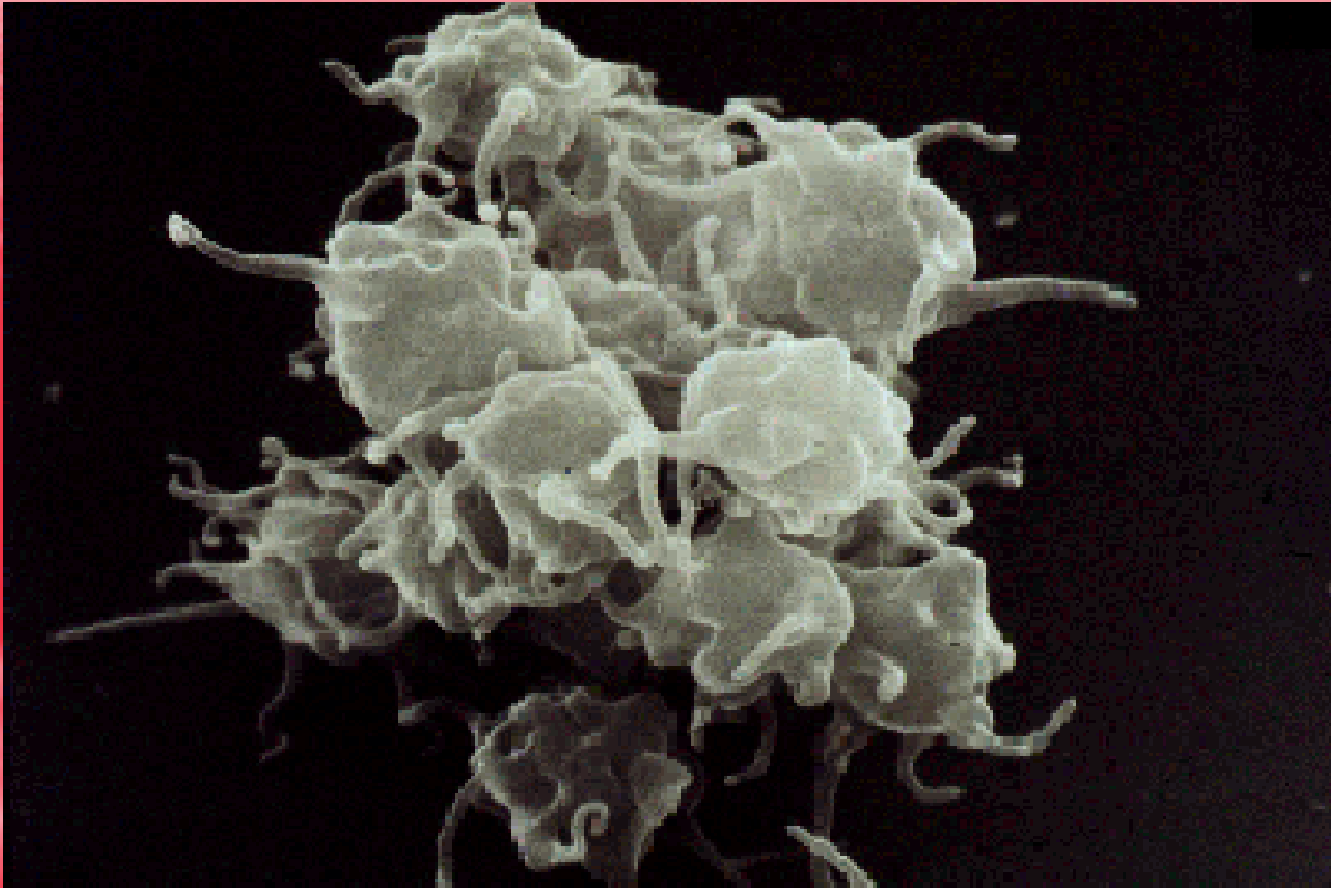


# The Story of the Platelet Clump

Wayne Hall  
MTN Network Laboratory  
Magee-Womens Research Institute  
Pittsburgh, PA

# Reason for Alarm?

Platelet Clumping was occurring  
in multiple African sites 1-2 times  
per week in the months  
September to November, 2010



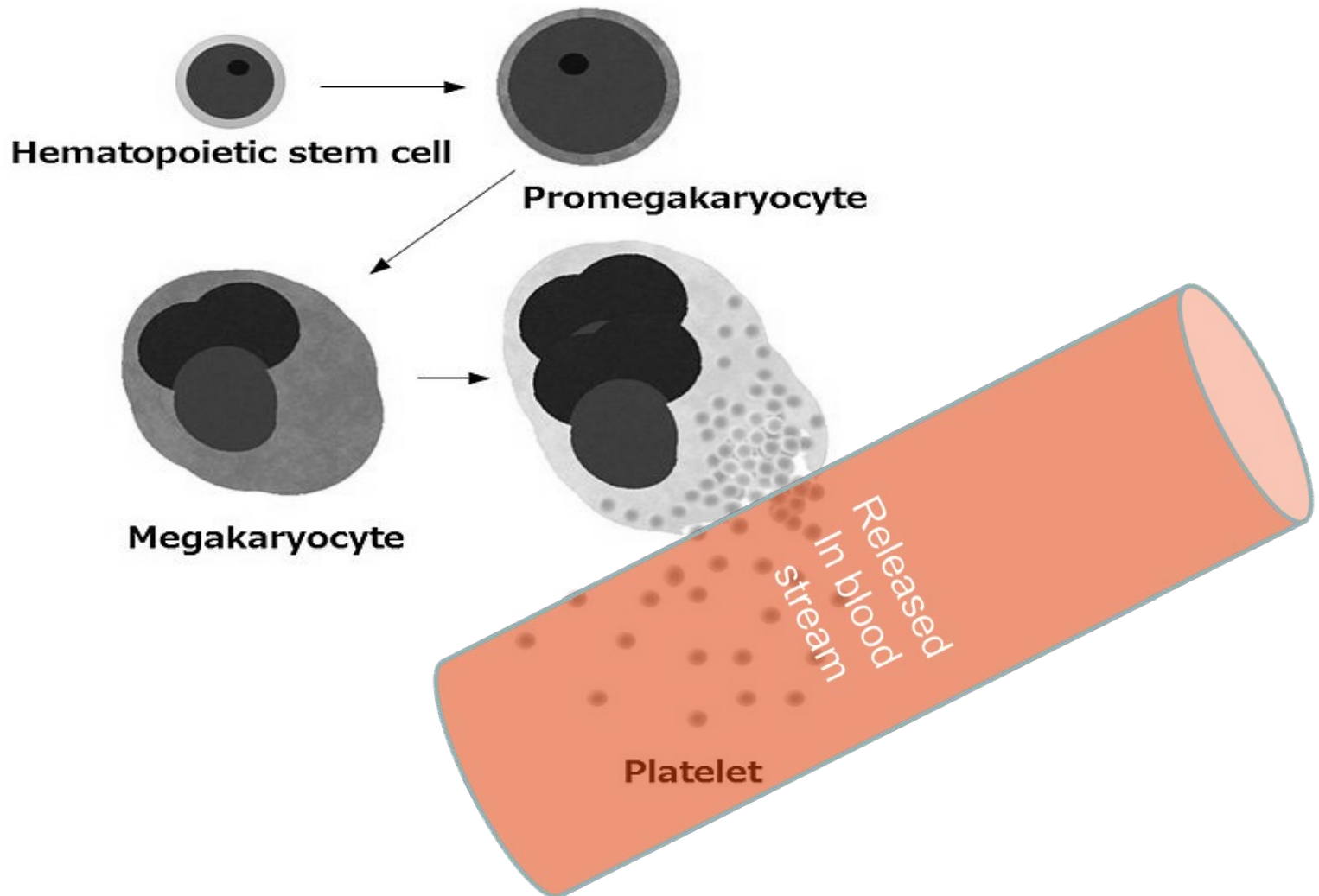
The Culprit!!

