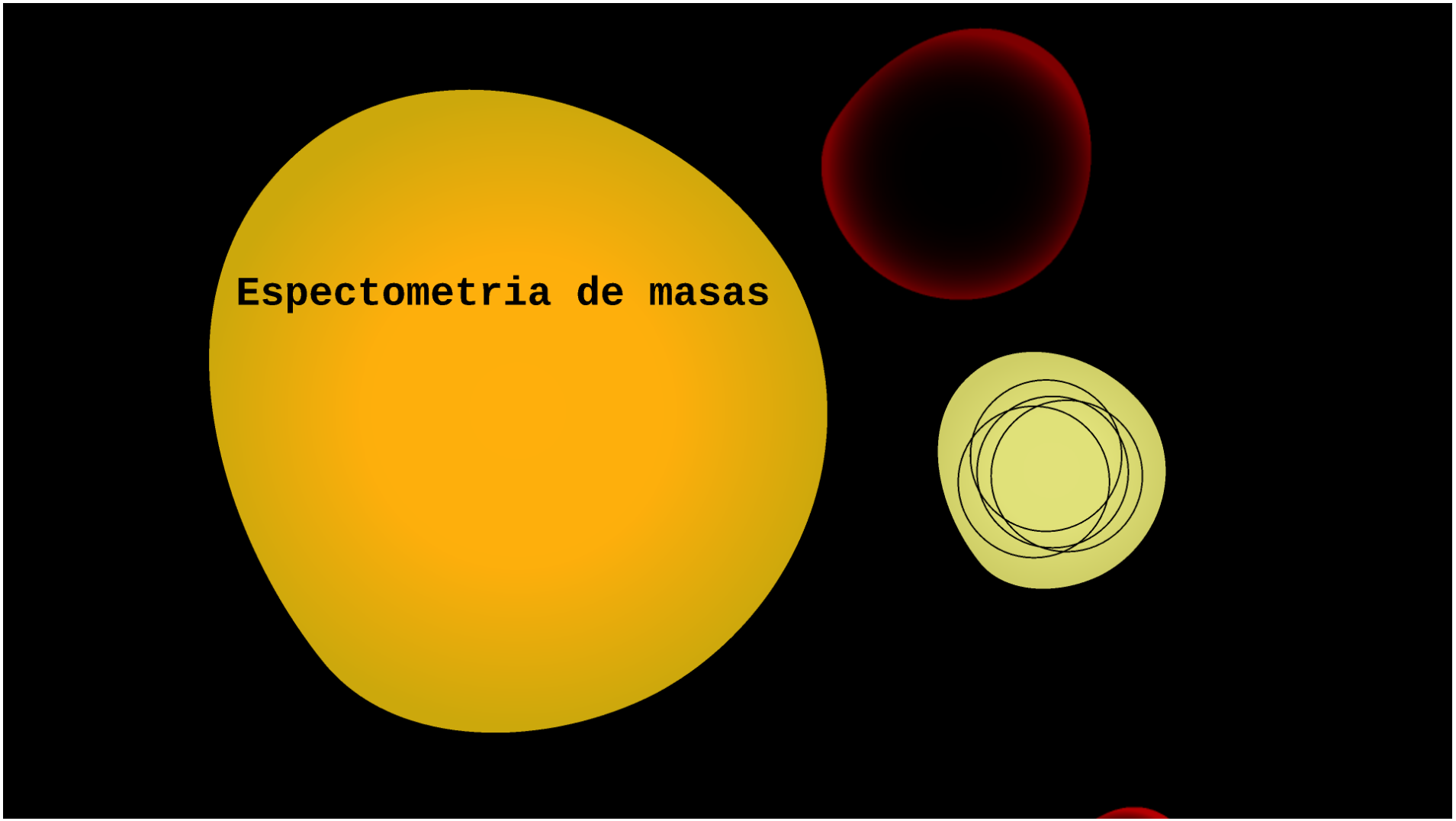
Espectometria de masas

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Proteotyping SARS-CoV-2 Virus from Nasopharyngeal Swabs: A Proof-of-Concept Focused on a 3 Min Mass Spectrometry Window

Duarte Gouveia,[¶] Guylaine Miotello,[¶] Fabrice Gallais, Jean-Charles Gaillard, Stéphanie Debroas, Laurent Bellanger, Jean-Philippe Lavigne, Albert Sotto, Lucia Grenga, Olivier Pible, and Jean Armengaud[*]

