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ADVANCED PRACTICE PROVIDER

Live Webinar

June 15, 2022

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The Advanced Practice Provider webinar series created and coordinated by Tammy Triglianos, DNP, ANP-BC, AOCNP, in partnership with the UNC Lineberger Cancer Network

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UNC Lineberger Cancer Network

ADVANCED PRACTICE PROVIDER

June 15, 2022

Laboratory Workup for Transfusion Reactions

Mariama Evans, MD

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OUR PRESENTER

Mariama Evans, MD

Mariama Evans, MD is the 2021-2022 Blood Bank and Transfusion Medicine Fellow at UNC's Department of Pathology and Laboratory Medicine. She completed her residency in anatomic and clinical pathology at UNC from 2017-2021.

Her current responsibilities include clinical service for apheresis patients and administrative tasks related to transfusion reactions and red blood cell antibodies. She is also a 2009 graduate of the University of North Carolina at Chapel Hill and received her medical degree from the George Washington School of Medicine in 2017.

In her free time she currently serves on the Executive Board of UNC's Resident Diversity Initiative (RDI), helping to recruit trainees from underrepresented groups to the institution.

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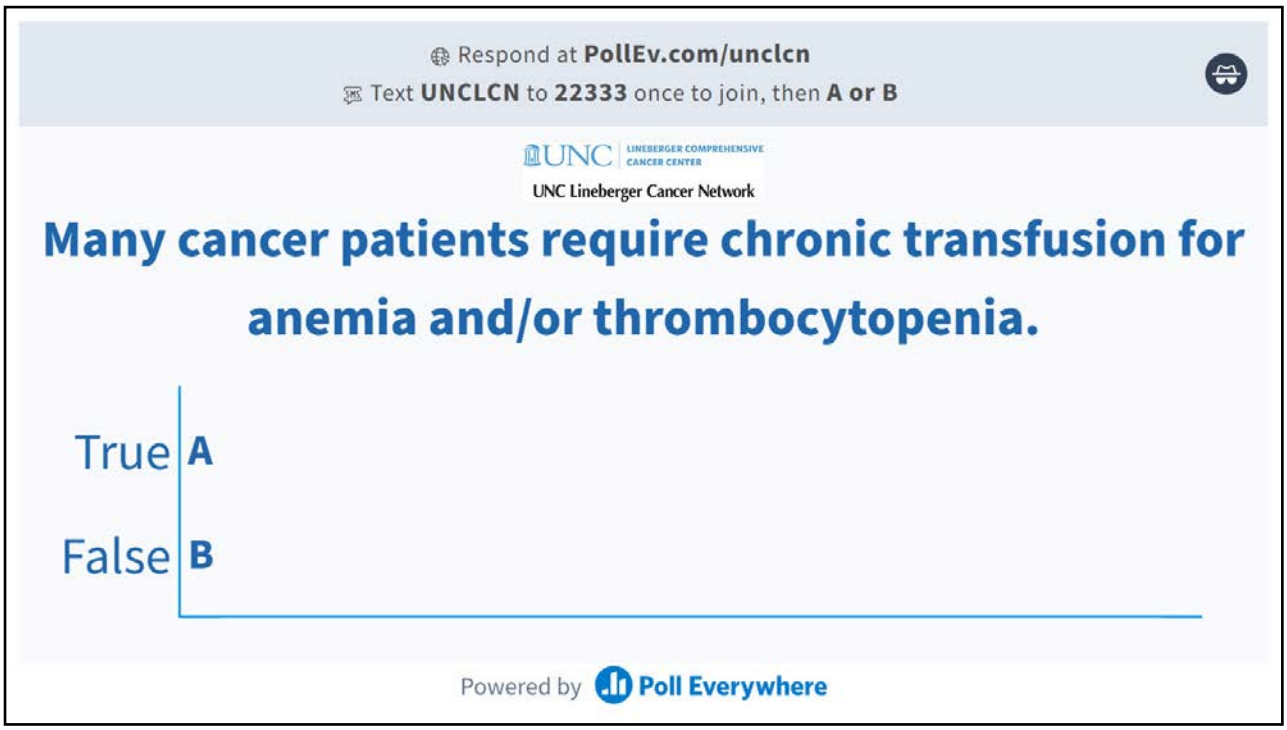

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DISCLOSURES

This activity has been planned and implemented under the sole supervision of the Course Director, William A. Wood, MD, MPH, in association with the UNC Office of Continuing Professional Development (CPD). The course director and CPD staff have no relevant financial relationships with ineligible companies as defined by the ACCME.

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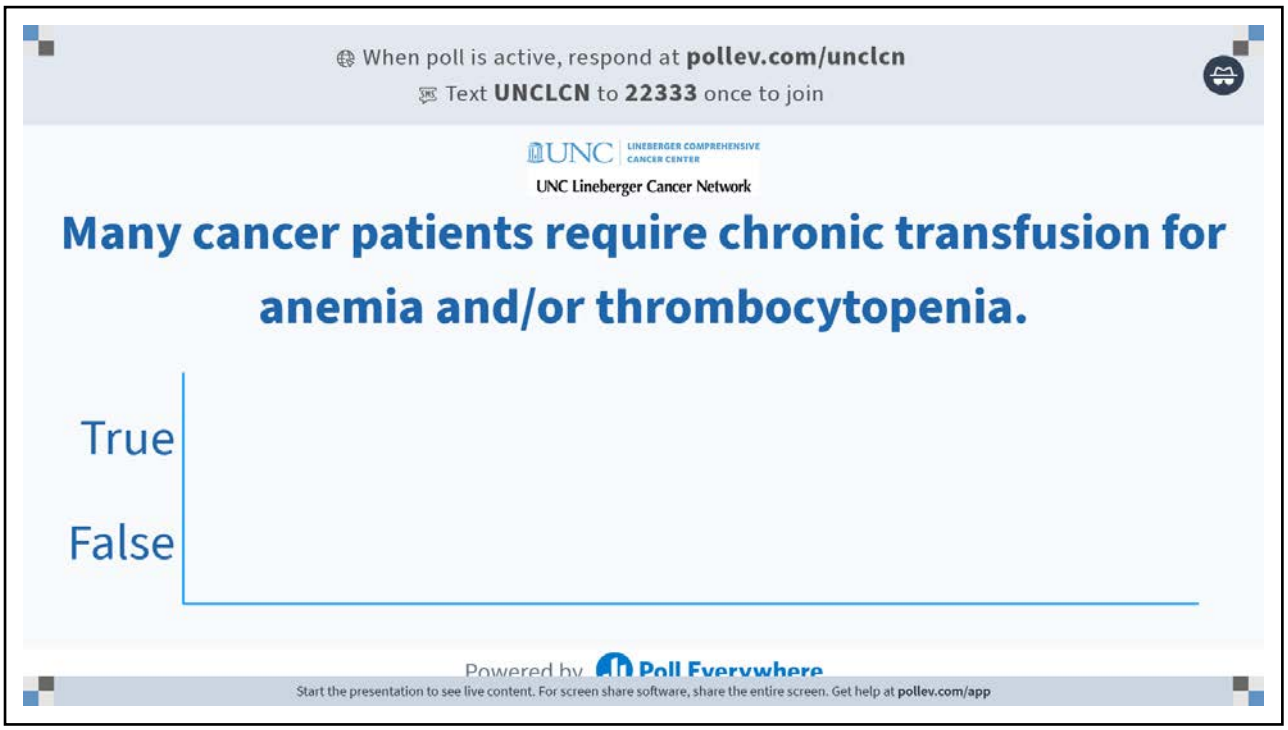

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LABORATORY WORKUP OF TRANSFUSION REACTIONS

Mariama Evans, MD
UNC Blood Bank and Transfusion Medicine Fellow
June 15, 2022

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I have no financial disclosures or conflicts of interest relating to the subject matter of this presentation.

