Application of Gold Nanoparticle-Based Colorimetric Methods in Virus Detection: A Narrative Review

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Abstract. Virus infections are one of the leading causes of rising global health threats. Increasing virus infections will encourage the development of faster and more accurate diagnostic methods. Gold nanoparticle biosensors have the potential to become a simple and rapid colorimetric-based diagnostic method. This study aims to determine the sensitivity and selectivity of gold nanoparticle-based colorimetric methods as an alternative for the rapid detection of several types of viruses that cause infection in humans. This narrative review was developed based on articles found from search engines ACS Publication and PubMed using the keywords ("gold nanoparticles") AND colorimetric detection AND virus, which resulted in 494 articles. The inclusion criteria included articles published from 2016 to 2020, full-text articles, origin articles, and research articles. The exclusion criteria were as follows: articles about viruses that did not cause infection in humans, articles that were not available in full text, and articles on metal nanoparticles other than gold, and 10 articles were selected for review. The results showed there were some colorimetric detection methods for viruses, with a detection limit of up to 1 pg/ml and a detection time of 10–30 min. Based on the results of a narrative review, this method is expected to be further developed due to its high sensitivity and selectivity of detection, particularly for SARS-CoV-2 detection.

Keywords: Virus · Human · Colorimetric Detection · Gold Nanoparticles

1 Introduction

Viruses are one of the most common causes of human illness, with symptoms ranging from mild to severe to fatal. Viral infection causes severe morbidity and mortality in humans [1]. Virus outbreaks worldwide necessitate the development of rapid and precise diagnostic methods [2]. Early detection of the virus helps in targeted therapy, prolongs the treatment period, reduces treatment costs and morbidity, and reduces transmission, all of which contributes to the prevention and lead to more effective management and control of treatment disease [3].

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Colorimetric sensors based on metal nanoparticles may be suitable for the development of simple and rapid colorimetric diagnostic tests, with gold is generally preferred over other metals [4]. The application of gold nanoparticles as a colorimetric sensor is based on the aggregation and disaggregation of gold nanoparticles in response to the detection of specific targets. Reduced particles distance leads to aggregation, which changes the optical properties of gold nanoparticles such as a shift in the plasmon band or a significant color change from red to blue-violet visible to the naked eye [5–8]. The operating principles of these sensors are based on changes in the refractive index of the medium around gold nanoparticles caused by analyte molecule attached to the particle surface or other modifications of the ligand on the particle surface. Another widely used mechanism is the aggregation of gold nanoparticles in colloidal systems caused by the hybridization of specific functional groups such as oligonucleotides, which allows the recognition and selective binding of gold nanoparticles with complementary functions [9]. In the visible absorption spectrum of the solution, the SPR of the spherical gold colloid occurs at ~520 nm, depending on the size of metal particles [10]. Surface Plasmon Resonance (SPR) is a phenomenon that occurs when electrons in a metal surface layer are exited at a specific angle of incidence by photons of incident light traveling parallel to the metal surface [11].

Colorimetric detection with the naked eye is widely accepted due to its simplicity and utility. Colorimetric approaches based on metal nanoparticles, in particular, have received a lot of attention because of their low cost, simplicity, and convenience, as well as the unique optical properties of metal nanoparticles. Gold nanoparticles are commonly used for colorimetric analysis due to their high extinction coefficient and distance-dependent optical properties. To date, gold nanoparticle-based colorimetric assays have been widely used to detect DNA, proteins, metal ions, and tiny molecules [12]. There has been no review of research results that in-depth discusses the use of gold nanoparticles in the colorimetric method to detect viruses with a range of articles published in 2016–2020. Therefore, this narrative review aims to determine the sensitivity and selectivity of gold nanoparticle-based colorimetric methods as an alternative for the rapid detection of viruses that cause infection in humans.

2 Methods

The articles were searched in the database of ACS Publications and PubMed, which covered articles published from 2016 to 2020. The terms ("gold nanoparticles") AND colorimetric detection AND virus were used in the search process, which resulted in 494 articles. The inclusion criteria included articles published from 2016 to 2020, full-text articles, origin articles, and research articles about the use of gold nanoparticles to detect various types of viruses that can infect humans using colorimetric methods. The exclusion criteria were as follows: articles about viruses that did not cause infection in humans, articles that were not available in full text, and articles on metal nanoparticles other than gold, and 10 articles were selected for review.

