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"Optimal" Algorithms for Serological and Molecular Typing for Finding The Best Match Donor

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VdA

Systems:

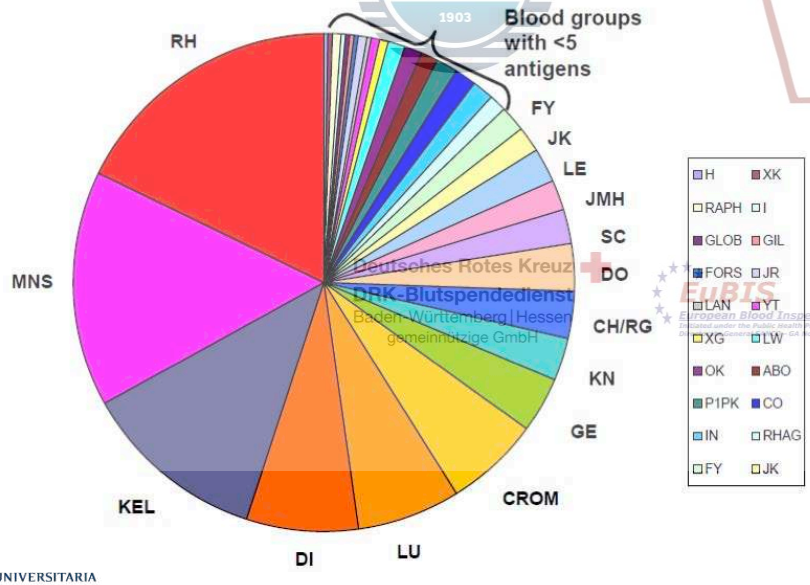
38 Blood Group Systems

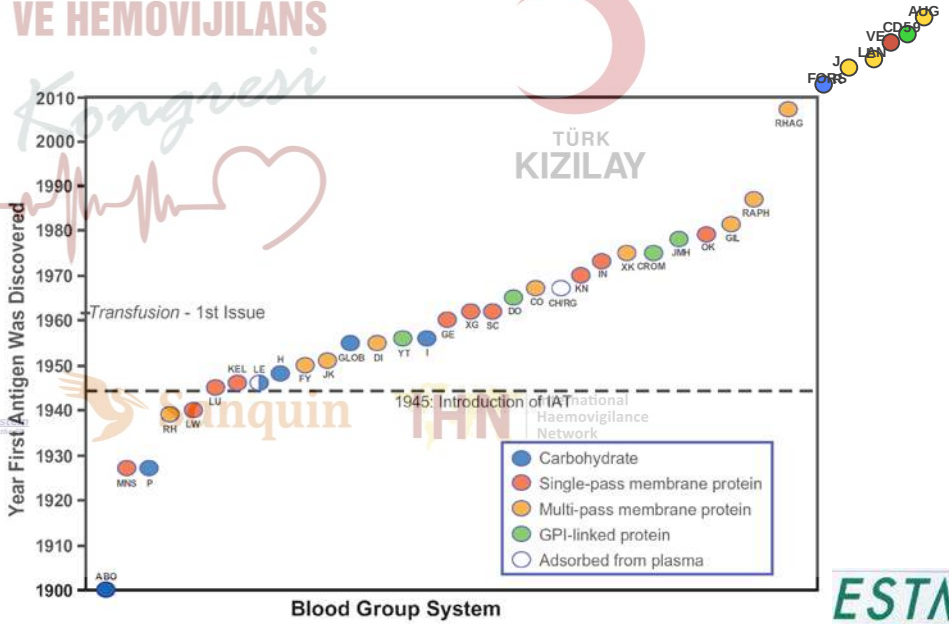
360 Antigens

45 Genes

>2000 Alleles

Collections (200 series)
High incidence antigens (901 series)
Low incidence antigens (700 series)





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Serological testing

- Discovered more than 100 years ago
- Improvements related to the origin of antibodies (monoclonal vs polyclonal), matrix for the reaction (solid phase and gel test vs tube test), automation in testing, reading, interpretation of the results
- It remains the basis for the majority of blood typing needs

Molecular testing

- DNA assays leading to the "prediction" of the RBC antigen phenotypes
- Results highly correlate with testing of the RBCs with a specific antibody
- A field with more than a decade of experience
- DNA arrays mainly available in specialized blood centers or in large hospitals

are they different stuff ?

