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Testing the capacity of the NBDRP EX30701

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Testing the capacity of the NBDRP – EX30701

Introduction

The National Biological Dosimetry Response Plan (NBDRP) is currently comprised of four core laboratories (Health Canada (HC), Defence Research and Development Canada–Ottawa (DRDC), McMaster University (MU), and Atomic Energy of Canada Limited (AECL)) that are capable of providing radiation biological dose estimates using the dicentric chromosome assay (DCA).

As indicated in the CRTI-06-0146RD charter**,** the existing biological dosimetry capacity in Canada will be greatly enhanced by conducting ongoing training and exercising of the four core laboratories. Under the charter, section 3.2: Project Risk Analysis and Risk Management Plan**,** HC (NBDRP lead), it was stated that loss of key personnel could compromise the biodosimetry capacity of the existing network. This exercise (EX30701) was designed to maintain a vigilant level of DCA training and scoring ability for all new and existing employees. The goal of this exercise was to broaden the expertise base within the core laboratories and assess the current scoring ability of all identified employees within the core laboratories. In this exercise we also plan to test whether using a "DCA Quick Scan" method, devised by AECL, versus the actual "DCA Full Triage" method greatly increases the scoring speed and still maintain a comparable level of accuracy

In addition, the core laboratories standardized and incorporated an additional routine biodosimetry method, the cytokinesis block micronucleus assay (CBMN), into the NBDRP to further expand Canada's rapid response capabilities for screening large volumes of samples. This involves standardizing the method across laboratories, establishing dose response curves and implementation of the assay in exercises. Although not radiation specific, this assay readily detects radiation-induced chromosomal damage, requires less training for scoring and, in a mass casualty scenario, could be better suited than the DCA for screening large volumes of samples.

Despite the increased expertise and capacity, the NBDRP would still be incapable of responding to a mass casualty R/N incident with timely dose estimates if patient numbers exceeded 500 individuals. The information provided by biological dosimetry is critical for use in medical triage and the diagnosis of casualties, in order to reduce immediate and/or long term effects and to also distinguish the "worried well" from those who have been exposed and require medical intervention (See section 3.1.1 and 3.1.2 of the CRTI-06-0146RD charter). The DCA has been used for over 30 years by HC to estimate exposure to radiation and has been proven to be a very reliable method throughout the world.

Finally, to ensure QA/QC and privacy of donor information, all samples were bar-coded in the current exercise to allow the core laboratories, if they chose, to test their ability to efficiently utilize their barcode readers in a simulated emergency and eventually allow incorporation of bar-coded samples into the DCA standard operating procedure for the NBDRP.

Scenario

The exercise was initiated on December 7, 2007. The scenario for over-exposure involved a radioactive package being received at a post office with 40 people potentially being exposed. The presence of radioactive material was confirmed by the Hazmat team.

Blood Collection

All donors were volunteers that willingly responded to an advertising call for participation in a research proposal approved by the HC Research Ethics Board. In total 40 blood samples were collected. Blood was drawn into 4mL lithium–heparnized vacutainer tubes from each of 7 individuals at HC.

In Vitro Irradiation of Blood Samples

Once all 40 samples were collected, they were randomly irradiated at 10 different doses so that each laboratory received samples irradiated with the same doses. The samples were blinded so the dose received could not be identified. Each sample was irradiated with a dose between 0 and 4 Gy with ^{137}Cs using a Gammacell 40 at a dose rate of 0.83 Gy/min. The irradiation and sample blinding were done by a third party.

Communications with the NBDRP Network Laboratories

On receiving a call from the person acting as a physician in this exercise, HC called the other three core laboratories to inform them of the accident and to prepare them for receiving the samples.