3 Results and Discussion

3.1 Development of Virus Detection with Gold Nanoparticle-Based Colorimetric Method

Virus detection using colorimetric methods based on gold nanoparticles has been studied over the last five years. Pathogen detection is critical in medical applications. There are several methods to detect viruses, one of which is nanosensor technology. Various recognition molecules are being examined for sensor device development due to the need for rapid diagnosis in more stable, cheap, and selective nanosensor technology. Over the last few decades, nanosensors have become an important tool for viral analyte identification and clinical diagnosis [13].

Colorimetric nanosensors rely on changes in SPR caused by changes in nanoparticle size and shape, particularly gold nanoparticles, which change color rapidly when their size is modified [14]. Gold nanoparticles (AuNPs) have unique optical properties in terms of their size, environment, and dispersion state. AuNP-based colorimetric assays have been used in chemical and biological applications for their versatility and simplicity [15]. Several recent studies have attempted to use AuNPs' unique properties to develop enhanced virus detection techniques. Developed tests differ significantly in design and fundamental principles of virus detection methods. Virus detection methods are based on colorimetric methods for the detection of several groups of viruses that infect humans (Table 1).

There are 9 types of viruses, which belong to 6 different families (Table 1). Some of these viruses have been reported to be detected by colorimetric methods based on gold nanoparticles. The physicochemical and optical properties of metallic nanomaterials are highly dependent on the size and shape of the gold nanoparticles [16, 17], so it is important to control and determine the size, size distribution, and shape of the nanoparticles [18–20].

3.2 Gold Nanoparticle System

Gold Nanoparticles (NPs) can be functionalized to target specific virus antigens, improve selective diagnosis, and kill infectious agents. Compared to other metals, gold nanoparticles have good biocompatibility, low price, easy synthesis, and potential for surface functionalization [21]. The gold nanoparticle system is reported to play a role in colorimetric virus detection by exploiting its uniqueness (Table 1). Most of the gold nanoparticles produced are spherical (Table 1), but in the research of Xu et al., and Ahmed et al., bipyramidal and non-spherical nanoparticles were obtained. The optical properties of non-spherical nanoparticles differ from their physical dimensions. Non-spherical nanoparticles are unique because their optical properties are easy to adjust with their size and shape. Different from gold nanoparticles, the resonant frequency of non-spherical nanoparticles can be set in a broad range from blue to near-infrared, and their surface plasmon resonance (SPR) can be tuned to a specific wavelength or spectral regions for specific applications. Gold's non-spherical nanoparticle structure and high level of biocompatibility show potential of use in a wide range of biological applications (optical labeling for biosensor events and biomedical labeling) [31].

3.3 Virus Type and Detection Target

Virus species have different specific targets. In the application of gold nanoparticle-based colorimetric methods for virus detection, specific targets of each virus species are used as detection targets. SARS-CoV-2, MERS-CoV, H5N1, H7N9, Rift Valley fever virus, HPV 16, HPV 18, Norovirus, and Enterovirus 71 are classified by virus family. SARS-CoV-2 and MERS-CoV belong to the coronaviridae, H5N1 and H7N9 belong to the family Orthomyxoviridae, Rift Valley fever virus belongs to the Penuiviridae, and HPV 16 and HPV 18 belong to the Papillomaviridae family, Norovirus is a member of the Caliciviridae family, and Enterovirus 71 is a member of the Picornaviridae family.

3.3.1 Coronaviridae

The family of Coronaviridae includes Coronavirinae and Torovirinae subfamilies. Coronavirinae subfamily members are common in animals and frequently cause only minor respiratory or intestinal illnesses. MERS-CoV is a severe respiratory infection that originated in the Middle East. The virus, known as MERS-CoV (Middle East Respiratory Syndrome Coronavirus), is still causing sporadic episodes of severe respiratory illness [32].

