TBIA101 LABELED WITH ^{68}GA AS POTENTIAL TB AGENT

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ISORBE 16: 22 March 2013

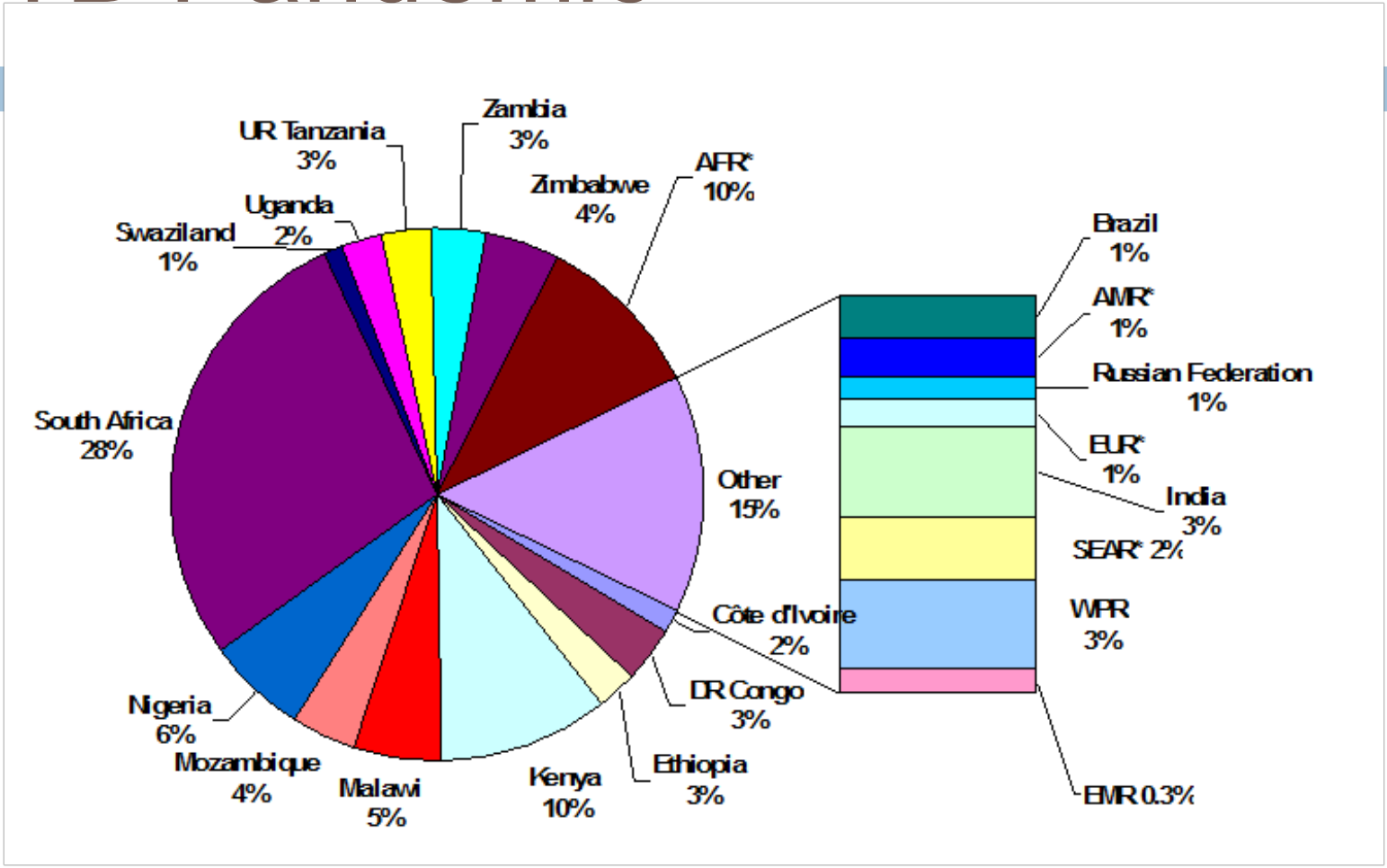
Why Focus on TB

" To fight AIDS we must do more to fight TB"

Nelson Mandela

15th International HIV/AIDS Conference, Bangkok, Thailand July 15, 2004

TB Pandemic



Geographical distribution of estimated HIV-positive TB cases, 2006.
For each country or region, the number of incident TB cases arising in people with HIV is shown as a percentage of the global total of such cases.
AFR* is all countries in the WHO African Region except those shown separately; AMR* excludes Brazil; EUR* excludes the Russian Federation; SEAR* excludes India.