Antibody-based typing	DNA-based typing
≤1-h turnaround	24-h turnaround
Manual/ semi-automation	Automated
Fresh RBCs required	Any cell source*
Existing equipment	Specialized equipment and environment
Direct detection of antigen expression	Indirect "predicted" antigen expression
Interference from transfused RBCs or bound IgG	No interference from transfused RBCs or bound IgG
No reagents for some clinically significant antigens	Type for any antigen whose genetic basis is known
Weak/variable antigen expression may be missed	Detection of weak antigen expression
Low resolution	High resolution possible

Blood group genotyping

blood® 25 APRIL 2019 | VOLUME 133, NUMBER 17

Connie M. Westhoff

- Indicate requirements to implement the activity (in terms of lab environment, staff, number of tests)
- Define situations in which molecular typing is appropriate
- Describe algorithms for integration of serological and molecular techniques
- Give directions on interpretation and management of results
- Focus on quality standards

DNA-based typing

- ORGANIZATION
- METHODS (techniques, technologies, LIS)
- DONORS (who-what-when)
- PATIENTS (who-what-when)
- RESULTS MANAGEMENT
- QUALITY REQUIREMENTS

Organization

It is recommended that laboratories performing molecular immunohematology investigations for erythrocyte and platelet antigens:

- **belong to authorized and accredited Transfusion Facilities**
- **have a laboratory head with at least five years of experience in advanced immunohematology and at least two years in molecular immunohematology**

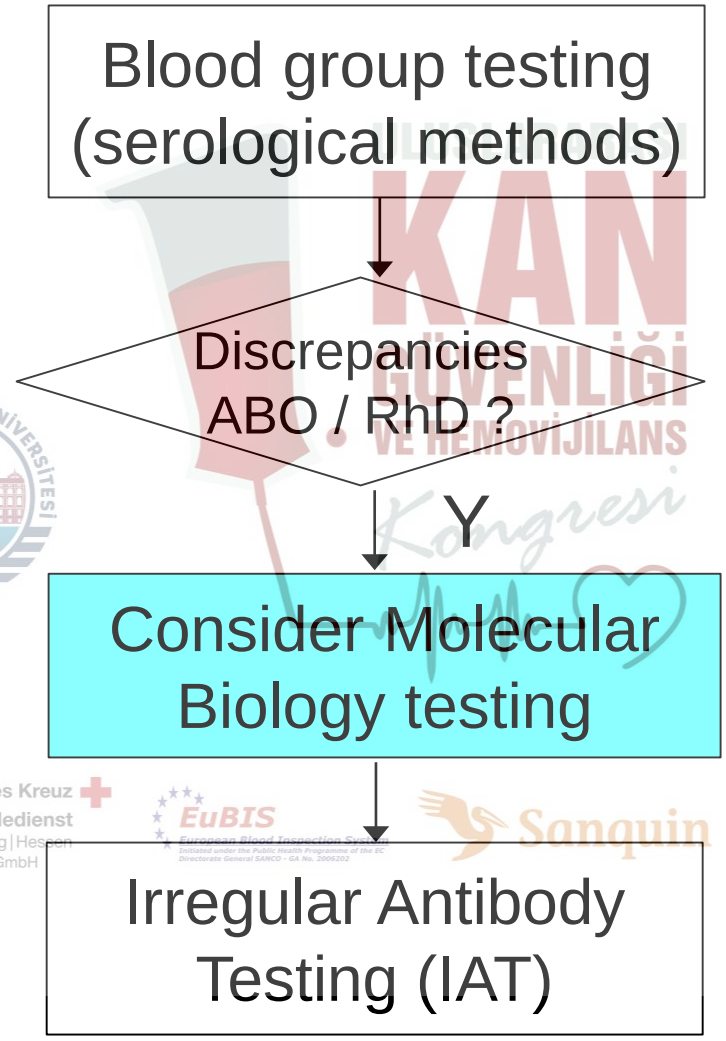
A **Molecular Reference IH Laboratory** is defined when it:

- performs at least **500 molecular typings/year** on patient and donor samples
- uses **two different molecular methods** for typing the main blood group systems.

A common scenario in a common Hospital Blood Bank.....

- Transfusion request for anemic patient
- T&S protocol
- Blood group testing (serological methods)

