A TARGETED LIPID–PROTEIN ASSAY ENABLES EARLY DETECTION OF OVARIAN CANCER IN SYMPTOMATIC PATIENTS

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MSACL Mass Spectrometry & Advances in the Clinical Lab

Delayed Diagnosis for Ovarian Cancer Results in Poor Patient Outcomes

- Vague, non-specific symptoms for ovarian cancer (OC) overlap with benign conditions
- ~85% of OC patients are symptomatic at early-stages, but average time to diagnosis in the U.S. is 9 months
- 5-year survival rates for early-stage disease are 92%, compared to just 32% for late-stage disease; early diagnosis is critical
- Standard-of-care biomarkers lack sensitivity & specificity to triage symptomatic women (ref 1-4)

Symptomatic women
A higher-prevalence population for earlier triage

	Weeks 1–8	Weeks 9–12	Weeks 13–24	Weeks 25–44+
Current Journey	Symptom onset	PCP/OB-GYN visit	Multiple tests, imaging, specialty care	GynOnc referral, additional blood testing, imaging, surgery, final diagnosis
AOA-Assisted Journey	Symptom onset	PCP/OB-GYN visit, AOA Blood test	Imaging, GynOnc referral, confirmation of diagnosis	

Faster Diagnosis
Higher Survival Rate

Translating Discovery Work to a Targeted Assay

DISCOVERY LIPIDOMICS
~1,000
features pre-filtered from >5k detected
Two Discovery Cohorts
n = 872 (ref 5)

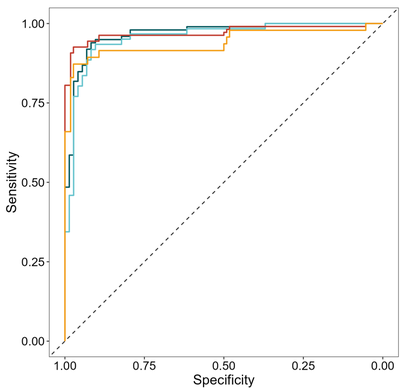
STAGED FILTERING
- Identification confidence (~200 excluded)
- Detection reproducibility (~200 excluded)
- Predictive capability (~350 excluded)
- QqQ feasibility (~150 excluded)

LIPID MRM ASSAY DEVELOPMENT
~100
features carried forward into assay development

LIPID DOWN-SELECTION
- Independent cohort testing
- Directionality
- Predictive capability
- Identification confidence

TARGETED ASSAY
9 targeted markers
7 lipids
2 proteins

Assay Performance is Maintained in an Independent Hold-Out Set



	Training set AUC with 95% CI	Hold-out set AUC with 95% CI
All-stage (I/II/III/IV) OC	0.97 (0.95–1.00)	0.97 (0.94–0.99)
Early-stage (I/II) OC	0.94 (0.89–0.99)	0.96 (0.92–0.99)

COHORT COMPOSITION

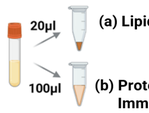
Cohort	Early-stage OC (I/II)	Late-stage OC (III/IV)	Symptomatic Controls	Total
Training set	47	62	112	221
Hold-out set	61	38	73	172

Sample Extraction and Targeted Assay

Sample Collection

Cohorts include serum samples from various conditions, including normal, benign, and different stages and subtypes of ovarian cancer

Sample Preparation

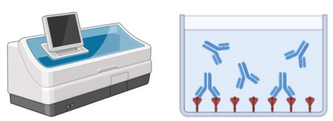


Patient-derived samples were processed for detection of lipids by LC-MS and proteins by immunoassay.

