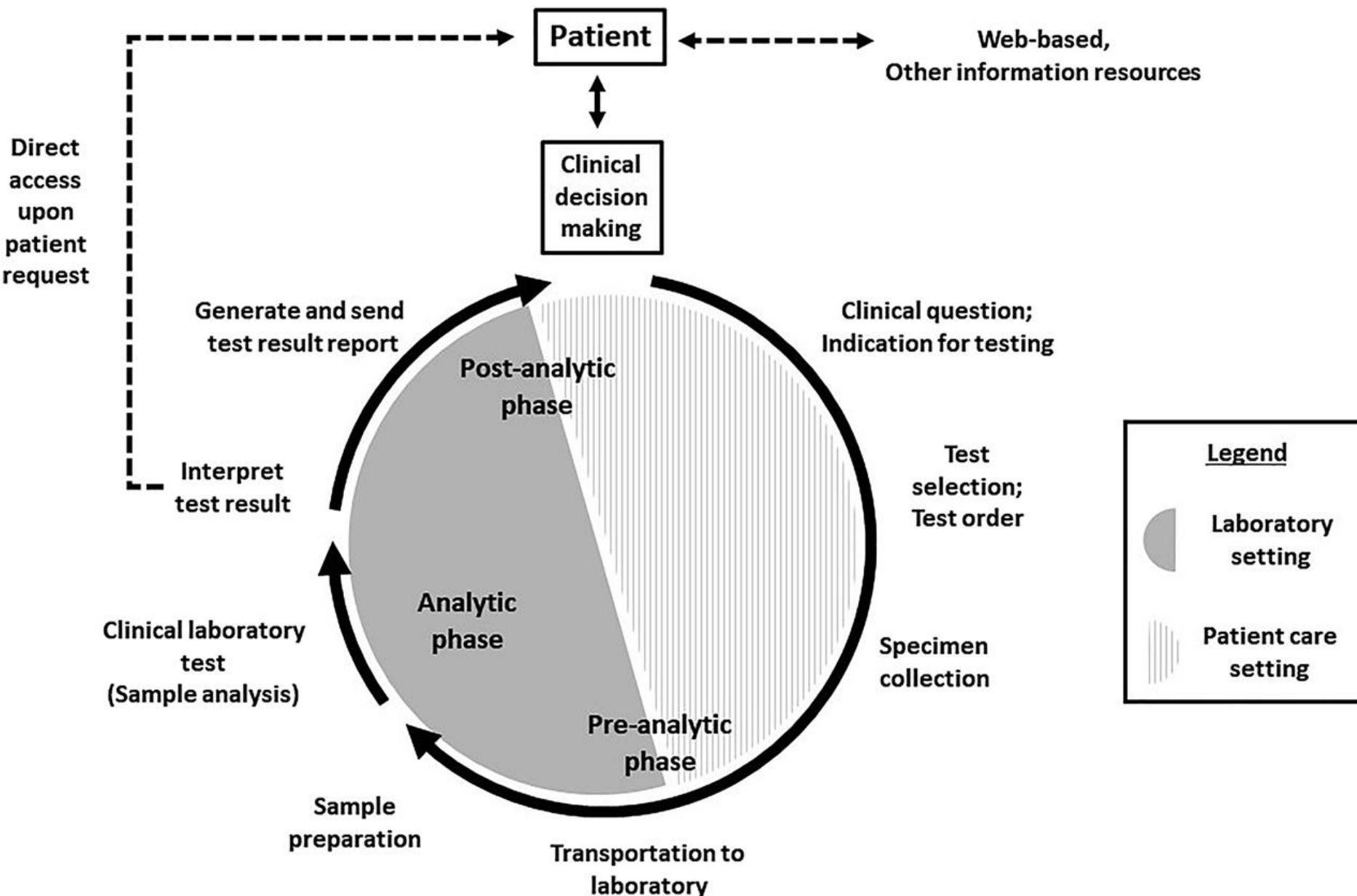


De basofiel: futiel of miskend?

Critically Appraised Topic
Bram Decru

O.l.v. Dr. Criel M. en Apr. Geerts I.





Vragen?

- 1. Hoe wordt basofilie gedefinieerd?**
- 2. Wat zijn de meettechnieken? Met welke gebreken?**
- 3. Wat is de diagnostische waarde van basofilie?**
- 4. Dient er een diagnostische uitwerking plaats te vinden in
geval van basofilie?**



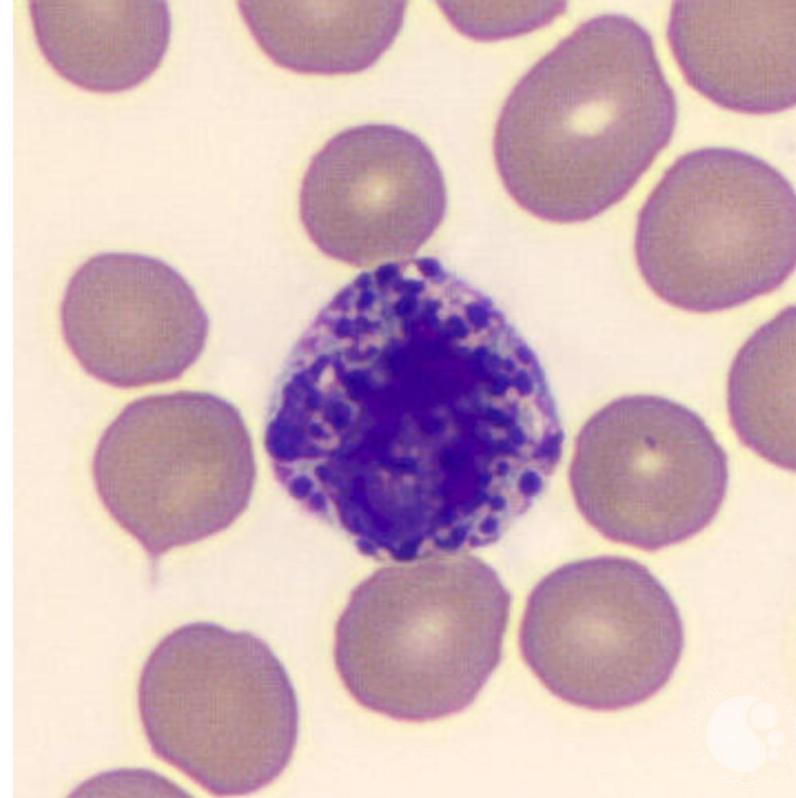
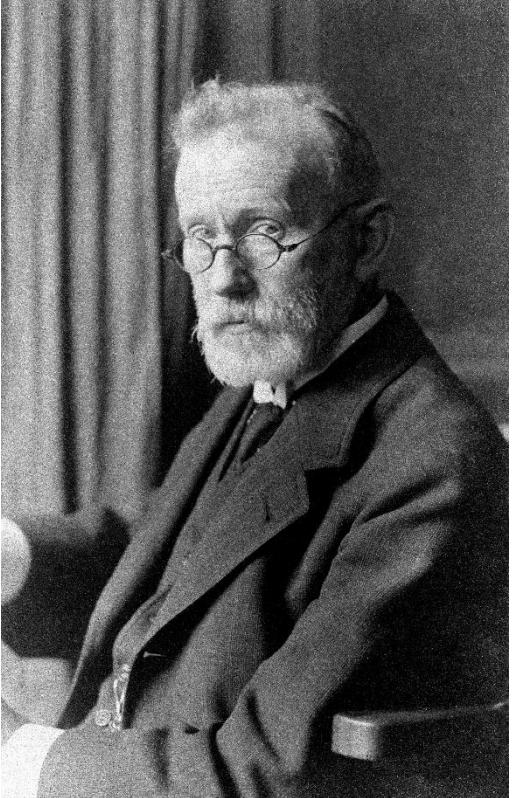
Indeling

Inleiding

- Definitie basofilie
- Analytiek
- Diagnostiek
- Conclusie



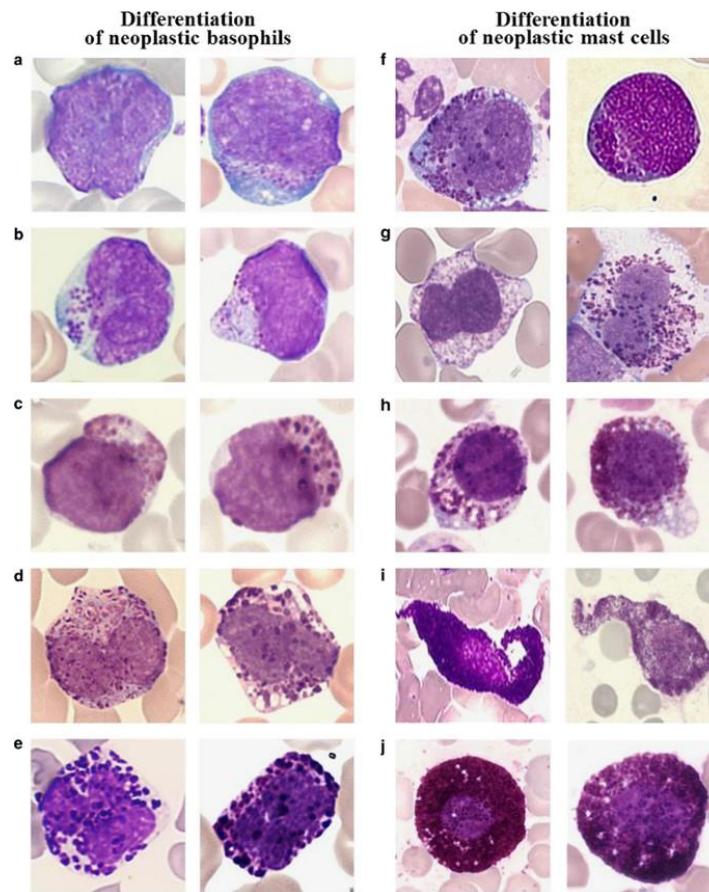
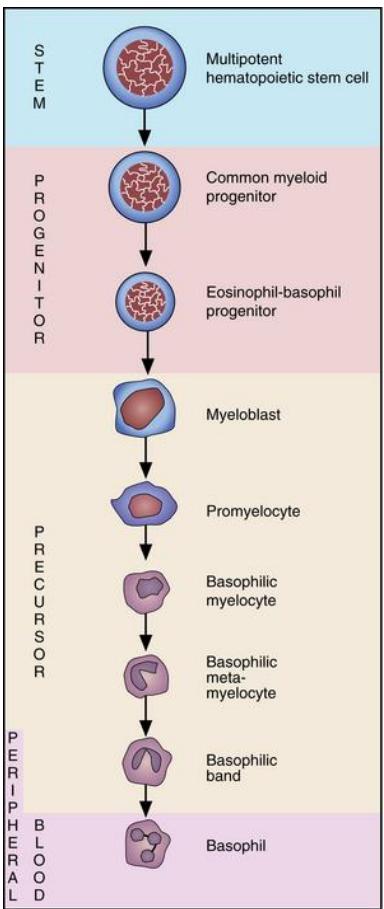
Inleiding



