ATR-IR spectroscopy application to diagnostic screening of advanced endometriosis

Izabela Kokot[1*], Sylwester Mazurek[2*], Agnieszka Piwowar[3], Roman Szostak[2], Marcin Jędryka[4,5], Ewa Maria Kratz[1]

[1] Department of Laboratory Diagnostics, Division of Laboratory Diagnostics, Faculty of Pharmacy, Wroclaw Medical University, Borowska Street 211A, 50-556 Wroclaw, Poland

[2] Faculty of Chemistry, University of Wrocław, F. Joliot-Curie 14, 50-383 Wrocław, Poland

[3] Department of Toxicology, Faculty of Pharmacy, Wroclaw Medical University, Borowska Street 211, 50-556 Wroclaw, Poland

[4] Department of Oncology, Gynecological Oncology Clinic, Faculty of Medicine, Hirszfeld Square 12, 53-413 Wroclaw Medical University, Wroclaw, Poland

[5] Department of Oncological Gynecology, Wroclaw Comprehensive Cancer Center, Hirszfeld Square 12, 53-413 Wroclaw, Poland

Correspondence to: Izabela Kokot: izabela.kokot@umed.wroc.pl or Sylwester Mazurek: sylwester.mazurek@chem.uni.wroc.pl

Supporting Informations

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Table S1. Serum parameters

	Endometriosis *n* = 29	Non-endometriosis *n* = 24	Control *n* = 18
	Mean ± SD	**Mean ± SD**	**Mean ± SD**
Estradiol [pg/mL]	83.63 ± 83.93	96.59 ± 81.74	77.52 ± 65.17
PRL [ng/mL]	29.01 ± 18.71	27.02 ± 18.62	12.84 ± 4.86
CA 125 [U/mL]	113.41 ± 129.42	23.64 ± 17.39	14.28 ± 7.40
IgG [mg/dL]	1065.46 ± 286.73	1078.22 ± 298.25	1237.28 ± 215.91
TAS [mmol/L]	1.50 ± 0.18	1.61 ± 0.27	1.60 ± 0.22
hsCRP [mg/L]	14.29 ± 19.45	13.88 ± 19.96	0.96 ± 1.19
Glucose [mg/dL]	77.79 ± 25.97	69.79 ± 17.91	86.24 ± 10.63
Total protein [g/dL]	6.89 ± 1.32	6.61 ± 0.68	6.77 ± 0.38
Albumin [g/dL]	4.36 ± 0.64	4.13 ± 0.32	4.26 ± 0.19
T-BIL [mg/dL]	0.54 ± 0.37	0.65 ± 0.45	0.59 ± 0.24
Calcium [mg/dL]	9.62 ± 1.15	9.35 ± 0.49	9.29 ± 0.25
Magnesium [mg/dL]	2.44 ± 0.37	2.47 ± 0.18	2.22 ± 0.14
Iron [μg/dL]	104.10 ± 60.76	107.21 ± 74.27	105.89 ± 38.24
T-CHOL [mg/dL]	197.83 ± 48.31	181.50 ± 28.66	190.67 ± 36.63
HDL [mg/dL]	53.79 ± 12.84	53.96 ± 10.38	55.44 ± 12.52
TG [mg/dL]	105.62 ± 44.41	111.79 ± 78.38	102.06 ± 42.21
Uric Acid [mg/dL]	4.78 ± 1.02	4.85 ± 1.38	4.72 ± 0.95
LDL [mg/dL]	122.86 ± 37.35	105.08 ± 23.54	114.72 ± 30.54
hsIL-1β [pg/ml]	0.57 ± 0.39	0.56 ± 0.46	0.27 ± 0.26
IL-6 [pg/ml]	19.33 ± 43.69	18.05 ± 34.09	1.47 ± 1.48
FRAP [mmol/L]	1.11 ± 0.26	1.18 ± 0.30	0.95 ± 0.22
AOPP [μmol/L]	235.16 ± 150.41	181.78 ± 156.00	105.16 ± 49.24
YKL-40 [ng/ml]	685.22 ± 1246.58	403.29 ± 934.76	104.12 ± 154.07
SIRT3 [ng/mL]	12.32 ± 7.59	11.11 ± 6.80	11.22 ± 6.03
SIRT5 [ng/mL]	8.21 ± 4.41	9.61 ± 5.72	8.49 ± 6.04
SIRT6 [ng/mL]	12.32 ± 16.84	15.88 ± 18.74	8.45 ± 10.95
Telomerase [ng/mL]	1.61 ± 1.88	1.82 ± 2.96	0.39 ± 0.52

AOPP — advanced protein oxidation products; CA 125 — carcinoma antigen 125; FRAP — ferric reducing antioxidant power; HDL — high-density lipoprotein cholesterol; hsCRP — high sensitive C-reactive protein; hsIL-1β — high sensitive interleukin 1β; IgG — immunoglobulin G; IL-6 — interleukin 6; LDL — low-density lipoprotein cholesterol; PRL — prolactin; SIRT3 — sirtuin 3; SIRT5 — sirtuin 5; SIRT6 — sirtuin 6; TAS — total antioxidant status; TG — triglycerides; T-BIL — total bilirubin; T-CHOL — total cholesterol; YKL-40 — chitinase-3-like protein 1.

Table S2. Confusion matrix for PLS-DA models after iPLS variable selection

Data set		Calibration			Cross-validation		
		Actual C	Actual E	Actual NE	Actual C	Actual E	Actual NE
Biochemical parameters	Predicted as C	17	3	2	17	3	3
	Predicted as E	0	21	0	0	20	2
	Predicted as NE	1	2	18	1	3	15
ATR spectra	Predicted as C	17	0	1	15	1	1
	Predicted as E	0	28	0	1	26	1
	Predicted as NE	0	1	23	1	2	22
Fused data	Predicted as C	18	1	1	18	2	1
	Predicted as E	0	25	1	0	24	3
	Predicted as NE	0	3	22	0	3	20

E – endometriosis, NE – non-endometriosis, C – control group of healthy women.

