

Supplementary Material

Diagnostic potential of nanoparticle aided assays for MUC16 and MUC1 glycovariants in ovarian cancer

Authors: Shruti Jain, Nimrah Nadeem, Benjamin Ulfenborg, Maria Mäkelä, Shamima Afrin Ruma, Joonas Terävä, Kaisa Huhtinen, Janne Leivo, Björg Kristjansdottir, Kim Pettersson, Karin Sundfeldt, Kamlesh Gidwani

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Subgroup	Malignant	Non-malignant	Marker	AUC	SN (%) at						95% CI	at 90-100% SP		
					75% SP	90% SP	95% SP	97% SP	98% SP	99% SP		pAUC	95% CI	
All patients	85 (67=M, 18=BL)	187 (58=H, 129=B)	Single marker	CA125 IA	0.518	23.5	14.1	12.9	8.2	8.2	1.2	0.445 - 0.591	0.527	0.497 - 0.563
				CA15-3 IA	0.677	48.2	31.8	25.9	17.6	14.1	11.8	0.606 - 0.748	0.598	0.552 - 0.651
				MUC16 MGL	0.753	60	44.7	28.2	22.4	11.8	2.4	0.690 - 0.816	0.618	0.557 - 0.689
				MUC16 STn	0.784	67.1	51.8	35.3	31.8	27.1	12.9	0.724 - 0.845	0.656	0.594 - 0.728
				MUC1 STn	0.893	84.7	77.6	48.2	36.5	25.9	20	0.852 - 0.934	0.721	0.635 - 0.812
				MUC1 Tn	0.904	85.9	74.1	63.5	61.2	51.8	29.4	0.864 - 0.944	0.789	0.721 - 0.858
			Combination	CA125 IA + CA15-3 IA	0.709	61.2	35.3	25.9	21.2	18.8	12.9	0.640 - 0.778	0.608	0.558 - 0.665
	MUC1 STn + MUC1 Tn	0.924	91.8	80.0	70.6	58.8	54.1	18.8	0.889 - 0.959	0.799	0.728 - 0.870			
All patients	67	187 (58=H, 129=B)	Single marker	CA125 IA	0.548	26.9	16.4	14.9	9.0	9	1.5	0.470 - 0.627	0.534	0.499 - 0.576
Excluding borderline				CA15-3 IA	0.723	55.2	37.3	29.9	22.4	17.9	14.9	0.649 - 0.798	0.622	0.567 - 0.683
				MUC16 MGL	0.763	64.2	47.8	32.8	25.4	11.9	3	0.693 - 0.833	0.633	0.566 - 0.709
				MUC16 STn	0.830	76.1	62.7	44.8	40.3	34.3	16.4	0.769 - 0.891	0.701	0.628 - 0.778
				MUC1 STn	0.896	85.1	80.6	52.2	38.8	29.9	25.4	0.851 - 0.941	0.739	0.651 - 0.833
				MUC1 Tn	0.926	89.6	80.6	71.6	70.1	62.7	37.3	0.886 - 0.966	0.830	0.760 - 0.900
			Combination	CA125 IA + CA15-3 IA	0.741	65.7	41.8	32.8	28.4	25.4	16.4	0.667 - 0.816	0.634	0.578 - 0.699
		MUC1 STn + MUC1 Tn	0.934	92.5	83.6	77.6	71.6	68.7	23.9	0.897 - 0.971	0.835	0.763 - 0.903		
Postmenopausal	64 (54=M, 10=BL)	144 (40=H, 104=B)	Single marker	CA125 IA	0.491	23.4	15.6	14.1	9.4	1.6	1.6	0.404 - 0.578	NA	
				CA15-3 IA	0.682	46.9	34.4	26.6	17.2	14.1	12.5	0.601 - 0.764	0.600	0.546 - 0.662
				MUC16 MGL	0.744	59.4	51.6	32.8	25.0	10.9	4.7	0.668 - 0.821	0.635	0.561 - 0.721
				MUC16 STn	0.816	71.9	59.4	50.0	39.1	31.3	14.1	0.751 - 0.880	0.698	0.620 - 0.781
				MUC1 STn	0.914	89.1	81.3	56.3	50.0	35.9	29.7	0.873 - 0.956	0.764	0.668 - 0.859
				MUC1 Tn	0.927	90.6	82.8	75.0	68.8	67.2	64.1	0.884 - 0.969	0.851	0.783 - 0.915
			Combination	CA125 IA + CA15-3 IA	0.732	64.1	40.6	28.1	23.4	18.8	12.5	0.656 - 0.809	0.613	0.557 - 0.683
		MUC1 STn + MUC1 Tn	0.942	92.2	82.8	73.4	70.3	70.3	67.2	0.905 - 0.979	0.854	0.787 - 0.921		
Postmenopausal	54	144 (40=H, 104=B)	Single marker	CA125 IA	0.508	25.9	16.7	14.8	9.3	1.9	1.9	0.416 - 0.601	0.532	0.495 - 0.581
Excluding borderline				CA15-3 IA	0.715	50	37.0	29.6	20.4	16.7	14.8	0.632 - 0.798	0.612	0.554 - 0.679
				MUC16 MGL	0.756	63	53.7	37.0	27.8	11.1	5.6	0.674 - 0.839	0.649	0.569 - 0.736

