Dilutions and dilution factors

Parts, volumes, serial steps, and the reported result

Adding diluent lowers concentration. The **dilution factor (DF)** is final volume divided by specimen volume; DF = 5 gives one fifth of the original concentration.

One specimen part in five final parts

Here, **1:5 means specimen:final volume**: one specimen part plus four diluent parts. A written **1 + 5** makes six parts, DF = 6. If a method uses specimen:diluent notation, follow its stated volumes.

Specimen **100 µL**	Diluent **100 µL**	Diluent **100 µL**	Diluent **100 µL**	Diluent **100 µL**

500 µL final volume = 100 µL specimen + 400 µL diluent
Equal-volume schematic. µL = microliter.

Volumes and factor

Dilution factor
DF = final volume ÷ specimen volume

Specimen volume = final volume ÷ DF

Diluent volume = final − specimen volume

Prepare 1:10, final 1.0 mL.
Specimen = 1.0 ÷ 10 = 0.10 mL.
Diluent = 1.0 − 0.10 = 0.90 mL.
mL = milliliter; 1 mL = 1000 µL.

Concentration and result

Diluted concentration
Original concentration ÷ DF

Original concentration
Diluted concentration × DF

A 1:5 aliquot measures 84 U/L.
84 × 5 = **420 U/L** in the original specimen. U/L = units per liter.

The method must recover the expected result after dilution; the choice of diluent can affect that recovery.

Serial dilutions multiply

Original specimen → 1:5 step **Total DF = 5** → 1:4 step from that mixture **Total DF = 5 × 4 = 20**

Each step uses the well-mixed preceding tube. A result of 12 mg/dL in the second tube gives 12 × 20 = **240 mg/dL** originally. mg/dL = milligrams per deciliter. Each step dilutes the preceding mixture, so the factors multiply.

> **Before reporting: apply each factor once.** Check whether the analyzer or laboratory information system has already applied the factor. An automatically corrected 420 U/L stays 420 U/L. The measured aliquot must fall within the method's analytical measuring interval, the range it can measure directly. The calculated result must also fit the verified extended interval. Use the approved diluent and ratios; an extra dilution cannot extend reporting beyond those limits or reliably remove interference.

Common QC rule violations

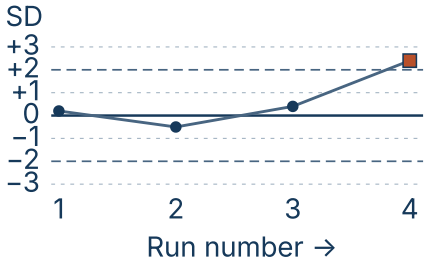
Six statistical rules and the limits of their interpretation

Quality control (QC) compares a control result with its established mean and **standard deviation (SD)**, a measure of usual variation. Random error increases scatter; systematic error shifts results toward one side. The patterns below suggest which type to investigate.

Vertical position = (result − mean) ÷ SD, using each level's own target. Lines: mean (0), ±1, ±2, ±3 SD. Squares meet the named rule; circles give context.

1_{2s}

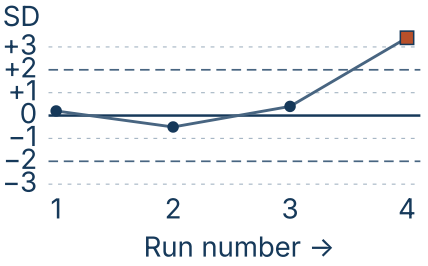
One result beyond ±2 SD.



Classic warning. Inspect the selected rejection rules.

1_{3s}

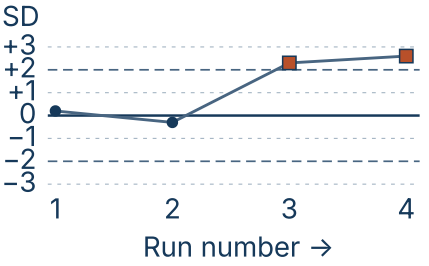
One result beyond ±3 SD.



Rejection. May reflect random or systematic error.

