

Chapter 2

Isolation and Identification of *Campylobacter* spp. in Poultry

Omar A. Oyarzabal and Heriberto Fernández

Abstract Poultry products, especially chicken meat, continue to be important sources of campylobacteriosis in humans. This chapter reviews the current methods used for the isolation and identification of *Campylobacter* spp. from chicken products. Emphasis is placed on the enrichment protocols, plate media, and most used, practical confirmation methods. The incorporation of molecular techniques and some of the methodologies used in some Latin American countries to detect *Campylobacter* spp. from poultry are summarized. Finally, some perspectives in future trends are provided.

Keywords Isolation • Identification • Culture media • Rapid methods • Molecular methods • Poultry • Food samples

2.1 Introduction

Campylobacteriosis is the generic name for the disease produced by bacteria belonging to the genus *Campylobacter*. Within the genus *Campylobacter* there are several bacterial species that can produce disease in humans and domestic animals (Man 2011), but *Campylobacter jejuni* and *Campylobacter coli* are the most important species from the public health stand point and are responsible for almost 98 % of all the confirmed human cases of campylobacteriosis (Gilliss et al. 2013).

The epidemiology of campylobacteriosis is complex and there are still several factors that are not well understood, even in developed countries. There are several

Authors do not endorse any particular assay or manufactures of assays discussed in the chapter.

O.A. Oyarzabal (✉)

University of Vermont Extension, St. Albans 05478-1866, VT, USA

e-mail: oaoyarzabal@gmail.com

H. Fernández

Institute of Clinical Microbiology, Universidad Austral de Chile, Valdivia, Chile

e-mail: hfernand@uach.cl

risk factors that have not been fully elucidated but that may prove to be important in the interpretation of the geographical variations (Ailes et al. 2012) and even within ethnic groups (Quinlan 2013) in the appearance of this disease. The incidence of campylobacteriosis varies according to countries. For instance, the reported incidence in the USA is 14 cases every 100,000 people, while the incidence in Australia is more than 100 cases every 100,000 people and excluding New South Wales (Anonymous 2014). In South America, there is also a variation in the prevalence of this disease by country, and although there are no consistent figures, this disease continues to have a major impact in public health (Fernández 2011).

The actual reported number of cases represents only confirmed cases and therefore there are many more cases that go underreported annually. Yet, with this incomplete reporting system campylobacteriosis represent one of the most important bacterial diseases transmitted by foods worldwide.

The foods most commonly implicated in cases of campylobacteriosis are meats, especially poultry meat and giblets, raw milk, and raw oysters. This chapter will focus on the methods most commonly used for the isolation of *Campylobacter* spp. from poultry meat, with emphasis on the methods suggested by food regulatory agencies from developed countries. The section about identification will focus on the protocols that are most commonly used in food microbiology laboratories and with special emphasis on the methods based on the identification using polymerase chain reaction (PCR) protocols.

2.2 Isolation

2.2.1 Isolation from Fecal Material of Live Chickens

Campylobacteriosis is largely considered as a foodborne disease. Poultry meat, primarily chicken meat, is an important source of transmission of *Campylobacter*. Some estimates suggest that up to 80 % of all cases of human campylobacteriosis are attributed to the transmission by chicken meat worldwide (Bahrdorff et al. 2013). Poultry meat gets contaminated during processing, at the slaughter house, at different stages. Defeathering and evisceration are important steps at which contamination with feathers/skin and intestinal content from the birds will occur. The subsequent full elimination of the bacteria from the meat is not completely achievable throughout the rest of the processing steps, and *Campylobacter* can survive (López et al. 2003) through storage and contaminate the kitchen of end users at home.

The isolation of *Campylobacter* from feces in commercial poultry farms is important for epidemiological studies of this agent. There is an extensive scientific literature on the methods for isolation of *Campylobacter* from fecal material and the best approach is the use of direct plating of feces on selective agar plates and the subsequent incubation of the plates at 42 °C under microaerobic conditions and for up

to 48 h. In South America, a modified protocol includes a pre-enrichment step of 24 h and a subsequent transfer to selective plates for up to 48 h. This protocol has been used to isolate *Campylobacter* from backyard hens and chickens in Southern Chile, with results showing a prevalence of 23–77 % of *Campylobacter* (Fernández 1992; Fernández et al. 1993; Fernández and Torres 2000). The pre-enrichment step increases the isolation rate by 20 % (Fernández 1992). With the same protocol, the prevalence of *Campylobacter* in poultry in a low-income community in Buenos Aires, Argentina was 40 % (López et al. 2003), whereas in Southern Brazil the prevalence of *Campylobacter* in 26 small, family farms with mixed flocks for meat and eggs production was 26 % (Gomes et al. 2006). In a study comparing direct plating versus pre-enrichment in 22 broiler flocks aged 3–5 weeks in Brazil, Kuana et al. (2008) found no statistical differences between the pre-enrichment and direct plating methods. However, the total rate of positive flocks detected by the pre-enrichment method amounted to 99.0 % (95/96), compared to 97.9 % (94/96) in direct plating.

2.2.2 Isolation from Poultry Products

2.2.2.1 Enrichment of Food Samples

The isolation of *Campylobacter* from foods is based on the enrichment of the samples in selective broths, the transfer of the enriched sample to selective agar plates and the identification of presumptive colonies grown on agar plates. This isolation protocol relies heavily on the use of selective agents and a high incubation temperature (42 °C) to reduce the competition from other microorganisms, mainly bacteria and yeasts, in the samples. It is important to keep in mind that high temperatures should be used only when suspecting the presence of thermotolerant species of *Campylobacter*, which are *C. jejuni*, *C. coli*, *C. lari*, and some strains of *C. upsaliensis* (Gharst et al. 2013).

If other, non-thermotolerant *Campylobacter* species are known or suspected in the samples, it is recommended that the isolation procedure be performed with incubation temperatures of 37 °C. However, the most important species in foods are *C. jejuni* and *C. coli* and most isolation protocols can be performed at incubation temperatures of 42 °C, especially in poultry samples. Some enrichment protocols suggest an initial temperature of 37 °C for the first 3–4 h of enrichment to help potentially injured *Campylobacter* cells to recover, but there are no scientific works or any important studies that justify the use of this initial temperature, or that suggest that a significantly larger proportion of samples will become positive if this variation in the protocol is included. The Cape Town Protocol (Lastovica 2006) utilizes an initial isolation temperature of 37 °C, presumptive colonies are reincubated at both 37 and 42 °C, allowing isolation of thermophilic and non-thermophilic *Campylobacter* spp. from chicken meat.

