OPEN ACCESS

GAVIN PUBLISHERS

Infectious Diseases Diagnosis & Treatment

Review Article

Reem A. et al. Infect Dis Diag Treat 4: 158.
DOI: 10.29011/2577-1515.100158

Application of Rolling Circle Amplification (RCA) to Detect the Pathogens of Infectious Diseases

Alariqi Reem[1*], Maged Almezgagi[2], Fadhl Al-Shaebi[3,4], Wadee Adbullah AL-Shehari[1], Jay Prakash Prasad Kumal[5]

[1]Department of Microbiology, Harbin Medical University, Harbin, China

[2]Department of Immunology, Medical College of Qinghai University, Qinghai Xining, China

[3]Department of Immunology, Hebei Medical University, Shijiazhuang, Hebei, China

[4]Faculty of Medicine and Health Sciences, Thamar University, Dhamar, Yemen

[5]Department of Human Anatomy, Harbin Medical University, 157 Baojian Road, Harbin 150081, China

[*]**Corresponding author:** Reem Alariqi, Department of Microbiology, Harbin Medical University, Harbin, China

Citation: Reem A, Almezgagi M, Al-Shaebi F, Al-Shehari WA, Kumal JPP (2020) Application of Rolling Circle Amplification (RCA) to Detect the Pathogens of Infectious Diseases. Infect Dis Diag Treat 4: 158. DOI: 10.29011/2577-1515.100158

Received Date: 26 August, 2020; **Accepted Date:** 02 September, 2020; **Published Date:** 10 September, 2020

Abstract

The appearance of new dangerous pathogens in recent years has caused severe outbreaks such as SARS-CoV-2 in 2019. Immediate medical attention, at the point of care, is needed, because of the height mortality rate; furthermore, a precise early infection diagnosis can prevent severe deterioration in patients, may surround a pandemic or limits, and besieges its consequences. The Rolling Circle Amplification (RCA) is a biotechnological polymerase chain reaction method that can be used to quantify and clonally amplify DNA or RNA. It was used to develop extremely sensitive diagnostic methods for diverse goals, counting nucleic acids (DNA, RNA), cells, proteins and small molecules. Importantly, RCA has been known to offer a more sensitive, reliable and reproducible identification of pathogens with low abundance and may be a better option for potential clinical uses than quantitative polymerase chain reaction PCR. Here, we summarized the potential benefits of RCA in the clinical diagnosis of infectious diseases, including viral infection, bacterial infection, parasite disease and fungal infection. We were noted that RCA offers a more sensitive, reliable and reproducible identification of pathogens with low abundance and maybe a better option for potential clinical uses than quantitative polymerase chain reaction (PCR).

Keywords: Rolling circle amplification (RCA), Infectious disease, Polymerase chain reaction (PCR)

Abbreviations: RCA: Rolling Circle Amplification; PCR: Polymerase Chain Reaction; HIV: Human Immunodeficiency Virus; SARS-CoV-2: Severe Acute Respiratory Syndrome-Corona Virus 2; HPV: Human Papilloma Virus

Introduction

Pathogens are the main causative agent of the illness in infectious disease [1]. Sometimes, because of latency infection, the pathogen concentration is too low in the sample, traditional 32 methods such as ELISA and blood smear cannot determine the pathogen such as Immunodeficiency Virus (HIV), tuberculosis, Ebola virus, malaria. High-sensitivity techniques accurate, rapidly and cheap are necessary to detect pathogens [2]. Accurate measurements of pathogen-related nucleic acids are becoming more and more important than ever before at the request of a precise medicine. RCA is an isothermal enzymatic method that is commonly used for create long single strands of DAN and RNA, and it can retain current clinical diagnoses in molecular biology, materials science and medicine, it was commonly used for the test of sputum ,saliva and blood samples [3]. In the evolution of specific detection methods for a range of diagnostic nucleic acid targets, this approach was harnessed as an easy and efficient form of signal amplification [4]. RCA is an advanced biotechnology, a too short circular oligonucleotide (e.g. 25-100 nucleotides in length) serves as a basis for DNA or RNA polymerase, creating long repetitive product sequences that function as replicated versions of the circle sequence [5]. Developed several RCA-based systems detect a range of targets, including RNA, DNA, protein, SNP, pathogens, micromolecules and cytokines [3]. Furthermore this technique is used in the diagnosis of infectious human disease for example (virus, bacteria, fungi, and parasite). It also help to identify virus infect plants such as Gemini viruses [6]. Badna viruses [7]. In addition, the technique used to detect MicroRNAs that act as a modern biomarker for various illnesses [8].

