

The Effects of Ionizing Irradiation on Liquid, Dried, and Absorbed DNA Extracts With and Without Preservatives

Margaret Kline, Jim Puhl, and Marc Desrosiers

National Institute of Standards and Technology

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All materials used in this study were purchased by NIST

X Ray screening of DNA Samples?



X Ray Package screening machines are found in more places than airport security lines these days.

It's "rumored" that shipping companies such as UPS and FedEx randomly screen shipped packages.

Could shipping DNA /evidence to other laboratories be a problem?

When we are conducting interlaboratory studies are the results bias due to shipping conditions?

Are there steps we can take to avoid changes to the samples during shipment (if they exist)?

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Mail Irradiation

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- In October 2001, the deadly micro-organism anthrax was found in mail sent to various news agencies and to the offices of U.S. Congressmen. As a precaution, the U.S. Postal Service, with assistance from FBI and national public health experts, began irradiating mail to kill potentially present anthrax spores.
- This applied to mail sent to Government offices.

Experiment Parameters

DNA extracts at 2 concentrations

2.0 ng/ μ L with and without stabilizer

0.2 ng/ μ L with and without stabilizer

- Stored in Tubes

Tube A (medical-grade polypropylene)

Wet and Dry (with and without stabilizer)

Tube B (polypropylene)

Wet and Dry (with and without stabilizer)

Tube C (perfluoroalkoxy fluoropolymer, PFA)

Wet and Dry (with and without stabilizer)

- Stored as “Stains” on paper:

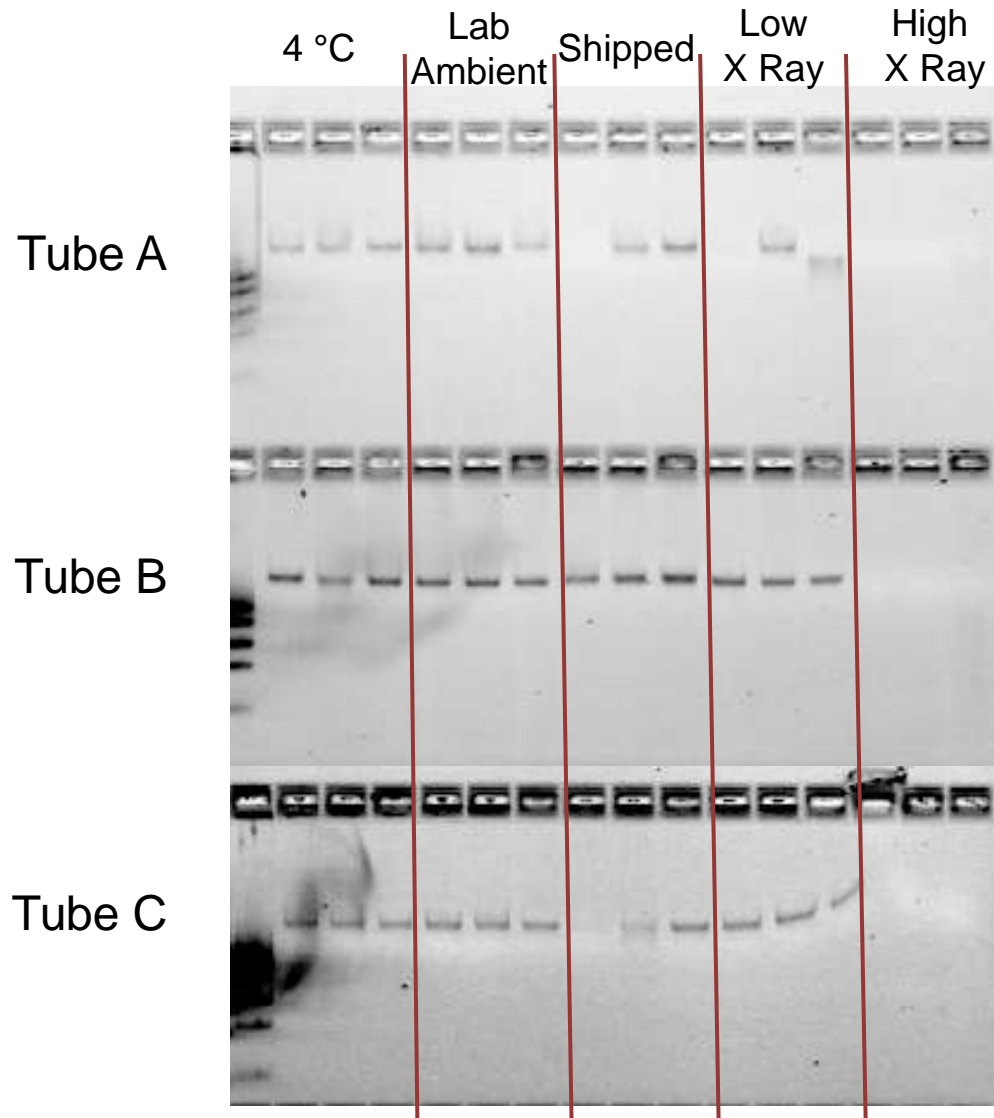
FTA: 2.0 ng/ μ L (treated paper)

