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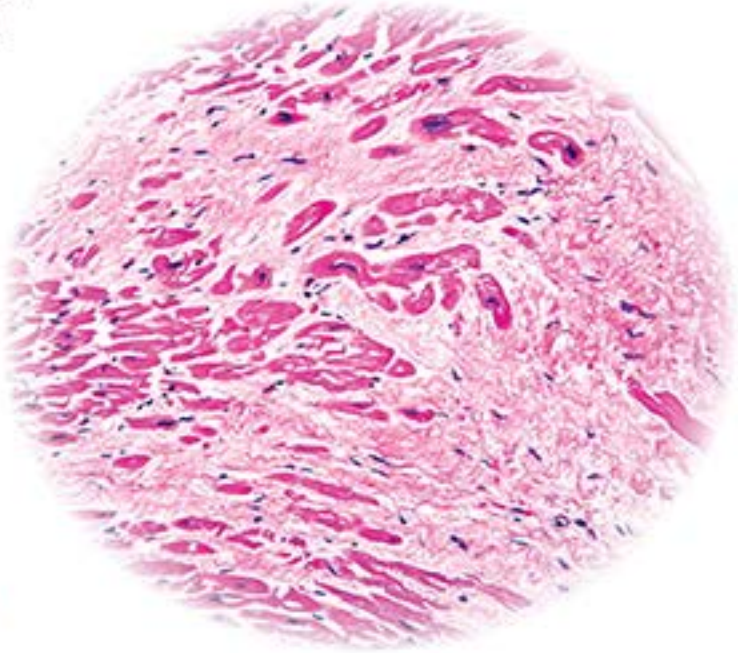



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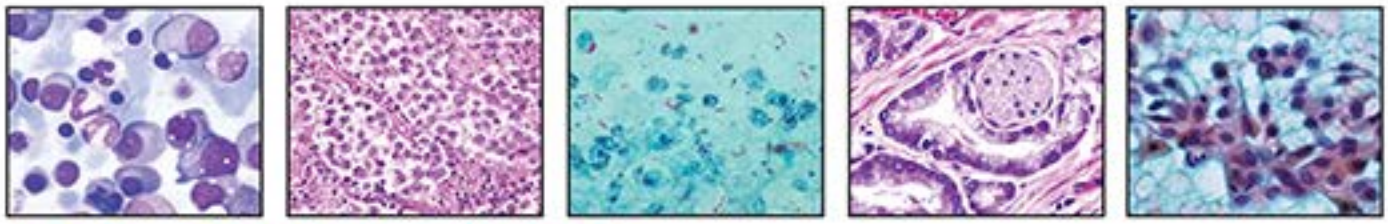

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Competencies (Outcomes)

CORE (MUST KNOW) NON-CORE (DESIRABLE TO KNOW) INTEGRATION ADDITIONAL

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EXERCISE

24 Counting of Blood Cells

Competencies (Outcomes)

CORE (MUST KNOW) | NON-CORE (DESIRABLE TO KNOW) | INTEGRATION | ADDITIONAL

- To estimate Hb, RBC, TLC, RBC indices, DLC, blood groups, BT/CT (Integration PY2.11=Physiology).
- To describe steps for reticulocyte and platelet count (Integration PY2.13=Physiology).
- To learn the principle, techniques and interpretation of counting of WBCs, RBCs, platelets and eosinophils, and to know the conditions causing their abnormal values *versus* normal range of these blood cells.

Counting of circulating blood cells is a basic screening blood test and includes counting of leucocytes (total and differential, i.e. TLC, DLC), red cells and platelets, and sometimes, eosinophils. However, the term *complete blood count(CBC)* is commonly used these days for the following panel of tests determined by electronic particle counter:

1. *Evaluation of WBCs*: TLC, DLC
2. *Evaluation of RBCs*: RBC count, haemoglobin, haematocrit (Hct), red cell indices (MCV, MCH, MCHC, red cell distribution width or RDW)
3. *Evaluation of platelets*: Platelet count, mean platelet volume (MPV), and platelet distribution width (PDW).

WBC COUNT

This is determination of number of white blood cells per µL of blood.

