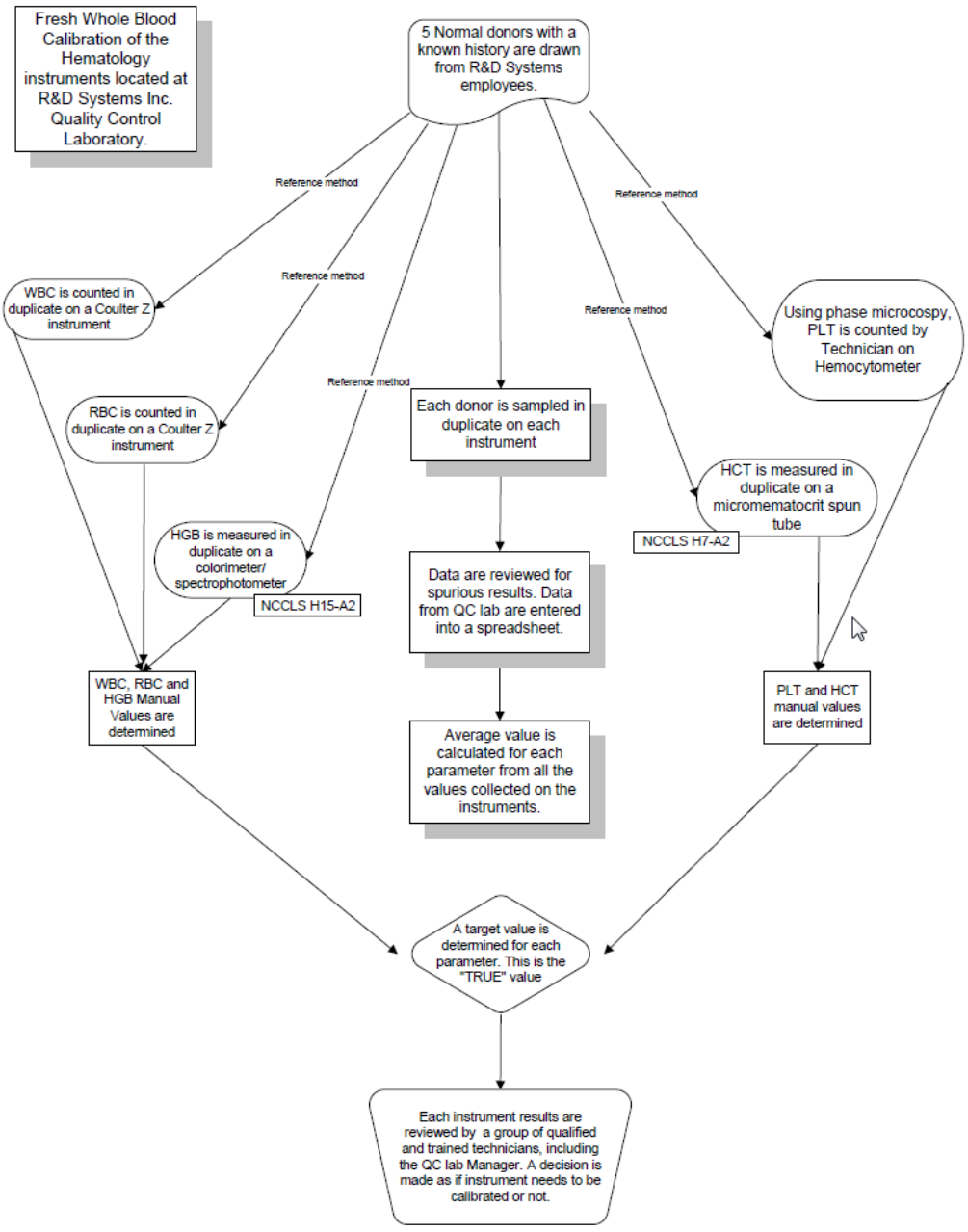
DECLARATION of TRACEABILITY and UNCERTAINTY
HORIBA Medical calibrator

The purpose of this document is to describe the metrological traceability of values assigned to HORIBA Medical calibrator: **ABX Minocal** Ref 2032002 and to estimate the calibrator assigned value uncertainty component.

