Pre-analytic and Analytic Laboratory Issues in Coagulation Testing

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Serious consequences due to errors in routine coagulation testing.

Falsely prolonged results

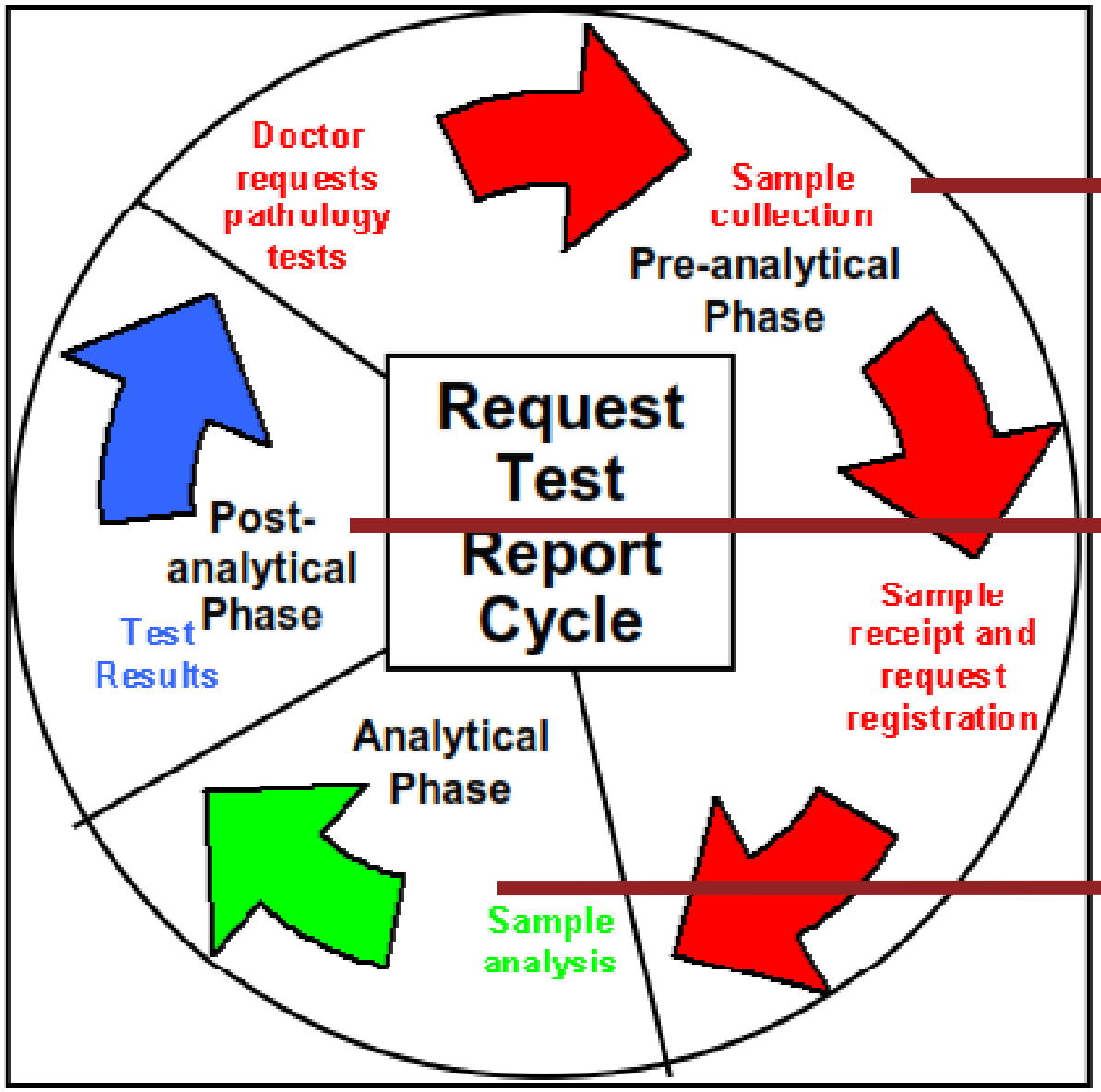
- further costly and time consuming investigations,
- unnecessarily delay invasive procedures, and
- raise unnecessary anxiety in the patient

