MODIFICATION OF HUMAN BLOOD IRRADIATION TECHNIQUE WITH THE RADON GAS: *INVITRO* STUDY

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ABSTRACT

The aim of this study is to design radon irradiation technique in the field of hematology for an invitro study. In addition, deposit of alpha particles into the human blood surface and its effects on the thrombocytopenia estimated using nuclear track detectors (NTDs). In this technique, amount of radon gas (2210±5.1Bq/m^3) collected in a tight PVC container wit h the appropriate engineering dimension using two sources of radium (5µCi). Blood samples (10 male and 10 female) a nd CR-39NTDs (40 pieces) are exposed to radon gas at various exposure time. Complete blood test and the computer sc anning for each piece of CR-39NTDs before and after exposure has done. The results show that the present technique has a good efficiency (≈96%) to the invitro exposure of human blood. When the radon gas moved on the surface of bloo d sample, alpha tracks registered into CR-39NTDs. Thus, this technique improved that the comparative method to evalu ate alpha particle density into exposure blood samples is an effective way; this depended on the geometry of design and the sensitivity of CR-39NTDs to track registration. Radon detector version 7 (RAD7) used to make a certain suitability of CR-39NTDs. Amount of radon concentration losses during the exposure process, in the present work it was variable from 0.41% to 1.4%. Radon concentration effected on the thrombocytopenia; this depended on time of exposure and alp ha energy loss into the blood and CR-39 through the atomic displacements. At the time of exposure of 10 minutes, rate of absorption dose was 2.255±0.11µSv (39%), and the platelet (PLT) cont reduced rapidly (high effected on reduce PLT), this makes thrombocytopenia.

ABSTRAK

Tujuan kajian ini adalah untuk merancang teknik iradiasi radon dalam bidang hematologi untuk kajian invitro. Selain i tu, deposit zarah alfa ke permukaan darah manusia dan pengaruhnya terhadap thrombocytopenia estimasi menggunak an pengesan lagu nuklear (NTDs). Dengan teknik ini, jumlah gas radon (2.210 ± 5.1Bq/m3) dihimpunkan dalam bekas PVC rapat dengan dimensi teknik yang tepat dengan menggunakan dua sumber radium (5µCi). Darah sampel (10 lelaki dan 10 wanita) dan CR-39NTDs (40 buah) yang terkena gas radon pada berbagai masa bukaan. ujian darah lengkap d an pengimbasan komputer untuk setiap potongan CR-39NTDs sebelum dan selepas kenalan telah dilakukan. Keputusa n kajian menunjukkan bahawa teknik ini mempunyai kecekapan yang baik (≈ 96%) ke paparan invitro darah manusia. Bila gas radon bergerak pada permukaan sampel darah, alfa trek berdaftar ke CR-39NTDs. Dengan demikian, teknik i ni diperbaiki bahawa kaedah perbandingan untuk menilai kepadatan zarah alpha menjadi sampel darah pendedahan a dalah cara yang berkesan, hal ini bergantung pada geometri dari desain dan sensitiviti CR-39NTDs untuk mengesan pe ndaftaran. Radon versi pengesan 7 (RAD7) digunakan untuk membuat kesesuaian tertentu CR-39NTDs. Jumlah kerugia n kepekatan radon semasa proses paparan, dalam karya ini itu variable dari 0,41% menjadi 1,4%. kepekatan Radon b erpengaruh pada thrombocytopenia, hal ini bergantung pada masa paparan dan kehilangan tenaga alfa ke dalam dara h dan CR-39 melalui perpindahan atom. Pada saat pendedahan dari 10 minit, tahap penyerapan dosis 2,255 ± 0.11µSv (39%), dan platelet (PLT) cont berkurang dengan cepat (berpengaruh tinggi pada mengurangkan PLT), ini membuat th rombocytopenia

Keywords: Human blood; CR-39 NTDs; Radon-222; Alpha particles; Invitro study

INTRODUCTION

Radon (^{222}Rn) is a natural radioactive gas, emitting from radium (^{226}Ra) decay. Three radioactive isotopes ^{218}Po, ^{214}Po and ^{210}Po are generating from radon decay, the doses from radon, however, are contributed to by the radon decay products (daughters) rather than by the gas itself. Thus, these isotopes will make risks on the human internal and external organs such as Lung, trachea (inhalation) and the skin (deposits). The exposure due to deform in DNA, blood components and improve the cancers (Pecaut *et.al.*, 2002; Hamza *et.al.*, 2009).