				MUC16 STn	0.846	77.8	68.5	59.3	46.3	37	16.7	0.779 - 0.913	0.737	0.651 - 0.826
				MUC1 STn	0.918	90.7	85.2	59.3	51.9	37	31.5	0.873 - 0.963	0.776	0.675 - 0.875
				MUC1 Tn	0.944	92.6	88.9	81.5	75.9	75.9	72.2	0.903 - 0.986	0.887	0.821 - 0.945
			Combination	CA125 IA + CA15-3 IA	0.751	68.5	40.7	29.6	25.9	22.2	14.8	0.672 - 0.831	0.624	0.560 - 0.699
				MUC1 STn + MUC1 Tn	0.945	92.6	87.0	79.6	79.6	79.6	75.9	0.904 - 0.987	0.888	0.823 - 0.948
Early stage EOC	56	187	Single marker	CA125 IA	0.482	17.9	8.9	8.9	7.1	7.1	0	0.399 - 0.564	NA	
	(38=M, 18=BL)	(58=H, 129=B)		CA15-3 IA	0.647	48.2	32.1	25.0	16.1	12.5	12.5	0.558 - 0.736	0.596	0.542 - 0.659
				MUC16 MGL	0.751	58.9	41.1	21.4	16.1	10.7	0	0.680 - 0.821	0.594	0.532 - 0.670
				MUC16 STn	0.754	60.7	44.6	28.6	26.8	23.2	10.7	0.680 - 0.828	0.631	0.565 - 0.703
				MUC1 STn	0.909	87.5	76.8	48.2	39.3	25	16.1	0.870 - 0.947	0.721	0.631 - 0.816
				MUC1 Tn	0.891	83.9	71.4	58.9	57.1	46.4	23.2	0.842 - 0.940	0.767	0.692 - 0.845
			Combination	CA125 IA + CA15-3 IA	0.711	53.6	39.3	30.4	25.0	21.4	12.5	0.631 - 0.790	0.625	0.566 - 0.692
				MUC1 STn + MUC1 Tn	0.920	91.1	76.8	64.3	55.4	48.2	16.1	0.881 - 0.959	0.780	0.703 - 0.861
Early stage EOC	38	187	Single marker	CA125 IA	0.519	21.1	10.5	10.5	7.9	7.9	0	0.424 - 0.614	0.516	0.482 - 0.563
Excluding borderline		(58=H, 129=B)		CA15-3 IA	0.714	60.5	42.1	31.6	23.7	18.4	18.4	0.611 - 0.816	0.638	0.565 - 0.720
				MUC16 MGL	0.767	65.8	44.7	26.3	18.4	10.5	0	0.684 - 0.850	0.610	0.538 - 0.696
				MUC16 STn	0.819	73.7	60.5	42.1	39.5	34.2	15.8	0.739 - 0.900	0.697	0.616 - 0.784
				MUC1 STn	0.922	89.5	81.6	55.3	44.7	31.6	23.7	0.882 - 0.962	0.753	0.656 - 0.853
				MUC1 Tn	0.925	89.5	81.6	71.1	71.1	63.2	34.2	0.876 - 0.974	0.828	0.745 - 0.908
			Combination	CA125 IA + CA15-3 IA	0.775	68.4	50.0	42.1	31.6	31.6	21.1	0.685 - 0.865	0.672	0.597 - 0.757
				MUC1 STn + MUC1 Tn	0.935	92.1	84.2	73.7	68.4	65.8	23.7	0.893 - 0.977	0.826	0.745 - 0.907