Development and principle of RCA

RCA has been systematically investigating the development of responsive identification methods for DNA , RNA, single nucleotide, DNA methylation, polymorphism (SNP), small

molecules , cells and proteins since its discovery in the early 1990s [9]. The RCA idea is based on a circle's main structure, this idea is applied effectively by living cells in the replication of viral genomes and circular plasmids. The DNA polymerase copies the ring of DNA. This characteristic of the circles indicates that the polymerase enzyme, if created at the same point by a primer on circular DNA, Generate a single stranded DNA concatemer by going around the DNA minicircle and synthesizing its linear replicate continuously until the cycle is complete [10] (Figure 1).

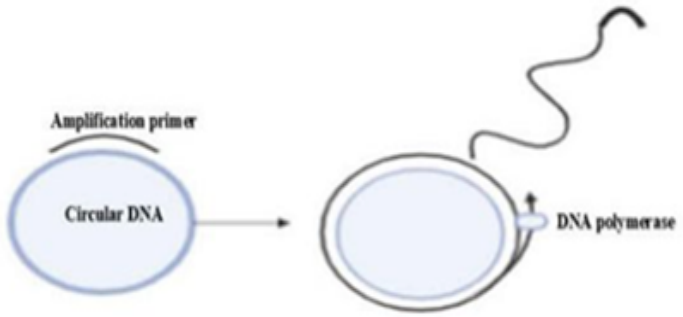

Figure 1: Illustration of the RCA process the generally accepted view of the RCA reaction that occurs in a free DNA minicircle using a single primer. In this case, the DNA polymerase displacement capability of the strand is assumed.

Advantage of RCA

Additionally, RCA has been shown to be a simple isothermal amplification method that does not require complex assay modeling and is typically stable against interference [11]. For the amplification process it needs no complicated heat cycling measures and advanced instruments only a simple heating device (e.g. a water bath or heating block) [12]. RCA can replication a circular DNA template covalently closed with many (up to thousands) complementary template copies into a single, long stranded DNA [13]. In addition, RCA amplicons can be viewed by a naked eye using some basic methods and/or filmed by a camera phone without using any detector probes [14]. Furthermore, the quantitative identification of RCA amplification is difficult to achieve [15] (Table 1).

Advantages	Disadvantages
RCA reactions are well resistant to many contaminants, allowing detection of target DNA and RNA molecules in crude mixtures.	Special instrumentation is needed
RCA reactions with padlock probes exhibit an excellent sequence specificity simple mechanism low cost of diagnostics	Difficult to achieve quantitative Detection

Table 1: Advantages and disadvantages of RCA.

Clinical use of RCA for the diagnosis of infectious diseases

Since the RCA technique was established, scientists tried to bring it in to clinical use [16]. The RCA is a rapid, sensitive and specific isothermal DNA amplification technique used for examination in some infectious diseases [17]. The PCR is a fast test tool and helpful analysis in some infectious diseases, but in other cases, it cannot be the diagnostic quality because of false positive results [18]. RCA can be conducted at mild temperatures and less sensitive than polymerase chain reaction PCR to certain errors such as sequence-dependent amplification bias [19]. Non-specific priming [20]. Mutation propagation [6]. RCA represents a better approach to PCR because of these benefits (Table 2).

