

# The species of Campy detection using antibody testing: a synopsis and comprehensive analysis

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### Abstract

**Context :** One of the main bacterial causes of foodborne illnesses is campylobacteriosis; infections caused by *Campylobacter* pose a serious risk to human health. It is challenging to diagnose campylobacteriosis since it necessitates the use of specialised culture methods and reputable laboratory facilities. However, *Campylobacter* antigen or antibody can be immediately detected by serological diagnostic assays that do not require a culture. The purpose of this systematic review and meta-analysis was to evaluate the diagnostic performance of serological tests that are used to identify distinct species of *Campylobacter* in various specimens.

**Techniques :** A thorough and methodical search for literature was conducted using MEDLINE, PubMed, Scopus, and Google Scholar to find publications published between 1999 and 2021 that discussed the diagnostic test accuracy of serological tests for the identification of *Campylobacter* species. The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines were followed in conducting this literature search. Only articles that satisfied the predetermined selection criteria were added to the meta-analysis. QUADAS-2 was used to evaluate the included papers' methodological quality in duplicate. Software called MetaDisc 1.4 was used to analyse the performance of the pooled tests.

**Findings :** The study had 13 publications in total. The test results were extracted, including the sensitivity, specificity, test efficiency (TE), negative predictive value (NPV), and positive predictive value (PPV). Next, an analysis was conducted on the serological tests for *Campylobacter* species, focusing on the pooled sensitivity, specificity, positive likelihood ratio, negative likelihood ratio, and diagnostic odds ratio. 7.0.3 and 99.8, 6 and 100, 36 and 100, 17.6 and 100, and 75.8 and 99.8 were the lowest

and highest recorded sensitivity, specificity, PPV, NPV, and TE, respectively. Significant heterogeneity was present. The combined values for LR+, LR-, DOR, specificity, and sensitivity were 86.7, 93.9, 15.4, 0.12, and 145.3, in that order. With an area under the curve (AUC) value of greater than 0.97, the overall diagnostic accuracy of serological tests in identifying *Campylobacter* species from various specimens was very good.

In conclusion, there is variability in the diagnostic test accuracy of serological tests used to rule out campylobacteriosis in various specimens. Nonetheless, these serological tests have extremely good pooled diagnostic test accuracy. It is therefore advised to use serological testing in situations where other culture- or molecular-based approaches are unavailable. There have been reports of ulcerative colitis and ase in Iran.<sup>11, 12</sup> Naturally, our study has certain numerical constraints. The in-patient data came from a single hospital's GILD ward.

Therefore, since many of these people proceed straight from our out-patient department to the surgical ward without admission to the GILD ward, this study may have underestimated disorders like gallstones and GI malignancies. Articles meeting the predetermined selection criteria were included in the meta-analysis, despite the fact that Shariati Hospital serves as a major referral facility for the entire nation and Tehran's population is multiethnic. QUADAS-2 was used to evaluate the included papers' methodological quality in duplicate. Software called MetaDisc 1.4 was used to analyse the performance of the pooled tests. low among Iranian men, in contrast to those in wealthy nations.

Despite being the most frequent cancer among women in Iran, the country's rates of breast cancer remain low when compared to other countries, particularly those in the US and Europe. Comparably, Iran has a very low incidence of cervical cancer—even lower than in low-risk nations like China, Kuwait, and Spain. We ought to design more extensive databases for in-patient data in the future, incorporating hospitals that are typical of every location in Iran.

You can also make use of information from the recently formed family physician network. Creating a suitable database for electronic data gathering in clinics and hospitals is a requirement for this. In conclusion, there is variability in the diagnostic test accuracy of serological tests used to rule out campylobacteriosis in various specimens. Nonetheless, these serological tests have extremely good pooled diagnostic test accuracy. It is therefore advised to use serological testing in situations where other culture- or molecular-based approaches are unavailable.

