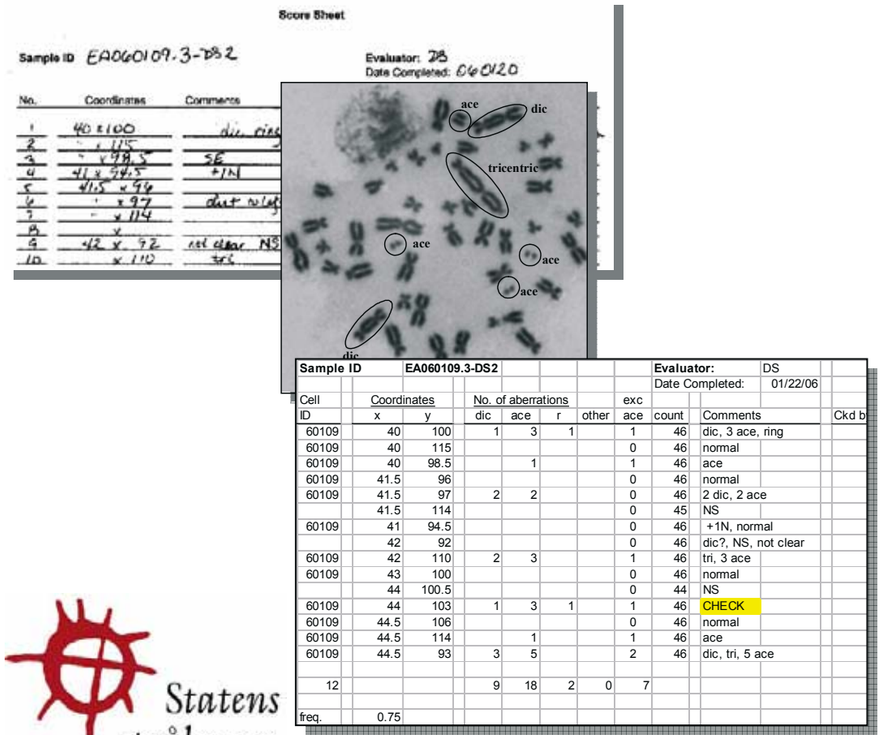
Efficient Training Technique for New Biodosimetry Evaluators

DANIELA STRICKLIN, EVA ARVIDSSON, ALICJA JAWORSKA



Score Sheet

Sample ID EA060109.3-DS2

Evaluator: DS
Date Completed: 060120

Sample ID	EA060109.3-DS2							Evaluator:	DS	
								Date Completed:	01/22/06	
Cell	Coordinates		No. of aberrations				exc			
ID	x	y	dic	ace	r	other	ace	count	Comments	Ckd b
60109	40	100	1	3	1		1	46	dic, 3 ace, ring	
60109	40	115					0	46	normal	
60109	40	98.5		1			1	46	ace	
60109	41.5	96					0	46	normal	
60109	41.5	97	2	2			0	46	2 dic, 2 ace	
	41.5	114					0	45	NS	
60109	41	94.5					0	46	+1N, normal	
	42	92					0	46	dic?, NS, not clear	
60109	42	110	2	3			1	46	tri, 3 ace	
60109	43	100					0	46	normal	
	44	100.5					0	44	NS	
60109	44	103	1	3	1		1	46	CHECK	
60109	44.5	106					0	46	normal	
60109	44.5	114		1			1	46	ace	
60109	44.5	93	3	5			2	46	dic, tri, 5 ace	
12			9	18	2	0	7			
freq.	0.75									

Statens strålevern
Norwegian Radiation Protection Authority

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Efficient Training Technique for New Biodosimetry Evaluators

Abstract
The dicentric assay, the current gold standard in biological dosimetry, requires a degree of technical competence. Expertise is usually developed by the evaluation of hundreds of metaphases, and is documented through establishment of a dose response curve, which is required by any laboratory performing biological dosimetry. Consistent evaluation of metaphases must be established for any new observer in a laboratory that should contribute to analyses. Discrepancies in scoring can seriously jeopardize the reliability of any assessment.

FOI together with the NRPA has conducted an inter-calibration exercise to establish comparable scoring criteria of aberrations. The exercise revealed specific aberrations that were difficult to identify and were consistent sources of uncertainty. This exercise further illustrated the need for a method report detailing the FOI lab's scoring criteria. The final outcome of this exercise was the development of a strategy for establishing technical competence in metaphase scoring in an efficient manner. The methods suggested here could be applied for the training of new or additional personnel. Comparable documentation of methods in other laboratories could facilitate more consistent scoring criteria among the biodosimetry community, a problem observed in previous international inter-comparisons. Better consistency among laboratories could provide an opportunity to reliably share the work load among different members of the biodosimetry community in the event of a mass casualty accident.

Keywords
biodosimetry, cytogenetics, dicentric assay, collaboration, chromosome damage

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Effektiv undervisningsteknik för ny biodosimetri utvärderingpersonal

Sammanfattning

Den dicentriska metoden, biodosimetrins gyllene standard, kräver en viss teknisk förmåga. Kunnandet utvecklas vanligtvis genom att hundratals metafaser utvärderas och dokumenteras genom att en laboratoriespecifik dosresponskurva etableras. Detta krävs för varje laboratorium som utför biologisk dosimetri. Det är viktigt att metafaser utvärderas konsekvent och helst utan variation av varje ny bedömare i ett laboratorium. Avvikelser i enskilda bedömningar kan allvarligt påverka pålitligheten i ett laboratoriums bedömning.

FOI har tillsammans med Strålevernet i Norge genomfört en kalibreringsövning för att etablera en jämförbar bedömningsgrund för aberrationer. Under övningen avslöjades några specifika aberrationer som var svåra att identifiera och som därmed oftare än andra ledde till osäkra resultat. Övningen illustrerade vidare behovet av att skriftligt dokumentera detaljer om FOIs bedömningskriterier. Övningen ledde till utvecklingen av en strategi för att på ett effektivt sätt etablera teknisk kompetens för att bedöma metafaser. De metoder som föreslås här kan appliceras för att på ett effektivt sätt träna upp ny personal. Liknande dokumentation av metoder i andra laboratorier kan möjliggöra en mer konsekvent bedömningsgrund mellan olika biodosimetrilaboratorier och därmed åtgärda ett problem som märkts i tidigare internationella jämförelser. Bättre överensstämmelse mellan laboratoriernas bedömningsgrunder kan öppna för möjligheten att enklare fördela prover mellan olika laboratorier i händelse av en större strålningsolycka.