	RCA		PCR
1	SARS-CoV-2	1	SARS-CoV-2
2	Ebola and Other Tropical Viruses (Dengue, and Zika)	2	Hepatitis B (Hepatitis B virus)
3	Influenza virus strains A and B	3	Acquired immunodeficiency syndrome(human immunodeficiency virus
4	Human immunodeficiency virus	4	Tuberculosis (Mycobacterium tuberculosis)
5	Mycobacterium tuberculosis	5	Human herpes (Equine herpes virus 1, Equine herpes virus 4
6	Vibrio parahaemolyticus	6	Syphilis (Treponema palladium)
7	Malaria (malaria parasites)	7	Tuberculosis (Mycobacterium tuberculosis)
8	Histoplasma capsulatum	8	Malaria (malaria parasites)

Table 2: Examples of infectious pathogens identified by RCA and PCR.

Viral infectious diseases

A viral infection happens when an organism is invaded with the virus and the infectious virus attaches themselves to susceptible cells then enters them [21]. Viruses only can survive within living cells of other species, but they are most commonly present in almost every ecosystem on Earth [22]. Rapid diagnosis plays a vital role in treating viral infection. A large number of scientists use RCA to diagnose human-related viruses, such as Severe Acute Respiratory Syndrome Corona Virus 2 (SARS-CoV-2), human herpes virus, Ebola virus human Influenza, and HIV.

Severe acute respiratory syndrome corona virus 2(SARS-CoV-2)

Corona virus 2 (SARS-CoV-2), the etiologic agent of Corona virus Disease 2019 (COVID-19) which began outbreak in

central China in December 2019, has quickly been a worldwide interest [23]. The respiratory droplets and routes between humans [24] transmit that. The time to death from the start of COVID-19 symptoms ranges from 6 days to 41 days with a mean of 14 days [25]. Consistent early diagnosis of infection will prevent patients from a serious worsening. Fast demand of SARS-CoV-2 complete genome, protocols for laboratories for real-time RT-PCR technique were published online in January 2020 for RNA-dependent RNA Polymerase (RdRp), envelope and nucleocapsid SARS-CoV-2 genes [26]. A study show that RCA analysis was applied to detect a synthetic complementary DNA of the coding sequence SARS-CoV-2 RdRp, reaching a detection limit of 0.4 fM with a complex detection range of 3 orders of magnitude and a total assay time of approx. 100 min. A standard for mathematics 2 was set up and evaluated to estimate assay efficiency. Surely, RCA has a potential approach for Covid 19 detection.

Ebola and Other Tropical Viruses (Dengue, and Zika)

Ebola virus (EBOV) is belong to the genus Ebola virus classified within the family Filoviridae [27]. EBOV is defined by a fast spread by exchange of body fluids or by close contact with infected persons, three weeks incubation periods and rapid death within 4 to 10 days from infection [28]. The most popular tool used for detecting EBOV is the electron microscopy, enzyme-linked immunosorbent assay, -time PCR , antigen check, and molecular testing (finding of viral RNA) [29].

All these methods however have analytical and practical limitations, which make none ideal once EBOV detection urgently. Interestingly, a simplified design of RCA assays can also be used for clinical detection of EBOV, along with tropical virus screen multiplexing; using a robust micro fluidic RCP enrichment (MRE) plan to get digital, analytic counts inside the sample. Two Padlock Probes (PLP) based Ebola detection assays were developed for the current research, and the multiplexing capability was demonstrated by simultaneous detection of EBOV, Dengue, and Zika. PLPs were conceived to target all three-virus genes [30].

Influenza virus strains A and B

Influenza is found within the family Orthomyxoviridae, with three genera infecting humans: influenza virus A, B, and C [31]. Influenza is a common cause of morbidity and mortality, and has a huge social and economic impact worldwide. Mortality rate always goes hand in hand with influenza outbreaks [32]. Studies with PLP / RCA already got established the framework for the production of diagnostic systems for the detection of influenza virus, the test had a detection limit of 18 viral RNA copies and reached 100% analytical and clinical specificity to identify and subtype differentials Influenza seasonal virus.

Human immunodeficiency virus

HIV are two Lentivirus species, which lead to HIV infection and are associated with depletion of CD4C, T-helper lymphocytes in the blood [33]. Early diagnosis is essential for a better treatment in HIV-infected patients as it encourages the estimated initiation of adequate treatment and decreases the risk of HIV transmission by3 to 5 times [34]. HIV nucleic acids play a significant part in scientific studies and clinical diagnosis. DNA nanomachine on gold nanoparticles pairing of RCA and DNA walker cascade amplification for ultra-sensitive for nucleic acid identification of HIV [15].