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LEARNING OBJECTIVES

- List the most common transfusion reactions and describe their manifestations.
- Explain what steps to take if a transfusion reaction is suspected.
- Discuss common laboratory techniques used to work up transfusion reactions.

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- Many cancer patients require chronic transfusion for **anemia** and/or **thrombocytopenia**
- Majority of platelet units administered to heme/onc patients to **stop or prevent bleeding complications**

Disease-associated	Treatment-associated
• **Hematologic malignancy** • **Bleeding** • Metastatic bone marrow infiltration • Anemia of chronic disease • Hypersplenism	• **Chemotherapy** • **Stem cell transplantation** • Radiation • Drug induced hemolysis

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- **Transfusion-associated circulatory overload (TACO)**
- **Transfusion-related acute lung injury (TRALI)**
- Transfusion-associated dyspnea (TAD)
- **Allergic reaction**
- Hypotensive transfusion reaction
- **Febrile non-hemolytic transfusion reaction (FNHTR)**
- **Acute hemolytic transfusion reaction (AHTR)**
- Delayed hemolytic transfusion reaction (DHTR)
- Delayed serologic transfusion reaction (DSTR)
- Transfusion-associated graft vs. host disease (TAGVHD)
- Post-transfusion purpura (PTP)
- Transfusion-transmitted infection (TTI)
  - **Transfusion-related sepsis**

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FREQUENCY OF REACTIONS

Most common

Allergic transfusion reaction	Platelets and plasma: 1 to 3% RBCs: 0.1 to 0.3%
Febrile non-hemolytic transfusion reaction (FNHTR)	≤1%
Transfusion-associated circulatory overload (TACO)	<1% of transfused patients (higher in hospitalized population)
Transfusion-related acute lung injury (TRALI)	<0.01%
Transfusion-related sepsis	≤0.002%
Acute hemolytic transfusion reaction (AHTR)	0.001%

Tobian, A. (2022). *Uptodate.*

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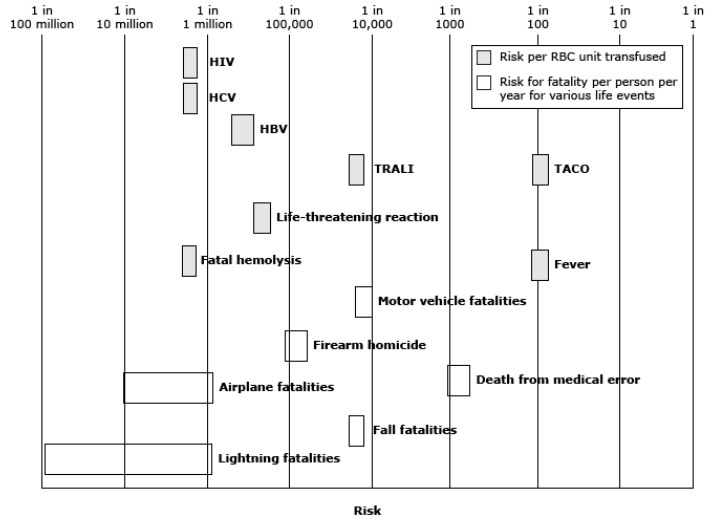
Frequency of adverse effects from RBC transfusion placed in context relative to the risks of other hazards



Tobian, A. (2022). *Uptodate.*

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ALLERGIC TRANSFUSION REACTION

- Range from **mild cutaneous symptoms → anaphylaxis**
- Most common
  - Urticarial (1%-3%)
  - Anaphylactic (≤ 0.005%)
- Hypersensitivity reaction to allergens in donor plasma
- Most frequently associated with **platelet transfusions**

2 or more of the following occurring during or within 4 hours of cessation of transfusion:

- Conjunctival edema
- Edema of lips, tongue and uvula
- Erythema and edema of the periorbital area
- Generalized flushing
- Hypotension
- Localized angioedema
- Maculopapular rash
- Pruritus (itching)
- Respiratory distress; bronchospasm
- Urticaria (hives)

https://www.cdc.gov/nhsn/pdfs/biovigilance/bv-hv-protocol-current.pdf

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FEBRILE NON-HEMOLYTIC TRANSFUSION REACTION (FNHTR)

- Due to leukoreduction, now less than 1% of reactions
  - Removes donor WBCs from cellular blood products
- These **WBCs can release cytokines** into the unit
  - IL-1,IL-6, IL-8, TNF-alpha
- More commonly seen in **red blood cell** (RBC) **and platelet transfusions**

Occurs during or within 4 hours of cessation of transfusion
AND EITHER
Fever (greater than or equal to 38°C/100.4°F oral and a change of at least 1°C/1.8°F) from pre-transfusion value
OR
Chills/rigors are present.

https://www.cdc.gov/nhsn/pdfs/biovigilance/bv-hv-protocol-current.pdf

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TRANSFUSION-RELATED SEPSIS

Blood Bank should be notified so blood products from this donor can be quarantined.

- **Fever** (≥ 38.5 C)
- Chills, rigors, hypotension
- Usually occurs within 4 hours of transfusion
- Source
  - Donor's **skin flora** (*S. aureus, S. epidermidis*)
  - RBC units from donors with **asymptomatic bacteremia** (*Yersinia, Pseudomonas, Serratia sp.*)
- Most commonly associated with **platelet units (stored at room temperature)**
- Blood product and patient samples sent for culture

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TRANSFUSION-RELATED ACUTE LUNG INJURY (TRALI)

- Rare
- Life threatening **non-cardiogenic pulmonary edema**
  - **Hypoxemia with bilateral lung infiltrates**
  - Hypotension, dyspnea, fever
- Recipient risk factors
  - Liver disease or transplantation, chronic alcohol abuse, shock, sepsis, current smoker, increased IL-8 levels, hematologic malignancy, massive transfusion

NO evidence of acute lung injury (ALI) prior to transfusion
AND
ALI onset during or within 6 hours of cessation of transfusion
AND
Hypoxemia defined by any of these methods:
- PaO2/FiO2 less than or equal to 300 mm Hg
- Oxygen saturation less than 90% on room air
- Other clinical evidence

AND
Radiographic evidence of bilateral infiltrates
AND
No evidence of left atrial hypertension (i.e., circulatory overload)

https://www.cdc.gov/nhsn/pdfs/biovigilance/bv-hv-protocol-current.pdf

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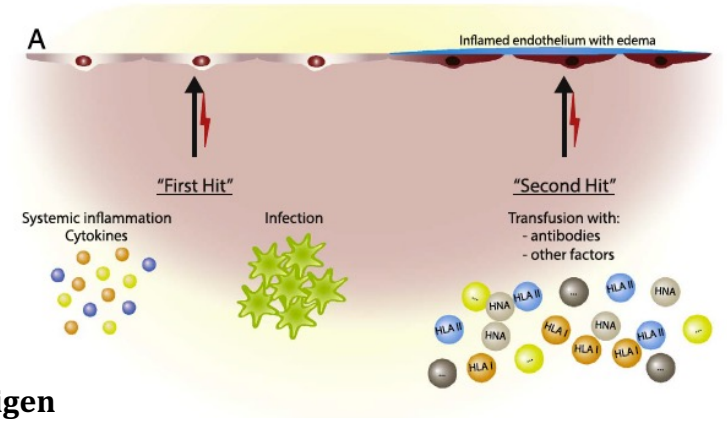
Fig. 1. a A scheme of the "two-hit" hypothesis. Any condition leading to a state of inflammation (upregulation adhesion molecules on endothelium, priming of leukocytes) can serve as a first hit. The second hit is the transfusion of blood products containing factors which results in activation of the endothelium and/or leukocytes and causes TRALI.



