Supplementary Information

Paper-based microchip electrophoresis for point-of-care hemoglobin testing

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Video S1. Real-time tracking of hemoglobin bands in HemeChip during electrophoresis process

Video S2. Step-by-step HemeChip test procedure and analysis of results

4. Materials and supplies used in HemeChip

HemeChip was originally designed and developed using a lamination approach, with Poly (methyl methacrylate) (PMMA) sheets from McMaster-Carr (Elmhurst, IL), and ePlastics (San Diego, CA) that were later laser-cut (VersaLASER VLS2.30) and laminated with 3M optically clear double-sided adhesive (DSA) purchased from iTapeStore (Scotch Plains, NJ). The injection molded HemeChip was developed in collaboration with Thogus Products (Avon Lake, OH). The Poly (methyl methacrylate) (PMMA) resin (Optix CA-41 FDA) for injection molding was procured from Plaskolite Inc. (Columbus, OH). The blotter pads were purchased from Helena Laboratories, Inc. (Beaumont, Texas). 1x Tris/Borate/EDTA (TBE) buffer solution (pH 8.3) was made from 10x TBE Buffer solution (Invitrogen™, Carlsbad, CA), diluted with deionized (DI) water (MilliQ Academic, Billerica, MA). Ultrapure DNase/RNase-free water was purchased from Thermofisher Scientific (Waltham, MA). Ultrapure grade Xylene Cyanol and the plastic-backed cellulose acetate sheets were purchased from VWR International LLC (Radnor, PA). Individually wrapped, sterile, stainless steel, disposable lancets were purchased from Med-Tex (Philadelphia, PA). The USB camera (ELP-USB500W02M) was purchased from eplcctv (Guangdong, China). The HemeChip Reader was developed in collaboration with Hemex Health (Portland, OR). The 3D CAD designs of the components developed for HemeChip were created using SolidWorks 3D CAD (Waltham, MA). Designs for the laser-machined component were created using CorelDRAW Suite X6 (Corel Corporation, Ottawa, Ontario).

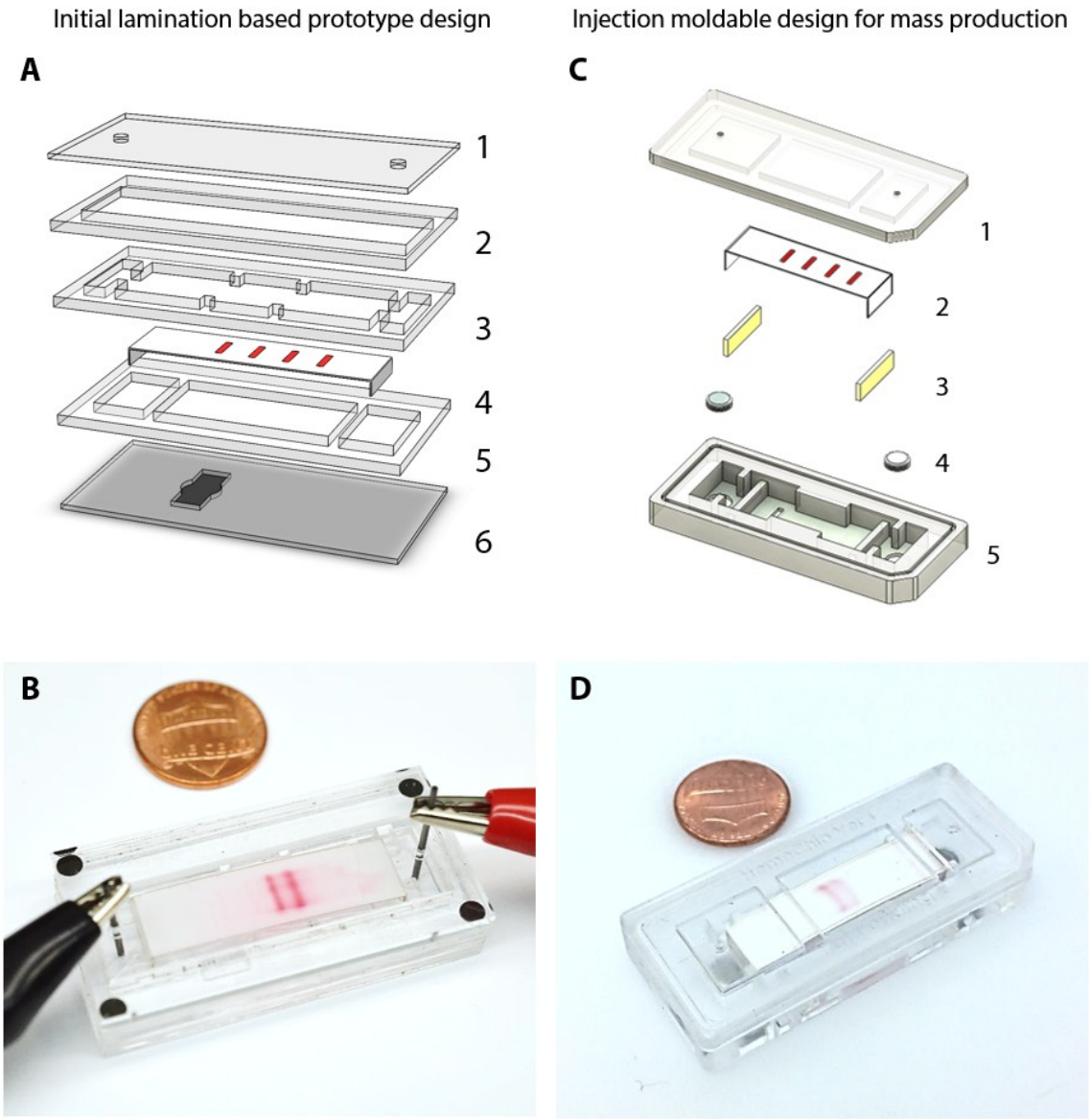

Fig. S1. Transforming HemeChip design for injection molded mass production.

(A) The first proof-of-concept design of the HemeChip utilized a lamination-based fabrication approach without integrated electrodes. This proof-of-concept prototype was made by laser micromachining and stacking five layers of PMMA sheets (1-3, 5&6) encompassing the cellulose acetate paper (4). **(B)** This lamination-based design helped us establish the proof-of-concept using manually inserted graphite electrodes (pencil leads) and an external power source. Separated hemoglobin bands are visible at the end of a proof-of-concept experiment. **(C)** Transformed mass-producible design of HemeChip includes a top (1) and bottom (5) injection molded Optix® CA-41 Polymethyl Methacrylate Acrylic (PMMA) parts, encompassing the cellulose acetate paper (2), blotting pads (3), and integrated stainless steel 316 electrodes (4). **(D)** An injection molded, assembled, and ultrasonic welded HemeChip cartridge at the end of a test run with visible hemoglobin bands.

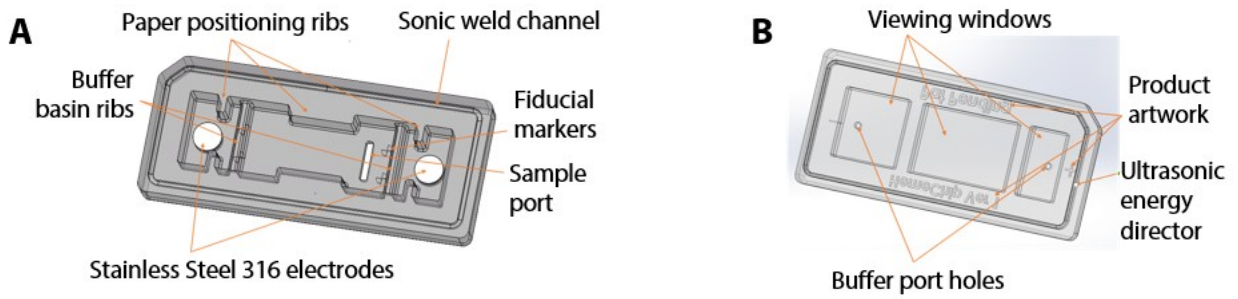

Fig. S2. Injection moldable HemeChip design with bottom and top plastic parts.
(A) 3D computer aided design (CAD) illustration of the bottom part. Design embeds the metal electrodes as well as the internal features necessary for buffer reserve, cellulose acetate paper positioning and aligning, ultrasonic weld groove, and a port for blood sample application. **(B)** 3D CAD design of the top part that contains the product artwork, ultrasonic energy director, viewing windows, and buffer loading ports.

5. Mass-production of HemeChip cartridges via Injection molding

5.1. Injection mold design

The HemeChip prototype design (**Fig. S1A&B**) has been transformed into an injection moldable design (**Fig. S1C&D**) with a plastic bottom and a top part (**Fig. S2A&B)** using a 1+1 mold. A 1+1 mold design is economic as it reduces the product cost due to less machine run time with reduced labor cost to produce each part. This is a very crucial aspect for mass producing a point-of-care (POC) single use cartridge as the cost per unit needs to be as low as possible. Optix® CA-41 Polymethyl Methacrylate Acrylic (PMMA) material was used in injection molding. The visual clarity of the Optix CA-41 is excellent. However, this visual clarity may be greatly impaired after the injection molding process due to surface finish of the mold (**Fig. S3A-C**). The visual clarity of the HemeChip is crucial, since the detection method is based on image acquisition and analysis, and any impairment of visual clarity will significantly impact the performance of the detection system. Visual clarity and light transmission of the injection molded parts significantly improved after the mold underwent aluminum oxide polishing, which improved the visual clarity of the finished HemeChip part to its desired level (**Fig. S3B&C**). Optical transmission for HemeChip components with both standard machine finishing and aluminum oxide finishing were tested and compared (**Fig. S3B**). The optical transmission for the HemeChip component was tested using VASE Ellipsometer (J.A. Woollam

Co., Inc., Lincoln, NE). The optical transmission was measured at an angle of 0° for wavelengths ranging from 300 ~ 1000 nm. The results showed that the HemeChip components produced in the aluminum oxide polished mold have a much higher optical transmission (up to 80%) compared to the standard machine polished mold. Optical clarity and light transmission significantly improved after the mold underwent aluminum oxide polishing (**Fig. S3B&C**).

