RESEARCH NOTE

Finger stick blood collection for gene expression profiling and storage of tempus blood RNA tubes [version 1; peer review: 3 approved with reservations]

Darawan Rinchai[1], Esperanza Anguiano[2], Phuong Nguyen[3], Damien Chaussabel[1]

[1]Systems Biology Department, Sidra Medical and Research Center, Doha
[2]The Jackson Laboratory for Genomic Medicine, Farmington
[3]Baylor Scott and White Health, Dallas

v1 **First published:** 15 Jun 2016, **5**:1385 (https://doi.org/10.12688/f1000research.8841.1)
Latest published: 03 Mar 2017, **5**:1385 (https://doi.org/10.12688/f1000research.8841.2)

Abstract

With this report we aim to make available a standard operating procedure (SOP) developed for RNA stabilization of small blood volumes collected via a finger stick. The anticipation that this procedure may be improved through peer-review and/or readers public comments is another element motivating the publication of this SOP. Procuring blood samples from human subjects can, among other uses, enable assessment of the immune status of an individual subject via the profiling of RNA abundance using technologies such as real time PCR, NanoString, microarrays or RNA-sequencing. It is often desirable to minimize blood volumes and employ methods that are the least invasive and can be practically implemented outside of clinical settings. Finger-stick blood samples are increasingly used for measurement of levels of pharmacological drugs and biological analytes. It is a simple and convenient procedure amenable for instance to field use or self-collection at home using a blood sample collection kit. Such methodologies should also enable the procurement of blood samples at high frequency for health or disease monitoring applications.

Keywords

Blood collection , Fingerstick , Gene expression , RNA , Tempus , Transcriptome

This article is included in the Sidra Medicine gateway.

Open Peer Review

Reviewer Status ? ? ✓

	Invited Reviewers		
	1	**2**	**3**
REVISED **version 2** published 03 Mar 2017			✓ report
version 1 published 15 Jun 2016	? report	? report	? report

1 **Angela E Vinturache**, University of Calgary, Calgary, Canada

2 **Jacqueline Margaret Cliff**, London School of Hygiene and Tropical Medicine, London, UK

3 **Eiliv Lund**, The Arctic University of Norway, Tromsø, Norway

Any reports and responses or comments on the article can be found at the end of the article.

Corresponding author: Darawan Rinchai (drinchai@sidra.org)

Competing interests: No competing interests were declared.

Grant information: DR and DC received support from the Qatar Foundation.
The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

How to cite this article: Rinchai D, Anguiano E, Nguyen P and Chaussabel D. **Finger stick blood collection for gene expression profiling and storage of tempus blood RNA tubes [version 1; peer review: 3 approved with reservations]** F1000Research 2016, **5**:1385 (https://doi.org/10.12688/f1000research.8841.1)

First published: 15 Jun 2016, **5**:1385 (https://doi.org/10.12688/f1000research.8841.1)

Introduction

Use of sample collection methods that are least invasive and that can be practically implemented outside of clinical settings, Finger-stick blood collection is used for a wide range of applications in routine clinical practice. It is for instance by this means that millions of individuals collect daily small blood volumes to monitor sugar levels.

More recently, availability of high throughput profiling technologies made it possible to measure simultaneously the abundance of tens of thousands of analytes. For instance, transcriptome profiling, which measures abundance of RNA on a genome-wide scale has become a mainstay in biomedical research settings[1–6]. This approach can be implemented through the use of technologies such as microarray and more recently RNA-sequencing. Robust and more cost-effective "meso-scale" profiling technologies, relying for instance on PCR or NanoString probes, can profile the abundance of hundreds of genes[7]. Blood transcriptome profiling has proven useful in generating high-resolution molecular phenotypes: to investigate pathogenesis of a wide range of diseases[8–11]; to develop biomarker signatures[12–15]; and to assess response to vaccines or therapies[7,16–20]. More recently, an approach consisting in correlating serial blood transcriptome signatures with clinical course of disease was described as a means to guide development and selection of novel therapeutic modalities in patients with systemic lupus erythematosus[1,21].

Transcriptome profiling studies have initially employed peripheral blood mononuclear cells (PBMCs)[11,22,23]. PBMCs are isolated by fractionation and are enriched in blood leukocytes. It is also a type of sample from which high quality RNA can be reliably obtained, which at the time was not the case of whole blood. However the PBMC preparation procedure involves multiple steps and important variations are introduced between the time of blood draw and preparation of the cell lysates[24]. Furthermore, it is a time consuming process that requires trained personnel and equipment and is not straightforward to implement in most clinical settings. Whole blood RNA stabilization systems have been adopted as they became available and are now widely used. However the vast majority of the studies carried out to date use relatively large volumes of venous blood[12,13,19,20,25]. Collection of small volumes of blood via finger sticks is especially indicated for high frequency sample collection to enable monitoring of the immune status of individuals in health and disease. Advantages of this collection modality stem from the fact that it is less invasive, faster and does not require a trained phlebotomist. Therefore it is more amenable to field applications and in home self-collection for proximity testing. A study by Obermoser *et al.,* employed this collection method to investigate transcriptome responses elicited by influenza and pneumococcal vaccines at 8 different time points in the 48 hours following vaccine administration[16]. A methods development article has also been published by Robinson *et al.,* demonstrating that RNA quality and gene expression data obtained from blood obtained via finger stick (70 µL) and venipuncture (2.5 mL) are highly comparable[26].

With this report, we aim to share our standard operating procedure for stabilization of RNA from 50 µl of blood collected via a finger stick. This SOP will be used specifically in a pregnancy monitoring study that will be conducted on the Thai-Myanmar border. This study will consist of measuring changes in blood transcript abundance in 400 women during the second and third trimester of their pregnancy. A complete description of this study will be provided elsewhere.

Narrative of the procedure

The procedure described in this article can be employed for serial blood collection in clinical or research laboratory settings as well as for in-home self-collection. A narrative is provided here, along with general remarks and considerations. A detailed point-by-point SOP follows.

Narrative: Tempus RNA tubes are designed for the collection of 3 ml of blood via venipuncture and contain 6 ml of a proprietary RNA stabilizing reagent. For the collection of 50 µl blood samples 100 µl of the RNA stabilizing reagent is aliquoted in microfuge tubes. Blood is collected with a plastic capillary straw. Immediately after collection, the tube is shaken vigorously to disrupt the blood cells. Lysis of blood cells occurs upon thoroughly mixing the blood drawn into the tube and the stabilizing reagent. Furthermore, RNases are inactivated and the freed RNA is selectively precipitated and thus further protected from degradation. Effective stabilization of the RNA ensures that the transcriptional profile is maintained and will accurately reflect the physiological state of the patient at the time of the blood draw. RNA properly collected in Tempus solution and stored at -20°C or -80°C will remain stable for minimum of 6 years[27].