903: 2.0 ng/ μ L with and without stabilizer

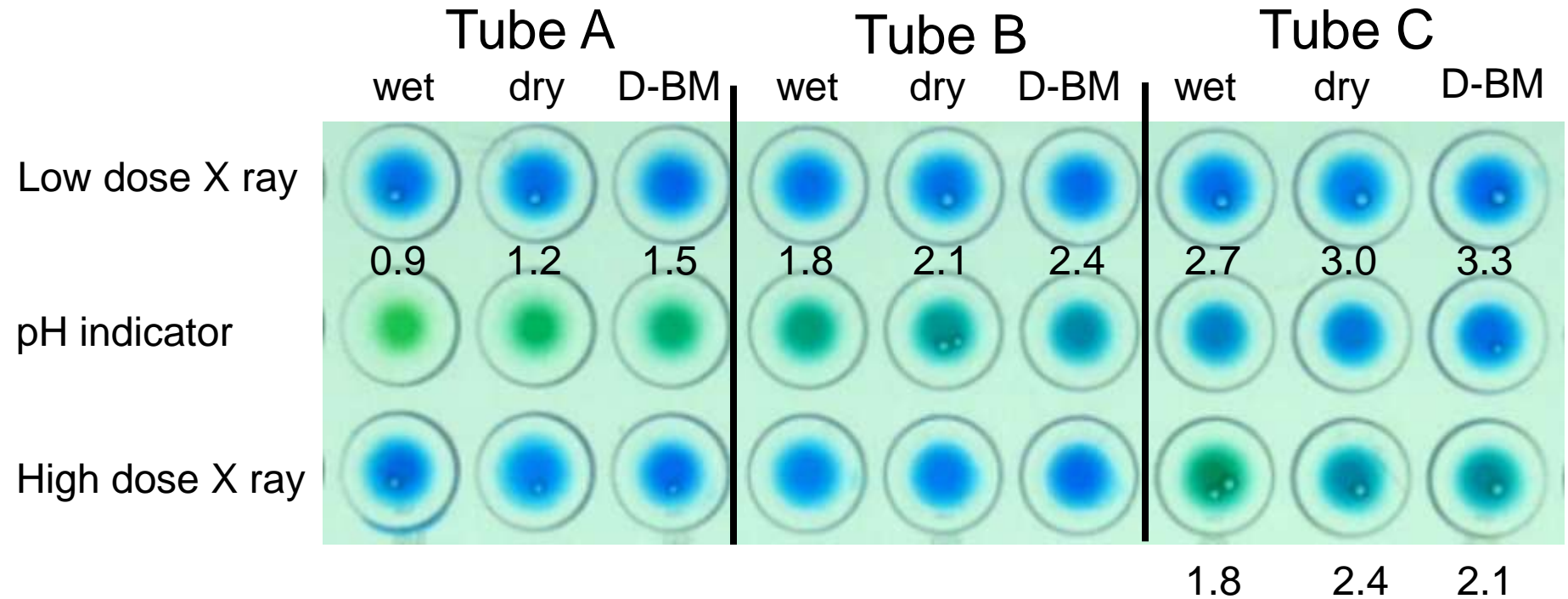
Treatments

- Control for Tubes:
 - Stored 4 °C
- Lab Ambient:
 - Stayed in the lab until processed
- Shipped:
 - Shipped UPS, returned UPS (?diagnostic x-rayed)
 - 15 days in transit to and from (Washington State Patrol)
- X Rayed:
 - Shipped UPS, X-rayed when received, returned UPS
 - 7 days in transit (Evidence Control Unit FBI, VA)
- High-Dose Sterilization X Ray:
 - U.S. Postal Service Government Mail

Flash Gel Results Post Treatment



Acid Formation in Irradiated Tubes



Tube C High irradiation dose DNA appears to have a lower pH than the other tubes.

Prior to STR amplification

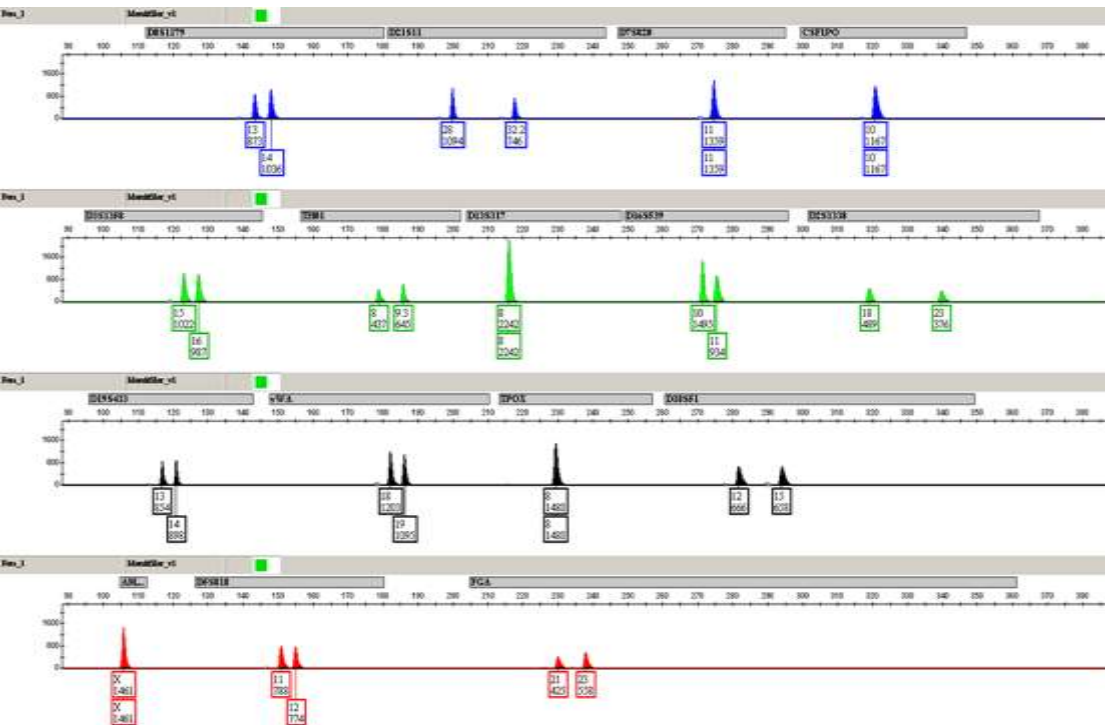
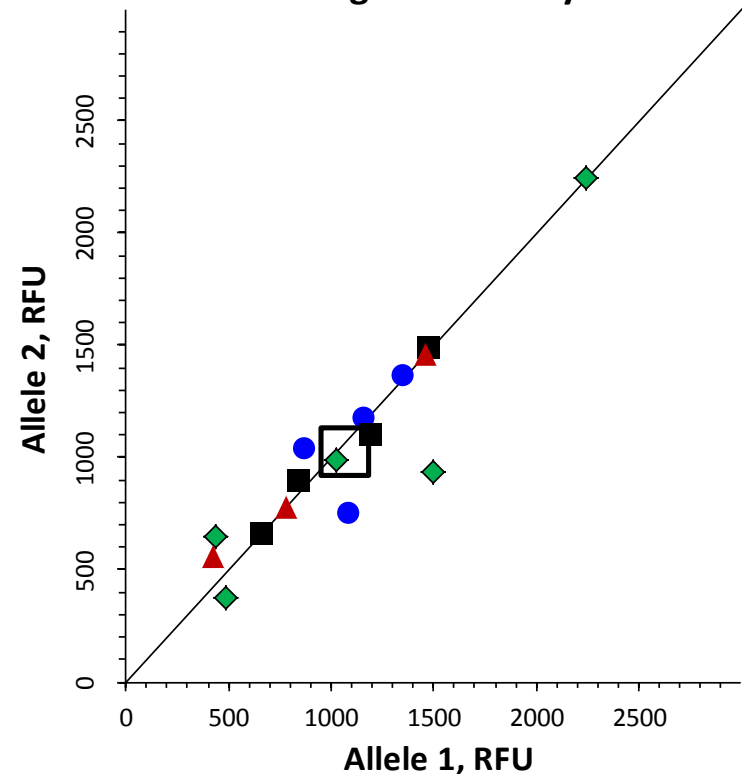
- All tube samples were centrifuged
- Liquid samples were volume checked
 - DI Water was added to samples to restore volume
- Dried samples were re-hydrated with 30 μL DI water
- Samples were rotated at room temperature for 2 h
- All samples were centrifuged
- Hi [DNA] samples were diluted 1 \rightarrow 5 with TE⁻⁴ buffer
- Amplify:
 - 2 μL of the diluted Hi [DNA] samples
 - 4 μL of the Lo [DNA] samples
 - Total PCR Reaction volume 10 μL

