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Special Considerations for Tracers Based on Proteins or Protein Fragments

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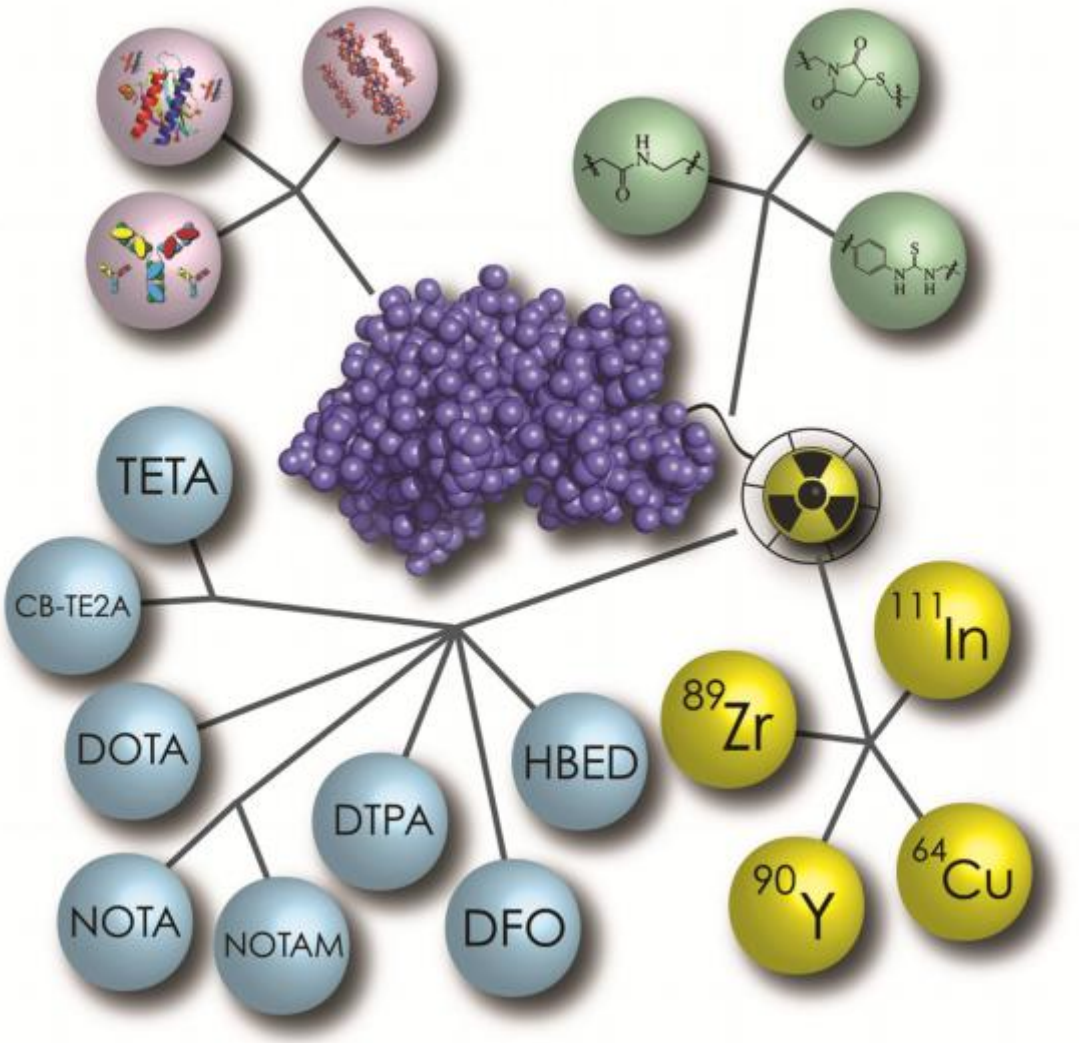
Disclosures

Serge K. Lyashchenko declares no conflicts of interest, real or apparent, and no financial interests in any company, product, or service mentioned in this program, including grants, employment, gifts, stock holdings, and honoraria.

Why Radiolabeled Proteins?