False-normal results prevent

- further evaluation of factor assays,
- unjustified risk of bleeding with invasive procedures

False low or high coagulation time in anticoagulant t/t monitoring

- incorrect dosing
- risk of thrombosis or bleeding depending

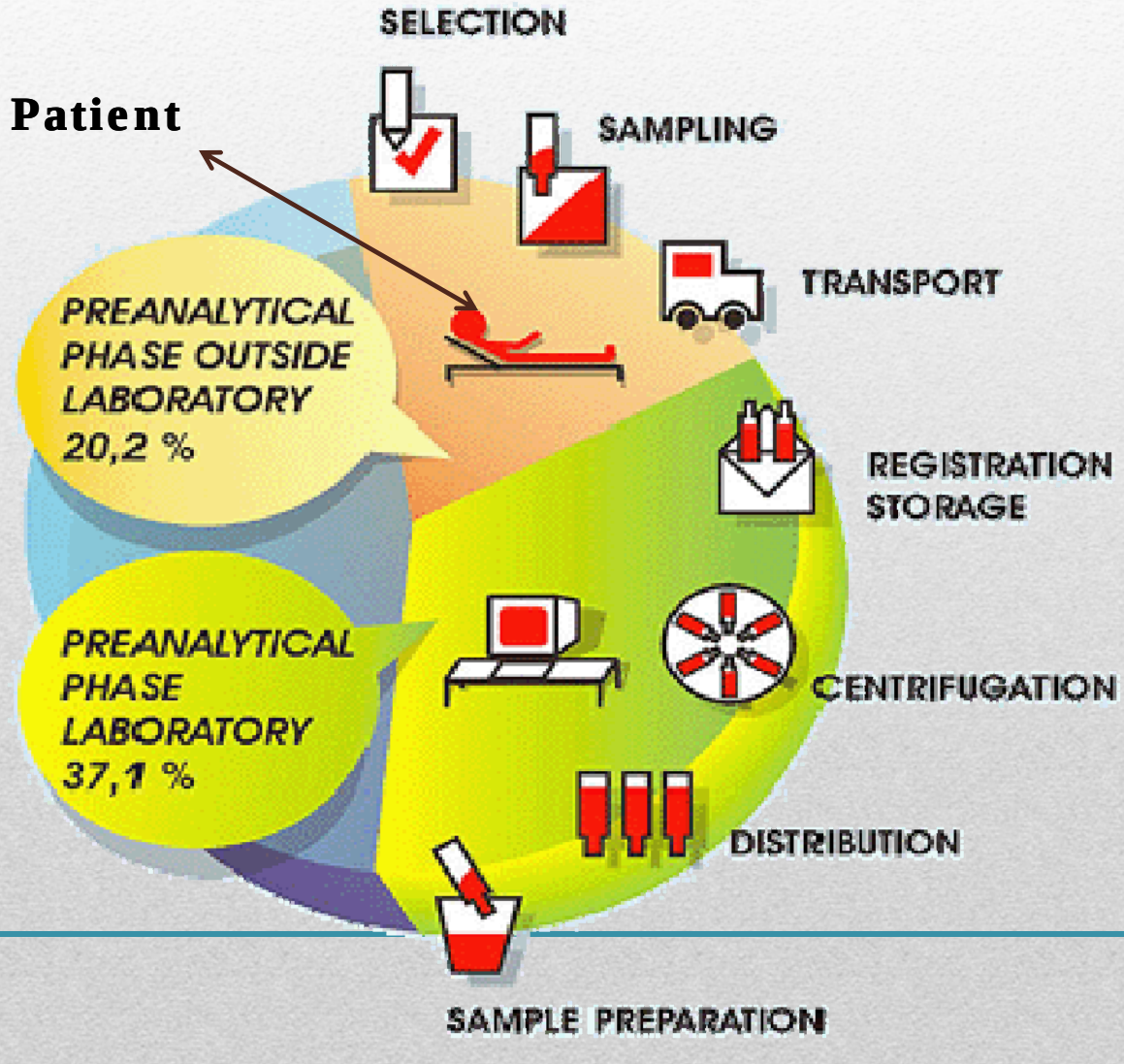


Pre-analytic issues

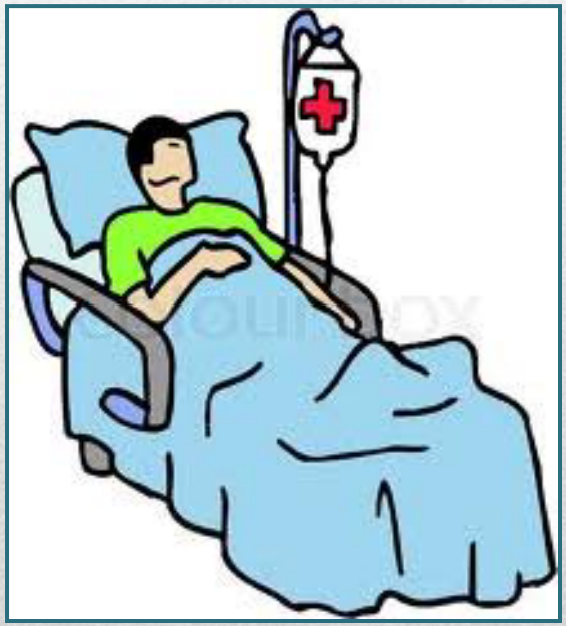
Post-analytic issues

Analytic issues

Pre-analytical Issues



Pre-analytical – Patient's History



- Aspirin – PFT
- Heparin – APTT
- Warfarin – PT/INR
- Contraceptives