2_{2s}

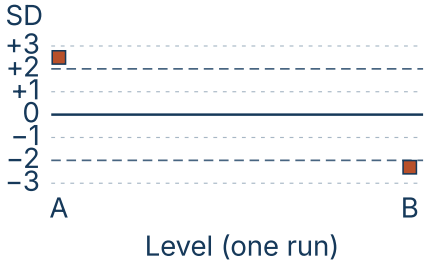
Two consecutive beyond the same 2 SD limit.



Rejection. Suggests systematic error.

R_{4s}

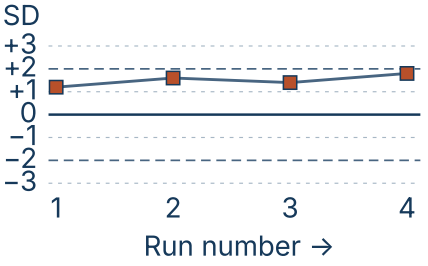
One > +2 SD, one < −2 SD, in one run.



Rejection: possible random error. Spread = 4.8 SD.

4_{1s}

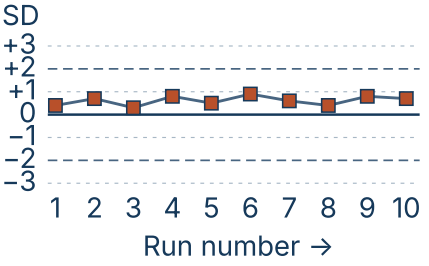
Four consecutive beyond the same 1 SD limit.



Rejection. Suggests systematic error.

10_{x}

Ten consecutive on one side of the mean.



Rejection. Suggests systematic error.

Examples use one level across runs, except R_{4s} (two levels, one run). The 2_{2s} rule also applies across levels within a run; 4_{1s} and 10_{x} can span levels and runs as the QC plan specifies.

Warning and rejection

In the classic Westgard scheme, 1_{2s} is a warning to check the rejection rules. Plans that evaluate every rule directly can detect the 4_{1s} and 10_{x} examples here even though no result exceeds 2 SD. The adopted plan defines rules, levels, runs, and look-back periods.

Respond to a rejection

Hold affected patient results. Use the adopted QC plan to investigate controls, reagents, calibration, and instrument performance. After correction and acceptable QC, assess which patient results need review or repeat testing. A passing repeat alone does not explain the original failure.

Iron study patterns

Deficiency, inflammation, mixed findings, and overload

Serum iron reflects circulating iron, while ferritin helps assess storage iron. Inflammation can trap iron in storage and raise ferritin, leaving little iron available for red cell production.

Total iron-binding capacity (TIBC) reflects transferrin, the main iron transport protein. **Transferrin saturation (TSAT)** is the percentage of that capacity occupied by iron.

TSAT (%) = serum iron ÷ TIBC × 100

Use the same concentration units for iron and TIBC.

Iron 30 µg/dL ÷ TIBC 300 µg/dL × 100 = **10% TSAT**. µg/dL = micrograms per deciliter. Low availability occurs in both deficiency and inflammation.

Typical adult patterns

↓ decreased; ↑ increased; **N** within the applicable reference interval. Compare with the laboratory's reference intervals; these are patterns, not diagnostic cutoffs.

Pattern	Serum iron	TIBC / transferrin	TSAT	Ferritin
Iron deficiency	↓	↑	↓	↓
Anemia of inflammation	↓	↓ or N	↓	N or ↑
Mixed deficiency + inflammation	↓	Variable; often N or ↓	↓	↓, N, or ↑
Iron overload	Often ↑	N or ↓	Usually ↑	Usually ↑

Why inflammation changes the panel

A low ferritin supports depleted stores. Normal or high ferritin cannot exclude deficiency during inflammation; liver injury can also raise it. Hepcidin, an iron-regulating hormone, reduces iron release from stores and absorption from the gut. Transferrin may also fall, so mixed deficiency can lose the expected high TIBC and low ferritin.

High ferritin alone does not establish overload. Persistent high TSAT strengthens that possibility; some overload disorders have normal TSAT. Further evaluation establishes the cause and tissue iron burden.

