Comparative study of irradiation of blood by MS9 and Try pan blue methods on blood storage

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Abstract:

Background: Transfusion-associated graft-versus-host disease (TA-GVHD) is a rare, but usually fatal, complication of transfusion. The risk associated with an individual transfusion depends on the number and viability of contaminating lymphocytes, the susceptibility of the patient's immune system to their engraftment and the degree of immunological disparity between donor and patient. The mainstay of prevention is gamma irradiation, which inactivates T lymphocytes whilst preserving the function of other blood cells.

Objective: Comparison between the two methods of assay of MS9 and Try Pan blue assay to study the effect of radiation on blood storage

Materials & Methods: Nine pints of blood had been taken from Notional Center for blood transfusion. From these pints, 255 samples were taken for test, 156 samples of blood exposed to high doses of gamma radiation of 15, 18, 20, 22, 24, 26, 28, and 30Gy. This radiation, obtained from ^{60}Co available in Hospital of Radiotherapy and Nuclear Medicine in Baghdad, and the other 99 samples were used as a control. Blood Components are count by try pan blue in a way that nine pints of blood were utilized and from each pint several samples were used, eight of them were exposed to radiation of doses 15, 18, 20, 22, 24, 26, 28, and 30Gy, assay of try pan blue was used for counting the No. of red blood cells & lymphocyte cells whenever testing. The other pint used as a control sample before and after irradiation immediately.

Results: From the results the assays by Try pan blue to count the viability of (lymphocyte cells and RBCs) was more accurate than assay by heamocounter MS9 (Haemocounter MS9 was used for automatic counts of blood cells) to know the period of the blood storage.

Keywords: Irradiation, MS9, Try pan blue, Blood storage

Introduction:

Graft versus host diseases (GVHD) is commonly seen after bone marrow transplantation [1, 2, 3]. It occurs when histo-incompatible viable lymphocytes are introduced into a host who is immunologically incapable of rejecting foreign cells. These lymphocytes recognize the recipient as foreign cells [4].

GVHD has been reported following transfusion of whole blood in patients with lymphoma, Hodgkin's disease, leukemia and neuroblastoma, most of whom had received immunosuppressive therapy (called transfusion associated GVHD "t-a GVHD") [5, 6], and also following transfusion of whole blood and blood products; this phenomenon was first reported by Hathaway et al, 1966 who described a syndrome of aplastic anemia, dermatitis, hepatosplenomegaly, and histiocytosis [7].

It is theoretically possible that any blood product containing viable lymphocytes could result in GVHD and, although rare this syndrome has been reported following transfusion of plasma to a child with Wiskott-Aldrich Syndrome [8,9], and following platelet transfusion in patients with Hodgkin's disease, (Parkman et al. 1974) [10].

There is no effective therapy for GVHD so prevention is of important role. Several drugs are used to prevent or minimize GVHD. These drugs include methotrexate, glucocorticoid, hormones (steroids), cyclosporine, and tacrolimus [11]. Another technique used to reduce the severity and incidence of GVHD is called T-lymphocyte depletion [12, 13]. Since T-lymphocytes are the primary effectors cells in GVHD, the donor blood can be depleted of T- cells by irradiation in an effort to minimize the immune attack [14].

Irradiation of blood products by gamma rays appears to be sufficient to prevent GVHD. Blood's cells are different in sensitivity to radiation where they are in their life cycle cells in division stage are most sensitive to radiation [13]. Photoelectric absorption is the most likely form of absorption at fairly low energy levels of the incident to γ - ray. The lower the energy of the photon the more likely it is to be absorbed by a photoelectric process [15].

The current study was conducted aiming to show the effect of high doses irradiation above 15 Gray on the time duration of blood storage & comparison between assay of Trypan blue on the viability of lymphocyte cells and RBCs (with MS9 "Heamocounter MS9 for automatic counts of blood cells")

Materials & Methods:

Materials of blood components Irradiation

Cobalt-60 unit, a device that emits γ - rays at 1.17 Mev and 1.33 Mev and has a high specific activity. The half-life is relatively short (5.26 years), so that treatment times to be increased by about 1% per month to correct for the decay. It is of course impossible to switch off the gamma emission from the cobalt-60, so that some means of interrupting the beam must be provided. The source is mounted within a massive head, which is made from lead and depleted uranium. This head will weight about

one tone for a 5000 ci source of cobalt- 60sec [16].

