

Is My Patient's Result Real? – Causes of POC/Lab Assay Interferences and Strategies to Avoid Them

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Hospital



Learning Objectives

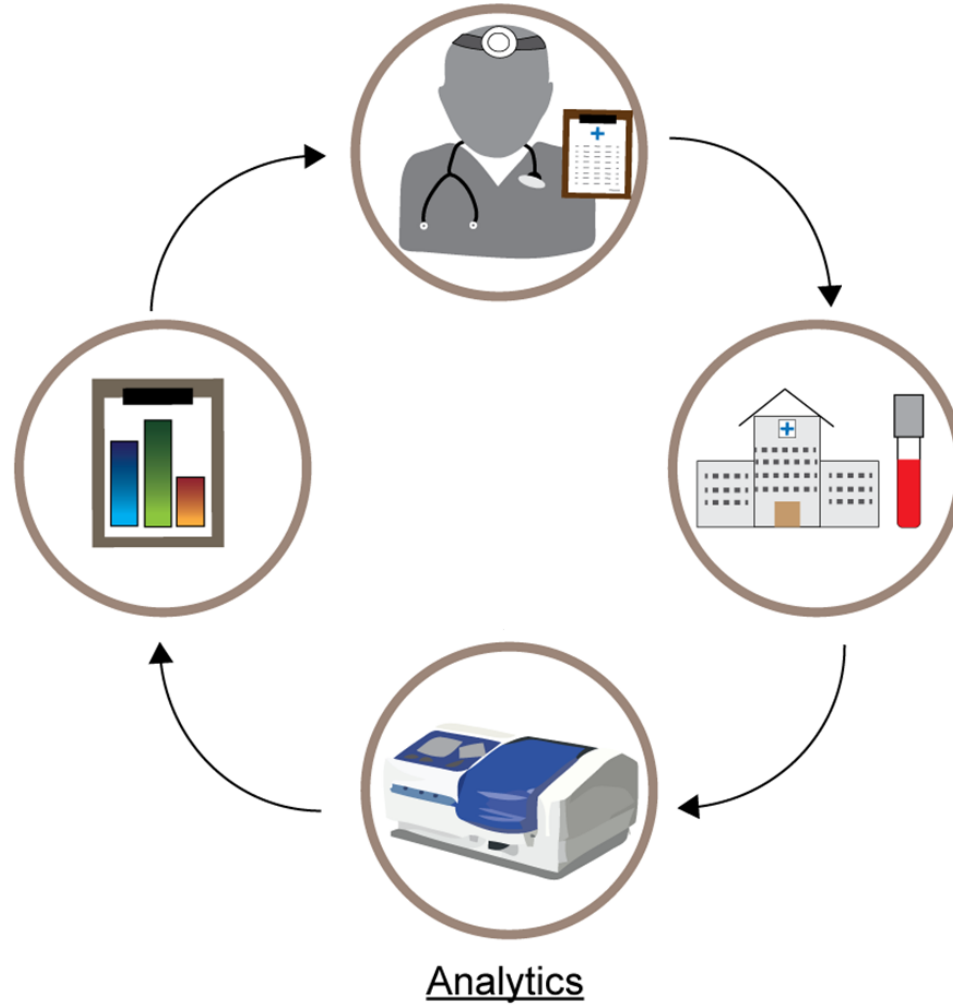
1. Review common interferences affecting POC/Lab assays
2. Explain mechanism of interference
3. Learn about strategies that mitigate interferences



Total Testing Process

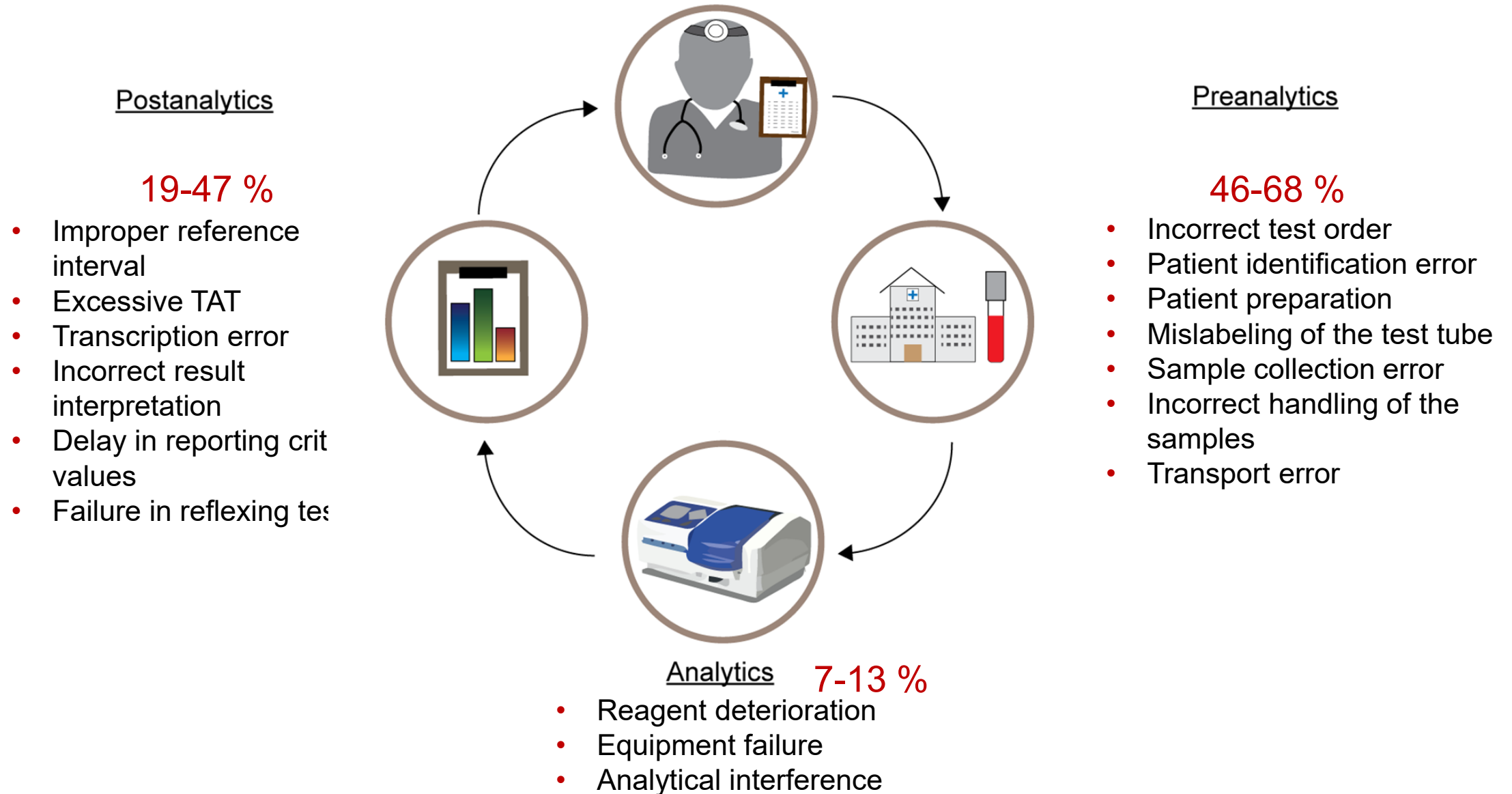
Postanalytics

Prenalytics

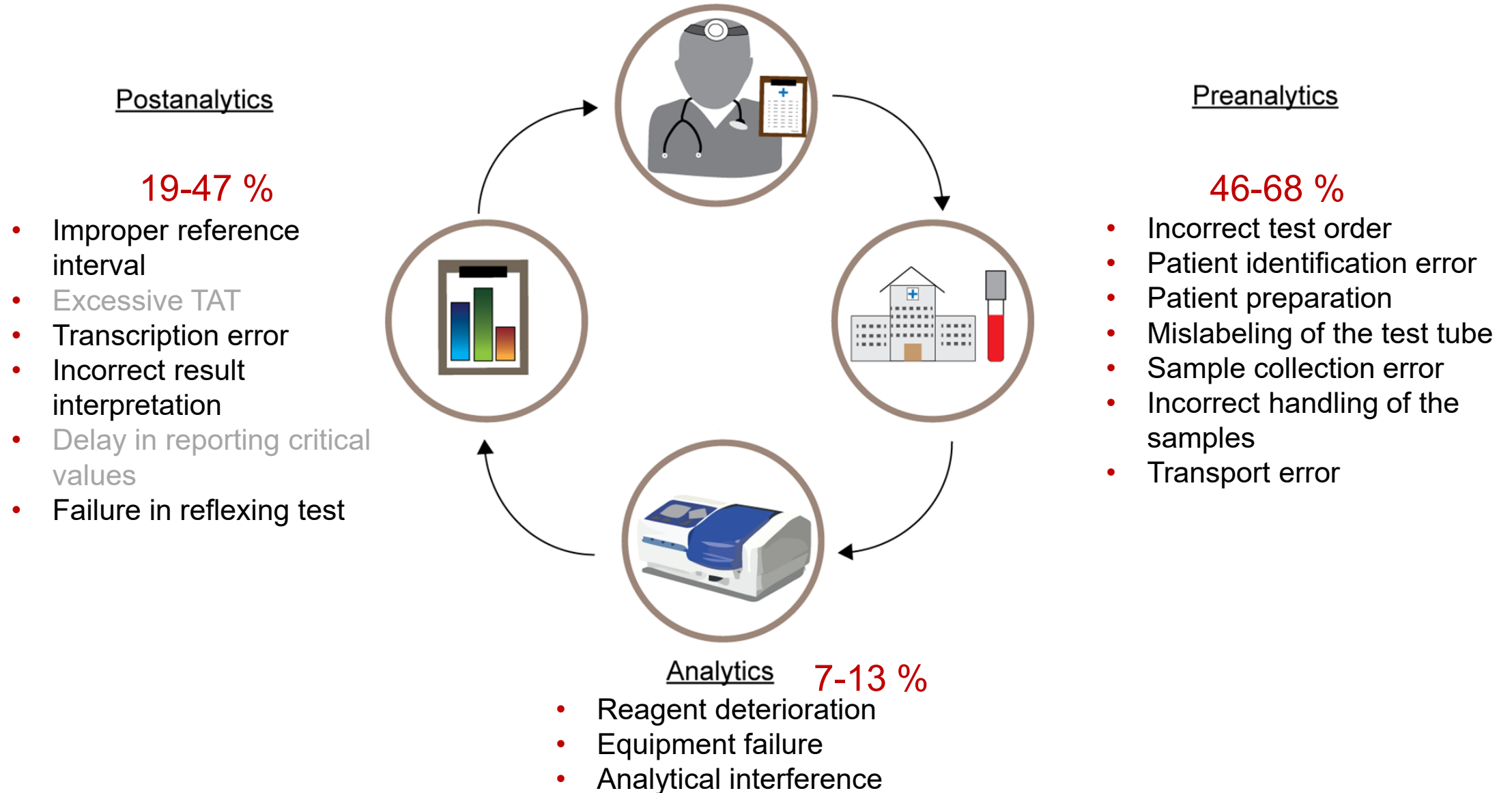


Analytics

Error sources and error rate in a clinical lab



Error sources and error rate in a clinical lab



Error sources in POC settings

Postanalytics

- Improper reference interval
- Excessive TAT
- Transcription error
- Incorrect result interpretation
- Delay in reporting critical values
- Failure in reflexing test
- **Transcription error (manual entry)**
- **Minimal expertise with what the result mean**



Analytics

- Reagent deterioration
- Equipment failure
- Analytical interference
- **Different performance than the lab**

Preanalytics

- Incorrect test order
- Patient identification error
- Patient preparation
- Mislabeling of the test tube
- Sample collection error
- Incorrect handling of the samples
- Transport error
- **Instrument being lost/broken**
- **Deviations from manufacturer instructions**
- **Testing performed by untrained operators**
- **QC not performed**
- **Excessive orders**
- **Difficulties with compliance and documentation**

Why POC testing is prone to error

Central Lab testing

- Structured lab environment
- One location with limited number of analyzers
- Tens of operators
 - trained in lab skills
- Priority = sample testing



Traffic in Wisconsin

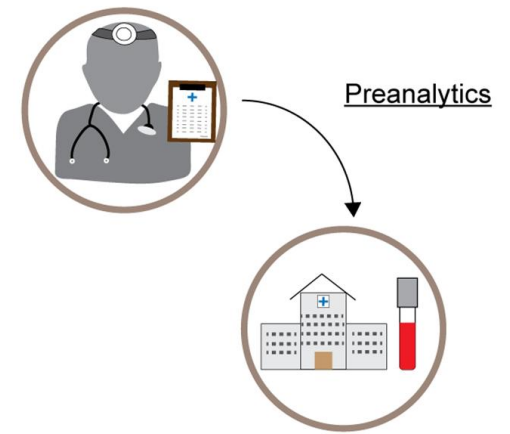
POC

- Chaotic hospital environment
- Multiple locations with hundreds of devices
- Hundreds of operators
 - not trained in lab skills
- Priority = patient care



Traffic in Atlanta

Pre-analytical variables



- Patient factors
 - Fasting, medications, time of the day, biological variation, stress, exercise, age, gender, pregnancy

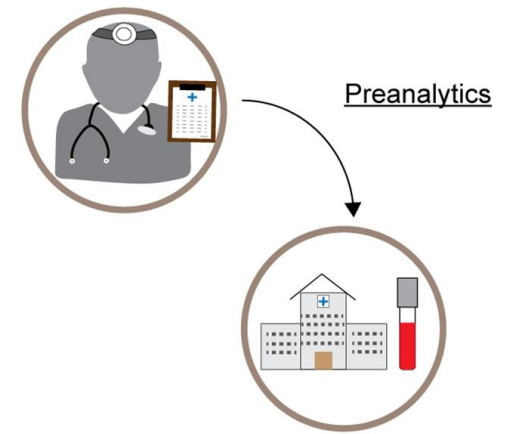


- Order and collection variables
 - Labeling of the specimen, specimen type, collection site, site preparation, tourniquet application time, order of draw, under filling of tubes, air bubbles



- Transport, storage, and processing
 - Storage temperature, light, travel/processing time

Pre-analytical variables



- **Patient factors**

- Fasting, medications, time of the day, biological variation, stress, exercise, age, gender, pregnancy



- **Order and collection variables**

- Labeling of the specimen, specimen type, collection site, site preparation, tourniquet application time, order of draw, under filling of tubes, air bubbles



- **Transport, storage, and processing**

- Storage temperature, light, travel/processing time

Unknown case

- A 44 year old woman with HTN, Asthma, COPD in home O2 who presents with 4 days of aphonia and SOB after self reported house fire
- CMP unremarkable except:

☒ All Rows			
		3/21/24 21:39	3/22/24 23:29
CHEMISTRY			
SODIUM-SERUM		141	140
POTASSIUM-SERUM		4.9	4.3
CHLORIDE-SERUM		108	108
CO2 CONTENT-SERUM		24	22
ANION GAP		11	10
GLUCOSE		134 ▲	92
UREA NITROGEN-SERUM		17	23 ▲
CREATININE		1.2 ▲	1.2 ▲
GLOMERULAR FILTRATION RATE ...		37 ▼ 📄	57 ▼ 📄
OSMO,CALCULATED		285	283

CMP: comprehensive metabolic panel

What could be the cause?

