Schirmer strip and conjunctival swab for viral detection on the ocular surface of adults: a scoping review

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Systematic Review

Keywords: virus, detection, Schirmer strip, conjunctival swab, ocular surface

Posted Date: February 9th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-113899/v2>

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Abstract

Purpose Schirmer Strips and Conjunctival swabs are used in ophthalmology for tears and fluids collection. During the COVID-19 pandemic, one of the biggest challenges is accurate diagnosis, and it is known that, in some cases, ocular manifestations are one of the first symptoms. In this context, this study has the objective of raising scientific evidence that highlights the use of Schirmer strips and conjunctival swabs as a method of sample collection for viral analysis to support future research on this theme.

Methods A literature search was conducted in the PubMed, Web of Science and BVS databases, following the Scoping Review protocol defined by Joanna Briggs Institute (JBI) after the guiding question “Is it possible to detect viruses on the ocular surface with Schirmer Test and/or conjunctival swab?”.

Results A total of 418 studies were identified, and after discerning analysis, 36 English written studies were selected. Three researchers analyzed studies after virus research, collection methods, and sample analysis. Publications were mainly on adenovirus, herpes simplex virus and SARS-CoV-2, and there is also evidence of ocular detection of more viruses types. Conjunctival swab analyzed through Polymerase Chain Reaction (PCR) or Reverse Transcriptase-PCR (RT-PCR) were the most used methods.

Conclusions Studies have generally been conducted to understand viral infection, to develop accurate diagnostic methods and to follow the patients’ response to treatment. Most studies were performed with a small number of patients and lacked clear definitions of collection time and viral persistence since the onset of diseases. Viruses can be detected on the ocular surface through the analysis of Schirmer strips and conjunctival swabs. However, additional studies with larger populations and time permanence are necessary to develop more assertive conclusions on the theme.

Introduction

Viruses are exclusively intracellular parasites and are also the smallest known infectious agents. Its mechanisms of disease are still not completely clear; however, there are direct factors that contribute to viral tropism: viral receptors in the host cell, specific cell line, and physical barriers that enable and/or inhibit infections. Once inside the cell, the virus may damage or destroy it through direct cytopathic effects, host antiviral immune responses, and/or transformations of the infected cells ⁽¹⁾.

It is known that the eye is a site for viral infections that might appear in the intra- or extraocular space without visible systemic reverberation and affect multiple structures with variable manifestations ^(2,3). Red eye, pain and blurred vision are one of the first clinical signs and symptoms of viral ocular impairment. Conjunctivitis is the topmost ocular infection in primary health care, given that 65-90% of cases are caused by adenovirus (AdV) and occasionally herpes simplex virus (HSV) or varicella zoster virus (VZV). Keratitis caused by HSV-1 is also commonly seen, and it is still the main cause of blindness succeeding infection in developed countries, presenting approximately 40 thousand new cases of visual

impairment every year worldwide ⁽⁴⁾. Viruses that are frequently associated with ocular or systemic complications are *Epstein Barr virus* (EBV), *Measles morbilivirus* and *Paramyxovirus* ⁽⁵⁾.

Viral diagnosis is made after clinical signs and symptoms and laboratory results that support medical hypotheses. There are a large variety of laboratory tests, but specificity and sensitivity change from one microorganism to another. The doctor in charge, based on clinical information and previous experiences, should decide between available options considering the patient's singularities. Cell culture and analysis of genetic material with samples collected from blood, mucosa or secretions are the main methods. In the eye, the most commonly used collection method is conjunctival swabs, but Schirmer strips have also shown good results.

Current scenario

The World Health Organization (WHO) declared COVID-19 a pandemic threat, because of that, the academic community has concentrated all efforts to solve the pandemic and give people's normal lives back. The Chinese Hero and ophthalmologist Li Wenliang, MD, first reported the possibility of a novel catastrophic virus, SARS-CoV-2, and now, it is already known that, in some cases, ocular manifestations are one of the first symptoms, and consequently, the eye may contribute to the understanding of COVID-19 pathophysiology ^(6,7). Since then, several studies on ocular manifestations of SARS-CoV-2 have been conducted, but virus collection methods and associations with the ocular surface are not clearly stated.

