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Isolation, Identification and Enumeration of *Campylobacter jejuni/coli/lari* from Poultry Rinse and Sponge Samples

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FSIS Official Methods can be found in the FSIS Microbiology Laboratory Guidebook (MLG). The MLG is updated to incorporate improvements in analytical methods. The latest version can be found at: http://www.fsis.usda.gov/Science/Microbiological_Lab_Guidebook/index.asp

Resources:

•USDA Microbiology Laboratory Guidebook (MLG) Chapter 41.01: Isolation, Identification and Enumeration of *Campylobacter jejuni/coli/lari* from Poultry Rinse and Sponge Samples.

Good practices remarks

- Campy-cefex plates should be spread out (not stacked) and sufficiently dried before placing into an airtight container to avoid spreading growth;
- Plates should be placed in an area that minimizes light exposure, as this can possibly affect the growth of *Camplylobacter*;
- A humectant, such as glycerol, should be used during incubation to remove excess moisture. This will limit confluent and swarming growth of *Camplylobacter;*
- Follow manufacturer instructions when using gas packs to obtain and maintain the proper microaerophilic conditions. It's critical to maintain microaerophilic atmosphere throughout analysis. Move quickly at each step to avoid *Camplylobacter* die-off.

Quality Controls

- Positive *Campylobacter* control: media inoculated with *Campylobacter* jejuni ATCC 33291 or equivalent. Confirm at least one isolate from positive control sample.
- Negative control: un-inoculated media

If all the samples are found to be negative, the controls may be stopped at the same point.

Control Culture Preparation

Day before analysis

- Streak the positive control for isolation onto a Trypticase Soy agar with 5% Sheep Blood plate (SBA) or equivalent;
- Utilize a MicroAero gas pack(s) or gas mixture consisiting of 85% nitrogen, 10% carbon dioxide, 5% oxygen;
- Incubate in a sealed container at 42±1°C for 21± 3h.

Control Culture Preparation

Day 1

- Analisi qualitativa: Using a 1µl loop inoculate from the prepared solution to either 30ml of Buffered Peptone Water (BPW) plus 30ml of double strenght blood free Bolton enrichment broth (2X BF-BEB) for rinsate analysis or 25ml of BPW plus 25ml of 2X BF-BEB for sponge analysis;
- Incubate controls with samples. Controls will be analyzed in the same manner as the samples

- Prepare a 0,5 McFarland suspension (approximately 10^8 cfu/ml) of the control strain in 0,85% saline. Mix tubes gently avoiding vigorous vortexing as introduction of excess oxygen could kill the *Campylobacter*;
- Analisi quantitativa: Streak a Campy-cefex plate for isolation from the prepared solution using a 1µl loop.

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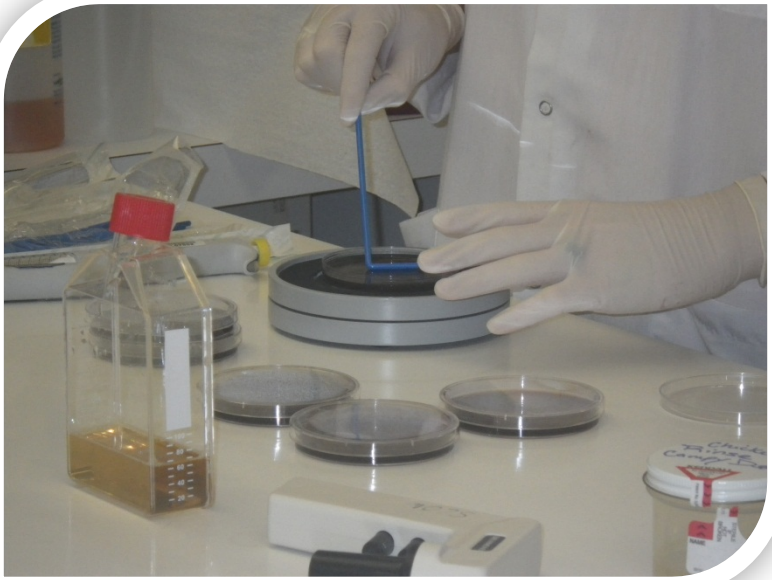
Samples are examined quantitatively for determining the number of colony forming units (CFU) and qualitatively for the presence or absence of the organism. These are prepared and conducted simultanously throughout the method.