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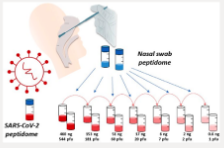
ABSTRACT: Rapid but yet sensitive, specific, and high-throughput detection of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in clinical samples is key to diagnose infected people and to better control the spread of the virus. Alternative methodologies to PCR and immunodiagnostics that would not require specific reagents are worthy to investigate not only for fighting the COVID-19 pandemic but also to detect other emergent pathogenic threats. Here, we propose the use of tandem mass spectrometry to detect SARS-CoV-2 marker peptides in nasopharyngeal swabs. We documented that the signal from the microbiota present in such samples is low and can be overlooked when interpreting shotgun proteomic data acquired on a restricted window of the peptidome landscape. In this proof-of-concept study, simili nasopharyngeal swabs spiked with different quantities of purified SARS-CoV-2 viral material were used to develop a nanoLC−MS/MS acquisition method, which was then successfully applied on COVID-19 clinical samples. We argue that peptides ADETQALPQR and GFYAQGSR from the nucleocapsid protein are of utmost interest as their signal is intense and their elution can be obtained within a 3 min window in the tested conditions. These results pave the way for the development of time-efficient viral diagnostic tests based on mass spectrometry.



KEYWORDS: *COVID-19, SARS-CoV-2, proteomics, peptides, mass spectrometry, viral protein detection; nasopharyngeal swab*