Schirmer test

The idea of collecting tears as a clinical test was first introduced by the German ophthalmologist Köster in 1900. The test consisted of the placement of filter paper on all extensions of the conjunctival sac while the nasal mucosa was stimulated to produce tearing caused by nasal irritation. The objective was to exhaust tear production to evaluate the function of lacrimal glands. Therefore, this test could take up to 90 minutes, becoming exhaustive and unviable for daily medical practice ⁽⁸⁾.

In 1903, Otto Schirmer, also a German ophthalmologist, shortened the size of the paper strips and quantified tear production for 5 minutes by three methods, each one analyzed a distinct tearing stimulation pathways: ocular and palpebral mucosa, nasal mucosa, and the retina ^(8,9). Since then, several modifications have been proposed on the Schirmer test; however, this test remains important in the quantification and standardization of tear volume. Currently, it is made with a filter paper strip 60 mm long and 5 mm wide, which is inserted in the temporal side of the conjunctival sac. The patient's eyes are closed, the strips are removed after 5 minutes, and the wet part is measured. Normal values are considered to be results greater than 15 mm, but they can vary mainly according to medication use, age and the presence of chronic diseases ^(10,11).

Conjunctival swab

Conjunctival swabs are the most commonly used methods for microbiological analysis because they allow the collection of cells and materials that are spread in the conjunctival sac instead of tears exclusively. The only exception are the in-office rapid antigen tests for AdV, since they are faster and they also present a good sensitivity and specificity, 89% and 94%, respectively ⁽¹²⁾. The method of collection is also very fast and simple: a swab with a cotton tip is gently passed in rotational movements on the conjunctival sac ⁽¹³⁾. Topic anesthesia can be used to make the procedure more comfortable, since there is no significant difference in the final results when samples are analyzed by polymerase chain reaction (PCR) methods ⁽¹⁴⁾. Proxymetacaine 0.5% is recommended for ocular surface anesthesia since it shows fewer bactericidal effects among the commercially available eye drops ⁽¹⁵⁾. It is also recommended to use sterile swabs, since calcium-containing swabs can inhibit polymerase activity ⁽¹⁶⁾.

In this context, this study has the objective of raising scientific evidence that highlights the use of Schirmer strips and conjunctival swabs as a method of virus collection on the ocular surface.

Methods

A literature review was conducted according to what was proposed by The Joanna Briggs Institute (JBI) on Scoping Reviews ⁽¹⁷⁾. All searches and publication access were completed in June 2020, and had no time limit.

The guiding question “Is it possible to detect virus on the ocular surface with Schirmer Test and/or conjunctival swab?” was defined for the selection and the search of the studies. This was built through the PCC strategy, which consists of a mnemonic for the words Population, Concept and Context. In this way, “P” was defined as adult patients (older than 18 years old), “C” as Schirmer Test and conjunctival swab, and the last “C” as all viruses.

For the literature search, the following descriptors, synonyms and key words were used: “adult patients”, “Schirmer test”, “conjunctival swab” and “virus”. The Boolean operators AND, NOT and OR were used between descriptors. Controlled descriptors were “Adult Patient(s)”, “Schirmer Test”, “Conjunctival Swab(s)” and “Virus”. Not controlled descriptors were “Adult(s)” OR “Patient(s)”, “Schirmer Strip(s)” and “Ocular Virus” OR “Viral Infection”.

The search was performed using the databases PubMed, Web of Science, and *Biblioteca Virtual em Saúde* (BVS). Included articles were only those written in English, published in indexed sources, and with quantitative or qualitative approaches, primary studies and reviews.

Insightful reading of the title, abstract and key words was performed to select the articles according to previously established inclusion and exclusion criteria. When the title, abstract and key words were not

sufficient, the full text was also analyzed. All articles were called studies, enumerated in chronological order, and evaluated by three different researchers. Recommendations by JBI were adapted for the study singularities and used for data extraction. This article followed the Preferred Reporting Items for Systematic reviews and Meta-Analyses extension for Scoping Reviews (PRISMA-ScR) guideline and checklist developed under EQUADOR (Enhancing the QUALity and Transparency Of health Research) network guidance ⁽¹⁸⁾.

Results

Following the database search, 418 potential studies were identified. After reading the title, abstract and key words, 79 studies were selected, and 27 were excluded because they were also found in different databases. The full texts of the 52 remaining articles were read, and 16 were excluded for not answering the guiding question. Using the described methodology, a literature search found 36 articles that met all criteria. This process is shown in Fig. 1.