Targeted Lipid MRM Assay (QqQ instruments)

SCIEX Citrine QqQ

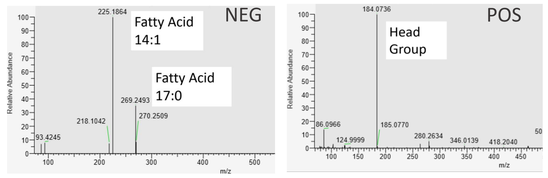
Clinically Validated Immunoassay (Cobas®)



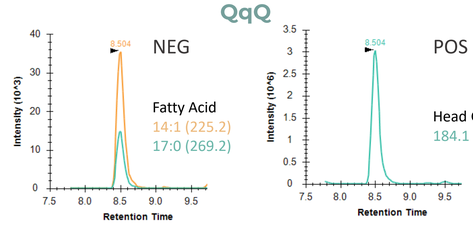
Analytical Characterization Underpins Classifier Confidence

- **Lipid identification** by matching HRMS to QqQ spectra. Example: 17:0-14:1 PC-d5 (Avanti, A85683)

HRMS

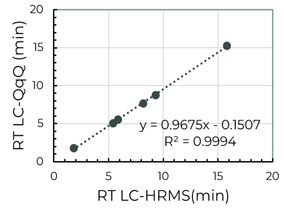


QqQ



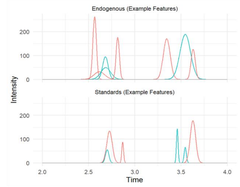
- **RT alignment** to confirm lipid detection on the QqQ MS.

RT anchor values for deuterated internal standards are used to align the HRMS and QqQ feature identification.



- **Ratiometric peak area** for relative quantification.

Each endogenous feature is normalized to a class-matched internal standard. Next step: develop a calibrated assay using species-level lipid standards.



- **Analytical performance of the LC-MRM candidate method**

Confidence in classifier performance is linked to analytical performance (ref 6). As part of development, we therefore characterized the analytical performance of our assay.
(1) Total imprecision was estimated by repeated measurements over 5 days.
(2) Stability upon freeze-thaw and during room temperature (RT) and 4°C storage was established at a limit of <10% change from reference.
(3) Linearity was examined with an admixture of ovarian cancer (OC) and normal human serum (NHS) pools at a ratio of 1:3 (Sample I), 1:1 (Sample II), 3:1 (Sample III). %Recovery in samples with <25% CV is reported.
(4) Functional sensitivity was evaluated by comparing measured signal in patient samples (N=~1,500) against the assay's lowest reliably measured signal (≤25% CV), established via serial dilution of NHS in analyte-free surrogate matrix.

Lipid ID	Imprecision	Functional Sensitivity		Lipid Stability (<10% loss from reference)			Linearity		
				NHS or OC			Recovery		
	Total CV	Maximal NHS dilution	Quantification in >95% patient samples	Room Temp	4 °C	Freeze-thaw	Sample I	Sample II	Sample III
Lipid 1	6.4%	1,000x	Yes	1 day	7 days	5 cycles	97.9%	95.5%	94.3%
Lipid 2	5.3%	1,000x	Yes	3 days (NHS) 2 days (OC)	7 days	5 cycles	96.1%	95.4%	94.4%
Lipid 3	2.1%	100x	Yes	3 days.	7 days	5 cycles	99.7%	98.8%	96.1%
Lipid 4	4.0%	100x	Yes	3 days (NHS) 0 day (OC)	7 days (NHS) 1 day (OC)	5 cycles	98.2%	99.0%	91.3%
Lipid 5	3.4%	10x	Yes	1 day	7 days	5 cycles	103.6%	100.3%	97.8%
Lipid 6	2.9%	10x	Yes	3 days (NHS) 2 days (OC)	7 days	5 cycles	102.1%	97.4%	92.0%
Lipid 7	4.0%	10x	Yes	3 days	7 days	5 cycles	99.9%	99.5%	93.7%

CONCLUSIONS

- Early detection of ovarian cancer is enabled with an LC-MRM method for 7 lipids.
- Analytical characterization of our assay confirmed low imprecision with high stability and linearity.
- Transition to external calibration and analytical validation is underway.

References: 1. Committee Opinion No. 477. Obstet Gynecol, 2011; 2. Bast et al. Cancer Epidemiol Biomarkers Prev. 2020; 3. Whitwell et al., Br J Cancer, 2020; 4. American Cancer Society; 5. Giles et al., Cancer Res Commun, 2025; 6. Grant et al, Clin Chem, 2014