Justification

Future R & D

- Develop imaging tests that are sensitive *and* specific for infection, either in general or for particular components (bacteria, cells, etc.)

Justification

- ^{18}F-FDG-PET has several advantages over other nuclear medicine techniques for the diagnosis of infectious diseases but it is ***non-specific***.

Responder

Sathekge et al., JNM 2011

Non-Responder

Justification

- The clinical application of ^{68}Ga-peptides, has shown success in a variety of tumours.
- There is interest in ^{68}Ga-DOTA-conjugated peptides PET/CT to assess infections.
- The fact that a synthesis of ^{68}Ga-DOTA-TBIA101 has not yet been reported, make it a very attractive target.

Aims

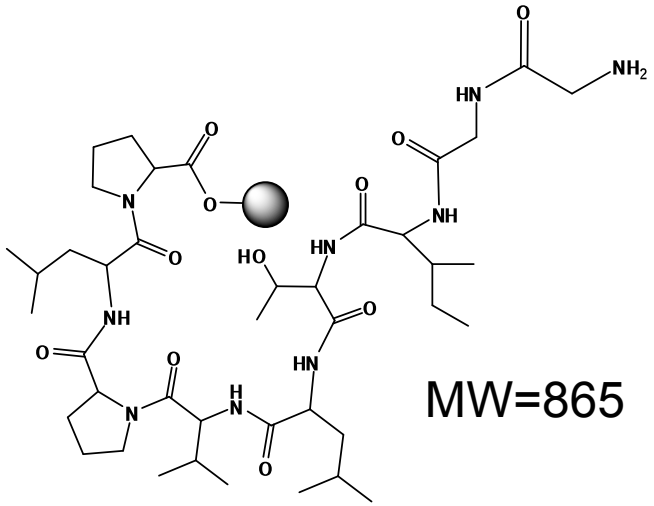
- **Successful labeling of ^{68}Ga-DOTA-TBIA101**
- To determine In vivo biodistribution of ^{68}Ga-DOTA-TBIA101 on:
  - **Healthy New Zealand white rabbits/mice,**
  - **Sterile inflammation induced and TB infected mice.**
- Discrimination of uptake between:
  - **Healthy animals,**
  - **Animals with sterile inflammation and**
  - **TB infected.**

TBIA101

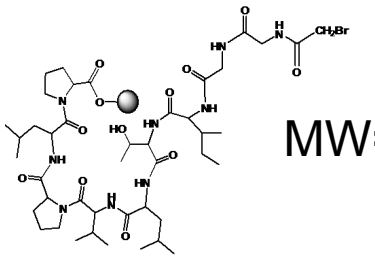
- Linear peptide synthesized using 8 amino acids (MW= 865.00 g/mol)
- The anti-tubecular activity test indicated TBIA101 to be more active against both normal TB and MDR TB
- The toxicity test has confirmed that it is safe to use it.
- The minimum inhibitory concentration (MIC) was indicated to be 32µg/ml against multi-drug resistant

Peptide synthesis

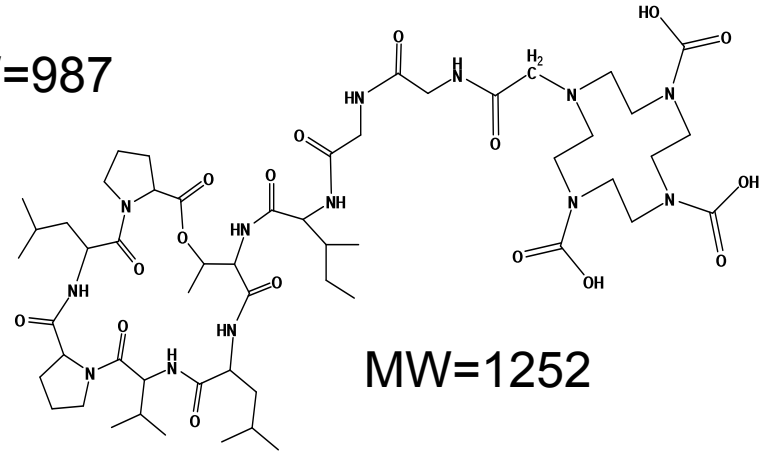
- Microwave peptide synthesizer
- Conjugation of DOTA to TBIA101 was performed manually



MW=865



MW=987



MW=1252

- analogue TBIA101 has one amine -selectively react with the DOTA

Purification

- Purified by RP-HPLC in > 99 % purity, RT: 17 min
- The analysis was achieved on a LCMS.
- Lyoplilized to yield compound as white powder (30 mg, 81 %)

Cold labelling

- **Compounds**

1mg/ml gallium trichloride- water

0.04 mg/ml DOTA-TBIA101- 50/50 Acetonitrile/water

- **mix**

100µl of gallium trichloride into a reaction vial (pH between 3.5 and 4)

- **Add and Shake**

25 µl DOTA-TBIA101 (vortex mixture for 30 sec)

- **Heat**

10 min at 100 ^{0}C.

