



Enabling Technologies for Rapid Point of Care Diagnostics

Howard J. Kirchick, Ph.D.
Director of Clinical Laboratory Affairs
ENCOMPASS Services
Biosite Incorporated

❖ Point of Care diagnostics should:

- use small volumes of whole blood
- be simple enough that any healthcare worker can be trained to run the test
 - require only a single step for completion
 - no calibration
 - minimal use of external controls
 - have self-contained QC
- provide quantitative, rapid (no more than 15-20 minutes), accurate results for a entire panel of markers simultaneously
- allow for multiple patients to be assayed without sacrificing time to result
- have a footprint no larger than a desktop phone
- be capable of interfacing with the hospital's LIS

Agenda

- ❖ **Marker Discovery Process**
- ❖ **Phage Display Antibody Process**
- ❖ **POCT System Technology and QC**
- ❖ **Introduction of the MultiMarker Index™ (MMX)**

Discovering New Markers

MARKER CANDIDATES

- Novel Markers
- Discovery Partners
- Licensing



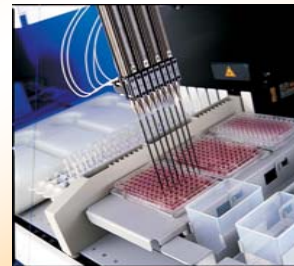
CLINICAL COLLABORATION

- Patient Medical History
- Blood Sample



MARKER VALIDATION

- High Throughput Immunoassay
- Data Analysis



Biomarker Sources

❖ Internal Research

- Thorough review of scientific literature to identify biomarkers involved in the disease process

❖ Discovery Collaborators

- Access to diagnostic licenses through collaborations with pharma and biotech companies

❖ External Licensing

- Accumulation of proprietary biomarker rights that are applicable to the program

MARKER CANDIDATES

- Novel Markers
- Discovery Partners
- Licensing



Clinical Collaborations

- ❖ **Identify diagnostic opportunity through market research and study of technical feasibility**
- ❖ **Recruit thought leaders in the field who are active in clinical research**
- ❖ **Refine product design based on input from thought leaders and additional market research**
- ❖ **Establish prospective clinical sample collection protocols at multiple sites targeting the appropriate patient population**

CLINICAL COLLABORATION

- Patient Medical History
- Blood Sample



Biomarker Validation

- ❖ Immunoassay reagents generated by internal antibody development are used to quantify potential biomarkers in patient samples.
- ❖ Combinations of biomarkers that provide the best discrimination of control vs. diseased patient populations are identified using Biosite's MMX™.
- ❖ Iterative process identifies panels with potential diagnostic utility.

MARKER VALIDATION

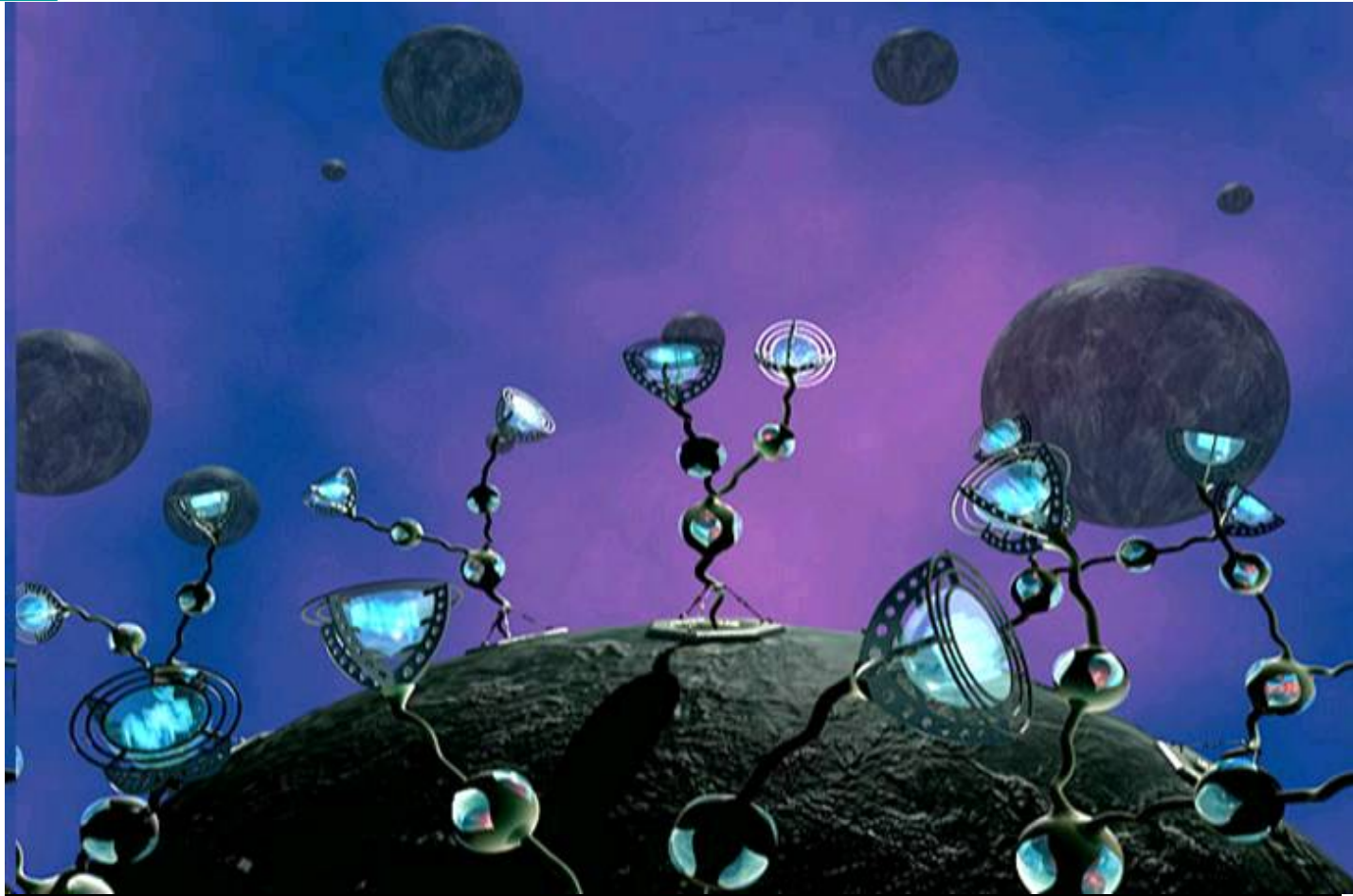
- High Throughput Immunoassay
- Data Analysis