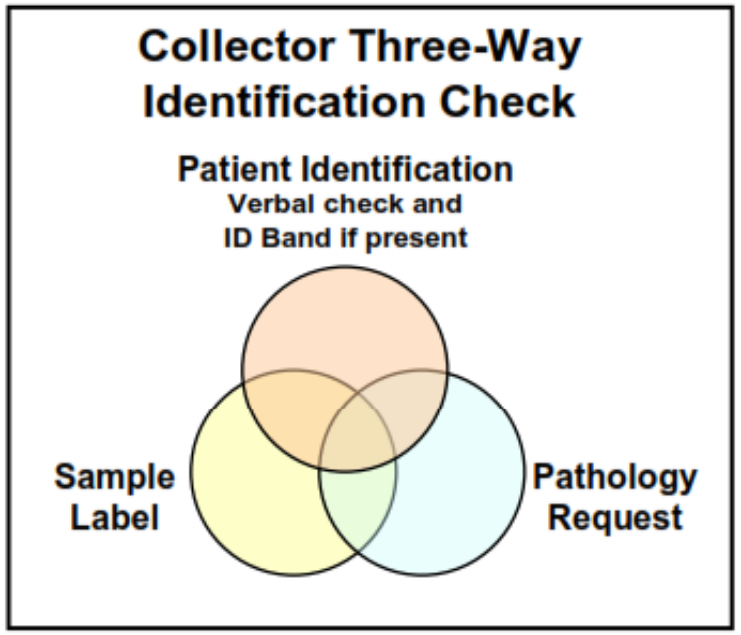
- FXIII – one month

- Smoking – Plt Act

Biological Variation

- Excess physical activity
- Stress
- Pregnancy

Positive Patient & Sample Identification



Outpatient setting - the principle of "double identifiers"

patients should identify themselves and some form of identification.

Hospital Settings

Case Number

electronic or bar-code methods

printing tube labels;

matching patient identification with 2 identifiers

patient's full name and

date of birth or

medical record number; and

collection date & time.