Purification

- **C18 Sep-Pak cartridge / column**

  Load sample and rinse with water

  Labeled product: 0.1% TFA/Acetonitrile

- The analysis was achieved on a MALDI-TOF-MS

- The labeled product was frozen and subsequently lyophilized.

In Vitro Affinity Assay

- Bacterial cells

Staphyluscoccus Aureus (Gram-positive)

Escherichia coli ATCC 25922 (Gram-negative)

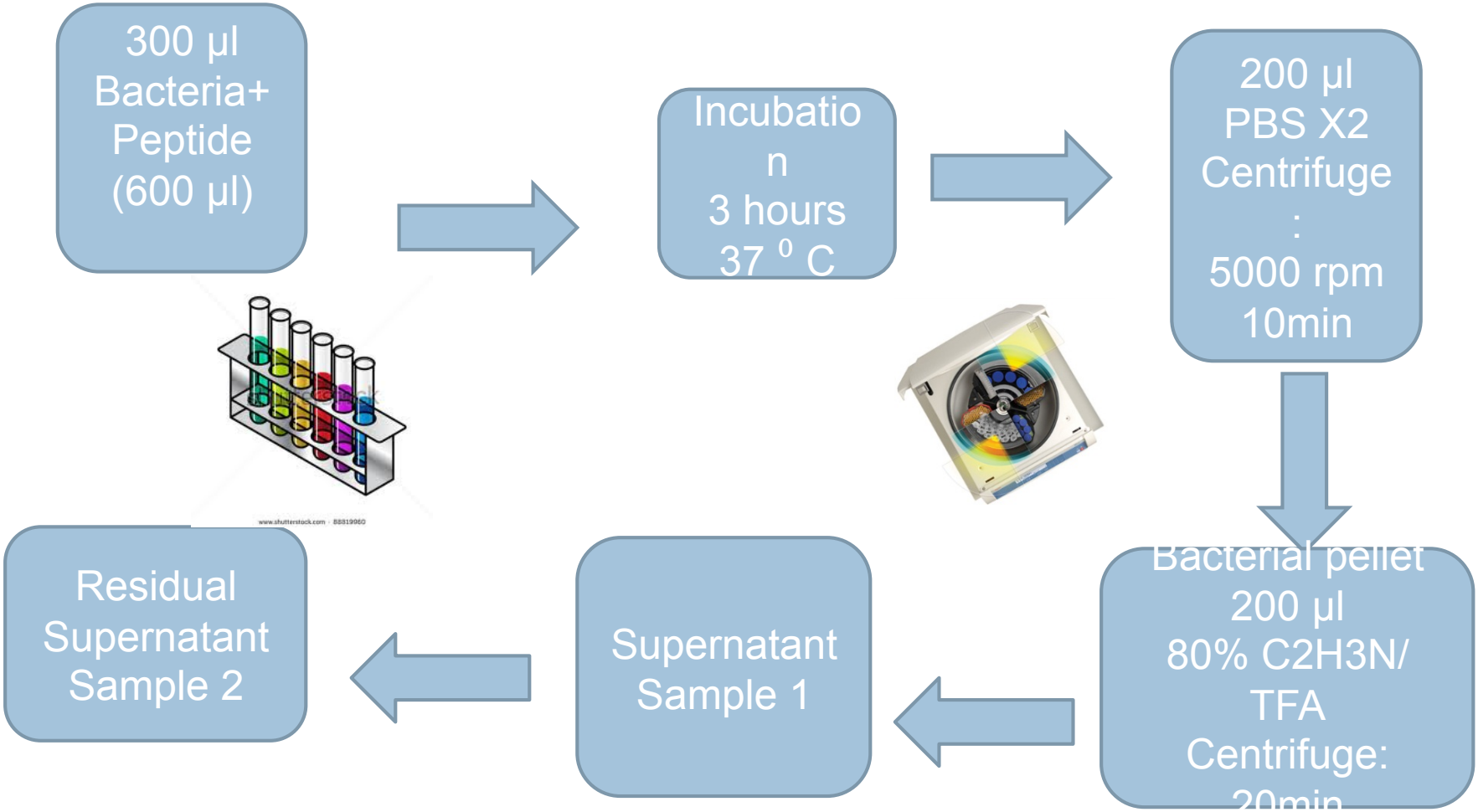
Bacteria cells -1.5 x 10^8 measured using the 0.5 McFarlands standard

