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## INTRODUCTION

- The plasma proteome profiling with monoclonal antibody based protein chips is a promising new tool in biomarker discovery that can be used to identify novel plasma markers in a wide variety of diseases.
- Biosystem International developed a monoclonal antibody library that has been selected to work in the “capture-inhibition” assay and recognizes proteins of the plasma proteome in their native conformation.
- Based on these monoclonal antibodies a novel system – QuantiPlasm69™ (QP69) – has been introduced by Radox Laboratories using 69 monoclonal antibodies (mAbs). Among other important attributes, the array measures the relative concentration of 69 reactive epitopes on different plasma proteins, simultaneously.
- The mAbs are immobilized on 9x9 mm ceramic chips and a biotinylated plasma protein tracer (prepared from human plasma) is competing with plasma proteins in the tested sample for mAb binding. The amount of the bound tracer is determined by a streptavidin-PO conjugate and a chemiluminescence substrate.

## AIMS

We aimed to evaluate the clinical use of the novel QP69 kit for identification of mAbs/plasma proteins able to discriminate between lung and prostate cancer patients and properly matched healthy controls.

## SUMMARY

- The QP69 kit is easy to handle, the workflow is simple, the assay time is reasonably short.
- The QP69 kit can be used for comparison of pooled plasma samples of patients and controls
  - the results of the pools could be validated in the case of lung cancer patients by individual measurements.
- The QP69 kit could identify 3 mAbs suitable for discrimination between lung cancer patients and age/gender-matched healthy controls.
- The combination of these markers with classical tumor markers (e.g. CEA, TPA) further enhanced their discriminative power.
- In the case of prostate cancer patients the QP69 kit could identify 3 promising mAbs for discrimination between patients and controls but their power should be tested by measuring individual samples.
- For this patient group the application of the QP300 kit could provide further possibilities, too.