Moitra et al. reported that the detection target of the SARS-CoV-2 virus is in the virus's N gene region, which is one of the three target gene sequences of the SARS-CoV virus, and the other two gene sequences are the RdRP framework (Orf1ab) and gene E. The gene is responsible for the open read of the envelope protein gene. The N gene of SARS-CoV-2 virus was selected as a detection target due to its low sensitivity to the N gene. This offers a lot of opportunities for biosensors targeting the SARSCoV2 N gene sequence to improve [22]. According to Kim et al., MERS-CoV virus detection targets two regions of MERS-CoV that encode an open reading frame (ORF)1a located upstream of the E protein gene (up E). These regions have a high detection sensitivity and are being examined for prospective preclinical screening [2].

3.3.2 Orthomyxoviridae

The Orthomyxoviridae family includes both human and animal pathogens. Virions range in size from spherical to filamentous with a diameter of about 100 nm. There are currently five known genera in the Orthomyoviridae family. Among them are the influenza A virus, influenza B virus, and influenza C virus that infect humans [33]. Ahmed et al. reported that the target detection of H5N1 is the H5N1 virus antigen.

The ultrasensitive detection of H5N1 is based on AP activity, and a colorimetric immunoassay has been developed [23]. On the other hand, H5N1 can be detected by targeting the H5N1 hemagglutination (HA) protein using a dual-enhanced colorimetric method for the visual detection of avian influenza viruses that is inexpensive, sensitive, specific, and cost-effective at time points. This is extendable to the development of therapeutic diagnostics and detection of other biomarkers based on nanozyme [24].

According to Zhang et al., H7N9 viral antigen is the focus for quick and sensitive detection. This colorimetric technique combines the advantages of high-efficiency magnetic bead-modifying enzymes and high-sensitivity cysteine/AuNP-based colorimetric assays [25].

3.3.3 Phenuiviridae

Rift Valley Fever Virus (RVF) is a single-stranded RNA virus with a negative orientation that belongs to the Phenuiviridae family of the Bunyavirales order. RVFV is currently only found in Sub-Saharan Africa, however, it has spread to the Arabian Peninsula. The virus causes abortion in pregnant animals and severe mortality in young animals when it infects ruminants. This virus can also infect humans, which causes a febrile illness that can be serious or fatal [34].

Zaher et al. reported that the target for the detection of RVF is RNA from the RVF virus. In the presence of RVFV RNA, unmodified gold nanoparticles (AuNPs) change color, resulting in a simple but sensitive experiment. The nanogold test yields qualitative data that demonstrate the presence of RVFV RNA in various sample types [26].

3.3.4 Papillomaviridae

The family Papillomaviridae is a small enveloped icosahedral virus that processes an 8 kb circular double-stranded DNA genome. The majority of the more than 150 HPV subtypes currently reported are associated with warts, which are benign tumors. It is important to note that HPV 16 and 18 are associated with cervical cancer [35].

Azizah et al. reported that the target of detection in HPV 16 is HPV 16 DNA as well as plasmid DNA containing HPV 16 and HPV 18. Total DNA from HPV 16 was extracted from serum plasma and showed blue lines on the UV-Vis spectrum when coupled with the AuNP probe [27]. Meanwhile, Kumvongpin et al. revealed in their work that HPV 16 and HPV 18 target DNA plasmids were employed in the detection limit of modified LAMP turbidity and LAMP-AuNP assays [28].

3.3.5 Caliciviridae

Calicivirus is a tiny, positive-stranded, enclosed RNA virus with icosahedral symmetry and a particle diameter of 2740 nm. Calicivirus has been isolated from humans. In cultivated cells, human norovirus is difficult to grow. Human norovirus is frequently replaced with mouse norovirus. According to the Centers for Disease Control and Prevention, norovirus is thought to be responsible for roughly half of all food-borne illnesses. Therefore, the majority of norovirus infections are not the result of direct contact with contaminated surfaces [36]. Khoris et al. discovered that colorimetric norovirus detection by bioassay was optimized to detect clinically isolated NoV using NoV-like particles (NovLP) [29].

