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

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Systematic Review

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Abstract

Purpose

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 developed in the PubMed, Web of Science and BVS databases and followed the Scoping Review protocol defined by Joana Brigs 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 studies published in English 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, but there is evidence of ocular detection on more viruses.

Discussion

Studies have generally been conducted to understand viral infection, to develop accurate diagnostic methods and to follow the patient’s response to treatment. Most studies were developed on 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.

Background

Viruses are exclusively intracellular parasites and are also the smallest infectious agents known [1]. 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 infection. Once inside the cell, the virus may damage or destroy it through direct cytophatic effects, host antiviral immune responses, and/or transformations of the infected cells [2].

It is known that the eye is a site for viral infection that might appear in the intra- or extraocular space without visible systemic reverberation and affect multiple structures with variable manifestations [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 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.

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 [6].

In 1903, Otto Schirmer, also a German ophthalmologist, shortened the size of the paper strips and quantified tear production for 5 minutes by three distinct methods: in method I, the patient remained blinking normally; in method II, the ocular surface was anesthetized with topical cocaine, and the nasal mucosa was stimulated; and in method III, the ocular surface was also anesthetized, but the patient kept looking for the sun during the test. These methods analyzed three tearing stimulation pathways: ocular and palpebral mucosa, nasal mucosa, and the retina [6, 7]. 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 realized 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 above 15 mm, but those can vary according principally to medication use, age and chronic diseases [8-10].

Conjunctival swab

Conjunctival swabs are the most commonly used method for microbiological analysis because they permit the collection of cells and materials dispersed in the conjunctival sac instead of tears alone [1]. 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 [11]. Topic anesthesia can be used to make the procedure more confortable, since there is no significant difference in the final result when samples are analyzed by

polymerase chain reaction (PCR) methods [12]. Proxymetacaine 0.5% is recommended once, and commercially available eye drops show fewer bactericidal effects [13].

Current scenario

The World Health Organization (WHO) recently declared COVID-19 a pandemic threat, because of that, the academic community has concentrated all forces 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 [14, 15]. Since then, several studies on ocular manifestations of SARS-CoV-2 have been published, but virus collection methods and associations with the ocular surface are not clearly stated. 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 [16]. All searches and publication access were completed in June 2020.

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, "C" as Schirmer Test and conjunctival swab, and the last "C" as virus.

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 BVS. Included articles were only 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 [17].

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 current decade, 2011-2020. The evolution of published articles on AdV, HSV and SARS-CoV-2 is represented in Fig. 2. 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. Most studies were on AdV [18-30] and HSV [21, 25, 28, 31-39], followed by SARS-CoV-2 [39-47]. VZV [31-33], CMV [31, 32, 48], EBV [31, 32], EBO [49, 50], HPV [51] and Zika virus [52] have also been identified on the ocular surface, but there are still few studies on this topic.

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 a pandemic, and the world's research efforts were completely directed to overcoming the pandemic. The linear evolution of published articles on AdV, HSV and SARS-CoV-2 is represented in Fig. 2. In contrast to SARS-CoV [53], SARS-CoV-2 RNA was found by RT-qPCR in conjunctival swabs [39-47], although samples were collected from a few patients, and only a low and varying percentage of them presented positive results and/or ocular symptoms [45]. Most of those studies were developed in China [39, 40, 42, 46], mainly because the country was the first epicenter of the disease.

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 [31-33].

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 [48]. No other studies on the topic were found in this review, probably because the incidence of CMV retinitis in the AIDS population significantly decreased with the introduction of effective antiretroviral therapy and early accurate diagnosis [54-57].

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 process that explains how liquids impregnate porous materials differently [58, 59]. Consequently, the samples collected represent two different materials: tears, cells and fluids dispersed in the conjunctival sac and tears and substances dissolved in it [1].

Samples were mainly analyzed by qPCR (quantitative PCR) or RT-qPCR (reverse transcriptase-qPCR), depending on the viral genetic material, DNA or RNA, respectively. PCR variations are widely used because they permit the replication and detection of low loads of viral DNA/RNA [60]. The point-of-care test was also compared with PCR effectiveness, sensitivity and specificity where high, but this field was only explored in AdV [29], probably because of epidemiological factors related to uncontrolled and fast spread of AdV conjunctivitis, which are decreasing due to the development of new drugs and treatment options [61].

