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SUPPLEMENTARY DATA

MINIREVIEW

High-specificity nucleic acid aptamers for detection of ovarian cancer protein biomarkers: Application in diagnostics

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Supplementary Table 1. Nucleic acid diagnostic aptamers developed for the detection of ovarian cancer clinical protein biomarkers CA125 and HE4 and corresponding conditions of *in vitro* selection and characterization**.**

Ovarian cancer protein biomarker	Target protein details	Aptamer type	Aptamer sequence (5'-3') *	Library and length of random region (N)	Number of cycles	Counter-selection	Binding characterization	Dissociation constant	Development of the diagnostic test	References
CA125	Recombinant 6xHis-tagged CA125	RNA	**CA125.1:** GGGAGACAAGAAUAAACGCUCA**AAAAUGCAUGGAGCGAAGGUGUGGG GGAUACCAACCGCGCCGUG**UUCGACAGGAGGCUCACAACAGGC	(2'-F-Py) modified ssRNA N = 45	**8** Incubation 30 min at RT	Yes (VEGF)	RT-qPCR SPR	**CA125.1:** 4.13 x 10^{-9} M	No	Lamberti et al, 2016
CA125	Purified CA125 from human ascites	DNA	**CA125_1:** FAM-AGCAGCACAGAGGTCAGATG**ACTAGCTCCGATCTTTCTTATCTAC**CCT ATGCGTGCTACCGTGAA **CA125_12:** FAM-AGCAGCACAGAGGTCAGATG**TGCCTTATTACTCTCTCCTGTTAAC**CCTATG CGTGCTACCGTGAA	5'FAM modified ssDNA N = 25	**4** Incubation 1 hour at RT	No	FA APCE	**CA125_1:** 207 ± 109 U/mL by FA and 80 ± 38 U/mL by APCE **CA125_12:** 118 ±123 U/mL by FA and 131 ± 93 U/mL	Yes	Scoville et al, 2017
CA125	Recombinant human 6xHis-tagged CA125	DNA	**rCAA-8:** GCGCGGATCCCGCGC**ACCACCACCACGACGCACGAGTACCCCGCG**CGCGC GAAGCTTGCG	Unmodified ssDNA N = 30	**10** Incubation 1 hour at 37 °C	Yes (BSA) Negative selection (Ni-NTA sepharose beads)	direct ELISA BLI	166 x 10^{-9} M	Yes	Gedi et al, 2018
CA125	Native CA125 from human ascites	DNA	**Apt 2.26** TAGGGAAGAGAAGGACATATGATTTTA GGGAAGAGAAGGACTTTTATGCCGCCTTGACTAGTA CATGACCACTTGA	Unmodified ssDNA N = 30	**6** Incubation 1 hour at 25 °C	No Negative selection (nitrocellulose membrane)	Membrane-based assessment of bound DNA Dot ELASA NALFA DPV	166 x 10^{-9} M	Yes	Triphati et al, 2020a
HE4	Human recombinant GST- tagged HE4	DNA	**A1: TTATCGTACGACAGTCATCCTACAC A3: CACAGTGCGTCACATTTAGGGCATT B10: CAGTGCGTGCTTATTGGCGTAGCGTC**	5' FAM modified ssDNA N = 25	**5** Incubation 30 min at 25 °C	Yes (GST)	APCE FA	**A1:** 2.2 x 10-6 M with FA and 390 x 10-9 M with APCE **A3:** 9.1 x 10-6 M with FA and 500 x 10-9 M with APCE **B10:** 280 x 10-9 M with FA and 870 x 10-9 M with APCE	Yes	Eaton et al, 2015

* Internal aptamer ID provided by authors and corresponding sequence; the sequence in bold indicate random region. The aptamer sequence listed above is the sequence synthesized, characterized and assayed post-SELEX; **CA125** - Cancer Antigen 125 ; **HE4** - Human Epididymis protein 4 ; **STIP1** - Stress-induced phosphoprotein 1 ; **VEGF** - Vascular Endothelial Growth Factor ; **GST** - glutathione-S-transferase ; **RT** - room temperature ; **RT-qPCR** - reverse transcriptase - quantitative polymerase chain reaction ; **BSA** - bovine serum albumin ; **SPR** - surface plasmon resonance ; **FA** - fluorescence anisotropy ; **APCE** - affinity probe capillary electrophoresis ; **ELASA** - Enzyme-linked aptamer sorbent assay ; **NALFA** - Nucleic acid lateral flow assay ; **DPV** - Differential pulse voltammetry ; **ELISA -** The enzyme-linked immunosorbent assay ; **BLI** – biolayer interferometry ; **n/a** – not available (not applicable)

Supplementary Table 2. Aptamer-based diagnostic tests for the detection of ovarian cancer protein biomarkers with the corresponding analytical performances.

