Granulocyte agglutination test

Granulocyte immunofluorescence test

Charlampos Pontikoglou

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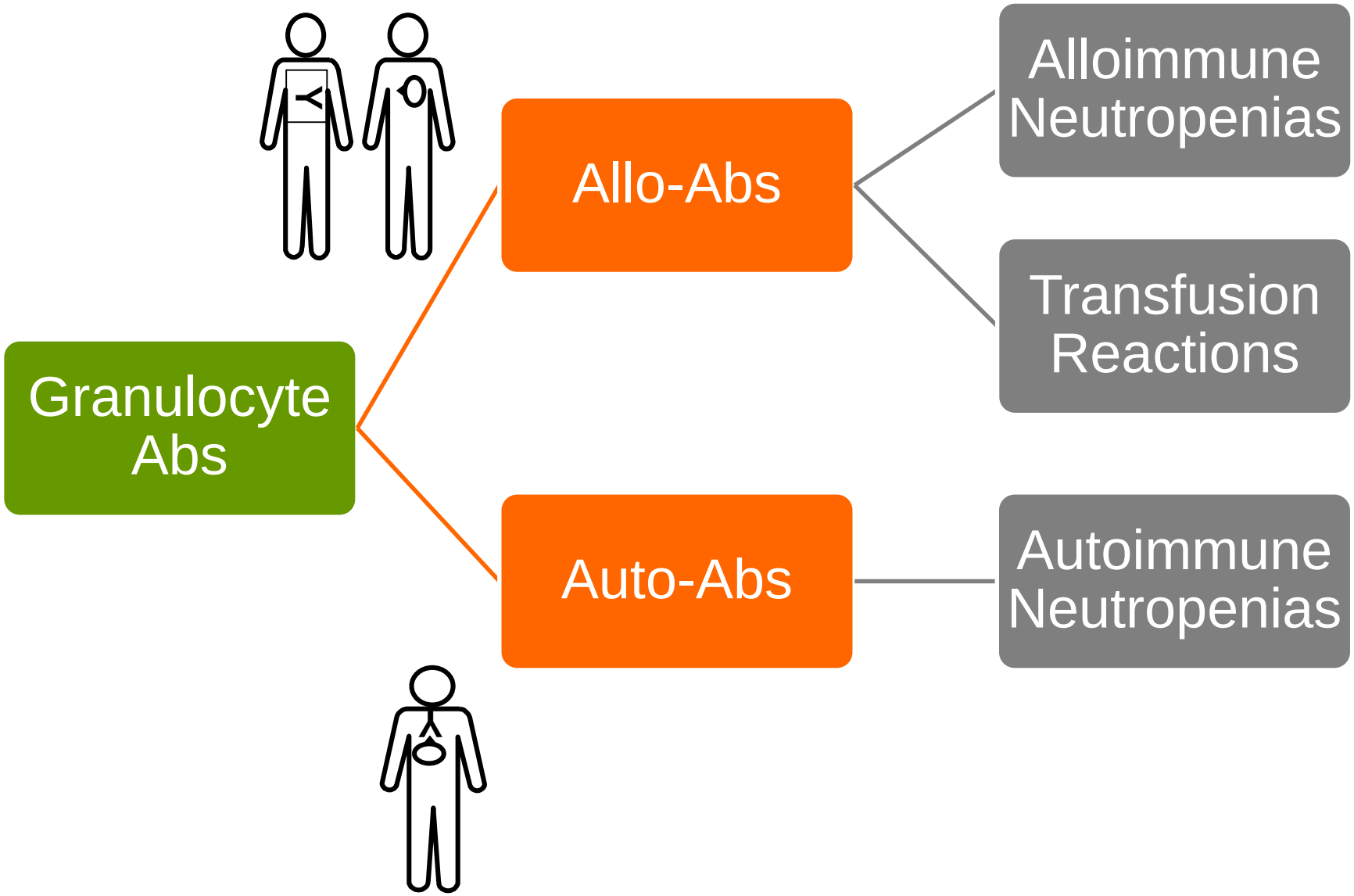

Granulocyte Abs
Allo-Abs
Auto-Abs
Alloimmune Neutropenias
Transfusion Reactions
Autoimmune Neutropenias

Granulocyte Antibody Detection

Direct Testing

- **Detection of neutrophil bound Abs**

Indirect Testing

- **Detection of circulating Abs**

Difficulties in Direct Testing

- The low numbers of autologous neutrophils consist a major obstacle of direct testing.
- High concentration of immune complexes in patient serum along with with activated autologous neutrophils ⇨ non-specific IgG binding
- Therefore a positive direct test can not be considered proof that anti granulocyte As are indeed present

Bux J. Transfus Med Rev. 1996

Granulocyte Antibody Screening

- The detection of neutrophil-reactive antibodies usually involves testing serum or plasma with a panel of test neutrophils using the granulocyte indirect immunofluorescence test (GIFT) and the granulocyte agglutination test (GAT)
- The combination of these assays appears to be the best approach for neutrophil antibody testing according to the 2nd International Granulocyte Workshop
- Initial testing may be done using a small random panel of un-typed, normal neutrophils.
- The specificity of any samples with positive reactions can then be characterized using a typed (phenotyped or genotyped) neutrophil panel.

Bux J et al., Transfusion 1997
Feng YL et al., ISBT Science Series 2011
Flesch B., ISBT Science Series 2020

Difficulties in antibody detection and identification

- Granulocytes are fragile cells:
  - They need to be freshly isolated for Ab testing
  - They need to be gently manipulated during isolation to avoid activation
- Modes of Ab binding
  - $F(ab)_2$ binding
  - FcR
  - Non-specific binding
  - Internalized Ig
- Differentiation between granulocyte-specific and anti-HLA antibodies