Traditionally, enrichment broths have been incubated under atmosphere containing a reduced oxygen level, usually atmospheres that are called “microaerobic” and are comprised of 5 % O₂, 10 % CO₂, and 85 % N₂. However, atmospheres with low oxygen levels are naturally generated in enrichment broths and therefore static incubation is enough to provide the adequate environment for *Campylobacter* cells to grow and multiply (Zhou et al. 2011).

Until recently, it was thought that the enrichment broths contained many different nutrients from which *Campylobacter* cells could grow. Yet, we now know that the presence of selective agents is more important than the nutrient composition of the broth for the successful isolation of *Campylobacter* spp. from food samples. For instance, buffered peptone water is sufficient for the isolation of *Campylobacter* from poultry meat (Oyarzabal et al. 2007, 2013). Among the selective agents for enrichment broths and plate media, cefoperazone (sodium salt) is the antibiotic most effective against competing bacteria present in the foods. Several enrichment broths incorporate, besides cefoperazone, vancomycin to control the growth of Gram-positive bacteria, trimethoprim, and amphotericin B as an antifungal agent. For many years, we have been suggesting the use of only cefoperazone, as a broad spectrum antibiotic, and amphotericin B. We use approximately 33 mg of cefoperazone and 4–10 mg of amphotericin B per liter of medium. It is difficult to predict how contaminated the food sample is, but this combination of antibiotics appears to be a good compromise for the isolation of *Campylobacter* spp. from poultry meat. An alternative to control high background flora is the addition of vancomycin at concentrations of 20 mg per liter, but we prefer to use filter membranes for the transfer of enriched samples to plate media and reduce the use of antibiotics (Speegle et al. 2009; Gharst et al. 2013). Some antibiotics used in antibiotic selective plates will suppress the growth of *Campylobacter* spp. (Lastovica, unpublished).

Due to an increase in the appearance of *Escherichia coli* strains expressing extended-spectrum beta-lactamase, some reports suggest the addition of tazobactam through the isolation procedure. This compound is more chemically stable than clavulanic acid or sulbactam; thus, tazobactam is more suitable for restoring the selectivity of CCDA (charcoal-cefoperazone-deoxycholate agar) and other media for the isolation of *Campylobacter* (Smith et al. 2015).

The traditional time for enrichment of samples is 48 h and the attempts to reduce the time to 24 h resulted in a larger proportion of samples identified as false negative (Liu et al. 2009; Oyarzabal et al. 2007). Yet, the transfer of enriched samples at 24 h will help identify the samples with higher number of naturally occurring *Campylobacter*. Using this methodology, Simaluiza et al. (2015) reported a prevalence of 62.7 % of *Campylobacter* positive samples in chicken livers for human consumption in Southern Ecuador.

The examination of enrichment broth at 24 h with PCR methods have not resulted in reliable identification. At 48 h, the use of PCR may have some benefits and some commercial systems, such as the BAX[®] (Dupont, Qualicon, Wilmington,

DE, USA) and iQ-Check™ (Bio-Rad Laboratories, Hercules, CA, USA) have been validated for detection at 48 h and for the use with poultry carcass rinse collected in chicken processing plants.

2.2.2.2 Growth on Selective Plates

In general, agar plates for isolation are based on the addition of either blood or charcoal. The original intention when adding blood or charcoal was to provide some substances that would reduce, or quench, oxygen in the medium. But nowadays these substances are usually added for differentiation purposes. A newer group of plate media are some chromogenic agars, but laboratories must buy premade media and therefore the cost of isolation increases substantially. In most countries, the plate most commonly used is CCDA (Bolton and Robertson 1982; Bolton and Coates 1983). This medium is one of the most economic alternatives for use in food microbiology laboratories and although identifying colonies may take some time, *Campylobacter* colonies have unique characteristics that make them be easily identifiable by trained personnel. Therefore, CCDA is a good differential plate for isolation purposes. The incubation time for plates is 48 h, although colonies can be identified at 36 h of incubation at 42 °C and under microaerobic conditions.

Other types of plates are those with the addition of blood. These plates have similar isolation efficiency as CCDA plates for isolation of *Campylobacter* from poultry products (Oyarzabal et al. 2005; Potturi-Venkata et al. 2007). In general, blood plates are supplemented with the some antibiotics incorporated in CCDA. In general, a personnel working in food microbiology laboratories like blood plates more because it is easier for them to learn how to identify presumptive *Campylobacter* colonies. The beta hemolysis from the growth of *Campylobacter* is a good selective way to identify presumptive *Campylobacter* colonies. However, this beta hemolysis is not unique to *Campylobacter* colonies and our experience indicates that charcoal-based plates are more reliable in the identification of presumptive *Campylobacter* colonies than blood-based plates. In addition, *Campylobacter* colonies tend to grow deeper than just the surface in blood plates due to the breakage of the agar surface during the streaking process. Figures 2.1 and 2.2 show the typical *Campylobacter* colonies in blood agar and CCDA plates respectively.