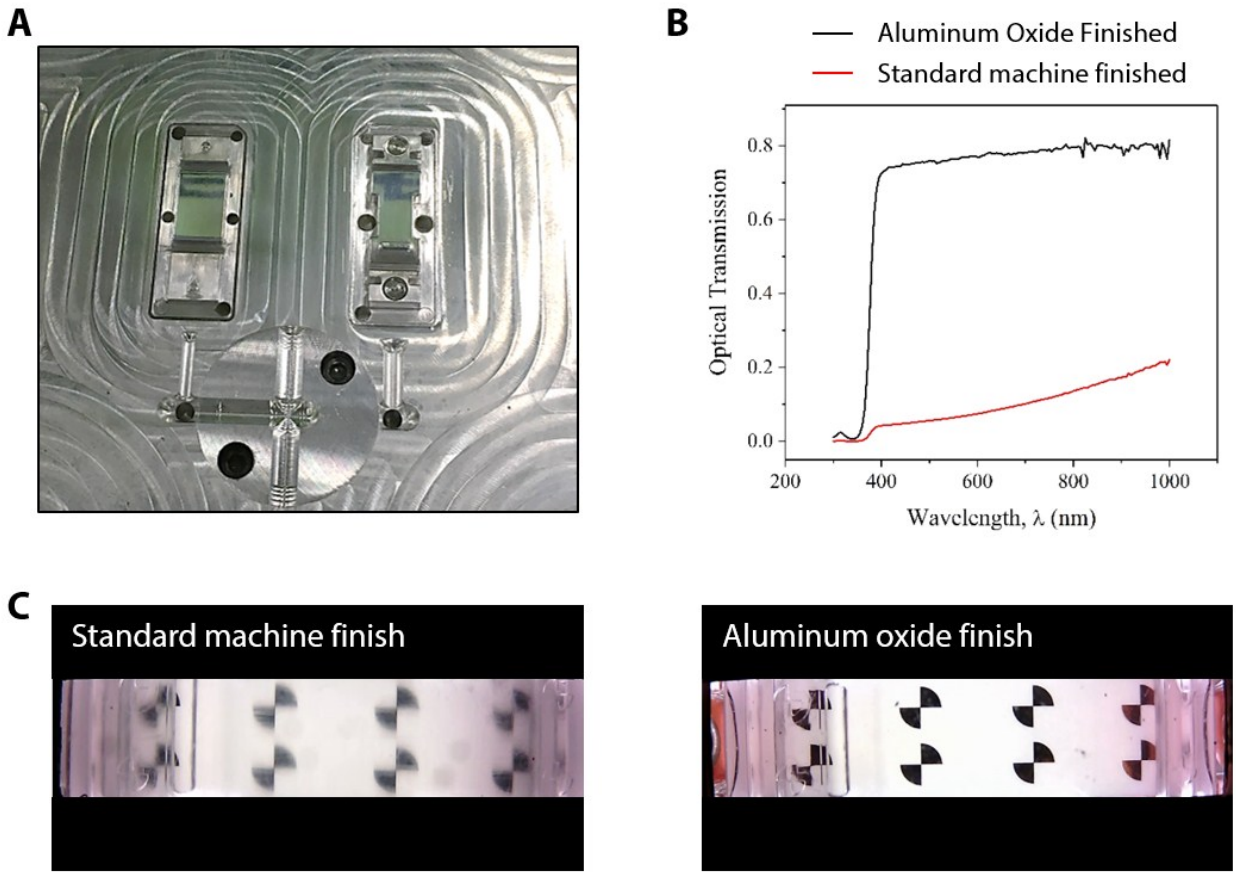

Fig. S3. Injection molding of HemeChip and effect of the mold finish on optical quality.
(A) HemeChip is fabricated using injection molding of general-purpose commercial grade PMMA. The figure shows HemeChip body parts just after injection molding process. The metal electrodes are embedded to the bottom part of the HemeChip during the injection molding process. **(B)** Optical transmission comparison between HemeChip parts made from standard machine finished mold (red line) and aluminum (oxide) finished mold (black line). **(C)** The optical clarity of the HemeChip with two different surface finished implemented to the mold. The left image shows the visual clarity for HemeChip where the mold was just machine finished. The right image shows the improved visual optical clarity after the mold has undergone through aluminum oxide polishing.

5.2. Injection molding process parameters and quality control

HemeChip parts were manufactured using a Vertical Injection Molding Machine (VIMM). For the HemeChip injection molding, the Optix CA-41 was dried with a desiccant dryer at 93 °C (200 °F). The top and the bottom parts of the HemeChip were processed following different process parameters since the thickness and the design complexity differ for these two parts of the HemeChip cartridges. The top part of HemeChip was processed at a melt temperature of 241 °C (465 °F), keeping the rate of injection at 3 grams per second. The mold surface temperature was kept at 82 °C (180 °F). Packing pressure was established at 71361 kN (10,350 psi) for 8 seconds. The processes then allows the mold to cool down for 15 seconds. The total process requires 45 seconds to complete. The bottom parts of the HemeChip were processed at a melt temperature of 244 °C (470° F), while maintaining an injection rate of 4.5 grams per second. The mold surface temperature was controlled at the same temperature as the top parts of HemeChip. Packing pressure was established at 75843 kN (11,000 psi) for 10 seconds. This process was given a cooling time of 20 seconds, with the overall cycle time at 70 seconds. The injection molded HemeChip cartridges are sealed via ultrasonic welding. As Optix CA-41 is a thermoplastic material, the use of ultrasonic welding was well suited. Ultrasonic welding is one of the most preferred welding methods in the industry for joining plastic or polymer components. Implementation of ultrasonic welding requires certain design considerations for the parts to be welded or joined. The parts to be ultrasonically welded require three main components in their design (**Fig. S2A&B**): **(i)** an energy director, usually closest to ultrasonic horn that focuses the ultrasonic energy and melts, **(ii)** a gap or groove that accommodate the additional material of the energy director after the energy director melts during the welding process, and **(iii)** a positive stop feature on the parts to be welded. The HemeChip cartridges were welded at an ultrasonic frequency of 32 kHz for a time of 0.6 seconds keeping the downward horn pressure at 55psi. The total machine cycle time to seal a fully assembled single HemeChip cartridge with the above-mentioned process parameters was 12 seconds. Total volume of PMMA material that was melted during ultrasonic welding was calculated as 0.0061 cc.

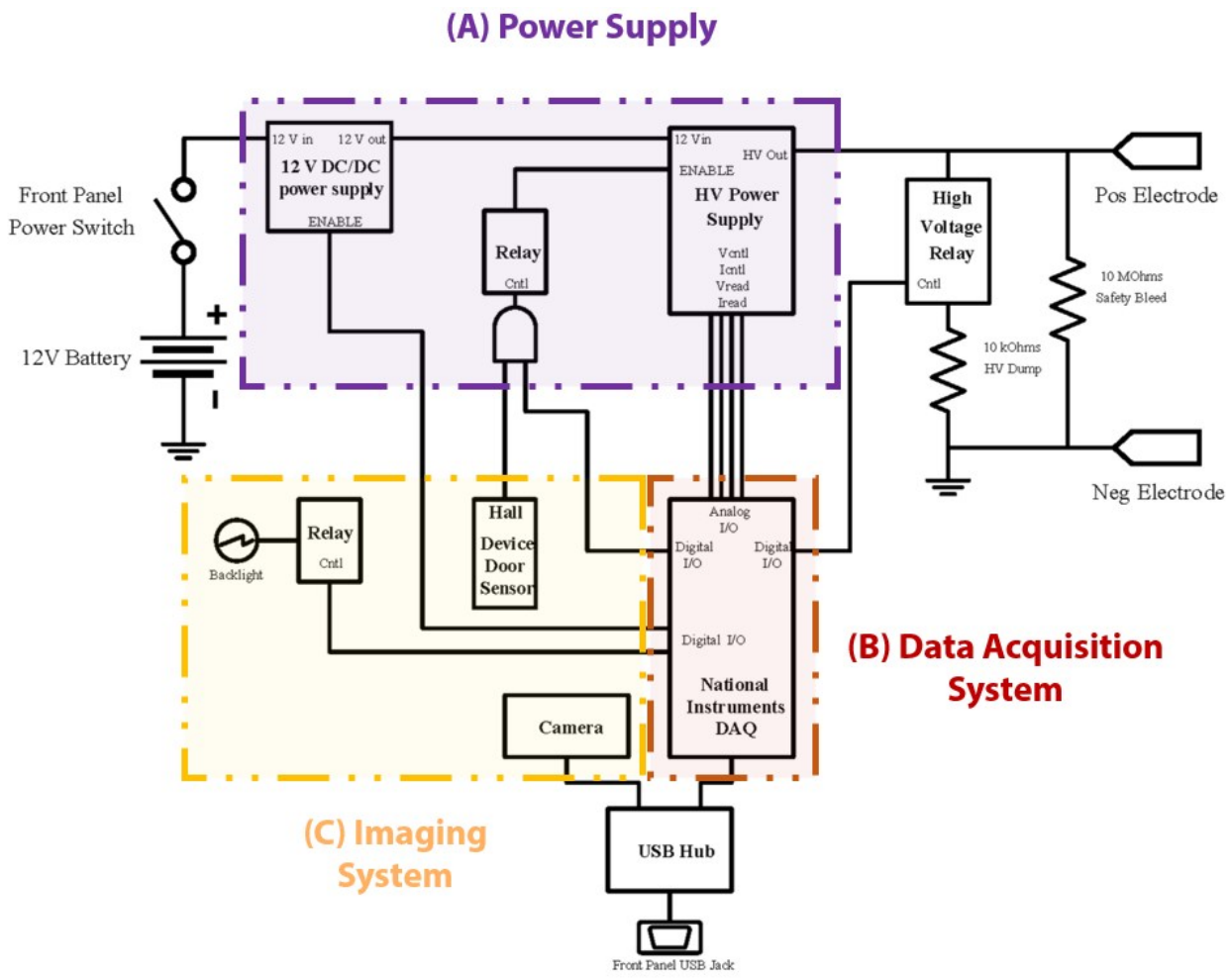

Fig. S4. Electronic circuit design of the HemeChip Reader.

HemeChip Reader consists of three major parts: **(A)** a rechargeable power supply, **(B)** a data acquisition system that collects current and voltage data for the duration of the test, and **(C)** an imaging system that records video and images for the duration of the test, which are transferred into an image processing software for analysis.

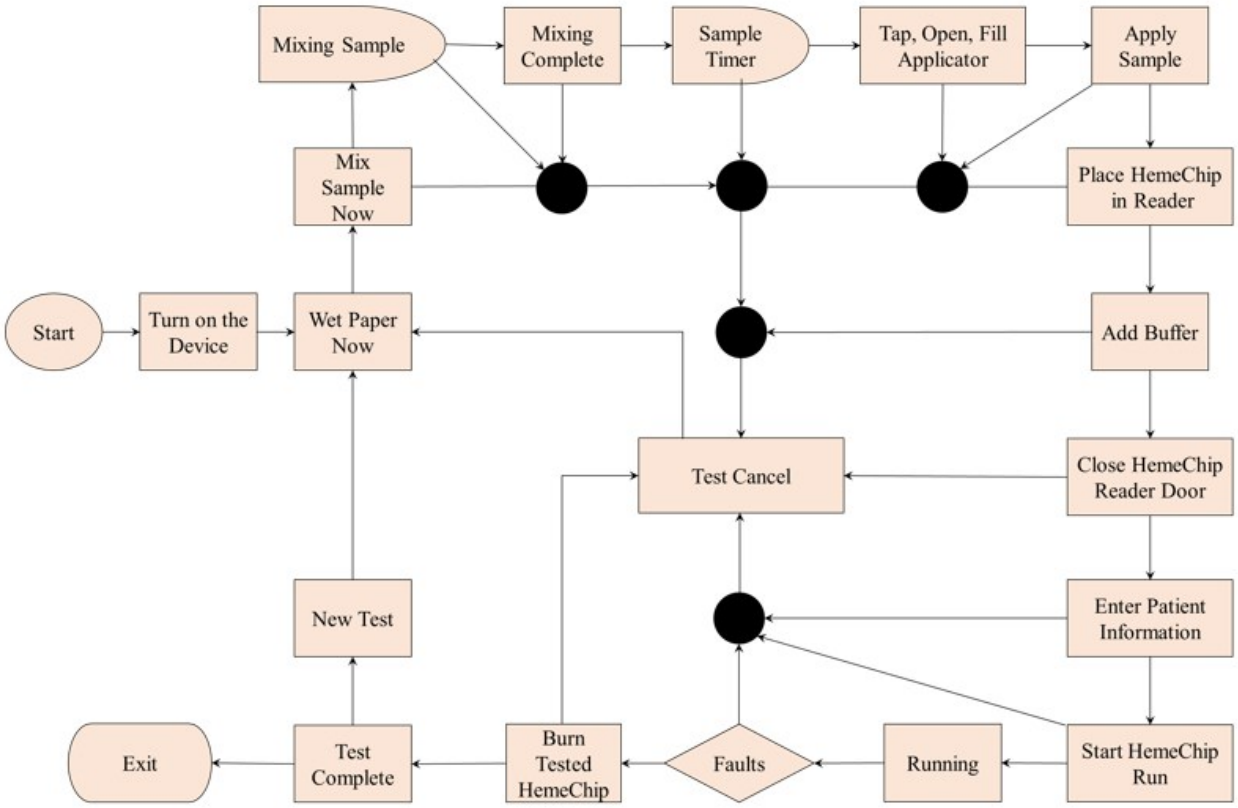

Fig. S5. Software algorithm for the HemeChip Reader user interface.

The software algorithm guides the step-by-step walkthrough of the test procedure, which is integrated, into the designed user interface.

