ORIGINAL ARTICLE

INTESTINAL RESEARCH

pISSN 1598-9100 • eISSN 2288-1956
https://doi.org/10.5217/ir.2024.00136
Intest Res 2025;23(2):213-224

# Diagnostic performance of noninvasive tests for cytomegalovirus ileocolitis: a systematic review and meta-analysis

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**Background/Aims:** Diagnosis of cytomegalovirus (CMV) ileocolitis traditionally requires colonoscopy with tissue biopsy. Due to potential complications in high-risk patients, there is growing interest in serum and stool tests for diagnosing this condition. We aimed to evaluate the diagnostic accuracy of these noninvasive tests compared to traditional gold standards. **Methods:** Two independent reviewers performed a comprehensive search on MEDLINE and Embase from inception up to October 1, 2023. Prospective and retrospective studies evaluating the performance of serum CMV polymerase chain reaction (PCR), serum CMV antigen (Ag), and stool CMV PCR in diagnosing CMV ileocolitis were included. Tissue histopathology or tissue CMV PCR served as reference standards. Diagnostic performances of each serum and stool test were calculated based on a meta-analysis using random-effects model. **Results:** A total of 30 studies, comprising 23 studies of serum CMV PCR, 9 of serum CMV Ag, and 7 of stool CMV PCR, were included. The pooled sensitivity, specificity, and area under summary receiver operating characteristic curves were 62% (95% confidence interval [CI], 51%–72%), 90% (95% CI, 79%–96%), and 0.81 for serum CMV PCR, 38% (95% CI, 26%–51%), 94% (95% CI, 70%–99%), and 0.56 for serum CMV Ag, and 53% (95% CI, 35%–70%), 91% (95% CI, 84%–95%), and 0.84 for stool CMV PCR. **Conclusions:** Serum and stool tests cannot replace colonoscopy for diagnosing CMV ileocolitis due to their low sensitivities but may be useful when colonoscopy is not feasible. Positive results can aid diagnosis, given their high specificities. Serum and/or stool CMV PCR are preferred over CMV Ag. **(Intest Res 2025;23:213-224)**

**Key Words:** Cytomegalovirus; Colitis; Enterocolitis; Sensitivity and specificity; Meta-analysis

## INTRODUCTION

Cytomegalovirus (CMV) is a double-stranded DNA virus in the human *Herpesviridae* family. This virus can persistently integrate into the DNA of host cells following initial infection and can be reactivated in response to various triggers, including immunosuppression and significant chronic inflammation.[1] While both acute CMV infection and subsequent reactivation are usually asymptomatic and self-limiting, some patients may develop CMV disease, characterized by clinical symptoms that vary based on the affected organ.[2] Gastrointestinal (GI) involvement is one common manifestation of CMV disease, with hematochezia and diarrhea being the most prevalent symptoms.[3] The risk factors for CMV GI infection include HIV (human immunodeficiency virus) infection with low CD4 count,[4-6] post-organ transplantation,[7-11] and taking immunosuppressive agents, especially corticosteroids.[12-15] Patients with inflammatory bowel disease (IBD) are particularly susceptible to CMV GI disease, mainly colitis, due to a combination of colonic inflammation, impaired immune function, and long-term immunosuppression from advanced therapy.[16,17] Additionally, there is growing evidence of CMV colitis in immunocompetent patients, especially in the elderly with multi-

**Received** August 27, 2024. **Revised** October 4, 2024.
**Accepted** October 8, 2024.
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ple comorbidities or severe illnesses.[14]

Tissue diagnosis, utilizing hematoxylin and eosin (H&E) staining, immunohistochemistry (IHC), and quantitative polymerase chain reaction (PCR) techniques, remains the most reliable method to diagnose CMV disease in the GI tract.[18] However, when performing endoscopy with biopsy may not be feasible in patients with severe medical illness, serum-based tests, such as CMV serology and PCR, and stool-based tests, such as stool CMV PCR, could be valuable alternatives. Although these tests may have relatively low diagnostic yield and serum-based tests may not be specific to intestinal disease,[19] they are feasible options, particularly when traditional methods are not achievable. Therefore, it is essential to know the performance and the limitations of these noninvasive diagnostic tests. Therefore, our study aimed to perform a systematic review and meta-analysis to assess the diagnostic accuracy of these noninvasive tests compared to tissue-based methods, including H&E staining, IHC staining, and tissue PCR across different susceptible patient populations.

## METHODS

This systematic review and meta-analysis adhered to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses statement guidelines (Supplementary Material).[20] This study was registered in the PROSPERO database with the registration number CRD42023402868.

### 1. Search Strategy and Study Selection

Electronic database searches of MEDLINE (OvidSP), Embase, and Cochrane Library were conducted from inception up to October 1, 2023, under the guidance of a trained medical librarian. Search terms were selected following the PICO format, with terms including "colitis" or "enteritis" and "cytomegalovirus" or "CMV" as populations, and "diagnosis," "sensitivity and specificity," "diagnostic accuracy," or "performance" as outcomes. Search terms were linked using "OR" to capture all relevant studies. To broaden the search, specific index or reference tests were not utilized in the search terms. The complete list of search terms for both databases can be found in the Supplementary Material. Exclusion criteria included case reports, review articles, small case series, and non-English publications.

