Table S1. Significant effects from two-way ANOVA of blue-channel intensity in the paper-based assay without glycerol.

A. Substrate formulation × serum volume, analyzed separately within each application/reading condition.

Serum	Condition	Significant effect	p
Commercial (Merck)	SI	Substrate formulation	<0.0001
Commercial (Merck)	SI	Serum volume	0.0002
Commercial (Merck)	SI	Substrate formulation × serum volume	0.0193
Commercial (Merck)	S20	Substrate formulation	<0.0001
Commercial (Merck)	S20	Serum volume	<0.0001
Commercial (Merck)	S20	Substrate formulation × serum volume	<0.0001
Commercial (Merck)	HI	Substrate formulation	<0.0001
Commercial (Merck)	H20	Substrate formulation	<0.0001
Female	SI	Substrate formulation	0.0455
Female	SI	Serum volume	0.0035
Female	S20	Substrate formulation	0.0055
Female	H20	Substrate formulation	0.0025
Male	SI	Substrate formulation	0.0139
Male	H20	Substrate formulation	0.0029

B. Substrate formulation × application/reading condition, analyzed separately at each serum volume.

Serum	Serum volume (μL)	Significant effect	p
Commercial (Merck)	4	Substrate formulation	<0.0001
Commercial (Merck)	4	Application/reading condition	<0.0001
Commercial (Merck)	4	Substrate formulation × application/reading condition	<0.0001
Commercial (Merck)	6	Substrate formulation	<0.0001
Commercial (Merck)	6	Application/reading condition	<0.0001
Commercial (Merck)	6	Substrate formulation × application/reading condition	<0.0001
Commercial (Merck)	8	Substrate formulation	<0.0001
Commercial (Merck)	8	Application/reading condition	<0.0001
Commercial (Merck)	8	Substrate formulation × application/reading condition	<0.0001
Female	4	Substrate formulation	0.0052
Female	4	Application/reading condition	0.0464

Female	4	Substrate formulation × application/reading condition	0.0208
Female	6	Substrate formulation	0.0169
Female	8	Substrate formulation	0.0249
Male	4	Substrate formulation	0.0337
Male	4	Application/reading condition	0.0453
Male	4	Substrate formulation × application/reading condition	0.0344
Male	6	Substrate formulation	0.0148
Male	8	Substrate formulation	0.0202

Only significant ANOVA effects ($p < 0.05$) are reported. Nonsignificant effects and post hoc comparisons are not included. Substrate formulation comprises eight combinations of substrate identity (AChCl or BChCl), concentration (30 or 60 mM), and substrate volume (4 or 8 μL). Serum volumes were 4, 6, and 8 μL. SI: dried substrate with immediate reading; S20: dried substrate with reading after 20 min; HI: substrate without prior drying with immediate reading; H20: substrate without prior drying with reading after 20 min. Ordinary two-way ANOVA was used for commercial serum. Repeated-measures two-way ANOVA, with measurements matched by donor for both factors and Geisser-Greenhouse correction, was used for female and male sera. Values are reported to four decimal places or as $p < 0.0001$.

Table S2. Significant effects from two-way ANOVA of blue-channel intensity in the paper-based assay containing 5% glycerol.

A. Substrate formulation × serum volume, analyzed separately within each application/reading condition.

Serum	Condition	Significant effect	p
Commercial (Merck)	SI	Substrate formulation	<0.0001
Commercial (Merck)	SI	Serum volume	0.0034
Commercial (Merck)	SI	Substrate formulation × serum volume	0.0409
Commercial (Merck)	S20	Substrate formulation	<0.0001
Commercial (Merck)	S20	Serum volume	0.0219
Commercial (Merck)	HI	Substrate formulation	0.0011
Commercial (Merck)	H20	Substrate formulation	<0.0001
Female	SI	Substrate formulation	0.0458
Female	S20	Substrate formulation	0.0256
Female	S20	Serum volume	0.0144
Female	HI	Substrate formulation	0.0322
Female	H20	Substrate formulation	0.0029
Male	SI	Substrate formulation	0.0376
Male	SI	Serum volume	0.0112
Male	S20	Substrate formulation	0.0038
Male	S20	Serum volume	0.0223
Male	H20	Substrate formulation	0.0040
Male	H20	Serum volume	0.0069

B. Substrate formulation × application/reading condition, analyzed separately at each serum volume.

Serum	Serum volume (μL)	Significant effect	p
Commercial (Merck)	4	Substrate formulation	<0.0001
Commercial (Merck)	4	Application/reading condition	<0.0001
Commercial (Merck)	4	Substrate formulation × application/reading condition	0.0013
Commercial (Merck)	6	Substrate formulation	<0.0001
Commercial (Merck)	6	Application/reading condition	<0.0001
Commercial (Merck)	6	Substrate formulation × application/reading condition	<0.0001
Commercial (Merck)	8	Substrate formulation	0.0007
Commercial (Merck)	8	Application/reading condition	<0.0001

Commercial (Merck)	8	Substrate formulation × application/reading condition	<0.0001
Female	4	Substrate formulation	0.0077
Female	4	Application/reading condition	0.0023
Female	4	Substrate formulation × application/reading condition	0.0359
Female	6	Substrate formulation	0.0383
Female	6	Application/reading condition	0.0056
Female	8	Substrate formulation	0.0202
Female	8	Application/reading condition	0.0024
Male	4	Substrate formulation	0.0044
Male	4	Application/reading condition	0.0003
Male	6	Substrate formulation	0.0170
Male	6	Application/reading condition	0.0131
Male	8	Substrate formulation	0.0365
Male	8	Application/reading condition	0.0296

Only significant ANOVA effects ($p < 0.05$) are reported. Nonsignificant effects and post hoc comparisons are not included. Substrate formulation comprises eight combinations of substrate identity (AChCl or BChCl), concentration (30 or 60 mM), and substrate volume (4 or 8 μL). Serum volumes were 4, 6, and 8 μL. SI: dried substrate with immediate reading; S20: dried substrate with reading after 20 min; HI: substrate without prior drying with immediate reading; H20: substrate without prior drying with reading after 20 min. Ordinary two-way ANOVA was used for commercial serum. Repeated-measures two-way ANOVA with Geisser-Greenhouse correction was used for female and male sera. Values are reported to four decimal places or as $p < 0.0001$.