- A. Mislabeling of the test tube
- B. Transcription error
- C. Incorrect handling of the sample
- D. Incorrect age or gender
- E. Assay interference
- F. Wrong calculation

☒ All Rows		3/21/24 21:39	3/22/24 23:29
CHEMISTRY	 		
SODIUM-SERUM		141	140
POTASSIUM-SERUM		4.9	4.3
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UREA NITROGEN-SERUM		17	23 ▲
CREATININE		1.2 ▲	1.2 ▲
GLOMERULAR FILTRATION RATE ...		37 ▼ 	57 ▼ 
OSMO,CALCULATED		285	283

Unknown case

- A 44 year old woman with HTN, Asthma, COPD in home O2 who presents with 4 days of aphonia and SOB after self reported house fire
- CMP unremarkable except:

Doctor X: “This is for sure lab error and it raises safety concerns not only for this patient but for everyone else”

Lab director: “Looks like a lab error for sure. Probably a Beaker calculation error, which is scary”

☒ All Rows			
		3/21/24 21:39	3/22/24 23:29
CHEMISTRY	 		
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UREA NITROGEN-SERUM		17	23 ▲
CREATININE		1.2 ▲	1.2 ▲
GLOMERULAR FILTRATION RATE ...		37 ▼ 	57 ▼ 
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Could it be eGFR calculation?

Serum Creatinine: 1.2 ☒ mg/dL ☐ μ mol/L

Serum Cystatin C: mg/L

Age: 44 Years

Gender: ☐ Male ☒ Female

Standardized Assays: ☒ Yes ☐ No ☐ Not Sure

Adjust for body surface area: ☐ Yes ☒ No ☐ Not Sure

Calculate

Results

CKD-EPI creatinine equation (2021) 57 mL/min/1.73m²

☒ All Rows

CHEMISTRY

	3/21/24 21:39	3/22/24 23:29
SODIUM-SERUM	141	140
POTASSIUM-SERUM	4.9	4.3
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CO2 CONTENT-SERUM	24	22
ANION GAP	11	10
GLUCOSE	134 ▲	92
UREA NITROGEN-SERUM	17	23 ▲
CREATININE	1.2 ▲	1.2 ▲
GLOMERULAR FILTRATION RATE ...	37 ▼ 📄	57 ▼ 📄
OSMO,CALCULATED	285	283

Patient was admitted as a STAT pack patient

STAT pack patient:
emergency room patient that comes in under urgent conditions that initially doesn't have identification



Solution: implement a rule that prevents reporting eGFR from unknown gender or age

LIVING ROOM

Emergency U. Fortdodge

Unknown ⓘ, 115 y.o., 1/1/1910

MRN: 30003080

Code: Not on file (no ACP docs)

Name: Emergency U. Fortdodge

None

Isolation: None

Verify Allergies for this

Specimen Inquiry: 25G-255CA0003

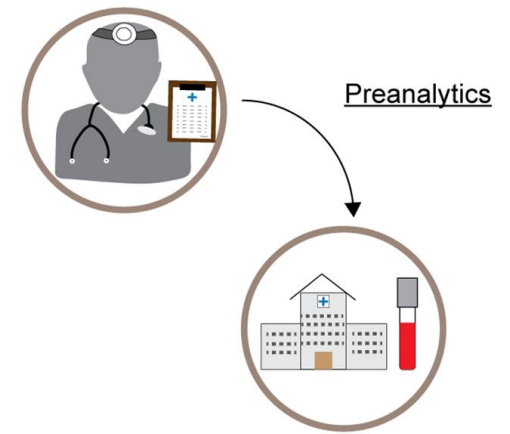
Documents Patient Inquiry

Specimen Inquiry

1	HEM				REMISOL		
1	ALB Adj. CA	10.0	mg/dL	8.9-10.3	REMISOL		
1	Anion Gap	12		1-13	REMISOL		
1	Osmo-Calc		mOsm/kg	275-300	REMISOL		275
1	eGFR		mL/min/1.73 mE2	>60	REMISOL		36

Comment:
Unable to calculate due to unconfirmed gender or age < 15: kidney failure

Pre-analytical variables



- Patient factors
 - Fasting, medications, time of the day, biological variation, stress, exercise, age, gender, pregnancy



- **Order and collection variables**
 - Labeling of the specimen, specimen type, collection site, site preparation, tourniquet application time, order of draw, under filling of tubes, air bubbles



- Transport, storage, and processing
 - Storage temperature, light, travel/processing time

Wrong blood in tube (WBIT) example #1

- Routine check up on a 40 year old patient resulted in a hospital admission for kidney failure

5m ago ☒ All Rows

Time Mark

	8/24/22 13:14	10/21/22 09:06	10/27/22 10:27
BASIC CHEM 1			
SODIUM (UWH)	138	137	137
POTASSIUM (UWH)	4.1	4.1	6.0 ▲
CHLORIDE (UWH)	109 ▲	109 ▲	109 ▲
CARBON DIOXIDE (UWH)	20 ▼	19 ▼	17 ▼
ANION GAP (UWH)	9	9	11
BUN (UWH)	12	11	28 ▲
CREATININE (UWH)	1.11 ▲	1.06 ▲	3.32 ▲
GLUCOSE (UWH)	99	95	106 ▲

Wrong blood in tube (WBIT) example #1

- Routine check up on a 40 year old patient resulted in a hospital admission for kidney failure

5m ago	8/24/22 13:14	10/21/22 09:06	10/27/22 10:27	10/27/22 18:46	10/27/22 22:06	10/28/22 06:09
BASIC CHEM 1						
SODIUM (UWH)	138	137	137	138	138	138
POTASSIUM (UWH)	4.1	4.1	6.0 ▲	4.2	4.3	4.2
CHLORIDE (UWH)	109 ▲	109 ▲	109 ▲	105	105	107
CARBON DIOXIDE (UWH)	20 ▼	19 ▼	17 ▼	24	26	25
ANION GAP (UWH)	9	9	11	9	7	6 ▼
BUN (UWH)	12	11	28 ▲	12	12	11
CREATININE (UWH)	1.11 ▲	1.06 ▲	3.32 ▲	0.86	0.84	0.77
GLUCOSE (UWH)	99	95	106 ▲	117 ▲	94	81

Potential solutions for WBIT – delta checks

- Laboratory information system (LIS) tool that compares a new patient test result with their previous result in order to detect test results changes greater than expected
- The difference is calculated, as an absolute value or a percentage change, and compared to a set threshold often over specified time span
- Examples:
 - Na ± 10 (7 days)
 - Creatinine ± 1 (7 days)
 - Calcium $\pm 20\%$ (7 days)

Wrong blood in tube (WBIT) example #2

- Clinical team concerned for liver failure

Legend: ☒ All Rows <1m ago Time Mark 7/12/24 21:04 7/13/24 14:56 7/14/24 02:01 7/15/24 02:42

CHEMISTRY

SODIUM-SERUM	139	130 ▼	136	137
POTASSIUM-SERUM	4.8	5.8 ▲	4.8	4.6
CHLORIDE-SERUM	110	95 ▼	109	111
CO2 CONTENT-SERUM	21 ▼	19 ▼	17 ▼	17 ▼
ANION GAP	8	16 ▲	10	9
GLUCOSE	160 ▲	312 ▲	112	97
UREA NITROGEN-SERUM	21	90 ▲	32 ▲	30 ▲
CREATININE	1.6 ▲	2.5 ▲	2.1 ▲	1.9 ▲
BILIRUBIN,TOTAL-SERUM	2.0 ▲	0.9	1.2	1.4
BILIRUBIN,DIRECT-SERUM	0.4	0.4	0.2	0.3
AST (SGOT)	37	953 ▲	32	22
ALT (SGPT)	21	1,209 ▲	18	13 ▼
ALKALINE PHOSPHATASE SERUM	103	580 ▲	98	97

- Delta check did not flag (difference <1)
- Sample repeated and gave same results

Wrong

- Clinical

☒ All Rows <1m ago Time Mark 7/12/24 21:04 7/13/24 14:56

CHEMISTRY

	SODIUM-SERUM	139	130 ▼
☒	POTASSIUM-SERUM	4.8	5.8 ▲
	CHLORIDE-SERUM	110	95 ▼
CI	CO2 CONTENT-SERUM	21 ▼	19 ▼
SC	ANION GAP	8	16 ▲
PC	GLUCOSE	160 ▲	312 ▲
CH	UREA NITROGEN-SERUM	21	90 ▲
CC	CREATININE	1.6 ▲	2.5 ▲
AN			
GL			
UF	BILIRUBIN,TOTAL-SERUM	2.0 ▲	0.9
CF	BILIRUBIN,DIRECT-SERUM	0.4	0.4
BIL	AST (SGOT)	37	953 ▲
BIL	ALT (SGPT)	21	1,209 ▲
AS			
AL	ALKALINE PHOSPHATASE SERUM	103	580 ▲
AL			

Sample #2

- Delta check did not flag (difference <1)
- Sample repeated and gave same results

Wrong blood in tube (WBIT) example #2

- Clinical team concerned for liver failure

Time Mark 7/12/24 21:04 7/13/24 14:56 7/14/24 02:01 7/15/24 02:42

CHEMISTRY

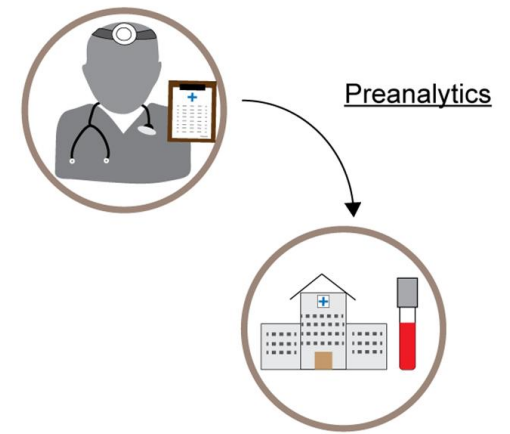
SODIUM-SERUM	139	130 ▼	136	137
POTASSIUM-SERUM	4.8	5.8 ▲	4.8	4.6
CHLORIDE-SERUM	110	95 ▼	109	111
CO2 CONTENT-SERUM	21 ▼	19 ▼	17 ▼	17 ▼
ANION GAP	8	16 ▲	10	9
GLUCOSE	160 ▲	312 ▲	112	97
UREA NITROGEN-SERUM	21	90 ▲	32 ▲	30 ▲
CREATININE	1.6 ▲	2.5 ▲	2.1 ▲	1.9 ▲
BILIRUBIN,TOTAL-SERUM	2.0 ▲	0.9	1.2	1.4
BILIRUBIN,DIRECT-SERUM	0.4	0.4	0.2	0.3
AST (SGOT)	37	953 ▲	32	22
ALT (SGPT)	21	1,209 ▲	18	13 ▼
ALKALINE PHOSPHATASE SERUM	103	580 ▲	98	97

- Delta check did not flag (difference <1)
- Sample repeated and gave same results

- The blood typing on blood was performed and this patient is O positive but the blood typing showed B positive

Contaminations

- Samples drawn from line (TPN, KCl, Heparin)
 - Elevated electrolytes, e.g. K: 8 mmol/L
 - Glucose, e.g. 400 mg/dL
 - APTT >320 sec
- Other normal serum components abnormally low
- Common causes of repeat contamination
 - Infusion not paused
 - Line not flushed
 - Waste tube not collected
- **All orders on draw should be cancelled in presence of suspected contamination**



Contamination - example

Ca flagged as Delta Check

34m ago ☒ All Rows

Time Mark

	9/13/22 18:44	9/14/22 06:45
BASIC CHEM 1		
SODIUM (UWH)	127 ▼	137
POTASSIUM (UWH)	4.7	2.6 ▼
CHLORIDE (UWH)	91 ▼	118 ▲
CARBON DIOXIDE (UWH)	28	15 ▼
ANION GAP (UWH)	8	4 ▼
BUN (UWH)	16	8 ▼
CREATININE (UWH)	0.50 ▼	0.24 ▼
GLUCOSE (UWH)	98	55 ▼
BASIC CHEM 2		
ALBUMIN (UWH)		1.9 ▼
CALCIUM (UWH)	9.6	4.5 ▼
MAGNESIUM (UWH)		1.1 ▼
PROTEIN, TOTAL (UWH)		3.1 ▼
GI/LIVER		
ALKALINE PHOSPHATASE (...)		32 ▼
ALT/SGPT (UWH)		15
AST/SGOT (UWH)		17
BILIRUBIN, TOTAL (UWH)		0.5
LDH (UWH)		182
LIPIDS/CV RISK		
CHOLESTEROL (UWH)		77
TRIGLYCERIDES (UWH)		21
HDL CHOLESTEROL (UWH)		34 ▼
LDL CHOLESTEROL (UWH)		39
CHOLESTEROL, NON-HDL (U...)		43