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. The results of the analyzed studies were substantial, but most were developed on a small number of patients and lacked clear definitions of collection time and viral persistence since the onset of diseases. Additional studies with larger populations and time permanence are necessary to develop more assertive conclusions on the theme.

Declarations

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Conflicts of interest

The authors have no conflicts of interest to declare.

Availability of data and material

Not applicable

Code availability

Not applicable

Author's contributions

Luís Expedito Sabage, Alessadra Mazzo and Luiz Fernando Manzoni Lourençone designed the work, acquired the data, contributed to the analysis and interpretation of data, and drafted the paper. Josmar Sabage and Taylor Endrigo Toscano Olivo contributed to the analysis and interpretation of data and revised the paper. All authors approved the final version.

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Figures

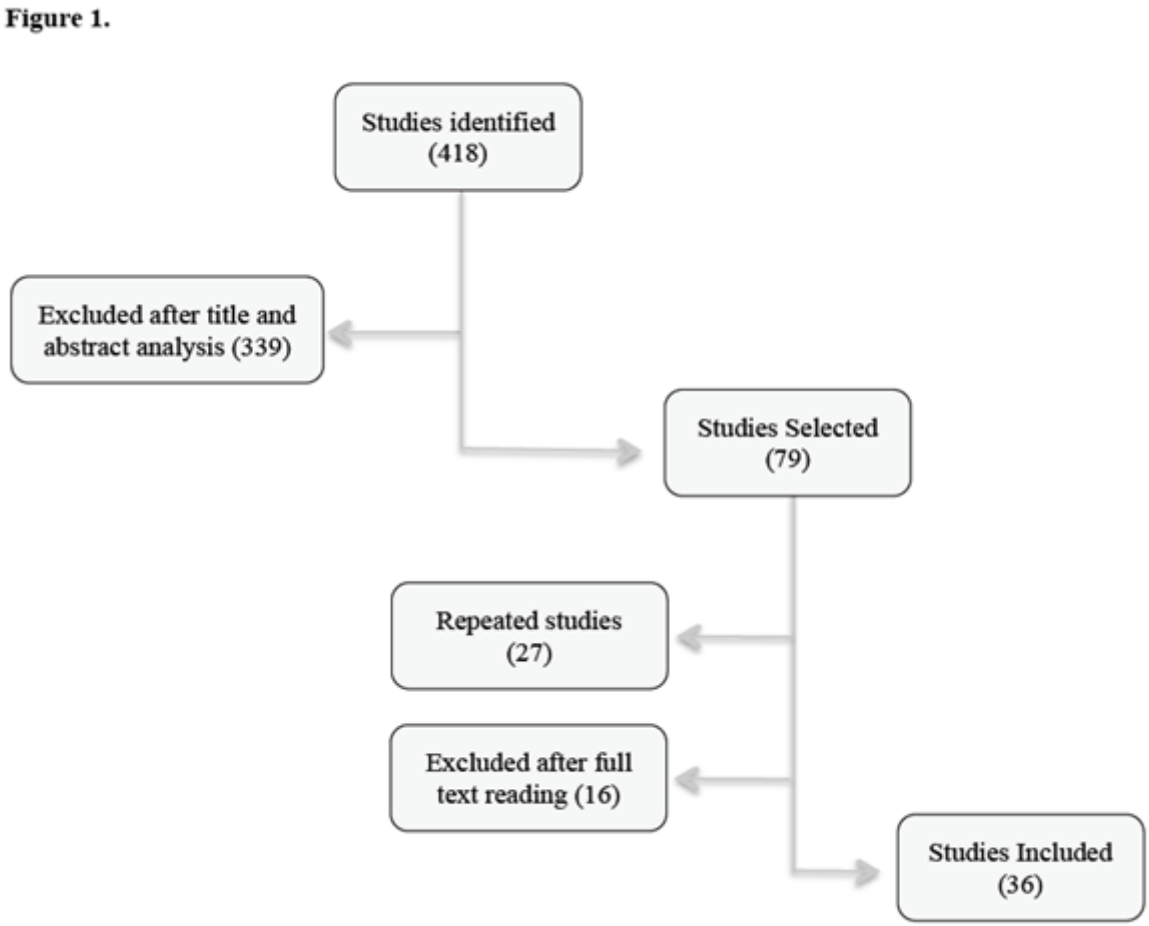

Figure 1

Process of inclusion

Figure 2.