Supplemental Table 1 – Data compilation of cyst fluid validation cohort (n=272).

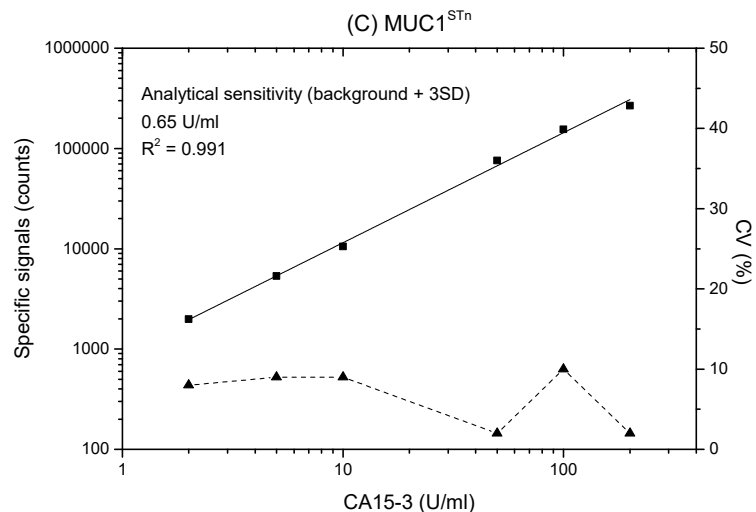
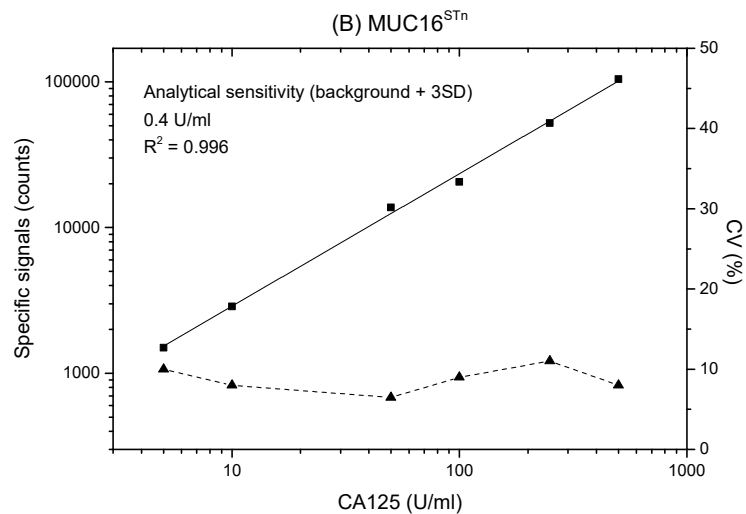
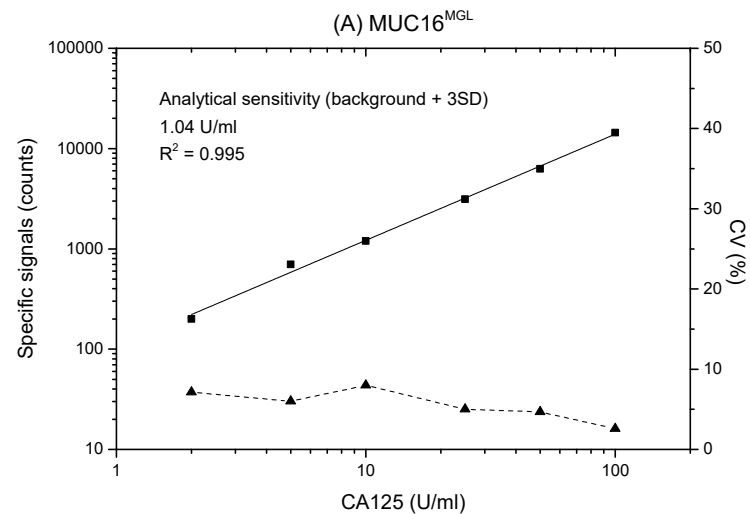
Data information: Area under the curve (AUC) along with 95% confidence interval (CI) and sensitivity (SN) at 75, 90, 95, 97, 98 and 99% specificity (SP) is reported. Corrected partial AUC (pAUC) and 95% CI is reported for SP 90-100%. 'NA' is the partial AUC correction not defined for ROC curves below the diagonal. Abbreviations: M, Malignant; BL, Borderline; H, Healthy; B, Benign.

