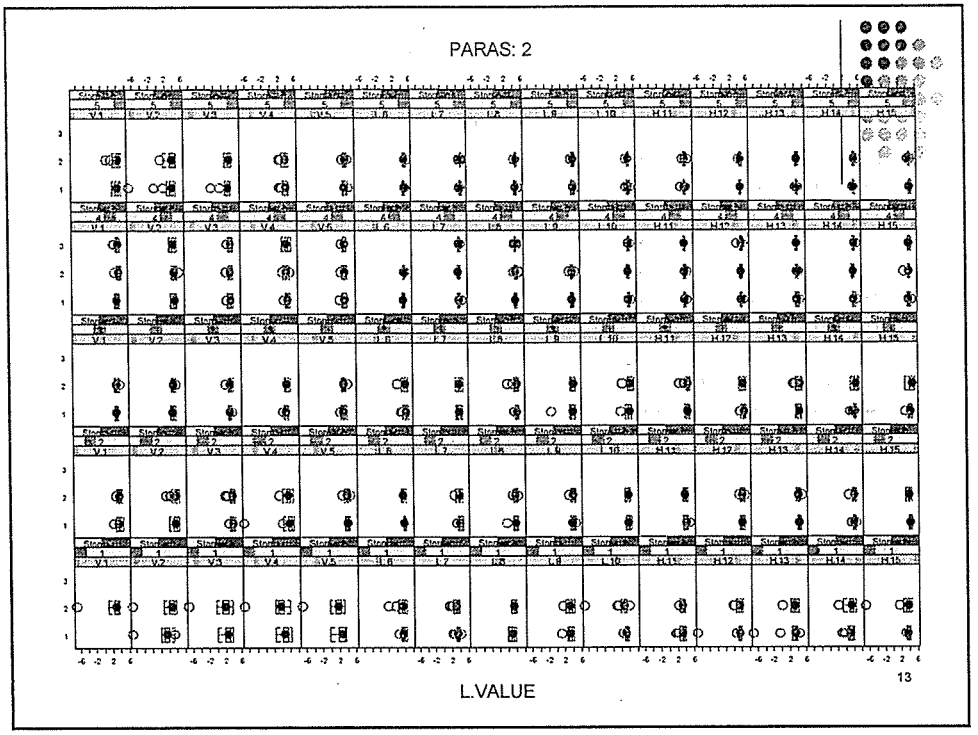
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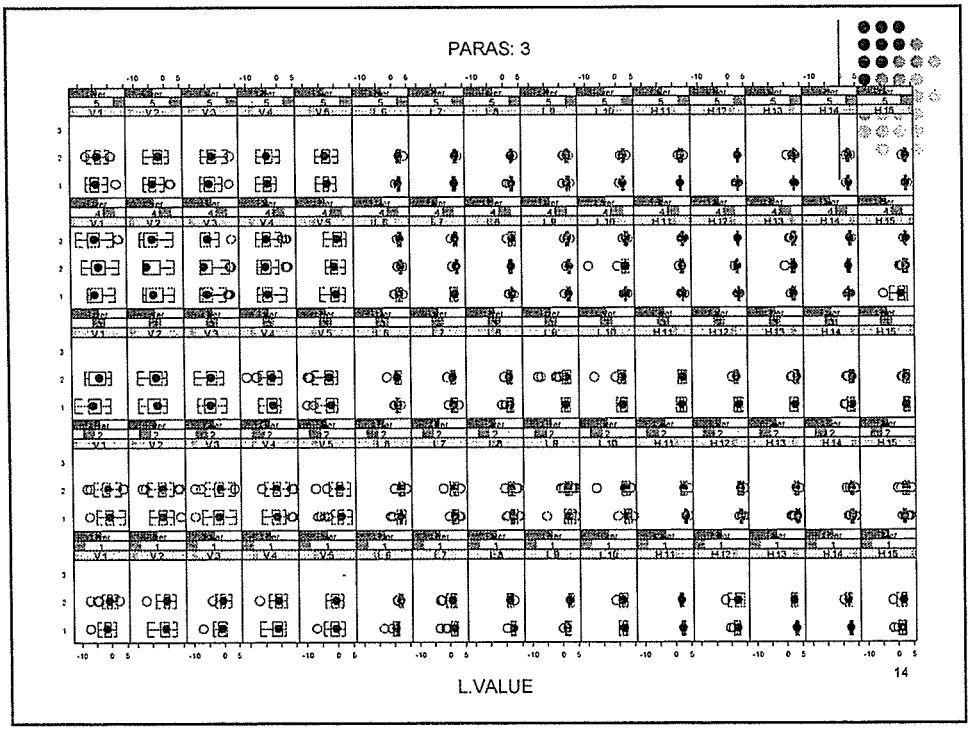
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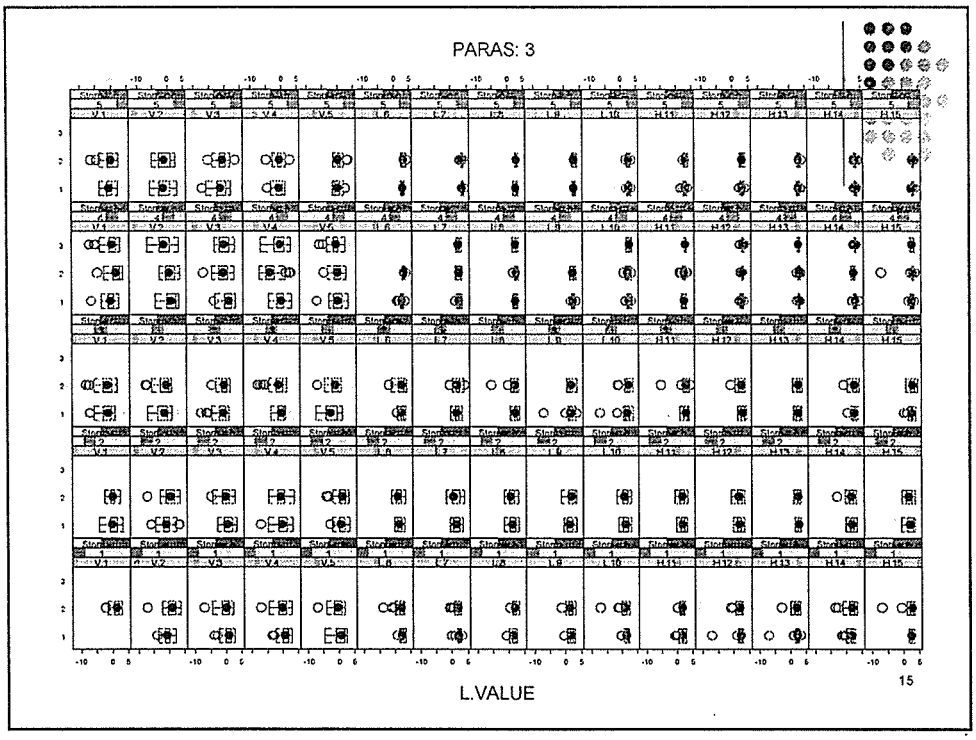
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INTERNATIONAL VALIDATION OF THE *IN VIVO* RODENT ALKALINE COMET ASSAY FOR THE DETECTION OF GENOTOXIC CARCINOGENS (VERSION 11)

