

**Immunology & Immunogenetics**

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03/03/2026

Dear Immunology Service Users,

**Lymphocyte subsets absolute counts: notification of change in method & instrument**

**What is changing?** The Immunology & Immunogenetics laboratory at North Bristol Trust is implementing a new method for counting lymphocyte subsets (T, B and NK lymphocyte populations) in the peripheral blood. Lymphocyte subsets are typically used for investigating and monitoring patients with suspected or confirmed primary and secondary immunodeficiencies; this includes monitoring of patients with retroviral disease (CD4 counts), B cell depletion therapies (e.g., Rituximab) and post-stem cell transplant monitoring.

The laboratory will be switching from the current method and instrument (Becton Dickinson Multitest /TruCount method using the FACSLyric flow cytometer) to the **Beckman Coulter Life Sciences Tetra reagents using the Aquios CL flow cytometer**.

The change in assay system is part of the Beckman Coulter Managed Equipment Service (MES) now being implemented across the three NHS organisations in Bristol and Bath and should increase our testing capacity whilst providing efficiency savings within the laboratory.

**Will I notice any differences to my results?** Lymphocyte subsets will be reported in the same units (% and  $\times 10^9$  cells/L). The age-dependent reference ranges will remain the same and can be found using the following link:  
<https://www.nbt.nhs.uk/sites/default/files/Immunology%20Age%20related%20reference%20ranges%20Jan%202015.pdf>

In addition to the subsets currently reported (CD3+ T cells, CD4+ T helper cells, CD8+ T cytotoxic cells, CD19+ B cells & CD16+/CD56+ NK cells) we will also now be reporting a **CD4/CD8 ratio**. The ranges for the CD4/CD8 ratio parameter are listed in the appendix at the end of this communication.

**Bias:** Method comparison indicates that the new method generates lower subset cell counts (negative bias) compared to the current methods. Negative bias was not statistically significant and/or of a low magnitude ( $\leq \sim 15\%$ ) at lower counts (Lymph count  $\leq 1.2$ , CD3+ T count  $\leq 1.0$ , CD4+ T count  $< 0.6$ , CD8+ T count  $< 0.4$ , CD19+ B count  $< 0.3$ , CD56+ NK count  $< 0.2$ ). At subset counts above these limits a more pronounced negative bias can be observed.

The negative bias on the new method is significantly more pronounced at higher cell counts and should not impact the investigation or monitoring of suspected or confirmed immunodeficiency. The clinical impact of this bias should be limited,



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as illustrated by the outcome of the qualitative comparison which demonstrated overall 88% concordance between methods (83% - 92%, dependent on subset examined).

We would recommend that any patients having lymphocyte subsets count monitoring have baseline levels determined using the new method after the date below.

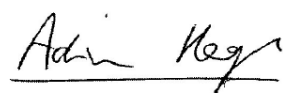
During the switchover, please consider that any reductions in lymphocyte subset counts at the middle and top of the measurement range could be the consequence of the change in method and may not be a consequence of genuine physiological changes.

**When is the change occurring?** The new lymphocyte subsets method will go live on Monday **30<sup>th</sup> March 2026**.

We will be sending a separate communication concerning blood sample stability and laboratory acceptance criteria.

Please feel free to contact me if you wish to discuss this matter further.

Kind regards



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## Appendix

### CD4 / CD8 ratio reference intervals:

| Parameter                                   | Neonatal  | 1 wk - 2 month | 2 - 5 month | 5 - 9 month | 9 - 15 month | 15 - 24 month | 2 - 5 yr  | 5 - 10 yr | 10 - 16 yr | Adults    |
|---|-----------|----------------|-------------|-------------|--------------|---------------|-----------|-----------|------------|-----------|
| CD4 / CD8 ratio (median)                    | 1.8       | 3.8            | 2.7         | 2.5         | 2.4          | 1.9           | 1.6       | 1.2       | 1.7        | 1.9       |
| CD4 / CD8 ratio (range 5th - 95th centiles) | 1.0 - 2.6 | 1.3 - 6.3      | 1.7 - 3.9   | 1.6 - 3.8   | 1.3 - 3.9    | 0.9 - 3.7     | 0.9 - 2.9 | 0.9 - 2.9 | 0.9 - 3.4  | 1.0 - 3.6 |

**Reference:** Comans-Bitter WM, de Groot R, van den Beemd R, Neijens HJ, Hop WC, Groeneveld K, Hooijkaas H, van Dongen JJ. Immunophenotyping of blood lymphocytes in childhood. Reference values for lymphocyte subpopulations. *J Pediatr.* 1997 Mar;130(3):388-93.



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