1. Activated endothelium in the patient (**PRIMING**)
2. **Anti-human leukocyte antigen (HLA) or Anti-neutrophil antibodies** in the **DONOR**
   - Donor history of pregnancy (exposed to foreign HLA antigens)

Morsing, K. H., van Buul, J. D., Peters, A. L., & Vlaar, A. J. (2018). *Blood Reviews*, *32*(1), 1-7.

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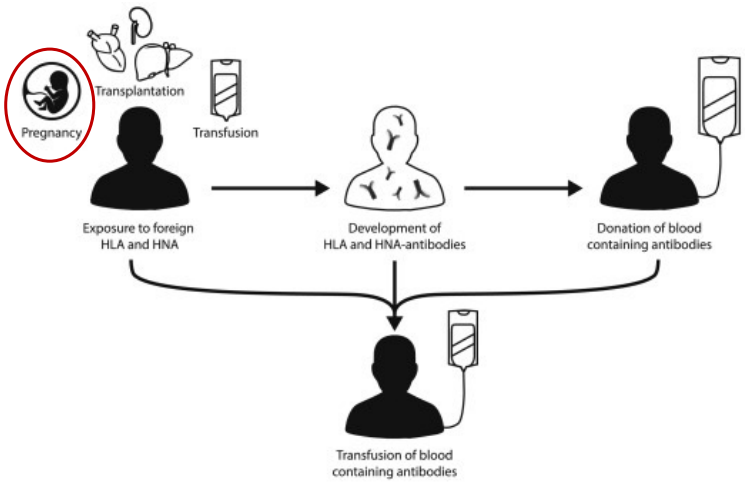
TRALI PATHOGENESIS



Donor

Recipient

Morsing, K. H., van Buul, J. D., Peters, A. L., & Vlaar, A. J. (2018). *Blood Reviews*, *32*(1), 1-7.

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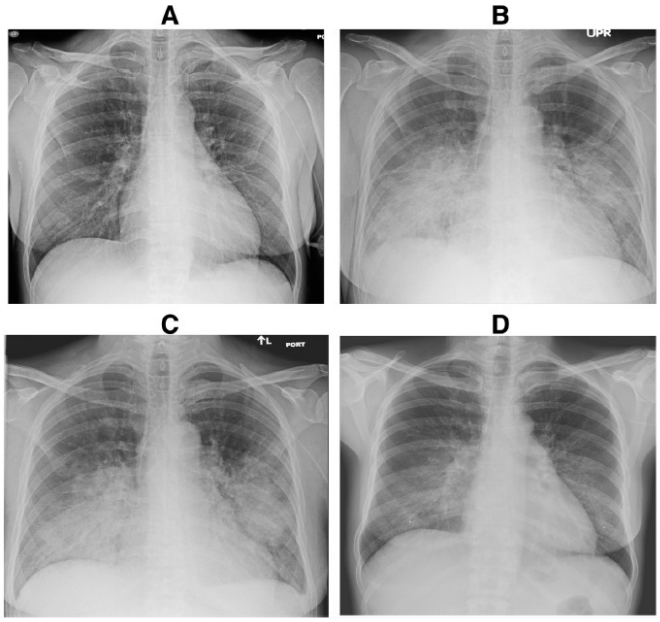

Carcano, C., et al. (2013). *Clinical Imaging*, *37*(6), 1020-1023.

Serial chest x-rays in a patient with TRALI

A. Pre-transfusion

B. 5 hours after transfusion

C. 40 hours after transfusion

D. 72 hours after transfusion – partial resolution

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TRANSFUSION ASSOCIATED CIRCULATORY OVERLOAD (TACO)

- <1% of transfused patients, though likely unreported
- **New onset or exacerbation of cardiogenic pulmonary edema** within 12 hours of transfusion
- Evidence of **acute respiratory distress**
  - Hypoxemia, tachypnea, dyspnea

+

- Radiographic or clinical **evidence of volume overload**
  - Crackles on lung exam, cough, frothy sputum, jugular venous distention (JVD), hypertension, peripheral edema
- **Elevated brain natriuretic peptide (BNP)**
- Changes not explained by underlying medical condition

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TACO V. TRALI

Transfusion Related Acute Lung Injury (TRALI)	Transfusion-Associated Circulatory Overload (TACO)
• **Acute dyspnea** • **Diffuse bilateral infiltrates**	

▪ **Fever**
▪ **Hypotension**
▪ Neutral or negative fluid balance
▪ BNP < 250
▪ **Transient leukopenia**
▪ Supportive treatment: O_2 supplementation, IV fluids, respiratory and pressor support as needed
	▪ Risk factors
	  ▪ Surgical or ICU setting
	  ▪ Rapid blood product administration
	  ▪ Impaired cardiac function
	  ▪ Positive fluid balance
	  ▪ Renal dysfunction
	▪ **Volume overload**
	  ▪ Physical exam: S3, JVD
	  ▪ **Elevated BNP (> 1200)**
	▪ **Hypertension**
	▪ Improves with **diuretics**

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ACUTE HEMOLYTIC TRANSFUSION REACTION (AHTR)

- **Immune-mediated** intravascular **hemolysis of incompatible RBCs** by patient antibodies
- Symptom **onset within 24 hrs** of transfusion
- Third most fatal transfusion reaction

Positive Direct Antiglobulin Test (DAT) + evidence of new alloantibody

2 or more of the following:
- Elevated bilirubin
- Decreased haptoglobin
- Increased lactate dehydrogenase (LDH)
- Hemoglobinuria
- **Inadequate bump in post-transfusion hemoglobin**
- **Discoloration of post-transfusion plasma**

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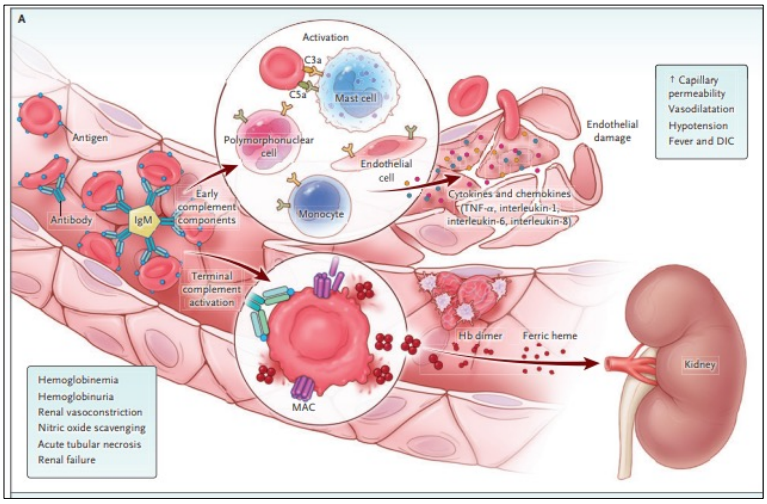
ACUTE HEMOLYTIC TRANSFUSION REACTION (AHTR)



Occurs during, or within 24 hours of cessation of transfusion with new onset of **ANY** of the following signs/symptoms:

- Back/flank pain
- Chills/rigors
- Disseminated intravascular coagulation (DIC)
- Epistaxis
- Fever
- Hematuria (gross visual hemolysis)
- Hypotension
- Oliguria/anuria
- Pain and/or oozing at IV site
- Renal failure

AND

2 or more of the following:

- Decreased fibrinogen
- Decreased haptoglobin
- Elevated bilirubin
- Elevated LDH
- Hemoglobinemia
- Hemoglobinuria
- Plasma discoloration c/w hemolysis
- Spherocytes on blood film

AND EITHER

(IMMUNE-MEDIATED)

Positive direct antiglobulin test (DAT) for anti-IgG or anti-C3

AND

Positive elution test with alloantibody present on the transfused red blood cells

OR

(NON-IMMUNE MEDIATED)

Serologic testing is negative, and physical cause (e.g., thermal, osmotic, mechanical, chemical) is confirmed.