Nyckelord

biodosimetri, cytogenetik, dicentriska metoden, samarbete, kromosom skada

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Glossary of Terms

The following glossary supports the terminology used in this document. Some of the definitions have been obtained or adapted from terms described in the ISO 2004 guidelines or the IAEA 1986 and 2001 guidelines. Other terms are described according to use in a practical manner at FOI and are further noted as lay terms.

acentric, ace
terminal or interstitial chromosome fragment

acentric ring
aberrant circular chromosome lacking a centromere that has resulted from the joining of two breaks on separate arms of the same chromosome

background frequency
spontaneous frequency of chromosome aberrations recorded in control samples or individuals

centric ring
aberrant circular chromosome containing a centromere that has resulted from the joining of two breaks on separate arms of the same chromosome

centromere
specialized constricted region of a chromosome that appears during mitosis joining together the chromatid pair

chromosome
structure that carries genetic information, 46 chromosomes are contained in normal human cells, they condense during nuclear division to form characteristic shapes

chromatid
either of the two strands of a duplicated chromosome that are joined by a centromere and separate during cell division to become individual chromosomes

cross-over
lay; term used in scoring at the FOI lab to refer to a chromosome with one arm of the chromosome overlaying the other arm, which is easy to mistake as a dicentric

dicentric, dic
aberrant chromosome bearing two centromeres derived from the joining of parts from two broken chromosomes

excess acentric
an acentric formed independently of a dicentric, tricentric, or centric ring chromosome aberration

inter-calibration
an exercise to facilitate evaluators learning to implement the same standards and judgments in scoring chromosomal aberrations

inter-comparison
a quality assurance exercise to ensure the ability of different laboratories to accurately evaluate samples

metaphase
stage of mitosis when the nuclear membrane is dissolved, the chromosomes are condensed to their minimum lengths and are aligned; for humans a complete metaphase contains 46 chromosomes and are otherwise considered "incomplete"

non-scoreable, NS
lay; metaphases that may not be included for analysis, either due to fewer than 46 chromosomes present, lack of accompanying acentrics to dicentric, tricentrics, or centric rings, or quality is too poor to reliably evaluate

quality assurance
planned and systematic actions necessary to provide adequate confidence that a process, measurement, or service will satisfy given requirements for quality in, for example, those specified in a license

scoreable
lay; complete metaphases that may be reliably included in an analysis

scoresheet
lay; the paper which is used during microscope evaluation and image capture for recording coordinates and notes

scoring
evaluation of metaphase chromosomes

service laboratory
laboratory performing biological dosimetry measurements

"snake eyes", se
lay; acentric fragments forming from interstitial deletions, technically referred to as *minutes*, but listed in our scoresheets as "se"

spreadsheet
lay; Excel tables where data from scoring is recorded and calculated

tricentric, tri
aberrant chromosome bearing three centromeres derived from the joining of parts from three broken chromosomes, for calculations tricentrics are counted as two dicentrics and must have two accompanying acentric fragments

triradial
three armed chromosome formed from interaction between a chromosome containing an isochromatid deletion with another chromosome having a chromatid deletion

Introduction

Background

Biodosimetry is the biological assessment of radiation dose in individuals and may be used to assess radiation exposure after an accident or in cases where an over-exposure is suspected and physical dosimetry is absent or uncertain. A reliable estimate of dose may be critical for making medical decisions, assessing the long-term health consequences, and for reassuring persons with non-significant exposures. Access to biodosimetry capabilities is important today as radiological sources are common in the medical setting, for diagnostics and treatment, as well as in the industrial setting, such as for application in sterilization.

Since radiological accidents are not extremely common, not all nations feel that it is economically justified to maintain biodosimetry competence. However, dependable access to biodosimetry capabilities is absolutely critical in the event of an accident. Moreover, in the event of a mass casualty event, the capacity of a single laboratory could be easily overwhelmed. Therefore, the sharing of competence and capabilities within the biodosimetry community is vital, so that any needs that do arise may be met to some degree.

The Norwegian Radiation Protection Authority (NRPA) which presently does not have a biodosimetry laboratory has been working together with the biodosimetry lab at the Swedish Defense Research Agency (FOI) to have access to such capabilities. Furthermore, since the standardized method for biodosimetry is time-consuming and can be technically demanding, an effort is being made to establish comparable evaluation competence at the NRPA. In an accident situation involving several individuals, this competence would serve to expand the capacity currently available at FOI and speed the overall assessment.

Dicentric Assay

While a great many markers and methods exist for biological radiation dose assessment, the evaluation of dicentric chromosomes which dates back to the early 1960's has been the most widely applied assay and is the most fully validated method available. Application of the dicentric assay over several decades and in nearly every radiation exposure accident has enabled assay optimization and has documented the merit and the limitations of the method. Today, the conventional dicentric assay has come under ISO standardization and has been incorporated into many radiological protection programs (IAEA 1986, 2001, ISO 2004).

In general, the dicentric assay is conducted by culturing blood lymphocytes, from an individual with a suspected exposure, for 48 hours to obtain metaphase chromosomes. The chromosomes of many metaphases, 500 - 1000 cells, are evaluated under a microscope for specific damage in the form of dicentric chromosomes, which are the result of chromosomal breakage from radiation interactions and subsequent abnormal rejoining. Dicentric chromosomes are the markers of choice for evaluation because they are easily identified, are quite specific to radiation, have low background frequency, and show a reproducible dose response relationship (Bauchinger 1984, Amundson 2001).

Technical Requirements

Biodosimetry laboratories that intend to perform the dicentric assay as a service for actual human evaluations must establish their lab as described in ISO regulations (ISO 2004) in order for their results to be considered legally defensible. This includes a large number of technical requirements, many of which are simply incorporating good laboratory practices

(GLP) and documenting protocols. Among the criteria for the establishment of a biodosimetry lab is the development of a dose response curve and documentation of all methods used for creating the curve, which the FOI lab has completed in the previous year (Stricklin 2005).

Other important criteria addressed in the ISO guidelines are the competent evaluation of metaphases, or *scoring*. Coding of slides, establishment of appropriate scoring techniques, and demonstration of scoring expertise are required. Only trained and experienced evaluators may contribute to a biodosimetry evaluation. The documentation of scoring expertise must be demonstrated in intra- and inter-laboratory comparisons, using established scoring criteria, and with such evaluations in agreement within 20% of the reference value. These measures are quite crucial for reproducible and accurate assessments due to the fact that manual evaluation is rather subjective in nature.