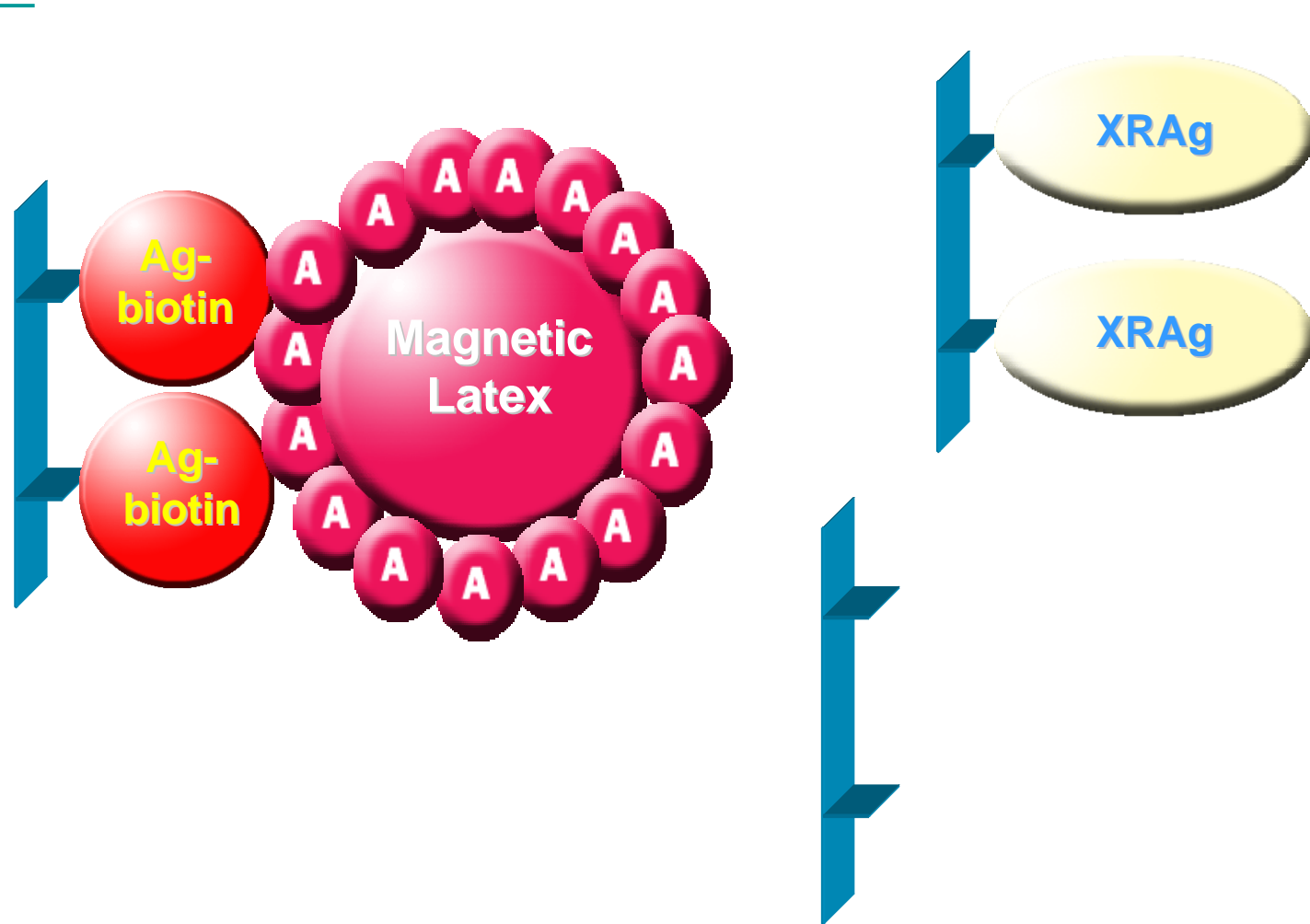


Phage Display Antibody Technology

Phage Display Technology



Selection of Omniconal[®] Ab (Step 3)





The Technology Behind the Tests

The Triage[®] System



The Triage MeterPlus Platform

❖ The Triage[®] MeterPlus

- **Manufactured by Biosite[®] Incorporated**
- **Runs individual Test Devices**
 - BNP
 - Cardiac markers
 - Shortness of breath panel
 - Drugs of abuse
- **Quantitative Results in approximately 15 minutes**
- **Built-in QC, QA functions**
- **Portable system**
- **Stored Memory, Printed and Displayed Results**
- **Hospital Information System Interface**
- **Lab Information System Interface**

Assay Procedure

What the User Sees

Step 1



**Add whole blood to
protein chip**

Step 2



**Insert protein chip
into instrument**

Step 3



Read results

Microfluidics of the Test Device

– What the user doesn't see

Sample Port

Sample enters here

Reaction Chamber

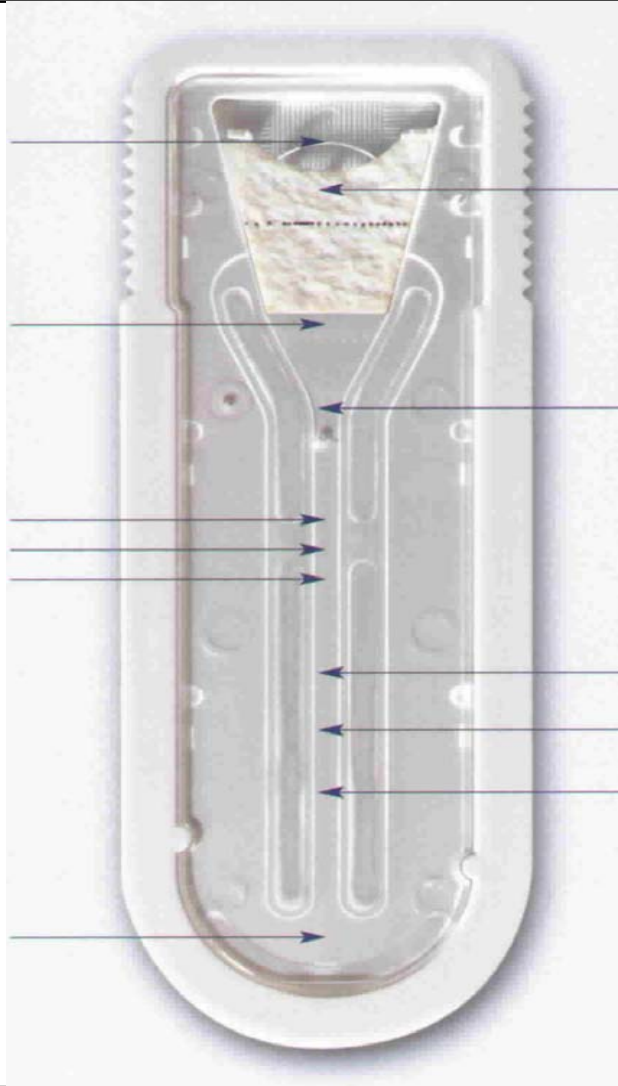
A small fraction of the plasma sample mixes with the dried reagents