When nuclear radiations (Alpha, Beta & Gamma) enter the body, some of their energy will lost in collisions with the body's atoms. Ejected electron loses energy by causing additional ionization; deflected alpha goes on to cause additional ionization. The major characteristic of the organ's atomic interactions is the stripping away of electrons from atoms in the body. This removal of electrons called ionization, therefore, alpha, beta, and gamma radiation often called ionizing radiation .The ionizations and their effects on cells cause the biological effects of radiation, and the body cells depend on individual atoms working together(Pohl-Rüling *et.al*., 1990).

Alpha particle energy relatively heavy, positively charged particles and fully absorbed within the first 20 micrometers of an exposed tissue mass. Therefore, all the radiation energy will be absorbed in a very small volume of tissue immediately surrounding each particle. Alpha particles have such limited penetrating ability that the maximum range for the highest energy alpha particle in tissue is less than 100 micrometers (Somosy, 2000).

When the human body exposed to radiation doses, it will cause injury to tissue systems from free radical damage. Exposure can be acute or chronic; this depends on total dose, dose rate, distribution of dose and the susceptibility of the patient to the radiation. Tissue systems with greater rates of cell division, such as the hematopoietic and gastrointestinal systems, In addition, platelet levels at the time of diagnosis could be a useful prognostic factor in lung cancer. Thus, designing and modification in radon irradiation technique in the field of hematology for an invitro study considered an interest scientific research (Geoffrey, 1998; Francisco et al, 2009; Pecaut *et al*, 2002).

As known, blood accounts for 8% of the human body weight with an average density of approximately 1.060 kg/m^3 (3-4) .The average adult has a blood volume of roughly five liters, , composed of plasma and several kinds of cells; these formed emends of the blood are erythrocytes (red blood cells), leukocytes (white blood cells), and thrombocytes (platelets). By volume, the red blood cells constitute about 45% of whole blood, the plasma about 54.3%, and white cells about 0.7%. Therefore, important parameters should be taken into account during design or modify any radon exposure technique, such as; Radon's parameters: half time, concentration, detecting method, controlling, range of alpha particles, restricted energy loss and the detector efficiency. Blood parameters: Mixing of blood components to get freshly drawn blood (see Fig.1), laboratory's temperature/ humidity, blood age and blood air exposure.

Decrease of platelets count in the blood due to a thrombocytopenia, which can result in poor blood clotting. Thrombocytopenia usually defined as less than 150,000 platelets per cubic millimeter of blood (Krailadsiri *et. al*., 2001). In this research, new exposure technique is presents to study the effects of accumulation of alpha particles on the surface of human blood samples on thrombocytopenia using a nuclear track detector type CR-39.

RESEARCH METHODOLOGIES

Research Materials

a. CR-39 Nuclear Track Detectors (NTDs)

It is a C12H18O7 polymer with a density of 1.31 g/cm3 and thickness of 700 µm cut into 1×1.5 cm, laser engraved code (Fig.2). They are commercially produced by Intercast Europe SRL (Parma-Italy). The efficiency of the CR-39 NTDs reduces with the time of storage (Ismail 2009). Thus, the calibration process is carried out to determine its efficiency (Ismail & Jaafar, 2009).

b. RAD7

Radon detector version 7 (RAD7), DURRIFGE CO., Serial number: 2495, date of calibration: 17 November 2009, was used for accurate results of the long-term measurements and for the calibration process.

Figure1: Two blood tubes. Tube A: after standing, tube B: contains freshly drawn blood

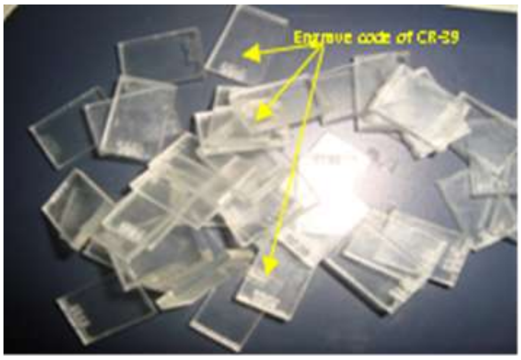

Figure 2: Laser-engraved code of the CR-39 NTDs; Intercast Europe SRL.