Transportation of Blood Samples

In a real scenario, the first contact laboratory would provide the instructions (Annex A) for collecting and shipping blood samples. In this exercise scenario, instructions were sent to the acting physician although the samples were collected at HC and distributed to the remaining three laboratories. The samples were shipped to MU and AECL by Purolator overnight express and were received the following day. DRDC picked up their samples at HC and started culturing the same day. An instruction form was sent with each shipment (Annex B)

Results of Analysis of Blood Samples

Each of the four laboratories processed their 10 samples and reported back to HC where the results were compiled. All trained scorers at each laboratory analysed all 10 samples. For full triage, each scorer analyzed 50 cells or 30 dicentrics, ensuring that each cell had 46 centromeres. The results from triage scoring at each laboratory are shown in Figure 1 along with the shaded ±0.5 Gy range. Each symbol represents the results from one scorer analysing 50 cells. Scorers from the same laboratory are shown in the same colour. Figure 2 shows the results of the same

samples after only 10 cells were scored to examine the accuracy of scoring with fewer cells. Nine of the scorers (HC and AECL) also used a "Quick Scan" method devised by AECL, in which individual chromosomes are not counted but the whole cell is examined for damage. The scorer takes a quick look at each cell, and if the cell appears to be complete with no damage, it is scored as normal. If damage is observed in the cell, the scorer takes the time to examine the damage closely and enumerate it. Fifty cells are still analyzed unless 5 dicentrics are found in less than 20 cells. Once 5 dicentrics are observed, scoring is stopped. The results from this scoring are shown in Figure 3. Another scoring system was generated by combining the 10 spread and Quick Scan methods: the Quick Scan method was used as described with the added caveat that if no damage was detected in the first 10 spreads, scoring was stopped. A summary of the five scoring scenarios are found in Table 1. The scoring time for each method was also tabulated by one laboratory, as shown in Table 2. The time for the combined Quick Scan/10 spread method was estimated based on available data.