Two investigators (T.C. and O.S.) independently screened titles and abstracts for eligibility using Rayyan (Cambridge, MA, USA), an online tool for systematic review management. The articles that match the predefined inclusion criteria were reviewed in full. Required inclusion criteria consisted of: (1) prospective or retrospective case-control or cross-sectional designs; (2) patients with CMV ileocolitis diagnosed by lower GI tract endoscopy; and (3) a tissue-based technique, CMV PCR, H&E stain, or IHC stain, was performed, along with at least one noninvasive method to detect CMV viremia or reactivation. Exclusion criteria included case reports, review articles, and non-English publications. Studies with 5 or fewer patients or insufficient data for true positive and true negative calculations were also excluded. Disagreement was resolved through discussion with the third reviewer (J.L.).

### 2. Study Endpoints

The primary objective of this study was to evaluate the diagnostic performance of noninvasive tests in predicting CMV ileocolitis. The tests included blood-based assays, namely CMV PCR and CMV phosphoprotein 65 (pp65) antigen (Ag), and stool assay for CMV PCR. For the PCR test, serum and stool underwent nucleic acid extraction, amplification, and quantitation to determine viral load. Detection of CMV pp65 Ag involved immunocytochemical detection of CMV immediate early Ag in blood leukocytes. Due to variability among studies, thresholds for positive PCR and Ag techniques could not be standardized. Tissue diagnosis from endoscopy served as the gold standard through positive tissue histopathology, identified by characteristic cytomegalic changes with intranuclear and/or intracellular inclusion bodies on H&E sections or identification of CMV-specific Ag by IHC. Positive tissue CMV PCR, tissue extracted DNA into the amplification mix, followed by PCR analysis, was also considered a standard reference. Subgroup analysis of study design and publication type was performed to assess potential sources of heterogeneity. Analyses based on the reference standard and comorbidity were performed to determine diagnostic accuracy in specific situations.

### 3. Study Quality Assessment

We assessed the quality of included studies using the Assessment of Diagnostic Accuracy Studies Version 2 (QUADAS-2) checklist,[21] which divides the assessment into 2 domains: risk of bias and concerns regarding applicability. Both areas encompass patient selection, index tests, and reference standards. The risk of bias assessment includes an additional domain: flow and timing. We evaluated and graded each domain as low, high, or unclear. Discrepancies in quality assessment were resolved through consensus among the investigators.

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### 4. Statistical Analysis

Pooled estimates of sensitivity, specificity, positive and negative likelihood ratios, diagnostic odds ratio, and the area under the summary receiver operating characteristic (AUSROC) curves were calculated, along with the corresponding 95% confidence intervals (CI). The coupled forest plots were sorted in hierarchical format by increasing sensitivity to allow visual assessment for the threshold effect. Heterogeneity across the studies was assessed by the $I^2$ value. Sensitivity analyses were conducted to determine the robustness of the results. Publication bias was evaluated through Deek's asymmetrical funnel plot method.[22] The diagnostic performance of all tests was analyzed using Stata version 17.0 (College Station, TX, USA), employing a random-effects model.

## RESULTS

### 1. Study Search Results and Characteristics

From an initial pool of 4,553 citations identified through a database search, 132 were deemed eligible for full-length review after removing duplicates and excluding based on title and abstract screening. Ultimately, 30 citations met the inclusion and exclusion criteria for the final analysis, as depicted in Fig. 1. These comprised 12 prospective[23-34] and 18 retrospective[35-52] studies, encompassing 26 full manuscripts and 4 abstracts.[27,38,44,46] These studies included patients with varying comorbidities, including IBD in 19 studies, with 12 focusing solely on ulcerative colitis and 10 specifically on active ulcerative colitis. The remaining were post-hematopoietic stem cell transplant (HSCT) conditions (4 studies), and other populations at risk for CMV infection (5 studies). The gold standard tissue diagnostic methods varied among the studies, with tissue CMV IHC used in 18 studies, CMV PCR in 16, and H&E staining in 8. The index tests evaluated were serum CMV PCR in 23 studies, CMV Ag detection in 9 studies, and stool CMV PCR in 7 studies. The cutoff value of serum CMV PCR varied across studies, with limits of detection ranging from 45 to 500 copies/mL. Only 1 study assessed the diagnostic accuracy of combined blood and stool detection.[32] The details of all included studies are shown in Table 1.