Radiation Sources

Two radium (^{226}Ra) source with the activity 5µCi use to combination of radon gas into the PVC cylinder chamber (length = 21cm; diameter= 7cm), it is used as a controlling radon gas (radon concentration is changeable) to blood exposure.

Radiation Dosimeter

The geometry form of radiation dosimeter type VICTOREEN, model 491-30, USA makes it consider a suitable dosimeter for the present technique; it used to know the average radon dose inside the irradiation chamber (PVC chamber). And for the calibration, digital dosimeter type RAM DA3-2000, model 2-0033-10, RCTEM INDUSTRIES C€ used, as shown in Fig.3.

c. Water bath and chemical solutions

Etching systems consist on etch track detectors at 6Normality of NaOH at 70C^{o}, distiller water and water bath "GOTECH TESTING MACHINES INC" model GT-7039-M, 220 volt, 50Hz.

d. Microscope Image and Scanning

The etched tracks observed using an optical microscope fitted with an objective lens of 400-time magnifications. At this magnification, counting field covers an area (=0.10912 mm^2). The microscope image viewed with a high-quality monochrome charge coupled device (CCD) TV camera, which connected to a PC-based image analyzer, as shown in Fig.4. Measurements carried out under magnification of 400*X* for a total scanned area of the detector equal to 1.5 cm^2.

Figure 3 : Radiation dosimeters (a): RAM DA3-2000 and (b): VICTOREEN.

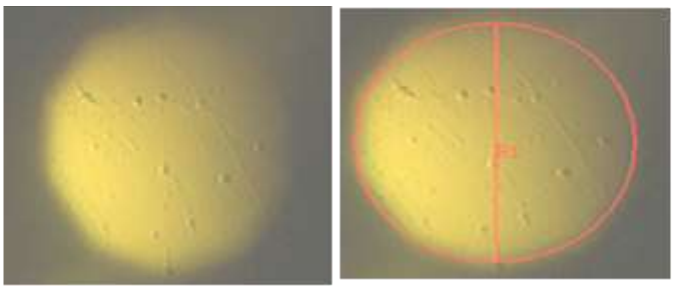

Figure 4: Alpha track register by CR-39NTDs (Area 0.101912 mm^2)

e. Blood Sample

Blood samples (2.5 ml) collected by venipuncture into heparinized syringes for 20 male and 20 female (20- 52 years old) using established blood-borne pathogen/biohazard safety protocols. This study conducted with the cooperation of the wellness center of Universiti Sains Malaysia (USM). Blood samples are prepared inside a plastic dish (diameter = 1. 42cm: depth =0. 5cm) to the exposure of radon gas.

f. Blood Analysis Machine

Sysmex, K-Series range KX-21, as shown in Fig.5, is a fully automated hematology analyzer, easily fitting into any laboratory and ideal as a backup analyzer to the Sysmex full differential analyzer system. In addition, it has interesting features such as ease of system operation and maintenance, fully automatic sample aspiration, dilution & analysis for 18 parameter test results; compact instrument 'footprint,' daily maintenance is automatically performed at every start up and shut down, suitable for central laboratories and emergency rooms or physician's office laboratories.

Figure 5: Sysmex, K-Series range KX-21 equipped with a computer to keep patient information

EXPERIMENT PROCEDURE

Procedures of Irradiation Technique

Specifically, the concept and idea of present design (radon exposure technique to an invitro study of the human blood) come from explain radiation effects on blood components via evaluating alpha particle density, range and restricted energy loss of alpha particles into the blood samples. For investigating that purpose, alpha tracks into the surface of CR-39NTDs used as a correlation and relative method. Therefore, thickness (=0.5cm), volume (=0.5ml) and place of blood samples justified with the thickness and place of CR-39NTDs, as shown in Fig. 6. Thus, by knowing the number of monuments belonging to the surface of the detector can find out the amount of alpha particle incidents on a blood.

