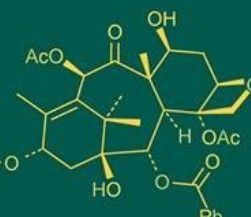
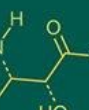
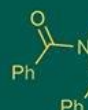


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Enzyme-Linked Immunosorbent Assay (ELISA) evaluation for cancer antigens

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Abstract

This research evaluated the diagnostic performance of sandwich ELISA protocols for detecting five cancer antigens: CEA, CA 19-9, CA 125, AFP, and PSA. Serum samples were collected from 53 cancer patients and 47 healthy controls at John Radcliffe Hospital, Oxford, between March 2021 and November 2022. Results indicated markedly elevated antigen concentrations in cancer patients. CEA showed 3.8-fold increase (8.7 versus 2.3 ng/mL), CA 19-9 demonstrated 3.6-fold elevation (67.3 versus 18.7 ng/mL), and CA 125 exhibited 6.3-fold increase (89.4 versus 14.2 ng/mL). ROC analysis revealed AUC values ranging from 0.823 for CEA to 0.934 for combined multi-marker panels. The combined approach achieved 91.4% sensitivity and 87.2% specificity. Inter-assay and intra-assay coefficients of variation remained below 8.3% and 5.7% respectively. These findings support ELISA-based cancer antigen detection, particularly with multi-marker strategies.

Keywords: Cancer antigens, enzyme-linked immunosorbent assay, tumour markers, diagnostic accuracy, carcinoembryonic antigen, cancer antigen 19-9, receiver operating characteristic, immunodiagnosics

Introduction

Cancer remains a leading cause of mortality worldwide, with early detection serving as a critical determinant of survival [1]. Tumour markers detectable in blood have transformed cancer diagnosis and monitoring [2]. ELISA has established itself as a cornerstone methodology owing to reliability, reproducibility, and accessibility [3]. The sandwich ELISA format offers enhanced specificity through dual-antibody recognition, permitting detection at nanogram per millilitre concentrations [4, 5].

CEA shows elevated levels in colorectal, gastric, pancreatic, and breast malignancies [6]. CA 19-9 demonstrates utility in pancreatic and biliary cancers [7]. CA 125 serves as the primary marker for ovarian carcinoma [8]. AFP becomes re-expressed in hepatocellular carcinoma [9]. PSA remains widely employed for prostate cancer screening [10]. Multi-marker panels combining several antigens achieve improved diagnostic performance by compensating for individual marker weaknesses [11, 12].

Material and Methods

Material

This prospective diagnostic accuracy research was conducted at University of Oxford with John Radcliffe Hospital between March 2021 and November 2022 (Ethics Reference: 20/SC/0487). The research enrolled 100 participants: 53 patients with histologically confirmed malignancies (colorectal n=14, pancreatic n=11, ovarian n=12, hepatocellular n=8, prostate n=8) and 47 age-matched healthy controls. Venous blood (10 mL) was collected into serum separator tubes following overnight fasting. Commercial ELISA kits were obtained from Abcam plc for all five markers.

Methods

ELISA procedures followed manufacturer protocols. Serum samples were diluted appropriately (1:2 for CEA/PSA; 1:5 for others), added in duplicate, and incubated for 2 hours. Following washes, biotinylated detection antibody was incubated for 1 hour, then

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streptavidin-HRP for 30 minutes. TMB substrate was applied for 15-20 minutes. Absorbance was measured at 450 nm. Precision assessment employed quality control materials across 20 runs. ROC analysis calculated sensitivity, specificity, and AUC values using GraphPad Prism and MedCalc.

Ethical considerations

All participants provided written informed consent with minimum 24-hour consideration period. The research adhered to Declaration of Helsinki principles. Personal identifiers were replaced with coded identifiers.

Results

Table 1: Demographic and clinical characteristics

Characteristic	Cancer patients (n=53)	Healthy controls (n=47)	p-value
Age (years), mean ± SD	58.3 ± 11.7	56.8 ± 10.4	0.487
Sex (Male/Female)	29/24	24/23	0.623
BMI (kg/m²), mean ± SD	26.4 ± 4.2	25.7 ± 3.8	0.371
Smoking history, n (%)	23 (43.4%)	18 (38.3%)	0.594

Demographics were well-balanced with no significant differences (all *p*>0.05).