Issued by: the Validation Management Team (VMT)

Date: November 2, 2006 revised

Add a 3 R section 1

Notes:

1. Will likely need to specify shelf life for some solutions as we reconcile lab-specific protocols

A. PURPOSE OF THIS DOCUMENT

This document is provided to clarify the conduct of an international validation study to evaluate the ability of the *in vivo* rodent alkaline Comet assay to identify genotoxic carcinogens, as a potential replacement for the *in vivo* rodent hepatocyte unscheduled DNA synthesis (UDS) assay. A study protocol will be developed by the testing facilities based on the information provided in this document.

B. ASSURANCE OF DATA QUALITY

The study will be conducted in facilities that are Good Laboratory Practice compliant. Consistency between raw data and a final report is the responsibility of each testing facility. The VMT may review the data for consistency, if deemed necessary.

C. ANIMAL WELFARE

Appropriate national and/or international regulations on animal welfare must be followed.

D. TESTING PROCEDURE

1. MATERIALS AND METHODS

1.1. Test substances and positive/negative controls

1.1.1. Test substance

With the exception of ethyl methanesulfonate (EMS), test substances will be supplied to each testing facility by the VMT. When coded substances are supplied, appropriate safety information will be provided in a sealed envelope to be opened only by an appropriate individual within the organization who is not involved in the study and/or in the case of

an emergency. If opened, appropriate documentation and justification will need to be provided to the VMT.

1.1.2. Test substance preparation

Each test substance will be dissolved or suspended with an appropriate solvent/vehicle just before administration (see section 1.1.4.).

1.1.3. Test Substance (also the positive control in future phases of the validation study)

EMS (CAS No. 62-50-0); the source and lot number to be used will be provided by the VMT. EMS will be dissolved in physiological saline just before administration (within 2 hour).

1.1.4. Negative control (solvent/vehicle)

Solvents/vehicles for test substance preparation will be used as negative controls. An appropriate solvent/vehicle for a test substance may be indicated by the VMT. In the absence of instruction from the VMT, an appropriate solvent/vehicle will be chosen for each test substance by the testing facility in the following order: physiological saline, 0.5% w/v sodium carboxymethylcellulose aqua solution, corn oil. The source and lot of the corn oil will be specified by the VMT.

1.2. Test animals

1.2.1. Species

Although either rats or mice can be used in this assay, the validation study will use rats. The rat is the species most commonly used in toxicological studies and is the preferred species in the *in vivo* rodent hepatocyte UDS assay.

1.2.2. Sex

In order to allow for a direct comparison with the rat hepatocyte UDS assay, males will be used.

1.2.3. Strain

Rat: Crl:CD (SD)

1.2.4. Source

Charles River Laboratories, Inc.

1.2.5. Age

At the time of purchase: 6-8 weeks of age (body weight 150g - 250g)

At the time of dosing: 7-9 weeks of age

1.2.6. Body weight

1.2.7. The weight variation of animals should be +/- 20% of the mean weight at the time of dosing.

Number of animals in each dose group at each sampling time

5 males for the validation study We will decide afterwards based upon power calculation.