Day 0 — Control Culture Preparation

	Quantitative analysis	Qualitative analysis
Day 1	Spread Campy-cefex plates with 250µl and 100µl. Incubate for 48±2h at 42°C	Add 2X BF-BEB to rinse or sponge sample in vented flask or bag. Incubate for 48±2h at 42°C
Day 3	Pick representatives colony types. Examine through microscopy. Latex agglutination. Calculate CFU	Streak from flask or bag to Campy-cefex Incubate for 48±2h at 42°C
Day 5	Pick representatives colony types. Examine through microscopy. Latex agglutination.	

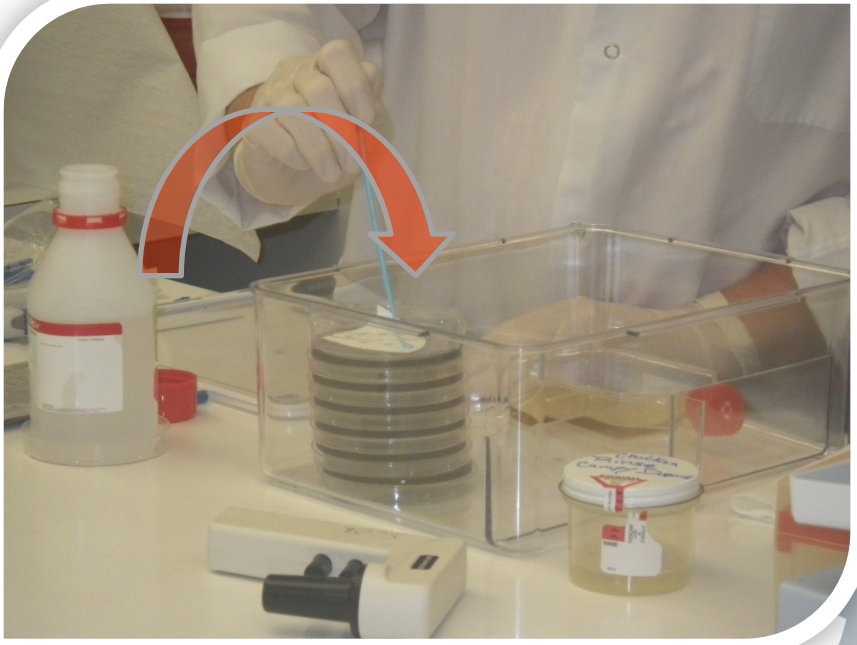
Quantitative sample preparation and Plating (Day 1)

- A portion of the rinsate from a chicken rinsed in 400ml BPW is submitted to the laboratory. Carcass sponge samples arrive to the laboratory containing 25ml of BPW;
- Gently mix rinses or hand squeeze sponges several time to ensure an even distribution of organism;
- Pipette approximately 250µl each onto 4 Campy–cefex plates (1ml) and spread inoculum evenly over plates;
- Pipette 100µl each onto 2 Campy–cefex plates and spread inoculum evenly over plates.



Quantitative sample preparation and Plating (Day 1)

- To prevent swarming growth, add 4-5 drops of a humectant, such as glycerol, to a sheet or filter paper and place in ad open dish in the cointainer to be used for incubation
- Incubate inoculated Campy-cefex plates, controls, flasks and sponges at 42±1°C for 48±2h in sealed container with appropriate microaerophilic conditions.



Qualitative sample preparation and Plating (Day 1)

Carcass Sponges:

•Add 25 ml of 2X BF-BEB to the sponge sample (that contains 25ml of BPW). Mix thoroughly by squeezing by hand several times to ensure an even distribution of organism. Incubate for 48±2h at 42°C.

Environmental Sponges:

•Use 10 ml DE Buffer or equivalent. Add 20 ml Buffered Peptone Water (BPW) and then add 30 ml 2X BF-BEB to the bag containing the sponge. Mix thoroughly by squeezing by hand several times to ensure an even distribution of organism. Incubate for 48±2h at 42°C.

Rinses:

•Add 30 ml of 2X BF-BEB to 30 ml rinse in sterile bag, vented flask or equivalent and mix gently to ensure an even distribution of the rinsate sample and broth. Incubate for 48±2h at 42°C.

