Quality Assurance in Hematology Laboratory

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August 21, 2017

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Learning Objectives

- Quality Assurance and Quality Control
- Blood Cell Morphology
- Quality control for Hb Elechtrophoresis
- Quality control for Coagulation Tests

What Is Quality?

"Quality is doing the right things and doing those things right"

" Quality is everyone's responsibility"

Quality Control vs. Quality Assurance

- Quality Control is method control
- Quality Assurance is process control
- Quality System is the process of building quality into the entire system

Introduction

- QA is the sum of all those activities in which the laboratory is engaged to ensure that information generated by laboratory is correct.
- QA is not restricted to the development and retention of quality control charts but rather includes all aspects of laboratory activities that affects the results produced, from the choice of methods, to the education of personnel, to the handling of specimens and reporting results.
- The real purpose of QA activities is to determine how correct or incorrect the results emanating from the lab are, and to allow those managing the lab to determine whether or not the lab is fulfilling its functions satisfactorily.

Introduction – cont'

- 3 major activities of QA :

1) **Preventive** – those activities that are done prior to the examination of the specimen or sample and that are intended to establish systems conducive to accuracy in analytical testing (eg : preventive maintenance and calibration of instruments, testing of media, orientation and training of personnel)

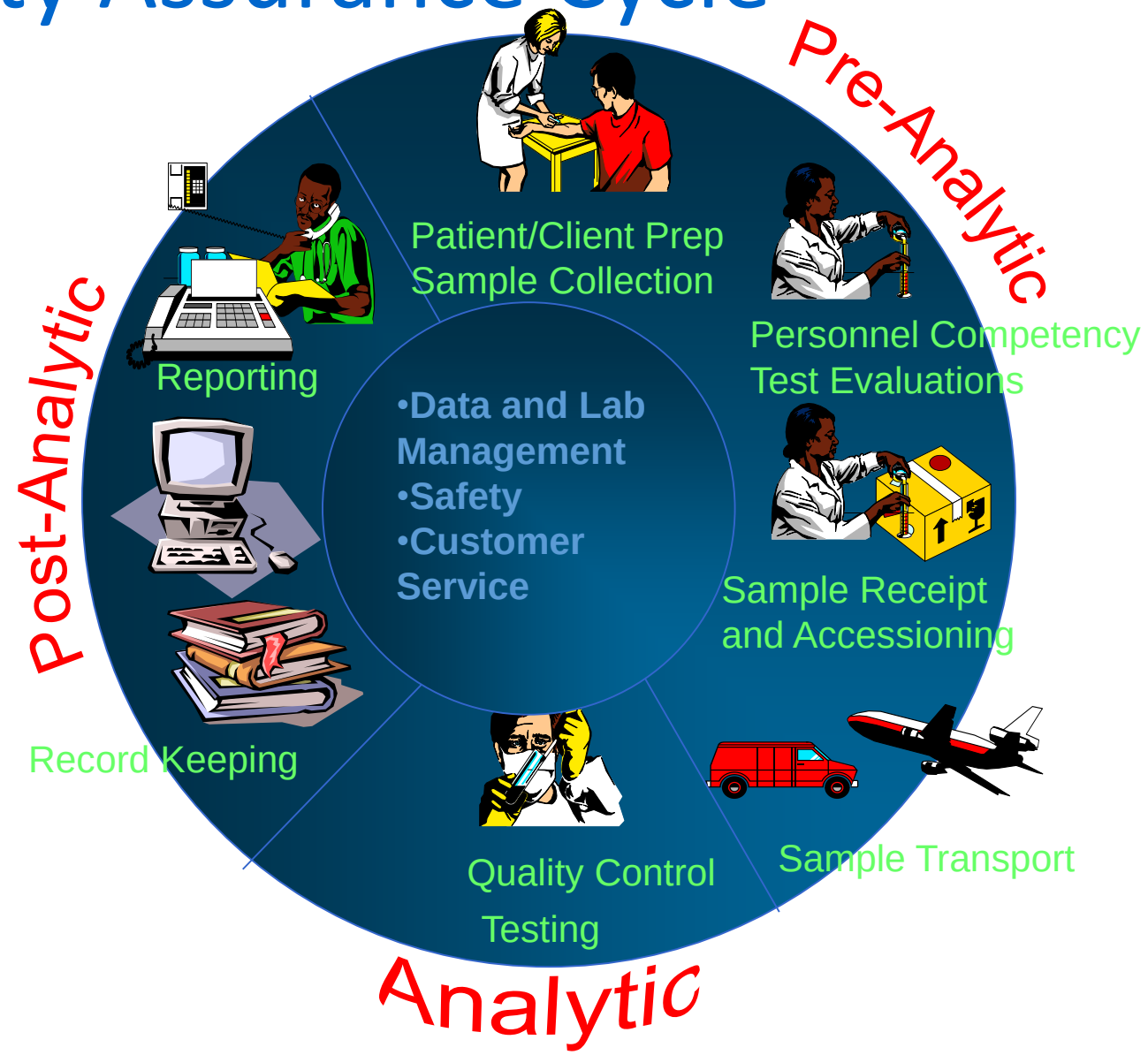
2) **Assessment** – those activities that are done during testing to determine whether the test systems are performing correctly (eg : the use of standard and controls, maintenance of control charts)

3) **Corrective** – those activities that are done, when error is detected, to correct the system (eg : equipment troubleshooting, recalibration of instruments)

The Quality Assurance Targets

- **Pre analytical Process**
  - Pat. preparation
  - Specimen collection, Anticoagulant ,Labeling ,Storage, Transportation
- **Analytical Process**
  - Test method/procedure, Reagant
  - IQC,EQC
- **Post analytical Process**
  - Review of Pat. Results ,Posting of Pat. Results
  - Maintenance of Pat.records
  - Maintaining of all documents

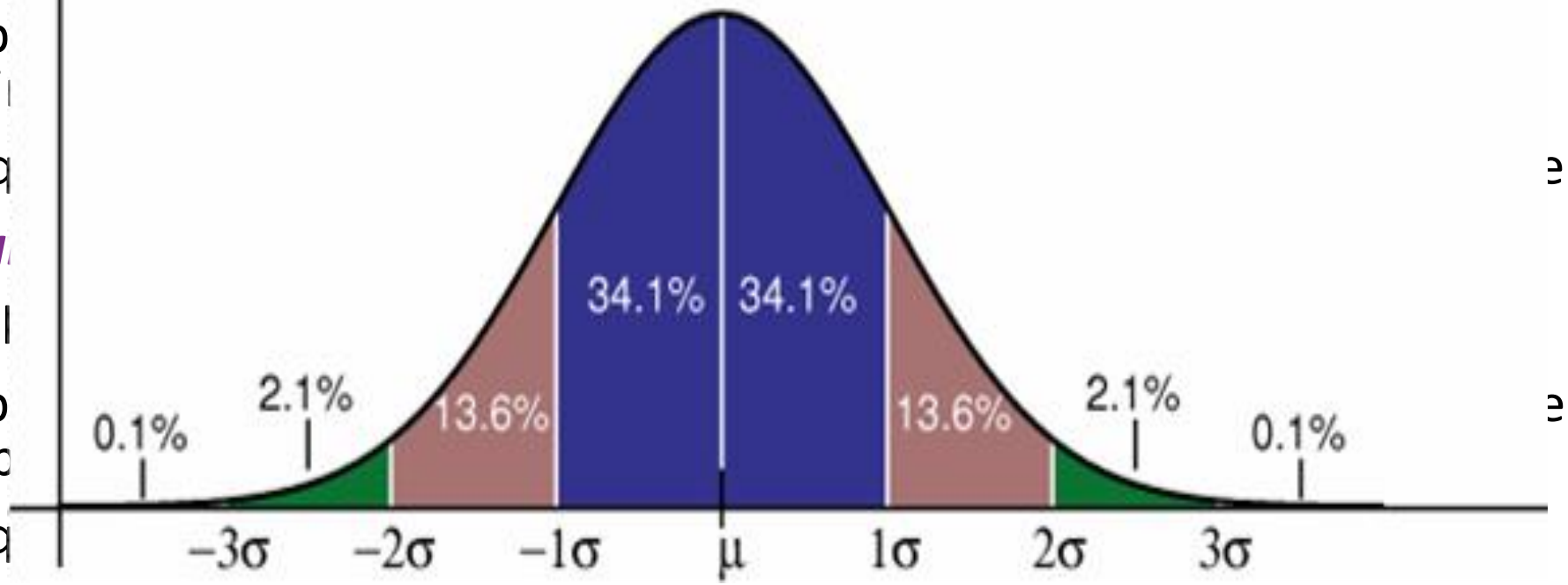
The Quality Assurance Cycle



Some Key Definitions Used In Quality Assurance

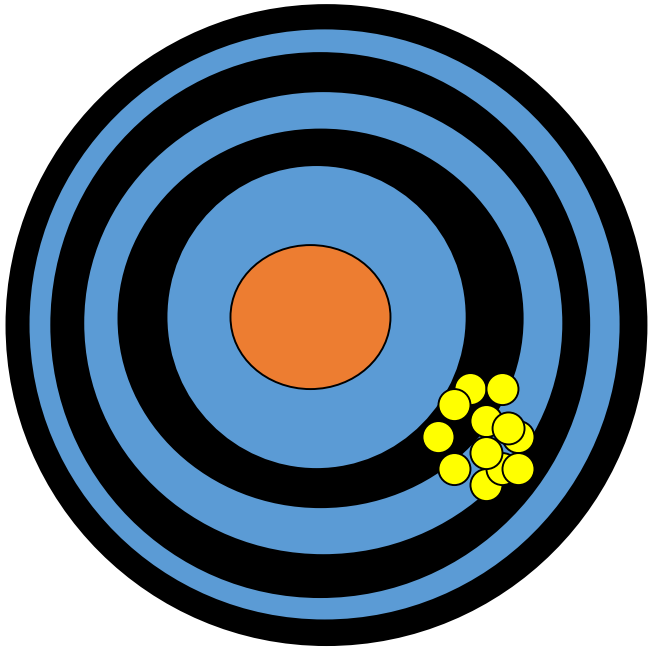
- ***Precision***

- reproducibility of a results, whether accurate or inaccurate within a define frame time
- can b
  speci
- The q error
- ***Accu***
- the cl
- can b ed by
  indep
- The q