Modified from Lucas G. 3rd Neutropenia Network Conference Heraklion 2008

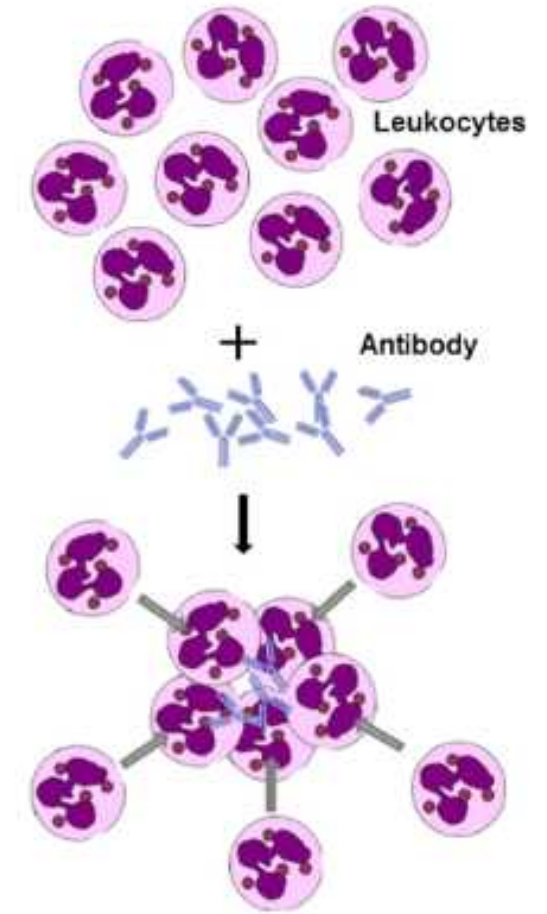
Time from venipuncture to cell isolation
Impact on granulocyte-reactive antibody testing

- Because of the short life span and the fragility of granulocytes, it is generally recommended to perform cell isolation and antibody testing immediately after blood donation.

- According to a recent study though a delay of up to 4 hours between venipuncture and neutrophil isolation does not impact on the accuracy of immunofluorescence, whereas agglutination is not influenced even if neutrophil isolation takes place 24 hours following blood donation

Schonbacher M et al.,Clin Biochem 2019

Granulocyte agglutination test (GAT)



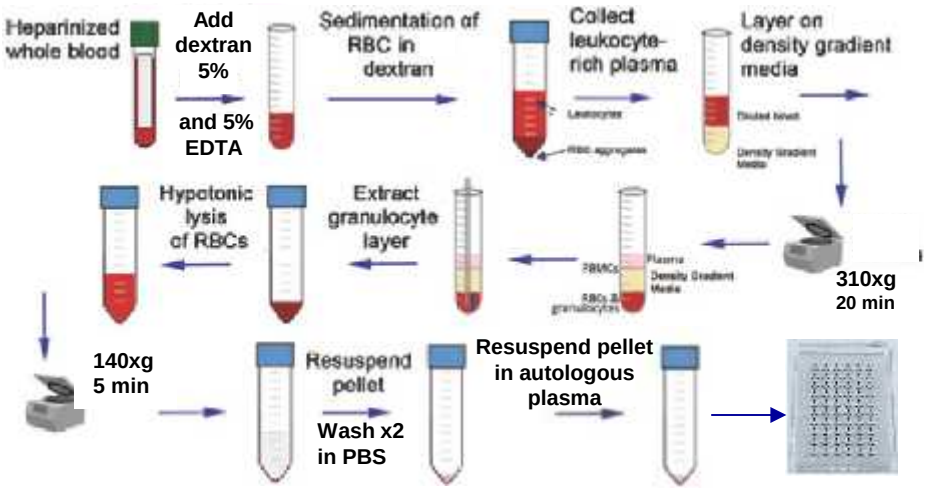
Leukocytes actively migrate towards the antibody to form aggregates

- *Lalezari et al* in 1960 were the first to use GAT to detect potent leukoagglutinins in the maternal serum in a family with multiple cases of neonatal neutropenia

- It is based on the intrinsic property of granulocytes to migrate towards each other and aggregate in the presence of activating corresponding antibodies

Flesch B., ISBT Science Series 2020

Granulocyte agglutination test (GAT)



Reil A et al., Methods Mol Biol. 2015
Photo: Courtesy of Dr. Mavroudi I.

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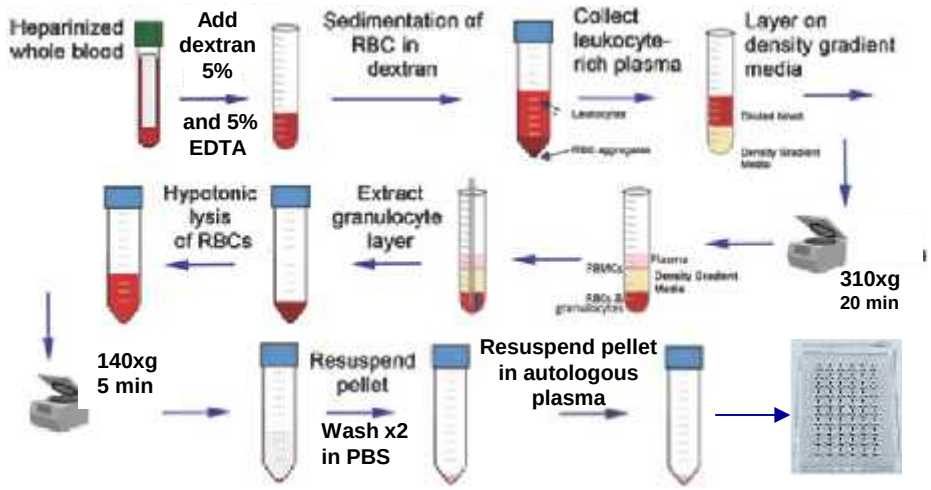

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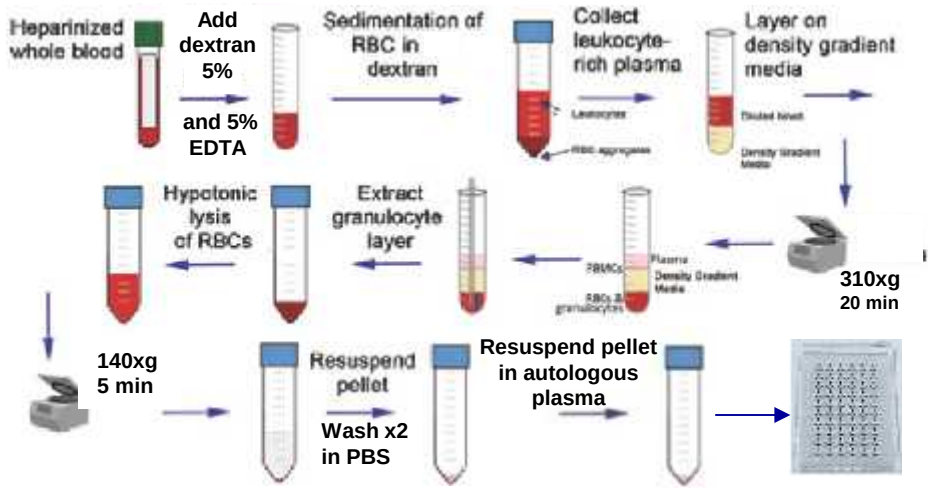