Papillomavirus type 16

Papillomaviruses have been classified as a separate family, Papillomaviridae [35]. Nearly 15 of the more than 100 types of HPVs identified so far are identified as high-risk types, which are closely related to the development of cervical carcinoma, while HPV type 16 (HPV16) is the dominant high-risk type globally [36]. A study analyzed HPV16 containing the root of plasmid and the replication proceed in epithelial cell .In vitro replication method raw extracts from distinguished epithelial cells used, with purified the DNA helicase E1 and the auxiliary factor E2. Furthermore, high molecular - weight DNA, well-defined circular replication products, was synthesized in a way that based on the origin, E1 and E2. The high-molecular - weight DNA was transformed to a linear DNA unit-length by treatment with restrictive enzymes that once cleaved the plasmid, suggesting that a concatemeric DNA was formed by rolling circle replication. Potential molecular mechanisms are presented for HPV16 rolling circle replication, and its physiological applicability in cervical viral carcinogenesis [37].

Bacterial infectious diseases

A bacterial infection is a growth of a contagious bacterial organism inside or on the individual. Bacteria can cause infection in any part of the body. Food poisoning, Pneumonia and meningitis are an example of disease caused by pathogen bacteria. Bacterial diseases are sometimes difficult to recognize due to specific biological phases of a particular pathogen, where the concentration of pathogens is very small, RCA illustrates high sensitivity to low DNA in a very small amount of pathogen.

Mycobacterium tuberculosis

TB is one of the world's top 10 causes of death, and the leading cause of a single infectious agent (above HIV / AIDS). Millions of people tend to fall ill with TB every year. In 2017, TB caused 1.3 million deaths [38]. Therefore, a new diagnostic approach for under low-resource healthcare facilities should also be evaluated. At China, Capital Medical University, Beijing Chest Hospital, they try to confirm the diagnostic accuracy of Hyperbranched Rolling Circle Amplification (HRCA). The padlock probe of HRCA, designed according on a sequence (IS6110) specific for MTC strains, was used to diagnose Mycobacterium Tuberculosis (Mtb), HRCA showed excellent sensitivity and ability to reproduce purified DNA (740 aM). With RCA's performance enhancement and progress, we can diagnose TB more easily and reliably in the future [39].

Vibrio parahaemolyticus

Vibrio parahaemolyticus is a human gastroenteritis causative agent found in seas where temperatures reach 15 ° C,

The prevalence of V. parahaemolyticus and elevated infection rates have thus historically In United States, Europe , South America and Asia then outbreaks were identified [40]. Quick and specific identification of the bacteria in fresh food specimen is a global challenge; they have developed a visualization technique for identification (Dual-Apt & CA-RCA) using aptameric, a single-stranded nucleic acid sequence with its closeness and 167 accuracy with that of an antibody. Fortunately, false negative results happened in the classical RCA because it provide less than 103 amplification rates, which are not appropriate for detection [41]. The Dual Apt & CA-RCA, which offers visible naked-eye results directly, provides fast-visualized identification of V. parahaemolyticus. Many advantages together make Strategy a suitable way to detect on-site.

Parasitic infectious disease (Malaria)

Infectious malaria causes signs also include headaches, fever, fatigue, vomiting and tiredness. Malaria is an infectious disease transmitted by mosquitoes that bite humans and other animals Malaria is a widespread tropical disease that has killed over half a million people around the world [42]. After RCA of the generated DNA circle in the presence of biotin conjugated deoxynucleotides the outcome was long tandem repeat products that was display calorimetrically to binding of Horse Radish Peroxidase (HRP) and added of 3,3′,5,5′-Tetramethylbenzidine that Has been converted to a blue colored product by HRP. The assay was directly quantitative, specific for Plasmodium parasites, and allowed detection of plasmodium infection in a single drop of saliva from 35 out of 35 infected individuals tested [43].