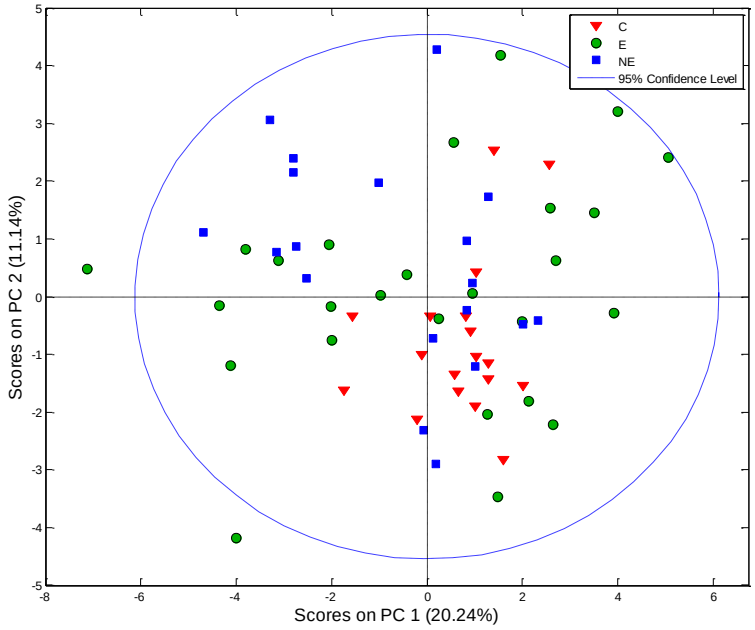


Figure S1. PCA scores based on biochemical parameters of serum samples. E – endometriosis, NE – non-endometriosis, C – control group of healthy women.

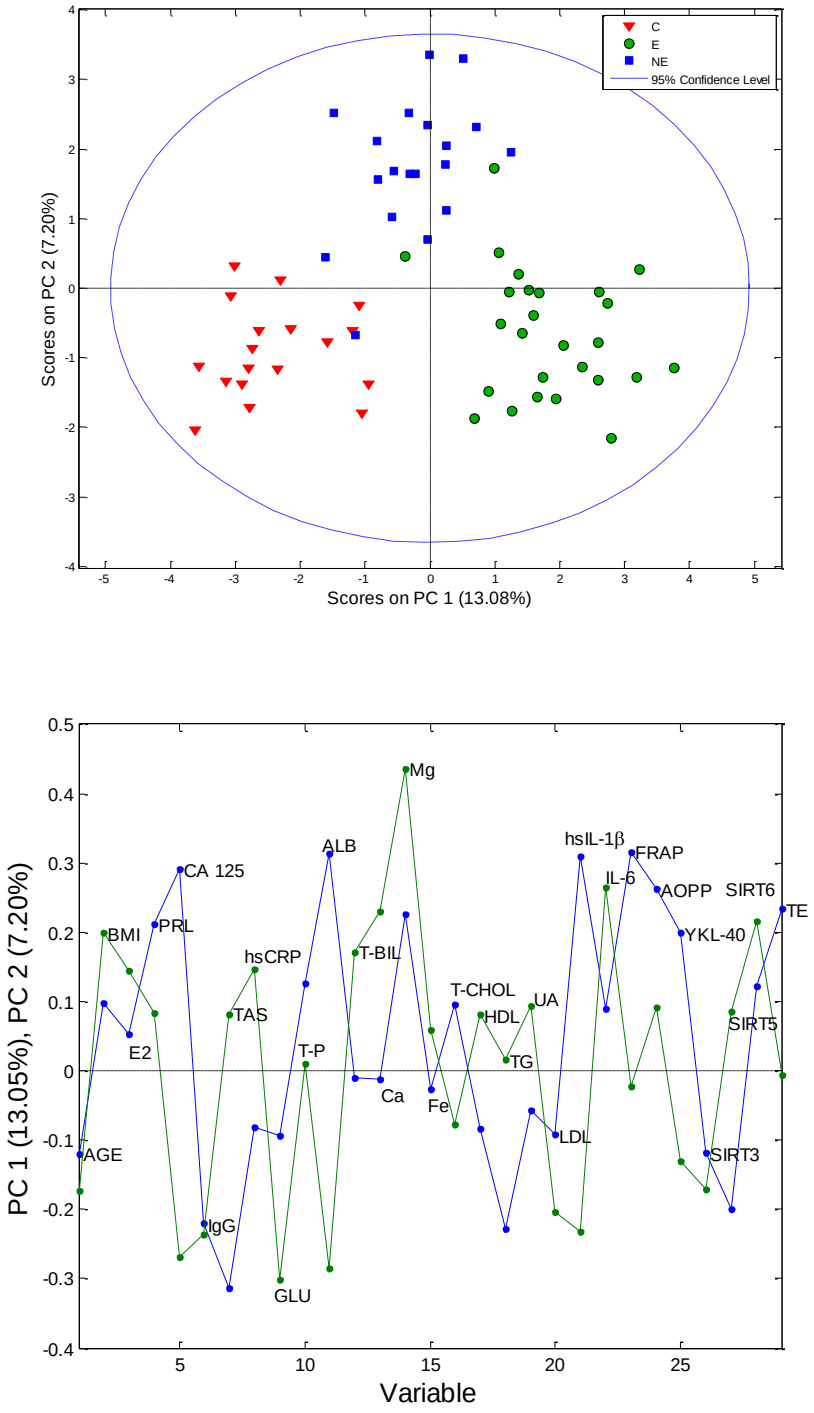


Figure S2. PCA of biochemical serum parameters obtained with GLSW pre-treatment; scores (top) and loadings (bottom). E – endometriosis, NE – non-endometriosis, C – control group of healthy women. ALB — albumin; AOPP — advanced protein oxidation products; BMI — body mass index; Ca — calcium; CA 125 — carcinoma antigen 125; E2 – estradiol; Fe — iron; FRAP — ferric reducing antioxidant power; GLSW — general least squares weighting; GLU — glucose; HDL — high-density lipoprotein cholesterol; hsCRP — high sensitive C-reactive protein; hsIL-1β — high sensitive interleukin 1β; IgG — immunoglobulin G; IL-6 — interleukin 6; LDL — low-density lipoprotein cholesterol; Mg — magnesium; PRL — prolactin; SIRT3 — sirtuin 3; SIRT5 — sirtuin 5; SIRT6 — sirtuin 6; TAS — total antioxidant status; TE — telomerase; TG — triglycerides; T-P — total protein; T-BIL — total bilirubin; T-CHOL — total cholesterol; UA — uric acid; YKL-40 — chitinase-3-like protein 1.