## Biosystem International



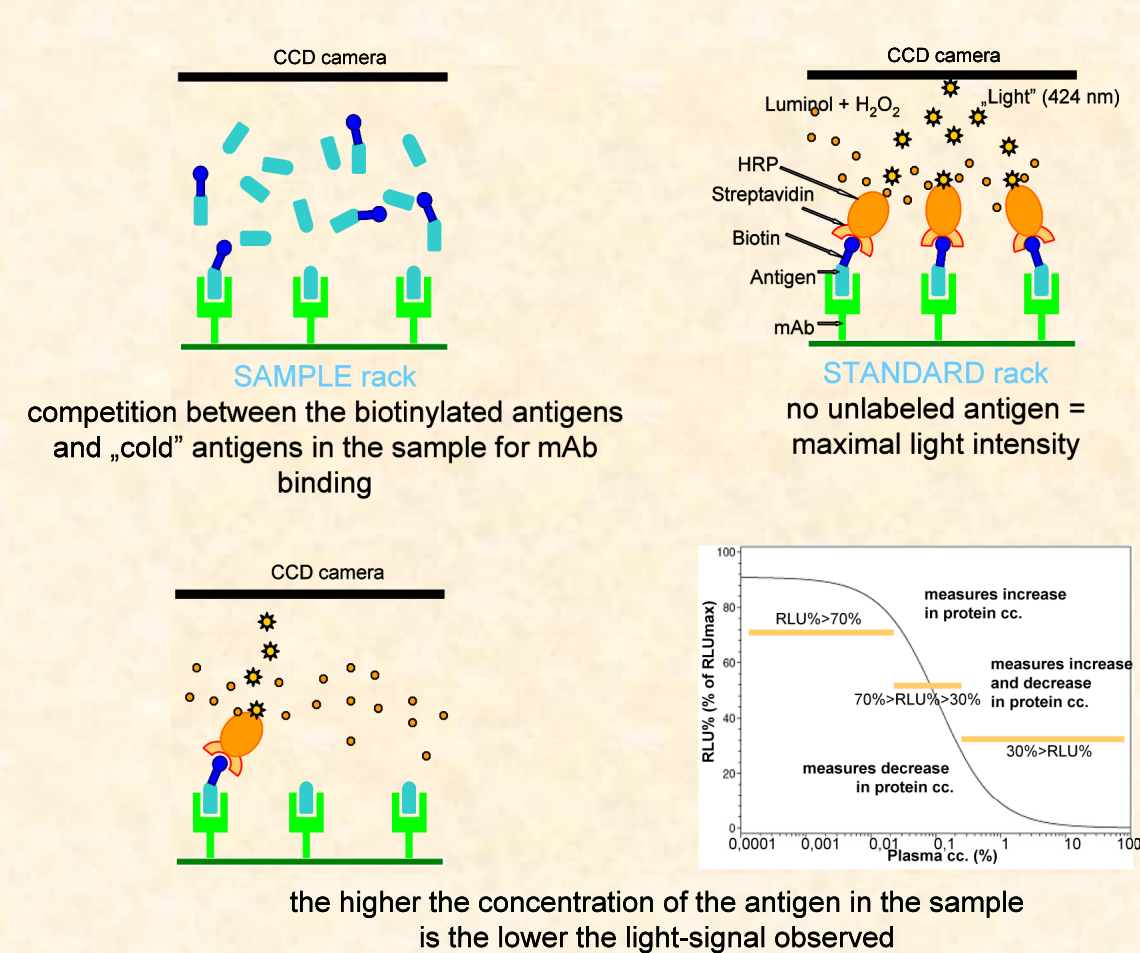
## Radox laboratories

Development of the QP69 mAb-based protein chip assay for the Evidence Investigator analyzer