Methods

There are two methods:

1. Visual haemacytometer method
2. Electronic method

Visual Haemacytometer Method

Principle This is counting of WBCs in a calibrated chamber by diluting of blood to 1:20 dilution with diluent which causes lysis of RBCs and stains WBCs.

Diluting fluid Turk's fluid is used which has the following composition:

Glacial acetic acid	:	3.0 ml
1% Aqueous gentian violet	:	2.0 ml
Distilled water	:	195 ml

Procedure

- Suck anticoagulated blood or blood from finger prick up to mark 0.5 in WBC pipette **(Fig. 24.1, A)**.
- Wipe tip and outside of the pipette.

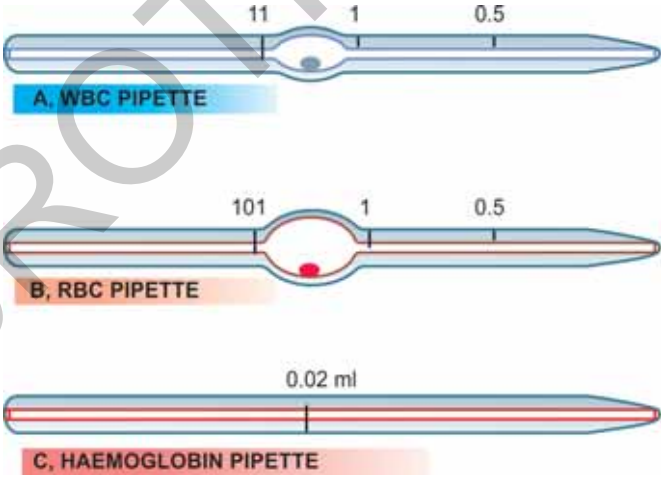

FIGURE 24.1 Pipettes for WBC (A) and RBC counting (B) contrasted with haemoglobin pipette (C).

- Draw diluting fluid up to mark 11 in the WBC pipette to get dilution of 1:20.
- Mix well by rotating the pipette for 2-3 minutes.
- Charge the Neubauer's chamber (haemacytometer) after discarding 1-2 drops of the mixture from the WBC pipette.
- Allow the cells to settle down for 2 minutes.
- Count the WBCs under low power (10X) objective lens of microscope in 4 large corner squares **(Fig. 24.2)**. Count the cells lying on left and lower lines while ignoring those on its right and upper lines.

Calculations*

Volume of area in which WBCs counted in 4 corner squares = $[(1 \times 1 \times 0.1) \times 4]$ mm^3
= 0.4 mm^3

*For calculation of count of WBCs, RBCs and platelets using Neubauer's chamber, remember that the dimensions of corner squares of the chamber are 1 mm each side and depth 0.1 mm. Volume = Length × Breadth × Depth

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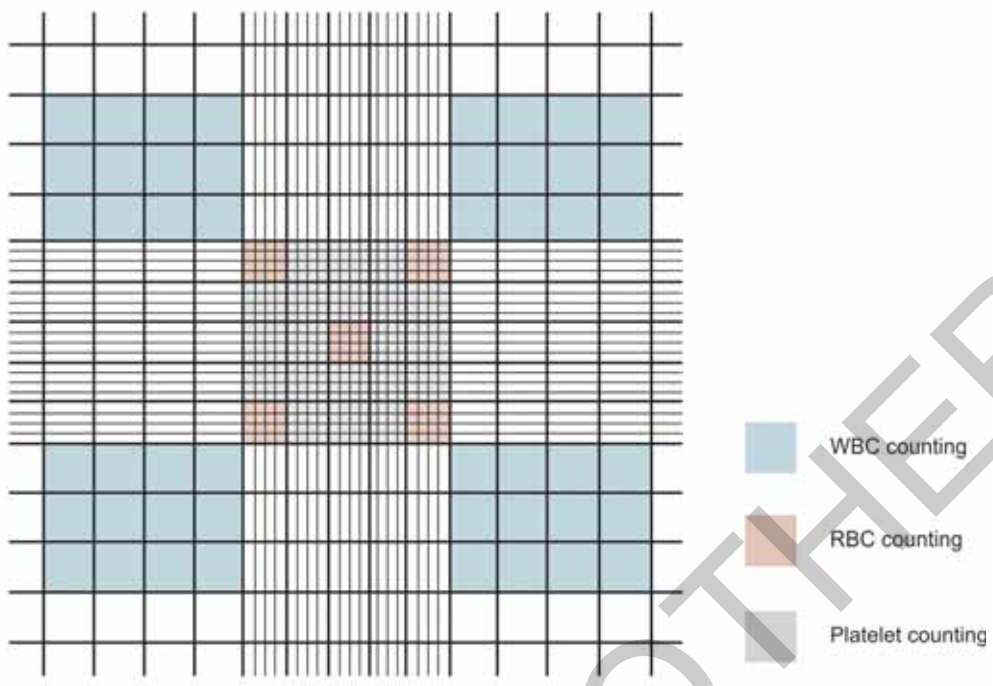

