

# **Gene expression profiles are different in venous and capillary blood: implications for vaccine studies**

**<sup>1</sup>Stein DF, <sup>2</sup>O'Connor D\*, <sup>2</sup>Blohmke CJ, <sup>2</sup>Sadarangani M, <sup>2</sup>Pollard AJ**

## Affiliations:

<sup>1</sup>School of Clinical Medicine, University of Cambridge

<sup>2</sup>Oxford Vaccine Group, Department of Paediatrics, University of Oxford, and the NIHR Oxford Biomedical Research Centre, Oxford, United Kingdom.

\*Corresponding Author: Daniel O'Connor, Oxford Vaccine Group, Department of Paediatrics, Centre for Clinical Vaccinology & Tropical Medicine (CCVTM), The Churchill Hospital, Old Road, OX3 7LE, United Kingdom.

Tel/Fax: +44 (0)1865 857420

Email: [daniel.oconnor@paediatrics.ox.ac.uk](mailto:daniel.oconnor@paediatrics.ox.ac.uk)

## Abstract

**Background:** Detailed analysis of the immunological pathways leading to robust vaccine responses has become possible with the application of systems biology, including transcriptomic analysis. Venous blood is usually obtained for such studies but others have obtained capillary blood (e.g. finger-prick). Capillary samples are practically advantageous, especially in children.

**Methods:** The aim of this study was to compare gene expression profiles in venous and capillary blood before, 12 hours and 24 hours after vaccination with 23-valent pneumococcal polysaccharide or trivalent inactivated seasonal influenza vaccines.

**Results:** Gene expression at baseline was markedly different between venous and capillary samples, with 4940 genes differentially expressed, and followed a different pattern of changes after vaccination. At baseline, multiple pathways were upregulated in venous compared to capillary blood, including transforming growth factor-beta receptor signalling and toll-like receptor cascades. After vaccination with the influenza vaccine, there was enrichment for T and NK cell related signatures in capillary blood, and monocyte signatures in venous blood. By contrast, after vaccination with the pneumococcal vaccination, there was enrichment of dendritic cells, monocytes and interferon related signatures in capillary blood, whilst at 24h there was enrichment for T and NK cell related signatures in venous blood.

**Conclusions:** These data show differences between venous and capillary gene expression both at baseline, and post vaccination, which may impact on the conclusions regarding immunological mechanisms drawn from studies using these different sampling methodologies.

**Keywords:** Genomics; Vaccines; Systems Biology; Immunity.

## Introduction

Vaccines are an unrivalled intervention in terms of impact on morbidity and mortality due to infectious disease, but the immunological processes determining protection following vaccination are not fully understood [1]. The use of high-throughput technology allowing different pathways and their interactions to be examined holistically, and subsequent integration and analysis of the data produced has been used to investigate transcriptional changes in response to vaccination in systems biology analyses. Analysis of such changes have identified gene expression signatures that correlated with immunogenicity following yellow fever and influenza vaccination [2-4].

The comprehensive data thus generated provide a novel approach to interrogate the molecular mechanisms underlying host responses to infection and vaccination and provide a new tool in the development of vaccines. One of the most cost-effective methods of analysing changes in the transcriptome is the use of RNA micro-arrays. Transcriptional data provide a snapshot of the genome wide expression profile at a specific point in time allowing conclusions about the molecular host responses to an immunological stimulus to be drawn if an analysis of global gene expression changes is performed before and after vaccination [2]. While useful, this approach produces thousands of data points, which can make extraction of coherent information difficult. Gene set enrichment, where catalogues of annotated sets of genes are used to interrogate the data, has allowed the extraction of biologically meaningful and objective information, leading to advances in understanding of changes in response to vaccination [5].