3.3.6 Picornaviridae

On electron microscopy, the enveloped Picornavirus virion, 30 nm in diameter with icosahedral symmetry, appears smooth and rounded in outline. The capsid contains 60 copies of each of the four polypeptides VP1, 2, 3, and 4, with one polyprotein being cleaved [37]. Picornaviruses have been linked to the gastrointestinal tract, respiratory tract, nervous tissue, and muscles disorders, among other organs. Based on tissue affinity, picornaviruses are classified into five genera. For example, those that affect the gastrointestinal tract are classified in the genus Enteroviruses. Poliovirus is a member of the

Table 1. Description of gold nanoparticle-based virus detection by colorimetric method

Family/Virus[a]	Gold Nanoparticle System		Colorimetric Method	Detection Limit of Virus-Specific Target	Time (minutes)	References
	Shape	Size				
Coronaviridae						
SARS-CoV-2	Spherical	< 30 nm	Conjugation with gold nanoparticles enveloped in antisense oligonucleotides (ASOs).	0,18 ng/μL of SARS-CoV-2 RNA	10	[22]
MERS-CoV	Spherical	19 nm	Disulfide bonds generated by thiolation probe hybridization serve as targets for preventing AuNP aggregation and minimizing color change.	1 pmol/μL of 30 MERS-CoV DNA base pairs	10	[2]
Orthomyxoviridae						
H5N1	Bipyramid	ND[b]	For ultra-sensitive colorimetric detection, a very uniform gold nanobipyramid (Au-NBP) is used.	1 pg/mL of H5N1 virus antigen	ND[b]	[23]
H5N1	Non-spherical	10 nm	Enhanced dual colorimetric immunoassay.	1,11 pg/mL of H5N1 protein HA	ND[b]	[24]
H7N9	Spherical	13 nm	Ultrasensitive colorimetry that combines the ease of enzyme-induced metallization with the high specificity of enzyme-associated immunosorbent tests.	25 pg/mL of H7N9 virus antigen	ND[b]	[25]
Phenuiviridae						
RVF	Spherical	20 nm	Unmodified AuNPs	10 copies of RNA RVFV/reaction	30	[26]

(*continued*)

Table 1. (*continued*)

Family/Virus[a]	Gold Nanoparticle System		Colorimetric Method	Detection Limit of Virus-Specific Target	Time (minutes)	References
	Shape	Size				
Papillomaviridae						
HPV 16	Spherical	30 nm	DNA-conjugated AuNPs	ND[b]	30	[27]
HPV 16	Spherical	12,44 ± 1,21 nm	LAMP-AuNP-probe combination	10^2 copies of DNA plasmid HPV 16	ND[b]	[28]
HPV 18	Spherical	12,44 ± 1,21 nm	LAMP-AuNP-probe combination	10^0 copies of DNA plasmid HPV 18	ND[b]	[28]
Caliciviridae						
Norovirus	Spherical	12 nm	In colorimetric bioassays, silver ion-bound gold nanoparticles (Au/Ag NPs) had increased peroxidase activity.	10,8 pg/mL of NoV-LPs (NoV-like particles)	ND[b]	[29]
Picornaviridae						
Enterovirus 71	Spherical	ND[b]	High efficiency of magnetic enrichment and enzyme catalytic activity.	0,65 ng/mL of virion EV71	10	[30]

[a] SARS-CoV, severe acute respiratory syndrome Coronavirus 2; MERS-CoV, middle east respiratory syndrome coronavirus; H5N1, influenza A virus subtype H5N1; H7N9, influenza A virus subtype H7N9; RVF, Rift Valley fever virus; HPV, human papillomavirus. [b] ND, Non-Definition.

Enterovirus genus. The genus Enterovirus, which causes common colds, is also part of the Picornaviridae family. Furthermore, the genus Enterovirus includes Enterovirus 71, which causes hand, foot, and mouth disease (HFMD) in children. Finally, the Picornaviridae family includes EMCV (encephalomyocarditis virus) and HAV (hepatitis A virus) [38].

Xiong et al. reported that the detection target of enterovirus 71 was the EV71 virion. Using the effects of enzymatic reaction amplification and GNP plasmon signals, EV71 virions can be easily detected with ultra-sensitive and good quantitation capabilities [30].