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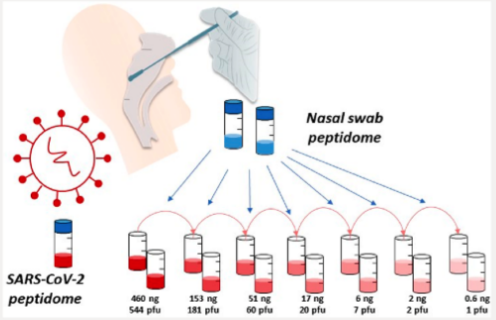

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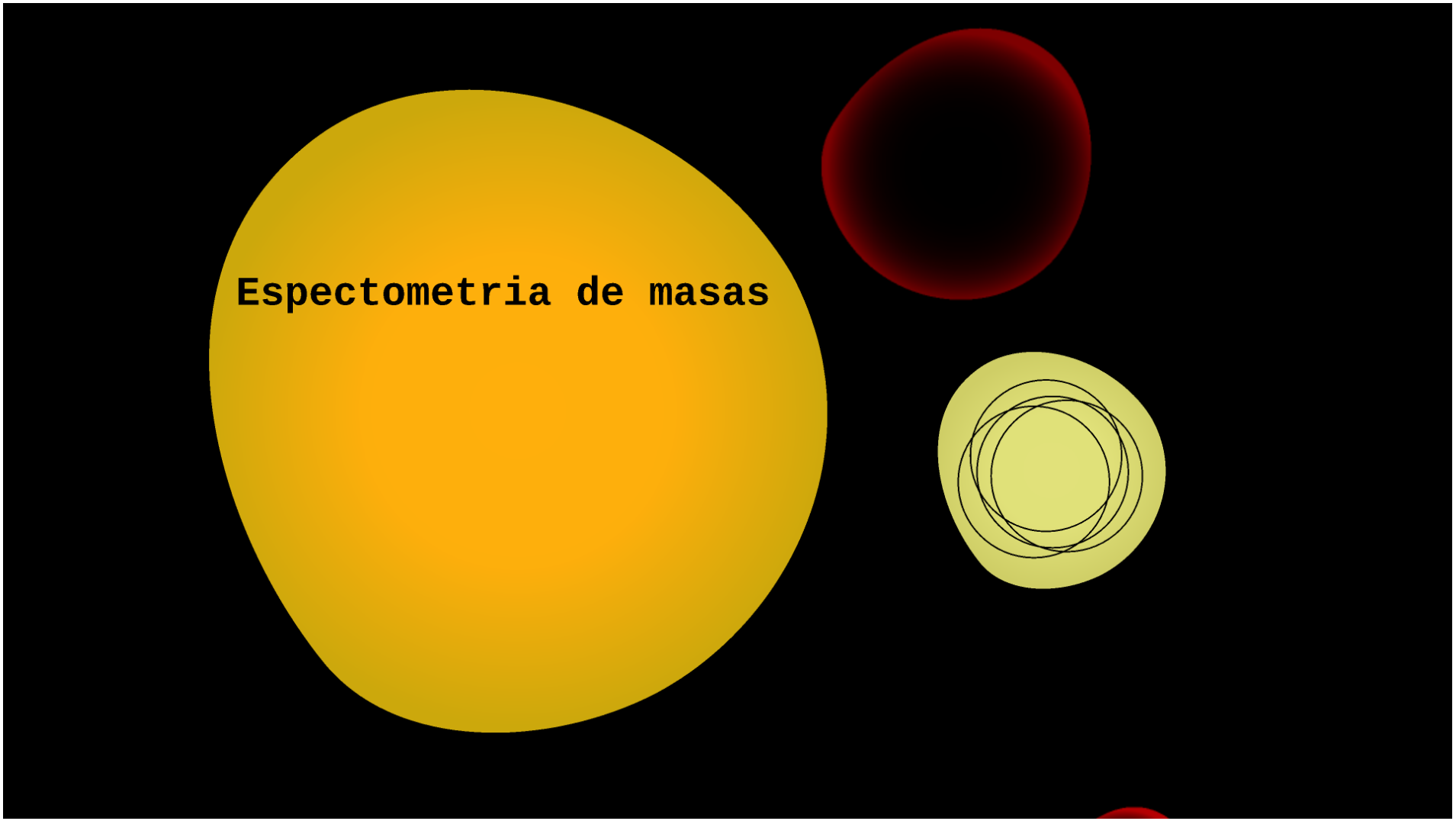
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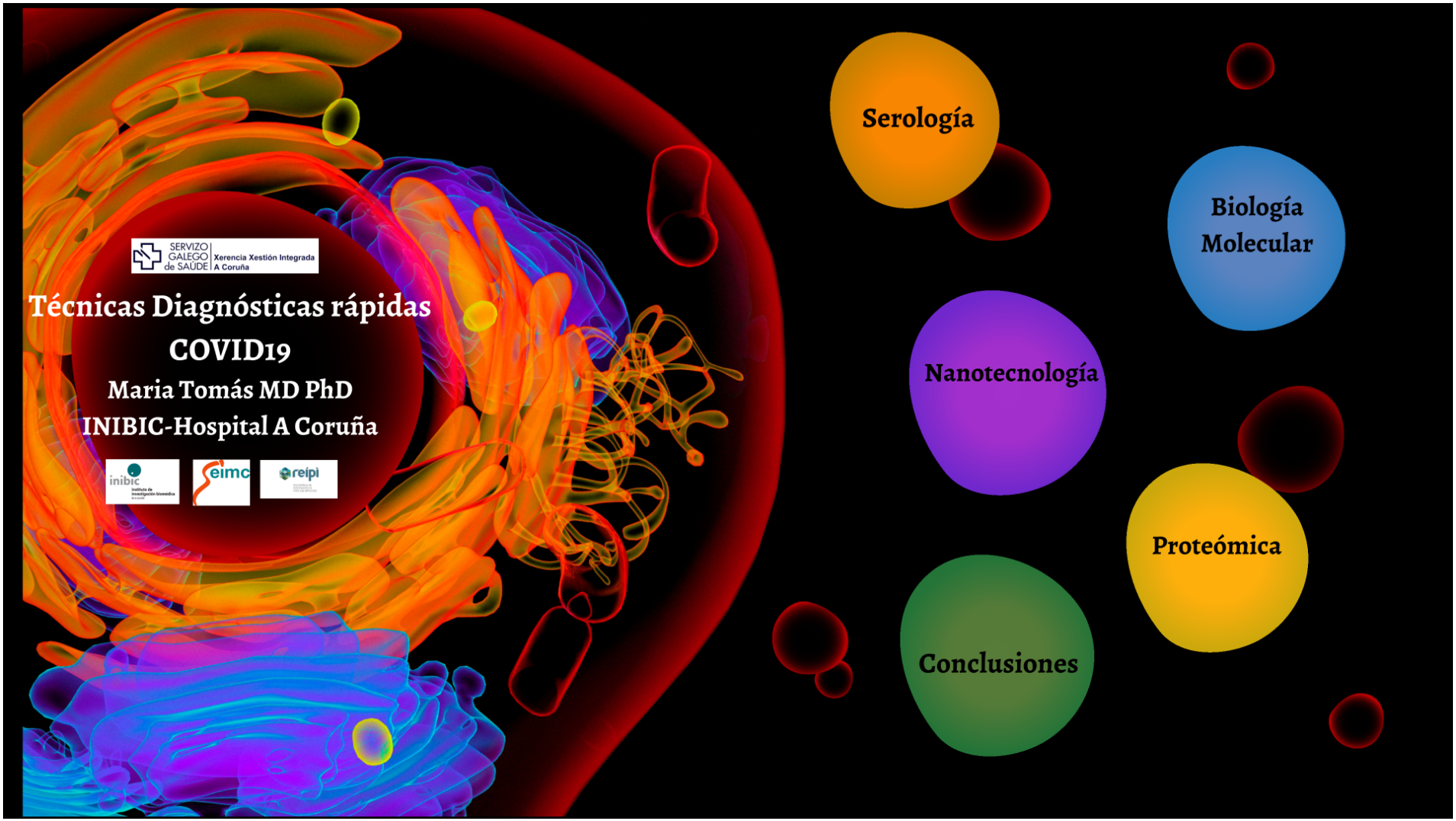
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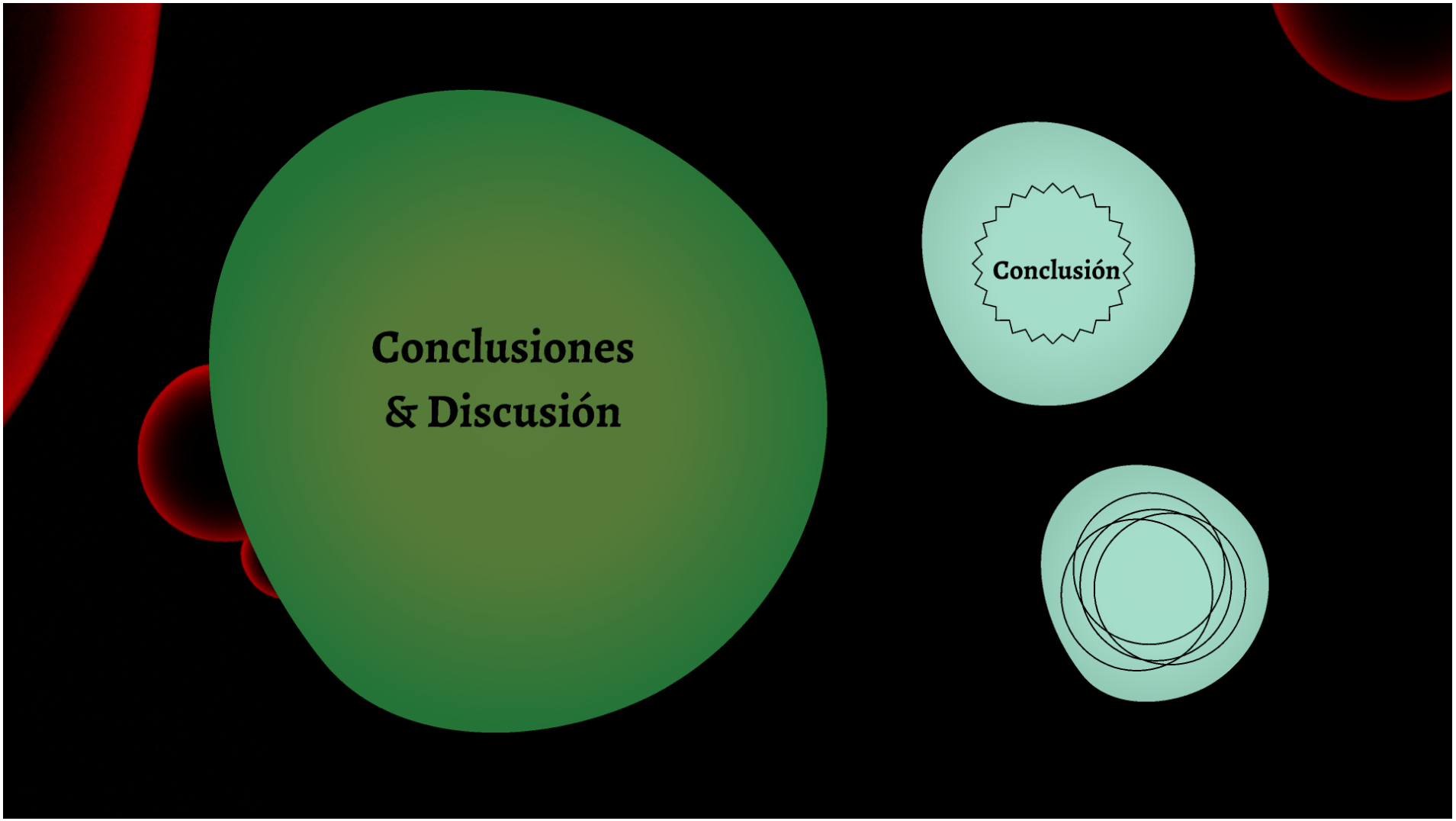
