

# Laboratory Investigation in Hematology

How to decide what a number is worth

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College of Medicine · Clinical Hematology · Lecture 1

These slides are an outline only. Read the accompanying material with them,  
along with the casebook and the question banks.





## Lecture outline

# What we will cover

- Part I — Before you believe a number: why we test, where the pipeline fails, and how to reason from a result.
- Part II — The tests themselves: the CBC, the film, the panels, coagulation, transfusion and advanced diagnostics.
- One framework runs through both: the five stations every result passes before it reaches you.
- The reference intervals used here are local. So is much of the evidence.



By the end of this lecture

## Learning outcomes

- State the question a test is meant to answer before ordering it.
- Locate a surprising result on the five-station pipeline.
- Recognise and resolve the common pre-analytic and analytic artefacts.
- Explain what a reference interval is, and why ours is not Jeddah's.
- Distinguish sensitivity and specificity from predictive value.
- Use a result to update a probability rather than to make a diagnosis.

Part I

# Before you believe a number



## Two questions

# Which question are you asking the laboratory?

### I suspect a diagnosis. Let me confirm it.

- You already have a clinical theory.
- The test moves you toward it or away from it.
- صح عليك

### I don't know what is going on. Let me order tests and see.

- There is no theory to test.
- The panel is a substitute for thinking.
- غلط ويا خسارة

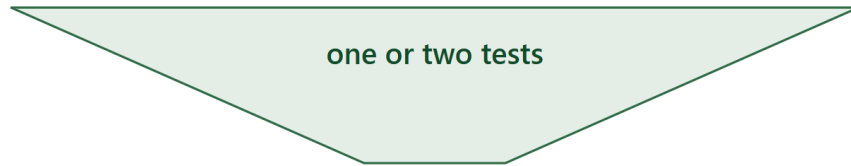


## STATION 1 • THE QUESTION

# Two different questions — and they cost differently

### **"I suspect this. Confirm it."**

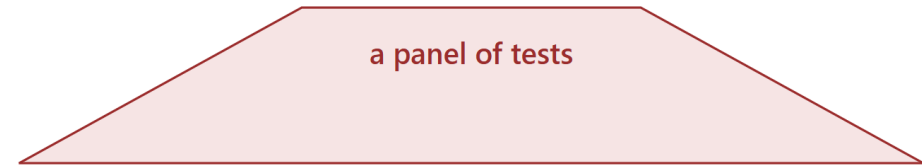
You already have a diagnosis in mind.



**A clear answer either way**

### **"Something is wrong. Find it."**

You are searching, not testing an idea.



**More results. More of them abnormal.**

The wider you cast, the more "abnormals" you catch that were never disease.



The rule for this lecture

## On ordering tests

*Use the laboratory to test a theory generated by clinical data. Do not use the laboratory to generate the theory.*

Everything that follows is a consequence of this sentence



## The spine of this lecture

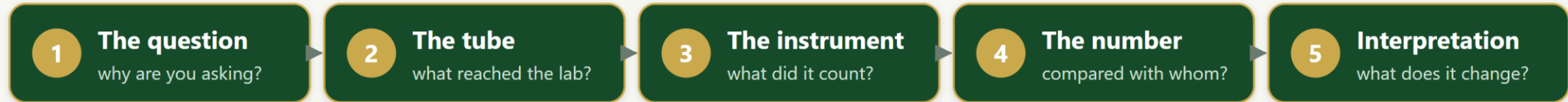
# Five stations between the patient and the number

|                   | What it is             | How it fails                                |
|-------------------|------------------------|---------------------------------------------|
| 01 The question   | Why you are testing    | A panel ordered instead of a hypothesis     |
| 02 The tube       | Pre-analytic           | A perfect measurement of the wrong material |
| 03 The instrument | Analytic               | The right sample, the wrong counting rule   |
| 04 The number     | The reference interval | Someone else's normal                       |
| 05 The meaning    | Interpretation         | A true result, believed too much            |



## THE SPINE OF THIS LECTURE

# Five places between your patient and the number



The patient



The number

**A result can fail at any one of the five. Ask which one before you believe it.**



Station 1 · The question

## What a test costs when it answers nothing

- Money — the direct cost of the assay, and the smallest of the four.
- Cascade — an unexplained abnormal recruits a repeat, an image, a referral.
- Harm — somewhere at the end of a cascade sits a biopsy or a treatment.
- Attention — a page of numbers you did not need hides the one that mattered.
- Our own audits of serum B12 and folate ordering both ended in the same recommendation: explicit ordering guidance.



## STATION 1 • THE QUESTION

# What a test costs when it answers nothing

### 1 Money

Paid by the patient or the system, for information nobody uses.

### 2 Time

A result you wait for is a decision you delay.

### 3 Harm

Needles, blood volume, and anxiety while waiting.

### 4 The next test

One odd result pulls in the next, and the next.

**Before you order: say what you expect, and what you will do with either answer.**



Before you order

## Two sentences, out loud

Say what result you expect. Then say what you will do differently if the result is the opposite. If you cannot answer the second, you are at station 1 without a question.



## The platelet count that was never low

- A well 34-year-old, pre-operative: Hb 13.9, WBC 6.4, platelets  $34 \times 10^9/L$ .
- No bruising, no bleeding, no petechiae, no drugs, normal examination.
- Surgery cancelled. Haematology referral. Someone writes “ITP” and steroids start to look reasonable.
- Nothing about this patient is thrombocytopenic.



## EDTA-dependent pseudothrombocytopenia

# The artefact, and the two-minute resolution

### What happened

- EDTA lets clinically silent antibodies agglutinate platelets.
- The analyser counts a clump as one particle, or as none.
- Roughly one to two per thousand hospital patients.

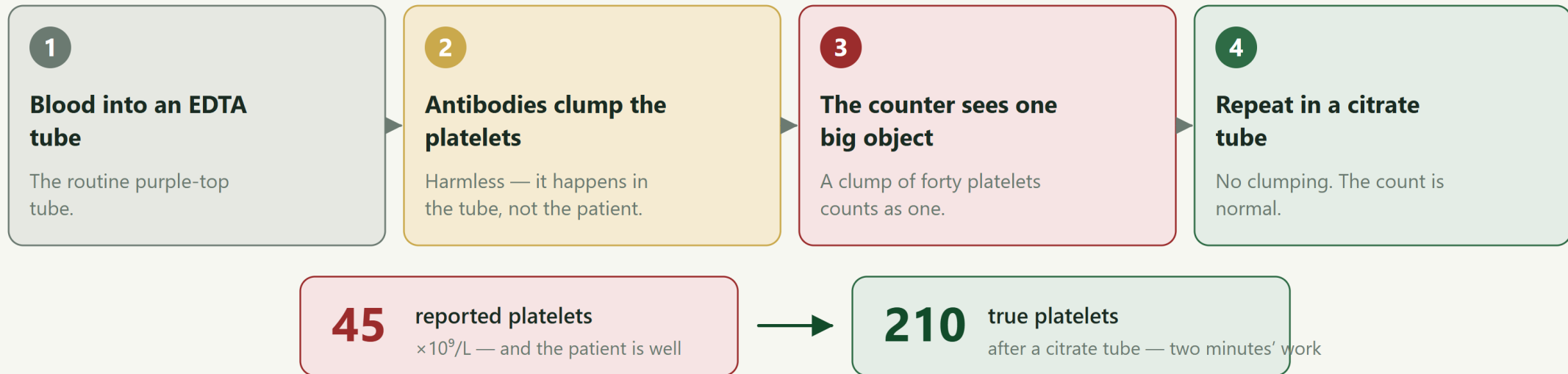
### What to do

- Look at the film — clumps are unmistakable.
- Repeat the count in citrate or heparin, well mixed.
- The film did not add a test. It audited station 2.



