Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
Disposable and low-cost pen-like sensor incorporating nucleic-acid amplification based lateral-flow assay for at-home tests of communicable pathogens

Xiao Lu, Haosi Lin, Xianzhen Feng, Grace CY. Lui, I-Ming Hsing

A R T I C L E   I N F O

Keywords:
At-home test
SARS-CoV-2
Loop-mediated isothermal amplification
One-pot loop probe-mediated isothermal amplification reaction
Lateral-flow assay

A B S T R A C T

Rapid at-home test is a good alternative to the gold standard quantitative polymerase chain reaction (qPCR) for early identification and management of infected individuals in pandemic. However, the currently available at-home rapid antigen kits and nucleic acid tests (NATs) are prone to false results. Although some CRISPR-mediated NATs enhanced accuracy, long turnaround time (ca. 1 h) and aerosol contamination due to additional open-lid reaction hinder their applicability for self-tests. Moreover, the accuracy of at-home NATs is also impacted by interference of sample matrix due to lack of sample purification. Here we report a Fast, Low-cost, Aerosol contamination-free and Sensitive molecular assay for at-Home tests of communicable pathogens (FLASH). The integrated platform enabled sample-to-result SARS-CoV-2 RNA detection in 20-30 min achieving a sensitivity of 0.5 copies/μL in a blinded experiment with a high accuracy comparable with the qPCR. Its prototype consists of two disposable pen-like instruments for single-step sample preparation and contamination-free NATs, respectively. The simplified workflow of the FLASH enabled detection to be readily conducted by untrained users for at-home tests. All in all, the FLASH prototype demonstrates itself to be a promising home-use assay platform for effective mitigation of the pandemic.

1. Introduction

The spread of the latest variant of SARS-CoV-2 with increased transmissibility has raised the demand for higher detection capacity and efficiency of pathogen tests (Poudel et al., 2022) for pandemic mitigation. Apart from the gold standard reverse-transcription quantitative polymerase chain reaction (RT-qPCR), (Mackay et al., 2002; Peto et al., 2020), rapid antigen tests (RAT) and rapid nucleic acid tests (NATs) are efficient alternatives for fast identification and management of the infected individuals (Blairon et al., 2020; Dinnes et al., 2021; Peto 2021; Peto et al., 2020). However, RAT kits suffer from lower sensitivity due to the lack of signal amplification (Nagura-Ikeda et al., 2020; Uribe-Alvarez et al., 2021). On the other hand, rapid NATs techniques such as loop-mediated isothermal amplification (LAMP) display higher sensitivity, nevertheless vulnerable for non-specific nucleic acid amplification in clinical utilities (Iacobucci 2020; Joung et al., 2020; Regalado 2021; Uribe-Alvarez et al., 2021). Although CRISPR-associated lateral-flow assays (LFA) have been developed recently for NATs with enhanced specificity (Broughton et al., 2020; Joung et al., 2020), the open-lid operation for LFA readouts worsened the aerosol-contamination problem as well as prolonged the turnaround time (ca. 1 h). Additionally, these approaches are limited by the interfering substances in the sample matrix due to the lack of the nucleic acid purification and enrichment (Dinnes et al., 2021).

Previously, a one-pot loop probe-mediated isothermal amplification reaction (oLAMP) was developed by our group (Lu et al., 2022). Unlike other multi-step NATs, we introduced a fluorophore-quencher-labeled loop probe which acts as a target-specific primer and as a reporter that is cleaved by a nicking endonuclease in the presence of the target. Thus, oLAMP enabled one-pot and target-specific concurrent signal generation by endonuclease cleavage during reaction, eliminating the non-specific response in conventional LAMP. Notably, oLAMP displayed high amplification efficiency due to shorter amplicon length, enabling faster response and shorter turnaround time. However, oLAMP still

* Corresponding author.
E-mail address: kehsing@ust.hk (I.-M. Hsing).

