DNA Extract was from filter paper stored at room temperature

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ABSTRACT

Preservation of samples requiring cold-chain storage is an often unavoidable challenge especially when doing laboratory work outside the western world. Samples are a precious commodity and it is imperative to maintain their viability. One method for collection and storage of samples in a liquid form (blood, saliva, serum) at room temperature is on filter paper cards like the Cellabs Tropbio Filter Paper Blood Collection Disks™. Methods and protocols exist for the use and recovery of whole blood from filter paper discs. This protocol describes the wash and recovery of DNA extracted from whole blood using the Qiagen DNeasy Extraction kit and dried on filter paper, re-suspended after several months of room temperature storage and use in conventional PCR.

This protocol was used to suspend DNA extracted from the wholeblood of Dogs in Cairo, Egypt. The DNA was extracted using the Qiagen DNEasy Extraction Kit and placed on Cellabs Tropbio Filter Paper Blood Collection Disks™ (filter paper). The DNA dried on the filter paper overnight and was stored in plastic self-closure baggies for several months before being washed off and used in Conventional PCR. The use case described here is of a dog who was PCR positive for *Babesia* spp. using a sample of whole blood washed from the filter paper as well as dried DNA from filter paper.

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DNA Extraction, Egypt, Field work, Tropbio filter paper, room temperature DNA storage

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MATERIALS TEXT

QIAgen DNeasy Blood and Tissue Kit, 50 rxn **Qiagen Catalog #69504** Step 3.1

QIAmp DNA Investigator Handbook **Qiagen Catalog #56504**

Tropbio Filter Paper Blood Collection Disks™ **Contributed by users Catalog #FP**

Taq PCR Master Mix Kit **Qiagen Catalog #201445**

Tris base **Sigma Aldrich Catalog #T1503**

Gel Red Nucleic Acid Gel Stain **Biotium Catalog ##41003** Step 10

1kb ladder **Biotium Catalog #99962**

General:
Pipettes, tips, hood, tabletop centrifuge, microcentrifuge tubes, rocking platform, thermal cycler, gel electrophoresis rig, UV light box, gloves, sterile 1L glass bottle, balance, autoclave, pH meter, distilled water

Whole blood collection from animal

1 Using a sterile syringe and needle, collect **1-5 mL** of whole blood from animal's forelimb. Immediately express the contents of the syringe into a new, clean EDTA tube and invert several times to mix.

Whole blood storage

2 IF EXTRACTING DNA ON THE SAME DAY AS BLOOD COLLECTION - Place EDTA tube containing fresh blood on ice or at **4 °C** until ready to extract.

IF EXTRACTING DNA AT A LATER DATE FROM BLOOD COLLECTION- Place EDTA tube containing fresh blood in the freezer at **-20 °C** until ready to extract; sample will need to be thawed and brought to room temperature before DNA extraction can begin.

DNA Extraction

3 **If using blood frozen in EDTA, bring it fully to room temperature before beginning to extract**

Set up a clean workspace in a biosafety cabinet or other appropriate laboratory space for working with biological material and DNA

3.1 Materials from the QIAgen DNeasy Blood and Tissue Kit, 50 rxn **Qiagen Catalog #69504** will be used following the 'FTA Guthrie Card' Instructions from the

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QIAamp DNA Investigator Kit **Qiagen Catalog #56504** with some modifications

3.2 3h

Modifications to FTA Guthrie Card Instructions:

1) **Protocol Step 4 states:** Place a **1.5 mL** microcentrifuge tube in a thermomixer or heated orbital incubator with shaking at **900 rpm, 56°C** *for* **01:00:00**

Instead: Place the **1.5 mL** tube in a thermomixer or heated orbital incubator and incubate at **900 rpm, 56°C** for **02:00:00**

2) **Protocol Step 17 states:** Apply **20-100 µl** Buffer ATE or distilled water to the center of the membrane.

Instead: Apply **100 µl** of Buffer AE to wash DNA into microcentrifuge tube

4) **Protocol Step 18:** Close the lid and incubate at room temperature for 1 minute. Centrifuge at full speed (20,000 xg; 14,000 rpm) for 1 minute.

Instead: Close the lid and incubate at room temperature for 5 minute. Centrifuge at full speed (20,000 xg; 14,000 rpm) for 1 minute.