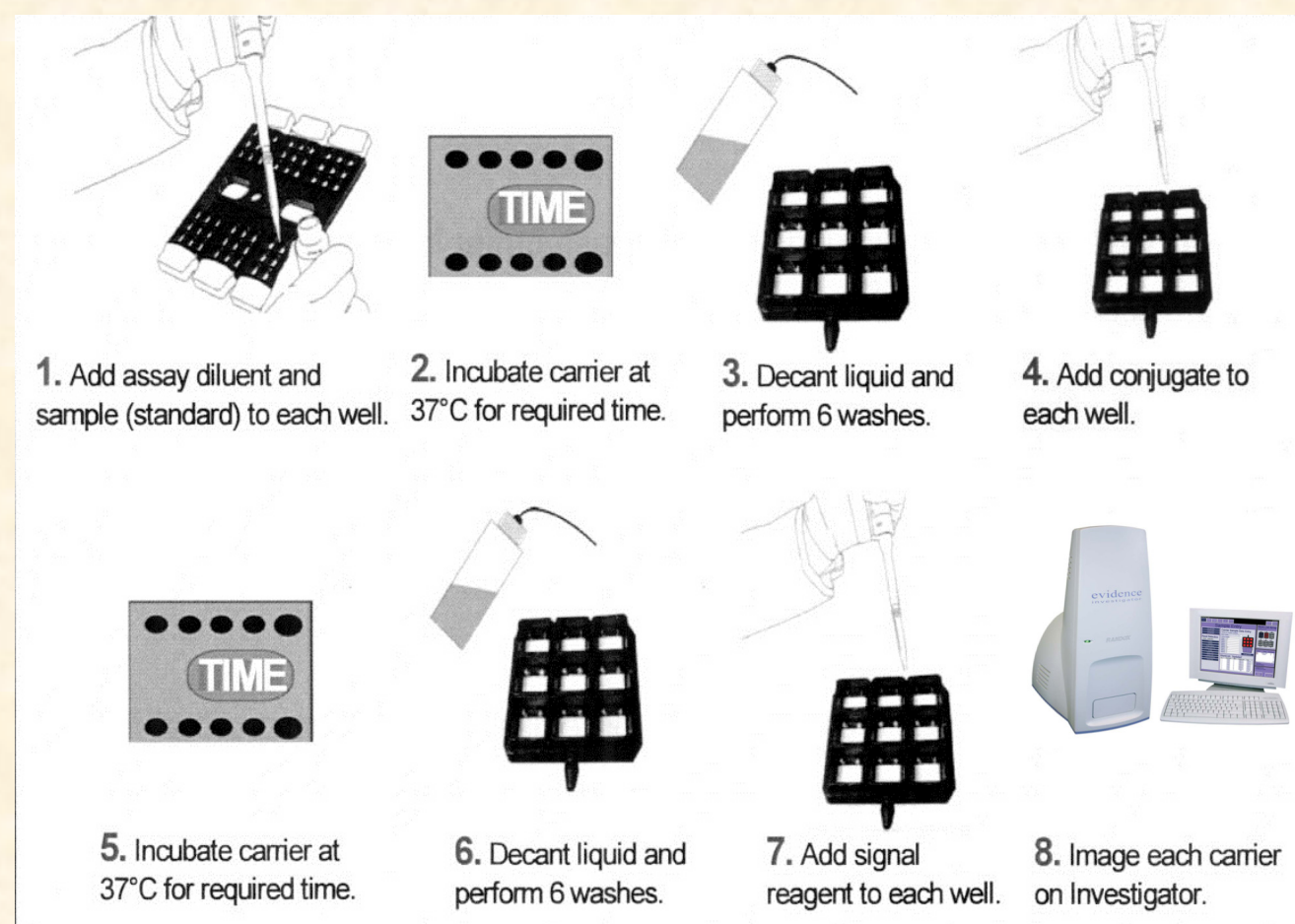
## University of Debrecen

- Analytical evaluation of the QP69 assay
- Clinical evaluation of the QP69 assay
  - Collection of plasma and serum samples of cancer patients and controls
    - Lung cancer: 150 patients
    - Prostate cancer: 150 patients
    - Controls: 300 healthy individuals
  - Collection of clinical and histopathological data (50-70 parameters) of patients
  - Measurement of clinical chemistry tests (24 analytes) and classical tumor markers (15 markers) in serum of patients and controls
  - Evaluate individual and pooled patient/control samples using the QP69 kit
  - Data analysis – with the help of Adware Research and Biosystems International