- Compounds (20-100 μM)

TBIA101

DOTA-TBIA101
Ga-DOTA-TBIA101

In Vitro Affinity Assay



In Vitro Affinity Assay

- Sandwich method

0.5 µl was spotted onto a MALDI stainless steel plate

α-cyano-4-hydroxycinnamic acid matrix

MALDI-TOF-MS

Matrix-Assisted Laser Desorption/Ionization

In Vitro Affinity Assay

	Staphylococcus aur. ATCC 25923 / *Positive*	*Escherichia coli* ATCC 25922 / *Negative*
	Compound binding/compound internalization	
TBIA101	**+ / +**	**+ / +**
DOTA–TBIA101	**+ / –**	**– / –**
Ga-DOTA–TBIA101	**+↑↑ / +↑↑**	**– / –**

+	Positive binding or internalization
—	Negative binding or internalization
+↑↑→→	binding at high concentration levels

In Vitro Affinity Assay

- Affinity binding constants (K)

K= 1/ [(peptide)0- (peptide)F

(peptide)0- is the intensity of peptide without bacteria (control)

(peptide)f- [intensity of sample1 + intensity sample 2]

Compound	SA	E.coli
TBIA101	276.4 μM	108.5 μM
DOTA-TBIA101	142.0 μM	-
Ga-DOTA-TBIA101	1690.0 μM	-

$^{68}Ge/^{68}Ga$ generator

- ^{68}Ga is a positron emitter for positron emission tomography (PET).

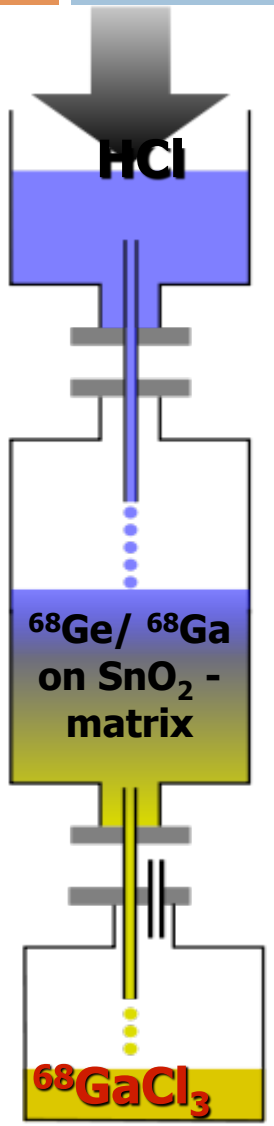
$^{69}Ga(p,2n)^{68}Ge$

^{68}Ga ($T_{1/2}$ =68 min)

^{68}Ge ($T_{1/2}$=271 days)

- The $^{68}Ge/^{68}Ga$ generator can be used for up to a year.

$^{68}Ge/^{68}Ga$ Generator



Technical details

Manufacturer: iThemba LABS

Dimensions: 127x 180 mm

Weight: 26 kg

Calibration: 10 - 50 mCi

- Modified SnO_2 glass column
- HCl (0.6 M)
  Elution efficiency >80 %
- ^{68}Ge breakthrough < 0.002
- Metal content < 0.3 ppm
- Fractionated elution
- Cation-exchange Cartridge
- Anion-exchange cartridge

Concept of ^{68}Ga-Radiolabelling

^{68}Ga obtained from ^{68}Ge/^{68}Ga-generator

fully automated module/Manually

↓

Chelators

(DOTA, NOTA, HBED, TRAP)-(80-95°C), CP256

^{68}Ga in buffered solution

↓

Separation of free ^{68}Ga from

^{68}Ga-DOTA-peptide
(SPE or HPLC)