Objectives

FOI has previously addressed many of the criteria described in the ISO guidelines for performing biological dosimetry. While specific criteria for scoring chromosomal aberrations exist in the laboratory, such criteria have not previously been described in detail. Therefore, specific and comprehensive guidance on the scoring criteria used in our laboratory will be described here.

Guidance on development of scoring criteria and for training competence, i.e. training a new evaluator or observer, has not been addressed in either the IAEA or ISO guidelines more than the requisition of scoring criteria and inter-comparisons. In our experience, the establishment of technical expertise in scoring can take a long time, up to six months, and requires numerous evaluations. Furthermore, the evaluation of different quality metaphases can greatly impact the results of an evaluation even by highly qualified observers. To address both of these points, another aim in this work is to establish more efficient and precise methods for training expertise in scoring. These methods have been developed in part by an inter-calibration exercise conducted together with the NRPA.

Methods

Preparation of Samples

Standard procedures for the dicentric assay were used for the acquisition of the samples utilized in this exercise, and are described in detail previously (Stricklin 2005). In short, *in vitro* radiation experiments of human blood lymphocytes were conducted for the preparation of a dose response curve for gamma radiation. Lymphocytes were cultured for 48 hours, followed by arrest, to obtain metaphase spreads for evaluation of chromosomal damage. Samples were dropped onto slides and stained with fresh Giemsa. In contrast to previous work, mounting media and cover slips are placed on the slides that shall be evaluated so that they may be archived and reviewed at later dates. Mounting further preserves the slides during evaluation, preventing scratches, smearing of stain, and accumulation of dirt.

Evaluation of Metaphases

Coding

The method for coding slides has varied during the development of the dose response curve. An initial coding scheme indicating precise parameters concerning the sample were used.

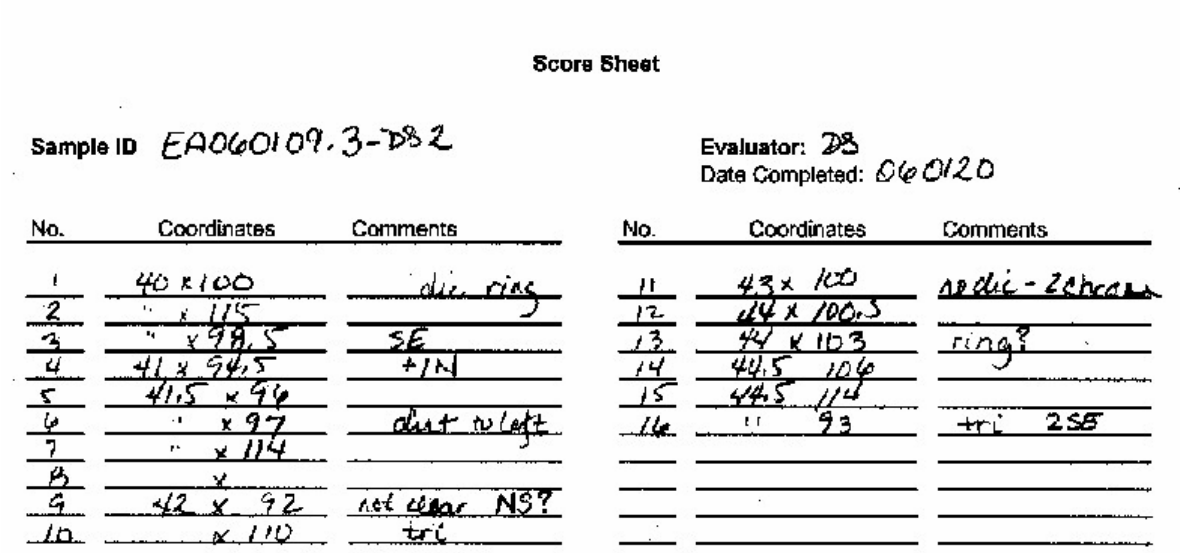
After methods were well established, a coding scheme that is quite anonymous, and can be used for blinded work has now been adapted. A sample and slide is simply coded by the date, sample number, and initials of the person who conducted the assay, with all specifics for the sample being recorded in the lab notebook. An example *sample number* would be **EA060109.3**. This indicates the 3rd sample prepared by Eva Arvidsson with the culture beginning on the 9th of January, 2006. A *slide* may be further coded with another set of initials and number to indicate the person who should evaluate the slide and which replicate slide. **EA060109.3-DS2** indicates the second replicate slide of the sample EA060109.3 to be evaluated by Daniela Stricklin. Finally, an additional code, either an **a** or **b**, is placed behind the sample number if another culture is conducted in parallel, for example premature chromosome condensation, PCC. An example is **EA060109.3a** and **EA060109.3b** would refer to dicentric assay and PCC culture samples, respectively.

Metaphase Acquisition

Slides are placed under the microscope with the frosted edge or label to the right. A note must be placed on the scoresheet if the slide label is placed to the left. Slides are scanned under the microscope typically at 40x to find metaphases for optimal acquisition of metaphases. If the slide is less dense or denser, 20x or 100x may be used for acquisition of metaphases, respectively, given that it is noted on the scoresheet. The slide is scanned systematically up and down rows as observed on the microscope ruler, moving only 0.5 mm between rows. Under 40x using the Leica DMR which has been utilized for all of the work at FOI to date, a slide may be scanned down row 34 for example and moved to row 34.5 and scanned up without missing metaphases and without duplication of metaphases, if one is careful. Many metaphases are found a bit to the side and must be centered for evaluation under higher magnification. Therefore, after a metaphase is acquired, the slide must be readjusted to the row position before one can continue scanning.

As good quality metaphases are identified, oil is applied to the slide if needed, the metaphase centered, and viewed at 100x. Images of the metaphases are captured at 100x using a Leica DMR microscope equipped with a Leica DC 200 camera and software which is imported into Photoshop 6.0. Images are saved using the metaphase coordinates (ex. 345x1015, note that decimals are not included in the file name), and notes are recorded on a scoresheet for any unusual appearances. If dicentrics (**dic**) or any other aberrations are observed, these are noted. Small acentric (**ace**) fragments, which are technically minutes, have been noted as **se**, "snake eyes". These are sometimes mistaken for dirt when analyzing the images, and notation can be helpful to prevent having to return to the microscope often to double check. Likewise, when dirt is present, this is noted so as not to be mistaken for a fragment in the picture. Any chromosomes falling outside the view of the image that shall be acquired are noted as +xN, for example **+1N** is noted for one normal chromosome outside the view of the camera. This minimizes the number of times an extra picture must be acquired. However, if many chromosomes or any aberrations fall outside the view of the camera, an additional image should be acquired. Occasionally, one or more chromosomes can be observed underneath a nearby cell. In such cases, a note should be taken and these metaphases may be included if the overlapping cell is transparent enough that an evaluation of the chromosome can be made. Further comments can be recorded for each observer's preference for clarification to minimize returning to the microscope.