The device (present in hospital of radiotherapy and nuclear medicine of Baghdad) components are,

Ficoll; which is lymphocyte separation medium, it had a density greater than lymphocytes but less than red cells, it was used to isolate lymphocyte, **Microscope;** used to count the cells and the viable cells will remain without colour, and **Trypan blue** to stain the non-viable cells and the viable cells remain without colour [11,17, 18]

RPMI 1640 culture medium

The **Centrifuge** was used to isolate the lymphocytes, when the blood was diluted by RPMI and Ficoll, then centrifugation with a rate of 2500 rpm for 20 min., lymphocytes settled at the interface of the medium and Ficoll. Lymphocytes were isolated by using pasture pipette and put in another sterile tube [2, 17].

Isolation of lymphocyte and blood cells: The whole blood was defibrinated by shaking with glass beads and the resulting clot removed. The blood was diluted by RPMI (1640) with ratio 1:1and then layered on top of a tube half full of Ficoll with ratio 1:1. Also Ficoll had a density greater than that of lymphocyte but less than that of red cells and granulocytes (macrophages).

After centrifugation with a rate of 2500 rpm for 20min, the red cells and PMNs passed down through the Ficoll to form a pellet at the bottom of the tube while lymphocytes settled at the interface of the medium and Ficoll. Lymphocytes and RBC were isolated by using pasture pipette and put in another sterile tube. They are diluted by RPMI (1640) again with ratio 1:1, and centrifuged for 10 min with a rate of 2000 rpm. This washing was repeated for three times in order to remove the Ficoll completely and obtained clear lymphocytes [19,20] Ten μL of this suspension was taken (Trypan blue and lymphocytes cells or RBCs) either to the hemocytometer chamber or on cover glass and count the cells. Non viable cells will stain blue.

$$\text{Cells viability \%} = \left(\frac{\text{No. of viable cells}}{\text{No. of viable} \times \text{No. of dead cells}} \right) \times 100$$

Such technique is based on that the trypan blue will stain the non-viable cells and the viable cells will remain without colour. Trypan blue has a greater affinity for serum protein than for cellular proteins. It should be mentioned that cells in Trypan blue solution should not be incubated longer than 15 minutes as viable cells might begin to take up [19,20].

The material of the study was by using nine pints of peripheral blood taken from the National Center for Blood Transfusion to be used in this study.

Results & Discussion:

Irradiation of cellular blood components prevents donor lymphocytes proliferating against host tissues. The absolute lymphocyte count is the first value to show any change after a significant radiation exposure without adverse affect on the function of red cells, neutrophils, and platelets. During the study it had seen that the viability of lymphocytes will decrease gradually with increase of irradiation dose till the viability became less than the half. Any dose of irradiation from 15 to 30 GY is strange because; at these points the lymphocytes will decrease significantly in counts, viability, and proliferation without clearly affecting the other blood elements (Table 1, Figure 1 & 2). This is in agreement with Sanders et al (3) who studied growth and development in children after bone marrow transplantation and in agreement with the findings reported by Hasan [18] who assess the viability of blood components after irradiation.

Table 1: The lymphocyte cell percent difference decrease in control and after different radiation dosage exposure for both methods.

	MS9 [19] (Lymph. %)	Try pan Blue (Lymph. %)
Control	49.91±4.15 (45.25-53.19)	49.33±0.58 (49.00-50.00)
15 Gray	48.09±12.29 (36.55-61.02)	44.33±2.08 (42.00-46.00)*#
18 Gray $	46.03±1.45 (44.44-47.27)	40.33±2.52 (38.00-43.00)*
20 Gray $	43.98±0.58 (43.54-44.64)	35.00±1.00 (34.00-36.00)*#
22 Gray $	41.15±3.10 (37.37-43.49)	30.33±4.16 (27.00-35.00)*
24 Gray $	37.96±0.27 (37.65-38.14)	25.00±3.00 (22.00-28.00)*
26 Gray $	35.07±2.52 (32.17-36.74)	20.33±1.53 (19.00-22.00)*
28 Gray $	31.91±0.16 (31.81-32.09)	15.67±2.08 (14.00-18.00)*#
30 Gray $	3.33±0.02 (3.31-3.36)	3.00±1.00 (2.00-4.00)*#

*Significant difference from control using t-test at level of 0.05.
Significant difference from previous (lower) dosage of radiation using t-test at level of 0.05.
$ Significant difference of MS9 [19] from Try pan Blue method using t-test at level of 0.05.