- Biomolecule
  - highly specific
  - less toxic
  - clinical need
- Radionuclide
  - PET
  - SPECT
  - Therapy
- Chelator
  - TETA
  - DOTA
  - DFO
- Linkage
  - Peptide
  - Thiourea
  - Thio-maleimide



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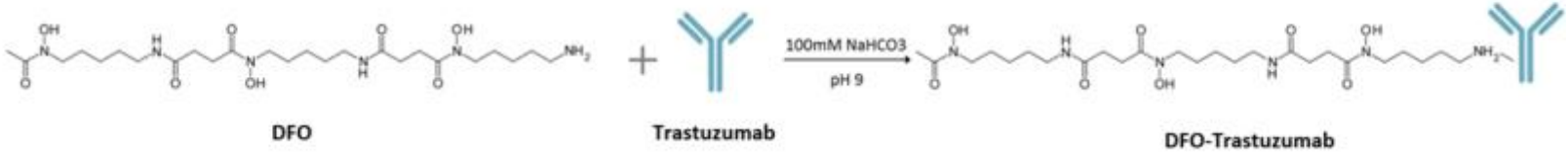
Zeglis, *et al. Dalton Transactions* 2011, 40: 6168-6195.

Presentation Overview

- Manufacturing Considerations and Experiences:
  - Protein conjugation
  - Radiolabeling
  - Dispensing
- Regulatory considerations
- Q&A

Conjugation Process

- Buffer exchange to appropriate buffer
- Incubation
- Purification buffer exchange
- Quality control
- Vialing and storage



Buffer Exchange Process

Tangential Flow Filtration

Considerations

- Easy-to-use
- Minimal manipulation
- Conjugations of up to 2gm of material are possible
- Single-use sterile, apyrogenic assemblies are available
- Emulsifiers in the formulation may be a problem

Conjugated Proteins
Unique QC Specifications

- Protein concentration (UV spectrophotometry)
- Number of chelates (radioisotopic dilution)

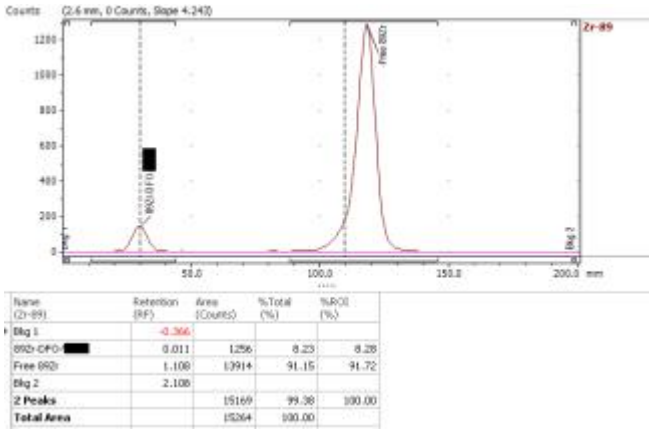
Protein Concentration Determination

- Determination of protein amount post initial buffer exchange
- Determination of conjugated protein yield
- Calculations are based on Beer-Lambert Law
  - Absorbance = extinction coefficient x concentration x travel distance
- Accurate response when using 0.2-1.0 mg/mL protein concentration
  - Dilution may be required

Chen Y, Methods Mol Biol.2013;1045:267-73. doi: 10.1007/978-1-62703-541-5_16

Why Number Of Chelate Sites is Important?

- Not enough chelates may result in suboptimal radionuclide incorporation efficiency
- Too many chelates may change protein behavior in-vivo
- Radioisotopic dilution method
  - Does not provide information on chelator distribution within protein species
  - Assumes minimal effect of the radioactive isotope species



Name (Zr-89)	Retention (RF)	Area (Counts)	%Total (%)	%ROI (%)
Bkg 1	-0.366			
89Zr-DFO-[redacted]	0.011	1256	8.23	8.28
Free 89Zr	1.108	13914	91.15	91.72
Bkg 2	2.108			
2 Peaks		15169	99.38	100.00
Total Area		15264	100.00	

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Lewis, MR, et al, Cancer Biother Radiopharm 2001 Dec;16(6):483-94

Protein Conjugation Experiences

- How much chelator to use?
  - As little as possible to allow sufficient radionuclide incorporation
- Storage (<-6o⁰C)
- Stability testing (depends on individual IND)

Radionuclide Availability

Diagnostic

- **^{89}Zr (78.4hours)**
- **^{124}I (100.4 hours)**
- ^{86}Y (14.74 hours)
- **^{64}Cu (12.69 hours)**
- ^{43}Sc (3.89hours)
- ^{44}Sc (3.97 hours)