Concept of ^{68}Ga-Radiolabelling

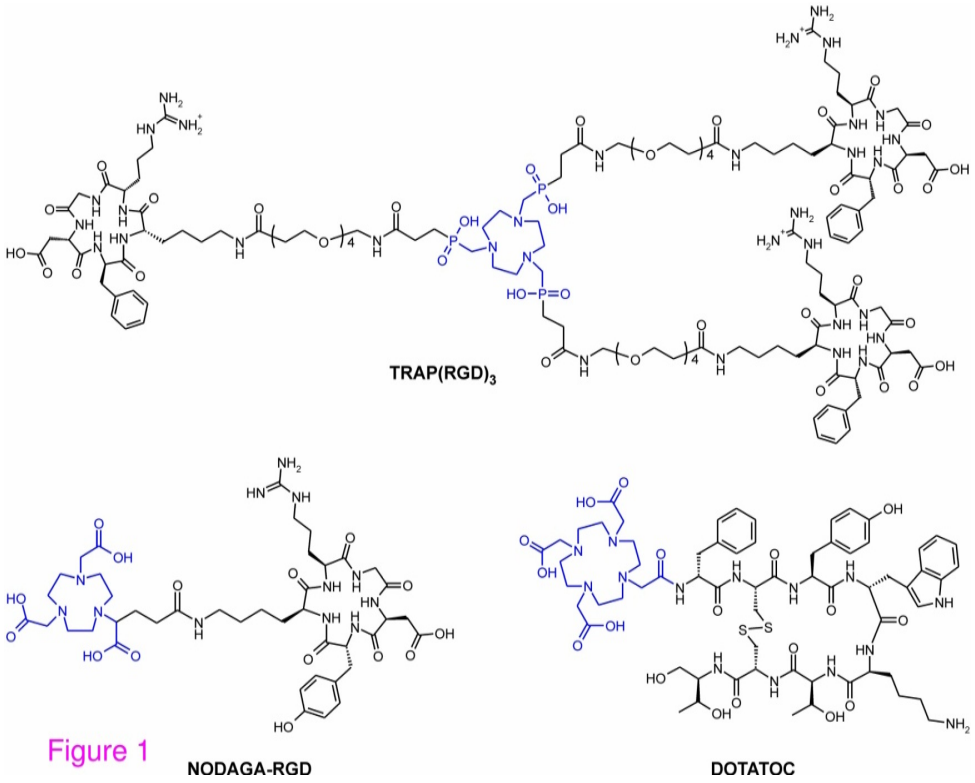
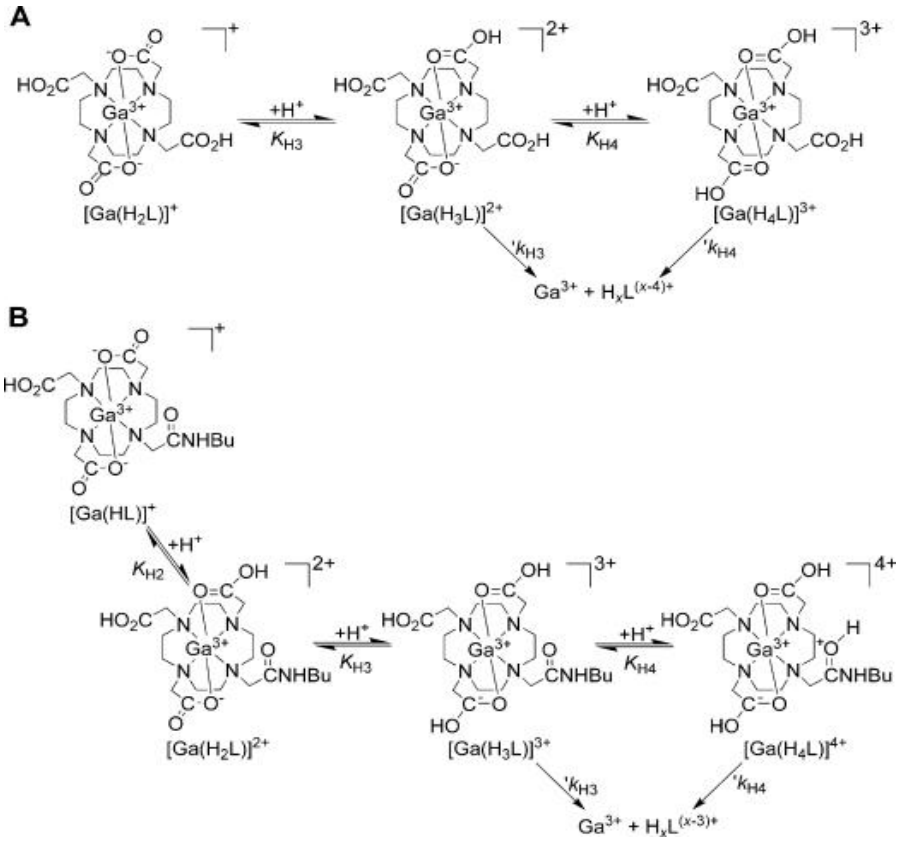