Blood Group Testing



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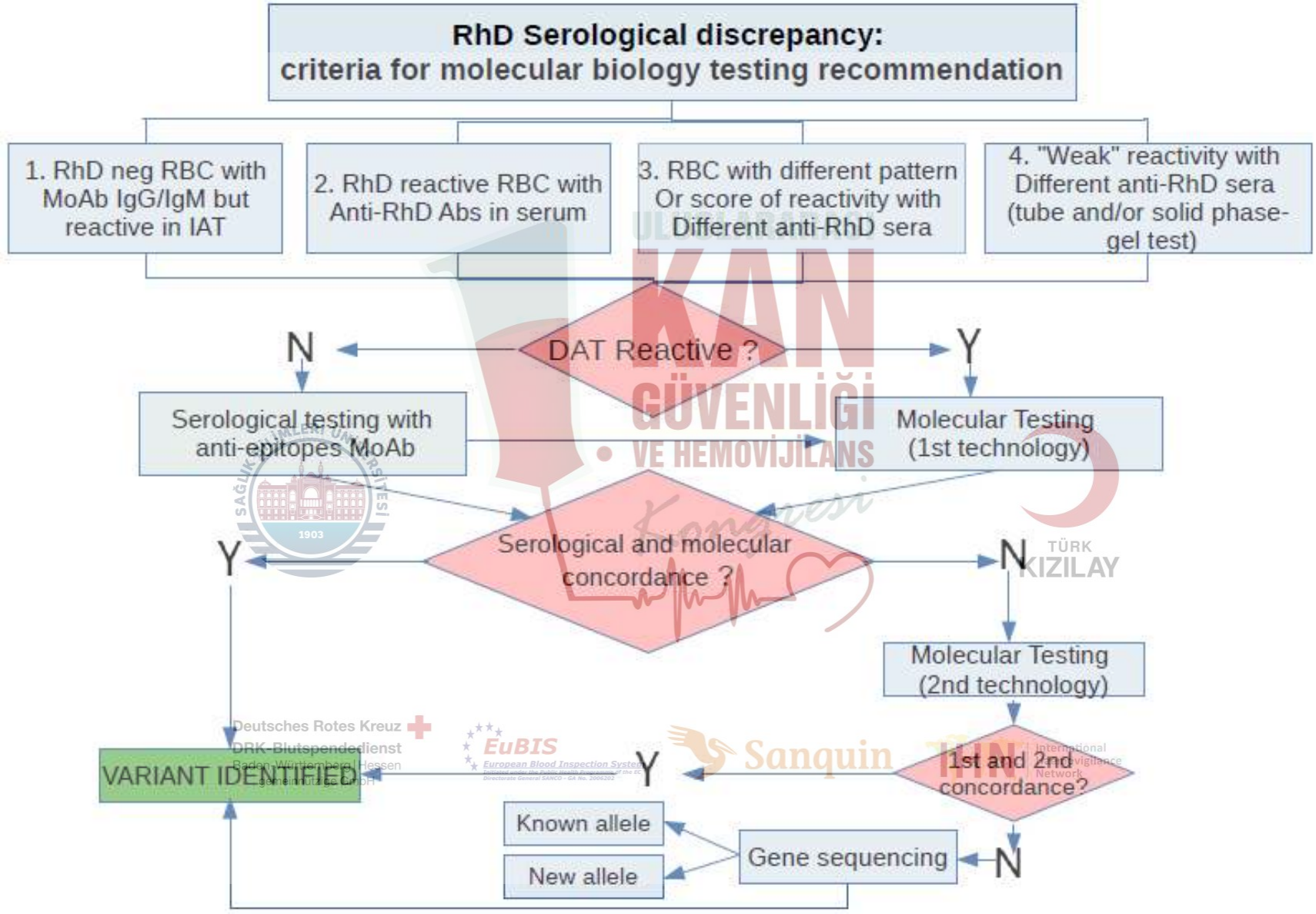
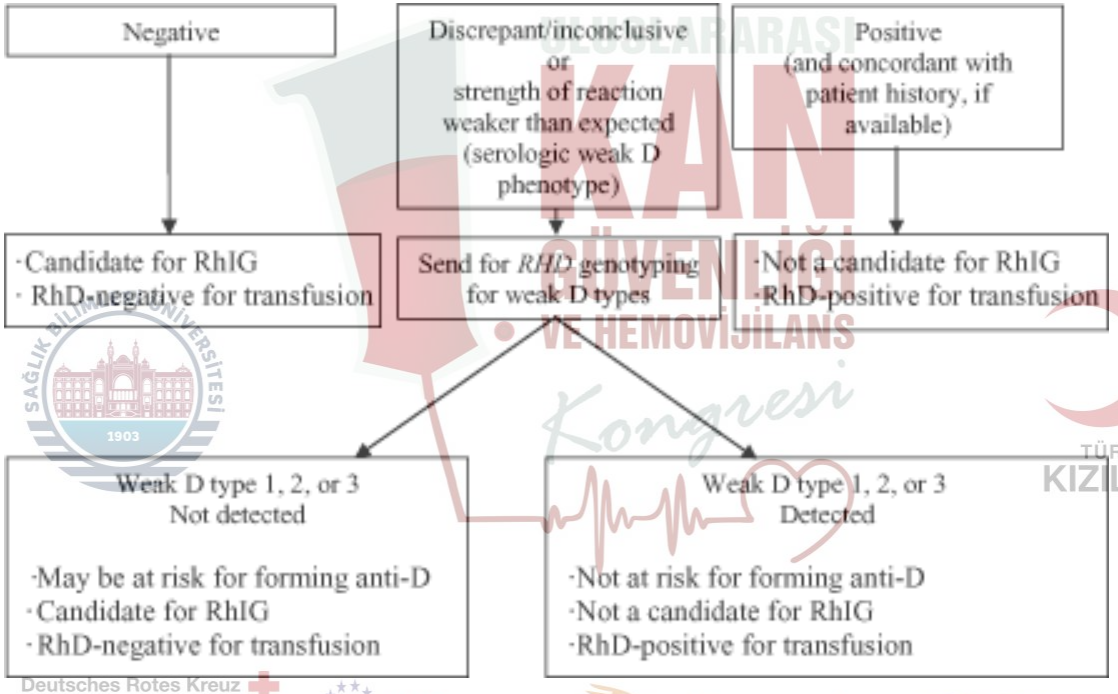
ABO genotyping not generally recommended but useful to....