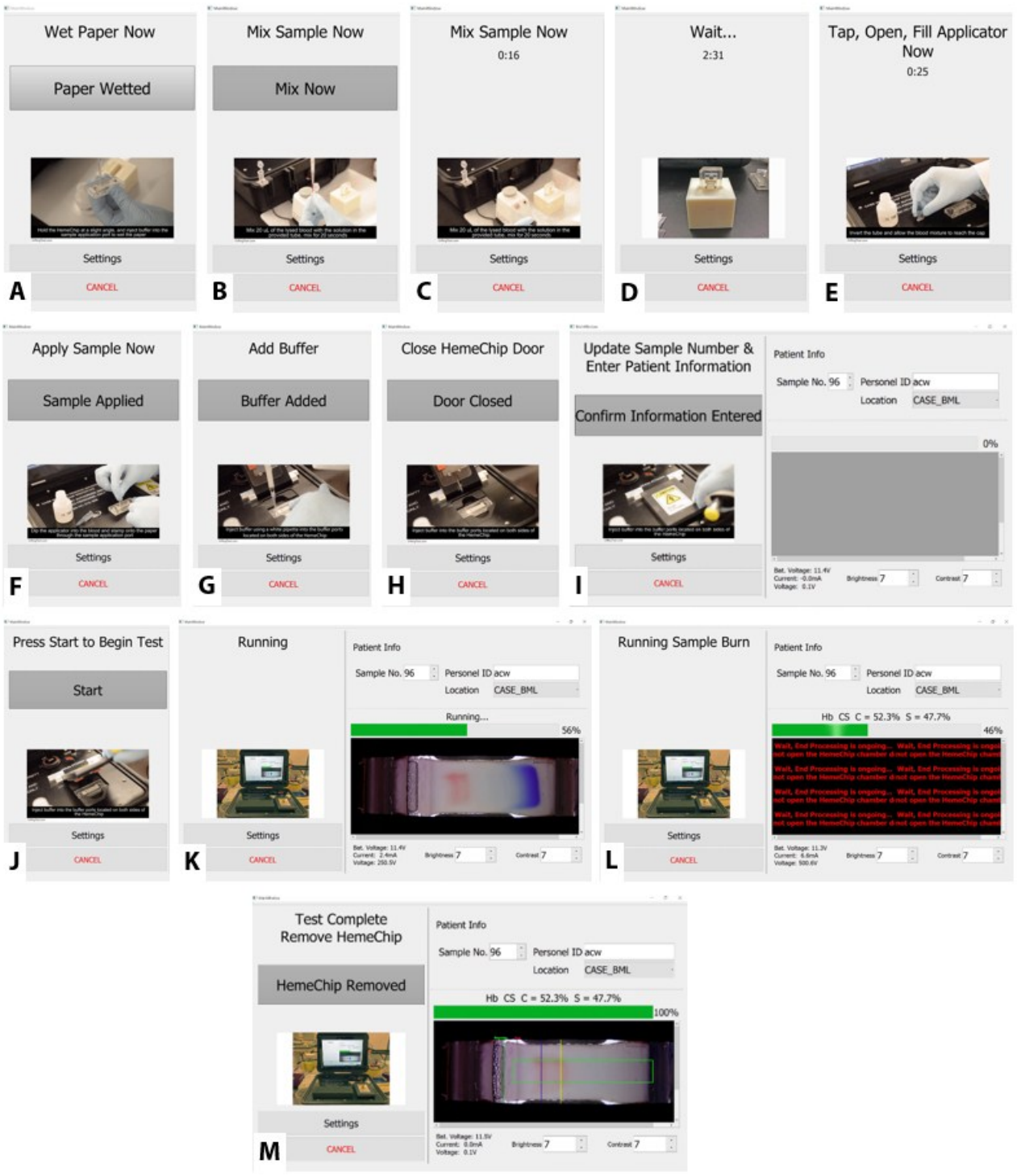

Fig. S6. Screen views of the step-by-step instructions provided by the HemeChip user interface.

The protocol integrated user interface guides the user through the test with visual, animated instructions. This step-by-step guidance facilitates a rigorous control of the test steps and reduces human error. This user guidance also ensures that a minimally trained person can run the HemeChip test with ease. **(A–D)** The steps of HemeChip cartridge and sample preparation are demonstrated. **(E–H)** The steps for sample application and HemeChip test initiation are

demonstrated. **(I–J)** The steps for logging sample information and starting the test. **(K)** A HemeChip test is started. The blue marker and the separating Hb types are visible in the frame. **(L)** The HemeChip test ends. **(M)** At the end of the test, the detected Hb type(s), their relative percentages are displayed on the result screen.

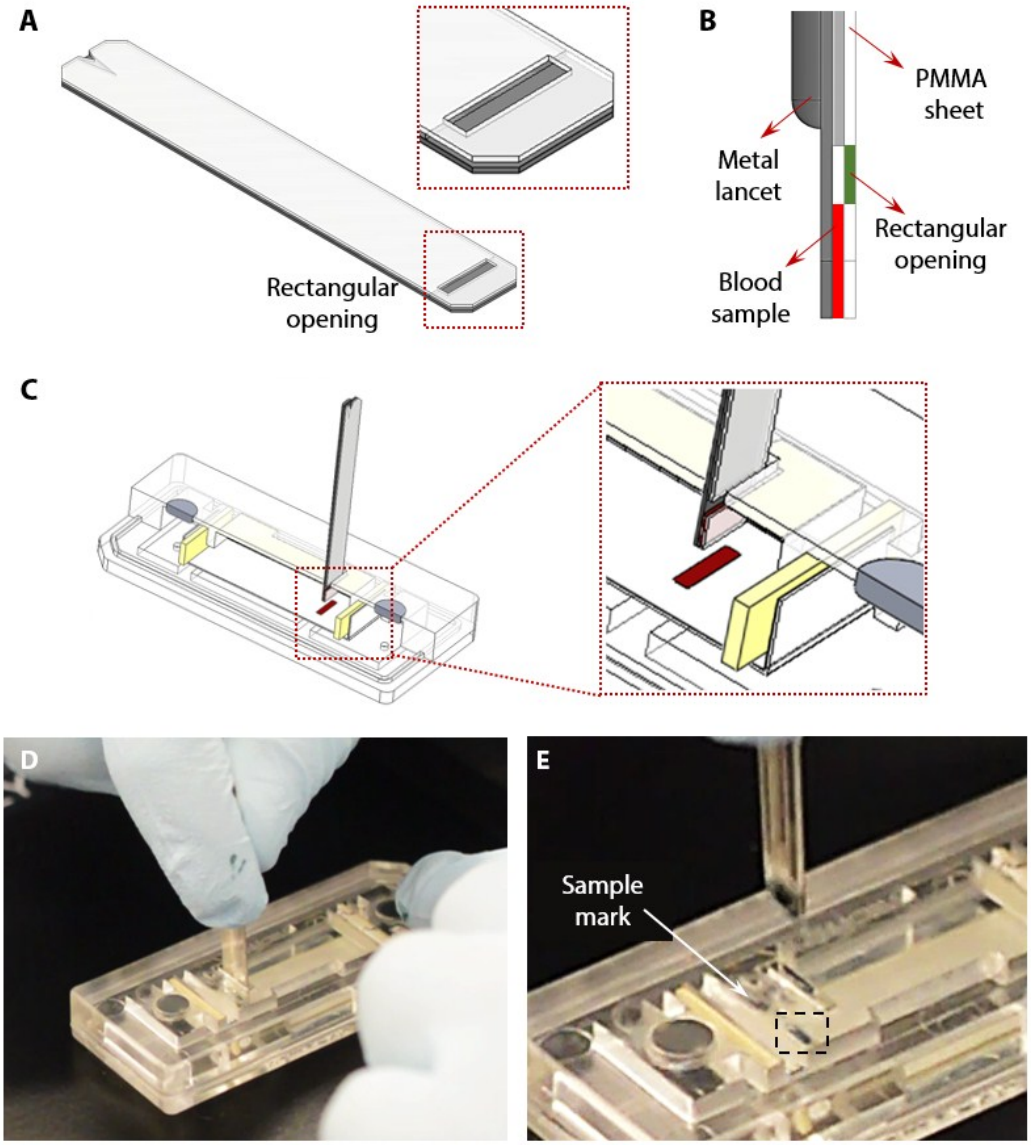

Fig. S7. Micro-applicator design and precise application of blood samples into HemeChip.
3D CAD design of the custom developed capillary micro-applicator. A zoomed-in view of the application end is shown in the inset. **(B)** An illustration showing the application end of the micro-applicator. **(C)** A 3D CAD representation of the blood sample application process. The sample loaded micro-applicator in inserted into the HemeChip through the sample loading port, located at the bottom of the HemeChip. A close-up view shows the application mark after the micro-applicator has applied blood sample on the cellulose acetate strip. **(D)** User is shown applying a blood sample into HemeChip cartridge. **(E)** Sample application mark is visible on the cellulose acetate paper strip.

6. Micro-applicator design and operation

We designed a capillary-based micro-applicator to apply blood samples into HemeChip cartridge (**Fig. S7**). This simple, easy-to-use component ensures a controlled and repeatable application of whole blood sample, and facilitates repeatable and reliable test results. The micro-applicator consists of a metal lancet and a PMMA sheet attached using double sided adhesive (DSA) (**Fig. S7A**). The spacing between the metal and the PMMA sheet is 150 µm. When the micro-applicator is dipped into the blood sample, it loads and retains a specific amount of the sample (**Fig. S7B**). A rectangular opening micro-machined onto the PMMA part of the micro-applicator (**Fig. S7B**) ensures this controlled amount of sample loading. The sample loading ports, located at the bottom of the HemeChip (**Fig. S7C**), are designed to provide just enough space to insert the micro-applicator (**Fig. S7D**), thus ensuring vertical alignment of the micro-applicator during sample application process. This design improves the accuracy and consistency of the application of blood samples at the same spot (**Fig. S7E**).

7. HemeChip test procedure

7.1. HemeChip test kit

The consumables and accessories needed for the sample preparation are as follows (**Fig. S8**):

1. A 20 µL capillary blood collection tube, this tube is used to collect exactly 20 µL of blood needed for the test, from either a heel or finger prick.
2. A 1.5 mL tube containing 40 µL of lysing solution premixed with the blue marker (Xylene Cyanol). The resulting lysing solution to blood ratio is 2:1.
3. A custom made applicator, which is dipped in the lysed blood mixture and applied to the surface of the cellulose acetate strip inside the HemeChip.

4. 50 µL, and 200 µL fixed volume pipettes are provided to minimize user error. The 50 µL pipette is used to wet the cellulose acetate strip in the first step of the HemeChip preparation, and the 200 µL is used to load the buffer into the buffer ports just prior to starting the test.

5. A battery-operated mini vortexer that is lightweight and portable, and is powered by four AA batteries.

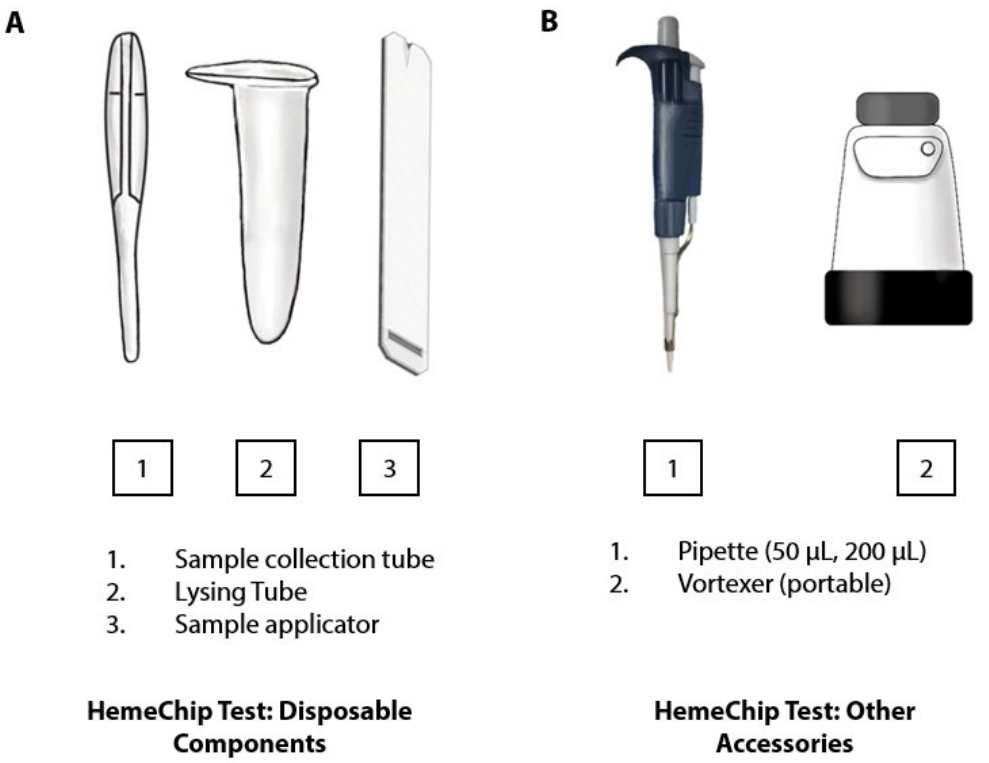

Fig. S8. HemeChip test kit components.