# Outline:

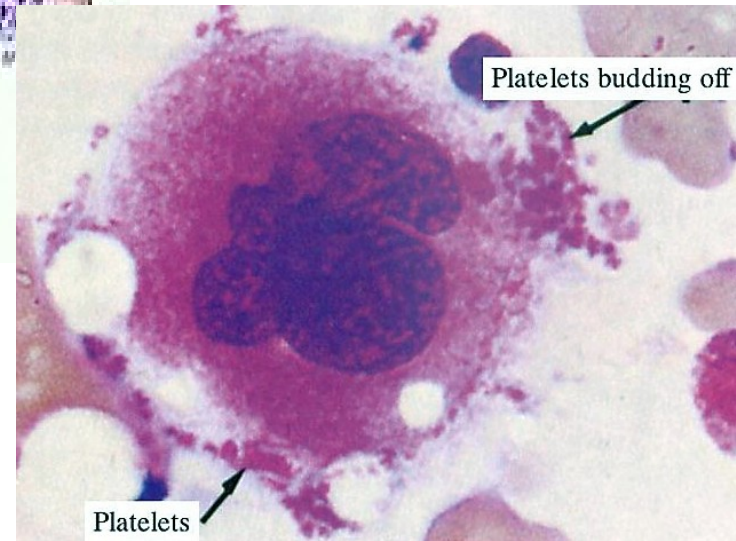
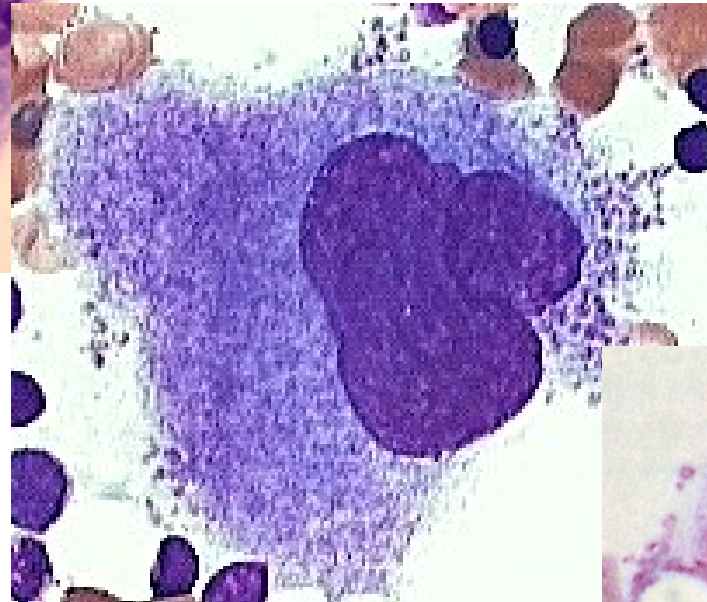
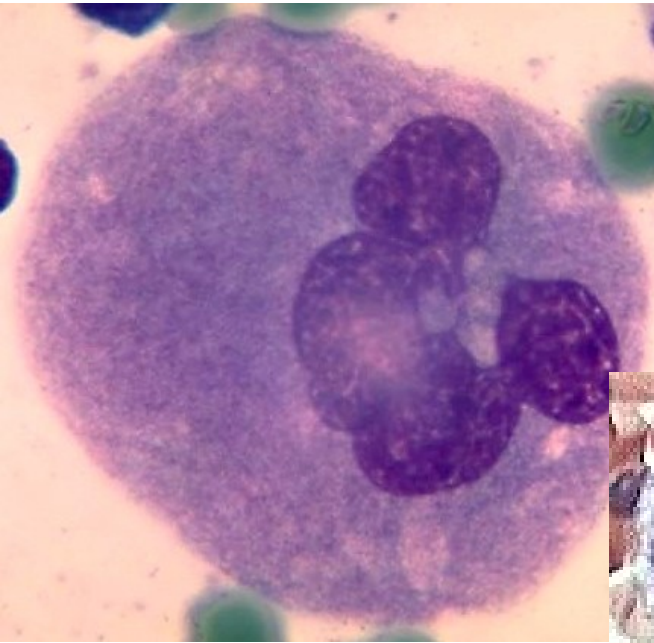
1. Development of Platelets & their Function
2. Laboratory Evaluation of Platelets in Clinical Trials
3. Platelet Clumping
4. Platelet Histograms & Pictures
5. Resolution to Platelet Clumping in EDTA tubes
6. Is platelet clumping a reason for Alarm?
7. Conclusions
8. Questions

# The Birth of a Platelet

in the bone marrow



What is this?



