

IMMUNOCHROMATOGRAPHIC ANALYSIS: ASSESSMENT AND PROSPECTS FOR APPLICATION IN THE DIAGNOSIS OF ROTAVIRUS AND ADENOVIRUS INFECTIONS

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ABSTRACT

The objective of this review was to provide information on immunochromatographic assays (ICA) relevant to the diagnosis of human adenovirus (HAdV) and rotavirus (HRV) infections. HAdVs and HRVs cause respiratory and gastrointestinal diseases in children and are characterized by strong inflammatory responses. Virological diagnostic methods include virus isolation in cell cultures, molecular-biological techniques, and immunological assays. These methods are considered the gold standard; however, obtaining results requires a significant amount of time. The development of reverse transcription polymerase chain reaction (RT-PCR) has greatly facilitated the diagnosis of viral infections due to its high specificity and sensitivity. However, the complexity of the reaction protocol and the need for additional laboratory equipment make RT-PCR unsuitable for point-of-care testing. Immunochromatographic analysis, due to its low cost, simple sample preparation, and ease of use, represents an excellent alternative for bedside diagnostics. In studies on other infections, ICA has demonstrated the absence of cross-reactivity, high reproducibility, and acceptable sensitivity compared to enzyme-linked immunosorbent assay (ELISA) and RT-PCR. In conclusion, ICA test systems may be useful in clinical practice for the rapid detection of rotavirus and adenovirus infections. However, the possibility of false-positive results and differences in subtype identification across various diagnostic methods should be taken into account.

Keywords: rotavirus, adenovirus, diagnosis, polymerase chain reaction, ELISA, immunochromatographic analysis, colloidal gold.

INTRODUCTION

The incidence of rotavirus (HRV) and adenovirus (HAdV) infections in young children is extremely high in developing countries [1]. HRV is the primary causative agent of severe diarrhea in this age group, leading to a significant number of hospitalizations and fatalities, as well as considerable economic burdens on healthcare systems. HRV accounts for approximately 2 million hospitalizations and 450,000 deaths of children worldwide, with the highest mortality rates recorded in Asia and Africa [2]. Another significant etiological factor in infants and young children is HAdV. Hospital-based studies have established a link between adenoviral infection and gastrointestinal disease. In recent years, adenovirus has played an increasingly important role among children who have undergone bone marrow transplantation. Infection with this virus can result in severe complications, increased mortality risk, and prolonged disease duration. Given these factors, the accurate and timely identification of the causative pathogen remains a critical issue in viral infections [3].

Timely and effective antiviral interventions require accessible, convenient, and rapid diagnostic methods. Currently, polymerase chain reaction (PCR) and enzyme-linked immunosorbent assay (ELISA) are the most widely used techniques for diagnosing viral infections. However, their multi-step protocols and the need for specialized laboratory equipment make them less effective for large-scale screenings. In contrast, immunochromatographic assays (ICA) is a one-step method that can be performed without additional equipment or reagents [4]. These technical characteristics make ICA well-suited for rapid testing and easy visual interpretation of results in point-of-care settings [5]. Additional advantages of ICA include low cost, long shelf life, and simple sample preparation [6, 7].

In recent years, ICA technology has been increasingly utilized not only in infectious disease diagnostics but also in

food safety monitoring. The scope of ICA applications has significantly expanded with advancements in nanotechnology. As improvements in sensitivity and performance continue, ICA is emerging as a key approach for pathogen detection. This review discusses the fundamental principles and classification of ICA technology, examines current trends in human rotavirus (HRV) and human adenovirus (HAdV) diagnostics, and highlights recent advancements aimed at enhancing the sensitivity of ICA test systems.

Diagnosis of Adenovirus and Rotavirus Infections

Diagnosis of HAdV and HRV infections relies on standard virological, molecular, and immunological methods. Virological methods include virus isolation and culture, electron microscopy, and serological tests [8]. Molecular methods primarily involve various types of PCR screening [9]. Immunological approaches based on antigen–antibody interactions include immunofluorescence assay (IFA), enzyme-linked immunosorbent assay (ELISA), and ICA [10–12].