Figure 1



Radiolabeling

- **Eluate**

  $^{68}Ge/^{68}Ga$ generator -10 ml 0.6 M HCl (fractionated Elution) (95 % 68Ga-activity in 2 ml)

- **Mix**

  2 ml ^{68}Ga with 0.575 ml 2.5 M sodium acetate into a reaction vial- adjust pH to 3,5 - 4

- **Add and Shake**

  50 µg DOTA-TBIA101 / vortex mixture for 30 sec

- **Heat**

  for 10min > 90 ^{0}C

Purification and QC

- **C18 Sep-Pak cartridge / column**

  Load sample and rinse with Saline

  Labeled product: Ethanol/Saline [50:50]

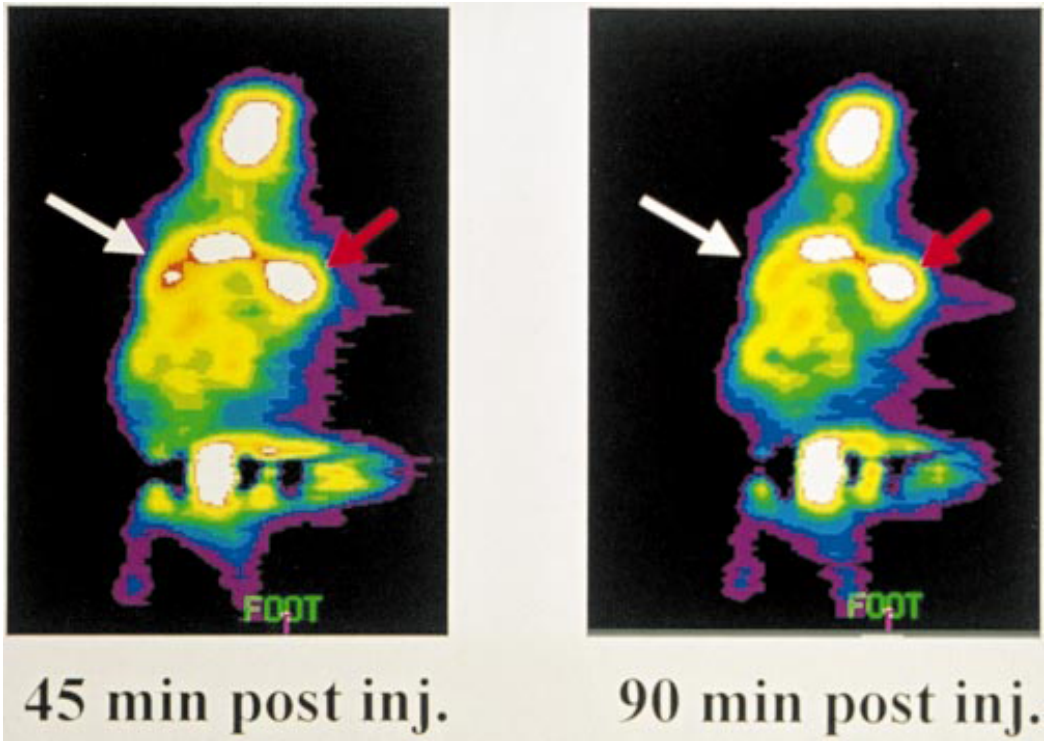
- **QC- ITLC strips (0.1 M Citrate)**

  Free ^{68}Ga activity - top

  labelled peptide- bottom

Preclinical Studies

- Imaging will be done from 1 April
- Following the departmental protocol



Results

Achieved

- Successful radiolabeling (LE: 70 ± 10% , purity 99 ± 0.5 %)
- TBIA101, DOTA-TBIA101 and Ga-DOTA-TBIA101 showed high binding affinity to Staphylococcus aureus

Conclusion

- Most attractive is the $^{68}Ge/^{68}Ga$ generator can be used for up to a year
- ^{68}Ga-DOTA-TBIA101 PET/CT is a first promising radiopharmaceutical for infection/ inflammation imaging.
- ^{68}Ga-Dota-TBIA101 is a potential radiolabelled tracer for TB imaging

THANK YOU