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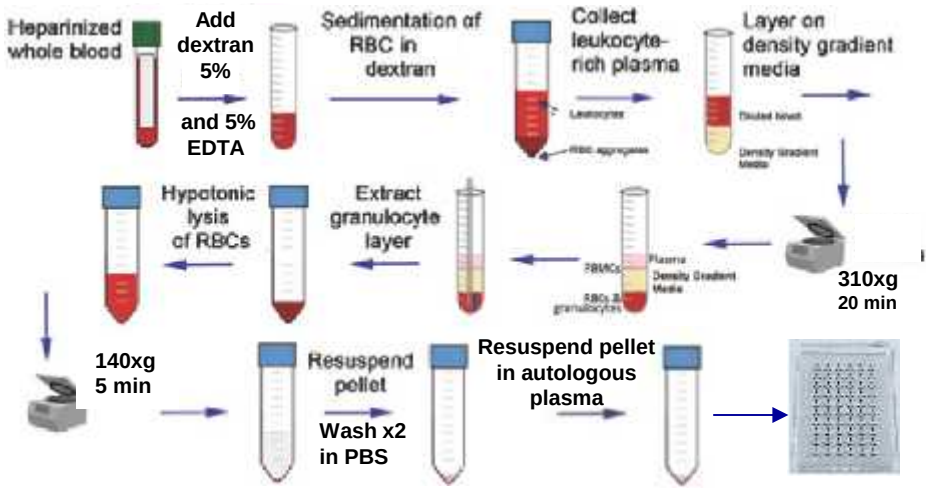

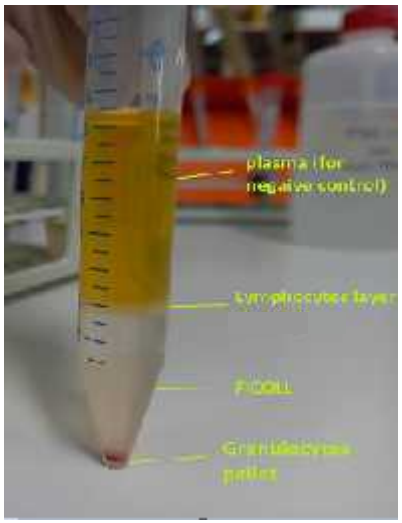

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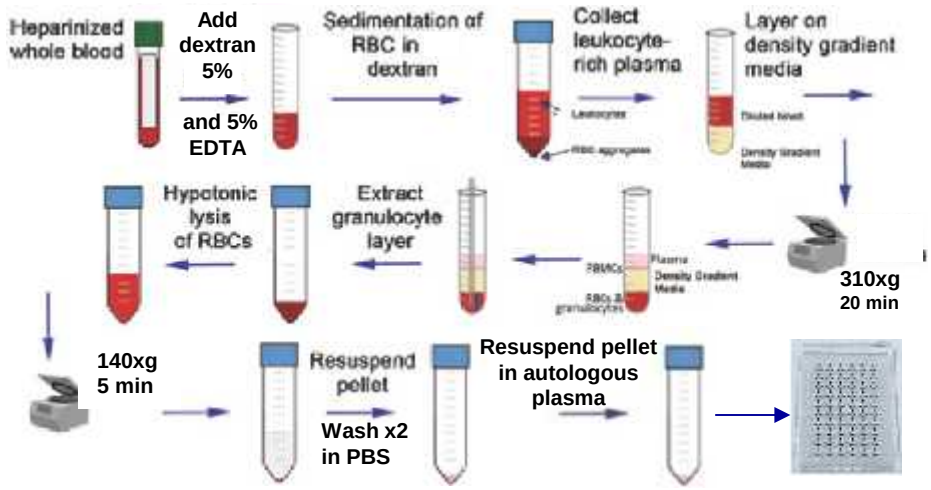

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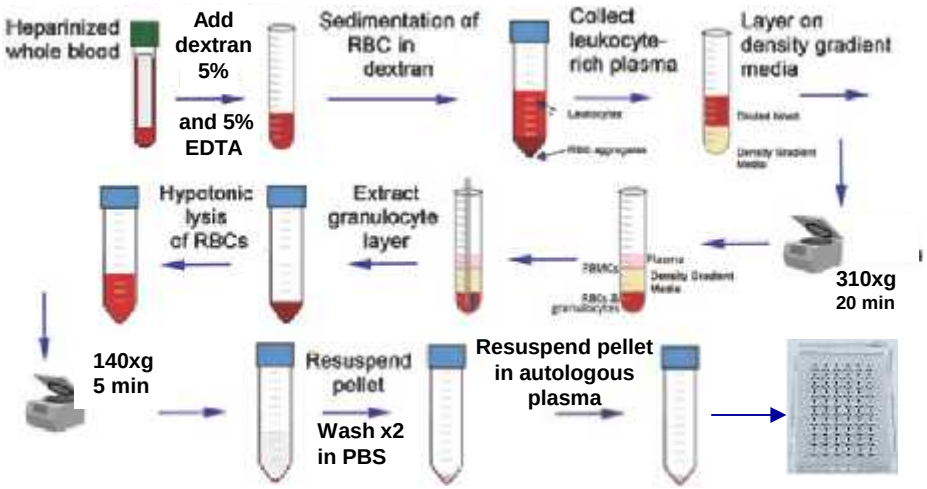