Sample Collection

Very Important

- **Site of collection**
  - No finger prick
  - Avoid Central line – heparinized
- Untraumatic venipuncture
- Prolonged use of a tourniquet
- Right gauge **needle** - hemolysis/aPlt
  - 21G recommended
- **Volume of sample**
  - Low – high anticoagulant
  - High – low anticoagulant

Sample Collection

Very Important

- **Anticoagulant of choice - 3.8% or 3.2% Sodium Citrate**
  - 3.2 % Preferred - due to stability and closeness to the plasma osmolality

- Anticoagulant/blood ratio is critical (1:9)
  - *Exact* amount of blood must be drawn.
  - No short draws are acceptable- falsely increase results
  - *CLSI guideline is 90 % of calibrated volume*
- Purpose of the anticoagulant is to bind or chelate calcium to prevent clotting of specimen

Order of Sample Collection

- 3.2% Sodium citrate (1:9)- Coagulation
- Plane (no additive) – Serum studies
- Thrombin based clot activat - Chemistry
- Heparin – FCM, Cytogenetics
- K2/K3 EDTA – CBC
- K2 EDTA – Bl bank
- Sodium citrate (1:4)- ESR
- Sod Fluoride+ Pot Oxalate – Bl Sugar
- Acid Citrate Dextrose sol A

Correction Formula: High Hematocrits

CLSI - Adjust anticoagulant ratio for hematocrits > 55%

Formula to calculate amount of anticoagulant

$$\frac{(100-Hct)*V}{(595-Hct)} = C$$

Where: C= volume of sodium citrate

V=volume of whole blood drawn

Hct= patient's hematocrit

Example:

If hematocrit of 60%, and blood is to be drawn into a 2.7 mL blue top-tube.

Patients Hct= 60% & V= 2.7 mL

$$\frac{(100-60) * 2.7mL}{(595-60)} = 0.2\ mL$$

Sample Collection

Mixing of sample

- Vigorous – Hemolysis – false short PT/PTT
    - Platelet activation – false short PT/PTT
- Inadequate – clot formation - false short PT/PTT
- Mix thoroughly but gently by 3 to 6 end-over-end tube inversions

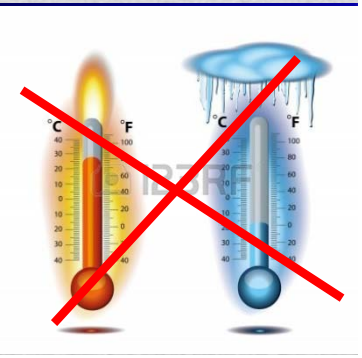
- **Order of draw -** coagulation tube is always first
  - except for blood culture.
- PT/INR and APTT not affected by first tube drawn

- Winged blood collection set for venipuncture
- **a discard tube** should be drawn first.

- Blood should never be transferred from one tube to other

Sample Transport

- Non-refrigerated at ambient temperature (15-22°C)
- in as short a time as possible.
- Ideally, routine coagulation tests – within 4 hrs
- APTT testing for unfractionated heparin monitoring
  process within 1 hr
- Avoid extremes of temp (ie, both refrigerated or high)
- Delays in transport may affect - labile factors (FV, FVIII)
- local centrifugation and separation of plasma followed by **freezing** and **frozen transport**



Sample Processing - Centrifugation

15°C-22°C

1500g

15-20 min

Rule of "15"

Swing-out bucket rotor should be used

- *"Double centrifuge"* to ensure platelet-free preparations prior to freezing for **LA testing**
- Before processing - check for clot formation/hemolysis

Issues related to Storage

- Frozen at -20^{0}C for up to two weeks or
  -70^{0}C for up to six months
- Inadequately thawed - inhomogeneous sampling
  - Cryoprecipitate portion would be selectively sampled
    - very high levels of FVIII:C, VWF, Fibrinogen or FXIII.
  - Cryo poor part
    - very low levels of FVIII:C, VWF, Fibrinogen or FXIII.