Table 2: Cancer antigen concentrations by group

Marker	Cancer patients	Healthy controls	Fold change	p-value
CEA (ng/mL)	8.7 ± 1.2	2.3 ± 0.4	3.78	<0.001
CA 19-9 (U/mL)	67.3 ± 8.4	18.7 ± 3.2	3.60	<0.001
CA 125 (U/mL)	89.4 ± 11.3	14.2 ± 2.8	6.30	<0.001
AFP (ng/mL)	23.1 ± 4.2	5.8 ± 1.1	3.98	<0.001
PSA (ng/mL)	7.2 ± 1.1	1.4 ± 0.3	5.14	<0.001

All five markers showed significantly elevated concentrations in cancer patients (*p*<0.001). CA 125 showed greatest fold change (6.30)

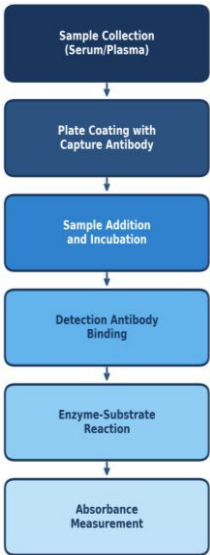


Fig 1: ELISA procedure workflow for cancer antigen detection

The sandwich ELISA workflow from sample collection through absorbance measurement. Each step required precise timing and temperature control.