## STATION 2 • THE TUBE

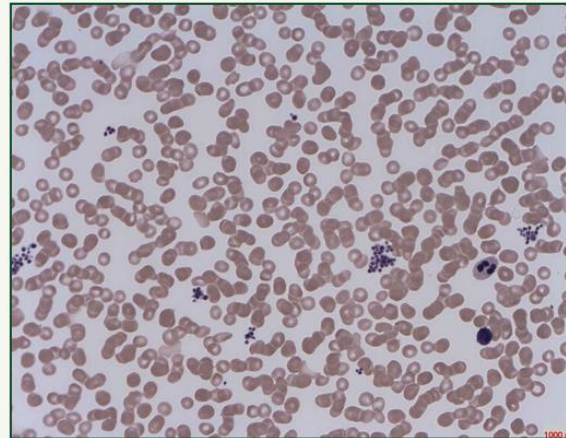
# The platelet count that was never low



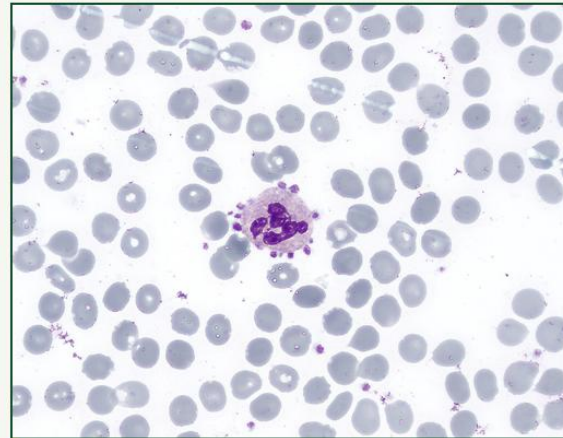


STATION 2 • ON THE FILM

## What the clumping actually looks like



**Platelets in a clump**  
counted as one — the count reads low



**Platelets round a white cell**  
same false low count

Images: Wikimedia Commons — Platelet Clumping 8 (CC BY-SA 4.0); Platelet Satellitism (CC BY-SA 4.0). Full credits in CREDITS.md.



Station 2

# The other pre-analytic classics

|                           | What it does to the result                                             |
|---------------------------|------------------------------------------------------------------------|
| Under-filled citrate tube | Too much anticoagulant per unit plasma — PT and aPTT prolong           |
| Clotted specimen          | Consumed platelets and factors — low count, long times, both false     |
| Drip-arm sample           | Diluted by whatever is running into that vein                          |
| Delay and temperature     | Potassium leaks, labile factors decay, cells swell and MCV rises       |
| Haemolysed sample         | Raises LDH and potassium — imitates the hemolysis you were testing for |



### Station 3 · The instrument

## What the analyser is actually counting

- The machine cannot see a platelet. It sees a particle of a certain size crossing an aperture, and applies a rule.
- Impedance sizes the particle; anything in the platelet window is called a platelet.
- The rule is simple, fast, and wrong whenever something else is the wrong size.
- Pseudothrombocytosis is therefore an analytic error, not a pre-analytic one — a different tube will not fix it.



### Station 3

## When the counting rule meets the wrong particle

|                                         | Reported as            |
|-----------------------------------------|------------------------|
| Schistocytes and red cell fragments     | Falsely high platelets |
| Severe microcytosis                     | Falsely high platelets |
| Leukaemic blast fragments               | Falsely high platelets |
| Cryoglobulin, bacteria, fungal elements | Falsely high platelets |
| Giant platelets                         | Falsely low platelets  |
| Platelet clumps                         | Falsely low platelets  |



### STATION 3 • THE INSTRUMENT

## The machine never sees a cell. It measures a signal.

#### Impedance — size by volume



A cell crossing a hole changes the current.  
Taller pulse = bigger cell.

#### Light scatter — size and inside



Laser light bounces off each cell.  
Where it lands sorts the cell.

#### When the rule meets the wrong particle

- A giant platelet  
→ sized as a red cell
- A red cell with a nucleus  
→ counted as a white cell
- A clump of platelets  
→ counted as one

The counting rule is right. The particle is wrong. Look at the film.

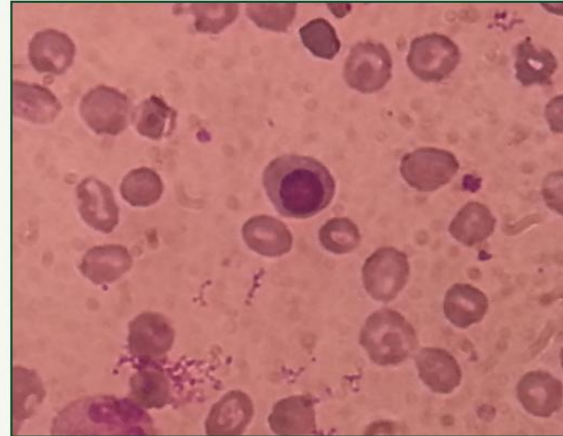


### STATION 3 • ON THE FILM

## Two particles the counting rule was not written for



**A giant platelet**  
as big as a red cell, so sized as one



**A red cell with a nucleus**  
counted as a white cell

Images: Wikimedia Commons — Giant Platelet, Peripheral Blood Smear (6032662354) (CC BY 2.0); Nucleated red blood cell (RBC) in a Wright's stained peripheral blood smear (PBS) microscopy of an aplastic anemia patient (CC BY-SA 4.0). Full credits in CREDITS.md.



A question for you

## The citrate problem

- Why do patients with polycythaemia — especially extreme polycythaemia — have falsely prolonged PT and aPTT?
- The tube holds a fixed volume of citrate in a 9:1 ratio, and the citrate dissolves in the plasma, not the whole sample.
- Raise the haematocrit and the plasma fraction shrinks, so the same citrate is concentrated in less plasma.
- The added calcium is partly consumed neutralising the excess, and the clot forms late.
- The patient's coagulation is normal. The tube was wrong.



The fix

## Correct the citrate to the haematocrit

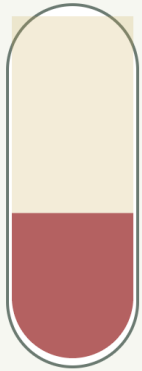
$C = (1.85 \times 10^{-3}) \times (100 - \text{Hct}) \times V \text{ blood}$ . Above a haematocrit of 55%, a 4.5 mL draw at Hct 70 needs 0.25 mL of citrate rather than the standard 0.50 mL.



## STATION 2 · THE TUBE

# Why a very high haematocrit gives a long clotting time

Normal



45% cells · plenty of plasma

Polycythaemia



70% cells · little plasma

The citrate in the tube is a fixed amount.

With less plasma, that same citrate is now too strong for it. In the analyser the sample is over-anticoagulated, and the PT and aPTT come back long — from the tube, not the patient.

**The fix: ask the lab to re-draw with the citrate corrected to the haematocrit**

$$C = (1.85 \times 10^{-3}) \times (100 - \text{Hct}) \times \text{volume of blood}$$

**Above about 55% haematocrit, correct it.**



## What “normal range” actually means

- A reference interval is the middle 95% of a reference population — not the range of health.
- By construction, one healthy person in twenty falls outside it.
- The people at the edges were not ill. They were the edges.
- Almost every over-investigation you will cause begins by forgetting this.



Station 4

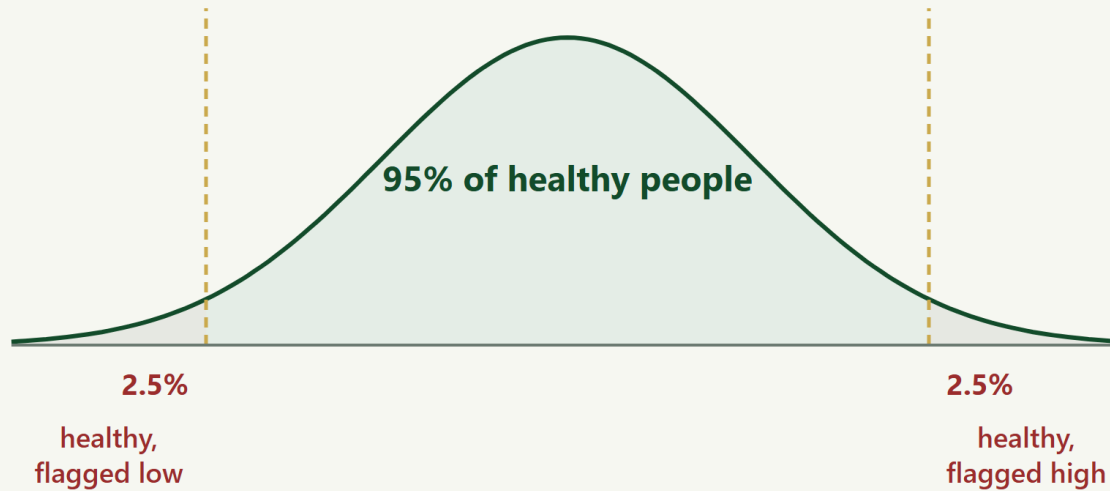
# Order more tests, find more “abnormals”

|    | Chance of at least one abnormal |
|----|---------------------------------|
| 1  | 5.0%                            |
| 5  | 22.6%                           |
| 10 | 40.1%                           |
| 20 | 64.2%                           |
| 30 | 78.5%                           |



#### STATION 4 • THE NUMBER

“Normal range” means the middle 95% of healthy people



**An abnormal flag is not a disease. It is a position on this curve.**

#### Order more, find more “abnormals”

1 test      **5%**      chance of one flag

7 tests      **30%**      chance of at least one

20 tests      **64%**      chance of at least one

...in a perfectly healthy person.