"A basophil is 10–16 µm in diameter with pale blue cytoplasm containing purple-black secondary granules. These granules are water soluble and may dissolve on staining leaving clear areas in the cytoplasm. The nucleus is segmented but is often obscured by basophilic granules which may vary in number, size and shape." (ICSH 2015)



Inleiding





Inleiding

Roles of the major effector mediators produced by basophils in allergic disease: e.g. allergic asthma



Histamine

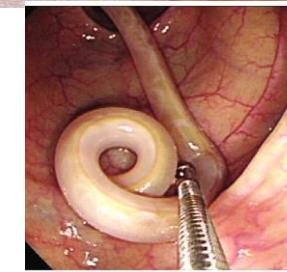
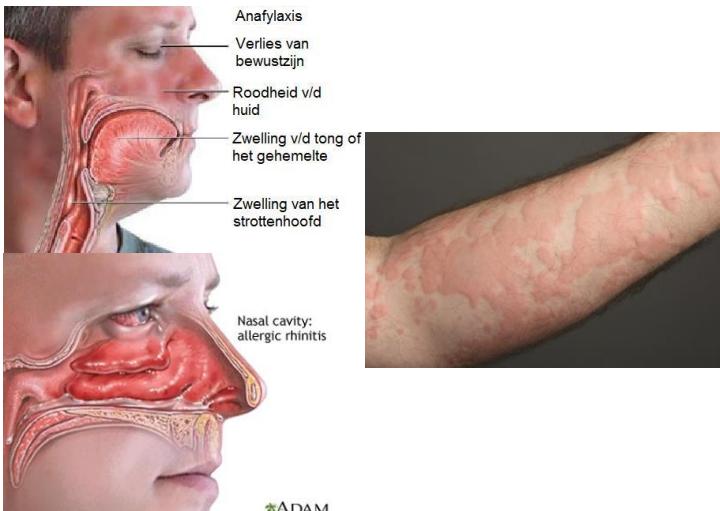


- increase vascular permeability
- cause smooth muscle contraction

IL-4, IL-13



- promotes Th2 lymphocyte differentiation
- promotes IgE production



Imelda omringt je met zorg



Indeling

Inleiding

Definitie basofilie

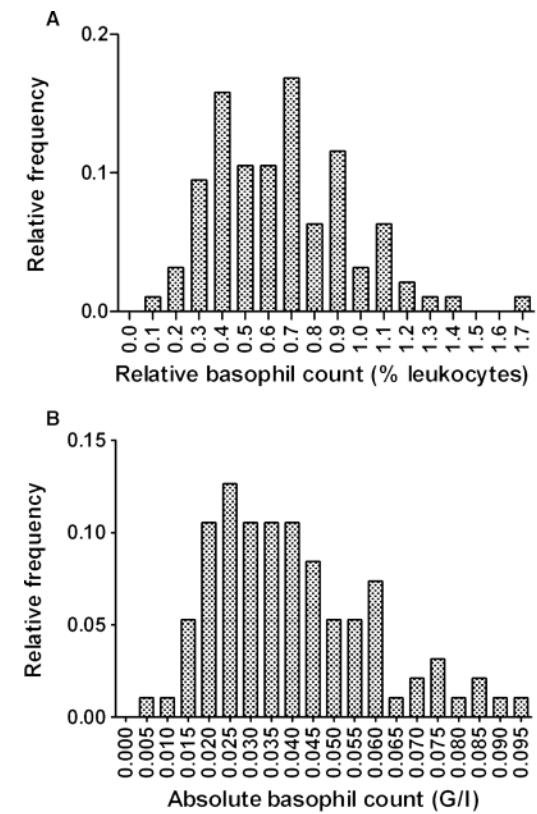
Analytiek

Diagnostiek

Conclusie

Definitie basofilie

- CLSI
 - Centrale 95% van referentiepopulatie
 - $0,014 - 0,087 \times 10^9/L$ (Ducrest *et al.*, 2005)
 - $0,01 - 0,09 \times 10^9/L$ (Arbiol-Roca *et al.*, 2018)
 - $0,1 \times 10^9/L$
 - $0,2 \times 10^9/L$ (Tietz, 1983)
 - $>1 \times 10^9/L = \text{hyperbasofilie}$ (Valent *et al.*, 2017)
- Absolute \Leftrightarrow relatieve



Frequency distribution of peripheral blood basophils. Allergy, Volume: 60, Issue: 11, Pages: 1446-1450, First published: 03 October 2005, DOI: (10.1111/j.1365-2995.2005.00910.x)



Indeling

Inleiding

Definitie basofilie

Analytiek

Diagnostiek

Conclusie



Analytiek

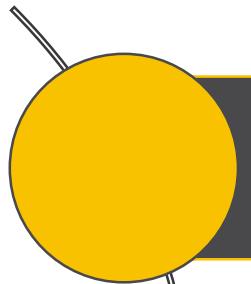
- Pre-Analytiek
 - K2 EDTA tube
 - Perifeer bloed uitstrijkje bij voorkeur <2-4h
- Biologische variabiliteit

| TYPE | MEDIAN CV ESTIMATE | LOWER CI LIMIT | HIGHER CI LIMIT | LAST UPDATED | TOOLS |
|-----------------|--------------------|----------------|-----------------|-------------------------|----------------------|
| Between-subject | 26.3 | 22.1 | 33.9 | 2020-05-05 11:02:28 UTC | <button>APS</button> |
| Within-subject | 12.4 | 11.4 | 32.0 | 2021-08-06 11:28:09 UTC | <button>RCV</button> |

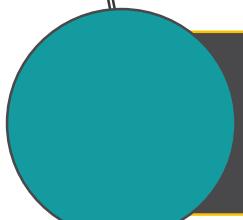
Bron: EFLM. <https://biologicalvariation.eu/search?q=basophil>



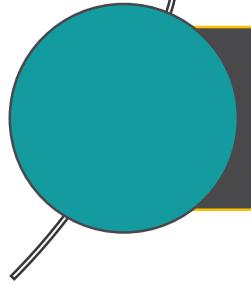
Analytiek



microscopie



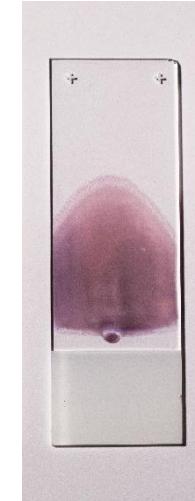
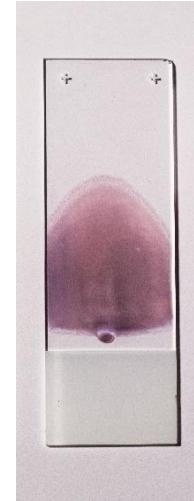
automatische celtellers



immunoflowcytometrie

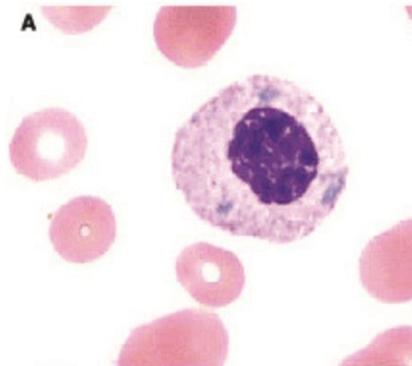
Analytiek

- Microscopie
 - Referentiemethode
 - Gebreken
 - Interpretatie
 - Cel distributie
 - Statistische error

