White Paper

From Farm to Fork, The Right Response at the Right Time

Rapid Immunodetection of *Campylobacter* Enables Earlier Detection of Contamination at Poultry Farms and Processing Plants

It is estimated that one in three people in industrialized countries may be affected by foodborne illnesses per year (1). According to the European Food Safety Authority (EFSA), campylobacteriosis, caused by the pathogen *Campylobacter*, is the most frequently reported cause of food-related illness in the European Union with an estimated nine million cases each year (2). The EFSA has calculated the cost of campylobacteriosis to public health systems and lost productivity in the EU to be around EUR 2.4 billion per year. In the United States, the Centers for Disease Control (CDC) estimates that campylobacteriosis affects more than 1.3 million persons every year (3).

There is an increasing awareness of and demand for *Campylobacter* testing in food. To protect consumers from this public health threat, regulations and policies are being implemented to shift the focus from contamination response to prevention.

The EU has adopted an integrated approach to food safety from the "farm to the fork". The approach consists of both risk assessment and risk management measures. Similarly, the US has seen a sweeping reform of its food safety laws with the Food Safety Modernization Act of 2011. The act represents a paradigm shift to prevention by establishing a modern system of food safety protection based not on reacting to problems but rather on preventing them from happening in the first place.

Initiatives targeting *Campylobacter* are similar to those instituted in recent years to better prevent *Salmonella* contamination. Coordinated approaches for controlling *Salmonella* in poultry have been hailed as a success and it is hoped that efforts focused on *Campylobacter* will deliver equivalent results. *Salmonella* cases continue to decline in EU member states and most members states met their reduction goals for prevalence in poultry in 2013 (4).

The *Campylobacter* Challenge

Campylobacter can be introduced to poultry flocks in a number of ways. Contaminated feed, insects or bacteria from human interaction can introduce the pathogen, causing the *Campylobacter* status of an entire flock to change from negative to positive within a few days.

To identify risk of contamination, it is critical to screen live chickens for *Campylobacter*; this enables segregation of contaminated flocks at the farm, ahead of slaughter. While testing methods do exist, standard microbiological testing and real-time PCR require samples to be analyzed in a laboratory setting by trained personnel, not directly on the farm, and can take up to four days to obtain results. Since the *Campylobacter* status of an entire flock can change so quickly, such results may be of limited predictive value. For the most up-to-date information, testing for *Campylobacter* in chicken should be done as close as possible to slaughter.

During the slaughter, plucking and evisceration can lead to contamination of carcasses; if a *Campylobacter*-positive flock is slaughtered, it is likely that a large number of carcasses will become contaminated. According to a 2008 survey, most slaughterhouses in the EU are highly likely to have *Campylobacter*-positive broilers as starting material (5).

A number of measures can be taken to protect consumers from campylobacteriosis and include scheduled slaughter, logistic slaughter or after-slaughter treatment such as disinfection with chlorinated water, which is used in the US, or steam treatment. Disinfection using chlorine is not perceived as a positive approach; consumer sentiment in the EU opposes imports of "chlorine chicken" (6).

Scheduled slaughter requires identification of flocks positive for *Campylobacter* and subjecting carcasses from these flocks to *Campylobacter*-reducing measures. Flocks must be sampled prior to slaughter, with results from the testing available before transport to the slaughterhouse. For scheduled slaughter, the samples should be taken and test results delivered as close to slaughter as possible.

With logistical slaughter, flocks are slaughtered in order of contamination severity with negative flocks prioritized after positive flocks; the intention is to prevent cross-contamination of carcasses. While this approach has been widely adopted to better control *Salmonella*, a study of the process has indicated that surprising levels of contamination still occur (7).

A contributing factor may be that the time between testing flocks at the farm and delivery to the slaughterhouse can be two weeks or more. Even if testing is performed within days of slaughter, flocks may become positive during the interim.