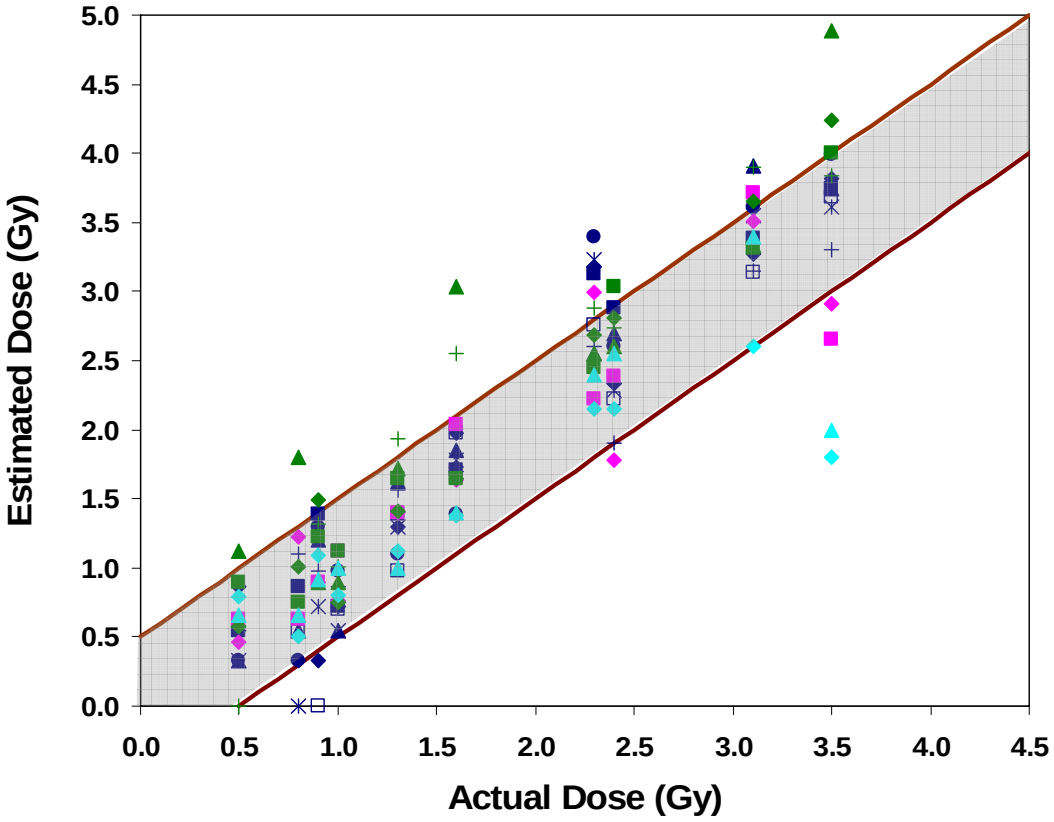

Figure 1: Dose estimates compared to the actual doses delivered after full triage scoring.

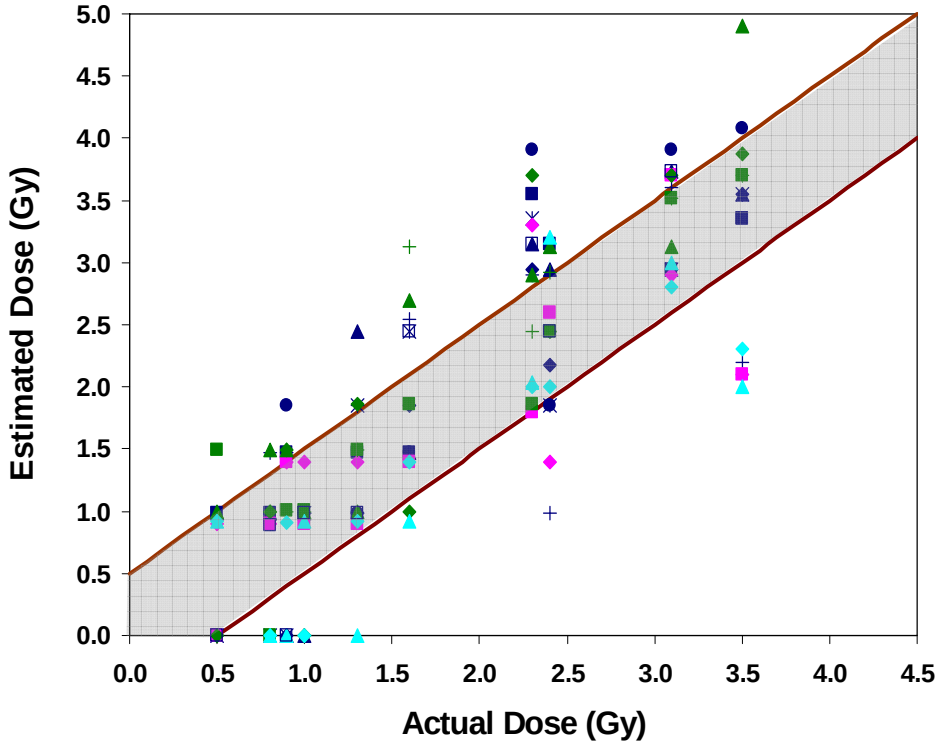

Figure 2: Dose estimates compared to the actual doses delivered after 10 spreads.