Viewing the Results

Since samples were sent in duplicate, we have summarized the results by plotting the peak height of allele 1 (X-axis) against the peak height of Allele 2 (Y-axis).

The displayed electropherogram is translated into the plot shown below. Using this technique you can see the peak imbalance associated with this sample. The average over all loci is represented as the open square.

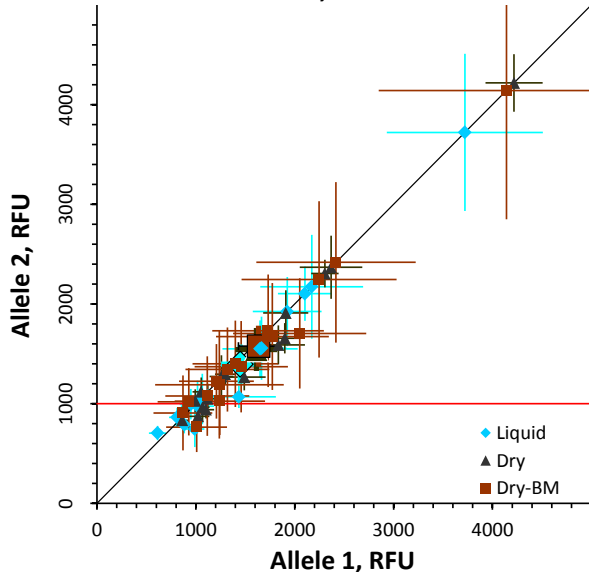
Peak Height Summary



4 °C Control Sample Results

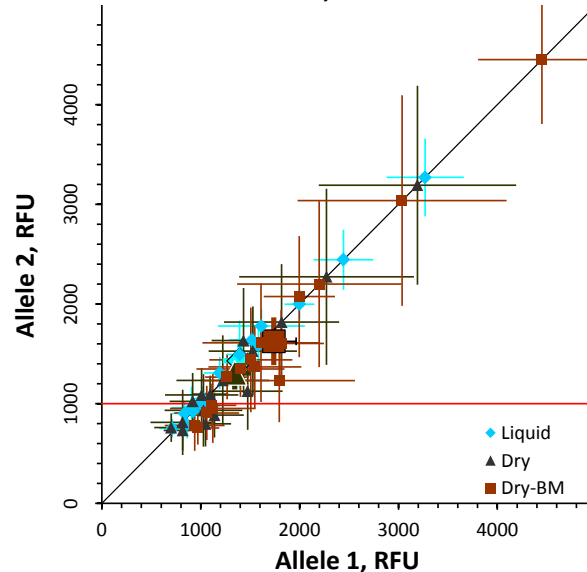
21 % volume loss

Tube A, 4 °C



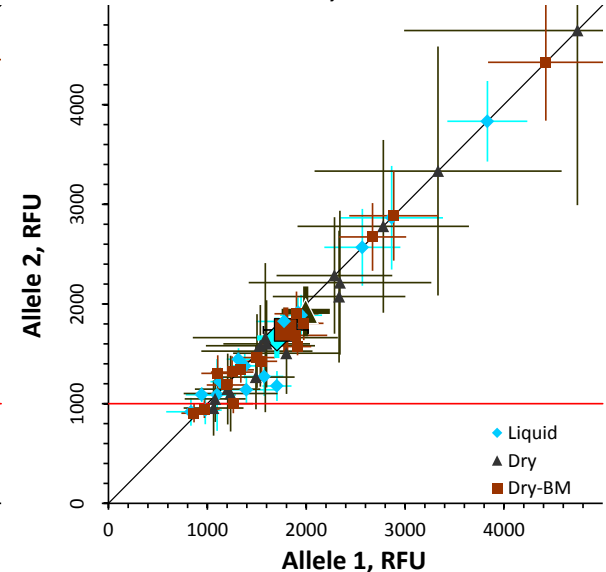
0 % volume loss

Tube B, 4 °C



0 % volume loss

Tube C, 4 °C



The peak heights of allele 1 are plotted on the X-axis
The peak heights of allele 2 are plotted on the Y-axis
Error bars span from the minimum to the maximum.
The large symbols represent the average over all loci
There is little difference between the tube types at 4 °C
except for the 21 % volume loss of Tube A.