### 2. Diagnostic Performance of Noninvasive Tests for Detecting CMV Ileocolitis

A total of 27 studies investigated CMV ileocolitis using blood-based tests, with 22 studies utilizing serum CMV PCR and 9 studies using CMV Ag detection. The gold standard for diagnosis included tissue CMV IHC, PCR, or H&E staining. The results are shown in Table 2. In total, 2,272 tests were assessed for serum CMV PCR, yielding a pooled sensitivity of 62% (95% CI, 51%–72%), a pooled specificity of 90% (95% CI, 79%–96%), and an AUSROC of 0.81 (0.77–0.84). For serum CMV Ag detection, which was evaluated in 699 tests, the sensitivity was notably lower at 38% (95% CI, 26%–51%) with a specificity of 94% (95% CI, 70%–99%), and an AUSROC of 0.56 (0–1). Seven studies with 417 tests using stool CMV PCR, showed a sensitivity of 53% (95% CI, 35%–70%), specificity of 91% (95% CI,

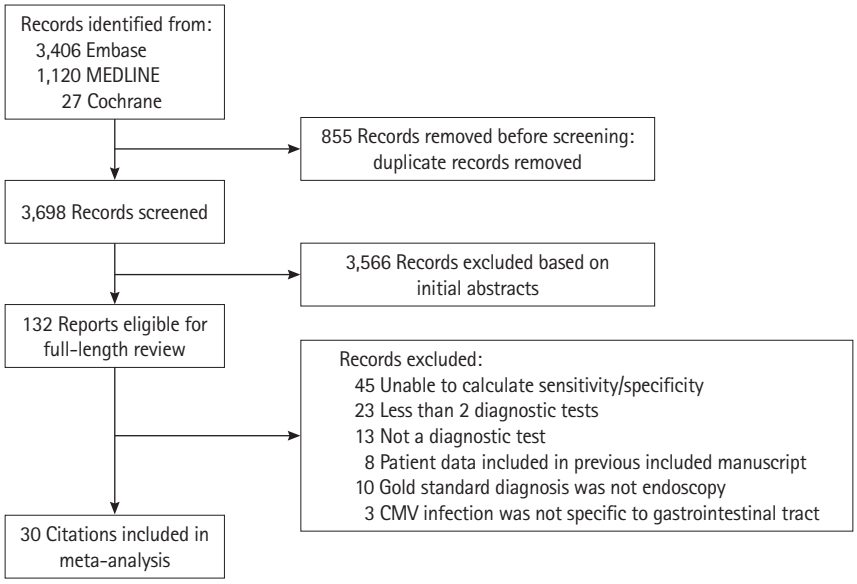


**Fig. 1.** PRISMA flow diagram for database search, including Embase, MEDLINE, and Cochrane Library. CMV, cytomegalovirus.

**Table 1.** Patient and Study Characteristics

| First author (year) | Year of collection | Country | Study design | Comorbidity | Gold standard tissue diagnosis | Index test | True positive / false negative/ total sample size |
|---|---|---|---|---|---|---|---|
| Alacam (2020)[35] | 2016–2018 | Turkey | Retrospective | Any (include IBD) | IHC | Serum PCR | 16/3/76 |
| Ambelil (2019)[36] | 2014–2015 | USA | Retrospective | Any | H&E, PCR | Serum PCR | 6/19/87 |
| Bhutani (2014)[37] | 2005–2011 | USA | Retrospective | Post-HSCT | PCR | Serum PCR | 28/3/252 |
| Boo (2011)[38] | 2000–2010 | South Korea | Retrospective[a] | UC | IHC | Serum PCR | 26/37/159 |
| | | | | | | Serum Ag | 29/34/159 |
| Ciccocioppo (2015)[24] | 2011–2013 | Italy | Prospective | IBD | PCR | Serum PCR | 6/7/40 |
| Cohen (2022)[39] | 2013–2018 | USA | Retrospective | Severe UC | IHC, H&E | Serum PCR | 11/2/206 |
| Dimitroulia (2006)[23] | 2000–2003 | Greece | Prospective | IBD | PCR | Serum PCR | 23/5/85 |
| Domènech (2008)[26] | 2000–2003 | Spain | Prospective | UC | IHC, PCR | Serum Ag | 6/0/19 |
| Fukuchi (2013)[25] | 2010–2011 | Japan | Prospective | UC | IHC, PCR | Serum Ag | 1/14/51 |
| Ganzenmueller (2012)[27] | 2012 | Germany | Prospective[a] | Immunosuppressed | PCR | Stool PCR | 8/6/53 |
| Hazir-Konya (2021)[28] | 2017 | Turkey | Prospective | Any (non-IBD) | IHC | Serum PCR | 0/3/155 |
| Herfarth (2010)[29] | 2007–2008 | USA | Prospective | IBD | PCR | Stool PCR | 5/1/21 |
| Inokuchi (2014)[40] | 2004–2011 | Japan | Retrospective | UC | IHC | Serum Ag | 9/14/49 |
| Kim (2014)[41] | 2001–2012 | South Korea | Retrospective | UC | IHC, H&E | Serum PCR | 27/34/229 |
| | | | | | | Serum Ag | 39/44/229 |
| Mavropoulou (2019)[42] | 2005–2016 | Germany | Retrospective | IBD | IHC, PCR, H&E | Serum PCR | 15/8/47 |
| | | | | Post-HSCT | IHC, PCR, H&E | Serum PCR | 16/4/61 |
| Okahara (2017)[43] | 2011–2015 | Japan | Retrospective | UC | PCR | Serum Ag | 4/5/26 |
| Prachasitthisak (2016)[30] | 2015–2016 | Thailand | Prospective | Immunosuppressed | PCR | Stool PCR | 8/6/53 |
| Roblin (2011)[31] | 2009 | France | Prospective | UC | PCR | Serum PCR | 3/13/42 |
| Sattayalertyanyong (2023)[32] | 2020–2021 | Thailand | Prospective | Any (include IBD) | IHC, H&E | Serum PCR | 18/9/122 |
| | | | | | | Stool PCR | 19/8/122 |
| Serra-Ruiz (2023)[44] | 2017–2022 | Spain | Retrospective[a] | IBD | IHC | Serum PCR | 13/12/89 |
| Sun (2015)[33] | 2013–2014 | China | Prospective | Post-HSCT | IHC, H&E | Serum PCR | 6/1/58 |
| | | | | | | Stool PCR | 2/5/58 |
| Thörn (2015)[34] | 2010–2013 | Sweden | Prospective | IBD, IBS | PCR | Serum PCR | 3/5/67 |
| | | | | | | Stool PCR | 2/5/67 |
| Tun (2019)[45] | 2011–2014 | Germany | Retrospective | IBD | PCR | Serum PCR | 6/5/20 |
| Wang (2019)[46] | 2014–2018 | USA | Retrospective[a] | Any | IHC | Serum PCR | 6/2/42 |
| Yang (2017)[47] | 2010–2015 | China | Retrospective | UC | IHC | Serum PCR | 12/12/50 |
| | | | | | | Serum Ag | 13/12/50 |
| Yoshino (2007)[48] | 2003–2006 | Japan | Retrospective | UC | H&E | Serum PCR | 4/0/30 |
| Yoshino (2011)[49] | 2003–2008 | Japan | Retrospective | Refractory UC | PCR | Serum Ag | 7/24/64 |
| Zagórowicz (2016)[50] | 2005–2012 | Poland | Retrospective | Inpatient UC | IHC | Serum PCR | 20/4/52 |
| | | | | | | Serum Ag | 2/7/19 |
| Zagórowicz (2018)[51] | 2009–2016 | Poland | Retrospective | UC | IHC | Serum PCR | 24/26/234 |
| Zavrelova (2018)[52] | 2008–2015 | Czech | Retrospective | Post-HSCT | IHC | Serum PCR | 4/2/69 |
| | | | | | | Stool PCR | 1/5/69 |