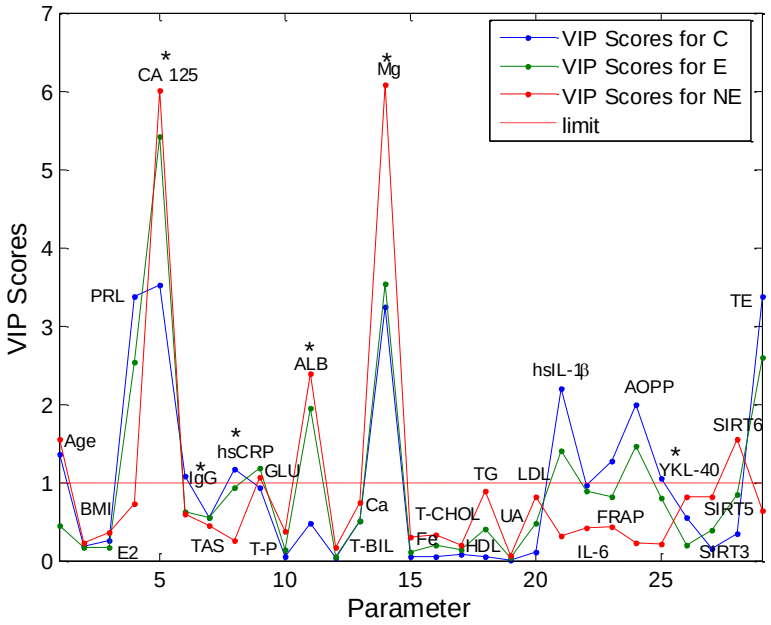


Figure S3. VIP scores for PLS-DA modeling of biochemical data of serum samples; * indicates variables selected by the iPLS. E – endometriosis, NE – non-endometriosis, C – control group of healthy women. ALB — albumin; AOPP — advanced protein oxidation products; BMI — body mass index; Ca — calcium; CA 125 — carcinoma antigen 125; E2 – estradiol; Fe — iron; FRAP — ferric reducing antioxidant power; GLU — glucose; HDL — high-density lipoprotein cholesterol; hsCRP — high sensitive C-reactive protein; hsIL-1β — high sensitive interleukin 1β; IgG — immunoglobulin G; IL-6 — interleukin 6; LDL — low-density lipoprotein cholesterol; Mg — magnesium; PRL — prolactin; SIRT3 — sirtuin 3; SIRT5 — sirtuin 5; SIRT6 — sirtuin 6; TAS — total antioxidant status; TE — telomerase; TG — triglycerides; T-P — total protein; T-BIL — total bilirubin; T-CHOL — total cholesterol; UA — uric acid; YKL-40 — Chitinase-3-like protein 1.