Therapeutic

- Beta
  - **^{177}Lu (6.65days)**
  - **^{131}I (8.02 days)**
  - **^{90}Y (2.66days)**
  - ^{67}Cu (2.57days)
  - ^{47}Sc (3.35 days)
- Alpha
  - ^{225}Ac (9.95days)
  - ^{213}Bi (45.6minutes)
  - ^{212}Pb (10.64 hours)
  - ^{211}At (7.21hours)

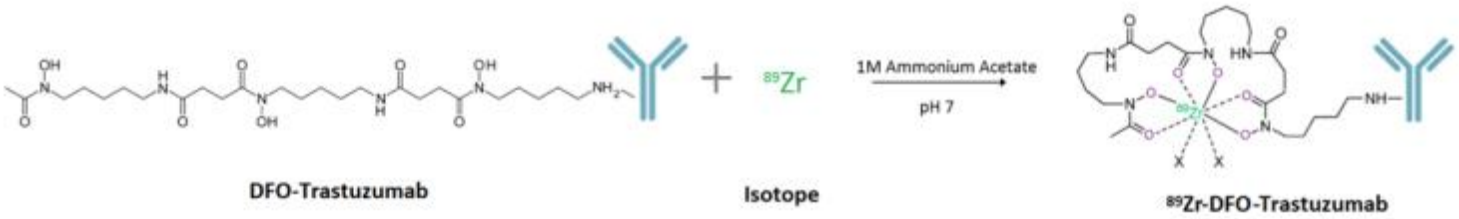
Radionuclide Related Experiences

- Dose calibrator radioactivity measurement
  - Dial setting ^{89}Zr 465 to 517
  - Dial setting ^{124}I 570 to 494 (487), with copper dipper

- Radiopharmaceutical vs. radiochemical raw material testing

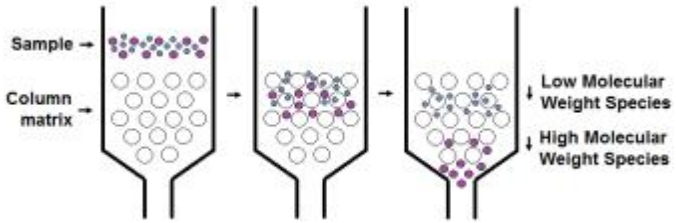
Beattie, BJ, et al, (2014) *PLoS ONE*, 9 (9), art. no. e0106868

Radiolabeling and Quality Control



Radiolabeling Considerations

Desalting Gel Purification



Considerations

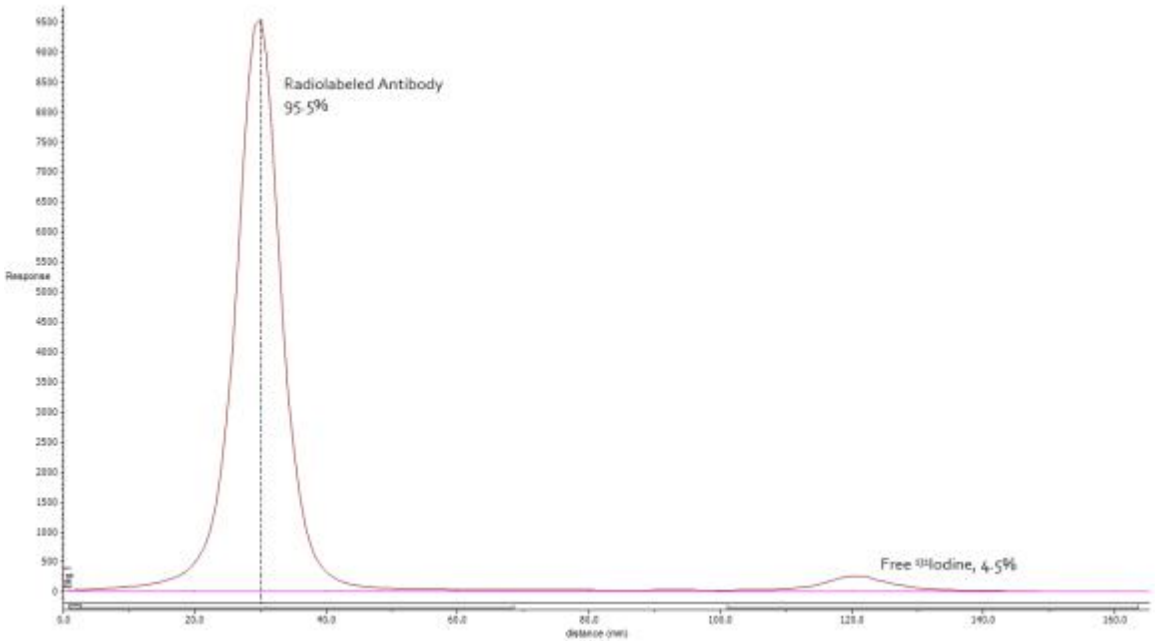
- Quick and easy-to-use
- Volume and protein mass limitations
- Manual process