The antibiotics used in plate media are the same and at the same concentrations of those antibiotics used in enrichment media. In some cases, vancomycin could be added if the sample is suspected to have a large contamination with background microflora. The cefoperazone/amphotericin B has worked well for the authors in the isolation of *Campylobacter* from poultry products (Williams and Oyarzabal 2012). Yet, a simple modification during the transfer of enrichment media to agar plates can make a large impact in the reduction of antibiotics used in the enrichment step. This modification includes the use of filter membranes with pores of 0.45 or 0.65 µm. Several different variations of these filter membranes have been used for more than 50 years in the isolation of *Campylobacter* in veterinary (Plumer et al. 1962) and clinical samples, and in some cases the membranes were used on agar plates without

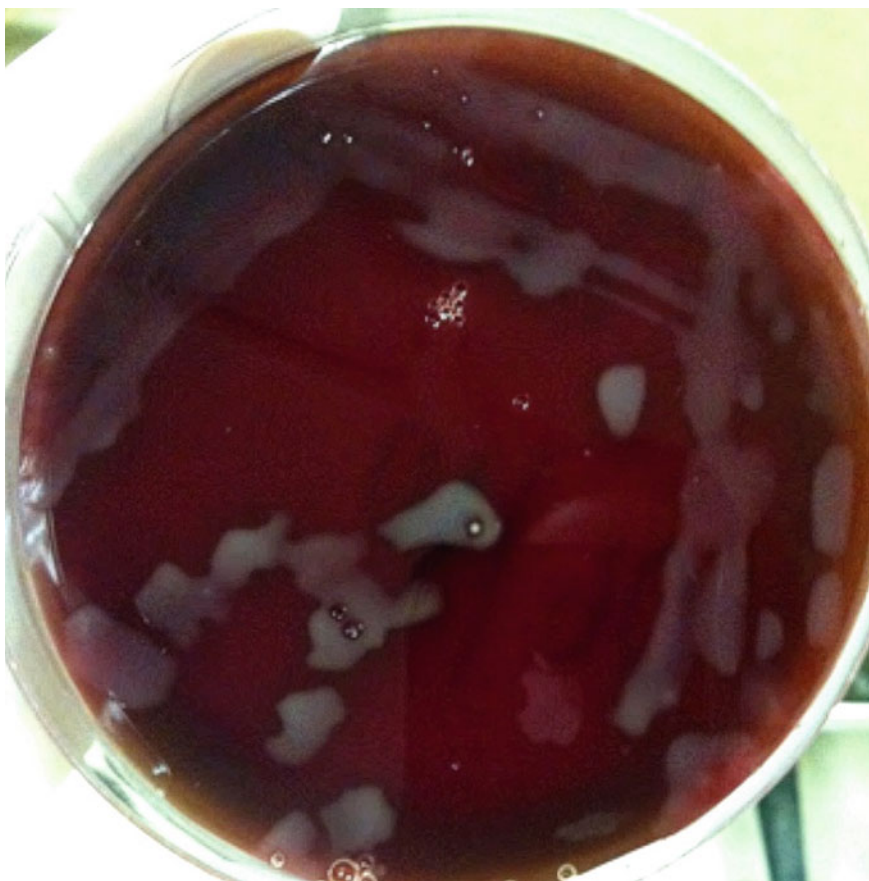


Fig. 2.1 Typical *Campylobacter* colonies in blood agar

any selective agents (Steele and McDermott 1978; Lastovica 2006). However, the use of these filters to isolate *Campylobacter* spp. from food samples did not start until the 1990s (Baggerman and Koster 1992). In our laboratories, we started the use of filter membranes in 2008 with very good results (Speegle et al. 2009). Some recent publications have also highlighted the practicality and usefulness of these filter membranes to isolate *Campylobacter* (Bi 2013).

These filters allow for food particles and large cells to be retained on the surface while the smaller, mobile *Campylobacter* cells pass through. We place one filter on top of an agar plate (charcoal- or blood-based), deposit approximately 100 μ l of the enriched broth on top of the filter, and wait approximately 15–20 min before removing the filter with disinfected tweezers. Filter membranes with pores of 0.65 μ m are adequate to isolate *Campylobacter* spp. and we prefer the use of selective media with at least 33 mg/L of cefoperazone to inhibit the growth of contaminating bacteria that can still pass through these filters (Speegle et al. 2009).

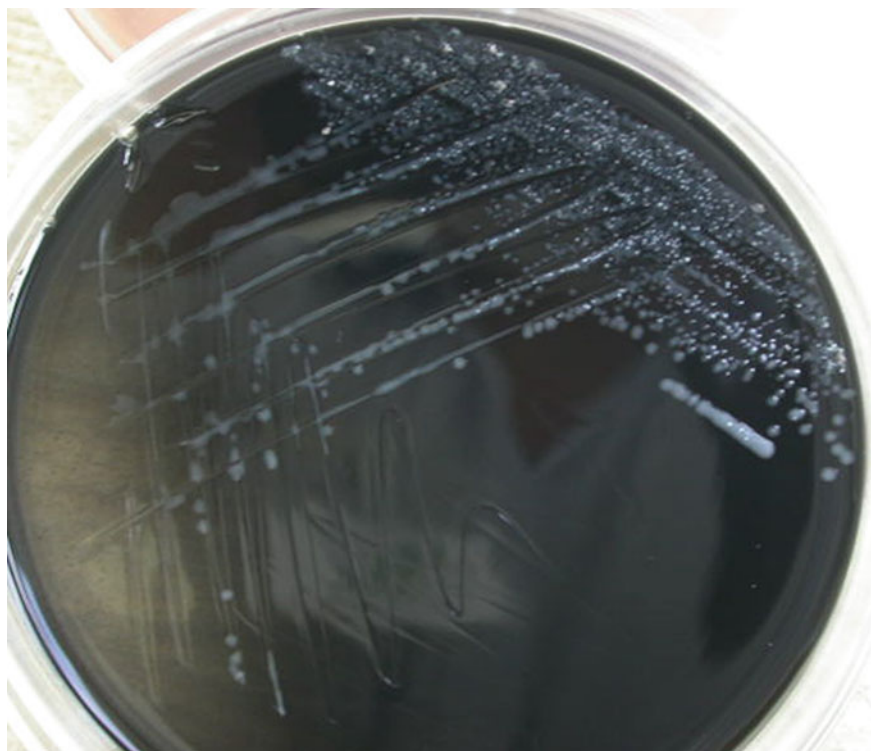


Fig. 2.2 *Campylobacter* colonies on CCDA plates

In general, these filter membranes help improve the efficacy of isolation of plate media irrespective of the formulation of the media (Chon et al. 2012). Figures 2.3 and 2.4 show the filtration procedure and the obtained colonies after incubation.