- Lab tech called the unit suspecting that it was a contamination but was told these results are real and should be released
- Luckily a comment was added : *please redraw to verify*

1	Calcium	4.5	mg/dL	✓	⚠	8.4-10.2	✓		9.6
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Contamination - example

Ca flagged as Delta Check

34m ago			All Rows		9/13/22 18:44	9/14/22 06:45
Time Mark			BASIC CHEM 1			
GLUCOSE (UWH)			98	55	▼	📄
BASIC CHEM 2						
ALBUMIN (UWH)				1.9	▼	📄
CALCIUM (UWH)			9.6	4.5	▼	📄
MAGNESIUM (UWH)				1.1	▼	📄
PROTEIN, TOTAL (UWH)				3.1	▼	📄
GI/LIVER						
ALKALINE PHOSPHATASE (...)				32	▼	📄
ALT/SGPT (UWH)				15		📄
AST/SGOT (UWH)				17		📄
HDL CHOLESTEROL (UWH)				34	▼	📄
LDL CHOLESTEROL (UWH)				39		📄
CHOLESTEROL, NON-HDL (U...)				43		📄

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34m ago ☒ All Rows

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BASIC CHEM 1			
SODIUM (UWH)	127 ▼	137	128 ▼
POTASSIUM (UWH)	4.7	2.6 ▼	4.2
CHLORIDE (UWH)	91 ▼	118 ▲	93 ▼
CARBON DIOXIDE (UWH)	28	15 ▼	28
ANION GAP (UWH)	8	4 ▼	7
BUN (UWH)	16	8 ▼	12
CREATININE (UWH)	0.50 ▼	0.24 ▼	0.50 ▼
GLUCOSE (UWH)	98	55 ▼	89
BASIC CHEM 2			
ALBUMIN (UWH)		1.9 ▼	4.1
CALCIUM (UWH)	9.6	4.5 ▼	9.3
MAGNESIUM (UWH)		1.1 ▼	2.1
PROTEIN, TOTAL (UWH)		3.1 ▼	6.8
GI/LIVER			
ALKALINE PHOSPHATASE (...)		32 ▼	75
ALT/SGPT (UWH)		15	30
AST/SGOT (UWH)		17	26
BILIRUBIN, TOTAL (UWH)		0.5	1.2
LDH (UWH)		182	220
LIPIDS/CV RISK			
CHOLESTEROL (UWH)		77	169
TRIGLYCERIDES (UWH)		21	45
HDL CHOLESTEROL (UWH)		34 ▼	76
LDL CHOLESTEROL (UWH)		39	84
CHOLESTEROL, NON-HDL (U...		43	93

- Lab tech called the unit suspecting that it was a contamination but was told these results are real and should be released
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1	Calcium	4.5	mg/dL	✓	⚠	8.4-10.2	✓	9.6
---	---------	-----	-------	---	---	----------	---	-----

Incorrect order of draw may lead to contamination

Color	Additive	Type	Sample tests
BLUE	Citrate	Plasma	Coagulation
RED	None	Serum	Chemistry tests
GREEN	Li Heparin	Plasma/ Whole blood	Electrolytes, BGAS (syringe), STAT lab
PURPLE	K ₂ EDTA	Plasma/ Whole blood	CBC, A1C
GREY	NaF/K oxalate	Plasma	Glucose, lactate

- Order of filling blood tubes is essential
 - 1) blood culture 2) citrate tubes
 - 3) Serum, Li hep 4) EDTA 5) Grey
- If proper order of draw is not followed, contamination of next tube with additive may occur



Discrepant chemistry results

- 59- year old male came in for a check up of his kidney function. On labs earlier today, he was found to have severe hyperkalemia, severe hypocalcemia. He was contacted and advised to go to the ED

TEST	8/9/2023 at 08:20 (primary care)
Sodium (mmol/L)	143
Potassium (mmol/L)	>10
Chloride (mmol/L)	103
CO2 (mmol/L)	22
BUN (mg/dL)	18
Creatinine (mg/dL)	1.08
Glucose (mg/dL)	97
Calcium (mg/dL)	<2.5

What do you think happened here?

- A. Real result, patient has high K and very low Calcium
- B. Hemolysis
- C. Delay in sample processing
- D. Transport error
- E. Wrong tube was drawn

Discrepant chemistry results

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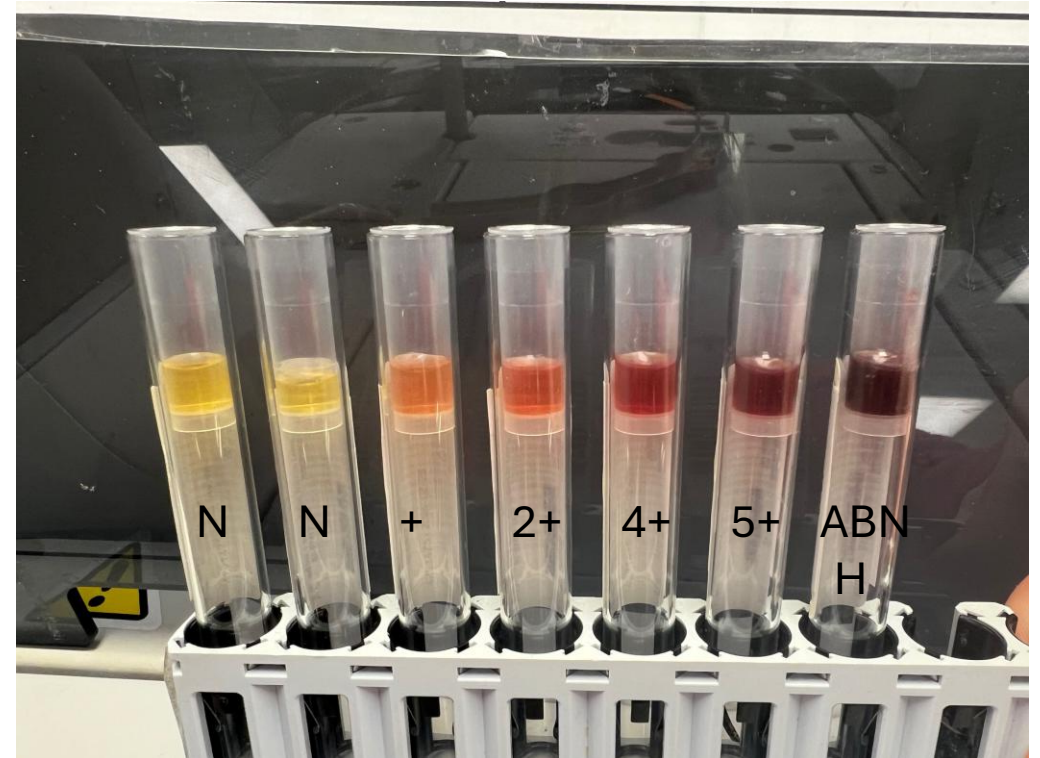
TEST	8/9/2023 at 08:20 (primary care)	8/9/2023 at 12:30 (ED)
Sodium (mmol/L)	143	142
Potassium (mmol/L)	>10	3.7
Chloride (mmol/L)	103	109
CO2 (mmol/L)	22	24
BUN (mg/dL)	18	14
Creatinine (mg/dL)	1.08	1.07
Glucose (mg/dL)	97	142
Calcium (mg/dL)	<2.5	9.5

- CMP was drawn into a K₂EDTA and then poured into a heparin tube
- Safety report submitted, patient credited and additional flags in LIS implemented

Hemolysis



- In vitro hemolysis: (occurs with turbulent flow, often with slow fill, high pressure on syringe, “milking” the finger during finger puncture)
- Several causes of discrepant results
 - Cellular release
 - Analytical interference
 - Proteolysis
- Results increase or decrease
- Hemolysis in whole blood (POCT) often unable to be assessed



K (3.4 – 5.1 mmol/L):	3.7-----	4.0 -----	6.8
AST (13-39 U/L) :	18 -----	22 -----	38.5
LDH (91 – 180 U/L):	153 -----	223 -----	952

Big thanks to Calvin!

Pseudohyperkalemia

- Traumatic draw
 - In vitro hemolysis -> increased K, LDH, AST
- In-vivo hemolysis
 - Autoimmune hemolytic anemia
 - Mechanical shearing of RBC (ECMO patients)
- Tourniquet application time (>2 min)
- Repeated fist clenching leads to potassium release from muscles
- K₃EDTA contamination due to wrong order of draw
- Transport
 - Temperature, pneumatic tube

POTASSIUM (mmol/L)						
Room Temperature						
Patient	Initial	2 Hour	4 Hour	6 Hour	12 Hour	24 Hour
1	4.13	4.04	4.08	4.03	4.03	4.11
2	3.88	3.73	3.81	3.97	3.82	4.25
3	3.77	3.59	3.47	3.54	3.48	3.63
4	3.42	3.31	3.45	3.44	3.37	3.73
5	4.22	4.16	4.23	4.16	4.03	4.23
6	4.33	4.13	4.06	4.15	4.02	4.27
7	3.7	3.53	3.51	3.57	3.41	3.63
8	4.14	3.95	4.03	3.97	3.97	4.33
9	3.66	3.51	3.48	3.43	3.38	3.33
10	4.17	4.06	3.9	3.89	3.7	3.71
Refrigerated						
Patient	Initial	2 Hour	4 Hour	6 Hour	12 Hour	24 Hour
11	3.82	3.91	4.19	4.43	5.17	7.23
12	4.13	4.19	4.22	4.65	5.3	7.6
13	4.35	4.56	4.85	5.09	6.26	8.29
14	3.7	3.86	4.19	4.54	5.9	8.46
15	4.41	4.53	4.43	4.89	6.02	9.15
16	4.02	4.2	4.72	5.1	6.87	10
17	4.15	4.23	4.68	4.92	5.96	8.27
18	4.49	4.87	5.14	5.29	6.42	9.68
19	4.05	4.2	4.41	4.66	5.38	8.02
20	4.33	4.49	4.85	5.1	6.05	9.33

Pseudohyperkalemia vs Reverse pseudohyperkalemia

Pseudohyperkalemia:

- Platelets release potassium when clotting occurs
 - Serum K > plasma K (if patient has thrombocytosis, order plasma K)

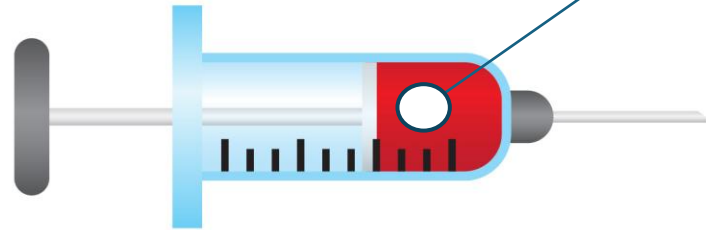
Reverse pseudohyperkalemia:

- WBC in patient with leukocytosis, release potassium when li heparin tube is used and sent to the lab via pneumatic tube
 - Plasma K > serum K (for patients with high WBC and K, order serum K)

K in PST	8.13 (3.5 – 5.1 mmol/L)
K in WB heparinized syringe	8.4 (3.5-5.1 mmol/L)
K in SST	5.1 (3.5 – 5.1 mmol/L)
WBC	264.7 (4.5-11.0 x 10 ⁹ /L)

Exposure to air

- A pH blood is generally stable unless the specimen is exposed to air, causing a loss of CO_2 and increase in pH
- An increase in pH will decrease ionized calcium
- Air bubbles elevate pO_2 value and decrease pCO_2



Air Bubble:
 $\text{pO}_2 \sim 150 \text{ mmHg}$
 $\text{pCO}_2 \sim 0 \text{ mm Hg}$

0.5-1% size bubble can cause significant error!