https://doi.org/10.1016/j.biosx.2022.100248
Received 27 April 2022; Received in revised form 12 August 2022; Accepted 27 August 2022
Available online 5 September 2022
2590-1370/© 2022 The Author(s). Published by Elsevier B.V. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).
relied on laboratory equipment for its fluorescence readout and was not suitable for at-home tests. In this study, we developed an integrated platform termed FLASH (Fast, Low-cost, Aerosol-contamination free, and Sensitive molecular assay for at-Home tests of communicable pathogens) (Fig. 1). It maintains the high specificity and rapid signal response of oLAMP while utilizing the visible LFA readout for at-home NATs. In the FLASH system, the closed-tube design of the Detection-Pen for oLAMP-based LFA eliminates the risk of aerosol contamination. In addition, we designed a syringe-based SampleDirect-Pen for room-temperature nucleic acid purification and enrichment. Hence, the interference of the sample matrix was removed, and a single-copy sensitivity was achieved. Integrating these two pens, nucleic acid purification, pre-concentration, amplification, and visual detection can be carried out on the FLASH platform with very limited user intervention. After 20–30 min of assay time including 15–25 min of incubation on a low-cost heating block, the result can be visually observed. The user operation is comparable with self-test RAT kits while achieving high accuracy comparable with the gold standard RT-qPCR.

2. Materials and methods

2.1. Development of oLAMP-based lateral-flow assay (oLAMP-LFA) for SARS-CoV-2 viral RNA detection

The primer and probe design, loop probe cleavage assay and oLAMP reaction were all conducted according to our previous work (Lu et al., 2022). The primers and probes used in this study listed in Table S1 were purchased from Generay. The SARS-CoV-2 viral RNA were obtained from EVAg (https://www.european-virus-archive.com/) (Table S2). Protease K, Nb.BssSI nicking endonuclease, Nuclease P1, deoxy-nucleotide mix (dNTPs mix), NEBuffer 3.1 were purchased from New England Biolabs. First, 5 μL RNA sample was added into 15 μL oLAMP reaction mix containing 4 U Bst 2.0 Polymerase, 10 U Nb.BssSI, 1 × NEBuffer 3.1, 1.4 mM dNTPs mix, 0.2 μM forward primer (FP), 0.2 μM backward primer (BP), 1.6 μM FAM-Biotin-labeled loop probe (LP) and 1.6 μM assistant probe (AP), and then the reaction was incubated at 60 °C for 25 min. After incubation, the 20 μL oLAMP reaction buffer was diluted by adding 100 μL assay buffer (TwistDx) containing 11% Polyethylene glycol (PEG) 4000 (Merck). The mixture was then tested by lateral-flow strips (TwistDx) and the results were visualized after 3 min. A single line close to the sample pad reveals a negative result, whereas two lines indicate a positive result (Fig. 1).

2.2. Design and fabrication of FLASH platform

Our FLASH platform (Fig. 1) containing SampleDirect-Pen and Detection-Pen was designed using Sharp3D for iPad. The extraction block, metal bath of the incubator and mold for Ecoflex® membrane fabrication were 3D-printed (SimpNeed 3D and Miradur Group), and all the files of 3D-printed components can be found at https://github.com/xluaq/FLASH-housing.git. Apart from the 3D-printed metal bath, the incubator also consisted of a ceramic heater, a temperature controller, and a negative temperature coefficient thermistor (NTC thermistor) for temperature sensing (Jiangsu Xinghe Electronic Technology Co., Ltd). The incubator was powered by a 5-V power supply.
To fabricate the SampleDirect-Pen, a piece of polyethersulfone (PES) membrane (0.22 μm pore size, 2-mm diameter) (Millipore) was compressed onto the tip of a 1-mL syringe (Becton Dickinson) (see Fig. 1 sample preparation) and secured by a 3D-printed cap. The sample extraction reagent (0.5 mM lipopeptide (Sigma), 50 mM KCl, 0.1 mg/mL SDS and 0.05 M Tris-HCl, and 1.5% (V/V) Tween 20, final volume 1 mL) was lyophilized in the SampleDirect-Pen directly for 24 h.