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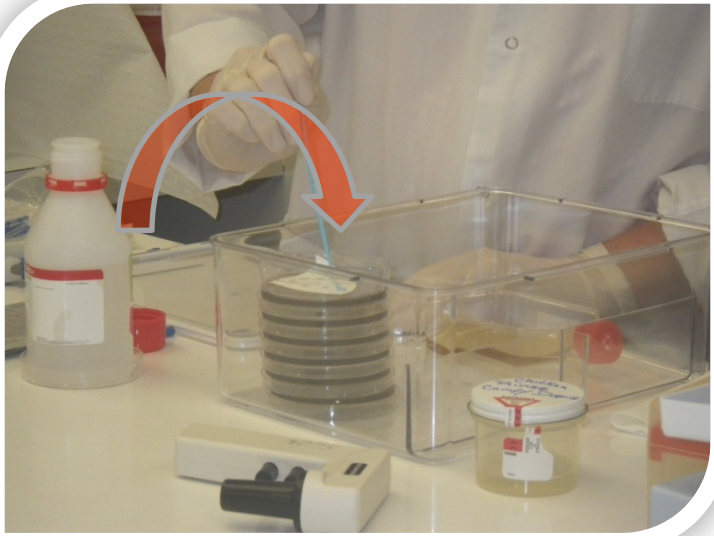
Qualitative Sample Plating (Day 3)

- Following incubation, streak one loop from each flask onto Campy-cefex plates for isolation for each sample.
- Incubate plates at 42±1°C for 48±2h in sealed container with appropriate microaerobic conditions and humectant.



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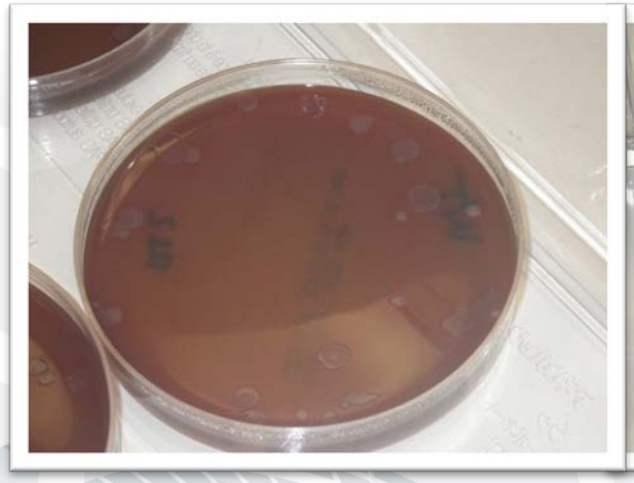
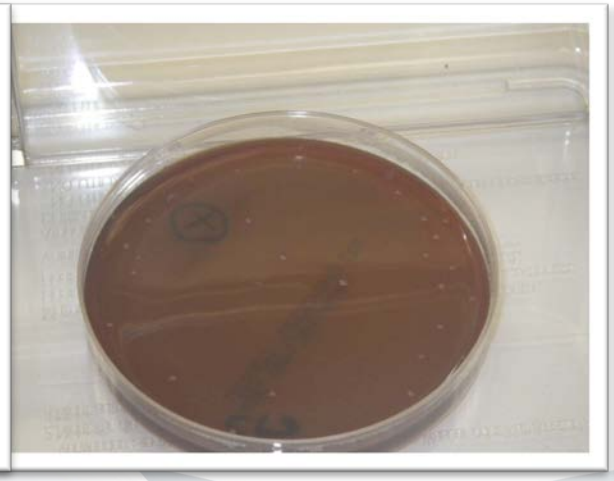
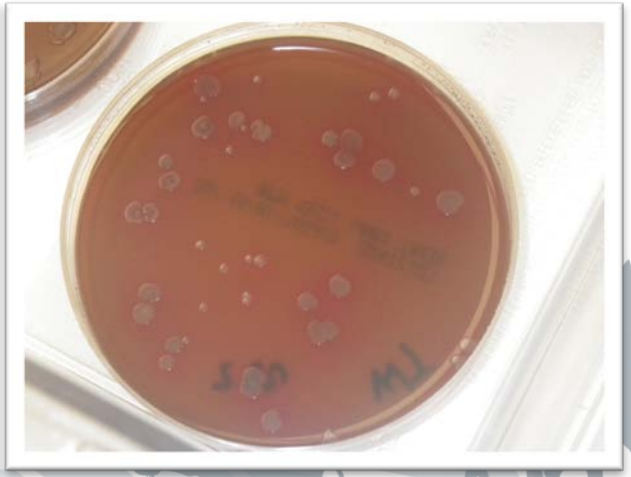
Quantitative Examination of Colonies (Day 3)

- Pick 5 colonies (if available) proportionally representative of all typical colony types from one or more plates for confirmation.
- If there are mixed confirmation results among the colonies of one perceived type (the colonies look the same but some confirm and other do not), pick up to a maximum of 10 colonies representing that type (if available).
- If the last dilution has an average >50 suspect colonies per plate, pick 10% of the average number of colonies up to a maximim of 10 colonies representing that type (if available).

Typical colonies:

- Traslucent or mucoid, glistening and pink in color.
- Flat or slightly raised.
- May vary significantly in size.