Megakaryocyte

# What fact is incorrect about Platelets?

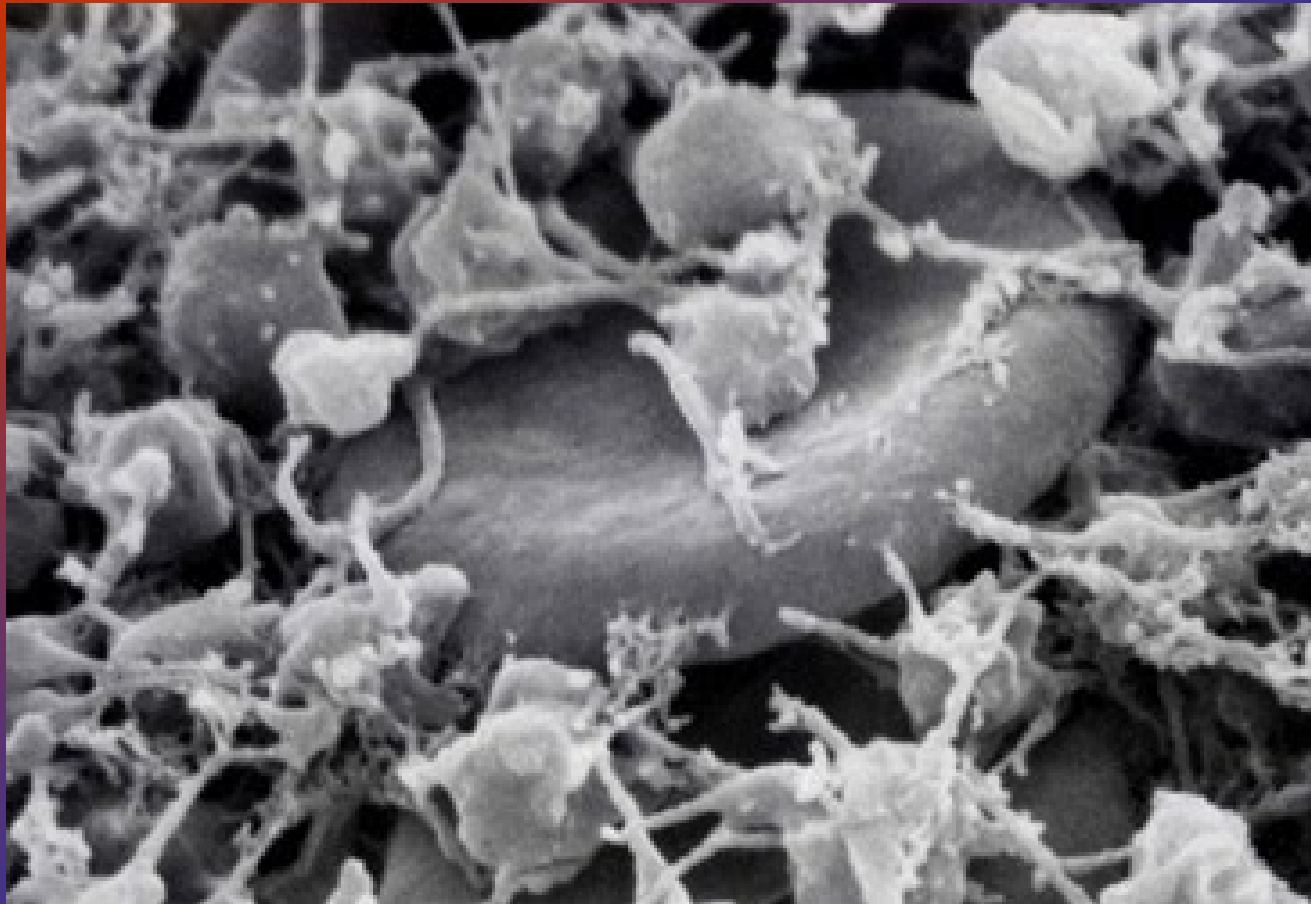
1. **Cytoplasmic fragments from Megakaryocytes formed in the bone marrow**
2. **Does not contain a nucleus or any DNA (contains enzymes & mitochondria)**
3. **One-Third (not 1/8<sup>th</sup>) of the total blood platelets can be found in the spleen**
4. **Life span of a platelet is 5-7 days**
5. **Normal Count ~ 140,000 to 440,000**
6. **30,000 to 50,000 platelets released in blood daily**

# Primary Platelet Functions

also called Primary Hemostasis

- **Maintenance of normal Hemostasis**
  - the opposite of hemostasis is hemorrhage
- **Adhere to the injury site**
  - due to chemical signal released by smooth muscle damage
  - exposed collagen fibers attracts platelets
- **Clump together (aggregate) with other platelets**
- **Release compounds that stimulate further aggregation**