Animal maintenance

Animals will be reared under appropriate housing and feeding conditions according to the standard operating procedures (SOP) in each testing facility, consistent with Section C "Animal Welfare".

1.2.7.1. Diet

Animals will be fed *ad libitum* with a commercially available pellet diet.

1.2.7.2. Water

Animals will be given free access to tap water *ad libitum.*

1.2.8. Animal quarantine and acclimation

Animals will be quarantined and acclimated for at least 5 days prior to the start of the study, according to SOPs in each testing facility. Only healthy animals approved by the Study Director and/or the Animal Facility Veterinarian will be used.

1.2.9. Animal identification and group assignment

Animals will be identified uniquely and assigned to groups by randomization on the basis of body weight according to the SOP in each testing facility.

1.3. Preparation of Comet assay solutions

The following solutions will be prepared, consistent with laboratory SOPs, unless otherwise specified.

1.3.1. 1.0-1.5% (w/v) standard agarose gel for the bottom layer (if used)

Regular melting agarose) will be dissolved at 1.0-1.5% (w/v) in Dulbecco's phosphate buffer (Ca^{++}, Mg^{++} free and phenol free) by heating in a microwave.

1.3.2. 0.5 % (w/v) low-melting agarose (Cambrex AG5897) gel for the cell-containing layer and, if used, a top layer

Low-melting agarose will be dissolved at 0.5% (w/v) in Dulbecco's phosphate buffer (Ca^{++}, Mg^{++} free and phenol free) by heating in a microwave. During the study this solution will be kept at 37-45°C and discarded afterward. note – check EDTA

1.3.3. Lysing solution

The lysing solution will consist of 100 mM EDTA (disodium), 2.5 M sodium chloride, and 10 mM tris hydroxymethyl aminomethane in purified water, with the pH adjusted to 10.0 with 1 M sodium hydroxide and/or hydrochloric acid. This solution may be refrigerated at <10°C until use. On the same day of use, 1 % (v/v) of triton-X100 and 10 % (v/v) DMSO will be added to this solution and the complete lysing solution will be refrigerated at <10°C for at least 30 minutes prior to use.

1.3.4. Alkaline solution for unwinding and electrophoresis

The alkaline solution consists of 300 mM sodium hydroxide and 1 mM EDTA (disodium) in purified water, pH >13. This solution will be refrigerated at <10°C until use. The pH of the solution will be measured just prior to use.

1.3.5. Neutralization solution

The neutralization solution consists of 0.4 M tris hydroxymethyl aminomethane in purified water, pH 7.5. This solution will be either refrigerated at <10°C or stored consistent with manufacturer's specifications until use.

1.3.6. Mincing buffer

The mincing and/or homogenization buffer consists of 20 mM EDTA (disodium) and 10% DMSO in Hank's Balanced Salt Solution (HBSS) (Ca^{++}, Mg^{++} free, and phenol red free if available), pH 7.5 (DMSO will be added immediately before use). This solution will be refrigerated at <10°C until use.

1.3.7. Staining solution

The fluorescent DNA stain is SYBR Gold (Invitrogen-Molecular Probes), prepared and used according to the manufacturer's specifications.

1.4. Comet assay procedure

1.4.1. Experimental design

Compound	Dose (mg/kg)	Number of animals
Vehicle (negative control)	0	5
EMS	50?	5
Test compound	Low (1/4)	5
Test compound	Medium (1/2)	5

Test compound	high	5

* Low dose level will be 50% of the high dose.

Dose Selection

In general, in the absence of VMT directions, the high dose level of a test compound will be selected as the dose producing signs of toxicity such that a higher dose level, based on the same dosing regimen, would be expected to produce mortality,or an unacceptable level of animal distress.

Selection of doses will be based on the toxicity of the test substance but will not exceed 2000 mg/kg.

1.4.2. Administration to animals

The test substance will be administered twice orally by gavage, 21 hours apart. The dosage volume will be 0.1 mL per 10 g body weight in rats on the basis of the animal weight just before administration.

1.4.3. Measurement of body weight and examination of animal conditions

Individual body weights will be measured in accordance with local SOPs and just prior to administration (the weight at this time will be used to determine the volume of each substance administered). The clinical signs of the animals will be observed from just after dosing to just before tissue removal with an appropriate interval according to the SOP in each testing facility.

1.4.4. Tissue sampling

Animals will be humanely killed, consistent with Section C "Animal Welfare". The stomach and portions of the liver will be removed. Tissues will be placed into ice-cold mincing buffer, rinsed sufficiently with the cold mincing buffer to remove residual blood (more rinses would likely be needed if exsanguination is not used), and stored on ice until processed. Histopath insert from same liver lobe, minimal possible for stomach

1.4.5. Preparation of single cells

The liver and the stomach will be processed as follows:

Liver: A portion of the left lateral lobe of the liver will be removed and washed in the cold mincing buffer until as much blood as possible has been removed. The size of the portion will be at the discretion of the laboratory but will be standardized. The portion will be minced with a pair of fine scissors to release the cells. The cell suspension will be

stored on ice for 15-30 seconds to allow large clumps to settle (or, the cell suspension will be strained through a Cell Strainer to remove lumps and the remaining suspension will be placed on ice), and the supernatant will be used to prepare comet slides.

