



Ecole Nationale
Vétérinaire de Lyon

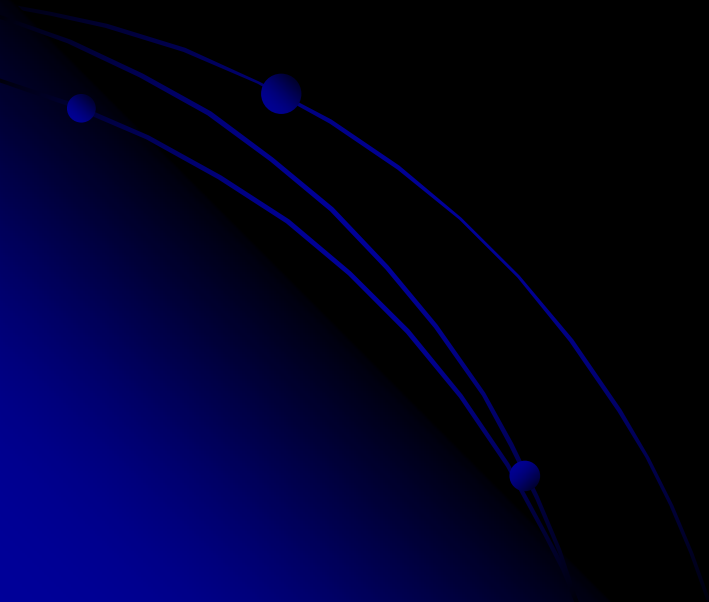
**Report of the
Task Force on Zoonoses Data Collection
on technical specifications for monitoring and
reporting of verotoxigenic *Escherichia coli* (VTEC)
in animals and food¹**



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. Sample collection for animals

Type and detail of sample



■ Hide swabs of cattle:

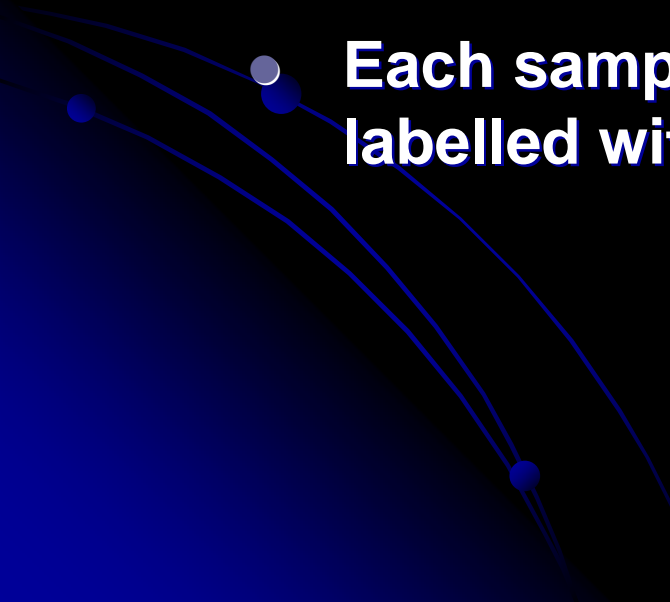
- Swab to be taken **from the brisket area** of the animal after exsanguination and prior to de-hiding.
- Use a **pre-moistened sponge swab** (Polyurethane sponges, 100mm x 100mm x 10mm)
- The sponge should be rubbed an area of **400 cm² area delineated by a sterile template, 10 times in a vertical direction and 10 times in a horizontal direction.**]
- The bag should then be re-inverted over the sponge and resealed.
- All swabs must be placed in separate bags **avoiding cross contamination.**

■ *What pressure should be used?*

■ **Fleece samples:**

- Samples to taken from the **brisket area of the animal before pelt removal.**
- A sterile stomacher bag is inverted over one hand so that the inside of the bag can be used **to grasp a handful of fleece/wool.**
- **A sharp sterile scissors is used to cut at least 10 g of fleece into the bag.** The bag is re-inverted and then secured with an elastic band.

Sample information

- **All relevant information should be recorded on a sampling form produced by the competent authority** to enable the data requirements in section “Reporting” to be fulfilled.
 - **Each sample and its sample form labelled with a unique number.**
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Transport of samples to the laboratory

All samples stored preferably **between 2 and 8°C** and free of external contamination during storage and transportation.