#### Station 4

## The interval belongs to a population, not to humanity

- Every interval carries hidden conditions: age, sex, ancestry, physiological state, environment.
- Isolated neutropenia by the printed interval is common in this region, and often constitutional rather than any disease.
- Applying an interval derived elsewhere converts a normal person into a haematology referral.



Station 4 · Where you stand

## The reference range is local — and here it is 2,270 m up

- Abha sits at roughly 2,270 m. Jeddah is at sea level. Same patient, same analyser, same assay — different normal.
- Hypobaric hypoxia contracts plasma volume, then expands red cell mass. Both raise the haemoglobin concentration.
- Haemoglobin and red cell indices differ systematically with altitude across regions of Saudi Arabia, and the effect interacts with age.
- International practice adds roughly 0.8 g/dL to the anaemia threshold at 2,000 m and 1.3 g/dL at 2,500 m.



Six months at altitude · measured by CO rebreathing

## Newcomers versus long-term residents

|                             | Newcomers     | Residents     | p       |
|-----------------------------|---------------|---------------|---------|
| Haemoglobin mass (g/kg LBM) | 15.65         | 18.01         | < 0.001 |
| Red cell volume (mL/kg LBM) | 48.0          | 54.2          | < 0.001 |
| Blood volume (mL/kg LBM)    | 98.9          | 109.8         | < 0.001 |
| Haemoglobin concentration   | no difference | no difference | 0.200   |
| Haematocrit                 | no difference | no difference | 0.952   |



The point of that table

## Concentration is not quantity

*Two groups whose oxygen-carrying capacity differs by 13% had the same haemoglobin and the same haematocrit.*

You are being shown a ratio and thinking about a mass



## STATION 4 · WHERE YOU STAND

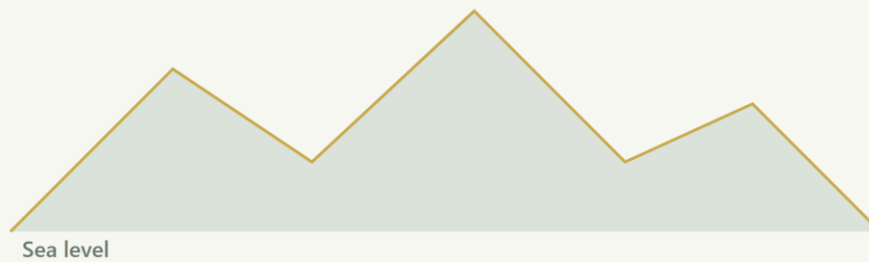
# The reference range is local — and here it is 2,270 m up

### The generic correction

add 0.8 g/dL at 2,000 m · 1.3 g/dL at 2,500 m

Better than nothing. Measuring your own population is better.

Abha · 2,270 m



Healthy men here: 13.5 – 18.3 g/dL

Oxygen-carrying capacity differed by 13%. The haemoglobin and the haematocrit were the same.

### Measured directly, by CO rebreathing

37 healthy men, six months at altitude

|                           | Newcomers | Residents |           |
|---------------------------|-----------|-----------|-----------|
| Haemoglobin mass LBM      | 15.65     | 18.01     | p < 0.001 |
| Red cell volume mL/kg LBM | 48.0      | 54.2      | p < 0.001 |
| Blood volume L/kg LBM     | 98.9      | 109.8     | p < 0.001 |
| Haemoglobin concentration | —         | —         | p = 0.200 |
| Haematocrit               | —         | —         | p = 0.952 |

no significant difference in either concentration



Station 5 · Interpretation

## Four numbers that describe any test

01

### Sensitivity

Of those with disease, the proportion called positive. Negative result rules out.

02

### Specificity

Of those without disease, the proportion called negative. Positive result rules in.

03

### PPV

Of those testing positive, the proportion who have the disease. Moves with prevalence.

04

### NPV

Of those testing negative, the proportion truly free of disease. Moves with prevalence.



## Station 5

# Two distinctions worth keeping

- Sensitivity and specificity belong to the test. Predictive values belong to the patient.
- The same test has an excellent PPV in the clinic and a poor one in a screening population.
- Precision is not accuracy: an instrument out of calibration is often exquisitely precise.
- It gives you the same wrong number, with great confidence.



## STATION 5 · INTERPRETATION

# Four numbers, and which way each one reads

| The patient   |                 |                |
|---------------|-----------------|----------------|
|               | Has the disease | Does not       |
| Test positive | True positive   | False positive |
| Test negative | False negative  | True negative  |

### Read across instead

PPV — my test is positive; how likely is disease?

NPV — my test is negative; how likely am I clear?

These two depend on how common the disease is.

**Sensitivity — read down**  
of those WITH disease, how many test positive

**Specificity — read down**  
of those WITHOUT, how many test negative

**Sensitivity and specificity belong to the test. PPV and NPV belong to your patient.**



## Bayes at the bedside

- A test result updates a probability you already held. Hold none, and the result cannot mean anything.
- $LR+ = \text{sensitivity} \div (1 - \text{specificity})$ .  $LR- = (1 - \text{sensitivity}) \div \text{specificity}$ .
- A D-dimer with sensitivity 98% and specificity 40% gives  $LR- = 0.05$  and  $LR+ = 1.63$ .
- Sensitive, not specific — and that asymmetry is the whole clinical story.



Worked example · suspected pulmonary embolism

# The same test, two patients

|                 | D-dimer negative      | D-dimer positive |
|-----------------|-----------------------|------------------|
| 15% (low risk)  | 0.87% — safe to stop  | 22.4% — image    |
| 30% (moderate)  | 2.1% — borderline     | 41.1% — image    |
| 60% (high risk) | 7.0% — not low enough | 71.0% — image    |



Why a positive D-dimer diagnoses nothing

## An LR+ of 1.63 is a feeble multiplier

Fibrin degradation products rise in infection, malignancy, trauma, surgery, pregnancy, liver disease and simple old age. A positive D-dimer means “I could not rule this out” — it is an instruction to image, never a diagnosis.



## STATION 5 • INTERPRETATION

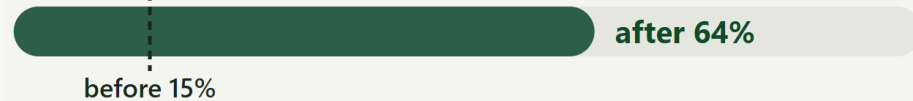
# A test moves a probability you already had

**D-dimer positive • LR+ 1.63**



A feeble multiplier. You have barely learned anything.

**A strong test • LR+ 10**



The same starting point, moved somewhere useful.

### Why the D-dimer diagnoses nothing

Fibrin degradation products rise in infection, surgery, cancer, pregnancy and old age. Almost anything raises it.

Its value is the other direction: a negative D-dimer in a low-risk patient is genuinely reassuring.

**Ask what you believed before the test. A result updates that; it does not replace it.**



Carry forward

## Part I in five lines

- Order a test to answer a question you have already formed.
- When a result surprises you, ask which station failed — not whether it is true.
- One healthy person in twenty falls outside any reference interval, by design.
- At 2,270 m the normal range is genuinely different, and concentration is not quantity.
- A result updates a probability. It does not replace one.

Part II

# The tests themselves



## The map

# Four questions, and the panels that answer them

01

### The counts

Too high, too low, right size, marrow responding? CBC, film, reticulocytes.

02

### The causes

Enough iron? Red cells destroyed? Which haemoglobins? Iron studies, hemolysis panel, electrophoresis.

03

### Hemostasis

Which arm is broken, and is a factor missing or blocked? PT, aPTT, TT, mixing studies.

04

### Transfusion and malignancy

Can this unit be given? What is this clone? Group and screen, DAT, flow, cytogenetics.



## PART II · THE MAP

# Four questions, and the panels that answer them

### The counts

01

Too high, too low, right size? Is the marrow responding?

---

CBC · blood film ·  
reticulocytes

### The causes

02

Enough iron? Are cells being destroyed? Which haemoglobins?

---

iron studies · hemolysis panel  
· electrophoresis

### Hemostasis

03

Which arm is broken — and is a factor missing or blocked?

---

PT · aPTT · TT · mixing studies

### Transfusion & malignancy

04

Can this unit be given? What is this clone?

---

group & screen · DAT · flow ·  
cytogenetics

**Learn the question each panel answers, and you will not need to memorise the panel.**



Station 3, made concrete

## What the analyser actually does

- Impedance — a cell crossing an aperture raises resistance; pulse height gives volume, pulse count gives number. This is where MCV comes from.
- Light scatter and fluorescence — size, granularity and nucleic acid content. This gives the differential, and counts reticulocytes by their residual RNA.
- Spectrophotometry — haemoglobin measured chemically on lysed blood. The one red cell parameter neither counted nor sized.