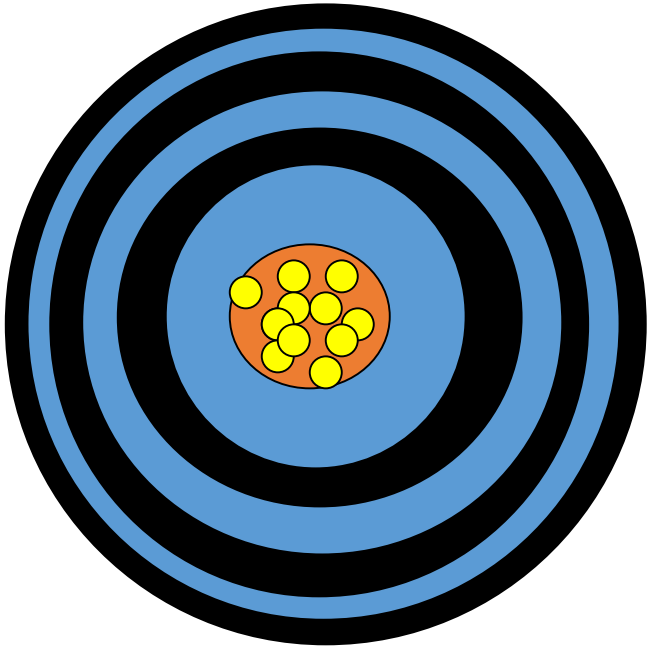


Precision and Accuracy

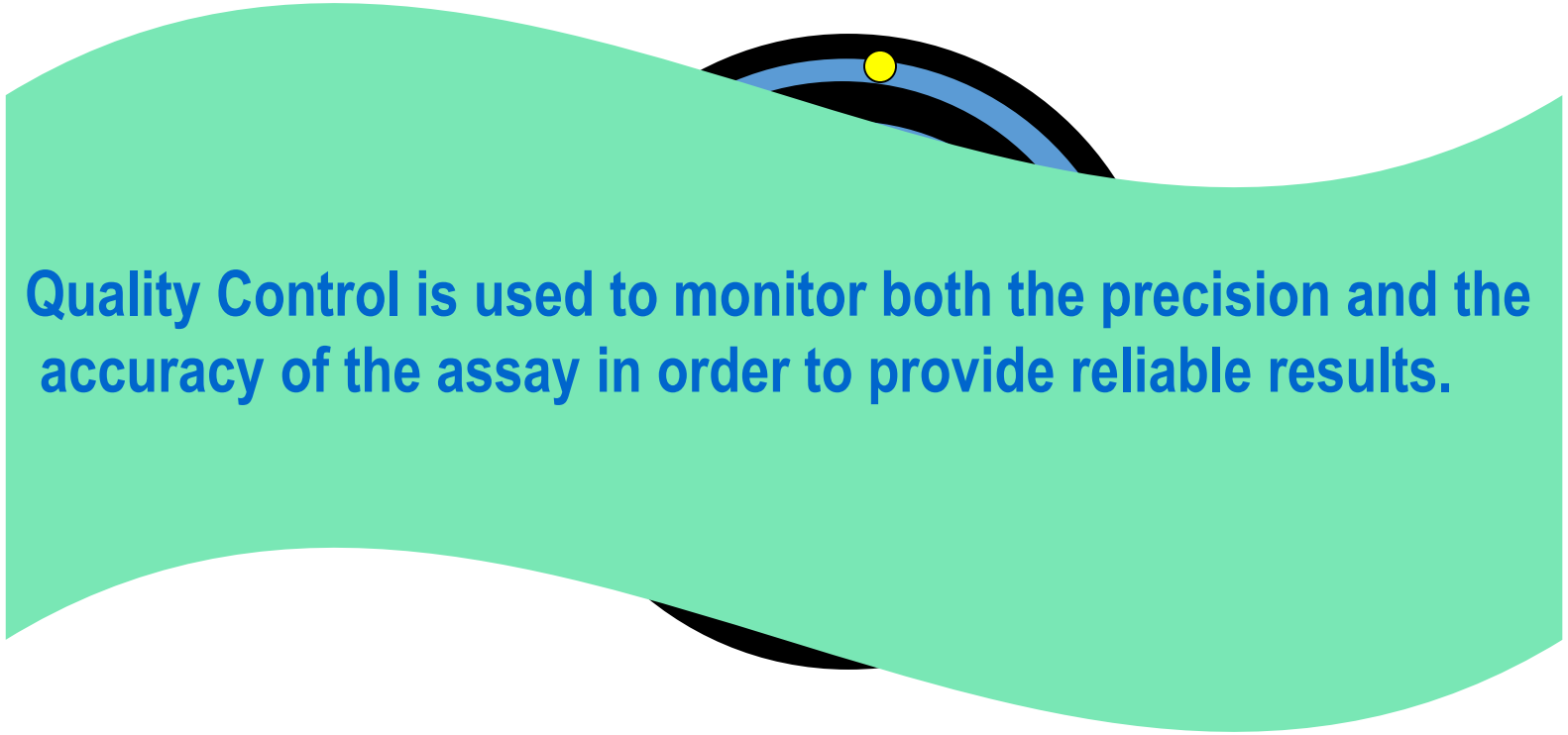
Precise and inaccurate



Precise and accurate



Imprecise and inaccurate



- In any test, inaccuracy, imprecision or both can occur as a result of using unreliable standards, controls or reagents, incorrect instrument calibration or poor laboratory technique

Some Key Definitions Used In Quality Assurance

- **Specificity:** Measures only the analyte of interest
- **Linearity:** The ability of a test to obtain results that are directly proportional to the analyte concentration
- **Limits:** The upper and lower limits of detection of the assay
- **Range:** The interval between the upper and lower limits of detection
- **Robustness:** A measure of how much a test or assay is affected by small variations in methodology

QA in Haematology Laboratory

- Quality assurance must ensure adequate control of the pre-analytical and postanalytical phases (i.e. from test selection and specimen collection t… …of an informative report) as well as the analytica… …nostic errors occur a… result of untoward… …bjective is to achiev…
- 4 compo…

1. Docum…
2. Standa… …perating Proced…
3. Quality Control samples
4. External Quality Assessment Scheme

The ultimate goal of quality system is to obtain test results that are Reliable, relevant, reproducible& As accurate as possible

Preventive Maintenance

- Refer to your instrument manual to determine which, if any, preventive maintenance procedures are required for your instrument. Most instruments do not require routine preventive maintenance, but there are cleaning and replacement procedures available for troubleshooting purposes.

Equipment Maintenance Log

Name of Equipment		Manufacturer's contact details:	
Label:		Date of purchase:	10/15/2016
Serial number:		Person responsible for equipment:	
Manufacturer:		Date put into service:	10/23/2016

Date:	Maintenance Description	Maintenance performed by:	Date of validation before put into service:	Validation performed by:	Next maintenance planned on (date):	Remarks:

Maintaining a Log

- It is essential that you maintain a log documenting your instrument's use. This will assist you both in the laboratory routine and when you need service. Use your log book and your instrument certification documents to keep your system's history current.

Technologist Review

- An experienced medical technologist who reviews the data from an instrument can detect possible malfunctions or irregularities. Technologist review includes assessing the reasonableness of results, investigating questionable cases, knowing when to repeat an analysis, knowing how to interpret QC results, knowing how to interpret calibration recommendations, and when there is a problem, knowing how to define and solve it. These are important skills necessary for any QC program.

مشخصات شناسنامه دستگاههای شمارشگر سلولی خودکار

۱-تاریخ خرید
۲- تاریخ نصب ، کالیبراسیون و نام فرد راه اندازی کننده
۳- تاریخ شروع بکار
۳-کارخانه و کشور سازنده
۴- مدل و شماره سریال دستگاه
۵- شرایط دستگاه هنگام خرید (نو ، مستعمل ، بازسازی شده)
نکته : علاوه بر شناسنامه ، موارد زیر نیز برای هر سل کانتر می بایست بصورت مکتوب در آزمایشگاه موجود باشد .
۶- نام وآدرس شرکت پشتیبان
۷- نام کاربر یا کاربران دستگاه
۸- مشخصات و نحوه آموزش کاربر دستگاه توسط شرکت پشتیبان
۹- میزان عدم دقت دستگاه در هنگام نصب

شناسنامه تجهیزات

نوع دستگاه			
کارخانه	مدل	کشور سازنده	شماره سریال
شرکت پشتیبان	محل استقرار	کاربران ویژه	کد شناسایی
تاریخ رسید به آزمایشگاه	تاریخ راه اندازی در بخش	شرایط دستگاه در موقع تحویل	ویژگی خاص
تجهیزات مرتبط	تلفن تماس با شرکت پشتیبان	سایر	

جهت اطمینان از دقت و صحت کار دستگاه شمارشگر سلولی چه مستنداتی باید در آزمایشگاه موجود باشد؟

سوابق نگه داری

- شسستشو روزانه–هفتگی–ماهیانه
- تعویض محلولها
- تعمیرات

سوابق کنترل کیفی

- چارت کنترلی
- روش اماری t-Britin
- آزمون دوتائی
- عدم دقت ماهیانه

سوابق کالیبراسیون

- پس از تعمیر
- با تغییر شماره سریال محلولها
- نتایج غیرقابل قبول QC
- حداقل هر ۶ ماه

Documentation- Critical values in Hematology

- Critical values are life-threatening results that require immediate notification to the patient's healthcare provider.
- The implementation of a reporting system for critical values is a good laboratory practice. It increases safety and reduces the risk of harm to a patient, and is required by most laboratory accreditation organizations

Critical values -ISO:15189

- 5.8
  - Reporting of results
- 5.8.2 C)

The laboratory shall ensure that critical results, where applicable are communicated effectively and meets the users' needs

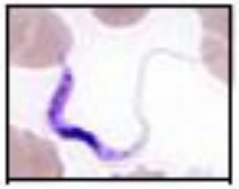
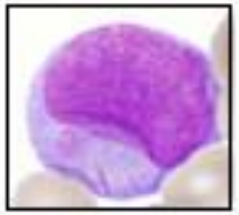
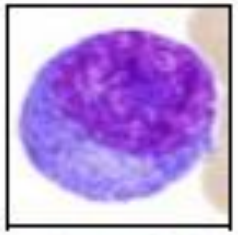
International Journal of Laboratory Hematology
The Official journal of the International Society for Laboratory Hematology
ORIGINAL ARTICLE INTERNATIONAL JOURNAL OF LABORATORY HEMATOLOGY

Critical values in Hematology

A. MCFARLANE*,†, B. ASLAN*,†, A. RABY*,†, G. BOURNER‡, R. PADMORE§

Table 2. Summary of the critical values for common hematology parameters

Parameter	Lower limit	Upper limit
Leukocyte count ($\times 10^9$/L)		
Median	2.0	40
5–95th percentile	0.5–2.5	20–50
N	154	158
Hemoglobin (g/L)		
Median	69	200
5–95th percentile	50–90	180–200
N	179	145
Platelet count ($\times 10^9$/L)		
Median	50	1000
5th–95th percentile	20–70	750–1000
N	177	143