There are few chromogenic agar plates that are already commercially available in the USA, Europe, and Latin American countries. Some of these plates have been validated for isolation of *Campylobacter* spp., primarily from meat, poultry meat, carcass rinse, and environment samples (Table 2.1), and all current chromogenic agars have been found to be equally sensitive to traditional plates for identification of *Campylobacter* spp. from food samples (Ahmed et al. 2012; Seliwiorstow et al. 2014; Teramura et al. 2015). The first chromogenic agar that appeared in the market was CampyFood ID agar (bioMerieux, Marcy l'Etoile, France), a plate that has performed similarly to CCDA for the isolation of *Campylobacter* spp. from naturally contaminated poultry samples. Yet, there may be other bacterial species growing on the plate and therefore this is not completely differential (Habib et al. 2008; Habib et al. 2011). In a study performed in Chile, CampyFood ID agar had a higher isolation rate than mCCDA in chicken meat, with a percentage of positive samples of 83 % for CampyFood Agar and 67 % for mCCDA (Fernández-Riquelme 2011). Figure 2.5 shows *Campylobacter* colonies in CampyFood ID agar (red colonies).

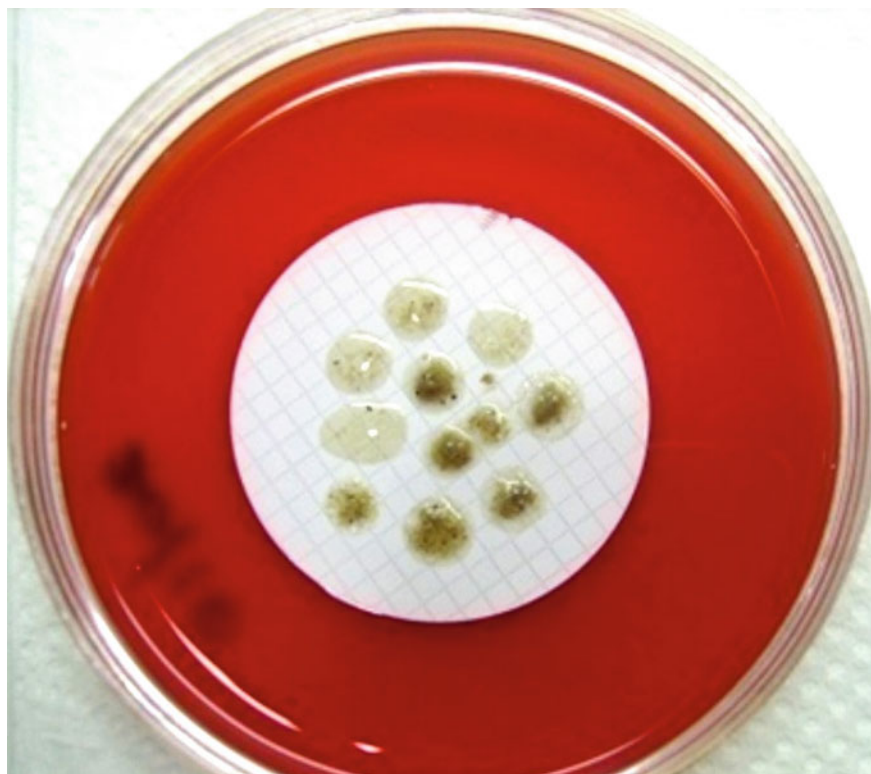


Fig. 2.3 Filtration procedure before incubation with filter membranes with pores of 0.65 μm in selective media

All of the media, broth and plates, used for isolation of *Campylobacter* have been modifications of media developed more than 30 years ago when generating microaerobic conditions in microbiology laboratories was more challenging than in current times. All of these media had the addition of substances that bind to oxygen to help produce a microaerobic environment that allows for *Campylobacter* to grow and to protect the cells from hydrogen peroxide. For instance, the addition of blood and charcoal to media was done with the intention of reducing the oxygen level in the media throughout the isolation process. Other substances that were commonly added were sodium metabisulfite, sodium pyruvate (which is supposed to also be a source of energy) and ferrous sulfate. However, the addition of blood or charcoal provides for the needed oxygen quenching substances and the added “differential” properties to the media to easily visualize the colonies on the plates. We have found that Brucella agar and even tryptic soy agar not supplemented with charcoal or blood are equally efficient for the isolation of *Campylobacter* from enriched samples. However, without a differential substance most colonies look similar and it is very difficult to identify presumptive *Campylobacter* colonies.