Fungus infectious disease (Histoplasma capsulatum)

Histoplasma is a dimorphic fungus which causes histoplasmosis which is respiratory and systemic diseases [44]. Data have shown that the RCA technique with the padlock probe is acceptable to the quick and accurate detection of H. Capsulatum isolated from clinical specimens of human hosts and bats. The RCA tests was developed in this study to cover all three currently recognized varieties of H. Capsulatum and is a useful tool for clinical and veterinary strains that could be indicated for differential diagnosis of diseases with similar clinical characteristics In our study, we compared sequences of the rDNA ITS spacer region of H. Capsulatum to develop a padlock probe. The method was tested with negative controls of closely related fungal species causing similar symptoms to those of H. Capsulatum with the aim of establishing a fast, accurate and particular diagnosis for this fungus [45].

Perspectives

RCA is an isothermal enzymatic Technique widely used for too long single-stranded production RNA and DNA [46]. Now, it had been used in many areas, such as, molecular biology, materials science, and medicine. It may also be a possible surveillance tool for diseases such as cancer [47]. RCA's benefits lie in many ways RCA reaction and its attractively clear process require low cost [17]. Diagnosis and make it easy to miniaturize and automate in high-performance experiments [17]. Furthermore, it offers patients great advantages in a simple, fast and easy diagnosis, it has read out quicker than serological or pathogenic culture tests. In contrast to the PCR, which requires a thermal cycler and the most able DNA polymerases RCA can be performed at a constant temperature of room temperature in a solution, on a solid base or in a mixed biological environment. (e.g., on the cell surface or inside a cell) [48]. Most significantly, RCA does not require cycling the temperature in expensive instrumentation, as it is needed with the commonly used DNA diagnostics based on PCR. This is particularly helpful in poor regions during epidemics where hundreds of samples need to be checked quickly [49]. The RCA is now also a possible tool for diagnosing infectious disease.

References

1. Lipkin WI (2013) The changing face of pathogen discovery and surveillance. Nature Reviews Microbiology 11: 133-141.
2. Li H, Bai R, Zhao Z, Tao L, Ma M, et al. (2018) Application of droplet digital PCR to detect the pathogens of infectious diseases. Bioscience reports 38.
3. Gu L, Yan W, Liu L, Wang S, Zhang X, et al. (2018) Research progress on rolling circle amplification (RCA)-based biomedical sensing. Pharmaceuticals 11: 35.
4. Chen J, Baker YR, Brown A, El-sagheer A, Brown T (2018) Enzyme-free synthesis of cyclic single-stranded DNA constructs containing a single triazole, amide or phosphoramidate backbone linkage and their use 227 as templates for rolling circle amplification and Nano flower formation. Chemical science 9: 8110-8120.
5. Mohsen MG, Kool ET (2016) The discovery of rolling circle amplification and rolling circle transcription. Accounts of chemical research 49: 2540-2550.
6. Haible D, Kober S, Jeske H (2006) Rolling circle amplification revolutionizes diagnosis and genomics of Gemini viruses. Journal of virological methods 135: 9-16.
7. Sukal AC, Kidanemariam DB, Dale JL, Harding RM, James AP, et al. (2019) Assessment and optimization of rolling circle amplification protocols for the detection and characterization of badnaviruses. Virology 529: 73-80.
8. Wang S, Lu S, Zhao J, Ye J, Huang J, et al. (2019) An electric potential modulated cascade of catalyzed hairpin assembly and rolling chain amplification for microRNA detection. Biosensors and Bioelectronics 126: 565-571.
9. Ali MM, Li F, Zhang Z, Zhang K, Kang DK, et al. (2014) Rolling circle amplification: a versatile tool for chemical biology, materials science and medicine. Chem Soc Rev 43: 3324-41.
10. Kornberg A, T Baker, DNA replication. WH. H. Freeman, New York, 1992.
11. Soares RR, Neumann F, Caneira CRF, Madaboosi N, Ciftci S, et al. (2019) Silica bead-based microfluidic device with integrated photodiodes for the rapid capture and detection of rolling circle amplification products in the femtomolar range. Biosensors and Bioelectronics 128: 68-75.
12. Gao M, Lian H, Yu L, Gong M, Ma L, et al. (2019) Rolling circle amplification integrated with suspension bead array for ultrasensitive multiplex immune detection of tumor markers. Analytica Chimica Acta 1048: 75-84.