Three Internal Controls

Independent high control zones and a zero control confirm that the test has been completed correctly

Waste Reservoir

The majority of the sample acts as a wash and collects in the perimeter of the device



Blood Filter

Cells are separated from plasma, eliminating the need for centrifugation

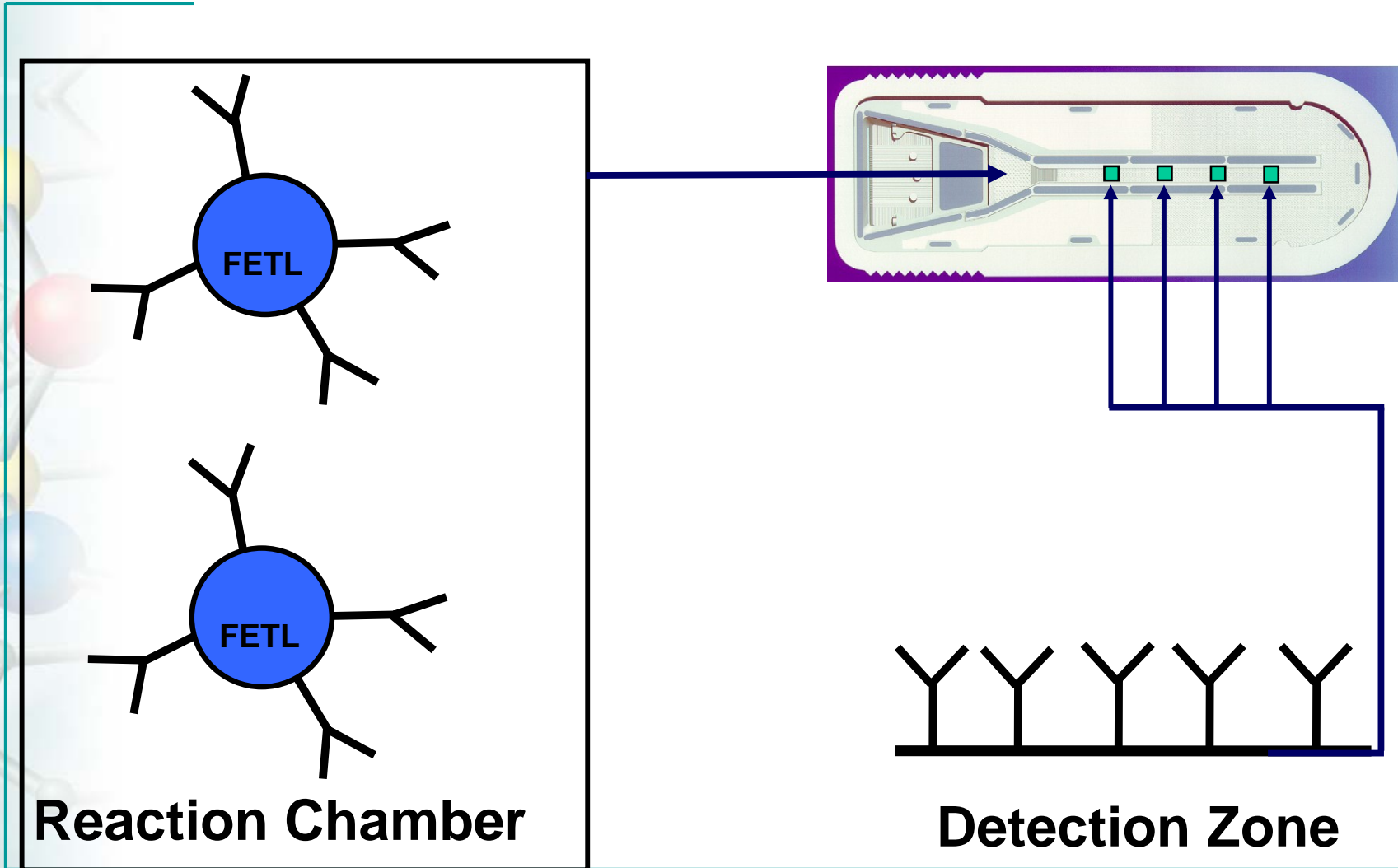
Time Gate

A hydrophobic surface acts as a time barrier and ensures an appropriate reaction time

Assay Zones

The assay analytes and the fluorescent-tagged antibodies are captured on separate zones of the device