FIGURE 24.2 Improved Neubauer's chamber. Each corner and central large square have an arm of 1 mm while the chamber has depth of 0.1 mm. Counting areas for WBCs, RBCs and platelets are depicted by different colours diagrammatically though the improved Neubauer's chamber does not have any such colours.

$$\text{Number of WBCs in 0.4 mm}^3 = n$$

$$\text{Number of WBCs in 1 mm}^3 = \frac{n \times 10}{4}$$

$$\text{Dilution factor} = 20$$

$$\therefore \text{ Number of WBCs per mm}^3 (\mu L) = \frac{n \times 10}{4} \times 20$$

$$= n \times 50$$

Where n is the total number of WBCs counted in 4 corner squares.

Precautions

i. The work bench must be free of vibrations and chamber should not be exposed to heat.
ii. The cover glass should be of special thickness and should have perfectly flat surface.
iii. The chamber area should be completely filled leaving no air bubbles or debris in the chamber area.
iv. The fluid should not overflow to the moat surrounding the ruled area on the chamber.

Electronic Method

Electronic counter is based on the principle of aperture impedance method, or light scattering technology, or both. In this, particles passing through a chamber in single file scatter the light and convert by a detector into pulses that are proportionate to the size of the cells, which are then counted electronically. A lysate is used to lyse red cells so as to count WBCs.

Advantages

i. Easy and rapid method.
ii. Time saving method.
iii. Very large number of cells is counted rapidly.
iv. There is high level of precision.

Disadvantages

i. Costly equipment.
ii. Calibration error.
iii. Nucleated RBCs are counted as leucocytes.
iv. Platelet clumps counted as leucocytes.

Normal Range for WBC Count

Adults	:	4,000–11,000/μL
Infants at birth	:	10,000–26,000/μL
Children under 1 year	:	6,000–18,000/μL

Causes of Increased WBC Count (Leucocytosis)

i. Microbial infections, e.g. bacterial, viral, parasitic
ii. Leukaemias
iii. Leukaemoid reactions
iv. Pregnancy
v. Diabetic coma
vi. Administration of steroids

Causes of Decreased WBC Count (Leucopenia)

i. Aplastic anaemia
ii. Chemotherapy and radiotherapy
iii. Megalobastic anaemia
iv. Typhoid fever
v. Myelodysplastic syndromes

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RBC COUNT

This is defined as determination of the number of RBCs per µL of blood.

Methods for RBC Counts

1. Visual haemacytometer method
2. Electronic method

Visual Haemacytometer Method

Principle This is counting of RBCs in a calibrated chamber by dilution of blood to 1 in 200 dilution with a diluent which is isotonic to blood. The diluent used prevents clotting, clumping and rouleaux formation and does not destroy WBCs.

Diluting fluids Two types of diluting fluids are used for RBC counting: Hayem's fluid and Dacie's fluid.