General remarks: After over 10 years of use across a wide range of clinical settings RNA stabilization using tempus solution has in our hands proven robust and reliable. However there are a few things that we have learned that are worth sharing:

1) Finger stick: The finger is usually the preferred site for capillary testing in an adult patient. When samples are collected serially it is recommended to choose a different finger from the one used for the last procedure to prevent bruising. The sides of the heel are only used in pediatric and neonatal patients. The guidance given in Section 7.1 of the WHO guidelines on blood drawing: best practices in phlebotomy, can help decide whether to use a finger or heel-stick, and with the selection of an appropriately sized lancet[28].

2) Blood volumes: The volume can be adjusted depending on the application. Typical yield from 50 µl of blood is about 500 ng of total RNA. Procedures for RNA extraction and quality control will be shared in a separate publication (Anguiano E., Rinchai D., Tomei S., Chaussabel D., unpublished report). A study was conducted where as little as 15 µl of blood was collected, which was sufficient to run a high throughput Fluidigm PCR assay (Speake C., Whalen E., Gersuk V., Chaussabel D., Odegard JM., and Greenbaum CJ., unpublished report). Such small blood volumes can also be obtained serially from mice, which allow longitudinal monitoring of individual animals. In human studies, instead of using a capillary straw small blood volumes can also be collected and measured with a micropipette. The blood is then placed into

the microfuge tube containing the tempus solution. This can be done when collecting small volumes of blood from a finger stick or obtaining a small aliquot of blood from a larger venous blood draw.

3) Volume of RNA stabilization solution: The appropriate ratio of [Blood : RNA stabilizing reagent] is 1 volume of blood for 2 volumes of tempus solution (in our case 50 µl of blood in 100 µl of RNA stabilizing reagent). Loss in RNA quality and quantity will be observed if this ratio is not respected. Collecting more blood will actually result in decreased yields and RNA quality. In cases when the amount of blood collected is lower, the volume of tempus solution can be adjusted accordingly when feasible. The same ratio can be used when working with mouse blood collected from the tail vein using a similar procedure (as mentioned above blood volumes can be lowered to 15 µl). The volumetric ratio is usually lower when working with non-human primate species (e.g. 1:3, 1:4) and should be determined on a species-by-species basis (a 1:3 ratio is used when collecting blood from macaques[29]).

4) Sample mixing: This, after maintaining an appropriate blood: tempus solution ratio, is the second most critical aspect of the procedure, and a potential cause of sample failure. As mentioned above samples must be homogenized by thorough mixing in order to disrupt cells and release their RNA cargo. The RNA will precipitate in the tempus solution and in this form is protected from degradation by the RNAses that are present in the sample.

5) Temperature: RNA should remain in a precipitated state at "room temperature". Although refrigeration and freezing at the earliest possible time is recommended, based on our observations keeping the blood lysates at room temperature (25°C) for up to 24 hours should not affect RNA quality. Samples can be stored at 4°C (refrigerator or cold packs) for up to 48 hours, which can simplify the logistics associated with temporary storage, transfer and shipping of samples post-collection. Based on information provided by the manufacturer RNA should remain in a precipitated state as long as temperatures remain below 30°C. It may therefore be necessary to take precaution when working in warm climates.

6) Storage and shipping: By default samples are stored in the lab at -20°C. We have observed that the RNA yield for samples stored at -80°C is generally about half the yield of same blood samples stored at -20°C. Furthermore, we observed that plastic tempus tubes are made of will become brittle at temperatures lower than -20°C. Shipments are made on dry ice although for overnight shipping in cooler climates using ice packs should be sufficient (however testing using mock samples is recommended). When shipping "off the shelf" tempus tubes direct contact with dry ice should be avoided to prevent breakage. When shipping on dry ice the thickness of the walls of the polystyrene container holding the tubes along with the dry ice matters. The thinner the walls the faster the shipment will run out of dry ice. This is especially important to consider when contemplating longer transit times and/or warm weather conditions. Regarding biosafety, we have found the tempus solution to prevent growth of bacteria known for their resilience such as *Burkholderia pseudomallei* (Rinchai D, unpublished report), and thus conclude that threat of contamination via tempus blood lysates is likely to be low. However, appropriate testing should be carried out on a case-by-case basis and all procedures in the laboratory involving tempus lysates should be consistent with standard blood handling procedures.

Materials and methods

Reagents and equipment

- Tempus™ blood RNA tubes (ThermoFischer Scientific, Waltham, MA, USA; Product number 4342792; https://www.thermofisher.com/order/catalog/product/4342792)
- Capillary blood and tube assembly, untreated, 50 µl, thin design (Kabe Labortechnik, Nümbrecht, Germany (Product number GK100, http://www.kabe-labortechnik.de/download/kapillarblut_en.pdf)[30,31]
- Sterile blood lancets
- Alcohol pads
- Biohazard container
- Lab coat
- Gloves
- Ziploc-type biohazard bag and freezer box
- Adhesive bandages
- Sample collection tube labels

Precautions

- Personal protective equipment must be worn to prevent accidental exposure to blood and bloodborne pathogens [http://www.cdc.gov/niosh/topics/emres/ppe.html].
- Discard all blood collection materials and "sharps" in properly labeled biohazard containers approved for their disposal.
- Check that the liquid preservatives and anticoagulants in the collection tubes are clear and colorless. Do not use any tubes if they are discolored or contain precipitates.

Procedures

The procedures below are illustrated in Figure 1 and a demonstration video is available here: https://www.youtube.com/watch?v=NjY-OqjrzbY

1. Assemble equipment and supplies, then complete the Fingerstick Information Log by recording relevant information about blood collection such as patient name, patient identity number (patient ID), date of blood collection, frequency number of blood collection (Day 1st, Day 7th... Day 90th,…). Double check that the label on the collection tube matches with the patient ID.
2. Put on well-fitting gloves

Figure 1. Illustration for capillary blood sampling. This figure illustrates the different steps involved in capillary blood sampling via finger stick.