[a]Abstract.
IBD, inflammatory bowel disease; IHC, immunohistochemistry; PCR, polymerase chain reaction; H&E, hematoxylin and eosin staining; HSCT, hematopoietic stem cell transplantation; UC, ulcerative colitis; Ag, antigen.

https://doi.org/10.5217/ir.2024.00136 • Intest Res 2025;23(2):213-224

**Table 2.** Pooled Diagnostic Performance of Noninvasive Tests for Diagnosis of CMV Ileocolitis

| Diagnostic test | No. of studies | No. of test | $I^2$ | Pool sensitivity (95% CI) | $I^2$ | Pool specificity (95% CI) | $I^2$ | Pool PLR (95% CI) | Pool NLR (95% CI) | Pool DOR (95% CI) | AUSROC (95% CI) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Tissue CMV IHC or PCR or H&E as references | | | | | | | | | | | |
| Serum CMV PCR | 23 | 2,272 | 61.57 | 0.62 (0.51–0.72) | 64.12 | 0.90 (0.79–0.96) | 70.58 | 6.50 (3.20–13.20) | 0.41 (0.32–0.54) | 16 (7–34) | 0.81 (0.77–0.84) |
| Serum CMV Ag | 9 | 699 | 0.02 | 0.38 (0.26–0.51) | 59.06 | 0.94 (0.70–0.99) | 26.69 | 6.60 (1.30–33.10) | 0.66 (0.58–0.76) | 10 (2–49) | 0.56 (0–1.00) |
| Stool CMV PCR | 7 | 417 | 41.25 | 0.53 (0.35–0.70) | 43.90 | 0.91 (0.84–0.95) | 48.34 | 5.90 (2.70–12.90) | 0.52 (0.34–0.78) | 12 (4–35) | 0.84 (0–1.00) |
| Tissue histopathology (IHC and/or H&E) as a reference | | | | | | | | | | | |
| Serum CMV PCR | 14 | 1,571 | 66.61 | 0.64 (0.52–0.75) | 44.08 | 0.84 (0.70–0.93) | 86.35 | 4.10 (2.00–8.30) | 0.43 (0.31–0.59) | 10 (4–24) | 0.77 (0.73–0.80) |
| Serum CMV Ag | 5 | 539 | 0.02 | 0.44 (0.35–0.54) | 27.73 | 0.86 (0.51–0.97) | 71.39 | 3.10 (0.80–12.60) | 0.65 (0.54–0.79) | 5 (1–23) | 0.51 (0.47–0.55) |
| Stool CMV PCR | 4 | 276 | 0.05 | 0.48 (0.23–0.74) | 55.04 | 0.86 (0.78–0.91) | 40.44 | 3.40 (1.40–8.70) | 0.60 (0.34–1.08) | 6 (1–25) | 0.83 (0–1.00) |
| Tissue CMV PCR as a reference | | | | | | | | | | | |
| Serum CMV PCR | 6 | 506 | 23.78 | 0.59 (0.34–0.84) | 75.44 | 0.99 (0.76–1.00) | 5.32 | 61.70 (2.30–1,676) | 0.41 (0.22–0.76) | 150 (6–3,834) | 0.90 (0.87–0.92) |
| Serum CMV Ag | 2 | 90 | | 0.28 (0.16–0.43) | | 0.98 (0.87–1.00) | | | | | |
| Stool CMV PCR | 3 | 141 | 0 | 0.56 (0.33–0.77) | 28.76 | 0.97 (0.90–0.99) | 7.52 | 18.60 (5.70–61.00) | 0.45 (0.27–0.77) | 41 (10–168) | 0.95 (0–1.00) |