Morphology	Percent of labs reporting results as critical ($n = 174$)
	Malaria: 94%
	Parasites other than malaria: 84%
	Blast cells: 74%
	Sickle cells: 48%
	Schistocytes: 39%
	Plasma cells: 37%
	Spherocytes: 22%

Critical values – Hematology

Hematocrit	**< 21%**
	< 30% (< 1 month old)
Hemoglobin	**< 9.5 g/dL or > 25.0 g/dL (newborns 0-1 month)**
	< 7.0 g/dL or > 18.5 g/dL (non newborns)
Platelet Count	**< 40 x10^3/uL (Call at first occurrence and call again if most recent previous result was not critical).**
	< 10 x10^3/uL (call every time)
	> 1000 x10^3/uL (Call at first occurrence and call again if most recent previous result was not critical).
Sickle Cell Prep	**All positives on pre-ops**
WBC	**< 1.0 x10^3/uL (Call at first occurrence and call again if most recent previous result was not critical).**
	> 40.0 x10^3/uL (Call at first occurrence and call again if most recent previous result was not critical).
Absolute Neutrophil Count	**< 0.5 x10^3/uL**
WBC (CSF)	**> 10 / uL (> 1 year of age)**
	> 40 / uL (0-1 year of age)

Critical values – Coagulation Test

PT INR	**> 4.0**
PTT	**> 100 seconds**
Fibrinogen	**< 100 mg %**
Factor 8, Factor 9	**<15%**

Standardisation

- Standardisation in haematology is the concern of the International Council for Standardisation in Haematology (ICSH) and other international and national organisations, whose recommendations are published in haematology journals or are available from the organisations' websites

SOME ORGANISATIONS INVOLVED IN STANDARDISATION AND QUALITY MANAGEMENT SYSTEMS

Abbreviation	Organisation	Website
AENOR	Asociación Española de Normalización y Certificación	www.aenor.es
AFNOR	Association Française de Normalization	www.afnor.org
AMREF	African Medical and Research Foundation, Nairobi, Kenya	www.amref.org
ANCLSH	Asian Network for Clinical Laboratory Standardisation and Harmonization	www.ancls.org
ASQ	American Society of Quality	www.asq.org
BCSH	British Committee for Standards in Haematology	www.bcshguidelines.com
CAP	College of American Pathologists	www.cap.org
CEN	The European Committee for Standardisation	www.cen.eu
CLSI	Clinical and Laboratory Standards Institute	www.clsi.org
EQALM	European Organisation for External Quality Assurance Providers in Laboratory Medicine	www.eqalm.org
ICSH	International Council for Standardisation in Haematology	www.icsh.org
IFCC	International Federation of Clinical Chemistry and Laboratory Medicine	www.ifcc.org
INSTAND	Gesellschaft zur Förderung der Qualitätssicherung in medizinischen Laboratorien	www.instandev.de
IRMM	Institute of Reference Materials and Measurements	Irmm.jrc.ec.europa.eu
ISO	International Organisation for Standardisation	www.iso.org
JCAHO	Joint Commission for the Accreditation of Healthcare Organisations	www.jointcommission.org
PPTC	Pacific Paramedical Training Centre New Zealand	www.pptc.org.nz
RCPA	Royal College of Pathologists Australia	www.rcpaqap.com.au
UK NEQAS	United Kingdom National External Quality Assessment Service	www.ukneqas.org.uk
WHO	World Health Organisation EQAS for Haematology	www.who.int/diagnostics_laboratory/quality/haematology/en/index.html

Quality Assurance Procedures

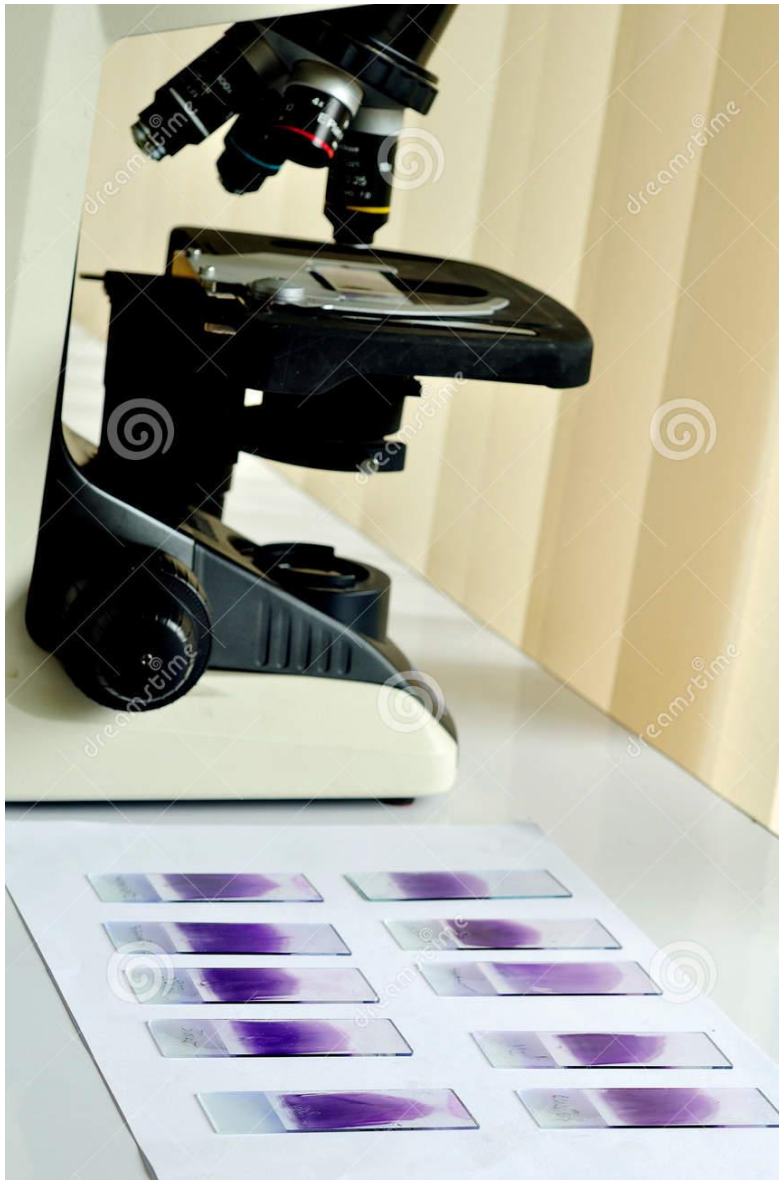
- The procedures that should be included in a QA programme vary with the tests undertaken, the instruments used, the size of the laboratory, the numbers of specimens handled, the computer facilities available and the amount of time that can be devoted to QA.
- At least some form of IQC must be undertaken and there must be participation in EQA for any test that a laboratory offers.
- Where no EQA programme is available the laboratory should establish some other means of assessing interlaboratory comparability, e.g. sample exchange with another laboratory.
- Some control procedures should be performed daily and other performance checks should be done at appropriate intervals.
- A review of performance is particularly important when there is a change in staff and after maintenance service or repair has been carried out on equipment.

Quality assurance programme

- **Correction system (At all times)**
  1. **Correction of blood film appearances with blood count**
     - **WBC count:** WBC (obj 40) x 2000
     - **PLT count:** PLT (obj100) x15000
     - **MCV →** micro/macro
     - **MCHC →** hypo/hyper
     - HCT= Hbx3 / Hb= RBCx3 / HCT=RBCx9

  2. Correction of blood count changes with clinical events

  For example, an unexpectedly higher or lower Hb might be explained by blood transfusion or haemorrhage, respectively.



Quality assurance programme

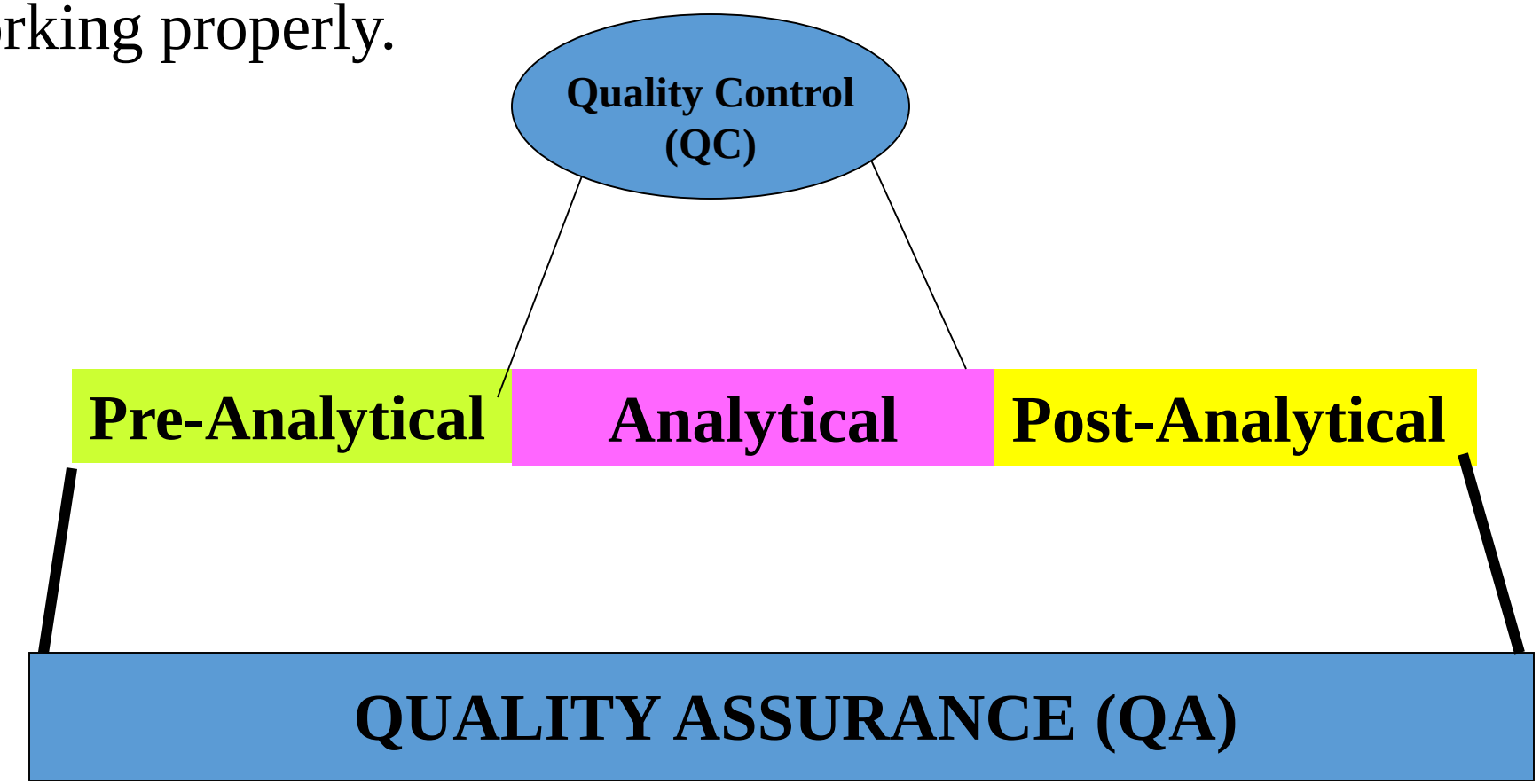
- **Daily**
  - Daily cleaning
  - Background counts
    - ✓ RBC: < 0/03 x 10^{12}/l
    - ✓ WBC: < 0/04 x 10^{9} / l
    - ✓ Hemoglobin: <0/2g/dl
    - ✓ Platelets: <5 x 10^{9} /l
  - Test on control specimen, control chart
  - Duplicate test s on a few of patients Spec.
  - Check test on a few patients from a previous batch
  - Delta test
  - Daily mean
  - Calibration Verification (T- Birittin)