Stomach: The stomach will be cut open and washed free from food using cold mincing buffer. The forestomach will be removed and discarded. The glandular stomach will be then placed into cold mincing buffer and incubated on ice for from 15 to 30 minutes. After incubation, the surface epithelia will be gently scraped two times using the a scalpel blade or a Teflon scrapper. This layer will be discarded and the gastric mucosa rinsed with the cold mincing buffer. The stomach epithelia will be carefully scraped 4-5 times (or more, if necessary) with a scalpel blade or Teflon scrapper to release the cells. The cell suspension will be stored on ice for 15-30 seconds to allow large clumps to settle (or, the cell suspension will be strained with a Cell Strainer to remove clumps and the remaining suspension will be placed on ice), and samples of the supernatant used to prepare comet slides.

1.4.6. Slide preparation

Comet slides will be prepared using laboratory specific procedures. The volume of the cell suspension added to 0.50% low melting agarose to make the slides will not decrease the percentage of low melting agarose by more than 10% (i.e., not below 0.45%) .

1.4.7. Lyses

Once prepared, the slides will be immersed in chilled lysing solution overnight in a refrigerator under a light proof condition. After completion of lysing, the slides will be rinsed in purified water or neutralization solution to remove residual detergent and salts prior to the alkali unwinding step.

1.4.8. Unwinding and electrophoresis

Slides will be randomly placed onto a platform of submarine-type electrophoresis unit and the electrophoresis solution added. A balanced design will be used (i.e., in each electrophoresis run, there should be the same number of slides from each animal in the study; see Attachment 1, an example of use to keep track of each slides during each electrophoresis run. Each laboratory will need to provide its own electrophoresis box chart, as different boxes can accommodate different numbers of slides). The electrophoresis solution will be poured until the surfaces of the slides are completely covered with the solution. The slides will be left to be unwind for 20 minutes. Next, the slides will be electrophoresed at 0.7 to 1 V/cm, with a constant voltage at approximately 0.30 A. The current at the start and end of the electrophoresis period should be recorded.

The temperature of the electrophoresis solution through unwinding and electrophoresis should be maintained at a constant temperature <10°C . The temperature of the electrophoresis solution at the start of unwinding, the start of electrophoresis, and the end of electrophoresis should be recorded. The electrophoresis duration should result in an average DNA migration in the negative control group of 1-15?% DNA in the tail.

1.4.9. Neutralization and dehydration of slides

After completion of electrophoresis, the slides will be immersed in the neutralization buffer for at least 5 minutes. All slides will be dehydrated by immersion into absolute ethanol (≥99.6%) for at least 5 minutes, allowed to air dry, and then stored until scored at room temperature, protected from humidity > 60 %. Once scored, slides should be retained and stored under low humidity conditions (e.g., in a desiccator) for potential rescoring.

1.4.10. DNA staining, comet visualization and analysis

Coded slides will be blind scored according to laboratory specific SOPs. The slides will be stained with SYBR Gold according to manufacturer's specifications. The comets will be measured via a digital (e.g. CCD) camera linked to an image analyzer system using a fluorescence microscope at magnification between 200X to 400X. For each sample (animal/tissue), fifty comets cells per slide will be analyzed, with 2 slides scored per sample. To be re-evaluated after stat analysis Heavily damaged cells exhibiting a microscopic image (commonly referred to as hedgehogs) consisting of small or non-existent head and large, diffuse tails will be excluded from data collection. Add pictures in an appendix – indicate if scorable by software then should be scored However, the frequency of such comets should be determined per sample, based on the visual scoring of 100 cells per sample. The comet endpoints collected will be % tail DNA, tail length in microns measured from the estimated edge of the head region closest to the anode, and, if possible for a particular image analysis system, Olive tail moment [= a measure of tail length (a distance between a center of head mass and a center of tail mass; microns) X a measure of DNA in tail (% tail DNA/100): Olive et al., 1990].

1.4.11. Neutral diffusion assay

To evaluate the extent of cytotoxicity associated with the treatment, one comet slide per sample will be used to evaluate the frequency of cells with low molecular weight DNA indicative of apoptosis or necrosis. After incubation in the lysis solution for 1 hour (± 5 minutes), the comet slide will be rinsed with purified water to remove residual detergent and salts. All slides will be dehydrated by immersion into absolute ethanol (≥99.6%) for

at least 5 minutes, allowed to air dry, and then stored until scored. Once scored, slides should be retained and stored under low humidity conditions (e.g., in a desiccator) for potential rescoring. Coded slides will be stained according to laboratory specific SOPs with SYBR Gold according to manufacturer's specifications. The frequency of cells with diffuse DNA (see Appendix 1) among 100 comets will be visually scored using a fluorescence microscope at magnification between 200X to 400X. add more information about classification and clarification and more pictures

1.4.12. Histopathology to revise

When a positive Comet assay response is obtained for a tissue, a sample histopathological assessment will be conducted to evaluate for the presence of examined for the tissue according to the SOP in each testing facility.