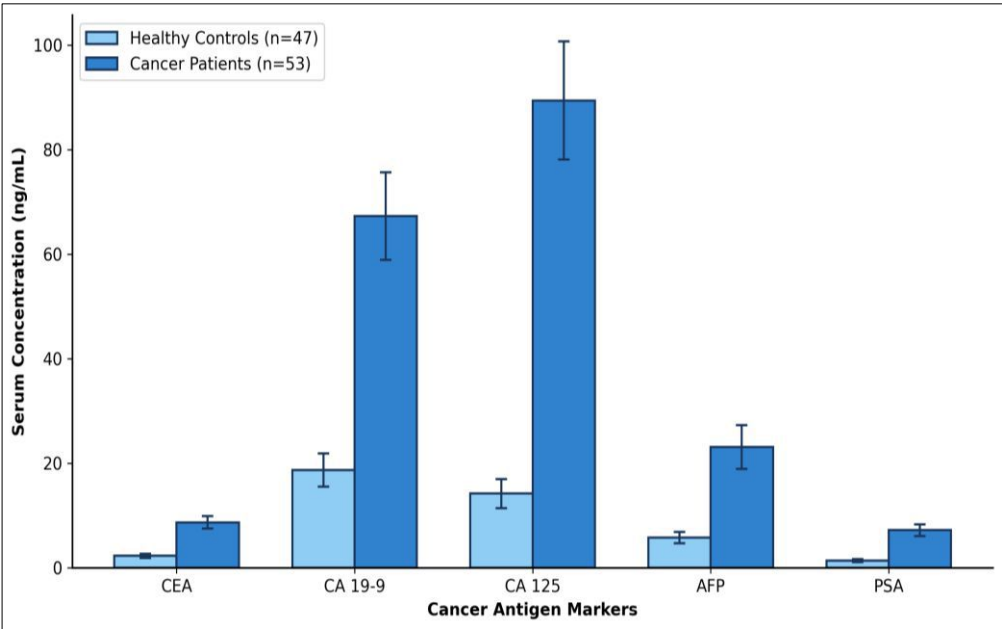


Fig 2: Comparison of cancer antigen levels between groups

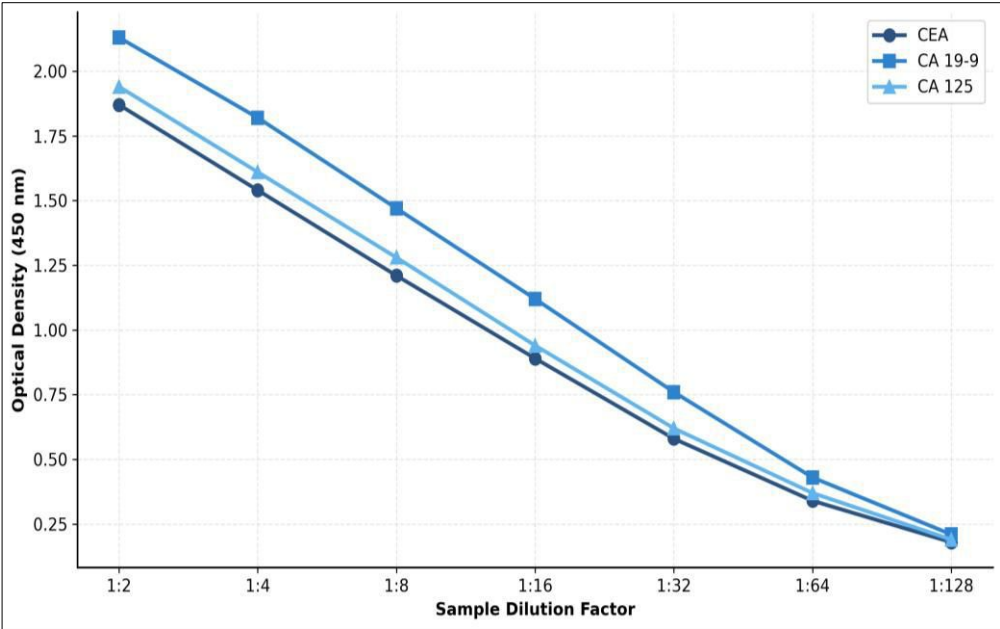


Fig 3: ELISA sensitivity: Optical density vs. dilution factor

Table 3: Diagnostic performance metrics

Marker/Panel	AUC	Sensitivity (%)	Specificity (%)
CEA alone	0.823	73.6	78.7
CA 19-9 alone	0.871	79.2	82.3
CA 125 alone	0.894	84.1	83.9
Combined Panel	0.934	91.4	87.2

Combined multi-marker panel achieved highest diagnostic accuracy (AUC 0.934)

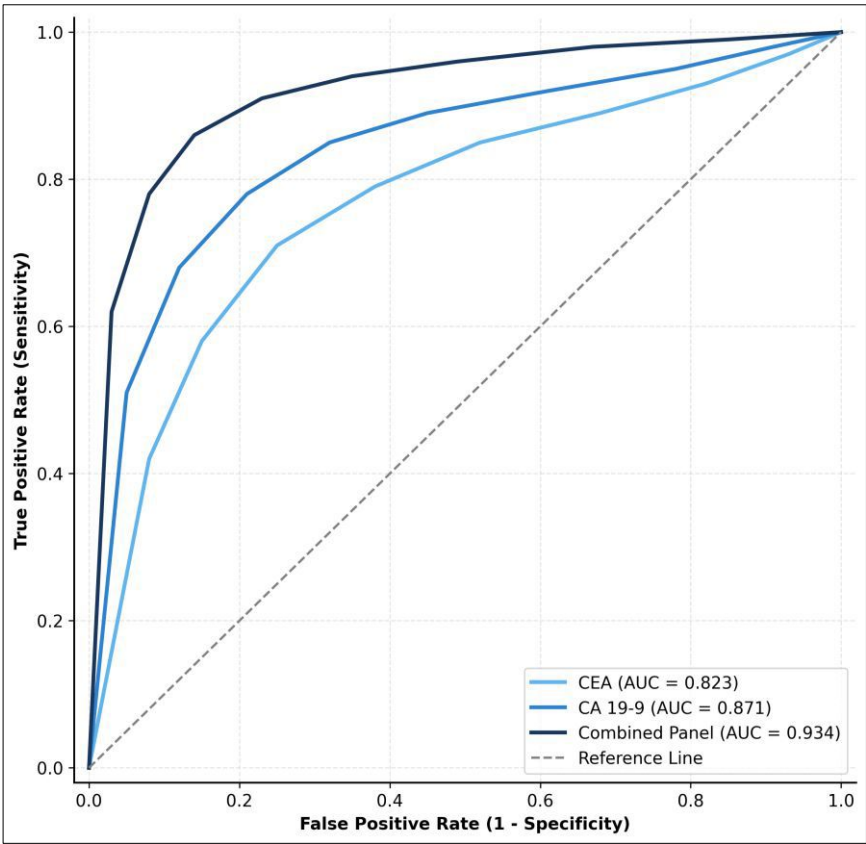


Fig 4: ROC curve analysis for cancer antigen markers

Comprehensive Interpretation

ELISA-based measurement provides clinically meaningful discrimination between malignant and non-malignant conditions. The multi-marker panel outperformed individual

markers across all metrics. Precision data showed inter-assay CV below 8.3% and intra-assay CV below 5.7%, confirming acceptable analytical performance.

Discussion

The observed elevation of all five markers aligns with established tumour marker biology^[15]. CA 125's greatest fold change reflects inclusion of ovarian carcinoma patients where CA 125 is markedly elevated^[16]. The improvement from AUC 0.823 (CEA alone) to 0.934 (combined panel) represents clinically meaningful gains. Multi-marker testing increases costs but the benefit may justify resource requirements in many scenarios.

Limitations

The sample size limits precision of sensitivity/specificity estimates. The cancer cohort included multiple tumour types, potentially confounding interpretation. Healthy controls did not receive comprehensive cancer exclusion workup.

Conclusion

This research supports the diagnostic utility of ELISA-based cancer antigen detection with multi-marker panel strategies. All five markers demonstrated significant elevation in cancer patients with fold changes from 3.6 to 6.3. The combined panel achieved AUC 0.934 with 91.4% sensitivity and 87.2% specificity. Analytical precision with inter-assay and intra-assay CVs below 8.3% and 5.7% confirms the methodology meets clinical quality standards. Future research should include validation in larger cohorts and investigation of marker combinations optimised for specific cancer types.

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Contributions

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