3. Choose one of the subject's fingers from which blood will be collected. The middle or the ring finger is the best choice for finger stick collection. Avoid the thumb and pinkie finger, fingers with thick calluses, that are injured or swollen and fingers with tight rings as they may constrict blood flow.
4. Prepare the puncture site by warming the area. If the subject is particularly cold have the subject wash hands under warm water to stimulate blood flow. In addition it may be necessary to warm the area with a moist towel for five to ten minutes.
5. Wipe the fingertip with the alcohol pad and allow to air-dry completely without blowing or wiping off the alcohol.
6. To stimulate blood flow, you may shake or gently knead the subject's hand from palm to fingertip. Blood will also flow better if the hand is kept lower, approximately at the level of the subject's waist.
7. Hold finger and press lancet firmly against the side of the center of the finger, with lancet oriented perpendicular to the fingerprint grooves.
8. Discard lancet in an appropriate container.

9. Release pressure and allow a full drop of blood to collect on finger. If necessary, gently knead the palm only to stimulate blood flow.

10. Wipe away the first drop of blood with a sterile gauze pad because it may be contaminated with tissue fluid or debris (sloughing skin).

11. Collect blood sample into the capillary tube.

    a. Hold the capillary and micro-tube assembly horizontally, and touch the tip of the capillary to the blood drop.

    b. The blood will be pulled into the tube via capillary action.

    c. Be sure to allow the capillary to fill end-to-end to allow collection of accurate blood volume.

    d. To expel the sample from the capillary, place the capillary and micro-tube assembly vertically and firmly tap the bottom of the tube. Remove capillary tube together with cap assembly system and discard in the appropriate biohazard container.

    e. **It is important to maintain the appropriate blood sample to tempus solution ratio.** A volume of 50 µl of blood should be added to the 100 µl of tempus solution. If necessary the volume of solution can be adjusted to the available or desired volume of blood; e.g. for 15 µl of blood use 30 µl of tempus solution.

    f. Close the micro-tubes, making sure that the cap is pressed down firmly to avoid any spillage during sample homogenization.

    g. To prevent clotting, blood samples should be collected within 30 seconds of performing the finger stick. Clotted samples will not be usable.

12. Have the subject apply pressure to the puncture site using sterile gauze pad until bleeding has stopped and apply a bandage. Do not use the alcohol pad as contact of an open wound with alcohol would be painful for the subject.

13. Mix the blood sample and preservative thoroughly by holding the top of the tube between thumb and index of one hand and **flicking the tube vigorously for 20 seconds with the index finger of the other hand (Figure 1)**.

14. If not already in place stick pre-printed label with sample information on the sample tube.

15. Place sample tube in appropriate container (e.g. freezer box).

16. The sample should be kept cold at 4°C and transferred to a -20°C freezer as soon as possible for long-term storage. Note that RNA integrity is preserved when samples are kept at "room temperature" for a few hours as long as the temperature does not rise above 30°C.

17. For local transportation samples can be kept in a freezer box containing ice or ice packs. For international shipping samples can be kept on dry ice in a freezer box.

Author contributions

DR: participated in development and testing of the protocol, in the shooting of the demonstration video; drafted the manuscript. **EA:** participated in development and testing of the protocol; edited the manuscript. **PN:** participated in testing protocol, reviewed the manuscript. **DC:** participated in development of the protocol, shooting of the demonstration video; assisted with the drafting of the manuscript.

Competing interests

No competing interests were declared.

Grant information

DR and DC received support from the Qatar Foundation.

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Acknowledgements

We would like to thank Dr. David Furman for his participation during the shooting of the demonstration video, Dr. Sara Tomei for her help with obtaining the necessary supplies and Benaroya Research Institute members (DCRP, Genomics and bioinformatics teams) for their input.

References

1. Banchereau R, Hong S, Cantarel B, *et al.*: **Personalized Immunomonitoring Uncovers Molecular Networks that Stratify Lupus Patients.** *Cell.* 2016; **165**(3): 551–65.
PubMed Abstract | Publisher Full Text

2. Chaussabel D, Pulendran B: **A vision and a prescription for big data-enabled medicine.** *Nat Immunol.* 2015; **16**(5): 435–9.
PubMed Abstract | Publisher Full Text

3. Joshi AD, Andersson C, Buch S, *et al.*: **Four Susceptibility Loci for Gallstone Disease Identified in a Meta-analysis of Genome-wide Association Studies.** *Gastroenterology.* 2016; pii: S0016-5085(16)30110-X.
PubMed Abstract | Publisher Full Text

4. Linsley PS, Chaussabel D, Speake C: **The Relationship of Immune Cell Signatures to Patient Survival Varies within and between Tumor Types.** *PLoS One.* 2015; **10**(9): e0138726.
PubMed Abstract | Publisher Full Text | Free Full Text

5. Linsley PS, Speake C, Whalen E, *et al.*: **Copy number loss of the interferon gene cluster in melanomas is linked to reduced T cell infiltrate and poor patient prognosis.** *PLoS One.* 2014; **9**(10): e109760.
PubMed Abstract | Publisher Full Text | Free Full Text

6. Furman D, Davis MM: **New approaches to understanding the immune response to vaccination and infection.** *Vaccine.* 2015; **33**(40): 5271–81.
PubMed Abstract | Publisher Full Text | Free Full Text

7. Nielsen T, Wallden B, Schaper C, *et al.*: **Analytical validation of the PAM50-based Prosigna Breast Cancer Prognostic Gene Signature Assay and nCounter Analysis System using formalin-fixed paraffin-embedded breast tumor specimens.** *BMC Cancer.* 2014; **14**: 177.
PubMed Abstract | Publisher Full Text | Free Full Text

8. Ramilo O, Allman W, Chung W, *et al.*: **Gene expression patterns in blood leukocytes discriminate patients with acute infections.** *Blood.* 2007; **109**(5): 2066–77.
PubMed Abstract | Publisher Full Text | Free Full Text

9. Bennett L, Palucka AK, Arce E, *et al.*: **Interferon and granulopoiesis signatures in systemic lupus erythematosus blood.** *J Exp Med.* 2003; **197**(6): 711–23.
PubMed Abstract | Publisher Full Text | Free Full Text

10. Pascual V, Chaussabel D, Banchereau J: **A genomic approach to human autoimmune diseases.** *Annu Rev Immunol.* 2010; **28**: 535–71.
PubMed Abstract | Publisher Full Text | Free Full Text

11. Tang BM, McLean AS, Dawes IW, *et al.*: **Gene-expression profiling of peripheral blood mononuclear cells in sepsis.** *Crit Care Med.* 2009; **37**(3): 882–8.
PubMed Abstract | Publisher Full Text