A sample scoresheet is provided in Appendix I. However, a short example is shown in Figure 1 to demonstrate the possible notations for comments.



Score Sheet

Sample ID EA060109.3-DS2

Evaluator: DS
Date Completed: 060120

No.	Coordinates	Comments
1	40 x 100	dic. ring
2	" x 115	
3	" x 99.5	SE
4	41 x 94.5	+1N
5	41.5 x 96	
6	" x 97	dust to left
7	" x 114	
8	x	
9	42 x 92	not clear NS?
10	x 110	tri
11	43 x 100	2 dic - 2 chrom
12	44 x 100.5	
13	44 x 103	ring?
14	44.5 106	
15	44.5 114	
16	" 93	tri 2SE

Figure 1. Example data recorded in a scoresheet.

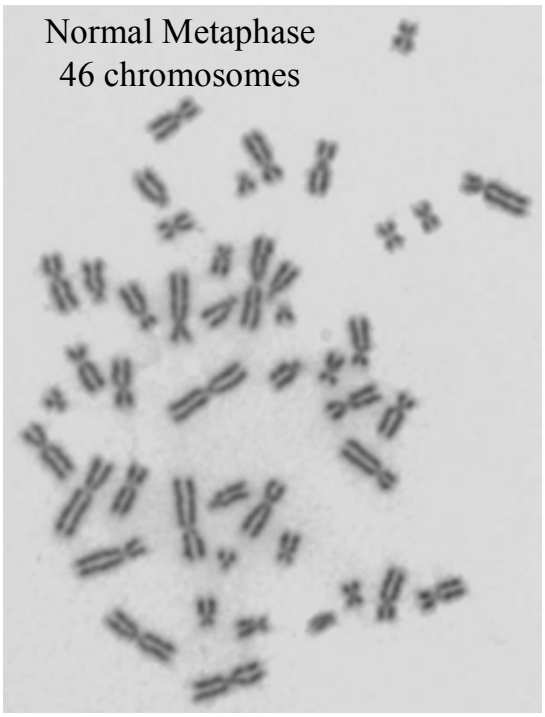
Scoring

Standards for metaphase evaluation were previously developed over time, approximately 6 months, via concurrent evaluations, comparisons, and discussions between scorers. The following paragraphs detail our methods for scoring.

The images are usually opened with the Paint software program which allows one to mark on the image easily and quickly. Marking the centromeres is an effective way of quantifying chromosomes in a metaphase, and an illustration is shown in Figure 7 below. Metaphase spreads are analyzed by counting 46 centromeres or chromosomes and taking note of dicentrics, accompanying acentrics, excess acentrics, centric rings, and any other aberrations. The number of centromeres should always be 46; however, they may sometimes be difficult to distinguish as the morphology or appearance of the chromosomes can vary greatly between metaphase spreads. Some chromosomes can appear more condensed and short or elongated and thin, causing arms not to be separated and centromeres more difficult to distinguish. Chromosome arms occasionally appear completely separated also making centromeres difficult or even impossible to identify. A variety of metaphases illustrating different chromosome morphologies are provided in Appendix II. The variation in shape can arise from either the length of time at which a cell has been in metaphase when it is arrested or from subtle variations in culture conditions. At any rate, the morphology and stain intensity can be useful in evaluating metaphases as well, for example in identifying the difference between an extra centromere (dicentric) and a situation where the long arms of a chromosome cross-over. This type of chromosome is discussed in more detail below and is illustrated in Figure 9.

Counting the pieces in a metaphase and determining what is clear often helps to determine whether an unclear piece is a fragment or chromosome. Dicentrics and centric rings should each have one accompanying fragment. Tricentrics are counted as two dicentrics and should likewise have two accompanying acentric fragments. Tricentrics are counted under the dicentric column but should be noted under the "comments" column. Triradials and one-armed fragments are considered chromatid damage and are not quantified but should also be noted in the comments. Other information should be included in the comments section of the spreadsheet. For example, any chromosomes counted outside the view of the image as noted on the scoresheet should likewise be noted in the comments on the spreadsheet. Acentric

rings are also recorded under comments. A few example metaphases and aberrations are illustrated in Figures 2-5 below.



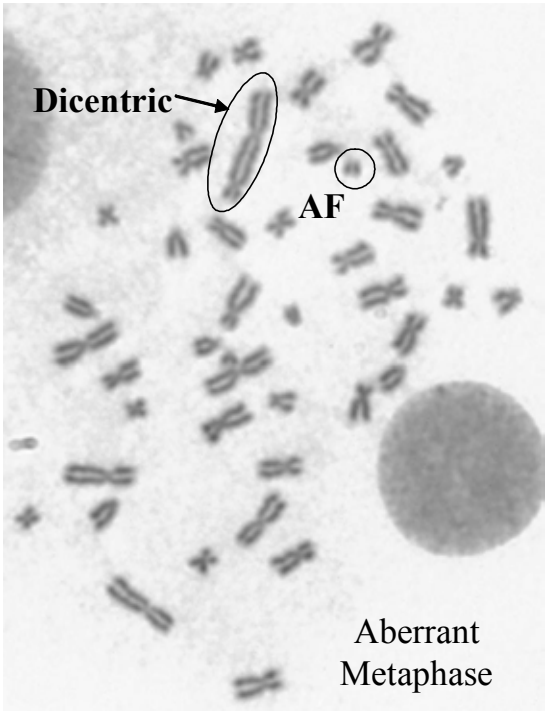
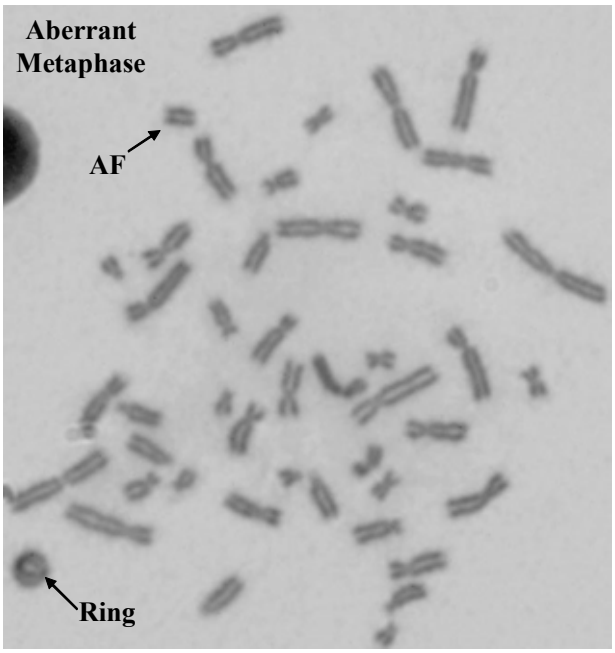