13. Neumann F, Hernandez-Neuta I, Grabbe M, Madaboodi N, Albert J, et al. (2018) Padlock probe assay for detection and subtyping of seasonal influenza. Clinical chemistry 64: 1704-1712.

14. Xie L, Wang T, Huang T, Hou W, Huang G, et al. (2014) Dew inspired breathing-based detection of genetic point mutation visualized by naked eye. Scientific reports 4: 6300.

15. Zheng J, Ji X, Du M, Tian S, He Z (2018) Rational construction of a DNA nanomachine for HIV nucleic acid ultrasensitive sensing. Nanoscale 10: 17206-17211.

16. Angell C (2020) Co-opting Rolling Circle Amplification to Create Lower Cost DNA Nanostructures. UC San Diego.

17. Javaheri Tehrani S, Aliabadian M, Fata A, Najafzadeh MJ, et al. (2014) Rolling Circle Amplification (RCA): an approach for quick detection and identification of fungal species. Journal of Mycology Research 1: 55-62.

18. Hall Sedlak R, KR Jerome (2014) The potential advantages of digital PCR for clinical virology diagnostics. Expert Review of Molecular Diagnostics 14: 501-507.

19. Joffroy B, Uca YO, Presern D, Doye JPK, Schmidt TL, et al. (2018) Rolling circle amplification shows a sinusoidal template length-dependent amplification bias. Nucleic acids research 46: 538-545.

20. Khalafalla AI, Ramadan RO, Rector A, Barakat S (2017) Investigation on papillomavirus infection in dromedary camels in Al-Ahsa, Saudi Arabia. Open Veterinary Journal 7: 174-179.

21. Lawrence CM, Menon S, Eilers BJ, Bothner B, Khayat R, et al. (2009) Structural and functional studies of archaeal viruses. Journal of Biological Chemistry 284: 12599-12603.

22. Robert A, F Edwards (2004) Viral metagenomics. Nat Rev Microbiol 3: 86-289.

23. Yongjian Z, Xie J, Huang F, Cao L, et al. (2020) Association between short-term exposure to air pollution and COVID-19 infection: Evidence from China. Science of the total environment 727: 138704.

24. Organization WH (2020) Modes of transmission of virus causing COVID-19: implications for IPC precaution recommendations: scientific brief. 2020 World Health 272 Organization.

25. Wang W, J Tang, F Wei (2020) Updated understanding of the outbreak of 2019 novel coronavirus (2019□nCoV) in Wuhan, China. Journal of medical virology 92: 441-447.

26. Vermeiren C, Marchand-Senecal X, Sheldrake E, Bulir D, Smieja M, et al. (2020) Comparison of Copan Eswab and FLOQswab for COVID-19 PCR diagnosis: working around a supply shortage. Journal of Clinical Microbiology 58: 669-620.

27. Kuhn JH, Becker S, Ebihara H, Geisbart TW, Jhonson KM, et al. (2010) Proposal for a revised taxonomy of the family Filoviridae: classification, names of taxa and viruses, and virus abbreviations. Archives of virology 155: 2083-2103.

28. Ohimain EI (2016) Recent advances in the development of vaccines for Ebola virus disease. Virus research 211: 174-185.

29. Broadhurst MJ, TJ Brooks, NR Pollock (2016) Diagnosis of Ebola virus disease: past, present, and future. Clinical microbiology reviews 29: 773-793.

30. Ciftci S, Neumann F, Abdurahman F, Appelberg KS, Mirazimi A, et al. (2020) Digital Rolling Circle Amplification–Based Detection of Ebola and Other Tropical Viruses. The Journal of Molecular Diagnostics 22: 272-283.

31. Webster RG, Bean WJ, Gorman OT, Chambers TM, Kawaoka Y (1992) Evolution and ecology of influenza A viruses. Microbiology and molecular biology reviews 56: 152-179.