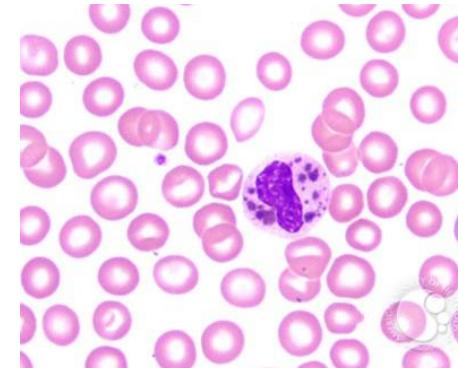


Analytiek

- Microscopie
 - Referentiemethode
 - Gebreken
 - Interpretatie



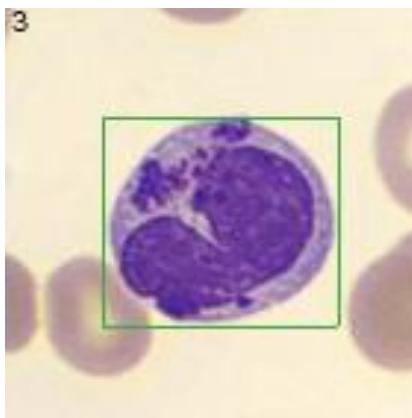
Pseudo Pelger Huet



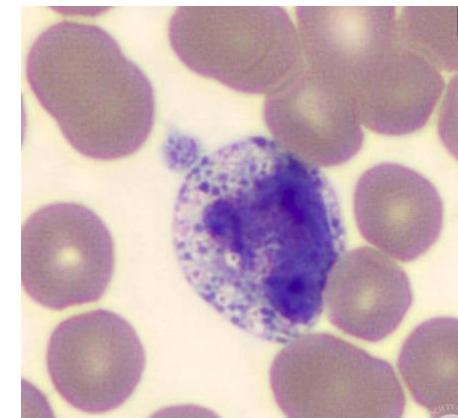
Chediak highashi



Immature eosinofiel met gemengde granules



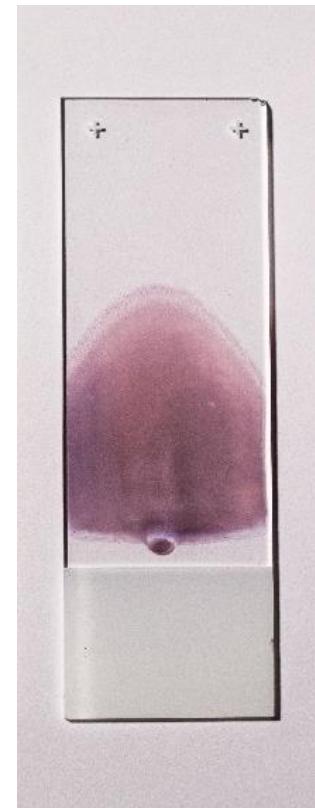
Dysplastische ontkorrelde basofiel in AML MDS



Ontkorreling ten gevolge van kleuring

Analytiek

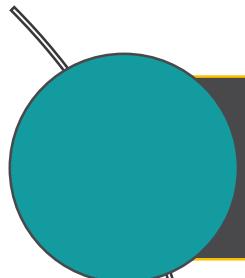
- Microscopie
 - Referentiemethode
 - Gebreken
 - Interpretatie
 - Cel distributie
 - Statistische error
 - 3 basofielen/100
 - WBC: $6 \times 10^9/L$
 - $\rightarrow 0,06 \Leftrightarrow 0,54 \times 10^9/L$



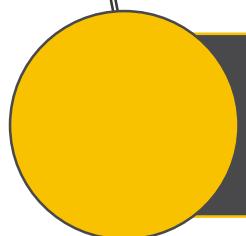
| d | N = 100 | N = 200 | N = 500 | N = 1000 |
|-----|---------|---------|---------|----------|
| 0 | 0-2 | 0-2 | 0-1 | 0-1 |
| 1 | 0-6 | 0-4 | 0-3 | 0-2 |
| 2 | 0-8 | 0-6 | 0-4 | 1-4 |
| 3 | 0-9 | 1-7 | 1-5 | 2-5 |
| 4 | 1-10 | 1-8 | 2-7 | 2-6 |
| 5 | 1-12 | 2-10 | 3-8 | 3-7 |
| 6 | 2-13 | 3-11 | 4-9 | 4-8 |
| 7 | 2-14 | 3-12 | 4-10 | 5-9 |
| 8 | 3-16 | 4-13 | 5-11 | 6-10 |
| 9 | 4-17 | 5-14 | 6-12 | 7-11 |
| 10 | 4-18 | 6-16 | 7-13 | 8-13 |
| 15 | 8-24 | 10-21 | 11-19 | 12-18 |
| 20 | 12-30 | 14-27 | 16-24 | 17-23 |
| 25 | 16-35 | 19-32 | 21-30 | 22-28 |
| 30 | 21-40 | 23-37 | 26-35 | 27-33 |
| 35 | 25-46 | 28-43 | 30-40 | 32-39 |
| 40 | 30-51 | 33-48 | 35-45 | 36-44 |
| 45 | 35-56 | 37-53 | 40-50 | 41-49 |
| 50 | 39-61 | 42-58 | 45-55 | 46-54 |
| 75 | 65-84 | 68-81 | 70-79 | 72-78 |
| 100 | 96-100 | 98-100 | 99-100 | 99-100 |



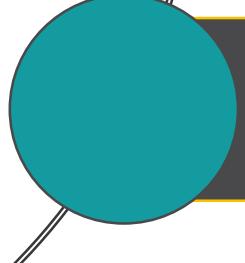
Analytiek



microscopie



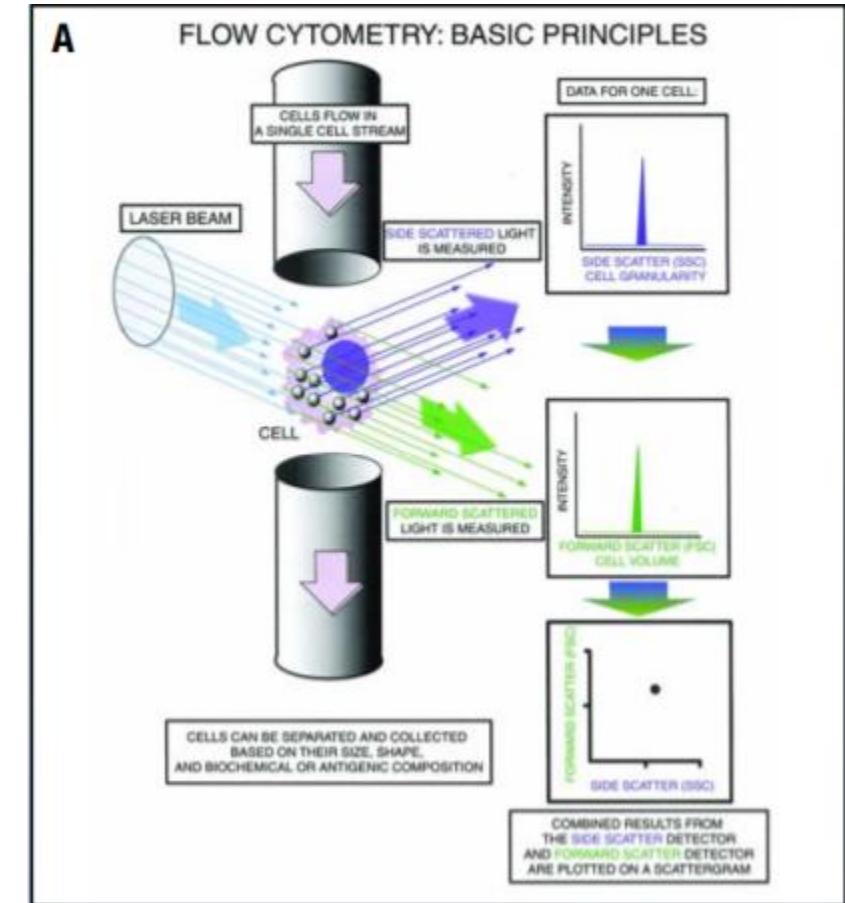
automatische celtellers



immunoflowcytometrie

Analytiek

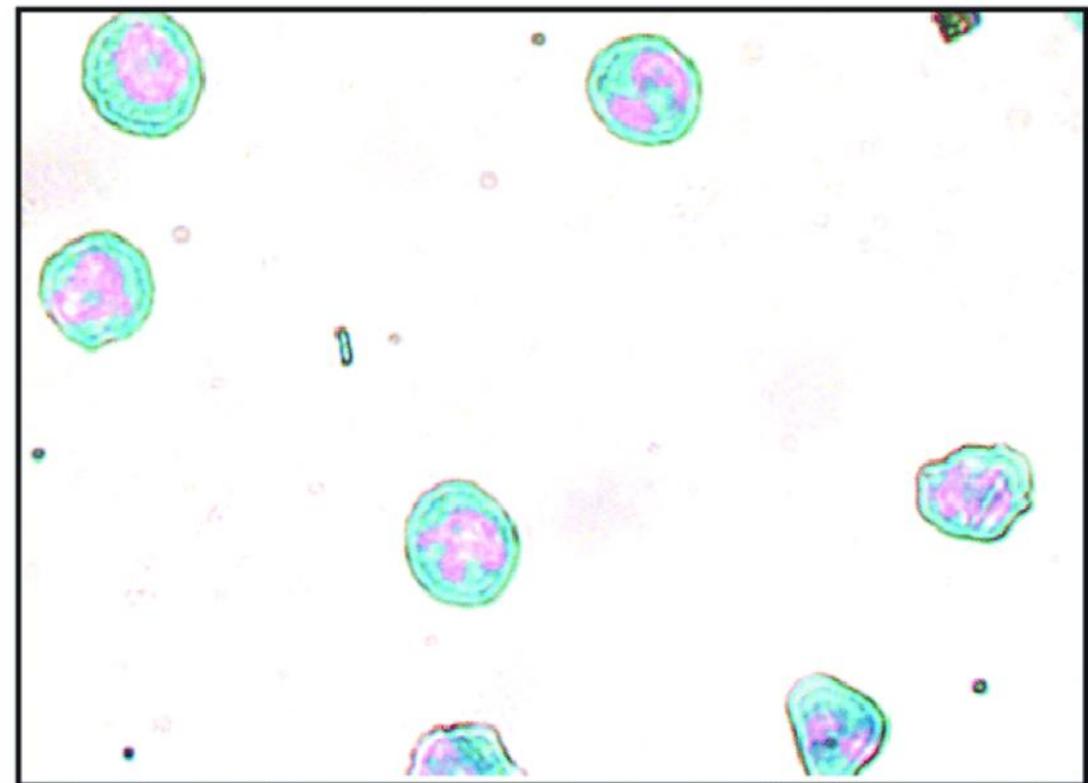
- Algemeen
 - Cel grootte
 - Cel complexiteit
 - RNA/DNA
- Basofielkanaal



