Title:

Indirect ELISA-based detection of histatin 3 and cystatin D for the forensic identification of human saliva

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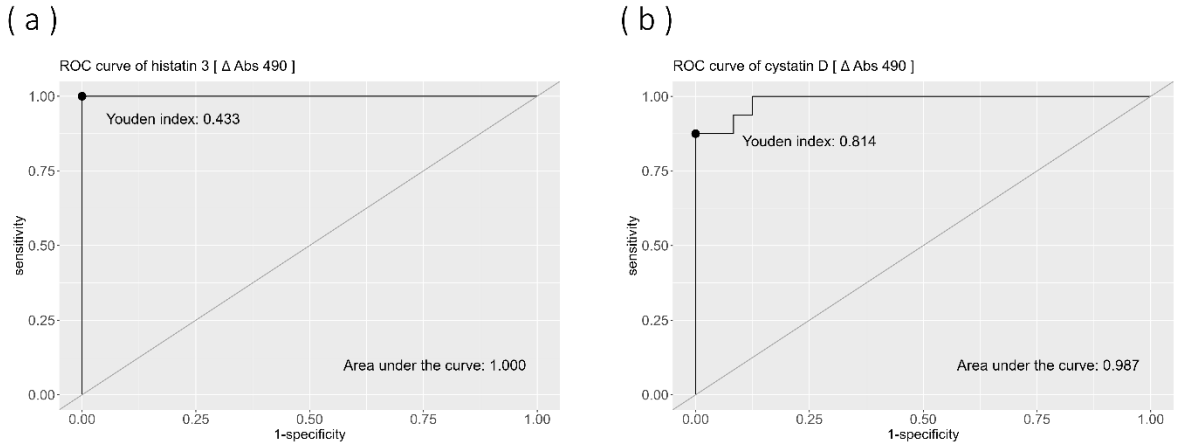
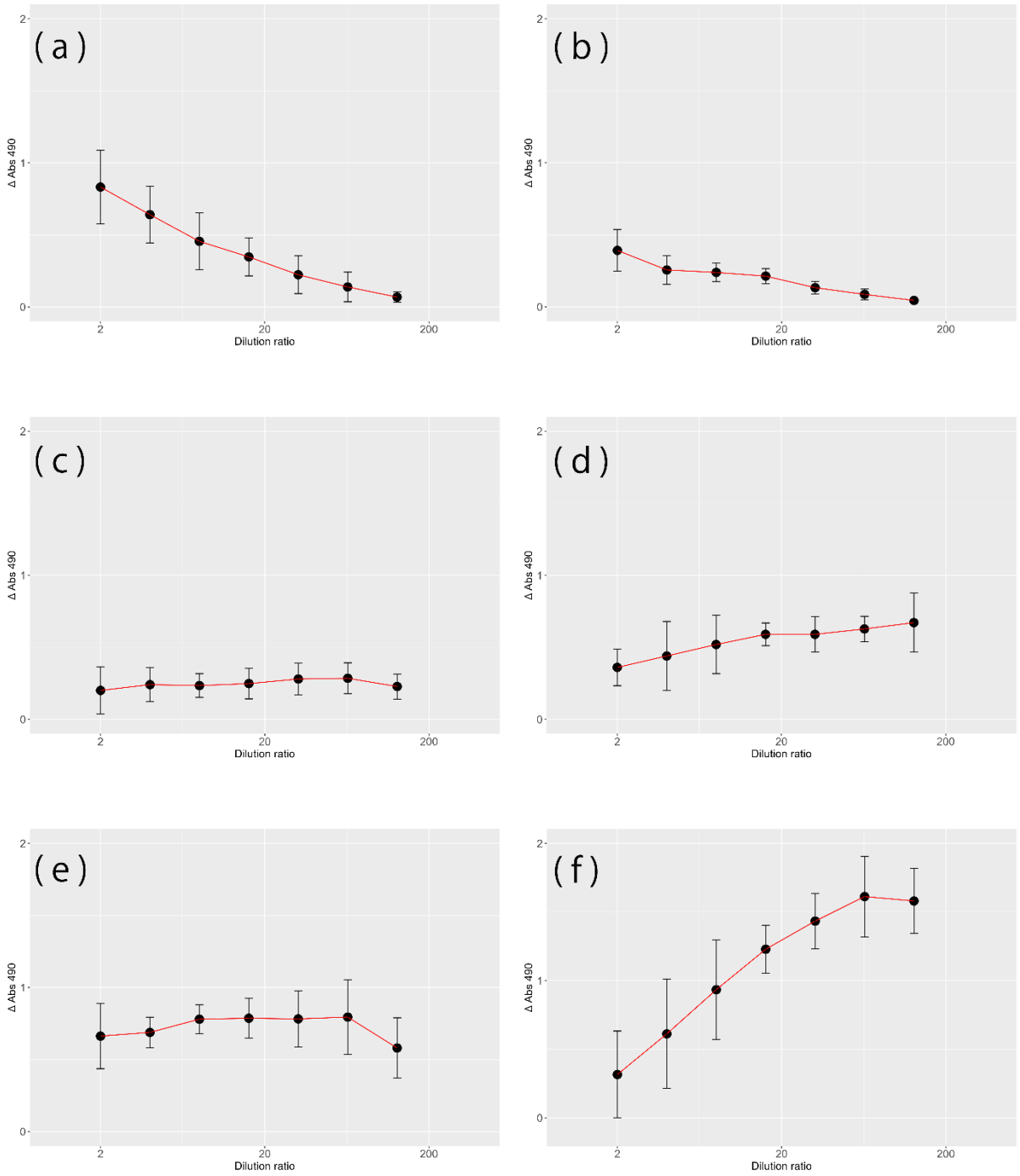


