

## **HRS Documentation Report**

### ***Flow Cytometry Results from the 2016 Venous Blood Study in the 2016 Health and Retirement Study***

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## Introduction

This document contains information on the flow cytometry data collected as part of the HRS 2016 Venous Blood Study (VBS).

Procedures for collection, assay, and descriptions of other blood-based variables are included in the documentation - *Venous blood collection and assay protocol in the 2016 Health and Retirement Study*. <https://hrs.isr.umich.edu/publications/biblio/9065>. The earlier document provided initial flow cytometry results for about 1,000 cases. This document contains additional information on our approach to flow cytometry and outlines in more detail the subsets characterized as well as descriptive information on flow cytometry for the entire 2016 VBS sample.

Additional detail on the effects of collection, processing, storage of cells and analysis of flow cytometry results are provided in:

Thyagarajan B, Barcelo H, Crimmins E, Weir D, Minnerath S, Vivek S, Faul J. Effect of delayed cell processing and cryopreservation on immunophenotyping in multicenter population studies. *J Immunol Methods*. 2018 Dec; 463:61-70. doi: 10.1016/j.jim.2018.09.007. Epub 2018 Sep 14. PubMed PMID: 30222961.

Barcelo H, Faul J, Crimmins E, Thyagarajan B. A Practical Cryopreservation and Staining Protocol for Immunophenotyping in Population Studies. *Curr Protoc Cytom*. 2018 Apr;84(1):e35. doi: 10.1002/cpcy.35. PubMed PMID: 30040214; PubMed Central PMCID: PMC6060642.

Hunter-Schlichting D, Lane J, Cole B, Flaten Z, Barcelo H, Ramasubramanian R, Cassidy E, Faul J, Crimmins E, Pankratz N, Thyagarajan B. Validation of a hybrid approach to standardize immunophenotyping analysis in large population studies: The Health and Retirement Study. *Scientific Reports* 2020; 10(1): 8759. PMID: 32472068; PMCID: PMC7260195.

## Subsample Weights

*Sample Weights for the 2016 Venous Blood Study – Full sample (PVBSWGTR)*

Respondents with at least one valid venous blood result (VBS16VALID) were assigned a VBS weight. The weights were adjusted for the differential probabilities of participation by dividing the HRS 2016 sample weight by the predicted probability of having a valid venous blood result among community-dwelling 2016 HRS respondents born prior to 1960, excluding all members of the LBB cohort. The resulting interim weight was trimmed at the 1st and 99th percentiles and was then post stratified back to the entire 2016 HRS sample born prior to 1960 by age, sex, and race/ethnicity. Analysts should use *PVBSWGTR* to weight the data included with this release.

## Flow Cytometry Assays

We used multiparameter flow cytometry to obtain counts and percentages for the distributions of 24 different types of T-cells, B-cells, Natural Killer (NK) cells, monocytes, and dendritic cells

(DC). Flow cytometry results are available for approximately 9300 respondents for any given cell subset.

The immune cell subsets were identified using minor modifications to the standardized protocol published by the Human Immunology Project (Maecker HT, McCoy JP, Nussenblatt R. Standardizing immunophenotyping for the Human Immunology Project. Nat Rev Immunol. 2012 Feb 17;12(3):191-200). The modifications include addition of CD95 to more definitively identify different subsets of T cells. One vial of cryopreserved mononuclear cells containing ~4 million cells was thawed and cells were incubated at 37°C in RPMI media for 1 hour. The cells were centrifuged at 1200rpm for 10 min at room temperature. The cells were resuspended in 1X PBS and stained using the two antibody cocktails as outlined in the tables below. The cells were kept on ice until analysis. All flow cytometry measurements were performed on a LSRII or a Fortessa X20 flow cytometer (BD Biosciences, San Diego, CA). (Thyagarajan et al. 2018)

We present data as both % cells and as absolute counts.

% T-cells, % B-cells were defined as % of events in the live lymphocytes gate after removing doublets. Percentages of T and B cell subsets were defined as % of the parent population (e.g.) %CD4+ T cells was defined as a % of total T cells.

