

# Multiplexed microELISA Used for Monitoring Renal Cell Carcinoma

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## Abstract:

Renal cell carcinoma accounts for approximately 2-3% of all adult malignancies. It does not respond well to radiation or chemotherapy; therefore surgery remains the most effective treatment. The objective of this study is to monitor renal cell carcinoma pre and post surgery (radical nephrectomy) to detect recurrence or advancement of the disease using multiplexed microELISAs.

Through a collaborative project with Dr. Ali Ben-Jacob of the Cache Valley Cancer Treatment Clinic, a sample was taken one-week pre surgery to measure multiple tumor markers and cytokine levels using the Quansys Cancer Array and the Q-Plex™ Human Cytokine Array. The tumor markers that were measured include AFP, CEA, PSA, IL-6, hCGB, CA125, Prolactin (PRL), and CA19-9. The cytokines that were measured include IL-1α, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-13, IFNγ, TNFα, and TNFβ. A follow-up sample was taken one-month post surgery to observe differences in the protein level expressions.

The pre surgery sample yielded a CA19-9 level of 20 U/ml, and IL-6 of 90 pg/ml. The post surgery sample yielded a CA19-9 level of 20 U/ml and IL-6 of <2 pg/ml. Cancerous levels of CA19-9 are expected to be >37 U/ml. The CA19-9 level detected is expected in normal sera. Research has shown that IL-6 could be a potential marker for renal cell carcinoma. Normal levels of IL-6 are expected to range between 5-8 pg/ml.

Responses of IL-6 between pre and post surgery samples indicate a definite decrease in IL-6 following the surgical removal of the tumor and kidney. This suggests IL-6 as being a potential marker for renal cell carcinoma. The multiplexed microELISAs prove to be effective tools for monitoring renal cell carcinoma.

## Introduction:

Renal cell carcinoma accounts for approximately 2-3% of all adult malignancies. It is characterized by a lack of early warning signs, resistance to radiation and chemotherapy, and infrequent but reproducible responses to immunotherapy agents such as interferon alpha and interleukin 2 (IL-2), therefore surgery remains the most effective treatment. Approximately 36,000 new cases of renal cell carcinoma are reported in the United States every year, with 12,500 deaths. The objective of this study is to monitor renal cell carcinoma pre and post surgery (radical nephrectomy) to detect recurrence or advancement of the disease using multiplexed microELISAs.

## Materials and Methods:

Using two of Quansys' multiplexed ELISA kits, the Quansys Cancer Array and the Q-Plex™ Human Cytokine Array, the samples taken pre and post surgery were analyzed simply in a 96-well multiplexed ELISA format. See figure 2. The Quansys multiplex ELISA technology involves capture antibody absorbed to the bottom of each well of a 96-well plate in a multiplexed fashion. Antigen is then captured by each specific antibody, following which, biotinylated detection antibody recognizes and binds to the antigen. Lastly, a response is observed by a chemiluminescent reaction of streptavidin-HRP and substrate. Each sample was run in triplicate on the 96-well plate and analyzed for AFP, CEA, PSA, IL-6, hCGB, CA125, Prolactin (PRL), and CA19-9 with the Quansys Cancer Array and IL-1α, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-13, IFNγ, TNFα, and TNFβ with the Q-Plex™ Human Cytokine Array. The response given was a chemiluminescent reaction and an image was captured using a CCD camera. See Figure 1.

## Results:

Two responses were seen in the results of the test. Using the Quansys Cancer Array, a response from CA19-9 and IL-6 were observed. Using the Q-Plex™ Human Cytokine Array, a response from IL-6 was observed. The responses observed from the other analytes tested showed no response and were at background levels. See Figure 3. The pre surgery sample yielded a CA19-9 level of 20 U/ml, and IL-6 of 90 pg/ml. The post surgery sample yielded a CA19-9 level of 20 U/ml and IL-6 of <2 pg/ml. Cancerous levels of CA19-9 are expected to be >37 U/ml. The CA19-9 level detected is expected in normal sera. Research has shown that IL-6 could be a potential marker for renal cell carcinoma. Normal levels of IL-6 are expected to range between 5-8 pg/ml.

## Conclusion:

Monitoring renal cell carcinoma pre and post surgery to detect recurrence or advancement of the disease using Quansys' multiplexed microELISAs has proven to be effective by accurately quantitating IL-6 levels. There was a definite decrease in the IL-6 response following surgical removal of the tumor and kidney. This suggests IL-6 as being a potential marker for renal cell carcinoma. The responses observed from the other analytes tested showed no response and were at background levels. The multiplexed microELISA's prove to be effective tools for monitoring renal cell carcinoma.