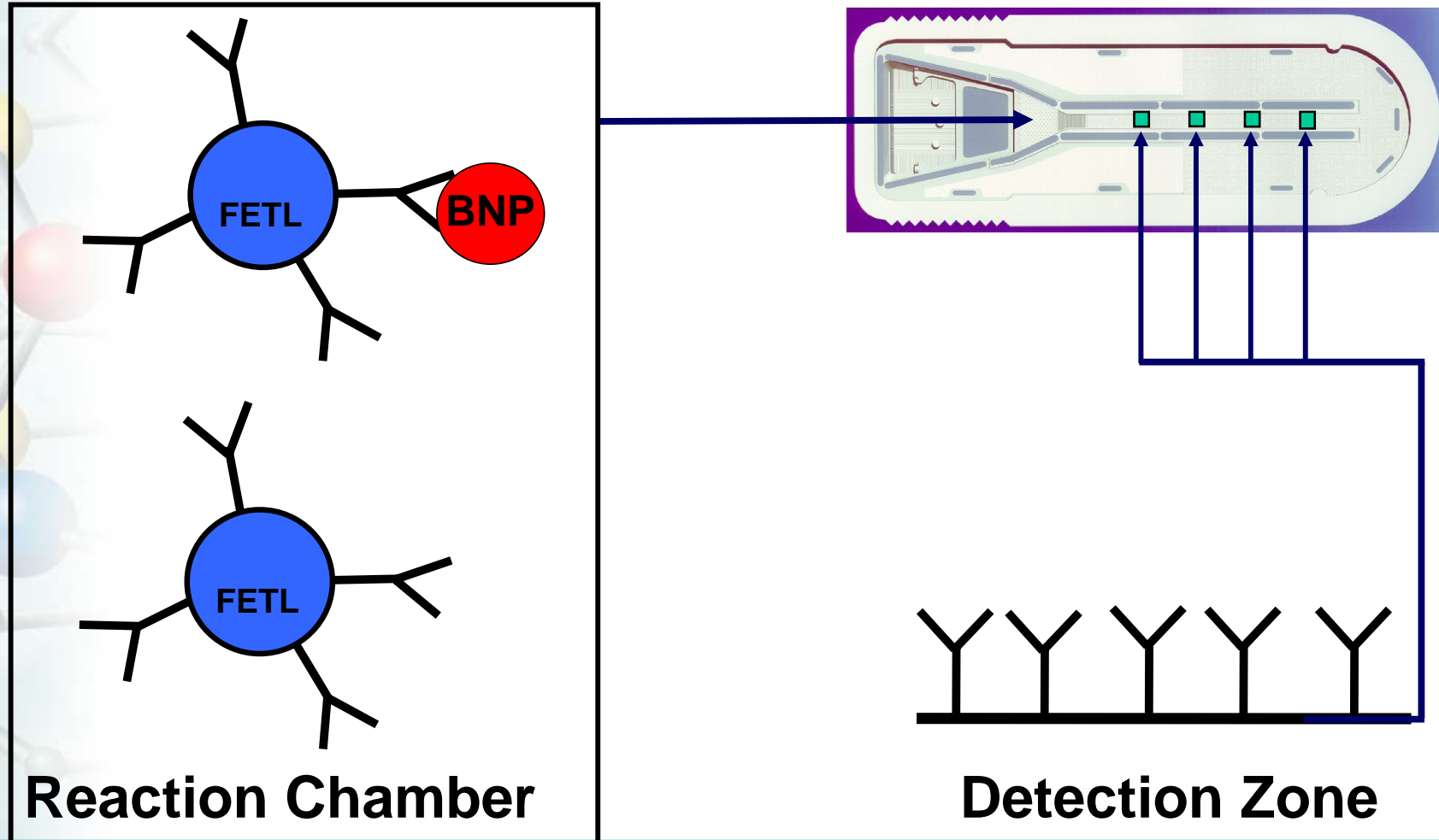
Test Device Components



Immunometric “Reverse Sandwich” Assay

Incubation with Detector Antibody

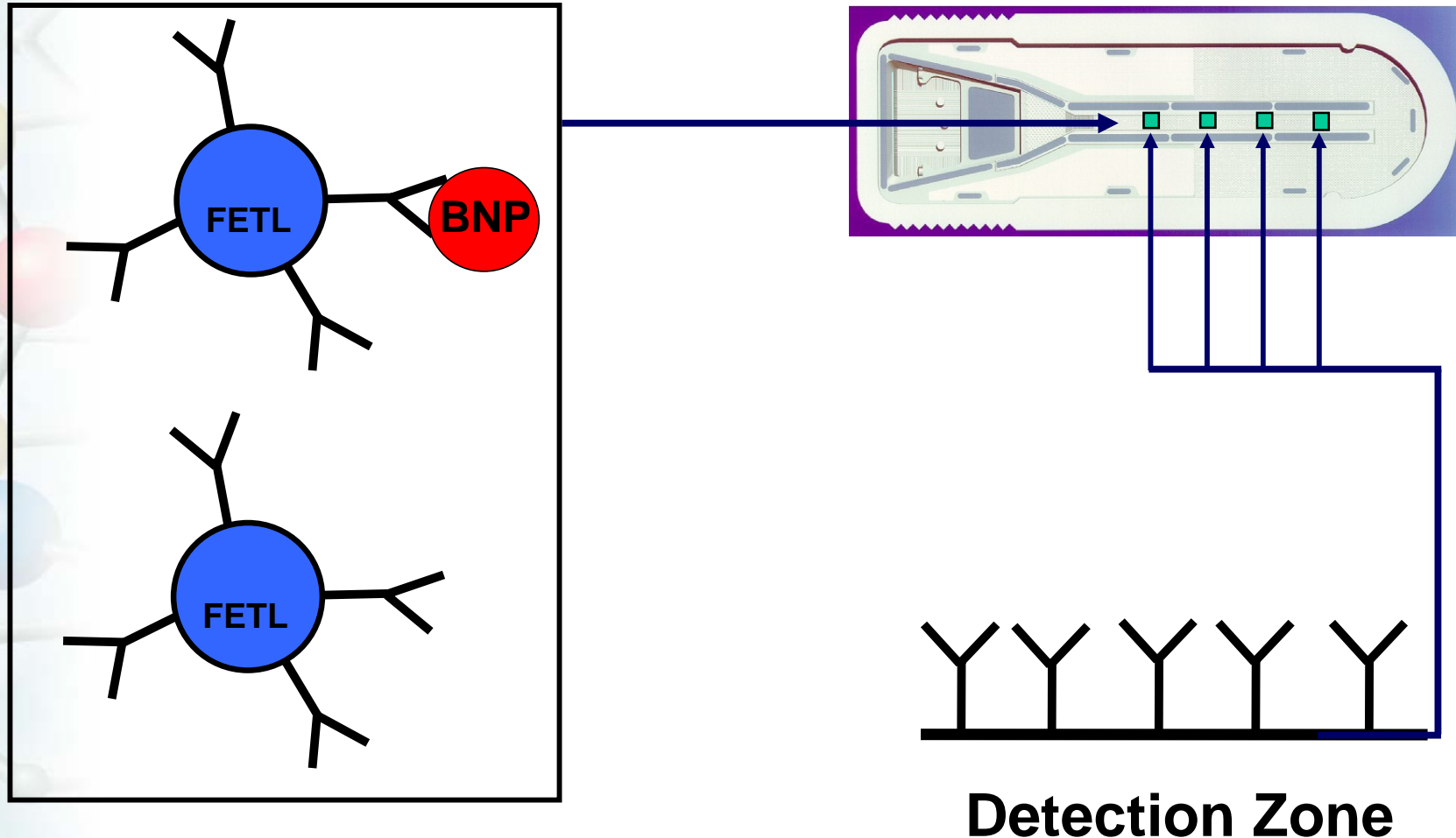
Analyte in sample binds to specific FETL-associated antibody during incubation in the reaction chamber.