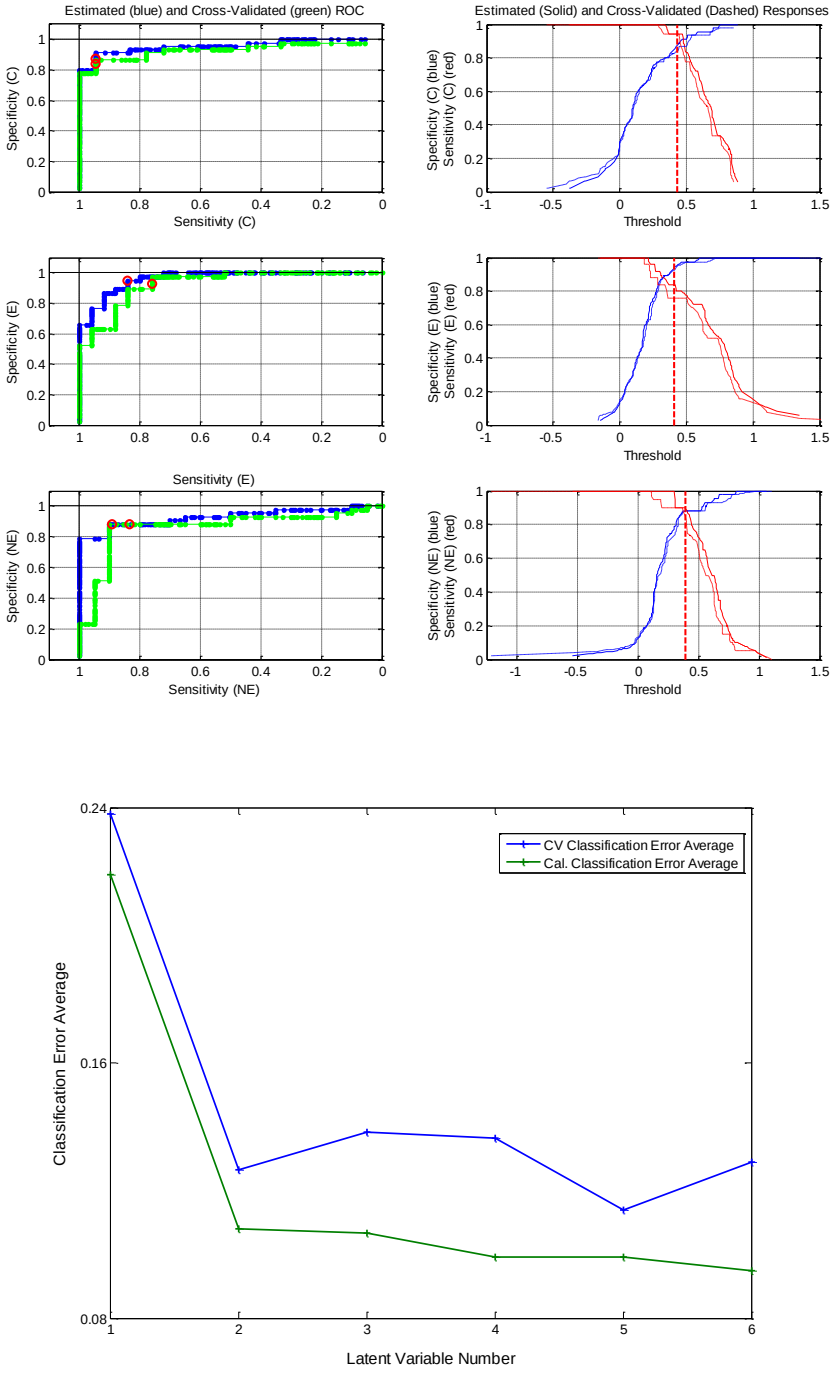


Figure S4. PLS-DA of serum biochemical parameters; ROC plots for three groups of samples (top); calibration and cross-validation errors for the model constructed applying variables selected by iPLS (bottom). E – endometriosis, NE – non-endometriosis, C – control group of healthy women.

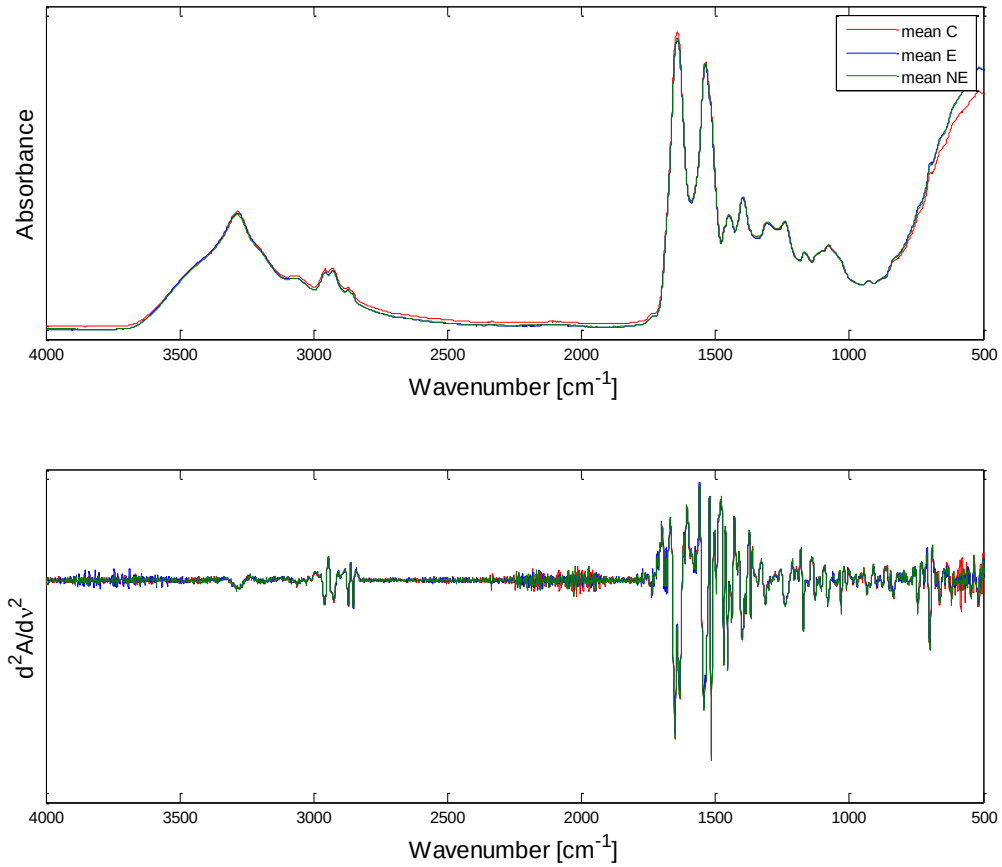


Figure S5. Average FTIR ATR spectra of serum samples for three studied groups (top) and second derivatives of spectra (bottom). E – endometriosis, NE – non-endometriosis, C – control group of healthy women.

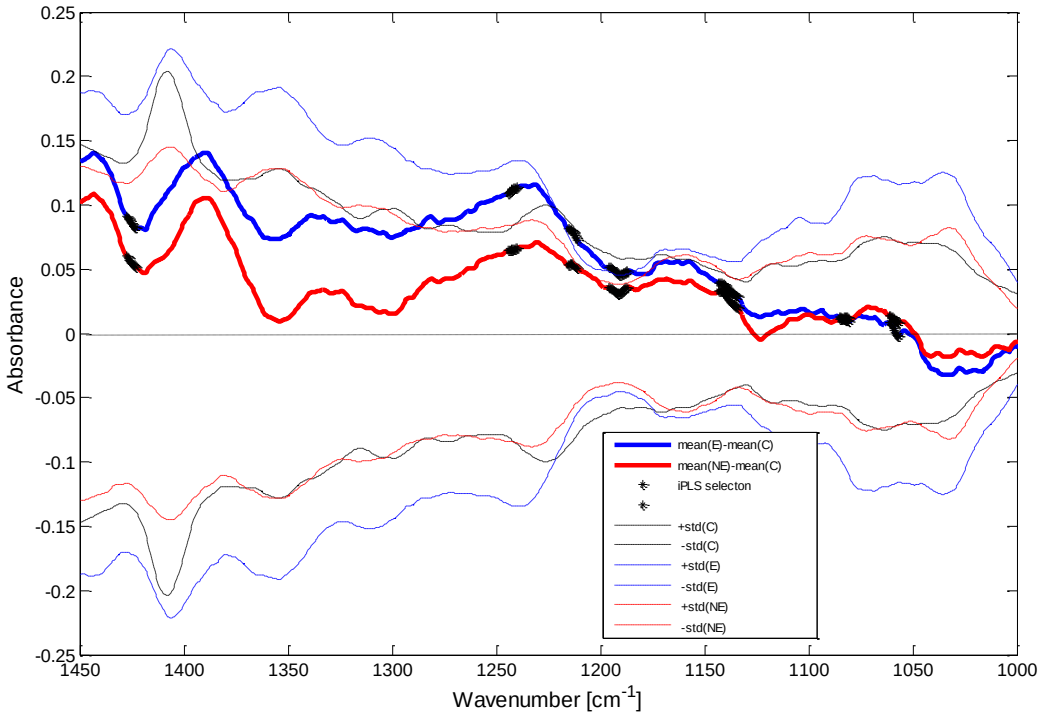


Figure S6. Difference spectra between the mean of sera samples with +/- standard deviation of signals' intensities. E – endometriosis, NE – non-endometriosis, C – control group of healthy women.