Internal Quality Control (IQC)

- based on monitoring the hematology tests procedures that are performed in the lab
- includes measurements on specially prepared materials, and repeated measurements on routine specimens, statistical analysis and day by day data obtained from the tests which have been routinely carried out
- is intended to ensure that there is continual evaluation of the reliability of the work of the lab and that control is exercised over the release of the results
- it is primarily a check of precision but not necessarily accuracy

Quality Control (QC)

- measures that must be included during each assay to verify that the test is working properly.



Implementing a QC Program –Quantitative Tests

- Select high quality controls
- Collect at least 20 control values over a period of 20-30 days for each level of control
- Perform statistical analysis
- Develop Levey-Jennings chart
- Monitor control values using the Levey-Jennings chart and/or Westgard rules
- Take immediate corrective action, if needed
- Record actions taken

Establishing QC Ranges

- Each laboratory must establish a test-specific acceptable range of quality control values for each type of analyzer.
- If the QC results are out of range, investigate if there is a problem with the control material. Try a new vial or lot number of the commercial product.
- Laboratories are required to verify control mean and expected ranges when using commercial controls.

Standard Deviation and Probability

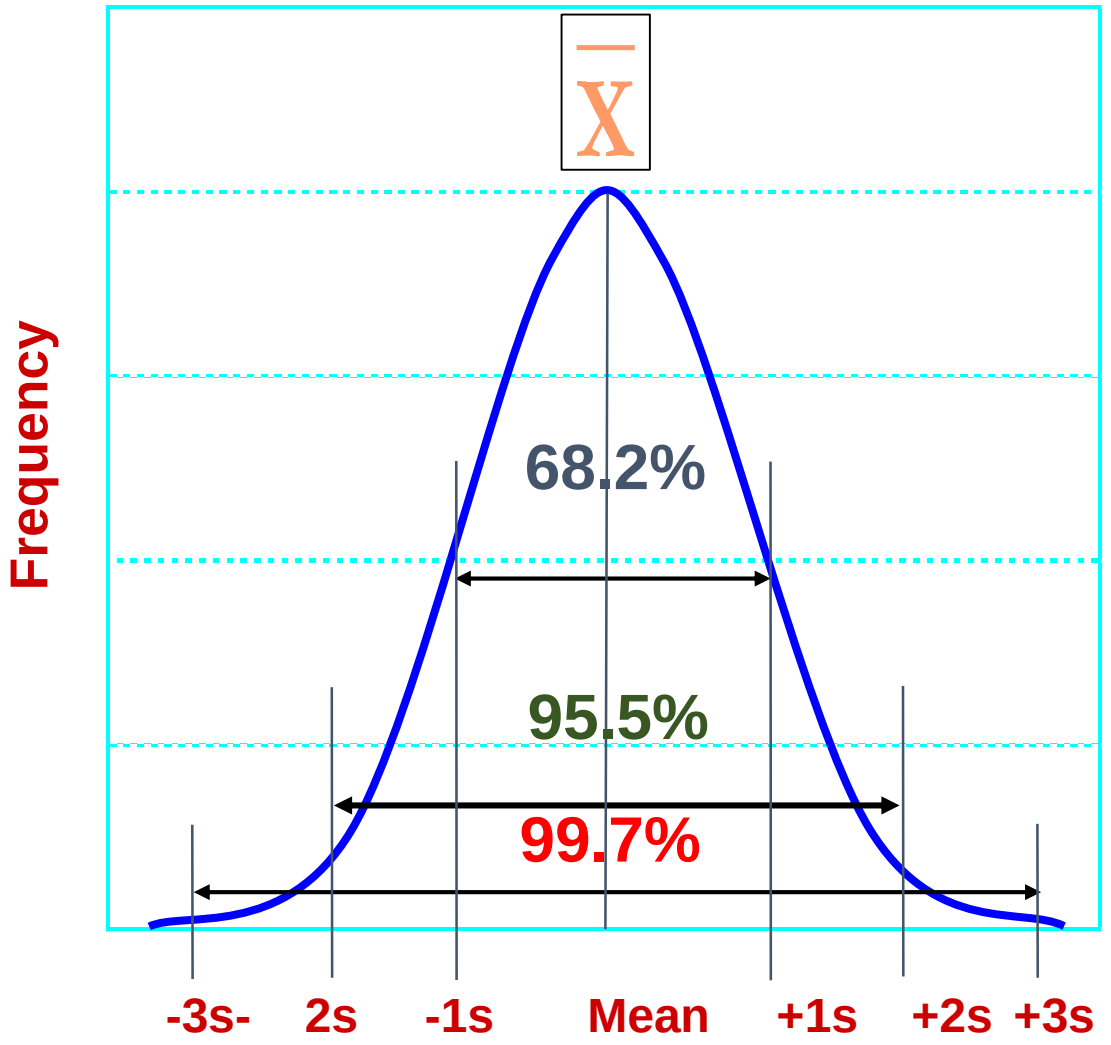
- In general, laboratories use the +/- 2 SD criteria for the limits of the acceptable range for a test
- When the QC measurement falls within that range, there is 95.5% confidence that the measurement is correct
- Only 4.5% of the time will a value fall outside of that range due to chance; more likely it will be due to error

Levey-Jennings Chart

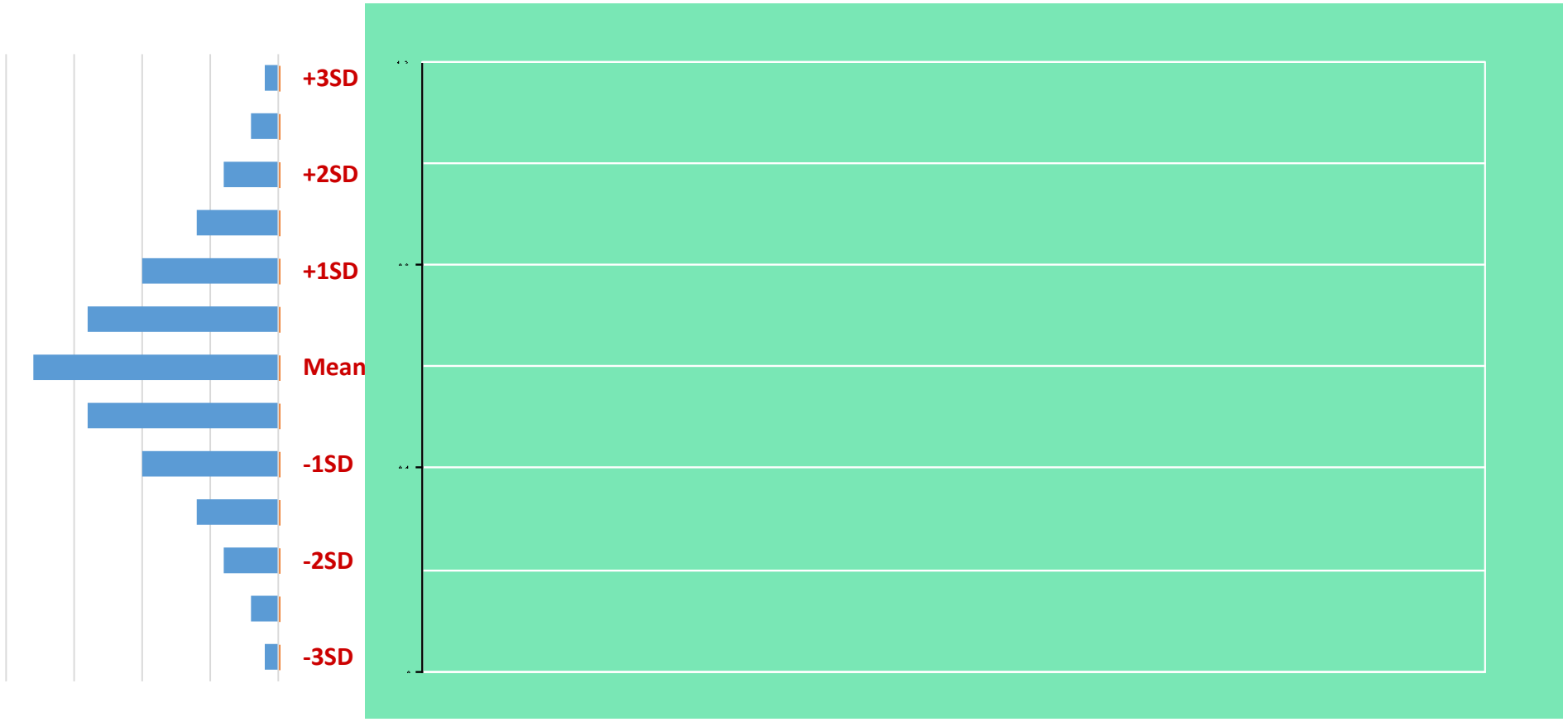
- A graphical method for displaying control results and evaluating whether a procedure is in-control or out-of-control
- Control values are plotted versus time
- Lines are drawn from point to point to accent any trends, shifts, or random excursions