Subgroup	Malignant	Non-malignant	Marker	AUC	SN (%) at						at 90-100% SP			
					75% SP	90% SP	95% SP	97% SP	98% SP	99% SP	95% CI	pAUC	95% CI	
All patients	85 (67=M, 18=BL)	187 (58=H, 129=B)	Single marker	CA125 IA	0.805	74.1	52.9	48.2	30.6	18.8	2.4	0.746 - 0.864	0.668	0.603 - 0.740
				CA15-3 IA	0.721	52.9	45.9	38.8	38.8	30.6	0.651 - 0.791	0.677	0.623 - 0.733	
				HE4 IA	0.747	65.9	47.1	38.8	32.9	18.8	0.679 - 0.815	0.666	0.610 - 0.726	
				MUC16 MGL	0.813	72.9	57.6	47.1	36.5	23.5	0.754 - 0.872	0.694	0.634 - 0.767	
				MUC16 STn	0.811	75.3	63.5	57.6	45.9	22.4	0.748 - 0.874	0.743	0.675 - 0.810	
				MUC1 STn	0.816	68.2	57.6	57.6	49.4	21.2	0.756 - 0.875	0.741	0.679 - 0.803	
				Combination	CA125 IA + HE4 IA	0.753	64.7	49.4	36.5	31.8	28.2	21.2	0.686 - 0.820	0.664
	MUC16 STn + MUC1 STn	0.837	74.1	62.4	57.6	54.1	45.9	22.4	0.781 - 0.893	0.739	0.676 - 0.801			
All patients	67	187	Single marker	CA125 IA	0.848	80.6	65.7	59.7	38.8	23.9	3	0.789 - 0.908	0.715	0.636 - 0.797
Excluding borderline		(58=H, 129=B)		CA15-3 IA	0.792	65.7	58.2	49.3	49.3	49.3	38.8	0.722 - 0.862	0.732	0.672 - 0.797
				HE4 IA	0.818	76.1	58.2	47.8	40.3	23.9	0.752 - 0.884	0.711	0.646 - 0.779	
				MUC16 MGL	0.856	80.6	70.1	58.2	46.3	29.9	0.798 - 0.915	0.748	0.675 - 0.824	
				MUC16 STn	0.883	86.6	74.6	73.1	58.2	28.4	0.826 - 0.939	0.804	0.727 - 0.875	
				MUC1 STn	0.872	76.1	70.1	70.1	61.2	26.9	0.818 - 0.927	0.798	0.728 - 0.865	
				Combination	CA125 IA + HE4 IA	0.825	76.1	61.2	44.8	38.8	26.9	0.761 - 0.890	0.709	0.640 - 0.781
	MUC16 STn + MUC1 STn	0.896	80.6	73.1	70.1	67.2	28.4	0.850 - 0.942	0.803	0.732 - 0.871				
Postmenopausal	64 (54=M, 10=BL)	144 (40=H, 104=B)	Single marker	CA125 IA	0.810	75	59.4	50.0	20.3	14.1	3.1	0.742 - 0.878	0.674	0.588 - 0.771
				CA15-3 IA	0.754	57.8	50.0	45.3	45.3	34.4	0.676 - 0.832	0.702	0.638 - 0.770	
				HE4 IA	0.788	68.8	48.4	40.6	37.5	29.7	0.719 - 0.858	0.675	0.613 - 0.744	
				MUC16 MGL	0.831	73.4	70.3	50.0	42.2	26.6	0.764 - 0.898	0.720	0.639 - 0.809	
				MUC16 STn	0.842	79.7	67.2	57.8	51.6	26.6	0.775 - 0.909	0.756	0.674 - 0.833	
				MUC1 STn	0.869	76.6	76.6	64.1	64.1	25	0.811 - 0.928	0.789	0.712 - 0.863	
				Combination	CA125 IA + HE4 IA	0.792	70.3	50.0	39.1	34.4	28.1	0.723 - 0.861	0.670	0.607 - 0.743
	MUC16 STn + MUC1 STn	0.886	87.5	68.8	65.6	62.5	28.1	0.833 - 0.940	0.787	0.707 - 0.863				
Postmenopausal	54	144	Single marker	CA125 IA	0.849	81.5	68.5	57.4	24.1	16.7	3.7	0.783 - 0.916	0.706	0.606 - 0.811
Excluding borderline		(40=H, 104=B)		CA15-3 IA	0.794	66.7	59.3	53.7	53.7	44.4	40.7	0.715 - 0.874	0.745	0.676 - 0.817
				HE4 IA	0.833	75.9	55.6	46.3	42.6	35.2	0.766 - 0.901	0.704	0.635 - 0.783	