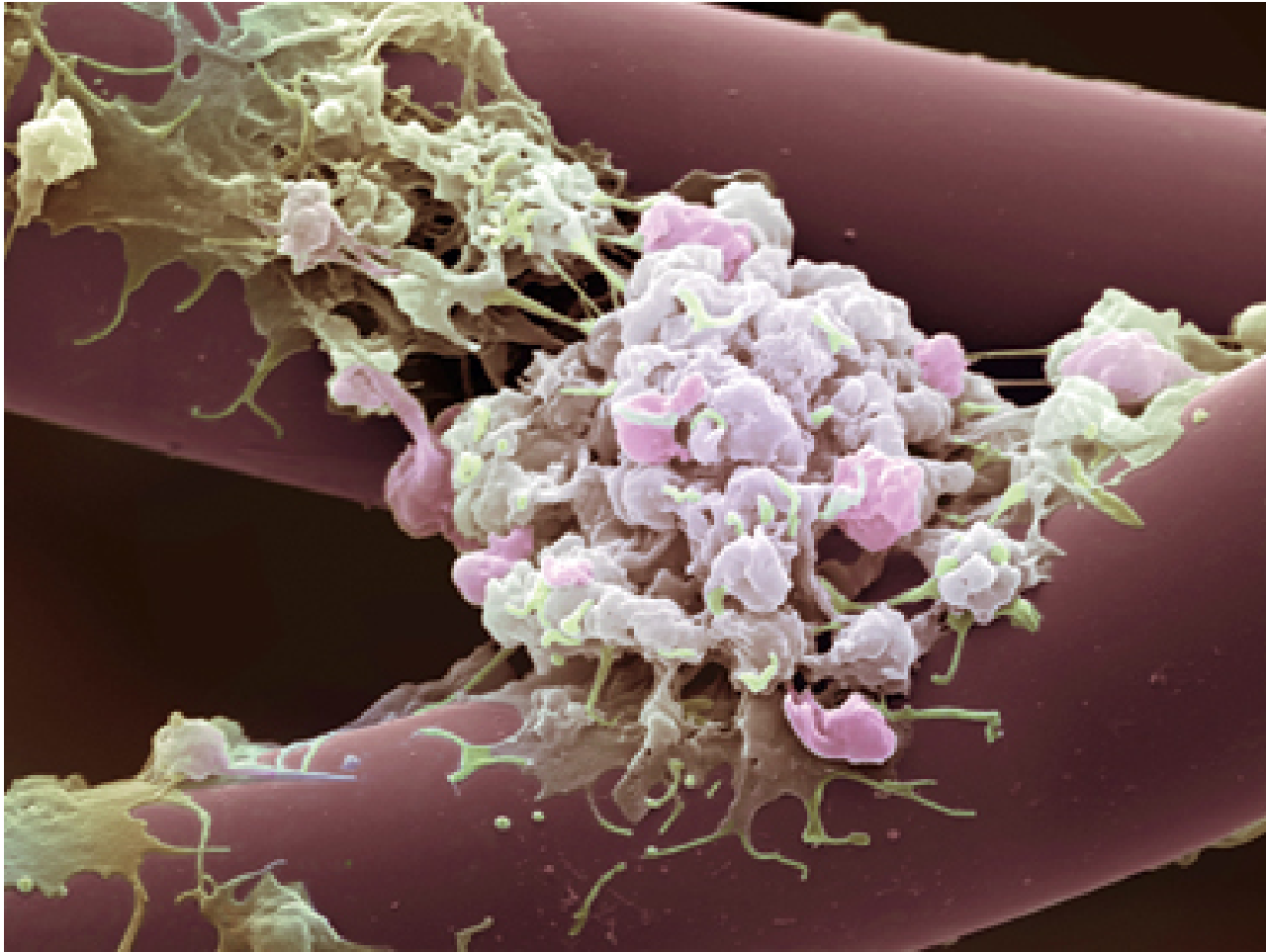




Platelets adhering to tissue

# Secondary Hemostasis

- Platelets support the Coagulation Cascade
- Formation of fibrin strains that are woven through the loose platelet plug forming a fibrin net



The Platelet Plug

# Laboratory Evaluation of Platelets in Clinical Trials

- To accurately assess the platelet concentration in the peripheral blood
- To distinguish low platelet counts that can potentially be harmful to participants.
- To Identify participants with a platelet anomaly that would defer them from the study.

# In Voice, what is the Platelet count Exclusion Criteria?

- A. Platelet count below 75,000/mm<sup>3</sup>**
- B. Platelet count below 100,000/mm<sup>3</sup>**
- C. Platelet count below 125,000/mm<sup>3</sup>**
- D. Platelet count below 150,000/mm<sup>3</sup>**

In ASPIRE (MTN-020), what is the Platelet count Exclusion Criteria?

- A. Platelet count below 75,000/mm<sup>3</sup>**
- B. Platelet count below 100,000/mm<sup>3</sup>**
- C. Platelet count below 125,000/mm<sup>3</sup>**
- D. Platelet count below 150,000/mm<sup>3</sup>**

# Exclusion Criteria for Clinical Trials

**In VOICE (MTN-003) platelet count below 100,000/mm<sup>3</sup>**

**In ASPIRE (MTN 020) platelet count below 125,000/mm<sup>3</sup>\***

\*Based on the Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events Version 1.0, December, 2004 (Clarification dated August 2009)

# Sticky Situations

## Platelet clumping in EDTA tubes

- creates illusion of low platelet count (pseudo-thrombocytopenia )
- Possibly will effect WBC count
- Creates extra work to resolve the problem
- Not a good candidate for enrollment



# **Causes for Clumped Platelets**

- **Faulty Phlebotomy practice**
- **Cold Agglutinins**
- **EDTA-Dependent Auto antibodies**
- **Unknown**

# Faulty Phlebotomy Practice:

...transferring blood into EDTA tube

## **SIDE NOTE ABOUT BLOOD DRAWS:**

Make sure that your phlebotomists ID each participant every time they are taken into a new room....even if they just performed testing with that participant in another area!!!

– Insufficient tube mixing

**These practices are preventable with good phlebotomy technique!!**

# Cold Agglutinins

- An antibody that attaches to platelets at temperatures colder than 34°C.
- Can be triggered by cold weather, change of environment, poor circulation
  - Warm the EDTA tube for 15-20 minutes in a 37 degree water bath or heat block, quickly vortex, and run immediately.

# EDTA-Dependent Auto-antibodies

- Rare condition that occurs when an IgG antibody forms in the presence of EDTA
- Antibody coats the platelets, the platelets can rosette around segmented neutrophils, bands, and sometimes around monocyte.
- Slide may show platelet satellites (satellitism)
- Redraw blood into a Sodium Citrate tube and run on analyzer. Beware to account for the dilution factor (usually multiply result by 1.1).

# Unknown

Unfortunately, not all platelet clumping issues can be resolved.

If no reason can be determined at screening, this is not a good candidate to enroll!!