Absolute counts of T and B-cells were calculated by multiplying the % cells and the total lymphocyte count (obtained from the complete blood count (CBC)) and expressed as  $10^9/L$ .

% monocytes, %NK cells and % DCs were defined as % peripheral blood mononuclear cells (PBMCs). Percentages of monocytes, NK cells and DC subsets were defined as % of the parent population (e.g.) %CD16+ monocytes was defined as a % of total monocytes.

Absolute counts of monocytes, NK cells and DCs were calculated by multiplying the % cells with the total PBMC count. Total PBMC count was calculated as total [white blood cell count – (neutrophil count+eosinophil count+basophil count)]. The absolute count was expressed as  $10^9/L$ .

| <b>T cells</b>                    | <b>Percent Calculation</b>               |
|-----------------------------------|------------------------------------------|
| CD4+ T cells                      | CD4+ T cells/total T cells*100           |
| CD4+ T cells: Central Memory (CM) | CD4+ CM T cells/total CD4+ T cells*100   |
| CD4+ T cells: (TemRA)             | CD4+ TemRA T cells/total CD4+T cells*100 |

| <b>Panel 1 (T cells and B cells)</b> |        |              |                    |                    |
|--------------------------------------|--------|--------------|--------------------|--------------------|
| Marker                               | Clone  | Fluorochrome | Provider (cat #)   | Volume/sample (µl) |
| brilliant stain buffer               | NA     | NA           | BD (659611)        | 50                 |
| viability dye                        | NA     | FVS 570 (PE) | BD (564995)        | 12                 |
| CD3                                  | UCHT1  | APC          | BD (555335)        | 4                  |
| HLA-DR                               | G46-6  | PE-CF594     | BD (562331)        | 1                  |
| CD19                                 | SJ25C1 | PE-Cy7       | BD (557835)        | 1                  |
| CD27                                 | O323   | FITC         | Biolegend (302806) | 2.5                |
| CD8                                  | RPA-T8 | BUV395       | BD (563796)        | 1                  |
| IgD                                  | IA6-2  | BUV737       | BD (564687)        | 1                  |
| CCR7                                 | G043H7 | BV421        | Biolegend (353208) | 2.5                |
| CD28                                 | CD28.2 | BV510        | Biolegend (302936) | 2.5                |
| CD45RA                               | HI100  | BV711        | Biolegend (304138) | 2.5                |
| CD4                                  | RPA-T4 | APC-Cy7      | BD (557871)        | 1                  |

| <b>Panel 2 (monocytes, DC, NK)</b> |          |              |                  |                     |
|------------------------------------|----------|--------------|------------------|---------------------|
| Marker                             | Clone    | Fluorochrome | Provider (cat #) | Volume/ sample (ul) |
| brilliant stain buffer             | NA       | NA           | BD (659611)      | 50                  |
| viability dye                      | NA       | FVS 570 (PE) | BD (564995)      | 12                  |
| CD3                                | UCHT1    | APC          | BD (555335)      | 4                   |
| HLA-DR                             | G46-6    | PE-CF594     | BD (562331)      | 1                   |
| CD19                               | SJ25C1   | PE-Cy7       | BD (557835)      | 1                   |
| CD11c                              |          | BB515        | BD (564490)      | 1                   |
| CD20                               | 2H7      | BUV395       | BD (563781)      | 1                   |
| CD16                               | 3G8      | BUV737       | BD (564433)      | 1                   |
| CD56                               | NCAM16.2 | BV421        | BD (562751)      | 1                   |
| CD14                               | MOP9     | BV510        | BD (563079)      | 1                   |
| CD123                              | 9F5      | BV711        | BD (563161)      | 1                   |
| CD45                               | 2D1      | APC-Cy7      | BD (560178)      | 0.5                 |