Panch, Montemayor-Garcia, & Klein. (2019). NEJM, 381(2), 150-162.
https://www.cdc.gov/nhsn/pdfs/biovigilance/bv-hv-protocol-current.pdf

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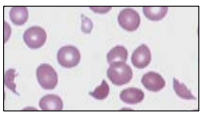
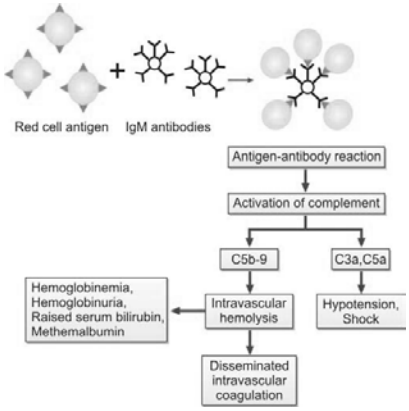
DISSEMINATED INTRAVASCULAR COAGULATION (DIC)

- **Consumption of coagulation factors and platelets**
  - **Elevated** Prothrombin Time (**PT**), Partial Thromboplastin Time (**PTT**), **D-Dimer**
  - **Decreased fibrinogen**
  - **Schistocytes** on peripheral smear



- Supportive care
  - Aggressive hydration, pressors, ventilatory support, blood products (if indicated)



Dg, D. (2019). *Bioscience.com.pk*
Maslak, P. (2008). *American Society of Hematology Image Bank.*

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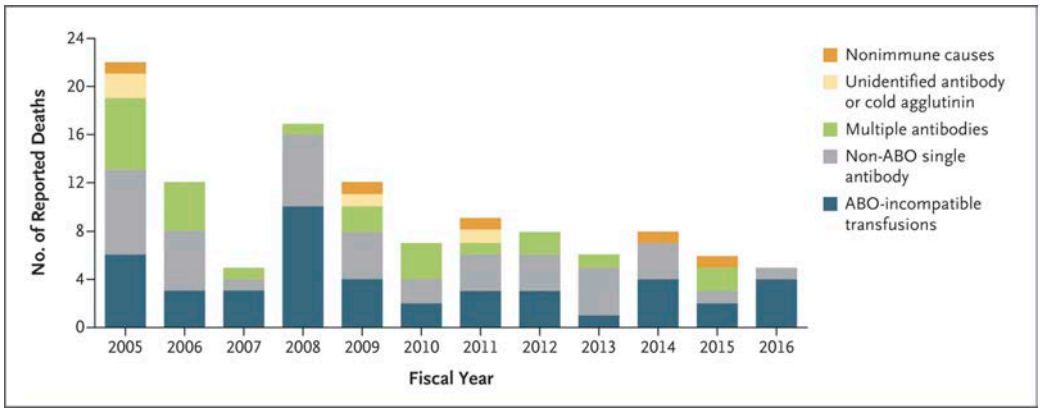
- There has been an overall decline in deaths related to hemolytic transfusion reactions.



Panch, Montemayor-Garcia, & Klein. (2019). NEJM, 381(2), 150-162.

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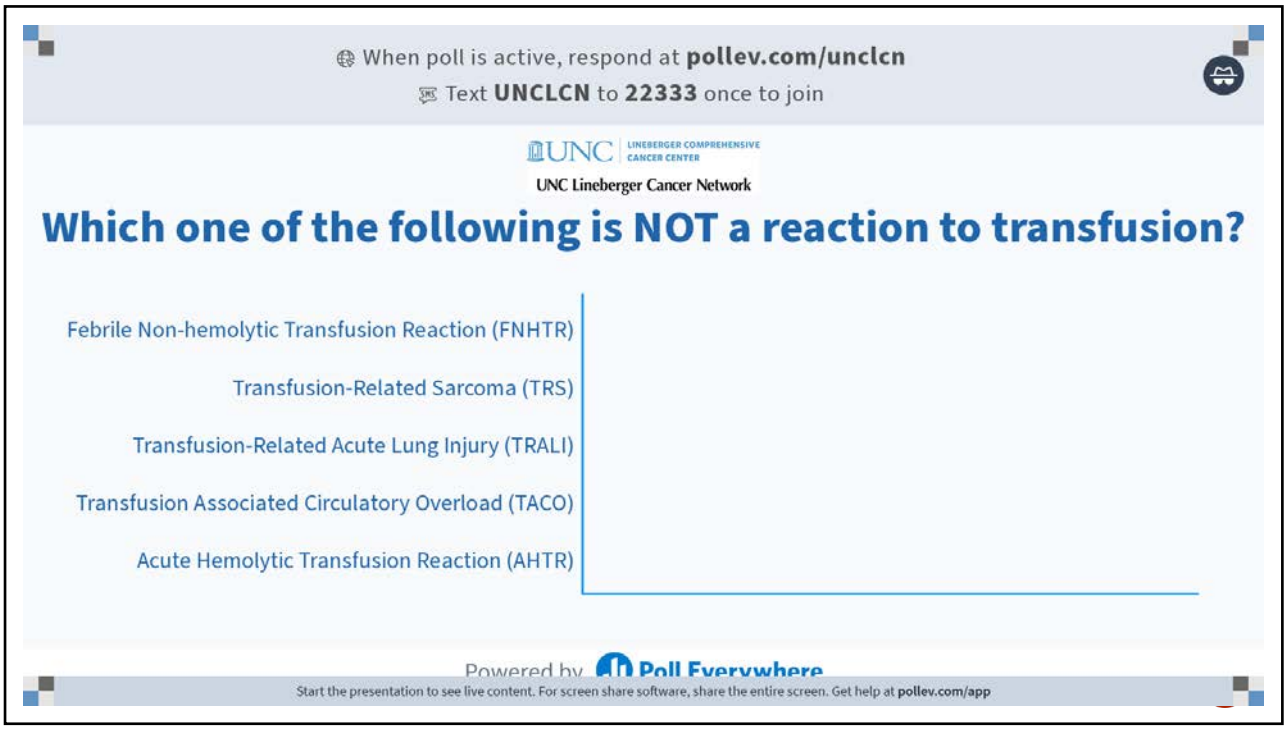

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TRANSFUSION-ASSOCIATED FATALITIES

- The number of **reported** fatalities is fairly low.
- As of 2019, **TACO** is the leading cause of transfusion related mortality.