For the Detection-Pen, we integrated a 1-mL syringe with a lateral-flow strip secured onto the plunger, a cap made of sealing membrane, and a 3D-printed tube with 2 chambers. We fabricated the sealing membrane using the Ecoflex® material following the instruction of the manufacturer (Ecoflex® 00–30, Smooth-On). 0.2 mL of Ecoflex® solution was injected into the 3D-printed mold and incubated at 55 °C for solidification.

2.3. Construction and validation of SampleDirect-Pen and Detection-Pen for SARS-CoV-2 detection

Serial dilutions of SARS-CoV-2 N gene pseudo-virus (Beyotime) were spiked into artificial saliva (pH 6.8, Phygene) to prepare the simulated samples. 20 μL oLAMP reaction and 100 μL assay buffer were added into the two chambers of the 3D-printed tube respectively. 1 mL simulated sample was added into the SampleDirect-Pen which was then manually injected through the PES membrane at the bottom of the chamber. The PES membrane containing released nucleic acids was added into the oLAMP reaction directly in lieu of solution samples. The lateral-flow assay part of the syringe was sealed by Ecoflex® sealing membrane, and the 3D-printed tube was attached at the bottom of the pen. Then, the Detection-Pen was incubated in water bath at 60 °C for 25 min. The two solutions were mixed by inverting the Detection-Pen and shaking it. Once the solutions were fully mixed, the plunge was pressed thus injecting the strip into the tube. The oLAMP reaction procedure and data analysis were performed as stated earlier in section 2.1. Two commercial RNA extraction approaches were conducted following the instruction of manufacturers (Qiagen and SalivaDirect™) in the control group for comparison.

2.4. Validation of integrated FLASH platform for SARS-CoV-2 RNA detection

SARS-CoV-2 simulated samples were prepared by adding serial diluted SARS-CoV-2 inactive virus (ATCC VR-01986) into artificial saliva containing 20 μg/mL of human total RNA (Thermofisher) and 50 ng/mL of human genomic DNA (Sigma). Sample preparation and detection was conducted as stated in section 2.3 using SampleDirect-Pen and Detection-Pen, except that the Detection-Pen was incubated on the reaction block (Fig. 1) instead of water bath. In the blinded experiment, the SARS-CoV-2 detection using FLASH was verified by RT-qPCR (TAKARA Bio) with Qiagen viral RNA extraction kits according to the instructions of the manufacturer on QuantStudio™ 5 (Thermofisher).

2.5. Data analysis

The test line intensities of lateral-flow strips were obtained by ImageJ according to the previous research (Parolo et al., 2020) (see supplementary data 1.2 for the protocol). The comparison of endpoint test line intensities was performed using one-way ANOVA with Dunnett’s post-test. Error bars in all plots represent one relative standard deviation (n = 3). The threshold distinguishing the negatives and the positives in Fig. 4D was determined as m+3σ, where m and σ are the mean and standard deviation of the test line intensities of 60 no-template controls in this study, respectively, and a customized mini-program running on the WeChat application (see Movie S1, constructed by Chongqing Yijingtai Big Data co. Ltd) was also employed for result interpretation in Fig. 4D. All the statistical analysis and plottings were conducted using GraphPad 8. All illustrations were drawn using BioRender.

Supplementary data related to this article can be found at https://doi.org/10.1016/j.biosx.2022.100248.