32. Organization WH (2005) Recommendations for the production and control of influenza vaccine (inactivated). WHO Technical Report Series 927: 99-134.

33. Spiegel H, Herbst H, Niedobitek G, Foss HD, Stein H (1992) Follicular dendritic cells are a major reservoir for human immunodeficiency virus type 1 in lymphoid tissues facilitating infection of CD4+ T- helper cells. The American journal of pathology 140: 15.

34. Medicine, ECOtASFR (2015) Human immunodeficiency virus (HIV) and infertility treatment: a committee opinion. Fertility and Sterility 104: e1-e8.

35. Bernard HU, Burk RD, Chen Z, Doorslaer KV, Hausen HZ, et al. (2010) Classification of papillomaviruses (PVs) based on 189 PV types and proposal of taxonomic amendments. Virology 401: 70-79.

36. Parkin DM, Almont M, Bruni L, Clifford G, Curado MP, et al. (2008) Burden and trends of type-specific human papillomavirus infections and related diseases in the Latin America and Caribbean region. Vaccine 26: 1-15.

37. Kusumoto□Matsuo R, T Kanda, I Kukimoto (2011) Rolling circle replication of human papillomavirus type 16 DNA in epithelial cell extracts. Genes to Cells 16: 23-33.

38. Organization WH (2018) Meeting of the implementation Core Group of WHO Global Task Force on latent TB infection and country stakeholders on implementation tools and joint TB and HIV programming to scale up TB preventive treatment: Geneva, 14-16 November 2018. World Health Organization.

39. Liu Y, Guo YL, Jiang GL, Zhou SJ, Sun Q, et al. (2013) Application of hyperbranched rolling circle amplification for direct detection of mycobacterium tuberculosis in clinical sputum specimens. PloS one 8: e64583.

40. Witherall L, Wagley S, Butler C, Tyler CR, Temperton B, et al. (2019) Genome Sequences of Four Vibrio parahaemolyticus Strains Isolated from the English Channel and the River Thames. Microbiology Resource Announcements 8.

41. Song S, Wang X, Xu K, Xia G, Yang X, et al. (2019) Visualized detection of Vibrio parahaemolyticus in food samples using dual-functional aptamers and cut-assisted rolling circle amplification. Journal of agricultural and food chemistry 67: 1244-1253.

42. Organization WH, World malaria report 2015. 2016: World Health Organization.

43. Hede MS, Fjelstrup S, Lotsch F, Zoleko RM, Klicpera A, et al. (2018) Detection of the malaria causing plasmodium parasite in saliva from infected patients using topoisomerase I activity as a biomarker. Scientific reports 8: 1-12.

44. Pitangui N, Sardhi JCO, Silva JF, Benaducci T, Silva RAM, et al. (2012) Adhesion of Histoplasma capsulatum to pneumocytes and biofilm formation on an abiotic surface. Biofouling 28: 711-718.

45. Furuie JL, Sun J, Nascimento MMF, Gomes RR, Hoog SG, et al. (2016) Molecular identification of H istoplasma capsulatum using rolling circle amplification. Mycoses 59: 12-19.

46. Ali MM, Li F, Zhang Z, Zhang K, Kong DK, et al. (2014) Rolling circle amplification: a versatile tool for chemical biology, materials science and medicine. Chemical Society Reviews 43: 3324-3341.

47. Konry T, Smolina I, Yarmush JM, Irimia D, Yarmush ML, et al. (2011) Ultrasensitive detection of low-abundance surface-marker protein using isothermal rolling circle amplification in a microfluidic nanoliter platform. Small 7: 395-400.

48. Vo-Dinh T (1995) SERS chemical sensors and biosensors: new tools for environmental and biological analysis. Sensors and Actuators B: chemical 29: 183-189.

49. Rodrigues AM, Najafzadeh MJ, Hoog GS, Camargo JP, et al. (2015) Rapid identification of emerging human-pathogenic Sporothrix species with rolling circle amplification. Frontiers in microbiology 6: 1385.