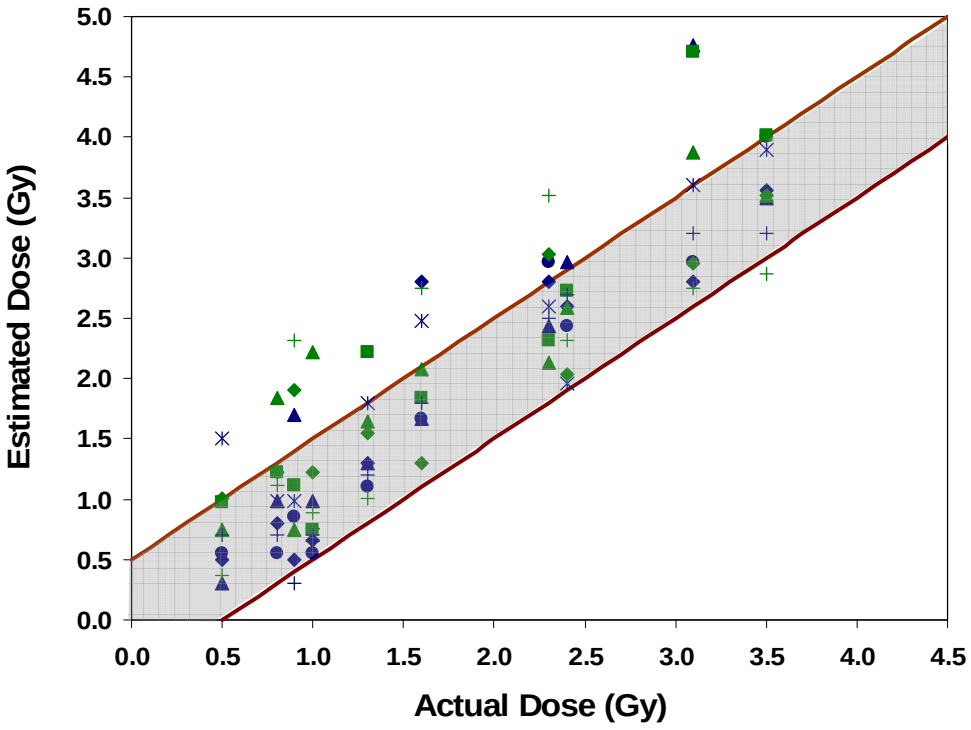

Figure 3: Dose estimates compared to the actual doses delivered using Quick Scan criteria.