Immunometric “Reverse Sandwich” Assay

Detection Zone Migration

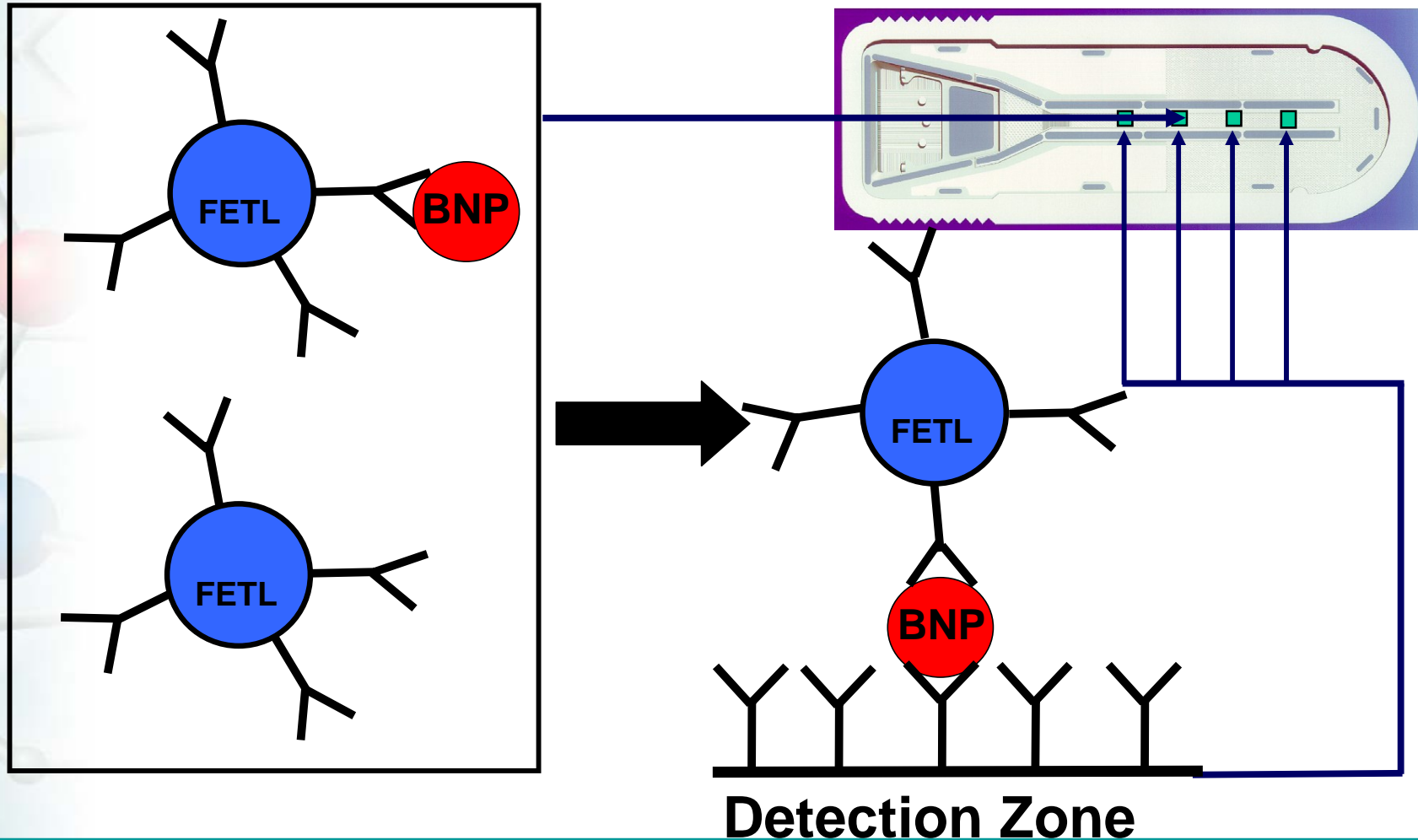
When the Time Gate is broken, bound and unbound FETL enter the Detection Zone.



Immunometric “Reverse Sandwich” Assay

Complex binding to Capture Antibody

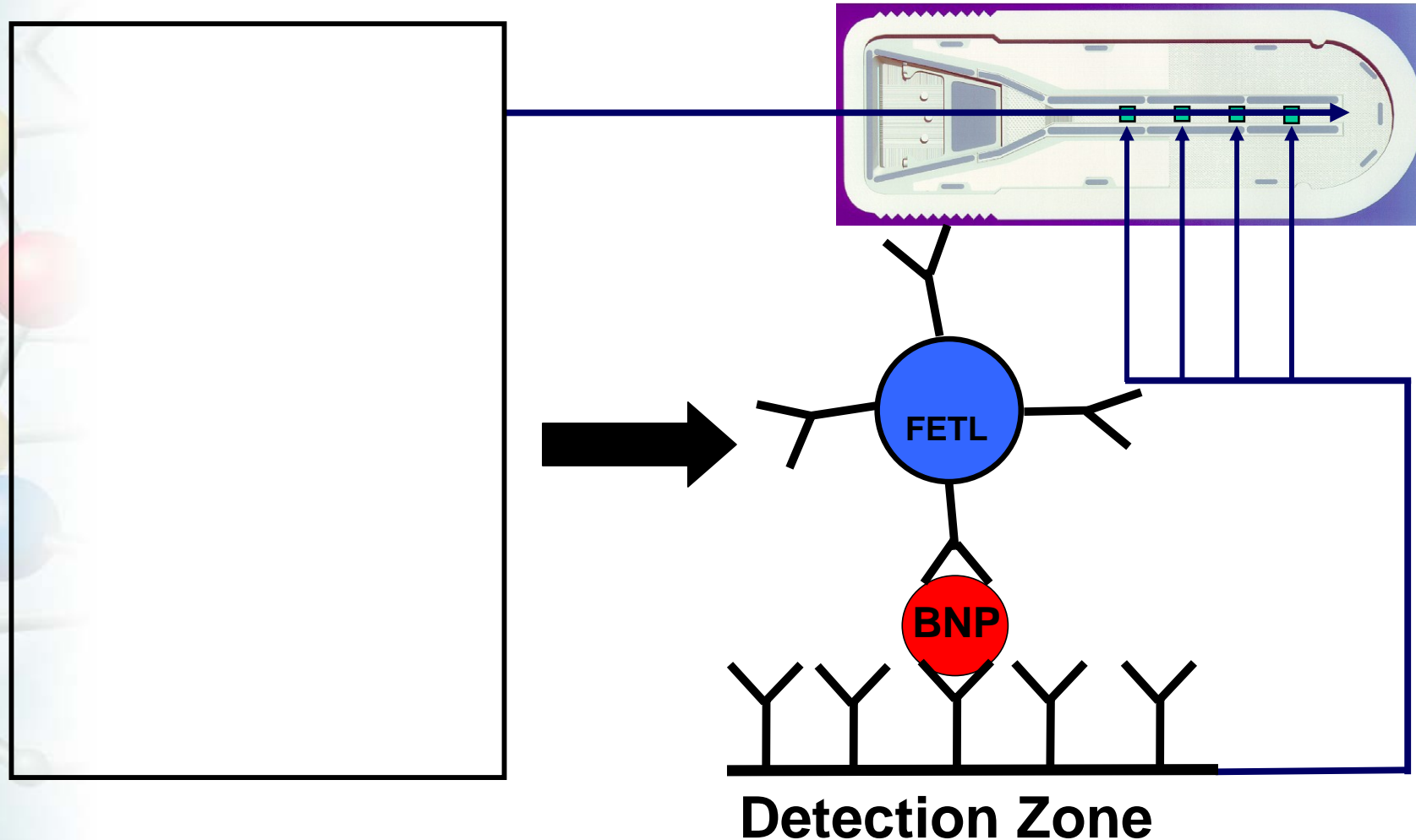
When an analyte-bound FETL crosses an immobilized Detection Zone antibody specific to the analyte, it is captured at that spot.