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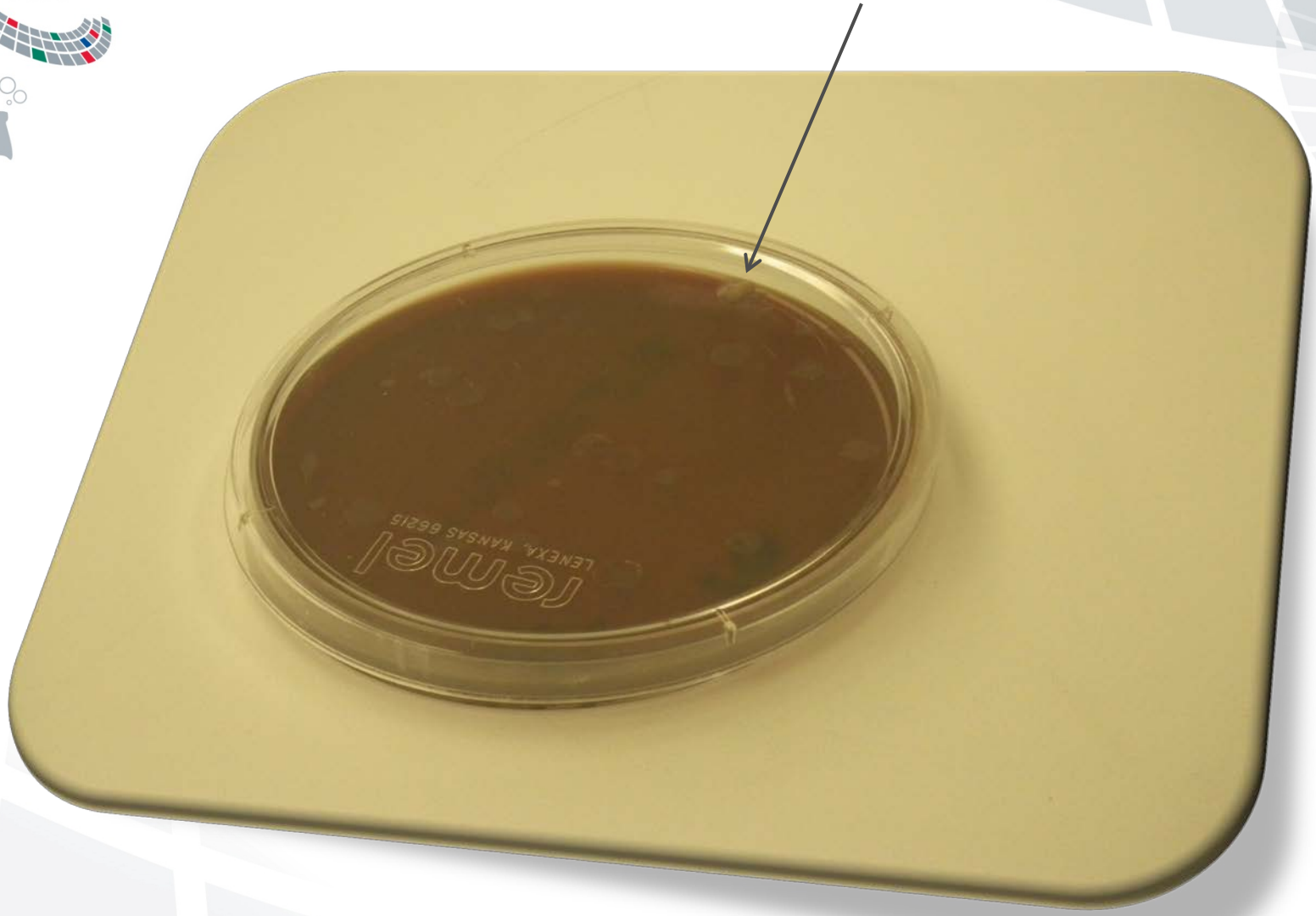

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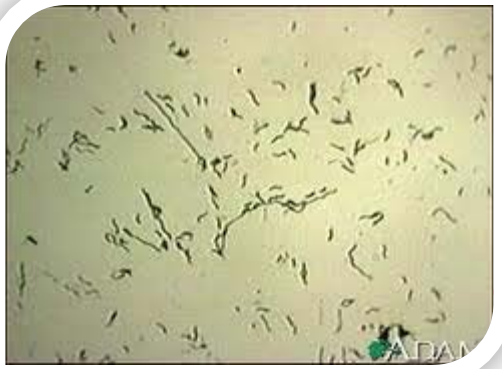
Quantitative Confirmation of Colonies (Day 3)

- Suspend each suspect colony in a drop of sterile 0,85% saline on a microscope slide.
- Cover with a glass cover slip and examine using oil immersion phase contrast microscopy and read immediately.
- Suspension demonstrating typical *Campylobacter* corkscrew morphology and darting motility are regarded as presuntive positive.