Protocols for the exploration of transcriptional changes have not been standardised between studies, sample types, timing and processing. Some studies have used whole blood from venepuncture whereas others use PBMCs from the same type of venous sample and these samples differ significantly [1-3, 6]. It has been suggested that whole blood may result in reduced detection sensitivity, and that prolonged handling of PBMC samples is associated with artifactual changes. Capillary blood has been used as a more convenient sample to obtain, particularly in studies of children. This means that studies using different sample types and processing and analysis methods cannot easily be compared. As gene expression data tend to be susceptible to sample

processing and handling, sampling methods may impact on the conclusions drawn from transcriptomic studies, leading to potentially redundant functional experiments. It is thus important to know whether venous and capillary samples produce results that are comparable. However, the difference in gene expression between capillary and venous blood samples has not been previously studied. In the present study we directly compared data derived from samples obtained simultaneously from individuals by venepuncture (venous) and fingerprick (capillary), which are publically available as a resource from the Gene Expression Omnibus (GEO) database, and investigated differential gene expression before and after vaccination, between these methods [7].

## Methods

### Data Source

The raw sample and control data used by Obermoser *et al* were downloaded from the Gene Expression Omnibus (GEO) database (Accession: GSE30101) [7].

### Study design and participants

The study population has been described in detail previously<sup>7</sup>. Briefly, healthy adult volunteers aged 18-64 years received the seasonal influenza vaccine (Fluzone, Sanofi Pasteur), pneumococcal polysaccharide vaccine (Pneumovax23, Merck) or saline control (Table 1). Blood was obtained at various time points, including four time points where both fingerprick (capillary) and venepuncture (venous) samples were taken simultaneously from the same subject (7 days pre-vaccination [-7d], at the time of vaccination [0d], and 12- and 24-hours post-vaccination [12h, 24h]), providing an opportunity to compare transcript expression between the two sample types.

### Table 1:

### Data Processing

Microarray data were background subtracted, and a force positive modification used so that a log<sub>2</sub> transformation could be performed, followed by a robust spline normalisation of the data (R package Lumi) [8]. The data were filtered to only include probes that were detected in greater than 65% of all the samples in the analysis, to exclude inconsistently detected probes ( $p < 0.05$ ; R Package Genefilter).

### Data Analysis: Fold Change

Paired analysis was conducted to compare transcript expression in capillary and venous blood derived from the same individual. The baseline samples (-7d, 0d) from all study groups – saline, influenza and pneumococcal vaccine groups were analysed. The pre- (0d) to post-vaccination fold changes were then tested using a paired t-test between sample types. Comparison of venous against capillary was done using a paired t-test, and pre-, post-vaccination changes were calculated using a linear regression. The changes were compared between venous and capillary with a paired t-test. Only samples with a pair were tested; un-paired samples were not analysed. These tests were fit using the lmFit and eBayes functions (R Package Limma) [9]. A

p-value of  $\leq 0.001$  and an absolute fold-difference between venous and capillary or between pre- and post-vaccination in expression of greater than 1.25 was used to select differentially expressed transcripts. To find total genes differentially expressed at baseline, the total numbers from -7d and 0d were added and replicates removed, whilst transcripts consistently differentially expressed were calculated using genes differentially expressed at both -7d and 0d. To compare numbers of transcripts between venous and capillary samples, a McNemar's test was performed. Bland-Altman plots containing all genes differentially expressed post-vaccination were used to examine whether the transcript expression changes were comparable when measured in venous and capillary blood.

### **Pathway Analysis**

Pathway analysis was performed on differentially expressed transcripts using the publically available analysis tool InnateDB [10]. Pathways were considered differentially expressed if the false-discovery rate was  $< 0.05$ . Two pathway analyses were performed at baseline: an analysis using total genes differentially expressed between venous and capillary blood, and a split analysis using only the genes higher in either venous or capillary blood.

### **Gene Set Enrichment Analysis**

Gene Set Enrichment Analysis (GSEA) was performed using R. Gene lists were ranked by log<sub>2</sub> FC and analysed for enrichment of blood transcriptional modules (BTMs) previously described by Li et al [11, 12]. Nominal p-values for Normalised Enrichment Scores (NES) were calculated using 1000 random gene set iterations and corrected for multiple testing using the Benjamini-Hochberg (BH) method. Gene lists used included the comparisons of capillary against venous at baseline (day 0) and capillary against venous fold change after vaccination.

## Results

### Investigation of Variability in Expression

To examine the variability in expression levels at a global level between venous and capillary samples, numbers of detected probes, mean probe intensity distributions, and distributions of variance were produced (Supplementary figure 1). These showed that while there was a higher number of probes detected on average in venous than capillary samples, variance and mean probe intensity were similar between venous and capillary blood.

