EU VII FP PROJECT "VITAL"

STANDARD OPERATING PROCEDURE

SOP VITAL 009

Sampling and virus concentration from pork meat and liver tissue

PERFORMED:	REVISED:	APPROVED:
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— WARNING —

Adenoviruses, Noroviruses, and Hepatitis A and E viruses are viral pathogens. All samples and controls shall be handled by trained staff in a laboratory with appropriate equipment. Staff must be fully vaccinated against Hepatitis A and poliovirus. Persons using this SOP must be familiar with normal virology laboratory practice. This SOP does not presume to address fully all of the safety issues associated with its use. It is the responsibility of the user to establish appropriate safety and health practices and to ensure compliance with any national regulatory conditions.

AIM

To obtain a supernatant from 250 mg of pork meat or liver tissue to be used for nucleic acids extraction.

PRINCIPLE

This protocol is based on the protocol described in Bouwknegt et al., 2007[1]. Two hundred fifty mg of pork meat or liver tissue including positive process controls is disrupted in a specific lysis buffer and the supernatant is taken for nucleic acids extraction.

[1] Bouwknegt M, Lodder-Verschoor F, van der Poel WH, Rutjes SA, de Roda Husman AM. (2007). Hepatitis E virus RNA in commercial porcine livers in The Netherlands. J Food Prot. 70: 2889-2895.

EQUIPMENT

- Micropipettes of a range of sizes, 1000µl, 200µl and 20µl
- Micropipette tips of a range of sizes, 1000µl, 200µl and 20µl
- Gloves
- Mechanical disrupter (e.g. Ribolyser Cell disrupter),
- Centrifuge and microcentrifuge tubes/bottles of a range of sizes, 2 ml, 15 ml, 50 ml. 2-ml autoclavable tubes with screw caps with rubber seal ring are necessary for liver and meat disruption. The exact selection of tubes required will depend on the centrifuges and rotors available in each laboratory.
- Centrifuge (7,000 x g)
- Refrigerator (4°C ± 3°C)
- Freezer (-70ºC)

REAGENTS

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles.

- Buffer RLT (RNeasy Midi Kit, QIAGEN)
- 14.3 M β-mercaptoethanol (β-ME)* (stock solutions are usually 14.3 M)

  β-ME must be added to Lysis Buffer before use. β-ME is toxic; dispense in a fume hood and wear appropriate protective clothing. Add 10 µl of 14.3 M β-ME per 1 ml of Lysis Buffer.

  Sterile zirconia beads (BIOspec products, cat. no. 11079110zx)

PROCEDURE

1. Sampling:

1. Take aseptically the liver or meat sample into a sterile plastic bag and seal it.
2. Label plastic bag for traceability, introducing, at least, the following details:
   Analyst
   Date of sampling
   Slaughterhouse
   Place (if possible take a photo of the place where the sample was recollected).
   Reference number (for traceability, use the same number for the rest of the analysis process)
3. Maintain the sample at 4ºC ± 3ºC (max. 24 h).

2. Virus Concentration

1. Wash zirconia beads thoroughly by repeated rinsing with water in a beaker until the water remains clear. After final rinsing discard the water.
2. Add 2.5 g of beads into autoclavable microcentrifuge tubes and tighten the screw caps loosely.
3. Autoclave tubes for 15 min at 121 ºC.
4. Take 1 cm^3 of liver tissue from three different inner-liver locations or at least 1 cm^3 of pork meat from three different locations and homogenize manually using surgical blades. Make sure to use new surgical blades for each sample.
5. Homogenize with sterile and RNAse free* mortar and pestle in 4 ml of RLT buffer RLT (containing 1:100 β-mercaptoethanol)
   * To remove RNAses, heat the mortar and pestles overnight at 150-200 ºC
6. Weight and transfer, using a RNAse-free disposable spoon, 250 mg of homogenized pork meat or liver tissue into the microcentrifuge tube containing 1 ml Lysis RLT (containing 0.14M β-mercaptoethanol) and zirconia beads.
7. Add 10 µl of the positive process control virus to the tube.
8. Transfer the microcentrifuge tube into a mechanical disruptor (such as Ribolyser Cell disrupter).
9. Apply the disrupter at the speed of 4 ms^{-1} for 40 s.
10. Repeat the step 10. Avoid overheating of the sample, and possibly use beater in cold room.
11. Allow the tube to stand for 5 min at room temperature.
12. Centrifuge the tubes for 20 min at 10,000 × g in a table top centrifuge
13. Transfer ~800 µl of the aqueous phase between the fat and debris to a new 2 ml eppendorf tube by piercing the fat with the tip or with a sterile Pasteur pipet (avoid the transfer of liver or pork meat debris
14. Repeat steps 13 and 14
15. Use the suspension immediately for RNA extraction.