A CRITICAL EVALUATION OF ELISA TESTS USED TO DETECT ANTIBODIES AGAINST CAMPYLOBACTER IN HUMANS

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Abstract: *Campylobacter infection is one of the most common causes of foodborne illness in the European Union. It is frequently followed by different sequelae, such as neuropathies and reactive arthritis. Diagnosis is made mainly by stool culture which means a lot of work, time and money. ELISAs, as serological tests are well suited for diagnosis's sequelae or for identifying infection from stool sample with non-viable bacteria. At present, international consensus regarding ELISAs for campylobacter in humans exists. It was identified 17 studies validating such assays in a literature review. The best assay that was validated is the one developed "in-house" and which was used especially for research purposes, then for routine diagnosis. Taken into account the burden of the disease and the possible severity of campylobacter infection, it would be useful, for surveillance and diagnosis, to develop a standardized and commercially available ELISA assay.*

INTRODUCTION

During the last couple of years, in many regions of the world, the number of *Campylobacter* infections increased. This could reveal a real increase or a heightened concern regarding the disease. *Campylobacter* infections are being considered the most common cause of bacterial gastroenteritis.

Many *Campylobacter* species are involved in human infections, but by far, the most frequent is *Campylobacter jejuni*. A population study made in Great Britain (1) demonstrated that *C. jejuni* is frequently isolated from chickens and broilers than *Campylobacter coli* and is also the cause of human confirmed cases in 93% of diseases. *Campylobacteriosis* is primarily zoonotic, which major source seems to be the manipulation and the ingestion of poultry meat insufficiently cooked. Unpasteurised milk and contaminated drinking water could be other sources of infection, occasionally resulting in large food and waterborne outbreaks.(2,3) Also, direct contact between infected animals and humans or between humans and infected humans can determinate the transmission of the disease.(4)

Campylobacter infection is usually a self-limiting disease, in many cases it presents not symptoms at all.(5,6) For example, in a Dutch population study (7), more then 95% of young adults showed, by serology, evidence of a *Campylobacter* infection in their past, and the fact that almost all of these were asymptomatic was demonstrated by comparing the estimated number of clinical diseases.(5) Contrary to this, *campylobacteriosis* has a very high burden in Europe due to the high number of affected people but, also, because of the fact that some post-infection sequelae, for example *Guillain-Barré* syndrome (GBS) can determine paralysis and even death.(7)

PURPOSE

The gold standard for the diagnosis of *Campylobacter* infection is stool culture. Regarding the patients with disease sequelae (e.g. reactive arthritis or GBS), this way of setting up the diagnosis became in several times difficult or even impossible. The reason is that, culturing is not performed because only a few cases were discovered in the acute phase or, if it is done, the result could be negative for different reasons.(8) The alternative diagnosis methods, such as ELISA assays technique have been proposed, taken into account the importance of *Campylobacter* burden and last but not least, the high proportion of patients who gain sequelae and need a proper management of the diagnosis.

ELISAs used to analyse the serum from humans are generally based on crude antigen preparations, which derive from acid-glycine extracts, or whole-cell sonicates. It was demonstrated that patients with *Campylobacter* infections exhibit an acute increase of IgG, IgA and IgM antibody levels against whole-cell antigens of *Campylobacter*. This increase is followed in the next 6 weeks by a rapid decrease to the original levels. Therefore, in order to determine the recent infection with *Campylobacter* using assays based on antibody detection, it is recommended to detect at least 2-antibody immunoglobulin's classes.

The objectives of the article are the following: to give an image of the documented use of ELISAs for the detection of *Campylobacter* immunoglobulin in humans' sera, to show the characteristics of the identified ELISA tests, and to identify techniques and ways in which these can be used worldwide.

MATERIALS AND METHODS

There was performed a literature search in the US National Library of Medicine, National Institutes of Health (PubMed.gov) and Research Gate (www.researchgate.net), for identifying as much as possible *Campylobacter* ELISAs tests. The title/keyword/abstract fields were verified for the words: *"serology", "antibody", "campylobacter", "infection"* and *"patient"*. Because *C. jejuni* is almost all the time the cause of gastroenteritis, I focused the search on specific ELISAs.

To identify the existence of commercially available ELISAs which could not have been yet evaluated in a published research, using the words *"Campylobacter ELISA antibody assay"*, an Internet search was done.