Air bubble can affect electrolytes on a lab analyzer

- Patient arrived to ED with dizziness, hypotension and bradycardia
- Initial K by POCT at 13:30 was 6.4 mmol/L

	2/21/25 13:31	2/21/25 16:13
CHEMISTRY		
POTASSIUM-SERUM	6.0 ▲ 📄	2.3 ▼

Air bubble can affect electrolytes on a lab analyzer

- Patient arrived to ED with dizziness, hypotension and bradycardia
- Initial K by POCT at 13:30 was 6.4 mmol/L

	2/21/25 13:31	2/21/25 16:13	2/22/25 00:34	2/22/25 04:30	2/22/25 10:41	2/22/25 17:51	2/23/25 07:10	2/23/25 10:41
CHEMISTRY								
POTASSIUM-SERUM	6.0 ▲	2.3 ▼	6.8 ▲	6.5 ▲	6.6 ▲	5.9 ▲	7.6 ▲	4.7

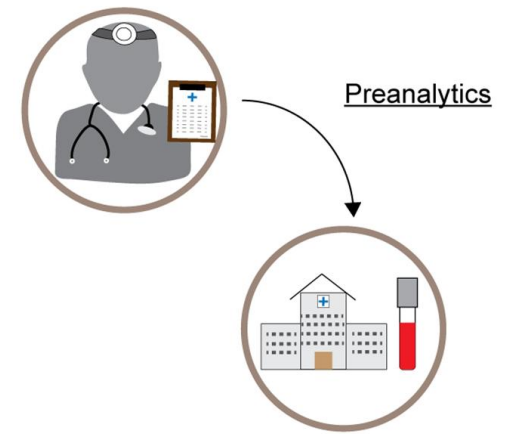
- Hospital physicians acted on 2.3 K with repletion which worsened the patient true hyperkalemia and precipitating EKG changes
- Solution: repeat implausible values automatically
 - K <2.3 and >6.5
 - Glucose < 40
 - Sodium <110 and Chloride <70

Specimen type (arterial, venous or capillary blood)

- Arteries: Peripheral arteries can be accessed when needed
 - To assess Oxygenation status : pO_2 , pCO_2 , pH
- Veins: Easy to access, few differences from arterial blood
 - **Venous blood: Reference sample for most laboratory testing**
 - Easy to obtain (compared to arterial)
 - Defined by ADA as reference type for diabetes diagnosis
- Capillaries: cannot be directly accessed (too small)
 - “Capillary blood” is mixture of arterial blood, venous blood, intracellular fluid (cells break during capillary puncture), and interstitial fluid (not blood, fluid that bathes tissue)
 - K, AST, LDH several fold higher



Pre-analytical variables



- Patient factors
 - Fasting, medications, time of the day, biological variation, stress, exercise, age, gender, pregnancy



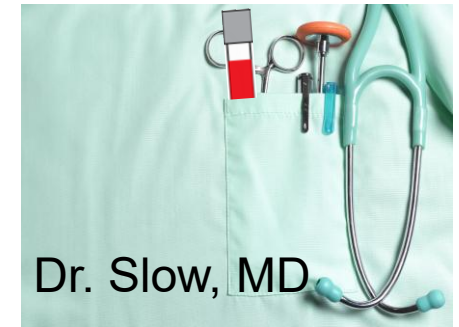
- Order and collection variables
 - Labeling of the specimen, specimen type, collection site, site preparation, tourniquet application time, order of draw, under filling of tubes, air bubbles



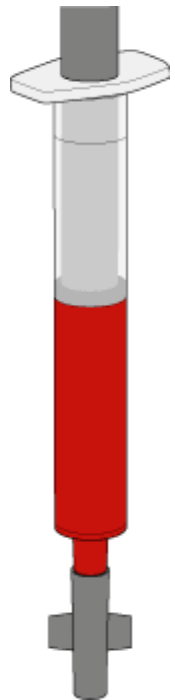
- **Transport, storage, and processing**
 - Storage temperature, light, travel/processing time

Delay in blood gas testing

Pocket syndrome



- Continued cellular metabolism in sample causes:



- ↓ pO_2 since oxygen will still be consumed ($\sim 20 \text{ mmHg/hour}$)
- ↑ pCO_2 since carbon dioxide will still be produced ($\sim 5 \text{ mmHg/hour}$)
- ↓ pH primarily due to the change in pCO_2 and glycolysis ($\sim 0.05/\text{hour}$)
- ↑ Ca^{2+} since the change in pH will influence the binding of Ca^{2+} to protein
- ↓ Glu since glucose will be metabolized
- ↑ Lac due to glycolysis

Stability of common tests in unspun whole blood

20 non-fasting healthy adults



- 30% male
- age 23 to 57 years



6

Tubes/participant

venous blood collected into 3-ml plastic Becton Dickinson (BD) Vacutainer™ plasma separated tubes (PST).

Reference:

T0: centrifuged immediately at 4000 g for 3 min at room temperature (RT) and analyzed

5 other tubes: Stored at Room Temperature or 4 °C

Centrifuge times 2, 4, 6, 12, and 24 hours

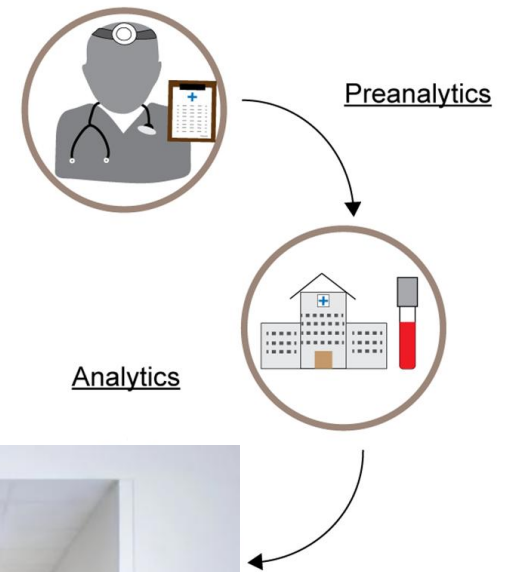
CLIA 2024				
Analyte	Concentration, mean	Allowable Total Error	Stability at 21 °C (hr)	Stability at 4 °C (hr)
Albumin	4.3 g/dL	± 8%	24	24
Total Calcium	9.7 mg/dL	± 1.0 mg/dL	24	24
Chloride	106.0 mmol/L	± 5%	24	24
Bicarbonate	25.8 mmol/L	± 20%	24	24
FT4	0.9 ng/dL	± 20%	24	24
Glucose	96.1 mg/dL	± 8%	<2	4
K	4.0 mmol/L	± 0.3 mmol/L	6	2
Mg	2.0 mg/dL	± 15%	24	24
Na	139.6 mmol/L	± 4.0 mmol/L	24	12
Phosphate	3.2 mg/dL	± 10%	4	24
Total Protein	7.6 g/dL	± 8%	24	24

GLUCOSE (mg/dL)						
Room Temperature						
Patient	Initial	2 Hour	4 Hour	6 Hour	12 Hour	24 Hour
1	100.8	93.2	88.5	82.2	65.3	39.3
2	90.5	83.8	80	77.6	66.5	46.9
3	89.1	82.5	78.4	73.1	56.2	26.4
4	89.5	79.8	67.8	67.9	46.6	24.8
5	83.1	78.2	71.9	67.9	43.6	27.8
6	96.3	88.3	78.9	81.3	66	39.9
7	75.5	70.5	68.6	66.4	53.1	33
8	92.9	85.7	79	76.4	59	38.1
9	100	91.2	84.6	80	58.7	30.2
10	102.7	96.7	86	84.1	55	29.9
Refrigerated						
Patient	Initial	2 Hour	4 Hour	6 Hour	12 Hour	24 Hour
11	94.7	91.8	90.7	89.7	84.4	72.8
12	67.2	65	62.4	61.9	58.1	46.1
13	91.5	89.9	87.8	83.9	78.8	67.4
14	85.5	88.9	86	82.5	77.2	70.9
15	140	137.5	135.8	134.4	130	122.4
16	105.6	101.7	98.2	100.8	90.9	72.9
17	87.5	79.1	83.9	80	79.3	62.6
18	94.3	91.3	91.3	90.4	84.9	70.7
19	135.1	134.2	129.8	131.6	123.5	113.3
20	100.6	97.3	96	93.2	88.9	76

Big thanks to Dr. Ospina-Romero!

Analytical variables

- Equipment/instrumentation
- Reagents
- Calibrators
- Calculation methods
- Lot to lot variability
- Instrument to instrument variability
- Interfering substances



Analytical performance of POC devices

- POC devices often differ in technology when compared to lab instruments
 - Smaller in size (space is often an issue)
 - Some devices are portable
 - Small volume
 - Faster turnaround
- Performance characteristics
 - Unique biases
 - Imprecision
 - Therefore, separate PT surveys
- Clinical management of patients may need to be different

Imprecision for POCT instrument differs from Lab

- Whole blood creatinine measured amperometrically using POC device

Product/ Level	N	Mean	SD	CV%	Allowable Total Error (CLIA limit)
QC L1	10	0.4 mg/dL	0.0	0.0%	±10% or ±0.2
QC L3	10	3.4 mg/dL	0.12	3.4%	±10% or ±0.2
Patient sample	10	0.6 mg/dL	0.05	8.1%	±10% or ±0.2

-
- Serum creatinine using enzymatic assay (core lab)

Product/ Level	N	Mean	SD	CV%	Allowable Total Error (CLIA limit)
QC L1	20	0.87 mg/dL	0.009	1.1%	±10% or ±0.2
QC L3	20	6.33 mg/dL	0.036	0.6%	±10% or ±0.2
Patient sample	20	0.86 mg/dL	0.007	0.8%	±10% or ±0.2

POC devices may generate different results

- Comparison of WB hsTroponin to Serum hsTroponin resulted in few falsely elevated troponin

Sex	Age	POC hsTnl 1	POC hsTnl 2	Chem hsTnl 1	Chem hsTnl 2		Sex	Age	POC hsTnl 1	POC hsTnl 2	Chem hsTnl 1	Chem hsTnl 2
F	41	<2.9	<2.9	<2.0	<2.0		M	23	<2.9	<2.9	2.3	2.3
F	66	<2.9	<2.9	2.4	2.4		M	42	<2.9	3.2	3.2	3.2
F	51	<2.9	<2.9	<2.0	<2.0		M	61	<2.9	<2.9	<2.0	<2.0
F	50	<2.9	<2.9	9.5	9.1		M	25	<2.9	<2.9	<2.0	2.0
F	29	<2.9	<2.9	<2.0	<2.0		M	62	2.9	3.6	2.8	2.9
F	46	<2.9	<2.9	2.6	2.6		M	49	2.9	3.3	2.9	2.8
F	24	<2.9	<2.9	<2.0	<2.0		M	38	2.9	3.8	3.1	3.1
F	32	<2.9	<2.9	<2.0	<2.0		M	31	3.1	<2.9	2.0	2.0
F	69	<2.9	<2.9	2.0	2.0		M	33	3.1	4.3	2.9	2.8
F	48	<2.9	<2.9	2.2	2.2		M	19	3.2	<2.9	2.6	2.5
F	63	<2.9	<2.9	<2.0	<2.0		M	26	3.4	3.6	2.9	2.7
F	42	<2.9	<2.9	<2.0	<2.0		M	45	3.6	3.5	3.7	3.6
F	27	<2.9	<2.9	<2.0	<2.0		M	44	3.7	<2.9	2.7	2.7
F	48	3	<2.9	<2.0	<2.0		M	35	4.2	5	4.6	4.5
F	60	3	3.6	2.1	2.0		M	55	4.4	3.3	2.8	2.9
F	39	3.8	4.1	3.6	3.4		M	32	4.7	4	4.8	4.8
F	64	5.5	4.3	4.7	4.6		M	62	6	4.1	2.3	2.3
F	51	6.1	6	6.2	6.1		M	46	7.2	8	6.5	6.2
F	62	6.5	5.3	5.5	5.4		M	68	7.6	7.5	6.4	6.3
F	63	7.5	8.1	7.4	7.4		M	49	31.2	31.9	8.2	7.9
F	23	16.8	14.6	3.3	3.3							
F	68	22.7	22.4	9.2	8.9							

Analytical interferences depend on POC methodology

- Potentiometry/Amperometry (BGAS and whole blood chemistry)
- Boronate affinity/Immunoassays (A1C)
- Electrochemistry (glucose meter)
- Immunoassays (Troponin)
- Test strips and lateral-flow testing (Drug of abuse, COVID, hCG)

And that's how you analyze blood! 🤒



Analyzing blood and urine provides key information about the functioning of the endocrine system.

ERproductions Ltd/Blend Images LLC

The best method for measuring oxygen saturation in a patient with methemoglobinemia is ...

- A. Any hand-held blood gas device
- B. Blood gas device with CO-oximetry
- C. Pulse oximetry

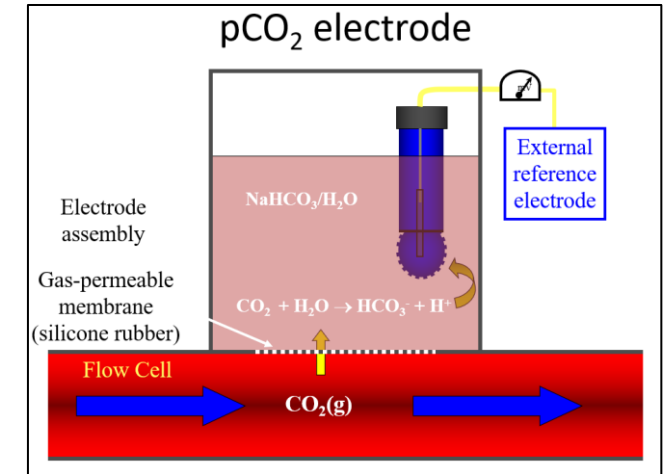
Blood Gas Analysis

Parameter	Normal value
pH	7.36-7.44 (a) 7.31-7.41 (v)
pCO ₂	34-46 mmHg (a) 40-52 mmHg (v)
pO ₂	80-90 mmHg (a) 25-40 mmHg (v)
sO ₂ (oxygen saturation)	95-98% (a) 60-80% (v)