3.4 Detection Method, Sensitivity, and Selectivity

The principle of the colorimetric detection method is based on colloidal nanoparticles that have been stabilized and modified with a biomolecular probe, with binding between the target biomolecule and the probe that can induce aggregation and thus cause color changes [39]. The sensors developed are used to monitor different levels of analytes based on antigen/antibody, DNA/RNA, and aptamer/protein interactions. Colorimetric

methods based on antigen-antibody interactions and enzyme catalysis as well as colorimetric methods based on DNA/RNA-based colorimetric sensors and aptamers used in detecting virus-specific targets are described in the presentation of the detection method, sensitivity, and selectivity subsections.

3.4.1 Virus Detection Method Based on Colorimetric Sensor Based on Antigen-Antibody Interaction and Enzyme Catalysis

Colorimetric virus detection methods based on antigen-antibody interactions and enzyme catalysis work by binding antibodies to specific antigens present on the cell surface and forming colored complexes, or they can be detected as markers of the presence or detection of biological agents in samples [40]. Natural enzymes are widely used as antibody labeling agents to produce meaningful color results that are visible to the human eye. In colorimetric immunoassays, natural enzymes such as horseradish peroxidase (HRP) and alkaline phosphatase (ALP) are commonly used. However, advances in nanomaterials technology have made it possible to replace natural enzymes with artificial enzymes based on nanoparticles [24].

Xu et al. described the detection of the H5N1 influenza virus using a sandwiched immunocomplex (Ab1-antigen-Ab2) formed by antigen-antibody interactions between capture antibodies (Ab1), the H5N1 target antigen, and the detection antibody (Ab2). The formed sandwiched immunocomplex will be conjugated with alkaline phosphatase (ALP) through biotin-avidin interaction. Alkaline phosphatase hydrolyzes 4-aminophenyl phosphate to 4-aminophenol, which is utilized to reduce silver nitrate to silver monomers, was placed on the surface of bipyramid gold nanoparticles, resulting in a blue shift in the LSPR peak. Its color shifts from brown or red to green. The color of the solution changed from brownish red to green, dark blue, and dark red depending on the thickness of the silver deposition layer on the surface of the bipyramid gold nanoparticles and the concentration of H5N1 virus antigen. While the blue shift of the LSPR peak occurs at 755 nm to 550 nm. The positive detection result for the presence of the H5N1 influenza virus is based on the formation of an immunocomplex sandwich in the presence of a target antigen, so without an H5N1 target antigen, there is no blue shift of the LSPR peak and the color of the solution does not change. When the selectivity of the H5N1 virus antigen detection method was compared to that of alpha-fetoprotein (AFP), carcinoembryonic antigen (CEA), human serum albumin (HSA), and H7N9 virus testing, the method utilized was found to be quite selective. The detection method's sensitivity is based on color changes in the solution that may be recognized as a reaction to different quantities of H5N1 virus antigen, allowing it to be utilized for semi-quantitative detection of H5N1 virus antigen in the test sample. The detection limit for the H5N1 virus antigen was 1 pg/mL and a linear range at a concentration of 1 pg/mL to 2.5 ng/mL with more varied color changes for visual detection [23].

Ahmed et al. reported on the detection of H5N1 influenza virus based on enhanced dual colorimetric immunoassay with in situ syntheses of gold nanoparticles from virus-specific antibodies and gold ion solution using 3,3',5,5'-tetramethylbenzidine (TMBZ) as the main chemical product for synthesizing gold nanoparticles with the resulting solution color are bluish-green. The resulting color is more intense when TMBZ/H2O2 is added because the synthesized gold nanoparticles have a positive charge. The positively

charged gold nanoparticles have a high catalytic activity, which allows them to produce a more intense blue color. The addition of TMBZ converts gold ions into nanostructures. Positive results in the form of color development were obtained only using the H5N1 virus target, gold ion conjugated with specific antibodies, and in the presence of tetramethylbenzidine (TMBZ). The detection method's sensitivity is evidenced by a continuous increase in absorbance with a linear response, indicating that the detection method may provide a good and stable response with viral concentrations ranging from 10 pg/mL to 10 g/mL and a detection limit value of 1.11 pg/mL. The selectivity of the detection method against the H5N1 virus target was compared to testing methods against other virus strains, namely H7N9, H7N8, H5N2, and H1N1. Significant changes were observed in the H5N1 virus target when compared to other virus strains, indicating that this method is quite selective to detect H5N1 virus targets [24].