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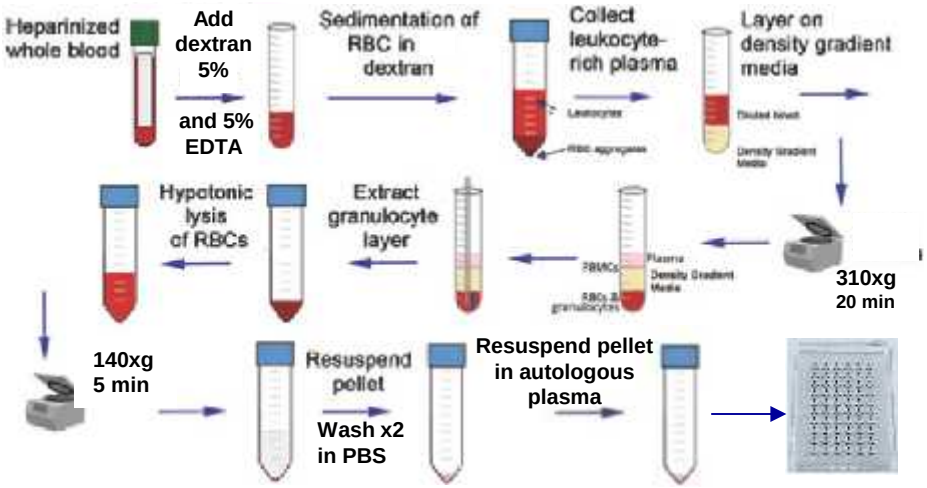

- To prevent evaporation during incubation, pipette one drop of paraffin oil into each well of a terrasaki plate
- Perform all tests in duplicate.
- Pipette 2 µL granulocyte suspension (cell concentration 5×10^3/µl) per well
- Add 6 µl of patient serum to each well
- Incubate for 4-6 h at 30 °C and evaluate using an inverted microscope

Reil A et al., Methods Mol Biol. 2015
Photos: Courtesy of Dr. Mavroudi I.

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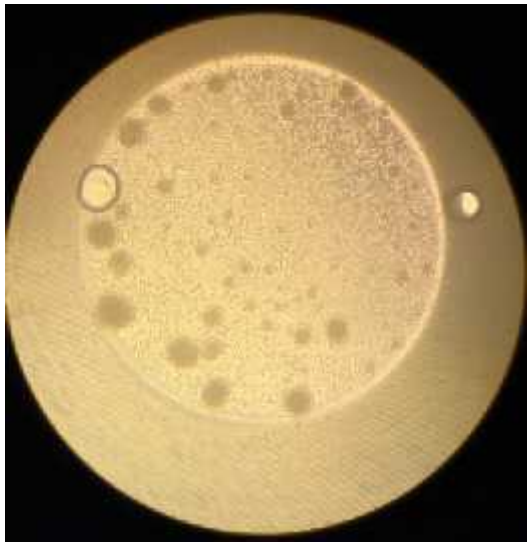

Granulocyte aggregates

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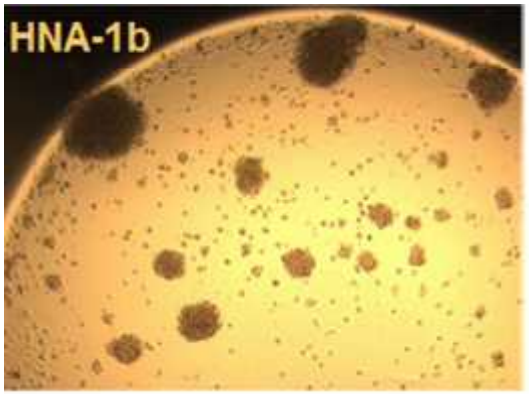
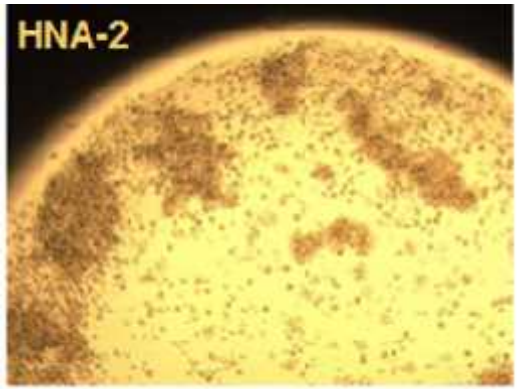
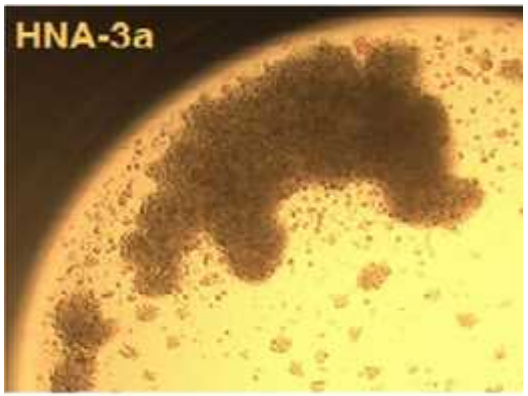
Photo: Courtesy of Dr. Mavroudi I.

Granulocyte agglutination test (GAT)

- Because neutrophil activation is an active process, test cells for the GAT cannot be treated by PFA
- GAT is the method of choice for the detection of HNA-3a antibodies which exhibit high aggregating capacity
- GAT also detects HNA-1a, -1b, -1c, HNA-2, HNA-3b and anti-HLA-A2 Abs
- Antibodies without aggregating capacity will produce negative results
- In case of a positive result, the antibody specificity may be deduced by experienced personnel according to the typing of the donor and the shape of the agglutinates







Flesch B., ISBT Science Series 2020

Granulocyte immunofluorescence test (GIFT)

- GIFT was developed in the late 1970s
- It is based on the detection of the fluorescence staining of granulocyte antigens by fluorescence-labelled antibodies
- Almost all HNA antibodies are reactive in the GIFT
- The GIFT is generally more sensitive than GAT with the exception of HNA3 antibodies that are only weakly reactive