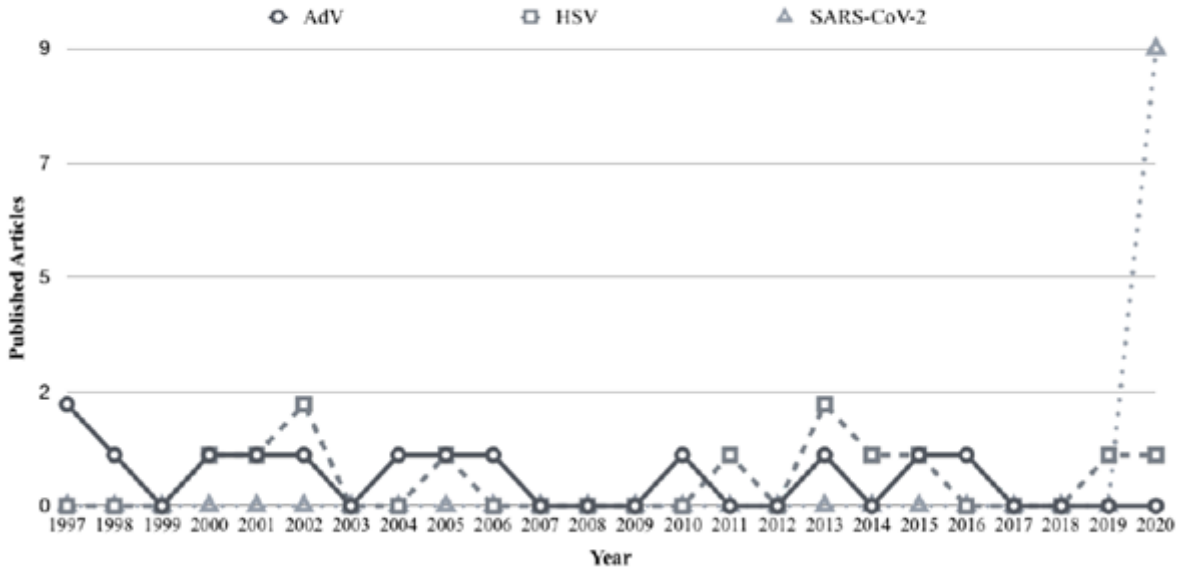

Figure 2

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

Figure 3.

Year	Country	Purpose	Methodology
1997	Japan	Compare IC and EIA tests for AdV detection	Experimental Quantitative Study
1997	Germany	Evaluate type-specific primers for AdV	Experimental Quantitative Study
1998	India	Develop and evaluate nested PCR as a tool for detecting AdV from conjunctival swabs	Descriptive Observational Study
1999	USA	Determine the genetic stability of EBO-Z, and whether additional strains of EBO virus were circulating during Kikwit outbreak	Experimental Quantitative Study
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
2000	UK	Develop a multiplex PCR for the detection of AdV, HSV, and Chlamydia trachomatis in conjunctival swabs	Experimental Quantitative Study
2001	Taiwan	Evaluate the sensitivity and applicability of PCR and RT-PCR diagnoses for keratoconjunctivitis associated with viral infection	Experimental Quantitative Study
2001	Netherlands	Develop a longitudinal analysis of VZV DNA on the ocular surface of patients with herpes zoster ophthalmicus	Experimental Quantitative Study
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
2002	Austria	Investigate a rapid and sensitive PCR-based assay for the detection of adenoviral infections	Experimental Quantitative Study
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
2004	Singapore	Determine the prevalence of virus in bodily excretions, and time of seroconversion in discharged patients with SARS	Experimental Quantitative Study
2004	Brazil	Develop a rapid protocol to detect AdV in eye swab	Descriptive Quantitative Observational Study
2005	UK	Determine if AdV persists on the ocular surface following adenoviral conjunctivitis	Experimental Quantitative Study
2006	Japan	Establish a method of quantitative detection and rapid identification of AdV	Observational Quantitative Study
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
2011	Japan	Investigate if ICP0 of HSV-1 is detectable in the tear fluid of patients with HEK	Observational Quantitative Study
2013	Korea	Analyze the methodological efficacy of the PCR assay for HSV-1 detection in tears	Experimental Quantitative Study
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
2014	Spain	Evaluate the usefulness of PCR as a rapid diagnostic method compared with the viral culture, and	Experimental Quantitative Study

Figure 3

Year of publication, country of development, purpose and methodology of all the included studies

Figure 4.