Figure 2 and 3. The picture above to the left represents a normal metaphase containing 46 normal chromosomes. The picture to the right shows an abnormal metaphase containing radiation damage illustrating a dicentric chromosome and its accompanying acentric fragment (AF). These pictures are taken from FOI's *in vitro* dose response experiments, control and 4 Gy irradiated samples, respectively.



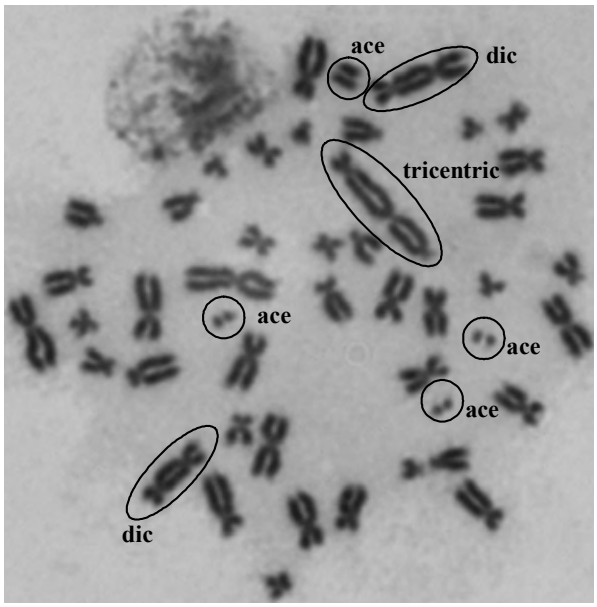


Figure 4 and 5. The picture above to the left represents an abnormal metaphase illustrating a ring chromosome and its accompanying acentric fragment (AF). The picture above to the right represents an abnormal metaphase illustrating a tricentric chromosome. Two dicentrics and a total of four acentrics are also shown in this picture. The pictures are taken from FOI's *in vitro* dose response experiments, 4 and 5 Gy irradiated samples, respectively.

These data are recorded on spreadsheets in Excel along with the coordinates, sample and slide ID. Cells that are not distinct, not containing 46 chromosomes, or containing dicentrics, tricentrics, or centric rings without accompanying acentrics are considered *non-scoreable* (**NS**). Cells that are not distinct include those that are too dense and have too many, i.e. more than two sets, of overlapping chromosomes. Non-scoreable cells are listed in the spreadsheet with comments and chromosome number but no frequencies are recorded and they are not included in the total number of cells evaluated. In our experience, the number of non-scoreable metaphases varies greatly between preparations and range from 2-20%. However, the number of cells containing dicentrics without acentrics, which can be an indication of second division metaphases, should not be more than 1% per slide (~1 to 2 cells per 500 cells).

Bias can occur if cells are repeatedly marked non-scoreable. Therefore, it is important to make a concerted effort to evaluate all metaphases. Uncertain metaphases should be reviewed under the microscope. However, if metaphases are still unclear and can not be evaluated confidently, one should mark them as non-scoreable. If too many non-scoreable cells are present, then one must be aware of the added uncertainty present in the evaluation.

Excess acentrics are calculated after the evaluation in an additional column that subtracts the number of dicentrics and centric rings which also should have accompanying fragments from the acentrics counted in the cell. These numbers should be 0 or positive. Any cells with negative numbers must be marked non-scoreable and removed from the calculation. In practice, the first column has a numeric marker that is removed from the row of non-scoreable cells. In the end of the analysis, the column is counted to determine the total of scoreable cells. The columns for each aberration are added together to obtain a total for each. The frequency of dicentrics with accompanying fragments is determined by division of the total number of cells by the number of dicentrics observed. Other aberration frequencies have greater uncertainty associated with them due to difficulty in their identification, and therefore have not been included in the curve to date. These data are, however, important for record keeping and may be used for further analysis at a later date.

A sample spreadsheet is included in Appendix I, and an example of the data recorded in a spreadsheet is illustrated in Figure 6.

Note: All methods described in this report are based on manual methods and equipment used at FOI. Other methods of evaluation exist as well as other equipment which greatly facilitates evaluation techniques. For example, automated instrumentation and software, such as a metaphase finder, is able scan an entire slide, automatically aquiring metaphases. This type of system also may incorporate image recognition software which sorts pairs of chromosomes automatically, identifying any chromosomes or pieces that do not have matches. These systems also have pros and cons but generally yield more reproducible results with minimal subjective uncertainty.

Sample ID		EA060109.3-DS2						Evaluator:	DS	
								Date Completed:	01/22/06	
Cell	Coordinates		No. of aberrations				exc			
ID	x	y	dic	ace	r	other	ace	count	Comments	Ckd by
60109	40	100	1	3	1		1	46	dic, 3 ace, ring	
60109	40	115					0	46	normal	
60109	40	98.5		1			1	46	ace	
60109	41.5	96					0	46	normal	
60109	41.5	97	2	2			0	46	2 dic, 2 ace	
	41.5	114					0	45	NS	
60109	41	94.5					0	46	+1N, normal	
	42	92					0	46	dic?, NS, not clear	
60109	42	110	2	3			1	46	tri, 3 ace	
60109	43	100					0	46	normal	
	44	100.5					0	44	NS	
60109	44	103	1	3	1		1	46	CHECK	
60109	44.5	106					0	46	normal	
60109	44.5	114		1			1	46	ace	
60109	44.5	93	3	5			2	46	dic, tri, 5 ace	
12			9	18	2	0	7			
freq.	0.75									

Figure 6. Sample data recorded in a spreadsheet.

Inter-Calibration of Evaluation Techniques

In the event of a significant radiological event, the potential exists for the NRPA to participate in biodosimetry evaluations together with FOI. An exercise towards calibrating evaluation techniques between a potential evaluator at the NRPA and those at FOI has been conducted so that such cooperation could be conducted reliably.