**Table 1: Markers used to determine 24 cell subsets using flow cytometry**

| CELL TYPE                           | MARKERS                                      | Variable Name          |                       |
|-------------------------------------|----------------------------------------------|------------------------|-----------------------|
|                                     |                                              | Percentages            | Counts                |
| <b>T cells</b>                      | CD3+ CD19-                                   | PTcell_pct             | PTcell_count          |
| CD4+ T cells                        | CD3+ CD19- CD8- CD4+                         | PCD4T_pct              | PCD4T_count           |
| CD4+ T cells: Central Memory (CM)   | CD3+ CD19- CD8- CD4+ CD45RA- CCR7+ CD28+     | PCD4M_pct              | PCD4M_count           |
| CD4+ T cells: (TemRA)               | CD3+ CD19- CD8- CD4+ CD45RA+ CCR7- CD28-     | PCD4TEMRA_pct          | PCD4TEMRA_count       |
| CD4+ T cells: Effector memory (Tem) | CD3+ CD19- CD8- CD4+ CD45RA- CCR7- CD28-     | PCD4TEM_pct            | PCD4TEM_count         |
| CD4+ T cells: Naïve                 | CD3+ CD19- CD8+ CD4+ CD45RA+ CCR7+ CD28+     | PCD4N_pct              | PCD4N_count           |
| CD8+ T cells                        | CD3+ CD19- CD8+ CD4-                         | PCD8T_pct              | PCD8T_count           |
| CD8+ T cells: Central Memory (CM)   | CD3+ CD19- CD8+ CD4- CD45RA- CCR7+ CD28+     | PCD8M_pct              | PCD8M_count           |
| CD8+ T cells: (TemRA)               | CD3+ CD19- CD8+ CD4- CD45RA+ CCR7- CD28-     | PCD8TEMRA_pct          | PCD8TEMRA_count       |
| CD8+ T cells: Effector Memory (Tem) | CD3+ CD19- CD8+ CD4- CD45RA- CCR7- CD28-     | PCD8TEM_pct            | PCD8TEM_count         |
| CD8+ T cells: Naïve                 | CD3+ CD19- CD8+ CD4- CD45RA+ CCR7+ CD28+     | PCD8N_pct              | PCD8N_count           |
| <b>B cells</b>                      | CD3- CD19+                                   | PBcell_pct             | PBcell_count          |
| IgD+ memory B cells                 | CD3- CD19+ IgD+ CD27+                        | PIgD_Plus_MemB_pct     | PIgD_Plus_MemB_count  |
| IgD- memory B cells                 | CD3- CD19+ IgD- CD27+                        | PIgD_Minus_MemB_pct    | PIgD_Minus_MemB_count |
| Naïve B cells                       | CD3- CD19+ IgD+ CD27-                        | PNaiveB_pct            | PNaiveB_count         |
| <b>Natural Killer (NK) Cells</b>    | CD3- CD19- CD20- CD14- CD16+ CD56+           | PNK_pct <sup>1</sup>   | PNK_count             |
| NK Cells: CD56HI                    | CD3- CD19- CD20- CD14- CD16+ CD56HI          | PNKHI_pct              | PNKHI_count           |
| NK Cells: CD56LO                    | CD3- CD19- CD20- CD14- CD16+ CD56LO          | PNKLO_pct              | PNKLO_count           |
| <b>Monocytes</b>                    | CD3- CD19- CD20- CD14+                       | PMONO_pct <sup>2</sup> | PMONO_count           |
| CD16- monocytes                     | CD3- CD19- CD20- CD14+ CD16-                 | PMONO16Minus_pct       | PMONO16Minus_count    |
| CD16+ monocytes                     | CD3- CD19- CD20- CD14+ CD16+                 | PMONO16Plus_pct        | PMONO16Plus_count     |
| <b>Dendritic cells</b>              | CD3- CD19- CD20- CD14- HLA-DR+               | PDC_pct <sup>3</sup>   | PDC_count             |
| Myeloid Dendritic cells (DC-M)      | CD3- CD19- CD20- CD14- HLA-DR+ CD11c+ CD123- | PDCm_pct               | PDCm_count            |
| Plasmacytoid Dendritic cells (DC-P) | CD3- CD19- CD20- CD14- HLA-DR+ CD11c- CD123+ | PDCp_pct               | PDCp_count            |