				MUC16 MGL	0.865	79.6	75.9	57.4	50.0	40.7	31.5	0.801 - 0.929	0.758	0.668 - 0.848
				MUC16 STn	0.889	87	75.9	68.5	61.1	57.4	31.5	0.828 - 0.950	0.801	0.714 - 0.882
				MUC1 STn	0.899	79.6	79.6	74.1	74.1	70.4	29.6	0.844 - 0.953	0.828	0.748 - 0.902
			Combination	CA125 IA + HE4 IA	0.839	77.8	61.1	44.4	40.7	37	33.3	0.773 - 0.905	0.699	0.629 - 0.781
				MUC16 STn + MUC1 STn	0.918	90.7	75.9	75.9	72.2	72.2	33.3	0.873 - 0.963	0.828	0.743 - 0.902
Premenopausal	21	43	Single marker	CA125 IA	0.764	71.4	33.3	23.8	23.8	23.8	23.8	0.643 - 0.885	0.614	0.524 - 0.732
	(13=M, 8=BL)	(18=H, 25=B)		CA15-3 IA	0.612	38.1	28.6	23.8	19.0	19	19	0.462 - 0.763	0.592	0.507 - 0.698
				HE4 IA	0.637	47.6	33.3	33.3	33.3	9.5	9.5	0.481 - 0.794	0.620	0.524 - 0.747
				MUC16 MGL	0.725	66.7	28.6	28.6	28.6	28.6	23.8	0.590 - 0.860	0.621	0.526 - 0.738
				MUC16 STn	0.712	61.9	52.4	42.9	42.9	42.9	42.9	0.559 - 0.865	0.721	0.610 - 0.834
				MUC1 STn	0.602	42.9	38.1	19.0	19.0	19	19	0.442 - 0.762	0.598	0.506 - 0.721
			Combination	CA125 IA + HE4 IA	0.753	61.9	33.3	28.6	23.8	23.8	23.8	0.630 - 0.876	0.620	0.526 - 0.729
				MUC16 STn + MUC1 STn	0.769	61.9	52.4	42.9	42.9	42.9	42.9	0.636 - 0.901	0.720	0.609 - 0.834
Early stage EOC	56	187	Single marker	CA125 IA	0.723	60.7	33.9	26.8	16.1	10.7	0	0.646 - 0.801	0.584	0.533 - 0.652
	(38=M, 18=BL)	(58=H, 129=B)		CA15-3 IA	0.639	39.3	32.1	25.0	25.0	25	19.6	0.551 - 0.727	0.604	0.549 - 0.667
				HE4 IA	0.649	53.6	30.4	21.4	16.1	12.5	7.1	0.561 - 0.737	0.578	0.527 - 0.638
				MUC16 MGL	0.732	58.9	39.3	28.6	21.4	16.1	12.5	0.653 - 0.811	0.605	0.548 - 0.681
				MUC16 STn	0.736	64.3	48.2	41.1	32.1	28.6	12.5	0.651 - 0.820	0.667	0.599 - 0.741
				MUC1 STn	0.753	57.1	44.6	44.6	37.5	32.1	12.5	0.674 - 0.832	0.679	0.611 - 0.751
			Combination	CA125 IA + HE4 IA	0.658	51.8	32.1	19.6	12.5	8.9	7.1	0.571 - 0.744	0.578	0.524 - 0.640
				MUC16 STn + MUC1 STn	0.771	60.7	48.2	44.6	41.1	32.1	10.7	0.695 - 0.847	0.680	0.613 - 0.752
Early stage EOC Excluding borderline	38	187	Single marker	CA125 IA	0.761	65.8	47.4	36.8	23.7	15.8	0	0.670 - 0.852	0.627	0.558 - 0.714
		(58=H, 129=B)		CA15-3 IA	0.725	55.3	47.4	36.8	36.8	36.8	28.9	0.625 - 0.825	0.666	0.591 - 0.748
				HE4 IA	0.727	65.8	42.1	28.9	21.1	15.8	10.5	0.628 - 0.826	0.616	0.548 - 0.695
				MUC16 MGL	0.769	65.8	52.6	39.5	31.6	23.7	18.4	0.679 - 0.859	0.657	0.582 - 0.746
				MUC16 STn	0.825	78.9	60.5	60.5	47.4	42.1	18.4	0.739 - 0.912	0.739	0.657 - 0.824
				MUC1 STn	0.823	65.8	60.5	60.5	52.6	44.7	18.4	0.743 - 0.904	0.749	0.665 - 0.834
			Combination	CA125 IA + HE4 IA	0.738	65.8	44.7	26.3	15.8	13.2	10.5	0.641 - 0.836	0.617	0.542 - 0.699
				MUC16 STn + MUC1 STn	0.843	65.8	60.5	60.5	57.9	44.7	15.8	0.771 - 0.915	0.750	0.668 - 0.835