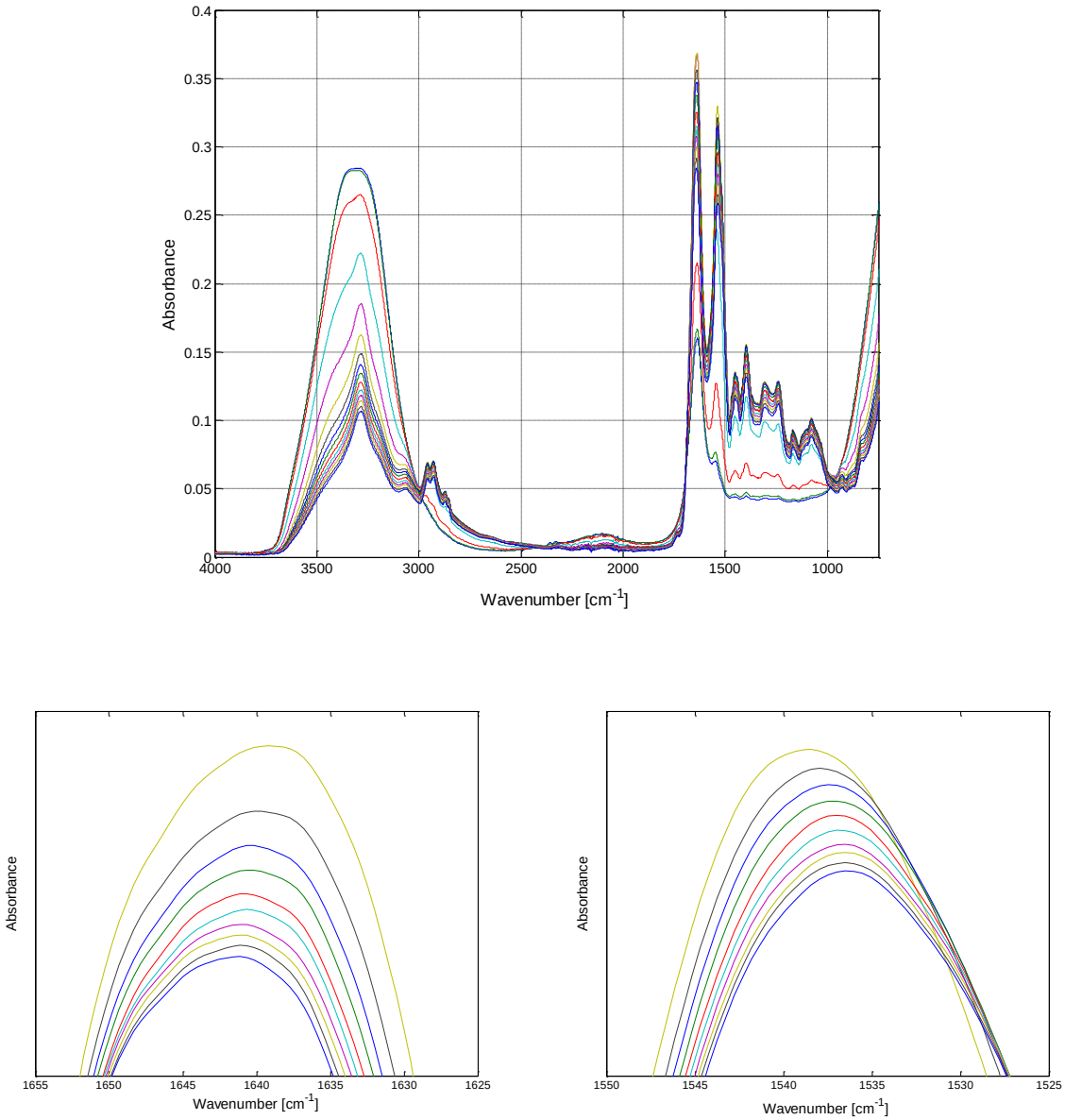


Figure S7. IR spectra of serum sample dried on an ATR crystal collected in 10-min. intervals (top); change of the peak position of the Amide I (left) and Amide II (right) bands during thin film formation (bottom). E – endometriosis, NE – non-endometriosis, C – control group of healthy women.