3.3

The DNA is recovered at the final step of the extraction process when buffer 'AE' is used. Take care not to accidentally discard the flow-thru of this step, the AE wash product contains the actual DNA

Prepare Filter Paper 5m

4 5m Wear clean gloves and remove individual filter paper disk with 'ears' (each Cellabs Tropbio Filter Paper Blood Collection Disks™- Disk has 6 individual ears attached to it) from the sheet.

Filter paper will need a clean, dry, flat area to dry **Overnight** after application of the DNA extract, ensure an appropriate bench space is prepared before removing Filter Paper from packaging.

4.1 use a sharpie or other permanent marker to write your sample ID on the center part of the disk

4.2 Bring the DNA extract to room temperature

Apply DNA to Filter Paper 5s

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5 Vortex or invert microcentrifuge tube containing DNA extract for **00:00:05** to mix DNA evenly. 5s

On each 'ear' of the filter paper disk, pipette **6 µl** of DNA extract

5.1 5s

Allow Filter Paper to dry **Overnight** , or longer on a clean, dry, flat surface

The remaining DNA in the microcentrifuge tube can be discarded or stored in a freezer.

Filter Paper Storage

6 After drying overnight, dried filter paper can be placed individually in self-closing plastic bags and sealed tightly. The individual plastic bags can be stored in a larger self-closing plastic bag containing a pelleted desiccant to minimize the risk of moisture getting to the filter paper.

Dried filterpaper cards sealed in individual plastic bags

Dried filter paper stored in individual plastic bags with silica

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6.1 DNA on filter paper can be stored at room temperature for up to 6 months

Prepare to wash DNA from Filter Paper - make Tris-HCL 3h

3h

7 10mM Tris-HCL, pH 8.5 is used to wash dried DNA from filter paper.

To make 500 mL 10mM Tris-HCL, pH 8.5 wash do the following:

7.1 to make 500mL 10mM Tris-HCL, pH 8.5 (Starting with 121.1 g/mol tris powder)

measure 0.6055 g tris base powder and pour into a sterile glass bottle

add about 300mL DI water to the bottle with the tris base powder

7.2 Use a pH meter to adjust the pH of the tris base in water until a pH of 8.5 is achieved.

7.3 once the appropriate pH is achieved, add DI water to bring total volume to 500mL

7.4 Loosely place a cap on the glass bottle containing the tris base and autoclave it on settings appropriate for your machine

7.5 ⚠

Allow tris base to cool to room temperature following completion of autoclave

Wash Extracted DNA from filter paper

8 In a clean, sterile 2mL microcentrifuge tube, place one whole filter paper 'ear' containing dried DNA extract.

8.1 Pipette 150µL of sterile Tris HCL @pH 8.5 (made in step 7 above) into the microcentrifuge tube containing the filter paper 'ear'

8.2 ensure the filter paper ear is completely submerged in the liquid, close lid, vortex thoroughly for 20 seconds

8.3 Ensure the filter paper ear is submerged following vortex, with lid closed, place tube on rocking platform at room temperature, 500rpm for 2 hours

8.4 following 2 hours of rocking, the DNA will be suspended in the eluate in the tube.

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Polymerase Chain Reaction

9 Prepare a clean and sterile bench space for PCR

9.1 Prepare mastermix following the protocol for the Qiagen *Taq*PCR Master Mix Kit (Catalog #201445).

MasterMix:

A	B
Qiagen MasterMix	50 µL
Forward Primer	3µL
Reverse Primer	3µL
Sample	10µL
Water	34µL
Total	100 µL

thaw (if necessary) and vortex the microcentrifuge tube with the washed DNA Sample before adding it to the mastermix

use 10µL of the washed DNA (from step 9 of this protocol) as the 'sample' in the mastermix

Thermal Cycle

9.2 Thermal Cycle

lid 105°C, 95°C 3min, 95°C 30sec, 60°C 30 sec 72°C 45sec, repeat 34x, 72°C 10min, 4°C infinite hold or placed in 4°C refrigerator until gel electrophoresis

Gel Electrophoresis

10 2% Agarose Gel Electrophoresis with Biotium Gel Red

Gel Red Nucleic Acid Gel

Stain **Biotium Catalog ##41003**

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