For the exercise presented, sets of pictures of metaphases obtained from the dicentric assay cell cultures from two different dose points (4 and 5 Gy) were provided to the NRPA. These pcturese were previously evaluated for FOI's dose response curve. The pictures were accompanyied by the scoresheets containing comments from observations under the microscope at the time pictures were acquired. Actual evaluation data was not provided. The pictures were evaluated by the NRPA participant together with the comments from the scoresheets for all aberrations, including dicentrics, acentrics, rings, and tricentrics. Standard methods for inclusion of spreads were applied on all analyses, such as only spreads containing 46 chromosomes and those that could be reasonably distinguished, otherwise cells were marked as non-scoreable. The results were recorded on spreadsheets along with sample ID, coordinates for the metaphases, and the resulting frequency of dicentrics with accompanying acentrics.

The evaluations obtained by the NRPA were compared with those recorded from FOI's evaluations. Each incongruous assessment was reviewed by both parties together. A decision in the judgment of the existence or not of such aberrations was made for each case. A sample of the data evaluated during this exercise is provided in Appendix III.

Results and Discussion

Inter-Calibration

The result from this exercise is intended to yield evaluations by both NRPA and FOI observers that are more analogous in the future. The summary of the results for the exercise are listed in Table 1.

Table 1: Summary of Calibration Exercise

Sample	Dose	Total #	# Cells Evaluated		Frequency of aberrations	
	Gy	Cells	NRPA	FOI	NRPA	FOI
dsea050117	5	64	52	53	1.4	1.8
EA1002aDS2	4	68	52	52	0.92	1.25
EA1002aEA2	4	70	67	62	0.94	1.24

The number of cells evaluated by each party as compared to the total number of cells available indicates good agreement in the number of cells considered scoreable by each group. The frequency of aberrations obtained for each sample by each party is listed. Each of the cells that were evaluated differently were reviewed together to identify the differences in judgments. The exercise elucidated specific types of aberrations, such as dicentrics with a centromere occurring close to the end of the chromosome, illustrated in Figure 7, and those with centromeres very close together in the middle of the chromosome, illustrated in Figure 8, that were consistently overlooked by one or the other of the observers. Also, counting tricentrics as two dicentrics, another source of incongruence, was missed in the original transfer of scoring criteria but will be used in future evaluations. Future exercises should provide more consistent results and will be tested in a Nordic inter-comparison exercise in the future.

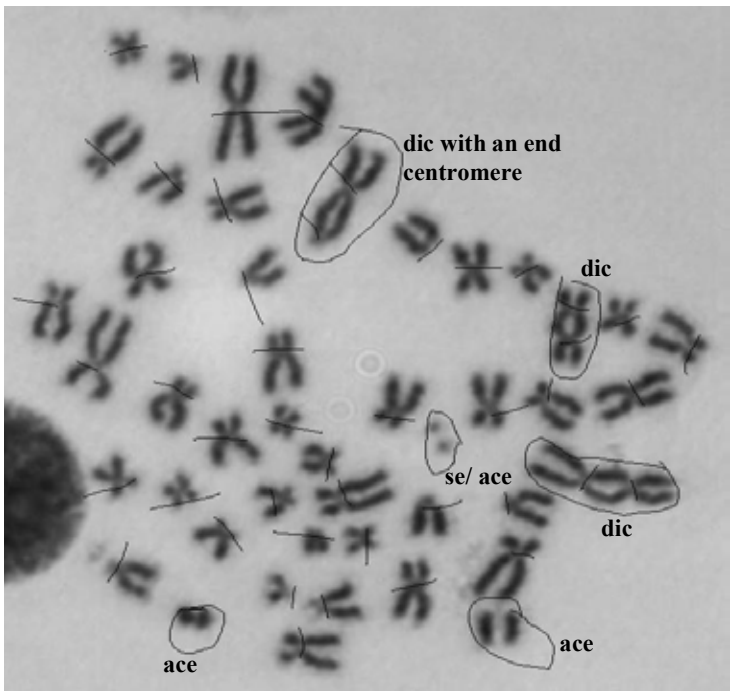


Figure 7. A metaphase with three dicentrics (dic) and acentric fragments (ace) is shown above. The dicentric towards the top of the picture illustrates a second centromere occurring at the end of the chromosome, which may be difficult to distinguish. The picture further illustrates our method of counting and marking centromeres and aberrations during scoring. This picture is taken from FOI's *in vitro* dose response experiments, 5 Gy irradiated sample.

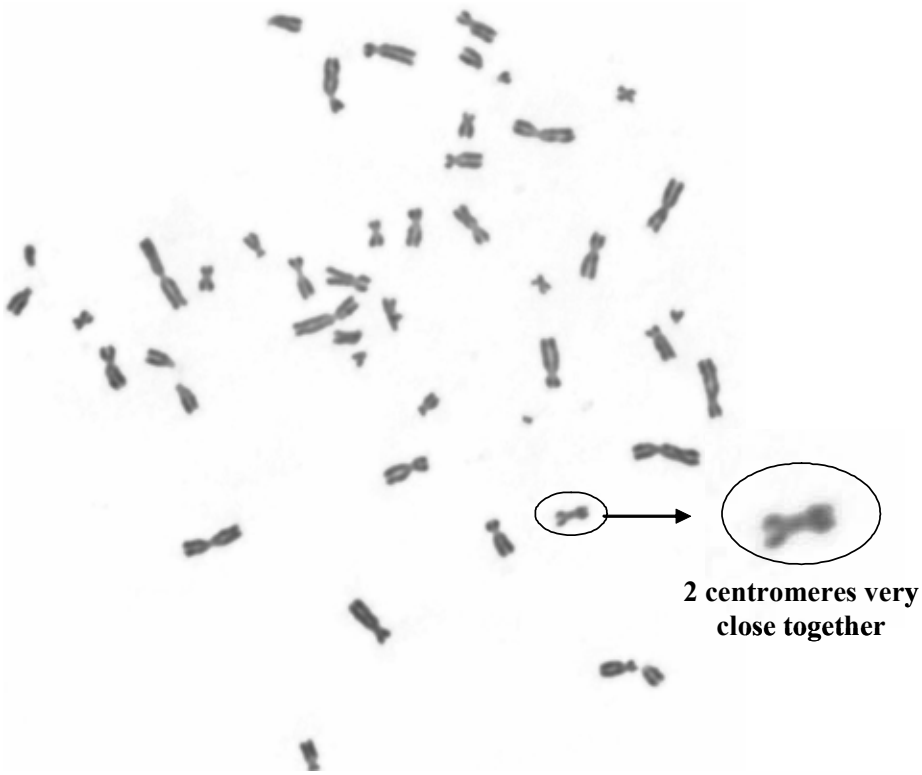


Figure 8. The picture illustrates a metaphase with a dicentric (dic) that is difficult to distinguish. The dicentric contains two centromeres that occur very close together in the middle of the chromosome, sometimes referred to as a knot, and may be difficult to distinguish. This picture is taken from FOI's *in vitro* dose response experiments, 4 Gy irradiated sample.