**Keywords :** *Campylobacter; Diagnosis; Sensitivity; Specificity; Serological test*

### INTRODUCTION

34 species make up the gram-negative, non-spore-forming genus *Campylobacter* [1]. The two most well-known species are *C. jejuni* and *C. coli*, which cause gastroenteritis in humans, but more species are also becoming known [2]. From a metabolic perspective, low oxygen tension environments (5% O<sub>2</sub>, 10% CO<sub>2</sub>, and 85% N<sub>2</sub>) are ideal for the survival and growth of microaerophilic bacteria [3]. One of the most common bacteria that cause foodborne infections worldwide is *Campylobacter*, and it mostly causes gastroenteritis. Eighty to eighty-five percent of human infections are caused by *C. jejuni*, while the remainder instances are primarily linked to *C. coli* [4]. Because it impacts the health of both humans and animals, it is a global public health concern.

The frequency of *Campylobacter* species from various sources is periodically rising sharply as a result of human-animal contact [4]. The existing data indicate that *Campylobacter* infection is endemic throughout Africa, Asia, and the Middle East, despite the fact that epidemiological data from these locations are currently lacking [5]. To cultivate *Campylobacter* Species (SP), advanced microbiological methods are needed, and the process takes longer than 48 hours. Apart from microbiological methods, Polymerase Chain Reaction (PCR) is another approach that can be used to detect *Campylobacter* spp. However, this method also necessitates well-established testing laboratories. These factors account for the extremely low detection rates of *Campylobacter* spp. in underdeveloped nations relative to high-income nations [3]. From various samples, the *Campylobacter* antigen can be directly detected using serological testing. Serological tests do not require complex laboratories or a lot of time, in contrast to microbiological and genetic methods for detecting *Campylobacter* spp. [6]. Furthermore, there is no special laboratory setup required to perform these serological assays, making them simple to do. There is insufficient information available about the combined diagnostic accuracy of the tests, despite the possibility that serological approaches could be diagnostically significant for clinical decision making. In light of the potential significance of the results for policy makers, this study set out to ascertain the pooled diagnostic test accuracy of serological tests for *Campylobacter* spp. from various specimens.

### Methods

#### Eligibility Criteria

This study covered publications that discussed the sensitivity and specificity of serological testing for *Campylobacter* species. Sample size, appropriate statistical measurement, and the use of culture or a combination of culture and qPCR as the gold standard are examples of quality markers. For this review, studies with a minimum of 60 samples, cross-sectional studies, and surveillances with a response rate of more than 80% were included.

#### Sources of Information and Search Techniques

From 1999 to March 17, 2021, MEDLINE articles were searched using PubMed, Scopus, and Google Scholar. To find more material, manual searches and certain articles' reference lists were also employed. Two search iterations were conducted, utilising Medical Subject Headings (MeSH) terms and key words such as serological testing, sensitivity, specificity, and *Campylobacter* species.

#### Process of Study Selection and Data Collection

Every article that was identified was exported to the EndNote 20 library. Reading the work's title, abstract, and final review came first in the screening process. The articles were evaluated independently for inclusion. In a similar vein, each of the two writers separately gathered data from the listed publications. Discussions were used to settle disagreements on the data items.

#### Definitions of Data Items

Any commercial serological test examined for the identification of *Campylobacter* species from specimens was considered an index test. The reference test was a standard culture, and samples that tested positive for the reference test were deemed true positives; those that tested negative were deemed true negatives. The reference test could be performed with or without other tests, such as the index test. Terms like sensitivity, specificity, positive likelihood ratio (LR+), negative likelihood ratio (LR-), diagnostic odds ratio (DOR), and hierarchical summary receiver operating characteristic (HSROC) curve that are associated with diagnostic test accuracy are described and defined in detail in reference [7].

#### Bias and Applicability Risk

The QUADAS-2 tool was utilised to evaluate the methodological

quality of the included publications in duplicate. This instrument is used to examine the diagnostic test accuracy (DTA) [7, 8]. The tool comprises three domains for applicability judgement and four domains for risk of bias judgement. A study is deemed to have “low risk of bias” if it has “low” ratings across the board for bias and applicability. A study is classified as having “high risk or unclear risk of bias/applicability” if it has a “high” or “unclear” rating across multiple domains [8].

