IFCC Recommendation for Reporting Blood Glucose Results & Sources of Error in Glucose POCT

Ellis Jacobs, Ph.D., DABCC

New York University School of Medicine
Bellevue Hospital Center
New York, New York

Reporting of Glucose Concentration

- ADA Recommendation
  - In terms of glucose in venous plasma
- WHO Recommendation
  - In terms of glucose in whole blood

Activity & Molality of Glucose

- Biosensors respond to activity of glucose
- Activity assumed to be equal to molality
- Activity is related to chemical potential – kJ/mol
- Molality = amount/unit water mass = mmol/kg H2O

Mass Concentration of Water

- Average erythrocyte cytoplasm = 0.71 kg/L
- Whole/hemolyzed blood = 0.84 kg/L
- Plasma = 0.93 kg/L
- Aqueous Calibrators = 0.99 kg/L

Note: Normal is defined as Hct= 0.43 and proteins and lipids in plasma within reference ranges

Direct Reading Glucose Biosensors

- Detect Activity in specimen (Blood or Plasma)
- Use aqueous calibrators to provide “relative molality” results
- Need to correct for differences in water concentration:
  - Blood: 0.99/0.84 = 1.18
  - Plasma: 0.99/0.93 = 1.06

Reporting of Glucose Concentration

- ADA Recommendation
  - In terms of glucose in venous plasma
- WHO Recommendation
  - In terms of glucose in whole blood
- ~11% Difference (Plasma > Blood)

Relationship Between Whole Blood and Plasma Glucose Concentration

Whole Blood Glucose =

[Plasma Glucose] x [1.0 - (.0024 x % Hematocrit)]