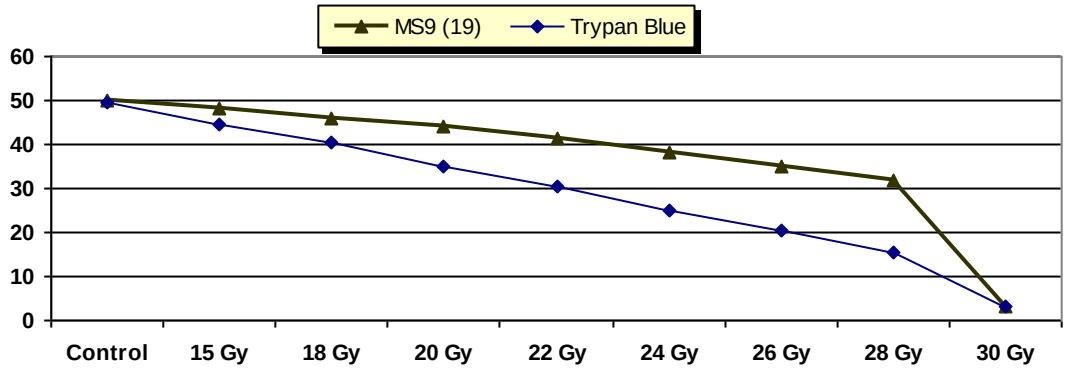

Figure 1: The mean lymphocyte cell percent difference change in control and after different radiation dosage for both methods.

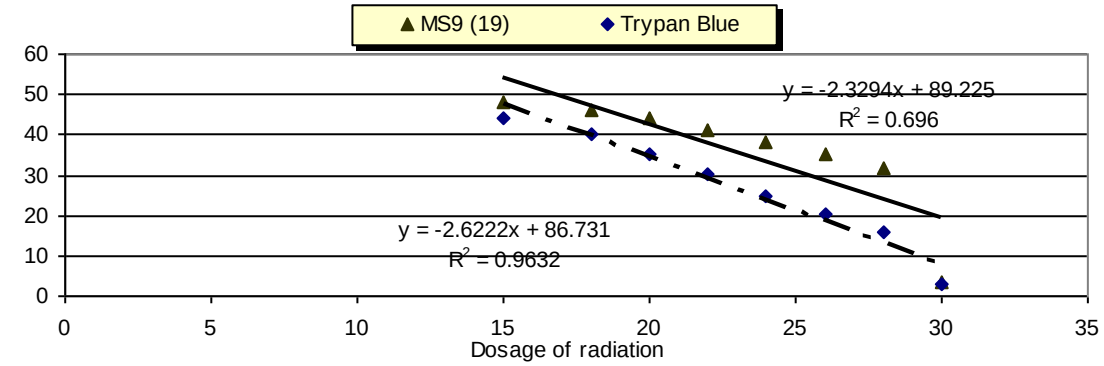

Figure 2: The correlation between mean lymphocyte cell percent difference change and different radiation dosage for the two methods (r=0.84, P=0.0001 for MS9 [19] versus r=0.98, P=0.0001 for Try pan Blue).

The sample of control is considered expired after passing 25 days of storing blood when the count of red blood cells reach to about 45-50 % [13], According to this scientific fact when account of RBC for each sample after irradiation reach to about (45-50 %) the blood is considered expired by two methods so the count of the red blood cells (RBCs) after irradiation of all dosages including control is similar, as shown in (**Table 2, Figure 3** & 4). This is in agreement with radiation effect reported by other researchers in the field [4,8,16,18].