- resolve patient and blood donor typing discrepancies
- determine the original blood type of patients massively transfused
- determine the original blood type of transplant recipients (also by testing a buccal sample)
- *confirm A 2 subgroup in kidney donors who may have been transfused or whose RBCs give discordant reactivity in serologic testing with anti-A 1 reagents.*


RhD Serological discrepancy:
criteria for molecular biology testing recommendation
1. RhD neg RBC with MoAb IgG/IgM but reactive in IAT
2. RhD reactive RBC with Anti-RhD Abs in serum
3. RBC with different pattern Or score of reactivity with Different anti-RhD sera
4. "Weak" reactivity with Different anti-RhD sera (tube and/or solid phase-gel test)
DAT Reactive ?
N
Y
Serological testing with anti-epitopes MoAb
Molecular Testing (1st technology)
Serological and molecular concordance ?
Y
N
Molecular Testing (2nd technology)
1st and 2nd concordance?
Y
N
VARIANT IDENTIFIED
Known allele
New allele
Gene sequencing

Work group on RHD genotyping: Algorithm for resolving serologic weak D



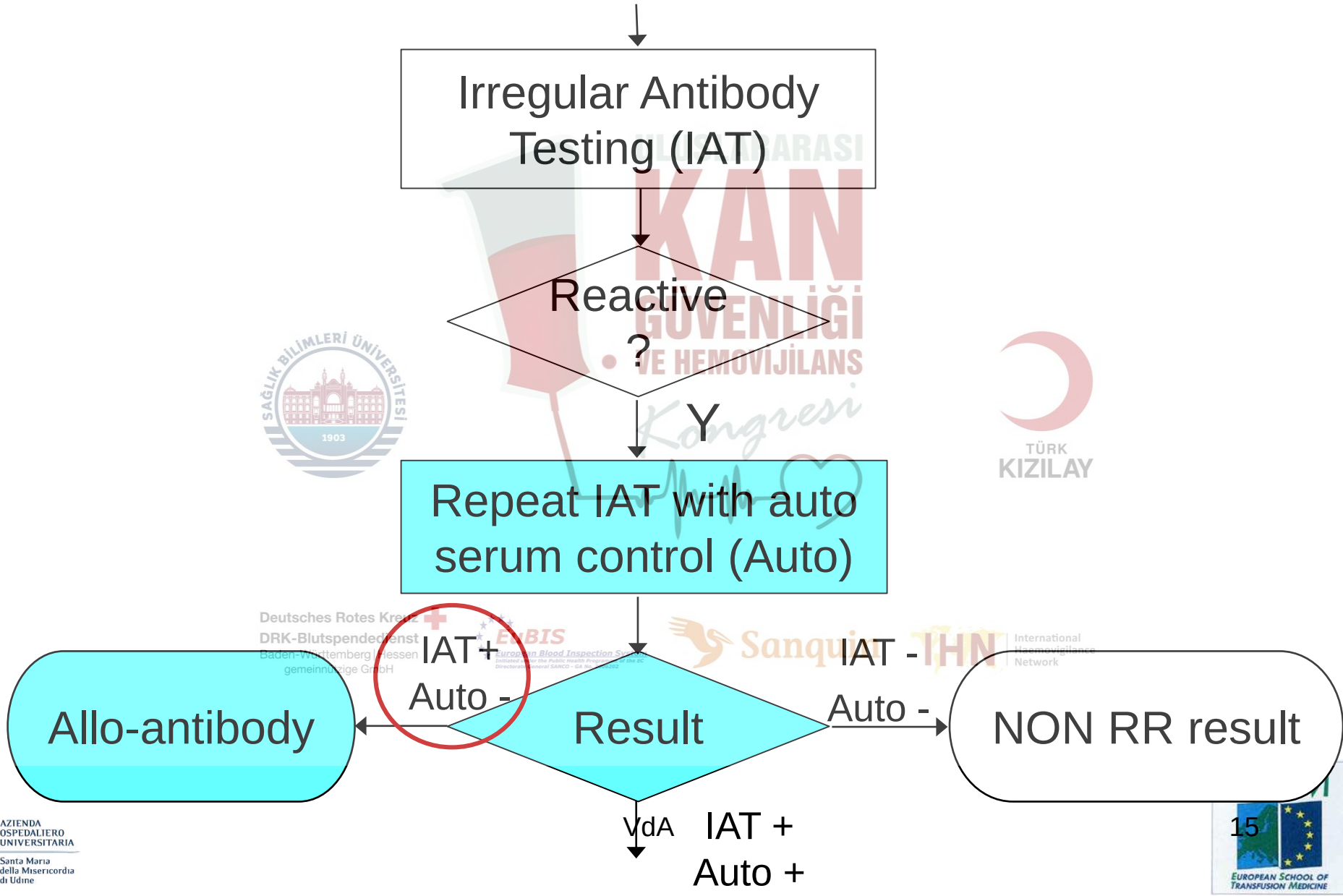
Adoption of RHD genotyping in clinical practice would be more effective with basic cost-effective tests designed to identify the most prevalent and clinical relevant genotypes