№	Virus	Method of Collection	Method of Analysis
1	AdV	CS	IC and EIA
2	AdV	CS	PCR
3	AdV / EV70 / CA24v	CS	Direct smear, PCR and virus isolation
4	EBO	CS	RT-PCR
5	CMV	CS	Immunofluorescence and PCR
6	AdV / HSV	CS	PCR
7	AdV / EV70 / CA24v	CS	PCR, RT-PCR, culture isolation and neutralization test
8	VZV / HSV	CS	PCR
9	EBV-1 / EBV-2 / CMV / VZV / HSV	ST	PCR
10	AdV	CS	PCR
11	HSV-1 / HSV-2 / VZV / CMV / EBV / HHV-6	ST	PCR
12	SARS-CoV	CS	PCR
13	AdV	CS	PCR
14	AdV / HSV	ST and CS	PCR
15	AdV	CS	PCR
16	AdV	CS	High-density resequencing microarray and PCR
17	HSV-1	ST	Immunoblot analysis
18	HSV	ST	PCR
19	HSV-1 / HSV-2 / AdV	CS	PCR
20	HSV	CS	PCR
21	HSV	ST	PCR and ELISA
22	AdV	CS	PCR and point-of-care test
23	AdV / EV	CS	PCR and RT-PCR
24	EBO	CS	RT-PCR
25	HPV	CS	PCR
26	Zika virus	CS	RT-PCR
27	HSV-1	CS	PCR
28	SARS-CoV-2	CS	RT-PCR
29	SARS-CoV-2 / HSV-1 / HHV-6B	CS	RT-PCR and PCR
30	SARS-CoV-2	CS	RT-PCR
31	SARS-CoV-2	CS	RT-PCR
32	SARS-CoV-2	CS	RT-PCR
33	SARS-CoV-2	CS	RT-PCR
35	SARS-CoV-2	CS	RT-PCR
36	SARS-CoV-2	CS	RT-PCR

*Study 34 was not included in this table because it is a narrative review
CS = conjunctival swab; ST = Schirmer test

Figure 4

Researched virus and methods of sample collection and analysis

Figure 5.

№	Virus	№ of Patients Recruited	№ of Samples	Result (+ or -)
1	AdV	130	130	+
2	AdV	68	68	+
3	Adv / EV70 / CA24v	20	20	+ / - / -
4	EBO	7	38	+
5	CMV	13	60	+
6	AdV / HSV	541	805	+ / +
7	AdV / EV70 / CA24v	113	113	+ / - / -
8	VZV, HSV	21	246	+ / -
9	EBV-1 / EBV-2 / CMV / VZV / HSV	54	54	+ / + / - / - / -
10	AdV	15	15	+
11	HSV-1 / HSV-2 / VZV / CMV / EBV / HHV-6	93	186	+ / + / + / + / + / +
12	SARS-CoV	64	126	-
13	AdV	7	7	+
14	AdV / HSV	30	90	+ / -
15	AdV	133	133	+
16	AdV	38	114	+
17	HSV-1	18	18	+
18	HSV	115	115	+
19	HSV-1 / HSV-2 / AdV	541	541	+ / + / +
20	HSV	188	188	+
21	HSV	82	82	+
22	AdV	109	109	+
23	AdV / EV	23	23	+ / -
24	EBO	112	92	-
25	HPV	21	42	+
26	Zika virus	29	58	+
27	HSV-1	110	220	+
28	SARS-CoV-2	1	8	+
29	SARS-CoV-2 / HSV-1 / HHV-6B	1	20	+ / + / +
30	SARS-CoV-2	45	45	+
31	SARS-CoV-2	2	4	+
32	SARS-CoV-2	1	2	+
33	SARS-CoV-2	36	72	+
35	SARS-CoV-2	33	66	+
36	SARS-CoV-2	1	2	+

*Study 34 was not included in this table since it is a narrative review

Figure 5

Researched virus, number of patients recruited, total of samples, and final results