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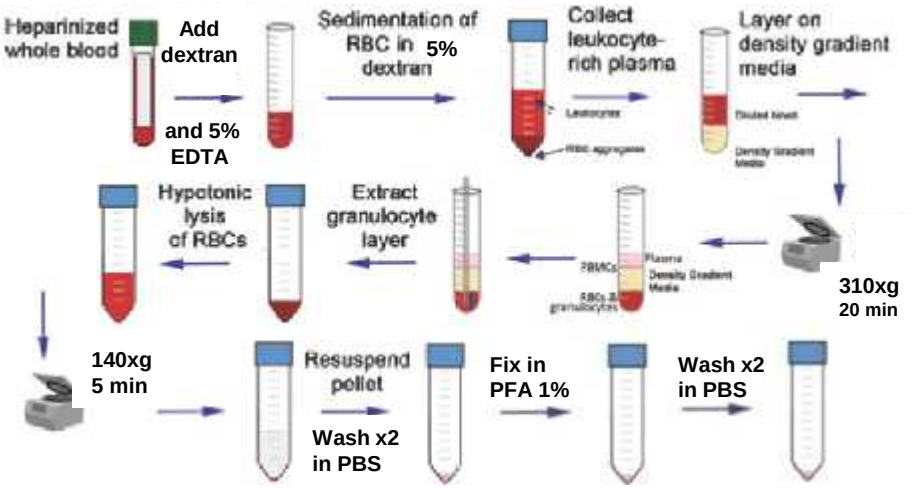

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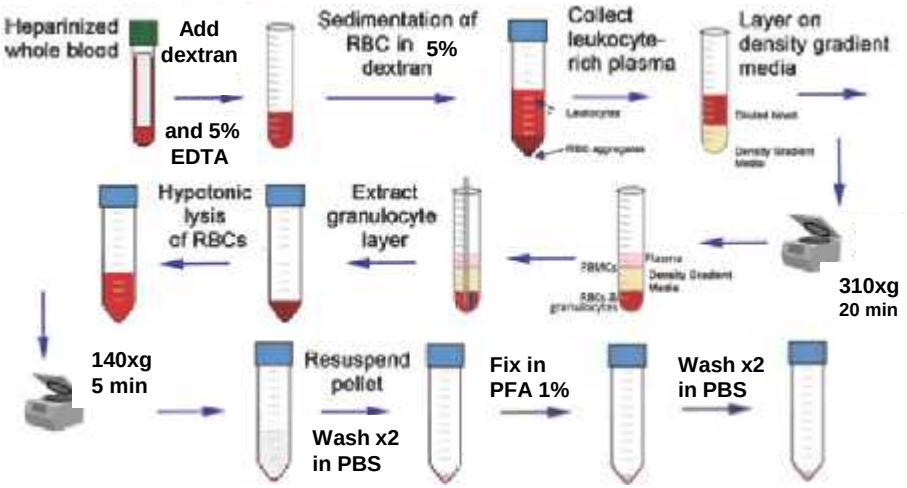

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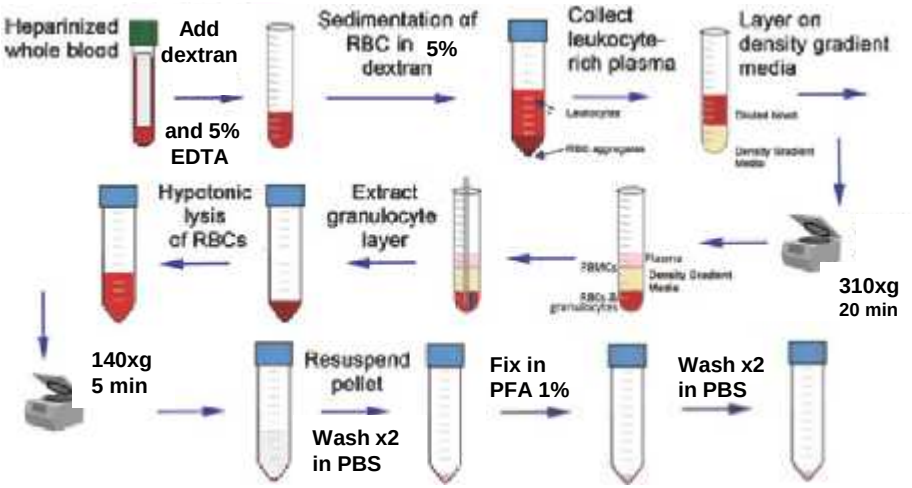

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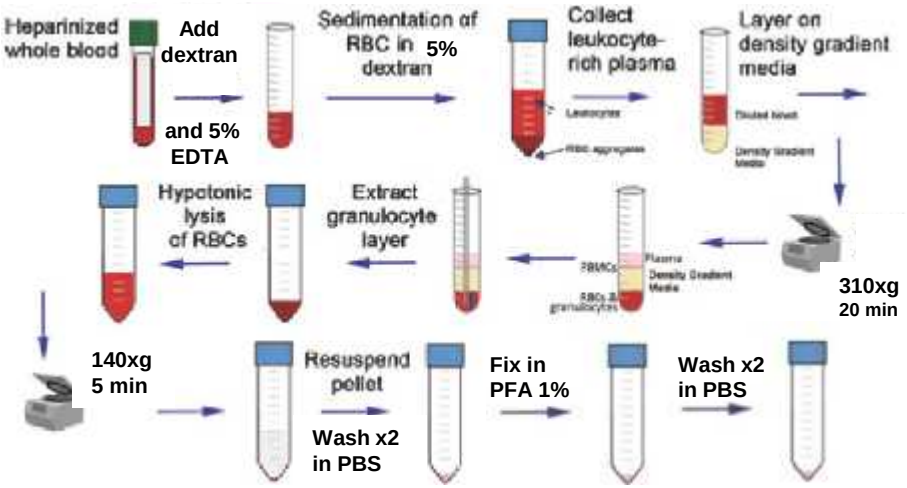