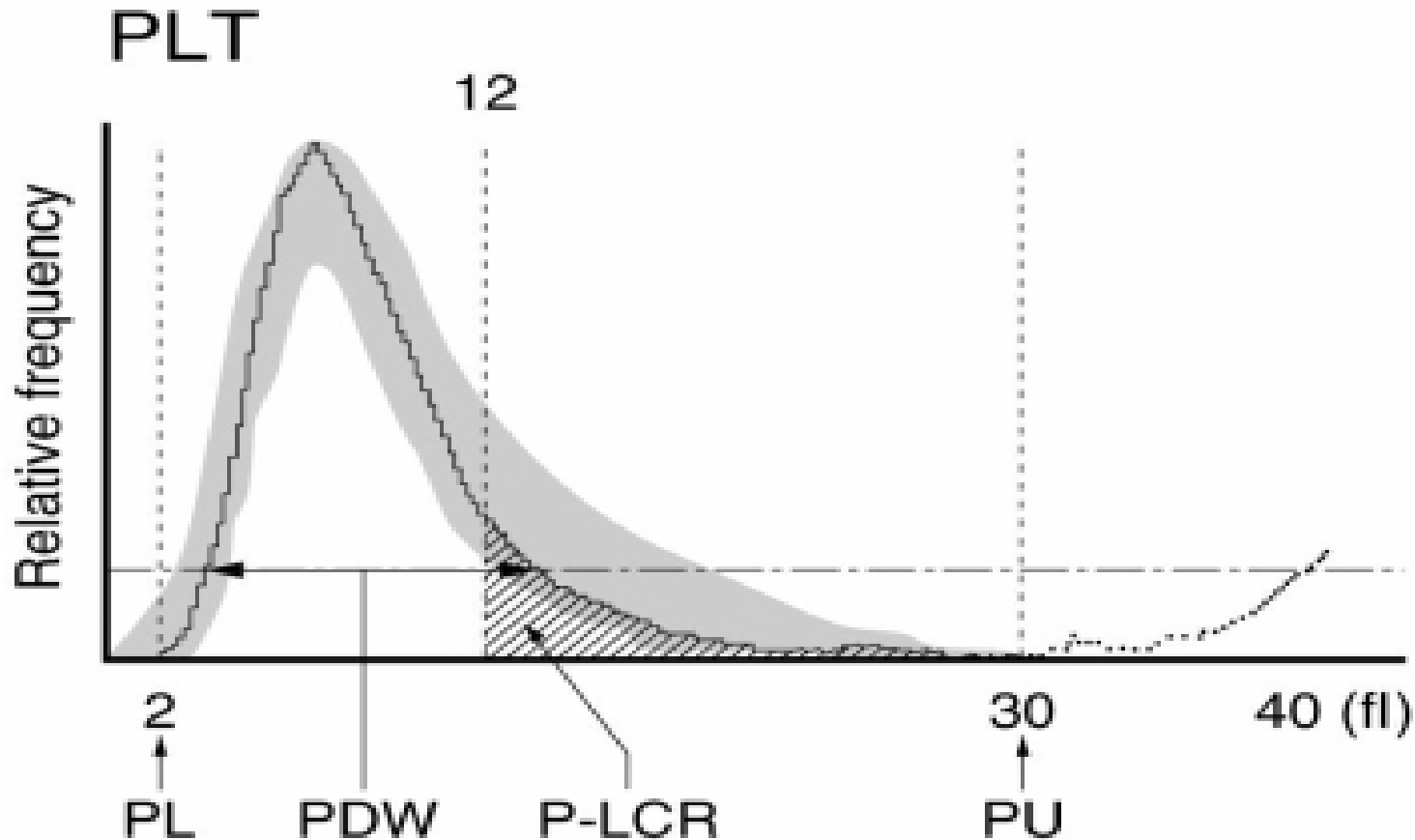
# How are Platelet Clumps found?

- Analyzer flag for platelet clumps or giant platelets.
- Histogram will indicate an abnormal distribution curve
- Abnormal RBC Parameters: Cold agglutinins to Platelets & RBCs may cause indices such as MCHC (generally greater than 37) to be affected.
- Slide Review

# Cell Analyzer Flags for Platelet Clumps or Giant Platelets

- All Platelet Clump and Giant Platelet flags will require a slide review
- The slide review will have both a WBC & Platelet estimate performed.

# Platelet Histogram



Platelet size histogram:

PL = the lower size threshold for the measurement of platelets

PU = the upper size threshold for the measurement of platelets

PDW= Platelet Distribution Width

P-LCR= Percentage of Large Cell Ratio



# Report:

Patient ID  
Gender  
Location  
Physician  
Date of Birth  
Comments:

Last Name  
Seq #  
Age

First Name  
Drawn Date  
User Field 1  
User Field 2  
User Field 3

| Report Name | All Parameters             | Last Modified: | 02/15/2008 | By:                 | LabAdmin |                            |
|-------------|----------------------------|----------------|------------|---------------------|----------|----------------------------|
| WBC         | 8.7 R 10 <sup>9</sup> /L   | RBC            | 4.82       | 10 <sup>12</sup> /L | PLT      | 133 R L 10 <sup>9</sup> /L |
| NE %        | 52.1 %                     | HGB            | 14.5       | g/dL                | MPV      | 11.9 R H fL                |
| LY %        | 40.7 %                     | HCT            | 0.414      | L/L                 |          |                            |
| MO %        | 5.1 %                      | MCV            | 85.9       | fL                  |          |                            |
| EO %        | 1.9 %                      | MCH            | 30.1       | pg                  |          |                            |
| BA %        | 0.2 L %                    | MCHC           | 35.0       | g/dL                |          |                            |
| NE #        | 4.6 R 10 <sup>9</sup> /L   | RDW            | 14.4 H %   |                     |          |                            |
| LY #        | 3.5 R H 10 <sup>9</sup> /L | RET %          |            |                     |          |                            |
| MO #        | 0.4 R 10 <sup>9</sup> /L   | RET #          |            |                     |          |                            |
| EO #        | 0.2 R 10 <sup>9</sup> /L   |                |            |                     |          |                            |
| BA #        | 0.0 R 10 <sup>9</sup> /L   |                |            |                     |          |                            |

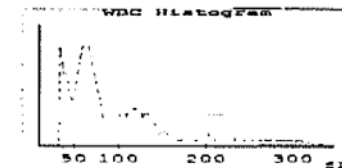
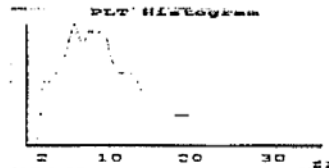
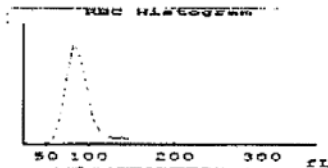
08 OCT 2010  
SIGNED: *[Signature]*