3. Results and discussion

3.1. Development of oLAMP-based lateral-flow assay (oLAMP-LFA) for SARS-CoV-2 viral RNA detection

To achieve accurate and visible nucleic acid tests (NATs), we integrated our previously published oLAMP reaction (Lu et al., 2022) with lateral-flow assays (LFA) as signal readout, allowing user-oriented and visible NAT for pathogens detection. The inner loop-probe primer in oLAMP was modified into a FAM-biotin-labeled loop-probe. The stem of loop-probe consisted of an inactive Nb.BssSI nicking endonuclease recognition site containing a mismatch at the last base to avoid undesired cleavage (Fig. 2A). During the oLAMP reaction (Fig. 2B), amplicons containing Nb.BssSI nicking endonuclease recognition site and FAM-biotin dual-label are generated from the loop-probe through the extension reaction. Then the amplicon is nicked by Nb.BssSI nicking endonuclease and the biotin label is thus released from the FAM-containing amplicon. As shown in Fig. 2B, in a positive test, the FAM-labeled long DNA products bind with Au-nanoparticles-labeled anti-FAM antibody and are subsequently captured by the anti-anti-FAM antibody immobilized at test line presenting a red band, while the excess of intact loop-probe presents a band at the control line. In a negative analysis, without the cleaved products generated by Nb.BssSI nicking enzyme, all the FAM-biotin-labeled loop-probes are captured by the streptavidin at the control line, showing a single band indicative of the negative result. Also, to avoid a non-specific band on the test line in a no template control (NTC) analysis, PEG 4000 was added to the assay buffer supplied by the HybriDetect1 later flow test kit (Tang et al., 2016) to a final concentration of 11% as per the optimization experiments shown in Fig. S1. Then we verified the feasibility of the nicking enzyme-mediated signal generation using this optimal LFA condition. The results in Fig. 2A(III) and Fig. S2 display the robustness and specificity of nicking enzyme cleavage process via LFA, where the test line only appears when the nicking endonuclease recognition site on the loop-probe is activated (reaction IV of Fig. 2A).

After above-mentioned optimization, the oLAMP-LFA was utilized for SARS-CoV-2 viral RNA detection. Using 5 μL of sample inputs and 25-min incubation time, we demonstrated that oLAMP-LFA was capable of testing SARS-CoV-2 RNA from 10^6 copies/μL to 1 copy/μL (Fig. 2C and Fig. S3A). Notably, oLAMP-LFA can identify SARS-CoV-2 RNA of mean viral load during infections (Zheng et al., 2020) within 10 min (Fig. 2D and Fig. S3B) in a lab environment, achieving sensitive performance of 1000 copies/μL recommended by WHO for Covid-19 rapid tests (WHO 2020). Moreover, oLAMP-LFA is a SARS-CoV-2 specific method with no cross-reactivity with 6 common pathogens (list in Table S2) with various degrees of homology in the clinical respiratory samples (Fig. 2E and Fig. S3C). The interfering substance test demonstrated that oLAMP-LFA is robust in the presence of 3 inhibitors with common concentrations in clinical samples, while significant inhibition was observed in viscous samples with high concentration of mucin (Fig. S4 and Table S3).