12. Mejias A, Dimo B, Suarez NM, *et al.*: **Whole blood gene expression profiles to assess pathogenesis and disease severity in infants with respiratory syncytial virus infection.** *PLoS Med.* 2013; **10**(11): e1001549.
PubMed Abstract | Publisher Full Text | Free Full Text

13. Berry MP, Graham CM, McNab FW, *et al.*: **An interferon-inducible neutrophil-driven blood transcriptional signature in human tuberculosis.** *Nature.* 2010; **466**(7309): 973–7.
PubMed Abstract | Publisher Full Text | Free Full Text

14. Martinez-Llordella M, Lozano JJ, Puig-Pey I, *et al.*: **Using transcriptional profiling to develop a diagnostic test of operational tolerance in liver transplant recipients.** *J Clin Invest.* 2008; **118**(8): 2845–57.
PubMed Abstract | Publisher Full Text | Free Full Text

15. Newell KA, Asare A, Kirk AD, *et al.*: **Identification of a B cell signature associated with renal transplant tolerance in humans.** *J Clin Invest.* 2010; **120**(6): 1836–47.
PubMed Abstract | Publisher Full Text | Free Full Text

16. Obermoser G, Presnell S, Domico K, *et al.*: **Systems scale interactive exploration reveals quantitative and qualitative differences in response to influenza and pneumococcal vaccines.** *Immunity.* 2013; **38**(4): 831–44.
PubMed Abstract | Publisher Full Text | Free Full Text

17. Gaucher D, Therrien R, Kettaf N, *et al.*: **Yellow fever vaccine induces integrated multilineage and polyfunctional immune responses.** *J Exp Med.* 2008; **205**(13): 3119–31.
PubMed Abstract | Publisher Full Text | Free Full Text

18. Hecker M, Hartmann C, Kandulski O, *et al.*: **Interferon-beta therapy in multiple sclerosis: the short-term and long-term effects on the patients' individual gene expression in peripheral blood.** *Mol Neurobiol.* 2013; **48**(3): 737–56.
PubMed Abstract | Publisher Full Text

19. Oswald M, Curran ME, Lamberth SL, *et al.*: **Modular analysis of peripheral blood gene expression in rheumatoid arthritis captures reproducible gene expression changes in tumor necrosis factor responders.** *Arthritis Rheumatol.* 2015; **67**(2): 344–51.
PubMed Abstract | Publisher Full Text | Free Full Text

20. Querec TD, Akondy RS, Lee EK, *et al.*: **Systems biology approach predicts immunogenicity of the yellow fever vaccine in humans.** *Nat Immunol.* 2009; **10**(1): 116–25.
PubMed Abstract | Publisher Full Text | Free Full Text

21. Jourde-Chiche N, Chiche L, Chaussabel D: **Introducing a New Dimension to Molecular Disease Classifications.** *Trends Mol Med.* 2016; **22**(6): 451–53, pii: S1471-4914(16)30009-0.
PubMed Abstract | Publisher Full Text

22. Kaizer EC, Glaser CL, Chaussabel D, *et al.*: **Gene expression in peripheral blood mononuclear cells from children with diabetes.** *J Clin Endocrinol Metab.* 2007; **92**(9): 3705–11.
PubMed Abstract | Publisher Full Text

23. Allantaz F, Chaussabel D, Stichweh D, *et al.*: **Blood leukocyte microarrays to diagnose systemic onset juvenile idiopathic arthritis and follow the response to IL-1 blockade.** *J Exp Med.* 2007; **204**(9): 2131–44.
PubMed Abstract | Publisher Full Text | Free Full Text

24. Debey-Pascher S, Eggle D, Schultze JL: **RNA stabilization of peripheral blood and profiling by bead chip analysis.** *Methods Mol Biol.* 2009; **496**: 175–210.
PubMed Abstract | Publisher Full Text

25. Pankla R, Buddhisa S, Berry M, *et al.*: **Genomic transcriptional profiling identifies a candidate blood biomarker signature for the diagnosis of septicemic melioidosis.** *Genome Biol.* 2009; **10**(11): R127.
PubMed Abstract | Publisher Full Text | Free Full Text

26. Robison EH, Mondala TS, Williams AR, *et al.*: **Whole genome transcript profiling from fingerstick blood samples: a comparison and feasibility study.** *BMC Genomics.* 2009; **10**: 617.
PubMed Abstract | Publisher Full Text | Free Full Text

27. Duale N, Lipkin WI, Briese T, *et al.*: **Long-term storage of blood RNA collected in RNA stabilizing Tempus tubes in a large biobank--evaluation of RNA quality and stability.** *BMC Res Notes.* 2014; **7**: 633.
PubMed Abstract | Publisher Full Text | Free Full Text

28. WHO Guidelines Approved by the Guidelines Review Committee: **WHO Guidelines on Drawing Blood: Best Practices in Phlebotomy.** Geneva, 2010.
PubMed Abstract

29. Skinner JA, Zurawski SM, Sugimoto C, *et al.*: **Immunologic characterization of a rhesus macaque H1N1 challenge model for candidate influenza virus vaccine assessment.** *Clin Vaccine Immunol.* 2014; **21**(12): 1668–80.
PubMed Abstract | Publisher Full Text | Free Full Text

30. RAM Scientific: **SAFE-T-FILL® Capillary Blood Collection Tubes.**
Reference Source

31. KABE Labortechnik: **The capillary blood collection sets.**
Reference Source

Open Peer Review

Current Peer Review Status: ? ? ?

Version 1

Reviewer Report 14 October 2016

https://doi.org/10.5256/f1000research.9516.r16392

?

Eiliv Lund
Department of Community Medicine, The Arctic University of Norway, Tromsø, Norway

This manuscript describes an important approach to transcriptomics that could be important for future research. It is remarkable that modern technology can give mRNA profiles based on microgram of mRNA. The SOP is important, but some parts of it confuse me.

First of all, this company is related to Tempus. Can the method be extended to other products? This is important since PAX is as much used as Tempus.

Secondly, I am a bit surprised that -20 degrees is the primary choice versus -70 degrees since all medical biobanks use -70 degrees. The explanation of plastic that is brittle does not sound convincing for future use of the technology.

Third, how long is the optimal mixing time before freezing? You state 20 seconds in the illustration while you also write that it can be stored for 24 hours at normal temperatures.

The technology is very interesting. Since there are no comparisons of outcomes for different procedures nor any quality measures of mRNA it may look more like a recipe.

Competing Interests: No competing interests were disclosed.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 23 Feb 2017

Darawan Rinchai, Sidra Medical and Research Center, Doha

We thank the reviewer for their precious spent reviewing our manuscript. Please see our point by point response below.