Finally, another common mistake in new observers that was conveyed prior to the exercise was that of mistaking a chromosome with one arm overlaying the other, or a "cross-over", as a dicentric. These are often difficult to judge and may be determined by other evidence in the cell, such as the presence or absence of a distinct acentric and all other chromosomes clearly defined and normal. Furthermore, a judgment can be made based on other evidence such as the length of the arms and the density and appearance of the questionable centromere. An illustration can be seen in Figure 9. Evidence that this chromosome does not contain two centromeres can be gained by observing the lengths of the pieces, i.e. the arms should be approximately the same length, however, since one arm stretches over the other, it appears shorter.

Figure 9. This picture illustrates a chromosome with its long arms crossing, which we refer to as a cross-over.

Methodology for Development of Technical Competence in Biodosimetry

The initial outcome of the inter-calibration was the realization that many details actually exist in our scoring criteria that are not easily conveyed verbally. Therefore, the need for a comprehensive description together with illustrations as provided above was identified. The exercise also illustrated the utility of having a well-established data set for training purposes. This enables very efficient practice for a new observer. The exercise further identified specific aberrations or phenomena that are common sources of error. With these specifics in mind, fewer errors will occur in scoring and are important to highlight during training. Otherwise, an observer may score hundreds of cells before encountering a specific phenomenon and at which time it may or may not be recognized and judged correctly. Therefore, with the guidance and methods described here, an observer could obtain technical

competence with focused training in a matter of weeks rather than by experience alone which can require months of work. The methodology for training technical competence in biodosimetry is outlined below.

1. Although it is not mandatory, we suggest that a trainee first review guidance for biodosimetry methods and establish understanding in the process and culture methods before beginning on actual evaluations.
    a. Example literature: ISO 2004, IAEA 1986, 2001, FOI Dicentric Assay report (Stricklin 2005) or analogous internal laboratory documentation.
2. The trainee should then read the guidance, i.e. documentation provided in this document, for evaluation of metaphase spreads for biodosimetry purposes.
3. The new evaluator can then begin on an inter-calibration exercise by being provided with a previously established data set; thoroughly reviewed and evaluated metaphases.
    a. The set should contain a significant number (~300) of a variety of highly exposed cells. For example, our data set contains samples from 4 and 5 Gy exposed cells, so that many different aberrations can be observed, requiring more intense focus on establishing judgment.
    b. The data set should also contain a variety of metaphase qualities to establish versatility in evaluations.
4. After evaluation of the data set is completed by the trainee, the incongruous evaluations as compared to what has been previously recorded should be reviewed at the same time by both the trainee and a well-established evaluator.
    a. Discussions and explanations during this process are critical for establishing congruent judgments in future evaluations.
5. The initial inter-calibration can be followed by another exercise conducted in a similar fashion, depending on the outcome of the first exercise.
    a. If the confidence level is high after the initial calibration exercise, an inter-comparison can now be conducted to verify the competence obtained.
        i. The dose level for this data should be moderate which tests both the identification of aberrations and that of normal, but perhaps unclear cells. Sometimes a bias towards an over-estimate of aberrations can occur after evaluation of highly exposed cells.
        ii. A significant number (~300) and variety of cells should again be used for the exercise.
    b. If the confidence level is low, another inter-calibration exercise is advised before proceeding.
6. When an inter-comparison demonstrates agreement between the new evaluator and the experienced evaluator within at most 20% (ISO 2004), but preferably within 10% agreement, the new evaluator may actively participate in evaluations.

Summary and Future

The NRPA and FOI have conducted a calibration exercise to make consistent evaluation techniques between the persons analyzing samples from the dicentric assay for biodosimetry assessments. This work is the first step towards making it possible to share samples for analyses between the two institutes. The next step in this work will be to prepare coded slides for evaluation at the NRPA and subsequent review of the pictures from those metaphases by persons at FOI. The equipment at the NRPA is different from that at FOI and could result in

significant differences in the images acquired. Therefore, it is important to evaluate whether the same judgments made from FOI's pictures can be made during the analysis of those pictures acquired at the NRPA. Then, a mounted slide containing only a few metaphases will be shared by both parties and analyzed both at the NRPA and at FOI for an inter-comparison exercise. It is important to note that the continuation of this work is necessary to maintain the competence gained during these exercises.

The hope in this work is to provide an efficient method for training the competence for metaphase evaluations for biodosimetry purposes. In the immediate future, the methods described in this report will be used to train additional expertise at FOI, as well as for technical expertise at the University of Defence in the Czech Republic as part of a technical arrangement. Any future training at FOI will also rely on the documentation provided here for establishing scoring competence.

Documentation of such methods as presented here from other laboratories and similar strategies for training could facilitate more consistent scoring criteria among the biodosimetry community, a problem observed in previous international inter-comparisons (Lloyd 1987, Roy 2004). Hence, these methods could be useful in the future for establishing inter-comparability between cooperating labs, so that such labs can reliably contribute to cases mutually, as is the goal with the work between FOI and the NRPA. Improved consistency among biodosimetry laboratories could further provide an opportunity to reliably share the work load among different members of the biodosimetry community in the event of a mass casualty accident.