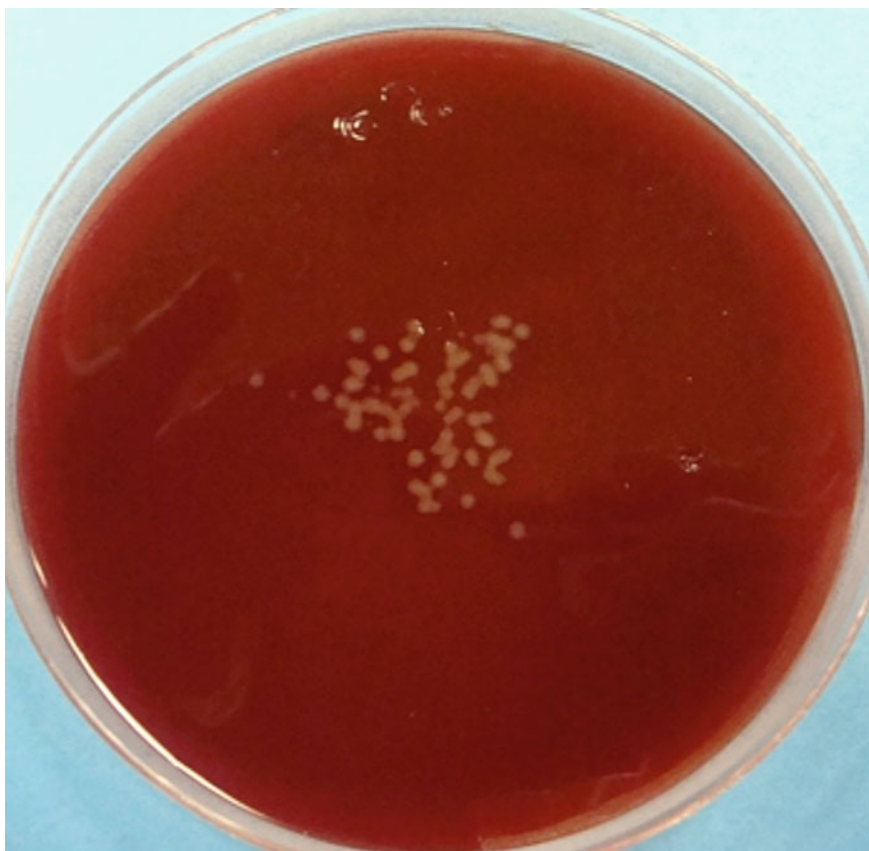


Fig. 2.4 Obtained colonies after filtration procedure and incubation over 48 h

Different substances have been added to plate media to generate microaerobic conditions on the surface of the plate. One of these substances, Oxyrase[®] (Oxyrase, Inc. Mansfield, Ohio), is an enzyme system that help produce anaerobic conditions in a wide variety of bacteriological broth media. However, as stated in previous section in this chapter, microaerobic conditions are naturally created in broth media and therefore the addition of any oxygen quenching substance is less important than the addition of selective agents that allow for the suppression of competing bacteria and for *Campylobacter* to multiply to detectable numbers. The addition of this enzyme system for plate media appears to be more appropriate, but the media have to be poured on special plates (OxyDish[™]), which makes the isolation procedure more expensive. We are not aware of any microbiology laboratory using Oxyrase[®] for the routine isolation of *Campylobacter* spp.

Table 2.1 Methods for detection of *Campylobacter* spp. from foods that have received validation by AOAC International^a

Type of method	Method name	Manufacturer	Validated matrices
PCR-based	BAX [®] System Real-Time PCR Assay for <i>Campylobacter jejuni</i> , <i>coli</i> , and <i>lari</i>	DuPont Nutrition and Health Diagnostics	Feces on cloacae swabs (levels above 100 cfu/g). Ready-to-eat turkey product and chicken (25 g), or carcass rinses (30 mL)
	<i>Campylobacter</i> real-time PCR	Eurofins Genescan	Chicken raw meat, feces on cloacae swabs, disposal shoe covers with chicken feces
	iQ-Check [™] <i>Campylobacter</i> real-time PCR	Bio-Rad Laboratories	Chicken carcass rinse (30 mL), turkey carcass sponge, raw ground chicken (25 g)
ELISA-based	VIDAS [®] <i>Campylobacter</i> (CAM)	BioMérieux	Meat products (25 g) and production environment samples. Fresh raw pork, raw chicken breast, processed chicken nuggets (25 g), chicken carcass rinse, turkey carcass sampled with sponge
Chromogenic agars	CampyFood Agar (CFA)	BioMérieux	Meat, poultry products 25 g, and production environment samples. Fresh raw pork, raw chicken breast, processed chicken nuggets (25 g), chicken carcass rinse, turkey carcass sampled with sponge
	CASA [®] (<i>Campylobacter</i> Selective Agar) for enumeration of <i>Campylobacter</i> spp	BioMérieux	Meat products, poultry products, and environmental samples
	RAPID [®] <i>Campylobacter</i> /Agar	Bio-Rad Laboratories	Meat products, and meat product and production environment samples
	Brilliance [™] CampyCount Agar	Oxoid Ltd, part of Thermo Fisher Scientific	Poultry products
Lateral flow	Singlepath [®] <i>Campylobacter</i>	Merck KGaA	Raw ground chicken, raw ground turkey (25 g), pasteurized milk
	Veriflow [™] <i>Campylobacter</i>	Invisible Sentinel, Inc	Chicken carcass rinse

^aThese methods target *Campylobacter jejuni*, *C. coli*, *C. lari*, or *Campylobacter* spp. The table has been modified from the Validated Test Kit table available at the website of the U.S. Department of Agriculture

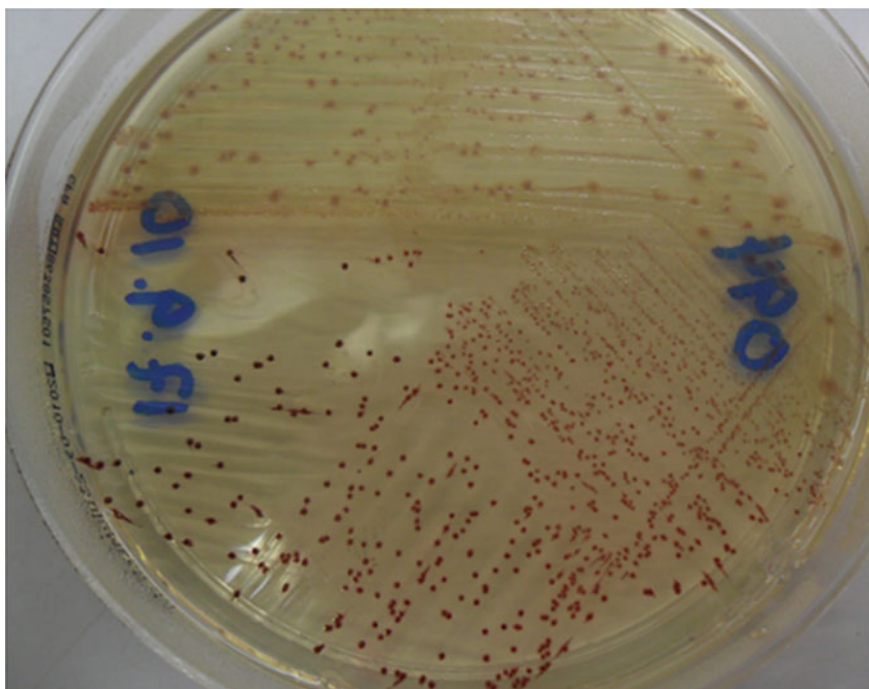


Fig. 2.5 *Campylobacter* colonies in CampyFood ID agar (red colonies)

2.3 Identification

It is important to remember that presumptive colonies on agar plates must be confirmed through a method that has been validated when reporting the results from samples that are under regulatory compliance, such as the performance standard for *Campylobacter* in whole chicken carcasses in processing plants inspected by the Food Safety and Inspection Service of the U.S. Department of Agriculture in the USA.