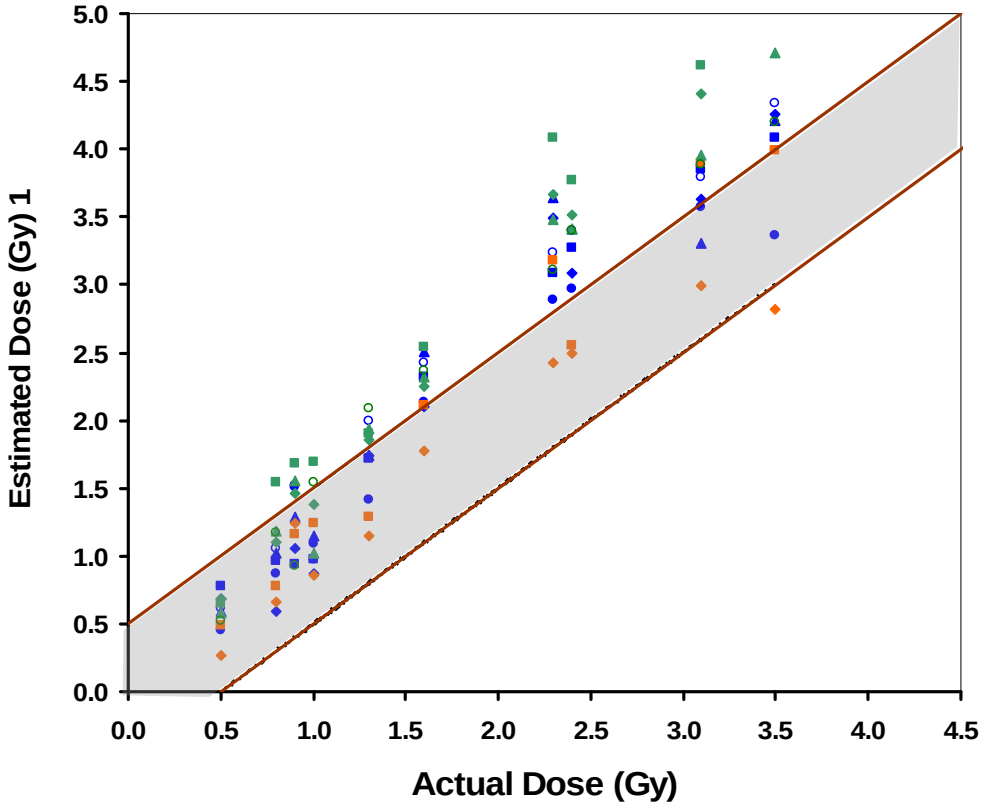

Figure 4: Dose estimates derived using the CBMN Assay.

Table 1. Comparison of scoring methods

Method	% within 0.5 Gy	% over estimates	% underestimates
Full Triage	83%	13%	4%
20 spreads	74%	14%	12%
10 spreads	61%	24%	15%
Quick Scan	81%	18%	1%
QS(10 spreads)	72%	18%	10%*
CBMN (200 BN cells)	57%	42%	1%*

*all underestimates were on samples receiving 1 Gy or less

Table 2. Scoring time for Full Triage vs. Quick Scan

Method	Ave. time to score 10 slides
Full Triage	**1265 minutes**
Quick Scan	**200 minutes**
Quick Scan (10 spreads)	**184 minutes**
CBMN (200 BN cells)	**135 minutes**

Discussion

Ten blind irradiated samples were sent to each of the four reference laboratories of the National Biological Dose Response Plan. Samples were scored for the dicentric chromosome assay and the CBMN assay. Using the DCA, cells were analysed for either 50 cells or 30 dicentrics (and rings) according to Full Triage biological dosimetry standard. Dose estimates were also determined after scoring 10 and 20 cells. In addition, a quick scan method was used. The results of the full triage method were the most accurate with 83% of the dose estimates falling within 0.5 Gy of the delivered dose. This is a lower success rate than in our previous exercise (88%), but in that exercise, each laboratory produced one dose estimate per sample. In this exercise, each individual was tested for their accuracy, including new staff with less experience. For comparison, in the previous exercises, the 40 samples were scored by 10 individuals, while in this exercise, 15 scorers each scored the same10 samples for an equivalent of 150 samples being scored.

As a strategy to decrease the scoring time to increase the throughput for biological dosimetry, decreasing the number of cells analysed has been considered. Decreasing the number of cells scored, but still ensuring the presence of 46 centromeres, reduced the scoring time but also the accuracy. Scoring 20 cells reduced the accuracy to 74% whereas scoring only 10 samples resulted in an even greater loss in accuracy (61% within 0.5 Gy).

The Quick Scan method devised by AECL, however, greatly increased the speed, reducing the average time to analyze 10 slides from 1265 minutes to 200 minutes while maintaining a high level of accuracy (81% within 0.5 Gy). It should also be noted that of the 19% of samples that were not within 0.5Gy, only 1% were underestimates. With this method, however, it was observed that the high dose samples were very quick to score although inherently less accurate compared to the DCA since 5 dicentrics were found in few cells. The samples that took the longest to score were the very low doses. By using the Quick Scan method but stopping after 10 spreads if no damage was found resulted in an additional reduction in scoring time. Some accuracy was lost, however this was predominantly on the lower doses as all underestimates were samples receiving less than 1.0 Gy. This dose level is of little importance when dealing with a mass casualty situation, as there would be no medical intervention after this level of exposure.