Zhang et al., described the detection of the H7N9 influenza virus by ultrasensitive colorimetric assay by modifying alkaline phosphatase (ALP) and detecting antibody (Ab2) on a magnetic bead (MB), resulting in Ab2-ALP-MB. This improves detection sensitivity because the changes to the magnetic bead (MB) have been modified to catch large amounts of alkaline phosphatase (ALP) and detect antibody (Ab2), allowing for the detection of extremely small amounts of virus. Immune complexes occur between the capture antibody, H7N9 target antigen, and Ab2-ALP-MB when an H7N9 target antigen is present. A shift in the color of the solution from red to blue indicates a positive result for the presence of the H7N9 target antigen. Alkaline phosphatase (ALP) is responsible for the color change response by dephosphorylating ascorbic acid 2-phosphate (AA-P) to ascorbic acid (AA). Ascorbic acid (AA) is used as a reducing agent for cystine to cysteine, which can cause gold nanoparticles to aggregate, causing the solution's color to shift from red to blue. In the absence of the H7N9 target antigen, an immunocomplex will not develop, and the color of the solution will not change. The color change reaction to an increase in the absorbance and concentration of the H7N9 target antigen in the range of 5 pg/mL to 150 pg/mL, with a detection limit value of 25 pg/mL that can still be observed with the naked eye, indicates the sensitivity of the detection method. The detection method's selectivity against the H7N9 virus antigen target was compared to testing methods against other antigens such as alpha-fetoprotein (AFP), prostate-specific antigen (PSA), human enterovirus 71 (EV71), H9N2, and IgG, with substantial signal and color changes. This approach has a high selectivity for detecting the H7N9 virus antigen target, indicating that it can detect the H7N9 virus antigen target [25].

Khoris et al. reported the detection of Norovirus by sensing mechanism with an increase in color intensity through enhanced catalytic activity by combining gold nanoparticles with silver ions into Au/Ag NPs in a colorimetric bioassay. The two concepts underlying colorimetric sensing based on Au/Ag NPs are the captures of virus targets in the form of NoV-like particles (NoV-LPs) using a gold probe, and the enhancement of catalytic activity using silver ions. The first step in the detection procedure was the immobilization of anti-NoV antibodies (NS14 Ab) in plate wells. The target virus was then placed in the sensing chamber. To form a sandwich structure, anti-NoV (NS14 Ab)-Au NP antibodies were combined with the NS14 Ab combination and the target virus. To improve the catalytic activity, silver ions and hydroquinone were added to create in-situ silver-coated gold nanoparticle structures. Then, a solution of H2O2 and

tetramethylbenzidine (TMB) was added, which intensified the blue color of the mixed solution of Au/Ag NP and the target virus. After that, H2SO4 was added to stop the reaction, and the final color of the solution was yellow, which would be more concentrated if the concentration of the target virus was higher. Monitoring the color change of the solution based on the concentration of NoV-LP results in a linear relationship between the concentration and the color of the resulting solution, indicating the sensitivity of the detection method. The selectivity of the NoVLP target detection method as a Norovirus model was compared to the test method for biological agents such as influenza virus and BSA. The results showed that only the wells containing NoVLP had a solid yellow color and no NS14 AbAu NPs. They are bound to viruses or other proteins due to the specificity of the antibodies used. This colorimetric immunoassay showed high sensitivity and selectivity for norovirus detection, with a detection limit of 10.8 pg/mL ranging from 1 pg/mL to 100 ng/mL [29].