Table 3: Transfusion-Associated Fatalities by Complication, FY2015 – FY2019

Complication	FY15 No.	FY15 %	FY16 No.	FY16 %	FY17 No.	FY17 %	FY18 No.	FY18 %	FY19 No.	FY19 %	Total No.	Total %
Anaphylaxis	2	5%	5	12%	3	8%	2	6%	2	5%	**14**	**7%**
Contamination	5	14%	5	12%	7	19%	7	23%	1	2%	**25**	**13%**
HTR (ABO)	2	5%	4	9%	1	3%	2	6%	4	9%	**13**	**7%**
HTR (Non-ABO)	4	11%	1	2%	6	16%	4	13%	11	25%	**26**	**14%**
Hypotensive Reaction	1	3%	1	2%	0	0%	0	0%	0	0%	**2**	**1%**
TACO	11	30%	19	44%	11	30%	12	39%	12	27%	**65**	**34%**
TRALI**	12	32%	8	19%	9	24%	4	13%	12	27%	**45**	**23%**
Transfusion Reaction, Type Not Determined	0	0%	0	0%	0	0%	0	0%	2	5%	**2**	**1%**

https://www.fda.gov/media/147628/download

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SIGNS AND SYMPTOMS OF TRANSFUSION REACTIONS

- Fever
- Chills/rigors
- Nausea/vomiting
- Respiratory distress
- Blood pressure alterations
- Abdominal, chest, flank or back pain
- Pain at infusion site
- Skin manifestations: urticaria, rash, flushing, pruritus, edema
- Cardiac arrhythmias
- Jaundice or hemoglobinuria
- Abnormal bleeding
- Oliguria/anuria
- Renal failure
- Anxiety
- Headache
- Paresthesia/tetany
- Anaphylaxis
- DIC
- Death

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TEMPERATURE INCREASE & TIMING

- Serious and fatal reactions may occur quickly.
- Importance of frequent vital signs, including **pre- and post-transfusion**

Presenting WITH Fever	
Acute	Delayed
Acute Hemolytic Febrile Non-hemolytic Tx-related Sepsis TRALI	Delayed Hemolytic TA-GVHD
Presenting WITHOUT Fever	
Acute	Delayed
Allergic Hypotensive Tx-associated Dyspnea TACO Acute Pain	Delayed Serologic Post-transf Purpura Iron overload

https://www.bbguy.org/education/videos/txrxnworkup/

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If a transfusion reaction is suspected…

- **STOP the transfusion**
- Maintain IV access
- Assess patient and treat the reaction
- **Clerical check**: confirm right patient, right unit
- Inform the blood bank
  - Obtain patient's **post-transfusion samples**
  - Return remaining blood product and tubing

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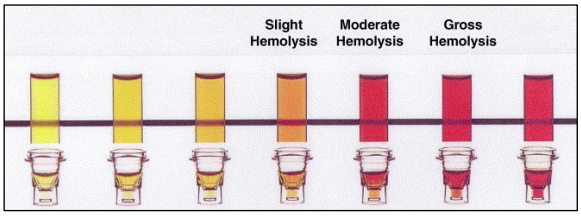
TRANSFUSION REACTION WORK-UP

- **Clerical check** – "right patient, right unit"
- Visual check for **hemolysis**
  - Hemoglobinemia
- **ABO-Rh typing, Direct Antiglobulin Test** (DAT)
  - Additional testing if concern for alloantibody
- Gram stain and culture of returned product, if indicated
  - High fever, hypotension



Dugan, L., Leech, L., Speroni, K. G., & Corriher, J. (2005). *Journal of Emergency Nursing, 31*(4), 338-345.

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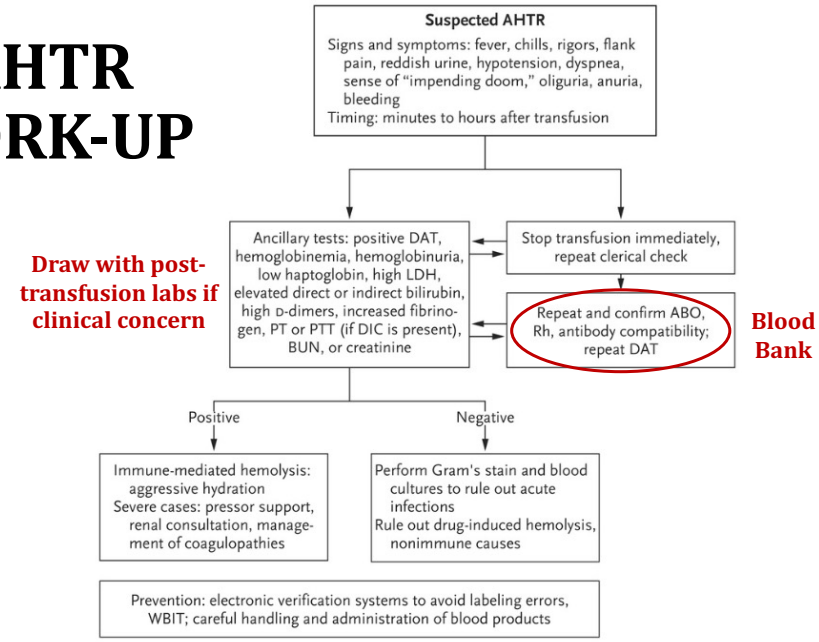
AHTR WORK-UP



Draw with post-transfusion labs if clinical concern

Blood Bank

Panch, Montemayor-Garcia, & Klein. (2019). NEJM, 381(2), 150-162.

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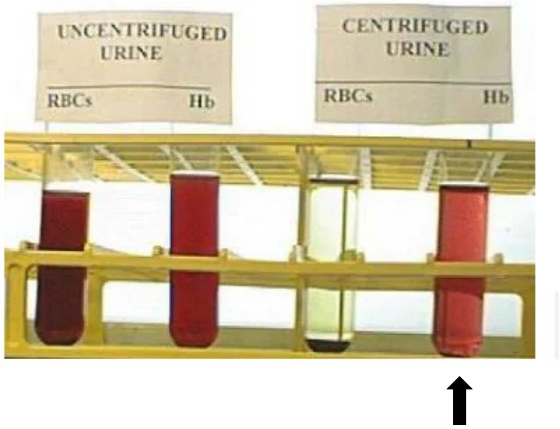
AHTR WORK-UP

- Hemoglobinuria
  - Urine remains red/darkened after centrifugation
  - **Excess free hemoglobin** present in urine
  - Suggests intravascular hemolysis



https://www.medscape.com/answers/206885-108630/how-is-hematuria-and-hemoglobinuria-distinguished-in-the-evaluation-of-transfusion-reactions

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ABO TYPING

- **FORWARD TYPE**
  - Uses reagents containing a known antibody to **determine ABO antigens present on patient RBCs**
- **REVERSE TYPE**
  - Uses reagent RBCs of a known ABO type to **test patient plasma for its expected ABO antibodies**

https://www.grifols.com/en/view-news/-/news/grifols-expands-its-blood-typing-solutions-portfolio-in-the-united-states-with-antisera-reagents