## STATION 3 • MADE CONCRETE

# Three ways the analyser sorts a cell

### Impedance

A cell crossing a small hole briefly blocks a current.

---

Gives size — and therefore MCV.

### Light scatter

A laser hits the cell; detectors sit at two angles.

---

Forward = how big. Side = how grainy inside.

### Staining

Dyes bind to particular contents before counting.

---

Separates the five kinds of white cell.

**None of these is looking at a cell. Each measures a signal and applies a rule.**

When the rule meets a particle it was not written for, the number is wrong — and the film shows why.



Know which is which

## Measured versus derived

### Measured directly

- Haemoglobin
- Red cell count
- MCV
- White cell count
- Platelet count

### Calculated from those

- $Hct = MCV \times RBC$
- $MCH = Hb \div RBC$
- $MCHC = Hb \div Hct$
- RDW = width of the volume distribution
- A derived value inherits every error of its parents.



## KNOW WHICH IS WHICH

**Some numbers are measured. Others are worked out.**

### Measured directly

- Haemoglobin
- Red cell count
- MCV (cell size)
- White cell count
- Platelet count

### Calculated from those

- Haematocrit =  $\text{RBC} \times \text{MCV}$
- MCH =  $\text{Hb} \div \text{RBC}$
- MCHC =  $\text{Hb} \div \text{Hct}$

**A calculated number carries every error of the numbers it came from. If the MCV is wrong, the haematocrit is wrong too.**



## The core test

# Reading a CBC — and where each number misleads

|               | Range                             | Where it misleads                                                 |
|---------------|-----------------------------------|-------------------------------------------------------------------|
| Hb            | M 13.5–18.3 (Abha) · F 12–17 g/dL | A concentration, not a mass — moves with plasma volume            |
| MCV           | 80–100 fL                         | An average conceals a mixed population; cold agglutinins raise it |
| MCHC          | 32–36 g/dL                        | Above 36 — spherocytes, cold agglutinins, or optical interference |
| RDW           | 11.5–14.5%                        | Rises early in iron deficiency; normal in thalassaemia trait      |
| Reticulocytes | 0.5–2.5%                          | A proportion of a shrunken denominator — use the absolute count   |
| Platelets     | 150–400 ×10 <sup>9</sup> /L       | The most artefact-prone number on the page                        |



## THE CORE TEST

# Reading a CBC — and where each number misleads

### Reading the CBC

The panel, the red-cell indices, and what each number tells you

| COMPLETE BLOOD COUNT |       |           |
|----------------------|-------|-----------|
| Test                 | Value | Reference |
| WBC                  | 7.4   | 4.0-11.0  |
| Hemoglobin           | 14.2  | 13-17     |
| Hematocrit           | 42    | 40-50     |
| MCV                  | 88    | 80-100    |
| MCH                  | 29    | 27-33     |
| MCHC                 | 33    | 32-36     |
| RDW                  | 13.0  | 11.5-14.5 |
| Platelets            | 250   | 150-400   |

#### Three questions every CBC answers

1. Are the RED CELLS adequate? → hemoglobin / hematocrit
2. What SIZE are the red cells? → MCV (micro / normo / macro)
3. Are WBCs & PLATELETS normal? → counts + differential

#### MCV

average cell SIZE

low = microcytic  
high = macrocytic

#### MCH / MCHC

hemoglobin CONTENT

low = hypochromic  
(pale cells)

#### RDW

size VARIABILITY

high = mixed sizes  
(anisocytosis)

#### Worked example

Hb 9.0 ; MCV 72 ; RDW 17 :

Microcytic anemia with high size-variability —  
classic for iron deficiency. Confirm with ferritin.



## The two-number method

# Anaemia, in two questions

### Size — the MCV

- Microcytic below 80 fL
- Normocytic 80–100 fL
- Macrocytic above 100 fL
- That single split does most of the work.

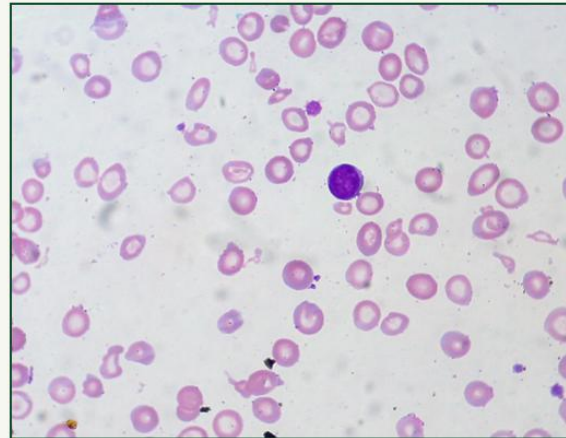
### Response — the reticulocytes

- High: marrow replacing losses — bleeding or hemolysis
- Low or inappropriately normal: the marrow is the problem
- Order it with the CBC, not after it.

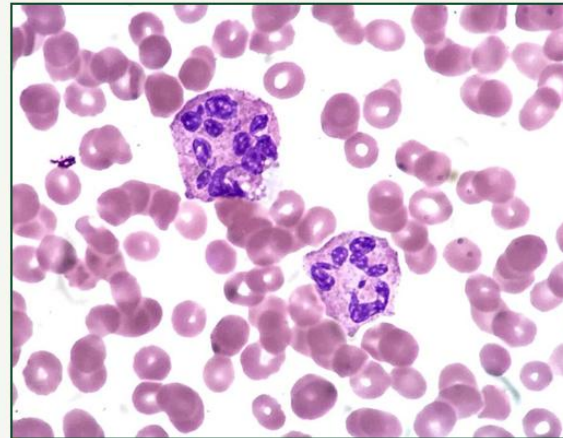


## THE TWO-NUMBER METHOD

# Size, on the film



**Small and pale**  
iron deficiency



**A neutrophil with 5+ lobes**  
B12 or folate deficiency

Images: Wikimedia Commons — Iron-deficiency Anemia, Peripheral Blood Smear (4422704616) (CC BY 2.0); Hypersegmented Neutrophils (36831145373) (CC BY 2.0). Full credits in CREDITS.md.



The most under-ordered test

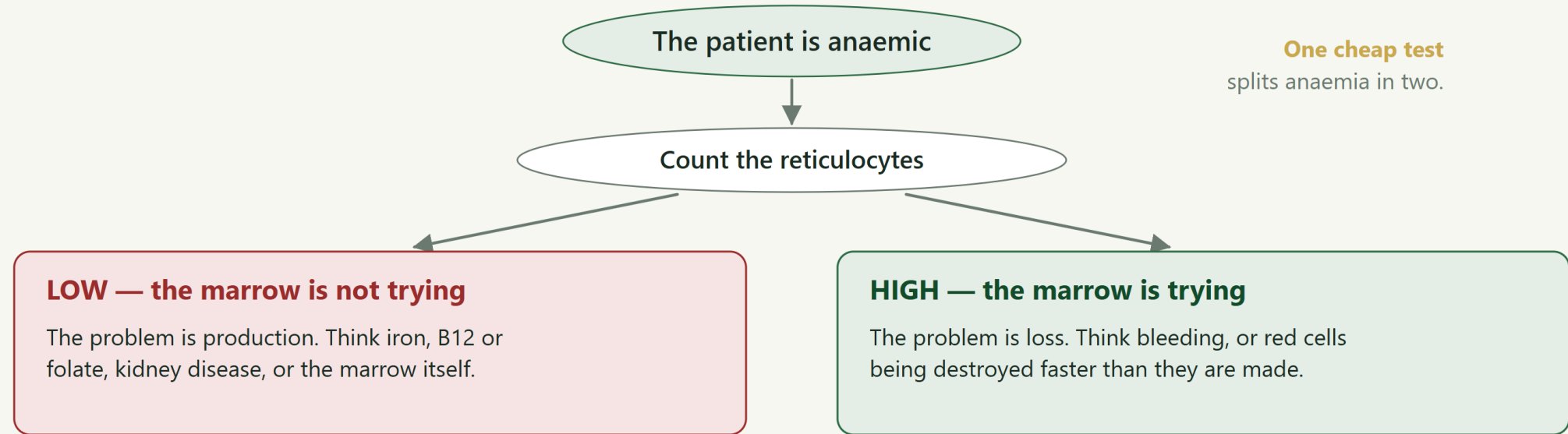
## Reticulocytes: is the marrow trying?