Radiolabeled Proteins
Key QC Specifications

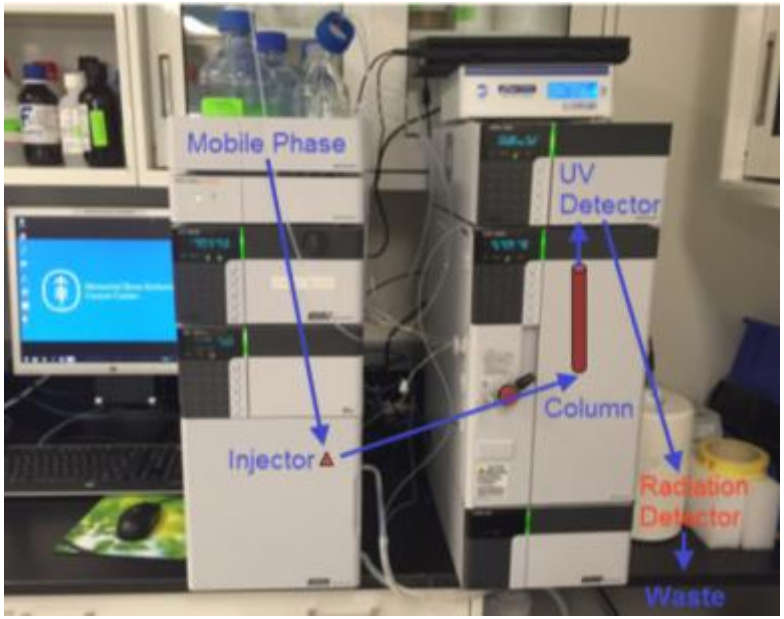
- Radionuclide incorporation (radiochemical purity by radio- TLC)
- Protein integrity (radiochemical purity by SEC-HPLC)
- Immunoreactivity (antigen binding assay)

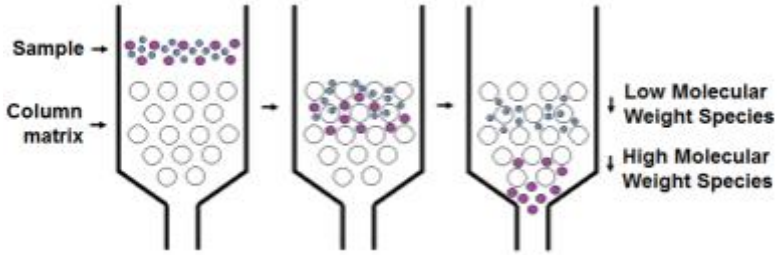
Radiochemical Purity by (r-TLC)

- Measures amount of unincorporated radionuclide



Radiolabeled Protein Integrity (Radiochemical Purity by SEC-HPLC)