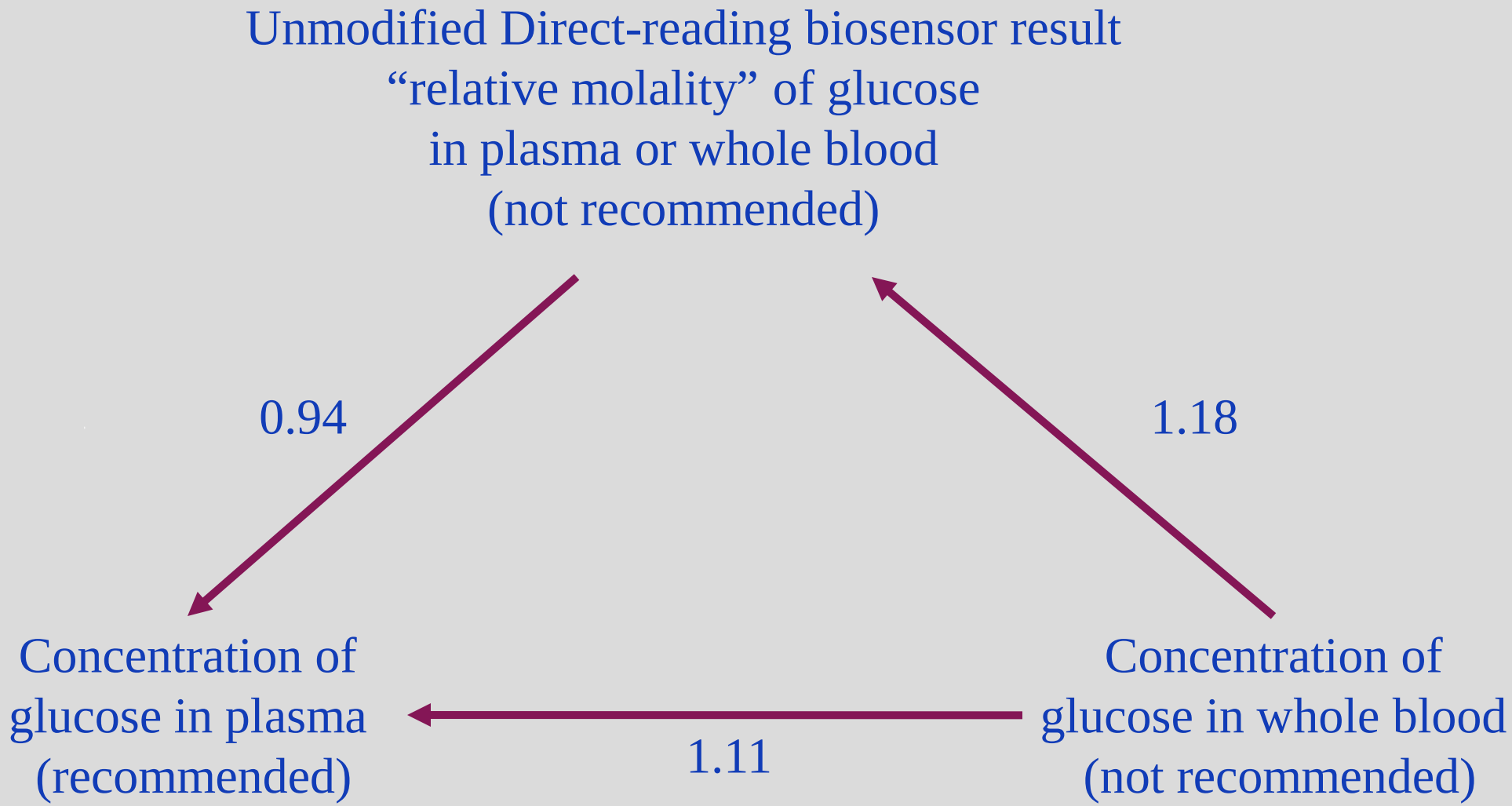
or

Whole Blood Glucose = [Plasma Glucose] x 0.892

or

Whole Blood Glucose = [Plasma Glucose] ÷ 1.12

Conversion Factors for Different Quantities of Glucose



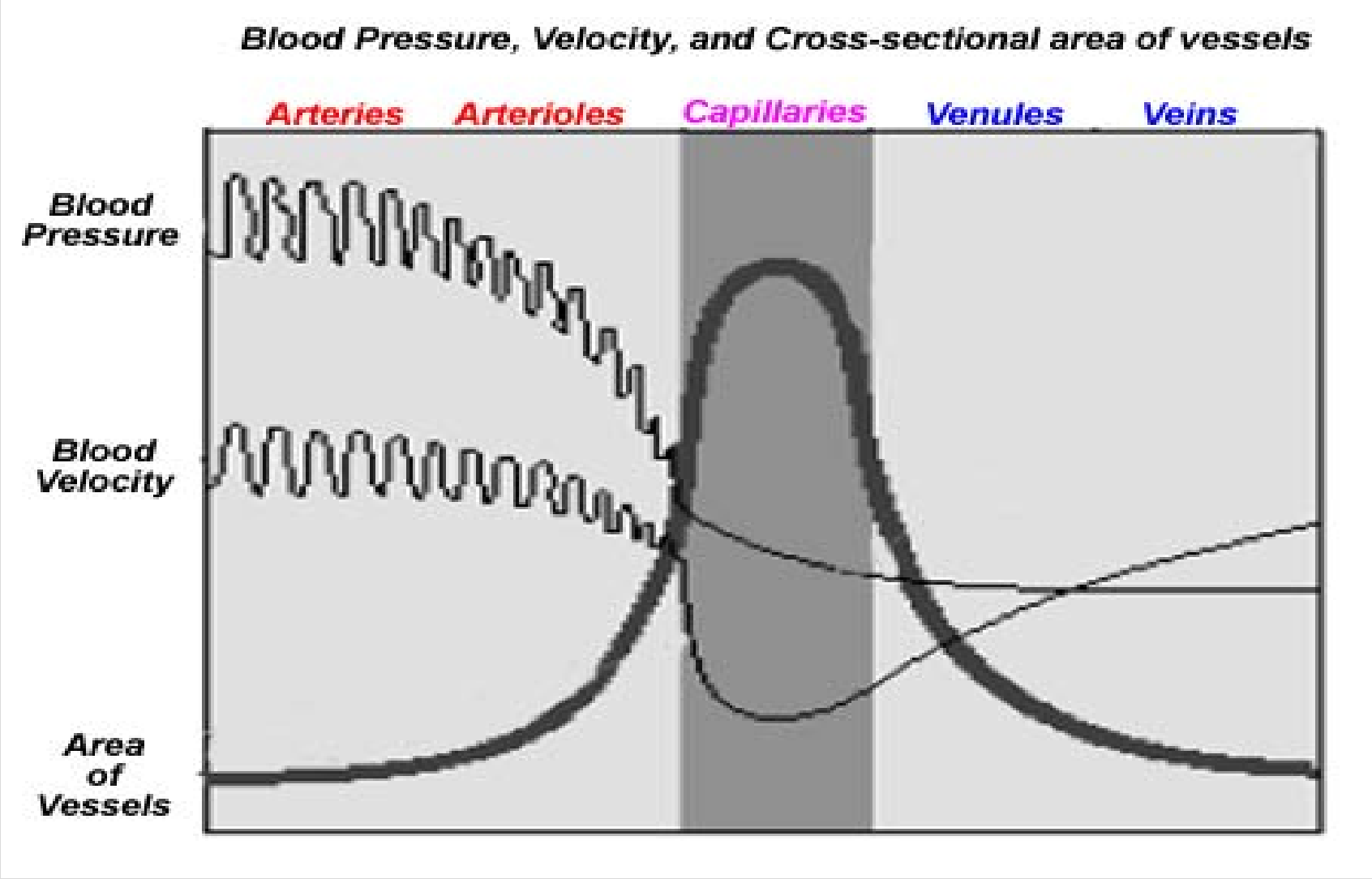
Phases of Analytical Testing

- Preanalytical
- Analytical
- Post Analytical

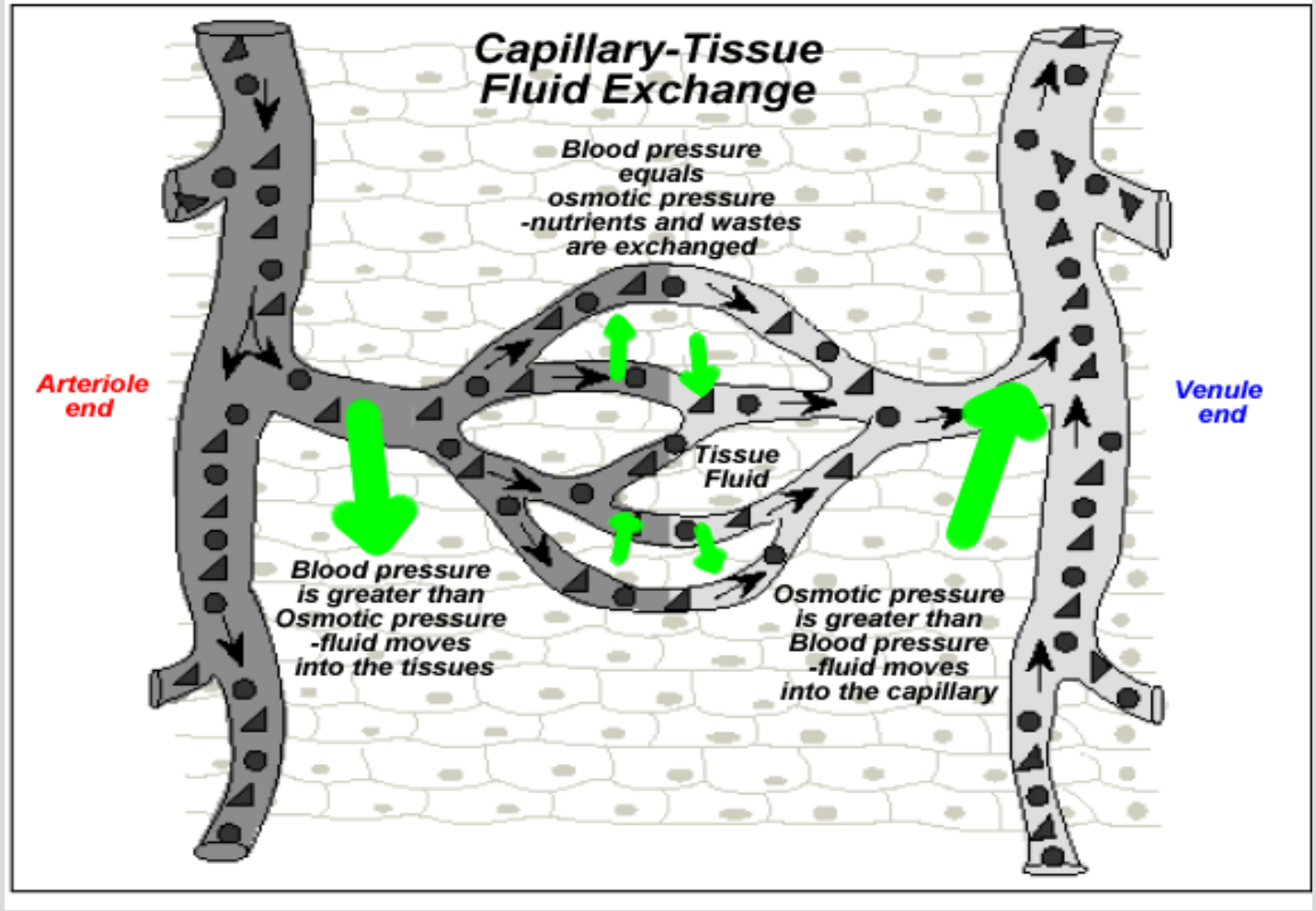
Preanalytical Sources of Variance in Bedside Glucose Testing

- Sample source
- Sample type
- Timing
- Additives
- Glycolysis
- Hematocrit Effect
- Interfering Substances

Effect of Branching of Arteries into Capillaries



Capillary-Tissue Fluid Exchange



Patient Factors Which Influence Capillary Glucose Testing

- Poor peripheral circulation
- Hypo/Hypervolemia
- Exercise