This manuscript describes an important approach to transcriptomics that could be important for future research. It is remarkable that modern technology can give mRNA profiles based on microgram of mRNA. The SOP is important, but some parts of it confuse me.

First of all, this company is related to Tempus. Can the method be extended to other products? This is important since PAX is as much used as Tempus.
Authors: *Our choice of the tempus system over PaxGene is based on side-by-side comparisons we have performed over 10 years ago. We have checked in publications whether PAXgene were used for small volume blood collection. We have found only a publication by Carrol ED. et al., BMC Immunol 2007 where the PAXgene Blood RNA System kit protocol was modified for used with small, sick children. These investigators aliquoted 860 uL of PAXgene reagent into microtubes and then added 300 uL whole blood to maintain the same recommended proportions as in the PAXgene evacuated tube system. Total RNA yield was between 1,114 and 2,950 ng. This blood volume is not compatible with finger stick blood collection but it is possible that a protocols very similar to the one we are publishing here could be derived for use with PAXgene solution instead of Tempus.*

*However, there are many studies have compared the "**Differences in RNA quality and yield between PAXgene™ and Tempus™".** Here are some selected publications;*

1. *Asare AL. et al., BMC Genomics 2008 have reported that "the tempus system has higher mean yields, improved RNA purity based on OD 260/230 ratios, less degradation based on GAPDH 3′/5 ratios, and a higher number of expressed transcripts based on Percent Present Calls [PMID:18847473].*
2. *Nikula T. et al., Transl Res 2013 have compared the performance of Tempus and PAXgene systems by using 2 RNA amplification protocols and high-density microarrays. "The m icroarray analysis showed acceptable correlation within and between the RNA preservation methods, but altogether 443 transcripts were differentially expressed between RNA samples preserved in TEMPUS and PAXgene tubes". However, the TEMPUS gene expression profile more closely resembles the PBMC than the PAXgene [PMID:23138105].*
3. *Häntzsch M. et al., PLoS One 2014. That have compared the 3 PAXgene and 3 Tempus tubes samples which collected from participants of the LIFE study with and without acute myocardial infarction (AMI). They extracted RNA with 4 manual protocols from Qiagen (PAXgene Blood miRNA Kit), Life Technologies (MagMAX for Stabilized Blood Tubes RNA Isolation Kit), and Norgen Biotek (Norgen Preserved Blood RNA Purification Kit I and Kit II), and 2 (semi-)automated protocols on the QIAsymphony (Qiagen) and MagMAX Express-96 Magnetic Particle Processor (Life Technologies). The results showed that RNA yields were highest using the Norgen Kit I with Tempus tubes and lowest using the Norgen Kit II with PAXgene [PMID:25469788].*

Thus, choice of RNA stabilizing reagent used to preserve samples can indeed be important and affect subsequent RNA quantity and quality [(1) -(3)]. We have added sentences below to our introduction;

"Whole blood RNA stabilization systems; PAXgene™ (Qiagen) and Tempus™ (Life Technologies) have been adopted as they became available and are now widely used. Several studies have compared the performance of these 2 commercial kits and found differences in gene expression profiles, RNA quality and yield [Asare AL. et al., BMC Genomics 2008., Nikula T. et al., Transl Res 2013., Häntzsch M. et al., PLoS One 2014]. Reported yields and quality of RNA stabilized in Tempus solution was generally greater. Thus, the choice of RNA stabilizing reagent used to

preserve samples can indeed be important and affect subsequent RNA quantity and quality. Our choice of the tempus system over PaxGene dates from side-by-side comparisons we have performed over 10 years ago."

Secondly, I am a bit surprised that -20 degrees is the primary choice versus -70 degrees since all medical biobanks use -70 degrees. The explanation of plastic that is brittle does not sound convincing for future use of the technology.
Authors: This point was also addressed in response to Dr. Cliff's comment: *Regarding to the optimal temperature for long term RNA storage. The preliminary data that we are referring to comparing the yield of RNA from samples that were storage at -20 °C or -80 °C are shown below.*

Table 1: Preliminary results for comparison of -20 °C and -80 °C storage conditions

Sample ID	Storage Temp (°C)	RNA yield (ug)	RNA RIN
S1	-20	8.59	8.2
S2	-20	8.69	8.0
S3	-80	3.04	9.0
S4	-80	2.81	8.9
NC		0.05	N/A

Note: Two whole blood samples collected in Tempus RNA Tubes were combined into 50 mL conical tubes and then mixed thoroughly by inversion. Two aliquots of 4 mL each were stored at -80ºC or -20ºC for 3 days. Samples were subsequently processed using modified MagMAX for Stabilized Blood Tubes RNA Extraction Kit (adjusted for input volume during homogenization step only). Purified RNA was assessed for concentration and integrity using NanoDrop and BioAnalyzer, respectively. Negative control is a 1X PBS processed through RNA extraction workflow.

The result shows that samples stored at -20°C have greater RNA yield compared to those stored at -80°C, but that quality was similar under both storage conditions. It is conceivable that over the long term integrity however would be better maintained at the lower temperature. We feel sharing these preliminary results at this point could be helpful to some readers although more extensive investigation is required, especially with long-term storage – although yields and QC results will obviously not be available for some time.

However, when using off the shelf vacuutainer tempus tubes it has indeed been our experience that tubes in direct contact with dry ice can break during transportation and will shatter if dropped to the ground. Recommendation for -20°C storage were made by the manufacturer on this basis. However, this is not a concern when using microtubes in which small volumes of blood in tempus solution are stored. The appropriate sentences were added into the manuscript (6) storage and shipping).

Third, how long is the optimal mixing time before freezing? You state 20 seconds in the illustration while you also write that it can be stored for 24 hours at normal temperatures.
Authors: *The 20 seconds mixing step is necessary and should always be performed. It should occur immediately after blood collection. This is important because it will allow precipitation of the RNA, which is then protected from degradation. We have clarified this point in the manuscript.*

Furthermore, in a recent paper by Speake C, et al., Clin Exp Immunol 2016 in which we report the implementation of a procedure using 15 ul of fingerstick for in home blood collections we

demonstrated that quality of RNA was similar when tubes were flicked, pipetted, or vortexed, but was reduced when samples were not mixed at all.

Subsequently to this homogenization step we recommend that samples be stored:

- *at room temperature for a few hours;*
- *at 4°C for no longer than 48 hours;*
- *at -20°C or -80°C freezer for long-term storage.*

It should be noted that per the manufacturer samples could be stored for 5 days at room temperature but in our experience this can lead to significant loss in RNA integrity and it is generally best to refrigerate or freeze as soon as feasible.

