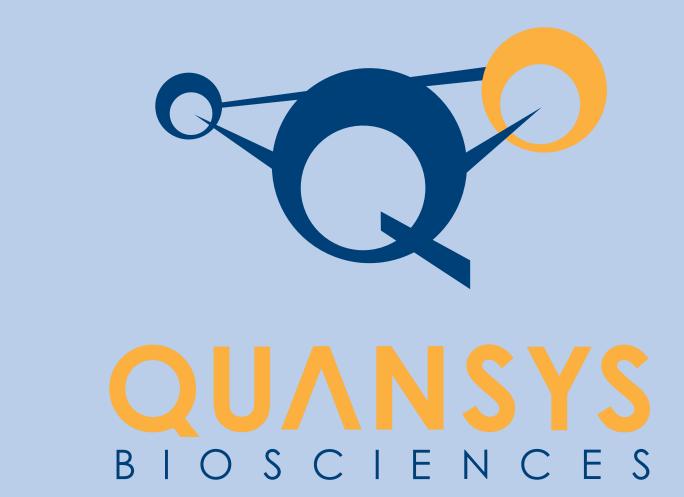
LB-179

Use of Protein Arrays to Identify the Sialylation of Cancer Markers



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Abstract:

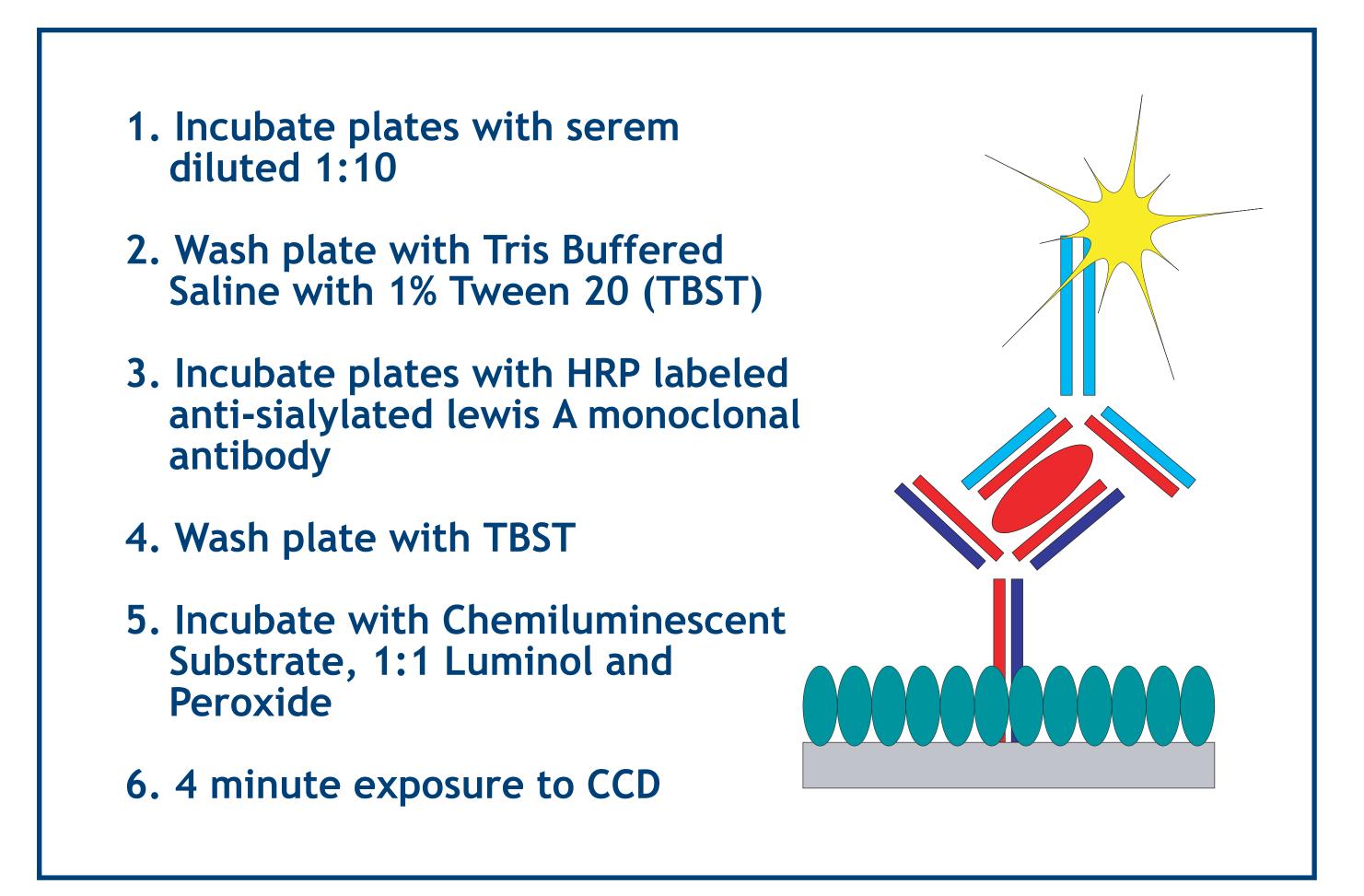
The level of expression of sialylated carbohydrate moieties is strongly correlated with patient survival and metastasis in cancer. The presence of sialylation is involved in the extravasation of cells from the bloodstream by binding to selectins. Though we know that sialylation is important to cancer progression, the types of proteins which are being sialylated have not been fully characterized. The objective of this study was to establish a multiplex assay which can be used to specifically monitor protein sialylation for use in cancer research. To develop this assay, we first identified which kind of proteins were being sialylated. We started by screening common cancer markers which would also be expected to be elevated during various cancers. We present here the markers we have found to be commonly sialylated.