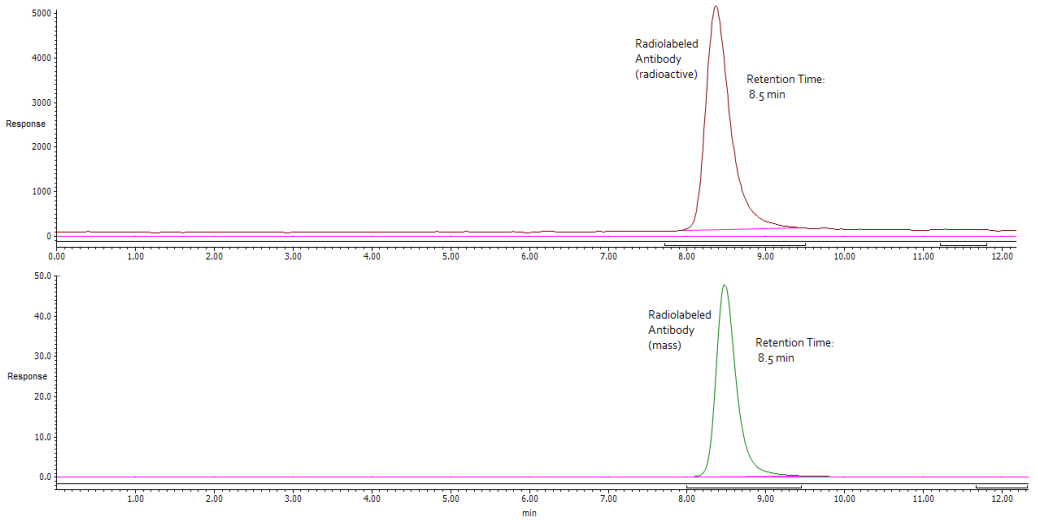




Radiochemical Purity by SEC-HPLC

Factors affecting results

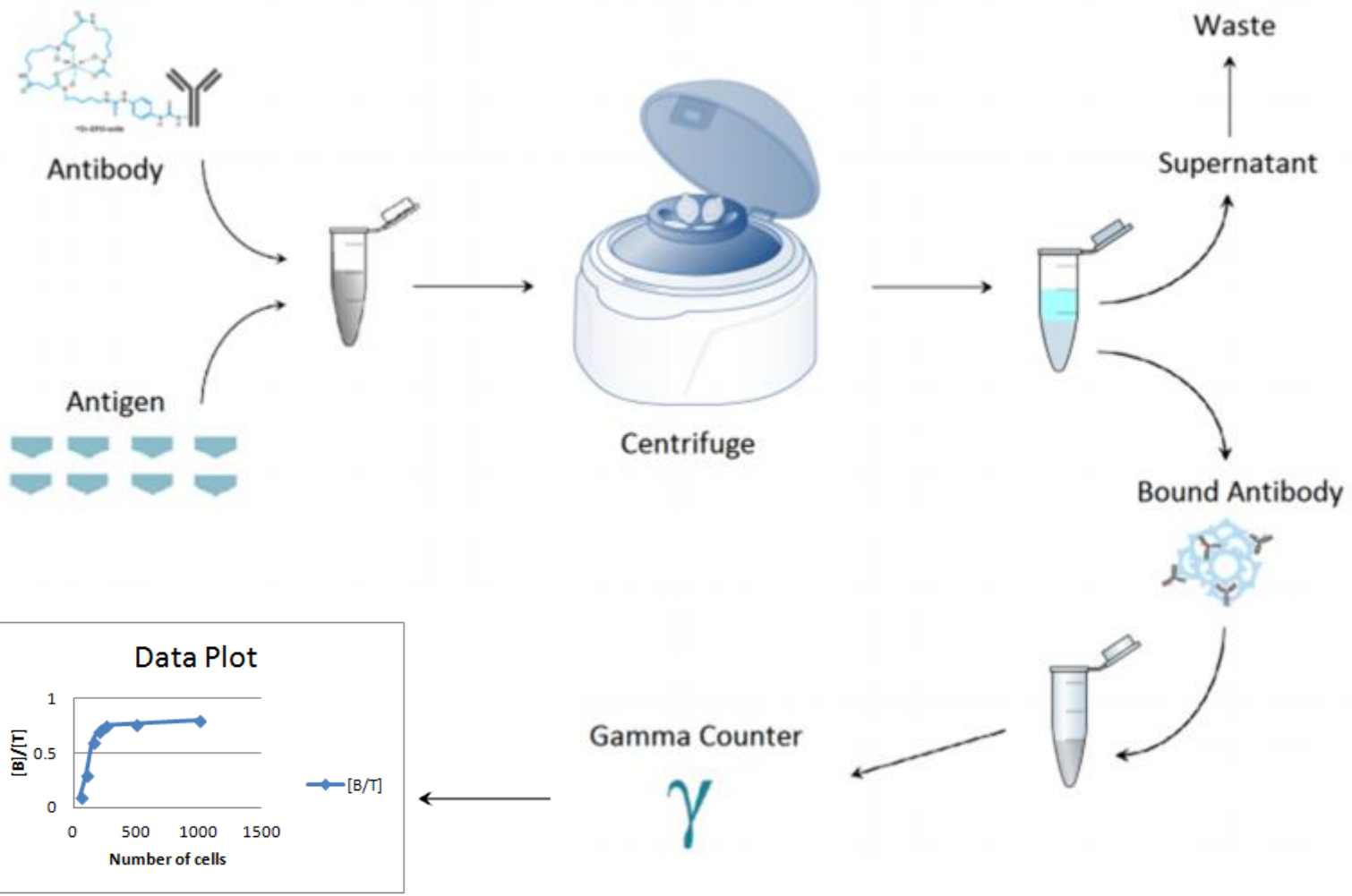
- Column
- Radioactivity detector
- Formulation