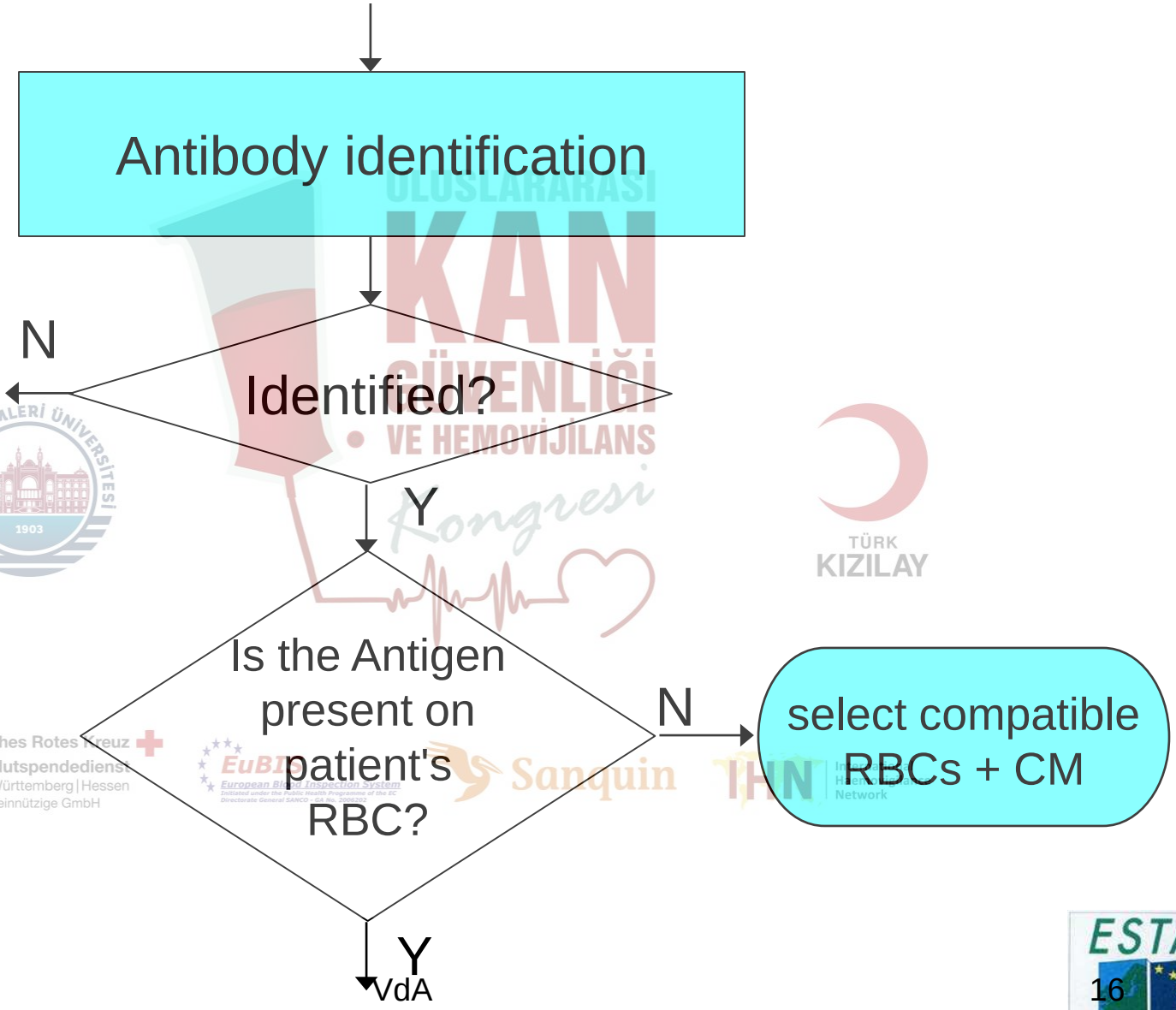
Sandler s.g., Flegel W.A., Westhoff C.M., et al..
Transfusion 2015; 55 (3): 680-689

Non-invasive fetal RHD genotyping

- it allows to target antenatal anti-D prophylaxis to only Rh-negative women carrying a Rh-positive fetus (60 % in Europe)
- it avoids unnecessary use anti-RhD Ig (shortage) in Rh-negative pregnant women carrying Rh-negative fetuses (40%)

Antibody testing




Antibody identification
N
Identified?
Y
Is the Antigen present on patient's RBC?
N
select compatible RBCs + CM
Y
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Only alloantibodies....