Xiong et al. used a colorimetric immunoassay to detect enterovirus 71 by reducing gold ions to gold nanoparticles. Controlling the enzyme's efficient magnetic concentration and high catalytic activity allows for colorimetric detection. The assay was modified with a biotinylated secondary antibody via contact with biotin-streptavidin and involved immunomagnetic bead production, EV71 virion capture, and antigen-antibody-mediated enzymatic coupling. Enzymes covalently bound to the secondary antibody are bound within the viral bead to form a sandwich structure immune complex. This enzyme catalyzes the hydrolysis of hydrogen peroxide (H2O2), influencing gold ion reduction and the shape of gold nanoparticles produced, resulting in a noticeable shift in the color of the viral sample. It was observed that the color change of the detection solution changed from red to magenta and blue, and there was a change in the redshift absorption spectrum that was directly proportional to the concentration of EV71. The sensitivity of the detection method was indicated by the change in color of the gold nanoparticle solution which was red to purple and then to blue as the concentration of EV71 increased. The immunoassay has a detection limit of 0.65 ng/mL, indicating that it can identify EV71 virions with good sensitivity. The detection method's selectivity for the target Enterovirus 71 virion was compared to that of Coxsackievirus A2 (CVA2), Coxsackievirus A4 (CVA4), Coxsackievirus A6 (CVA6), Coxsackievirus A16 (CVA16), and human intestinal cytopathic uncommon virus (ECHO18). Only the target virus sample exhibited blue-violet aggregation of gold nanoparticles, while the reference sample showed red dispersion of gold nanoparticles, implying that the color change solution for the target EV71 virus is based on a biologically specific interaction between EV71 and its specific antibody [30].

3.4.2 Virus Detection Method Based on DNA/RNA Based Colorimetric Sensor and Aptamer

DNA/RNA-based and aptamer-based colorimetric virus detection methods offer new possibilities for detecting a variety of target analytes by using Au NP as a signal transducer. The detection event can be easily converted into a color change dependent on the aptamer using SPR, resulting in a red solution with well-dispersed AuNP and blue aggregated AuNP (or purple). Furthermore, color changes can be detected with the naked eye without the use of sophisticated tools. [41].

Moitra et al. reported the detection of SARS-CoV-2 virus using gold nanoparticles coated with a thiol modified antisense oligonucleotide (ASO) differentially functionalized to exchange the surface capping agent of citrate stabilized gold nanoparticles. It consists of 4 ASO, namely ASO 1 and ASO 3 functioned with a thiol group at the 5 ends, and ASO 2 and ASO 4 functioned with a thiol group at the 3 ends. SARS-CoV-2 RNA will clump together, causing a rise in absorbance at 660 nm with a redshift of 40 nm, which will be visible as a color change from purple to dark blue. RNaseH increases this, resulting in the deposition of gold nanoparticles that can be seen with the naked eye. When the detection method for SARS-CoV-2 was compared to the test method for MERS-CoV, the results revealed that aggregation occurred only when target RNA encoding the SARSCoV2 virus N gene was present. The increased sensitivity of AuASOmix nanoparticles was monitored at specific concentrations of SARS-CoV-2 RNA (1 ng/μl) and varying incubation temperatures, reaching optimal sensitivity at 37 °C. Due to the constant increase in absorbance in the linear response, the detection method used provided a good and stable response of AuASOmix to SARSCoV2 RNA. The detection limit of SARS-CoV-2 RNA was 0.18 ng/μL, the dynamic range was 0.23 ng/μL, and the color of the solution changed from purple to dark blue [22].

Kim et al. reported the detection of the MERS-CoV virus using a gold nanoparticle-based colorimetric approach that used a pair of thiol-modified probes at the 3' and 5' ends to target the partial (30 bp) genomic region of MERS-CoV virus containing the upE protein and open reading frame (ORF). A thiol-modified probe pair then forms double-stranded DNA with the detection target and forms a disulfide-induced continuum complex. The sulfur-rich complex can protect the stability of gold nanoparticles against salt-induced aggregation because the sulfur groups at the ends of the double-stranded DNA are mediated by covalent bonds with the gold surface, as a result, the color of the solution remains red. If there is no target DNA, the probes will interact with each other because it is induced by disulfide, which cannot protect the stability of the gold nanoparticles so salt-induced aggregation occurs which causes a color change from red to blue. Salt can interfere with the interaction of charges around the gold nanoparticles and promote attractive forces between the particles. Positive detection results of the presence of the MERS-CoV virus were indicated by inhibition of gold nanoparticle aggregation by forming a double-stranded DNA complex between a pair of probes and their detection target. By performing focused detection of two sections of the MERS-CoV virus, the resulting detection approach for MERS-CoV diagnosis has been explored for prospective preclinical screening, and it has good sensitivity. The detection limit for the partial genomic region (30 bp) of the MERS CoV virus was 1 pmol/μL in the absence of gold nanoparticle aggregation, so the color of the solution remained red [2].