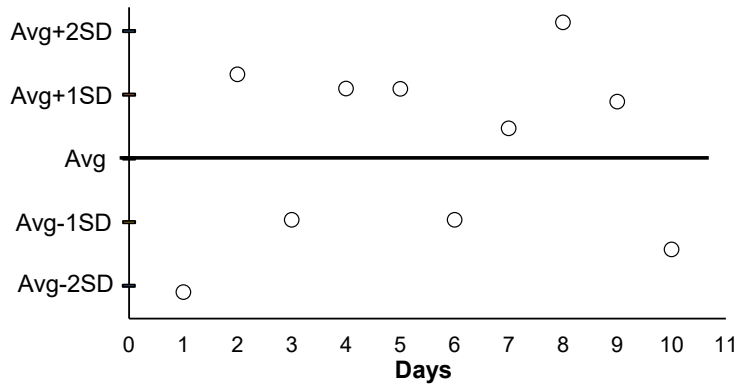
Early stage EOC	29	144	Single marker	CA125 IA	0.752	65.5	48.3	34.5	13.8	10.3	0	0.646 - 0.858	0.619	0.539 - 0.722
Excluding borderline		(40=H, 104=B)		CA15-3 IA	0.702	51.7	44.8	41.4	41.4	31	31	0.579 - 0.824	0.679	0.592 - 0.775
Excluding premenopausal				HE4 IA	0.745	62.1	37.9	24.1	20.7	13.8	10.3	0.641 - 0.848	0.591	0.520 - 0.682
				MUC16 MGL	0.773	62.1	58.6	37.9	31.0	20.7	17.2	0.669 - 0.876	0.662	0.573 - 0.768
				MUC16 STn	0.834	79.3	62.1	55.2	48.3	44.8	20.7	0.737 - 0.930	0.736	0.639 - 0.840
				MUC1 STn	0.849	69	69.0	62.1	62.1	58.6	20.7	0.763 - 0.935	0.770	0.674 - 0.867
			Combination	CA125 IA + HE4 IA	0.754	69	44.8	24.1	17.2	10.3	10.3	0.652 - 0.856	0.586	0.516 - 0.683
				MUC16 STn + MUC1 STn	0.868	82.8	62.1	62.1	62.1	58.6	20.7	0.794 - 0.943	0.765	0.668 - 0.863
Late stage EOC	29	187	Single marker	CA125 IA	0.963	100	89.7	89.7	58.6	34.5	6.9	0.938 - 0.987	0.830	0.723 - 0.925
	(29=M, 0=BL)	(58=H, 129=B)		CA15-3 IA	0.880	79.3	72.4	65.5	65.5	65.5	51.7	0.804 - 0.955	0.818	0.727 - 0.902
				HE4 IA	0.936	89.7	79.3	72.4	65.5	65.5	41.4	0.892 - 0.979	0.834	0.747 - 0.916
				MUC16 MGL	0.970	100	93.1	82.8	65.5	62.1	44.8	0.949 - 0.992	0.866	0.776 - 0.945
				MUC16 STn	0.958	96.6	93.1	89.7	72.4	69	41.4	0.911 - 1.000	0.889	0.801 - 0.961
				MUC1 STn	0.937	89.7	82.8	82.8	72.4	69	37.9	0.883 - 0.990	0.861	0.773 - 0.937
			Combination	CA125 IA + HE4 IA	0.943	89.7	82.8	69.0	65.5	65.5	48.3	0.905 - 0.982	0.835	0.745 - 0.916
				MUC16 STn + MUC1 STn	0.977	100	96.6	79.3	72.4	69	41.4	0.960 - 0.994	0.882	0.789 - 0.956