Composition of Hayem's fluid

Mercuric chloride	:	0.25 g
Sodium chloride	:	0.5 g
Sodium sulphate	:	2.5 g
Distilled water	:	100 ml

Composition of Dacie's fluid

40% Formaldehyde	:	5ml
3% Trisodium citrate	:	495 ml

Procedure

- Draw anticoagulated blood or blood from finger prick up to mark 0.5 in RBC pipette **(Fig. 24.1, B)**.
- Wipe tip and outside of the pipette.
- Draw diluting fluid up to the mark 101 in the RBC pipette.
- Mix well by rotating the pipette for 2-3 minutes.
- Charge the Neubauer's chamber after discarding 1-2 drops of mixture from the RBC pipette.
- Allow the cells to settle down for 2 minutes.
- Count RBCs under high power 40X objective lens of microscope in 80 tiny squares (5 × 16 tiny squares) in the centre of the chamber as shown in **Figure 24.2**.

Calculations

Volume of area in which RBCs counted in 5 squares

$$= \left[\left(\frac{1}{5}\times\frac{1}{5}\times 0.1\right)\times 5\right] mm^3$$

$$= \frac{1}{50}$$

$$\therefore \text{ Number of RBCs in volume } \frac{1}{50} mm^3 = n$$

$$\text{Number of RBCs in 1 } mm^3 = n \times 50$$

$$\text{Dilution factor} = 200$$

$$\therefore \text{ RBC count per } mm^3(\mu L) = n \times 50 \times 200$$

$$= n \times 10{,}000$$

Where n is the number of RBCs counted in 5 small squares.

Electronic Method

Principle Principle of electronic method for counting RBCs is the same as for WBCs. But unlike WBC counting, no lysate is used; instead anticoagulated blood is diluted with particle-free diluting fluid such as physiological saline or phosphate buffer saline.

Advantages

i. Easy and rapid method.
ii. Many thousands of cells are counted compared to fewer cells counted in manual method.

Disadvantages

i. Costly equipment.
ii. Calibration error.
iii. Altered composition of diluent causes erroneous results.
iv. Giant platelets are counted as RBCs.
v. High WBC count alters results.

Normal Range for RBC Count

Males	:	5.0–6.0 million/µL (5.5 ± 0.5 million/µL)
Females	:	4.5–5.5 million/µL (5 ± 0.5 million/µL)
Children	:	4.0–5.0 million/µL (4.5 ± 0.5 million/µL)

Cause of Decreased RBC Count

i. Anaemia

Cause of Increased RBC Count

i. Polycythemia

PLATELET COUNT

Platelets are thin discs 2-4 µm in diameter. They function in haemostasis, in maintaining vascular integrity and in the process of blood coagulation. Their lifespan is 7-10 days.

Methods for Counting Platelets

1. Visual methods
2. Electronic method

Visual Methods

Type of blood used Use only venous blood as the blood obtained from finger prick causes clumping of platelets.

Diluting fluid 1% ammonium oxalate is prepared as under:

Ammonium oxalate	:	1 g
Distilled water	:	100 ml

Filter it and keep in a refrigerator at 4°C.

Procedure

- Using an RBC pipette, prepare a 1:20 dilution **(Fig. 24.1, B)**.
- Mix for 2 minutes, charge the Neubauer's counting chamber.
- Place the charged Neubauer's chamber into a petri dish having a moist filter paper at bottom for allowing the platelets to settle down.

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- Count the platelets as for red cell count using 40X objective lens of microscope with reduced condenser aperture. Count platelets in the entire central 1 mm^2 area.
- If platelet count is low, then a WBC pipette can be used for charging the Neubauer's chamber.

Calculations

Volume of area in which platelets counted in 25 smaller squares
$= 1 \times 1 \times 0.1\ mm^3$
$= \frac{1}{10}\ mm^3$

$\therefore$ Number of platelets in $\frac{1}{10}\ mm^3 = n$
Number of platelets in 1 $mm^3 = n \times 10$
Dilution factor $= 20$
$= n \times 10 \times 20$
$\therefore$ Platelet count per mm^3 (µL) $= n \times 200$

A phase-contrast microscope can also be used for platelet counting which gives better results.

Rough Visual Method for Platelet Counting

Prepare a thin peripheral blood smear, stain it with any of the Romanowsky stain. Dry it and examine under high power lens of microscope. If you find one clump of platelet per high power field, then number of platelets is adequate; roughly each platelet under high power represents count of 25,000 platelets per mm^3.

Electronic Method

Principle Platelets can be counted by electronic particle counter method which implies electrical impedance principle as for counting RBCs.