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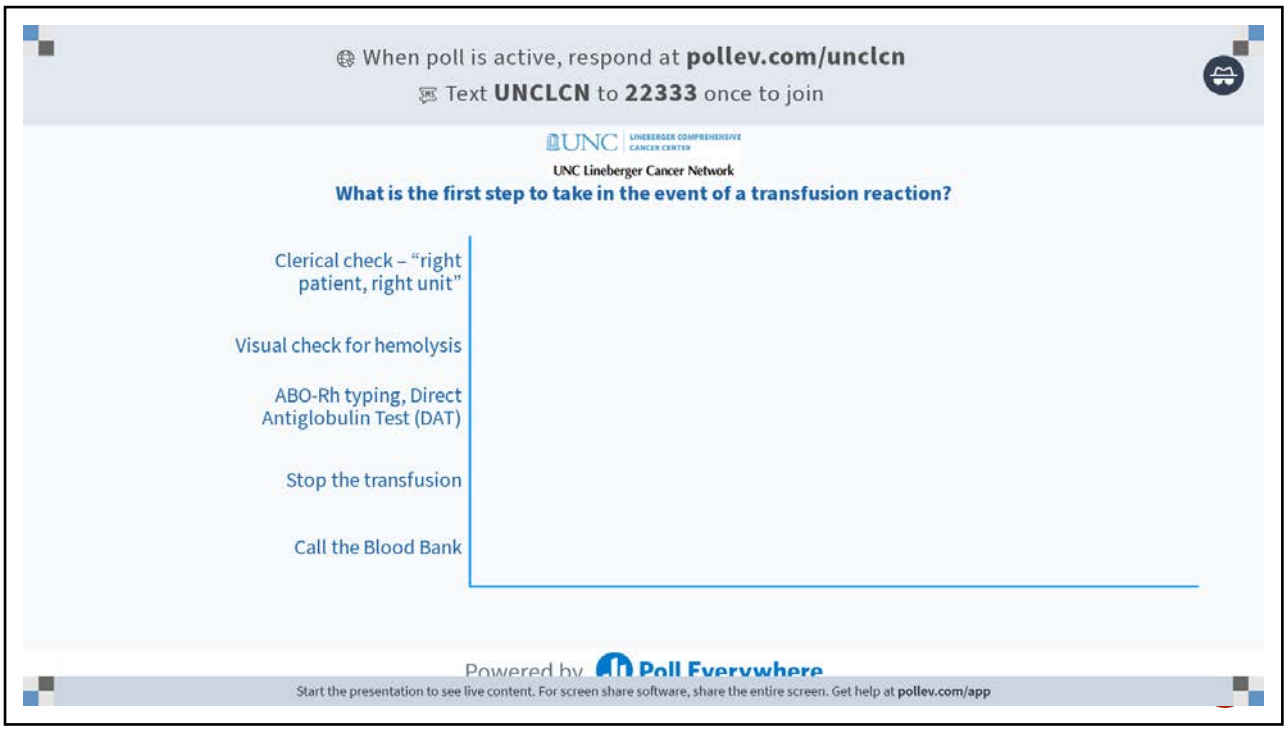

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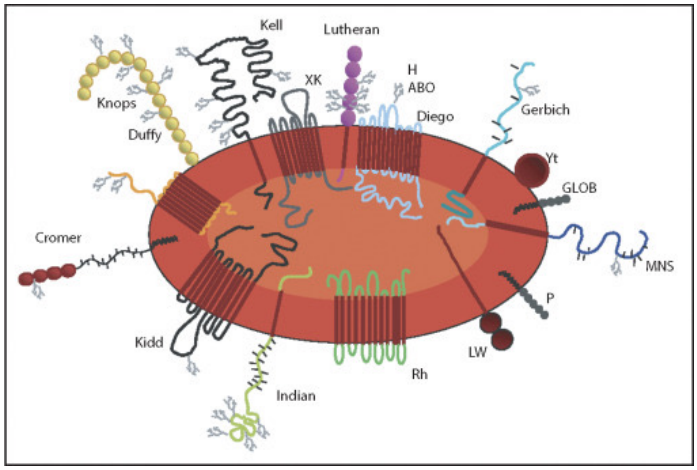
RBC ANTIGENS



- More than 700 RBC antigens reported
- Some are high frequency/essentially universal
  - 90-99% of individuals
- Antigen frequency often varies by ethnic origin
  - **Rh, Duffy, Kidd** blood groups

Tormey, C. A., & Hendrickson, J. E. (2019). *Blood, 133*(17), 1821-1830.

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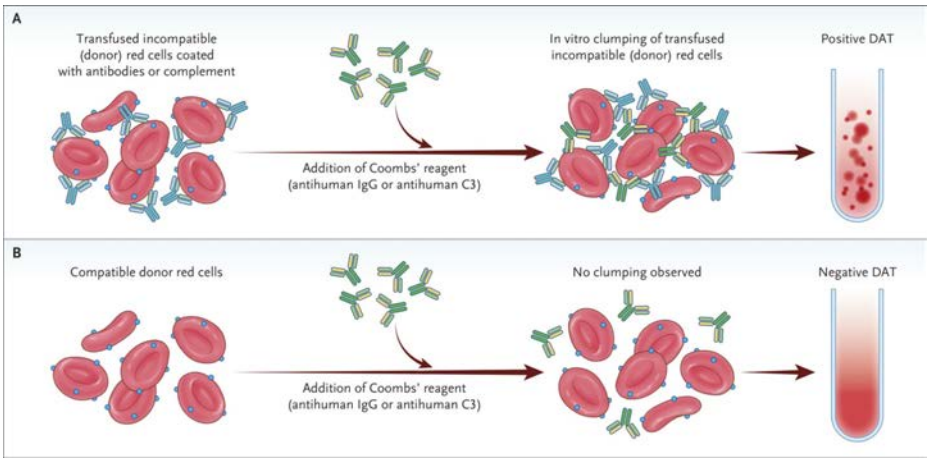
DIRECT ANTIGLOBULIN TEST (DAT)



Panch, Montemayor-Garcia, & Klein. (2019). NEJM, 381(2), 150-162.

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ELUTION

- Disrupts the attachment between RBCs and the coating antibodies
- Dissociated antibodies remain in a solution which can be tested for specificity

TABLE 35-39
Antibody Elution Techniques

Method	Principle*
Heat	Addition of heat changes equilibrium constant of antigen–antibody reaction, and antibodies are released
Freeze-thaw	Damage to the red cell membrane by hemolysis alters complementary fit between antigen and antibody
Acid (e.g., digitonin acid, glycine acid/EDTA)	At an acid pH, antigen and antibody become negatively charged and repulse each other
Organic solvents (e.g., chloroform, xylene, ether)	Disrupts lipid bilayer of red cell membrane, altering complementary fit and/or reversing selected attractive forces between antigen and antibody

EDTA, Ethylenediaminetetraacetic acid; *HDFN*, hemolytic disease of the fetus and newborn; *Ig*, immunoglobulin
*From Issitt and Anstee (1998).

McPherson & Pincus. (2016). Henry's clinical diagnosis and management by laboratory methods.

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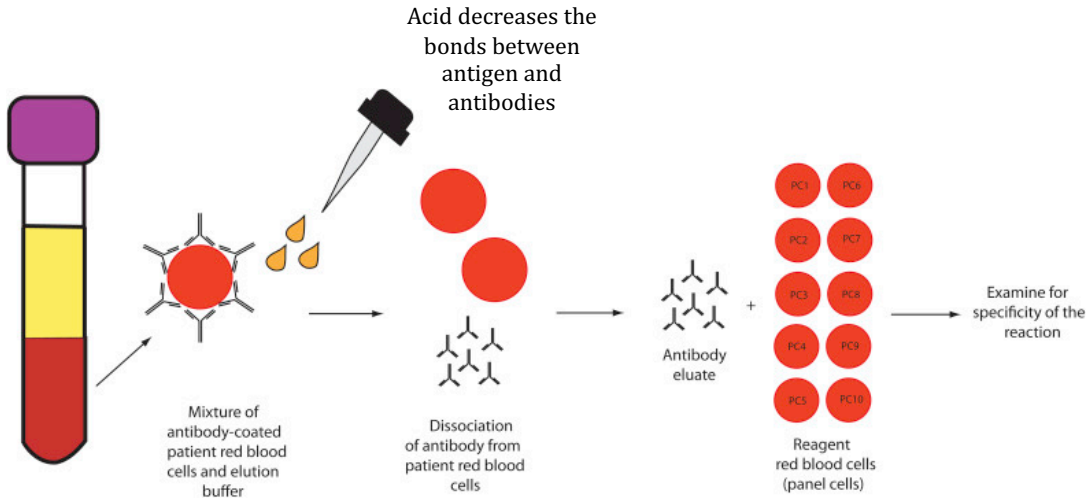
ELUTION