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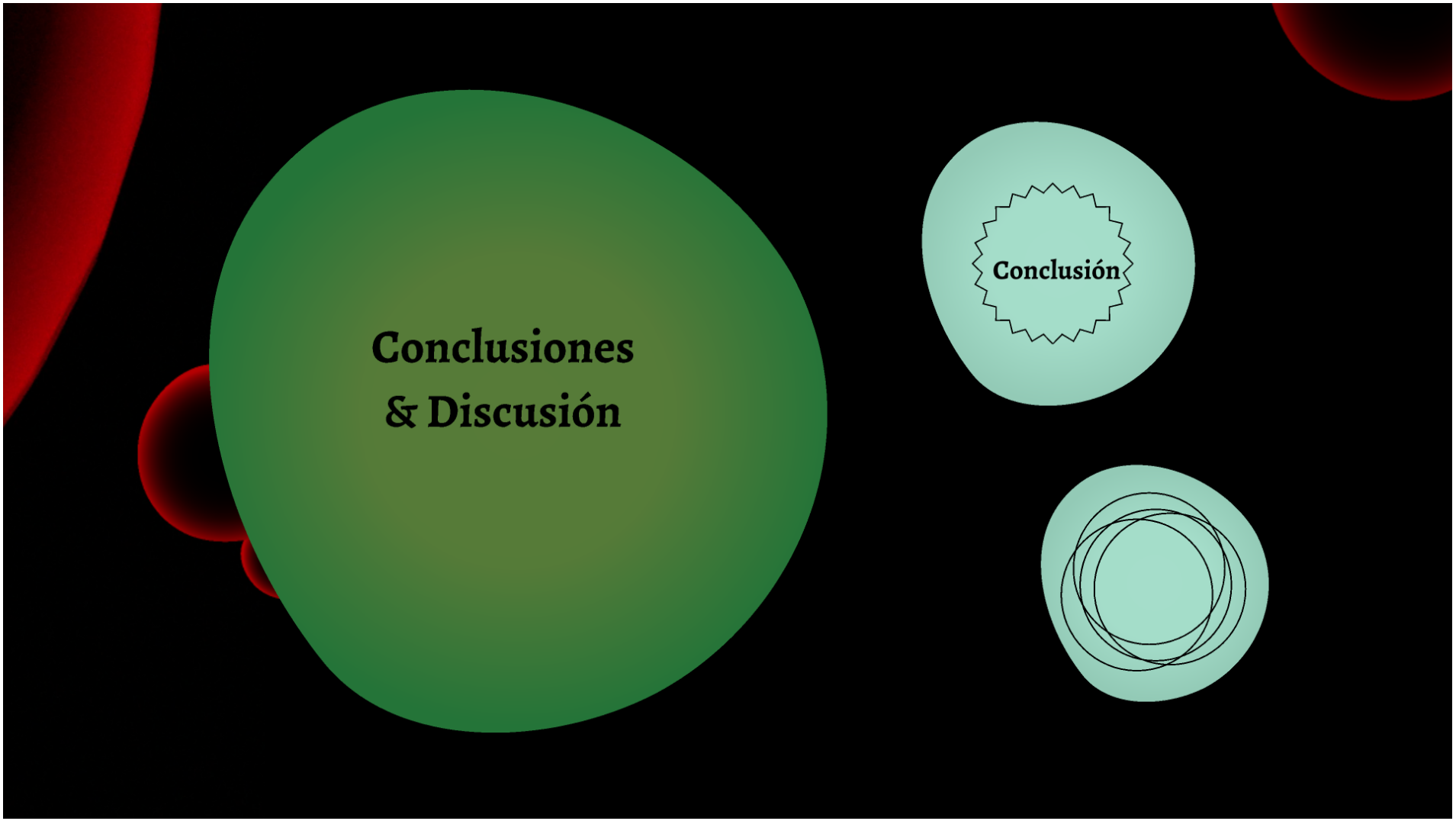
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Takk
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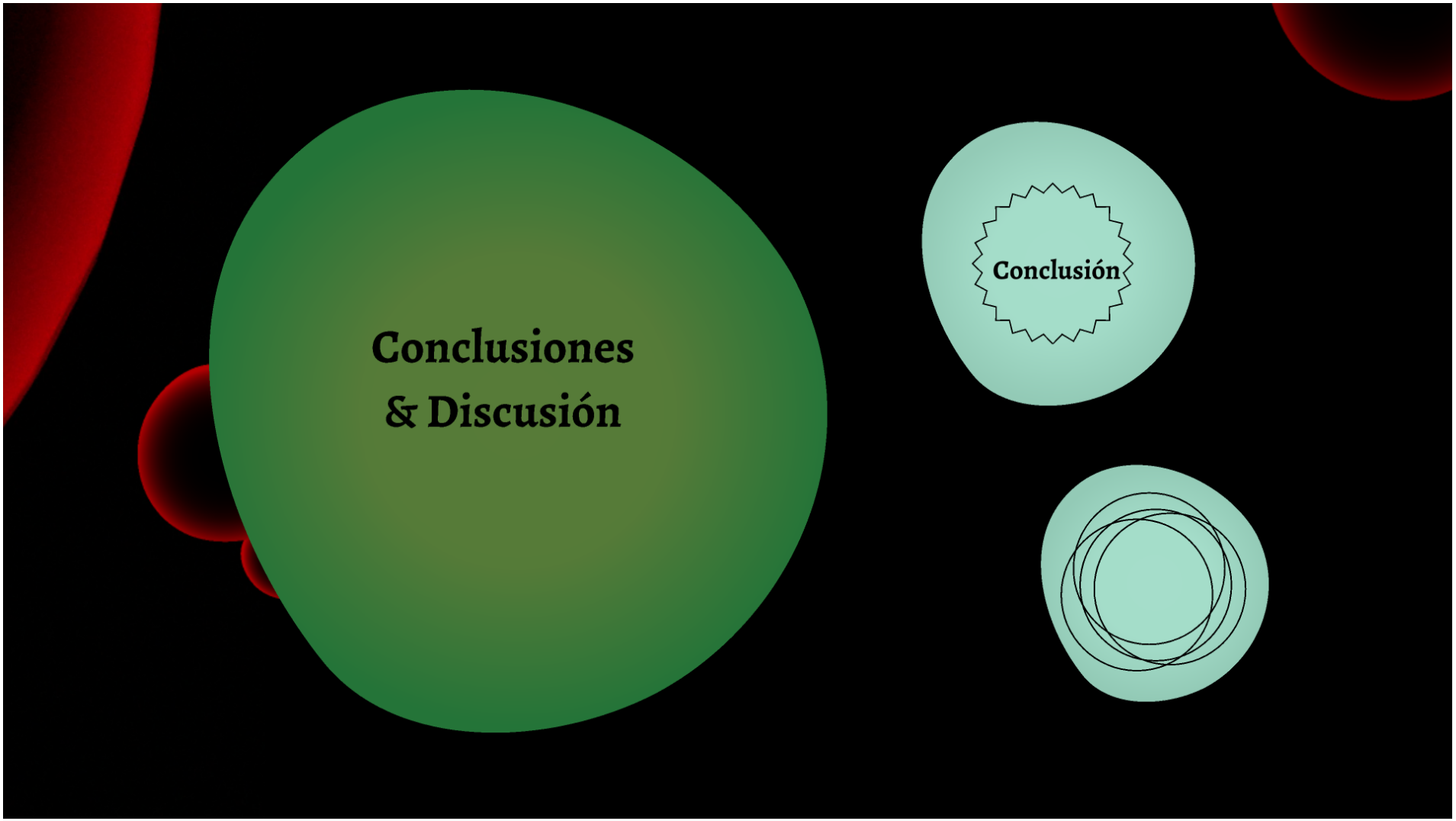
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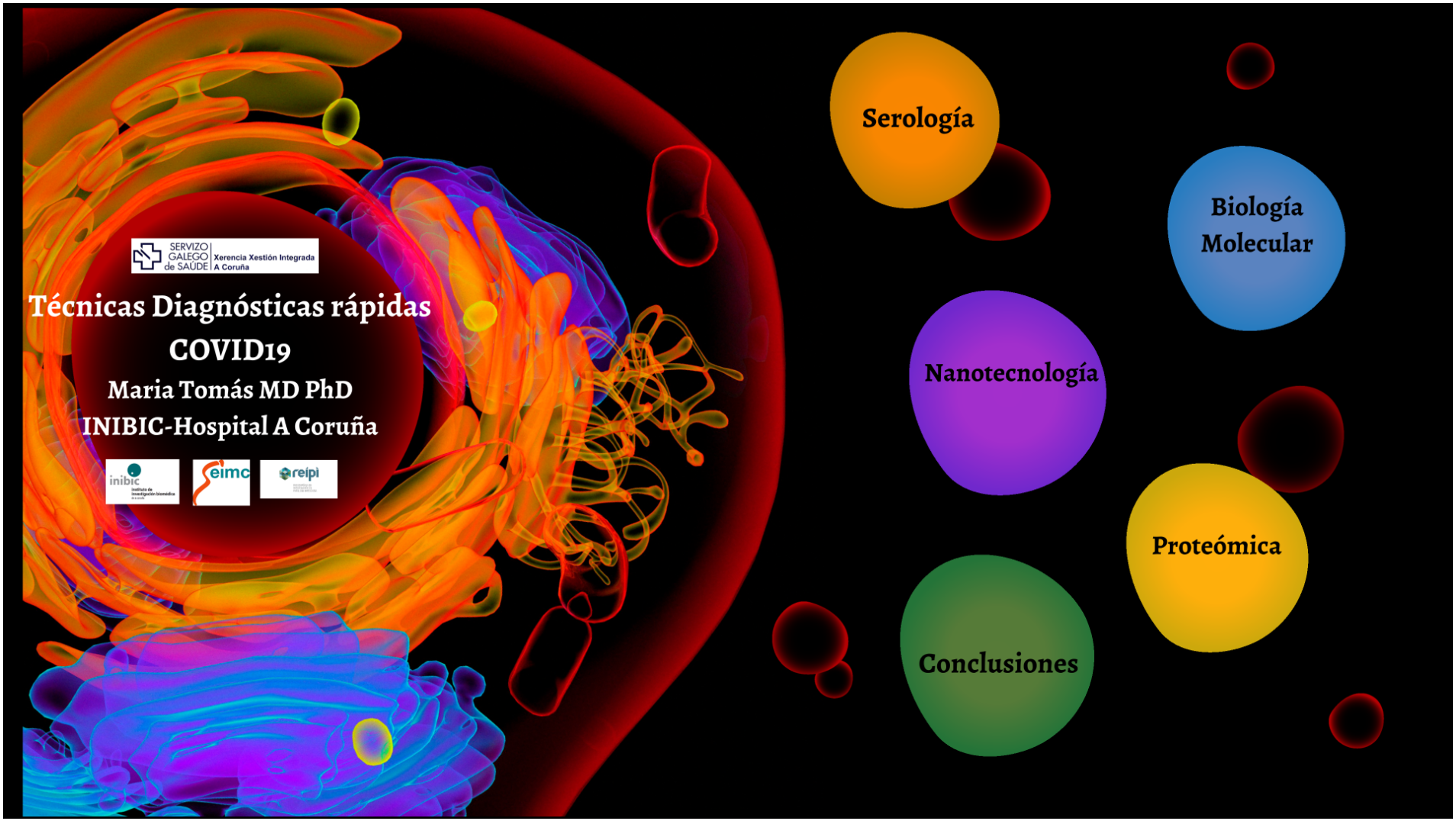
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