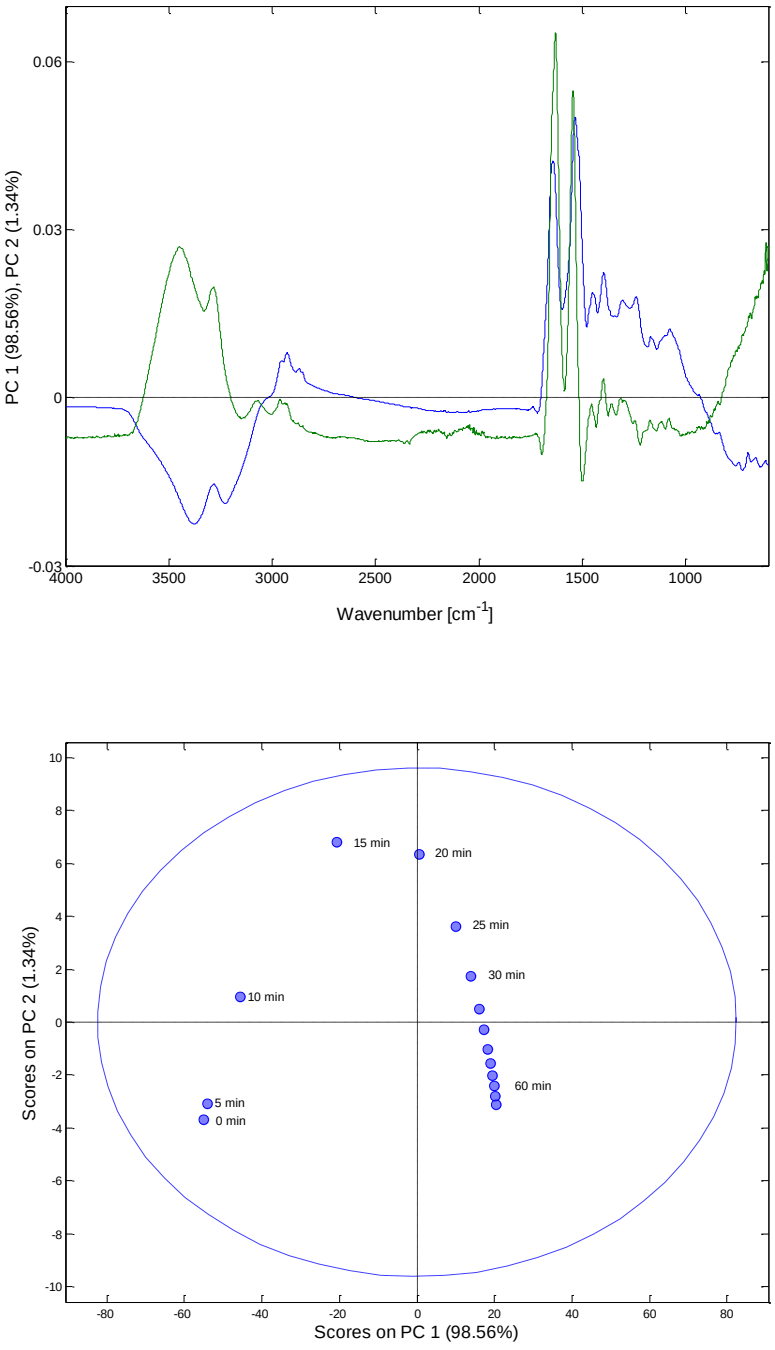


Figure S8. PCA for ATR spectra of serum sample during drying and thin film formation: loadings of two first principal components (top) and scores plots (bottom).

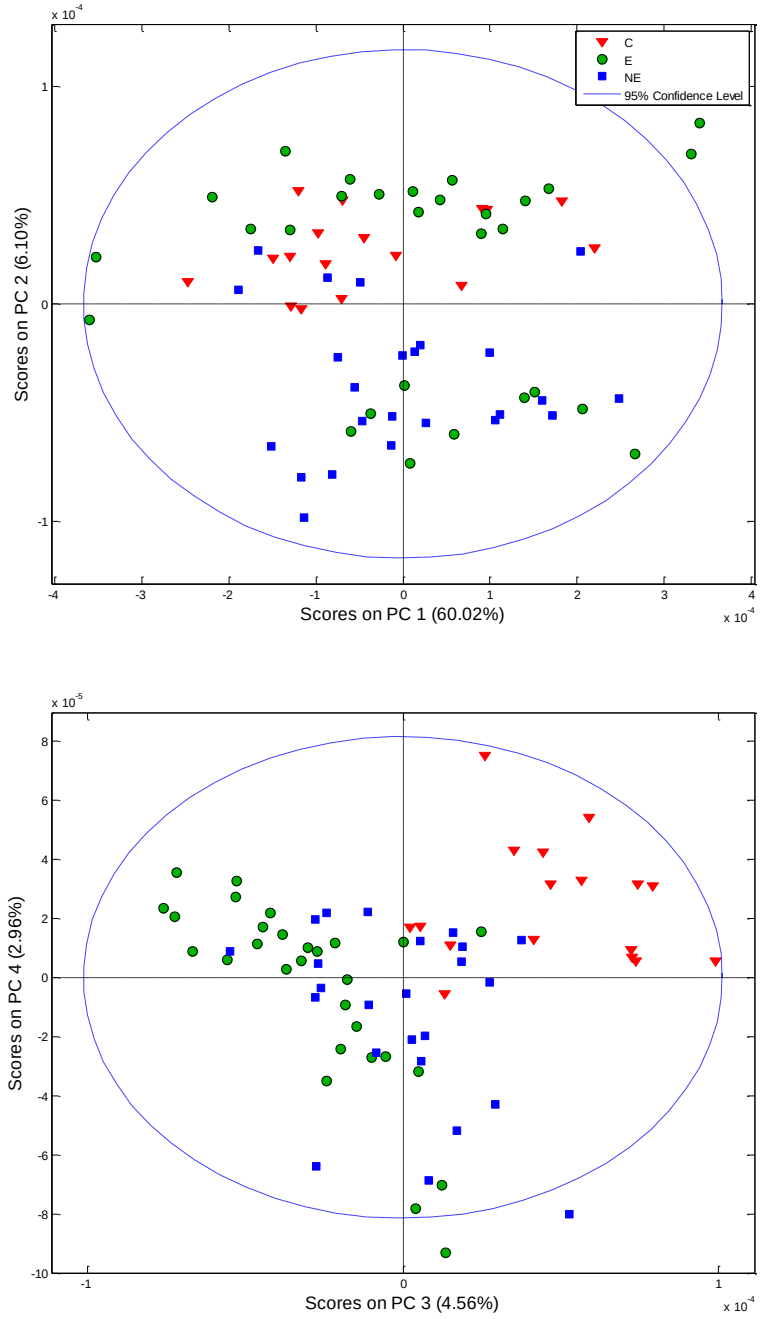


Figure S9. PCA scores for ATR spectra of serum samples obtained based on 700-1450 cm^{-1} spectral range; loadings of two first principal components (top) and third and fourth principal components (bottom). E – endometriosis, NE – non-endometriosis, C – control group of healthy women.