### Results Synthesis and Meta-Analysis

The MetaDisc 1.4 programme was used to carry out the analysis. This programme is a thorough and specialised test accuracy meta-analysis programme [9]. Sensitivity, specificity, diagnostic DOR, LR+, LR-, and the Summary Receiver Operating Characteristic (SROC) curve were the summary metrics used to assess the accuracy of diagnostic tests. At the assay level, these summary metrics were determined. Tables were used to summarise the results for sensitivity, specificity, DOR, LR+, and LR-. Forest plots were used to visually evaluate heterogeneity. The random effects technique was used since the Diagnostic Test Accuracy (DTA) naturally exhibits heterogeneity. To visualise the landscape of the serological tests, the SROC curve was also used. The random effects model was employed to reduce the impact of heterogeneity because the  $I^2$  was greater than 50%. The assays' DTA was calculated by evaluating the Area Under Curve (AUC).

## Results

### Data Selection and Study Characteristics

Articles were chosen in accordance with the flow diagram (Figure 1) of PRIMSA 2009 [10]. Seven additional papers were found through manual search after the first 126 articles were obtained from various databases. After that, 23 articles were deleted for being duplicated. Out of the 110 publications that were evaluated, 97 were eliminated because of incorrect titles and abstracts, irrelevant data, or inadequate data. Out of the 39 publications that met the data criterion, 26 were deemed ineligible for full text examination. Lastly, the meta-analysis had 13 publications that matched the eligibility criteria. A summary of the features of the research that were part of our meta-analysis can be found in (Table 1). The 13 publications, which were released between 1999 and 2021, detailed the results of 20 serological tests that were run on a total of 4207 specimens with various origins.

including skin samples from people, animals, and the environment, faeces, preputial wash, and sera. Numerous serological assays utilising various principles were included, such as enzyme immunoassay, immunochromatography, and complement fixation principles. The majority of the tests were enzyme immunoassays. The studies assessed the DTA of the serological assays using reference tests. The reference tests consist of either a combination of assays other than culture or a combination of culture and other assays. Individual Study Outcomes

A standard formula was used to determine the diagnostic accuracy of each test, taking into account the number of TP, TN, FP, and FN specimens. Positive predictive value (PPV) ( $TP/TP+FP$ ), negative predictive value (NPV) ( $TN/TN+FN$ ), specificity ( $TN/TN+FP$ ), sensitivity ( $TP/TP+FN$ ), and test efficiency (TE) ( $TP+TN/TP+TN+FP+FN$ ) of The specific assays are shown in (Table 1). The individual test results for the lowest and highest reported sensitivity, specificity, PPV, NPV, and TE were 17.6 and 100, 6 and 100, 36 and 100, 70.3 and 99.8, and 75.8 and 99, respectively. The complement fixation test, monoclonal antibody ELISA on preputial wash specimens, Ridascreen campylobacter enzyme immunoassay, and EIA-Foss enzyme immunoassay were shown to have the lowest sensitivity, specificity, PPV, NPV, and TE for individual tests, respectively. Conversely, monoclonal antibody ELISA on preputial wash specimens was shown to have the lowest specificity and test efficiency. As opposed to other tests, this one had the highest sensitivity (100%) of all. The best specificity and PPV (100%) were demonstrated by ProSpecT enzyme immunoassay, while ICA immunochromatography and EIA The high-est NPV was replaced by the enzyme immune assay. Overall, ProSpecT enzyme immunoassay performed better for TE. According to Table 1, the assays' average sensitivity, specificity, PPV, NPV, and TE were 84.7, 88.8, 82.2, 90.9, and 90.2.

In their evaluation of the DTA of two immunochromatography assays using 305 patient-collected stool specimens, Bessedé et al. [11] found that Ridaquick Campylobacter performed better than ImmunoCard STAT. The effectiveness of three tests that use the enzyme immunoassay principle—Meridian EIA, Remel EIA, and Meridian STAT!—was also assessed by Granato et al. [12]. Based on their findings, 485 stool specimens with  $TE \geq 96\%$  were successfully identified as belonging to a Campylobacter species by every test. ELISA displayed the lowest specificity (6%) and the highest specificity (%) in bovine preputial wash specimens.