In an evaluation of ICA for the rapid detection of adenoviral conjunctivitis, the sensitivity of the control and test kits was 89.8% and 98.3%, respectively, while the specificity of both kits was 100%. A significant difference was observed in the sensitivity of the two ELISA kits for samples that tested positive by PCR. The sensitivity of the test kit was 32–64 times higher than that of the control kit for both adenovirus types. These findings suggest that the new enhanced kit for detecting adenovirus in tears, including conjunctival exudates, is advantageous because it reduces patient discomfort during specimen collection and offers substantially higher sensitivity compared with the conventional ICA kit [13,14].

The HAdV ICA kit was evaluated using 138 nasopharyngeal specimens collected from patients at a clinic in Japan between January and June 2003. Patients had clinical manifestations of pharyngoconjunctival fever ($n = 38$) or exuda-

tive tonsillitis ($n = 100$). The ICA kit yielded positive results in 84% (116 of 138) of patients diagnosed at the bedside. The remaining ICA test extract solutions were transferred to maintenance medium and tested by in vitro diagnostics. The ICA demonstrated 95% sensitivity (116 of 122 patients) when compared with virus isolation as the reference standard, and 91% sensitivity (116 of 128 patients) when compared with PCR. All ICA-positive samples were confirmed positive by both isolation and PCR. Similarly, all isolation-positive samples were also PCR-positive [15]. Twenty-two ICA-negative samples were further tested by real-time PCR. Among these, six samples that were ICA-negative but isolation-positive contained between 3.8×10^7 and 2.5×10^9 HAdV genome copies/mL. Five samples positive only by PCR contained between 3.0×10^4 and 3.8×10^5 HAdV genome copies/mL, whereas one sample tested negative by real-time PCR. Taken together, these results indicate that the IC kit is a useful point-of-care diagnostic tool for HAdV infections, with 95% sensitivity compared with virus isolation. However, a negative result does not definitively exclude HAdV infection.

The sensitivity and specificity of a new ICA kit, ALSONIC® Adeno (Alfresa Pharma Co., Osaka, Japan), designed for the detection of HAdV in throat swabs, were evaluated against real-time PCR results. The sensitivity and specificity of the ICA were 92.2% (83/90) and 95.1% (58/61), respectively. The assay also demonstrated positive and negative predictive values of 96.5% (83/86) and 89.2% (58/65), respectively. These findings indicate that ALSONIC® Adeno is a reliable diagnostic tool for the acute phase of HAdV infection [16, 17, 18].

Fundamentals of the immunochromatographic assay principle

The immunochromatographic assay (ICA) method is based on a combination of antigen-antibody immunological interactions and chromatographic separation of sample components. The separation process relies on differences in the affinity of sample components for the mobile and stationary phases of the chromatographic system. Due to its high selectivity and efficiency, chromatographic separation (e.g., thin-layer chromatography) is widely used for detecting target molecules in complex mixtures. In ICA, antibodies are commonly employed as the primary component for detecting or quantifying immunogenic molecules [19]. In addition to antibodies, ICA can incorporate chemical substances with affinity for the target molecule. The method is applicable for detecting both low-molecular-weight compounds (e.g., drugs, hormones, herbicides) and high-molecular-weight substances (e.g., peptides, proteins, viruses, bacteria) [20].

The test system design consists of four main membrane components: a sample pad, a conjugate pad, a nitrocellulose membrane, and an absorbent pad. Assembly is performed by sequentially layering the sample pad, conjugate pad, and absorbent pad onto the nitrocellulose membrane (Fig. 1). The assembled structure is then laminated and cut into strips with a width of 3.5 mm [21]. A biological sample is applied to the sample pad, after which capillary forces drive the sample through the strip. Upon reaching the conjugate pad, labeled antibodies conjugated with colloidal gold bind to the target analyte (e.g., an antigen, if present), forming an antigen-antibody complex. The sample then migrates to the test

line, where immobilized antibodies specific to the target antigen capture the complex, resulting in a visible signal. A control line, located further along the strip, contains immobilized anti-species monoclonal antibodies that bind to the labeled antibodies, verifying proper test performance [22]. Depending on the detection system, visualization markers, and the test's specific application, ICA can be categorized into different formats. Based on the chemical detection mechanism, ICA is classified into the «sandwich» format, the competitive format, and the multiplex format.