**Table 2. Percentages - Cell Subsets: Descriptive Measures (Unweighted)**

| <b>Variable</b>                         | <b>N</b> | <b>Mean</b> | <b>Std Dev</b> | <b>Minimum</b> | <b>Maximum</b> |
|-----------------------------------------|----------|-------------|----------------|----------------|----------------|
| <b>T cells</b>                          | 9359     | 0.699       | 0.130          | 0.020          | 0.971          |
| CD4+ T cells                            | 9359     | 0.677       | 0.138          | 0.000          | 0.966          |
| CD4+ T cells: Central Memory (CM)       | 9313     | 0.366       | 0.123          | 0.000          | 0.823          |
| CD4+ T cells: TemRA                     | 9313     | 0.040       | 0.066          | 0.000          | 0.815          |
| CD4+ T cells: Tem                       | 6918     | 0.014       | 0.041          | 0.000          | 0.524          |
| CD4+ T cells: Naïve                     | 9313     | 0.430       | 0.178          | 0.000          | 0.947          |
| CD8+ T cells                            | 9359     | 0.248       | 0.119          | 0.014          | 0.929          |
| CD8+ T cells: Central Memory (CM)       | 7482     | 0.086       | 0.058          | 0.000          | 0.633          |
| CD8+ T cells: TemRA                     | 9314     | 0.458       | 0.221          | 0.000          | 0.994          |
| CD8+ T cells: Tem                       | 7476     | 0.021       | 0.044          | 0.000          | 0.553          |
| CD8+ T cells: Naïve                     | 9314     | 0.228       | 0.163          | 0.000          | 0.905          |
| <b>B cells</b>                          | 9359     | 0.071       | 0.055          | 0.000          | 0.959          |
| IgD+ memory B cells                     | 9336     | 0.130       | 0.099          | 0.000          | 0.858          |
| IgD- memory B cells                     | 9336     | 0.118       | 0.095          | 0.000          | 0.980          |
| Naïve B cells                           | 9336     | 0.645       | 0.174          | 0.000          | 0.978          |
| <b>Natural Killer (NK) Cells</b>        | 9092     | 0.035       | 0.024          | 0.000          | 0.338          |
| NK Cells: CD56HI                        | 9309     | 0.009       | 0.017          | 0.000          | 0.420          |
| NK Cells: CD56LO                        | 9309     | 0.929       | 0.080          | 0.002          | 1.000          |
| <b>Monocytes</b>                        | 9092     | 0.030       | 0.022          | 0.000          | 0.280          |
| CD16- monocytes                         | 9306     | 0.951       | 0.087          | 0.049          | 1.000          |
| CD16+ monocytes                         | 9306     | 0.041       | 0.050          | 0.000          | 0.951          |
| %Monocytes (2016 VBS file) <sup>1</sup> | 9597     | 0.085       | 0.024          | 0.000          | 0.435          |
| <b>Dendritic cells</b>                  | 9092     | 0.008       | 0.006          | 0.000          | 0.182          |
| Myeloid Dendritic cells (DC-M)          | 9308     | 0.716       | 0.125          | 0.021          | 0.986          |
| Plasmacytoid Dendritic cells (DC-P)     | 9308     | 0.156       | 0.094          | 0.001          | 0.902          |

<sup>1</sup> Monocyte percent comes from the complete blood count in the 2016 VBS data release (variable name for the monocyte %: pmono).