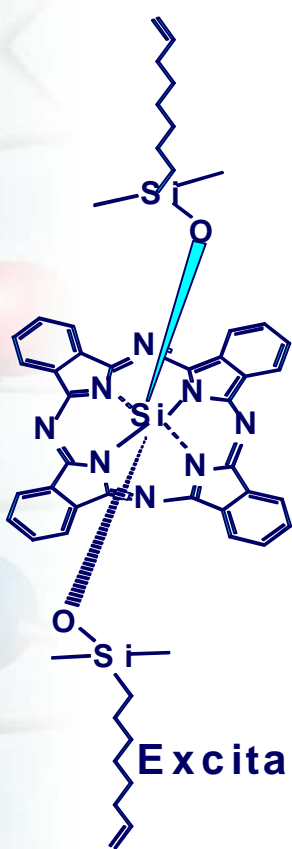
Immunometric “Reverse Sandwich” Assay

Detection Zone Wash

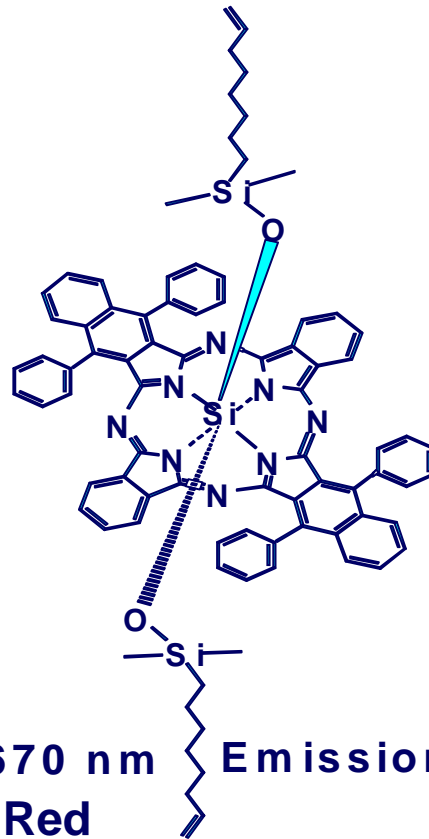
Unbound FETL progress down the Detection Zone to the Waste Reservoir. The remaining plasma continues to wash the Detection Zone.



Novel Fluorescent Dye Technology



Excitation = 670 nm
Red



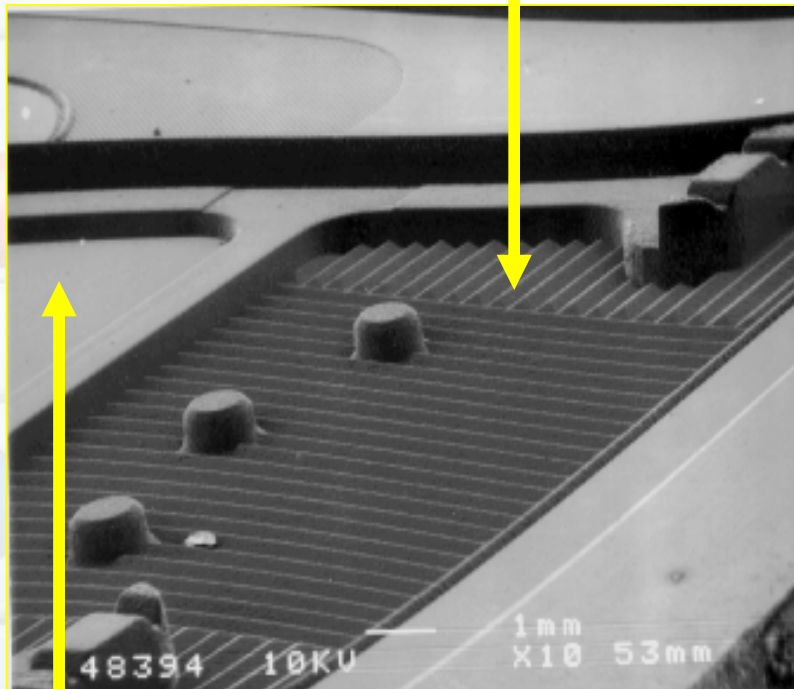
Emission = 760 nm
Near Infrared

- ❖ Utilizes fluorescence energy transfer
- ❖ High fluorescence intensity allows use of small reaction volume
- ❖ Dyes are axial substituted phthalocyanine derivatives

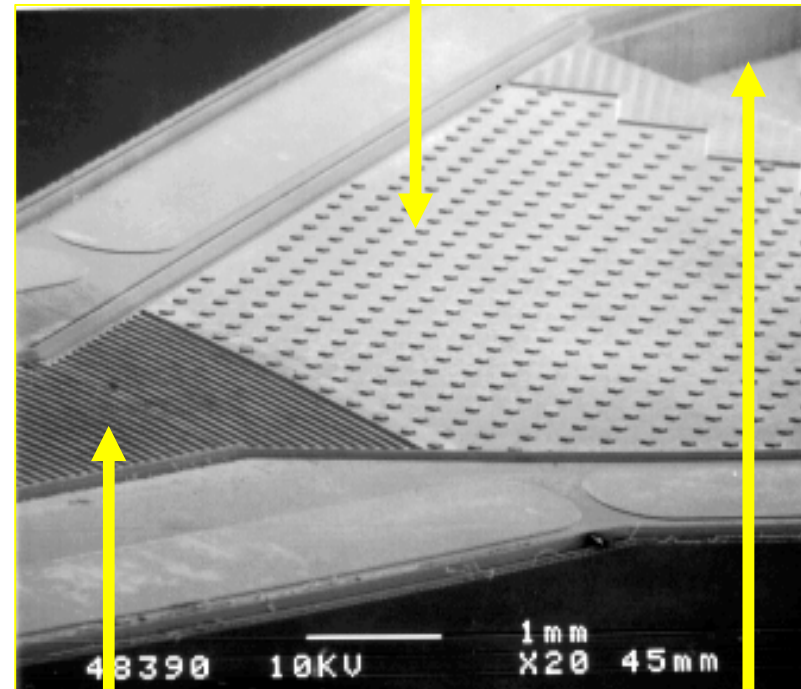
Blood and plasma absorb and fluoresce light at wavelengths < 600 nm