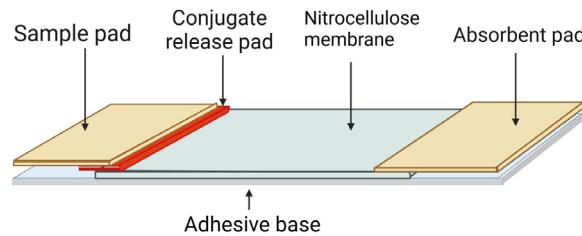


Figure 1. Design of the immunochromatographic test system

In the «sandwich» format, colloidal gold-conjugated specific antibodies are pre-deposited on the conjugate pad. The primary antibody against the antigen is immobilized on the test line, while a secondary antibody against the labeled antibody is immobilized on the control line. When the target antigen is present in the sample, it forms an antigen-antibody complex, which is captured by the immobilized antibodies at the test line, generating a visible signal (Fig. 2) [23].

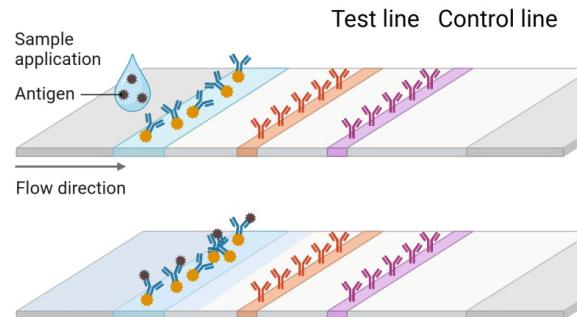


Figure 2. «Sandwich» version of the immunochromatographic test system

In the competitive format, specific antibodies conjugated to colloidal gold are also deposited on the conjugate pad. Unlike the sandwich format, the test line contains immobilized target antigen. Secondary antibodies against the labeled antibody are immobilized on the control line. If the target antigen is present in the sample, it competes with the pre-bound antigen on the test line, preventing the conjugated antibodies from binding. This results in the absence of a visual signal at the test line. Conversely, if the target antigen is absent in the sample, the conjugate binds to the immobilized antigen, generating a visible signal, which indicates a negative result (Fig. 3) [23]. The multiplex format is designed for the simultaneous detection of multiple target analytes. It involves test strips with multiple test lines, each corresponding to a different target species [24].

The most commonly used marker for visualization in ICA

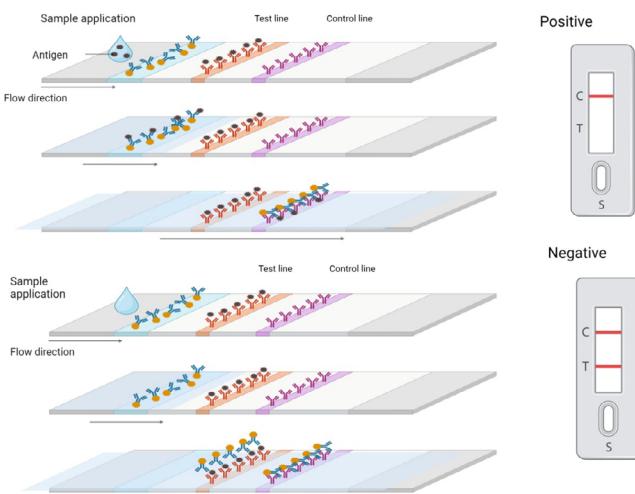


Figure 3. Competitive version of the immunochromatographic test system

is colloidal gold. Its widespread use is attributed to its unique optical properties, including strong light absorption at specific wavelengths and high conductivity due to surface electrons, as well as the ease of surface modification [25]. Colloidal gold nanoparticles can be synthesized in various shapes and sizes, making them indispensable for interactions with biomolecules and organelles [25]. Traditional ICA systems based on colloidal gold typically utilize gold nanoparticles ranging from 30 to 40 nm in diameter [26]. To enhance assay performance, new gold-containing nanomaterials with improved optical and colorimetric properties are being developed. These include gold nanoflowers, nanorods, and hybrid structures such as magnetic gold nanoparticles, which offer tunable size, shape, and surface modification capabilities [26,27].