Disadvantages

i. Equipment is costly.
ii. Calibration error.
iii. Debris counted as platelets.
iv. Heinz bodies and Howell–Jolly bodies can be counted as platelets.

Normal Platelet Count

150,000–400,000/µL

Conditions Causing Abnormal Platelet Counts

- Decreased count is termed thrombocytopenia.
- Increased count is termed thrombocytosis.

Conditions Causing Thrombocytopenia

1. *Impaired platelet production:*
i. Aplastic anaemia
ii. Acute leukaemias
iii. Myelofibrosis
iv. Marrow infiltration by malignancy
v. Drugs (e.g. chloramphenicol, thiazides, anti-cancer drugs)
vi. Chronic alcoholism

2. *Accelerated platelet destruction:*
i. Idiopathic thrombocytopenia (ITP)
ii. Systemic lupus erythematosus (SLE)
iii. AIDS
iv. Chronic lymphocytic leukaemia (CLL)
v. Disseminated intravascular coagulation (DIC)
vi. Drug-induced (e.g. sulfonamides, quinine, gold)
vii. Microangiopathic haemolytic anaemia
viii. Splenomegaly
ix. Massive transfusion of blood

Conditions Causing Thrombocytosis

i. Essential thrombocytosis
ii. Chronic infection
iii. Haemorrhage
iv. Postoperative state
v. Malignancy
vi. Postsplenectomy

ABSOLUTE EOSINOPHIL COUNT (AEC)

AEC is required for knowing the number of circulating eosinophils in blood in allergic conditions and other causes of eosinophilia.

Methods

There are two methods:
1. Improved Neubauer's chamber method
2. Fuchs–Rosenthal counting chamber method

Diluting Fluid

For both methods, Dunger's diluting fluid is used having the following composition:

Eosin	:	200 mg
Distilled water	:	90 ml
Acetone	:	10 ml

Eosin stains the eosinophil granules bright red, water is used to lyse red cells and acetone is meant for fixation of eosinophils.

Improved Neubauer Chamber Method

Principle This is counting of eosinophils in a calibrated chamber by diluting of blood to 1:10 dilution with diluent which causes lysis of RBCs and stains WBCs.

Procedure

- Suck anticoagulated blood up to mark 1 in WBC pipette **(Fig. 24.1, A)**.
- Wipe tip and outside of the pipette.
- Draw diluting fluid up to mark 11 in the WBC pipette to get dilution of 1:10.
- Mix well by rotating the pipette for 2–3 minutes.
- Charge the Neubauer's chamber after discarding 1-2 drops of the mixture from the WBC pipette.
- Allow the cells to settle down for 2 minutes.

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■ Count the eosinophils under low power (10X) objective lens of microscope in 4 large corner squares as for TLC **(Fig. 24.2)**. Eosinophils are identified by having brightly red granules.

Calculations Volume of 4 squares in which eosinophils counted

$= [(1 \times 1 \times 0.1) \times 4\ mm^3$
$= 0.4\ mm^3$

Number of eosinophils in 0.4 mm^3 = n

Number of eosinophils in 1 mm^3 = $\frac{n \times 10}{4}$

Dilution factor = 10

∴ Number of eosinophils per mm^3 (µL) = $\frac{n \times 10}{4} \times 10$

$= n \times 25$

Where n is the total number of eosinophils counted in 4 corner squares.

Fuchs-Rosenthal Counting Chamber Method

This is a preferred method in which the ruled area for counting is 16 small squares (1 mm each) and depth of chamber is 0.2 mm. Hence, more cells are counted and that gives more accurate result.

Normal Range for AEC

Adults: 40–400/µL

Causes of Abnormal AEC

Increased AEC: Eosinophilia
Decreased AEC: Eosinopenia

The conditions causing eosinophilia and eosinopenia are given in Exercise 27.

Key Questions for Viva Voce

Q. 1. How do we distinguish WBC, RBC and haemoglobin pipettes?
Ans. i) *WBC pipette* has a white bead in its bulb and the pipette has markings of 0.5 and 1 on capillary and 11 on the top of the bulb. ii) *RBC pipette* has a red bead in its bulb and its capillary has similar markings of 0.5 and 1 but the top of the bulb is marked 101. iii) *Haemoglobin pipette* has no bulb and its capillary has a marking of 0.02 ml or 20µL.