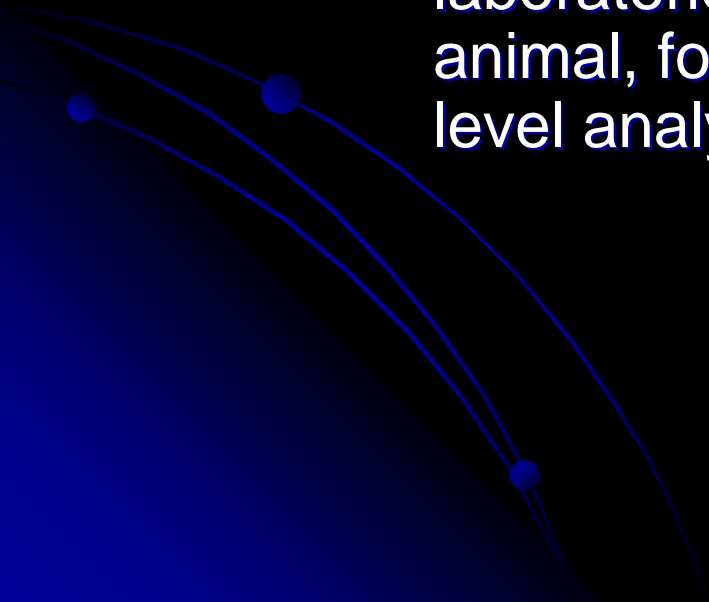
Appropriate to transport the samples back to the laboratory in **cooled boxes**.

Samples sent for analysis as quick as possible by fast mail or courier to laboratory, where it should be stored between 2 and 8°C until analyzed.

Sample analysis started within 80 hours after sampling

Laboratory analytical methods

Laboratories

- National Reference Laboratories for *E. coli* (NRLs)
 - **Designation of a limited number** of other laboratories involved in official control of animal, food and feed to perform the first level analyses.
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Detection and identification methods

Detection of VTEC O157

Food and feed

- The detection of VTEC O157 in all types of foods and feeding stuffs shall be done according to the horizontal **methods ISO 16654:2001**, which employs modified TSB supplemented with 20 mg/l Novobiocin as enrichment broth .
- **Other methods** for the isolation of *E.coli* O157 from food that have been validated against the ISO 16654:2001 in accordance with the ISO 16140:2003 and certified by a relevant body can be used.
 - **Possibility to also use alternative validated methods ??**

Detection and identification methods

Detection of VTEC O157

Hide and fleece

- The **ISO 16654:2001** in food and feed based on specific **immunomagnetic separation** enrichment, shall also be used for hide and fleece with the following **modifications, concerning the enrichment step:**
 - Cattle **hide** swabs: the hide sponge shall be mixed to 90 mL of **pre-warmed BPW and incubated at 41.5°C for 18 hours** .
 - Sheep **fleece** samples: 10g of fleece shall be mixed and homogenized in 90mL of pre-warmed BPW and incubated at **41.5°C±1°C for 18 hours**.

Detection of other VTEC serotypes associated with severe human disease

The non-O157 VTEC serogroups that should be considered are: O26, O103, O111 and O145.

Food and feed

Hide and fleece



Community Reference Laboratory for *E.coli*

Department of Food Safety and Veterinary Public Health
Unit of Foodborne Zoonoses and Veterinary Epidemiology
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A real-time PCR method for detection of Shiga toxin-producing *Escherichia coli* in food

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*The "STEC ad hoc Group" is a designated group of experts appointed by CEN to define a consensus protocol aimed at the detection of STEC in foodstuffs to be proposed as a standard to ISO. The "ad hoc group" members are listed in the next page. The activities of the group are co-ordinated by Stefano Morabito and the is group co-chaired by Jeppe Boel

- **the enrichment step.???**

- Food and feed ??

- (TSB) modified by the addition of 1.5 gr/l bile salts n. 3 and supplemented with either 16 mg/l of novobiocin or, for dairy products, 12 mg/l of acriflavin. When frozen samples are processed, pre warmed BPW without antibiotics is used as enrichment medium. Enrichment culture are incubated at $37^{\circ}\text{C}\pm 1^{\circ}\text{C}$ for 18.)

- Animal sample (hide and fleece)

- Same enrichment step than that used for detecting E. coli O157:H7 from these samples?? More convenient , false negative results??
- A more selective two-steps enrichment protocol has been recently proposed by Posse et al c, (TSB supplemented with 8 mg/l novobiocin and 16 mg/l vancomycin for a 6 hours pre-enrichment at 37°C , followed by the addition of 2 mg/l rifampicine, 1.5 g/l bile salts and 1.0 mg/l potassium tellurite and further incubation for 18 hours at 42°C (selective enrichment).

Storage of isolates

- All isolates should be sent to the **NRL** for verification of the typing and storage. Further typing that may be performed include:
- **H** typing
- **vtx** genes subtyping
- **eae** subtyping
- molecular typing by **PFGE**, performed according to the Pulse-net Europe protocol
- VTEC O157 **phagetyping**, performed at the Community Reference Laboratory (CRL)
- Typing scheme and methods **harmonized** with those used by **medical laboratories** for strains from human infections.
- Typing data shall be collected in a **database** for integration and comparison with the database of human strains (ECDC Foodborne and Waterborne Infections network, Pulse-net Europe).



Thank
you



For your



Attention