The ICA has gained widespread application due to its simplicity, rapid analysis time, and low cost. ICA-based test systems have demonstrated significant potential in environmental monitoring and food safety control [28]. Their reliability and efficiency have been confirmed in diagnosing myocardial infarction by detecting cardiac troponins [26] and human fatty acid-binding protein (hFABP) [29]. ICA is also extensively used for the diagnosis of bacterial, viral, and parasitic infections [30-34].

One of the key advantages of ICA test systems is their suitability for use in peripheral healthcare facilities, where access to specialized equipment and trained personnel may be limited. These settings often serve the majority of the population, particularly in rural areas with frequent power outages that can disrupt the storage conditions of biological reagents and diagnostic materials [35]. Another important benefit distinguishing ICA from ELISA is its ability to analyze small sample volumes, making it more practical for field applications [36].

However, ICA tests still face limitations in certain regions endemic to specific diseases, such as malaria. Some ICA tests require refrigeration of reagents and have a long incubation time of 18–24 hours before results become available. Additionally, more than 20 companies producing ICA-based malaria tests have not undergone rigorous validation, leading to inconsistencies in test performance outside research institutions [37,38].

The ICA integrates advancements in nanotechnology, immunology, and chromatography, offering ease of use, time efficiency, high sensitivity, and low cost. Among the various ICA formats, gold nanoparticles (AuNPs) are the most commonly used signal markers. However, their sensitivity and accuracy are constrained by the low brightness of the colorimetric signal and slow polyclonal antibody binding rates. To improve ICA performance, researchers have explored alternative nanomaterials, including quantum dots (QDs), metal-organic frameworks, time-resolved fluorescent microspheres, magnetic nanoparticles, and other cutting-edge technologies [39].

ICA variants can be classified based on the type of markers used, such as colloidal gold, QDs, chemiluminescent materials, magnetic markers, and nanostructured labels [21,40]. Novel detection techniques include absorption and fluorescence-based assays, as well as chemiluminescence, electrochemical and radiometric detection, thermal measurements, and mass spectrometry. Many of these methods require antibody- or antigen-conjugated chemical or enzymatic labels, but certain ICA variants allow for direct pathogen detection by leveraging its intrinsic properties [41,42].

Among emerging technologies, quantum dots (QDs) have shown great potential due to their high stability, large extinction coefficients, high quantum yield, and long fluorescence lifespan. These advantages make QDs ideal markers for developing ultrasensitive ICA test systems [43,44].

Magnetic nanoparticles (MNPs) are another promising innovation in diagnostic assays, drug tracking, targeted therapy, and magnetic resonance imaging (MRI) [45]. Unlike colloidal gold, MNPs can be easily separated using an external magnetic field, allowing for efficient target molecule concentration and extraction from samples. Additionally, MNPs serve as signal markers due to their distinctive bright brown coloration, further expanding their applications in rapid and highly specific ICA-based diagnostics [46].

CONCLUSION

Immunochromatographic assays (ICA) are effective and rapid tools for the primary diagnosis of rotavirus, adenovirus, and norovirus infections, particularly in settings with limited

laboratory resources. Despite their simplicity and speed, ICA tests have lower sensitivity compared to molecular methods, which requires confirmation of negative results using PCR or other highly sensitive molecular techniques. Therefore, ICA should be used as a screening tool, complemented by molecular diagnostics to enhance the accuracy of viral pathogen detection.

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COMPETING INTERESTS

The authors declare that they have no competing interests.