There are several available options for the identification of presumptive colonies on agar plates. Until approximately 20 years ago the use of few biochemical tests was still common for identification of strains to the genus and even species level. Over the years, the use of these biochemical tests for *Campylobacter* spp. has almost completely disappeared from food microbiology laboratories because of the lack of reliability of these techniques to identify strains to the species level. M'ikanatha et al. (2012) found considerable variation in laboratory protocols, detection methods, and isolation rates of *Campylobacter* in a study of 176 clinical laboratories. Some reference laboratories however, may still use some of these biochemical tests under strict protocols.

The current clinical and food microbiology laboratories use latex agglutination tests, ELISA and PCR tests to confirm colonies to the genus and sometimes to the

species level. The antibody-based techniques, such as the latex tests, lateral flow devices, and ELISA, can be used for the confirmation of isolates to the genus level but are not very robust for identification as the species level. For species identification, the polymerase chain reaction (PCR) technique has several advantages and has been employed for several years in the identification of *Campylobacter* spp. to the species level and in different laboratories. As it was mentioned earlier in this chapter, the species of importance are *C. jejuni* and *C. coli* and therefore a multiplex PCR with only two pair of primers can provide information to the species level for all common food isolates from chicken products.

2.3.1 Latex Agglutination Tests

These tests are based on polyclonal antibodies and have been in the market for more than 20 years. There have been several laboratories that have owned some of the antibodies that were developed in early 1990s. All these tests are based on the agglutination of *Campylobacter* cells in the presence of polyclonal antibodies that normally react with fluellin or other proteins present on the cell walls. The latex particles are covered by the antibodies (immunoglobulins) that usually react with *C. jejuni*, *C. coli*, and *C. lari*. The methodology for confirmation of isolates suggested in the Microbiology Laboratory Guidebook of the U.S. Department of Agriculture includes the use of a latex test and phase contrast microscopy, which is not commonly used in research laboratories (Anonymous 2013). Three commercially available latex agglutination tests are available and have been evaluated (Miller et al. 2008).

2.3.2 ELISA Tests

Most of the current ELISA tests in the market are for the confirmation of presumptive colonies isolated from clinical samples. Most of the food microbiology laboratories do not employ ELISA. One exception is the use of the VIDAS® *Campylobacter* (bioMérieux, Marcy l'Etoile, France), which is an immuno-based test that is almost completely automated and that has been validated for several food matrices and has been in use for several years (Liu et al. 2009; Reiter et al. 2010). In Chile, this system has been in use by some of the laboratories testing poultry products and was used in a study aimed at detecting the prevalence of *Campylobacter* spp. in chicken and turkey samples (Fernández-Riquelme 2011).

2.3.3 PCR Methods

There are several PCR assays for incorporation in food laboratories. Some of the PCR methods are commercially available and have a high level of automation. PCR

assays have several advantages that make them easy to incorporate in laboratories. One advantage is that samples can be treated with heat to stop the action of enzymes and still leave the DNA available for identification purposes. PCR assays are also very specific for identification of unique DNA fragment that allow for identification to the species level and even sometime at the infra-species level.

In the last 10 years, the cost of the DNA methods has decreased substantially and the protocols have been simplified considerably to allow for systems that are almost completely automated. There are some PCR assays that identify isolates only to the genus level and therefore the results are expressed as *Campylobacter* spp. As described, some of these assays are almost completely automated and their protocol include an enrichment step and the equipment necessary to perform the actual PCR, which is a real-time PCR assay in all the commercially available PCR systems. In this cases, the operator only have to load the sample and the reagents to the equipment to perform the assay. The enrichment of the sample is still an important step to allow for *Campylobacter* cells to multiply to detectable levels. Most PCR assays have a sensitivity of approximately 3 Log CFU/g or ml of samples. Therefore, an enrichment step is needed for the samples that carry low number of *Campylobacter* cells. The enrichment step also increases the chances of having live cells in the sample, which will increase the probability of confirming the presumptive result found from testing the enriched samples with the PCR assays. When performing PCR assays, the use of stringent protocols for handling the samples are important to minimize the probability of cross-contamination that can results in samples identified as positive by PCR but are not confirmed through the use of plate media. This type of problems occur more frequently with clinical sample, especially stool samples, where the sample may have had a large number of *Campylobacter* spp. but the handling of the samples (freezing/thawing, etc.) could result in the inactivation of the cells and lack of growth on plates but still a detection by PCR assays.

The use of multiplex PCR assays have allowed for the detection of more than one species of *Campylobacter* in the same poultry sample. In these cases, both *C. jejuni* and *C. coli* were detected in the samples after enrichment (Oyarzabal et al. 2007). This is not surprising due to the fact of the large number of *Campylobacter* cells colonizing live chickens. However, these findings do point out to the complexity of the epidemiology of *Campylobacter* in live chickens and the resulting contamination of food products. Several of the research multiplex PCR assays that have been used in our laboratories have been validated through a large number of samples tested in different studies and are relatively simple to incorporate (Linton et al. 1997; Cloak and Fratamico 2002; Oyarzabal et al. 2005, 2007; Persson and Olsen 2005; Zhou et al. 2011). Yet, like other molecular techniques, the incorporation of PCR requires the initial training of laboratory personnel and the investment in equipment for identification of the amplified products with methods other than the traditional gel electrophoresis protocols. To avoid staining gels with ethidium bromide, real-time PCR protocols are the best choice but they are more expensive than conventional PCR assays. In South American countries, PCR assays

have been incorporated in clinical and food microbiology laboratories, primarily in Chile, Brazil, and Costa Rica (Rivera et al. 2011; Silva et al. 2014; Zumbado-Gutiérrez et al. 2014).

2.4 Perspectives and Future Trends

There is a large body of research on the isolation and identification of *Campylobacter* spp. in poultry carcass rinses, with mainly samples collected in processing plants. As the methodology of testing for *Campylobacter* moves toward detection in retail samples, or in other segments in the farm-fork continuum, there will be a need to validate some of these methods for the new sample types or even for new matrices.

The use of chromogenic agars will increase as they provide a simpler system for detection, especially for small laboratories where there are limited resources or do not handle large volume of samples. But the trend in food microbiology laboratories is that of consolidation, with fewer laboratories with more automated equipment that can provide a very competitive price for testing and can deal with a very large number of samples.