CMV, cytomegalovirus; CI, confidence interval; PLR, positive likelihood ratio; NLR, negative likelihood ratio; DOR, diagnostic odds ratio; AUSROC, area under the summary receiver operating characteristic curve; IHC, immunohistochemistry; PCR, polymerase chain reaction; H&E, hematoxylin and eosin staining; Ag, antigen.

84%–95%), and an AUSROC of 0.84 (0–1). Visual analysis of sensitivity and specificity pairs using coupled-forest and AUSROC plots revealed no threshold effect (Fig. 2). Substantial heterogeneity was detected in serum CMV PCR ($I^2$ = 61.57).

We performed subgroup analyses for each noninvasive test using different reference standards, including tissue histopathology and tissue CMV PCR. As shown in Table 2, the overall diagnostic performances were not significantly different.

### 3. Comparison of Diagnostic Performance of Different Tests in the Same Studies

We performed a subgroup analysis by examining only studies that utilized more than 1 noninvasive test in a single study. The results are shown in Table 3. The serum CMV PCR diagnostic performance was comparable to CMV Ag in this subgroup.[38,41,47,50] In contrast, stool CMV PCR had significantly lower sensitivity than serum PCR (39% vs. 70%) but tended to have higher specificity (90% vs. 76%).[32-34,52] One study employed a combination of serum and stool CMV PCR, with tissue IHC serving as the reference standard, demonstrating a sensitivity of 81.5% when either one was positive, significantly higher than serum CMV PCR alone, and a specificity of 96% when both were positive, significantly higher than stool CMV PCR alone.[32]

### 4. Diagnostic Performance Based on IBD and Post-HSCT Subgroup

As shown in Table 4, of the 19 studies involving populations with IBD, 13 assessed serum CMV PCR. Overall, the pooled sensitivity was 56% (95% CI, 45%–67%), while the pooled specificity was 94% (95% CI, 82%–98%). The corresponding values for serum CMV Ag detection were 35% (95% CI, 22%–50%) and 94% (95% CI, 79%–99%), respectively. Only 1 study utilized stool PCR to detect CMV ileocolitis, using qualitative tissue PCR as the reference standard. The sensitivity and specificity were 83% (95% CI, 36%–99.6%), and 93% (95% CI, 68%–99.8%), respectively. In the subgroup of patients who underwent HSCT and were clinically suspected of CMV ileocolitis, data were available from only 4 studies. Here, the pooled sensitivity of serum CMV PCR was 85% (95% CI, 73%–92%) with a specificity of 47% (95% CI, 21%–74%). In contrast, the pooled sensitivity of stool PCR was only 23% (95% CI, 8%–52%), and the pooled specificity was 81% (95% CI, 72%–87%).