Study Shipped Land Carrier Irradiation ?

- Package screening X-ray machine by Carrier?
- **Film Badge dosimeter included in package**

– Dose “Not above background”

Study Low Dose Irradiation

- Carrier screening by X-ray machine?
- Shipped land carrier to and from Evidence Control Unit FBI, Quantico VA
- Package screened by X-ray machine at FBI
- **Film Badge dosimeter included in package**

– Dose “Not above background”

Tube A Sample Results

95 % volume loss

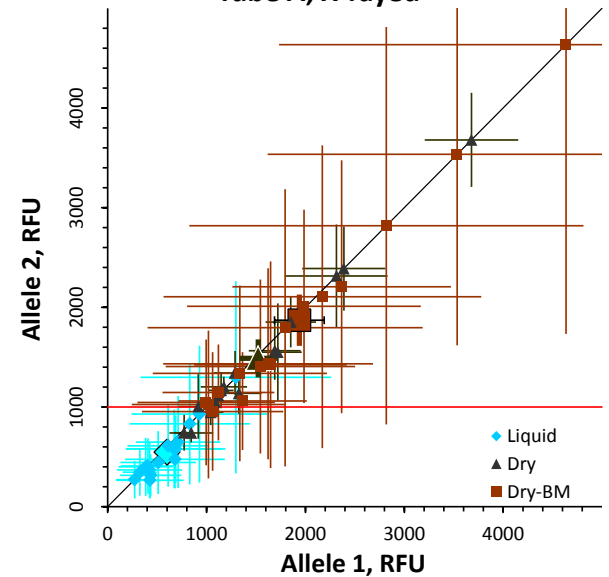
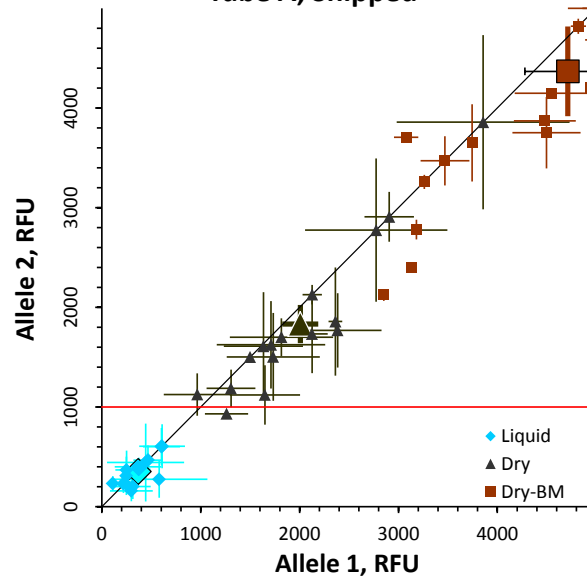
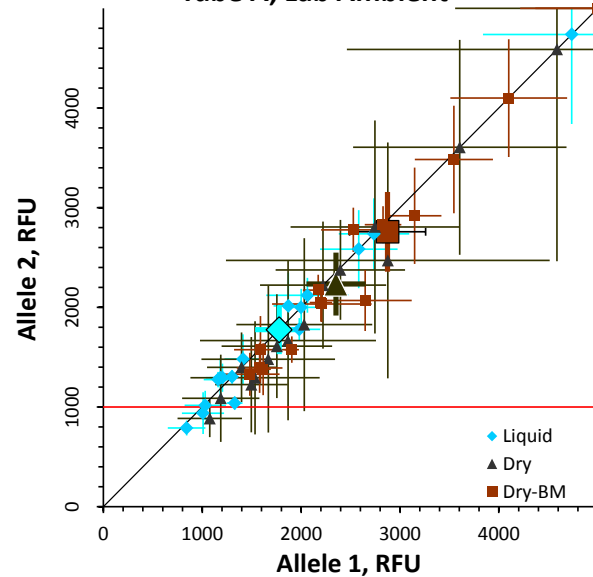
100 % volume loss