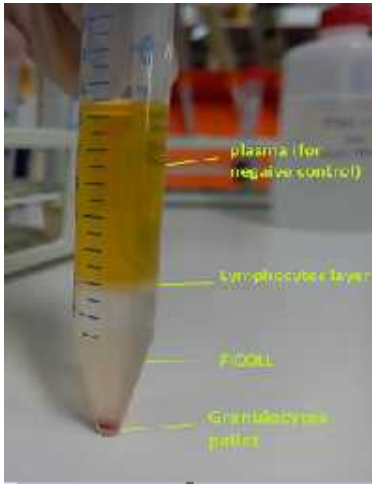

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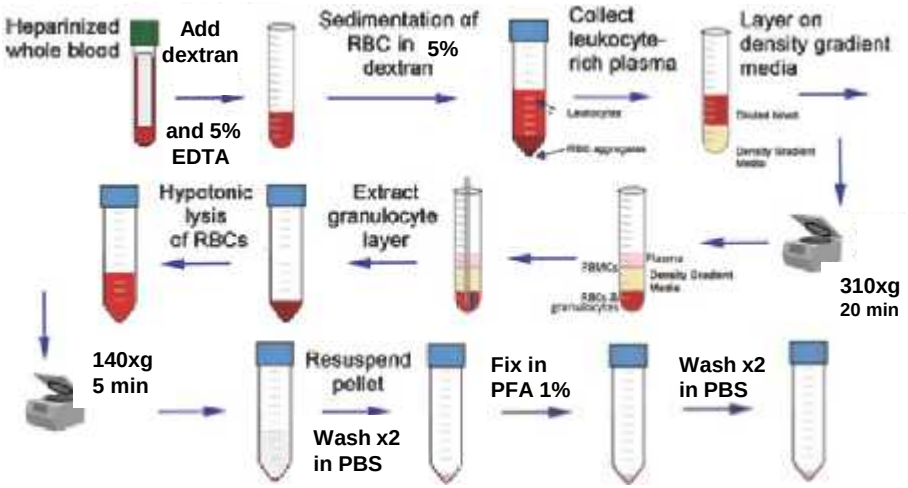

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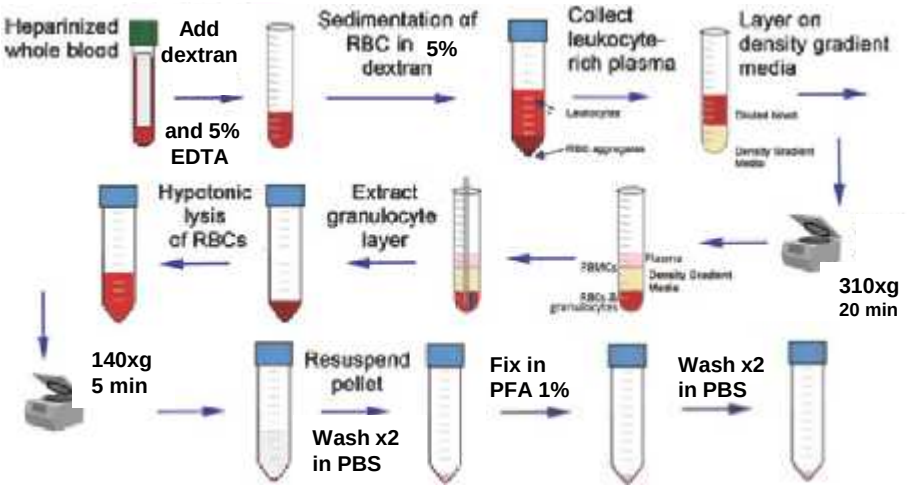
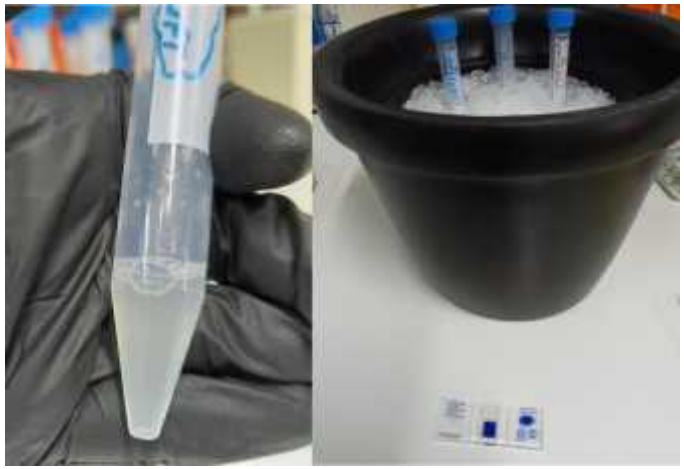
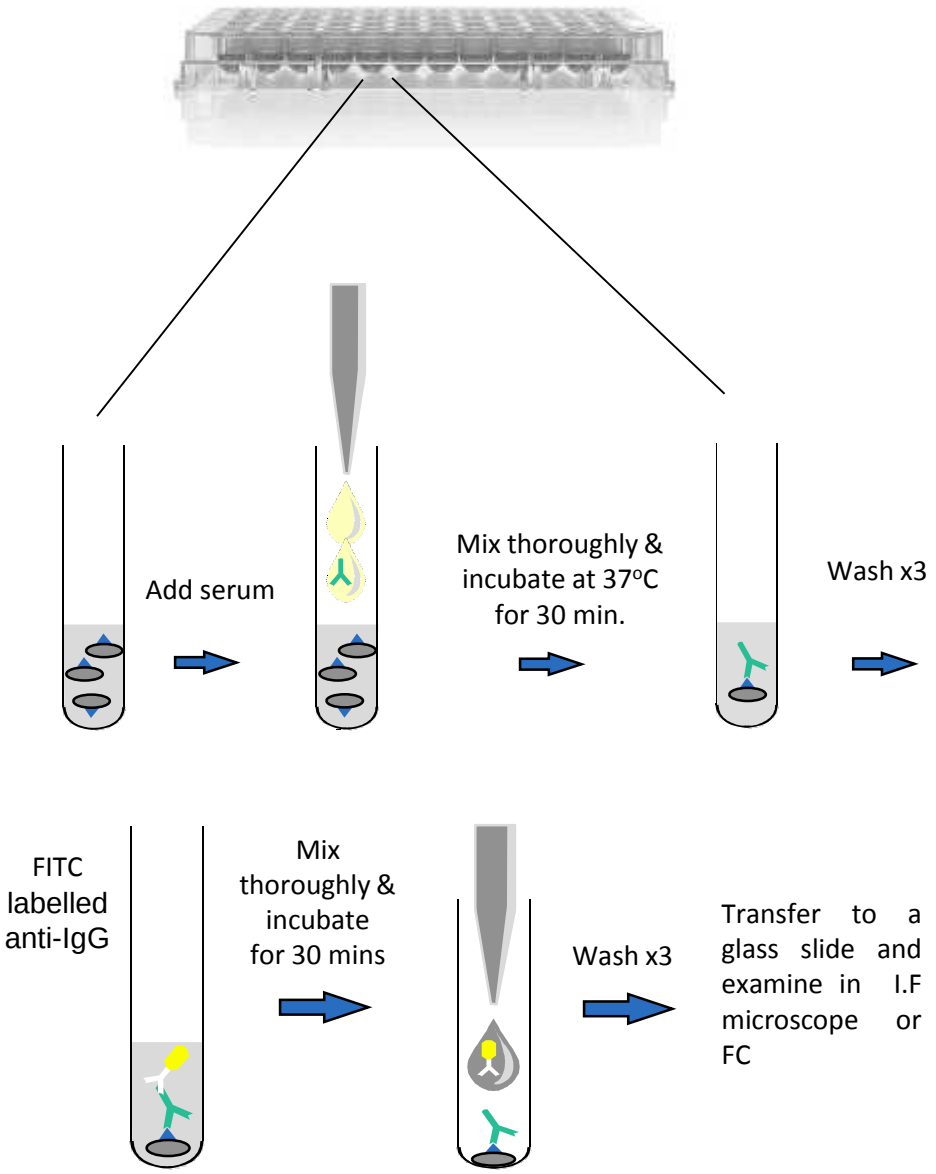