### 5. Comparison of Diagnostic Performance Based on Different Study Designs and Publication Types

To assess potential sources of heterogeneity, further subgroup

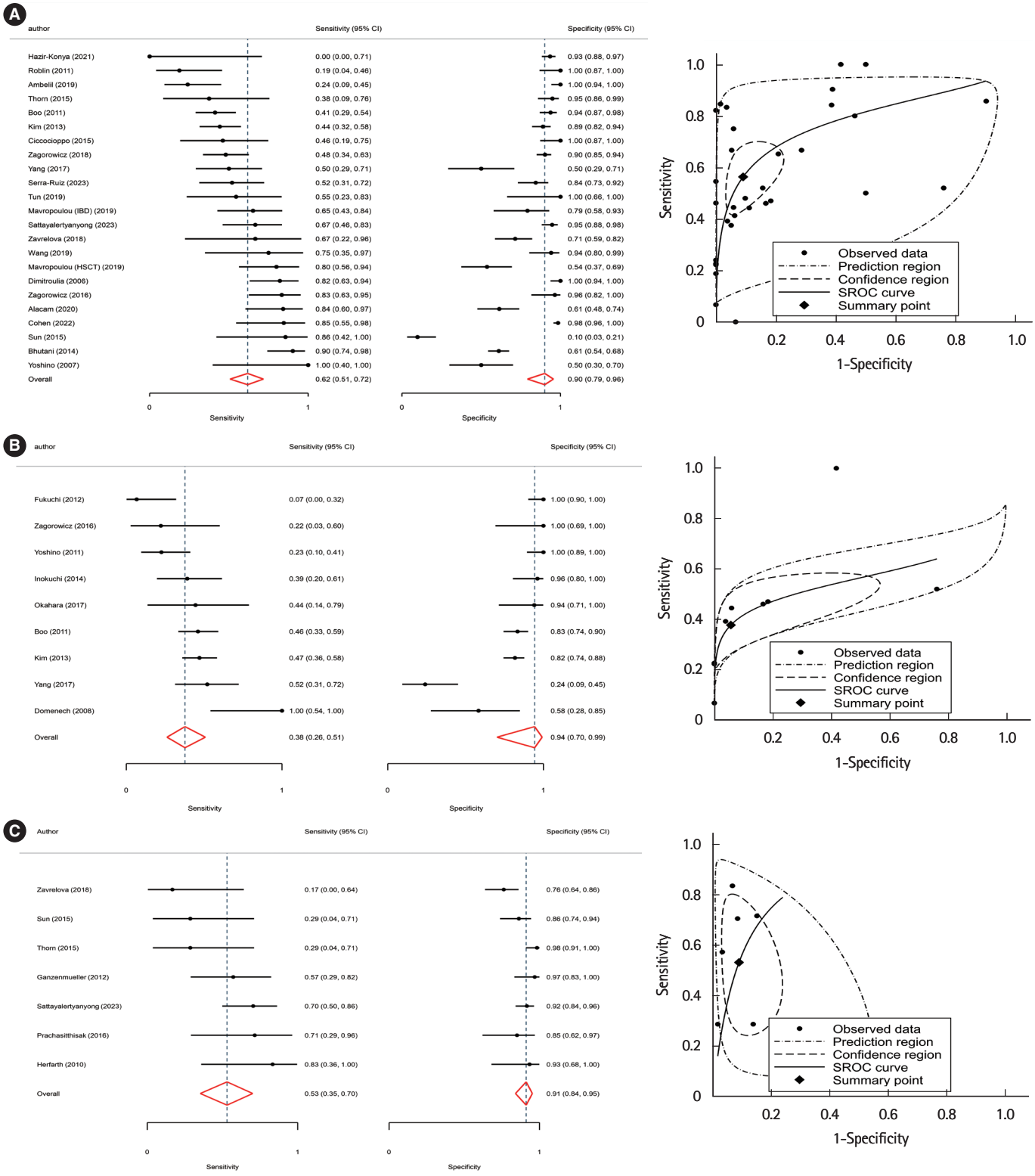


**Fig. 2.** Coupled forest plot and summary receiver operating characteristic (SROC) curve of serum CMV PCR (A), CMV antigen (B), and stool CMV PCR (C) to detect CMV ileocolitis. CMV, cytomegalovirus; PCR, polymerase chain reaction; CI, confidence interval.

analysis was conducted, categorized by study design and publication type. The results are shown in Supplementary Table 1.

Among studies using serum CMV PCR as the index test, 7 out of 23 were prospective studies, showing lower sensitivity (53%)

https://doi.org/10.5217/ir.2024.00136 • Intest Res 2025;23(2):213-224

**Table 3.** Pooled Sensitivities and Specificities of Each Noninvasive Test in the Studies Comparing Different Tests within the Same Studies

| Diagnostic tests | No. of studies | No. of tests | Pooled sensitivity (95% CI) | Pooled specificity (95% CI) |
|---|---|---|---|---|
| Two methods in a single study | | | | |
| Serum CMV PCR vs. serum CMV Ag | 4 | 490 (PCR), 457 (Ag) | 0.54 (0.36–0.70) vs. 0.46 (0.36–0.56) | 0.89 (0.67–0.97) vs. 0.80 (0.38–0.96) |
| Serum CMV PCR vs. stool CMV PCR | 4 | 316 | 0.70 (0.48–0.85) vs. 0.39 (0.16–0.68) | 0.76 (0.28–0.96) vs. 0.90 (0.79–0.95) |

CMV, cytomegalovirus; PCR, polymerase chain reaction; Ag, antigen; CI, confidence interval.

but higher specificity (97%) compared to overall and retrospective studies. This discrepancy could be from the study protocols, patient populations, and diagnostic methodologies, which may explain the observed heterogeneity. Studies examining serum CMV Ag and stool CMV PCR showed no differences in subgroup analysis.

### 6. Quality Assessment and Publication Bias

Supplementary Fig. 1 and Supplementary Table 2 illustrate the risk of bias assessment across the included studies. The domains of patient selection, index test, and flow and timing predominantly showed a low risk but with a noticeable portion of high risk and some unclear areas. The reference standard domain primarily indicates a low risk of bias, with minor segments of high and unclear risks. Regarding applicability concerns, most domains exhibited low concern suggesting strong applicability of the studies. Overall, most studies maintain a low risk of bias and concerns regarding applicability. Deek's funnel plot asymmetry tests showed no significant bias across the studies, with *P*-values of 0.99, 0.48, and 0.42 for serum CMV PCR, CMV Ag detection, and stool CMV PCR, respectively (Supplementary Fig. 2).

