ATION OF VET EOR OF CAMPYLOBACTER SPP. DETECTION elena Höök, al Veterinary Institute, Sweden Na





VIVALDI: VETERINARY VALIDATION OF Commission POINT OF CARE DETECTION INSTRUMENT (VETPOD)

- EU project within Horizon 2020: Innovation action
- January 2018 December 2020
- 7 partners from 5 member states (DK, SE, DE, FR, IT)
- Led from DTU in Copenhagen
- VETPOD lab-on-a-chip, based on LAMP technology
- Adapation and validation of VETPOD for detection of
 - Avian influensa
 - Salmonella
 - Campylobacter





VIVALDI: THE VETPOD SYSTEM

- LAMP: loop-mediated isothermal amplification
- Amplification of DNA in a constant temperature (60–65 °C)
- Cheaper and more rapid than PCR
- Lab-on-a-chip: reagents loaded on pre-fabricated chips
- Turbidity measured in real time with optical technique

Pre-storage of gelified reagents in a lab-on-a-foil system for rapid nucleic acid analysis

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(Previous paper on lab-on-a-chip from DTU, not part of the present project.)



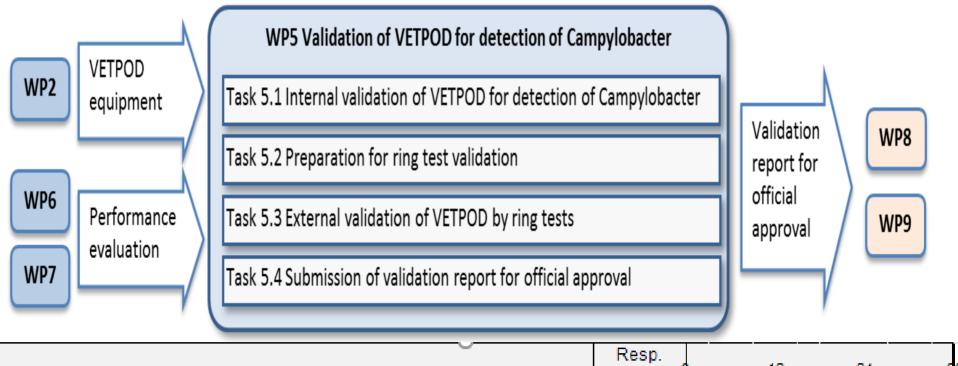
VIVALDI WORK PACKAGES

- WP1 Project Management (DTU)
- WP2 Providing VETPOD equipment for validation (DTU)
- WP3 Validation of VETPOD for detection of AIV (ANSES)
- WP4 Validation of VETPOD for detection of Salmonella (DTU)
- WP5 Validation of VETPOD for detection of Campylobacter (SVA)
- WP6 Implementation of VETPOD for monitoring of animal health (ACEL)
- WP7 Implementation of VETPOD for monitoring of food safety (QLAB)
- WP8 Business plan and market uptake (DIA)
- WP9 Dissemination and communication (DTU)
- WP10 Ethical issues



WP5 Objectives

- To perform internal and to organise an external validation ring test for validation the performance of the VETPOD.
- The internal validation and the ring test validation of the VETPOD system will be validated against the international standard provided by <u>ISO</u> (ISO 10272) for *Campylobacter*.



Work Plan			12	24	38
WP5	Validation of VETPOD for detection of Campylobacter	SVA			
Task 5.1	Internal validation of VETPOD for detection of Campylobacter	SVA			
Task 5.2	Preparation for ring test validation	SVA			
Task 5.3	External validation of VETPOD by ring tests	SVA]
Task 5.4	Submission of validation report for official approval	DTU			

WP5: VALIDATION OF VETPOD FOR DETECTION OF CAMPYLOBACTER SPP.