*Lab*

Cellular Interference

Suspect/Definitive Messages:  
NRBC

Platelet Clumps



# Report:

Report Name: All Parameters  
Last Modified: 02/15/2008 By: LabAdmin

|      |       |   |                    |       |       |                     |
|------|-------|---|--------------------|-------|-------|---------------------|
| WBC  | 7.4   | R | 10 <sup>9</sup> /L | RBC   | 5.07  | 10 <sup>12</sup> /L |
| NE % | ..... |   | %                  | HGB   | 15.1  | g/dL                |
| LY % | ..... |   | %                  | HCT   | 0.441 | L/L                 |
| MO % | ..... |   | %                  | MCV   | 87.1  | fL                  |
| EO % | ..... |   | %                  | MCH   | 29.9  | pg                  |
| BA % | ..... |   | %                  | MCHC  | 34.3  | g/dL                |
| NE # | ..... |   | 10 <sup>9</sup> /L | RDW   | 13.8  | %                   |
| LY # | ..... |   | 10 <sup>9</sup> /L |       |       |                     |
| MO # | ..... |   | 10 <sup>9</sup> /L | RET % |       |                     |
| EO # | ..... |   | 10 <sup>9</sup> /L | RET # |       |                     |
| BA # | ..... |   | 10 <sup>9</sup> /L |       |       |                     |

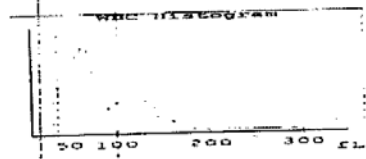
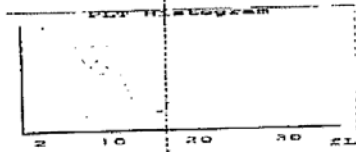
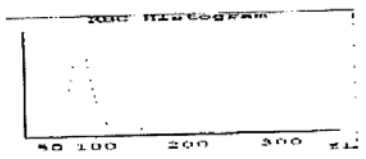
|     |     |     |                    |
|-----|-----|-----|--------------------|
| PLT | 46  | RaL | 10 <sup>9</sup> /L |
| MPV | 9.4 | V   | fL                 |

Suspect/Definitive Messages:  
NRBC

Platelet Clumps  
Thrombocytopenia *check*

Verify Diff  
Cellular Interference

15 OCT 2010



# Report:

|             |                    |                |                     |          |                        |
|-------------|--------------------|----------------|---------------------|----------|------------------------|
| Report Name | All Parameters     | Last Modified: | 02/15/2008          | By:      | LabAdmin               |
| WBC 7.2     | 10 <sup>9</sup> /L | RBC 4.83       | 10 <sup>12</sup> /L | PLT 148  | R L 10 <sup>9</sup> /L |
| NE % 51.3   | %                  | HGB 13.3       | L g/dL              | MPV 10.4 | R fL                   |
| LY % 39.9   | %                  | HCT 0.399      | L/L                 |          |                        |
| MO % 6.8    | %                  | MCV 82.6       | fL                  |          |                        |
| EO % 1.7    | %                  | MCH 27.5       | pg                  |          |                        |
| BA % 0.3    | %                  | MCHC 33.3      | g/dL                |          |                        |
|             |                    | RDW 13.3       | %                   |          |                        |
| NE # 3.7    | 10 <sup>9</sup> /L | RET %          |                     |          |                        |
| LY # 2.9    | 10 <sup>9</sup> /L | RET #          |                     |          |                        |
| MO # 0.5    | 10 <sup>9</sup> /L |                |                     |          |                        |
| EO # 0.1    | 10 <sup>9</sup> /L |                |                     |          |                        |
| BA # 0.0    | 10 <sup>9</sup> /L |                |                     |          |                        |

mm. NE 2  
mm. NE 1

Suspect/Definitive Messages:

93

98

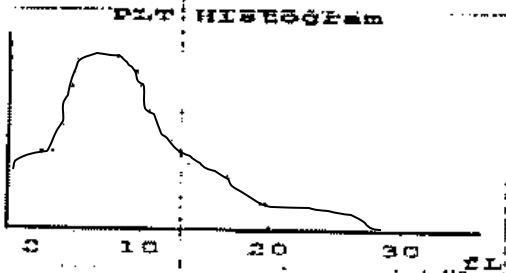
Giant Platelets

slide 180

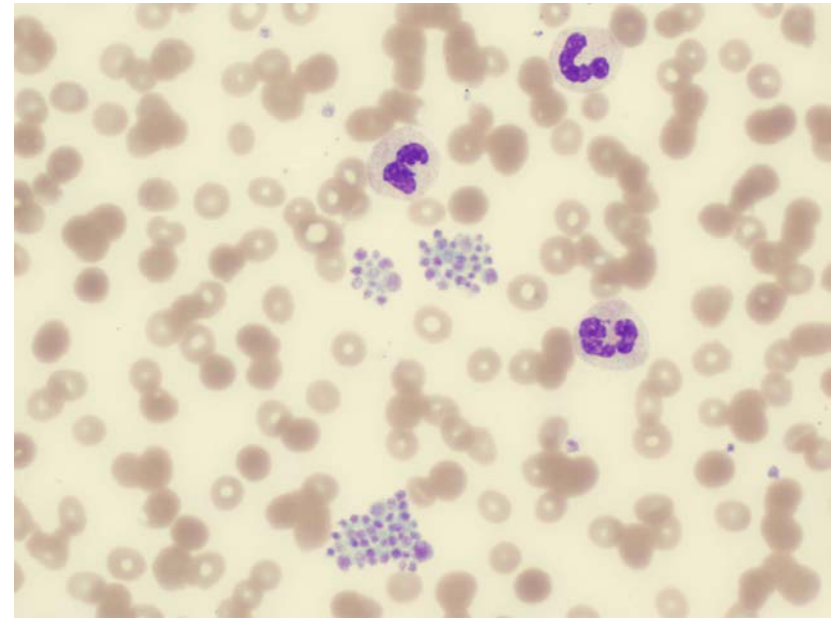
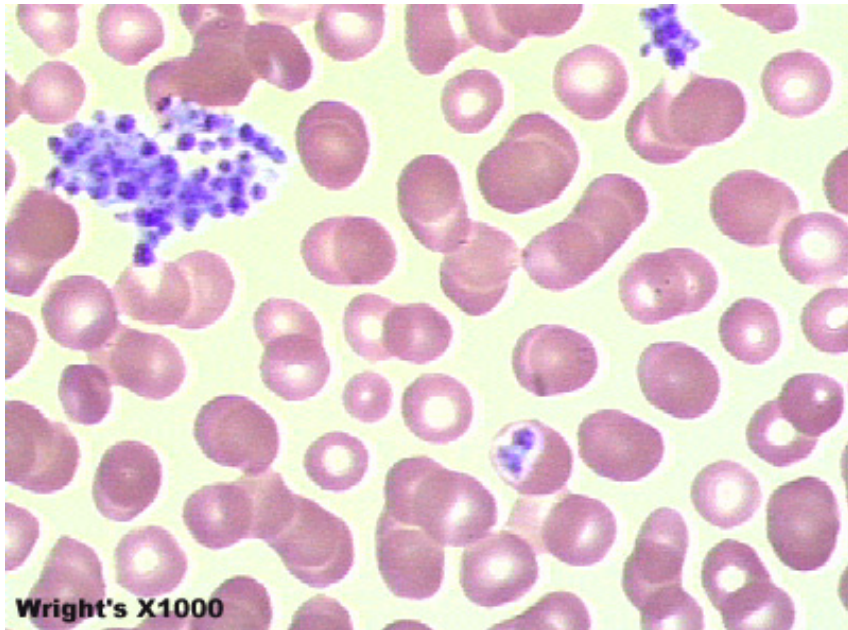
no clots