- Positions
- Stress
- Alchohol/Drugs
- Uremia

Special Medical Conditions

- Hypotension or shock
  - Pseudohypoglycemia may result from increased glucose extraction by the tissues because of low capillary flow and increased glucose transit time
- Change or pathology of capillary beds
  - Raynaud phenomenon
  - Peripheral vascular disease
  - Edema etc.
- Hyper-osmotic ketoacidosis
  - Pseudohypoglycemia may result from influx of fluid from the tissue and consumption of the glucose in the capillary bed

Analytical Sources of Error

- Technique
- Poor Instrument Maintenance
- Methodologies
- Interferences

Analytical Methods Utilized in Bedside Glucose Testing

- Reflectance Colorimetry
- Polarography
- Amperiometric
- Rate Spectrophotometry

Glucose Oxidase (GO)

$$\text{Glucose} + O_2 \xrightarrow{\text{GO}} \text{Gluconolactone} + H_2O_2$$

$$H_2O_2 + \text{Reduced Dye} \xrightarrow{\text{Peroxidase}} \text{Oxidized Dye} + H_2O$$

$$\text{GO} + \text{Glucose} \longleftrightarrow \text{Gluconolactone} + \text{GO-reduced}$$

$$\text{GO-reduced} + \text{Ferrocene (Oxid)} \longleftrightarrow \text{GO} + \text{Ferrocene (Red)}$$

$$\text{Ferrocene (Red)} \longrightarrow \text{Ferrocene (Oxid)} + e^-$$

Glucose Oxidase Limitations

- Ag, Hg, Cu inhibitors of GO
- Negative Interferences of Indicator Rxn
  - Ascorbic Acid
  - Bili, Uric Acid, Citric Acid, l-Dopa, Aldose
  - Sugars, Acetoacetic Acid, Creatinine, L-cysteine
- Positive Interferences
- O_2 concentration