**Table 3. Absolute counts - Cell Subsets: Descriptive Measures (Unweighted)**

| <b>Variable</b>                                          | <b>N</b> | <b>Mean</b> | <b>Std Dev</b> | <b>Minimum</b> | <b>Maximum</b> |
|----------------------------------------------------------|----------|-------------|----------------|----------------|----------------|
| <b>T cells</b>                                           | 9141     | 1.405       | 0.700          | 0.022          | 30.484         |
| CD4+ T cells                                             | 9141     | 0.947       | 0.473          | 0.000          | 10.508         |
| CD4+ T cells: Central Memory (CM)                        | 9095     | 0.339       | 0.197          | 0.000          | 4.505          |
| CD4+ T-cells: TemRA                                      | 9095     | 0.036       | 0.076          | 0.000          | 2.063          |
| CD4+ T cells: Tem                                        | 6760     | 0.012       | 0.045          | 0.000          | 1.201          |
| CD4+ T cells: Naïve                                      | 9095     | 0.427       | 0.314          | 0.000          | 3.595          |
| CD8+ T cells                                             | 9141     | 0.355       | 0.313          | 0.008          | 16.839         |
| CD8+ T cells: Central Memory (CM)                        | 7309     | 0.028       | 0.029          | 0.000          | 0.644          |
| CD8+ T cells: TemRA                                      | 9096     | 0.181       | 0.254          | 0.000          | 15.584         |
| CD8+ T cells: Tem                                        | 7305     | 0.009       | 0.027          | 0.000          | 0.738          |
| CD8+ T cells: Naïve                                      | 9096     | 0.070       | 0.068          | 0.000          | 1.037          |
| <b>B cells</b>                                           | 9141     | 0.194       | 2.491          | 0.000          | 192.806        |
| IgD+ memory B cells                                      | 9118     | 0.021       | 0.166          | 0.000          | 9.385          |
| IgD- memory B cells                                      | 9118     | 0.047       | 2.095          | 0.000          | 188.988        |
| Naïve B cells                                            | 9118     | 0.096       | 0.160          | 0.000          | 8.573          |
| <b>Natural Killer (NK) Cells</b>                         | 9092     | 0.226       | 0.167          | 0.000          | 3.078          |
| NK Cells: CD56HI                                         | 9091     | 0.002       | 0.003          | 0.000          | 0.078          |
| NK Cells: CD56LO                                         | 9091     | 0.212       | 0.160          | 0.000          | 2.593          |
| <b>Monocytes Flow Data</b>                               | 9092     | 0.199       | 0.159          | 0.001          | 1.898          |
| CD16- monocytes                                          | 9088     | 0.188       | 0.151          | 0.001          | 1.530          |
| CD16+ monocytes                                          | 9088     | 0.008       | 0.020          | 0.000          | 0.805          |
| Monocyte Count (2016 VBS file) <sup>1</sup>              | 9597     | 0.562       | 0.224          | 0.000          | 8.500          |
| CD16- monocytes based on VBS absolute count <sup>2</sup> | 9092     | 0.535       | 0.219          | 0.000          | 8.067          |
| CD16+ monocytes based on VBS absolute count <sup>2</sup> | 9092     | 0.023       | 0.031          | 0.000          | 0.653          |
| <b>Dendritic cells</b>                                   | 9092     | 0.051       | 0.083          | 0.000          | 6.846          |
| Myeloid Dendritic cells (DC-M)                           | 9090     | 0.037       | 0.039          | 0.000          | 2.074          |
| Plasmacytoid Dendritic cells (DC-P)                      | 9090     | 0.007       | 0.047          | 0.000          | 4.402          |

<sup>1</sup>For monocytes, we added 3 variables to this table - monocyte count, CD16-, and CD16+ - from the complete blood count data in the 2016 VBS data release (variable name for the monocyte count: pamon).

<sup>2</sup>To calculate the counts of CD16+ and CD16- monocytes for the CBC data we use the following formulas:

CD16+ absolute count = % CD16+\*absolute count of monocytes

CD16- absolute count = % CD16-\*absolute count of monocytes

## QC results

We provide results of the QC analysis done on about 200 blinded duplicates. Individual subsets do not show significant differences. We believe this indicates that our study procedures worked well and did not produce systematic bias. Large CVs between blinded duplicate samples were observed for some cell subsets that are present in very low proportions in peripheral blood. These are indicated with a \*.