## DISCUSSION

Our study represents the first systematic review and meta-analysis to evaluate the diagnostic ability of noninvasive tests, including serum and stool tests, for detecting CMV ileocolitis across various populations. A total of 30 studies were included. Serum CMV PCR demonstrated an acceptable pooled sensitivity of 60% and a high specificity of 90%. Serum CMV Ag exhibited significantly lower sensitivity than serum CMV PCR. Stool CMV PCR had comparable pooled diagnostic performance to serum CMV PCR. However, the stool tests showed substantially lower sensitivity but higher specificity when including only the studies performed both tests in the same populations. A combination of serum CMV PCR and stool CMV PCR enhanced the diagnostic performance, but the evidence is limited to only a single study. Subgroup analysis in patients with IBD shows satisfied diagnostic performances for noninvasive tests, with pooled sensitivity of 56% and specificity of 94% for serum CMV PCR and the corresponding values of 83% and 93%, respectively, for stool CMV PCR. On the other hand, in post-HSCT, serum CMV PCR achieved a high pooled sensitivity of 85% but a low pooled specificity of 47%, while stool CMV PCR showed poor diagnostic performance with a very low sensitivity of 23% and fair specificity of 81%.

The common sites of viral latency after primary CMV infection are circulating lymphocytes, monocytes, and polymorphonuclear leukocytes.[53] Therefore, CMV viremia is reasonably used as a diagnostic test for CMV GI disease. However, our results show that serum CMV PCR and Ag pooled sensitivity is not high, 60% and 38%, respectively. This could be explained by the fact that the GI tract is another site of viral latency, and local GI reactivation can occur without systematic reactivation.[14,47,53,54] Of note, the sensitivity also varied due to differences in the cutoff thresholds used for noninvasive tests and the number of inclusion bodies required for histopathological confirmation.[41] In contrast, the pooled specificity of both serum tests is high, 90% and 94% for serum CMV PCR and Ag, respectively. This result is initially unexpected because it is well known that CMV viremia commonly occurs in patients with CMV reactivation, particularly in an immunosuppressed state, without CMV GI disease.[55,56] However, in our systematic review, 17 of 23 studies evaluating serum CMV PCR and 7 of 9 studies evaluating serum CMV Ag reported a specificity above 70%. We hypothesized that these tests may become more specific in a setting with GI symptoms in high-risk patients for CMV reactivation. Some organizations, such as the Transplant Associated Virus Infections Forum, have accepted using serum CMV PCR to diagnose possible GI CMV disease

**Table 4.** Pooled Diagnostic Performance of Noninvasive Tests for Diagnosis of CMV ileocolitis in Patients with Inflammatory Bowel Diseases and Post-Hematopoietic Stem Cell Transplantation

| Diagnostic test | No. of studies | No. of test | $I^2$ | Pool sensitivity (95% CI) | $I^2$ | Pool specificity (95% CI) | $I^2$ | Pool PLR (95% CI) | Pool NLR (95% CI) | Pool DOR (95% CI) | AUSROC (95% CI) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| IBD using tissue CMV IHC or PCR or H&E as references | | | | | | | | | | | |
| Serum CMV PCR | 13 | 1,283 | 56.01 | 0.56 (0.45–0.67) | 55.77 | 0.94 (0.82–0.98) | 56.26 | 9.40 (3.30–26.60) | 0.45 (0.34–0.60) | 21 (6–70) | 0.79 (0.76–0.83) |
| Serum CMV Ag | 8 | 649 | 0.02 | 0.35 (0.22–0.50) | 63.09 | 0.94 (0.79–0.99) | 47.31 | 6.60 (0.58–0.76) | 0.66 (0.58–0.76) | 10 (2–49) | 0.56 (0–1.00) |
| Stool CMV PCR | 1 | 21 | | 0.83 (0.36–0.996) | | 0.93 (0.68–0.998) | | | | | |
| Post-HSCT using tissue CMV IHC or PCR or H&E as references | | | | | | | | | | | |
| Serum CMV PCR | 4 | 440 | 0.14 | 0.85 (0.73–0.92) | 1.11 | 0.47 (0.21–0.74) | 93.26 | 1.60 (0.90–2.70) | 0.33 (0.14–0.75) | 5 (1–18) | 0.84 (0.81–0.87) |
| Serum CMV Ag | 0 | 0 | | | | | | | | | |
| Stool CMV PCR | 2 | 127 | | 0.23 (0.08–0.52) | | 0.81 (0.72–0.87) | | | | | |

CMV, cytomegalovirus; CI, confidence interval; PLR, positive likelihood ratio; NLR, negative likelihood ratio; DOR, diagnostic odds ratio; AUSROC, area under the summary receiver operating characteristic curve; IBD, inflammatory bowel diseases; IHC, immunohistochemistry; PCR, polymerase chain reaction; H&E, hematoxylin and eosin staining; Ag, antigen; HSCT, hematopoietic stem cell transplantation.

in patients after solid organ transplantation.[57]

Stool CMV PCR has emerged as a promising noninvasive diagnostic method, potentially offering a better representation of ileocolonic infection by targeting the same affected organ, which may enhance the test's specificity. Although the overall pooled specificity in our study is 91%, comparable to but not greater than serum CMV PCR, the specificity appears to be higher, 90% for stool CMV PCR and 76% for serum CMV PCR, in the analysis including only the studies measuring the 2 tests in the same studies. The limitation of stool CMV PCR is its low sensitivity. Although the overall pooled sensitivity is 53%, comparable to serum CMV PCR, the pooled sensitivity is significantly lower in stool CMV PCR, 39% for stool CMV PCR and 70% for serum CMV PCR, when including only the studies with 2 tests performed in the same studies. The low sensitivity of stool CMV PCR could be due to various factors, including intermittent viral shedding, non-standardized specimen handling, timing of collection, and sample processing methods. The study by Gu et al.[58] compared different extraction methods and reported different diagnostic performances.