Frozen samples should be thawed and mixed at 37^{0}C water bath before testing.

Cold activation at 2-4^{0}C is not known to occur in 4 hrs.

Cold activation will result in activation of FVII and also FIX resulting in falsely decreasing the times in PT and APTT tests respectively.

Freeze-thawing Events

- Loss of some labile factors, notably FV and FVIII.
- Since it is not always clear how many times a sample has been thawed and refrozen prior to testing,
- Retesting using a fresh sample is always indicated if unexpected low factor results
- Unexplained abnormal result - a new specimen and repeat

Preparation of reagents

- Improper reconstitution of reagents.
- Reagent stability.
- Contamination of reagents.
- Lot variation.
- Wrong labeling of reagents.
- Deterioration of buffer.
- Stability of factor deficient plasma.
- **Correction studies**
- Adsorbed plasma - PT > 60s < 90s to ensure adequate adsorption and prevent over adsorption that leads to loss of FVIII and FV.
- Aged serum - activated factors 3 separate serum samples for mixing as there is a risk of activated factors clotting and correcting FVIII / FV deficiency.

Intra-laboratory handling, preparation and storage of samples

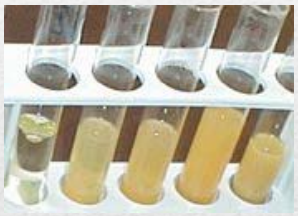
- Registration, identification
- Visual Inspection for – clots, Hemolysis, Lipemia etc
- Centrifugation
- Proper Distribution
- Storage (to analysis performed not-daily, for post-analysis if it is needed)

Intra-laboratory handling - Common problems

Typical errors	Consequences
native blood centrifugated before clotting	haemolysed sample, fibrin stand in serum, clogging
inappropriate melting of frozen specimens	concentration-gradient or precipitation, false result
inappropriate storage of samples in lab (sample ID lost, contamination, break down, instable components etc.)	false results contamination
clots in anticoagulated blood cryoglobulins	false results

Solution: New sample requested when necessary.

Common interferences

Typical errors	Consequences
in vitro haemolysis 	**•high K, LDH, HBDH** **•interference with many analytical procedures**
hyperlipidaemia 	**•pseudo-hyponatraemia** **•interference with many analytical procedures**
hyperbilirubinaemia 	**•interference with many analytical procedures**
drugs	**•interference with many analytical procedures**

Solution: New sample requested when possible.

Alternative methods used. Results commented.

Under-recognized Pre-analytical Issues

- Normal Reference Range (NRR) Derivations

  NRR = mean +/- 2SD → 5% outside NRR – False Abnormal

- International Normalized Ratio (INR)=(patient PT/MNPT)^{ISI}

  where MNPT= mean of normal PT, and ISI=international sensitivity index.

- WHO recommend the use of PT reagent that have an ISI between 0.9-1.7.
- PT reagents with ISI of less than 1.4 is good and quite easily available.

- Biological variations

  Physical activity

  Stress

-

Analytic Issues

- Instrument related – automated
  - Daily Controls:- 2 levels are adequate and a must.
  - It is recommended to run after

    every 40 samples in laboratories with heavy workload,

    Otherwise - beginning of the day,

    - at least once in each shift or

    - with each group of tests
  - Periodic maintainance
  - External Quality Assessment Programme – EQAS

- Clot formation based - Semiautomated instruments
  - The difference between duplicate tests should agree within 10% of their mean value.