One trend that is difficult to predict is the increase in small food processors across different regions. Some of these processors are small and have many limitations. If this trend continues, there will be a need for regional laboratories to capture the sampling coming from these small processing plants as they start to be scrutinized by regulatory agencies.

PCR assays and other versions of molecular techniques based on DNA detection will continue to expand and be incorporated in clinical and microbiology laboratories. Automation of the protocols, including sample handling and preparation, will increase and may even result in systems that can be adapted for the testing of small number of samples without significantly increase the cost. Different versions of microfluidic arrays have been generated in the past 10 years and some versions may find applicability in food microbiology laboratories. Most of these systems offer high sensitivity and reduced time for detection.

Sample validation and protocol standardization across different countries continues to be an area of expansion and challenges. And as we continue recreating food systems that provide local foods and are more segmented, there will be more challenges to the incorporation of testing methods that prioritize public health.

References

- Ahmed R, Leon-Velarde CG, Odumeru JA (2012) Evaluation of novel agars for the enumeration of *Campylobacter* spp. in poultry retail samples. *J Microbiol Methods* 28:304–310
- Ailes E, Scallan E, Berkelman RL, Kleinbaum DG, Tauxe RV, Moe CL (2012) Do differences in risk factors, medical care seeking, or medical practices explain the geographic variation in

- campylobacteriosis in foodborne diseases active surveillance network (FoodNet) sites? Clin Infect Dis 54:S464–S471
- Anonymous (2013) Isolation, identification, and enumeration of method for the enumeration of *Campylobacter jejuni/coliformi* from poultry rinse, sponge and raw poultry product samples. USDA FSIS Microbiology Laboratory Guidebook, Chapter 41.02. Available at [http://www.fsis.usda.gov/wps/wcm/connect/0273bc3d-2363-45b3-befb-1190c25f3c8b/MLG-41.pdf?MOD=](http://www.fsis.usda.gov/wps/wcm/connect/0273bc3d-2363-45b3-befb-1190c25f3c8b/MLG-41.pdf?MOD=AJPERES)
- AJPERES
- Anonymous (2014) National Notifiable Disease Surveillance System. Canberra, Australian Government Department of Health and Ageing. Available at <http://www9.health.gov.au/cda/Source/CDA-index.cfm>
- Baggerman WI, Koster T (1992) A comparison of enrichment and membrane filtration methods for the isolation of *Campylobacter* from fresh and frozen foods. Food Microbiol 9:87–94
- Bahrndorff S, Rangstrup-Christensen L, Nordentoft S, Hald B (2013) Foodborne disease prevention and broiler chickens with reduced *Campylobacter* infection. Emerg Infect Dis 19:425–428
- Bi S (2013) Comparison of various culture methods (Skirrow medium, a blood-free medium and a filtration system enriched in Bolton and Preston broths) for isolation of *Campylobacter* spp. from raw meat samples. Ann Microbiol 63:179–185
- Bolton FJ, Coates D (1983) Development of a blood-free *Campylobacter* medium: screening tests on basal media and supplements, and the ability of selected supplement to facilitate aerotolerance. J Appl Bacteriol 54:115–125
- Bolton FJ, Robertson L (1982) A selective medium for isolating *Campylobacter jejuni/coli*. J Clin Pathol 35:462–467
- Chon JW, Hyeon JY, Park JH, Song KY, Seo KH (2012) Comparison of 2 types of broths and 3 selective agars for the detection of *Campylobacter* species in whole-chicken carcass-rinse samples. Poult Sci 91:2382–2385
- Cloak OM, Fratafico PM (2002) A multiplex PCR for the differentiation of *Campylobacter jejuni* and *C. coli* from a swine processing facility and characterization of isolates by PFGE and antibiotic resistance profiles. J Food Prot 65:266–273
- Fernández H (1992) Increase of *Campylobacter* isolation rates using an enrichment medium. Rev Microbiol São Paulo 23:5–7
- Fernández H (2011) *Campylobacter* and Campylobacteriosis: a view from South America. Rev Peru Med Exp Salud Publica 28:121–127
- Fernández H, Salazar R, Landskron E (1993) Occurrence of thermotolerant species of *Campylobacter* in three groups of hens maintained under different environmental conditions. Rev Microbiol São Paulo 24:265–268
- Fernández-Riquelme A (2011) Detection of *Campylobacter* in poultry meat for human consumption and antimicrobial susceptibility. Dissertation, Universidad Austral de Chile
- Fernández H, Torres N (2000) Occurrence of *Campylobacter jejuni* and *Campylobacter coli* in three groups of hens of different geographic origin in Southern Chile. Arch Med Vet 32:241–244
- Gharst GA, Oyarzabal OA, Hussain SK (2013) Review of current methodologies to isolate and identify *Campylobacter* spp. from foods. J Microbiol Meth 95:84–92
- Gilliss D, Cronquist AB, Cartter M et al (2013) Incidence and trends of infection with pathogens transmitted commonly through food - foodborne diseases active surveillance network, 10 U.S. sites, 1996–2012. MMWR Morb Mortal Wkly Rep 62:283–287
- Gomes FR, Curcio BR, Ladeira SRL, Fernández H, Meireles MCA (2006) *Campylobacter jejuni* occurrence in chicken fecal samples from small properties in Pelotas, Southern of Brazil. Braz J Microbiol 37:375–378
- Habib I, Sampers I, Uyttendaele M, Berkvens D, De Zutter L (2008) Performance characteristics and estimation of measurement uncertainty of three plating procedures for *Campylobacter* enumeration in chicken meat. Food Microbiol 25:65–74