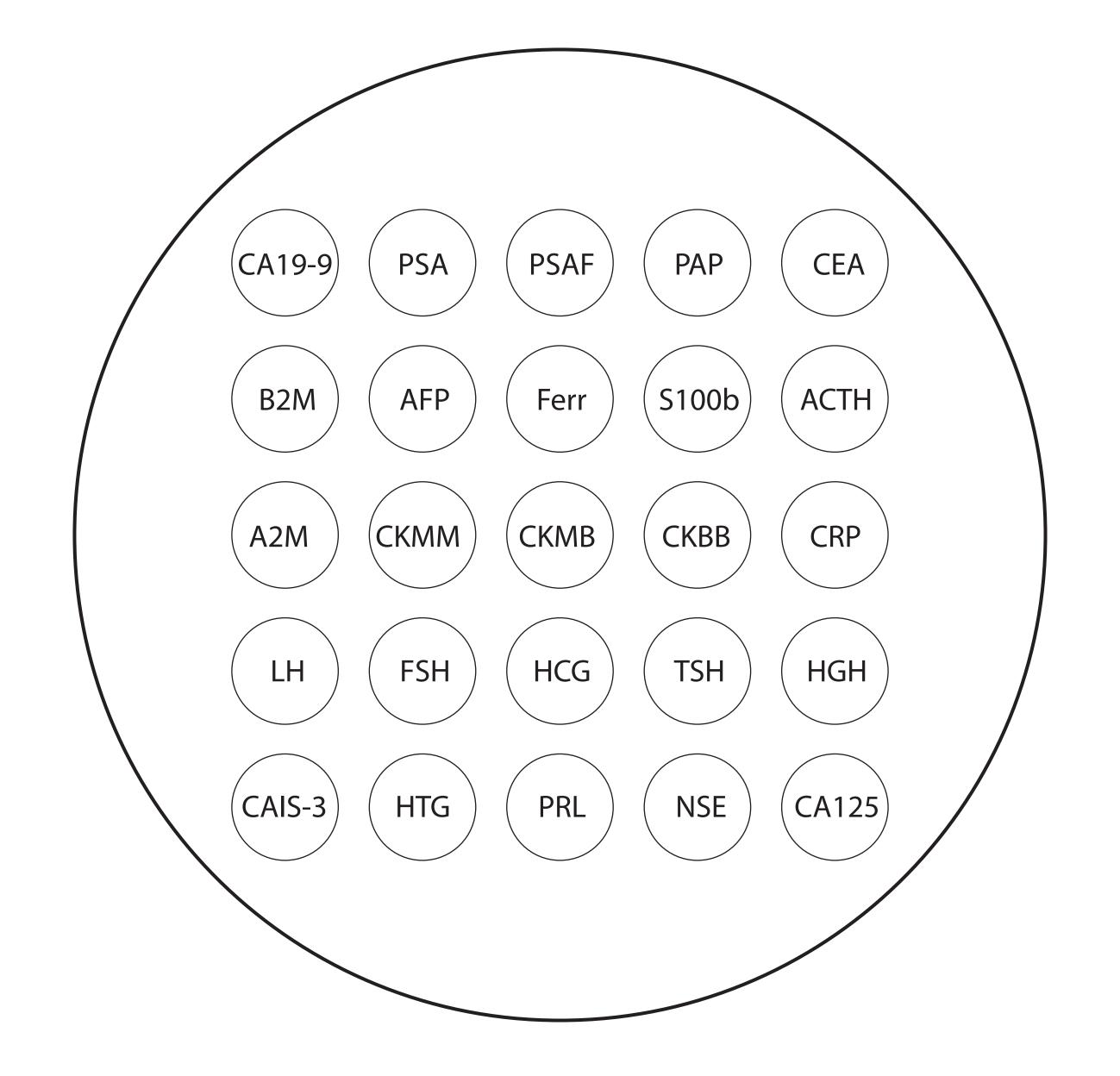
CA19-9 is a test for the sialylation described above. Using micro immunoassay applications, we analyzed serum samples with high reported CA19-9 values to determine what other types of cancer markers are known to be sialylated. To perform this assay, monoclonal antibodies previously validated with clinical cancer marker assays were printed into 96 well plates in a defined pattern. The markers tested include CA15-3, CA19-9, CA125, carcinoembryonic antigen (CEA), chorionic gonadotropin (HCG), beta-2-microglobulin (B2M), ferritin, prostate specific antigen (PSA), free PSA (PSAF), S100B, and alpha-fetoprotein (AFP). We also tested a panel of pituitary and thryroid hormones as a control group. This panel included luteinizing hormone (LH), follicle stimulating hormone (TSH), growth hormone (HGH), prolactin (PRL), and thyroglobulin (HTG). Next, we selected 84 serum samples to be assayed. These samples included 66 samples with high CA19-9 values and 18 samples with low CA19-9 values. With the monoclonal antibodies printed on the plates, 30 ul of each serum sample (diluted 1:10) was incubated for 1 hour. The plates were then incubated for 15 minutes with a single monoclonal antibody which reacts with sialylated Lewisa. Samples were then flooded with chemiluminescent substrate and the plate imaged with a CCD.

