COLLEGE of AMERICAN PATHOLOGISTS

**Educational Discussion: 2025-B Accuracy-Based Lipids**

## Variability in Direct HDL Methods Across Platforms

High-density lipoprotein cholesterol (HDL-C) remains a key component of cardiovascular risk assessment and is integral to clinical decision-making, including calculation of non-HDL cholesterol and use in cardiometabolic risk algorithms. While most clinical laboratories employ homogeneous (direct) HDL-C assays, method-to-method variability relative to the CDC reference measurement procedure (RMP) persists and may impact accuracy-based grading.

This participant summary evaluates observed bias across commonly used direct HDL-C assays—specifically Abbott, Beckman Coulter, and Roche platforms—relative to the CDC HDL-C reference method, which uses ultracentrifugation with heparin-manganese precipitation. Across recent accuracy-based program challenges:

- Both the Abbott Ultra HDL assay and the Beckman Coulter AU Direct HDL assays demonstrated close agreement with the CDC RMP in the HDL-C range of 30-53 mg/dL.
- The Roche HDL-C Gen.4 (HDLC4) assay, despite switching to a new reagent (Gen 4), consistently exhibited a negative bias, approximately 6% lower on average compared to the CDC RMP, even within the same clinically relevant concentration range. This systematic negative bias with the Roche HDLC4 method was reproducible across challenges and appears method-specific rather than sample-specific.

Key differences in analytical design to separate HDL likely contribute to the observed differences in HDL-C measurements. All three assays are classified as two-step homogeneous (direct) HDL-C methods that measure HDL by cholesterol esterase and oxidase enzymatic activity. However, in the initial step they utilize different approaches to sequester non-HDL apoB-containing lipoproteins (VLDL, LDL, chylomicrons). The Abbott Ultra HDL (Architect/Alinity) employs polyanions (such as dextran sulfate) and nonionic detergents for selective masking of non-HDL cholesterol. The Beckman Coulter AU Direct HDL assay utilizes cyclodextrins and polyanions to selectively inhibit non-HDL lipoproteins. The Roche HDL-C Gen.4 (HDLC4) assay uses nonionic detergents and monoclonal antibodies directed against apoB, the main apolipoprotein on LDL, VLDL, and chylomicrons, for selective inhibition. Potential contributors to the Roche bias include differences in the detergent formulation and immunochemical masking, which may affect the measurement of certain HDL subfractions and possibly calibration slope differences relative to the RMP.

Despite acceptable precision, why is such a small negative bias in HDL important? It may lead to systematic underestimation of HDL-C, particularly near clinical decision thresholds. It can influence non-HDL cholesterol calculations and cardiovascular risk stratification.

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