Generally, present technique (see Fig.7) consists of three parts; first part (A), is the PVC radon chamber radon, to collect radon gas inside a control chamber (i.e. radon concentration to be under control) two sources of radium were put inside the mentioned chamber. After 28 days of exposure, radon concentration is saturated, and then it used to blood exposure. Second part (B), this part included systematic of human blood and CR-39NTDs exposure to radon gas, radiation dosimeters (type VICTOREEN), Thermometer and the radon chamber (high =7cm, diameter 6 cm) (Ismail & Jaafar, 2009). Third part (C), include RAD7 that used to measure radon concentration during the period of exposure. Specifically, Rad7 use to demonstrate estimate that the CR39NTDs are in high efficiency. Continuity in the measurement of exposure and declined further comment are two ways; RAD7 and CR-39NTDs considered important points in this technique.

Blood samples (male and female) have put inside plastic dishes, then fixed temporary beside two pieces of CR-39 to exposure radon gas. Thus, radon exposure for different time (6, 10, 15, 20 minutes) done for the select human blood samples (20 male & 20 female). To mixing blood components and to avoid a plasma deposit above red blood, both; blood samples and CR-39 NTDs moved slowly (left to right, right, also appositively) without any effect on radon concentration, because the design supplied with the scroll move. Age of blood samples has taken into account, blood tests before and after irradiation was less than one hour.

In addition, before using radon exposure technique, and for control (to avoid the effects of Virus and infection on the results) , complete blood test for 20 human blood samples processed before and after selected time (6 , 10, 15 , 20 minutes) .

Exposure dose was 10 to 41 µSv/hr and for exposures to the radiation dose of the radon gas, whole blood samples transported at a temperature of (27±2.2 C^0) inside the technique. SRIM 2010 program used to calculate the range of alpha particle and restrict energy loss into the blood and CR-39NTDs.

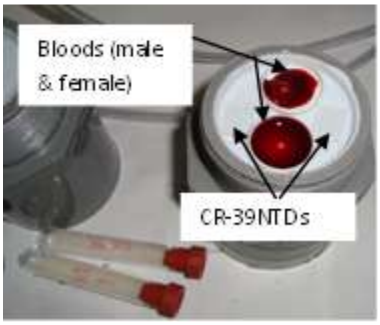

Figure 6: Systematic of human blood exposure to radon gas.

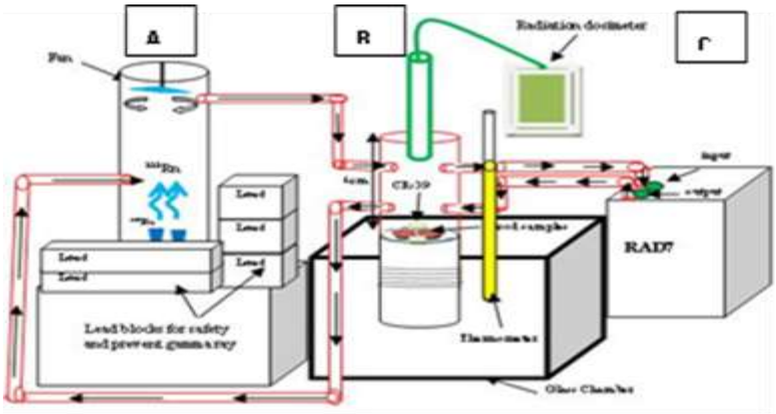

Figure 7: Schema diagram of human blood exposed invitro to radon gas (irradiation technique).

Etching and scanning process of CR-39 NTDs

The etching's systems consist on etch track detectors at six Normality of NaOH at 70C^o, distiller water and water bath "GOTECH TESTING MACHINES INC" model GT-7039-M, 220 volt, 50Hz. For scanning process, the etched tracks observed using an optical microscope fitted with a magnification of 50 X to 1000X. The microscope image viewed with a high-quality monochrome charge coupled device (CCD) TV camera, which connected to a PC-based image analyzer.

RESULTS AND DISCUSSION

The variation of the range of alpha particle was depended on the energy of it and the target density (blood or CR-39NTD), this depended on the restricted energy loss, as shown in Fig.8. High LET radiation of alpha particles, which emitted from ^{226}Ra, is more efficient in inducing biological damage because all the energy deposited within a short distance, causing dense ionization in the trajectory.

Average radon concentration (2198±5.1Bq/m^3) in PVC chamber ranged around 2193 to 2205 Bq/m^3, as shown in Fig. 9. This means that there is relatively a loss in radon concentration in each stage of exposure, because there is absorbed dose by CR-39NTDs and the blood sample, also the process of the mixing of blood samples affected on keep of radon concentration.