clumping present

13 OCT 2008

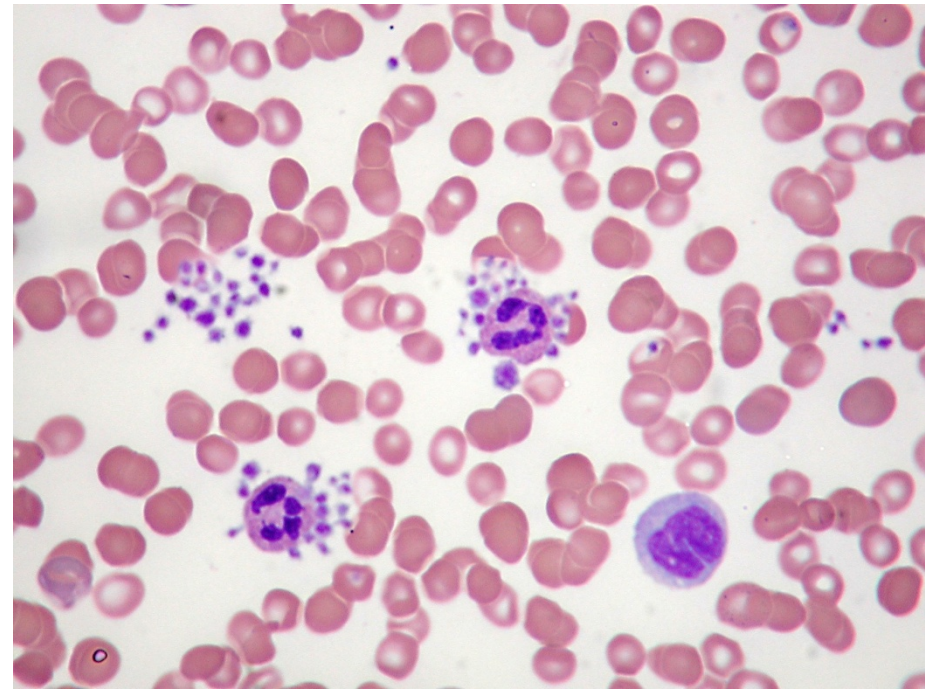
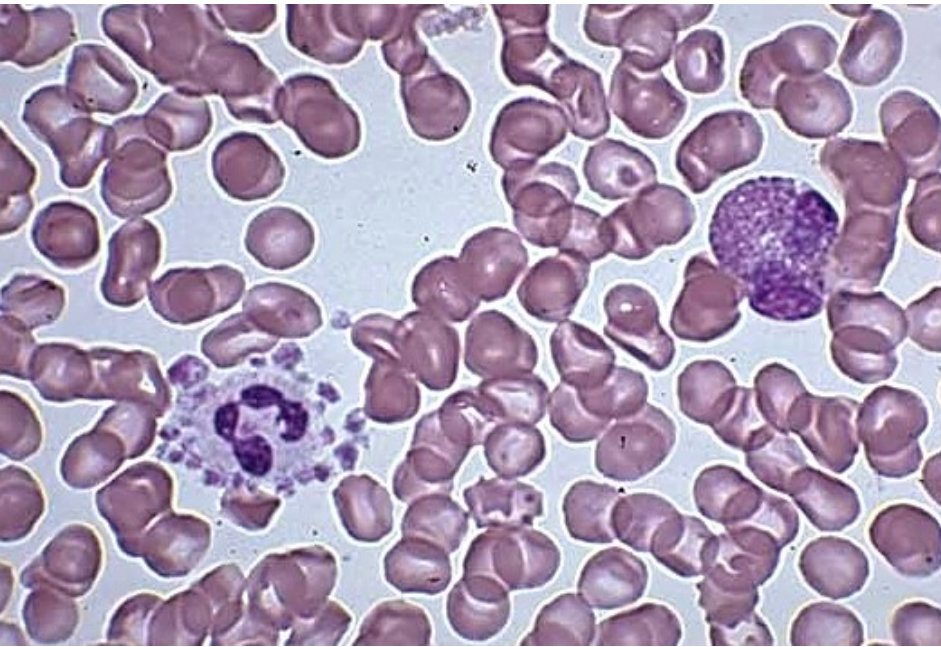


# Slide reviews



Wrights Stain: Platelet clumps

# Platelet Satellitism



Platelet Clumping due to antibody-mediated, EDTA-dependent phenomenon.