Almost regularly IgG o IgM antibodies, toward group specific) or common membrane antigens.

RBC allo-Ab formation remains a major problem mainly for chronically transfused patients (SCD or other hemoglobinopathies, MDS...)

Prevention: to reduce the overall burden of alloimmunization consider **extended blood type as a strategy**

Only alloantibodies....

- Approach generally successful for patients of similar ancestry to donor population or who are expected to have limited transfusion exposures.
- However, still many individuals who become sensitized towards one or more blood group antigens (comprising minor blood group systems) not assessed in routine blood testing.
- Overall RBC alloimmunization rates described in different studies and countries range from 2% to 6% *(Schonewille & Brand, Br J Haematol 2005; Tormey et al, Transfusion 2008; Stack & Tormey, Transfusion 2016, Karafin MS et al, BR j Haematol 2018)*

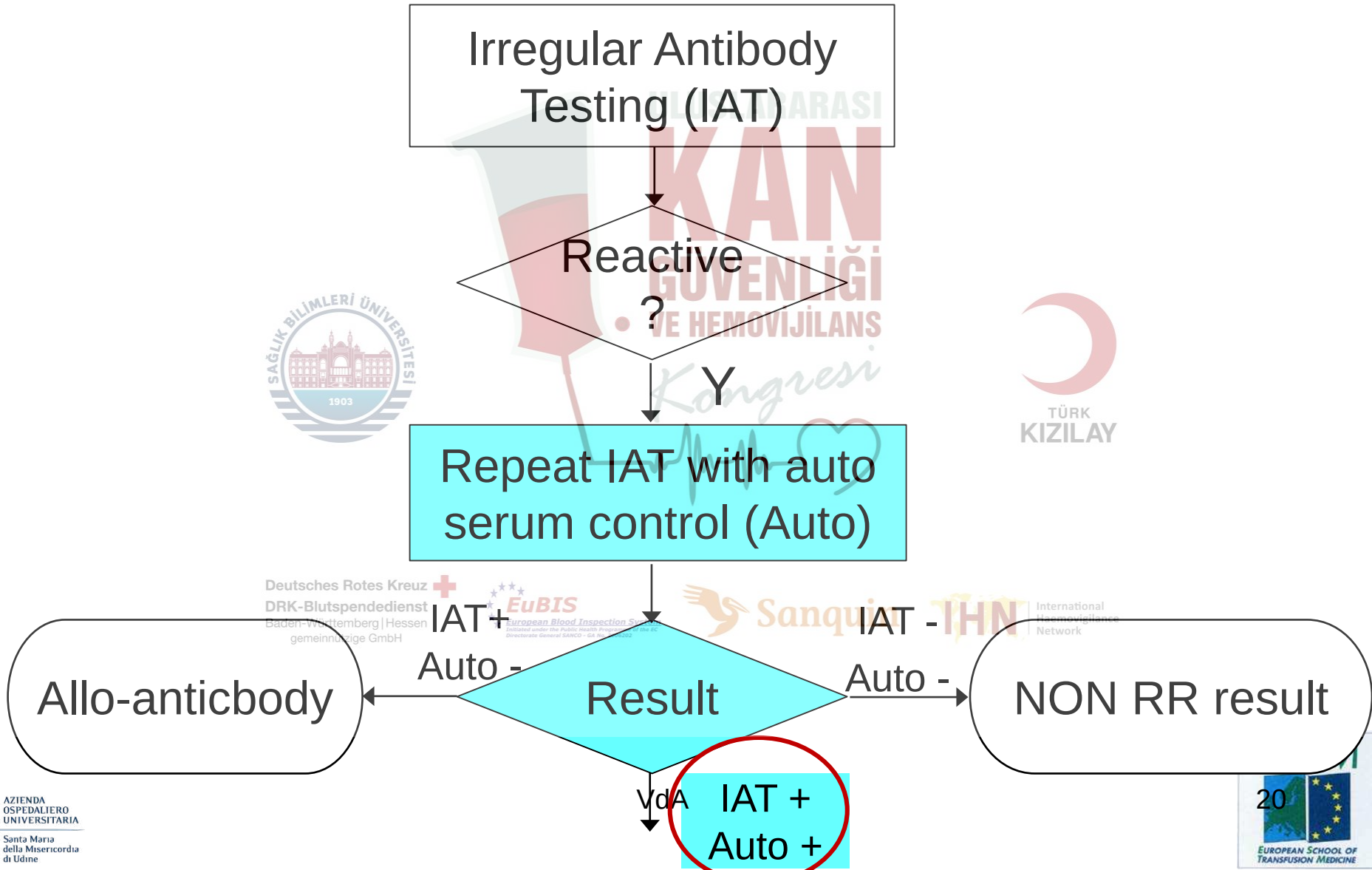
RECOMMENDATION FOR RBC SELECTION

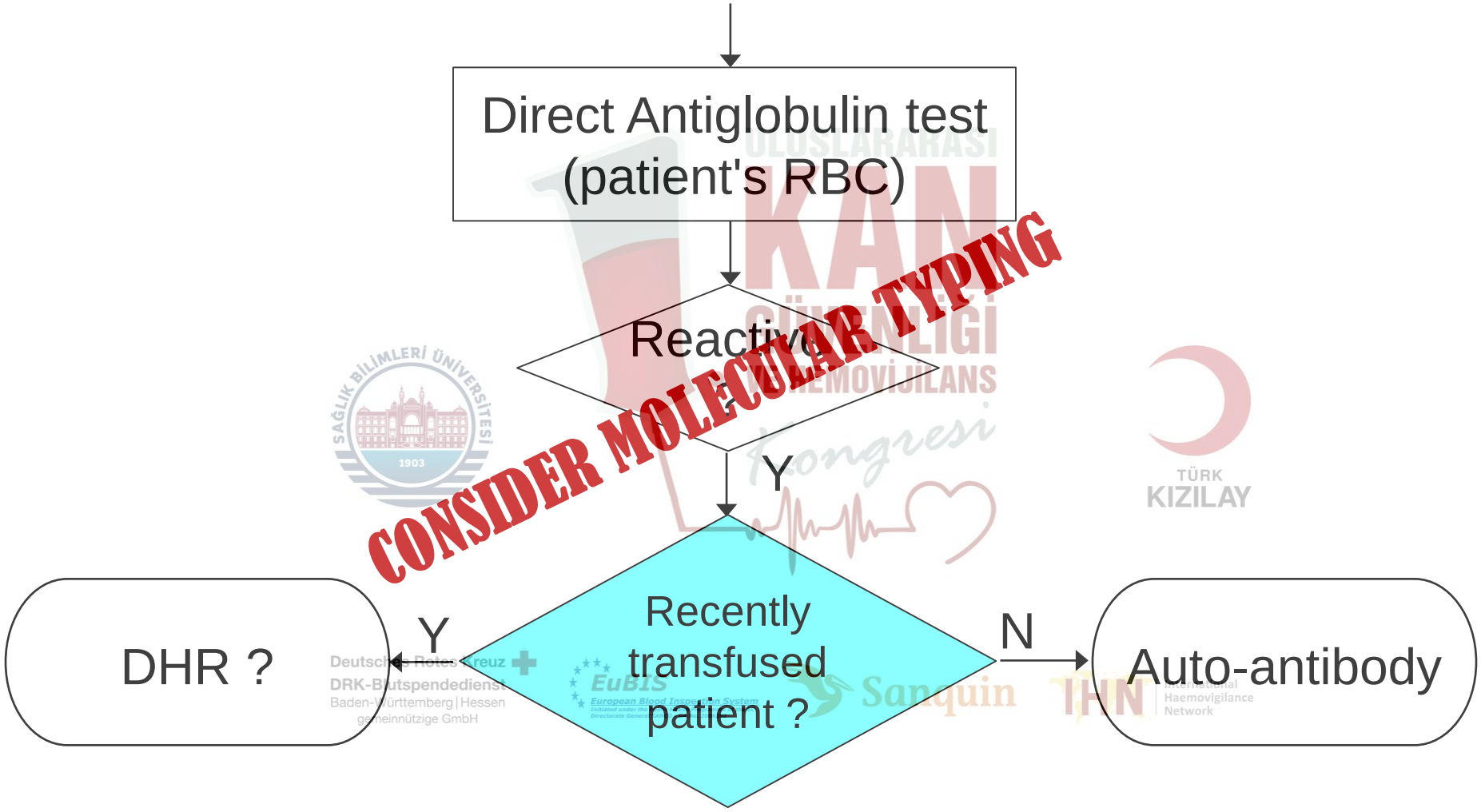
ANTIBODY	RED CELLS
Anti -A, -B, -M, -S, -s, -U, Lu^b, -K, -k, $-Kp^b$, -Fy, -Jk, Di	Negative for the Antigen
Anti-A1, -N, -P1, $-Wr^a$, $-C^w$, $-Kp^a$, $-Lu^a$, -Le	Compatible in IAT crossmatch at 37 °C
Chido/Rodgers, -Lw	Serologically *less* incompatible

Daniels G et al: Transfusion Medicine 2002, 12:287-95

SOMETIMES CHALLENGING.....