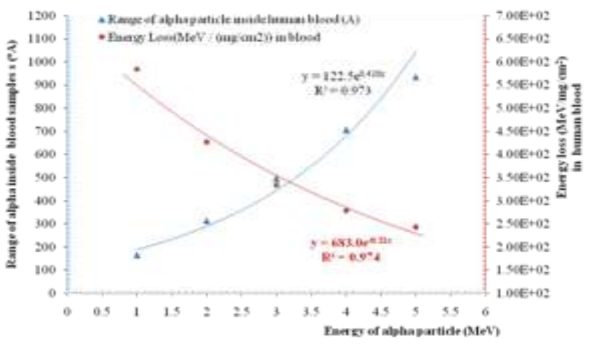

Figure 8: Reverse changes for each of the range and energy loss of alpha particle inside the CR-39 NTDs.

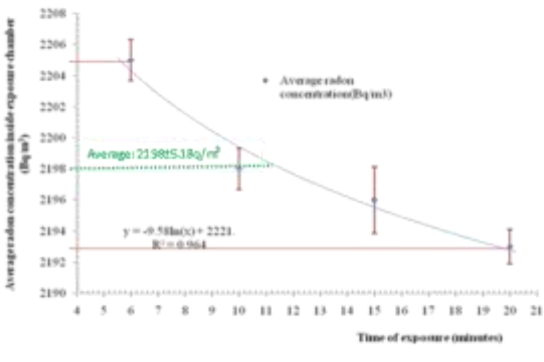

Figure 9: Changes of radon concentration with the time of exposure.

Average loss ratio of radon concentration variable from 0.413% to 1.404 %, this depends on time of exposure, as shown in Fig.10. This means that the efficiency of the present design that related to keeping radon concentration for blood exposure very good (= 96%). On the other hand, average absorbed dose by selected samples (CR-39NTDS +0. 5ml Blood sample) was enough for the selected time of exposure. Thus in Fig. 10, rate of radiation absorbed dose into the human blood samples is high at 20 minutes, so the effects on the thrombocytopenia high as shown in Fig.11. In fact, anyone can be exposure to that periods and that concentration of radon gas during workplace, so the present design considered an important system to knowledge the effect of radon gas on the blood component, especial platelet content.

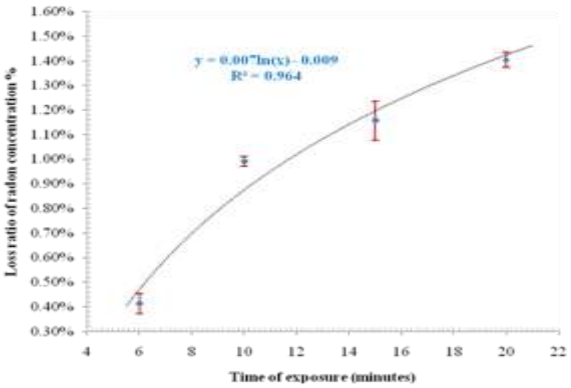

Figure 10: Percentage decreases of radon concentration with the stages of exposure during exposure.

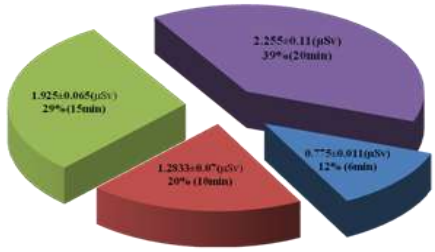

Figure 11: Rate and percentage occupation absorption dose into blood samples for various exposure times.

As well as, results of average exposure and absorbed radon concentration into CR-39NTDs and the human blood samples (male and Female) with the time of exposure are listed in Table 2. Composite of alpha particle into the surface of blood samples measured via measuring tracks of alpha particles into the surface of CR-39NTDs are observed markedly.

In addition, average absorbed dose via the exposure samples increased with increasing time of exposure, this made the number of platelet count decreasing relatively. The reason of this phenomenon due to high dose of alpha particle, this is making the greatest damage to the blood cells, that the alpha particles are heavy ions. Thus, the alpha particles will be losing most of their energy at a short distance in their trajectory for irradiation.

The effect of radon absorbed dose by the blood sample variable, because biological factors, which may influence the effect of alpha radiation on an individual include age and sex, as shown in Fig.12.