a= arterial , v=venous blood

Blood Gas Analysis

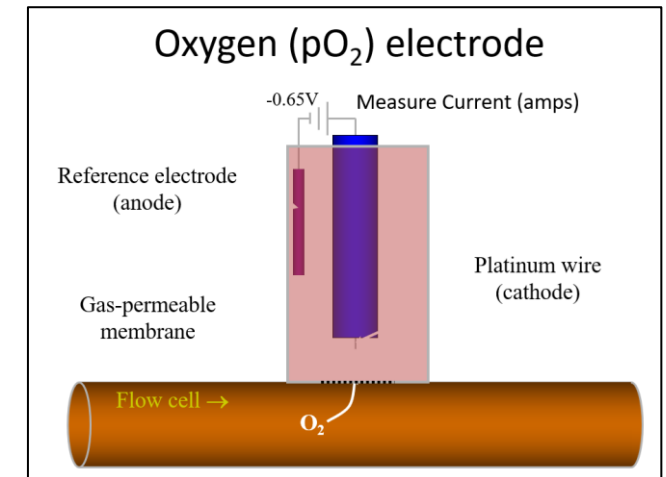
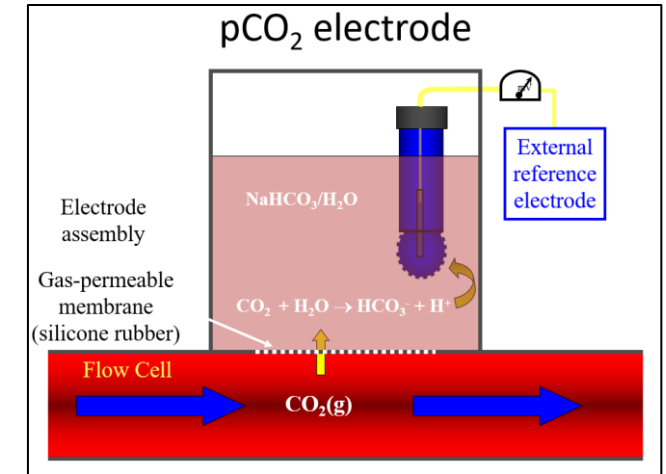
Parameter	Normal value	Method
pH	7.36-7.44 (a) 7.31-7.41 (v)	Potentiometry (pH electrode)
pCO ₂	34-46 mmHg (a) 40-52 mmHg (v)	Potentiometry (Severinghaus electrode)
pO ₂	80-90 mmHg (a) 25-40 mmHg (v)	
sO ₂ (oxygen saturation)	95-98% (a) 60-80% (v)	



a= arterial , v=venous blood

Blood Gas Analysis

Parameter	Normal value	Method
pH	7.36-7.44 (a) 7.31-7.41 (v)	Potentiometry (pH electrode)
pCO ₂	34-46 mmHg (a) 40-52 mmHg (v)	Potentiometry (Severinghaus electrode)
pO ₂	80-90 mmHg (a) 25-40 mmHg (v)	Amperometric (Clark electrode)
sO ₂ (oxygen saturation)	95-98% (a) 60-80% (v)	

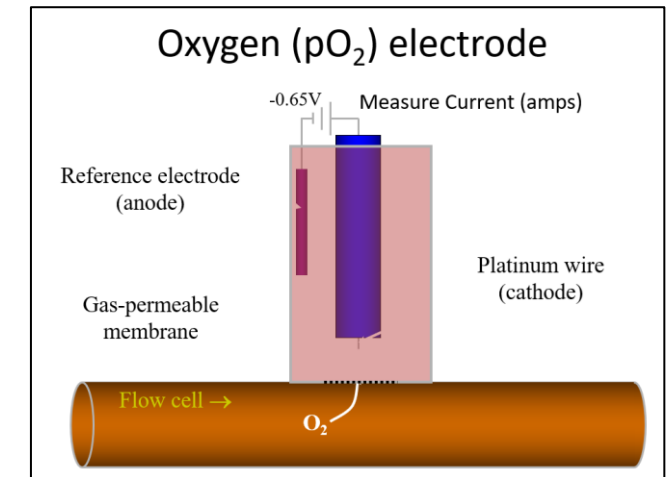
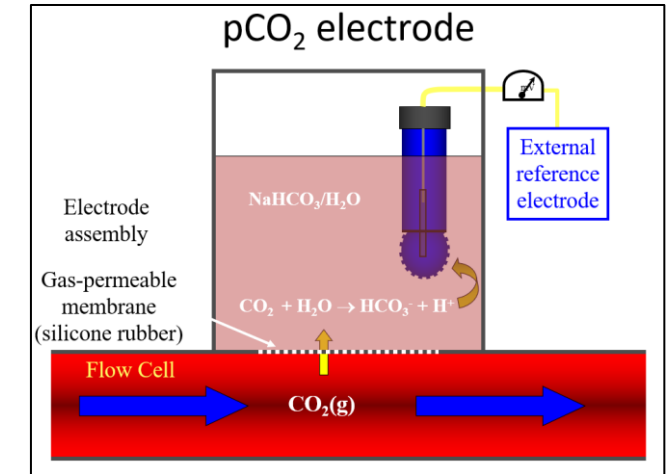


a= arterial , v=venous blood

Blood Gas Analysis

Parameter	Normal value	Method
pH	7.36-7.44 (a) 7.31-7.41 (v)	Potentiometry (pH electrode)
pCO ₂	34-46 mmHg (a) 40-52 mmHg (v)	Potentiometry (Severinghaus electrode)
pO ₂	80-90 mmHg (a) 25-40 mmHg (v)	Amperometric (Clark electrode)
sO ₂ (oxygen saturation)	95-98% (a) 60-80% (v)	Calculated from pH, pO ₂ and HCO ₃

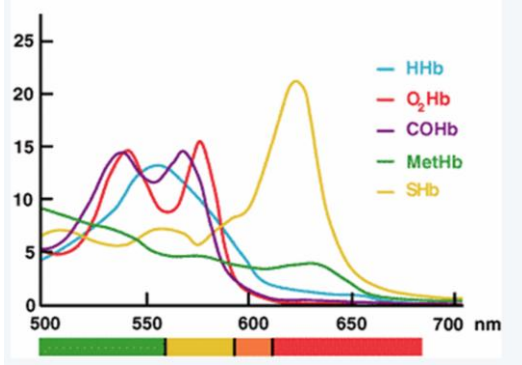
- Quite resistant to analytical interferences



a= arterial , v=venous blood

CO-oximetry (Co-ox) module

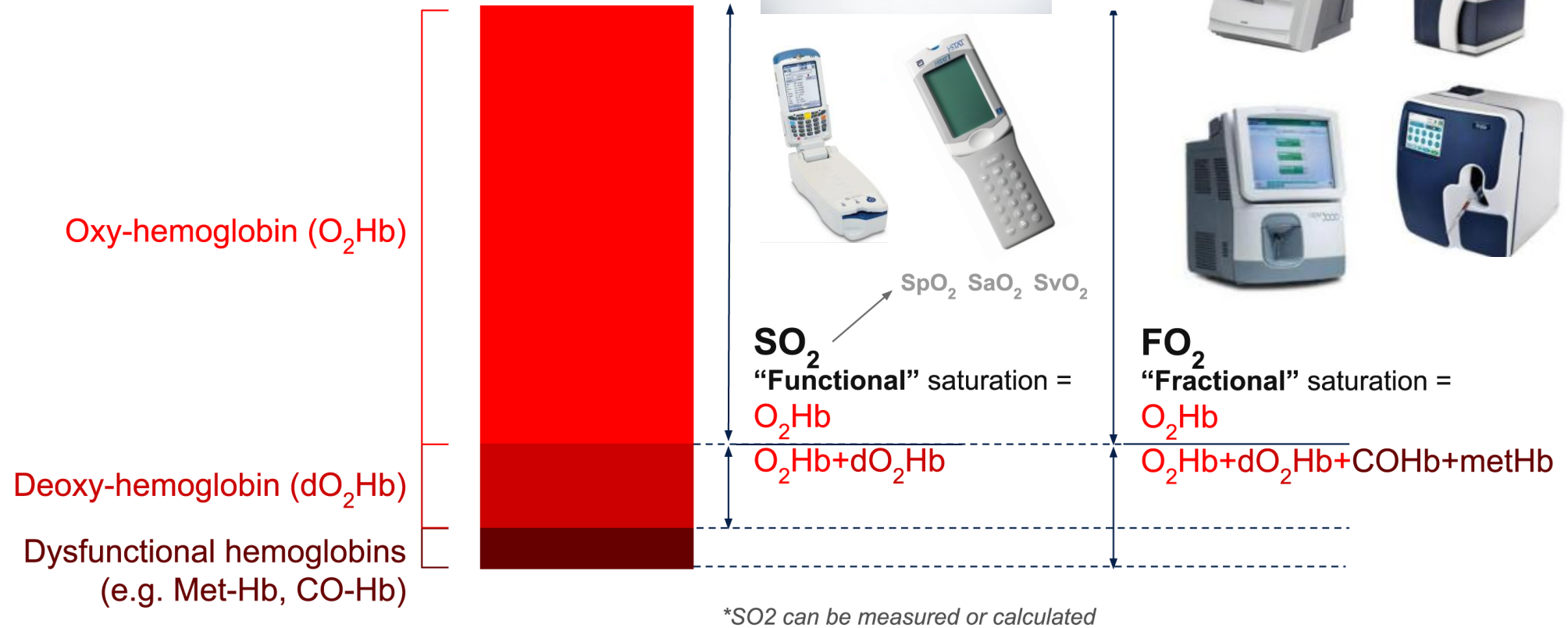
- Device that measures different hemoglobin species including dysfunctional species

Parameter	Normal value	Method
ctHb	13.6-17.2 g/dL (M) 11.6-15.6 g/dL (F)	Absorption spectroscopy 
% O ₂ Hb Oxyhemoglobin	95-98% (arterial) 60-85% (venous)	
% COHb Carboxyhemoglobin	<3% nonsmokers <9% smokers	
% MetHb Methemoglobin	<1.5%	
ctO ₂ (oxygen content)	15-22 ml/dL	Calculated from Hgb and SaO ₂

- Interferences: Methylene blue, Sulfhemoglobin, Hydroxycobalamin

$$ctO_2 = \alpha O_2 \times pO_2 + sO_2 \times (1 - FCOHb - FMetHb) \times ctHb$$

sO_2 vs FO_2Hb



Patient examples

Pt with CO toxicity from house fire

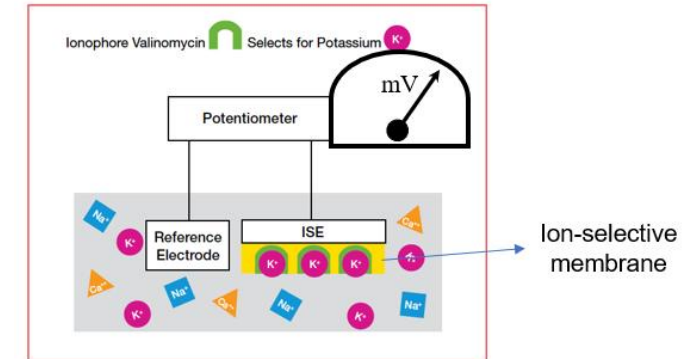
Test	Results (Ref Interval)
SpO ₂ by pulse oximeter	96% (95-100%)
Oxyhemoglobin (FO ₂ Hb)	↓ 38% (94-100%)
Carboxyhemoglobin (FCOHb)	↑ 35.5% (1.0 – 1.5%)
Methemoglobin (FMetHb)	0.3% (0.4 – 1.5%)

Pt with methemoglobin toxicity from drugs abuse

Test	Results (Ref Interval)
SpO ₂ by pulse oximeter	80% (95-100%)
Oxyhemoglobin (FO ₂ Hb)	↓ 21% (94-100%)
Carboxyhemoglobin (FCOHb)	0.0% (1.0 – 1.5%)
Methemoglobin (FMetHb)	↑ 76.1% (0.4 – 1.5%)

Chemistry tests in whole blood

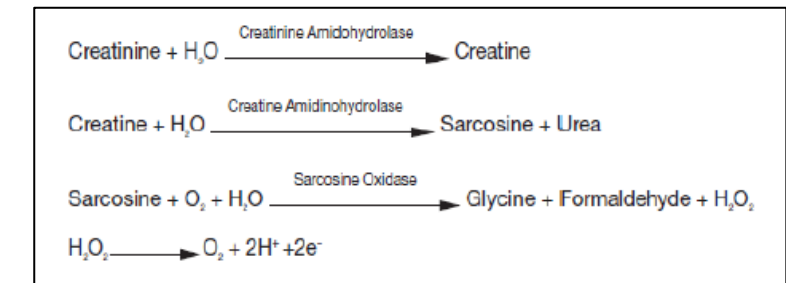
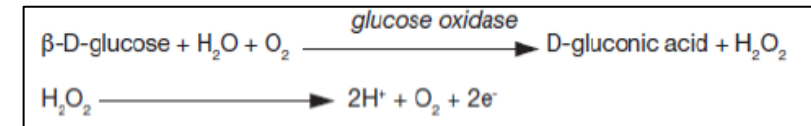
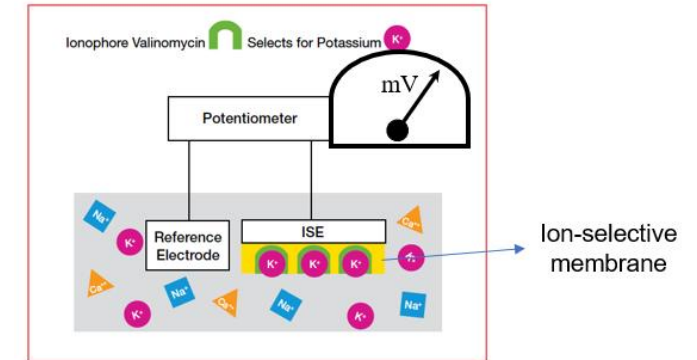
Parameter	Method	Interference*
Na, K, Cl, Ca	Ion selective electrode potentiometry via Nerst eq	Bromide, acetaminophen (Ca), salicylic acid (Cl) protein build up