### Comparison of Venous and Capillary samples at Baseline

At baseline (-7d and 0d), there were a total of 6641 transcripts with differential expression in capillary compared to venous samples, of which 4940 were consistent between the two baseline time points (See Figure 1A). Expression of 4095 transcripts were higher in venous blood, with 2984 consistent between the two timepoints, whilst 2546 were higher in capillary blood, with 1956 consistent between the two timepoints. Regression analysis from -7d to 0d showed no statistically significant differences, so 0d was subsequently used as baseline for pathway analysis.

Differences at 0d corresponded to 100 pathways that were differentially expressed between venous and capillary blood (Supplementary figure 2A). The majority of the pathways with differential enrichment, between venous and capillary blood, were upregulated in venous blood; only five pathways significantly were enriched in capillary compared blood, including the mRNA splicing and processing, gene expression – which encompasses transcription, mRNA processing and translation – and immune system pathways – which encompasses innate and adaptive immune system along with cytokine signalling (See Figure 1B). Those greater in venous blood include pathways representing the spliceosome, nonsense mediated decay, formation of a pool of free 40S subunits, GTP hydrolysis and joining of 60S subunit, cap-dependent translation initiation, eukaryotic translation, initiation and elongation (See Figure 1C). These pathways are sequentially active in the processing of RNA, post-transcriptional regulation and translation of proteins [13-16]. There were a number of other metabolic pathways differentially enriched, including mitochondrial translational pathways and those involved in organelle biogenesis and maintenance. The immune related pathways included Class I MHC mediated antigen

processing and presentation, antigen processing (ubiquitination and degradation), adaptive immune system – which includes T- and B-cell receptor signalling, major histocompatibility complex function, and other immune related signalling – and toll-like receptor (TLR) 2, 3, 4 and 6 cascades (See Figure 1D). Additionally, in the blood transcriptional module (BTM) analysis, modules involved in generic immune activation and CD4 lymphocyte cell cycle have greater enrichment in venous than capillary (Supplementary figure 2B).

These results were underlined by the different clustering behaviour between the samples when performing principal component analysis (PCA) (Supplementary figure 3), with 37% of the gene expression variance accounted for by the first principal component. This analysis showed some separation by batch on the second principal component (Supplementary figure 4), but one batch was split between venous and capillary, and separated on the first principal component. This showed that the batch effect does not appear to impact on the separation caused by the difference between venous and capillary samples.

See Figure 1

### **Comparison of Venous and Capillary Fold-Change (FC) Post Vaccination**

At 12 hours following influenza vaccination a total of five transcripts from the capillary samples were differentially expressed (DE) when compared with baseline, compared with 76 transcripts in the venous samples when compared with baseline. At 24 hours there were nine transcripts differentially expressed compared with baseline in the capillary samples, and 277 transcripts in the venous samples (Supplementary figure 5 and Supplementary figure 6). For the pneumococcal vaccine samples, at 12 hours a total of 239 transcripts from the capillary samples had a differential expression compared to baseline, compared to 269 transcripts differentially expressed in the venous samples. At 24 hours there were 70 transcripts differentially expressed compared to baseline in the capillary samples, and 280 transcripts in the venous samples (Table 2). A McNemar's test highlighted the disparities in differential gene expression findings in venous, compared with capillary, samples (influenza 12h: p-value =  $7.381 \times 10^{-15}$ , influenza 24h: p-value <  $2.2 \times 10^{-16}$ , pneumococcal 12h: p-value = 0.1389, pneumococcal 24h: p-value <  $2.2 \times 10^{-16}$ ).