Red Blood Cell Antibodies in Hematology/Oncology Patients | Oncohema Key

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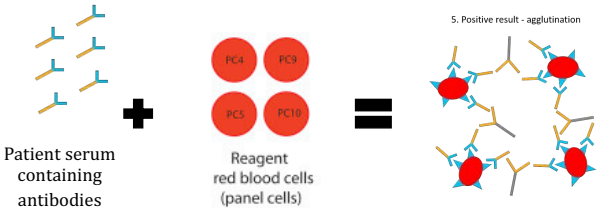
ANTIBODY SCREEN

- Screen patient's serum for **unexpected (non-ABO) antibodies** prior to anticipated transfusion
  - History of **exposure to non-self RBCs** via pregnancy or transfusion
  - Naturally occurring



Adapted from https://logicalbiological.com/wp-content/uploads/2019/05/Figure-1-Irreg-antibodies-V2-1.png
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ANTIBODY SCREEN

Patient serum incubated with **reagent RBCs** with a known antigen profile

Reagent Cell	Antigens expressed on reagent cell																									Patient test results		
	Rh							Kell						Duffy		Kidd		Lewis		P	MN				Lutheran		Xg			
	D	C	c	E	e	f	V	C^w	K	k	Kp^a	Kp^b	Js^a	Js^b	Fy^a	Fy^b	Jk^a	Jk^b	Le^a	Le^b	P_1	M	N	S	s	Lu^a	Lu^b	Xg^a	IS	AHG	CC
I	+	+	0	0	+	0	0	0	+	+	0	+	0	+	+	+	0	+	0	+	+	+	+	+	+	0	+	0	0	0	+
II	+	0	+	+	0	0	0	0	0	+	0	+	0	+	0	+	+	0	+	0	+	+	+	0	+	0	+	+	0	2+	

Positive screen (**agglutination**) requires further workup for identification of the antibody.

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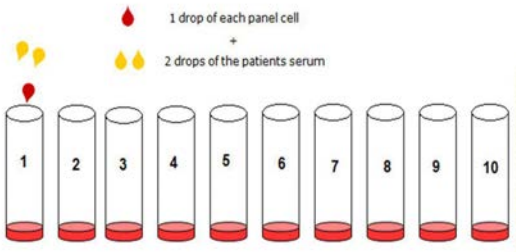
ANTIBODY IDENTIFICATION

- Patient serum tested against a larger panel of known RBC reagent cells
- Determines which RBC antigen(s) the alloantibody targets

WHY?

- Identify clinically significant antibodies to prevent or diagnose:
  - **Hemolytic transfusion reactions**
  - **Hemolytic disease of the fetus and newborn** (HDFN)
- Provide **antigen negative units for future transfusions**
- Clinical behavior varies depending on the antibody and its characteristics



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ANTIBODY IDENTIFICATION

- Federal Drug Administration (FDA) requires detection of the following antibodies:
  - **D, C, E, c, e, M, N, S, s, P1, Le^a, Le^b, K, k, Fy^a, Fy^b, Jk^a, and Jk^b**

Name *John Doe*

Hospital number *0001234.56*

Reagent RBCs of known phenotypes

		Rh								MNSs				P	Lewis		Lutheran		Kell*				Duffy		Kidd			Vial			
	Rh phenotype	D	C	E	c	e	f	Cw	V	M	N	S	s	P_1	Le^a	Le^b	Lu^a	Lu^b	K	k	Kp^a	Js^a	Fy^a	Fy^b	Jk^a	Jk^b	Xg^a		37C	AHG	CC
1	rr	0	0	0	+	+	+	0	0	+	0	+	0	+	+	0	0	+	0	+	0	0	+	+	+	0	+	1	0	1	
2	rr	0	0	0	+	+	+	0	0	+	+	0	+	+	0	+	0	+	+	+	0	0	0	+	0	+	+	2	0	3	
3	r'r	0	0	0	+	+	+	0	0	+	0	+	+	+	0	+	0	+	0	+	0	0	0	+	0	+	0	3	0	0	2
4	r''r	0	0	+	+	+	+	0	0	+	+	+	+	0	0	+	+	+	0	+	0	0	0	+	0	+	0	4	1	2	
5	rr	0	0	0	+	+	+	0	0	0	+	+	+	+	0	+	0	+	0	+	0	0	0	+	0	+	+	5	0	0	2
6	R_or	+	0	0	+	+	+	0	+	+	+	0	+	+	0	0	0	+	0	+	0	0	0	0	+	0	0	6	0	0	2
7	R_1R_1	+	+	0	0	+	+	0	0	+	0	+	+	0	+	0	0	+	0	+	0	0	0	+	+	+	0	7	0	0	2
8	R_1R_1	+	+	0	0	+	+	0	0	+	+	+	+	+	0	+	0	+	+	+	0	0	+	0	0	+	+	8	0	3	
9	$R_1R_1^w$	+	+	0	0	+	+	+	0	+	+	0	+	+	0	+	0	+	0	+	0	0	+	+	+	+	+	9	0	1	
10	R_2R_2	+	0	+	+	0	+	0	0	+	+	0	+	+	+	0	0	+	0	+	0	+	0	0	+	+	+	10	1	2	
11	rr	0	0	0	+	+	+	0	0	0	+	0	+	+	0	+	+	+	0	+	+	0	+	+	+	0	0	11	0	1	
																												Auto	0	0	2

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KEY POINTS

- Blood transfusions are safe but not without risk
- Importance of accurate symptom documentation and pre- and post-transfusion vital signs
  - Allergic reaction
  - Febrile non-hemolytic transfusion reaction (FNHTR)
  - Transfusion-related sepsis
  - Transfusion-related acute lung injury (TRALI)
  - Transfusion-associated circulatory overload (TACO)
  - Acute hemolytic transfusion reaction (AHTR)
- Complex antibody panel results may require additional sample for send out testing to reference laboratory
- Transfusion Medicine service can advise regarding diagnosis of transfusion reactions and special blood product needs for patients with RBC antibodies