The technology is very interesting. Since there are no comparisons of outcomes for different procedures nor any quality measures of mRNA it may look more like a recipe.
Authors: *We indeed tried to be pragmatic and publish a detailed SOP with sufficient level of details that it could be directly incorporated in a clinical protocol. Outcome comparisons with RNA quality and quantity measurements are presented in a paper that was published very recently and is now referred to in version 2 of our manuscript.*

Competing Interests: No competing interests were disclosed.

Reviewer Report 04 October 2016

https://doi.org/10.5256/f1000research.9516.r16014

?

Jacqueline Margaret Cliff
Department of Immunology and Infection, London School of Hygiene and Tropical Medicine, UK, London, UK

Thank you for the opportunity to review the manuscript by Rinchai *et al*, entitled “Finger stick blood collection for gene expression profiling and storage of tempus blood RNA tubes”.

Blood gene expression profiling has led to rapid advances in our understanding of a range of pathological conditions, including cancer, autoimmune disease and infectious disease. Blood RNA stabilisation tubes have greatly advanced the ease and standardisation of blood collection for such studies. Developing this methodology further by enabling reduced blood volume collection by non-trained phlebotomists or even study participants themselves would facilitate more detailed investigations at greater frequency and more pertinent time points, for example during times of disease exacerbation, and by removing the necessity to visit healthcare settings.

This article describes such a procedure, describing the collection of 50µl blood in a capillary tube, collected into Tempus Tube stabilisation reagent. This paper therefore provides an important contribution to the blood transcriptomics field. However, the manuscript could be improved if the following points are addressed.

1. The authors are suggesting that this sample collection method could be rolled out for home-based testing. However, the Tempus Tube RNA stabilisation reagent is hazardous according to the MSDS. This should be mentioned in the manuscript under the precautions section, whilst also addressing this limitation in terms of suitability for extensive roll-out for personal sample collection.

2. Whilst the manuscript, including the figure and the accompanying video, are very explicit about the blood sample collection into the capillary tube, the preparation of the sample collection tubes in advance is not described. Including this under the preparation steps would be very helpful, as from the video it is difficult to see the Tempus reagent already in the tube.

3. The paper would be substantially improved by the inclusion of some RNA quality data, although the authors indicate RNA extraction will be published in a separate manuscript. But it would be reassuring to see some quantity and quality indicators in this manuscript, or the inclusion of some downstream analysis data to demonstrate that reasonable quality RNA can be generated.

I agree with reviewer Angela Vinturache that the paper does not show much data regarding the storage of Tempus tubes as described in the paper title, except for a note about storage at -20^{o}C being better than -80^{o}C. In our experience, we get good yields of RNA from Tempus Tubes when samples have been stored at -80^{o}C, as normally recommended for long-term RNA storage, believed to be due to inactivation of RNAses. A comment on why -20^{o}C is better would be useful.

Overall I think this is an important manuscript which could stimulate the expansion of blood-based transcriptomics for disease analysis and treatment monitoring, and recommend that it is indexed, subject to addressing the reservations (particularly point 1) described above.

Competing Interests: No competing interests were disclosed.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 23 Feb 2017

Darawan Rinchai, Sidra Medical and Research Center, Doha

We thank the reviewer for their precious spent reviewing our manuscript. Please see our point by point response below.

Blood gene expression profiling has led to rapid advances in our understanding of a range of pathological conditions, including cancer, autoimmune disease and infectious disease. Blood RNA stabilisation tubes have greatly advanced the ease and standardisation of blood collection for such studies. Developing this methodology further by enabling reduced blood volume collection by non-trained phlebotomists or even study participants themselves would facilitate more detailed investigations at greater frequency and more pertinent time points, for example during times of disease exacerbation, and by removing the necessity to visit healthcare settings.

This article describes such a procedure, describing the collection of 50µl blood in a capillary tube, collected into Tempus Tube stabilisation reagent. This paper therefore provides an important contribution to the blood transcriptomics field. However, the manuscript could be improved if the following points are addressed.

1. The authors are suggesting that this sample collection method could be rolled out for home-based testing. However, the Tempus Tube RNA stabilisation reagent is hazardous according to the MSDS. This should be mentioned in the manuscript under the precautions section, whilst also addressing this limitation in terms of suitability for extensive roll-out for personal sample collection.
Authors: *We thank reviewer for this suggestion. RNA stabilizing reagent is indeed potentially hazardous and designed for research use only. We therefore added the paragraph below under the "precaution" and "general remarks" sections, respectively and as suggested discuss this point as well.*
"Tempus Tube RNA stabilization reagent is a potential health hazard; acute oral toxicity, skin corrosion/irritation and serious eye damage/eye irritation can occur upon contact (See MSDS for details)"
The hazardous nature of the tempus solution would make extensive roll out of the collection procedure described in this manuscript problematic and is at the moment clearly intended for research use under well controlled conditions, with preferably collection carried out by trained personnel. However, it should be noted that it has been-field tested for in-home self-collection in a limited number of subject over a period of 6 months without incident [Speake C., et al., Clin Exp Immunol 2016]. The fact that small volumes of solution are used may alleviate some concerns (30 microliters of solution for 15 microliters of blood in the above-mentioned study, vs 6 ml of solution for 3 ml of blood using "off the shelf" tempus collection tubes). However, other technical solutions in which liquids are better contained may indeed be preferable (e.g. microfluidics card, sponges), with one of the best example being the recently developed "DxCollect" system (DxTerity, Rancho Dominguez, CA).

2. Whilst the manuscript, including the figure and the accompanying video, are very explicit about the blood sample collection into the capillary tube, the preparation of the sample collection tubes in advance is not described. Including this under the preparation steps would be very helpful, as from the video it is difficult to see the Tempus reagent already in the tube.
Authors: *As suggested by the reviewer, we added a new section and figure (new Figure 1) for "Preparation of collection tubes" in "Materials and Methods" section. We provide the step-by-step procedure for aliquoting Tempus RNA stabilization and preparation of the blood collection tubes.*

3. The paper would be substantially improved by the inclusion of some RNA quality data, although the authors indicate RNA extraction will be published in a separate manuscript. But it would be reassuring to see some quantity and quality indicators in this manuscript, or the inclusion of some downstream analysis data to demonstrate that reasonable quality RNA can be generated.
Authors: *A proof of principle has been obtained with a study that we recently published, in which weekly in home self-finger stick blood collection (15 uL) was implemented for 13 subjects with type 1 diabetes and 14 controls for a period of 6 months [Speake C., et al., Clin Exp Immunol 2016]. A high degree of correlation between results obtained via fingerstick and a standard 3 mL venipuncture sample was observed. RNA yields obtained from blood volumes ranging from 10, 15, 20, and 25 µL indicated that those volumes were sufficient to generate the 100 ng of RNA needed for downstream high throughput real time-PCR. Furthermore, it was found that equivalent quantities of of RNA were obtained whether tubes were flicked, pipetted, or vortexed, but was reduced when samples were not mixed at all.*

Several sentences were added into part of introduction and the work referenced throughout, where appropriate.