Table 2: The red blood cells (RBCs) changes in control and after different radiation dosage exposure with percent different decrease (mean ± SD) and range (minimum-maximum) for both methods (when RBC reaches to (45-50 %) this means samples are expired).
So the % RBC for all samples including control is similar

	MS9 [19] (RBC %)	Try pan Blue (RBC %)
Control	44.83±3.13 (41.25-47.00)	49.33±1.15 (48.00-50.00)
15 Gray	54.19±3.82 (49.86-57.06)	49.67±1.53 (48.00-51.00)
18 Gray $	54.99±1.00 (53.89-55.84)	50.00±2.00 (48.00-52.00)
20 Gray $	55.00±0.45 (54.57-55.46)	49.67±0.58 (49.00-50.00)
22 Gray	55.05±0.52 (54.59-55.61)	50.00±5.00 (45.00-55.00)
24 Gray	54.99±0.40 (54.55-55.30)	50.00±4.58 (45.00-54.00)
26 Gray $	55.02±0.21 (54.83-55.24)	50.67±2.08 (49.00-53.00)
28 Gray	52.23±4.46 (47.08-54.88)	50.33±0.58 (50.00-51.00)
30 Gray $	54.95±1.08 (53.85-56.00)	51.00±1.00 (50.00-52.00)

-Data were presented as Mean±SD and range (Minimum-Maximum)

*Significant difference from control using t-test at level of 0.05.

Significant difference from previous (lower) dosage of radiation using t-test at level of 0.05.

$ Significant difference of MS9 [19] from Try pan Blue method using t-test at level of 0.05.

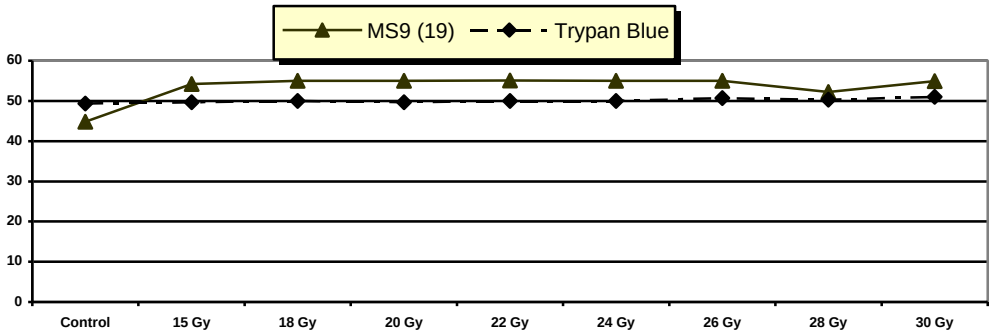

Figure 3: The mean red blood cell % difference change in control & after different dosage for both methods.

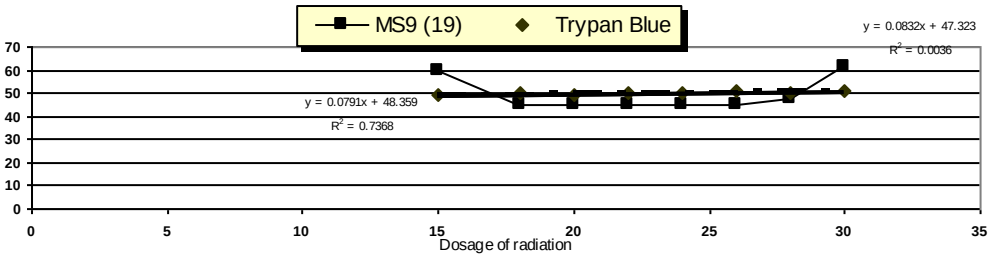

Figure 4: The correlation between mean red blood cell percent difference change and different radiation dosage for the two methods (r=0.06, P=0.879 for MS9 [19] versus r=0.86, P=0.0001 for Try pan Blue).

All doses of radiation between (15 to 30 Gy) had good effect on time of blood storage by two methods (MS9 and Trypan blue) but method of Trypan blue is more accurate than assay by heamocounter MS9 to count the viability of RBC and time of blood storage, as mentioned in (Table 3, Figure 5). This finding is the same as that reported by Sanders et al, Moore & Kitamural, and Shapiro et. Al of RBC changes after irradiation [3,11,13] .