Best to review manufacturer's information

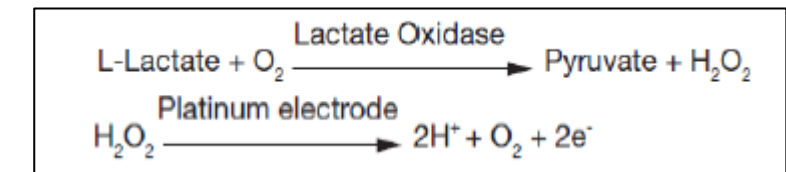
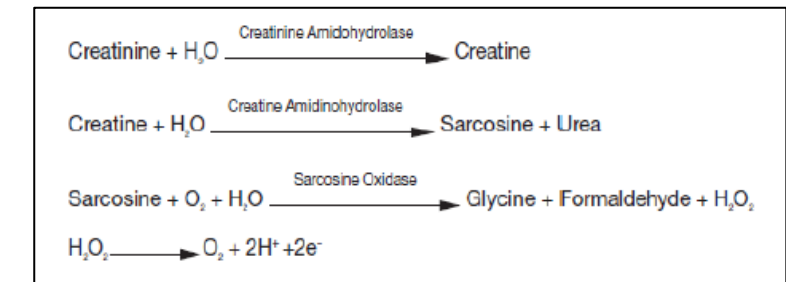
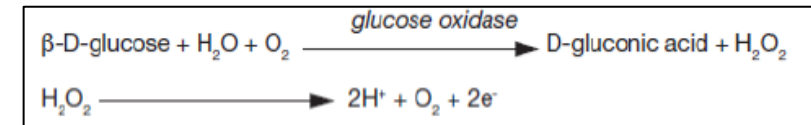
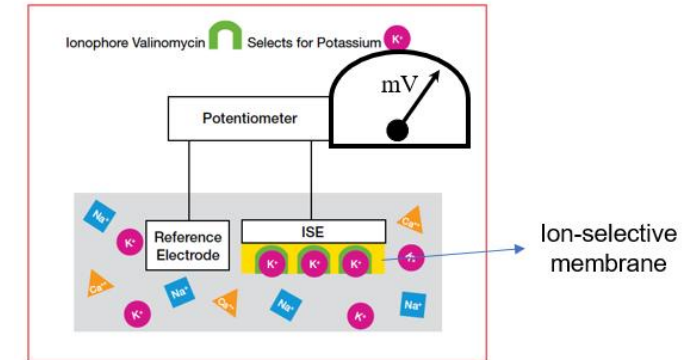
Chemistry tests in whole blood

Parameter	Method	Interference*
Na, K, Cl, Ca	Ion selective electrode potentiometry via Nerst eq	Bromide, acetaminophen (Ca), salicylic acid (Cl) protein build up
Glucose	Amperometric using an enzyme electrode with glucose oxidase	Ascorbic acid, Ethylene glycol metabolites
Creatinine	Amperometric using an enzyme electrode with sarcosine oxidase	Acetaminophen, Ascorbic acid



Chemistry tests in whole blood

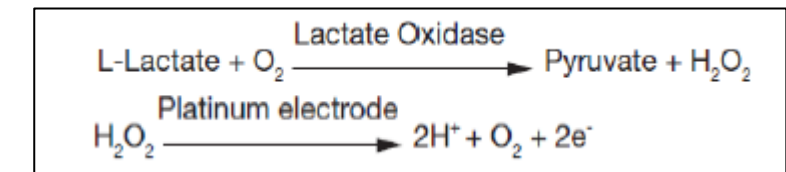
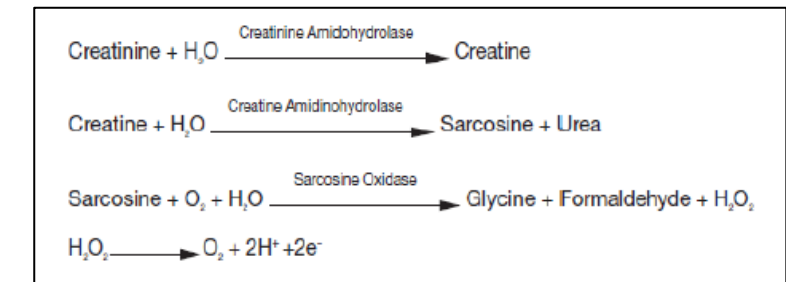
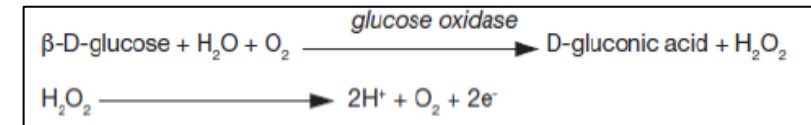
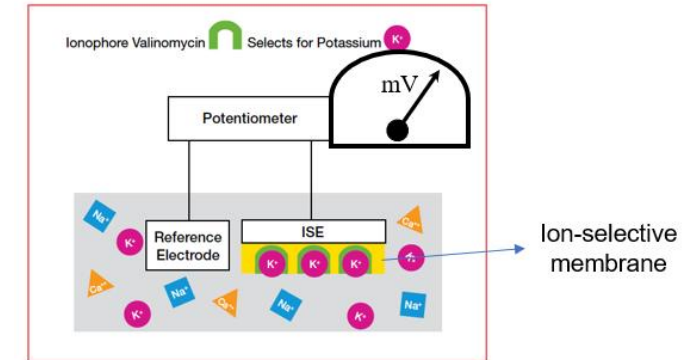
Parameter	Method	Interference*
Na, K, Cl, Ca	Ion selective electrode potentiometry via Nerst eq	Bromide, acetaminophen (Ca), salicylic acid (Cl) protein build up
Glucose	Amperometric using an enzyme electrode with glucose oxidase	Ascorbic acid, Ethylene glycol metabolites
Creatinine	Amperometric using an enzyme electrode with sarcosine oxidase	Acetaminophen, Ascorbic acid
Lactic acid	Amperometric using an enzyme electrode with lactate oxidase	Ethylene glycol metabolites, acetaminophen, ascorbic acid, bromide, hydroxyurea



Best to review manufacturer's information

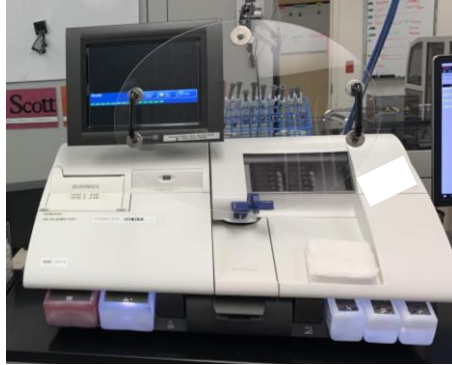
Chemistry tests in whole blood

Parameter	Method	Interference*
Na, K, Cl, Ca	Ion selective electrode potentiometry via Nerst eq	Bromide, acetaminophen (Ca), salicylic acid (Cl) protein build up
Glucose	Amperometric using an enzyme electrode with glucose oxidase	Ascorbic acid, Ethylene glycol metabolites
Creatinine	Amperometric using an enzyme electrode with sarcosine oxidase	Acetaminophen, Ascorbic acid
Lactic acid	Amperometric using an enzyme electrode with lactate oxidase	Ethylene glycol metabolites, acetaminophen, ascorbic acid, bromide, hydroxyurea



Best to review manufacturer's information

Potentiometry: Direct versus Indirect ISE



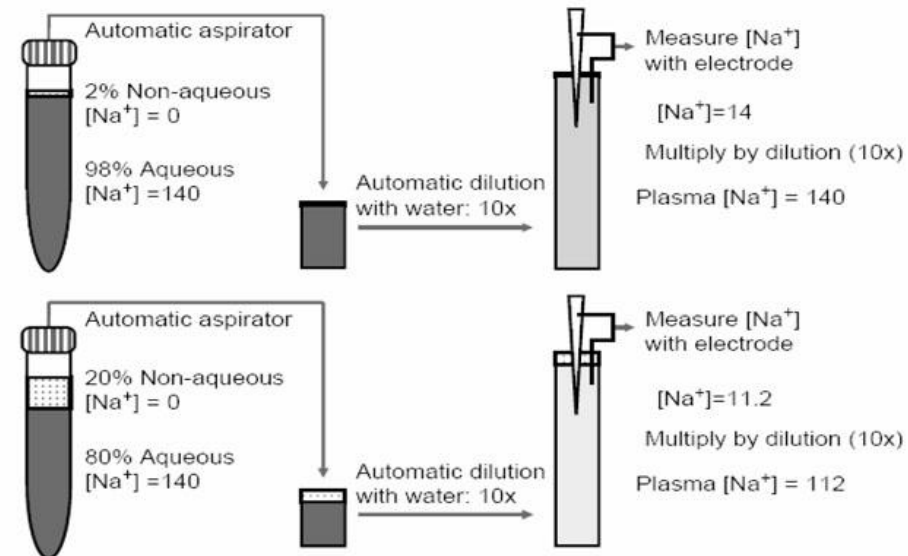
- Sample is not diluted prior to analysis
- BGAS analyzers: whole blood or plasma/serum
- Not impacted by composition of serum or plasma
 - Lipids, proteins



- Sample is diluted prior to analysis
- Automated chemistry analyzers: serum/plasma
- Impacted by composition of serum/plasma
 - Lipids, proteins
- **Pseudohyponatremia**

Pseudohyponatremia

- Each liter of plasma contains
 - ~930 ml water
 - ~70 ml proteins and lipids
- Electrolyte exclusion: high lipids or proteins reduce plasma water: thus plasma Na measured per liter of plasma is artificially low



Patient example: routine visit, viscous plasma

Indirect ISE	Results (Ref Interval)
Sodium	131 (136 – 145 mmol/L)
Potassium	4.7 (3.5 – 5.1 mmol/L)
Chloride	95 (98-107 mmol/L)
Total Protein	10.8 (6.4 – 8.3 g/dL)

Direct ISE (BGAS)	Results (Ref Interval)
Sodium	144* (136 – 145 mmol/L)
Potassium	5.1* (3.5 – 5.1 mmol/L)
Chloride	106* (98-107 mmol/L)

* Measured using direct potentiometry

Solutions to pseudohyponatremia

1. Ultracentrifuge lipemic samples

- HIL (hemolysis, icteric, lipemia) indices are measured on every chemistry test affected by these interferences

2. Implement a rule to flag high Total protein in a patient sample

- If Total protein >10 g/dL, run Na, Cl and K on Blood Gas analyzer
- Only works if Total protein was ordered



Point-Of-Care HbA1c assays

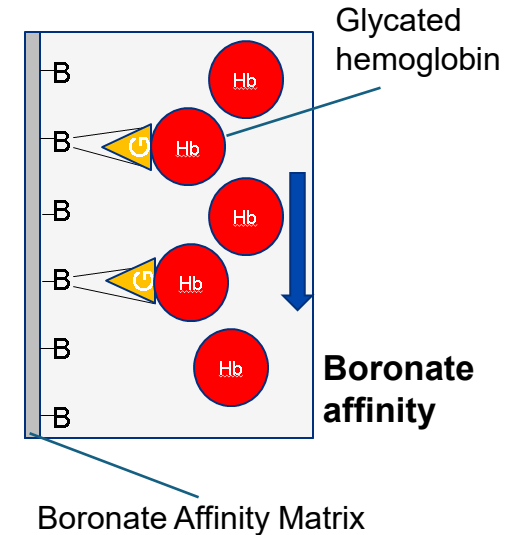
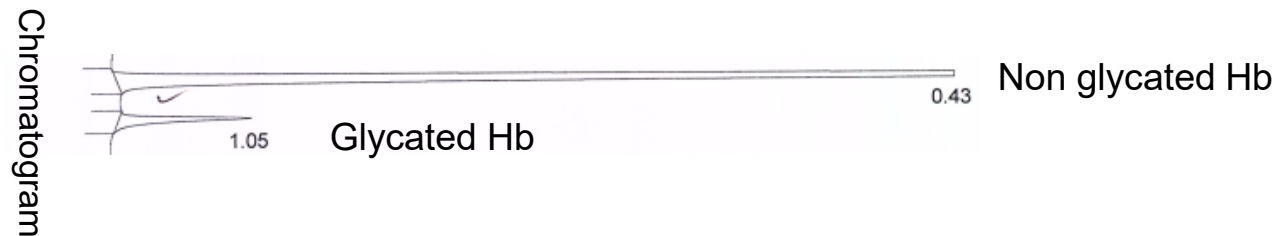
- Charge difference
 - High pressure liquid chromatography (HPLC)
 - Capillary electrophoresis
- Structure difference
 - Immunoassays
 - Boronate affinity
- Enzymatic assays
- Affinity chromatography



Assays to measure HbA1c based on structure

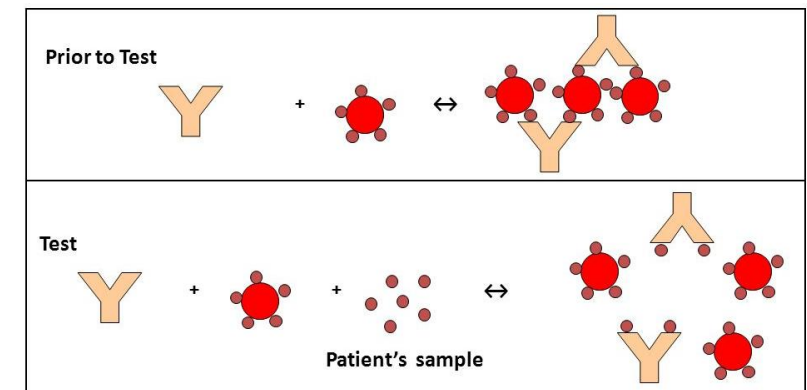
- **Boronate affinity chromatography**

- determines total glycated Hb (calculates HbA1c)
- does not measure labile fractions
- slightly overestimates A1C (binds A1a, A1b and 80% A1c)
- unable to detect Hb variants



- **Immunoassay**

- antibodies target the β -N terminal glycated amino acid (4-10 aa) (calculates A1C)
- unable to detect Hb variants
- interferences by rare Hb variants



Hb variants may change the RBC lifespan

- Heterozygous are clinically silent
- Homozygous can lead to hemolytic anemia and decreased RBC lifespan

Hemoglobin (Hb)	Average RBC Span (days)
Hb AA	120
Hb AS	93 ¹²
Hb AC	87 ¹³
Hb SS	17 ¹²
Hb SC	28 ¹³
Hb CC	29 ¹²
Hb S-Beta –thal	75 ¹²

One important issue with POC A1c- may report an incorrect value on a patient with sickle cell disease

Patient Age/Sex	HbA1c		D-100 Area% (Variants)	HPHENO History
		S/N 20016626 Lot # 10228788		
44/M	No value	4.4	F = 23.19, S = 64.07	Hgb SS inc. F
25/M	No value	4.2	C = 45.83, S = 44.76	Hgb SC
44/M	No value	4.2	C = 46.35, S = 44.29	Hgb SC
44/M	No value	4.2	C = 46.35, S = 44.29	Hgb SC
28/M	No value	4.4	S = 63.74	SA, inc. A2 /F
75/M	No value	4.7	S = 45.36, C = 45.21	SC
32/F	No value	4.1	S = 87.64	Hgb SS, prelim.
83/M	No value	<4.0	No value	Hgb SS

Thank you Karen Smith from Grady Hospital!

