Technical issues encounterd by comet users

3/9/2015 - 11:00-13:00

Assays performed regularly	%
alkaline comet assay	73
neutral comet assay	6
comet assay for assessing occupational exposure and human biomonitoring	23
comet assay for in vitro genotoxicity testing	35
comet assay for in vitro genotoxicity testing of nanoparticles	19
comet assay for clinical applications	15
in vivo comet assay for genotoxicity testing	27
in vivo comet assay for genotoxicity testing following the OECD guideline	13
comet assay to test the effect of nutrients	16
comet-based in vitro DNA repair assay	18
comet-based cellular DNA repair assay (following damage removal over time)	16
comet assay for genotoxicity testing in plants	12
comet assay applied to ecotoxicology	10
FISH comet assay	4
high throughput gel systems (12 - 96 gel systems)	18
high throughput scoring systems	11
semi-throughput scoring system (e.g. from Perceptive Instruments)	24
freely available scoring system	11

Cell preparation, gels, cell types

	%
gels coming off microscope slides	16
highly damaged cells (hedgehog or ghost cells) appearing in gels unexplained	16
see images resembling halos, rather than comets	10
comet tails going in different directions	7
detect big tails (representing non-specific enzyme/cleavage activity) when incubation cell/tissue extracts with non-exposed substrates	7
edge effect in minigels (in 12 or 96 gel system)	5
different DNA damage depending on the position of the gels (in 12 or 96 gel system)	3
Can you detect mitochondrial DNA damage with the comet assay?	30
How important is lysis time?	26
Special features of doing comet assay with sperm cells - storage, keeping background damage low, image quality (avoiding impurities) etc.	
In the case of sperm cells were more agressiv lysis is used wash slides before transferring from lysis solution to electrophoresis solution to avoid foaming of electrophoresis solution	
Special interest in eye tissues	

Electrophoresis

	%
Do you need to use the alkaline conditions to see single strand breaks?	12
Can the neutral comet assay be used to detect double strand breaks?	7
How important is lysis time?	26
How important is pH?	19
How important is voltage?	24
How important is current?	22
Does it matter if there are non-viable cells among those used in the comet assay?	18
Can you detect apoptotic cells with the comet assay?	26
Can you detect mitochondrial DNA damage with the comet assay?	30
How do I measure other kinds of damage in addition to strand breaks?	31
What is the best way to present my data; which image analysis parameter to use?	31
Which scoring systems are available: advantages and disadvantages?	37
Automated electrophoresis platform	
How do you keep the voltage/current constant during electrophoresis.How do you keep the electrophoresis tank under 10°C? Is this really important?	

Staining, scoring of slides

What is the best way to present my data; which image analysis parameter to use?	31
Which scoring systems are available: advantages and disadvantages?	37
Manuel scoring and semi-automated scoring results are not similar.	
Quick scoring, FPG on mini-gels	
High degree of automation is indeed needed	
In the context of a semi automated platform including gel preparation and accurate fast scoring, it is important to extend the "good" agreement between visual and automated scoring across systems.	
RELIABLE automatic scoring	
Quick scoring	
Quick scoring solutions to decrease the time for comet assay (electrophoresis, lysis, etc.)	
Quick scoring and transfer of scored data to Excel.	
Different outcomes in visual scoring when different individuals are scoring?	
Scoring of % DNA in tail or tail moment?	

Interpretation, statistics, calibration

What is the best way to present my data; which image analysis parameter to use?	31%
Detection of faint tails	
Uneven distribution of cells with tails (edge) and without tail (mid) on the slides	
Variability results between experiments	
Different results in whole blood/ PBMC from the same subjects	
Calculating mean or median of total comets scored for same sample (of2-3 replicates)	
How do I measure strand breaks in 96 well plate system	
Is it important to include a positive control with a known number of damages?	
Statistical evaluation of the results incomet assay performed in human lymphocytes.	
Any inter-lab differences in assay conduct and impact would be very useful to cover	
Not getting enough damage by H2O2, sometimes: and sometimes reproducibility problems with cell suspensions	
How to deal with high standard deviation in comet assay?	

Kind of damage

	%
Does it matter if there are non-viable cells among those used in the comet assay?	18
Can you detect apoptotic cells with the comet assay?	26
How do I measure other kinds of damage in addition to strand breaks?	31
Detect “negative” DNA repair activity	5
BER repair assay: how important is protein measurement, how to determine?	

Other issues

How to save money by replacing expensive chemicals with cheap ones	
How do you justify the results of nanoparticles for comet assay? In ecotoxicological studies should one use coated or un-coated NP?	
Application of comet assay with particulate matter extracts	
I would like to learn FISH comet assay, high throughput scoring systems and how do I measure strand breaks in 96 well plate system	