Supplemental Table 2 – Data compilation of serum validation cohort (n=272).

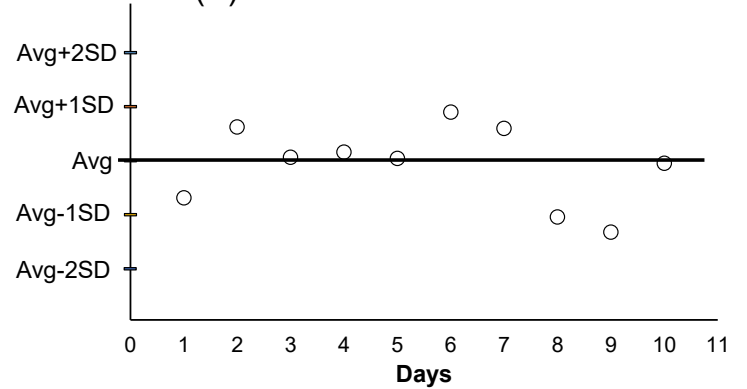


Supplemental Figure 1. Calibration curves (solid line) and precision profiles (dashed line) of (A) MUC16^{MGL}, (B) MUC16^{STn} and (C) MUC1^{STn} GV assay from cyst fluid and serum cohorts.

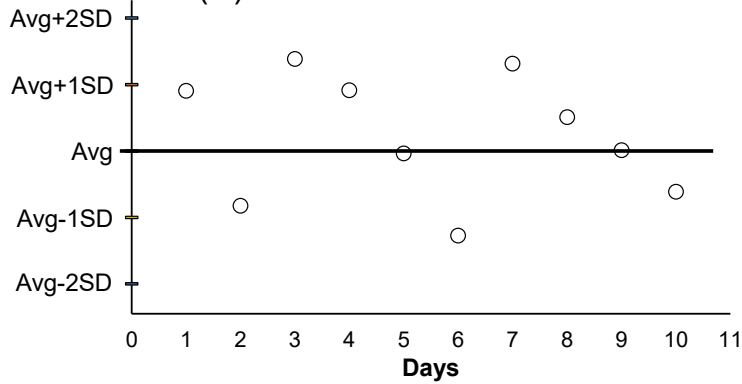
(A) MUC16^{MGL}: Control-1



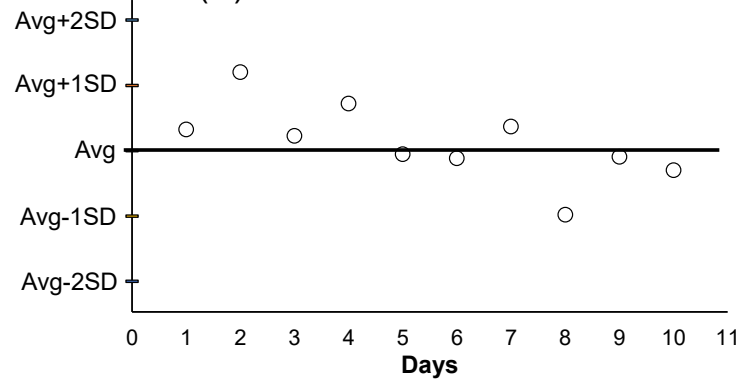
(B) MUC16^{MGL}: Control-2