- Habib I, Uyttendaele M, De Zutter L (2011) Evaluation of ISO 10272:2006 standard versus alternative enrichment and plating combinations for enumeration and detection of *Campylobacter* in chicken meat. *Food Microbiol* 28:1117–1123
- Kuana SL, Santos LR, Rodrigues LB, Borsoi A, Moraes HL, Salle CT, Nascimento VP (2008) Occurrence and characterization of *Campylobacter* in the Brazilian production and processing of broilers. *Av Dis* 52:680–684
- Lastovica AJ (2006) Emerging *Campylobacter* spp.: the tip of the iceberg. *Clin Microbiol News* 28:49–55
- Linton D, Lawson AJ, Owen RJ, Stanley J (1997) PCR detection, identification to species level, and fingerprinting of *Campylobacter jejuni* and *Campylobacter coli* direct from diarrheic samples. *J Clin Microbiol* 35:2568–2572
- Liu L, Hussain SK, Miller RS, Oyarzabal OA (2009) Efficacy of mini VIDAS for the detection of *Campylobacter* spp. from retail broiler meat enriched in Bolton broth with or without the supplementation of blood. *J Food Prot* 72:2428–2432
- López C, Agostini A, Giacoboni G, Cornero F, Tellechea D, Trinidad JJ (2003) Campylobacteriosis in a low-income community in Buenos Aires, Argentina. *Rev Sci Tech Off Int Epi* 22:1013–1020
- Man SM (2011) The clinical importance of emerging *Campylobacter* species. *Nat Rev Gastroenterol Hepatol* 8:669–685
- M'ikanatha NM, Dettinger LA, Perry A, Rogers P, Reynolds SM, Nachamkin I (2012) Culturing stool specimens for *Campylobacter* spp., Pennsylvania, USA. *Emerg Infect Dis* 18:484–487
- Miller RS, Speegle I, Oyarzabal O, Lastovica AJ (2008) Evaluation of three commercial latex agglutination tests for the identification of *Campylobacter* spp. *J Clin Microbiol* 46:3546–3547
- Oyarzabal OA, Backert S, Nagaraj M, Miller RS, Hussain SK, Oyarzabal EA (2007) Efficacy of supplemented buffered peptone water for the isolation of *Campylobacter jejuni* and *C. coli* from broiler retail products. *J Microbiol Methods* 69:129–136
- Oyarzabal OA, Macklin KS, Barbaree JM, Miller RS (2005) Evaluation of agar plates for direct enumeration of *Campylobacter* spp. from poultry carcass rinses. *Appl Environ Microbiol* 71:3351–3354
- Oyarzabal OA, Williams A, Zhou P, Samadpour M (2013) Improved protocol for isolation of *Campylobacter* spp. from retail broiler meat and use of pulsed field gel electrophoresis for the typing of isolates. *J Microbiol Meth* 95:76–83
- Persson S, Olsen KEP (2005) Multiplex PCR for identification of *Campylobacter coli* and *Campylobacter jejuni* from pure cultures and directly on stool samples. *J Med Microbiol* 54:1043–1047
- Plumer GL, Duvall WC, Shepler VM (1962) A preliminary report on a new technique for isolation of *Vibrio fetus* from carrier bulls. *Gen Vet* 52:110–122
- Potturi-Venkata LP, Backert S, Lastovica AJ, Vieira SL, Norton RA, Miller RS, Pierce S, Oyarzabal OA (2007) Evaluation of different plate media for direct cultivation of *Campylobacter* spp. from live broilers. *Poultry Sci* 86:1304–1311
- Quinlan JJ (2013) Foodborne illness incidence rates and food safety risks for populations of low socioeconomic status and minority race/ethnicity: A review of the literature. *Int J Environ Res Publ Hlth* 10:3634–3652
- Reiter MG, López C, Jordano R, Medina LM (2010) Comparative study of alternative methods for food safety control in poultry slaughterhouses. *Food Anal Meth* 3:253–260
- Rivera FN, Bustos BR, Montenegro HS, Sandoval MM, Castillo NJ, Fernández JH, Maturana RM, Delgado RL, Contreras SA, Chávez ND, Quevedo LI (2011) Genotyping and antibacterial resistance of *Campylobacter* spp strains isolated in children and in free range poultry. *Rev Chilena Infectol* 28:555–562
- Seliworstow T, Baré J, Verhaegen B, Uyttendaele M, de Zutter L (2014) Evaluation of a new chromogenic medium for direct enumeration of *Campylobacter* in poultry meat samples. *J Food Prot* 77:2111–2114
- Silva DT, Tejada TS, Cunha CC (2014) Occurrence of *Campylobacter* in poultry, meat chicken and human feces, and *cdt* genes research. *Arq Bras Med Vet Zootec* 66:297–304

- Simaluiza RJ, Toledo Z, Ochoa S, Fernández H (2015) The prevalence and antimicrobial resistance of *Campylobacter jejuni* and *Campylobacter coli* in chicken livers used for human consumption in Ecuador. *J Animal Vet Adv* 14:6–9
- Smith S, Meade J, McGill K, Gibbons J, Bolton D, Whyte P (2015) Restoring the selectivity of modified charcoal cefoperazone deoxycholate agar for the isolation of *Campylobacter* species using tazobactam, a beta-lactamase inhibitor. *Int J Food Microbiol* 210:131–135
- Speegle L, Miller ME, Backert S, Oyarzabal OA (2009) Use of cellulose filters to isolate *Campylobacter* spp. from naturally contaminated retail broiler meat. *J Food Prot* 72:2592–2596
- Steele TW, McDermott S (1978) *Campylobacter* enteritidis in South Australia. *Med J Aust* 2:404–406
- Teramura H, Iwasaki M, Ogihara H (2015) Development of a novel chromogenic medium for improved *Campylobacter* detection from poultry samples. *J Food Prot* 78:1624–1769
- Williams A, Oyarzabal OA (2012) Prevalence of *Campylobacter* spp. in skinless, boneless retail broiler meat from 2005 through 2011 in Alabama, USA. *BMC Microbiol* 12:184
- Zhou P, Hussain SK, Liles MR, Arias CR, Backert S, Kieninger J, Oyarzabal OA (2011) A simplified and cost-effective enrichment protocol for the isolation of *Campylobacter* spp. from retail broiler meat without microaerobic incubation. *BMC Microbiol* 11:175
- Zumbado-Gutiérrez L, Arévalo-Madrigal A, Donado-Godoy M, Romero-Zúñiga JJ (2014) Molecular diagnosis of *Campylobacter* in poultry chain intended for human consumption in Costa Rica. *Agron Mesoam* 25:357–363

Campylobacter spp. and Related Organisms in Poultry
Pathogen-Host Interactions, Diagnosis and
Epidemiology

Fonseca, B.B.; Fernandez, H.; Rossi, D.A. (Eds.)

2016, XII, 206 p. 19 illus., 17 illus. in color., Hardcover

ISBN: 978-3-319-29906-8