

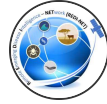


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F-3 FECES STORAGE

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DISCLAIMER

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ABSTRACT

This protocol describes feces storage.

OPEN ACCESS



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We use this protocol and it's working

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STORAGE PROCEDURE FOR
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GUIDELINES

OBJECTIVE

To outline steps for properly storing feces samples and nucleic acid samples purified from these samples.

SUMMARY/SCOPE

The overarching aim of the REDI-NET is to develop a collaborative laboratory network between domestic and international partnering institutions to address disease surveillance needs in order to effectively detect, predict and contain potentially emergent zoonosis. This SOP provides guidance on storage of fecal samples and their purified nucleic acid to preserve their integrity for downstream nucleic acid extraction and/or sequencing library preparation

MAINTENANCE OF EQUIPMENT

Decontaminate a PCR workstation by keeping the UV light on for  00:15:00 .

MATERIALS

EQUIPMENT AND MATERIALS

Note

NOTE: If product number is listed, please ensure use of this or equivalent product.

| A | B | C |
|---|---|------------------------|
| Equipment / Material | Description | Mfg / Product # |
| −80°C freezer | For sample storage | Locally sourced |
| Forceps | Clean, stainless | Locally sourced |
| Ice | To maintain cold chain during sample handling | Locally sourced |
| 96-Well Microfuge tube racks with cover | To hold microplates | Locally sourced |
| KingFisher™ 96 KF microplate | To store the sample | ThermoFisher, 97002540 |
| PCR Workstation | PCR cabinet with UV light | Locally sourced |
| Clear Adhesive Film | To seal the KingFisher™ 96 KF microplate | ThermoFisher, 4306311 |
| Adjustable micropipettes | To handle the samples | Locally sourced |
| Multi-channel micropipettes | 8- or 12- channel; to handle the sample | Locally sourced |
| Nuclease-free filter tips low-retention | To ensure appropriate sample handling | Locally sourced |
| Nuclease free microfuge tubes | 1.5 mL | Locally sourced |
| Saran wrap | Plastic wrap; to seal rack holding sample | Locally sourced |
| Permanent markers | To label tubes and microplates | Locally sourced |
| Data sheet | REDI-NET DCS T-3 Tick Storage | REDI-NET Data Portal |

SAFETY WARNINGS



RISKS AND PERSONAL PROTECTION:

Gloves should be worn all the time when handling samples.

STORAGE PROCEDURE FOR UNTREATED SAMPLE

1

Note

NOTES:

- Fecal samples need to be kept on cold chain all the time to prevent RNA degradation. The following procedure will apply only where -80 °C storage is feasible.
- If -80 °C storage is not possible, temporarily store the tick samples in a -20 °C freezer and follow feces sample processing SOP (REDI-NET SOP F-2 Feces Processing) as soon as possible for total nucleic acid extraction. Subsequently, use a portion of the total nucleic acid and reverse-transcribe RNA into cDNA for -20 °C storage. To do this, follow the initial steps of the feces sample testing SOP (REDI-NET SOP F-4 Feces Testing) cDNA Synthesis until finishing step 40.

Precool 96-well microfuge tube racks On ice .

- 2 Using preprinted adhesive labels or permanent markers, label 1.5 ml microfuge tubes with unique sample ID.
- 3 Transfer fecal sample into the corresponding pre-labeled 1.5 mL microfuge tubes and put it onto the microfuge tube rack On ice sequentially.
- 4 Once the rack is full or all feces samples have been completed, label the rack with a unique rack ID.
- 5 Close the rack lid tightly, secure with clear Saran wrap and immediately transfer to -80 °C freezer.

6 Update the freezer inventory so samples can be tracked properly.


STORAGE PROCEDURE FOR TOTAL NUCLEIC ACID

7



Note

NOTES:

- The following procedure is to properly store total nucleic acid extracted from feces samples (including negative controls) using the KingFisher nucleic acid purification system. The eluted total nucleic acid will be in either 96-well microplate (Flex model) or elution strip (Duo Prime model).
- Total nucleic acid samples need to be kept  On ice all the time to minimize RNA degradation.

In the clean PCR workstation, carefully transfer the eluted total nucleic acid to a 96-well PCR microplate, make sure to keep samples in the exact same locations corresponding to the rack where the original blood were stored.

Note

IMPORTANT: Mark the “A1” position of the 96-well microplate to prevent any mistakes on plate orientation.

8 Cover the 96-well PCR microplate with adhesive film to prevent spill over or contamination.

9 Label the film with a unique plate ID.

10 Immediately transfer the 96-well PCR microplate to  -80 °C freezer.

- 11 Update the freezer inventory so samples can be tracked properly.