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RESULTS

The results of the scientific literature search were: 210 kits of which only 17 (8%) founded to be relevant and also one single article for commercially available ELISA. Another 3 commercially available assays, without the first one, were identified by the Internet search.

Unfortunately almost all articles identified did not express extended information on the antigen used for testing. In two studies (9,10), there is mentioned the use of antibodies against recombinant proteins (specifically P18, P39 and PEB4). Because these assays had a high sensitivity and specificity and there is no evidence of cross-reactions with other bacteria (e.g. *Yersinia enterocolitica* and *Helicobacter pylori)*, these represent suitable candidates to be used in serologic studies. Also, because in the patients infected with *C. jejuni* strains, P18 and P39, present high seroconversion rates, it can be concluded that these proteins are the same across the strains. One article (11) analysed the importance of the presence of antibodies against the major protein of *Campylobacter* flagellum, the flagellin. Other researchers used surface antigens of *C. jejuni*, from a pool of several strains - expressing the most prevalent ones in the environment (12) or from only a particular strain.(8)

The assay sensitivity and specificity were reported in 16, respectively 14 studies. The values for sensitivity were between 18% and 100%, while specificity was between 68.4% and 100%. The *cut-off* value that defines *seropositivity* was determined by using different methods. One of them was 2 standard deviations above the optical density (OD) mean for each antibody (12), using the receiver operating characteristics (ROC) curve analyses at a specificity level of a 95%, arbitrary chosen from a group of healthy individuals.(8) Another different method used any measurement that was above reference antibody titres, also taken into account a group of healthy individuals.(13)

Healthy individuals or persons with another infection can use ELISA techniques in cases confirmed by *Campylobacter* culture, as well as in the situation in which we have a control group formed. Various situations were identified, such as: a group of patients with gastrointestinal infections or with suspected GBS was investigated using *ELISA Virion/Serion* (14); another 2 studies examined patients with diarrhoea, one without control group (15) and the other compared to healthy individuals; and some researchers (16) evaluated their assays on patients with GBS and Fischer's syndrome - neurologic sequelae. These patients had no *Campylobacter* infection confirmed by culture and were compared with a group formed by healthy individuals and/or persons with other neurologic diseases.

The total number of persons included in the reviewed studies varies between 40 (11) and 739 (8), with 13 of 17 articles presenting a study population including more than 100 persons. In the largest study (8), there were examined 220 patients (with and without complications), 482 healthy individuals and 40 persons with other gastrointestinal infections.

Many authors were interested in cross-reactivity with gastro-enteric bacteria (*Y. enterocolitica, Salmonella enterica, H. pylori* and *Legionella pneumophila*).(8,9,10,12,13). One of them (9) demonstrated that in 7.5% of patients with serological determination of infection with *H. pylori* or *L. pneumophila*, *campylobacter* antigens was also determined. The conclusion of the majority of the reviewed studies revealed the fact that antibodies against other pathogens are not significantly influenced by the results of ELISA in case of *Campylobacter* infection.

A number of 4 commercial ELISAs were identified, but background in technical information and scientific evaluation was found only for two: *Serion ELISA classic Campylobacter jejuni* and *recomWell Campylobacter ELISA* (Mikrogen, Neuried, Germania). The other two were: the *Human Campylobacter jejuni PEB1 ELISA* kit developed by Bio-Swamp and *FCAMP* sold by Mayo Medical Laboratories (Rochester, MN, USA).

DISCUSSIONS

In this literature review, a small number of articles were found to present the detection of *Campylobacter* antibodies in human sera using ELISAs. By these techniques, there were detected increased levels of immunoglobulins, as a response to *Campylobacter* proteins: recombinant proteins, flagellin proteins or crude surface proteins. Generally, a comparison of results between the reviewed studies was difficult because of the lack of information regarding *cut-off* and *seropositivity*' values and the different way of defining them.(9) A total number of 4 ELISA kits for *campylobacter* diagnosis commercially available were identified. Two of them were reviewed for this article with different results.(10,14) It was difficult and even not possible to find information or detailed evaluations regarding another commercial assays. This fact indicated that their use was not widespread. Regarding specificity, the two largest studies (8,12) shown similar levels.