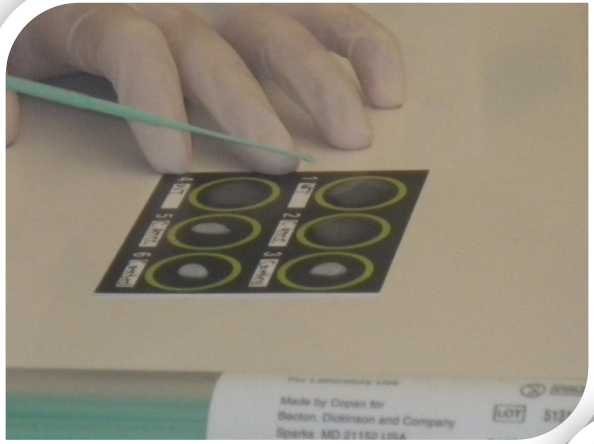
Important note:

Any delay in examining plates (after 48±2h) may result in culture microscopy appearing spherical or coccoid because the culture is old or has been exposed to air. These results would be recorded as degenerative or non-viable.



Latex Agglutination Immunoassay (Day 3)

After examination, confirm presumtive positives by testing an isolated colony using an appropriate *Campylobacter* latex agglutination kit.
Follow the manufacturer's instructions.



Calculating CFU

For sponge samples, CFU/ml is converted to CFU/cm^2 to reflect the surface area sampled. To report CFU/ml as CFU/cm^2, take CFU/ml, multiply by 25ml and divide by 100cm^2.

Count all typical colony types of *Campylobacter.* The countable range is 15-300 CFU.

To determine CFU if only one dilution is within countable range:

- If countable plates are the four 250µl plates, the CFU/ml is the sum of these plates
- If countable plates are the two 100µl plates, the CFU/ml is the average of these plates multiplied by 10
- If one or more of the plates in a particular dilution is not within the countable range, that dilution cannot be used to determine the exact CFU/ml. (Report as estimated CFU)

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Calculating CFU

If both dilutions are within the countable range:
CFU/ml= (sum of four 250µl plates + average of two 100µl plates x 10)/2

If the final result is > 300 CFU on each of the six plates record as: Too numerous to Count (TNTC)

Calculating CFU: Examples of estimated counts:1

If both dilutions are within the countable range

Take the sum of the four 250µl plates and the average of the two 100µl plates multiplied by 10 and calculate the average of the two results.

Dilution (colonies per plate)						
		1 ml (250 µl ea)			100µl	100µl
Colonies	150	100	75	80	30	19

Calculation for CFU/ml: 150+100+75+80=405

[(30 + 19)/2]x 10= 245

(405+245)/2=325 CFU/ml

Calculation for CFU/cm^2: (325 x 25 ml)/ 100cm^2 = 81 CFU/cm^2

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Calculating CFU

When plates have spreading growth of *Campylobacter j/c/l* colonies, calculate results as follows:

- If the colony spreader(s) are located on one or more of the four 250µl plates, determine the total CFU/ml using the 100µl plates;
- If the colony spreader(s) are located on one or both of the 100µl plates, determine the total CFU/ml using the sum of the 250µl plates;
- If the colony spreader(s) are located on both 250µl and 100µl plates, count only the isolated colonies and record the estimated count as > the calculated count of isolated colonies.

If all colonies of specific morphology confirm, 100% of colonies with that morphology are included in the count. If there are mixed confirmation result among colonies representing one specific colony type and 10 colonies representing that type were picked for confirmation, the total count for that colony type must be multiplied by the percentage of colonies of that type that confirmed.

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Qualitative Confirmation of Colonies (Day 5)

- Pick 5 colonies representative of all typical colony types from one or more plate for confirmation;
- Conduct microscopy examination (as performed on quantitative confirmation);
- Conduct latex agglutination (as performed on quantitative confirmation).
- Store *Campylobacter* isolates in Cryostor™ containing storage Media (Wang's Freezing/Storage Medium)

Note: If the sample is positive for the quantitative analysis, there is no need to repeat confirmatory testing for the sample when conducting the qualitative analysis.

WANG'S FREEZING/STORAGE MEDIUM

Brucella broth powder 28 g
Glycerol 200 ml
DI water 750 ml
Supplement
Lysed horse blood 5 ml/100 ml

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