The CBMN assay has been proposed as a possible screening tool in situations where large sample volumes are expected. While not radiation-specific, this assay is radiation-responsive and could provide a useful tool to screen out samples which did not receive a dose, and identify high priority samples for full DCA analysis. Incorporation of the CBMN assay into this exercise consisted of two phases. The first involved 12 scorers across 3 labs (HC, DRDC and AECL) assessing unknown samples from 6 donors at 8 doses. In essence, individual scorer and laboratory dose-response curves were generated for this assay. From this phase, lab-specific calibration curves were derived upon which to base biological dose estimates. In the second phase, 10 samples from the current exercise were assessed by 11 scorers across 3 labs (HC, DRDC and AECL) using the lab-specific algorithms to derive dose-estimates. Figure 4 depicts the results of this exercise. Compared to the DCA assay (and its variants), the CBMN only yielded 57% of dose-estimates were within 0.5 Gy of the actual dose. However, all but one of the samples outside this range were over-estimated doses (see Table 1). As indicated in Table 2, the CBMN assay is considerably more beneficial in relation to turnaround time for each sample relative to the DCA assay. While the accuracy results for the CBMN assay in this exercise were somewhat disappointing, it is likely that the accuracy will improve in the next exercise as most scorers involved in evaluating the CBMN results had never used this technique previously.

Conclusions:

Based on this exercise, it is recommended that for a pre-triage screening of large sample numbers, the Quick Scan method and/or the CBMN assay be used to quickly prioritize samples for further, more accurate analysis. Using this method, it is feasible to produce initial dose estimates for 150 individuals within a few hours of the samples being processed. With the initial processing time, initial dose estimates would be available within 4 days of exposure.

Overall, this exercise demonstrated an increased capacity for performing the DCA and CBMN for biological dosimetry, not only through an increasing number of qualified scorers but also through new scoring strategies. It also demonstrated the operability of the network and its ability to provide timely dose estimates for a large number of exposed individuals.

Lessons Learned

1. The reporting criteria were not clearly explained in the instruction sheet. The following will be added to the instructions: "Report the time sample was received, time for processing and scoring. Report the number of dicentrics (and rings if counting), the number of cells scored, the estimated dose from your standard curves with 95% confidence limits". This could be laid out in a format that could be filled in by the laboratories and returned to the lead laboratory.

2. The bar coding still needs some work. HC made up sheets of bar code labels to use during processing but found that too much information was in the code and it was confusing. It was decided just to print a sheet of sample numbers with bar codes and not tailor them to each step of the processing.

3. There was an error in the e-mail and fax number of the contact person at HC. These documents must be carefully reviewed before distribution.

4. An email/message should be sent to several members of the team at each laboratory in case of server breakdown, employee illness etc. upon shipment of the samples from the lead laboratory.

5. Neck of culture flasks should all face either to the right or left and labels should be applied such that they face the worker at all times for all samples. This should be standard for all the Network laboratories in the eventuality of working together at a satellite location in response to a large event.

6. A protocol should be drafted and distributed to guide laboratories on the preliminary reporting of suspected high doses (by Quick Scan, Full Triage or CBMN) prior to complete dose assessment by DCA.

7. Materials (e.g. BrdU, Hoechst, PHA, Colcemid, CytoB, FBS etc.) for biological dosimetry should be tested and ready for use at all times. This is already being done in some laboratories but if others are using these reagents frequently it may need to be noted.

8. Standard methods/guidelines should be prepared and distributed to Network laboratories for DCA and CBMN culturing and scoring criteria to clearly outline methodology to further minimize variations between laboratories. eg. scoring of centric vs acentric rings and requirements for an acentric with a dicentric in DCA, etc. It is very important that all the laboratories are preparing samples and scoring as similarly to each other as possible, especially for intercomparisons.

9. Guidelines are needed on emergency preparedness requirements for handling of sample volumes, i.e. for how many samples are laboratories required to keep reagents in stock. This is important in that if we are keeping reagents on hand and ready for use, how much to keep would be useful to know so that we are not keeping too much or too little on hand. Certain reagents such as PHA and Fetal Bovine Serum need to be re-qualified before use, potentially increasing the turn around time.