**Table 2:**

Correlation plots produced showed the general relationship between venous and capillary expression (Supplementary figure 7). However, Bland-Altman plots of the difference between transcript FC (DE transcripts only) measured in venous and capillary blood compared with the mean FC of these two sampling methods showed a lack of agreement, with an evident relationship between the difference in the FC measures of these methods and their mean (See Figure 2). In the influenza vaccine groups there was a moderate negative trend of differences, proportional to mean of the two measurements. Moreover, 24h after influenza vaccine the average of the differences between the methods was less than zero; meaning for these transcripts, on average, the log FC measured in capillary blood was higher than that measured in venous blood. Conversely, for the pneumococcal vaccine, there was a moderate positive trend of differences, with reference to mean of the two measurements; and for both 12h and 24h after vaccination the average of the differences between the methods was more than zero, indicating the average log FC in venous blood was higher than that measured in capillary blood (See Figure 2).

Multiple different biological pathways were enriched in fold-changes of transcript expression from baseline (Supplementary figure 8). There were a greater number of pathways differentially expressed in the venous than the capillary groups. This was true for both vaccines and at all times after vaccination. There were multiple immune pathways differentially expressed from baseline in the venous groups (more extensively analysed by Obermoser et al.)[7].

See Figure 2

### Comparison of Fold-Changes Between Venous and Capillary samples

Multiple genes had different fold-changes between venous and capillary samples (Table 3). There were multiple blood transcriptional modules (BTMs) differentially enriched between capillary and venous samples (Figure 3). At both 12h and 24h after vaccination with the influenza vaccination, the BTMs enriched in capillary blood represented T-cell activation and NK cell related patterns. In contrast, responses in venous blood were characterized by enrichment in monocyte and generic immune activation modules (Figure 3A and 3C). In contrast, while no BTMs were enriched in venous blood 12hrs following pneumococcal vaccination, capillary samples showed a moderate pattern of DCs, monocytes and interferon related signatures (Figure 3B). The responses were increased 24hrs after pneumococcal vaccination with inflammatory signals, monocytes, cell cycle and interferon responses strongly enriched in capillary blood, where as T and NK cell signatures were enriched in venous blood (Figure 3D).

A pathway over-representation analysis was also performed, using the differentially expressed genes detailed above. At 24h after pneumococcal or influenza vaccinations there were no pathways differentially expressed. At 12h after vaccination, there were multiple pathways enriched by differentially expressed genes. After influenza vaccination, these included multiple immune pathways, including interferon  $\alpha/\beta$  signalling, multiple toll-like receptor (TLR) related pathways including activated TLR3 and TLR4 signalling. Furthermore, multiple pathways related to mRNA transcript expression, including translational and transcriptional pathways, along with non-sense mediated decay pathways, had differential fold changes from baseline between venous and capillary blood (Supplementary figure 8A). Similarly, multiple pathways had differential fold changes following pneumococcal vaccination including immune pathways, cell trafficking and membrane trafficking pathways (Supplementary figure 8B).

Table 3:

See Figure 3

## Discussion

This study demonstrates a clear difference in gene expression between venous and capillary blood samples, both before and after vaccination with influenza and pneumococcal vaccines. Depending on which sample type is utilised, inferences about the molecular mechanisms of immunity may be different, leading to different conclusions regarding pathways to the most robust vaccine response and potentially modifying future vaccine development work. While vaccines are one of the most important public health interventions, we have limited understanding of the molecular mechanisms underpinning the development of vaccine-induced protection. High-throughput technologies enable investigation of the detailed responses to vaccination, yet issues such as standardisation exist, potentially leading to confounded or erroneous conclusions. Based on these analyses, further data directly comparing venous and capillary samples are required to enable future standardisation of sample type, which would help future study design.

At baseline, there were 4940 genes consistently differentially expressed between venous and capillary blood, with a majority of these higher in venous than capillary blood. This corresponds to 94 pathways that are enriched for genes with greater expression in venous blood, including a number of important translation-related, immune and cell trafficking pathways. However, only 2 blood transcriptional modules were differentially enriched between venous and capillary blood, with both modules, one involved in generic immune activation, the other in CD4 cell cycle, enriched in venous blood.

After vaccination, there were a greater number of genes differentially expressed in the venous than the capillary samples, possibly reflecting the difference in sample sizes of these groups. After influenza vaccination, there was enrichment of BTMs involved with T-cell proliferation and activation, and NK cell related modules in capillary blood. In venous blood, there was enrichment of monocyte and generic immune module activation modules. After pneumococcal vaccination, there was enrichment of interferon, dendritic cell, inflammatory and monocyte signatures in capillary blood. After 24h, there was also enrichment of T and NK cell signatures in venous blood. At all time points except 12h after influenza vaccination, interferon activation was enriched in capillary blood.