(A) Disposable components of the test kit are the sample collection tube, blood lysing tube, and the sample applicator, which come into contact with the blood sample. **(B)** A Pipette and a portable vortexer assist HemeChip test procedure.

7.2. Blood collection and HemeChip test protocol

1. Pipette 50 µL of the buffer solution onto the paper through the sample loading port, located at the bottom of the HemeChip cartridge. Then allow the paper to soak (**Fig. S9A**).

2. After administering a finger/ heel prick, touch the drop of blood with the capillary sample collection tube at a slight angle. Allow the tube to fill to the black line (**Fig. S9B**).

3. Squeeze the top part of the tube to empty the blood into the tube containing lysing solution (**Fig. S9C**).

4. Place the tube on top of the vortexer to mix the blood and lysing solution for 20 seconds (**Fig. S9D**).

5. Invert the tube containing the blood mixture. Tap the tube on a solid surface to allow the mixture to reach the cap. Next, keep the tube inverted and open the cap. Dip the tip of the applicator into the blood mixture in the cap (**Fig. S9E**).

6. Stamp the blood mixture in the applicator onto the paper through the sample loading port by gently touching the paper surface with the applicator, while making sure not to puncture the paper with the applicator (**Fig. S9F**).

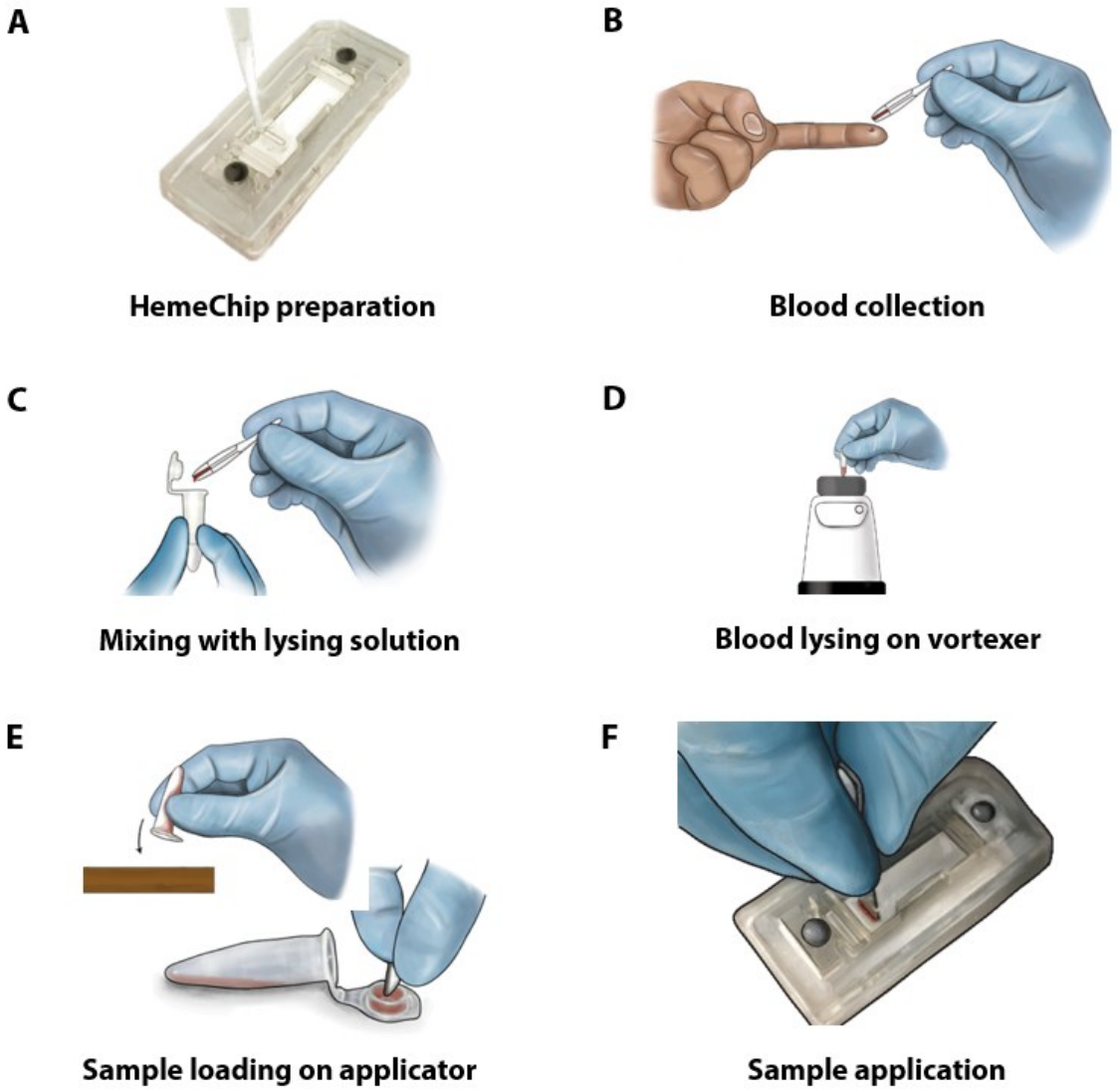

Fig. S9. Blood collection and HemeChip test protocol steps.

As a preparation to the HemeChip test, the HemeChip cellulose acetate paper in the cartridge is wetted with 50 µL of 1x TBE buffer. **(B)** In this step, the blood sample is collected from a person via a finger or heel prick. Then the blood sample collection tube is used to collect the blood sample (~ 20 µL). **(C)** The collected blood sample is then poured into the lysing tube. The lysing tube contains the lysing solution with Xylene Cyanol solution (the blue marker). **(D)** The blood is mixed with the lysing solution using a portable battery powered vortexer for effective lysing. **(E)** Before the sample application into the cartridge, the blood lysing tube is flipped upside down and tapped against the workbench, which results in accumulation of lysed blood sample in the tube cap. Then, the tube is opened, keeping the tube in the up-side down position and the lysed blood sample is loaded into the micro-applicator. **(F)** The processed blood sample loaded micro-applicator is inserted into the HemeChip and the sample is loaded onto the cellulose acetate strip inside the HemeChip. Next the cartridge is placed into the Reader chamber and the test is started. Then the Reader takes over, completes the test run and automatically analyzes the results.

8. HemeChip test result screens

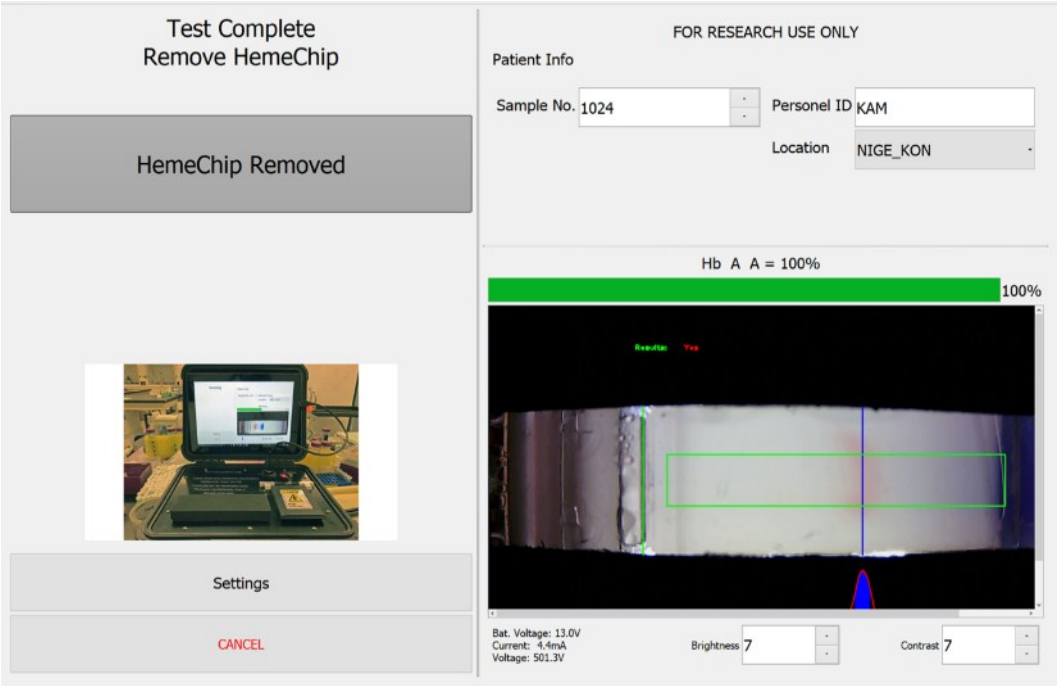

Fig. S10. HemeChip test result screen for no abnormal hemoglobin (Hb AA).

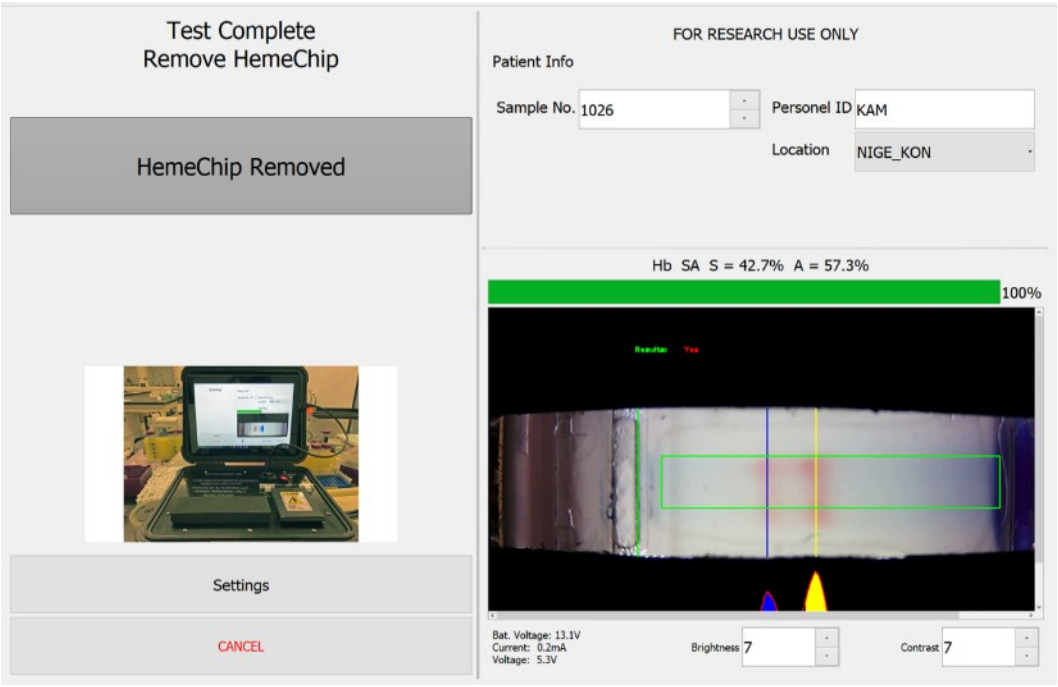

Fig. S11. HemeChip test result screen for SCD Trait (Hb AS).