The three researchers analyzed all studies after virus research, collection methods, and sample analysis. Most of them were experimental or observational studies developed in the United Kingdom (7/36). Studies on the topic started in 1997, but 55,6% were only published in the decade of 2011-2020.

Most studies were on AdV ⁽¹⁹⁻³¹⁾ and HSV ^(22, 26, 29, 32-40), followed by SARS-CoV-2 ⁽⁴⁰⁻⁴⁸⁾. VZV ⁽³²⁻³⁴⁾, Cytomegalovirus (CMV) ^(33, 34, 49), EBV ^(33, 34), Ebola (EBO) ^(50, 51), Human papillomavirus (HPV) ⁽⁵²⁾ and Zika virus ⁽⁵³⁾ have also been identified on the ocular surface. The evolution of published articles on AdV, HSV and SARS-CoV-2 is represented in Fig. 2. Enterovirus (EV), Coxsackievirus A24 variant (CA24v), and SARS-CoV were not found positive on the ocular samples by samples collected by conjunctival swab or Schirmer strips ^(21, 23, 54).

The preferred collection method was conjunctival swab alone, 29 out of 36 articles, while six studies used Schirmer strips, and only one of them compared both collection methods ⁽²⁶⁾. One of the studies was a narrative review about SARS-CoV-2 ⁽⁴⁶⁾. The majority of the samples were analyzed by PCR or Reverse Transcriptase-PCR (RT-PCR) with the exception of the studies number 1 ⁽¹⁹⁾ and 17 ⁽³⁵⁾, that used immunochromatography (IC) and enzyme immunoassay (EIA), and immunoblot analysis, respectively. Details of the results are specified in Fig. 3-5.

Discussion

A literature search found that viruses might be identified on the ocular surface through the analysis of conjunctival swabs or Schirmer strips, but studies mainly focused on AdV, HSV and SARS-CoV-2, while other viruses were still not deeply investigated.

Studies that focused on the detection of AdV and HSV were linearly developed over the last 20 years and randomly conducted all over the world. In contrast, in 2020, scientists exponentially published studies on

SARS-CoV-2 because the virus was first seen in the last days of 2019; in a few months, the WHO declared its disease as a pandemic, and the world's research efforts were completely directed to overcoming this pandemic. The evolution of published articles on AdV, HSV and SARS-CoV-2 is represented in Fig. 2.

In contrast to SARS-CoV ⁽⁵⁴⁾, SARS-CoV-2 RNA was found by RT-PCR in conjunctival swabs ⁽⁴⁰⁻⁴⁸⁾, although samples were collected from a few patients, and only a low and varying percentage of them presented positive results and/or ocular symptoms ⁽⁴⁶⁾. Most of these studies were developed in China ^(40, 41, 43, 47), mainly because the country was the first epicenter of the disease. In some cases, ocular manifestations are one of the first symptoms, and because of that, academic society believes the eye may contribute to the understanding of COVID-19 pathophysiology ^(6, 7). Recently, new studies proved the presence of in the conjunctiva, limbus, and cornea, with prominent staining in the superficial epithelium surface, key factors for the infection of SARS-CoV-2 in human cells ⁽⁵⁵⁾. Those results collaborate for the necessity of ocular protection in preventing the spread of viruses.

Research on viral screening was, most of the time, correlated with external ocular symptoms with the purpose of solving clinical doubts between pathogenic agents and of looking after methods for a fast accurate viral diagnosis. Studies on VZV, CMV and EBV were mostly combined with HSV to determine coinfection and differential diagnosis ⁽³²⁻³⁴⁾.

Only one study correlated viral detection on the ocular surface with intraocular symptoms. This study was published in 2000 and aimed to verify the efficacy of intravenous ganciclovir treatment in immunocompromised AIDS patients with CMV retinitis. The results showed a high clinical relevance for confirming and differentiating the diagnoses of CMV retinitis when ophthalmoscopic findings were associated with PCR methods on conjunctival swab samples ⁽⁴⁹⁾. No other studies on the topic were found in this review, probably because the incidence of CMV retinitis in the Acquired Immunodeficiency Syndrome (AIDS) population significantly decreased with the introduction of effective antiretroviral therapy and early accurate diagnosis ⁽⁵⁶⁻⁵⁹⁾.