3.2. Construction and validation of SampleDirect-Pen and Detection-Pen for SARS-CoV-2 detection

To simplify the procedure of sample preparation and minimize user errors, SampleDirect-Pen was designed, utilizing a novel sample extraction reagent lyophilized directly into the 1-mL syringe and a polyethersulfone (PES) membrane for nucleic acid purification and enrichment (Fig. 3A). PES membrane can capture nucleic acids from the sample matrix via non-covalent interaction with nucleic acid bases by π-π stacking and nucleic acid backbone by hydrogen bonding (Linnes et al., 2016). This design consolidates sample inactivation, extraction,
Fig. 2. The oLAMP-based lateral-flow assay (oLAMP-LFA) for SARS-CoV-2 viral RNA detection. (A) The structure of loop-probe and the signal-generation principle of loop probe cleavage assay. The FAM-Biotin-labeled loop-probe (LP) consists of the nicking recognized region (in red) which originally contains a mismatch to prevent untimely cleavage. Test line intensities after 60 min of LP cleavage reaction in the presence of (I) LP, (II) LP and its complementary sequence (III) LP, single-base mismatched complementary sequence and Nb.BssSI nicking endonuclease, (IV) LP, complementary sequence and Nb.BssSI, (V) LP and nuclease P1. (B) Schematic of the oLAMP-LFA test. The target gene is amplified with forward primers (FP), backward primers (BP), LPs, and assistant probes. The labeled oLAMP products were cleaved by the Nb.BssSI and the cleaved oLAMP products were detected by the lateral-flow strip. (C) The endpoint test line intensity histogram of the oLAMP-LFA in the presence of various concentrations of SARS-CoV-2 RNA inputs. (D) Test line intensity under different oLAMP incubation time for the detection of 1000 copies/μL of SARS-CoV-2 RNA. NTC, no template control (**: $P < 0.001$, ***: $P < 0.0001$, ns: not significant). (E) The endpoint test line intensity plot in the presence of different pathogens depicting the specificity towards SARS-CoV-2 viral RNA against other pathogens commonly found in clinical respiratory samples. (**: $P < 0.001$, ***: $P < 0.0001$, ns: not significant). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)
purification, and enrichment into a single-step process at room temperature that can be easily conducted by untrained users. Within the SampleDirect-Pen, oLAMP-LFA for SARS-CoV-2 RNA detection reached a sensitivity of 0.5 copies/μL (Fig. 3B and Fig. S5A). Meanwhile, usage of SampleDirect-Pen obviates the inhibition of mucin on oLAMP-LFA (Fig. S4 and Fig. S5B). Mucin is a common component of various human fluidic samples (Acuña and Juárez, 2021). It inhibits the DNA polymerase activity (Al-Soud et al., 2005) concurrently impacts the viscosity significantly, decreasing the flow rate in LFA and inhibiting the movement of analytes and Au-nanoparticles on the LFA strip (Kainz et al., 2021). The sample preparation workflow using SampleDirect-Pen ensured the robustness and sensitivity of oLAMP-LFA and eliminated the impacts from interfering substances in the saliva sample matrix such as mucin. Moreover, SampleDirect-Pen provides simpler and faster sample preparation than the two FDA approved approaches used in our experiments (see Table S4 for comparison).

The Ecoflex® membrane-sealed two-chamber Detection-Pen (Fig. S5C) was designed for preventing the risk of the aerosol contamination (Borst et al., 2004) during a typical LFA-based NAT including open-lid operation. Performance of SampleDirect-Pen and Detection-Pen in detecting SARS-CoV-2 viral RNA in artificial saliva was tested against off-device oLAMP-LFA test using SampleDirect-Pen. As shown in Fig. 3C and Fig. S5D the usage of the two pen-like devices enabled a sensitivity of 0.5 copies/μL which was better than that of the lab-based oLAMP-LFA using the two FDA approved sample preparation approaches.

SampleDirect-Pen and Detection-Pen also displayed good stability upon storage. SampleDirect-Pen containing lyophilized sample extraction reagents retained stable after being stored at 37 °C for 31 days during the accelerated stability test (Fig. S6), which was equivalent to 3-month storage at room temperature (Khazani et al., 2017). The Detection-Pen containing pre-mixed oLAMP reagent maintained its functionality after 5 freeze-thaw cycles at −20 °C (Fig. S7). Thus, we anticipate that these two pen-like sensors can tolerate the long-term transportation while retaining their performance, showing good potential for application on our self-test platform.

3.3. Construction and validation of integrated FLASH platform for SARS-CoV-2 detection

We then developed the integrated FLASH platform into a hand-held, low-cost and modular prototype utilizing the two disposable pen-like devices. Such design can further streamline the oLAMP-LFA workflow for operation by non-specialists for point-of-care application under various conditions such as in schools, homes, offices, etc. FLASH was configured with two blocks: a sample extraction block containing the SampleDirect-Pen and a waste box, and a reaction block containing a 60 °C incubator powered by a 5-V power supply and the Detection-Pen.
(Fig. 4A). The incubator comprises a 10-W ceramic heater, a 3D-printed metal bath, and a temperature controller containing a thermistor (Fig. S8). The disposable pen-like instruments cost around $6.05 per test, and the reusable part of the platform costs $5.24 (Table S5). A large portion of the costs belongs to commercial enzymes and 3D-printing services which can be significantly reduced upon large-scale manufacturing.