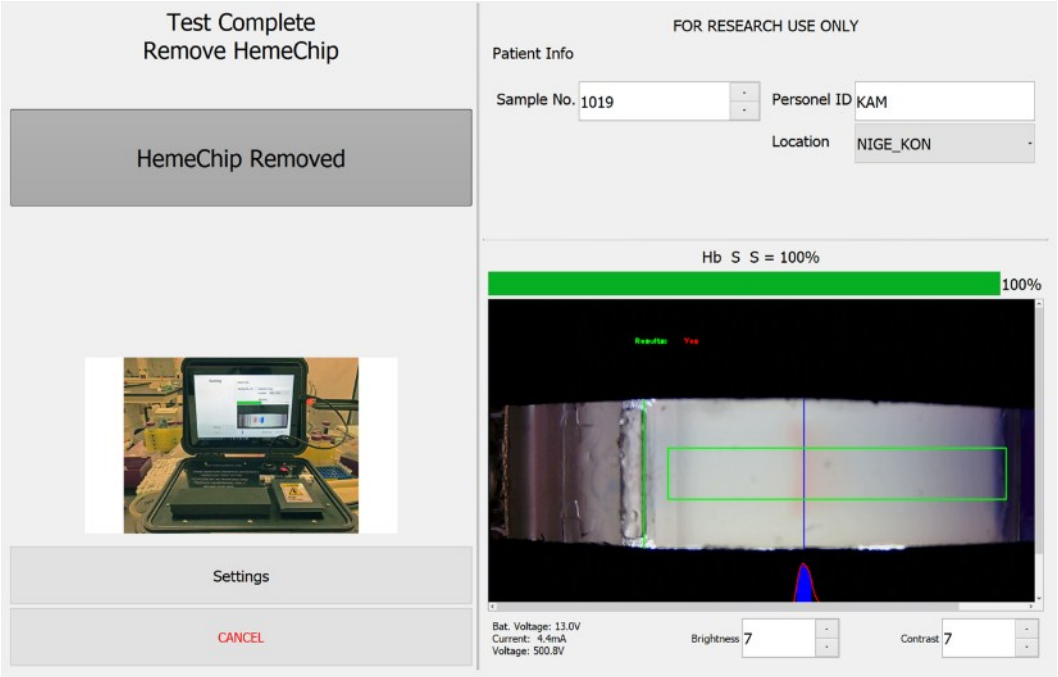

Fig. S12. HemeChip test result screen for SCD-SS (Hb SS).

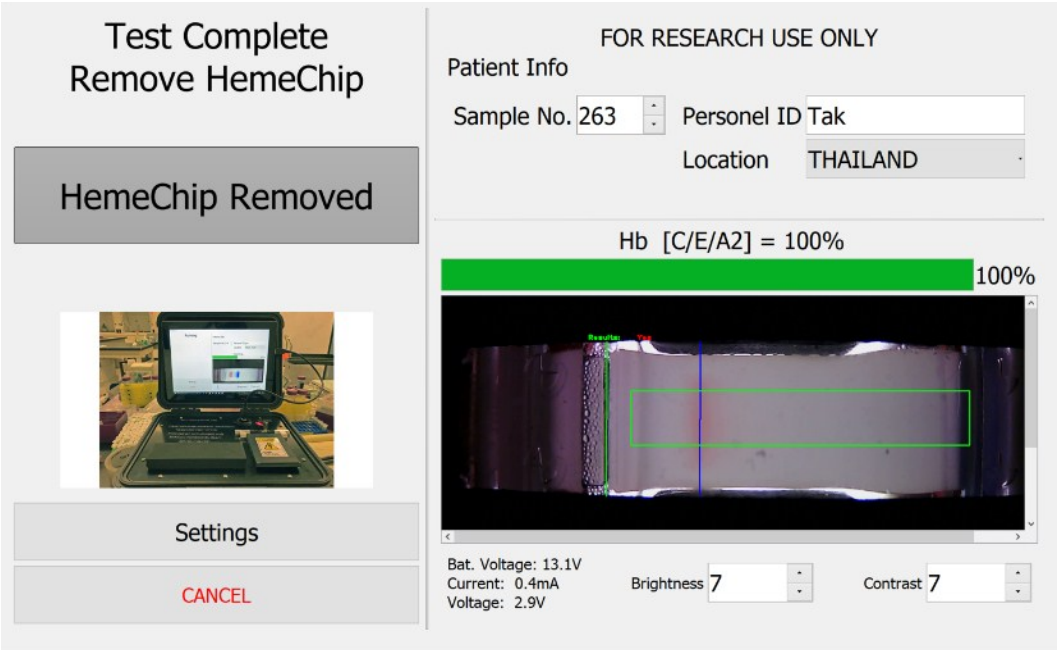

Fig. S13. HemeChip test result screen for Hb EE (Hb E Disease).

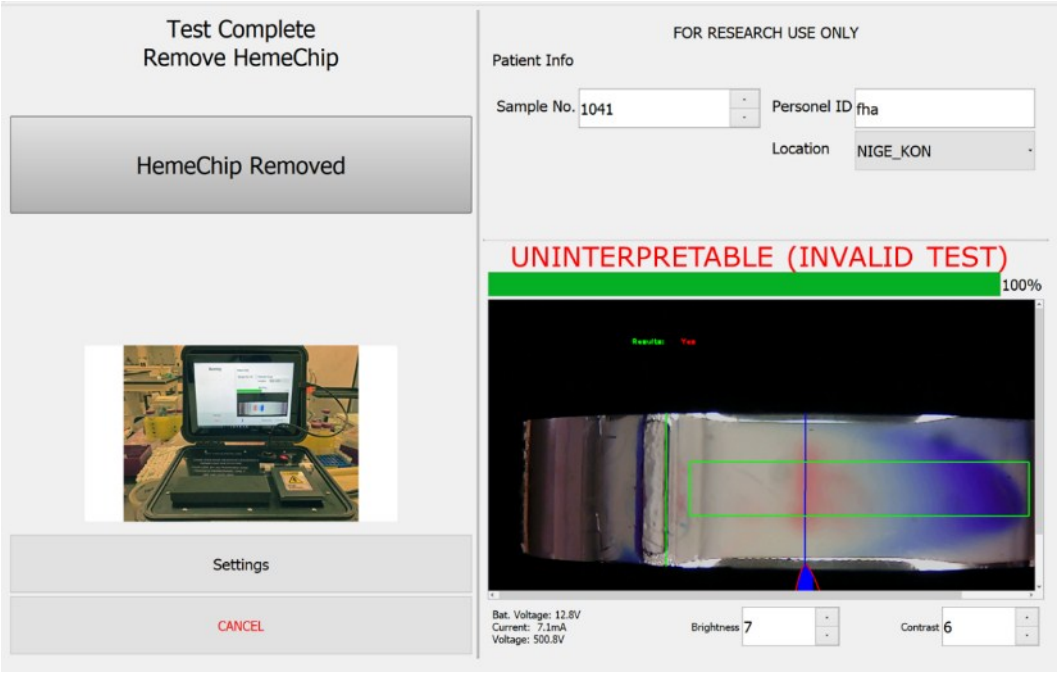

Fig. S14. HemeChip test result screen for an 'Uninterpretable' or invalid test.

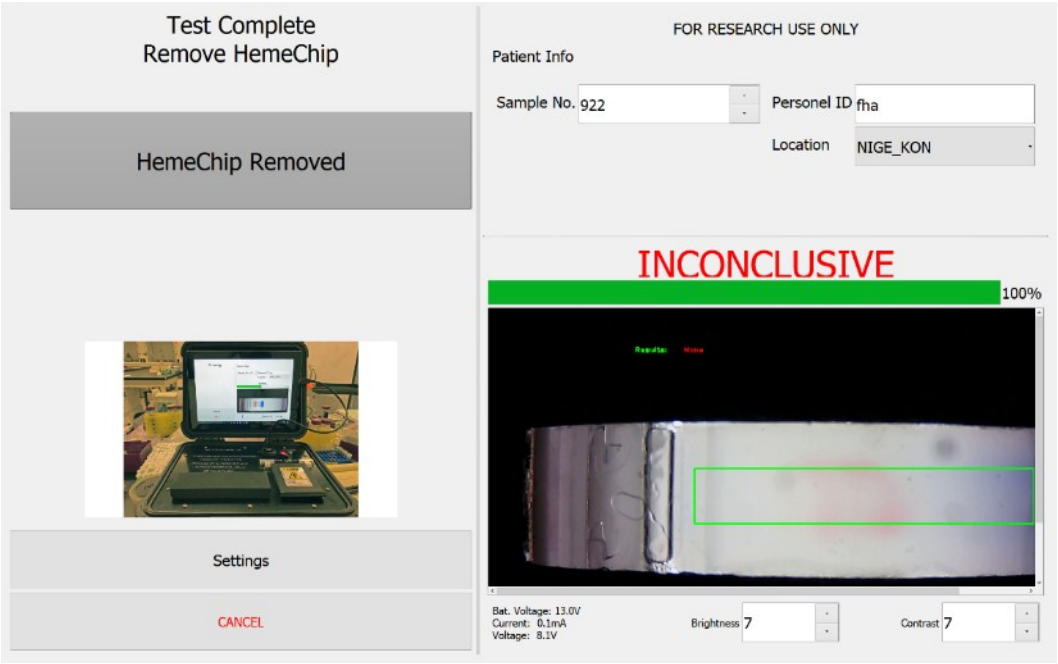

Fig. S15. HemeChip test result screen for an 'Inconclusive' test.

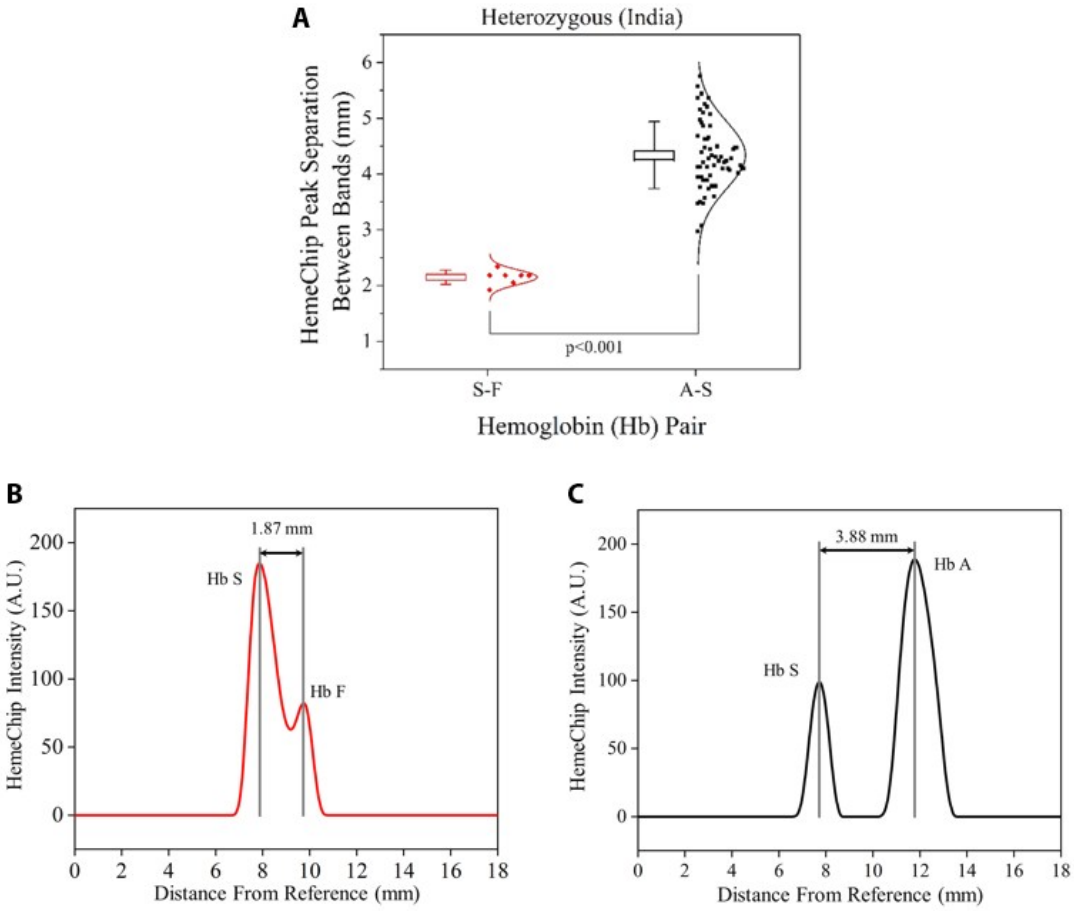

Fig. S16. Hemoglobin F separation and identification in HemeChip.