The most commonly used method for sample collection was conjunctival swabs. This method also collects conjunctival cells, while Schirmer strips allow only the collection of tears, since fluids pass to the filter paper because of gravity, viscosity, and capillary flow dynamics – the same physical processes that explain how liquids impregnate porous materials differently ^(60, 61). Consequently, the samples collected represent two different materials: tears, cells and fluids dispersed in the conjunctival sac and tears and substances dissolved in it.

Samples were mainly analyzed by PCR or RT-PCR, depending on the viral genetic material, DNA or RNA, respectively. PCR variations are widely used because they allow the replication and detection of low loads of viral DNA/RNA ⁽⁶²⁾. The point-of-care test was also compared with PCR effectiveness, sensitivity and specificity where high, but this field was only explored in AdV ⁽³⁰⁾, probably because of epidemiological factors related to uncontrolled and fast spread of AdV conjunctivitis ⁽⁶³⁾.

This review included a large number of articles, 36, from many different countries and time periods, fact that collaborated for a broad comprehension of how the detection of viruses on the ocular surface was worldwide studied in the last decades. The results of this article may contribute for future research by clarifying key concepts that might assist the design of future researches on ocular viruses.

In conclusion, viruses can be detected through the analysis of samples collected by Schirmer strips and conjunctival swabs, and studies were generally conducted to understand viral infection, to develop accurate diagnostic methods and to follow patients' response to treatment. However, this study has some limitations: only three databases were consulted, and new articles on the theme are constantly being published, which might exclude relevant outcomes. Moreover, among the analyzed studies, most were developed on a small number of patients and lacked clear definitions of collection time and viral persistence since the onset of the diseases. Additional studies with larger populations and time permanence are necessary to develop more assertive conclusions on this topic.

Declarations

Funding

Grant #2020/07365-8, São Paulo Research Foundation (FAPESP).

Author's contributions

LES, AM and LFML designed the work, acquired the data, contributed to the analysis and interpretation of data, and drafted the paper. JS and TETO contributed to the analysis and interpretation of data, and revised the paper. All authors approved the final version.