Immunoreactivity

- Ability of antibody to bind to the antigen
- Serves as a measure of efficacy
- Factors Affecting Immunoreactivity
  - Incorporation of chelator
  - Radionuclide incorporation
  - Site of incorporation
  - Conjugation and radiolabeling conditions
  - Autoradiolysis
  - Radiolabeled protein specific activity

Immunoreactivity Assay (RIA)

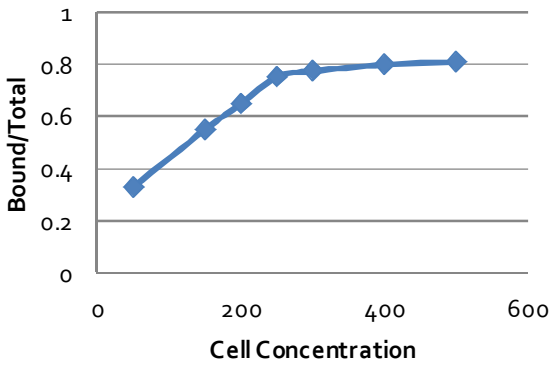


Cell Based RadioimmunoAssay

Conc.	CPM	CPM	CPM	Avg CPM	Avg - Blank
500	6885	6885	6885	6885	6885
400	6800	6800	6800	6800	6800
300	6600	6600	6600	6600	6600
250	6400	6400	6400	6400	6400
200	5525	5525	5525	5525	5525
150	4675	4675	4675	4675	4675
50	2800	2800	2800	2800	2800
5 (Blank)	0	0	0	0	0
STD	8500	8500	8500	8500	

Conc.	NIC	T/B
500	1	1.23
400	1.25	1.25
300	1.666667	1.29
250	2	1.33
200	2.5	1.54
150	3.333333	1.82
50	10	3.04

Y-Intercept	1.003
IR (%)	**99.7**



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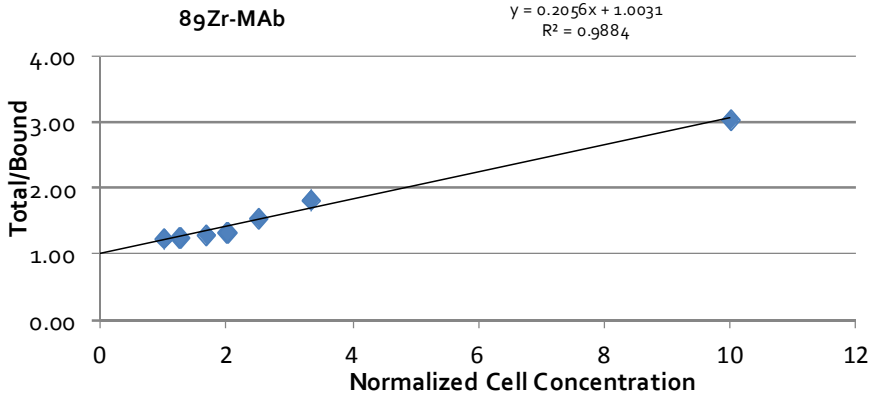

Lindmo T, et al. J Immunol Meth 1984

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Radioimmunoassay Considerations

Lindmo Limitations

- Reported calculated IRF value by itself is insufficient for evaluation purposes
- The method is prone to overestimation of true immunoreactivity
- Results may not be valid when the calculated theoretical value varies by more than 10% from the obtained experimental value