Post-analytic Issues

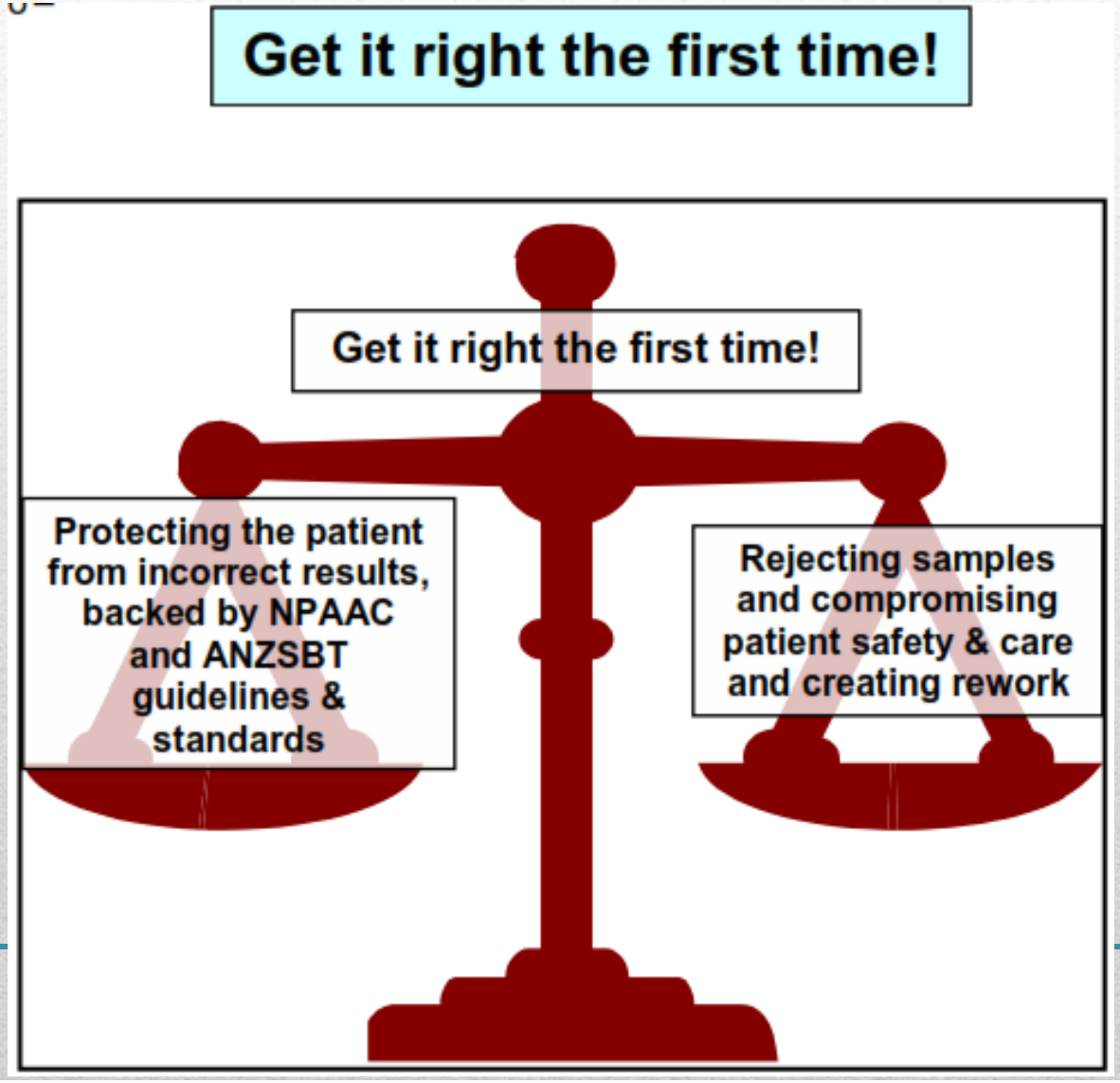
- **Reporting** :-
- report the results of PT and APTT on basis of **own NRR**
- INR is reported as such and not with a reference interval.

- **Results to wrong patients –**
- check patient's name, ID number, Case Number
- Minimum 2 should match

Record & Analyze the Errors

- Record & analyze the errors – Monthly & yearly

Take Home Massage




That's all Folks!