In contrast, the complement fixation test demonstrated the lowest sensitivity of 17.6% in identifying antibodies against

Campylobacter species from 153 sheep serum. sensitivity (100%) [18]. [19]. In stool specimens, the Prospect enzyme immunoassay demonstrated perfect specificity (100%) for identifying Campylobacter jejuni antibodies [22]. When compared to other immunoassays, the Prospect enzyme immunoassays had superior diagnostic accuracy with TE $\geq$ 89% [15,20,22], however the EIA-Foss enzyme immunoassay demonstrated the lowest diagnostic accuracy (TE: 70.3%) [17].

### Discussion

Few papers that describe the diagnostic performance of serological tests for Campylobacter spp. were found in our search of the literature. Just 13 papers were chosen for examination out of the 133 that were found using manual searches and databases. These papers primarily describe the outcomes of serological tests using complement fixation, immunochromatography, and enzyme immunoassay to detect Campylobacter spp. Most regions of the world are seeing an increase in campylobacter infections. The list of nationally notifiable diseases was expanded to include campylobacteriosis in 2015 [24]. However, due to the lack of a national surveillance programme and the irregular availability of culture for Campylobacter species in clinical and research settings, the true frequency of Campylobacter spp. is still not adequately presented [4].

Different serological assays are available to identify Campylobacter. These serological tests have lower costs and quicker turnaround times than culture-based approaches for detecting Campylobacter [25]. The sensitivity and specificity of a multi-center research based on stool antigen detection ranged from 79.6% to 87.6%, 95.9 to 99.5%, and 41.3 to 84.3% for the positive predictive value, respectively [26]. Variations in sample size, variations in the types of specimens utilised, and variations in the intrinsic accuracy of the various test procedures could all contribute to variations in sensitivity, specificity, and positive predictive value. API A promising identification method is the use of a Campy, Neisseria-Haemophilus (NH) identification card and matrix aided laser desorption ionization time of flight mass spectrometry (MALDI-TOF MS). technique for species of Campylobacter [27]. Among these tests, the monoclonal antibody ELISA test had a 100% sensitivity, while the MALDI-TOF mass spectrometry test had a 100% accuracy rate with a 98.3% sensitivity [27]. [18]. The primary challenge in diagnosing campylobacteriosis is that Campylobacter species identification

primarily depends on culture [28]. The susceptibility of Campylobacter to perish during handling and the challenge of identifying tiny colonies amid competing faecal flora restrict the accuracy of culture [29]. It is unknown how little Campylobacter can be grown in stool samples at this time. The relationship between the quantity of germs found by culture and culture-independent serological testing and clinical signs of diarrhoea [30, 31]. Understanding this estimate is useful for researching Campylobacter spp. asymptomatic carriage, particularly in endemic environments. According to Buss et al. [32], there was a range of 0.3-5 x 10<sup>6</sup> CFU/mL in the detection limits for Campylobacter in culture. The CAMPYLOBACTER QUIK CHEK™ test, an FDA-approved fast membrane-based EIA, has a detection threshold of 8.4 x 10<sup>4</sup> CFU/mL for C. jejuni and 7.7 x 10<sup>5</sup> CFU/mL for C. coli [32]. Microspheres that glow The limit of the labelled immunochromatographic test is 10<sup>6</sup> CFU/ml [33]. Because serologic assays can identify even very small amounts of Campylobacter spp. in a sample, they are particularly valuable in epidemiologic research and Campylobacter surveillance [34].

The results of this meta-analysis offer useful guidance on serological techniques that are independent of culture for the detection of Campylobacter species. Not only will this information be helpful for large and small diagnostic laboratories, but it will also yield unexpected results on underreported Campylobacter species. Excellent sensitivity, specificity, negative predictive value, positive predictive value, and test efficiency characterise these culture-independent serological techniques. The results imply that clinical decision-making should involve serological testing. Because these tests are inexpensive, quick to complete, and require simple laboratory settings, they may be more important to utilise than culture-dependent procedures, particularly in low- and middle-income nations where the application of molecular and culture techniques is limited.

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