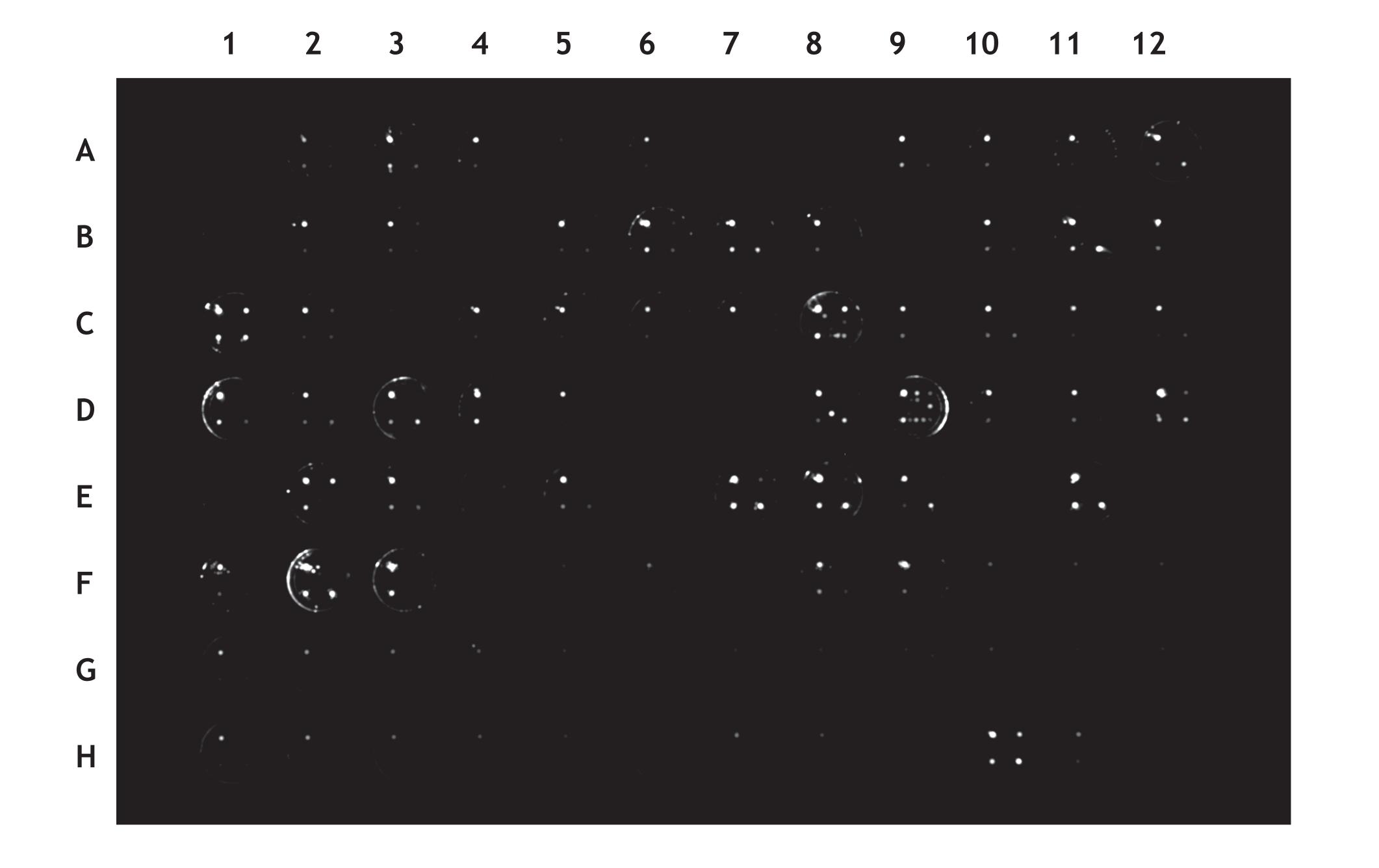
We found that there are multiple proteins from both the cancer marker group and the hormone group were commonly sialylated. These markers showing significant sialylation include CA15-3, CA125, CEA, hCG, S100beta, ferritin, Prolactin, TSH, hTG, AFP, and PSAF. The most commonly sialylated antigens were CA15-3, CA125, and CEA.

We also found many serum samples which had high CA19-9 responses though none of the markers tested were sialylated.

It is clear that many common cancer markers are sialylated and that there are more to be identified. Our next step is to use this sialylation test to determine the diagnostic usefulness of sialylation profiles and to better understand the role sialylation has in the progression of cancer.







Conclusion:

Using the Q-Plex Protein Array, we show that there are multiple natural sialyation targets, and that samples display varied profiles of sialylation.

Marker	Number of samples with sialyated marker	
CA15-3		49
CA125		35
Carcinoembryonic antigen		17
Adenocorticotropic hormone		11
Ferritin		8
S100-beta		3
Thyroglobulin		3
Prostate specific antig	gen (free form)	2
Prolactin		2
Neuron specific enola	se	2
C-reactive protein		2
Chorionic gonadotropi	in-beta	2
Alpha-fetoprotein		2
Follicle stimulaitng ho	ormone	1
Prostatic acid phospha	atase	0
Prostate specific antig	gen (complex form)	0
Beta-2-microglobulin		0
Alpha-2-macroglobulii	1	0
Creatine kinase MM		0
Creatine kinase MB		0
Creatine kinase BB		0
Leuteinizing hormone		0
Thyroid stimulating ho	ormone	0
Growth Hormone		0