With this integrated platform, we showed that the SARS-CoV-2 targets can be identified by 15-min incubation time with the WHO required 1000 copies/μL sensitivity for COVID-19 self-test kits (WHO 2020) (Fig. 4B and Fig. S9A) on a low-power point-of-care device. After verifying the performance of our miniaturized device, we then defined the sensitivity of FLASH as the lowest detectable concentration of inactivated SARS-CoV-2 viruses (ATCC VR-1986HK) in artificial saliva. FLASH platform using 25-min incubation (turnaround time ~ 30 min) achieved attomolar sensitivity (0.5 copies/μL) (Fig. 4C & Fig. S9B), comparable with commercially available nucleic acid-based self-test kits, while reducing the turnaround time by almost half. Moreover, the integrated FLASH system includes an intact sample extraction and enrichment process by the introduction of SampleDirect-Pen which is lacked in other rapid NATs, ensuring the robustness of performance in biological sample matrix (Table S6). This sensitivity is comparable with the limit of detection (LoD) required by WHO (1 copy/μL), US FDA (1 copy/μL), and China CDC (0.5 copies/μL) for gold-standard RT-qPCR testing.

Also, to avoid any inaccuracy introduced during LFA results interpretation, we developed a mini-program working on WeChat application (https://github.com/xluaq/FLASH.git). With the embedded camera on smartphones, this mini-program measures the intensity of test line and control line and automatically interprets the results into “positive” “negative” and “invalid” (Fig. 4D & Movie S1).

Then, the accuracy of FLASH was evaluated in comparison with the WHO-recommended gold standard RT-qPCR through a blinded experiment detecting 45 mock saliva samples, simulating a COVID-19 outbreak with 11% prevalence as in Kwun Tong, Hong Kong SAR in March 2022 (HKCHP 2022). The five positive samples were spiked with 1000, 100, 10, 1, 0.5 copies/μL of inactivated SARS-CoV-2 virus. The FLASH platform readily enables to screen and identify the 5 positive samples with low viral loads, achieving 100% consistent results, relative to the gold-standard RT-qPCR results (Fig. 4D & Table S7).

Finally, the analytical performance and turnaround time of FLASH were compared with the FDA EUA-approved RATs for SARS-CoV-2 self-testing (Table S8). With a user-friendly workflow and 30-min turnaround time, FLASH can identify Covid-19 infections at viral loads three orders of magnitude lower than LoDs of RATs. The viral titer of SARS-CoV-2 ranged from 1 copy/μL to 10^8 copies/μL (Fajnzylber et al., 2020) in an infection and fluctuates during the whole period of infection irrelevant to symptom severity (Zou et al., 2020). Currently, patients in self-quarantine are arranged to conduct routine self-tests using RAT for confirmation of their recovery. Low sensitivity of RAT has raised concerns about its reliability considering the low viral load period during infections. Thus, FLASH with single-copy sensitivity is a more reliable tool for monitoring the infection condition of self-quarantined patients.
4. Conclusion

In this paper, we reported FLASH, a low-cost molecular home test platform with attomolar sensitivity and 30-min turnaround time, utilizing disposable pen-like design and visible LFA readout. This system was able to detect the N gene of various SARS-CoV-2 variants (Table S9) with attomolar sensitivity. The turnaround time can be shortened to 20 min when the WHO recommended 1000 copies/μL sensitivity is used, including 2-min sample preparation, 15-min oLAMP reaction, and 3-min LFA readout. The concerns related to the false-negatives and false-positives of the currently available self-test techniques have limited their contribution. Here the FLASH platform enabled highly accurate and rapid NAT-based pathogen tests for at-home self-tests and thus is readily available for application in epidemic control.