Analytiek

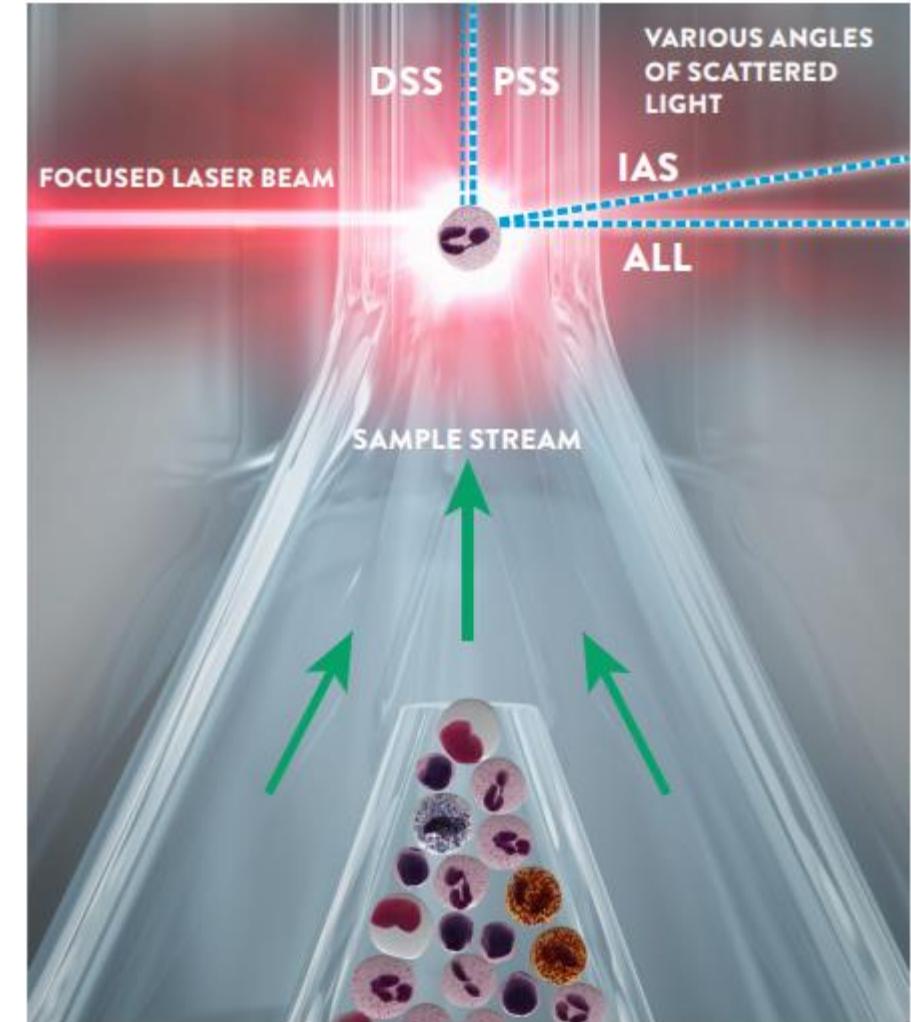
- Algemeen
- Basofielkanaal
 - Alcian blue

B. Purified blood basophils



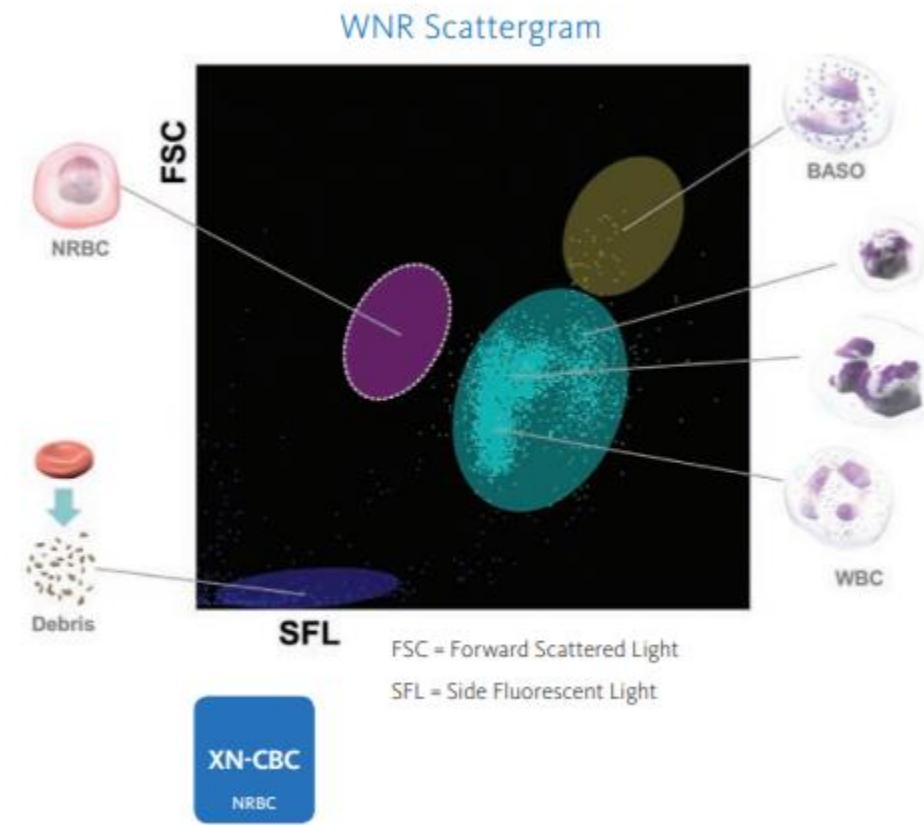
Analytiek

- Algemeen
- Basofielkanaal
 - Alcician blue
 - Lichtverstrooiing



Analytiek

- Algemeen
- Basofielkanaal
 - Alcacian blue
 - Lichtverstrooiing
 - Zure lysis
 - Pseudobasofilie



Analytiek

Opinion Paper

Johannes J. M. L. Hoffmann*

Basophil counting in hematology analyzers: time to discontinue?

| Hematology analyzer | Comparative method | n | Coefficient of correlation | Reference |
|---------------------------|--------------------------|-------|----------------------------|-----------|
| Abbott CELL-DYN Sapphire | Median of 4 analyzers | 202 | 0.32 | [24] |
| | Median of 5 analyzers | 349 | 0.37 | [62] |
| | Advia 2120 | 200 | 0.070 | [63] |
| | Sysmex XE-2100 | 200 | 0.132 | [63] |
| Abbott Alinity h | Abbott CELL-DYN Sapphire | 336 | 0.48 | [28] |
| | Sysmex XN-9000 | 314 | 0.681 | [64] |
| Beckman Coulter DxH-800 | Median of 4 analyzers | 202 | 0.14 | [24] |
| | Median of 5 analyzers | 349 | 0.62 | [62] |
| | CELL-DYN Sapphire | 135 | 0.049 | [65] |
| | CELL-DYN Sapphire | 332 | 0.275 | [66] |
| | Beckman Coulter LH 780 | 332 | 0.613 | [66] |
| Mindray BC-6800 | Sysmex XE-2100 | 477 | 0.314 | [67] |
| | ABX Pentra 120 | 1025 | 0.583 | [23] |
| | Siemens Advia 2120 | 195 | 0.589 | [68] |
| Roche Cobas m511 | Sysmex XN-10 | 1513 | 0.679 | [69] |
| Siemens Advia 120 or 2120 | Median of 4 analyzers | 202 | 0.04 | [24] |
| | Median of 5 analyzers | 349 | 0.82 | [62] |
| | Coulter Gen-S | 95 | 0.0239 | [29] |
| Sysmex XE-2100 | Median of 4 analyzers | 202 | 0.01 | [24] |
| Sysmex XE-5000 | Median of 5 analyzers | 349 | 0.67 | [62] |
| Sysmex XN series | Median of 5 analyzers | 349 | 0.80 | [62] |
| | Beckman Coulter LH-750 | 261 | 0.21 | [70] |
| | Beckman Coulter DxH-800 | 2142 | 0.583 | [71] |
| | Siemens Advia 2120 | 261 | 0.61 | [70] |
| Sysmex XE-2100 | 390 | 0.76 | [20] | |
| Sysmex XE-2100 | 160 | 0.787 | [72] | |
| Sysmex XE-2100 | 261 | 0.42 | [70] | |
| Sysmex XE-5000 | 241 | 0.818 | [73] | |

| Hematology analyzer | n | Coefficient of correlation | Reference |
|---------------------------|-----|----------------------------|-----------|
| Abbott CELL-DYN Sapphire | 200 | 0.265 | [63] |
| | 292 | 0.17 | [62] |
| | 125 | 0.159 | [65] |
| | 272 | 0.478 | [66] |
| | 156 | 0.30 | [74] |
| Abbott Alinity h | 346 | 0.38 | [28] |
| | 314 | 0.53 | [64] |
| ABX Pentra | 174 | 0.23 | [74] |
| Beckman Coulter DxH-800 | 292 | 0.26 | [62] |
| | 125 | 0.321 | [65] |
| | 272 | 0.581 | [66] |
| | 132 | 0.58 | [74] |
| | 102 | 0.444 | [71] |
| | 249 | 0.039 | [22] |
| Mindray BC-6800 | 134 | 0.56 | [74] |
| | 186 | 0.719 | [68] |
| Siemens Advia 120 or 2120 | 292 | 0.34 | [62] |
| | 200 | 0.485 | [63] |
| | 444 | 0.23 | [74] |
| Sysmex XE-2100 | 200 | 0.616 | [63] |
| | 663 | 0.63 | [74] |
| | 101 | 0.238 | [75] |
| Sysmex XN series | 390 | 0.70 | [20] |
| | 292 | 0.30 | [62] |
| | 122 | 0.21 | [74] |
| | 102 | 0.466 | [71] |
| | 261 | 0.08 | [70] |
| | 346 | 0.585 | [76] |

Hoffman et al, Cl. Chem. Lab. Med., 2021

Analytiek

Image 1 Gating strategy used to identify basophils by flow cytometry; 1 representative patient sample. Basophils are ...