- A reticulocyte is a red cell released with residual RNA. No other test asks whether the marrow is responding.
- The percentage misleads, because it is a proportion of a reduced red cell population.
- Absolute count = reticulocyte %  $\times$  red cell count. The cleanest single number.
- Reticulocyte production index above roughly 2 indicates an adequate response; below 2, inadequate.
- Reticulocyte haemoglobin content reports iron available now — useful where ferritin is least trustworthy.



## THE MOST UNDER-ORDERED TEST

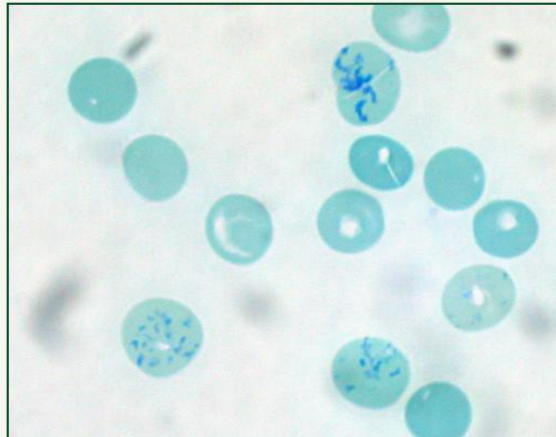
# Reticulocytes answer one question: is the marrow trying?





## THE MOST UNDER-ORDERED TEST

# Young red cells, stained so you can count them



### Reticulocytes

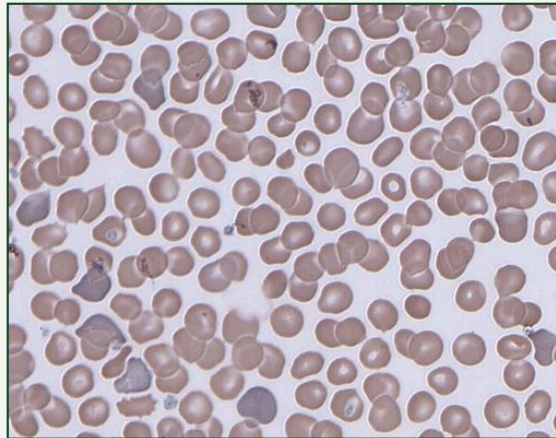
the blue mesh marks a young red cell

Images: Wikimedia Commons — Reticulocytes Human Blood Supravital Stain (CC BY 3.0). Full credits in CREDITS.md.



## THE MOST UNDER-ORDERED TEST

# The same young cells, on a routine stain



### Polychromasia

the larger, bluer cells are the young ones

Images: Wikimedia Commons — Polychromasia 1, Osaretin (CC BY-SA 4.0). Full credits in CREDITS.md.



Looking, not counting

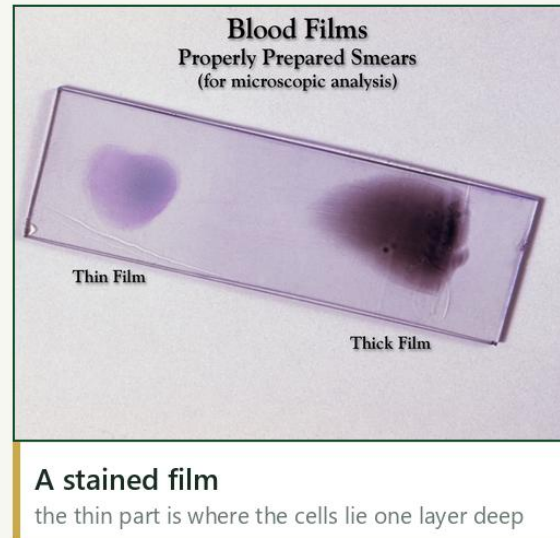
## The blood film

- The CBC counts cells; the film lets you look at them. Numbers say something is wrong; the film often says what.
- Request one for any unexpectedly abnormal CBC — especially any you are about to act on.
- Essential, not optional, in suspected hemolysis, leukemia or microangiopathy.
- An analyser flag is a request for a film, not a footnote.
- Describe four things in order: size, shape, colour, inclusions.



LOOKING, NOT COUNTING

## The film itself



Images: Wikimedia Commons — Blood film 01 (Public domain). Full credits in CREDITS.md.



Can't-miss morphologies

# One finding, one diagnosis

|                            | Think of                                       |
|----------------------------|------------------------------------------------|
| Schistocytes               | Microangiopathic hemolysis — TTP, HUS, DIC     |
| Spherocytes                | Hereditary spherocytosis; autoimmune hemolysis |
| Target cells               | Thalassaemia, liver disease, post-splenectomy  |
| Tear-drop cells            | Marrow fibrosis or infiltration                |
| Howell–Jolly bodies        | Hyposplenism, post-splenectomy                 |
| Blasts and Auer rods       | Acute leukemia                                 |
| Hypersegmented neutrophils | B12 or folate deficiency                       |



## CAN'T-MISS MORPHOLOGIES

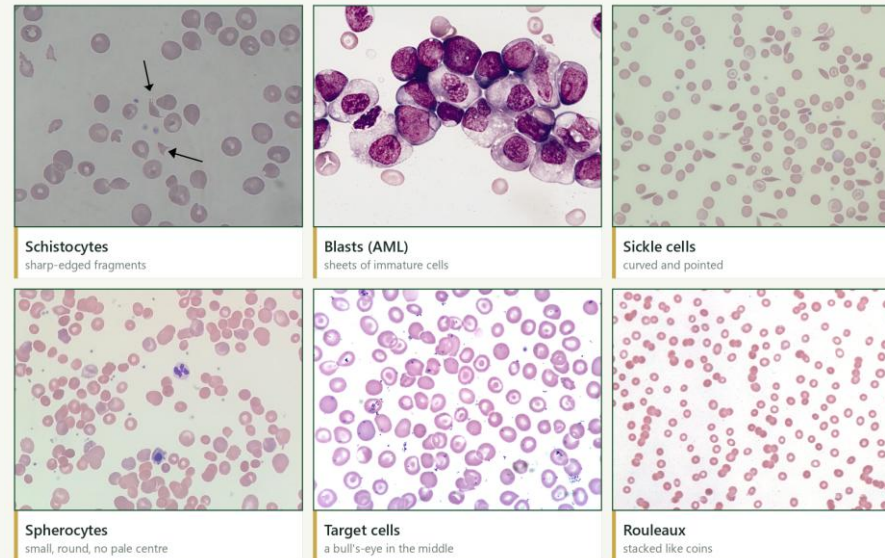
# The shapes, drawn

| Blood film — can't-miss morphologies                                                                                                                                  |                                                                                                                                                                  |                                                                                                                                                                 |                                                                                                                                                                 |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Schematic red-cell shapes and the diagnoses they point to                                                                                                             |                                                                                                                                                                  |                                                                                                                                                                 |                                                                                                                                                                 |
|  <p><b>Normal RBC</b><br/>central pallor -15<br/>reference</p>                       |  <p><b>Microcyte</b><br/>small, pale<br/>iron deficiency,<br/>thalassemia</p> |  <p><b>Macrocyte</b><br/>large, oval<br/>B12 / folate, liver, MDS</p>        |  <p><b>Spherocyte</b><br/>no central pallor<br/>HS, warm AIHA</p>            |
|  <p><b>Target cell</b><br/>bull's-eye<br/>thal, liver, HbC,<br/>post-splenectomy</p> |  <p><b>Sickle cell</b><br/>crescent<br/>sickle cell disease</p>               |  <p><b>Schistocyte</b><br/>fragment<br/>MAHA: TTP/HUS, DIC</p>               |  <p><b>Teardrop</b><br/>dacryocyte<br/>marrow fibrosis,<br/>infiltration</p> |
|  <p><b>Bite cell</b><br/>missing piece<br/>G6PD oxidative hemolysis</p>              |  <p><b>Howell-Jolly</b><br/>nuclear remnant<br/>asplenia / hyposplenism</p>   |  <p><b>Rouleaux</b><br/>stacked coins<br/>high paraprotein<br/>(myeloma)</p> |  <p><b>Agglutination</b><br/>clumping<br/>cold AIHA</p>                      |



## CAN'T-MISS MORPHOLOGIES

# The same shapes, down a microscope

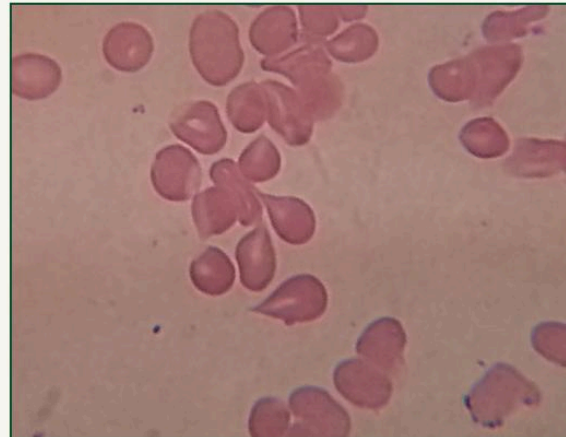


Images: Wikimedia Commons — Schizocyte smear 2009-12-22 (CC BY-SA 3.0); AML-M2 associated with a t(8;21) chromosome abnormality (Public domain); Sickle cell anemia smear (CC BY-SA 4.0); Warm autoimmune hemolytic anemia blood smear (CC BY-SA 4.0); Target Cells, Peripheral Blood Smear (39144139915) (CC BY 2.0); Rouleaux (CC BY-SA 3.0). Full credits in CREDITS.md.