Zaher et al., reported the detection of the Rift Valley fever virus (RVFV) by hybridization between the probe and RVFV target RNA, which can be used to detect the presence of nucleotides (RVFV target RNA) by completing the base sequence in the probe. Before interacting with the target RNA, hybridization was performed between phosphate-buffered saline (PBS) pH 7 and the 5′- ATGATGACATTAGAAGGGA-3′ probe that would be used in the nanogold assay. Nanogold assays rely on the use of a probe concentration to replace citrate ions in stabilizing gold nanoparticles when exposed to a salt (NaCl). The sample is declared positive if the probe binds to the target RNA which leads

to the surface dissociation of gold nanoparticles which one day will be able to aggregate when added with NaCl. The change in color of the solution from red to blue will be used to detect gold nanoparticles aggregation. If the probe is unable to directly connect to the virus RNA target, the sample is ruled negative, and the probe will continue to interact with the negative charge of the gold nanoparticles, stabilizing it and preventing aggregation and discoloration of the solution. The sensitivity of the detection method was tested by performing virus RNA dilution series ranging from 105 RNA copies to 100 RNA copies and showing a color change response to the dilution series used based on the aggregation of gold nanoparticles with a detection limit of 10 RNA copies in each reaction. The selectivity of the detection method for the RVFV RNA target was compared with the method on mosquitoes and RVFV serum-negative samples, with clear aggregation and color changes observed on the RVFV RNA target, indicating that this method has good selectivity for detecting RVFV RNA targets [26].

Azizah et al. reported on the detection of HPV 16 using an electrical-based sensor combined with an oligonucleotide probe to detect changes in charge when the HPV 16 target DNA attaches to the probe. In various molecular biology detection techniques, oligonucleotides serve as probe guides that, when combined with a probe or signal generator, can recognize the complementary DNA/RNA sequence. A visual change in the color of the solution from red to purple can occur in the presence of virus target DNA. When the AuNP-HPV oligo probe is hybridized with the DNA target of HPV 16, double-stranded DNA is produced, which destabilizes gold nanoparticles in the presence of NaCl, causing aggregation and the color to change to purple. If there is no virus DNA target, the AuNP-oligo probe will not aggregate and the solution will remain red so that this method can be used to detect the presence of HPV with good sensitivity and selectivity in a relatively fast time of 30 min, which can be a complement to previously available detection method [27].

Kumvongpin et al. reported that the detection of HPV 16 and HPV 18 viruses could be carried out with gold nanoparticles (AuNP) mounted on a single-stranded DNA probe in combination with Loop-mediated isothermal amplification (LAMP). The combination of LAMP and AuNP exhibited higher sensitivity and ease of vision in the detection of HPV 16 and HPV 18, compared to the modified turbidity LAMP, which was an alternate method for DNA detection with cloudy detection findings and not easily discernible with the naked eye. The combination of LAMP and AuNP has a 100% sensitivity and specificity with HPV 16 and HPV 18 positive clinical specimens, while HPV 16 and HPV 18 negative clinical specimens yielded negative results. The LAMP-AuNP method with detection limits on HPV 16 and HPV 18 obtained 10^2 and 10^0 copies of HPV 16 and HPV 18 plasmid DNA, respectively, showing that the LAMP-AuNP assay was up to 10 times more sensitive than the modified LAMP turbidity method [28].

4 Conclusion

A colorimetric method based on gold nanoparticles has good sensitivity and selectivity as an alternative for the rapid detection of viruses that cause infection in humans. Based on the results of this review, it is hoped that the application of the colorimetric method to each virus can be further developed, particularly in the detection of the SARS-CoV-2

virus, to help reduce the incidence of transmission and infection due to the virus, which was still high in Indonesia at the time of writing of this narrative review.

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