However, the initial experiment with RNA extraction of 50 uL blood collection protocol in Tempus solution, we obtained the average RNA yield was 1 ug, Purity of RNA; average of RNA integrity number (RIN) was ~7.5 and A260/A280 ratio of ~2.14. This concentration was enough for downstream assays; RNA sequencing, microarray or Fluidigm assay. These data will be included in our manuscript describing the RNA extraction procedures.

I agree with reviewer Angela Vinturache that the paper does not show much data regarding the storage of Tempus tubes as described in the paper title, except for a note about storage at -20^{o}C being better than -80^{o}C. In our experience, we get good yields of RNA from Tempus Tubes when samples have been stored at -80^{o}C, as normally recommended for long-term RNA storage, believed to be due to inactivation of RNAses. A comment on why -20^{o}C is better would be useful.
Authors: *We thank the reviewer for helping us to make our manuscript clearer. The following sentence has been added to the manuscript (6) Storage and shipping)*

"After collecting the blood sample should be kept cold at 4°C no longer than 48 hours and transferred to a -20°C or -80°C freezer for long-term storage. Data obtained using a limited set of samples frozen overnight showed that the RNA yield for samples stored at -80°C was about half the yield of same blood samples stored at -20°C, but was nevertheless still amply sufficient for downstream analyses. It should also be noted that the plastic tempus tubes are made of will become brittle at temperatures lower than -20°C. The effect of storage temperature on RNA yield and quality will have to be evaluated further, especially over extended periods of time where storage at lower temperatures might show benefits (see also referees comments and our response for more details)."

We intend to publish standard operating procedures for RNA extraction from tempus blood, which will include extensive QA/QC analyses under different storage conditions. The preliminary data that we are referring to when comparing the yield of RNA from samples that were storage at -20 °C or -80 °C are shown below.

Table 1: Preliminary results for comparison of -20 °C and -80 °C storage conditions

Sample ID	Storage Temp (°C)	RNA yield (ug)	RNA RIN
S1	-20	8.59	8.2
S2	-20	8.69	8.0
S3	-80	3.04	9.0
S4	-80	2.81	8.9
NC		0.05	N/A

Note: Two whole blood samples collected in Tempus RNA Tubes were combined into 50 mL conical tubes and then mixed thoroughly by inversion. Two aliquots of 4 mL each were stored at -80ºC or -20ºC for 3 days. Samples were subsequently processed using modified MagMAX for Stabilized Blood Tubes RNA Extraction Kit (adjusted for input volume during homogenization step only). Purified RNA was assessed for concentration and integrity using NanoDrop and BioAnalyzer, respectively. Negative control is a 1X PBS processed through RNA extraction workflow.

The result shows that samples stored at -20°C have greater RNA yield compared to those stored at -80°C, but that quality was similar under both storage conditions. We feel sharing these preliminary results as part of our point by point response could be helpful to some readers although more

extensive investigation is required, especially with long-term storage – although yields and QC results will obviously not be available for some time.

Competing Interests: No competing interests were disclosed.

Reviewer Report 29 September 2016

https://doi.org/10.5256/f1000research.9516.r15424

?

Angela E Vinturache
Department of Physiology and Pharmacology, University of Calgary, Calgary, AB, Canada

Thank you for the opportunity to review the manuscript Finger stick blood collection for gene expression profiling and storage of tempus blood RNA tubes by Rinchai D, Anguiano E, Nguyen P and Chaussabel D.
In this paper, the authors are proposing an SOP for finger stick blood collection for RNA profiling.
While the objective of this manuscript is worthwhile for the scientific world in proposing standardized lab methods, the paper would benefit from some improvement.
There is a disconnect between the title and the objective of the paper. Is the focus on the finger stick collection or on the tempus tube storage? This should be revised, especially considering that the authors intend to publish the RNA extraction in a separate paper.

Introduction:
First sentence is unclear. The introduction could be shortened considerably. A more concise and to the point argument on why we need this method, advantages and disadvantages can be made. Also, the authors should add supporting reasons for why their method should be published and how it is different from the others.
The authors mention that the method will be used in the future. Is this to understand they have no experience with the proposed method?
Why is mentioning where/what study this method will be used important? Do authors have any experience with non-pregnant patients? Does pregnancy bear any weight in sample collection and transportation?

Narrative of the procedure:
The figure is very useful for following the procedure step-by-step. Considering that a follow up paper proposed by the authors will discuss RNA extraction, all the practical points shared under 'General Remarks' could be moved to the respective manuscript. Since this paper proposes to discuss only the finger stick procedure, the discussion about the volumes of blood drawn is exhaustive and unwarranted for this particular manuscript. However, it is not clear if the tubes for the blood collection are the same as the tubes pre-prepared with the volume of RNA stabiliser for 50 µL of blood. Please provide some practical explanation on the procedure when the capillary straw is not filled (i.e. how to measure the blood volume). What type of capillary is to be used?

Precautions:

There is no mention of the possibility of sample contamination. For point 3 and 5, I suggest they add the reasoning for the selected choices in addition to the drawbacks of the other possibilities. Eliminate repetitions about blood flow and the ratio blood to solution. Point 16 is quite vague. As the matter is essential for the RNA quality, I invite the authors to expand the topic and share their experience, with numbers rather than “as soon as possible”. How much of those samples could be kept during transportation? Some information on authors’ experience with the bio-repository would be very welcome. The authors mention somewhere about using the method as in home self collection. Have they experimented that? It would be particularly interesting if they would provide details about how they plan quality assurance for these samples. Also, any particularities of this method of collection should be explained.

Competing Interests: No competing interests were disclosed.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 23 Feb 2017

Darawan Rinchai, Sidra Medical and Research Center, Doha

We thank the reviewer for their precious spent reviewing our manuscript. Please see our point by point response below.