Taken into account the articles reviewed, it appears that a method to detect immunoglobulins against *Campylobacter* antibodies in human serum and a candidate suitable for standardized and commercially available could be ELISA based on selected recombinant proteins. Such a technique, based on recombinant proteins P18 and P39 was found to be already available on the market. Its real diagnostic value is entirely known yet. A group of researchers (9), based on the same proteins, developed an *in-house* assay and concluded that this can detects previous *C. jejuni* infections with a high accuracy, and so, allowed a clear understanding of the *Campylobacter* role played in causing complications with late onset. To use pooled surface antigens for an ELISA may represent a problem in a larger setting because of significant local differences between circulating strains of *C. jejuni* and cross-reactions with similar gastrointestinal bacteria that may occur.

Supposing that the proteins P18 and P39 are the same across *C. jejuni* strains, an ELISA technique using these proteins would capture up to 90% of all *Campylobacter* reported. The numbers of isotypes of antibodies that must be included in the test consist in an additional factor to consider. The detection of IgG was found to be more specific than IgA or IgM in several studies published.(11,17). The most rapid IgA and IgM changes were observed to be in post-infection periods.(13)

The IgG levels are a less specific indicator of infection, but it can be still observed a few months after infection or even exposure.(17) IgA and IgM levels detection is important to be used in outbreak exercises; in spite of these, IgG detection may offer a solution in order to identify post-infection sequelae with a long-lasting life that in several times can be the best. In order to be a candidate test for being commercially available and easily used, the ELISA assay must be validated several times, using a suitable number of serum samples: known positive ones or, in order to test for cross-reactivity, from a group of healthy individuals or with other known gastrointestinal infections (e.g. *Y. enterocolitica* and *H. pylori*) (both negative). It was suggested that ideally, at least 100 patients with acute *campylobacter* infection must be used, 100 healthy individuals, 100 persons with sequelae related to *Campylobacter* infections (e.g. GBS, Fischer's syndrome and reactive arthritis) and another 100 patients with other

gastrointestinal bacterial infections. Regarding performance, sensitivity and specificity of the test always will depend on the purpose followed: for example, diagnosing the late-onset complications required a high specificity - at least 95%, while for research and also for purposes of a general diagnostic, it is acceptable even a lower specificity (e.g. 85%). The *cut-off* value can be determined for other ELISAs also by using many other methods. Two studies, found in this literature review defined *seropositivity* as being any reading above the geometric mean of optical density (OD) sera from healthy individuals plus 2 standard deviations of the mean.(12)

It was found evidence only for 4 *Campylobacter* ELISA kits in searching of Internet sources and scientific literature. Two of these, the *Human Campylobacter jejuni PEB1 ELISA kit* (Bio-Swamp) and *FCAMP* (Mayo Medical Laboratories) seem not to have been evaluated and presented in any published scientific study. Some researchers, using the *recomWell* kit (9), showed that IgA antibody detection registered a low sensitivity level (18%), but the detection of IgG antibody having a sensitivity level of 82%, was comparable to the level detected in most of the *in-house* assays reviewed in the article. However, the 89-100% specificity of the *Serion ELISA classic* was more comparable to previously reported *in-house* assays. Few published studies tried to compare *commercial* versus *in-house* ELISAs in order to use for detecting *H. pylori* (considered to be a *Campylobacter*-like organism for many years). So, some demonstrated similarities between *H. pylori in-house* ELISA (with 100% sensitivity and 89% specificity), and a *commercial* test (with 96% sensitivity and 89% specificity) (18), while others revealed no significant differences between *in-house* and other 8 *commercial* tests.(19) Currently, there is not substantial evidence demonstrating that the accuracy of a *commercial Campylobacter* ELISAs should be smaller than *in-house* tests usually have. It is definitely known that ELISA assays are among the most reliable and cheapest serological diagnostic methods for the infectious diseases. This is a technical point of view to develop an ELISA for the diagnosis in human sera - even on the basis of antigens that can be preserved across the two main human pathogenic species - but in this review, there was shown that most tests were developed and used *in-house*. Developing and implementing a standardized ELISA method to replace the existing *in-house* ones, will not be a priority for many countries where *Campylobacteriosis* is considered to be of small importance. These practical issues are minor, considering the obvious advantages of ELISA.

CONCLUSIONS

Standardization of ELISA and its *commercial* use in order to detect *Campylobacter* antibodies in human serum are unexplored topics yet. Most of the techniques reviewed were *in-house* ELISAs that did not use standardized antigens or, considered *seropositivity* using the *cut-off* levels. These ELISA assays were evaluated primarily in the same laboratory, where they were developed, and most of times using only a small number of sera.

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