## CAN'T-MISS MORPHOLOGIES

# Two more shapes, down a microscope



**Tear-drop cells**  
marrow fibrosis or infiltration



**Howell-Jolly bodies**  
a dark dot left in the cell — no spleen

Images: Wikimedia Commons — Tear Drop Cells in PBS Microscopy, Ajay Kumar Chaurasiya (CC BY-SA 4.0); Howell-Jolly smear 2010-11-17, Paulo Henrique Orlandi Mourao (CC BY-SA 3.0). Full credits in CREDITS.md.



One question per panel

## Iron studies — is there enough iron, and where is it?

|                           | Ferritin      | Serum iron | TIBC         | Transferrin sat. |
|---------------------------|---------------|------------|--------------|------------------|
| Iron deficiency           | Low           | Low        | High         | Low              |
| Anemia of chronic disease | Normal / high | Low        | Low          | Low / normal     |
| Iron overload             | High          | High       | Low / normal | High             |



ONE QUESTION PER PANEL

# Iron studies — is there enough iron, and where is it?

Iron studies — pattern recognition

The four-test pattern that separates the look-alike microcytic anemias

| Condition              | Serum iron | Ferritin   | TIBC   | Transferrin sat |
|------------------------|------------|------------|--------|-----------------|
| Iron deficiency        | ↓          | ↓↓         | ↑      | ↓               |
| Anemia of inflammation | ↓          | ↑ / normal | ↓      | normal / ↓      |
| Iron overload          | ↑          | ↑↑         | ↓      | ↑↑              |
| Thalassemia trait      | normal     | normal     | normal | normal          |

*Ferritin is the single most useful test — low ferritin is specific for iron deficiency (but rises with inflammation).*



One question per panel

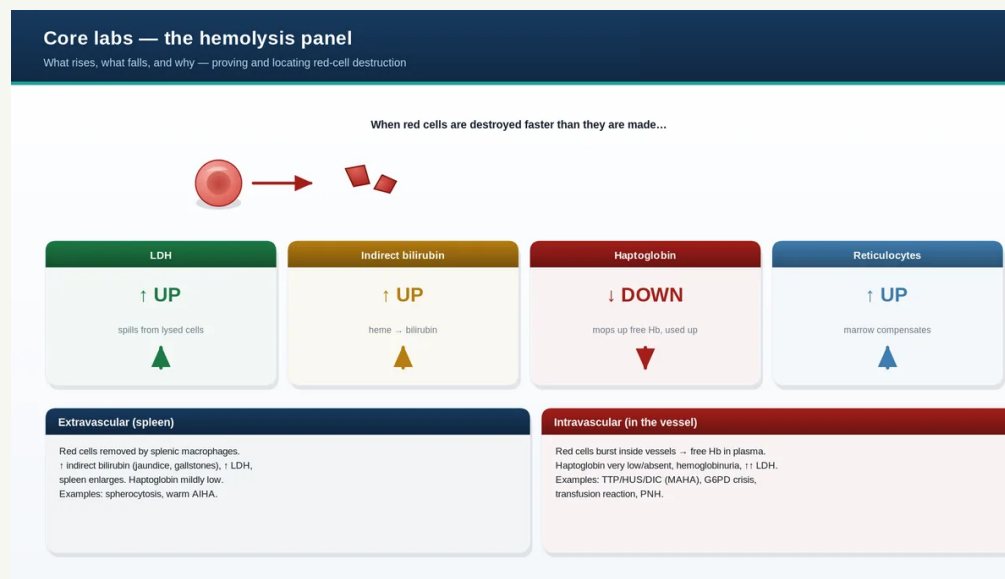
## The hemolysis panel — are red cells being destroyed?

- Proving hemolysis needs evidence of breakdown plus a marrow response.
- Breakdown raises LDH and unconjugated bilirubin, and consumes haptoglobin, which falls.
- The marrow responds with reticulocytosis — which is why the reticulocyte count belongs in this panel.
- The direct antiglobulin test then splits the differential: immune, or not.
- Trap: ferritin is an acute-phase reactant, so a normal ferritin can hide iron deficiency in a sick patient.



ONE QUESTION PER PANEL

## The hemolysis panel — are red cells being destroyed?





## Quantity versus quality

# Hemoglobin electrophoresis — which haemoglobins are present?

|      | Chains             | Adult %  | Note                                     |
|------|--------------------|----------|------------------------------------------|
| HbA  | $\alpha_2\beta_2$  | ~97%     | The main adult haemoglobin               |
| HbA2 | $\alpha_2\delta_2$ | 2.5–3.5% | Rises in beta-thalassaemia trait         |
| HbF  | $\alpha_2\gamma_2$ | < 1%     | Dominant in the fetus; falls after birth |



## Reading the pattern

# Two classic results — and what the test cannot do

### Beta-thalassaemia trait

- No abnormal variant — reduced production only
- Microcytosis with a raised HbA2
- Red cell count often high, not low

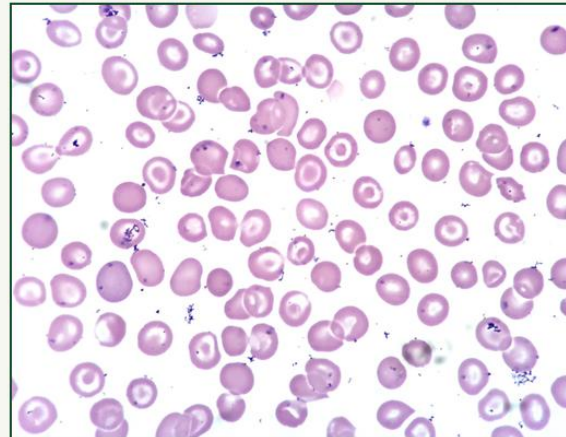
### What electrophoresis cannot tell you

- Cannot exclude alpha-thalassaemia trait — needs molecular testing
- Distorted by recent transfusion
- Iron deficiency lowers HbA2 and can hide a coexisting beta trait. Correct the iron, then repeat.

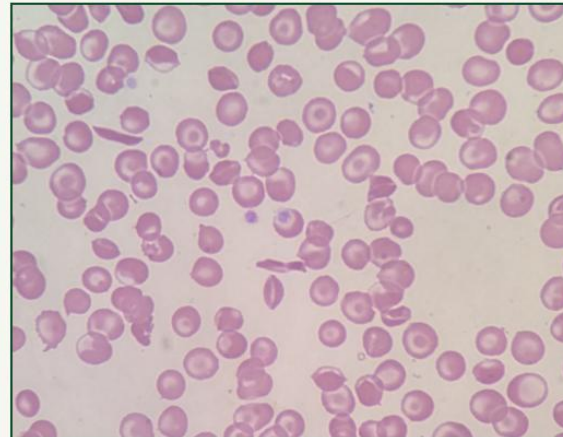


## READING THE PATTERN

# Two haemoglobin problems, on the film



**Target cells**  
common in thalassaemia



**Haemoglobin SC disease**  
targets alongside distorted cells

Images: Wikimedia Commons — Target Cells, Peripheral Blood Smear (39144139915) (CC BY 2.0); Blood film of Hemoglobin SC disease (CC BY-SA 4.0). Full credits in CREDITS.md.



### The third category

## Variants that change oxygen affinity

- Thalassaemia is a problem of quantity; sickle cell disease a problem of shape. There is a third group.
- High affinity — oxygen held too tightly, released poorly. Erythropoietin rises. Presents as unexplained erythrocytosis.
- Low affinity — oxygen released readily, saturation reads low. Presents as unexplained, asymptomatic hypoxaemia.
- Suspect one when erythrocytosis has a normal erythropoietin and no JAK2, or when hypoxaemia will not fit its story.