Standard Deviation and Probability

- For a set of data with a normal distribution, a value will fall within a range of:
  - +/- 1 SD 68.2% of the time
  - +/- 2 SD 95.5% of the time
  - +/- 3 SD 99.7% of the time

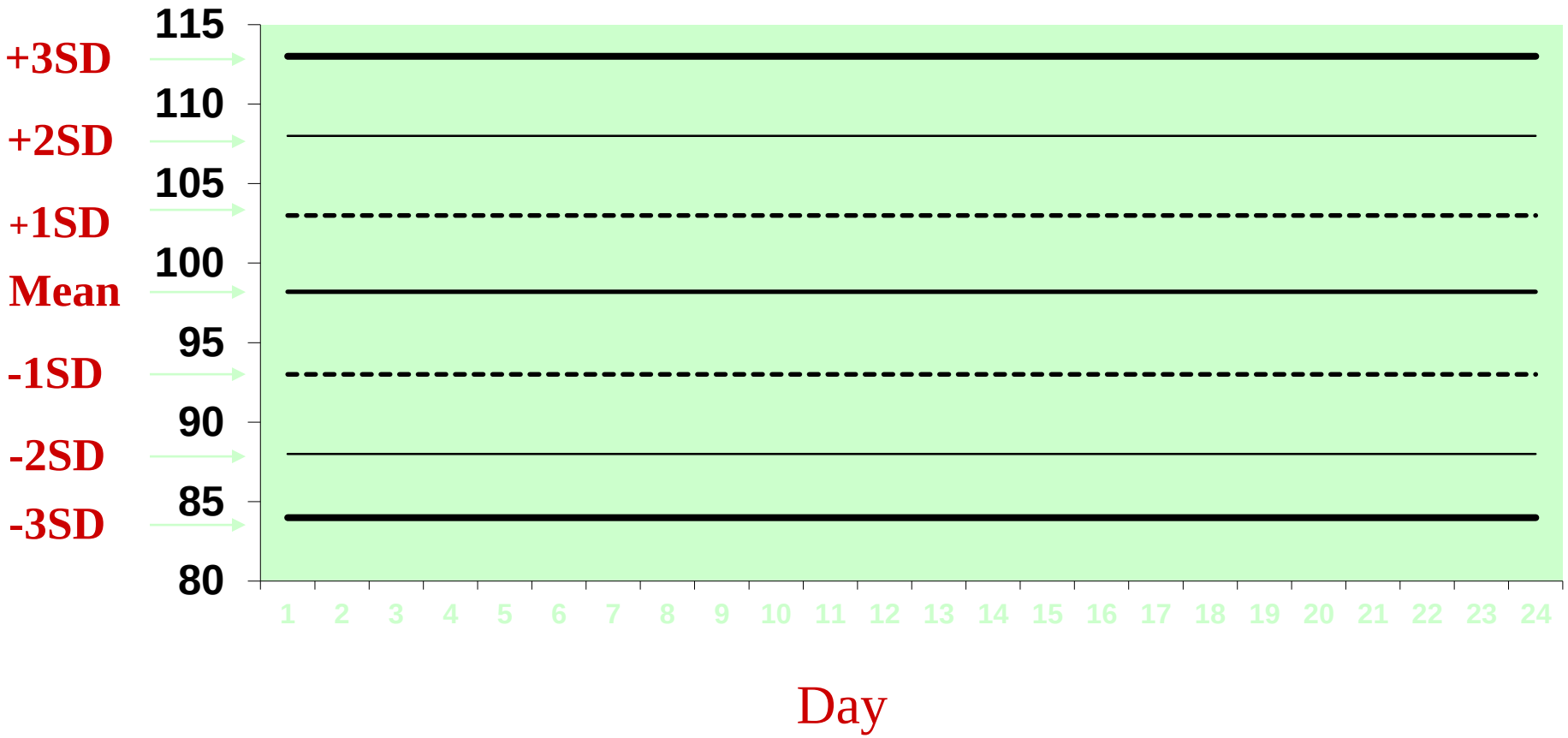


Leveay-Jennings Chart

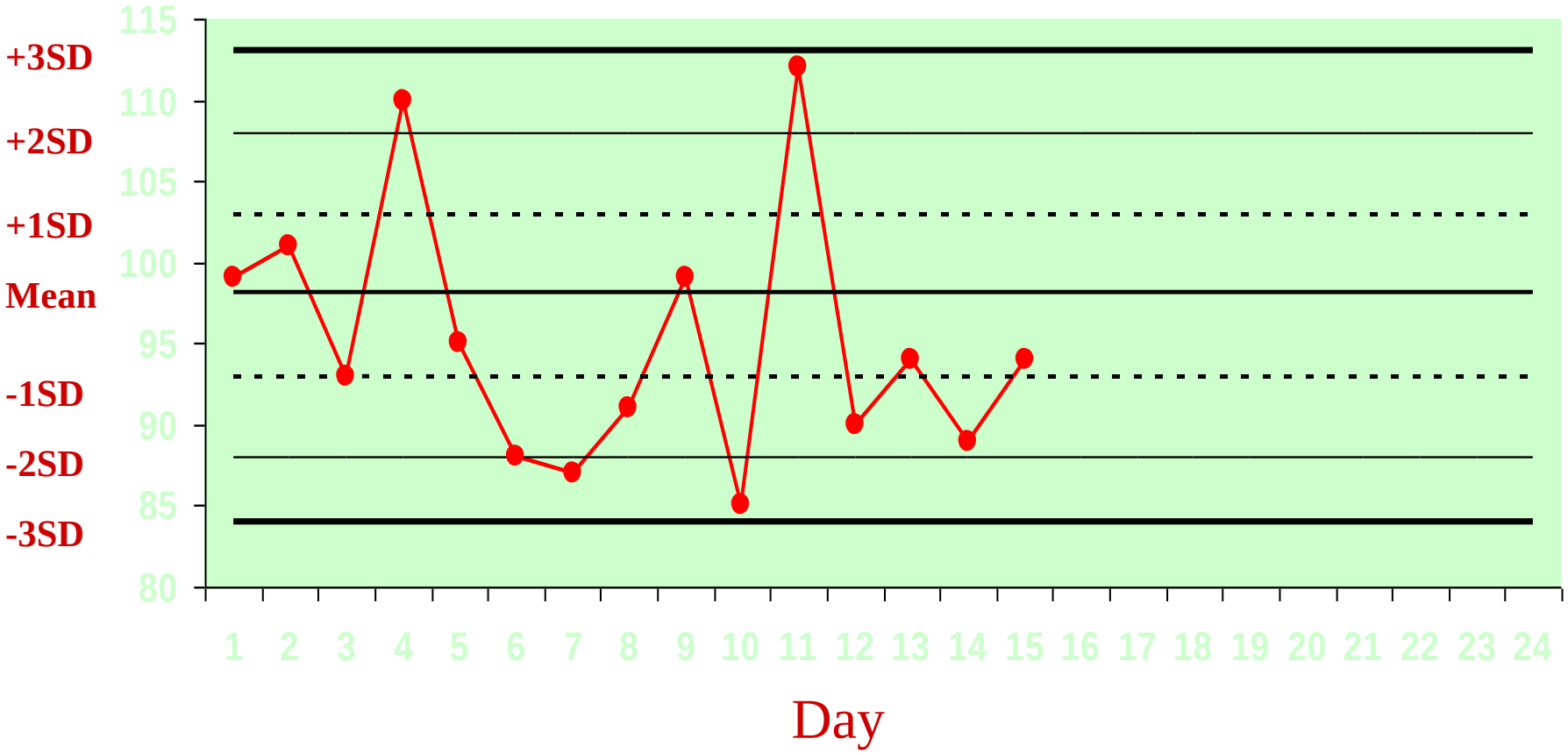


Levey-Jennings Chart

Calculate the Mean and Standard Deviation;
Record the Mean and +/- 1,2 and 3 SD Control Limits