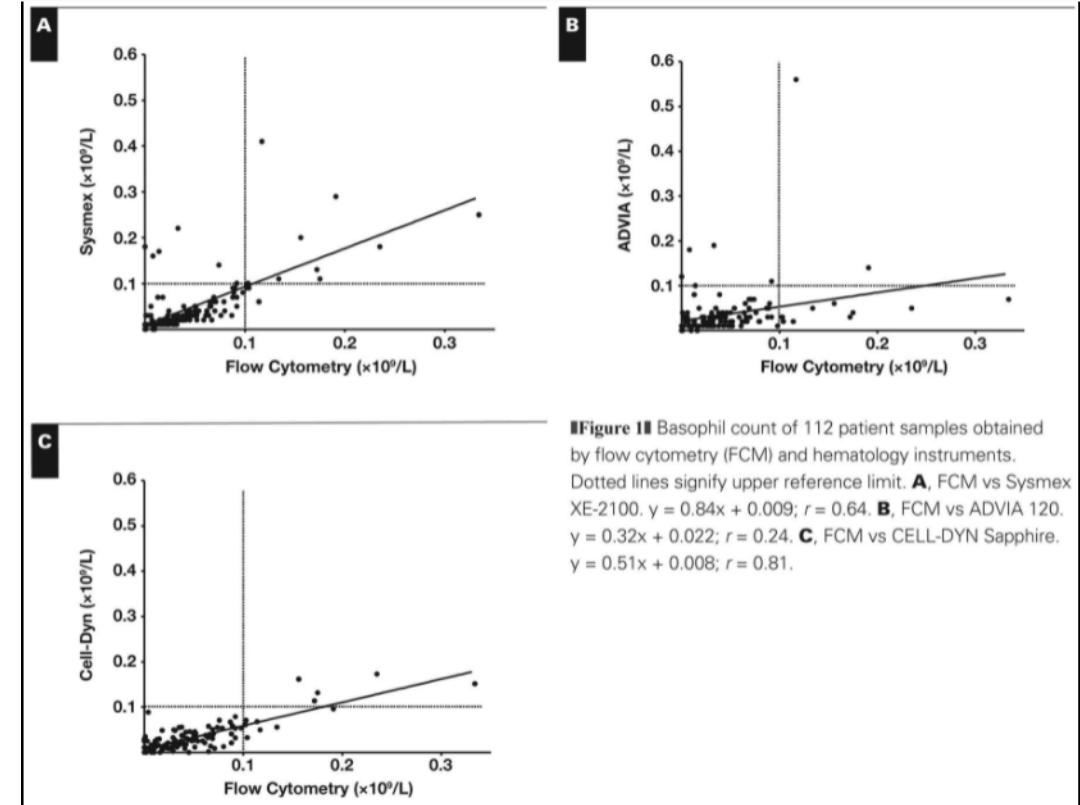
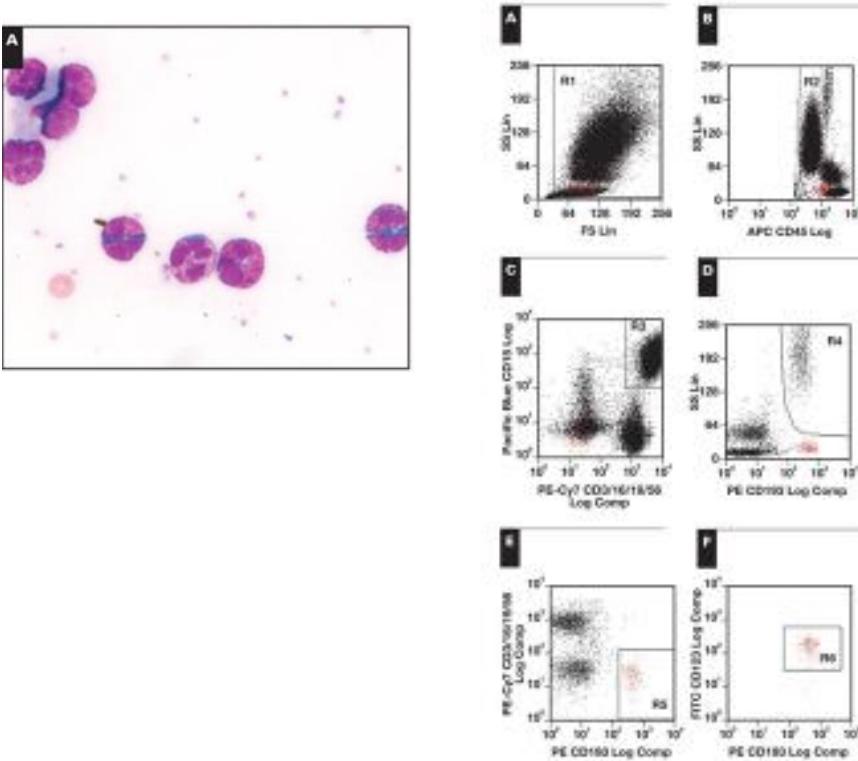
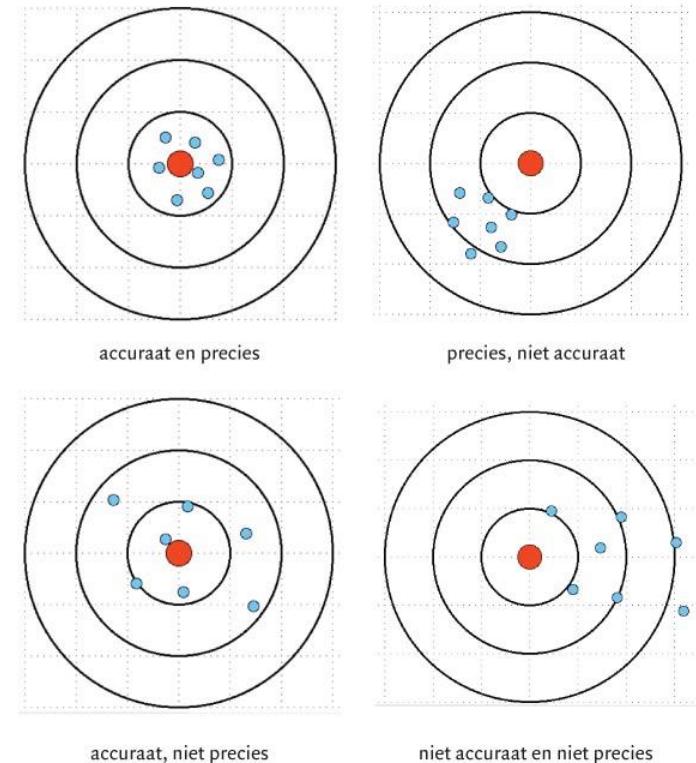


Figure 11 Basophil count of 112 patient samples obtained by flow cytometry (FCM) and hematology instruments. Dotted lines signify upper reference limit. **A**, FCM vs Sysmex XE-2100. $y = 0.84x + 0.009$; $r = 0.64$. **B**, FCM vs ADVIA 120. $y = 0.32x + 0.022$; $r = 0.24$. **C**, FCM vs CELL-DYN Sapphire. $y = 0.51x + 0.008$; $r = 0.81$.

Amundsen EK et al, Am J Clin Pathol, 2012

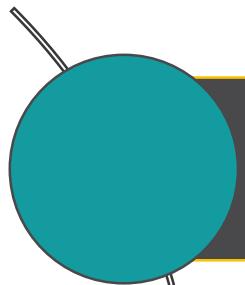
Analytiek

- Analytische variatie (EFLM)
 - Imprecisie 6,2%
 - Inaccuraatheid 7,3%
 - TE 17,5%
- QC samples: 5-10%
- ⇔ patient samples 20% (Vis et al)