Hexokinase (HK)

$$\text{Glucose} + \text{ATP} \xrightarrow[\text{Mg}]{\text{HK}} \text{Glucose-6-P} + \text{ADP}$$

$$\text{Glucose-6-P} + \text{NADP}^{+} \xrightarrow{\text{G-6-P-DH}} \text{6-Phosphogluconate} + \text{NADPH} + \text{H}^{+}$$

$$\text{Glucose-6-P} + \text{NAD}^{+} \xrightarrow[\text{Mg}]{\text{HK}} \text{6-Phosphogluconate} + \text{NADH} + \text{H}^{+}$$

$$\text{NADH} + \text{MTT} \xrightarrow{\text{Diaphorase}} \text{Formazan} + \text{NAD}^{+}$$

MTT – methylthiazolyldiphenyl tetrazolium

Hexokinase Specificity

- Not totally specific for beta-D-glucose, will react with other hexoses(fructose, mannose, glucosamine)

- Coupling reaction with G6P-DH enhances overall specificity

Glucose Dehydrogenase (GD - NAD)

$$\text{Glucose} + \text{NAD}^{+} \xleftrightarrow{\text{GD}} \text{Gluconolactone} + \text{NADH}$$

$$\text{NADH} + \text{MTT} \xleftrightarrow{\text{Diaphorase}} \text{Formazan} + \text{NAD}^{+}$$

$$\text{GD-NADH} + \text{PQ (Oxid)} \longleftrightarrow \text{GD-NAD}^{+} + \text{PQ (Red)}$$

$$\text{PQ (Red)} \longrightarrow \text{PQ (Oxid)} + e^{-}$$

NAD – nicotinamide adenine dinucleotide

MTT – methylthiazolyldiphenyl tetrazolium

PQ – Phenanthroline quinone

Glucose Dehydrogenase (GD - FAD)

$$\text{Glucose} + \text{GD-FAD}^{+} \longleftrightarrow \text{Gluconic Acid} + \text{GD-FADH}$$

$$\text{GD-FADH} + \text{Ferricyanide} \leftrightarrow \text{GD-FAD}^{+} + \text{Ferrocyanide (Red)}$$

$$\text{Ferrocyanide (Red)} \longrightarrow \text{Ferricyanide (Oxid)} + e^{-}$$

FAD – flavin adenine dinucleotide

Glucose Dehydrogenase (GD - PQQ)

$$\text{Glucose} + \text{GD-PQQ} \longleftrightarrow \text{Gluconic Acid} + \text{GD-PQQ}^{2-}$$

$$\text{GD-PQQ}^{2-} + 2\ \text{Ferricyanide} \longleftrightarrow \text{GD-PQQ} + 2\ \text{Ferrocyanide (Red)}$$

$$2\ \text{Ferrocyanide (Red)} \longrightarrow 2\ \text{Ferricyanide (Oxid)} + 2e^-$$

PQQ – pyrroloquinoline quinone

Glucose Dehydrogenase Advantages

- GDH-NAD Highly specific for Beta-D-glucose
- Not affected by Uric Acid, Bili & Ascorbic Acid
- Single Step Reaction
- High turnover rate

Glucose Dehydrogenase Disadvantages

- GDH-FAD Cross Reacts with d-Xylose
- GDH-PQQ Cross Reacts with
  - d-Xylose
  - Maltose (in some immunoglobulin preparations)
  - Galactose
  - Icodextrin (peritoneal dialysis solutions)