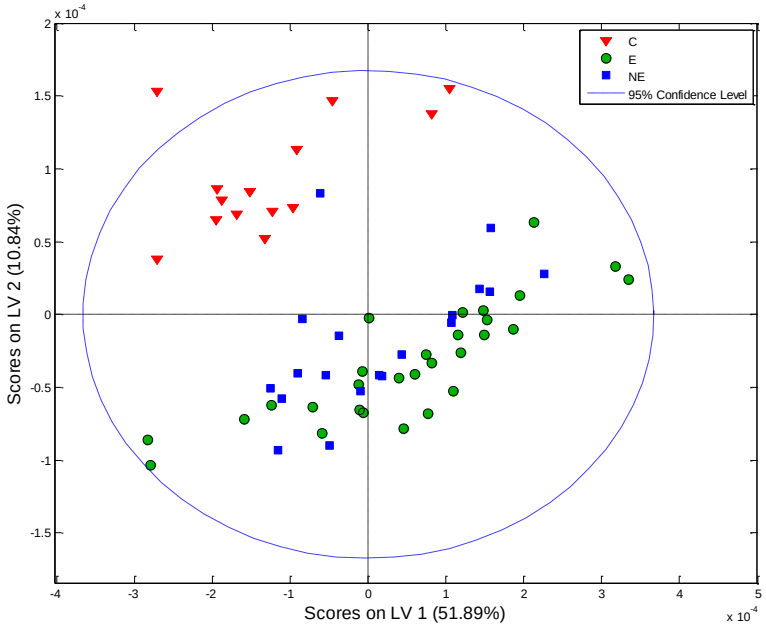


Figure S10. PLS-DA scores for ATR spectra of serum samples obtained based on 700-1450 cm^{-1} spectral range. E – endometriosis, NE – non-endometriosis, C – control group of healthy women.

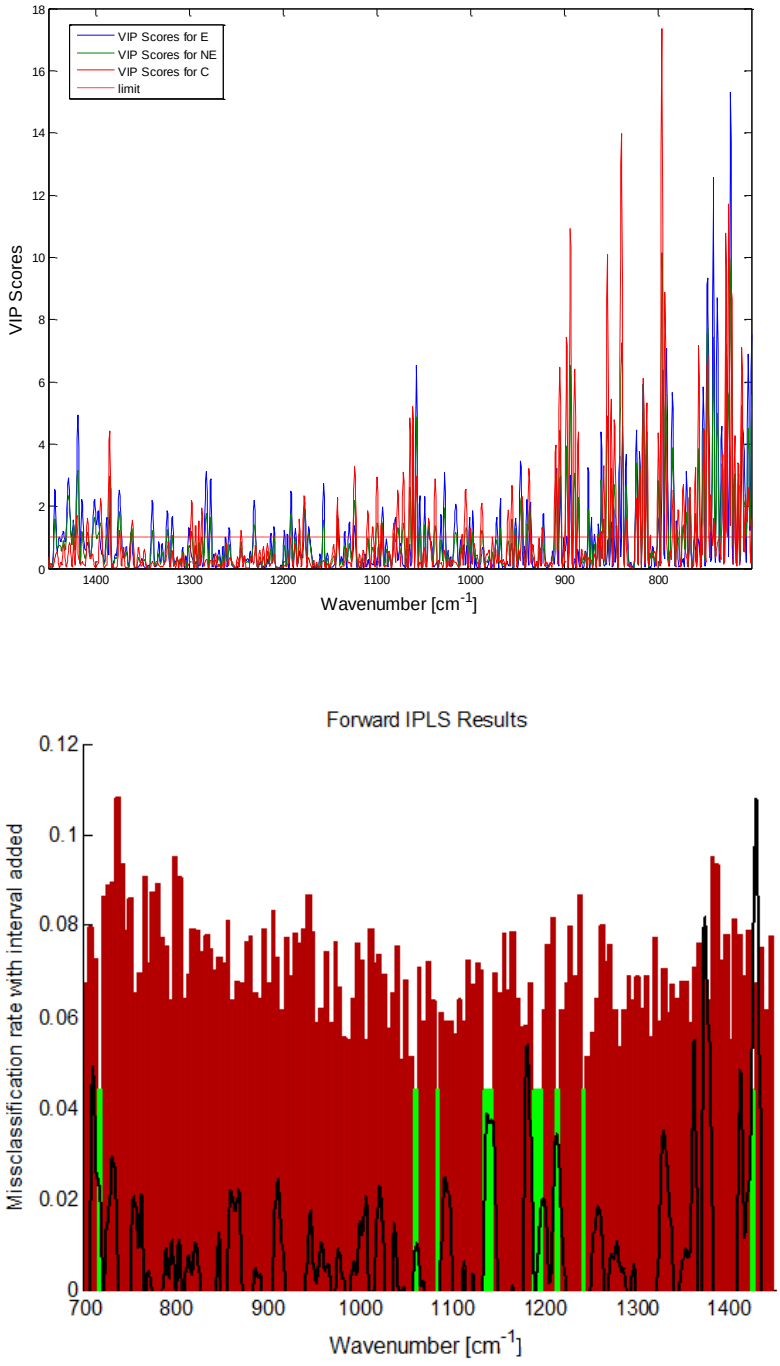
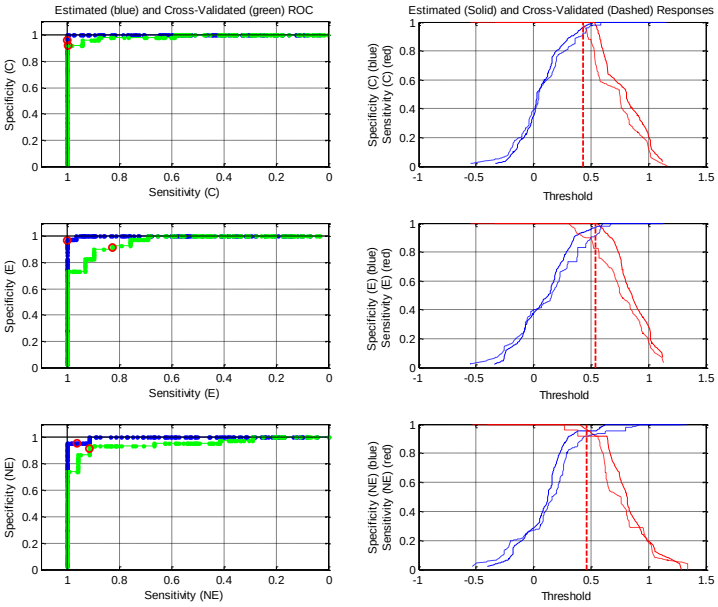