95 % volume loss

Tube A, Lab Ambient

Tube A, Shipped

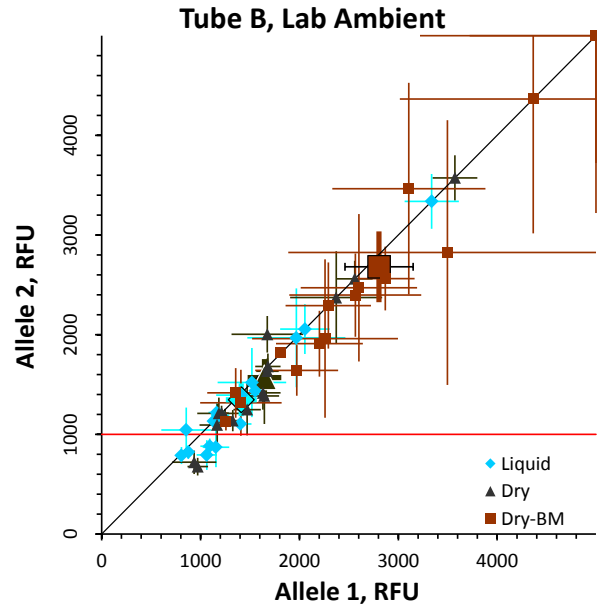
Tube A, X-rayed



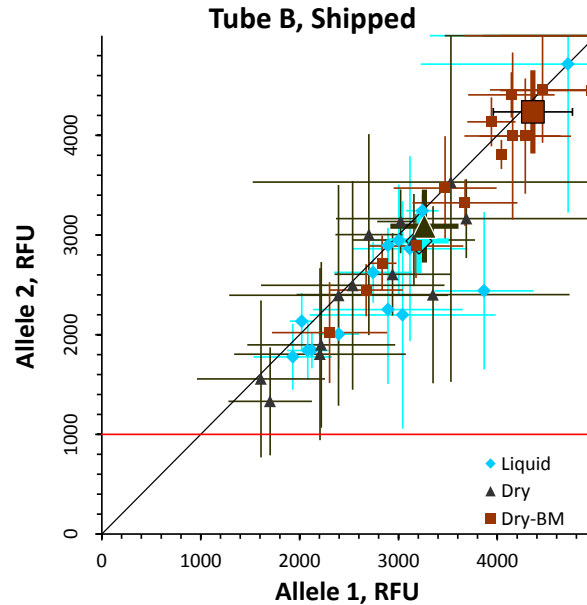
The peak heights of allele 1 are plotted on the X-axis
The peak heights of allele 2 are plotted on the Y-axis
Error bars span from the minimum to the maximum
The large symbols represent the average over all loci

Tube B Sample Results

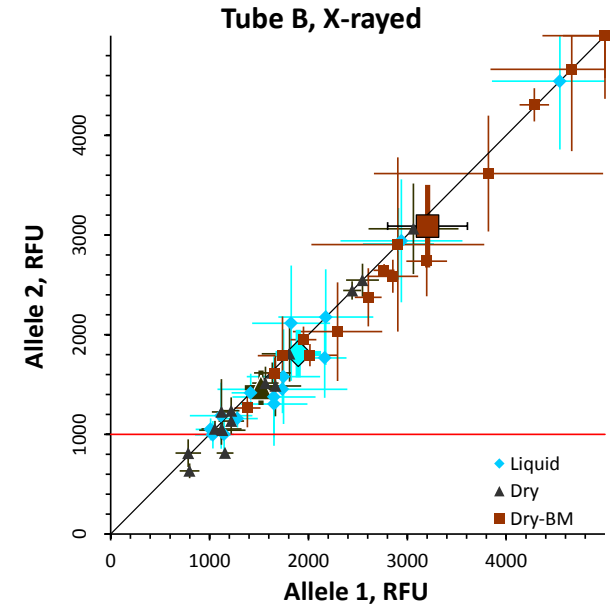
14 % volume loss



33 % volume loss



15 % volume loss



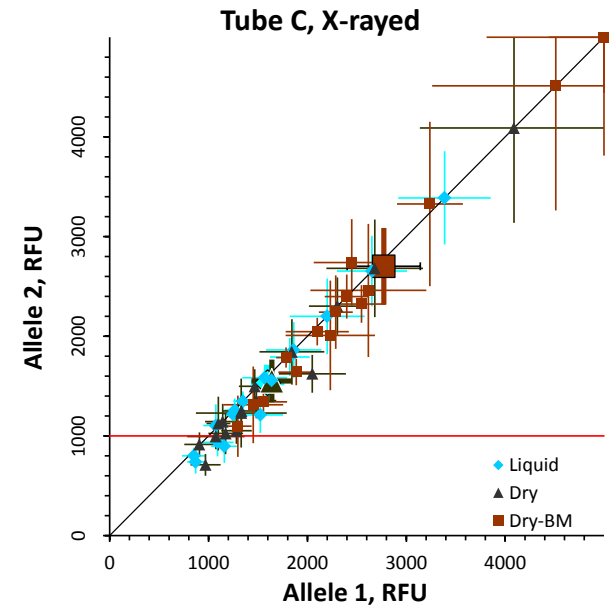
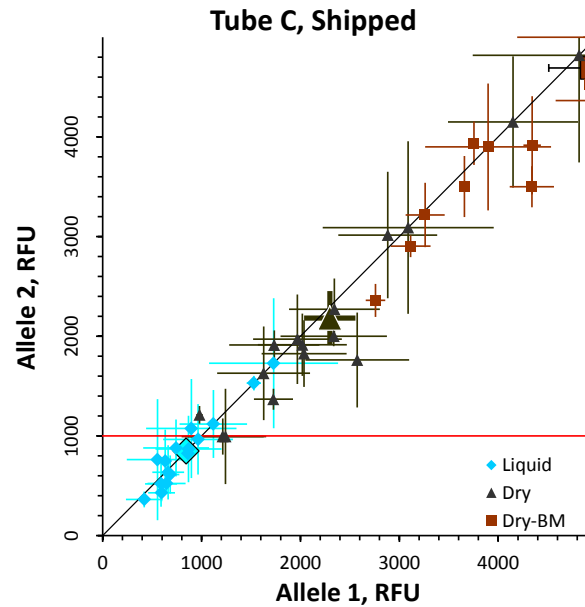
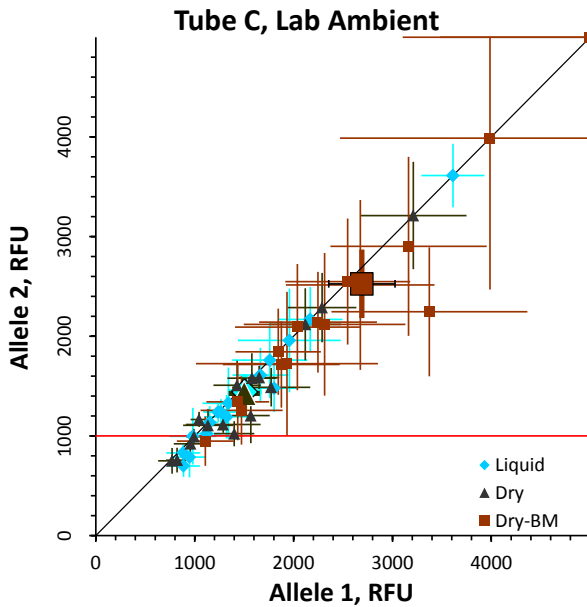
The peak heights of allele 1 are plotted on the X-axis
The peak heights of allele 2 are plotted on the Y-axis
Error bars span from the minimum to the maximum
The large symbols represent the average over all loci

Tube C Sample Results

0 % volume loss

1 % volume loss

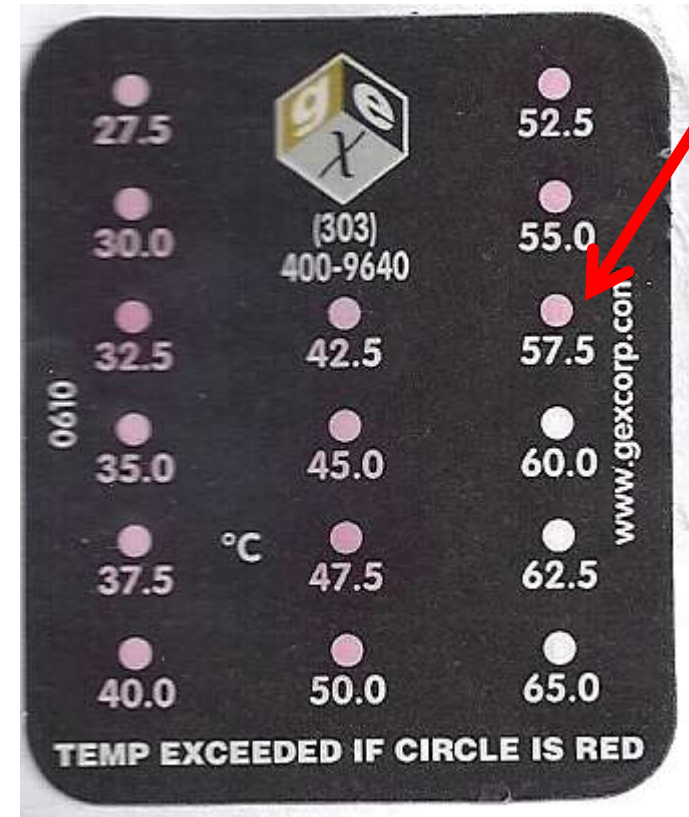
0 % volume loss



The peak heights of allele 1 are plotted on the X-axis
The peak heights of allele 2 are plotted on the Y-axis
Error bars span from the minimum to the maximum
The large symbols represent the average over all loci

Study High Dose Irradiation

- 5 MeV industrial x-ray beam
- Alanine dosimeter
 - (NIST reference class)
- Magazine Container
 - Dose (91 ±4) kGy
 - Max temp (57.5 ±1) °C
- Letter Mail Container
 - Dose (87 ±4) kGy
 - Max temp (62.5 ±1) °C



Sample Packages were at these temperatures for 20 to 30 minutes.

EPA: Health Effect of irradiation exposure



Gray (Gy)
 0.05-0.10
 0.50
 0.55
 0.70
 0.75
 0.90
 1.0
 4.0
 10
 20

Exposure (rem)	Health Effect	Time to Onset (without treatment)
5-10	changes in blood chemistry	
50	nausea	hours
55	fatigue	
70	vomiting	
75	hair loss	2-3 weeks
90	diarrhea	
100	hemorrhage	
400	possible death	within 2 months
1,000	destruction of intestinal lining	
	internal bleeding	
	and death	1-2 weeks
2,000	damage to central nervous system	
	loss of consciousness;	minutes
	and death	hours to days

Food Irradiation dosages

Applications

On the basis of the dose of radiation the application is generally divided into three main categories:

Low dose applications (up to 1 kGy)

- Sprout inhibition in bulbs and tubers 0.03-0.15 kGy
- Delay in fruit ripening 0.25-0.75 kGy
- Insect disinfestation including quarantine treatment and elimination of food borne parasites 0.07-1.00 kGy

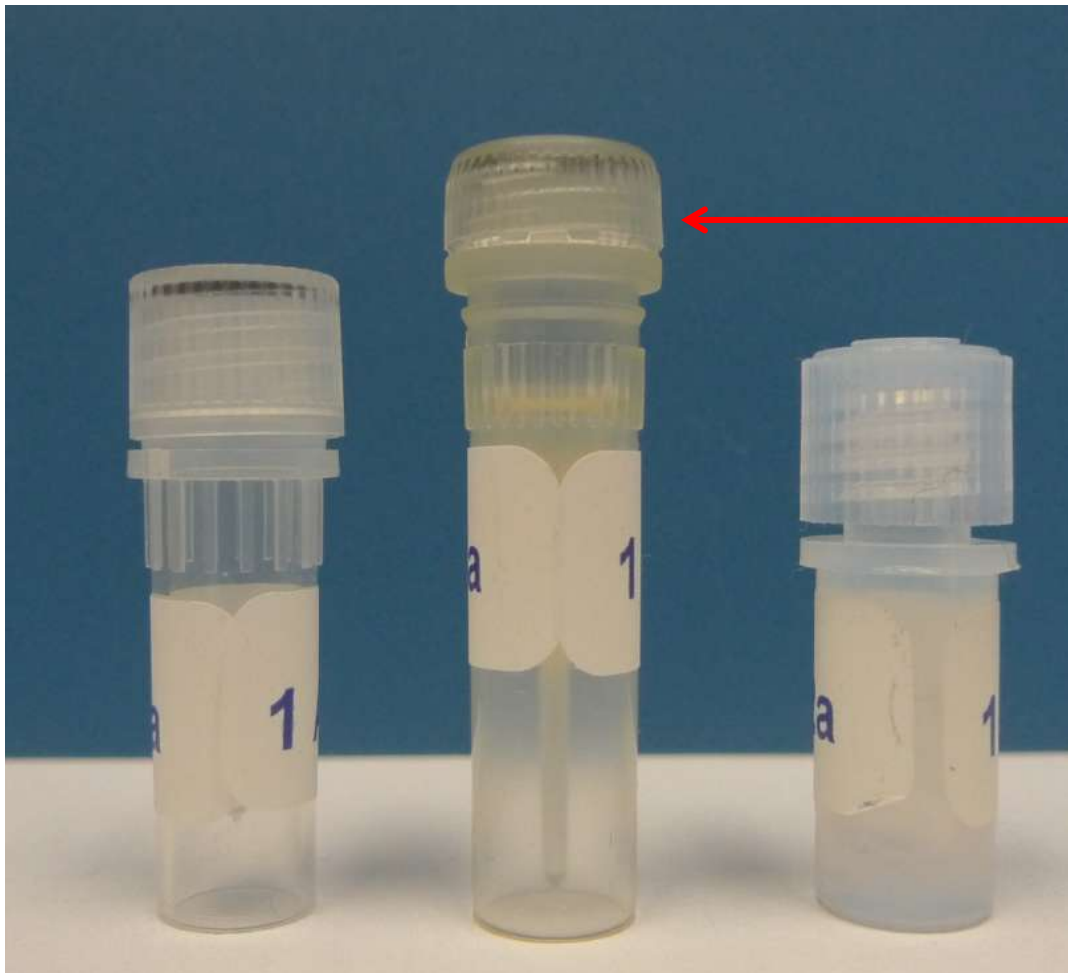
Medium dose applications (1 kGy to 10 kGy)

- Reduction of spoilage microbes to prolong shelf-life of meat, poultry and seafoods under refrigeration 1.50–3.00 kGy
- Reduction of pathogenic microbes in fresh and frozen meat, poultry and seafoods 3.00–7.00 kGy
- Reducing the number of microorganisms in spices to improve hygienic quality 10.00 kGy

High dose applications (above 10 kGy)

- Sterilization of packaged meat, poultry, and their products that are shelf stable without refrigeration 25.00-70.00 kGy
- Sterilization of Hospital diets 25.00-70.00 kGy
- Product improvement as increased juice yield or improved re-hydration

Discoloration of the tubes after high dose of irradiation



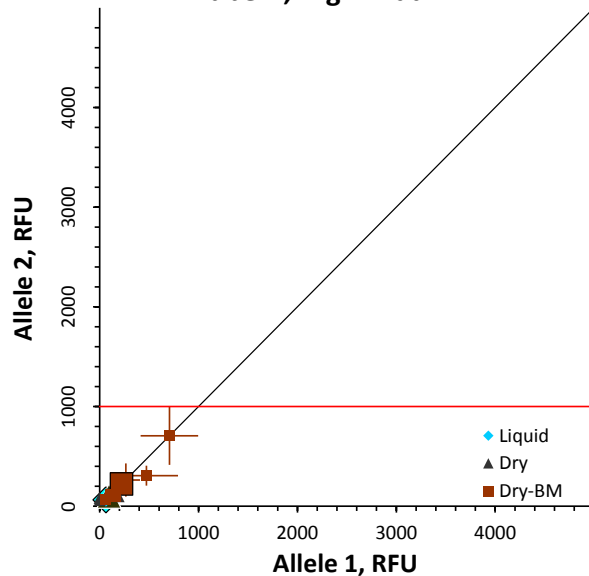
The discoloration of the Tube B was greater than the other tube materials

High dose of irradiation

High Irradiation Sample Results

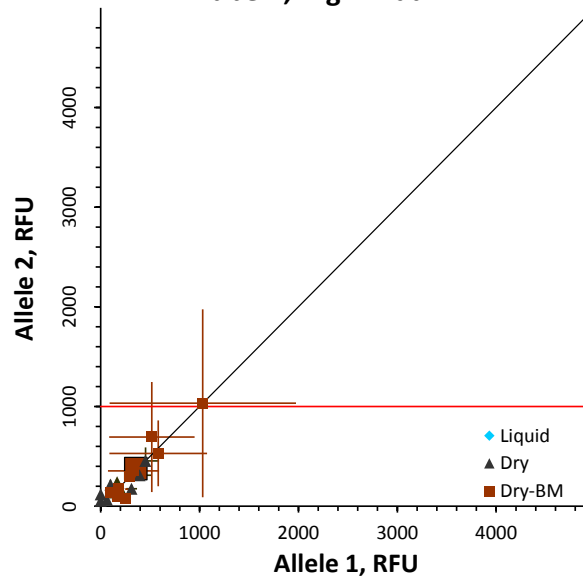
100 % volume loss

Tube A, High Irrad



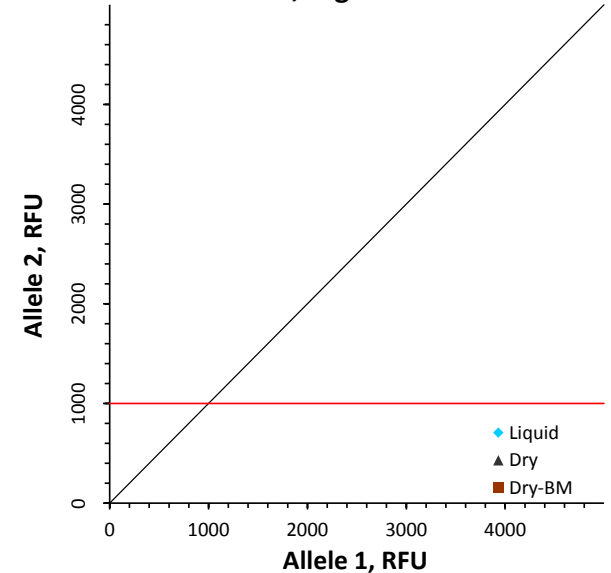
17 % volume loss

Tube B, High Irrad



19 % volume loss

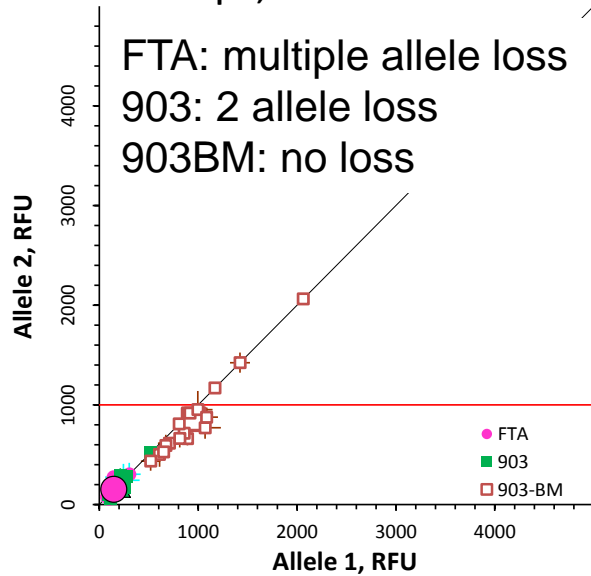
Tube C, High Irrad



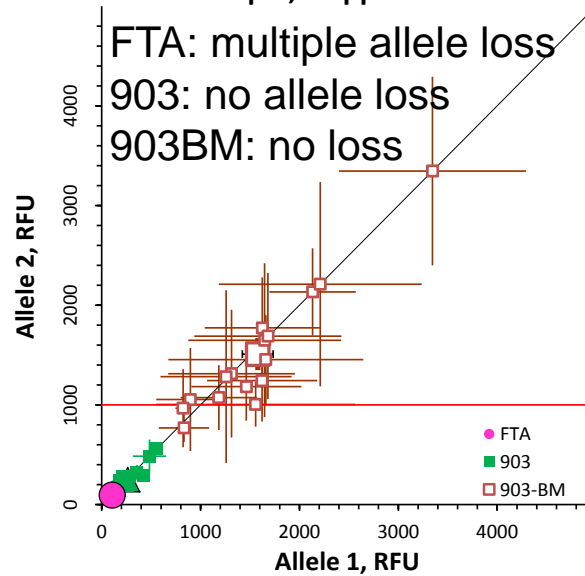
The peak heights of allele 1 are plotted on the X-axis
The peak heights of allele 2 are plotted on the Y-axis
Error bars span from the minimum to the maximum
The large symbols represent the average over all loci
No full profiles were obtained - only a few scattered alleles amplified.

Extracted DNA on paper

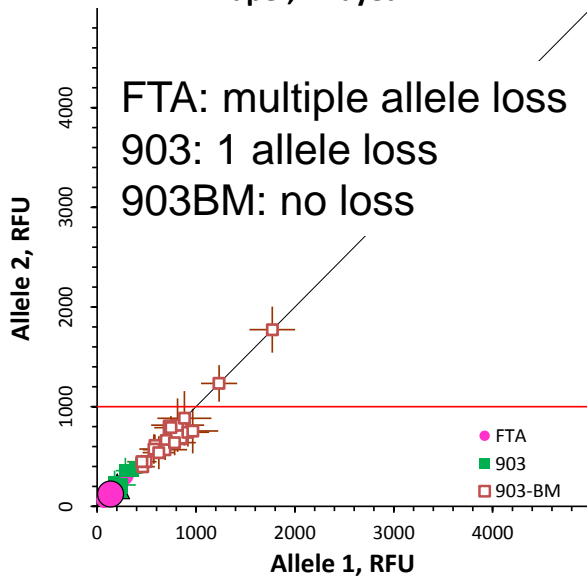
Paper, Lab Ambient



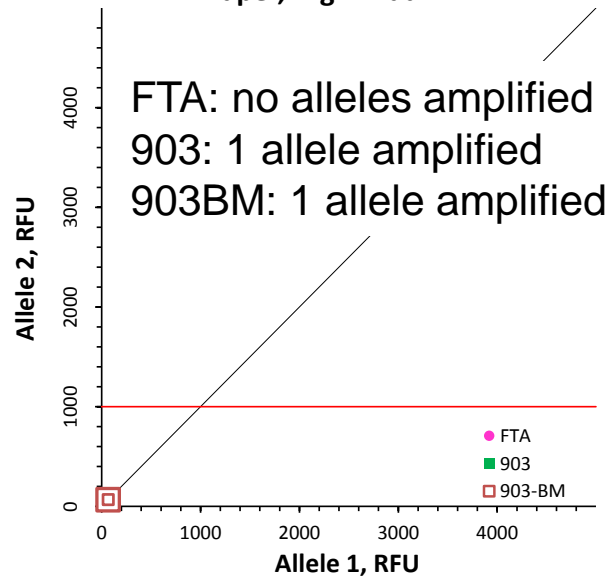
Paper, Shipped



Paper, X-rayed



Paper, High Irrad



FTA: 30 μ L of 2 ng/ μ L extracted DNA spotted

903: 30 μ L of 2 ng/ μ L extracted DNA spotted

903-BM: 30 μ L of 2 ng/ μ L extracted DNA spotted with stabilizer added

2 mm punches (\approx 1.6 ng) amplified in 25 μ L reaction volume.

Error bars span from the minimum to the maximum

The large symbols represent the average over all loci

Bottomline

- Tube packaging makes a difference
- Stabilizer doesn't hurt, may help
- Getting a typing result from paper stain requires more DNA than you think
- Low dose X Ray doesn't hurt
- High dose X Ray destroys

Thank you for your Attention!!



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Mike Coble

Kevin Kiesler

Dave Duewer

Email: Margaret.Kline@nist.gov