Reil A et al., Methods Mol Biol. 2015
Photos: Courtesy of Dr. Mavroudi I.

Granulocyte immunofluorescence test (GIFT)

- 40 µl of a cell suspension of 5000/µl are transferred to U-bottom Elisa plates
- 40µl of patient's serum are added and mixed manually with the cell suspension and incubated with the cell suspension for 30 min at 37°C
- Cells are washed thrice with PBS and spun down for 1 min at 275xg. Supernatant is discarded and cells are resuspended by vortex
- Cells are incubated with 40 µl of FITC-conjugated anti-IgG (1:50) for 30 min at RT in the dark

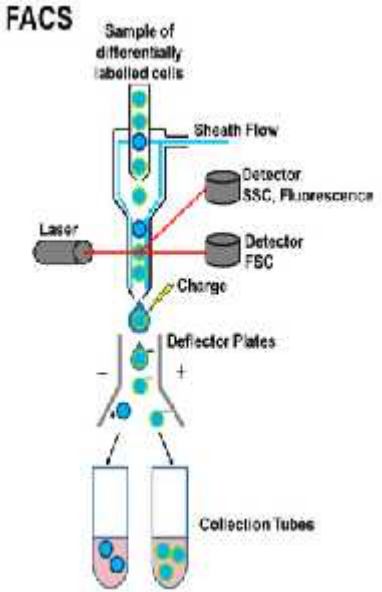


Reil A et al., Methods Mol Biol. 2015
Lucas G. 3rd Neutropenia Network Conference Heraklion 2008

Granulocyte immunofluorescence test (GIFT)

- Cells are washed thrice with PBS and resuspended in one drop of PBS-Glycerol (3:1)

- The suspension is transferred to a glass slide, mounted with a cover slip and examined with an IF microscope or FC



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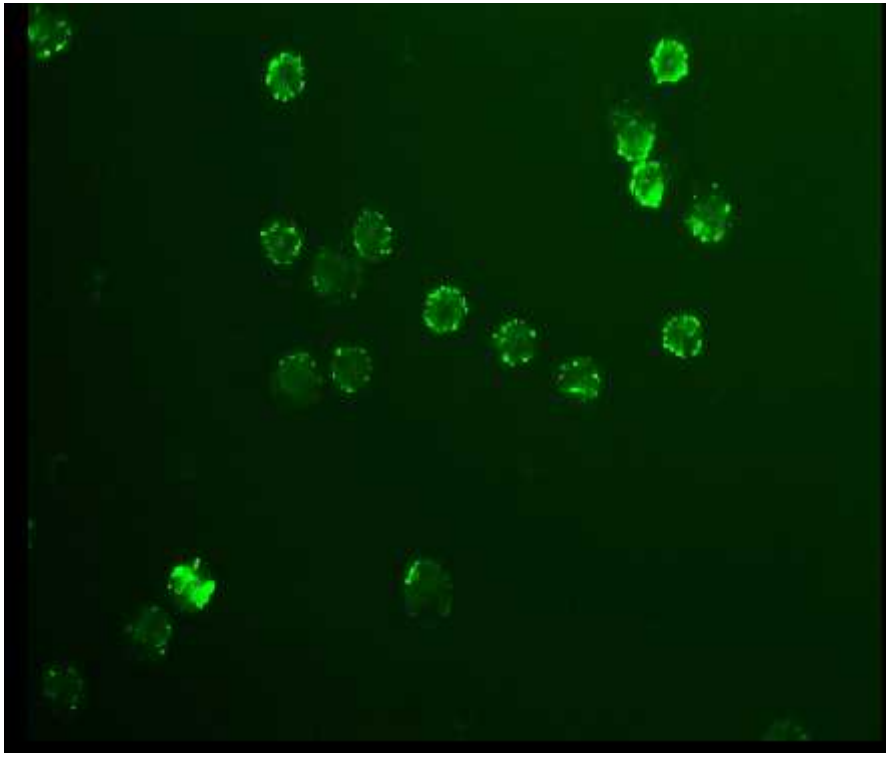
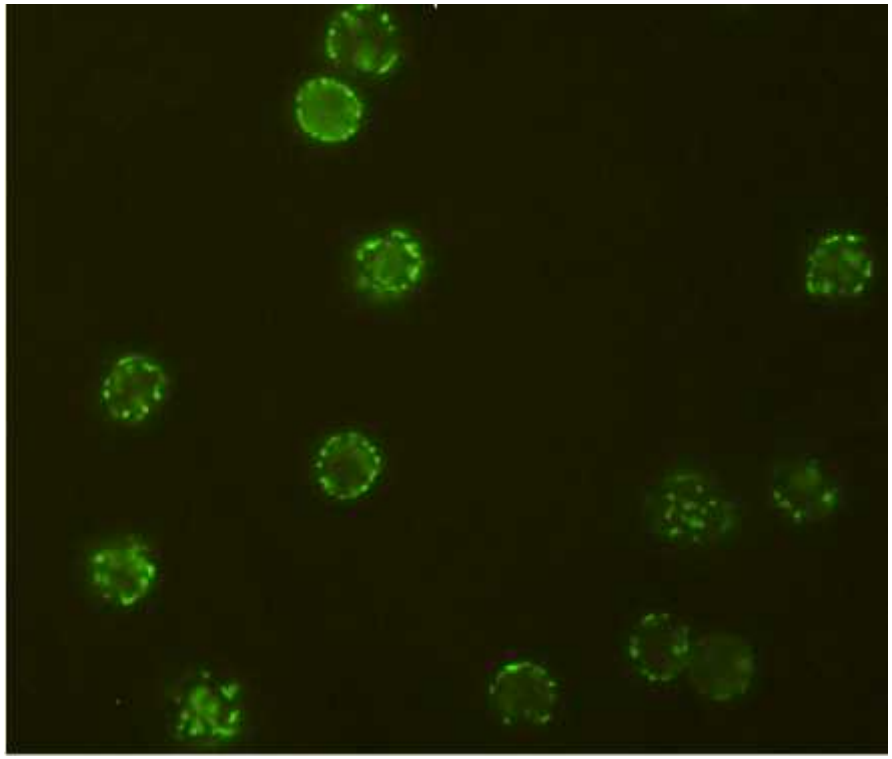
GIFT- Fluorescence Microscopy