Rapid Identification with Rapid Testing

Since *Campylobacter* can spread to an entire flock in a matter of days, testing with a rapid turnaround time is essential. Unfortunately, current *Campylobacter* detection methods have significant shortcomings that contribute to longer testing timeframes. Currently, the most commonly used techniques to test food products for *Campylobacter* are traditional methods based on culture media. The standard detection method involves enrichment for 48 hours, followed by isolation on selective agars, so that final identification results are available only after four to five days. Both culture steps have to be carried out in a microaerophilic environment. These methods are time-consuming as well as labor-intensive.

While real-time PCR is more rapid (about one hour) it requires capital-intensive instrumentation (e.g. a thermocycler) and trained personnel. The transport of samples to the lab can increase the risk of false negatives if transportation conditions are inadequate for *Campylobacter* survival.

Singlepath® lateral flow tests for the detection of *Campylobacter* allow the poultry industry to identify the presence of contamination much faster, enabling a more effective and timely response. Tests are optimized for use both on the farm and at the processing facility, from on-site pre-screening to release testing. The tests deliver reliable results without the need for expensive instrumentation or trained staff. Results are clearly displayed in a yes/no format within 20 minutes after sample application.

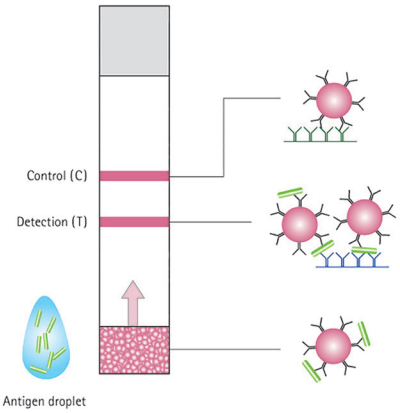

Fig. 1 (left – single line): Singlepath® negative test result.

Fig. 2 (right – double line): Singlepath® positive result. Tests are based on immunochromatographic principles and use antibody-linked colloidal gold particles to react specifically with its complementary antigenic determinant to provide a visual reaction read-out.

On-site Pre-screening

The Singlepath® Direct Campy Poultry kit for rapid point of use immunological screening of *Campylobacter* requires no prior enrichment step and delivers results within two hours. By elimination of the extended enrichment step, the speed of the test enables screening immediately prior to slaughter. This allows financially relevant logistical decisions, such as the separation of *Campylobacter* high-risk (> 10^7 CFU per g of feces) and low-risk flocks for slaughter, to be made on the basis of up-to-date information.

Fig. 3: The Singlepath® Direct Campy kit includes everything needed for on-site testing.

The easy-to-use "mini-laboratory" contains the test device, tubes, a pipette, sample buffer and sample diluent and includes a built-in control reaction for increased result reliability.

Comparison of Singlepath® Direct Campy with Traditional and PCR Methods

For research purposes, a poultry manufacturing site used the Singlepath® Direct Campy Poultry kit to identify contamination ahead of slaughter. Rapid detection of *Campylobacter* colonization in broiler chicken flocks was performed on-farm and in slaughter houses (see reference #9 for full-length article).

Experiments compared the results of the Singlepath® Direct Campy method versus a PCR method for detection of *Campylobacter* at slaughter and from boot swabs taken 10 days before slaughter. The Singlepath® test was also compared to a plate count method (CFA plates) for detection of *Campylobacter* from caecal droppings on-farm and at slaughter.

In comparison with results from standard plate count methods and PCR testing (control methods), the Singlepath®Direct Campy Poultry kit offered faster and on-site detection of *Campylobacter* in fecal samples within an hour of sampling instead of up to two days.

The poultry manufacturer concluded that the Singlepath®Direct Campy Poultry kit is a simple and rapid alternative to laboratory testing where regulations do not require PCR. In countries where standard methods are regulated, the test is a viable supplement to laboratory analysis. The test format significantly reduces time-to-result compared to traditional methods and allows on-site testing to be performed. Contamination can therefore be detected faster, and closer to the day of slaughter.