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Figures

Figure 1

Summary of the systematic searches and the processing of information

Figure 2

Time evolution of articles published on AdV, HSV and SARS-CoV-2

No	Year	Country	Purpose	Methodology
1 ⁽¹⁹⁾	1997	Japan	Compare IC and EIA tests for AdV detection	Experimental Quantitative Study
2 ⁽²⁰⁾	1997	Germany	Evaluate type-specific primers for AdV	Experimental Quantitative Study
3 ⁽²¹⁾	1998	India	Develop and evaluate nested PCR as a tool for detecting AdV from conjunctival swabs	Descriptive Observational Study
4 ⁽⁵⁰⁾	1999	USA	Determine the genetic stability of EBO-Z, and whether additional strains of EBO virus were circulating during Kikwit outbreak	Experimental Quantitative Study
5 ⁽⁴⁹⁾	2000	China	Describe the application of conjunctival swab with PCR and virus culture to confirm the diagnosis of CMV retinitis in AIDS patients	Prospective Longitudinal Quantitative Study
6 ⁽²²⁾	2000	UK	Develop a multiplex PCR for the detection of AdV, HSV, and Chlamydia trachomatis in conjunctival swabs	Experimental Quantitative Study
7 ⁽²³⁾	2001	Taiwan	Evaluate the sensitivity and applicability of PCR and RT-PCR diagnoses for keratoconjunctivitis associated with viral infection	Experimental Quantitative Study
8 ⁽³²⁾	2001	Netherlands	Develop a longitudinal analysis of VZV DNA on the ocular surface of patients with herpes zoster ophthalmicus	Experimental Quantitative Study
9 ⁽³³⁾	2002	UK	Determine whether ocular shedding of EBV in the tear film is peculiar to patients with Sjogren's syndrome, and whether co-infection with EBV occurs in the tear film	Experimental Quantitative Study
10 ⁽²⁴⁾	2002	Austria	Investigate a rapid and sensitive PCR-based assay for the detection of adenoviral infections	Experimental Quantitative Study
11 ⁽³⁴⁾	2002	France	Use a multiplex PCR to detect herpes viruses in tears from normal subjects and from patients with pathological conditions	Descriptive Quantitative Observational Study
12 ⁽⁵⁴⁾	2004	Singapore	Determine the prevalence of virus in bodily excretions, and time of seroconversion in discharged patients with SARS	Experimental Quantitative Study
13 ⁽²⁵⁾	2004	Brazil	Develop a rapid protocol to detect AdV in eye swab	Descriptive Quantitative Observational Study
14 ⁽²⁶⁾	2005	UK	Determine if AdV persists on the ocular surface following adenoviral conjunctivitis	Experimental Quantitative Study
15 ⁽²⁷⁾	2006	Japan	Establish a method of quantitative detection and rapid identification of AdV	Observational Quantitative Study
16 ⁽²⁸⁾	2010	China	Test if high-density resequencing microarray can be applied to detection of viruses in conjunctival swabs for patients with conjunctivitis	Experimental Quantitative Study
17 ⁽³⁵⁾	2011	Japan	Investigate if ICP0 of HSV-1 is detectable in the tear fluid of patients with HEK	Observational Quantitative Study
18 ⁽³⁶⁾	2013	Korea	Analyze the methodological efficacy of the PCR assay for HSV-1 detection in tears	Experimental Quantitative Study
19 ⁽³⁹⁾	2013	UK	Validate and introduce a simple boil extraction on dry swabs followed by amplification and real-time detection using 'in-house' assays for HSV and AdV with RNaseP as an internal control	Experimental Quantitative Study
20 ⁽³⁷⁾	2014	Spain	Evaluate the usefulness of PCR as a rapid diagnostic method compared with the viral culture, and to assess if conjunctival swabs samples were equivalent to corneal scrapings to diagnose of HK	Experimental Quantitative Study
21 ⁽³⁸⁾	2015	Japan	Investigate diagnostic efficacy of PCR and ELISA for HSV in tears	Nonrandomized Prospective Cross-Sectional Study
22 ⁽³⁰⁾	2015	UK	Estimate the diagnostic accuracy of the AdenoPlus point-of-care AdV test compared to PCR	Prospective Diagnostic Accuracy Study
23 ⁽³¹⁾	2016	India	Identify and characterize the viral etiological agents associated with keratoconjunctivitis	Retrospective Observational Study
24 ⁽⁵¹⁾	2016	UK	Report EBO virus RT-PCR data for body site and fluid samples from a large cohort of EBO virus survivors at clinic follow-up	Cross-Sectional Observational Study
25 ⁽⁵²⁾	2017	Germany	Evaluate a non-invasive detection method for HPV in ophthalmic pterygia	Observational Prospective Case Control Study
26 ⁽⁵³⁾	2017	Singapore	Check if Zika virus could be detected in human tears after the first week of infection	Descriptive Observational Study
27 ⁽³⁹⁾	2019	UK	Investigate the use of a corneal impression membrane for the detection of HSV-1	Experimental Quantitative Study
28 ⁽⁴¹⁾	2020	China	Report the ocular characteristics and the presence of viral RNA of SARS-CoV-2 in conjunctival swab specimens in a patient with confirmed Covid-19	Prospective Observational Case Study
29 ⁽⁴⁰⁾	2020	China	Detect SARS-CoV-2 in eye sample of one Covid-19 patient with obstruction of common lacrimal ducts	Prospective Observational Study
30 ⁽⁴²⁾	2020	India	Detect the presence of viral RNA of SARS-CoV-2 in conjunctival swab specimens of Covid-19 patients	Experimental Quantitative Study
31 ⁽⁴³⁾	2020	China	Describe the clinical spectrum of ocular symptoms and laboratory test in conjunctival swab samples	Cross-Sectional Study
32 ⁽⁴⁴⁾	2020	Canada	Present a case of Covid-19 with an initial medical presentation of keratoconjunctivitis	Descriptive Observational Case Study
33 ⁽⁴⁵⁾	2020	Spain	Evaluate the presence SARS-CoV-2 RNA in conjunctival swabs of Covid-19 patients	Cross-Sectional Study
34 ⁽⁴⁶⁾	2020	USA	Understand evidence about SARS-CoV-2 and ocular infection	Narrative Review
35 ⁽⁴⁷⁾	2020	China	Detect SARS-CoV-2 RNA in conjunctival swabs	Retrospective Observational Study
36 ⁽⁴⁸⁾	2020	France	Describe the multiplicity of ocular manifestations of Covid-19 patients	Descriptive Observational Study