Levey-Jennings Chart -
Record and Evaluate the Control Values

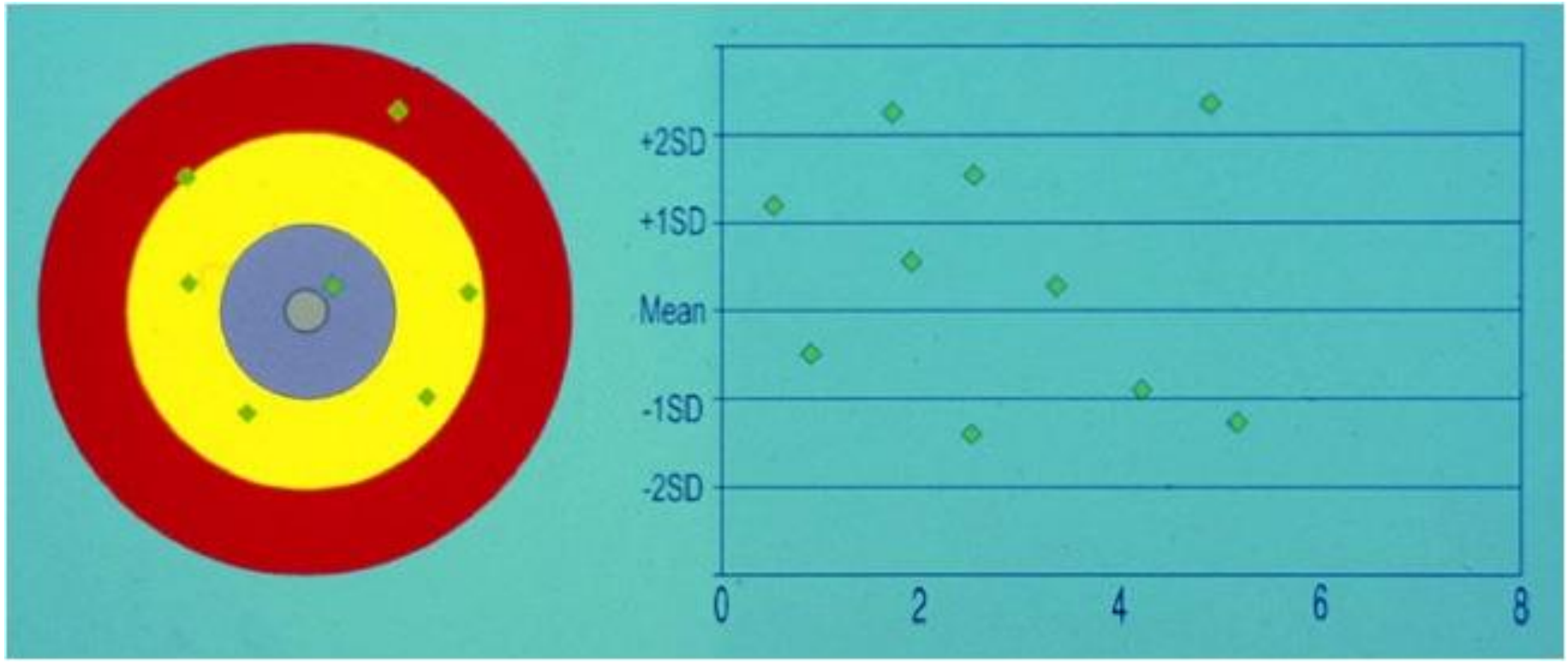


Accuracy and Precision

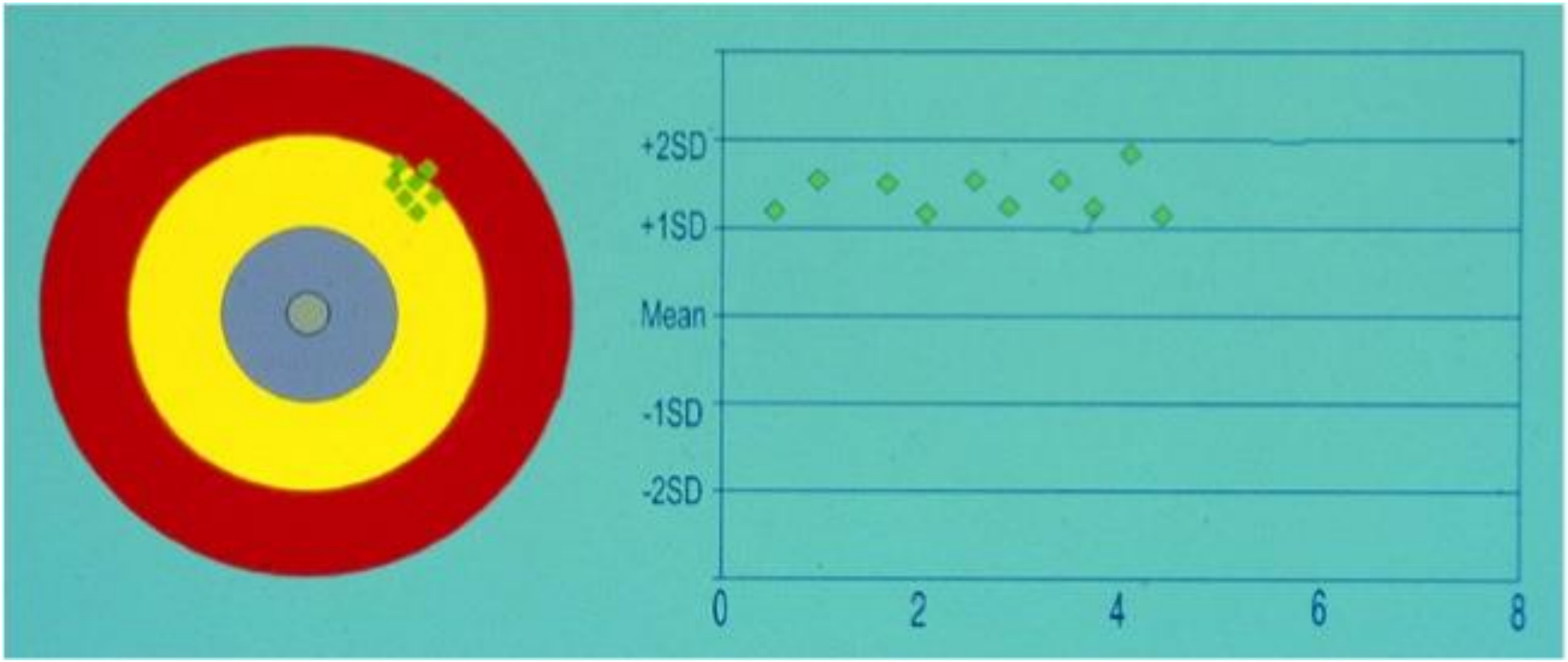
- The degree of fluctuation in the measurements is indicative of the "precision" of the assay. (reproducibility)
  - can be controlled by replicate tests, check tests on previously measured specimens and statistical evaluation of results
  - The quantification of precision is measured by CV , Indicator of Random error

- The closeness of measurements to the true value is indicative of the "accuracy" of the assay. can be checked by the use of reference materials
  - The quantification of accuracy is measured by mean
- Quality Control is used to monitor both the precision and the accuracy of the assay in order to provide reliable results.

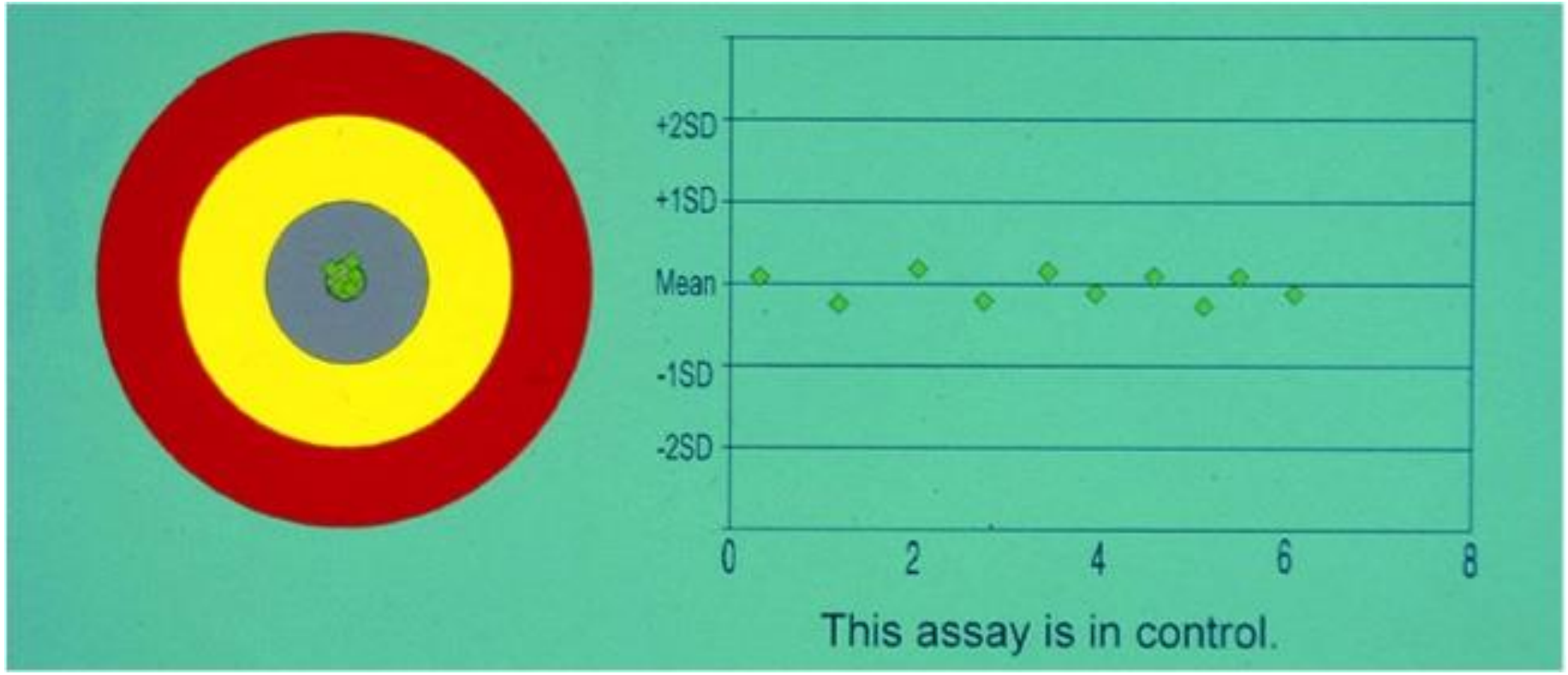
The following examples demonstrate some of these principles. The results are replicate samples assayed at different time points.



This test is both inaccurate and imprecise



This assay is inaccurate but precise. The assay is said to have a positive 'BIAS'



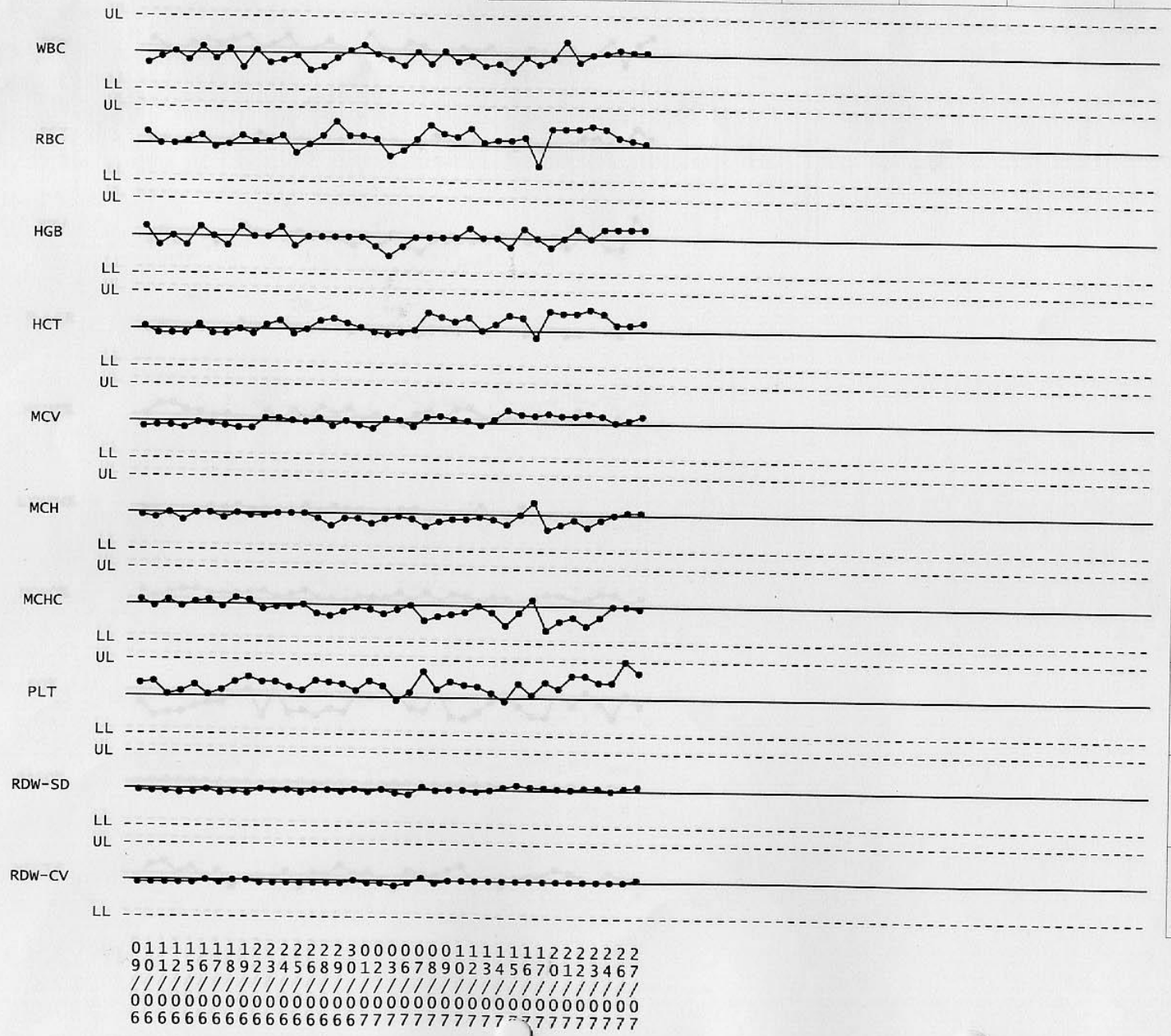
This assay is both accurate [i.e. no positive or negative bias] and precise [i.e. very little scatter of results about the mean value)