Need to add study acceptance criteria
Negative controls
Positive controls
Historical data

2. STATISTICS (redo based on more discussion among the few)

Different approaches for data analysis have been proposed for comet data generated across a range of test substance dose levels (Lovell et al. 1999; Hartmann et al. 2003; Wiklund and Agurell 2003). The primary endpoint of interest for DNA migration is the % tail DNA. However, other measures of DNA migration (tail length, Olive tail moment, and also categorical data, if available) will be analyzed also. In addition, the distribution of migration patterns among cells within an animal will be considered. The percentage of "hedgehogs" and of cells with low molecular weight DNA will also be evaluated as a function of treatment. The unit of analysis for a specific tissue is the individual animal. Each laboratory should make their own conclusion about the *in vivo* genotoxicity of a test substance using their standard approach.

What should be the criteria for a positive call – 3 fold increase above/below (?) control

3. DATA AND REPORTING

3.1.1. Treatment of results

Individual animal data and group summaries will be presented in a fixed tabular form that will be provided from the VMT.

3.1.2. Evaluation and interpretation of results

A positive response is defined as a statistically significant change in the % tail DNA in at least one dose group at a single sampling time in comparison with the negative control value. The positive control should produce a positive response, and if not, the study data will not be acceptable. Where a positive response is obtained in a test substance group, the investigator(s) will assess the possibility that a cytotoxic rather than a genotoxic effect is responsible based on the percentage of cells with low molecular weight DNA and histopathology. Positive results indicate that the test substance induce DNA damage in the target tissue(s) investigated. Negative results indicate that, under the test conditions used, the test substance does not induce DNA damage in vivo in the tissue(s) evaluated.

3.1.3. Study report

The study report from each testing facility will at least include the following information:

3.1.3.1. Test substance and positive/negative controls

Identification; CAS number; supplier; lot number; physical nature and purity; physiochemical property relevant to the conduct of the study, if known; justification for choice of vehicle; and solubility and stability of the substances in the solvent/vehicle, if known.

3.1.3.2. Test animals

Species/strain used; number, age and sex of animals; source, housing conditions, quarantine and acclimation procedure, and animal identification and group assignment procedure; individual weight of the animals on the day of receipt, at the end of the acclimation period, and before administration (at the time of grouping), including body weight range, mean and standard deviation for each group; and choice of tissue(s) and justification.

3.1.3.3. Reagents to prepare reagent solutions

Identification; supplier; lot number; and time limit for usage if known.

3.1.3.4. Test conditions

Data from range-finding study, if conducted; rationale for dose level selection; details of test substance preparation; details of the administration of the test substance; rationale for route of administration; methods for verifying that the test substance reached the general circulation or target tissue, if applicable; details of food and water quality; detailed description of treatment and sampling schedules; method of measurement of toxicity, including histopathology; detailed methods of single cell preparation; method of slide

preparation, including agarose concentration, lysis conditions, alkali conditions and pH, alkali unwinding time and temperature, electrophoresis conditions (pH, V/cm, mA, and temperature at the start of unwinding and the start and the end of electrophoresis) and staining procedure; criteria for scoring comets and number of comets analyzed per slide, per tissue and per animal; evaluation criteria; criteria for considering studies as positive, negative or equivocal.

3.1.3.5. Results

Signs of toxicity, including histopathology in the appropriate tissue(s) if applicable; individual and mean/median values for DNA migration (and ranges) and % cells with low molecular weight DNA and % hedgehogs in individual tissue, animal, and group; concurrent positive and negative control data; and statistical evaluation.

3.1.3.6. Discussion of the results and/or conclusion, as appropriate.

4. ARCHIVES AND REVIEW

The study report and all raw data (including slide samples and image data) from this study will be retained according to the SOP in each testing facility. All raw data will be submitted to the management team for review if required.

5. REFERENCES

Burlinson B, et al., 4th International Workgroup on Genotoxicity Testing: result of the in vivo comet assay workgroup (in preparation).

Collins AR, et al., Direct enzymatic detection of endogenous oxidative base damage in human lymphocyte DNA. Carcinogenesis, 14, 1733-1735, 1993.

Hartmann A, et al., Recommendation for conducting the *in vivo* alkaline Comet assay. Mutagenesis, 18(1), 45-51, 2003.

Lovell DP, G Thomas G, R Dubow., Issues related to the experimental design and subsequent statistical analysis of in vivo and in vitro comet studies. Teratog Carcinog Mutagen. 19(2), 109-119, 1999.

Olive PL, et al., Heterogeneity in radiation-induced DNA damage and repair in tumor and normal cell using the "comet" assay. Radiat. Res., 122, 86-94, 1990.

Tice RR et al., Single cell gel/Comet assay: guidelines for in vitro and in vivo genetic toxicology testing. Environ. Mol. Mutagen., 35, 206-221, 2000.