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ИММУНОХРОМАТОГРАФИЯЛЫҚ ТАЛДАУ: РОТАВИРУС ЖӘНЕ АДЕНОВИРУС ИНФЕКЦИЯЛАРДЫ ДИАГНОСТИКАДА ПАЙДАЛАНУ БОЛАШАҒЫ ЖӘНЕ БАҒАЛАУ

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ТҮЙИН

Әдеби шолу мақсаты адамның аденоцирустық (HAdV) және ротавирустық (HRV) инфекцияларың диагностикаға көзісты иммунохроматографиялық талдаулар туралы ақпаратты ұсыну болып табылады. HAdV және HRV балалардың тыныс алу және ас қорыту жүйесілерінің ауруларың тудырады және күшті қабыну реакцияларымен сипатталады. Вирусологиялық диагностика әдістеріне жатады выириусты жасуша дақылдары бойынша оқшаулау, молекулярлы-биологиялық және иммунологиялық әдістері. Қорсетілген әдістер алтын стандарт болып саналады, бірақ талдау қорытынды алу процесі көп уақыт алады. Кері транскрипциялық полимеразды тізбекті реакция (РТ-ПТР) әдістерінің дамуы оның жоғары ерекшелігі мен сезімталдығына байланысты вирустық инфекциялардың диагностикасын айттарлықтай женеілдетті. Дегенмен, құрделі реакция хаттамасы және қосымша зертханалық жабдықты пайдалану емдедушінің төсегінің жаһында аурухана жағдайында RT-ПТР қолдану мүмкін емес. Иммунохроматографиялық талдау (ИХТ) өз арзандылығына, үлгіні дайындау мен талдаудың қарапайымдылығына байланысты науқастың төсегінде инфекцияны диагностикалау үшін тамаша баламасы болып табылады. Басқа инфекциялар үлгі бойынша ИХТ иммуноферменттық талдау (ИФА) және RT-ПТР-мен салыстырғанда ешқандай айқаспалы реaktivтілікті, жоғары қайталануды және қолайлы сезімталдықты қорсетпеді. Қорытындысында, ИХА тест жүйелері ротавирустық және аденоцирустық инфекцияларды жылдам анықтау үшін клиникалық тәжірибеде пайдалы болуы мүмкін деп айтуга болады. Дегенмен, әртүрлі диагностикалық әдістерді пайдаланған кезде жалған оң нәтижелер алу мүмкіндігін және қосалқы типтеудегі айырмашылықтарды ескеру кажет.

Кілт сөздер: ротавирус, аденоцирус, диагностика, полимеразды тізбекті реакция, ELISA, иммунохроматографиялық талдау, коллоидті алтын.

ИММУНОХРОМАТОГРАФИЧЕСКИЙ АНАЛИЗ: ОЦЕНКА И ПЕРСПЕКТИВЫ ПРИМЕНЕНИЯ В ДИАГНОСТИКЕ РОТО- И АДЕНОВИРУСНОЙ ИНФЕКЦИЙ

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АННОТАЦИЯ

Целью обзора являлось предоставление информации по иммунохроматографическим анализам имеющее отношение к диагностике аденоцирусной (HAdV) и ротавирусной (HRV) инфекций человека. HAdV и HRV вызывают заболевание органов дыхания и пищеварительной системы детей и характеризуются сильными воспалительными реакциями. Вирусологические методы диагностики включают выделение вируса на клеточных культурах, молекулярно-биологические и иммунологические методы. Представленные методы считаются золотым стандартом, однако процесс получения результата занимает длительное время. Развитие методов полимеразной цепной реакции с обратной транскрипцией (RT-PCR) значительно упростило диагностику вирусных инфекций за счет высокой специфичности и чувствительности. Однако, сложный протокол реакции и использование дополнительного лабораторного оборудования делает невозможным применения RT-PCR в стационарных условиях у постели пациента. Иммунохроматографический анализ (ICA) вследствие своей дешевизны, простоты подготовки исследуемого образца и постановки анализа, является прекрасной альтернативной позволяющие диагностировать инфекцию у постели пациента. На примере других инфекций ИХА демонстрировала отсутствие перекрестной реактивности, высокую воспроизводимость и приемлемую чувствительность в сравнении с методами иммуноферментным анализом (ELISA) и RT-PCR. В заключении можно сказать, что ИХА тест-системы могут быть полезными в клинической практике для быстрого выявления ротавирусной и аденоцирусной инфекции. Однако следует учитывать вероятность ложноположительных результатов, а также различия в определении подтипов при использовании различных методов диагностики.

Keyword: ротавирус, аденоцирус, диагностика, полимеразная цепная реакция, ELISA, иммунохроматографический анализ, коллоидное золото.