## Percentages

|                                     | QC Pairs |      |                 |            |        |       |
|-------------------------------------|----------|------|-----------------|------------|--------|-------|
|                                     | N        | Mean | Within SD (Lab) | Between SD | Reliab | CV    |
| <b>T cells</b>                      | 194      | 0.73 | 0.10            | 0.10       | 0.89   | 3.53  |
| CD4+ T cells                        | 194      | 0.68 | 0.11            | 0.11       | 0.96   | 2.52  |
| CD4+ T cells: Central Memory (CM)   | 194      | 0.31 | 0.11            | 0.11       | 0.78   | 8.01  |
| CD4+ T-cells: TemRA*                | 194      | 0.02 | 0.04            | 0.04       | 0.92   | 34.64 |
| CD4+ T cells: Tem*                  | 114      | 0.01 | 0.02            | 0.02       | 0.98   | 31.18 |
| CD4+ T cells: Naïve                 | 194      | 0.51 | 0.18            | 0.18       | 0.86   | 6.60  |
| CD8+ T cells                        | 194      | 0.25 | 0.09            | 0.09       | 0.97   | 5.41  |
| CD8+ T cells: Central Memory (CM)   | 130      | 0.07 | 0.04            | 0.04       | 0.91   | 12.83 |
| CD8+ T cells: TemRA                 | 194      | 0.02 | 0.04            | 0.04       | 0.95   | 13.77 |
| CD8+ T cells: Tem*                  | 130      | 0.01 | 0.02            | 0.01       | 0.19   | 43.71 |
| CD8+ T cells: Naïve                 | 194      | 0.40 | 0.20            | 0.20       | 0.97   | 8.44  |
| <b>B cells*</b>                     | 194      | 0.07 | 0.04            | 0.04       | 0.88   | 15.89 |
| IgD+ memory B cells*                | 194      | 0.15 | 0.09            | 0.09       | 0.82   | 16.39 |
| IgD- memory B cells*                | 194      | 0.12 | 0.08            | 0.08       | 0.73   | 21.28 |
| Naive B cells                       | 194      | 0.63 | 0.16            | 0.16       | 0.90   | 6.50  |
| Natural Killer (NK) Cells           | 202      | 0.08 | 0.04            | 0.04       | 0.90   | 12.98 |
| NK Cells: CD56HI*                   | 202      | 0.01 | 0.01            | 0.01       | 0.66   | 32.94 |
| NK Cells: CD56LO                    | 202      | 0.96 | 0.04            | 0.04       | 0.67   | 1.32  |
| Monocytes*                          | 202      | 0.06 | 0.07            | 0.07       | 0.87   | 29.67 |
| CD16- monocytes                     | 202      | 0.96 | 0.04            | 0.04       | 0.45   | 1.58  |
| CD16+ monocytes*                    | 202      | 0.04 | 0.04            | 0.04       | 0.46   | 32.59 |
| Monocytes (2016 VBS file)           | 190      | 7.58 | 1.7             | 1.7        | 0.91   | 5.4   |
| Dendritic cells*                    | 202      | 0.01 | 0.01            | 0.01       | 0.66   | 23.63 |
| Myeloid Dendritic cells (DC-M)*     | 202      | 0.64 | 0.12            | 0.12       | 0.86   | 5.16  |
| Plasmacytoid Dendritic cells (DC-P) | 202      | 0.25 | 0.12            | 0.12       | 0.91   | 13.18 |

\* Large CVs (>15%) between blinded duplicate samples.