Two clocks

## The coagulation screen

### PT — extrinsic and common

- Factors VII, X, V, II, fibrinogen
- Prolonged first in liver disease, vitamin K deficiency, warfarin
- Because factor VII has the shortest half-life

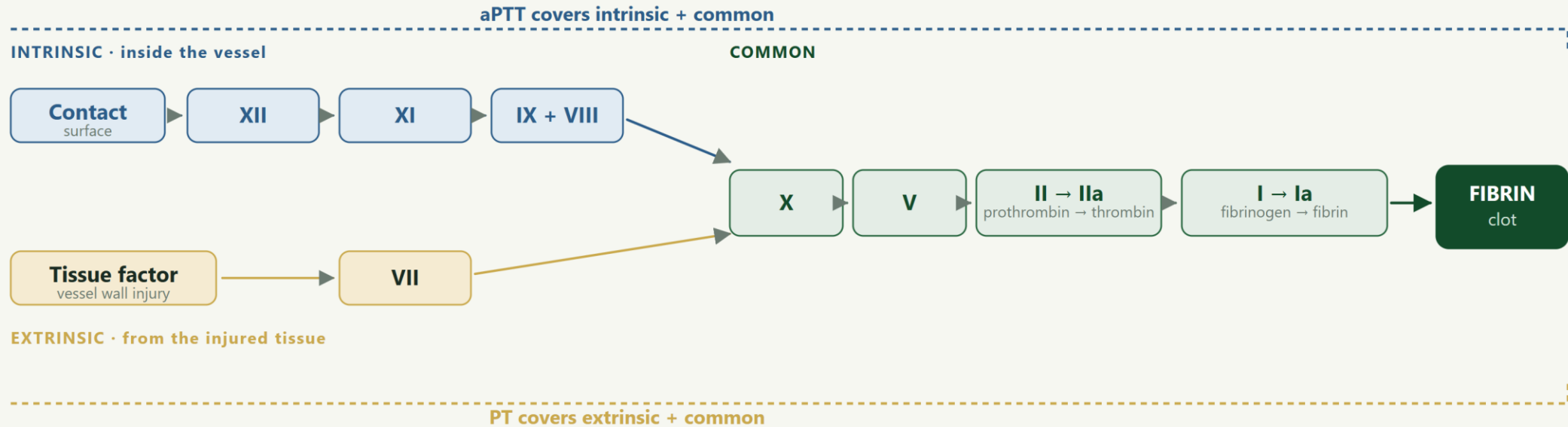
### aPTT — intrinsic and common

- Factors XII, XI, IX, VIII, X, V, II, fibrinogen
- Haemophilia A and B, vWD via factor VIII, heparin, lupus anticoagulant
- Neither clock sees factor XIII, platelet function or vWF activity.



## TWO CLOCKS

# Two arms, one common path — and the clock that covers each





Screen, mix, assay

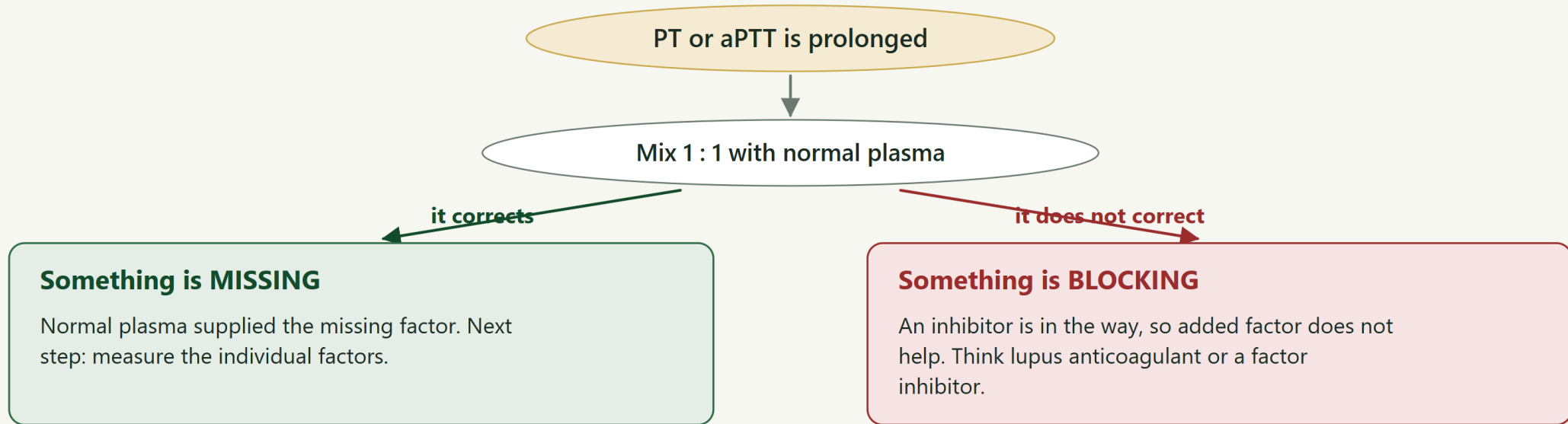
## Mixing studies — missing, or blocked?

- A prolonged clotting time has two explanations: something is missing, or something is blocking.
- Mix the patient's plasma 1:1 with normal plasma. The mix carries about 50% of every factor.
- Corrects — a factor is missing. Next step: specific factor assays.
- Does not correct — something is blocking. Next step: incubated mix, inhibitor titre, lupus anticoagulant testing.
- A factor VIII inhibitor is time and temperature dependent, so an immediate-only mix can miss it.



SCREEN, MIX, ASSAY

## A long clotting time has only two explanations





The whole lecture in miniature

# The D-dimer, station by station

|                   | What can go wrong                                                         |
|-------------------|---------------------------------------------------------------------------|
| 01 The question   | Ordered without a pre-test probability — the commonest error by far       |
| 02 The tube       | Citrate tube, correctly filled; a clot or under-fill invalidates it       |
| 03 The instrument | D-dimer units versus fibrinogen-equivalent units differ about twofold     |
| 04 The number     | The threshold is not universal — it rises with age; pregnancy has its own |
| 05 The meaning    | Sensitive, not specific. A positive means only “not excluded”             |



## THE WHOLE LECTURE IN MINIATURE

# The D-dimer, station by station

- 1 The question** Am I ruling out clot in someone unlikely to have one? Then it helps.
- 2 The tube** Citrate tube, correctly filled — an underfilled tube misleads.
- 3 The instrument** Different assays, different units. Know which your lab reports.
- 4 The number** The cut-off rises with age. A fixed cut-off over-calls in the elderly.
- 5 Interpretation** Negative in a low-risk patient rules out. Positive proves nothing.

**Five stations, one test. This is the habit the lecture is for.**



## Blood bank

# Transfusion serology — can this unit be given safely?

- Grouping — ABO and RhD determined twice: forward typing of cells, reverse typing of plasma. The two must agree.
- Antibody screen — patient plasma against reagent cells of known antigens, to find alloantibodies.
- Crossmatch — the final check against the actual unit; electronic where the screen is negative and history clean.
- Restrictive transfusion thresholds are now the default in stable patients.



## Two antiglobulin tests

# Direct versus indirect

### Direct (DAT)

- Antibodies already bound to the patient's red cells, in vivo.
- Autoimmune hemolysis, hemolytic disease of the newborn, transfusion reactions.
- Positive without hemolysis in some donors, the elderly, after IVIG, and with several drugs.

### Indirect (IAT)

- Antibodies free in the patient's plasma.
- The mechanism behind the antibody screen and the crossmatch.
- Interpret a DAT only next to retics, LDH, bilirubin and haptoglobin.

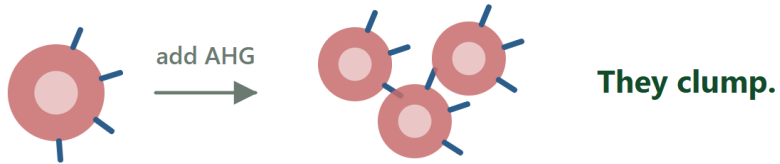


## TWO ANTIGLOBULIN TESTS

# Is the antibody on the cells, or still in the plasma?

### Direct (DAT) — antibody already on the cells

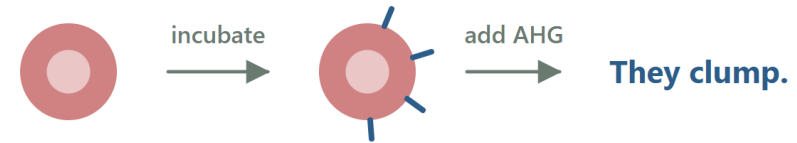
Take the patient's own red cells.



A positive DAT says the patient's cells are already coated — as in autoimmune haemolysis.

### Indirect (IAT) — antibody still in the plasma

Take the patient's plasma; add donor cells.