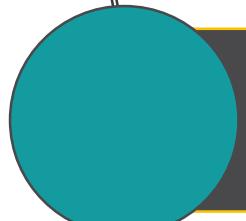




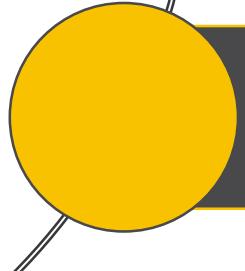
Analytiek



microscopie



automatische celtellers



immunoflowcytometrie

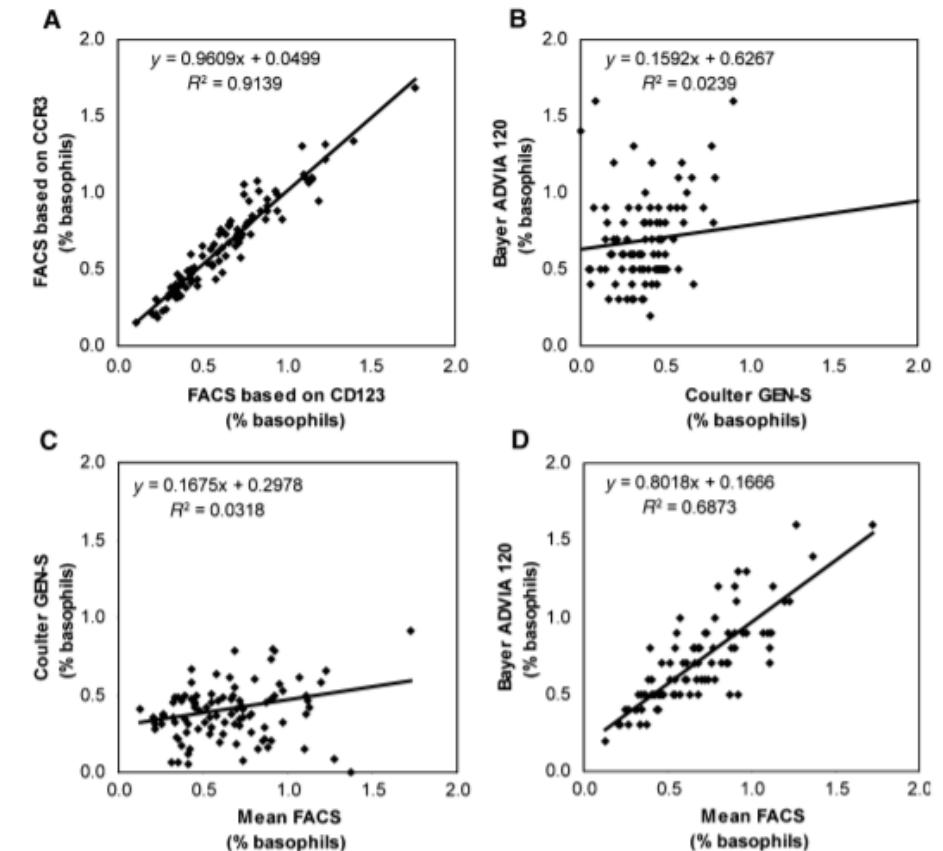
Analytiek

12.1 A Potential Alternative Reference Method

An alternative method to WBC differential counting that is potentially more accurate and time-efficient, is the use of monoclonal antibodies and multiparametric flow cytometry to identify the various WBC subsets and NRBC. This method uses antigenic properties of cells to define the lineage and can improve the statistical precision of cell counting, since 50 to 100 000 cells can be analyzed in minutes.³¹⁻³⁴

Multicolor flow cytometric immunophenotyping is more suited for the identification of low-frequency or morphologically indistinct cell subsets, such as basophils and dendritic cells.^{32,35-38}