## Absolute counts

|                                                           | QC Pairs |      |                 |            |        |       |
|-----------------------------------------------------------|----------|------|-----------------|------------|--------|-------|
|                                                           | N        | Mean | Within SD (Lab) | Between SD | Reliab | CV    |
| <b>T cells</b>                                            | 180      | 1.35 | 0.49            | 0.50       | 0.9695 | 5.02  |
| CD4+ T cells                                              | 181      | 0.92 | 0.38            | 0.38       | 0.9676 | 5.86  |
| CD4+ T cells: Central Memory (CM)                         | 180      | 0.28 | 0.15            | 0.15       | 0.8760 | 8.47  |
| CD8+ T cells: TemRA                                       | 180      | 0.10 | 0.10            | 0.10       | 0.9648 | 13.64 |
| CD4+ T cells: Tem*                                        | 110      | 0.00 | 0.01            | 0.01       | 0.9634 | 28.13 |
| CD4+ T cells: Naïve                                       | 180      | 0.48 | 0.29            | 0.30       | 0.9232 | 10.64 |
| CD8+ T cells                                              | 180      | 0.34 | 0.17            | 0.17       | 0.9639 | 8.04  |
| CD8+ T cells: Central Memory (CM)*                        | 126      | 0.02 | 0.01            | 0.01       | 0.9098 | 15.73 |
| CD8+ T cells: TemRA                                       | 180      | 0.10 | 0.10            | 0.10       | 0.9648 | 13.64 |
| CD8+ T cells: Tem*                                        | 126      | 0.00 | 0.00            | 0.00       | 0.3533 | 44.54 |
| CD8+ T cells: Naïve                                       | 180      | 0.14 | 0.12            | 0.12       | 0.9640 | 10.75 |
| <b>B cells*</b>                                           | 181      | 0.13 | 0.10            | 0.10       | 0.9260 | 16.36 |
| IgD+ memory B cells *                                     | 180      | 0.02 | 0.01            | 0.01       | 0.7855 | 20.18 |
| IgD- memory B cells *                                     | 180      | 0.01 | 0.01            | 0.01       | 0.8163 | 24.29 |
| Naïve B cells*                                            | 180      | 0.09 | 0.08            | 0.08       | 0.9376 | 19.14 |
| Natural Killer (NK) Cells                                 | 188      | 0.18 | 0.10            | 0.10       | 0.9078 | 13.63 |
| NK Cells: CD56HI *                                        | 188      | 0    | 0               | 0          | 0.7422 | 34.45 |
| NK Cells: CD56LO                                          | 188      | 0.17 | 0.10            | 0.10       | 0.9121 | 13.63 |
| Monocytes*                                                | 188      | 0.12 | 0.09            | 0.09       | 0.5933 | 30.28 |
| CD16- monocytes*                                          | 188      | 0.12 | 0.09            | 0.09       | 0.6048 | 30.14 |
| CD16+ monocytes *                                         | 188      | 0    | 0.01            | 0.01       | 0.3444 | 46.89 |
| Monocytes (2016 VBS file) <sup>1</sup>                    | 190      | 0.47 | 0.14            | 0.14       | 0.9045 | 6.2   |
| CD16- monocytes based on VBS absolute count <sup>2</sup>  | 188      | 0.46 | 0.14            | 0.14       | 0.8977 | 7.20  |
| CD16+ monocytes based on VBS absolute count <sup>2*</sup> | 188      | 0.02 | 0.02            | 0.02       | 0.4637 | 34.30 |
| Dendritic cells *                                         | 188      | 0.03 | 0.02            | 0.02       | 0.5684 | 24.16 |
| Myeloid Dendritic cells (DC-M) *                          | 188      | 0.02 | 0.01            | 0.01       | 0.6420 | 24.52 |
| Plasmacytoid Dendritic cells (DC-P)*                      | 188      | 0.01 | 0               | 0          | 0.6274 | 28.37 |

\* Large CVs (>15%) between blinded duplicate samples were observed for some cell subsets that are present in very low proportions in peripheral blood.

<sup>1</sup>For monocytes, we added 3 variables to this table - monocyte count, CD16-, and CD16+ - from the complete blood count data in the 2016 VBS data release (variable name for the monocyte count: pamon).

<sup>2</sup>To calculate the counts of CD16+ and CD16- monocytes for the CBC data we use the following formulas:

CD16+ absolute count = % CD16+\*absolute count of monocytes

CD16- absolute count = % CD16-\*absolute count of monocytes

## **Citing this Document**

Please include the following citation in any research reports, papers, or publications based on these data:

**In text:** "The HRS (Health and Retirement Study) is sponsored by the National Institute on Aging (NIA U01AG009740) and is conducted by the University of Michigan."

**In references:** " Thyagarajan B, Crimmins E, Faul J, Weir D. Flow Cytometry Results from the 2016 Venous Blood Study in the 2016 Health and Retirement Study. Ann Arbor, MI: Survey Research Center, Institute for Social Research, University of Michigan; 2021."