Similarly, after influenza vaccination, there were a number of interferon related pathways differentially expressed between venous and capillary blood. After

pneumococcal vaccination, the pathways included FcγR dependent phagocytosis, along with multiple pathways involved with cellular response to bacterial infection. Obermoser *et al.*, found that interferon signalling was one of the most important changes in the first 48 hours after vaccination with the trivalent inactivated influenza vaccine, as did Cao *et al.* and Nakaya *et al.*, again with the trivalent inactivated influenza vaccine [3, 7, 17]. Obermoser *et al.* also highlighted modules M5.1 and M4.3 as among those having the greatest enrichment at 1 day after vaccination. M5.1 – involved in T cell activation and signalling – was higher in venous blood at 24h after pneumococcal vaccination, while M4.3 – involved in myeloid cell receptors and transporters – was higher in capillary blood at 24h after pneumococcal vaccination, but higher in venous blood at 24h after influenza vaccination [7]. This is important, as it shows that one of the main differences in gene expression changes from baseline between venous and capillary samples is in a gene set key that has been used to characterise the immunogenicity of the influenza vaccine. Additionally, Obermoser *et al.* noted that one of the key changes after pneumococcal vaccination is in FcγR signalling, which is critical in the development of protective immunity against *Streptococcus pneumoniae*, via opsonophagocytosis [7].

A key question is whether the differences we observed, between venous and capillary blood, reflect real biological differences in gene expression between these blood sources, or whether these findings could be explained by sampling artefact. Physiologically, capillary blood is different from venous blood, with different levels of proteins, enzymatic activity and electrolytes, along with different changes after glucose intake, but this does not fully explain the differences seen [18-21].

Several studies have demonstrated that capillary and venous blood have different white blood cell counts, with capillary blood showing a higher leukocyte count than venous blood [22, 23]. Additionally, there is some evidence that venous and capillary blood are immunologically different, with higher levels of TNF-α in capillary blood at baseline and after stimulation, along with differences in the level of IL-10 [24, 25].

It is also possible that differences could be caused by the physical method of blood extraction leading to different gene expression, resulting in artefactual differences. However, it has been shown that the minimum time between the stress stimulus and mRNA production is greater than 15 minutes, longer than it would take for cell lysis after blood taking. Whilst capillary blood yields less RNA than venous blood, it produces equivalent quality RNA [26, 27].

This study is important in illuminating factors to be taken into account in designing future studies testing the immunogenicity of vaccines. Fingerprick blood samples can be taken more regularly, without a trained professional, and it is straightforward to carry out longitudinal, frequent sampling within the same individual. In contrast, obtaining venous blood requires a trained phlebotomist and can only be taken with limited frequency. In addition, fingerprick (or heelprick) samples are much less problematic in paediatric studies. This means that the findings presented in this paper have an important effect on the design of studies into the immunogenicity of vaccines in the paediatric setting.

There are some limitations to this study. The sample size used was small, reducing the ability to detect differences, and sample group sizes differed within the study. There is, additionally, a lack of corroborating data, and the study was not originally designed to illuminate causal processes, making causation difficult to ascertain.

This is a novel investigation, as no group has previously used this approach to compare venous and capillary blood, and yields results significant both for the basic physiological understanding of differences in gene expression, and for the design of future trials, as we show that capillary and venous blood were not equivalent for the investigation of gene expression changes post vaccination.

However, with comparisons at only four time-points, further investigation is vital. The ease of taking fingerprick samples makes it important that subsequent papers add fingerprick samples to their study protocol and compare them with venous samples to further investigate these differences, specifically pre- and post vaccination at multiple time points.

## **Acknowledgement**

We would like to acknowledge Damien Chaussabel for making the dataset (GSE30101) described in this article publicly available on the Gene Expression Omnibus database, without which this analysis would not have been possible.

359 **Authors' contributions**

360 DFS conducted the analysis presented in this manuscript, with input from CJB, MS,  
361 and DOC. DFS, DOC and AJP contributed to the conception and design of this work.  
362 All authors contributed to the interpretation and drafting of this manuscript.