When results do not agree

Compare with hemoglobin, red cell indices, C-reactive protein or another inflammation marker, and collection and iron-treatment history.

Soluble transferrin receptor may help when inflammation obscures ferritin, but increased red cell production also raises it and cutoffs vary by method. Reticulocyte hemoglobin shows the iron available to recently produced red cells. It can be low in either deficiency or inflammation. Diagnostic thresholds depend on the population and guideline.

PT and aPTT patterns

Screening patterns, specimen checks, and mixing studies

Prothrombin time (PT) and **activated partial thromboplastin time (aPTT)** measure clotting after different reagents activate citrated plasma. The factors shared by the two tests explain why either one or both times can be prolonged.

PT · tissue factor / extrinsic arm

VII

↓

aPTT · contact / intrinsic arm

XII → XI → IX + VIII

↓

Common: X + V → thrombin (activated II) → fibrin formation

Assay schematic: Roman numerals identify factors; V and VIII are cofactors. Thrombin cleaves fibrinogen to fibrin. Contact activation also needs prekallikrein and high-molecular-weight kininogen.

Patterns and possible explanations

Screening pattern	Possibilities to investigate	Interpretive limit
PT prolonged aPTT in range	VII deficiency/inhibitor; early vitamin K deficiency or warfarin.	Also possible with liver disease or direct factor Xa inhibitors.
aPTT prolonged PT in range	VIII, IX, XI, or contact-factor deficiency/inhibitor; heparin; lupus anticoagulant.	Contact-factor deficiency usually causes no bleeding. Lupus anticoagulant can accompany thrombosis.
Both prolonged	Common/multiple-factor deficiency: vitamin K deficiency, liver disease, or disseminated intravascular coagulation; anticoagulants.	Compare with platelets, fibrinogen, medications, and targeted tests.

Specimen and method checks

An underfilled tube leaves excess citrate for the plasma volume and may prolong times. Hematocrit above 55% also reduces plasma volume; adjust citrate under the procedure. Check for clots, heparin from a line draw, delays, and optical interference.

Anticoagulant effects depend on drug, concentration, and method. Normal times cannot exclude a direct oral anticoagulant.

What a mixing study shows

Equal volumes of patient and normal plasma supply missing factors. If the time corrects by the method's criteria, deficiency is more likely. Failure to correct suggests an inhibitor or drug. Some inhibitors become apparent only after incubation.

Mixing also dilutes inhibitors, so a weak inhibitor may be missed. Anticoagulant drugs can mimic an inhibitor pattern.

> Normal screening times do not exclude every bleeding disorder. They can occur in von Willebrand disease, platelet dysfunction, mild factor deficiencies, and factor XIII deficiency.

Full explanation and sources: latticemls.org/guides/pt-aptt/

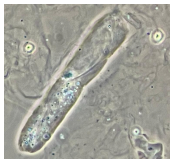
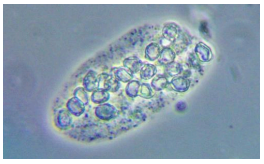
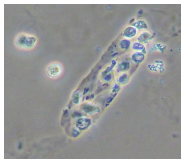
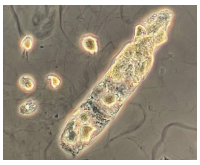
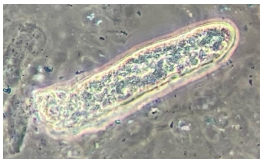
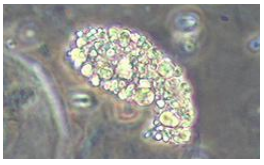
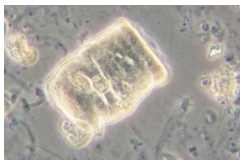
Source review: September 5, 2026 · LatticeMLS

Urine casts: phase contrast

Seven cast types, identifying features, and common look-alikes

Casts form in renal tubules in a uromodulin protein matrix. Identify the matrix first; loose cell clumps can mimic casts.

Phase contrast: makes faint structures visible through differences in refractive index. Bright edge halos are optical effects, not extra cast material.