XE-2100 Quality Control

Instrument ID	Level(Lot)	Lot No.	Exp. Day	Date From	To
XE-2100-1	Level2(Current)	QC-31320802	27/07/2003	09/06/2003	27/07/2003

Material:e-CHECK Number Of Plots:38
Mode:Closed

Print Date:27/07/2003
Time:16:10:42



UL=	7.14	SD=	0.105
Targ.=	6.61	Mean=	6.56
LL=	6.08	CV=	1.6
UL=	4.54	SD=	0.032
Targ.=	4.41	Mean=	4.43
LL=	4.28	CV=	0.7
UL=	13.4	SD=	0.08
Targ.=	13.0	Mean=	13.0
LL=	12.6	CV=	0.6
UL=	39.5	SD=	0.38
Targ.=	37.8	Mean=	38.0
LL=	36.1	CV=	1.0
UL=	89.1	SD=	0.51
Targ.=	85.7	Mean=	85.8
LL=	82.3	CV=	0.6
UL=	31.0	SD=	0.21
Targ.=	29.5	Mean=	29.3
LL=	28.0	CV=	0.7
UL=	36.1	SD=	0.34
Targ.=	34.4	Mean=	34.2
LL=	32.7	CV=	1.0
UL=	235	SD=	5.1
Targ.=	212	Mean=	220
LL=	189	CV=	2.3
UL=	46.9	SD=	0.31
Targ.=	42.6	Mean=	42.5
LL=	38.3	CV=	0.7
UL=	15.1	SD=	0.08
Targ.=	13.7	Mean=	13.7
LL=	12.3	CV=	0.6

Westgard Rules

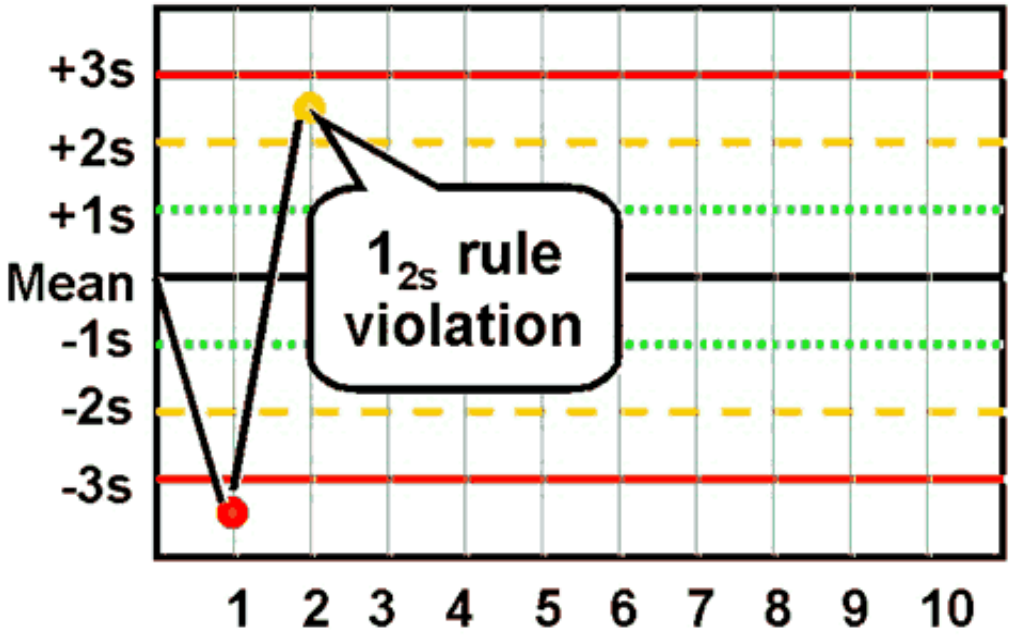
(Generally used where 2 levels of control material are analyzed per run)

- 1_{2S} rule
- 1_{3S} rule
- 2_{2S} rule
- R_{4S} rule
- 41S rule
- 10X rule
- 8x rule
- 12X rule

The preceding control rules are usually used with N's of 2or 4, which means they are appropriate when two different control materials are measured 1 or 2 times per material.

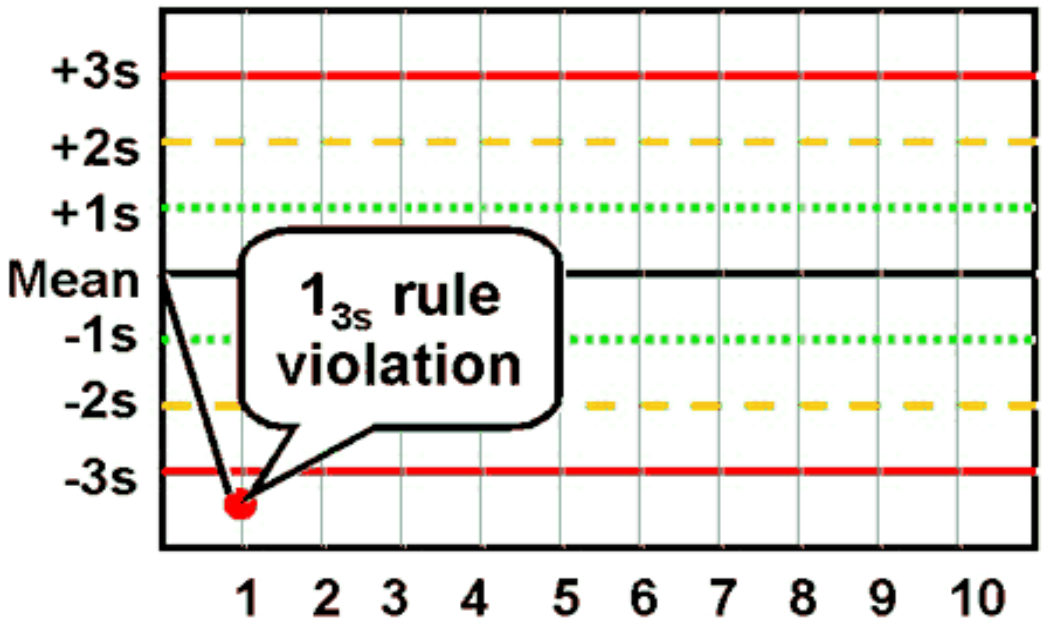
Westgard – 1_{2S} Rule

- "warning rule"
- One of two control results falls outside ±2SD
- Alerts tech to possible problems
- Not cause for rejecting a run
- Must then evaluate the 1_{3S} rule



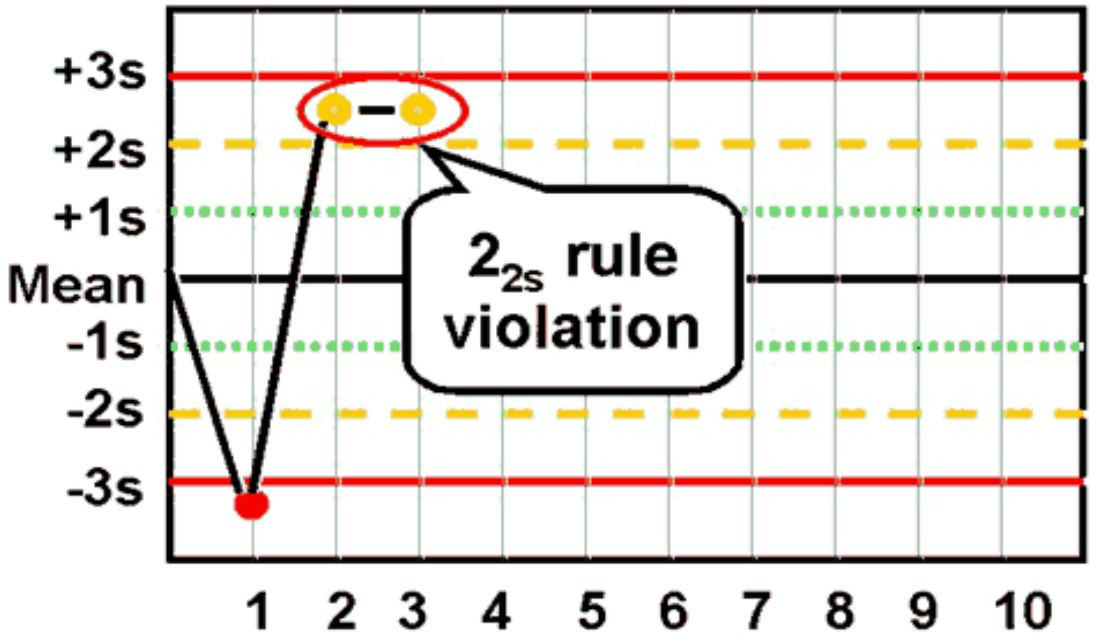
Westgard – 1_{3S} Rule

- If either of the two control results falls outside of ±3SD, rule is violated
- Run must be rejected
- If 1_{3S} not violated, check 2_{2S}