Postanalytical Causes of Variance

- Transcription Errors
- Communication

Evolution of POCT

Manual

Automation

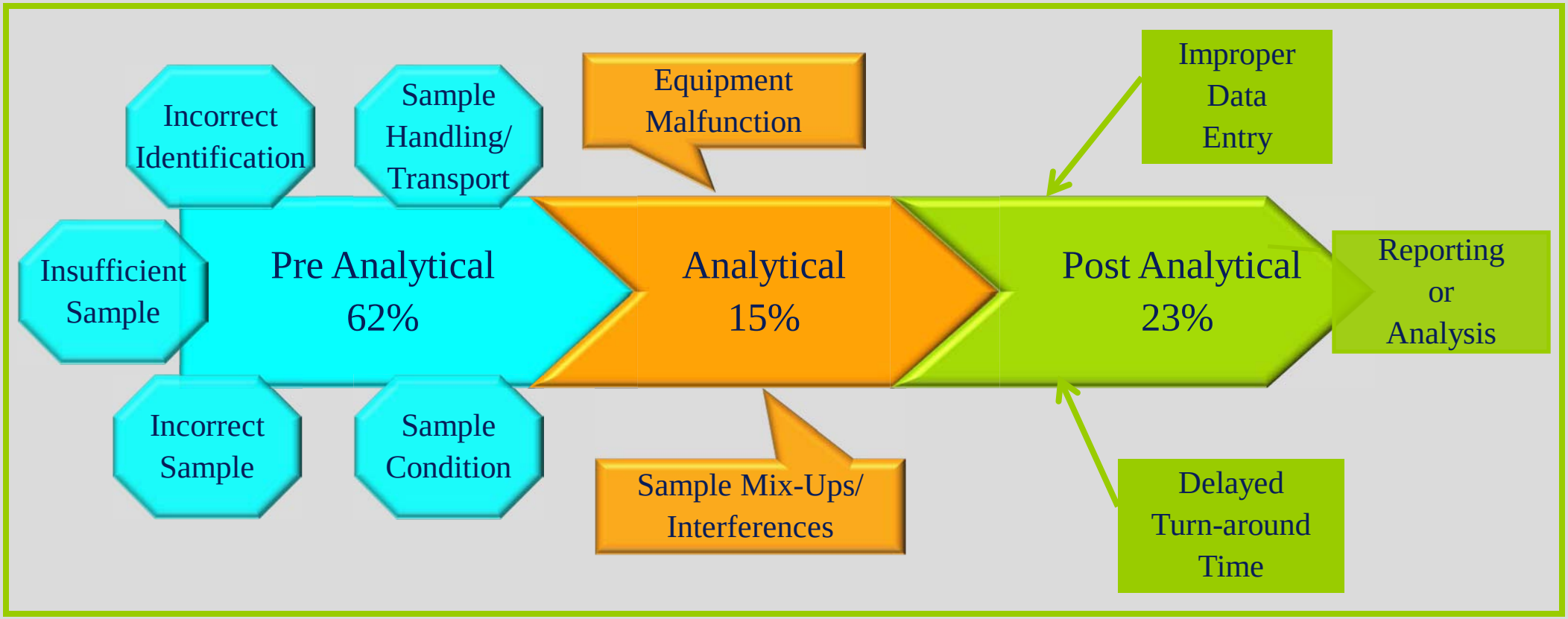
A process or system operating automatically

Autonomation

Intelligent automation – detects single defective operation and automatically stops

Ehrmeyer S, Lassig R. Clin Chem Lab Med 2007;45(6):766-773

Thinking in the POCT Box



As autononmation reduces errors in the box,

further reductions must occur outside the box.

Thinking Outside the POCT Box

- Pre-pre: Phsician must consider
  - What POCT is available?
  - What POCT will best serve the patient?
  - Will an immediate answer improve the patient's outcome?
- Post-post: Is the Physician?
  - Receptive to using an immediate POCT result
  - Able to interpret result in the patient's context
  - Amenable to initiating an immediate response

QUESTIONS