Antibody testing




Direct Antiglobulin test
(patient's RBC)
Reactive
Y
CONSIDER MOLECULAR TYPING
Recently transfused patient ?
Y
DHR ?
N
Auto-antibody

Patients

It is recommended to perform DNA-based typing in transfusion setting to:

- **Patients with discrepant typing**
- **Patients carrying weak/partial antigens**
- **Patients massively transfused**
- **Patients with autoimmune hemolytic anemia or RBC's coated with immunoglobulins**
- **Patients receiving monoclonal antibodies therapies**
- **Transplanted patients**

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Patients

DNA-based typing to determine **Red Cell or Platelet antigens** is also recommended in obstetric setting to advance diagnosis and evaluation of hemolytic disease of the fetus and newborn (**HDFN**) and fetal and neonatal alloimmune thrombocytopenia (**FNAIT**).

It is recommended to perform DNA-based typing in obstetric setting to:

- **Women carrying weak/partial D phenotypes to determine candidates to RhIg**
- **Fetuses to determine risk to HDFN and FNAIT**
- **Fathers to determine zygosity to RHD and HPA**

Blood Group Donors

It is recommended to perform DNA-based typing in the following settings:

- **Resolution of serological discrepancies**
- **Identification of weak/partial antigens**
- **Detection of rare antigens** and creation of **Rare Blood Banks**
- **Extended matching program**

It is recommended to confirm blood group typing on a second sample obtained independently, combining genetic and serologic methods for the two determinations.

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Blood Group Donors

When extended blood group genotyping is addressed to **optimize and manage deep inventories of multiple antigen-negative units**, the main immunogenic blood group systems **RH, KEL, MNS, FY, JK, LU**, should be typed.

Donors with particular phenotypes like **RhD-/C+ or E+** should be genotyped to avoid the presence of variants (DEL) and correctly label blood units.

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Blood Group Donors

The laboratory **should establish** in advance donors selection criteria for **large-scale molecular testing**, considering number of donations, age, ABO/Rh/Kell typing

Age	18-55 years old
ABO group	A/O
Rh phenotype	CCDee, ccdee, ccDee, ccDEE, CCdee, ccdEE, Ccdee, ccdEe
Kell phenotype	KK, kk
N. donations	≥ 2

Extended matching program

- Provide antigen-matched RBC transfusions to patients with SCD in Brazil
- molecular matching in 3 levels: RH and K matching; extended matching (RH, KEL, FY, JK, MNS, DI),without or with RHD and RHCE variant alleles.

  - clinical benefits to the patients with SCD,
  - reduces the rates of alloimmunization.
  - Improvements in the clinical outcomes (increase in their hemoglobin levels and reduction in % HbS and diminished frequency of transfusions).

Castilho L, Dinardo CL - Transfus Med Hemother. 2018

Extended matching program

- Retrospective review of records for SCD patients 18 months to 81 years of age covering two 5-year periods:
  - Period 1, no PAM, occasional leukoreduction
  - Period 2, consistent leukoreduction and extended PAM (Rh, Kell, S, Fy, Jk) for patients already alloimmunized

  Prevalence of initial and subsequent RBC alloimmunization in Period 2 lower than that in Period 1; overall prevalence remained high *(Campbell-Lee SA et al Transfusion. 2018)*

- Molecular matching for Rh and K reduces red blood cell alloimmunisation in patients with myelodysplastic syndrome *(Guelsin GA et al Blood Transfus. 2015)*

Extended matching program

Impact of Red Blood Cell Antigen Matching on Alloimmunization and Transfusion Complications in Patients with Sickle Cell Disease: A Systematic Review.

- No prospective randomized controlled trials.
- Low-quality evidence from observational cohort studies supports that alloimmunization prevalence can be decreased by extending serological RBC antigen matching
- Multicenter prospective randomized clinical trials are needed to determine best strategy

(Fasano et al. Transfus Med Rev. 2019)

In conclusion....Genotyping

Uses of DNA-based genotyping for Transfusion Medicine

Type patients for multiple antigens in a single assay
Type patients who have been recently transfused or RBCs coated with immunoglobulin
Type patients with autoimmune hemolytic anemia (to select antigen-negative RBCs for transfusion and adsorption of autoantibodies when searching for underlying alloantibodies)
Type patients receiving monoclonal antibody therapies that interfere with pretransfusion testing
Type RBCs when commercial antisera are not available
Type obstetric patients to identify weak D and partial D phenotypes to determine candidates for RhIg and to avoid use of limited RhD- blood)
Resolve blood group typing discrepancies
Determine paternal zygosity for RHD and HPA
Type fetus to determine risk for HDFN or FNAIT
Accurate Rh antigen matching in SCD

- Provides extended antigen profiles with one time testing
- Enables transfusion with antigen matched products
- Begin to consider prevention to alloimmunization

C.M. Westhoff, Blood (2019)


ULUSLARARASI
KAN
GÜVENLİĞİ
VE HEMOVİJİLANS
Kongresi
THANK YOU!!
SAĞLIK BİLİMLERİ ÜNİVERSİTESİ
1903
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