Immunoassay interferences:

Endogenous

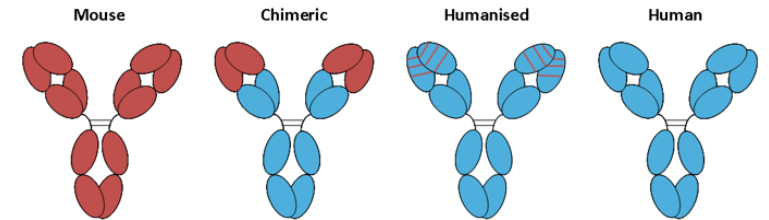
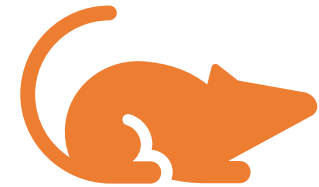
- Lipemia and hemolysis
- Antibodies
 - Autoantibodies
 - Heterophile antibodies
 - Macro-complexes
- Rheumatoid factors
- Hook effect
- Other binding proteins

Exogenous

- Therapeutic monoclonal antibodies
- Biotin
- Cross-reactive interference

Heterophile antibody

- Heterophilic antibodies are endogenous antibodies found in patients' blood which can bind to antibodies used as reagents for immunoassays
- Exposure to antigens from animals, especially mouse HAMA – human anti-mouse antibody
- Therapeutic antibodies given during cancer therapy may cause formation of heterophile antibodies
- Prevalence: 0.2-3.7%



Heterophile antibody- 1st case

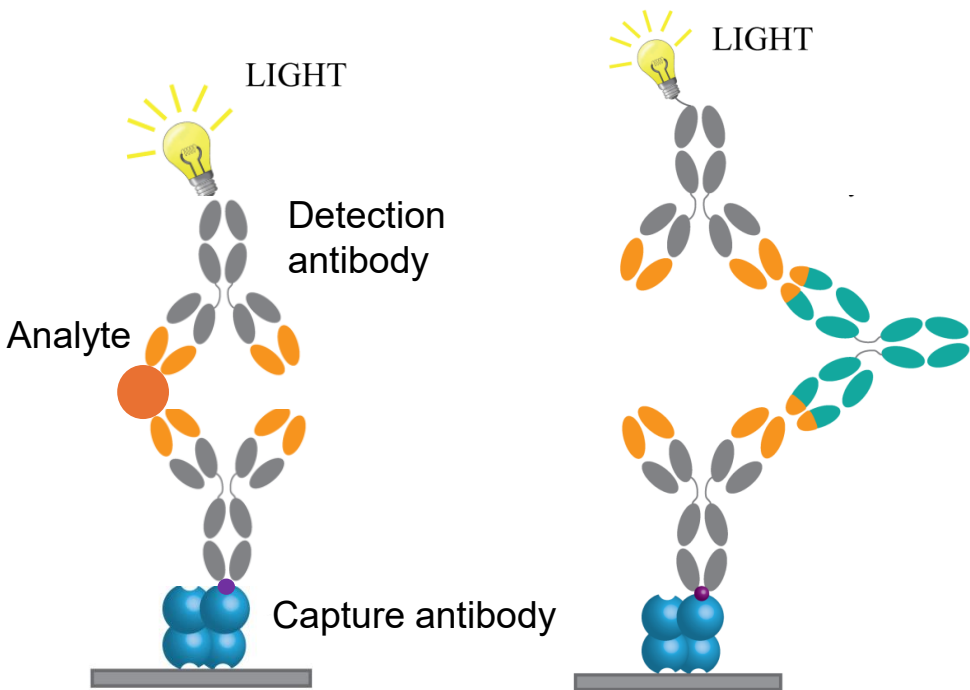
- Positive hCG (without pregnancy) was repeated 40 times, value between 250-350
- For 3 years, 22 y.o. patient had unnecessary chemotherapy, hysterectomy and thoracotomy after being misdiagnosed with a rare form of cancer (GTD)
- Pt. Awarded 15 million for misdiagnosis



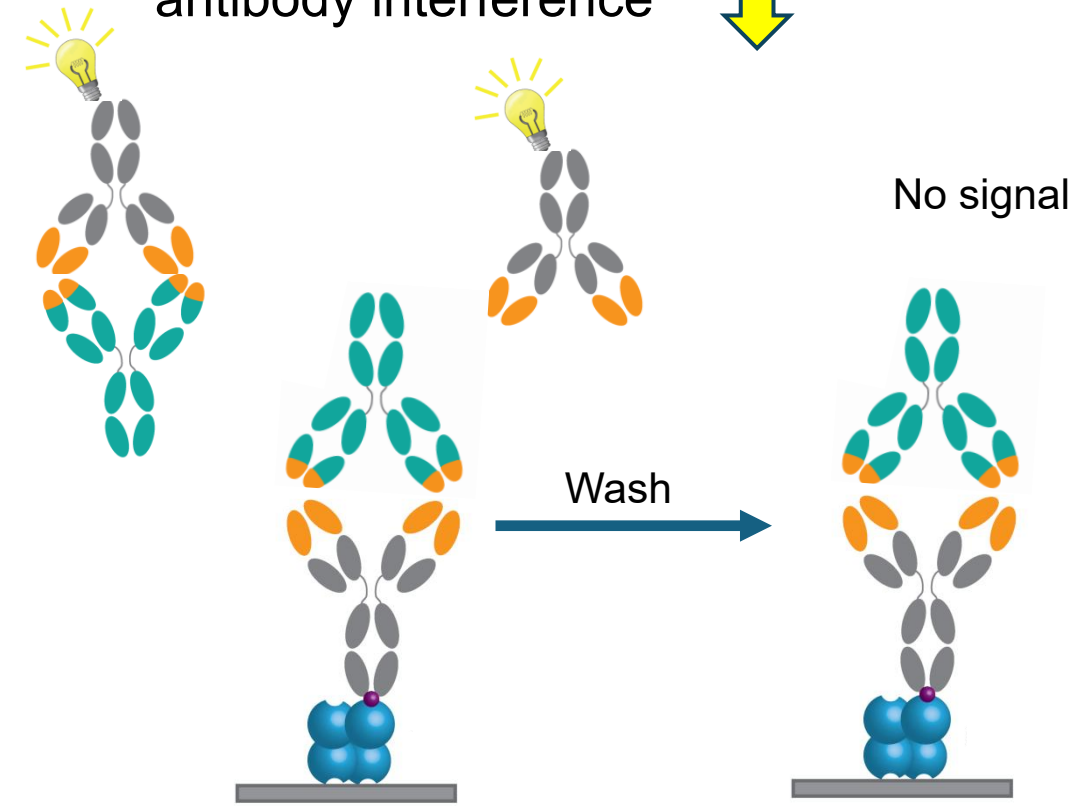
<https://www.seattlepi.com/local/article/Jury-awards-15-5-million-to-woman-misdiagnosed-1058509.php>

Heterophile antibody- mechanism of interference

Positive heterophile
antibody interference

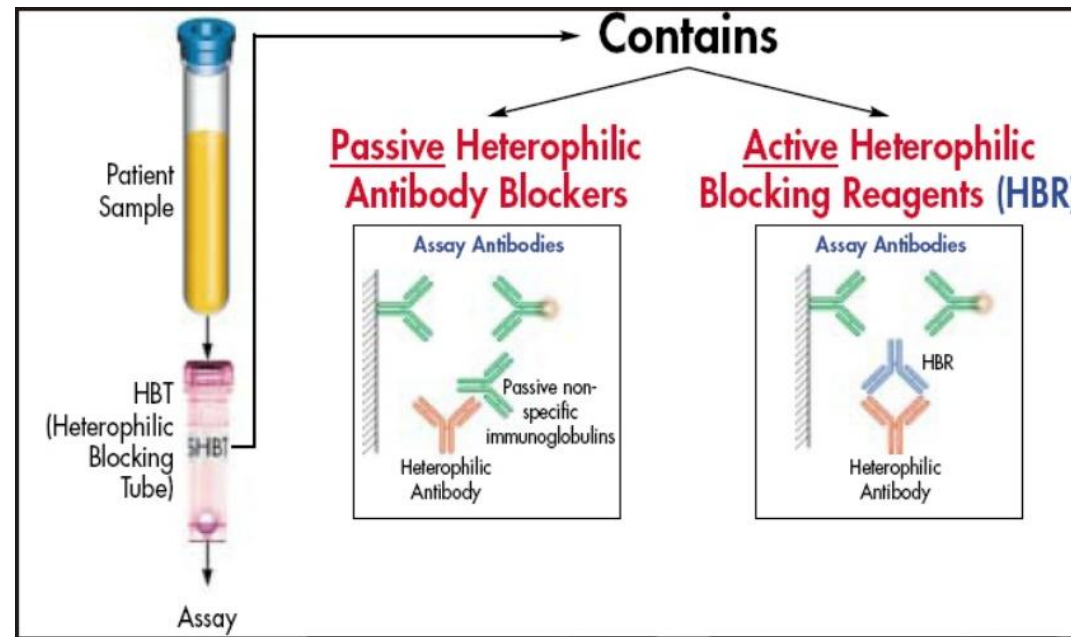


Negative heterophile
antibody interference



Heterophile antibody- solutions

- Use of a different assay
- Dilutions
- Monitor analyte in an alternative specimen
- Pretreatment with heterophile blocking tube (HBT) or heterophile blocking reagent (HBR)



Tricky troponins

- Patient presents to urgent care with chest pain on two separate occasions

	9/5/2018 0835	9/5/2018 1019	9/5/2018 1235	10/31/2021 1448	10/31/2021 1714	10/31/2021 1850
CARDIAC						
TROPONIN ED		<0.01 *	<0.01 *		<0.01 *	<0.01 *
TROPONIN POC	0.28 ↑			0.22 ↑		

Tricky troponins

- Patient presents to urgent care with chest pain on two separate occasions

	9/5/2018 0835	9/5/2018 1019	9/5/2018 1235	10/31/2021 1448	10/31/2021 1714	10/31/2021 1850
CARDIAC						
TROPONIN ED		<0.01 *	<0.01 *	<0.01	<0.01 *	<0.01 *
TROPONIN POC	0.28 ⬆			0.22 ⬆	0.21	0.19

- After heterophile blocking treatment : <0.03 <0.03 <0.03

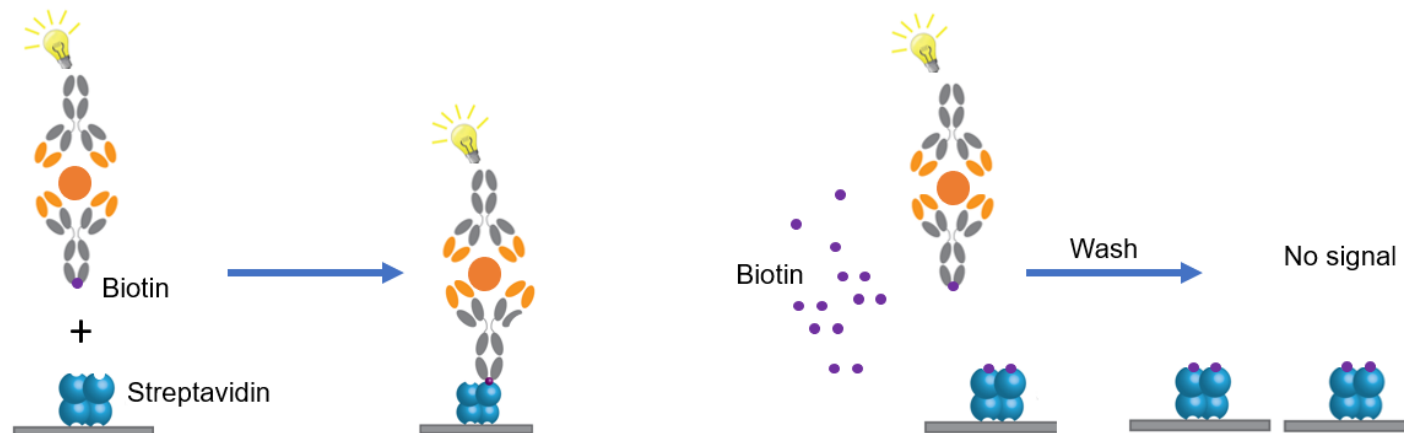
Analytical interference of cardiac troponins

UPDATE: The FDA Warns that Biotin May Interfere with Lab Tests: FDA Safety Communication

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This updated safety communication provides new information on biotin interference with certain troponin lab tests. For more information, refer to [Biotin Interference with Certain Troponin Lab Tests](#) for details on specific tests.