It seems that the dose cause no any significant damage. The blood does not become radioactive" and will not harm you or anyone around you. Gamma irradiation has a negative effect on the physiological K^+ / Na^+ gradient of erythrocytes that the increased permeability of the erythrocyte membrane to K^+ and Na^+ these results indicate in irradiated RBCs, alters the concentrations of intracellular prune nucleotides and increases haemolysis of stored RBCs. This means the radiation irradiation damage the red blood cell membrane sodium-potassium pump, causing leakage of potassium across the cell membrane into the plasma. Plasma potassium levels increase almost twofold within 24 hours. The same observations were reported by Silvergleid (1995) on transfusion–associated graft-versus–host disease changes and that of Hasan (2008) of blood component changes after irradiation [17,18]

In conclusion, the assays by Try pan blue to count the viability of (lymphocyte cells and RBCs) is more accurate than assay by heamocounter MS9 (Haemocounter MS9 was used for automatic counts of blood cells) to know the period of the blood storage. We recommended studying the effect of radiation on time of blood storage by using other new procedures that high technique to determine the times of storage of blood after irradiated to each dose of radiation or by using another sources of radiation such as UN instead of gamma–rays.

Table 3: The mean time of storage of blood for control after different exposure dosage by both methods.

Control	Mean time of storage\days for MS9 [19]	Mean time of storage\days for Try pan Blue
	25.33	24.67
15 Gray $	14.67*	12.67*#
18 Gray $	12.67*	10.67*#
20 Gray	10.67*	9.0*
22 Gray	9.67*	8.0*
24 Gray $	8.67*	7.0*
26 Gray	6.33*	5.67*#
28 Gray	5.33*	4.33*#
30 Gray $	4.33*	2.33*#

*Significant difference from control using t-test at level of 0.05, # Significant difference from previous dosage of radiation using t-test at level of 0.05, $ Significant difference of MS9 [19] from Try pan Blue method using t-test at level of 0.05.

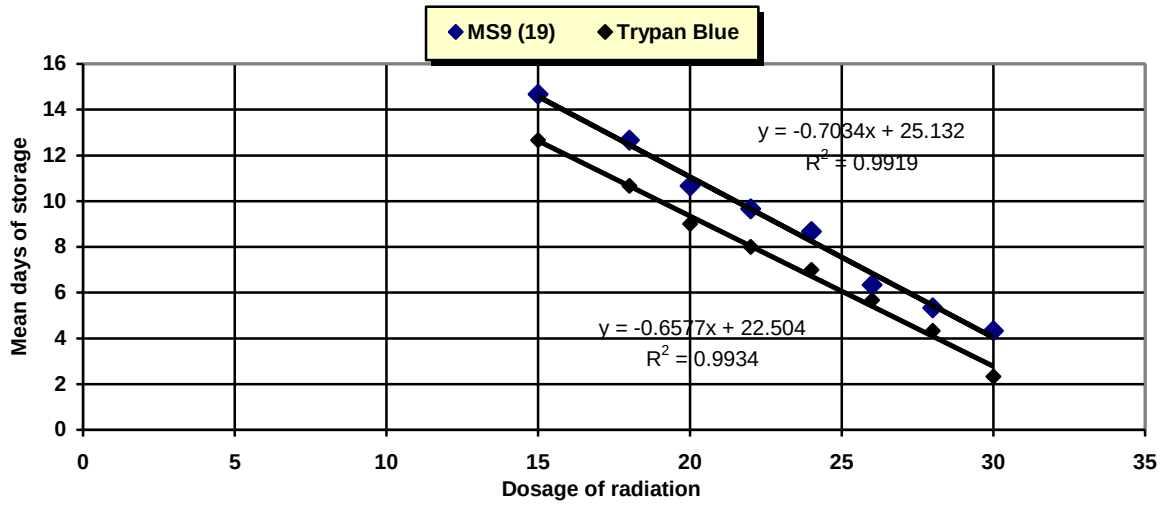

Figure 5: The correlation between mean time of storage of blood for and different radiation dosage exposure for the two methods (r=-0.996, P=0.0001 for MS9 [19] versus r=-0.997, P=0.0001 for Try pan Blue).

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