The diagnostic performance varied when divided into IBD and post-HSCT populations. Tandon et al.[59] conducted a meta-analysis of publications from 2004 to 2015 on IBD populations. The study evaluated the diagnostic accuracy of blood-based tests (either serum CMV PCR or pp65 Ag) using tissue CMV IHC or PCR as reference standard. Despite an excellent specificity of 99.9%, the overall sensitivity was only 50%, with serum CMV PCR at 60% and CMV Ag at 39.7%. These findings are consistent with the performance observed in our study. The sensitivity of stool CMV PCR in the IBD subgroup was higher than that of serum studies. However, the results are from a single study done in hospitalized patients due to severe disease activity, possibly with high viral load and contributing to the high chance of stool CMV PCR detection.[29] For the post-HSCT group, the high sensitivity of serum CMV PCR at 85% was likely due to severe immunosuppression, which might promote viremia, but this is not specific to ileocolitis. In contrast, stool tests performed poorly in this subgroup, likely because the virus may be confined in intestinal tissue without leaking through the mucosa, as seen in IBD, resulting in lower viral shedding. The other hypotheses were stool dilution and differences in the detection time window between fecal and tissue samples, which may further limit sensitivity.[33]

A key challenge is how to integrate these noninvasive tests into clinical practice. According to the results above, both serum and stool CMV PCR cannot replace colonoscopy with

https://doi.org/10.5217/ir.2024.00136 • Intest Res 2025;23(2):213-224

tissue biopsy due to their low sensitivities. However, they are possibly helpful when colonoscopy is not available because of their high specificities. In another scenario, these tests may be used as an initial screening to enhance the diagnostic yield of subsequent colonoscopies. This approach is supported by evidence in post-HSCT patients. In the study of Bhutani et al.[37] involving 252 post-HSCT patients, 31 were diagnosed with biopsy-proven CMV gastroenteritis. A strong association was found between CMV viremia and gastroenteritis, with viremia being detected a median of 9 days before the diagnosis in 28 of the 31 patients (90%). Given the high risks associated with either colonoscopy or ganciclovir side effects in post-HSCT patients who may have concurrent neutropenia, the decision to empirically treat CMV colitis or confirm with colonoscopy after a positive noninvasive test should be individualized. In patients with IBD, both serum and stool CMV PCR can be useful when there is clinical worsening given their considerable performance. If the test is positive, although early colonoscopy should still be recommended to confirm the diagnosis and grade disease severity, antiviral treatment should not be delayed if pathology results are expected to take time.

The strength of our study is that we conducted a meta-analysis using 2 large databases and performed several subgroup analyses, including different reference standards, populations, and types of publications. The results were robust even when different reference tests were used, suggesting strong reliability. Nonetheless, our study has some limitations. Firstly, although we include all populations at risk, the majority of them are individuals with IBD. Secondly, diagnostic cutoff values for each noninvasive test and the types of commercial assays varied across studies. We attempted to demonstrate the absence of threshold effect using a couple-forest plot. However, substantial heterogeneity was present, particularly in serum CMV PCR, which may complicate the interpretation of findings. Additionally, the number of biopsies and threshold for the reference standards varied and were not accounted for, especially tissue CMV PCR that may detect low levels of the virus and classify it as CMV ileocolitis without having significant symptoms. Lastly, there is an unclear risk of bias in certain areas of the studies reviewed.

In conclusion, noninvasive tests cannot replace colonoscopy with tissue biopsy for diagnosing CMV ileocolitis owing to their poor sensitivities. However, they can be valuable when colonoscopy is not feasible, as positive results can aid further management. Serum and/or stool CMV PCR tests should be prioritized over serum CMV Ag due to their higher pooled sensitivities. Combining serum and stool CMV PCR may improve diagnostic accuracy, but further research is warranted.

## ADDITIONAL INFORMATION

**Funding Source**
The authors received no financial support for the research, authorship, and/or publication of this article.

**Conflict of Interest**
No potential conflict of interest relevant to this article was reported.

**Data Availability Statement**
Data, analytic methods, and study materials are available to other researchers upon request.

**Author Contributions**
Conceptualization: Limsrivilai J. Data curation: Chaemsupaphan T, Sattayalertyanyong O. Formal analysis: Chaemsupaphan T. Investigation: Chaemsupaphan T, Sattayalertyanyong O, Limsrivilai J. Methodology: Chaemsupaphan T, Limsrivilai J. Supervision: Limsrivilai J. Writing - original draft: Chaemsupaphan T, Limsrivilai J. Writing - review & editing: Chaemsupaphan T, Limsrivilai J. Approval of final manuscript: all authors.

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**Supplementary Material**
Supplementary materials are available at the Intestinal Research website (https://www.irjournal.org).

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