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Appendix I

Sample Scoresheet

Sample ID

Evaluator:
Date Completed:

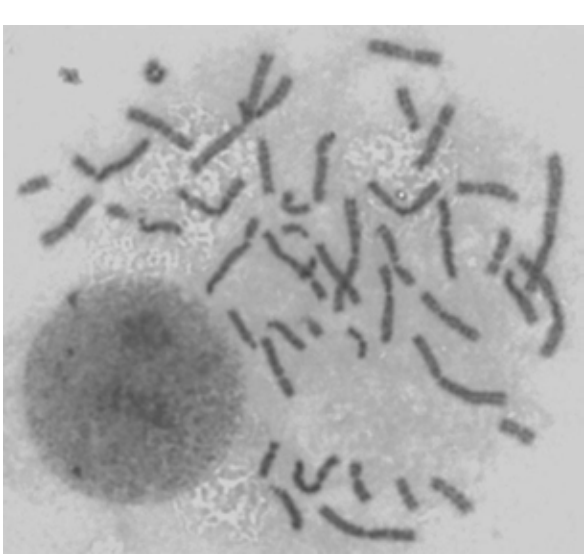
No.	Coordinates	Comments	No.	Coordinates	Comments

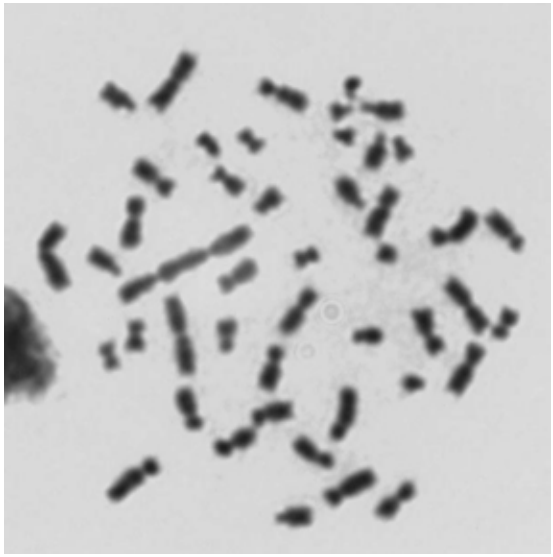
Sample Spreadsheet

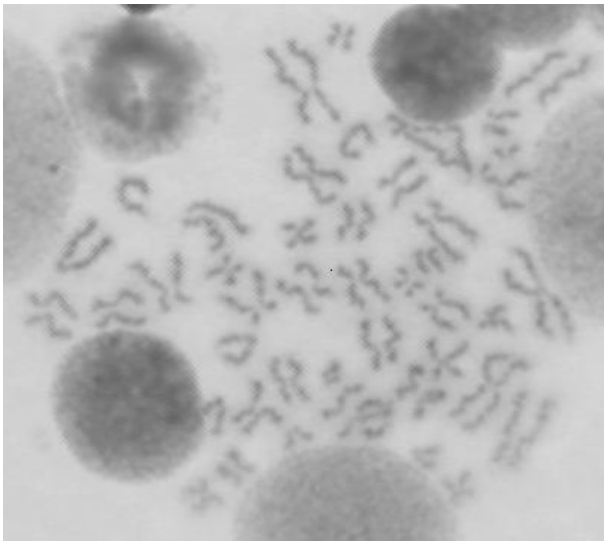
Sample ID								**Evaluator:**			
								Date Completed:			
Cell	Coordinates		No. of aberrations				exc				
ID	x	y	dic	ace	r	other	ace	count	Comments		Ckd by
							0				

Appendix II

Examples of Different Metaphase Morphology







These pictures illustrate some of the different shapes and morphologies that chromosomes can have. In such cases, identification of centromeres may be more difficult and evaluation is harder, requiring some scrutiny and judgment.

Appendix III

Sample Inter-calibration Data

Sample ID		**dsea050117**															
Cell	Coordinates		No. of aberrations						Number of aberrations								
ID	x	y	dic	ace	r	other	count	Comments	dic	ace	r	other	count	Comments		Ckd by	
	35	109				0	46	NS					46	NS		ds/aj	√
50117	36	95	2	3		1	46	CHECK	2	1			46			ds/aj	√
50117	36	110	3	3		0	46	3 dic, 3 ace	3	2			46			ds/aj	√
50117	36	93.5	1	1		0	46	dic, ace	1	1			45			ds/aj	√
50117	36	96.5	2	4		2	46	2 dic, 4 ace		2			44			ds/aj	reviewed
50117	37	110	3	8	1	4	46	3 dic, 8 ace, ring	2	4	1	1	45	NS? 1cross		ds/aj	reviewed
	37	109.9				0		NS						NS non-vissible centr		ds/aj	√
50117	39	105	1	8		7	46	dic, 8 ace	1	4			46			ds/aj	√
50117	39	116.5	2	6	1	3	46	2 dic, 6 ace, ring	1	3	1		46			ds/aj	reviewed
50117	40	100	3	3		0	46	3 dic, 3 ace	2				42	NS		ds/aj	reviewed
50117	40	115	1	3		2	46	dic, 3 ace, 2 ace	1			1 dic	46	NS?		ds/aj	√
50117	40	98.5	2	7	1	4	46	2 dic, 7 ace, ring	2	6	1		46			ds/aj	√
50117	41	96		3		3	46	3 ace		3			46			ds/aj	√
50117	41	97	3	3		0	46	3 dic, 3 ace					46	! 2dic??		ds/aj	reviewed
50117	41	114	1	1		0	46	dic, ace, +1N					44	NS		ds/aj	reviewed
50117	41	94.5	1	1		0	46	dic, ace	1				46			ds/aj	√
50117	42	92	3	3		0	46	3 dic, 3 ace	3	3			46			ds/aj	√
50117	42	110	1	3		2	46	dic, 3 ace	1	3			46			ds/aj	√
50117	43	100	2	4	1	1	46	2 dic, 4 ace, ring	2	3	1		46			ds/aj	√
50117	44	93	2	3		1	46	2 dic, 3 ace	2	2			46			ds/aj	√
50117	44	95	2	5		3	46	2 dic, 5 ace	2	2		1	46			ds/aj	√
50117	44	99	1	4	1	2	46	dic, 4 ace, ring		3	1		46			ds/aj	reviewed
50117	44	103	1	1		0	46	dic, ace					46			ds/aj	reviewed
50117	44	106	2	3		1	46	2 dic, 3 ace		1			46			ds/aj	reviewed
50117	44	114	1	2		1	46	dic, 2 ace					46			ds/aj	reviewed
50117	44	96.5	4	6		2	46	4 dic, 6 ace	1	4			46			ds/aj	reviewed
50117	44	100.5	3	6		3	46	3 dic, 6 ace, ace ring	1	4		1	46	1RING(AC)?		ds/aj	reviewed
50117	50	100.5	1	2		1	46	dic, 2 ace	1	2			46		hard	ds/aj	√
50117	50	108.5	1	3		2	46	dic, 3 ace	1	1			46			ds/aj	√
50117	45.5	107		4		4	46	3 ace, ace ring		3			46			ds/aj	√
	46.5	96				0		NS					46	NS diffuse		ds/aj	√
	46.5	97				0	44	NS		4		1	46	???tricetric		ds/aj	√