(A) Separation between hemoglobins A, F, and S can be used to distinguish Hb AS and Hb SF samples. The separation between Hb S and Hb F is significantly less than the separation between Hb A and Hb S. **(B)** A typical Hb SF result with 1.87 mm separation between S and F hemoglobin bands. **(C)** A typical Hb AS result with 3.88 mm separation between A and S hemoglobin bands.

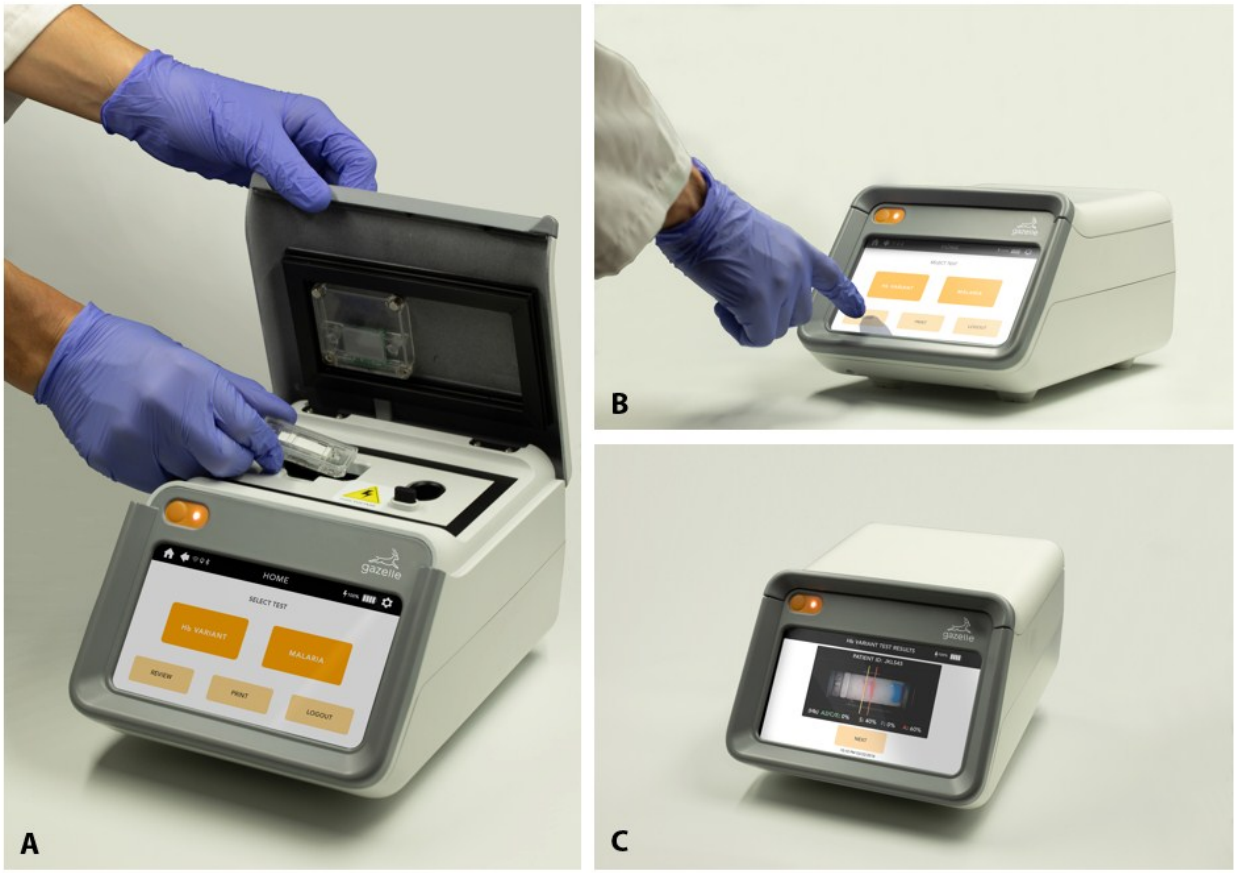

Fig. S17. New design of portable, affordable HemeChip reader: Gazelle Hb Variant™.

(A) The new reader design (Gazelle Hb Variant™, Hemex Health Inc., Portland, Oregon, USA) for HemeChip microchip electrophoresis is lightweight, battery operated, and has been developed and tested for tropical environments, with operating temperature range: 5°C - 45°C, operating relative humidity range: 5% - 95%, and storage temperature: -40°C -60°C. **(B)** Gazelle™ allows digital data entry, can store up to 1000 test results, and embodies GPS geo-location capability. Test reports can be printed wirelessly, transmitted in PDF format, or transmitted directly to the cloud. **(C)** Gazelle™ guides the user step-by-step through the test procedure, automatically interprets and objectively displays the test results to the user. *(Regulatory clearance pending)*

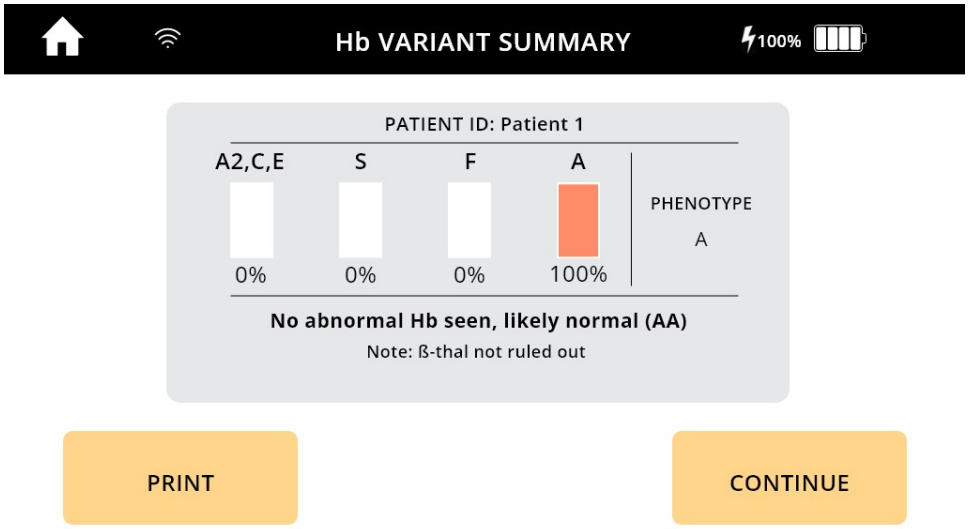

Fig. S18. Gazelle™ Hb Variant test result screen for Hb AA (normal).

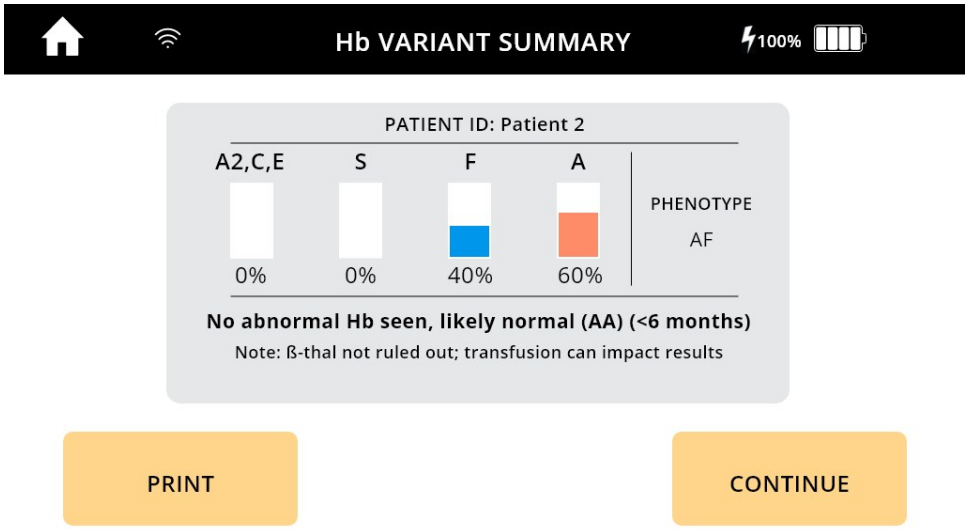

Fig. S19. Gazelle™ Hb Variant test result screen for Hb AA (normal) for a <6 months old infant.

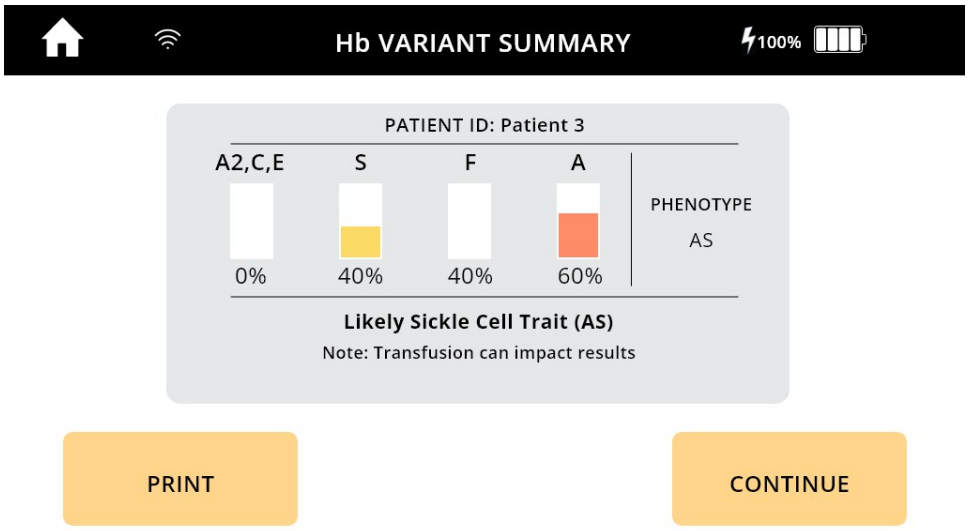

Fig. S20. Gazelle™ Hb Variant test result screen for Hb AS trait.

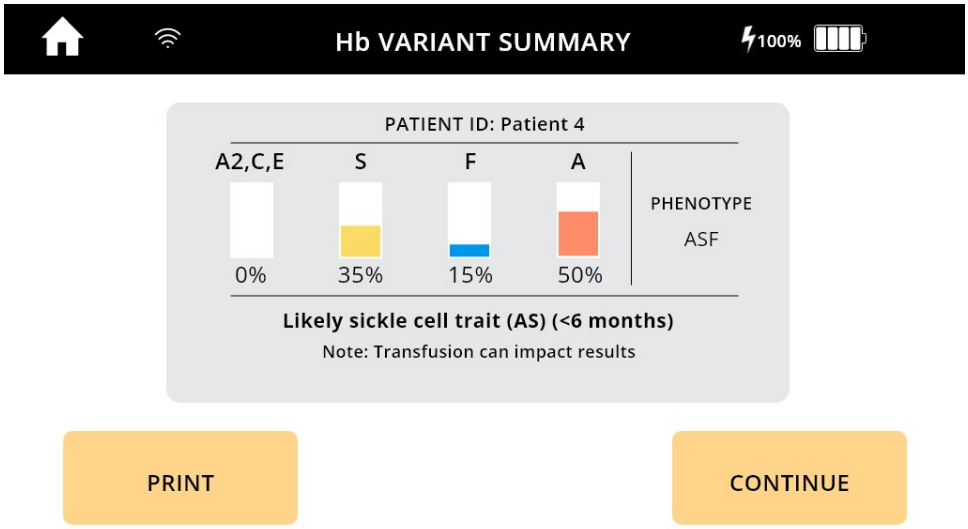

Fig. S21. Gazelle™ Hb Variant test result screen for Hb AS trait for a <6 months old infant.