Date Issued: November 5, 2019



According to FDA, in 2017, a patient with a high intake of supplemental biotin died following a troponin test that gave a falsely low result because the test was subject to biotin interference

Poppy Seed Dilemma

Pt. prescribed Lorazepam gets urine drug screen performed

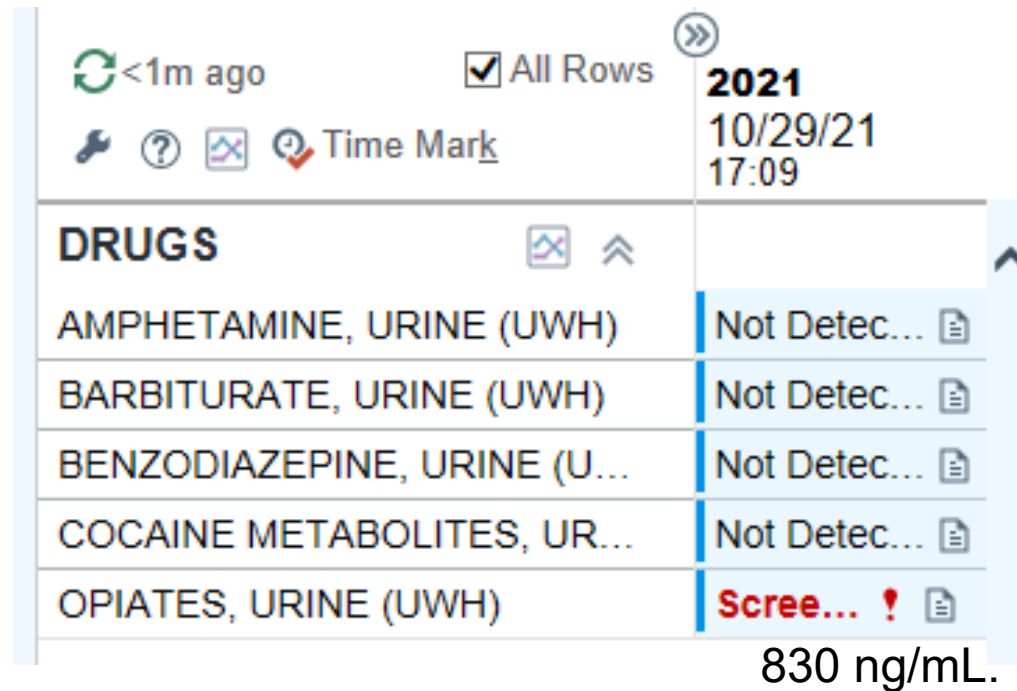
 <1m ago ☒ All Rows 		2021 10/29/21 17:09
    Time Mark		
DRUGS  		
AMPHETAMINE, URINE (UWH)		Not Detec... 
BARBITURATE, URINE (UWH)		Not Detec... 
BENZODIAZEPINE, URINE (U...		Not Detec... 
COCAINE METABOLITES, UR...		Not Detec... 
OPIATES, URINE (UWH)		Scree... ! 

830 ng/mL.

Urine Opiate : cutoff 300 ng/mL

Poppy Seed Dilemma

Pt. prescribed Lorazepam gets urine drug screen performed



 <1m ago ☒ All Rows 	
    Time Mark	
2021 10/29/21 17:09	
DRUGS  	
AMPHETAMINE, URINE (UWH)	Not Detec... 
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OPIATES, URINE (UWH)	Scree... ! 

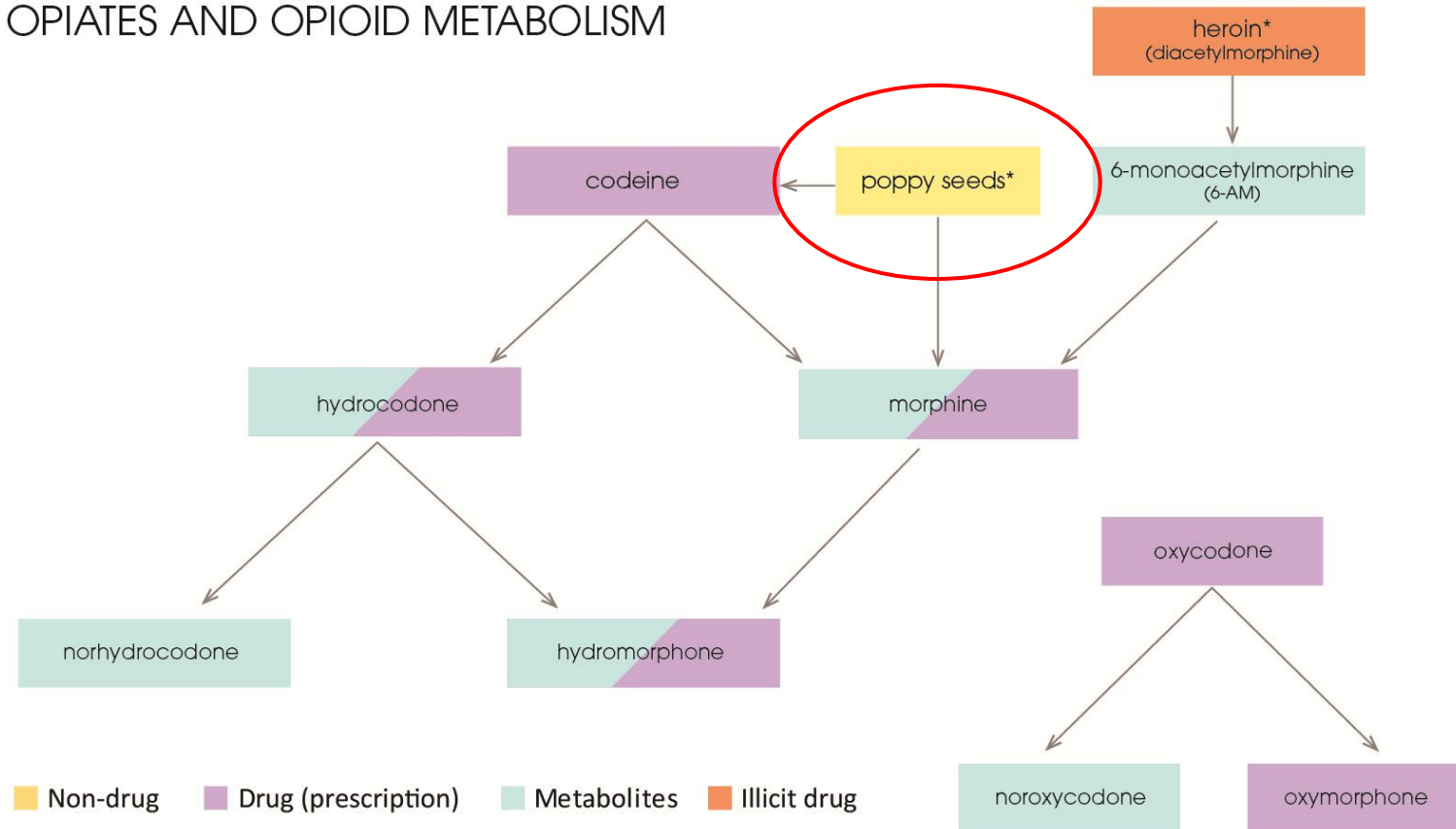
830 ng/mL.

Sample sent out to ARUP for confirmatory by Mass Spec : **positive for morphine**
Patient denied taking any illegal substances

Urine Opiate : cutoff 300 ng/mL

Doc X: It appears that the bread that this patient uses is coated in poppy seeds and she eats it most daily. Do you think this would be enough to explain the positive morphine result?

OPIATES AND OPIOID METABOLISM



Patient was retested and had Opiates undetectable

<https://www.aruplab.com/>

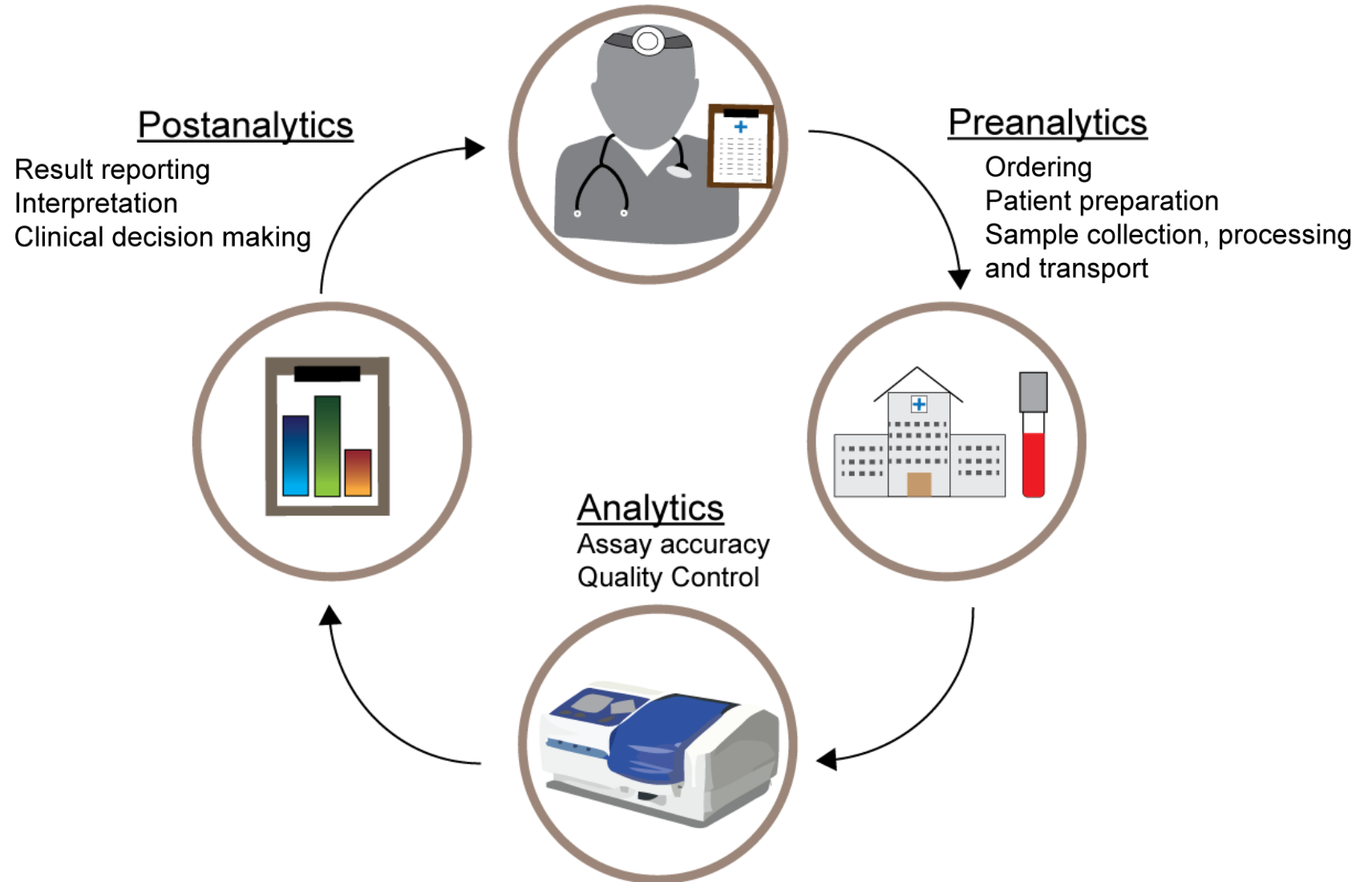
Post-analytical variables

- Result reporting
 - Manual entry
- Result interpretation
- Reference intervals
 - Different ranges for venous versus arterial blood, pediatric population, vendor specific ranges
- Critical values



Conclusion

If results do not match clinical picture, think about all the variables that can affect test result



EMORY



Grady



Thank you for listening!

kgalior@emory.edu

Pre-analytical variables for BGASes

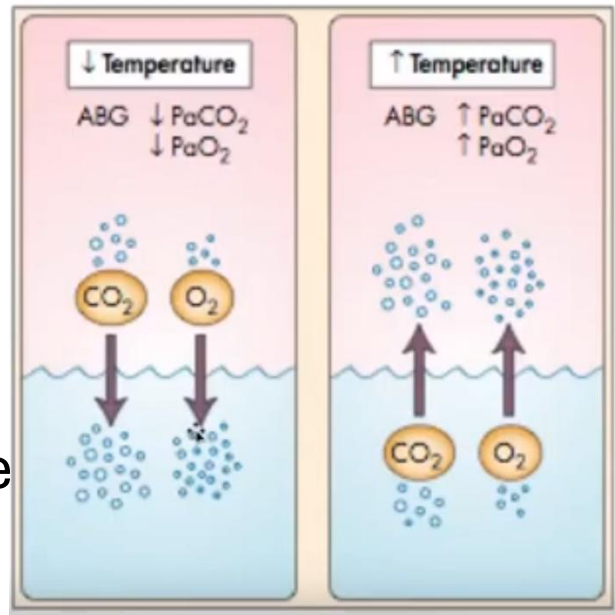
- Venous admixture
- Timing
- Metabolism
- Air bubbles
- Anticoagulant effects
- Temperature

Temperature

- ABG analyzer is controlled for normal body temperatures
- Affects PaCO_2 and HCO_3^-
- Increase in patient temp: $\uparrow \text{PaO}_2$ $\uparrow \text{PaCO}_2$ $\downarrow \text{pH}$
- Decrease in patient temp: $\downarrow \text{PaO}_2$ $\downarrow \text{PaCO}_2$ $\uparrow \text{pH}$

Gas phase

Liquid phase



Ideal gas law
 $PV=nRT$