Based on $3x10^7$ cfu/g LOD	%
Sensitivity	96%
Specificity	100%
False Negative Rate	4%
Positive Predictive Value	100%
Negative Predictive Value	97%

Summary of studies performed on-farm and in slaughterhouse

Release Testing

The Singlepath® *Campylobacter* kit is AOAC Research Institute approved and delivers clear yes/no results in just twenty minutes after sample application.

The majority of *Campylobacter* spp. have low biochemical activity, therefore, identification is difficult on phenotypic characteristics. The standard detection method is enrichment for 48 hours in a microaerophilic environment, followed by isolation on selective agars for 48 hours in a microaerophilic environment. Results are therefore only available after four days.

The Singlepath® *Campylobacter* kit, however, greatly reduces the time-to-result. Following 48-hour enrichment, a result is obtained on the heat-killed sample within twenty minutes.

For optimized enrichment of *Campylobacter,* granulated culture media is recommended. The media are specifically formulated to minimize dust formation, component separation and clumping – even under warm or humid conditions. Granulated media ensure greater solubility and homogeneity, which makes cultivation significantly easier and more efficient.

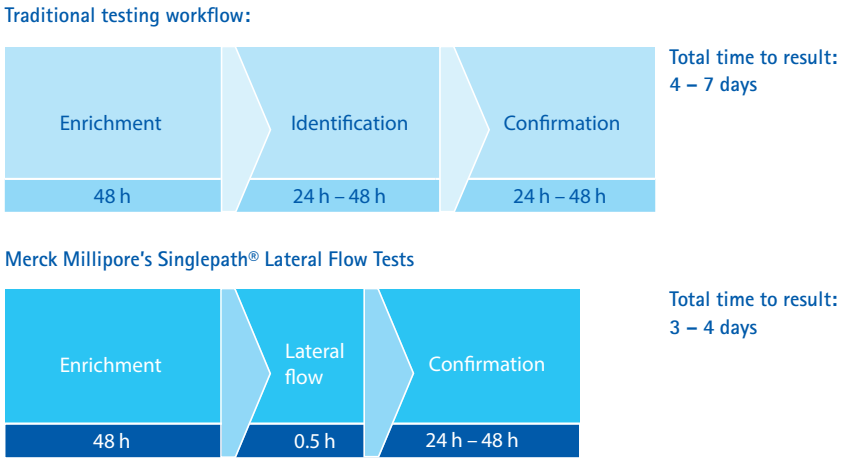

Fig. 4:
Comparison of traditional testing workflow and Merck Millipore's Singlepath® lateral flow tests.

Simple to use Singlepath® lateral flow tests for *Campylobacter* detection deliver results in just a few hours and can be used on the farm or at processing plants to help protect product integrity and reduce risk to consumers. These rapid methods are enabling the poultry industry to shift from contamination response to earlier detection and deliver on the promise of "farm to fork" protection as envisioned by regulatory authorities.

Accelerated Detection with Singlepath® Salmonella

Salmonellosis remains one of the most common and widely distributed foodborne diseases. The World Health Organization estimates that tens of millions of human cases occur worldwide every year and the disease results in more than one hundred thousand deaths (8). Despite improvements, *Salmonella* control in poultry remains essential.

Similar to *Campylobacter* testing, screening for the presence of *Salmonella* in foods by conventional methods is lengthy. Methods involve a three step technique: non-selective pre-enrichment (18-24 h), selective enrichment in (at least) two different selective broth media (24-48 h) followed by plating on (at least) two different selective/indicative agars (24-48 h). This leads to a total time for yes/no screening result of up to five days. For products where a positive release system is enforced, this means a delay of five days before release of the product.

How to Order

Singlepath® Campylobacter	1.04143.0001
Singlepath® Samonella	1.04140.0001
Singlepath® Direct Campy Poultry Kit	1.04155.0001

References

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