Assignment of Reference Values to Fresh Whole Blood



Hematology Reference Methods

Hematology analyzers in R&D Systems' Quality Assurance Laboratory are whole blood calibrated to values obtained using these standard reference methods. Whole blood samples drawn from normal, healthy donors are collected in EDTA anticoagulant and analyzed within six hours of collection.

WBC: A 1:500 dilution is prepared using a 200 mL Class A volumetric flask filled with isotonic diluent. 2.4 mL of diluent is removed. Sample is added to the flask using a 400 µL T.C. micropipet, followed by 2.0 mL lysing agent. Counting is performed on a Coulter Counter Z series instrument. All counts are corrected for coincidence.

RBC: A 1:50,000 dilution is prepared using a 1000 mL Class A volumetric flask filled with isotonic diluent. Sample is added to the flask using a 20 µL T.C. micropipet. Counting is performed on a Coulter Counter Z series instrument. All counts are corrected for coincidence.

HGB: A 1:251 dilution is prepared using a 100 mL Class A volumetric flask filled with the NCCLS recommended reagent for the hemoglobincyanide (cyanmethemoglobin) method (1). Sample is added to the flask using a 400 µL T.C. micropipet. The sample is filtered with a 0.2 µm filter immediately before reading. Readings are made at 540 nm in a colorimeter/spectrophotometer calibrated according to NCCLS H15-A3 and ICSH recommendations (1).

HCT: Plain glass microhematocrit tubes (not coated with anticoagulant) are filled with sample, sealed with sealing putty and centrifuged for 5 minutes in a microhematocrit centrifuge according to the NCCLS H7-A3 document (2). After centrifugation, the length of the whole column including the plasma, and the length of the red blood cell column, are viewed and measured using a microscope with graduated stage and an ocular micrometer. The hematocrit (packed cell volume) is calculated as the ratio of the two measurements. No correction is made for trapped plasma.

MCV: On some instruments MCV is the calibrated parameter instead of the HCT. The MCV is calculated from the HCT and RBC using the formula: **MCV = HCT × 10/RBC**

PLT: A 1:126 dilution is prepared using a 50 mL Class A volumetric flask filled with filtered 1% ammonium oxalate. Sample is added to the flask using a 400 µL T.C. micropipet. The dilution is plated onto a clean, dry Neubauer ruled phase type hemocytometer. The hemocytometer is left for 10 minutes in a humidified chamber. Using phase contrast optics, the platelets in the entire central square millimeter on both sides of the hemocytometer are counted. The two counts are averaged and multiplied by 1260 (dilution factor 126 × volume factor 10 = 1260).

BIBLIOGRAPHY

1. National Committee for Clinical Laboratory Standards. Reference and Selected Procedures for the Quantitative Determination of Hemoglobin in Blood: Approved Standard-Third Edition. NCCLS document H15-A3. Wayne, PA: NCCLS, 2000.
2. National Committee for Clinical Laboratory Standards. Procedure for Determining Packed Cell Volume by the Microhematocrit Method: Approved Standard, NCCLS document H7-A3. NCCLS, Wayne, PA: NCCLS, 2001.

Determination of uncertainty (calibrator component)

The uncertainty associated with the calibration of the HORIBA Medical analyzer with the **ABX Minocal** calibrator has been estimated by adding the following sources of uncertainty:

-Uncertainty of the equipment used to determine the reference values: flask, pipette, single aperture impedance counter (WBC, RBC), hemocytometer by phase-contrast (PLT), spectrophotometer (HGB) and hematocrit measurement (ruler).

Uncertainty as an absolute value:

Parameter	Uncertainty
WBC (G/L	**0.09**
RBC (T/L)	**0.03**
HGB (g / dL)	**0.06**
HCT (%)	**0.45**
PLT (G/L)	**5.4**

Determination of total uncertainty

Total uncertainty is defined as the amount of error associated with reported patient results by the HORIBA Medical hematology analyzers to reference methods when the analyzers are calibrated using the **ABX Minocal** calibrator.
Three elements contribute to total uncertainty:

- the calibration system (working calibrators, primary and secondary calibrators, reference measurement procedures…)
- the procedure (reagents, instruments, laboratory staff …)
- the sample

The overall expression of uncertainty is therefore:

$$u_{result} = \sqrt{u_{cal}^2 + u_{method}^2 + u_{sample}^2 + u_{other}^2}$$