Supplementary Fig. 1 Receiver operating characteristic (ROC) curve analysis of the delta values from various human body fluids.

ROC curves of the delta values from various human body fluid stain samples using (a) histatin 3 and (b) cystatin D markers are shown with Youden's indices. The x-axis and y-axis indicate the false-positive rate (1 − specificity) and the true positive rate (sensitivity), respectively.



Supplementary Fig. 2 Characteristics of changes in the delta values with a sample dilution series.

Stain samples were examined by indirect ELISA using (a, c, e) histatin 3 and (b, d, f) cystatin D markers. The delta values from 10-fold diluted saliva stain samples (a, b) were decreased in a sample dilution-dependent manner. The delta values from the saliva-blood (c, d) and saliva-vaginal secretions (e, f) mixed stain samples did not decrease depending on the sample dilution. The x-axis indicates the dilution ratio (log scale), and the y-axis indicates the delta absorbance values. Point data are shown as

the mean ± standard deviation (n = 4).

Supplementary Table 1 Qualitative results of the sensitivity experiments using the two thresholds.

Samples	n[a]	Number of positive samples			
		Histatin 3		Cystatin D	
		Δ Abs490[b] > AT[c] = 0.1	Δ Abs490 > DT[d] = 0.3	Δ Abs490 > AT = 0.1	Δ Abs490 > DT = 0.8
Saliva (undiluted)	4	4	4	4	4
Saliva (10-fold diluted)	4	4	4	4	0
Saliva (100-fold diluted)	4	3	0	1	0

[a] n: Number of samples.

[b] Δ Abs490: Delta values of absorbance at 490 nm.

[c] AT: Analytical threshold.

[d] DT: Discriminative threshold.

Supplementary Table 2 Qualitative results of the specificity experiments using the two thresholds.

Samples	n[a]	Number of positive samples			
		Histatin 3		Cystatin D	
		Δ Abs490[b] > AT[c] = 0.1	Δ Abs490 > DT[d] = 0.3	Δ Abs490 > AT = 0.1	Δ Abs490 > DT = 0.8
Saliva	16	16	16	16	14
Blood	4	3	0	4	0
Semen	4	0	0	0	0
VS[e]	4	0	0	1	0
Urine	4	0	0	1	0
Sweat	4	0	0	0	0
NS[f]	4	0	0	0	0

[a] n: Number of samples.

[b] Δ Abs490: Delta values of absorbance at 490 nm.

[c] AT: Analytical threshold.

[d] DT: Discriminative threshold.

[e] VS: Vaginal secretions.

[f] NS: Nasal secretions.

Supplementary Table 3 Qualitative results of the mixture experiments using the two thresholds.

Samples	n[a]	Number of positive samples			
		Histatin 3		Cystatin D	
		Δ Abs490[b] > AT[c] = 0.1	Δ Abs490 > DT[d] = 0.3	Δ Abs490 > AT = 0.1	Δ Abs490 > DT = 0.8
Saliva	4	4	4	4	3
Saliva-Blood	4	4	2	4	2
Saliva-VS[e]	4	4	4	4	4

[a] n: Number of samples.

[b] Δ Abs490: Delta values of absorbance at 490 nm.

[c] AT: Analytical threshold.

[d] DT: Discriminative threshold.

[e] VS: Vaginal secretions.