As well as, alpha particle density into the surface of CR-39NTDs and the human blood samples increased with the time of exposure as shown in Fig.13. Therefore, the mentioned design considered as an effect and suitable way to the *invitro* studies of hematology.

Table2: Human blood (0. 5ml) and CR-39NTDs exposed to the Radon (^{222}Rn) Gas; average exposed dose rate and radon concentration (=7.7±0.041) μSv/hr and (=2198±5.1) $Bq.m^{-3}$, respectively. Average laboratory temperature (=17.975±0.05) C^o.

Time of exposure (minutes)	**Average radon concentration (RAD7) (Bq/m^3) Inside exposure chamber**	**average density of alpha into the blood and CR-39NTDs ($track/cm^2$) per time of exposure**	**Average dose rate inside exposure chamber (μSv/hr)**	**Average absorbed dose by blood and CR-39NTDs (μSv)**	**Reduced value of PLT count ΔPLT(after and before exposure) Male:29 years old**	**Reduced value of PLT count ΔPLT(after and before exposure) Female:30 years old**
6	2205±18.15	**262.088±18.42**	7.75±0.01	0.775±0.011	9	7
10	2198±28.51	**284.166±22.06**	7.7+0.07	1.2833±0.07	28	18
15	2196±17.87	**326.065±22.8**	7.7+ 0.03	1.925±0.065	16	15
20	2193±23.12	**350.106±14.8**	7.65+0.14	2.255±0.11	30	21
Ave. ± SDV	**2198±5.1**		**7.7±0.041**			

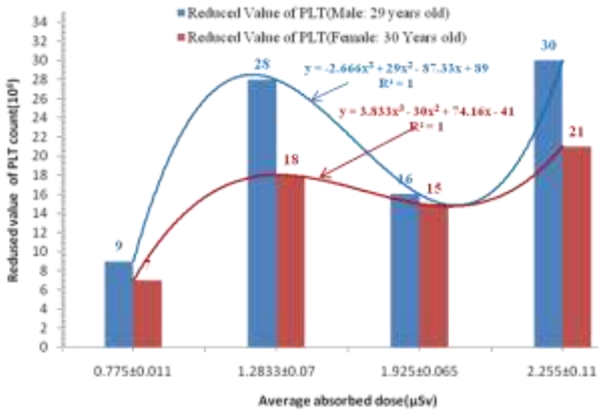

Figure 12: Relative change in reduced of platelet count (PLT) with average absorbed radon dose.

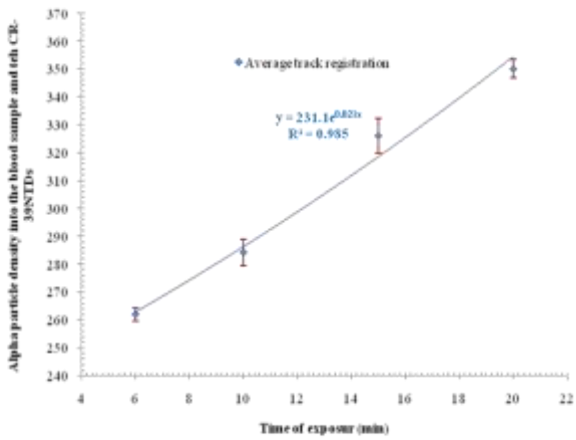

Figure 13: Increasing the deposition of alpha particles with the time of exposure

CONCLUSION

The distributional methods for alpha particles and radiation dose on the surface of CR-39NTDs and the completely human blood samples have been significantly improved through the design new system to exposure. In a new design, there was a little loss of radon concentration pursuant to several factors including the method of mixing components of blood sample and the quantity of absorbed dose. However, this did not affect on the efficiency (≈96%) of the present design.

The range of alpha particle in CR-39NTDs was less than the range of it in the human blood samples, depending on their density and restricted energy loss. Comparative study between CR-39NTDs and the human blood samples was a new technique for *invitro* studies of the blood ionization, especially to estimate ionization of alpha particle. Therefore, CR-39 NTDs considered as the most suitable nuclear detector to get alpha particle density deposited on the blood surface.

The present design is applicable in an invitro study of the blood ionization, decrease of the platelet count in each stage of exposure (different time of exposure), relatively, refers to an effect of radon absorption dose from 0.5ml of human blood on the blood components. In high time of exposure (=20minutes), the change value of the platelet count before and after exposure go to high (reduce of PLT go to high).

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