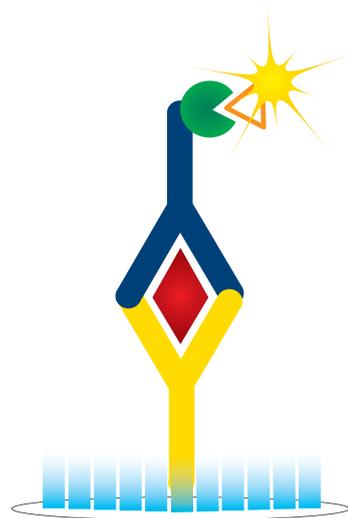


Figure 1: Sandwich ELISA

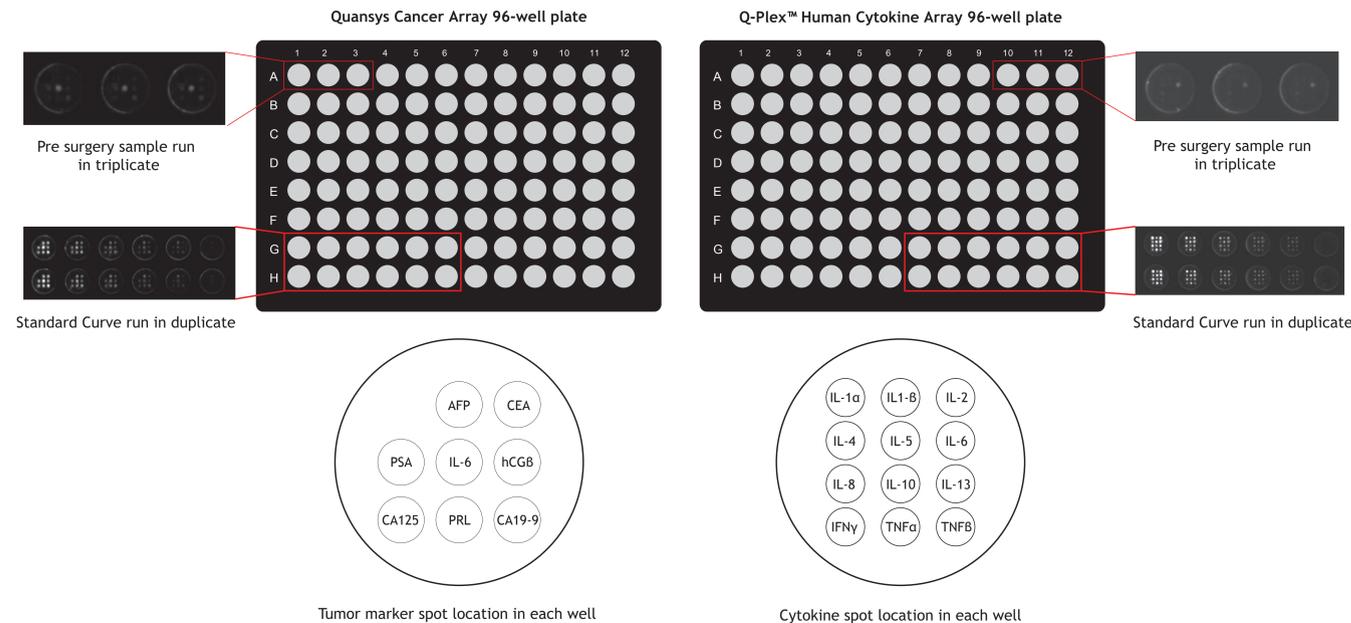


Figure 2: Outline of test set up using two of Quansys' multiplexed ELISAs

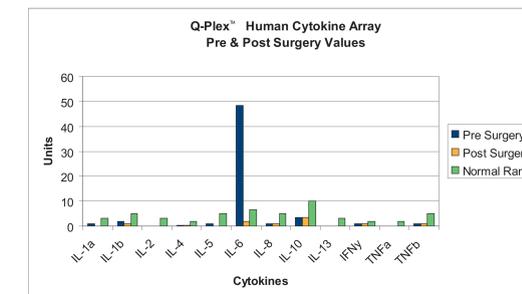
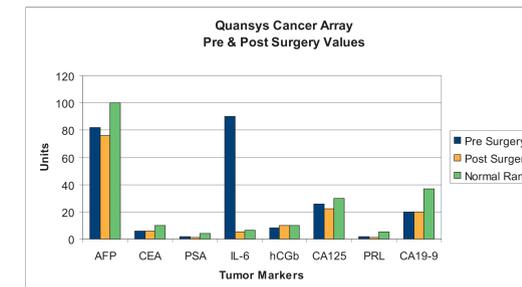


Figure 3: Responses observed from each of the analytes tested compared against pre surgery, post surgery, and the normal range expected in human sera.



Dissected tumor and kidney