Photo	Cast and identifying feature	Association and look-alike
	**Hyaline** · 400× Pale matrix with parallel sides; phase contrast makes the faint outline more visible.	Concentrated urine, exercise, or renal disease; a few may occur without disease. **Look-alike:** Mucus tapers and varies in width.
	**Red cell** · 400× Red cells held within a continuous matrix.	Bleeding within the nephron, especially from a glomerular source. **Look-alike:** Free red cell clumps have no enclosing cast matrix.
	**White cell** · 400× White cells in a matrix; look for granular cytoplasm and lobed nuclei.	Renal inflammation, including pyelonephritis and interstitial nephritis. **Look-alike:** Tubular cells or white cell clumps can resemble a cast.
	**Tubular epithelial** · 400× Tubular cells with single round nuclei and visible cytoplasm within a matrix.	Tubular injury. Degeneration can obscure the cell type. **Look-alike:** White cell and tubular cell casts overlap; compare nuclei, not size alone.
	**Granular** · 400× Coarse or fine granules fill the matrix as cells and proteins break down.	Numerous muddy brown casts support tubular injury. A few granular casts are less specific. **Look-alike:** Debris or crystal clumps lack a continuous cast outline.
	**Fatty** · 400× Refractile lipid droplets within a matrix; some show Maltese crosses in polarized light.	Lipiduria, often with heavy proteinuria. **Look-alike:** Starch also forms crosses; confirm droplets within a cast.
	**Waxy** · 100× Highly refractile, sharply outlined matrix; cracks, notches, and blunt ends.	Prolonged tubular stasis, often with advanced renal impairment. **Look-alike:** Fibers have irregular edges and lack a cast matrix.

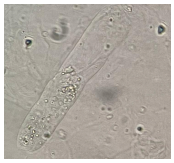
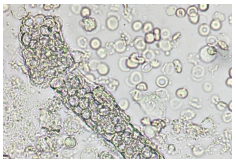
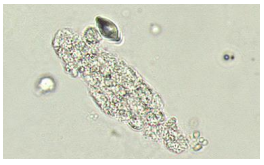
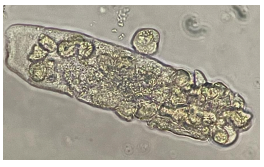
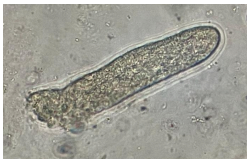
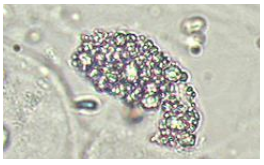
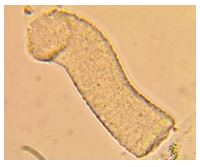
Scan low power; identify contents at higher power. Delay and dilute alkaline urine can destroy casts; centrifugation can break or lose them. Broad describes width, not contents. Interpret with other urine findings using the laboratory's reporting method.

Urine casts: brightfield

Seven cast patterns, identifying features, and common look-alikes

Casts form in renal tubules in a uromodulin protein matrix. Identify the matrix first; loose cell clumps can mimic casts.

Brightfield: transparent casts have little contrast. Reduced illumination helps reveal their outline; phase contrast can clarify cell detail.

Photo	Cast and identifying feature	Association and look-alike
	**Hyaline** · 400× Pale matrix with parallel sides; faint edges can disappear in strong illumination.	Concentrated urine, exercise, or renal disease; a few may occur without disease. **Look-alike:** Mucus tapers and varies in width.
	**Red cell** · 400× Red cells held within a continuous matrix.	Bleeding within the nephron, especially from a glomerular source. **Look-alike:** Free red cell clumps have no enclosing cast matrix.
	**White cell** · 400× Granular white cells in a matrix. Nuclear lobes may be difficult to resolve.	Renal inflammation, including pyelonephritis and interstitial nephritis. **Look-alike:** Tubular cells or white cell clumps can resemble a cast.
	**Epithelial / white cell** · 400× The source labels this as an epithelial/white-cell cast. Indistinct nuclei limit cell typing.	Tubular injury or renal inflammation; the cast alone cannot establish the cause. **Look-alike:** White cell and tubular cell casts overlap; compare nuclei, not size alone.
	**Granular** · 400× Coarse or fine granules fill the matrix as cells and proteins break down.	Numerous muddy brown casts support tubular injury. A few granular casts are less specific. **Look-alike:** Debris or crystal clumps lack a continuous cast outline.
	**Fatty** · 400× Refractile lipid droplets within a matrix; some show Maltese crosses in polarized light.	Lipiduria, often with heavy proteinuria. **Look-alike:** Starch also forms crosses; confirm droplets within a cast.
	**Waxy** · 400× Highly refractile, sharply outlined matrix; cracks, notches, and blunt ends.	Prolonged tubular stasis, often with advanced renal impairment. **Look-alike:** Fibers have irregular edges and lack a cast matrix.