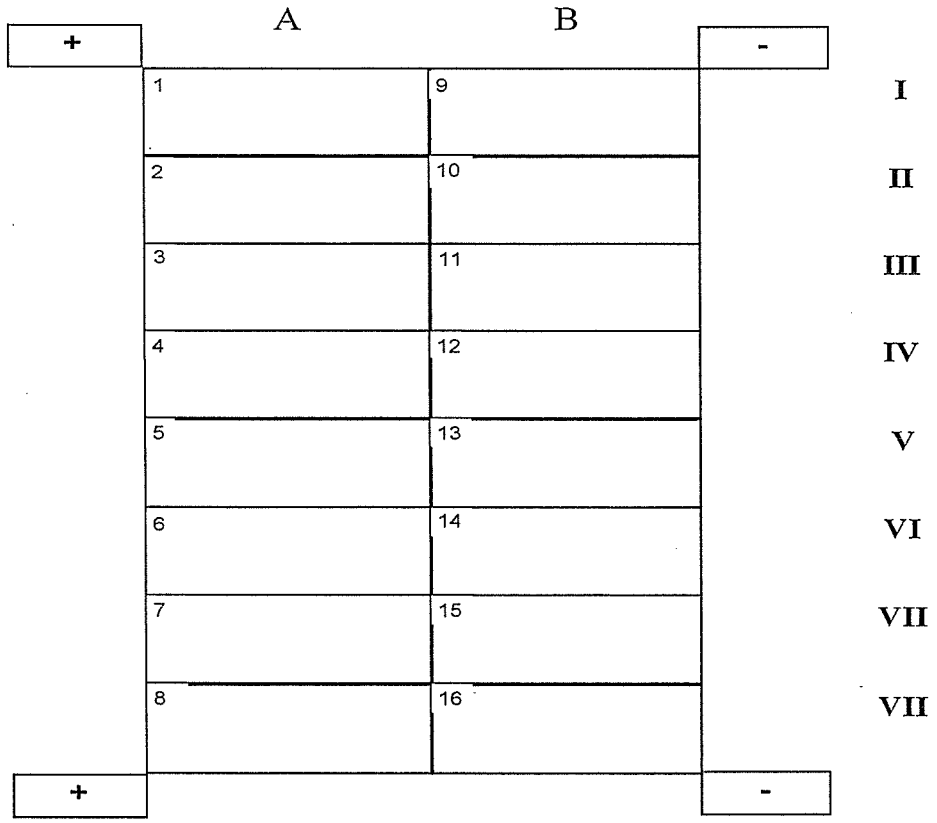
Wiklund SJ, E Agurell., Aspects of design and statistical analysis in the Comet assay. Mutagenesis 18(2):167-175, 2003.

Attachment 1:

SLIDES UNWINDING & ELECTROPHORESIS RECORDING SHEET

Electrophoresis Run #				**Initials & Date**
Approximate alkaline electrophoresis buffer volume in chamber				
Unwinding				
Time	Total	Start	End	
Buffer Temperature				
Electrophoresis				
Running time	Total	Start	End	
Volts				
Milliamperes				
Buffer Temperature				
Thermometer No.				
Electrophoresis chamber No.				
Power supply No.				

Diagram Electrophoresis Chamber



RED(+) 12 **BLACK(-)**

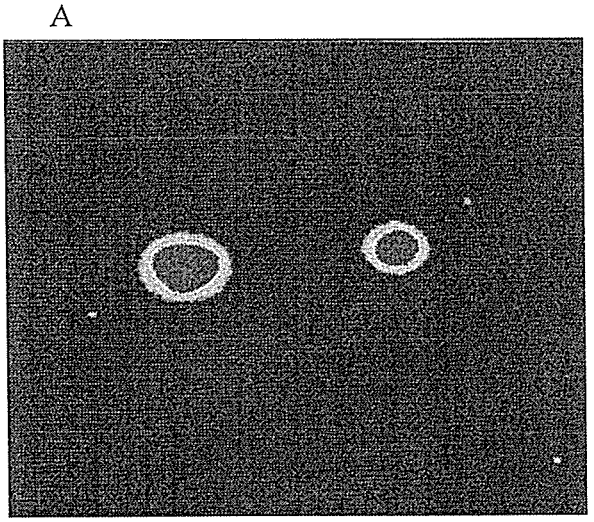
+	

Position of slide in

Appendix 1: rewrite

Photographs of cells with diffuse DNA indicative of low molecular weight DNA. Comet slides were removed from lysis after one to 24 hours, rinsed to remove detergents, stained with SYBRGreen, and examined at 250X magnification. Control cells with high molecular weight DNA are in picture A. The remaining 3 pictures (B, C) show a progression of cells with low molecular weight DNA, depending on when the slides were removed from lysis (B at 1 hr, C at 24 hrs). The cells with low molecular weight DNA were from an experiment in which maintaining cells on low serum for 73 hours induced either apoptosis or necrosis, depending on the cell line. Under the experimental conditions used, it was not possible to distinguish between apoptosis and necrosis based on the appearance of the cells with diffuse DNA.