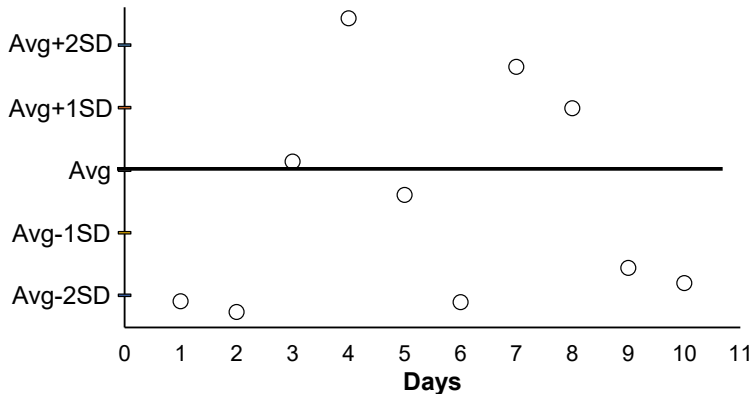
(C) MUC16^{STn}: Control-1



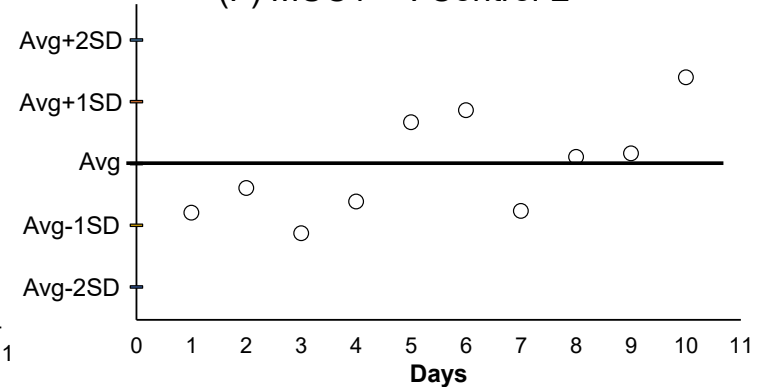
(D) MUC16^{STn}: Control-2



(E) MUC1^{STn}: Control-1



(F) MUC1^{STn}: Control-2



Supplemental Figure 2. Day-to-day variation of 2 controls (high & low) of the three GV assays. (A, B) MUC16^{MGL} (C, D) MUC16^{STn} (E, F) MUC1^{STn}. Variation is seen to be within 2 standard deviations (SD) of the average (Avg).