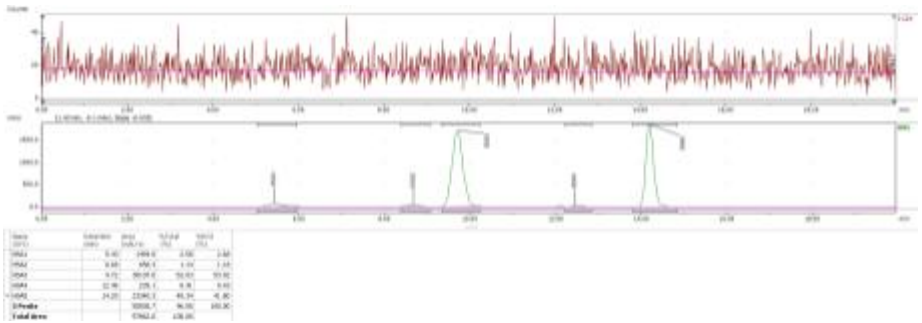
Experimental Factors

- Incubation time
- Protein mass
- Operator technique when separating the bound fraction from the unbound
- Sample dilution
- Gamma counter operation
- Degree of antigen expression on antigen expressing cells

Mattes MJ. Int J Cancer 1995

Formulation Considerations

- Radioprotection
- Stabilization
- Compatibility with intended route of administration
  - Volume
  - pH
  - Osmolality
  - Chemical Composition



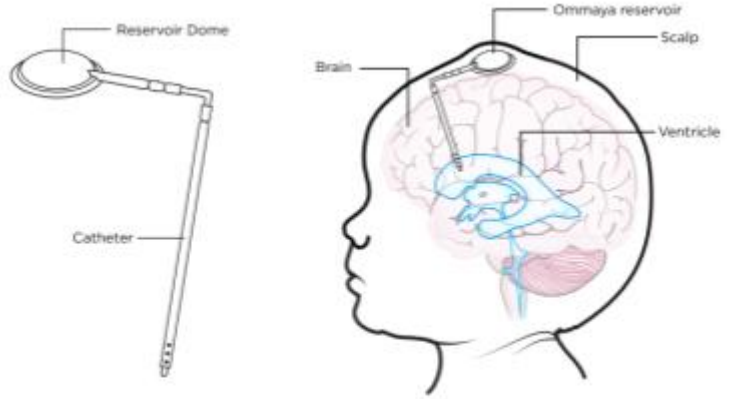


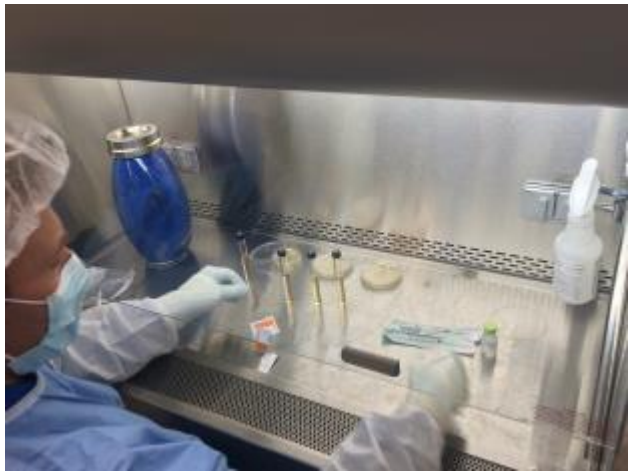
Figure 1: Ommaya reservoir

Figure 2: Placement of the Ommaya reservoir

https://www.mskcc.org/pdf/cancer-care/patient-education/about-your-ommaya-reservoir-placement-surgery

Patient Unit Dose Preparation

- Systemic administration requires the addition of the cold protein
  - Final protein mass may exceed microdose limits
  - Separate administration is preferred
  - Special regulatory consideration is required if the cold protein will not be marketed separately as a pharmaceutical



Additional Regulatory Considerations

- Which material to use for toxicology batches
  - Protein
  - Chelated protein
  - Chelated protein labeled with non-radioactive isotope
- Radiotherapeutic toxicology challenge
- Stability testing program

Comparison to Traditional PET

Similarities

- Stability limitations
  - Autoradiolysis
  - Radioactive decay
  - Post-release testing
  - Distribution
- QC represents entire batch of the final drug product
- Limited personnel and resources at smaller facilities

Differences

- Drug substance mass plays a significant role
- Longer radioactive half-life
  - Cleaning
  - Radioactive waste storage
- Various radionuclides used
  - Equipment (shielding)
  - Radiotherapeutics

Summary and Conclusion

- Radiolabeling process should be designed to preserve the protein integrity and efficacy as much as possible
- Radiolabeled proteins require special evaluation of efficacy of the radiolabeled portion of the protein
- As the field develops and expands, the unique nature of radiolabeled biologic compounds must be taken into account when implementing new regulations and guidance associated with radiopharmaceutical preparation

Special Thanks… MSK RMIP Core

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