- Validation against ISO 10272-1:2017
- Detection of Campylobacter spp. in
 - poultry meat
 - primary production samples from poultry
- Internal validation 2019
- External validation (ring test) 2020
- Apply for official approval by NordVal





WP5 – INTERNAL VALIDATION ACCORDING TO NORDVAL PROTOCOL & ISO 16140-2

- Sensitivity study: comparing the sensitivity of the VETPOD protocol to the reference method (ISO) using naturally (or artificially) contaminated samples
- Relative Level of Detection (RLOD) study: determining the RLOD of the VETPOD protocol using artificially contaminated samples
- Selectivity (inclusivity/exclusivity) study: confirming the selectivity of the VETPOD system using 50 target strains (*C. jejuni*, *C. coli* and possibly *C. lari*), and 30 non-target strains



RELEVANT MATRIX CATEGORIES AND TYPES ACCORDING TO NORDVAL (AND ISO 16140-2)

Matrix group	Category	Types (matrix)	Example of items	
Meat	Raw poultry and	Fresh meat	Carcasses, meat, cuts	
	ready-to-cook poultry products	(unprocessed)	Carcasses, swabs, rinsates	
			Minced meat, meat preparations	
		Ready-to-cook products (processed)	Seasoned chicken breast	
	Ready-to eat, ready to reheat meat poultry products (only in ISO	Cooked meat products	Cooked turkey filet	
		Fermented or dry meat products	Chicken sausage	
	16140-2, error in NordVal doc?)	Raw cured (smoked) (aw > 0.92)	Smoked turkey filet	
Primary production	Primary production samples (PPS)	Animal faeces	Swab samples (boot socks), faeces rectal	
samples (PPS)		Environmental samples and non-faeces	Dust samples, hygiene swabs, water from drinkers, litters, hatchery samples	



SENSITIVITY STUDY: MATRICES

- Raw poultry and ready-to-cook poultry products Matrix type: Fresh meat (unprocessed)
 - chicken skin
 - fresh chicken meat

Matrix type: Ready-to-cook products (processed)

- frozen seasoned chicken breasts
- Primary production samples

Matrix type: Animal faeces

- broiler caecal samples
- sock samples from broiler houses

Matrix type: Environmental samples and non-faeces

- sock samples from surroundings of broiler houses
- 20 samples of each matrix (= totally 120 samples), at least 30 of each category positive

RELATIVE LEVEL OF DETECTION (RLOD) STUDY

- One type for each category
 - Fresh/frozen chicken meat
 - Broiler caecal samples
- Artificial contamination, three levels of inoculation:
 - Negative (5)
 - Low level (at LOD_{50}) (20)
 - High level (at $LOD_{50} \times 2$) (5)
- All replicates are analysed with both methods, i.e. at least 30 samples of each type



SELECTIVITY STUDY

- Inclusivity study: at least 50 target strains
 - 30 Campylobacter jejuni
 - 20 Campylobacter coli
 - 10 Campylobacter lari (?)
- Exclusivity study: at least 30 non-target strains
 - Other *Campylobacter* species
 - Other species that share some properties with Campylobacter
 - Other species common in chicken intestines
- Carried out on the alternative method, i.e. VETPOD
- Doubtful results: test repeated with the reference method (ISO 10292-1:2017) included



STRAIN COLLECTION

- For inclusivity and exclusivity
- 50 target strains
- 30 non-target strains
- At SVA: mainly isolates from primary production
- From the National Food Agency in Sweden: food and water isolates
- Possibly NRL-Campylobacter isolates from the baseline survey in the EU 2008 (isolates from food and primary production)?



WP5 – RING TRIAL VALIDATION (ACCORDING TO ISO 5725-2 AND ISO 16140-2)

Participants:

 10 external laboratories from at least 5 (preferably 10) different organisations

Preparation of test samples:

- Matrix: chicken meat (?)
- 3 levels: negative, low (around LOD₅₀) and high, 8 replicates,
 2 methods = 48 samples to analyse for each laboratory