Scan low power; identify contents at higher power. Delay and dilute alkaline urine can destroy casts; centrifugation can break or lose them. Broad describes width, not contents. Interpret with other urine findings using the laboratory's reporting method.

Photos: Bert Grijsen, *Microscopic Urine Examination* (2026). CC BY-SA 4.0. Cropped; mixed cast rotated; colors unchanged. Magnifications are source values, not print scale.

Full explanation and sources: latticemls.org/guides/urine-casts-brightfield/

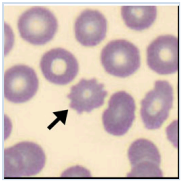
Source review: September 5, 2026 · LatticeMLS

Selected red cell shapes

Eight shape findings on a stained peripheral blood smear

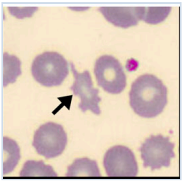
Red blood cell (RBC) shape is assessed where cells lie singly or just touch and normal central pallor remains visible. Thick areas distort shape and obscure pallor. Normal pallor occupies about one third of a cell's diameter.

May-Grünwald Giemsa-stained human blood smears. Letters and arrows identify source panels; crops are not at a common scale. Source patients had coronavirus disease 2019 (COVID-19); these shapes are nonspecific.



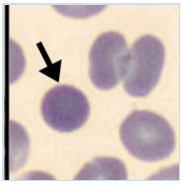
Echinocyte A

Short, evenly spaced projections. Often artifact; also uremia. Acanthocytes have irregular projections.



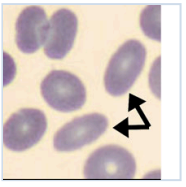
Acanthocyte B

Uneven projections of varied length. Severe liver disease; lipid disorders. Compare with regular echinocytes.



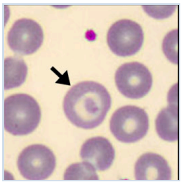
Spherocyte D

Dense, no central pallor (single arrow). Immune hemolysis; hereditary spherocytosis. Thick areas hide normal pallor.



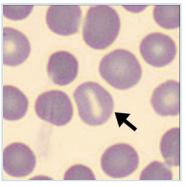
Elliptocyte D

Elongated ovals (paired arrows). Hereditary elliptocytosis; iron deficiency. Large ovals suggest megaloblastic change.



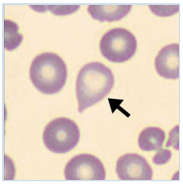
Target cell E

Stained center, pale ring, outer rim. Thalassemia; liver disease; hemoglobin variants. Uneven distribution suggests artifact.



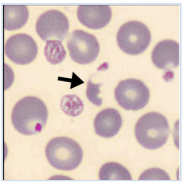
Stomatocyte F

Slit-like central pallor. Hereditary stomatocytosis; liver disease. Slow drying can mimic this.



Teardrop cell G

One tapered end; a dacrocyte. Marrow fibrosis/infiltration; megaloblastic anemia. Artifact tails often point the same way.



Schistocyte H

Angular or helmet-shaped fragment. Microangiopathic or mechanical damage. Follow counting and urgent-review policy.

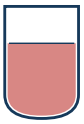
Schistocytes are red cell fragments. Their significance depends on the number present and the other smear findings; follow the laboratory's counting and urgent-review criteria.