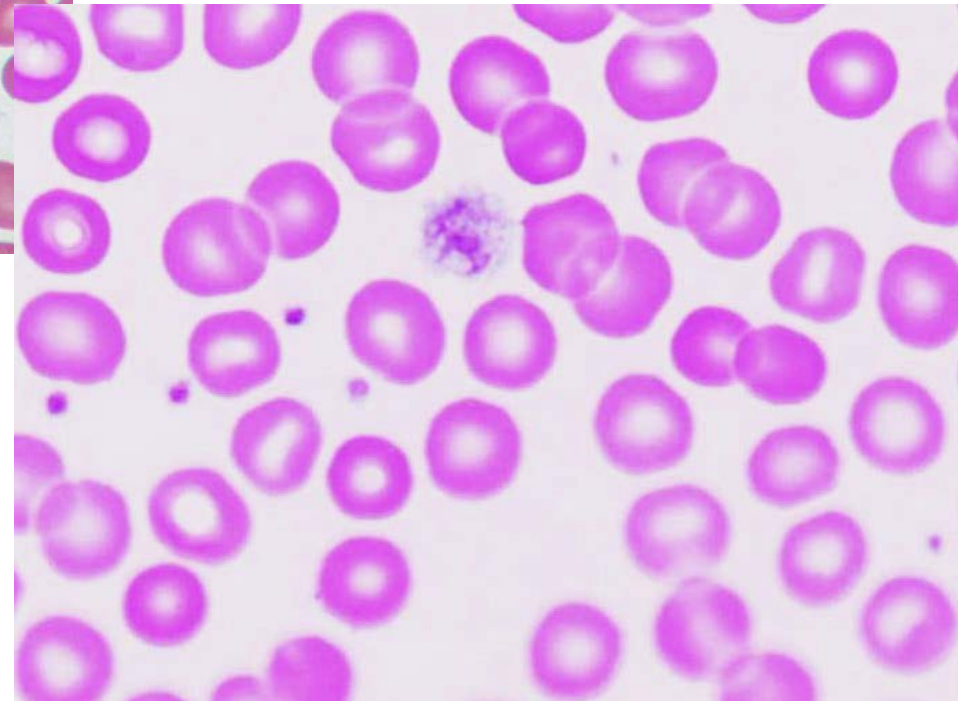
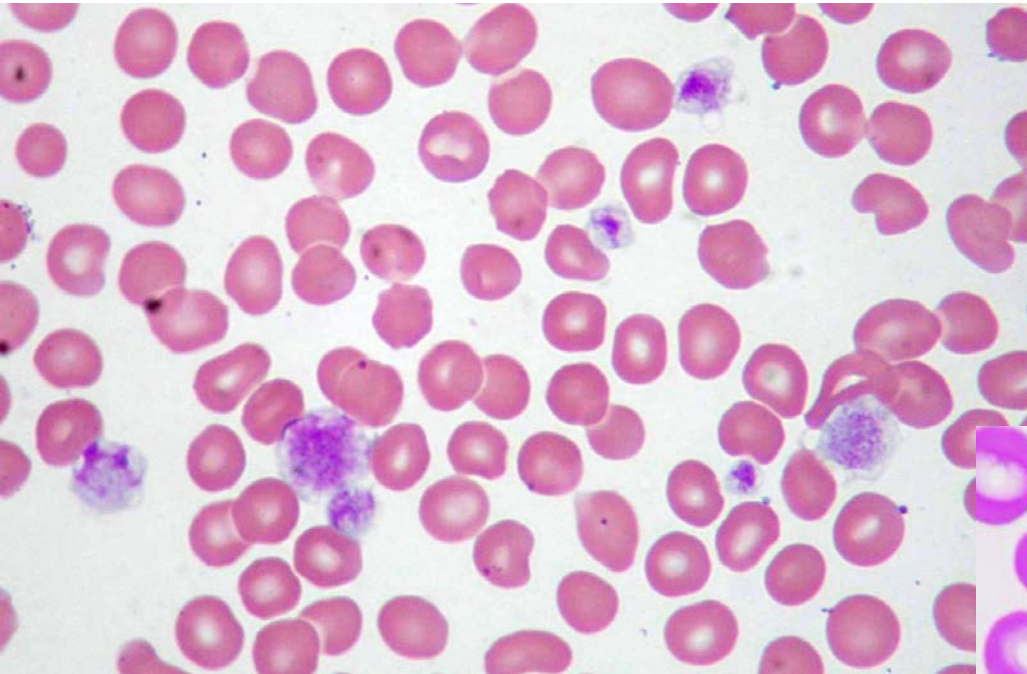
Note how the platelets do **not** satellite around the eosinophil & monocyte

# Giant Platelets

- Giant Platelets can cause some instruments to give a “clumped platelet error”.
- On a slide, a platelet larger than the size of the average red blood cell, assuming a normal MCV, is considered “giant”.
- Can affect the WBC count



# Giant Platelets



# Resolution to Platelet Clumping in EDTA tubes

## Instrument Flag & Errors:

Platelet Clumps, Giant Platelets, Rejected Platelet counts

1. Check for Clots (use 2 applicator sticks)
2. Vortex tube for 15-20 seconds and rerun
3. Pull a slide for a slide review
4. If clumping seen on slide, pre-warm tube, vortex, and rerun.
5. Redraw into a citrate (or ACD) and EDTA tube. Correct for dilution factor.
6. If a manual platelet count (by a cytometer) is not available, then estimate count and comment: Adequate, low, or high.
7. If the manual platelet count can be performed, then a finger stick is performed, and blood is directly introduced into a diluting fluid.



# Is Platelet Clumping a reason for Alarm?

- At present, there does not appear to be an abnormally high number reported from sites.
- New platelet clumpers are not being enrolled
- Recurrent clumpers are being drawn in citrate tubes.
- Your labs are handling the situations well!

Do we know why this happened  
last year from Winter to Spring?

**No**

Conjecture:

Tube Inequality

Colder weather than normal

Poor phlebotomy technique

# Conclusions

- Not all platelet clumping issues will be resolved (In Pittsburgh, LA, & Hawaii, approximately 10-15% of patient clumping in tubes goes unresolved).
- Upon screening, do not accept participants that have platelet clumps (unless due to poor phlebotomy technique, and the sample would be redrawn).
- Once enrolled, if clumping can not be resolved by drawing a citrate tube, then estimate platelet count from slide and determine if number is adequate.

# Questions