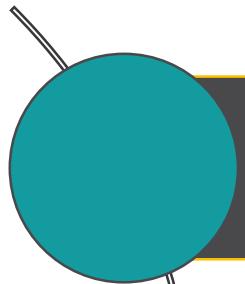
CLSI H20-A2



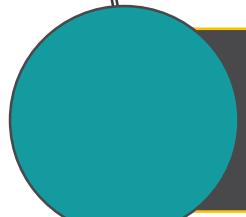
Ducrest et al, Allergy, 2005



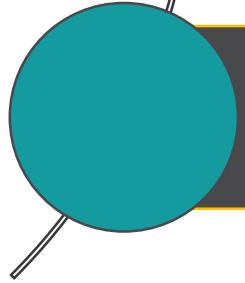
Analytiek



microscopie



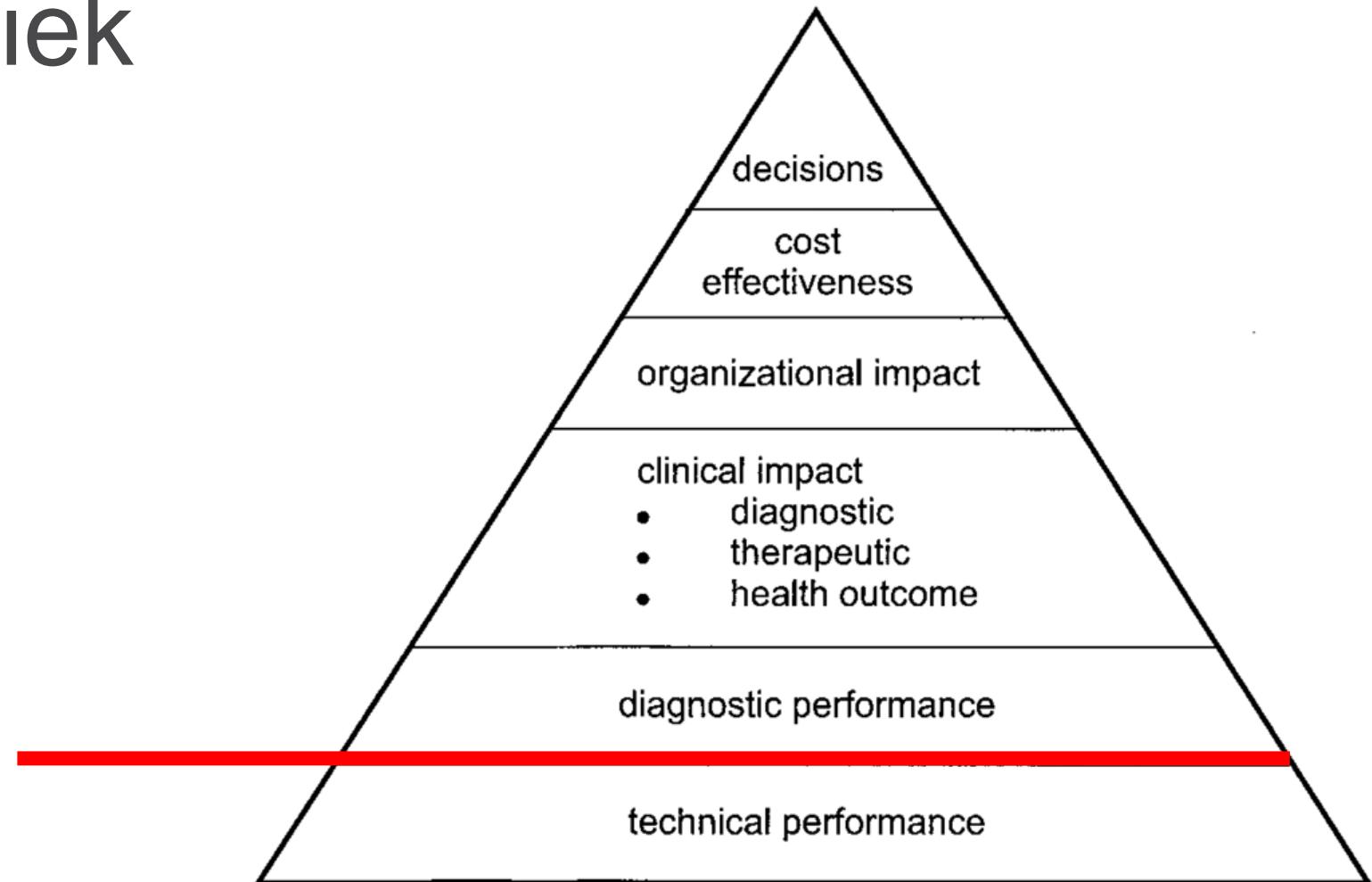
automatische celtellers

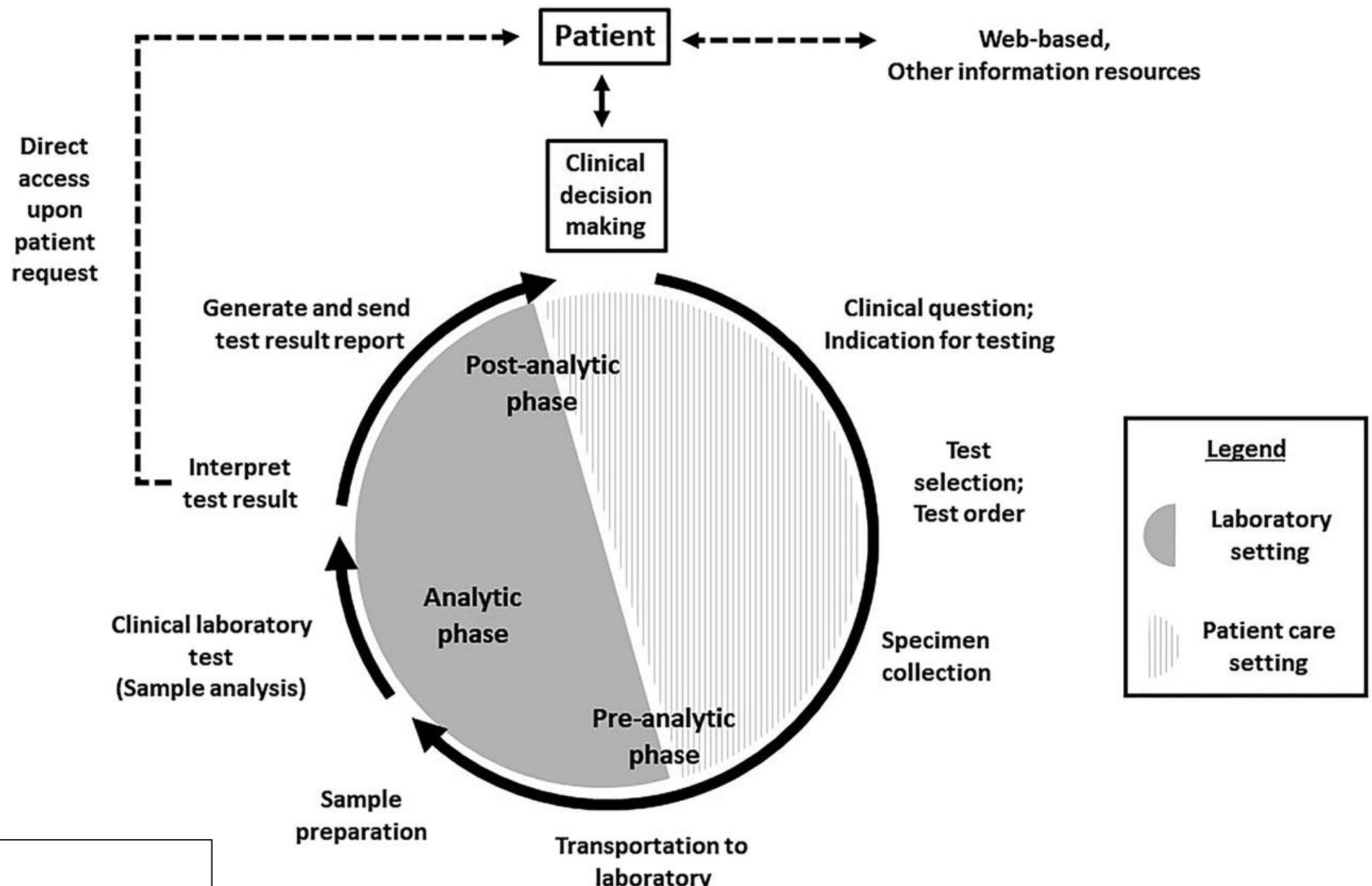


immunoflowcytometrie



Analytiek





Opinion Paper

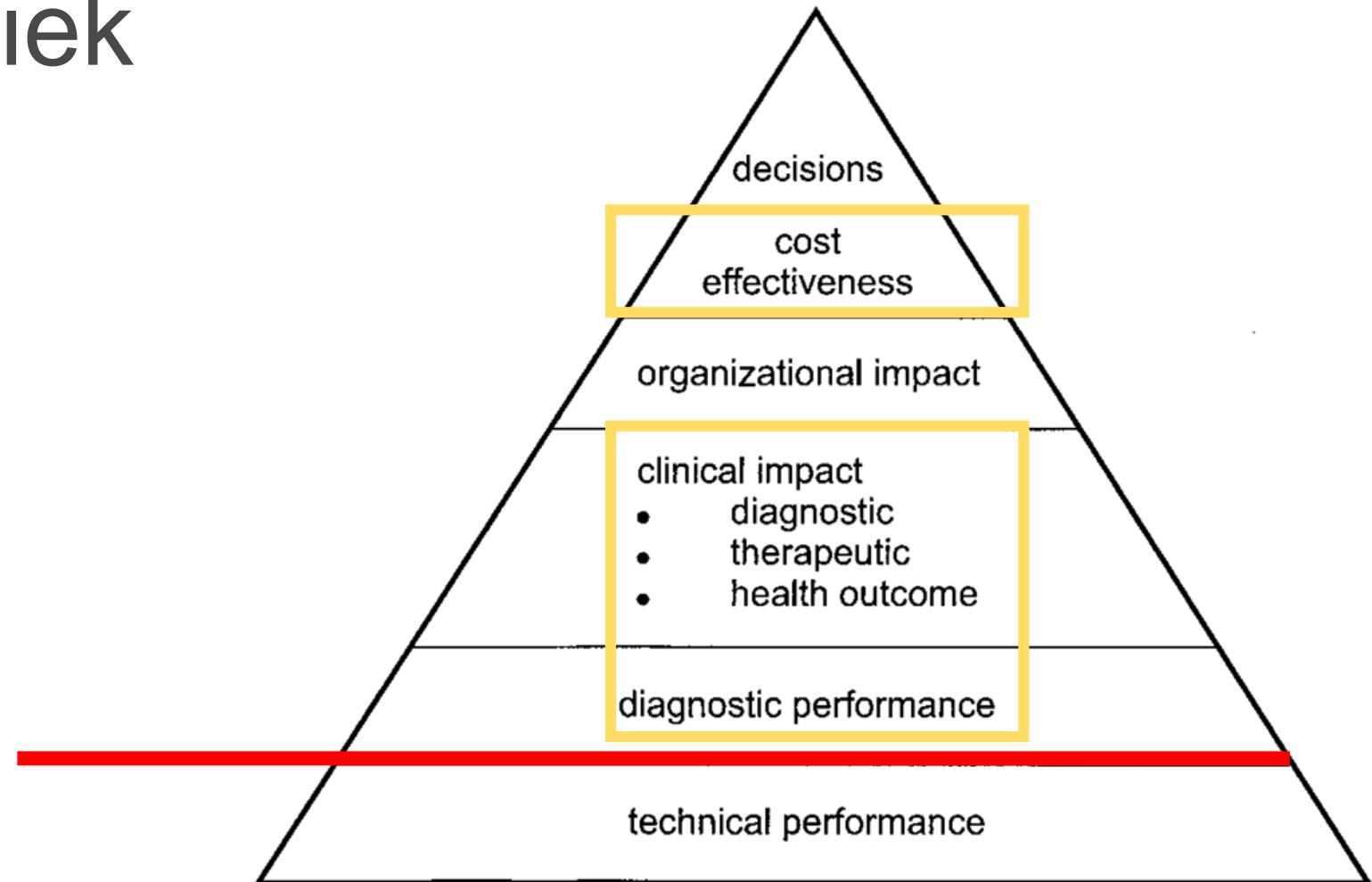
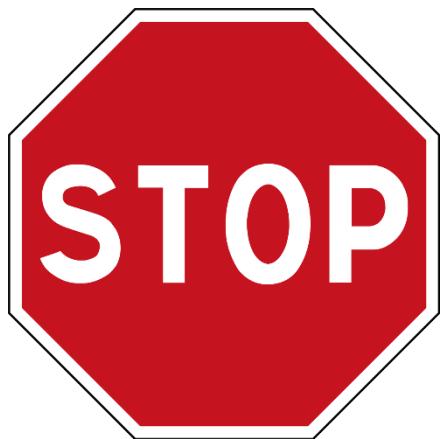
Johannes J. M. L. Hoffmann*

Basophil counting in hematology analyzers: time to discontinue?

Imelda omringt je met zorg



Analytiek





Indeling

- Inleiding
- Definitie basofilie
- Analytiek
- Diagnostiek
- Conclusie



Diagnostiek

- Pseudobasofilie
- Maligne basofilie
- Reactieve basofilie



Diagnostiek

- **Stap 1: Sluit pseudobasofilie uit**
 - Preanalytiek: correcte staalbewaring?
 - Analytiek celtellers :
 - Zure lysis? (WNR kanaal): atypische lymfocyten?
 - Common WBC channel : degranuleerde neutrofielen, NRBC, plaatjesaggregaten?

→ microscopie



Diagnostiek

- Microscopie?
 - $> 0,5 \times 10^9 /L \rightarrow$ smear (ISLH consensus rules)
 - $< 0,5 \times 10^9 /L$?
 - Pseudobasofilie?
 - Andere MPN kenmerken?
 - Leuko-erythroblastaire formules
 - Immature granulocyten
 - ...



Diagnostiek

- Stap 2: Sluit maligne basofilie uit
 - Concentratie?
 - $> 0,40 \times 10^9 /L$ (Smith et al. Am J Hematol, 2020)
 - $> 1,00 \times 10^9 /L$ (Valent et al. Leukemia, 2017)



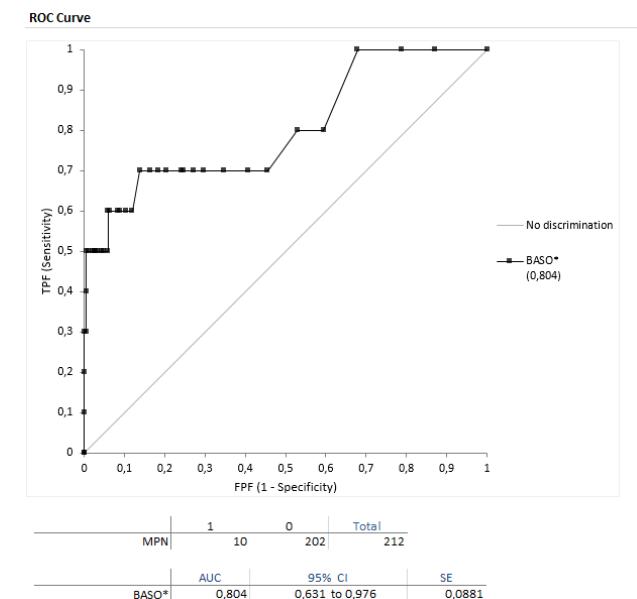
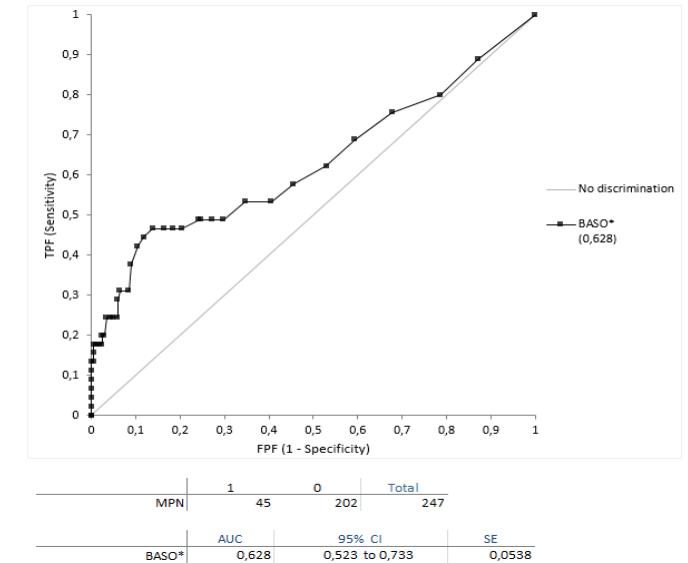
Diagnostiek