In this paper, the authors are proposing an SOP for finger stick blood collection for RNA profiling. While the objective of this manuscript is worthwhile for the scientific world in proposing standardized lab methods, the paper would benefit from some improvement. There is a disconnect between the title and the objective of the paper. Is the focus on the finger stick collection or on the tempus tube storage? This should be revised, especially considering that the authors intend to publish the RNA extraction in a separate paper.

Introduction:
First sentence is unclear. The introduction could be shortened considerably. A more concise and to the point argument on why we need this method, advantages and disadvantages can be made. Also, the authors should add supporting reasons for why their method should be published and how it is different from the others.
The authors mention that the method will be used in the future. Is this to understand they have no experience with the proposed method?
Why is mentioning where/what study this method will be used important? Do authors have any experience with non-pregnant patients? Does pregnancy bear any weight in sample collection and transportation?

Authors: *The introduction provides the rationale for adopting such a sample collection procedure. The benefits of this approach were mentioned in the third paragraph of the “Introduction”. We could not find a major disadvantage of this method, except quality and quantity of RNA may be affected if the protocol was to be adapted to be used collecting blood lower than 15 uL.*

As per the reviewer’s suggestion,
1) *We have edited the first paragraph to make it more clear and concise.*
2) A detailed standard operating procedure has not been provided elsewhere before, which is the

reason why we are publishing this report (and to gather feedback from other users/reviewers). We have first used this approach 6 years ago, with ensuing publications (Obermoser., et al. 2013), and more recently in a study in which samples were collected from diabetics and control subjects in home and on a weekly basis (Speake C., et al., Clin Exp Immunol 2016) – however those details have not been published before. The intent was to publish a ready to use SOP that could be readily incorporated in a clinical protocol. We have also added a sentence to clarify these points.
3) Pregnancy has no particular bearing on this procedure, only this is mentioned here as the paper is published as part of our "molecular profiling of pregnancy channel". We have added more information into this sentence.
"The standard operating procedure that we are sharing with this report will be published as part of our molecular profiling of pregnancy channel. Indeed, it was specifically developed for collection and stabilization of 50 µl of blood collected via a finger stick in a pregnancy monitoring study currently being conducted on the Thai-Myanmar border."

Narrative of the procedure:
The figure is very useful for following the procedure step-by-step. Considering that a follow up paper proposed by the authors will discuss RNA extraction, all the practical points shared under 'General Remarks' could be moved to the respective manuscript. Since this paper proposes to discuss only the finger stick procedure, the discussion about the volumes of blood drawn is exhaustive and unwarranted for this particular manuscript. However, it is not clear if the tubes for the blood collection are the same as the tubes pre-prepared with the volume of RNA stabiliser for 50 µL of blood. Please provide some practical explanation on the procedure when the capillary straw is not filled (i.e. how to measure the blood volume). What type of capillary is to be used?
Authors: *Discussion of volume is extensive and warranted as this is in our experience one of the main causes of failure during the extraction step; we now provide a procedure detailing "preparation of the sample collection tubes" (Please see "Material and Methods"); the capillary needs to be filled in order to collect appropriate volumes. Alternative methods, such as use of micropipettes is possible if preferred; other products are also available and could be tested for that purpose. We describe the method that in our hands produces the best results for our particular conditions.*

Precautions:
There is no mention of the possibility of sample contamination.
Authors: *Tempus™ Blood RNA stabilizing Reagent immediately lyse whole blood and stabilize RNA in single step. We have tested lysate in the solution by bacterial culture and found that the sample were still sterilized after blood collection. Therefore we never found contamination after blood collection. The only possibility of the contamination is from sterile/hygiene of blood collection. Personal protective equipment (PPE) is a key of sterile sample collection. We therefore added more details into point 1 of "Precaution" about the point of sample contamination as below.*

- *"Personal protective equipment (PPE) must be worn to prevent accidental exposure to blood and bloodborne pathogens, and to help of reducing contamination during sample collection [http://www.cdc.gov/niosh/topics/emres/ppe.html]."*

For point 3 and 5, I suggest they add the reasoning for the selected choices in addition to the drawbacks of the other possibilities. Eliminate repetitions about blood flow and the ratio blood to solution.
Authors: *Ideally choosing a finger for fingerstick blood collection is a finger of non-dominant hand which are generally less calloused. We also need to choose the fingers that less painful for subjects. Therefore, the best choice should be middle and ring finger. We don't recommend the thumb that may be calloused and has pulse and pinkie finger or index finger that is often calloused*

and potentially more sensitive to pain due to additional nerve ending.

We added this information in the manuscript.

Point 16 is quite vague. As the matter is essential for the RNA quality, I invite the authors to expand the topic and share their experience, with numbers rather than “as soon as possible”. How much of those samples could be kept during transportation? Some information on authors’ experience with the bio-repository would be very welcome.
Authors: *As the comment by reviewer, we have edited point #16 “The sample should be kept cold at 4°C no longer than 48 hours and transferred to a -20°C or -80°C freezer for long-term storage.”*

The authors mention somewhere about using the method as in home self collection. Have they experimented that? It would be particularly interesting if they would provide details about how they plan quality assurance for these samples. Also, any particularities of this method of collection should be explained.
Authors: *Since version 1 of our SOP came out in F1000Research (F1000Research 2016, 5:1385 (doi: 10.12688/f1000research.8841.1) we have published a paper describing results of a study in which weekly in home self-finger stick blood collection was undertaken by 13 subjects with type 1 diabetes and 14 controls for a period of 6 months [Speake C., et al., Clin Exp Immunol 2016]. Subjects returned an average of 24 out of 26 total weekly samples, and transcript data were successfully obtained for >99% of samples returned. A high degree of correlation between finger stick data and data from a standard 3 mL venipuncture sample was observed. RNA yields obtained from blood volumes ranging from 10, 15, 20, and 25 µL indicated that those volumes were sufficient to generate the 100 ng of RNA needed for downstream high throughput real time-PCR. [Speake C., et al., Clin Exp Immunol 2016]. However, the detailed procedure for finger stick blood collection and RNA stabilization employed in this and other studies has never been published.*

The paragraph above was included in the manuscript (Part of **Introduction**).

This point was also addressed in response to Dr. Cliff’s comment: In Our initial experiment with RNA extraction of 50 uL blood collection protocol in Tempus solution, we obtained the average RNA yield was 1 ug, Purity of RNA; average of RNA integrity number (RIN) was ~7.5 and A260/A280 ratio of ~2.14. Such yields and quality are sufficient for downstream assays; including RNA sequencing, microarray or Fluidigm. These data will be included in our manuscript describing RNA extraction procedures.

Competing Interests: No competing interests were disclosed.