IC: immunochromatography; EIA: enzyme immunoassay; EBO: Ebola virus; EBO-Z: EBO subtype Z; ICP0: infected cell protein 0; HEK: herpetic epithelial keratitis; HK: herpetic keratitis; ELISA: enzyme-linked immunosorbent assay

Figure 3

Identification of the included studies

Nº	Virus	Method of Collection	Method of Analysis	Nº of Patients Recruited	Nº of Samples	Result (+ or -)
1 ⁽¹⁹⁾	AdV	CS	IC and EIA	130	130	+
2 ⁽²⁰⁾	AdV	CS	PCR	68	68	+
3 ⁽²¹⁾	Adv / EV70 / CA24v	CS	Direct smear, PCR and virus isolation	20	20	+ / - / -
4 ⁽⁵⁰⁾	EBO	CS	RT-PCR	7	38	+
5 ⁽⁴⁹⁾	CMV	CS	Immunofluorescence and PCR	13	60	+
6 ⁽²²⁾	AdV / HSV	CS	PCR	541	805	+ / +
7 ⁽²³⁾	AdV / EV70 / CA24v	CS	PCR, RT-PCR, culture isolation and neutralization test	113	113	+ / - / -
8 ⁽²²⁾	VZV, HSV	CS	PCR	21	246	+ / -
9 ⁽³³⁾	EBV-1 / EBV-2 / CMV / VZV / HSV	ST	PCR	54	54	+ / + / - / - / -
10 ⁽²⁴⁾	AdV	CS	PCR	15	15	+
11 ⁽³⁴⁾	HSV-1 / HSV-2 / VZV / CMV / EBV / HHV-6	ST	PCR	93	186	+ / + / + / + / + / +
12 ⁽⁵⁴⁾	SARS-CoV	CS	PCR	64	126	-
13 ⁽²⁵⁾	AdV	CS	PCR	7	7	+
14 ⁽²⁶⁾	AdV / HSV	ST and CS	PCR	30	90	+ / -
15 ⁽²⁷⁾	AdV	CS	PCR	133	133	+
16 ⁽²⁸⁾	AdV	CS	High-density resequencing microarray and PCR	38	114	+
17 ⁽³⁵⁾	HSV-1	ST	Immunoblot analysis	18	18	+
18 ⁽²⁶⁾	HSV	ST	PCR	115	115	+
19 ⁽²⁹⁾	HSV-1 / HSV-2 / AdV	CS	PCR	541	541	+ / + / +
20 ⁽²⁷⁾	HSV	CS	PCR	188	188	+
21 ⁽³⁸⁾	HSV	ST	PCR and ELISA	82	82	+
22 ⁽³⁰⁾	AdV	CS	PCR and point-of-care test	109	109	+
23 ⁽³¹⁾	AdV / EV	CS	PCR and RT-PCR	23	23	+ / -
24 ⁽⁵¹⁾	EBO	CS	RT-PCR	112	92	-
25 ⁽⁵²⁾	HPV	CS	PCR	21	42	+
26 ⁽⁵³⁾	Zika virus	CS	RT-PCR	29	58	+
27 ⁽³⁹⁾	HSV-1	CS	PCR	110	220	+
28 ⁽⁴¹⁾	SARS-CoV-2	CS	RT-PCR	1	8	+
29 ⁽⁴⁰⁾	SARS-CoV-2 / HSV-1 / HHV-6B	CS	RT-PCR and PCR	1	20	+ / + / +
30 ⁽⁴²⁾	SARS-CoV-2	CS	RT-PCR	45	45	+
31 ⁽⁴³⁾	SARS-CoV-2	CS	RT-PCR	2	4	+
32 ⁽⁴⁴⁾	SARS-CoV-2	CS	RT-PCR	1	2	+
33 ⁽⁴⁵⁾	SARS-CoV-2	CS	RT-PCR	36	72	+
34 ⁽⁴⁶⁾	SARS-CoV-2	Narrative Review				
35 ⁽⁴⁷⁾	SARS-CoV-2	CS	RT-PCR	33	66	+
36 ⁽⁴⁸⁾	SARS-CoV-2	CS	RT-PCR	1	2	+

Figure 4

Main results of the included studies