Q. 2. Which squares in Neubauer's chamber are used for counting of WBCs, RBCs and platelets?
Ans. i) For WBC counting, four corner squares, each having area of 1 mm^2 are used. ii) For counting of RBCs, the central square which has further divisions into 25 smaller squares is used; in this four peripheral and one central smaller squares are used for counting. iii) For platelet counting, the entire 1 mm^2 area of the central 25 smaller squares is used for counting.

Q. 3. What are the dilutions used for counting of WBCs, RBCs and platelets?
Ans. These are: for WBC count 1:20, for RBC count 1:200 and for platelet count 1: 20.

Q. 4. What are the diluting fluids for counting of WBCs, RBCs and platelets?
Ans. These are: Turks fluid for WBC counting, Hayem's or Dacie's fluid for RBC count, and ammonium oxalate for platelet count.

Q. 5. Give three major causes for leucocytosis.
Ans. i) Bacterial infections, ii) leukaemias and leukaemoid reaction, iii) pregnancy.

Q. 6. Give three major causes of leucopenia.
Ans. i) Aplastic anaemia, ii) typhoid fever, iii) chemotherapy.

Q. 7. What are the major causes of thrombocytopenia?
Ans. i) Aplastic anaemia, ii) acute leukaemias, iii) ITP.

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FIFTH EDITION

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HIGHLIGHTS

As per Competency-based Medical Education Includes 8 sections containing 64 exercises prepared on the pattern of practical class of students: Basic Techniques in Pathology, General Principles of Pathology, Basic Diagnostic Cytology, Haematology, Lymphoid Tissues and Transfusion, Clinical and Applied Pathology, Pathology of Systems, Basics of Autopsy Pathology, and Appendices (Common Instruments in Pathology, Common Similes in Pathology, and Normal Values).

Colour-coded Stratification of Competencies Colour-coded list of competencies/outcomes as per CBME curriculum given in the beginning of each exercise for segregating them in accordance with their relative significance and requirement of the students as: *Core (Must Know)*, *Non-core (Desirable to Know)*, *Integrated Learning*, and *Additional Knowledge*.

Newer Images of High Quality Many gross and microscopic images added and several replaced with better quality, adding more value and clarity in understanding morphologic pathology for the beginner students.

Key Questions for Viva Voce Important short questions with their brief answers at the end of each exercise, based on the core content covered in that exercise. These are the usual questions which the students are often asked in their viva voce, in ward rounds or in clinic which is likely to help the students in facing exam confidently and for self-test.

Complimentary Online Learning Resource Includes two sections: a section on 10 structured CPCs (text and illustrations) representing different organ systems and common diseases, and the second section on Quick Review of Pathology Museum that includes images and legends of 108 gross specimens from pathology museum of common conditions for quick review of *must know* specimens for students.

Although the book is primarily prepared for undergraduate students of pathology, it is also expected to be useful for practicing clinicians and other students of medicine such as those pursuing course in Dentistry, Physiotherapy, Pharmacy, Nursing, Laboratory Technology and alternate systems of medicine.

Harsh Mohan MD FAMS FICPath FUICC

A Gold Medallist in academics from Panjab University, Dr Mohan has served as former founding Professor and Head, Department of Pathology at Government Medical College and Hospital, Chandigarh, India for about 22 years until his superannuation in July 2015. An alumnus of Post Graduate Institute of Medical Sciences (PGIMS), Rohtak, Haryana, India, he initially worked on the faculty of his alma mater in various capacities until 1993. Dr Mohan has over 40 years of rich professional experience in teaching and diagnostic pathology, has published over 300 scientific articles in national and international indexed journals, and has authored 7 books. He was Editor-in-Chief of Indian Journal of Pathology and Microbiology (2003-07), and President of Indian Association of Pathologists and Microbiologists (2008). Besides other awards and honours, he was conferred Haryana Vigyan Ratna (2010-11), in recognition of his outstanding contribution to medical science and education.

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