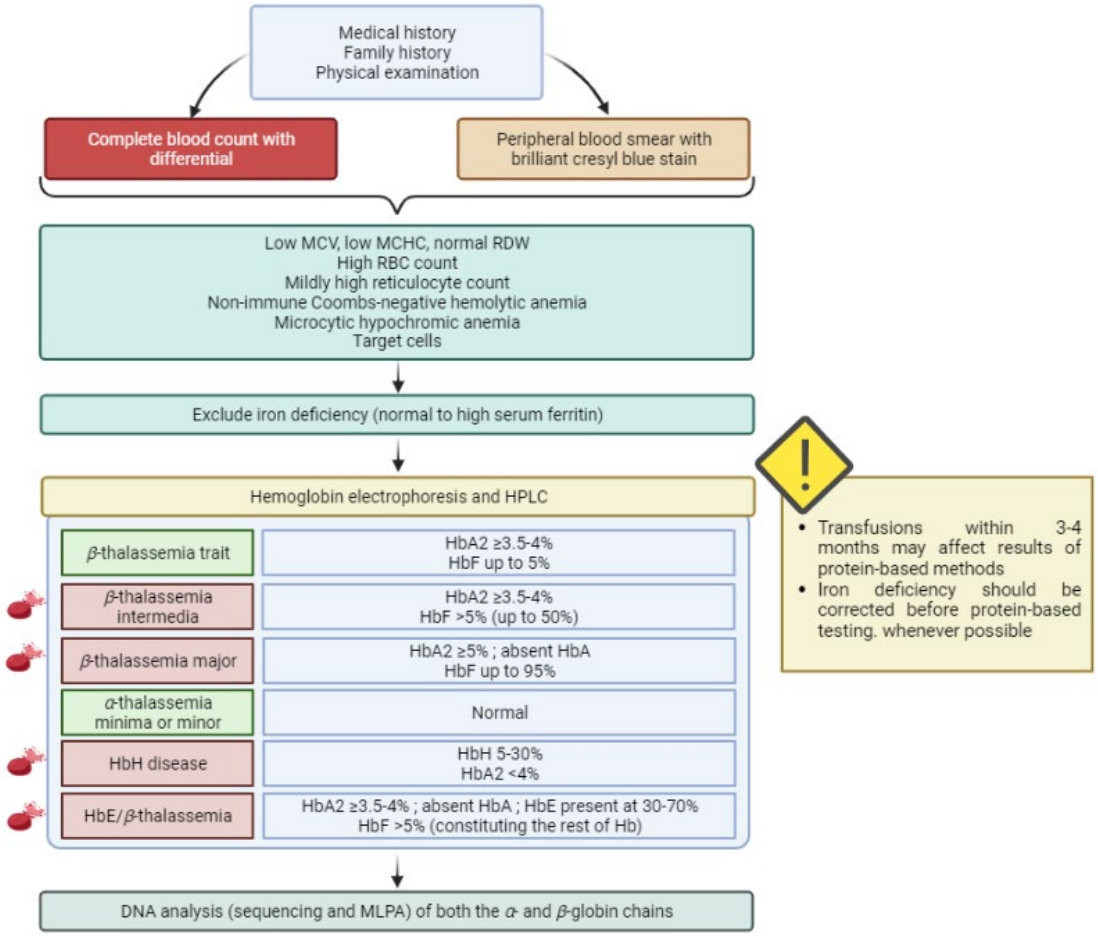

Supplementary Figure 1. Diagnostic testing workflow for thalassemia. Individuals suspected to have thalassemia, based on medical history, physical examination, or family history, should undergo testing with a complete blood count and peripheral blood smear. Some of the thalassemic conditions or traits (*β*-thalassemia minor and *α*-thalassemia minima or minor) are not associated with significant hemolysis while others are associated with non-immune hemolytic anemia. The common finding is microcytosis with varying degrees of anemia. Thalassemias presenting at birth are more likely to be *α*-thalassemias. *β*-thalassemias typically present later during the first 6 months of life as the concentrations of HbF decrease. After the neonatal period, the diagnosis of *α*-thalassemia minor or minima can only be made by DNA testing, which is generally a requirement to establish a diagnosis of HbH disease or less common forms of *β*-thalassemia. In individuals with *α*-thalassemia, protein studies, such as hemoglobin electrophoresis or HPLC, can show HbH or Hb Barts – typically in the neonatal period only. Protein studies are affected by blood transfusion, whereas DNA-based testing is not. Even for *β*-thalassemia where a diagnosis is possible based on hemoglobin electrophoresis and HPLC, we recommend performing full *β*-globin DNA analysis to ascertain the specific mutation. Carrying a mutation in *β*-globin does not preclude the possibility of an alteration in *α*-globin. Generally, in *β*-thalassemia, the more pronounced the degree of imbalance between *α*-globin and *β*-globin chains the more severe the anemia, the higher the need for blood transfusion, and the more serious the clinical morbidities. Lysing red blood cells indicate conditions where there is appreciable hemolysis, clinically or based on laboratory testing. HbF, fetal hemoglobin; MCV, mean

corpuscular volume; MCHC, mean corpuscular hemoglobin concentration; RDW, red cell distribution width; RBC, red blood cell; HPLC, high-performance liquid chromatography; MLPA, multiplex ligation-dependent probe amplification.