SEM of MicroCapillary Structure

Sample Addition Zone

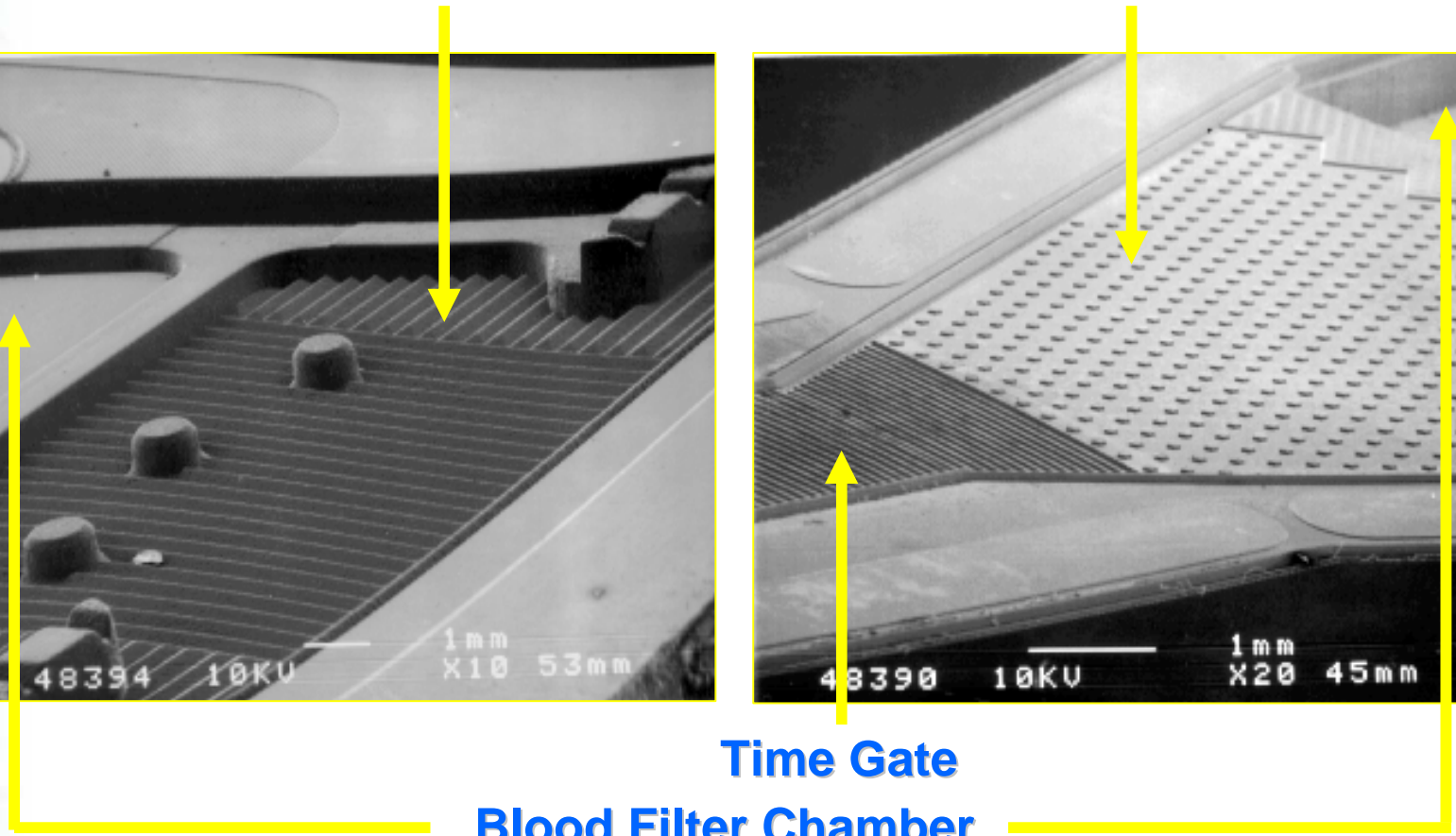


Reaction Chamber



Time Gate

Blood Filter Chamber





System and Assay QC

Meter Calibration

- ❖ **Meters are calibrated at the factory. The user can verify the meter calibration at any time by using the QC Device.**
- ❖ **The meter has an internal reference that is read every time a Test device is run in the meter.**
 - **Each day the QC Device is run, the Meter's internal reference is re-value assigned, effectively recalibrating the meter for the day.**

Device (Assay) Calibration

- ❖ **Each lot of Test Devices is calibrated at the factory. The calibration curves are downloaded to the Triage[®] Meter via an EEprom (reagent Code Chip[®] memory module) that is shipped with each kit of devices.**
 - **Each time a Test Device is run, the internal reference is checked and compared to the value assigned at the beginning of the day. The Test Device readings are adjusted based on the internal reference comparison.**

Meter QC - QC Device

- ❖ **Every day that testing is performed, the QC Device is run.**
 - **Cartridge identical to patient test cartridge**
 - **Mechanically handled same as patient test**
 - **True optical system check, not electronically simulated**
 - **Multiple zones of fluorescent chemistry**
 - **Three results – Pass or Fail**
 - **Instrument Calibration**
 - **Laser/Cartridge Alignment**
 - **Laser Functionality**
- ❖ **If any of the above tests fail or if the QC Device is not run at the beginning of the day, patient Test Devices cannot be run (lock out).**

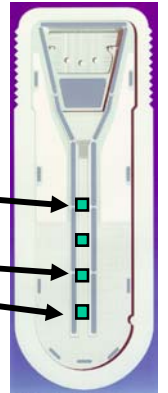
Additional Triage[®] Meter QC

- ❖ Each time a Test Device is run, the internal reference is checked to assure proper laser function.
 - If the internal reference check fails, the patient test result will not be reported. An error code will be displayed instead.
- ❖ The meter performs many electronic checks upon power up.
- ❖ Additional electronic checks are made each time a device is run.