CRedit authorship contribution statement

Xiao Lu: Conceptualization, Methodology, Investigation, Final analysis, Writing – original draft. Haoqi Lin: Investigation, Data curation, Formal analysis, Writing – original draft. Xianzhen Feng: Data curation, Investigation. Grace CY. Lui: Resources, Supervision. I-Ming Hsing: Conceptualization, Methodology, Supervision, Project administration, Funding acquisition, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgment

This work was financially supported by the Research Grants Council (CRF C6107-20G) and the Innovation and Technology Commission (MRP/077/20) of the Hong Kong Special Administrative Region. We thank European Virus Active-Global and Erasmus University Medical Center Rotterdam for their contributions on providing the viral RNA. We acknowledged Alan F. Rodríguez-Serrano, Asmita Veronica and Li Yue for manuscript editing.

Appendix B. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bios.2022.100248.

References

Acuña, M.J., Jaurez, R.P., 2021. Odontoestomatologia 38.
Al-Soud, W.A., Ouis, I.S., Li, D.Q., Ljungh, S., Wadstrom, T., 2005. FEMS Immunol. Med. Microbiol. 44 (2), 177–182.
Blairon, L., Wilmet, A., Beukinga, I., Tre-Hardy, M., 2020. J. Clin. Virol.: Off. Publ. Pan Am. Soc. Clin. Virol. 129, 104472.
Borres, A., Box, A.T., Fluit, A.C., 2004. Eur. J. Clin. Microbiol. Infect. Dis.: Off. Publ. Eur. Soc. Clin. Microbiol. 23 (4), 289–299.
Broughton, J.P., Deng, X., Yu, G., Fasching, C.L., Servellita, V., Singh, J., Miao, X., Streithorst, J.A., Granados, A., Sotomayor-Gonzalez, A., Zom, K., Gopez, H., Hu, E., Gu, W., Miller, S., Pan, C.V., Guevara, H., Wadford, D.A., Chen, J.S., Chiu, C.Y., 2020. Nat. Biotechnol. 38 (7), 870–874.
Dinnes, J., Deeks, J.J., Berhane, S., Taylor, M., Adriano, A., Davenport, C., Dittrich, S., Emperador, D., Takwoingi, Y., Cunningham, J., Reese, S., Domen, J., Dreitke, J., Ferrante di Ruffano, L., Harris, L.M., Price, M.J., Taylor-Phillips, S., Hoofn., L., Leeflang, M.M., Mclnnes, M.D., Spijker, R., Van den Brul, A., Cochrane, C.-D.T.A.G., 2021. Cochrane Database Syst. Rev. 3, CD017505.
Fajnýrber, J., Regan, J., Coten, K., Corry, H., Wong, C., Rosenthal, A., Worrall, D., Gignel, F., Piechocka-Trocha, A., Ayvsee, C., Fischinger, S., Chan, A., Flaberty, K.T., Hall, K., Dugan, M., Ryan, E.T., Gillespie, E., Chisthi, R., Li, Y., Jilg, N., Hanidzicz, D., Baron, R.M., Baden, L., Tsibris, A.M., Armstrong, K.A., Kuritzkes, D.R., Alter, G., Walker, B.D., Yu, X., Li, J.Z., Massachusetts Consortium for Pathogen, R., 2020. Nat. Commun. 11 (1), 5493.
HKCHP, 2022. Retrieved from https://www.chp.gov.hk/files/pdf/local_situation_covid-19_en.pdf. (Accessed 31 March 2022).
Iacobucci, G., 2020. BMJ 371, n4322.