Annex A: Blood Sample Collection and Shipping

- Analysis of chromosomal aberrations in human peripheral blood lymphocytes is the present day standard for the biological assessment of radiation exposure. To optimize the recovery of lymphocytes from the blood, it is very important that the blood be collected and shipped according to the protocol outlined below.
- Before blood samples are taken please notify HC so that we can prepare for arrival and pick up.
- All blood samples are to be collected into lithium heparin tubes (if not available sodium heparin tubes are acceptable), and are to contain at least 3 mL (ideally 2 x 5 mL tubes). Gently rock the tubes to ensure proper mixing. Label the tubes unambiguously using the coding system identified by the receiving laboratory.
- Package the blood sample carefully to prevent breakage of the tubes in transit. The blood should be maintained at approximately 20°C. **Blood samples must not be frozen.** One method of maintaining blood at room temperature is to place the tubes on a gel pack that has been allowed to stay at room temperature for several hours.
- Immediately following blood collection, ship the samples by **special transportation and use overnight air express so we can receive the blood early in the morning following sample collection.** Contact the laboratory to confirm the shipment and inform us of the **Way Bill** number. THIS IS IMPORTANT FOR TRACKING THE SAMPLES.
- For best results blood must be received within 24 h of sampling.
- For air transport, packaging and labelling should conform to the current International Air Transport Association (IATA) regulations. These require that blood samples be packed to conform to **United Nations Regulation 650** for biological substances. Saf-T-Pak manufactures packaging that meets these requirements (STP 210) (www.saftpak.com). Other packaging is acceptable providing it meets the requirements stated below.
- Packaging:
  - leak proof primary container (blood collection tube)
  - leak proof secondary container (e.g. Ziploc bag)
  - absorbent material placed between the primary and the secondary container
  - if purchased must be marked with **TC-125-1B** (e.g. STP 210 packaging)
  - if the shipper is making his own packaging, it must be a rigid outer packaging, and the exterior must be marked with **125-1B**
- Marking and labelling on outer package for air transport:
  - **name, address and telephone number of receiver and shipper**
  - **name, address and telephone number of person responsible if other than shipper**
  - **Biological substances, category B**
  - diamond shaped **UN3373 label**
  - **2 orientation arrows** placed on opposite sides of the package
  - **DO NOT X-RAY, DO NOT FREEZE**
- **An itemized list** of package contents must be placed between the secondary and outer packaging
- Waybill:
  - in "Description", enter only: **UN3373 Biological substances, category B**

Ship to: Health Canada
Consumer and Clinical Radiation Protection Bureau
775 Brookfield Road, PL 6303B
Ottawa, ON K1A 0K9
Phone: (613) 355-6028 Fax: (613) 941-1734

Annex B: EX30701 Exercise **December 3rd, 2007**

Instructions for Network Laboratories

Please find enclosed 10 randomly irradiated samples for biological dosimetry using the Dicentric Chromosome Assay and Cytokinesis Block Micronucleus Assay.

The samples are coded as follows:
E3VIAL1S01
E3VIAL1S02
E3VIAL1S03
E3VIAL1S04
E3VIAL1S05
E3VIAL1S06
E3VIAL1S07
E3VIAL1S08
E3VIAL1S09
E3VIAL1S10

N.B. VIAL# is different for every lab

Results should be faxed and e-mailed back to Health Canada for compilation and a report will be sent to the CRTI Secretariat
Fax: Attention Vinita Chauhan
(613)952-7584
E-mail : Vinita_Chauhan@HC-SC.GC.CA
A follow-up e-mail will be sent with a sample reporting sheet.

If you have any questions, please feel free to call me at **(613)-355-6028 (cell)** or **(613)-941-7263 (office)**
E-mail Ruth_Wilkins@hc-sc.gc.ca

Thank you for helping in processing these samples.

Ruth Wilkins
Health Canada
Consumer and Clinical Radiation Protection Bureau
775 Brookfield Road
Ottawa, Ontario

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