363

364

365 **Funding statement**

366 AJP is a Jenner Investigator and James Martin senior fellow. The authors received no  
367 financial support or other form of compensation related to the development of the  
368 manuscript.

369

370 **Conflict of interests**

371 The authors report no conflict of interests.

## 372 References

- 373 1. O'Connor D, Pollard AJ: **Characterizing vaccine responses using host**  
374 **genomic and transcriptomic analysis.** *Clin Infect Dis* 2013, **57**:860-869.
- 375 2. Querec T, Akondy R, Lee E, Cao W, Nakaya H, Teuwen D, Pirani A, Gernert  
376 K, Deng J, Marzolf B, et al: **Systems biology approach predicts**  
377 **immunogenicity of the yellow fever vaccine in humans.** *Nat Immunol*  
378 2009, **10**:116-125.
- 379 3. Nakaya HI, Wrammert J, Lee EK, Racioppi L, Marie-Kunze S, Haining WN,  
380 Means AR, Kasturi SP, Khan N, Li GM, et al: **Systems biology of**  
381 **vaccination for seasonal influenza in humans.** *Nat Immunol* 2011,  
382 **12**:786-795.
- 383 4. Nakaya HI, Clutterbuck E, Kazmin D, Wang L, Cortese M, Bosinger SE,  
384 Patel NB, Zak DE, Aderem A, Dong T, et al: **Systems biology of immunity**  
385 **to MF59-adjuvanted versus nonadjuvanted trivalent seasonal**  
386 **influenza vaccines in early childhood.** *Proc Natl Acad Sci U S A* 2016.
- 387 5. Haining WN, Wherry EJ: **Integrating genomic signatures for**  
388 **immunologic discovery.** *Immunity* 2010, **32**:152-161.
- 389 6. Bucasas KL, Franco LM, Shaw CA, Bray MS, Wells JM, Niño D, Arden N,  
390 Quarles JM, Couch RB, Belmont JW: **Early patterns of gene expression**  
391 **correlate with the humoral immune response to influenza**  
392 **vaccination in humans.** *J Infect Dis* 2011, **203**:921-929.
- 393 7. Obermoser G, Presnell S, Domico K, Xu H, Wang Y, Anguiano E, Thompson-  
394 Snipes L, Ranganathan R, Zeitner B, Bjork A, et al: **Systems scale**  
395 **interactive exploration reveals quantitative and qualitative**  
396 **differences in response to influenza and pneumococcal vaccines.**  
397 *Immunity* 2013, **38**:831-844.
- 398 8. Du P, Kibbe WA, Lin SM: **lumi: a pipeline for processing Illumina**  
399 **microarray.** *Bioinformatics* 2008, **24**:1547-1548.
- 400 9. Ritchie ME, Phipson B, Wu D, Hu Y, Law CW, Shi W, Smyth GK: **limma**  
401 **powers differential expression analyses for RNA-sequencing and**  
402 **microarray studies.** *Nucleic Acids Res* 2015, **43**:e47.
- 403 10. Breuer K, Foroushani AK, Laird MR, Chen C, Sribnaia A, Lo R, Winsor GL,  
404 Hancock RE, Brinkman FS, Lynn DJ: **InnateDB: systems biology of**  
405 **innate immunity and beyond--recent updates and continuing**  
406 **curation.** *Nucleic Acids Res* 2013, **41**:D1228-1233.
- 407 11. Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA,  
408 Paulovich A, Pomeroy SL, Golub TR, Lander ES, Mesirov JP: **Gene set**  
409 **enrichment analysis: a knowledge-based approach for interpreting**  
410 **genome-wide expression profiles.** *Proc Natl Acad Sci U S A* 2005,  
411 **102**:15545-15550.
- 412 12. Li S, Rouphael N, Duraisingham S, Romero-Steiner S, Presnell S, Davis C,  
413 Schmidt DS, Johnson SE, Milton A, Rajam G, et al: **Molecular signatures of**  
414 **antibody responses derived from a systems biology study of five**  
415 **human vaccines.** *Nat Immunol* 2013.
- 416 13. Warnasooriya C, Rueda D: **Single-molecule fluorescence-based studies**  
417 **on the dynamics, assembly and catalytic mechanism of the**  
418 **spliceosome.** *Biochem Soc Trans* 2014, **42**:1211-1218.