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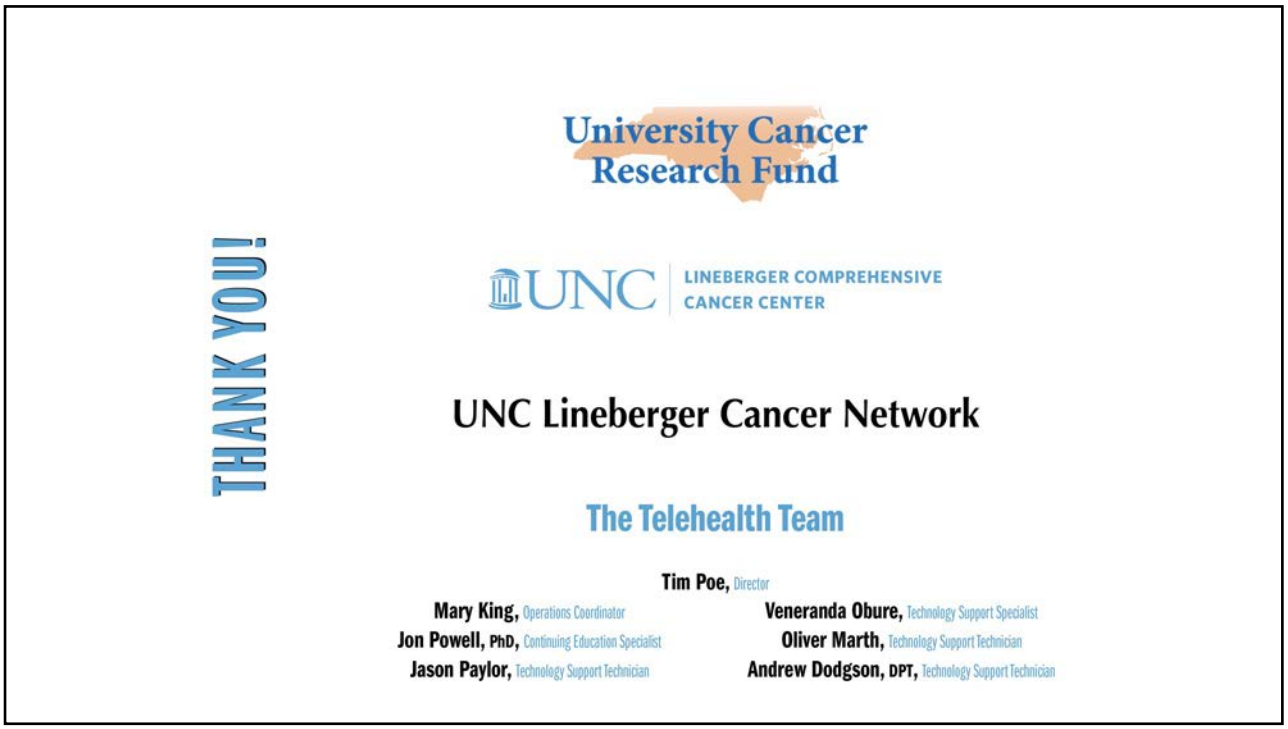
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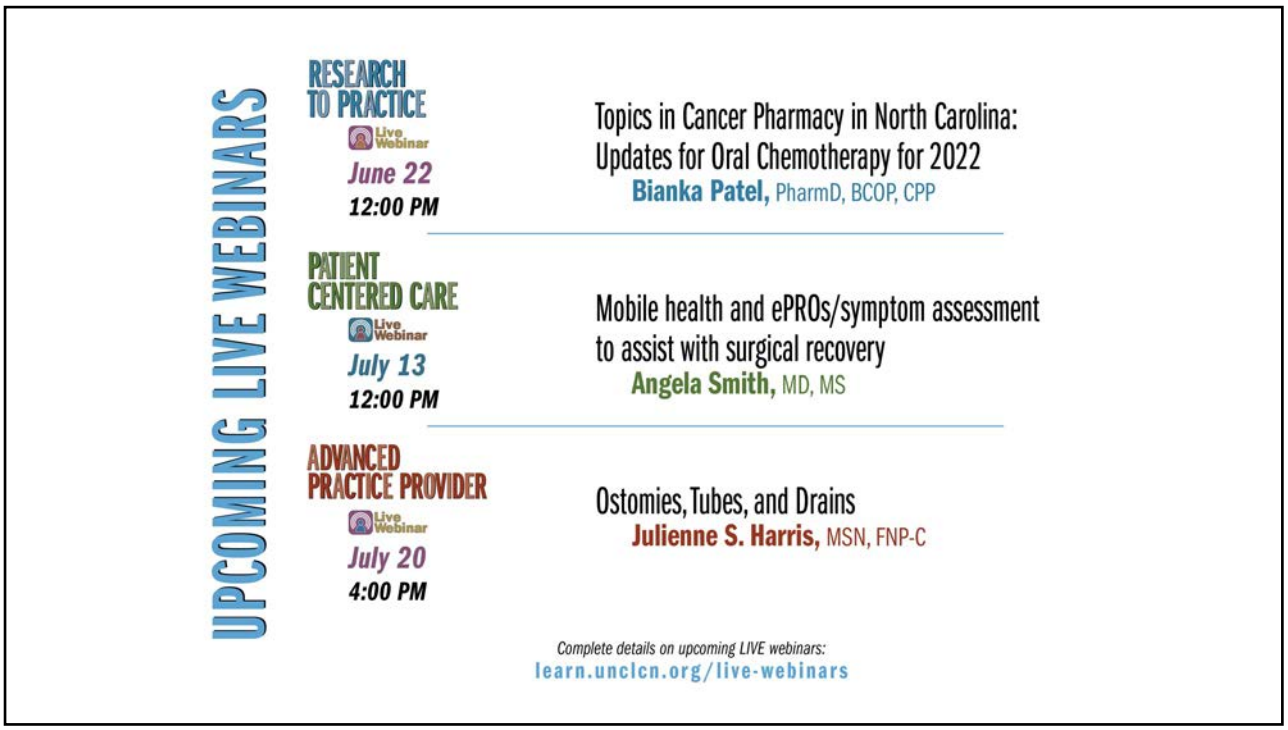
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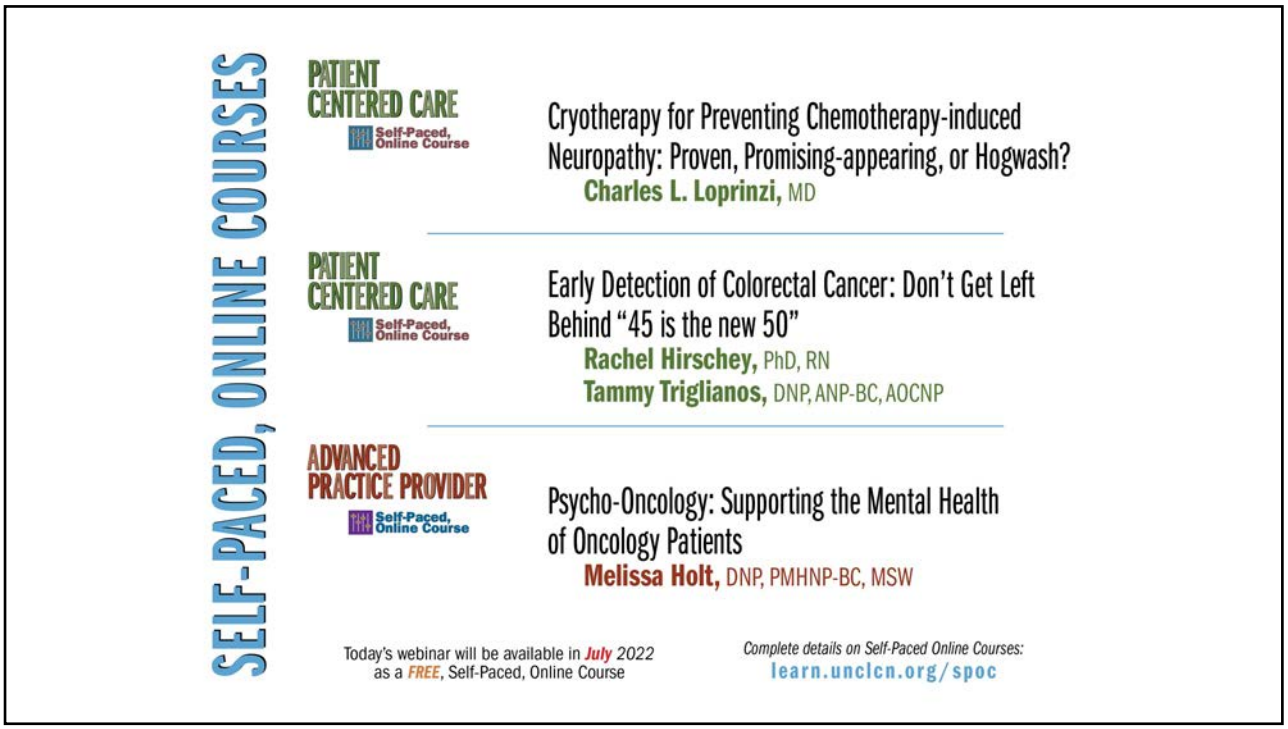
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