Cancer biomarker	Type of diagnostic test	Description	Aptamer	Aptamer sequence (5' - 3')	Limit of detection	Target protein detection range	Correlation with clinically relevant protein concentration**	Tested in human samples	Type of human samples included in the study	Tested on the ovarian cancer patients	Reference
CA125	Biosensor	Field effect transistor type aptasensor based on carboxylated multiwalled carbon nanotubes immobilized onto reduced graphene oxide film	DNA	TTATCGTACGACAGTCATCCTACAC*	5.0×10^{-10} U/mL	1 x 10−9 U/mL to 1U/mL	No	Yes	Serum	Yes (n=1)	Majd and Salimi, 2018
CA125	Fluorescence-based sandwich assay	A chip-based assay using a three-dimensional network of carbon nanotubes surface and anti-CA125 antibody-aptamer pair	DNA	5'-ACC ACC ACC ACG ACG CAC GAG TAC CCC GCG-6-FAM-3	10 pg/mL	1 pg mL/1 to 1 µg/mL	Yes	No	n/a	No	Gedi et al, 2018
CA125	Biosensor	Target induced strand displacement on electrospun Ag nanoparticles nanofibers, cyclic voltametry	DNA	TTATCGTACGACAGTCATCCTACAC*	0,0042 U/mL	0,01 to 350 U/mL	Yes	Yes	Serum	No	Farzin et al, 2019
CA125	Lateral flow assay	Aptamer-nanozyme lateral flow assay	DNA	TAGGGAAGAGAAGGACATATGATTTTA GGGAAGAGAAGGACTTTTATGCCGCCTTGACTAGTA CATGACCACTTGA (From Triphati et al, 2020a)	5.21 U/mL	7.5-200 U/mL	Yes	Yes	Serum	No	Triphati et al, 2020b
CA125	Aptamer-antigen-antibody sandwiched assay	Magnetic bead-amplified voltammetric detection with enzyme labels	DNA	SH -5′ ACTAGCTCCGATCTTTCTTATCTAC 3 (CA125_1 from Scoville et al. 2017)	0.08 U/mL	2 U/mL - 100 U/mL	Yes	Yes	Whole blood Serum	No	Sadasivam and Sendwitch, 2020
CA125	Biosensor	Aptasensor based on upconversion luminescence resonance energy transfer	DNA	5′-AAAAAACTCACTATAGGGAGACAAGAATAAACGCTC AA-3′	9.0×10^{-3} U/mL	0.01 to 100 U/mL	Yes	Yes	Serum	Yes (n=3)	Zhang et al, 2021
CA125 + STIP1	Biosensor	Aptasensor based on resonance light scattering intensity	DNA	**CA125 aptamer:** 5′-CGG CAC TCA CTC TTTGTT AAG TGG TCT GCT TCT TAA CCT TCA TAT CAA TTA CTT ACC CTAGTG GTG TGA TGT CGT ATG GAT G-3′ **STIP1 aptamer:** 5′-CAT CCA TAC GAC ATC ACA CCA CTA GGG TAA GTAATT GAT ATG AAG GTT AAG AAG CAG ACC ACT TAA CAA AGA GTGAGT GCC G-3′	n/a	0.1 to 2 U/mL for CA125 1 to 40 ng/mL	No	Yes	Serum	No	Chen et al, 2017
HE4	Biosensor	Aptasensor based on upconversion luminescence resonance energy transfer	DNA	5′–NH2-CACCATTATCGTACGACAGTCATC CTACACAATGGT-BHQ-1-3′ (Partially seqquence A1 from Eaton et al .2015)	0.021 ng/mL (buffer) 0.049 ng/mL (serum)	0.4 ng/mL to 7.0 ng/mL	n/a	Yes	Serum	No	Ma et al, 2021

CA125 - Cancer Antigen 125 ; **HE4** - Human Epididymis protein 4 ; **STIP1** - Stress-induced phosphoprotein 1 ; **n/a** - not applicable

***** aptamer initially developed against HE4 as a target

****** Clinical *cut off* value for the serum concentration of ovarian cancer biomarkers are CA125 > 35 U/mL; STIP1 > 55 ng/mL; HE4 > 60 pmol/L