14. Metze S, Herzog VA, Ruepp MD, Mühlemann O: **Comparison of EJC-enhanced and EJC-independent NMD in human cells reveals two partially redundant degradation pathways.** *RNA* 2013, **19**:1432-1448.
15. Martínez-Salas E, Lozano G, Fernandez-Chamorro J, Francisco-Velilla R, Galan A, Diaz R: **RNA-binding proteins impacting on internal initiation of translation.** *Int J Mol Sci* 2013, **14**:21705-21726.
16. Hinnebusch AG: **The scanning mechanism of eukaryotic translation initiation.** *Annu Rev Biochem* 2014, **83**:779-812.
17. Cao RG, Suarez NM, Obermoser G, Lopez SM, Flano E, Mertz SE, Albrecht RA, García-Sastre A, Mejias A, Xu H, et al: **Differences in antibody responses between trivalent inactivated influenza vaccine and live attenuated influenza vaccine correlate with the kinetics and magnitude of interferon signaling in children.** *J Infect Dis* 2014, **210**:224-233.
18. Kupke IR, Kather B, Zeugner S: **On the composition of capillary and venous blood serum.** *Clin Chim Acta* 1981, **112**:177-185.
19. Wurzinger S, Bratu M, Wonisch W, Wintersteiger R, Halwachs-Baumann G, Porta S: **Interdependency of the oxidizability of lipoproteins and peroxidase activity with base excess, HCO<sub>3</sub>, pH and magnesium in human venous and capillary blood.** *Life Sci* 2006, **78**:1754-1759.
20. Wittbrodt MT, Espinoza S, Millard-Stafford ML: **Biological variation of plasma osmolality obtained with capillary versus venous blood.** *Clin Chem Lab Med* 2015, **53**:1613-1619.
21. Colagiuri S, Sandbaek A, Carstensen B, Christensen J, Glumer C, Lauritzen T, Borch-Johnsen K: **Comparability of venous and capillary glucose measurements in blood.** *Diabet Med* 2003, **20**:953-956.
22. Yang ZW, Yang SH, Chen L, Qu J, Zhu J, Tang Z: **Comparison of blood counts in venous, fingertip and arterial blood and their measurement variation.** *Clin Lab Haematol* 2001, **23**:155-159.
23. Schalk E, Heim MU, Koenigsmann M, Jentsch-Ullrich K: **Use of capillary blood count parameters in adults.** *Vox Sang* 2007, **93**:348-353.
24. Eriksson M, Sartono E, Martins CL, Balé C, Garly ML, Whittle H, Aaby P, Pedersen BK, Yazdanbakhsh M, Erikstrup C, Benn CS: **A comparison of ex vivo cytokine production in venous and capillary blood.** *Clin Exp Immunol* 2007, **150**:469-476.
25. Faulkner SH, Spilsbury KL, Harvey J, Jackson A, Huang J, Platt M, Tok A, Nimmo MA: **The detection and measurement of interleukin-6 in venous and capillary blood samples, and in sweat collected at rest and during exercise.** *Eur J Appl Physiol* 2014, **114**:1207-1216.
26. Arner E, Daub CO, Vitting-Seerup K, Andersson R, Lilje B, Drabløs F, Lennartsson A, Rönnerblad M, Hrydziuszko O, Vitezic M, et al: **Transcribed enhancers lead waves of coordinated transcription in transitioning mammalian cells.** *Science* 2015, **347**:1010-1014.
27. Robison EH, Mondala TS, Williams AR, Head SR, Salomon DR, Kurian SM: **Whole genome transcript profiling from fingerstick blood samples: a comparison and feasibility study.** *BMC Genomics* 2009, **10**:617.



468  
469  
470  
471  
472  
473  
474  
475  
476  
477

## Supplementary

478  
479  
480

See Supplementary figure 1

481

See Supplementary figure 2:

482

See Supplementary figure 3: