



DNA Damage, PARP, and the Comet Assay

Society of Toxicology Exhibitor Hosted
Session

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Background

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- The Comet Assay is a single cell gel electrophoresis assay for evaluating DNA damage in cells
 - The premise is that damaged DNA becomes fragmented. Increasing amounts of DNA damage results in
 - Increased number of fragments
 - Smaller fragments based on gel electrophoresis.
 - Applicable for the analysis of either single strand or double strand DNA breaks.
- Applications
 - genotoxicity testing, human biomonitoring
 - cellular response to DNA damage, cancer risk assessment
 - cancer cell resistance to treatment

The Comet Assay

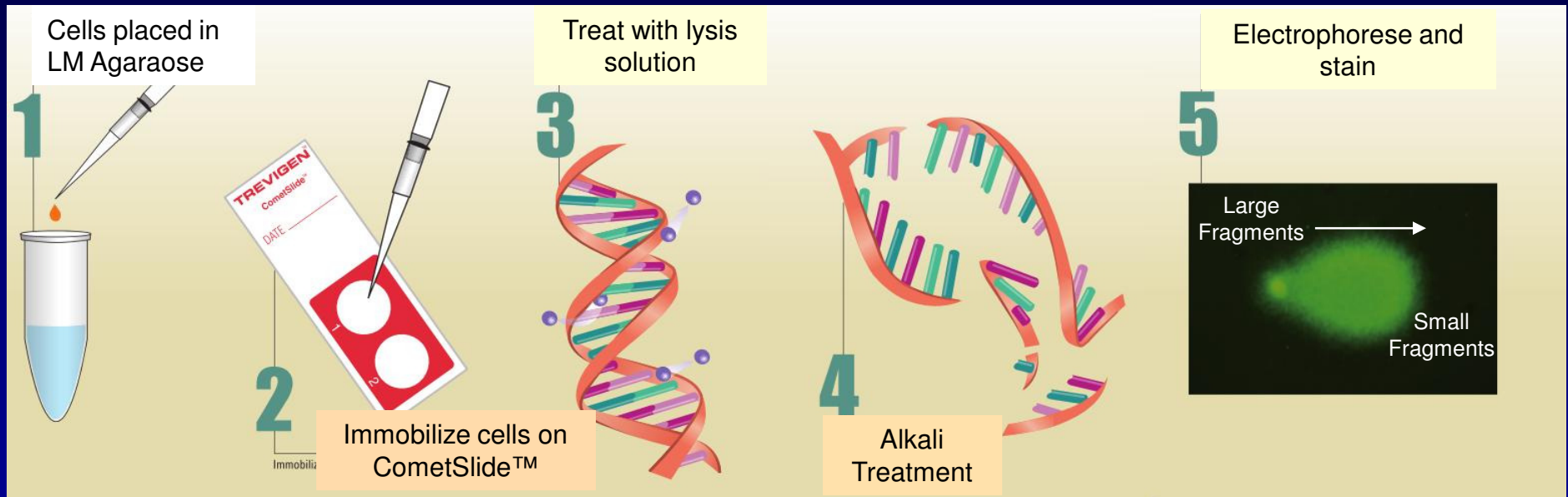
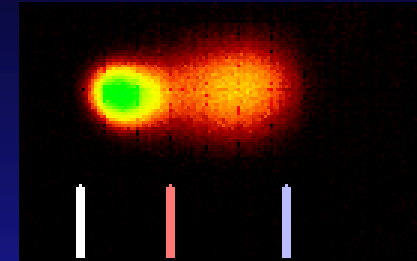


Figure 1.

There are Two CometAssay® Formats

- Alkaline Comet is performed under denaturing conditions and is used to analyze single strand breaks in DNA.
- Neutral Comet is performed under non denaturing conditions and is used to analyze double strand breaks in DNA.
- Analysis of DNA damaging agents

Comet Definitions



Percent DNA in the Tail

- The integrated tail intensity x 100 divided by the total integrated cell intensity for a normalized measure of the percent of total cell DNA found in the tail

Tail Moment

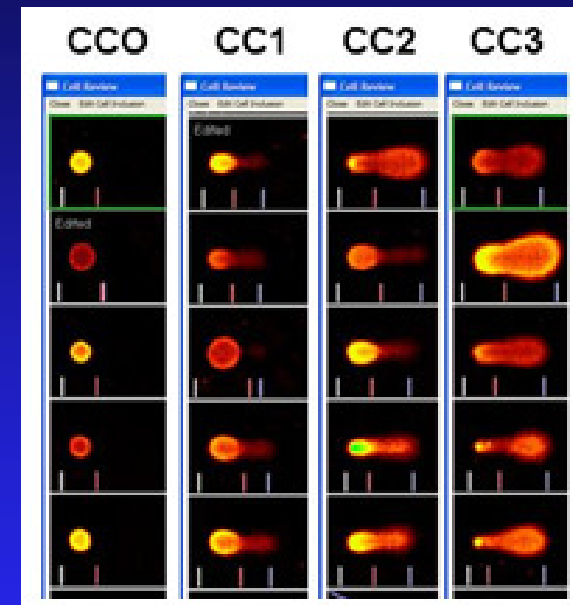
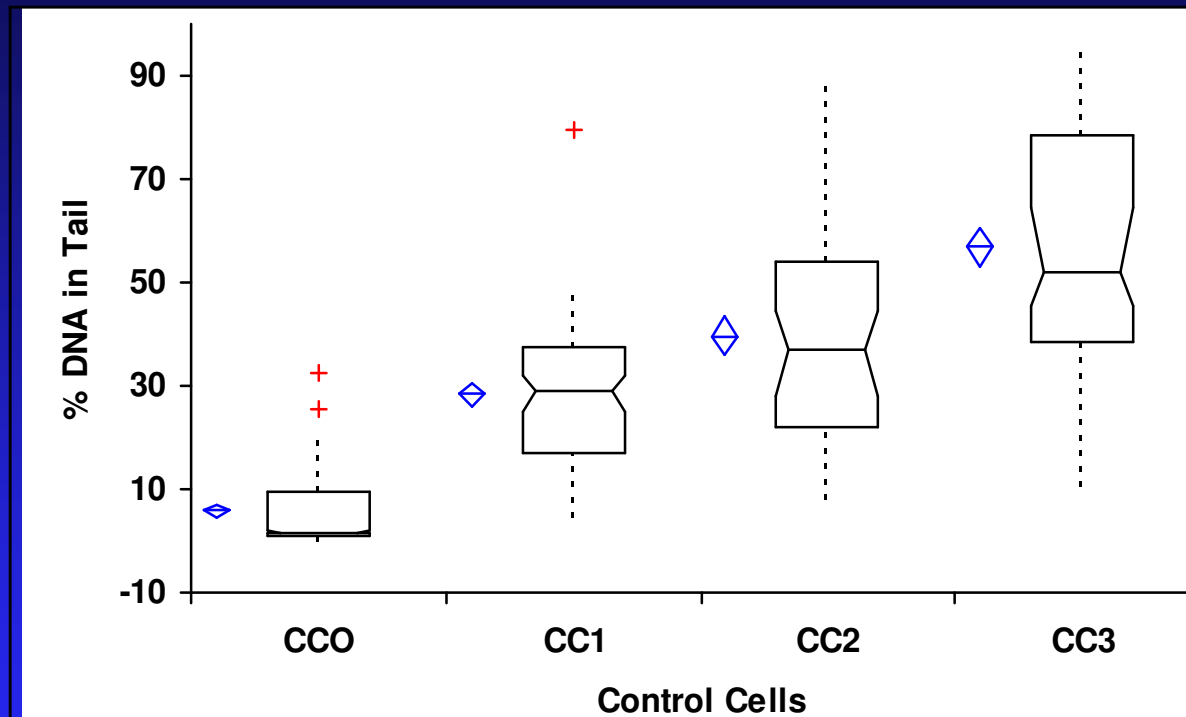
- The product of distance and normalized intensity integrated over the tail length, $\Sigma (L_x \cdot \% \text{DNA}_x)$
- a damage measure combining the amount of DNA in the tail with the distance of migration (severity of damage)

Comet Control Cells

Control Cells

- **Control Cells** to standardize and compare electrophoresis methods between users and laboratories.
 - Used as Standards to Develop Comet Electrophoresis system
 - Four unsynchronized suspension cell preparations with incremental increases of DNA damage
 - Designed for long term storage
 - CometAssay® Control Cells
 - cat# 4256-010-CC *for Alkaline Comet*
 - Neutral CometAssay® Control Cells
 - cat# 4257-010-NC *for Neutral Comet*

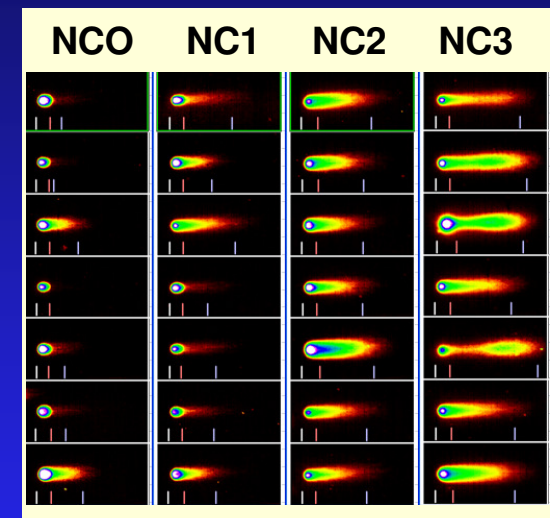
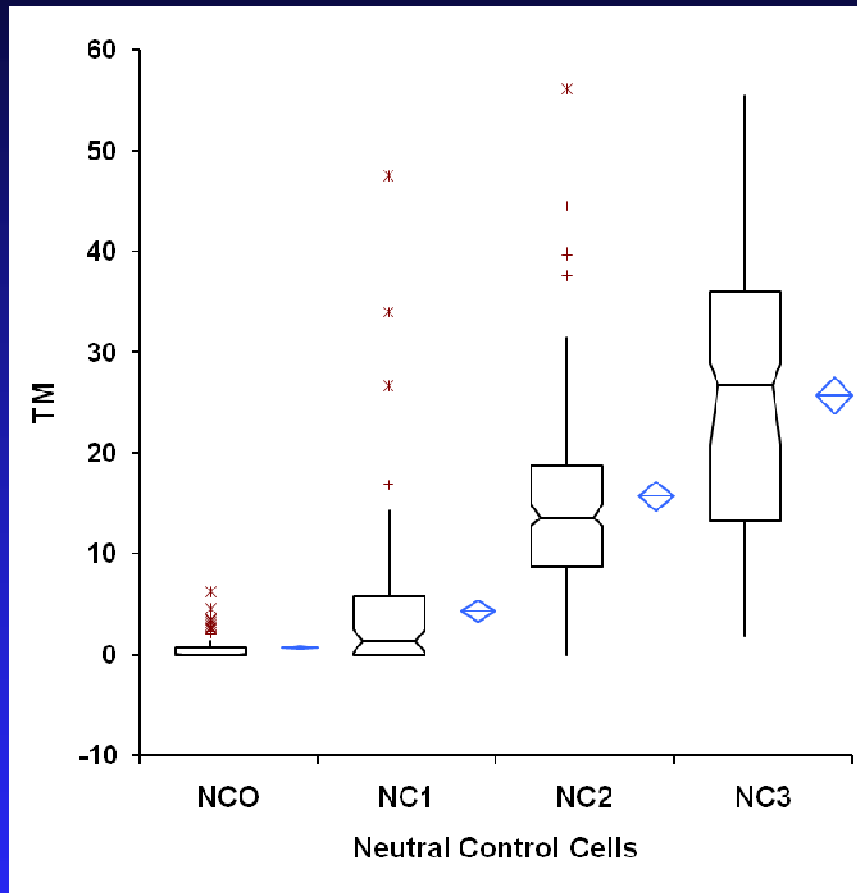
Specifications of Alkaline Control Cells



% DNA by Etoposide	n	Mean	SD	SE	75% CI of Mean	Median	IQR	75% CI of Median
CC0	50	5.757	7.7270	1.0928	4.485 to 7.029	1.640	8.925	1.290 to 2.230
CC1	50	28.374	14.0080	1.9810	26.068 to 30.680	28.990	20.313	25.180 to 31.840
CC2	50	39.736	21.8164	3.0853	36.144 to 43.328	37.050	32.183	27.790 to 44.630
CC3	50	56.800	23.5893	3.3360	52.916 to 60.683	51.905	40.240	45.460 to 64.390

Figure 2.

Specifications of Neutral Control Cells



TM by Bleomycin	n	Mean	SD	SE	75% CI	Median	IQR	75% CI
NCO	75	0.677	1.2410	0.1433	0.511 to 0.843	0.000	0.637	0.000 to 0.140
NC1	75	4.316	7.7817	0.8986	3.274 to 5.358	1.360	5.748	0.240 to 2.510
NC2	75	15.711	10.7829	1.2451	14.268 to 17.155	13.600	10.117	12.830 to 14.950
NC3	75	25.730	13.7918	1.5925	23.884 to 27.577	26.780	22.750	20.810 to 28.930

Figure 3.

Comet Electrophoresis System

Comet Electrophoresis System

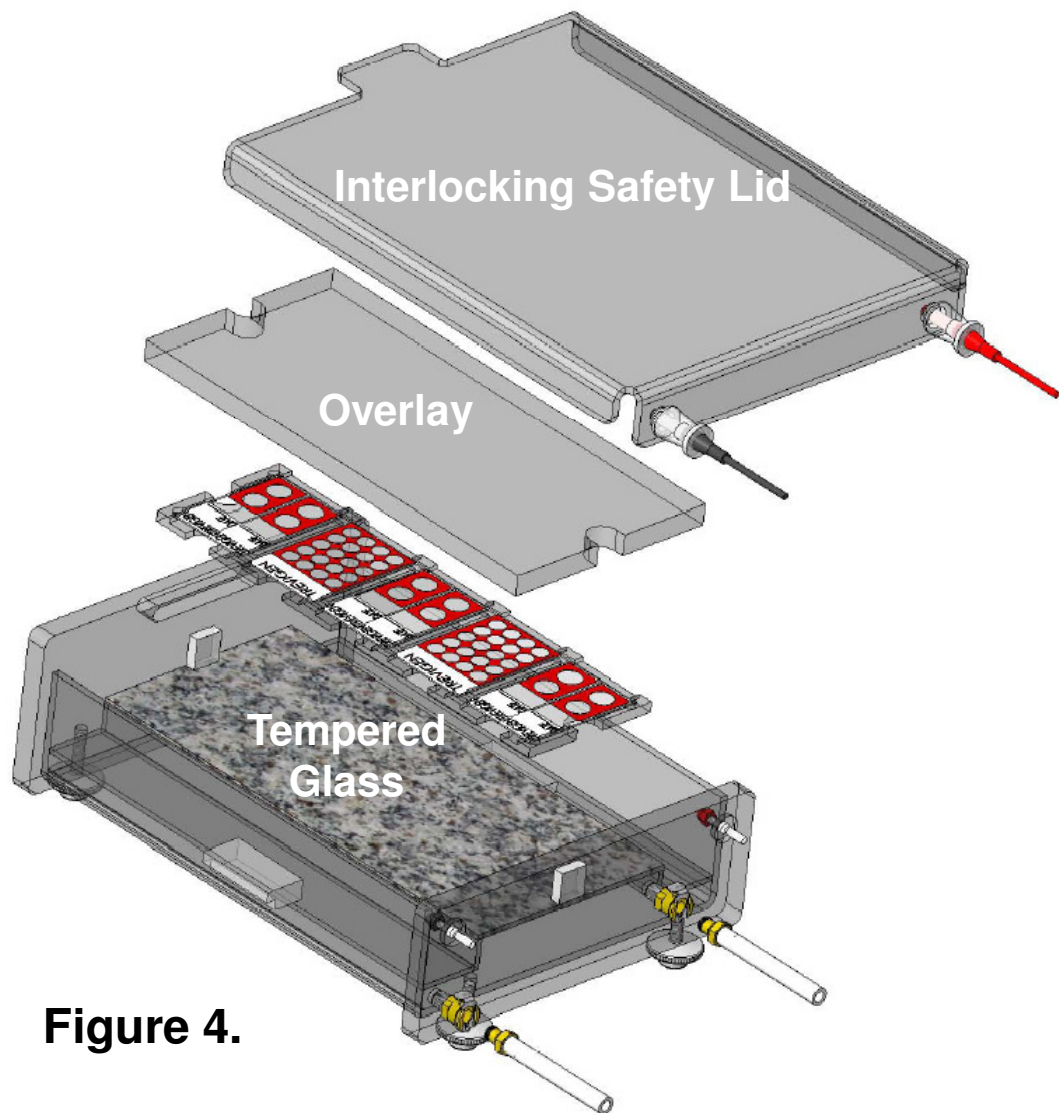
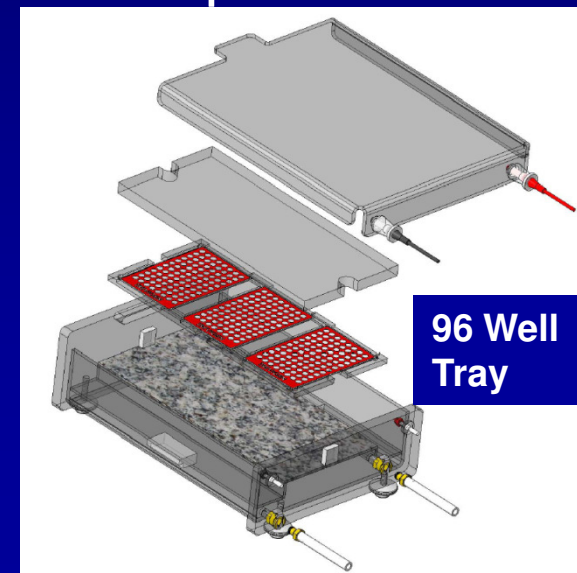


Figure 4.

- **2/20 well Tray**
 - Ten 2-well slides
 - Five 20-well slides
- **96 well Tray**
 - 3 slides - 288 samples



Factors that affect Comet electrophoresis

Problem:

1. Buffer height above slides dramatically affects Comet electrophoresis.
2. Variations in temperature are responsible for inconsistent Comet results.
3. Non-homogenous electric field create alters Comet tail parameters.

Solution:

1. Overlay plate (Fig. 4) assures identical run to run buffer height.
2. Cooling chamber and glass plate (Fig.4) assures consistent run to run temperatures.
3. Critical positioning of electrodes assures consistent electric field.

Demonstration of well to well consistency

- Tests performed on 96 well Comet slide
- 2 rows of 12 for each control cell population was applied a 96 well Comet slide.
- 30 cells per well analyzed
- Tail length and percent DNA in the tail were measured.
- Data shown in Fig.5.

Trevigen's Comet Electrophoresis System provides minimal well to well variation.

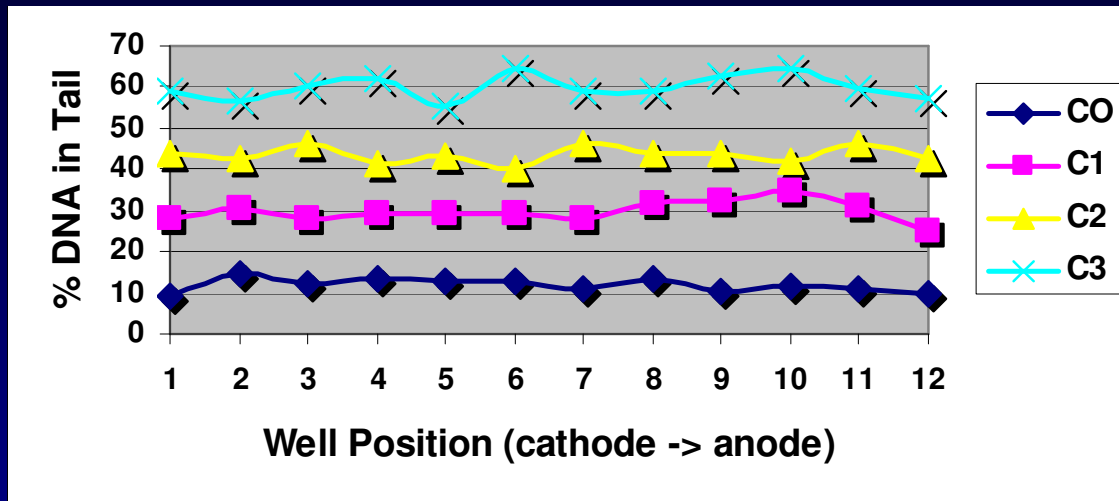
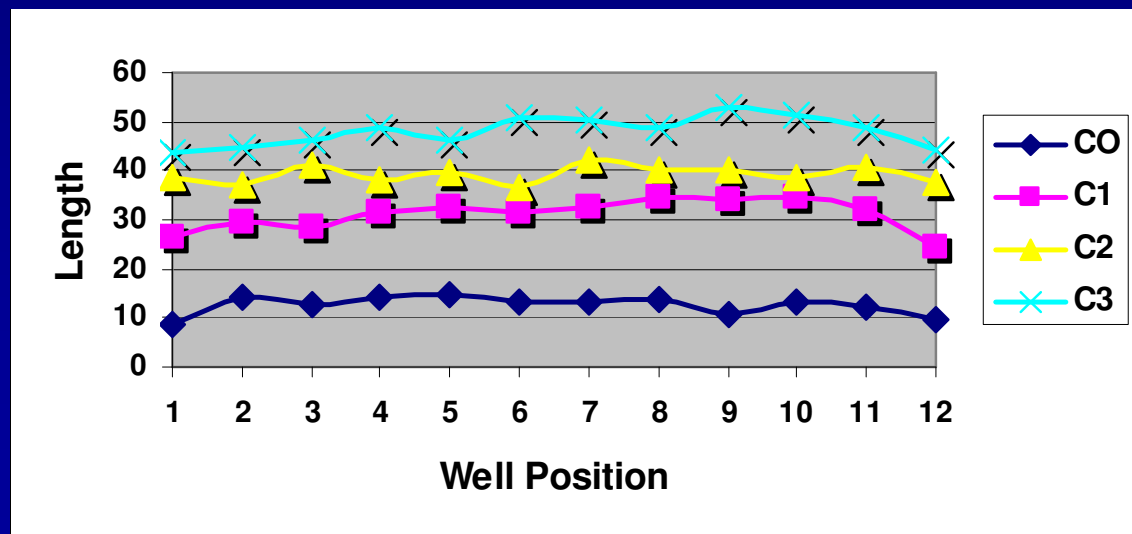


Figure 5.



Trevigen's Comet Electrophoresis System provides minimal well to well variation.

- Minimal well to well variation observed in both intra-and inter-unit experiments.
- System permits comparison of both intra and inter laboratory experiments.

Alkaline vs Neutral CometAssay®

Alkaline and Neutral Comet Assay Overview

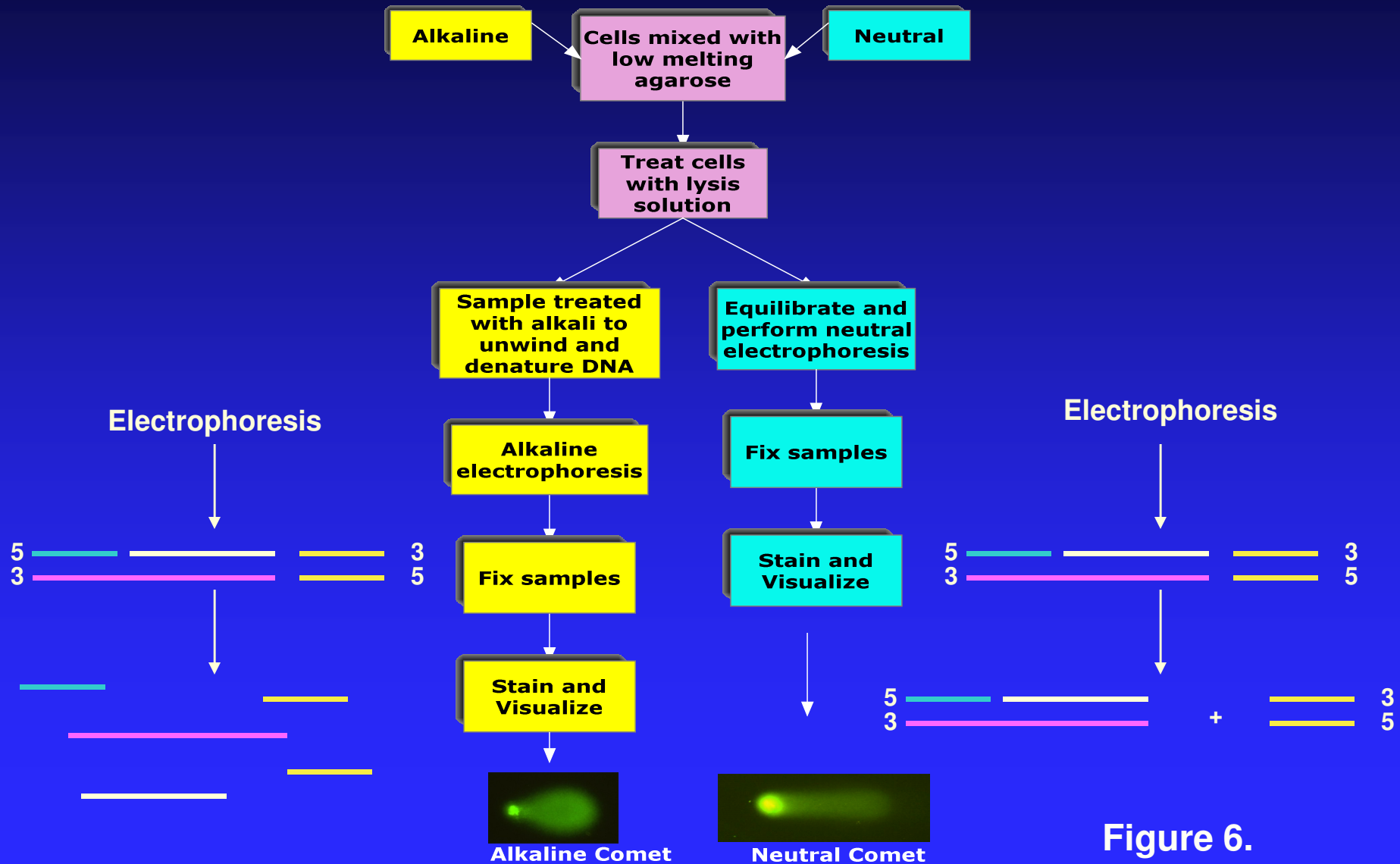


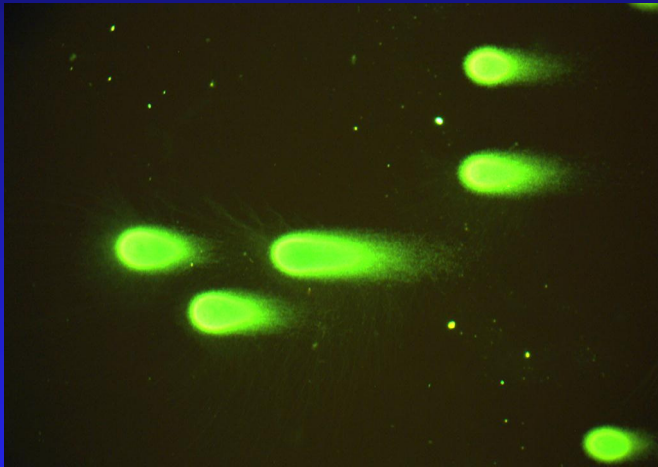
Figure 6.

The “Heads or Tails” of Neutral Comet

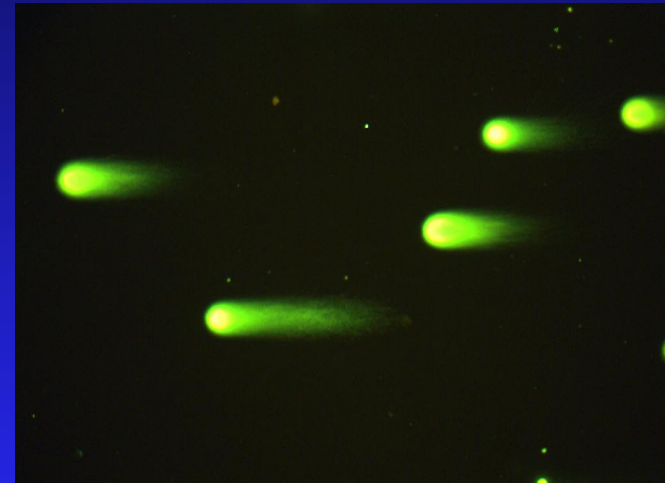
The biggest problem with neutral Comet is that it is frequently difficult to make “heads or tails” of the data.

Is it “Heads or Tails ?”

TBE Method



Tris Acetate Method



- Tris Borate EDTA electrophoresis is the traditional system but gives poor resolution between the head and tail.
- Tris-Acetate system provides better resolution.

Figure 7.

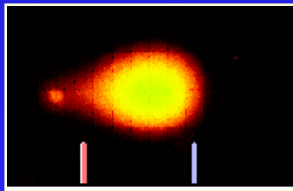
Neutral CometAssay® Buffer systems

- Neutral Comet electrophoresis is traditionally performed in a Tris-Borate EDTA buffer. In this buffer it is frequently difficult to differentiate between the comet head and tail.
- Tris-Acetate EDTA buffer systems provide better resolution of comet head and tail resulting in more accurate more reproducible Neutral Comet analysis

Damage Measurements

Alkaline Comet

- Single Strand Breaks
- Droplet shape
- Tail intensity increases but finite Tail length due to gel resolution



Neutral Comet

- Double Strand Breaks
- Elongated shape
- Both Tail intensity and Tail length increase of migration

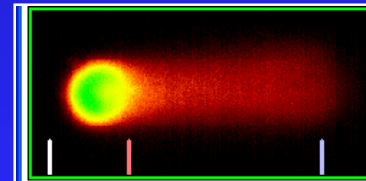
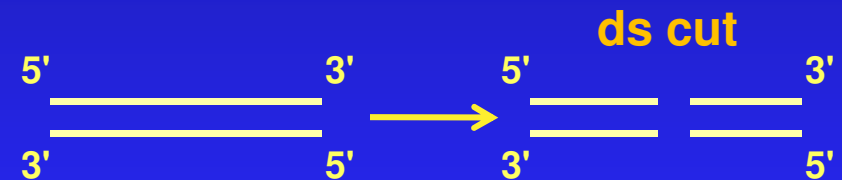
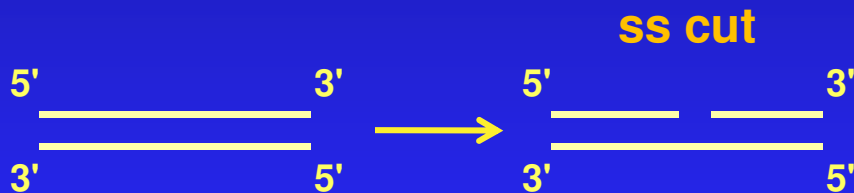


Figure 8.

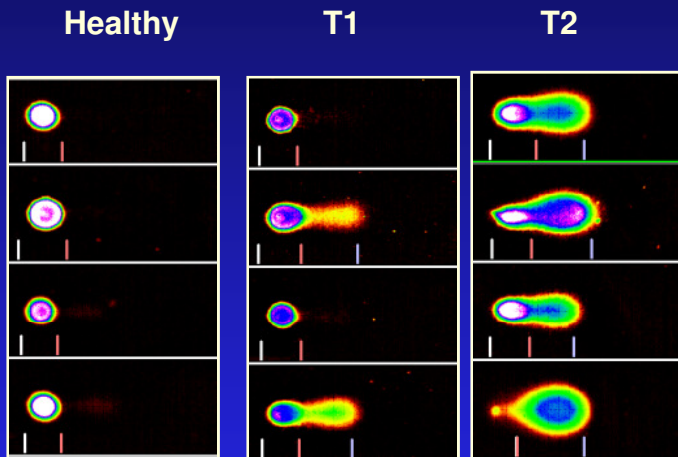
DNA Damaging Agents

- Hydrogen Peroxide
 - Single-strand cuts
- Bleomycin
 - Double-strand cuts

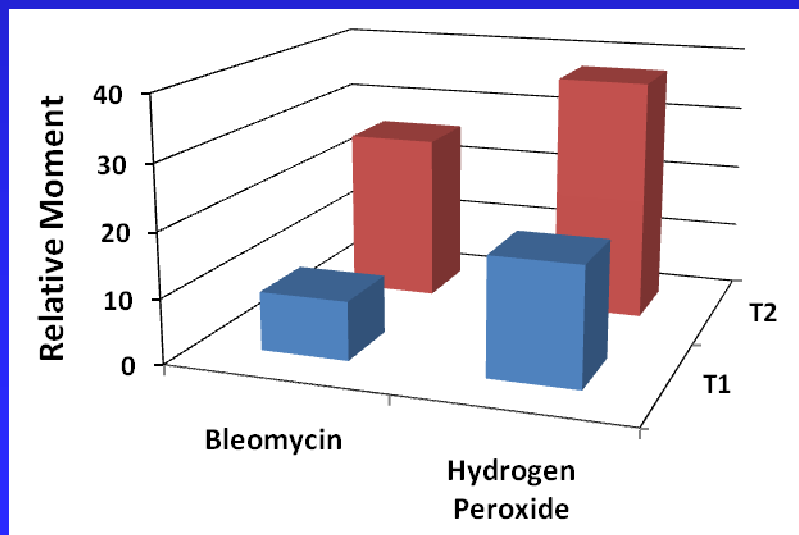
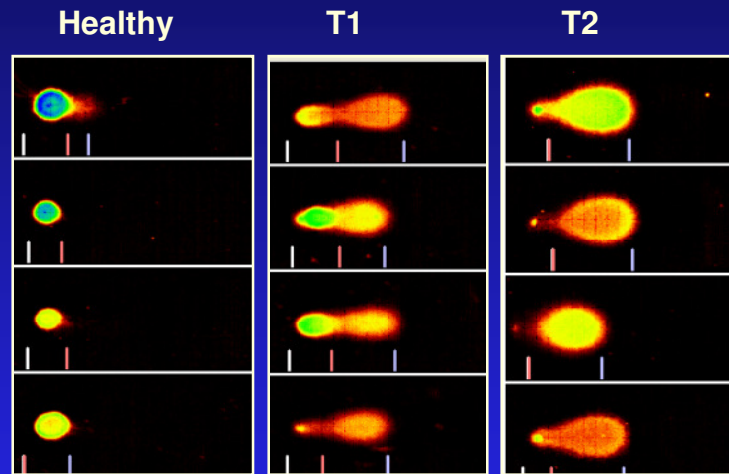


Alkaline Comet

Bleomycin



Hydrogen Peroxide

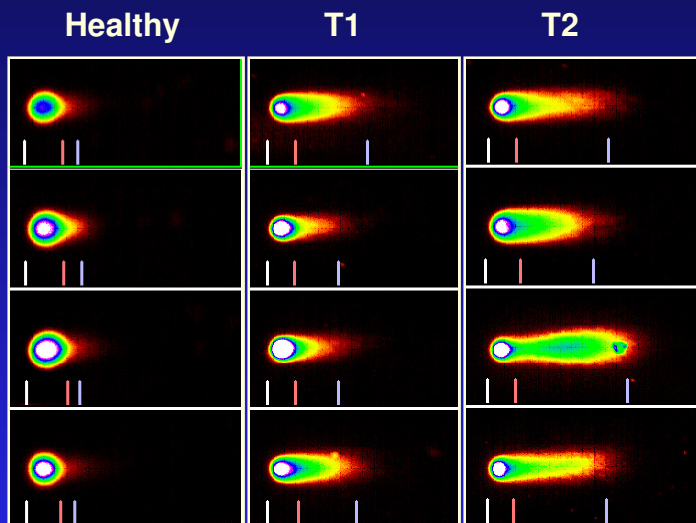


Increase in damage seen with increasing concentrations of Bleomycin and Hydrogen Peroxide

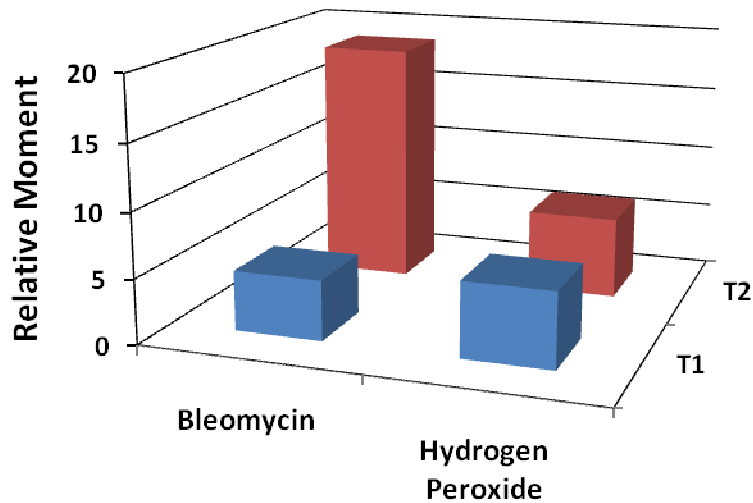
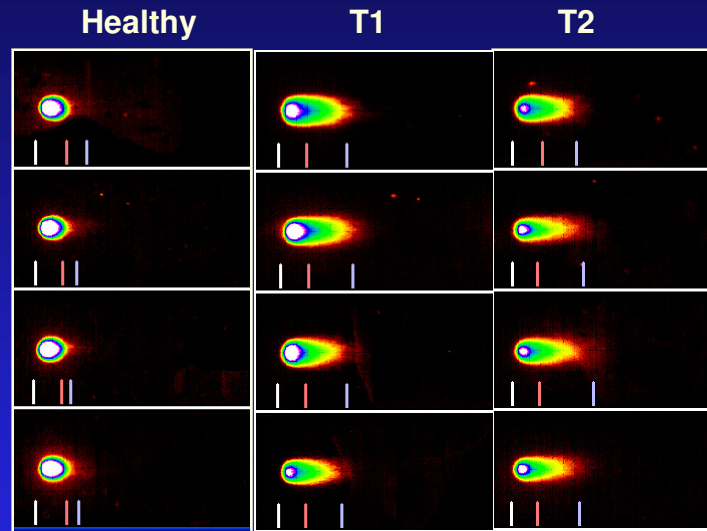
Figure 9.

Neutral Comet

Bleomycin



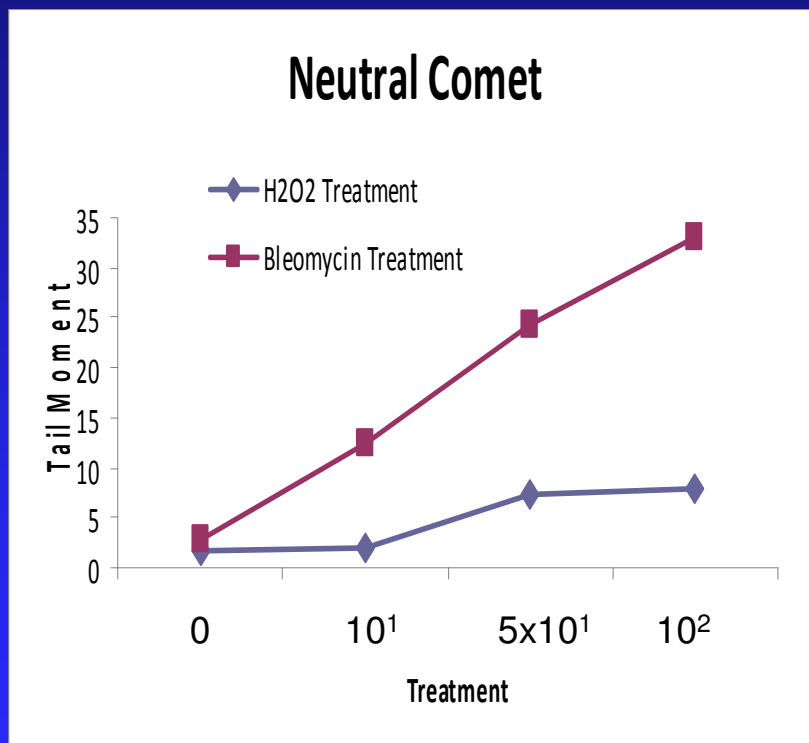
Hydrogen Peroxide



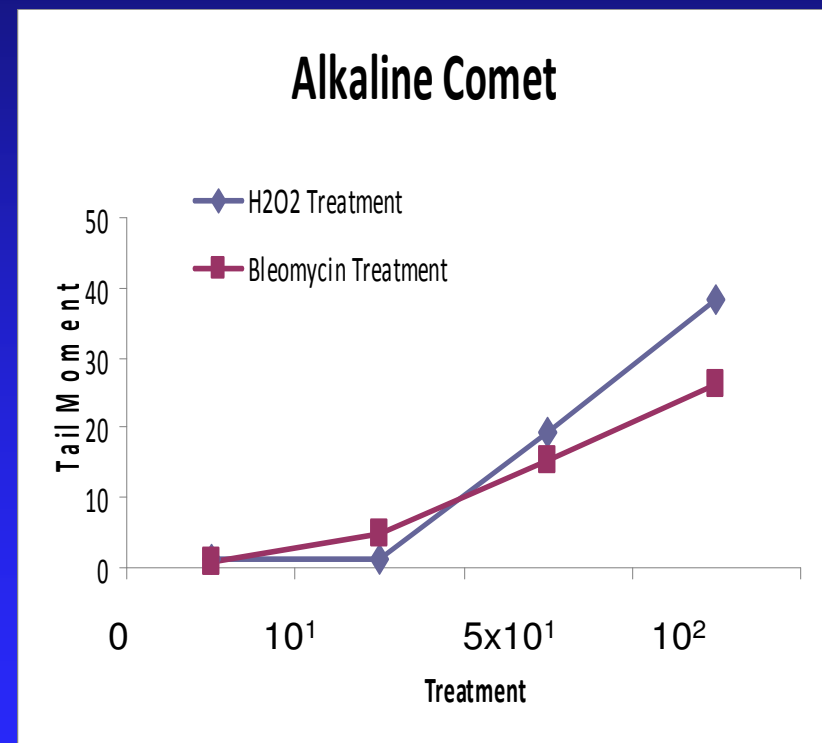
Increase in damage only seen with increasing concentration of Bleomycin

Figure 10.

Neutral Comet Differentiates Between Double and Single Strand DNA Damaging Agents



A.



B.

Figure 11.

Neutral Comet Differentiates Between Double and Single Strand DNA Damaging Agents

- **In the Neutral CometAssay® Comet tail parameters:**
 - show dose response behavior when cells are treated with double strand damaging agents. (Fig. 11, Panel A)
 - do not show dose response behavior when treated single strand DNA damaging agents (Fig .11, Panel A).
- **In the Alkaline CometAssay® Comet tail parameters:**
 - Show dose response behavior when treated with either single or double strand damaging agents. (Fig. 11, Panel B)

Application of the CometAssay® to Study DNA Repair

Two Regulators of Base Excision Repair

- PARP: Poly (ADP-Ribose) Polymerase binds to DNA strand breaks and polymerizes NAD⁺ into polymers of [ADP]-ribose (PAR) on itself and other DNA associated proteins. The PAR polymer recruits DNA repair proteins to the site of damage.
- PARG: Poly (ADP-Ribose) Glycohydrolase is for the degradation of the PAR polymer.

The PARP Pharmacodynamic Assay II is a Capture ELISA

In order to measure PAR levels in cells we developed a PARP Pharmacodynamic Assay.

- Lysates are prepared from lymphocytes, tissues or cultured cells.
- Free PAR and PAR bound to proteins is captured by anti-PAR monoclonal antibody attached to microtiter plates.
- Subsequently, captured PAR is quantified using a PAR directed rabbit polyclonal antibody.

PARP Pharmacodynamic Assay II

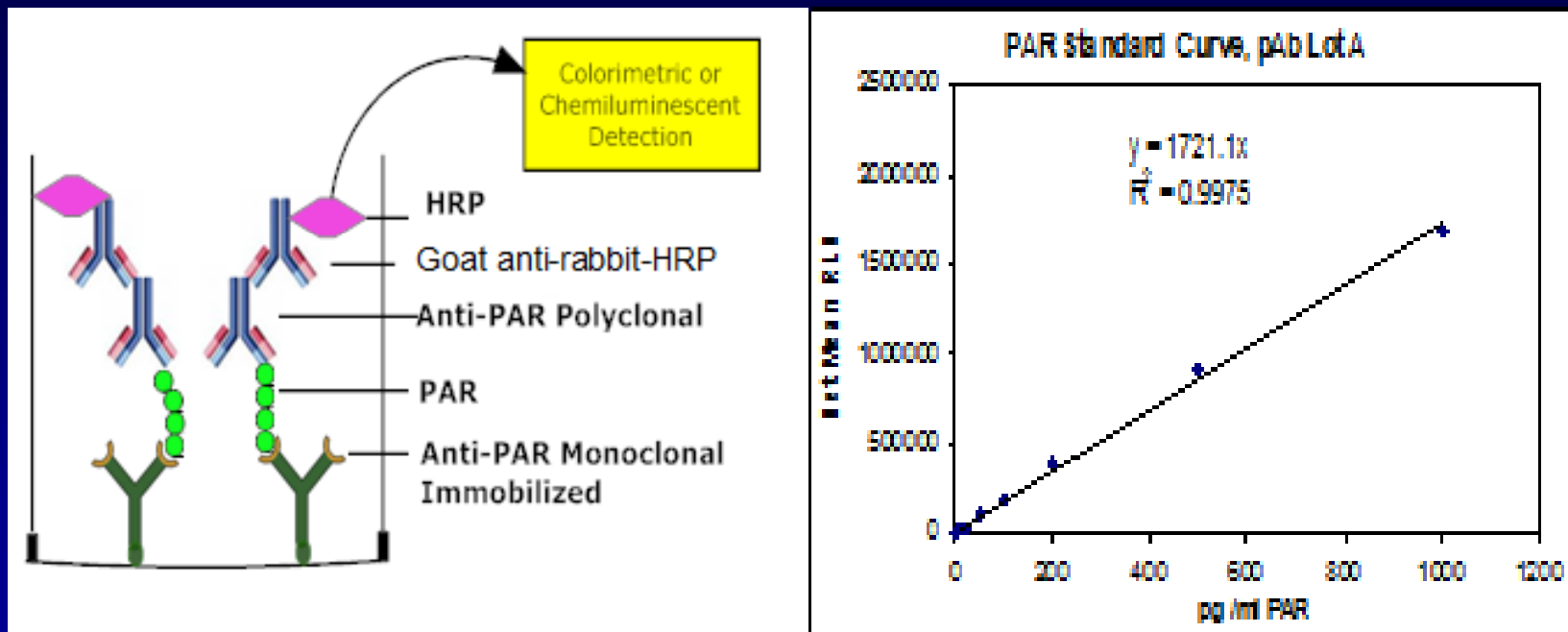


Figure 12.

Action of PARP and PARG

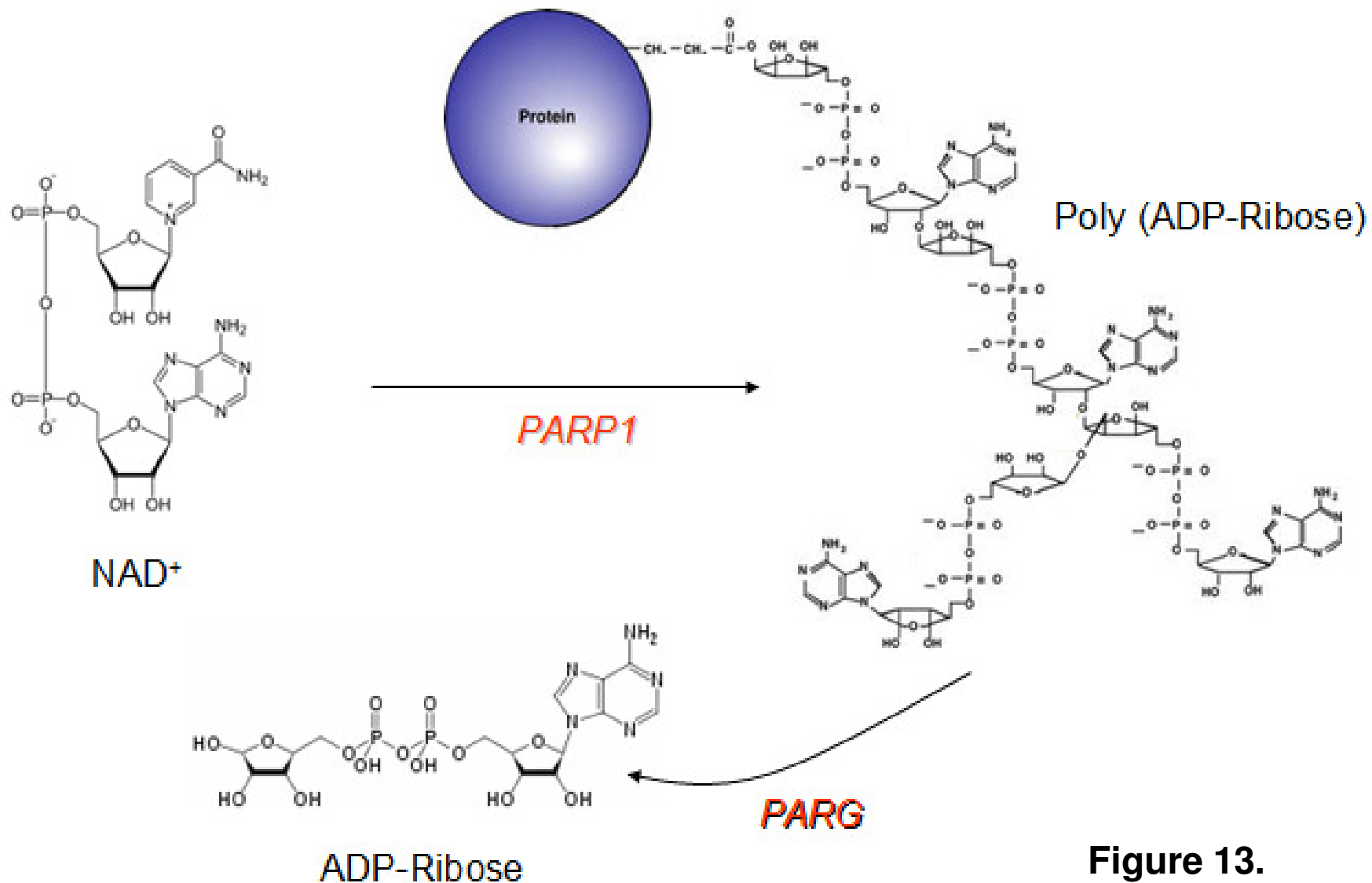


Figure 13.

The Role of PARP and PARG in Base Excision Repair

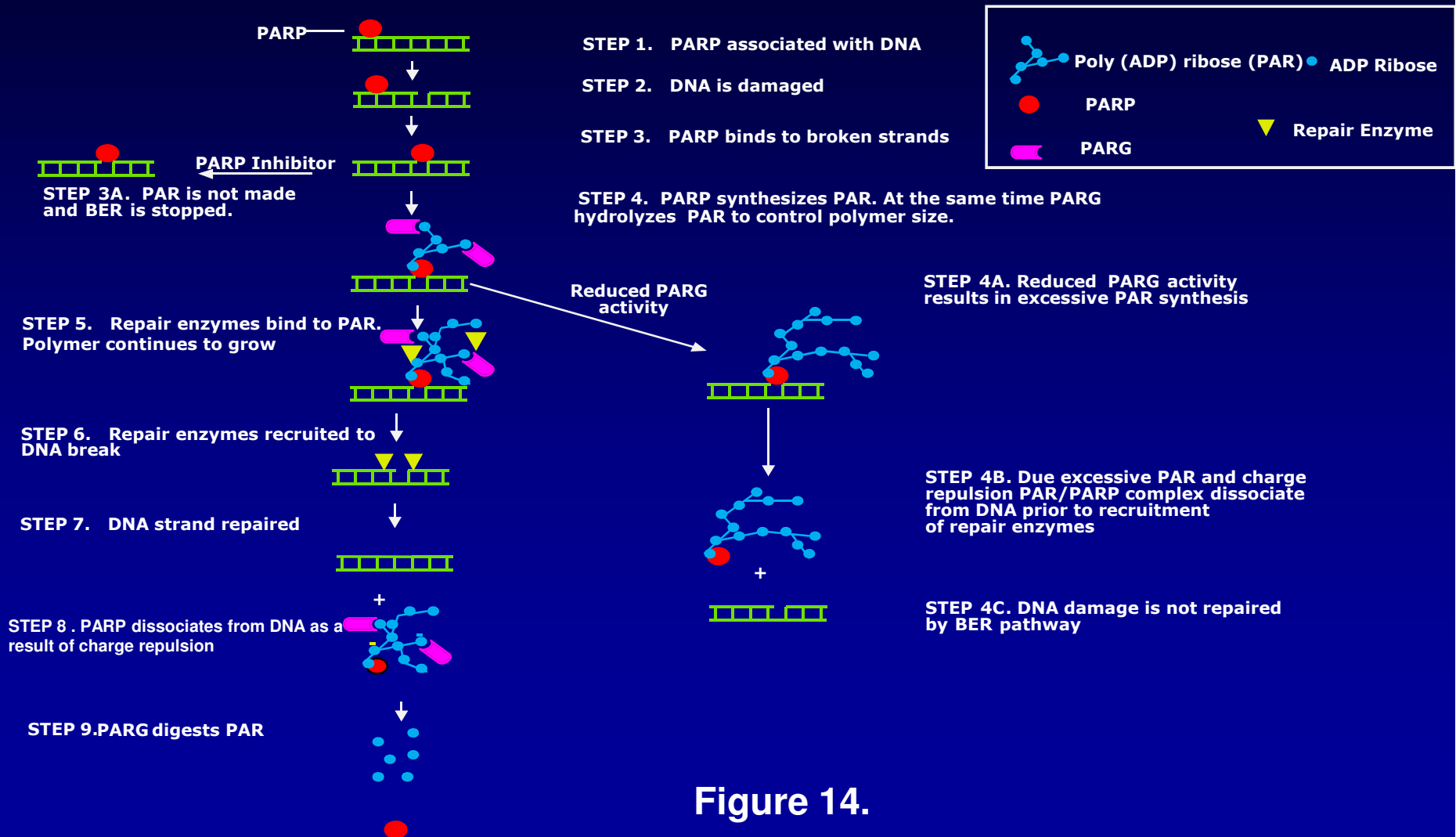


Figure 14.

Cells Deficient in Homologous Recombination are Sensitive to PARP Inhibitors

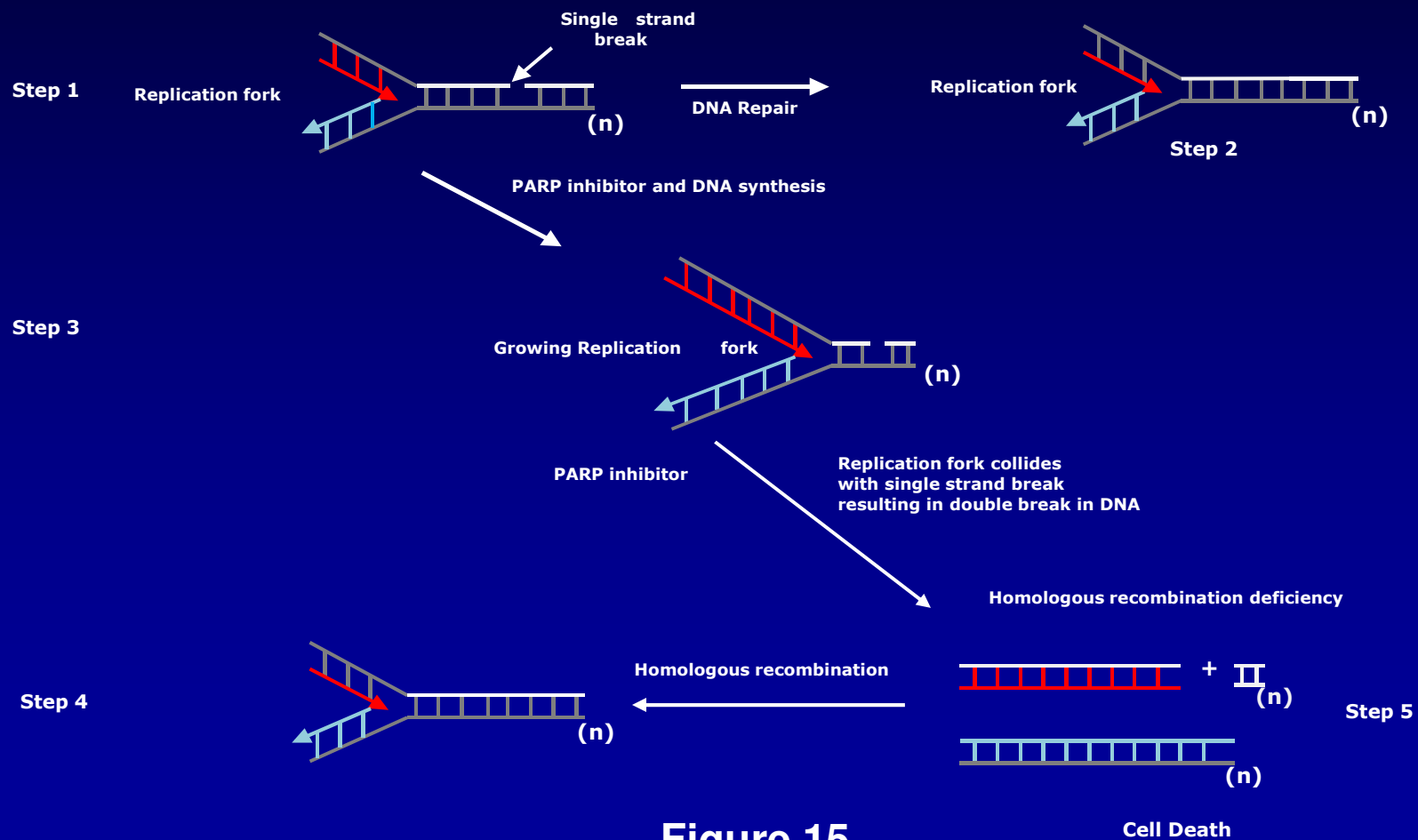


Figure 15.

Cell Treatment for PAR and Comet Analysis

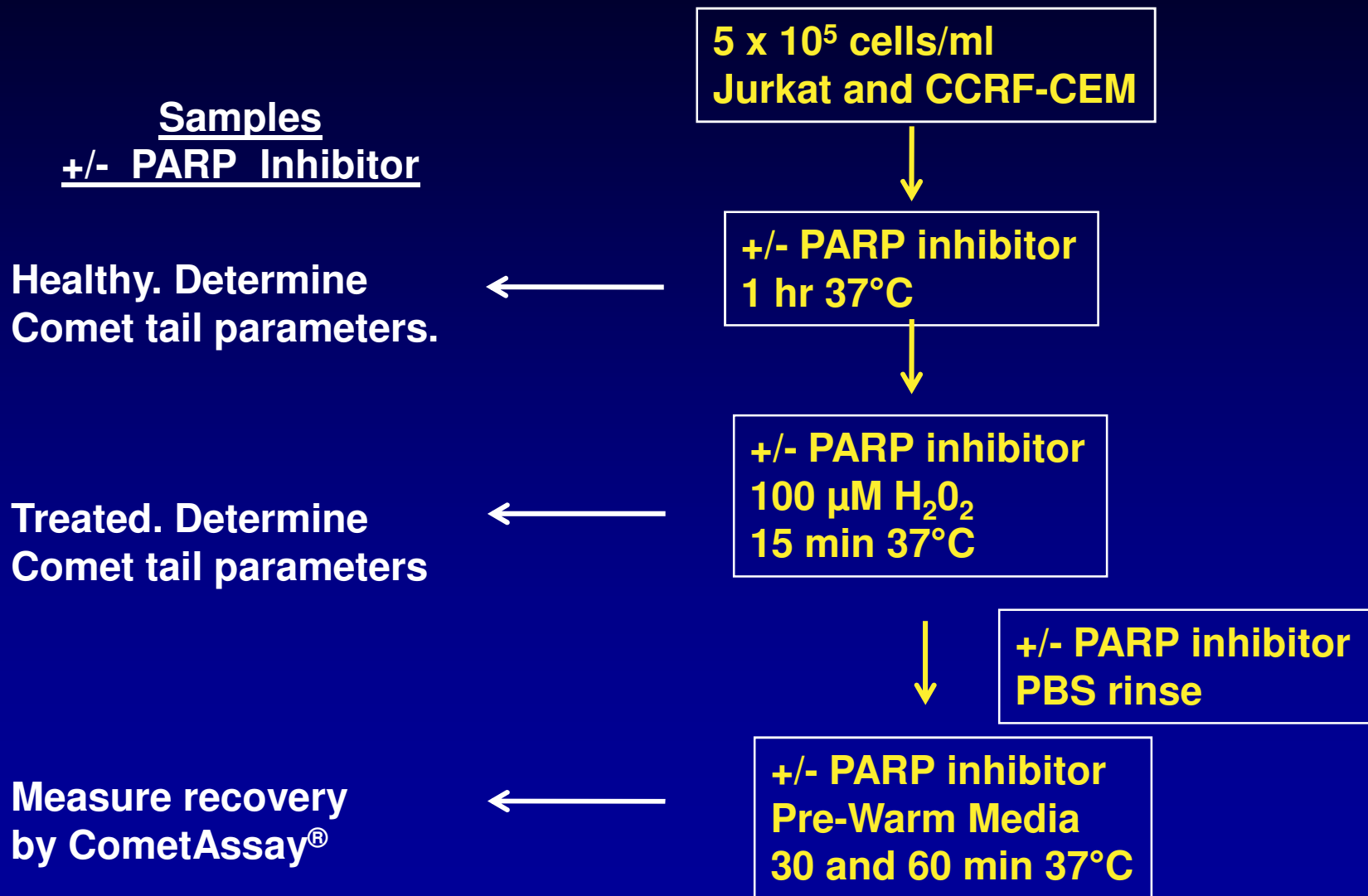
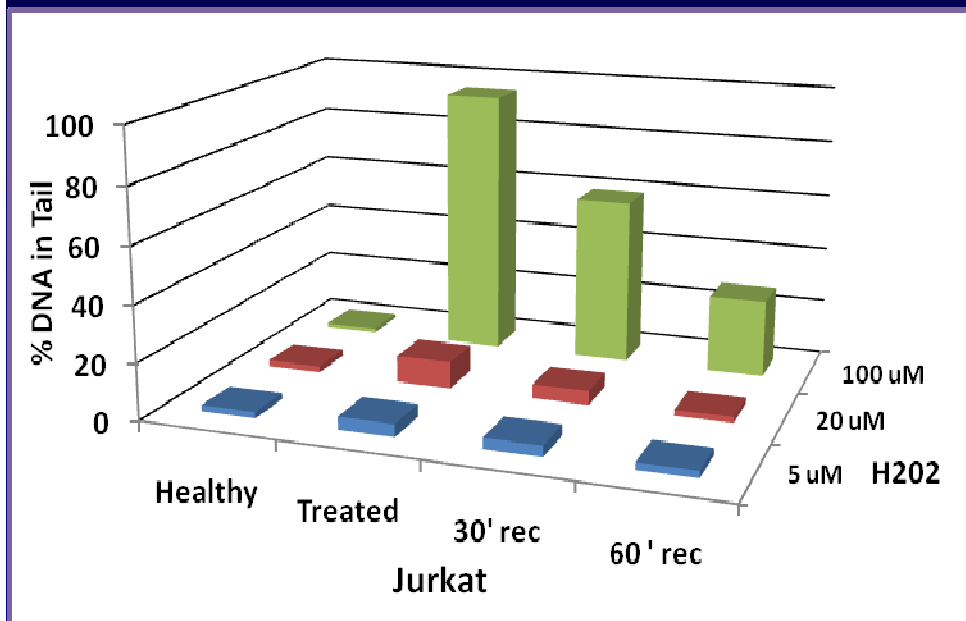


Figure 17.

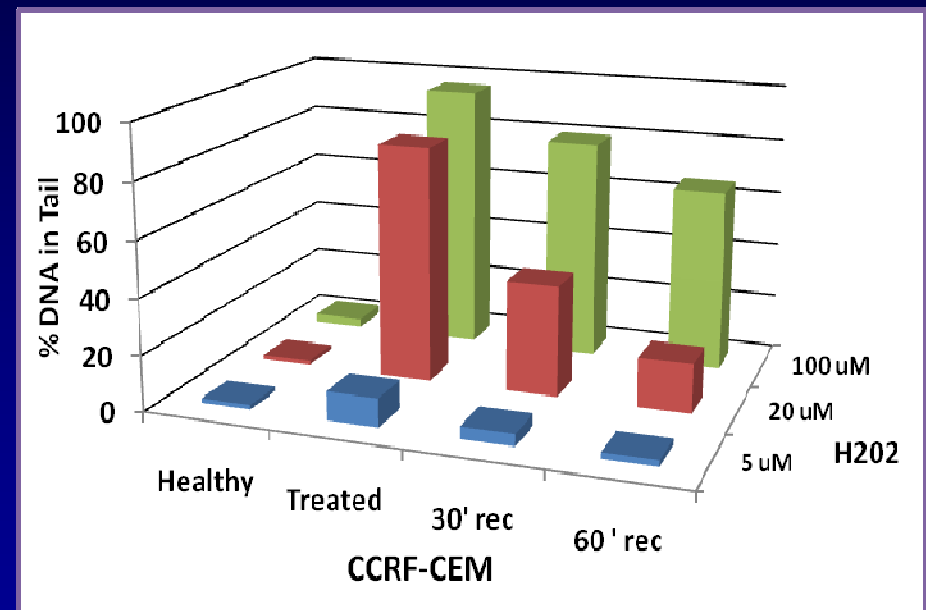
Do PAR Levels Reflect DNA Repair Capacity?

Panel A



Jurkatt-150 pg/m/ PAR

Panel B

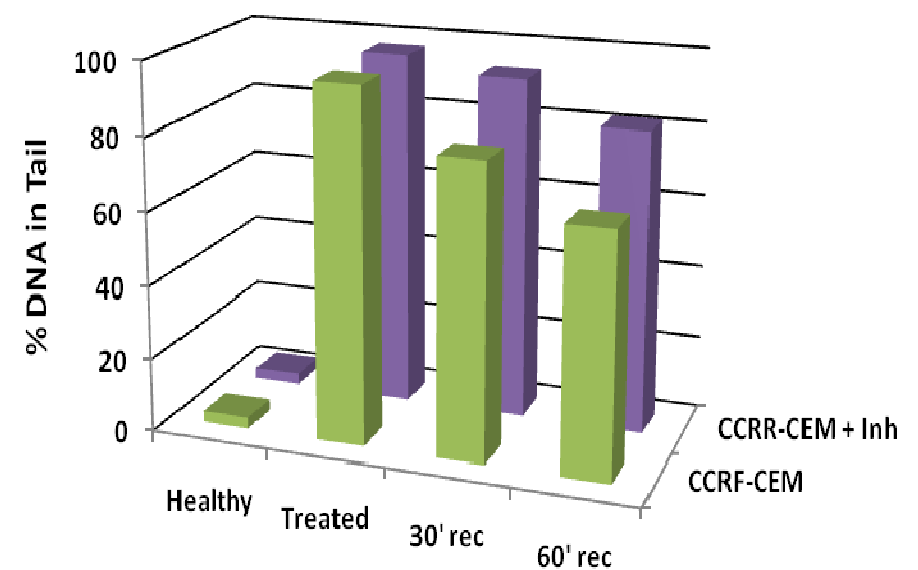
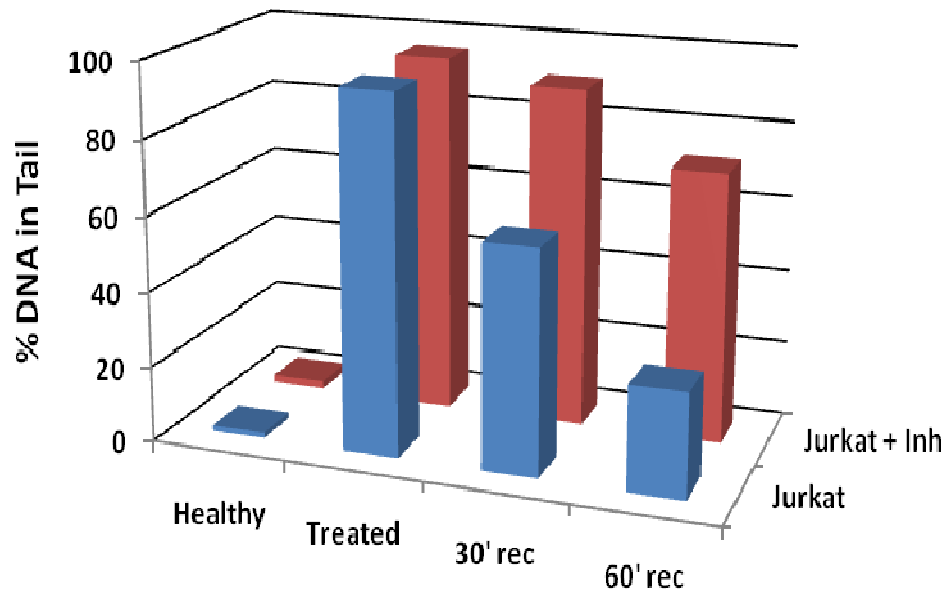


CCR-CEM PAR
below detection limits

DNA repair measured by the Comet Assay

Figure 18.

PARP inhibitor potentiates effect of Hydrogen Peroxide



Basal PAR Level: 150 pg/ml

Basal PAR Level: ND

DNA Repair measured by Comet Assay

Figure 19.

Relationship between PAR levels and DNA repair measured by the CometAssay®

- DNA repair levels measured by the CometAssay® appear to be related to cellular PAR levels. Cells with high PAR levels more readily repair their DNA compared to cells with low levels of PAR (Figure 18).
- PARP inhibitors potentiate the effect of Hydrogen Peroxide on DNA damage (Figure 19).

Standardized CometAssay® System



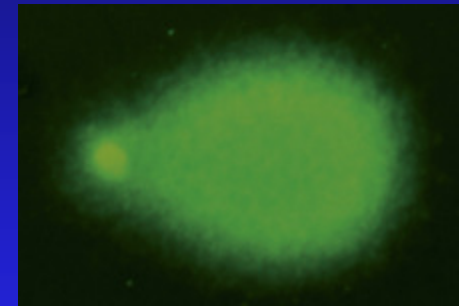
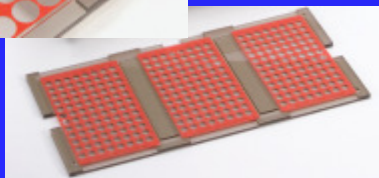
Instrumentation



Control Cells



Slides



Kits

Ordering Information

CometAssay® Kit	4250-050-K
CometAssay® Control Cells	4256-010-CC
Neutral CometAssay® Control Cells	4257-010-NC
CometAssay® Electrophoresis System	4250-050-ESK
CometAssay® Electrophoresis System Starter Kit	4250-050-ESK
PARP in vivo Pharmacodynamic Assay II	4520-096-K