This is the crossmatch and the antibody screen — will this unit be safe for this patient?

**Same reagent, two questions: what is already stuck on, versus what is still floating.**



Beyond the counter

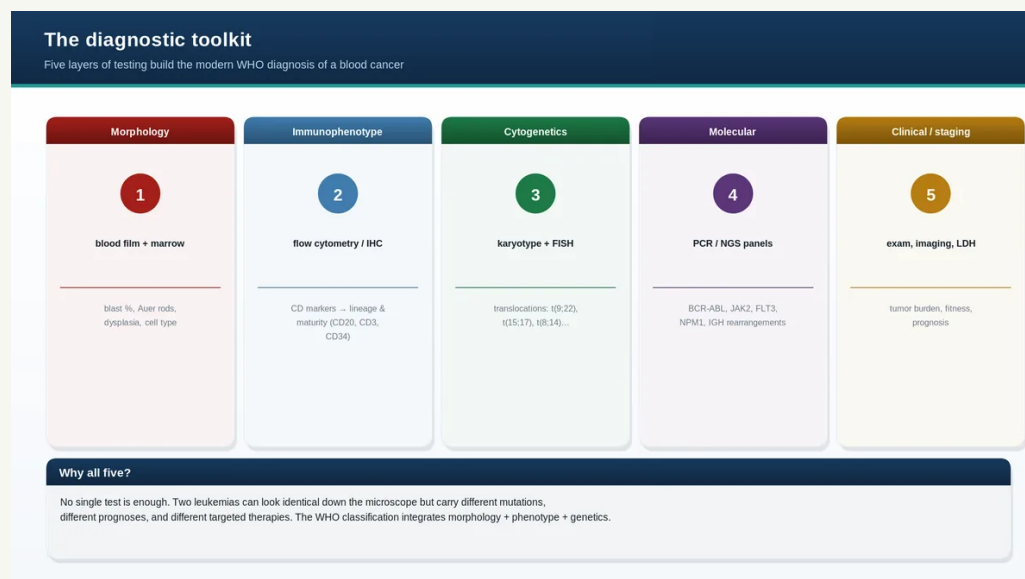
## Advanced diagnostics — which tool answers which question

|                   | The question it answers                    | Limitation                                           |
|-------------------|--------------------------------------------|------------------------------------------------------|
| Immunophenotyping | What lineage is this cell, and how mature? | Needs viable cells — a fresh specimen                |
| Karyotype         | What does the whole genome look like?      | Slow, needs dividing cells, low resolution           |
| FISH              | Is this specific rearrangement present?    | Only finds what you probed for                       |
| PCR               | Is this sequence present, and how much?    | Defined target; basis of residual disease monitoring |
| Sequencing        | Which mutations across many genes?         | Interpretation of variants of unknown significance   |



## BEYOND THE COUNTER

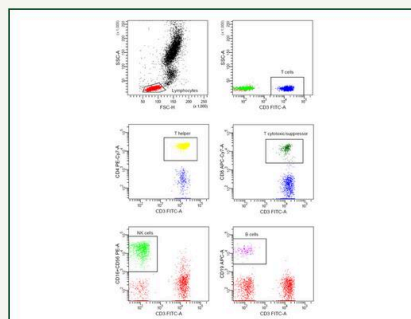
# Advanced diagnostics — which tool answers which question





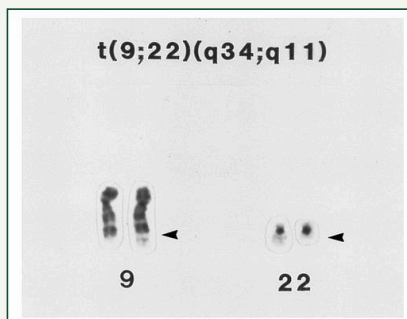
## BEYOND THE COUNTER

# What each tool actually shows



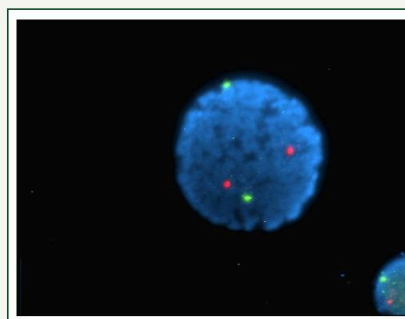
### Immunophenotyping

flow cytometry: cells gated by markers



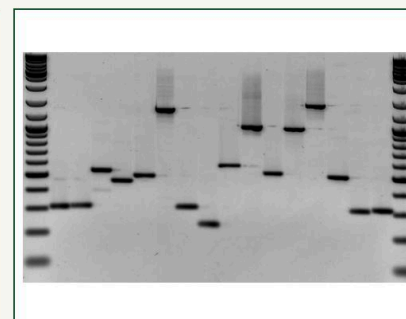
### Karyotype

a translocation, t(9;22)



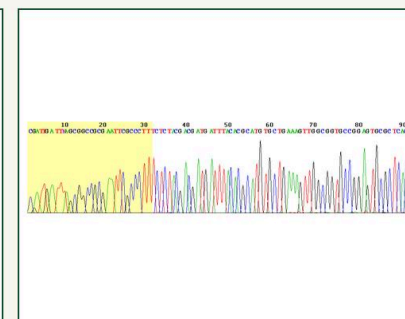
### FISH

coloured probes in one nucleus



### PCR

amplified products on a gel



### Sequencing

the read: one peak per base

Images: Wikimedia Commons — Flow Cytometry Chart, CharlotteHayden (CC BY-SA 4.0); Philadelphia chromosome, t(9;22) translocation, AFIP (public domain); FISH 13 21, Gregor1976 (public domain); PCR gel electrophoresis, Rkalendar (CC BY-SA 3.0); Sanger sequencing read display, Loris (public domain). Full credits in CREDITS.md.



## BEYOND THE COUNTER

# Watch how each of these actually works

[PCR — copying one piece of DNA many times](#) BioMan Biology · 3 min

[FISH — a coloured probe lands on one gene](#) CancerQuest, Emory University · 1 min

[Karyotype — laying the chromosomes out in order](#) Pathology Tests Explained · 3 min

[Flow cytometry — sorting cells by what is on them](#) Cell Signaling Technology · 3 min

[Sequencing — reading the letters themselves](#) Illumina · 5 min

Links open on YouTube. The same five are linked on the lecture page.



To end on

## What a result really is

*A laboratory result is a claim about your patient made by a machine that never met them.*

Knowing how much of that claim to believe is a clinical skill, not a laboratory one



Carry forward

## Part II in five lines

- Every panel answers one question. Learn the question, not the table.
- The film is the cheapest audit of the analyser you will ever order.
- Reticulocytes turn anaemia from a long differential into a short one.
- A normal coagulation screen never excludes a bleeding disorder.
- Genetics increasingly defines the entity, not merely supports it.



Where this comes from

# Selected sources · 1 of 2

|                               | Source                                                                  |
|-------------------------------|-------------------------------------------------------------------------|
| Reference intervals           | CLSI EP28-A3c, Defining, Establishing and Verifying Reference Intervals |
| Altitude and red cell indices | Alkhalidy HY et al. Int J Gen Med 2022;15:3559–65                       |
| Aging at altitude             | Alkhalidy H et al. Int J Gerontol 2021;15(2):160–4                      |
| Haemoglobin mass at altitude  | Alkhalidy H et al. Medicine 2025;104(23):e42762                         |
| Polycythaemia at altitude     | Alkhalidy H et al. Clin Lab 2024;70(6)                                  |
| Isolated neutropenia          | Alkhalidy HY et al. Int J Gen Med 2020;13:1373–9                        |
| Test-ordering audits          | Alasmari S et al. Ann Clin Lab Sci 2024;54(2):211–6                     |



Where this comes from

# Selected sources · 2 of 2

|                         | Source                                                           |
|-------------------------|------------------------------------------------------------------|
| Pseudothrombocytopenia  | Bizzaro N. Am J Hematol 1995;50(2):103–9                         |
| Citrate correction      | Marlar RA et al. Am J Clin Pathol 2006;126(3):400–5; CLSI H21-A5 |
| Bayes at the bedside    | Fagan TJ. N Engl J Med 1975;293(5):257                           |
| D-dimer characteristics | Stein PD et al. Ann Intern Med 2004;140(8):589–602               |
| Age-adjusted D-dimer    | Righini M et al. JAMA 2014;311(11):1117–24                       |
| Hb J-Auckland           | Asseri AA, ... Alkhalidy H. Hematology 2024;29(1):2405751        |
| JAK2 at altitude        | Alkhalidy HY et al. Int J Gen Med 2024;17:4551–8                 |

Discussion

# Questions

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