Figure S11. VIP scores of PLS-DA (top) and iPLS variable selection (bottom) for ATR data in the 700-1450 cm^{-1} spectral range. E – endometriosis, NE – non-endometriosis, C – control group of healthy women.



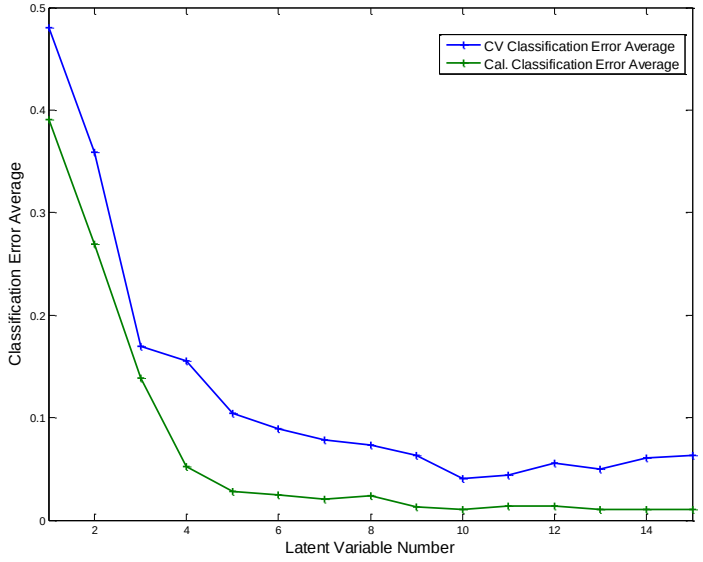


Figure S12. PLS-DA of ATR spectra of sera - ROC plots for all three groups of samples (top) and calibration/cross-validation errors for the model constructed applying variables selected by iPLS (bottom). E – endometriosis, NE – non-endometriosis, C – control group of healthy women.

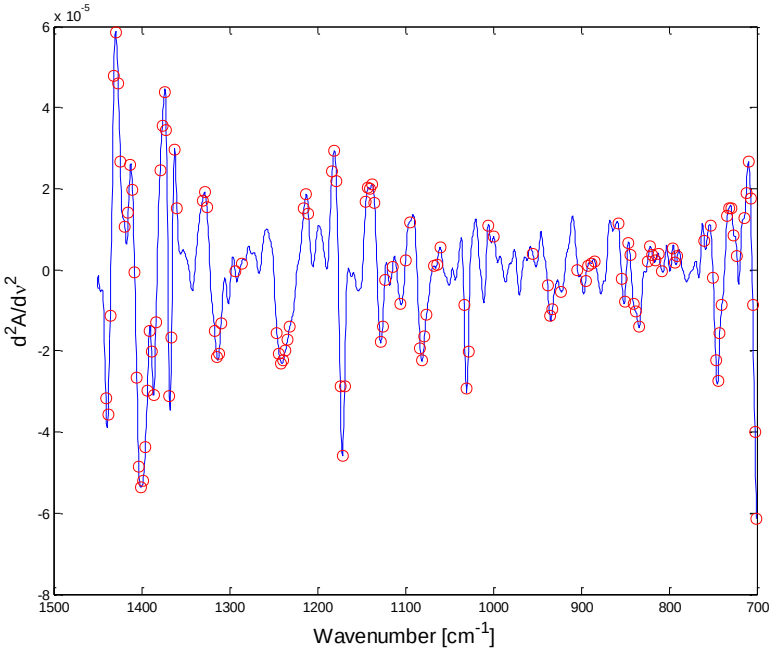


Figure S13. ATR inputs selected by PCA for fused data modeling.

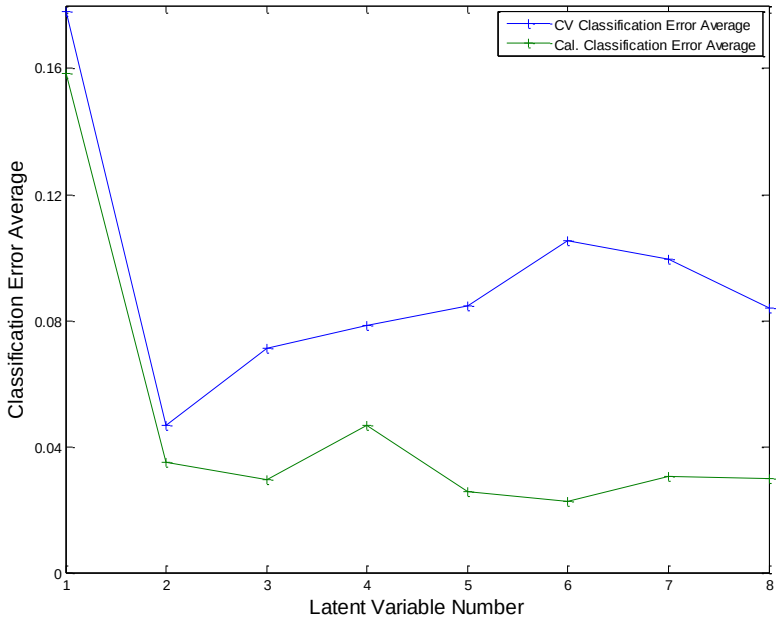


Figure S14. Errors of classification for PLS-DA model constructed on the basis of fused data.