- Imelda ziekenhuis maligne basofilie?
- Studiepopulatie
 - 2018-2019
 - >12 jaar
 - $>0,2 \times 10^9/L$ basofielen (Tietz)
 - *Nieuwe diagnose?*
- ROC

- 247 unique patients after exclusion
- 45 with MPN + 6 AML + 4 MDS
 - CML 8
 - CMML* 6
 - ET 11*
 - MDS/MPN 1 (MDS/MPN RS-t)
 - MPN 1
 - MF 7
 - PV 13*
- 1 patient had both ET/CMML, 1 ET/PV
- 10 new diagnoses + 1 AML
 - 2 ET
 - 3 PV
 - 4 CML
 - 1 CMML

Diagnostiek

- Imelda ziekenhuis maligne basofilie?
- Studiepopulatie
- ROC
 - Optimale cut-off: $0,36 \times 10^9/L$ (LR+ 3,39 LR- 0,48)
 - ~~Excluderende kracht~~
 - Aantonende kracht? Baso $>0,45 \times 10^9/L$
 - ~ smith et al, $0,46 \times 10^9/L$



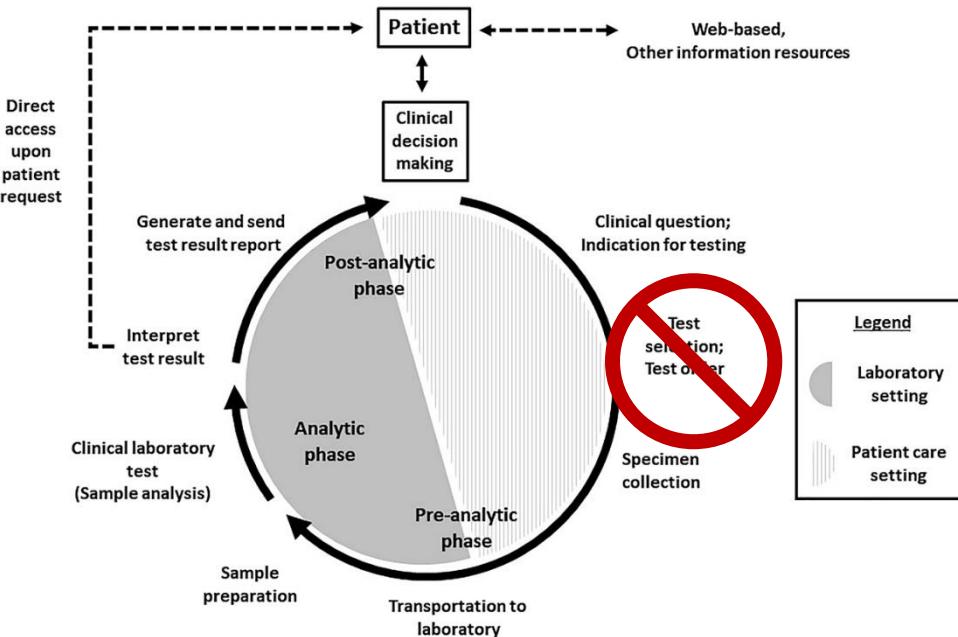


Diagnostiek

- Stap 2: Sluit maligne basofilie uit
 - Concentratie?
 - $> 0,4 \times 10^9 /L$ (Smith et al. Am J Hematol, 2020)
 - $> 1,0 \times 10^9 /L$ (Valent et al. Leukemia, 2017)
 - Andere argumenten voor MPN?

Diagnostiek

- Stap 2: Sluit maligne basofilie uit
 - Concentratie?
 - $> 0,4 \times 10^9 /L$ (Smith et al. Am J Hematol, 2020)
 - $> 1,0 \times 10^9 /L$ (Valent et al. Leukemia, 2017)
 - Andere argumenten voor MPN?



→Reflex om MPN diagnose te overwegen!
→~~basofielen aanvragen?~~
→~~Vervangt het een test?~~



Diagnostiek

- Stap 3: reactieve basofilie?
 - Fysiologie: accumulatie lokaal?
 - Allergie?
 - Correlatie met ernst symptomen rhinitis
 - Urticaria? Anafylaxie? ..
 - Parasitaire infecties?
 - Andere?
 - Crohn? Colitis?
 - Acute rejectie na longtransplantatie?
 - Diabetes, diabetische keto-acidose?
 - Variola, pokken, ijzerdeficiëntie?

→ Zwakke evidentië

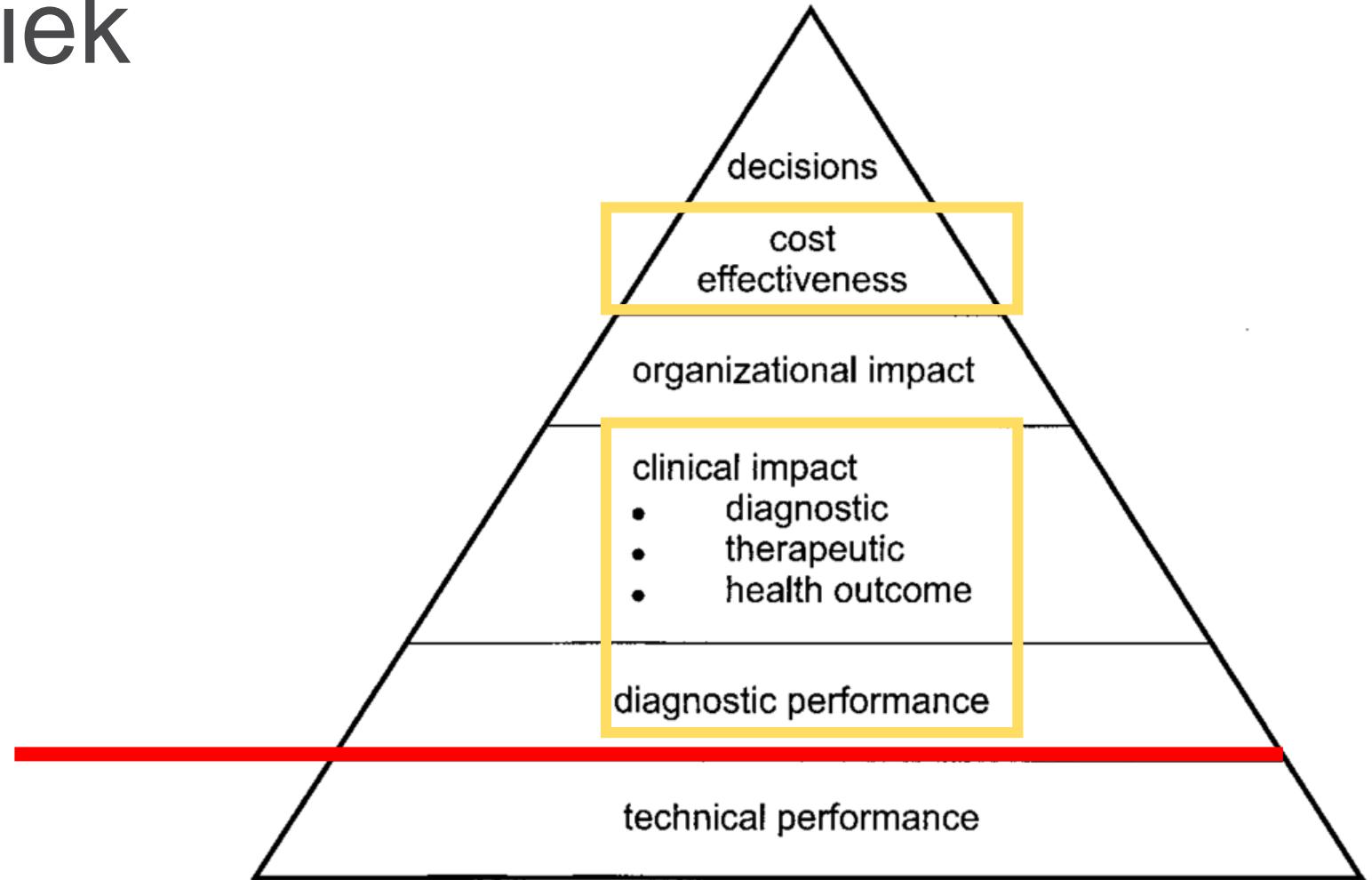
Diagnostiek

- Stoornis: actieve basomucozaal?
 - Biologie: accumulatie lokaal?
 - Allergie?
 - Correlatie met ernst symptomen rhinitis? Urticaria? Anafylaxie? ..
 - Paroxysmale infecties?
 - Andere?
 - Crohn? Colitis?
 - Acute rejectie na transplantatie?
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 - Variola, pokken, ijzerdeficiëntie?

Zwakke evidentië



Analytiek





Indeling

- Inleiding
- Definitie basofilie
- Analytiek
- Diagnostiek
- Conclusie



Conclusie

- Basofilie: cut-off onduidelijk
 - $>0,4 \times 10^9 /L \rightarrow$ MPN mogelijk?
- Meettechnieken → niet optimaal
 - Optimalisatiekost wenselijk?
- Autonome diagnostische waarde? Nihil



To do

- Feedback van hematologen
- Uitleg aan clinici
- Commentaar bij basofielconcentratie?