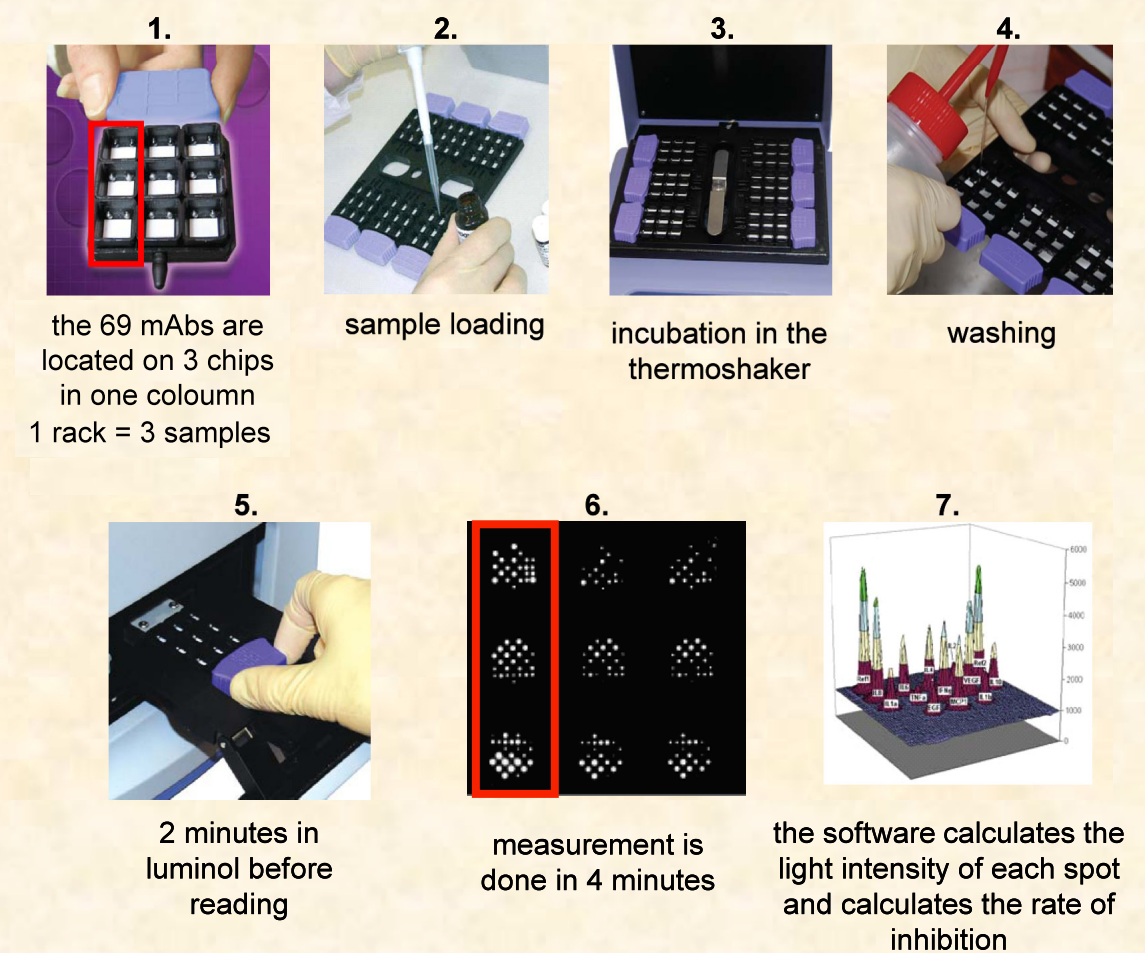
## The principle of the assay



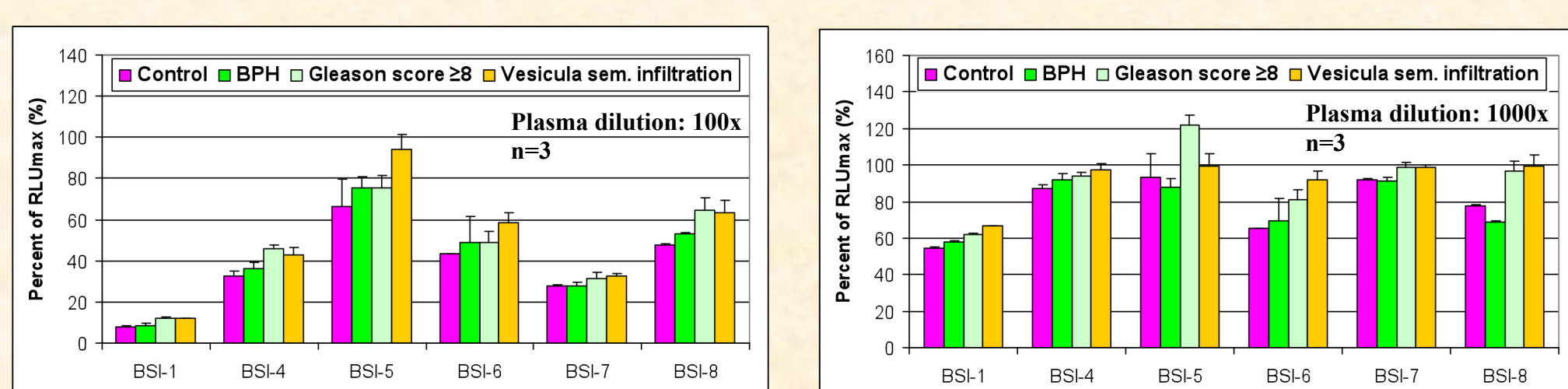
## The protocol of the assay



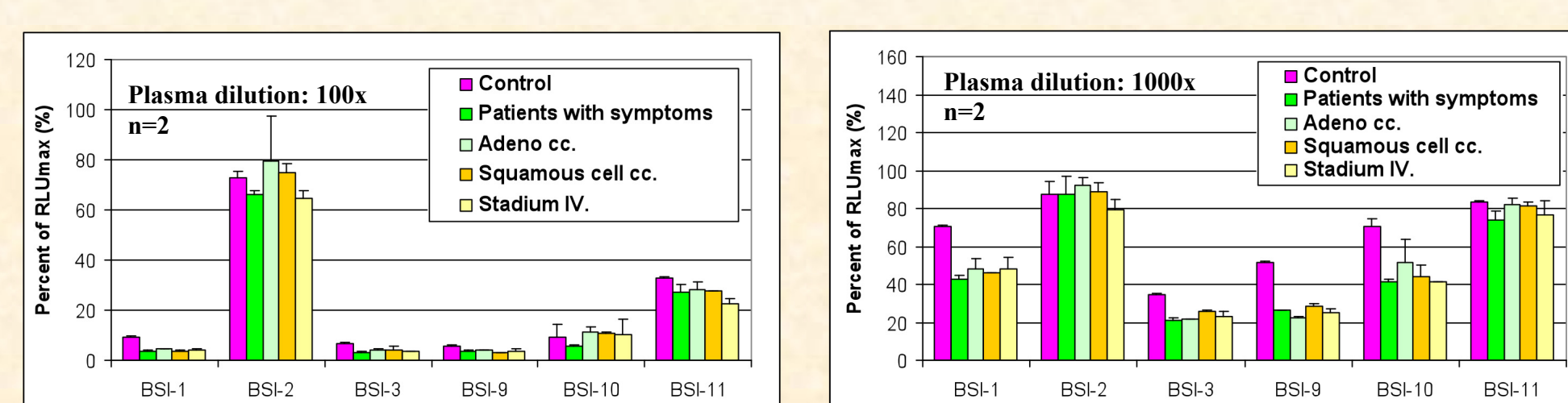
## Technical details



## Prostate cancer



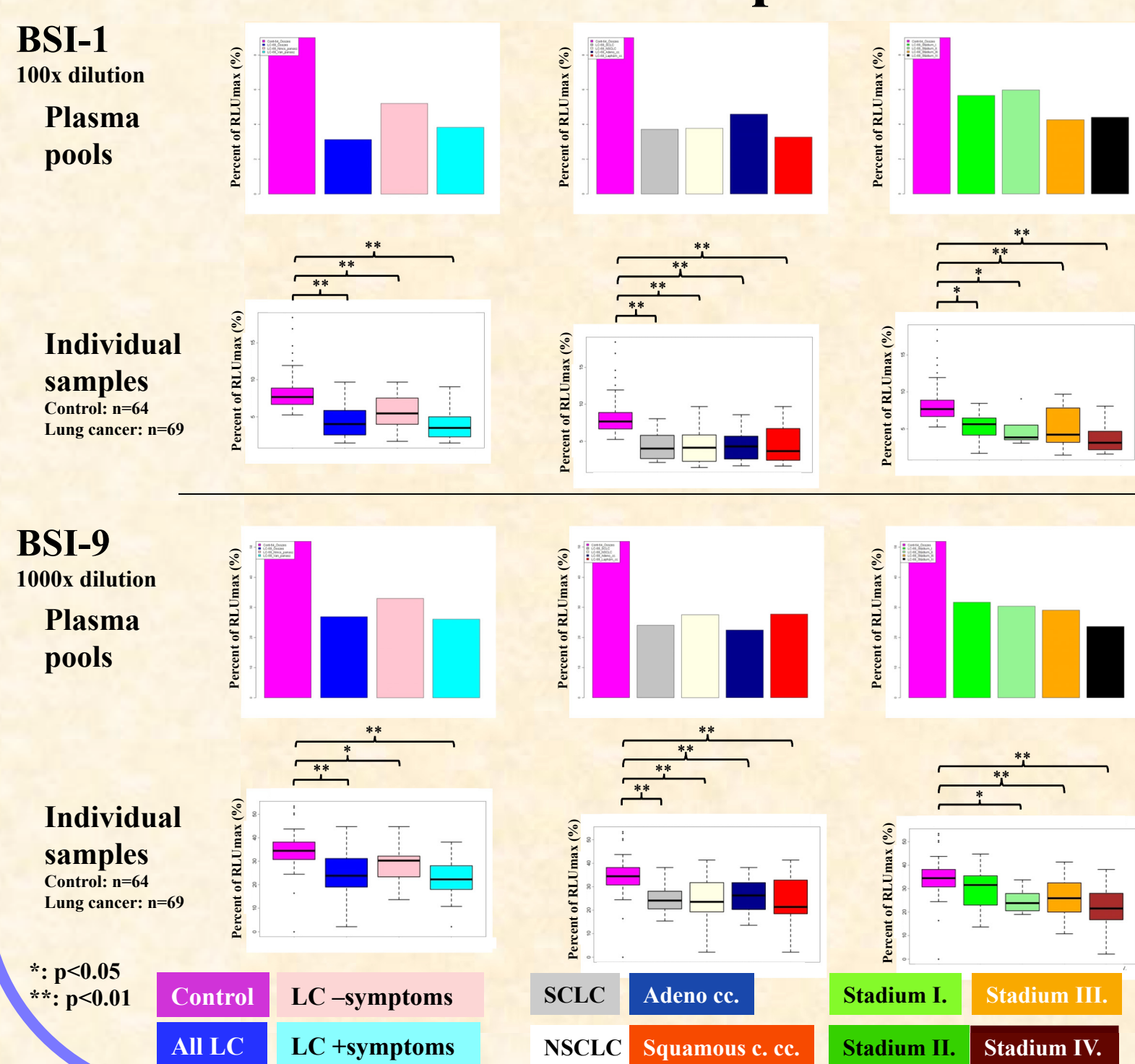
## Lung cancer



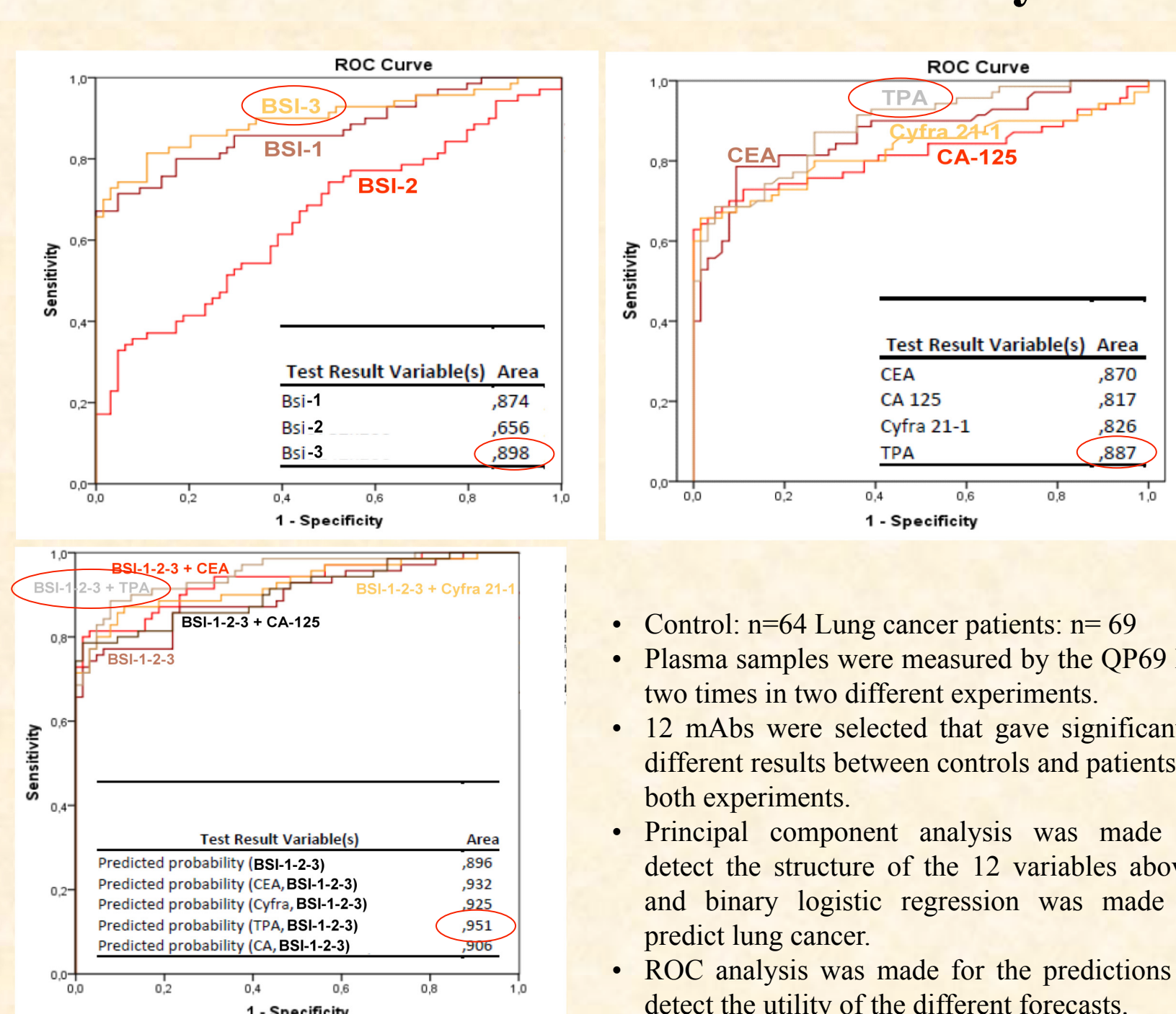
## Evaluation of plasma pools

- Based on clinical and pathological data patients were placed in different subgroups.
- Equal amounts of plasma were pooled from each member of a certain subgroup.
- The plasma pools of the different subgroups were evaluated by the QP69 kit 2-3-times.

## Comparison of plasma pools and individual samples



## Discriminative power of QP69 and classical tumor markers – ROC analysis



- Control: n=64 Lung cancer patients: n= 69
- Plasma samples were measured by the QP69 kit two times in two different experiments.
- 12 mAbs were selected that gave significantly different results between controls and patients in both experiments.
- Principal component analysis was made to detect the structure of the 12 variables above, and binary logistic regression was made to predict lung cancer.
- ROC analysis was made for the predictions to detect the utility of the different forecasts.

## Possible applications of the QP69 assay