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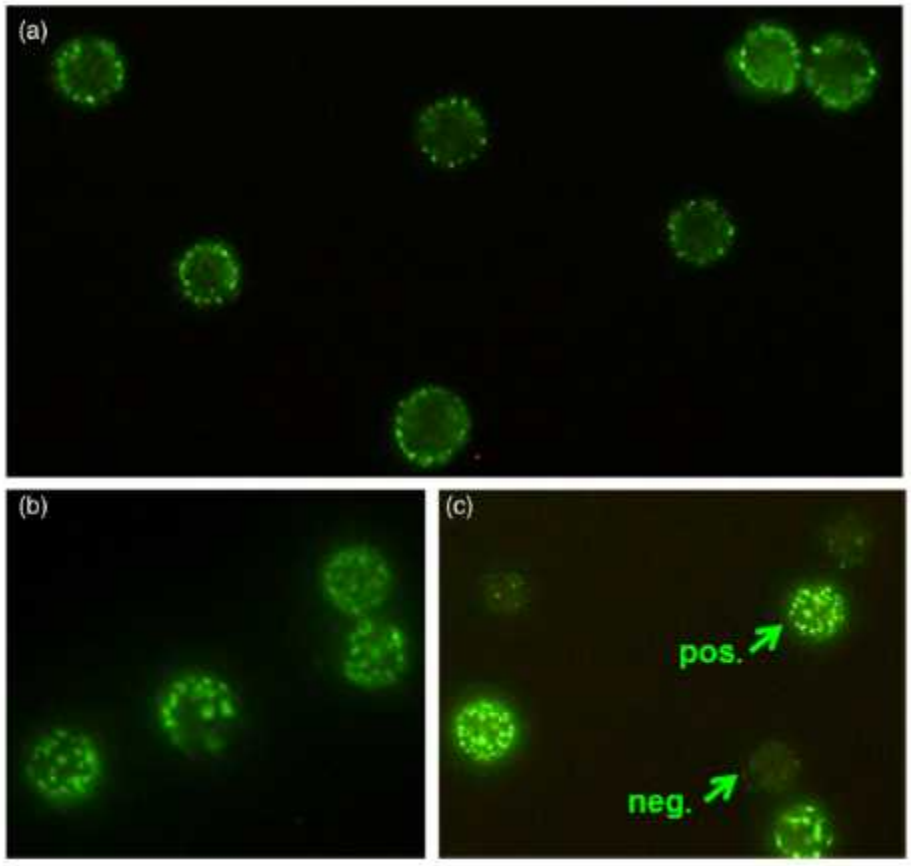
GIFT- Fluorescence Microscopy

- Specific fluorescence patterns may be associated with certain HNA-specificities
- A rather fine granular membrane fluorescence (a) indicates HNA-3, HNA-4 and HLA class I antibodies
- A more coarse granular fluorescence is typical for HNA-1 and HNA-2 Abs
- HNA-2 is expressed only on a proportion of neutrophils that may vary between 5 and nearly 100% and is characteristic for each individual



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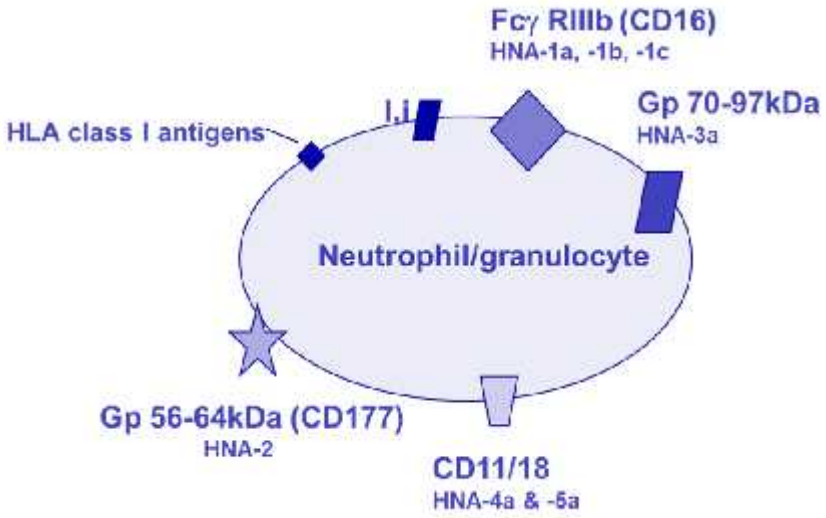
Lymphocyte Immubofluorescence Test

- Because both GIFT and GAT use intact test granulocytes, contaminating HLA Abs can interfere with the detection of specific granulocyte Abs
- Therefore, sera reactive in GIFT and GAT must be screened for HLA Abs
- Lymphocyte immunofluorescence test (LIFT) is performed in parallel with GIFT to detect anti-HLA class I Abs
- LIFT also detects anti-HNA-3 Abs



Bux J. Transfus Med Rev. 1996
Flesch B., ISBT Science Series 2020


Ευχαριστώ
Ευχαριστώ
thank you
danke
謝謝
ngiyabonga
teşekkür ederim
спасибо
dank je
gracias
tesekkur ederim
bedankt
dziękuje
mochchakkeram
go raibh maith agat
obrigado
sukriya
kop khun krap
arigato
dakujem
terima kasih
grazie
merci