Triage[®] Test Device Quality Control

❖ Besides the analyte detection zone, each Test Device has a:

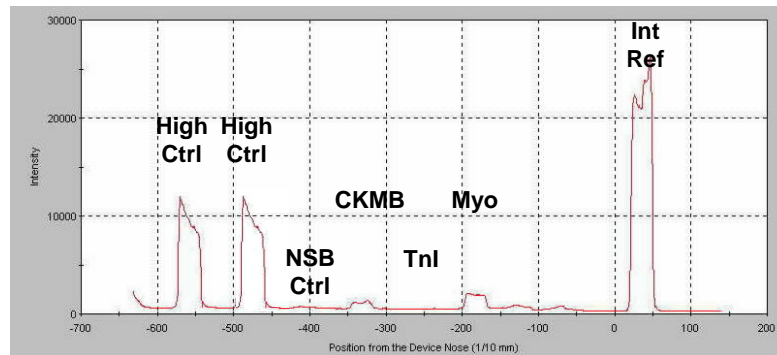
- Zero (non-specific binding) control
- Two immunoreactive assay controls



Triage[®] BNP Device

❖ The meter software checks:

- The position and geometry of all control and analyte specific reaction zones
- The baseline quality across the entire length of the device



Triage[®] Cardiac Device Scan

Triage[®] Test Device Quality Control (cont.)

- ❖ **The meter software checks:**

- **That sufficient sample was applied to the device**

- **That sufficient time for incubation and washing has occurred**

- ❖ **Biosite recommends that External QC (2 levels) be run when starting a new lot, for each subsequent shipment of a lot, or every 30 days a single shipment of a lot is in use.**

If any QC tests fail, an error message is generated and the patient result is not reported.

QC Lockouts

- ❖ **Invalid User ID**
 - Only valid users can operate the system
- ❖ **Lot Expiration Date Exceeded**
 - Expired reagents cannot be run
- ❖ **External Controls Not Run**
 - Controls must be run on new reagent lots and according to the frequency set by the Lab supervisor
- ❖ **QC Device Not Run**
 - The QC Device must run according to the frequency set by the Lab Supervisor
- ❖ **QC Device Failure**
 - All QC Device tests must meet specifications



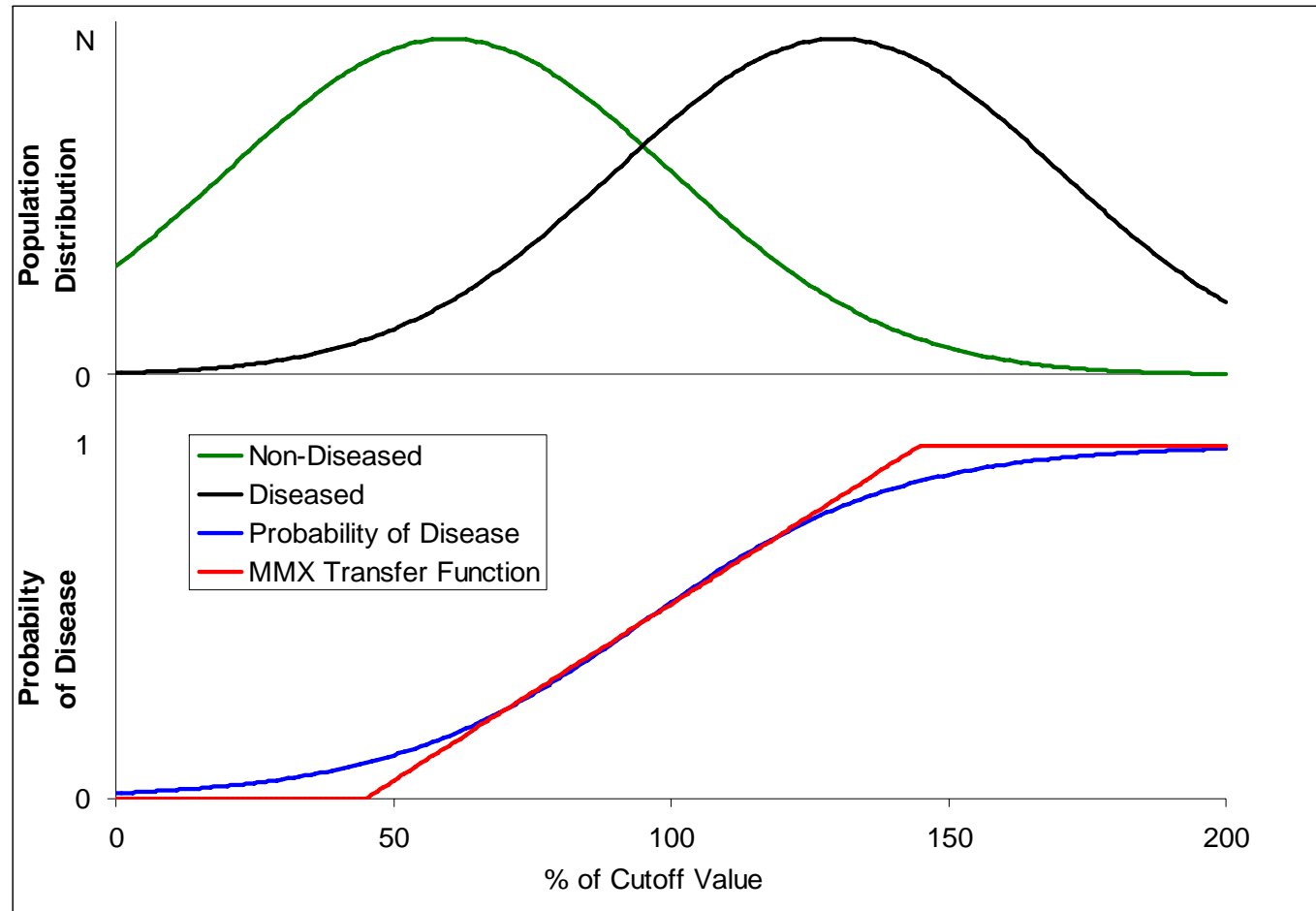
**Introduction of the
MultiMarker Index™
Concept**

MultiMarker Index™ - Motivation

- ❖ In complex disease states multiple markers are required due to complex disease etiology.
- ❖ Utilization of multiple markers can improve the diagnosis.
- ❖ Makes panels easier to interpret.



Probability of Disease



Conclusions

- ❖ **The technology to deliver rapid and accurate POC diagnostics that are easy to interpret, even for diseases with complex etiologies, exists today.**
- ❖ **Through the use of self-contained microfluidic devices that can run multiple simultaneous assays, and a compact analyzer that can be used by non-laboratory personnel to read and interpret the results of the assays, these goals can be achieved.**



Questions?