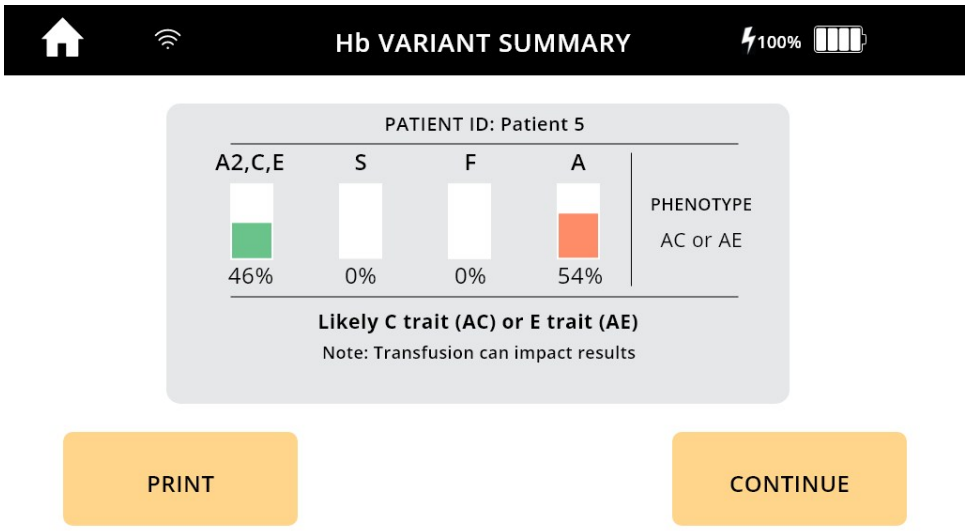

Fig. S22. Gazelle™ Hb Variant test result screen for Hb AC or Hb AE trait.

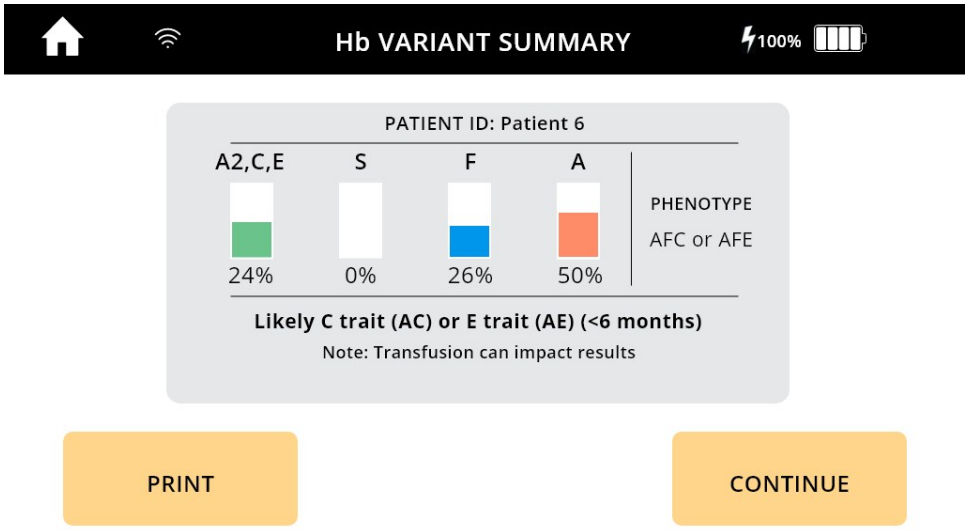

Fig. S23. Gazelle™ Hb Variant test result screen for Hb AC or Hb AE trait for a <6 months old infant.

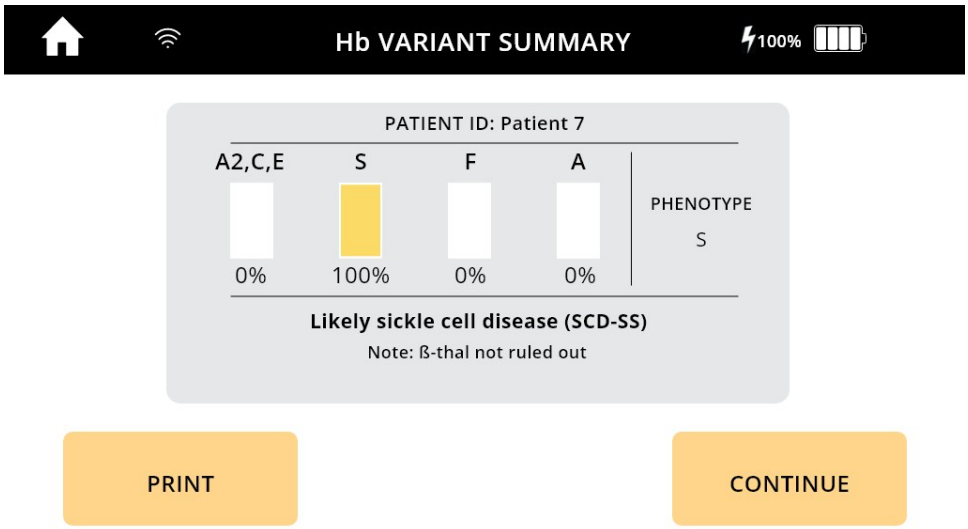

Fig. S24. Gazelle™ Hb Variant test result screen for Hb SS (SCD).

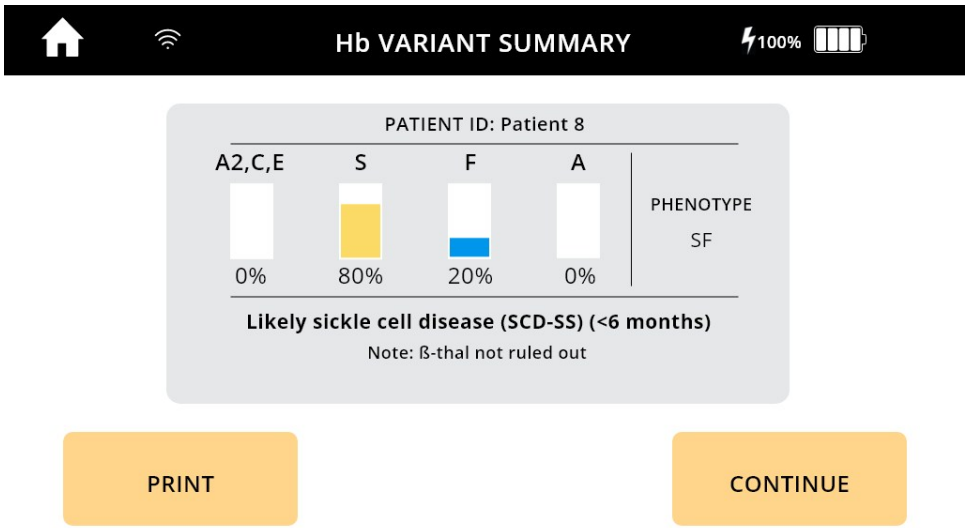

Fig. S25. Gazelle™ Hb Variant test result screen for Hb SS (SCD) for a <6 months old infant.

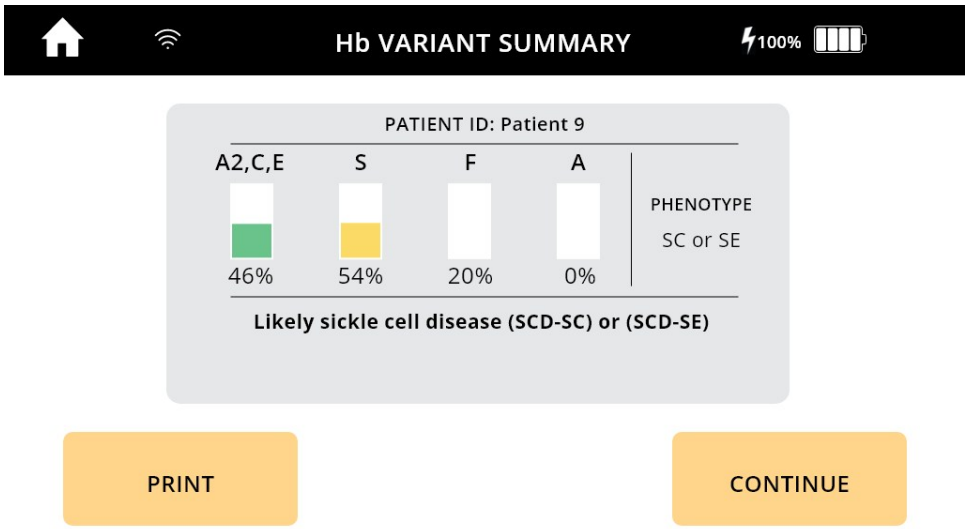

Fig. S26. Gazelle™ Hb Variant test result screen for Hb SC or SE.

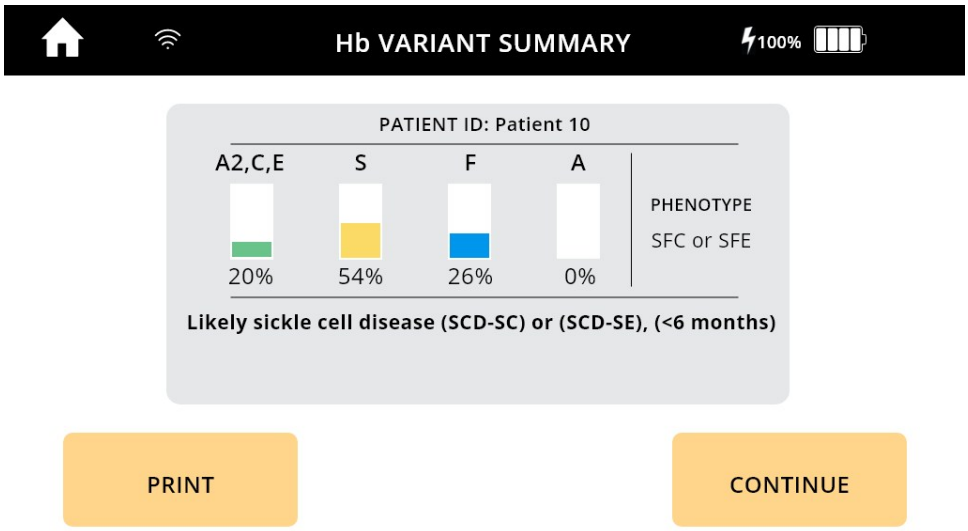

Fig. S27. Gazelle™ Hb Variant test result screen for Hb SC or Hb SE for a <6 months old infant.

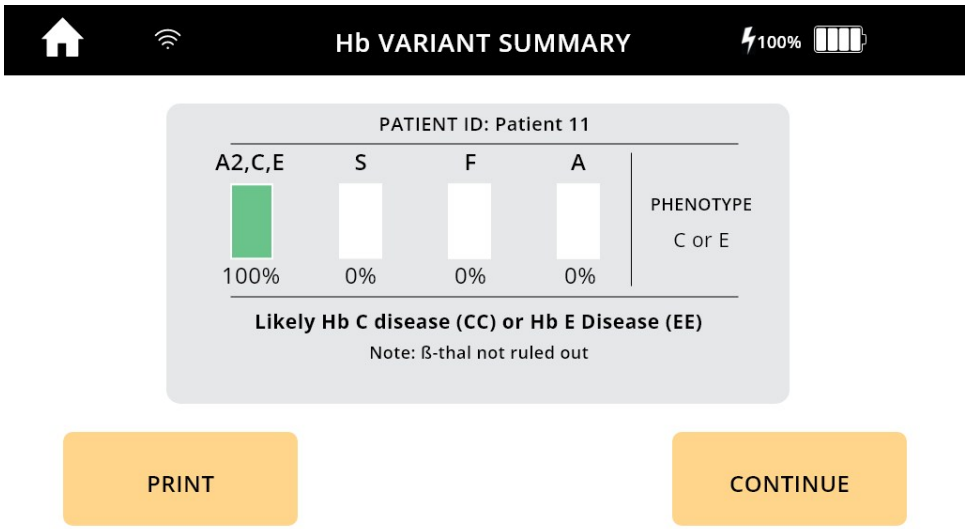

Fig. S28. Gazelle™ Hb Variant test result screen for Hb CC or EE.