Joung, J., Ladha, A., Saito, M., Kim, N.G., Woolley, A.E., Segel, M., Barretton, R.P.J., Ramu, A., Macrè, R.K., Faure, G., Ioannidi, E.I., Krajæski, R.N., Brunere, R., Huang, M.W., Yu, X.G., Li, J.Z., Walker, B.D., Hung, D.T., Greninger, A.L., Jerome, K.R., Gootenberg, J.S., Abasalyeey, O.O., Zhang, F., 2020. N. Engl. J. Med. 383 (15), 1492–1494.
Kainz, D.M., Breiner, B.J., Fruh, S.M., Hutzenlaub, T., Zengerle, R., Paust, N., 2021. Microsyst. Nanoeng. 7, 72.
Khazani, N.A., Noor, N.Z., Yeung Yean, C., Hasan, H., Suriya, S., Mohamad, S., 2017. J. Trop Med., 7210849.
Linnes, J.C., Rodriguez, N.M., Liu, L., Klapperich, C.M., 2016. Biomed. Microdevices 18 (2), 30.
Lu, X., Lee Yu, H., Lin, H., Cao, Y., He, J., Huing, L.M., 2022. Sensor. Actuator. B Chem. 357, 131385.
Mackay, I.M., Arden, K.E., Nitsche, A., 2002. Nucleic Acids Res. 30 (6), 1292–1305.
Nagarika-Ikeeda, M., Inai, K., Tabata, S., Miyoshi, K., Murahara, N., Mizuno, T., Horiuchi, M., Kato, K., Imoto, Y., Iwata, M., Mimura, S., Ito, T., Tamura, K., Kato, Y., 2020. J. Clin. Microbiol. 58 (9).
Parolo, C., Sena-Torralba, A., Bergua, J.F., Calacho, E., Fuentes-Chatu, C., Hu, L., Rivas, L., Alvarez-Duduk, R., Nguyen, N.E., Ginti, S., Quesada-Gonzalez, D., 2019. Microsyst. Nanoeng. 5, 72.
Peto, J., 2020. R. Soc. Open Sci. 8 (7), 201468.
Peto, J., Carpenter, J., Smith, G.D., Duffy, S., Houlston, R., Hunter, D.J., McPherson, K., Pearce, N., Romer, F., Sasienn, P., Turnbull, C., 2011. R. Soc. Open Sci. 7 (6), 20915.
Poudel, S., Ishak, A., Perez-Fernandez, J., Garcia, E., Leon-Figueroa, D.A., Romani, L., Bonilla-Aldana, D.K., Rodriguez-Morales, A.J., 2022. Trav. Med. Infect. Dis. 45, 102234.
Regalado, A., 2022. MIT Technol. Rev. 2021. Retrieved from https://www.technologyreview.com/2021/05/04/1024450/at-home-covid-test-review-accuracy-binaxnow-lucira-ellume/. (Accessed 31 March 2022).
Tang, R., Yang, H., Choi, J.K., Gong, Y., Hu, J., Feng, S., Pingguan-Murphy, B., Mei, Q., Xu, F., 2016. Talcanta 152, 269–276.
Uribe-Alvarez, C., Lam, Q., Baldwin, D.A., Chernoff, J., 2021. PLoS One 16 (5), e0250202.
WHO, 2020. Retrieved from https://www.who.int/publications/m/item/covid-19-target-product-profiles-for-priority-diagnostics-to-support-response-to-the-covid-19-pandemic-v1.0. (Accessed 31 March 2022).
Zheng, S., Fan, J., Yu, F., Feng, B., Lou, B., Zou, Q., Xie, G., Lin, S., Wang, R., Yang, X., Chen, W., Wang, Q., Zhang, D., Liu, Y., Gong, R., Ma, Z., Lu, S., Xiao, Y., Gu, Y., Zhang, J., Yao, H., Xu, K., Lu, X., Wei, G., Zhou, J., Fang, Q., Cai, H., Qiu, Y., Sheng, J., Chen, Y., Liang, T., 2020. BMJ 369, m1443.
Zou, L., Ruan, F., Huang, M., Liang, L., Huang, H., Hong, Z., Yu, J., Kang, M., Song, Y., Xia, J., Guo, Q., Song, T., He, J., Yen, H.L., Peiris, M., Wu, J., 2020. N. Engl. J. Med. 382 (12), 1177–1179.