A



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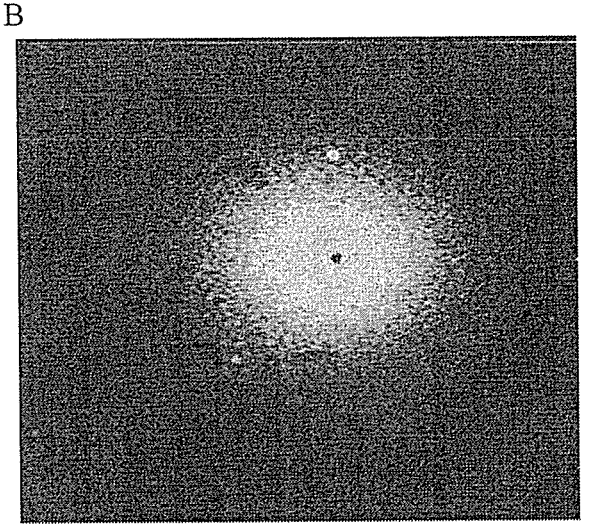
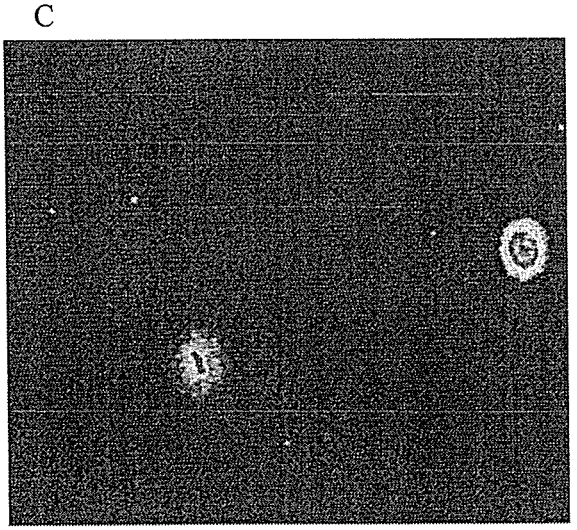


C



Draft Minutes

The 2nd International Validation Meeting on Comet Assay

Date: December 10-11, 2006
(December 10, 9:00 to 17:30, December 11, 9:00 to 15:00)
Venue: Tokyo University Komaba Campus,
Tokyo, Japan

Participants:
M. Hayashi (NIHS), T. Hartung (ECVAM), L. Schectman (FDA), R. Tice (NICEATM), Y. Uno (Mitsubishi Pharma Co.), H. Kojima (JaCVAM, NIHS), N. Asano (Nitto Denko Co.: MMS president), T. Ohmori (Kyoto Univ.), B. Burlinson (Huntingdon), P. Escobar (Invitrogen), M. Honma (NIHS), A. Kraynak (Merck), Y. Nakagawa (FDSC), M. Nakajima (Anpyo-Center), M. Ueda (Anpyo-Center), K. Yamakage (FDSC), Y. Ohno (NIHS), Y. Nakamura (LSG Co.: observer), N. Tanaka (FDSC: observer), Kui Lea Park (NITR, KFDA:obserber)

1 Welcome address

Dr. Ohno, who has grant for this validation study, welcomed everyone. Dr. Hayashi asked everyone to introduce himself or herself. After that, the following agenda items were discussed.

2 Data of the pre-validation study

Drs. Burlinson, Escobar, Kraynak, Yamakage and Nakajima presented each testing data of this pre-validation study with EMS. Participants felt that overall testing results were well validated. Dr. Escobar also reported results in a co-laboratory comparison study on the comet image analysis with SYBR Gold staining. There seemed no obvious difference among laboratories.

3. Protocol issues: discussion and conclusion

Protocol issues were discussed item by item on the version 10 protocol, and a revised version 11 protocol was prepared (see a MS word file **"protocol V11"**). Points to discussion were as follows: 1) need to add EDTA into low-melting

agarose in order to keep lower background (issued by FDSC): additional examination will be conducted in Invitrogen and HLS, 2) lost gel problem in unwinding and electrophoresis process under room temperature (issued by HLS), 3) should studies be conduct under GLP or just in GLP compliant laboratories?, 4) need to detect cross-linker type mutagens (issued by Dr. Tice), 5) the number of animals per group: need to analyze with power calculation after validation, 6) an average DNA migration in a negative control group: 1-15%? of % DNA in tail, 7) dehydration problem (issued by HLS), 8) recognition of hedgehog: >90% of % DNA in tail?, 9) need to conduct histopathology in validation studies, 10) statistics.

4. Time schedule proposal

Dr. Hayashi confirmed the time schedule in near future as follows: 1) each leading laboratory will send a EXCEL data sheet and an individual study protocol of this pre-validation study to Dr. Kojima ASAP, 2) Dr. Omori will analyze the data by the end of January, and then Dr. Hayashi will share the results with all participants (by the middle of February?), 3) Dr. Uno will send a protocol check sheet of this pre-validation study to leading laboratories, all testing laboratories will fill in each item column and then send it to Dr. Kojima by the end of this year, 4) Drs. Hayashi and Uno will revise a standard draft-final protocol for the further validation studies by the end of this year.