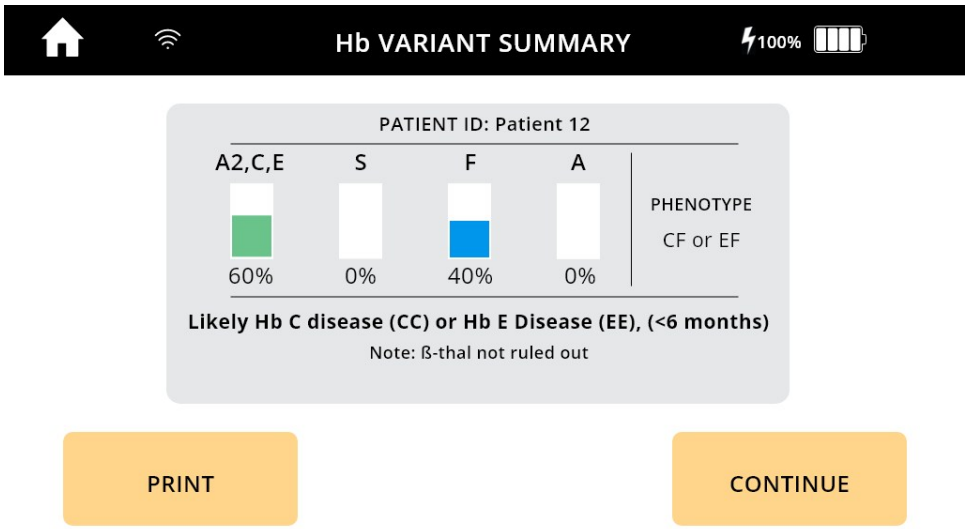

Fig. S29. Gazelle™ Hb Variant test result screen for Hb CC or Hb EE for a <6 months old infant.

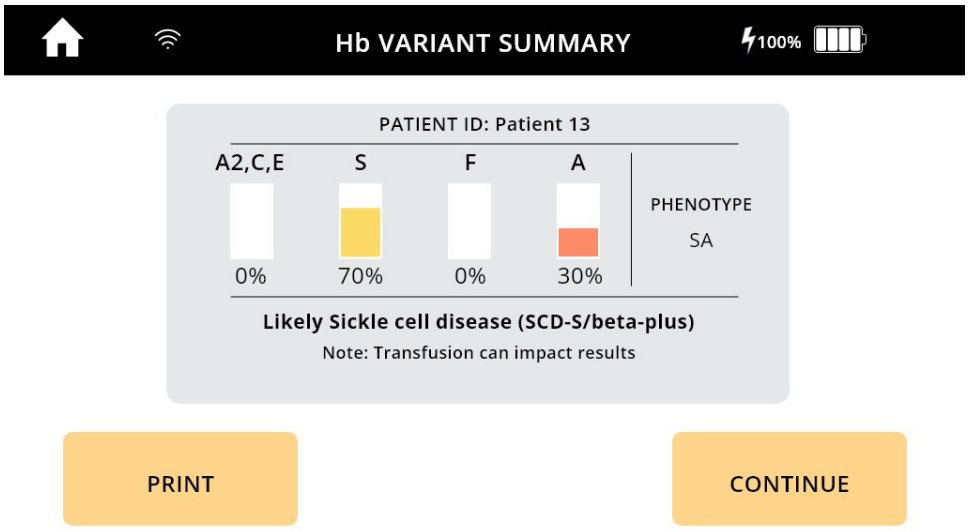

Fig. S30. Gazelle™ Hb Variant test result screen for Hb Sβ⁺.

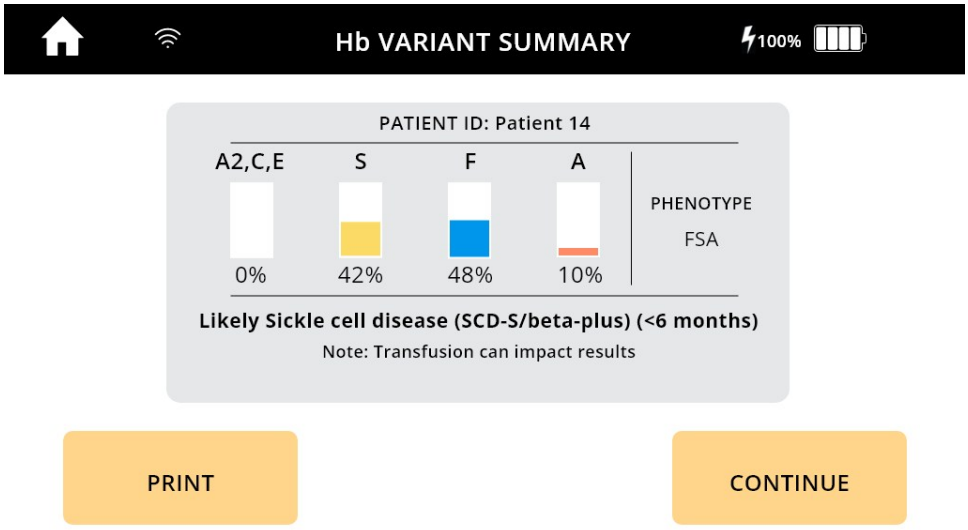

Fig. S31. Gazelle™ Hb Variant test result screen for Hb Sβ⁺ for <6 months old infant.

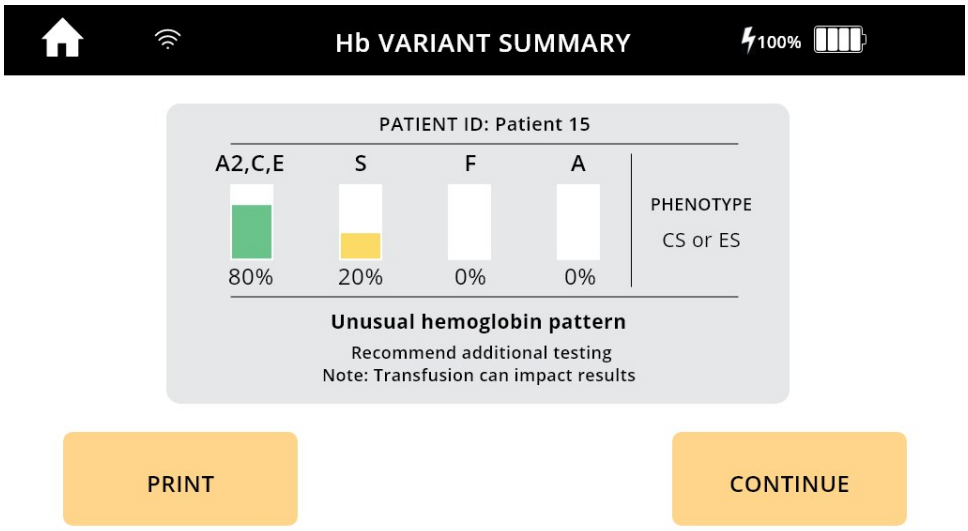

Fig. S32. Gazelle™ Hb Variant test result screen for an unusual hemoglobin pattern.

9. Comparison of HemeChip with existing and emerging techniques

Table S1. Comparison of HemeChip with standard laboratory methods for hemoglobin testing

	HemeChip (Microchip Electrophoresis)	Electrophoresis (IEF)	HPLC	Microscopy-based tests	Sickledex – Turbidity Test
Differentiates between trait and disease	**Yes**	Yes	Yes	No	No
Quantification	**Yes**	No	Yes	No	No
Operator skill required	**Low**	High	Medium	High	Medium / Low
Cost per test	**$2.00**	$5-10	$10-15	$3-$5	$0.50
Initial equipment cost	**<$700**	>$10,000	$30-80K	$2500	None
Time to result	**< 10 minutes**	24+ hours	24+ hours	24+ hours	6-10 min
Sensitivity	**High**	High	High	Poor sensitivity; Only detects SCD not trait or other hemoglobinopathies	Can't be used on infants under 6 months, can't differentiate trait/disease. Confounders such as severe anemia; Only detects HbS
Specificity	**High**	High	High		
Reference	**This article**	[1, 2]	[1, 2]	[3, 4]	[5]

Table S2. Comparison of HemeChip with emerging point-of-care technologies for hemoglobin testing

	HemeChip (Microchip Electrophoresis)	Paper-based hemoglobin solubility	Density-based red cell separation	SickleSCAN (Lateral flow immunoassay)	HemoTypeSC (Lateral flow immunoassay)
Hemoglobin types identified	**A, F, S, C/E/A_2**	A, S	A, S	A, S, C	A, S, C
Hb% quantification	**Yes**	No	No	No	No
SCD-SC identification	**Yes***	No	No	Yes	Yes
Hb EE, AE identification	**Yes***	No (as normal AA)	No (as normal AA)	No (as normal AA)	No (as normal AA)
Sβ-thal identification	**Yes (as SCD-SS)**	Yes (as SCD-SS)	No (as SCD trait)	Yes (as SCD-SS)	No (as SCD trait)
Differentiates between Trait and Disease	**Yes**	No	No	Yes	Yes
Newborn/infant testing	**Yes (best > 6 weeks)**	> 6 months only	> 6 months only	Yes	Yes
HbF identification and quantification	**Yes**	No	No	No	No
Automated interpretation of results	**Yes**	No	No	No	No
Digital test result storage	**Yes**	No	No	No	No
Wireless connectivity for data transfer	**Yes**	No	No	No	No
Works without uninterrupted power	**Yes**	Yes	No	Yes	Yes
Biological reagents	**No**	No	No	Yes (polyclonal antibodies)	Yes (monoclonal antibodies)
Temperature range	**5-45°C**	15-25°C	4-8°C	2-30°C	15-40°C
Required blood volume	**<1 µL**	20 µL	5 µL	5-10 µL	1.5 µL
Total test time: finger stick to results reported	**<10 minutes**	<35 minutes	<12 minutes	5-10 minutes	10 minutes (plus sample preparation time)
Cost per test	**$2.00**	$0.70	$0.50	$4.20	$2.00
References	**This article**	[6-8]	[9]	[10-15]	[12, 16-19]

*HemeChip reports hemoglobin C/E/A_2. Test location can be used to differentiate co-migrating hemoglobins C and E.

		Nigeria	**India**	**Thailand**
Prevalence estimate	**P**	**0.030****	**0.027*****	**0.087******
Width of the 95% CI: 10%	W	0.10	0.10	0.10
Sensitivity expected: 98%	SN	0.98	0.98	0.98
Specificity expected: 98%	SP	0.98	0.98	0.98
Statistical constant, 1.96 for 95% CI	Z_{alpha}	1.96	1.96	1.96
Number with disease, True Positive + False Negative	TP+FN	8	8	8
Sample size required for sensitivity*	N1	251	279	87
Number without disease, False Positive + True Negative	FP+TN	8	8	8
Sample size required for specificity*	N2	8	8	8
Minimum sample size (greater of N1 and N2):		**251**	**279**	**87**
Minimum sample size total:		**616**		

** N.M. Buderer, Statistical methodology: I. Incorporating the prevalence of disease into sample size calculation for sensitivity and specificity, Acad Emerg Med, 3 (1996), pp. 895–900*

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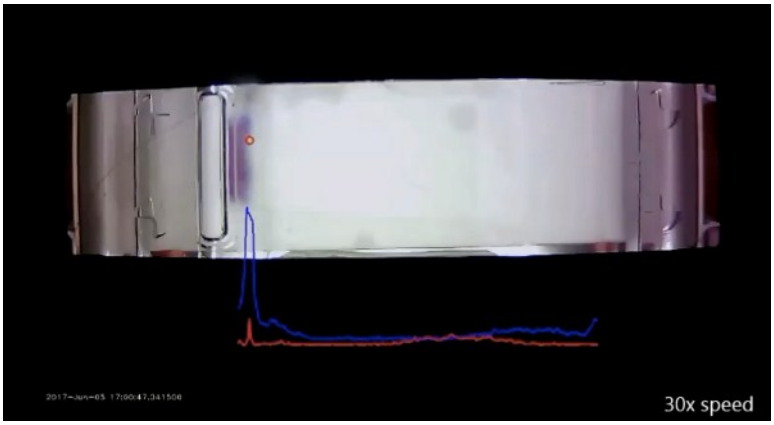
Table S3. Sample size calculation for clinical studies

10. Supplementary Videos



Video S1. Real-time tracking of hemoglobin bands in HemeChip during electrophoresis process

Video S2. Step-by-step HemeChip test procedure and analysis of results

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