> **Interpret across the smear.** Confirm findings across suitable fields and compare with the blood count and hemolysis studies. Delay and poor drying can alter shape. Associations and isolated cells do not establish a diagnosis.

Hemolysis, lipemia, and icterus

Appearance and interference in serum or plasma

Hemolysis, lipemia, and icterus can alter serum or plasma measurements. The same specimen may be acceptable for one test and unsuitable for another because each method responds differently to interference.



Hemolysis

Pink to red; free hemoglobin

Lipemia

Cloudy to milky; lipid particles

Icterus

Deep yellow to brown; bilirubin

Appearance schematics. Clear serum/plasma is usually pale yellow. Mixed interferences can obscure appearance; a visual estimate cannot determine acceptance.

Finding	How results may change	Practical implication
Hemolysis	Cell rupture releases potassium and lactate dehydrogenase. Free hemoglobin absorbs light and can affect chemical reactions.	Released potassium can raise results; other biases vary. Recollection may resolve collection damage. Hemolysis that occurred in the patient may persist after recollection.
Lipemia	Particles scatter light and affect sampling. Reduced plasma-water fraction affects methods that dilute the sample.	Indirect ion-selective electrodes can report falsely low sodium. Direct electrodes generally avoid this water-fraction effect. Lipid removal may also remove analyte.
Icterus	Bilirubin absorbs light and can react with assay components, including oxidants.	Effects differ between assays. A fresh draw may remain icteric; an alternate validated method may be needed.

What the interference index means

An instrument interference index estimates hemoglobin, bilirubin, or turbidity from optical readings. Its scale comes from the instrument method and cannot be inferred from color alone. A lipemia index is not a triglyceride concentration. Index scales and limits are not interchangeable between platforms.

Review each affected test

Compare the index with the manufacturer's interference data and the laboratory's verified limit for that test. Hold, qualify, recollect, or use a validated alternate method as the procedure directs. Document unresolved interference and communicate delays for urgent testing.

> Interference can raise or lower a result, depending on the assay. Acceptance limits and any dilution, blanking, or lipid-removal step require method-specific evidence. Controls can pass while an individual patient specimen remains affected.

Full explanation and sources: latticemls.org/guides/specimen-interference/

Source review: September 5, 2026 · LatticeMLS

DAT versus IAT

Where sensitization occurs and what the result can establish

The **direct antiglobulin test (DAT)** detects antibody or complement already coating the patient's red cells. The **indirect antiglobulin test (IAT)** tests whether antibodies in serum or plasma can coat test red cells during incubation.

Sensitization is antibody or complement coating a red cell. **Antihuman globulin (AHG)** reacts with the coating specified on the reagent label, often **immunoglobulin G (IgG)** and/or fragments of **complement component 3 (C3)**.

DAT · sensitized in the body

Patient red cells

IgG and/or C3 already attached

↓

AHG detects the existing coating

IAT · sensitized in the test

Patient serum or plasma + test cells

Antibodies bind during incubation

↓

AHG detects coating formed in the test

In a tube method, AHG bridges sensitized cells, causing agglutination (clumping). Washing removes unbound globulin that could neutralize AHG.

The diagram illustrates the tube method. Gel and solid-phase methods separate and detect reactions differently; specimen and reagent instructions govern the method used.

Comparison	DAT	IAT
Material tested	Patient red cells, commonly from anticoagulated blood.	Serum/plasma plus reagent cells, or donor cells for an antiglobulin crossmatch.
What is detected	Bound IgG and/or complement, per reagent.	Antibodies reactive with test-cell antigens under the assay conditions; commonly IgG.
Common uses	Investigation of immune hemolysis, transfusion reactions, and hemolytic disease of the fetus and newborn.	Antibody screening and identification, prenatal testing, and antiglobulin compatibility testing.

A positive DAT does not establish hemolysis. Compare bilirubin, lactate dehydrogenase, haptoglobin, and clinical findings. A negative DAT may miss a small amount of coating or a type the reagent does not detect.

A positive reaction alone does not identify the antibody. A negative IAT means no reaction was detected with those test cells and conditions; a weak antibody or one whose antigen is absent from the cells may be missed.