VMT discussed the future validation study plan in a VMT meeting this morning but could not decide yet. Points should be discussed for the future plan are as follows: 1) test compound selection considering chemical classes, 2) the total number of test compounds to reach a conclusion, 3) recruitment and selection of testing facilities, including technical transfer to new entries, 4) design of validation studies, 5) animal reduction, especially for positive/negative controls. Dr. Hartung commented that a lean design is preferable for validation studies, 20 test compounds, for example, are too small to calculate the sensitivity and specificity of a comet assay, and one resource to evaluate the performance without many new data may be to mix up the existing data. VMT will continue to discuss about above points thorough e-mail/tele-conference, and then inform all participants of a draft plan and ask them the feasibility.

5. *In vitro* comet assay

Dr. Honma presented a proposal of an *in vitro* comet assay protocol. A kick-off meeting (and a workshop) on the validation will (may?) be held on May 2007 together with a cell-transformation assay meeting.

Abbreviation

Anpyo-center	Biosafety Research Center, Foods, Drugs and Pesticides
ECVAM	European Center for the validation of alternative methods
FDA	Food and Drug Administration
FDSC	Food and Drug Safety Center
Huntingdon	Huntingdon Life Sciences
Invitrogen	BioReliance, invitrogen bioservices
JaCVAM	Japanese Center for the validation of alternative methods
MMS	Mammalian Mutagenicity Study
Merck	Merck Research Laboratories
NICEATM	Interagency Center for the Evaluation of Alternative Toxicological Methods
NIHS	National Institute of Health Sciences
OECD	Organisation for Economic Co-operation and Development
VMT	Validation Management Team

In vivo Alkaline Comet Assay International Validation Plan in 2007 - 2009 or 2010 * (Draft)

* Details are shown in a next slide

- Pre-validation & standby phase (2Q/2007 - 2Q/2008)
  - 5 lead-labs will examine 4 compounds
  - Recruit and select new participant laboratories for a definitive study (Additional 10 or more entries will be sufficient to progress further studies)

- Definitive validation phase (3Q/2008 - 3Q/2009 or 4Q/2010)
  - Step 1 (3Q/2008 - 3Q/2009) : 15≤ labs will examine total 20 compounds
    - ✓ Each compound will be examined in 1-lead lab and other 2≤ labs
    - ✓ If insufficient to conclude validity, Step 2 will be done
  - Step 2 (4Q/2009 - 4Q/2010) : 15≤ labs will examine further 20 compounds

- Review and mix up existing data to evaluate assay performance (2Q/2007 - 2Q/2008)

Schedule

(□: pre-validation & standby phase, ▨: definitive validation phase)

	2007				2008			
	1Q	2Q	3Q	4Q	1Q	2Q	3Q	4Q
Lead Lab 1	Fix protocol & schedule, & select compounds	Compound 1	Compound 2	Compound 3	Compounds 4	Data analysis & discussion (modification of future plans if necessary)	Compound 5	Compound 10
Lead Lab 2		Compound 1	Compound 2	Compound 3	Compounds 4		Compound 6	Compound 11
Lead Lab 3		Compound 1	Compound 2	Compound 3	Compounds 4		Compound 7	Compound 12
Lead Lab 4		Compound 1	Compound 2	Compound 3	Compounds 4		Compound 8	Compound 13
Lead Lab 5		Compound 1	Compound 2	Compound 3	Compounds 4		Compound 9	Compound 14
Lab 6	[redacted]	Announce a definitive validation study plan, recruit participant candidates & select participants for definitive a validation study					Compound 5	Compound 14
Lab 7							Compound 6	Compound 10
Lab 8							Compound 7	Compound 11
Lab 9							Compound 8	Compound 12
Lab 10							Compound 9	Compound 13
Lab 11							Compound 5	Compound 13
Lab 12							Compound 6	Compound 14
Lab 13							Compound 7	Compound 10
Lab 14							Compound 8	Compound 11
Lab 15							Compound 9	Compound 12

	2009				2010			
	1Q	2Q	3Q	4Q	1Q	2Q	3Q	4Q
Lead Lab 1	Compound 15	Compound 20	Data analysis, discussion & article submission if possible	25	30	35	40	Data analysis, discussion & article submission
Lead Lab 2	Compound 16	Compound 21		26	31	36	41	
Lead Lab 3	Compound 17	Compound 22		27	32	37	42	
Lead Lab 4	Compound 18	Compound 23		28	33	38	43	
Lead Lab 5	Compound 19	Compound 24		29	34	39	44	
Lab 6	Compound 17	Compound 20		25	30	35	40	
Lab 7	Compound 18	Compound 21		26	31	36	41	
Lab 8	Compound 19	Compound 22		27	32	37	42	
Lab 9	Compound 15	Compound 23		28	33	38	43	
Lab 10	Compound 16	Compound 24		29	34	39	44	
Lab 11	Compound 17	Compound 20		25	30	35	40	
Lab 12	Compound 18	Compound 21		26	31	36	41	
Lab 13	Compound 19	Compound 22		27	32	37	42	
Lab 14	Compound 15	Compound 23		28	33	38	43	
Lab 15	Compound 16	Compound 24		29	34	39	44	