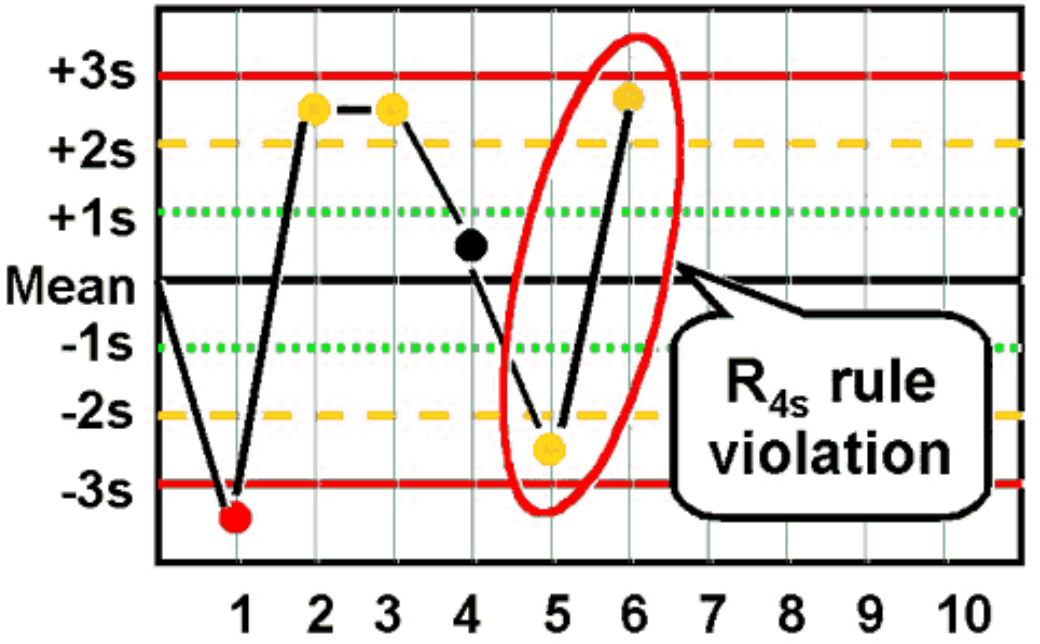
Westgard – 2_{2S} Rule

- 2 consecutive control values for the same level fall outside of ±2SD in the same direction, or
- Both controls in the same run exceed ±2SD
- Patient results cannot be reported
- Requires corrective action



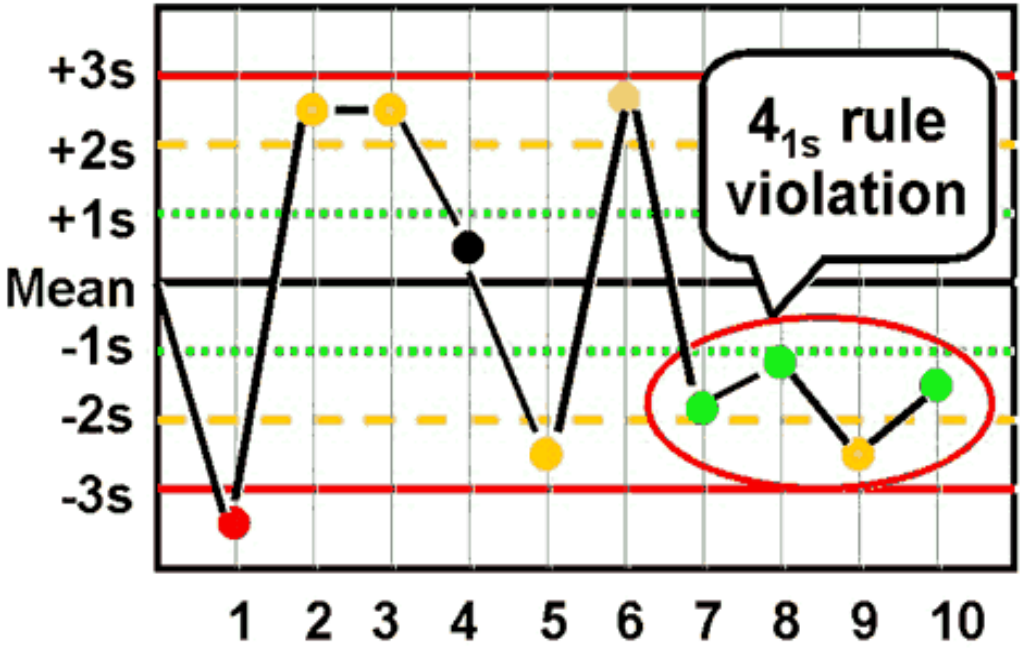
Westgard – R_{4S} Rule

- One control exceeds the mean by –2SD, and the other control exceeds the mean by +2SD
- The range between the two results will therefore exceed 4 SD
- Random error has occurred, test run must be reiected



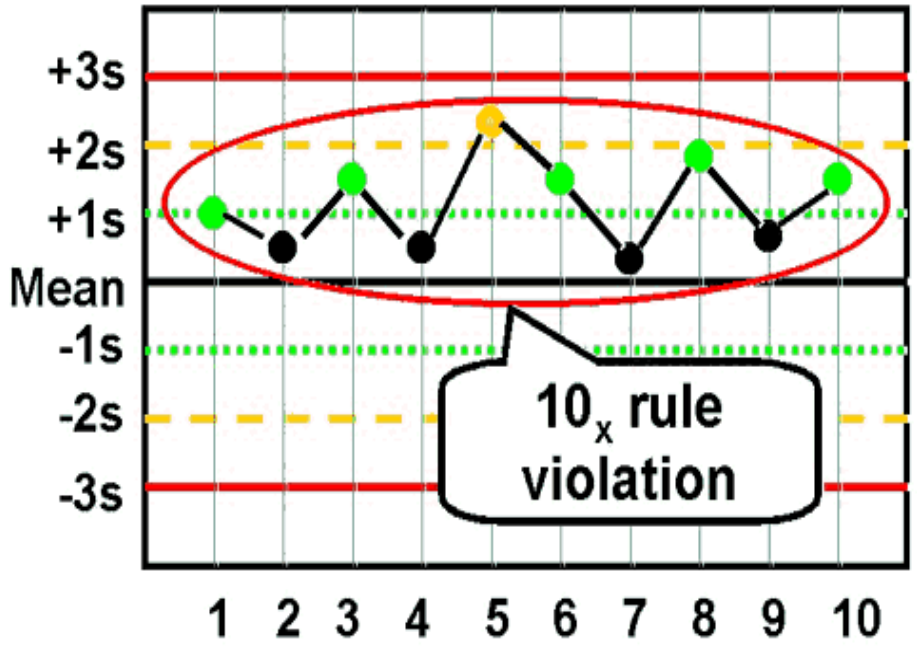
Westgard – 4_{1S} Rule

- Requires control data from previous runs
- Four consecutive QC results for one level of control are outside ±1SD, or
- Both levels of control have consecutive results that are outside ±1SD



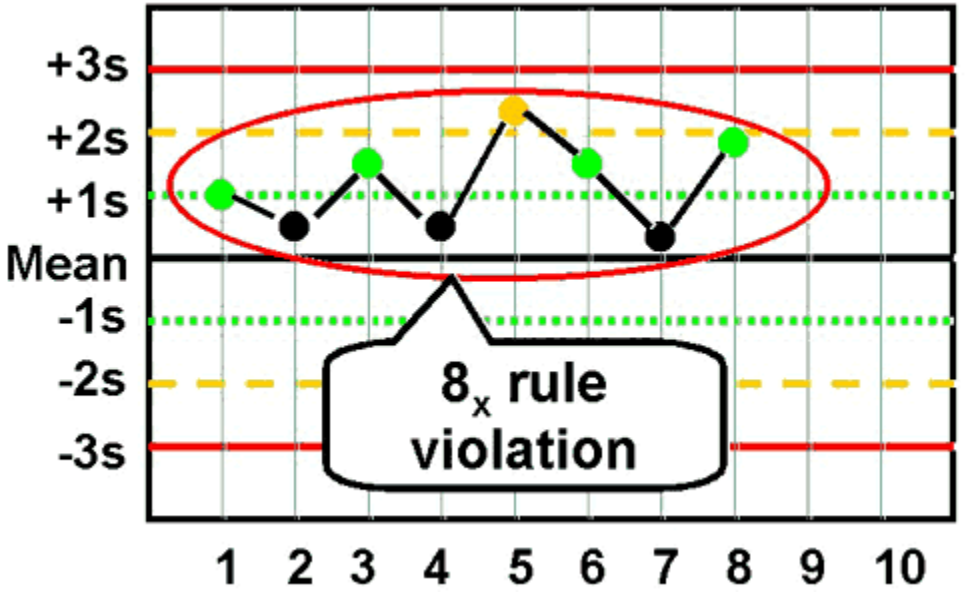
Westgard – 10_X Rule

- Requires control data from previous runs
- Ten consecutive QC results for one level of control are on one side of the mean, or
- Both levels of control have five consecutive results that are on the same side of the mean



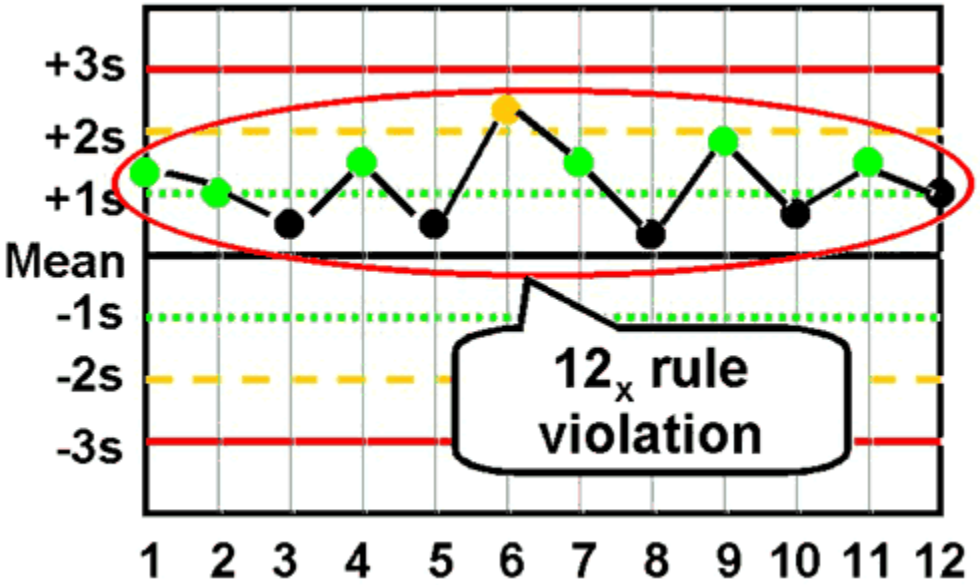
Westgard – 8_X Rule

- reject when 8 consecutive control measurements fall on one side of the mean.

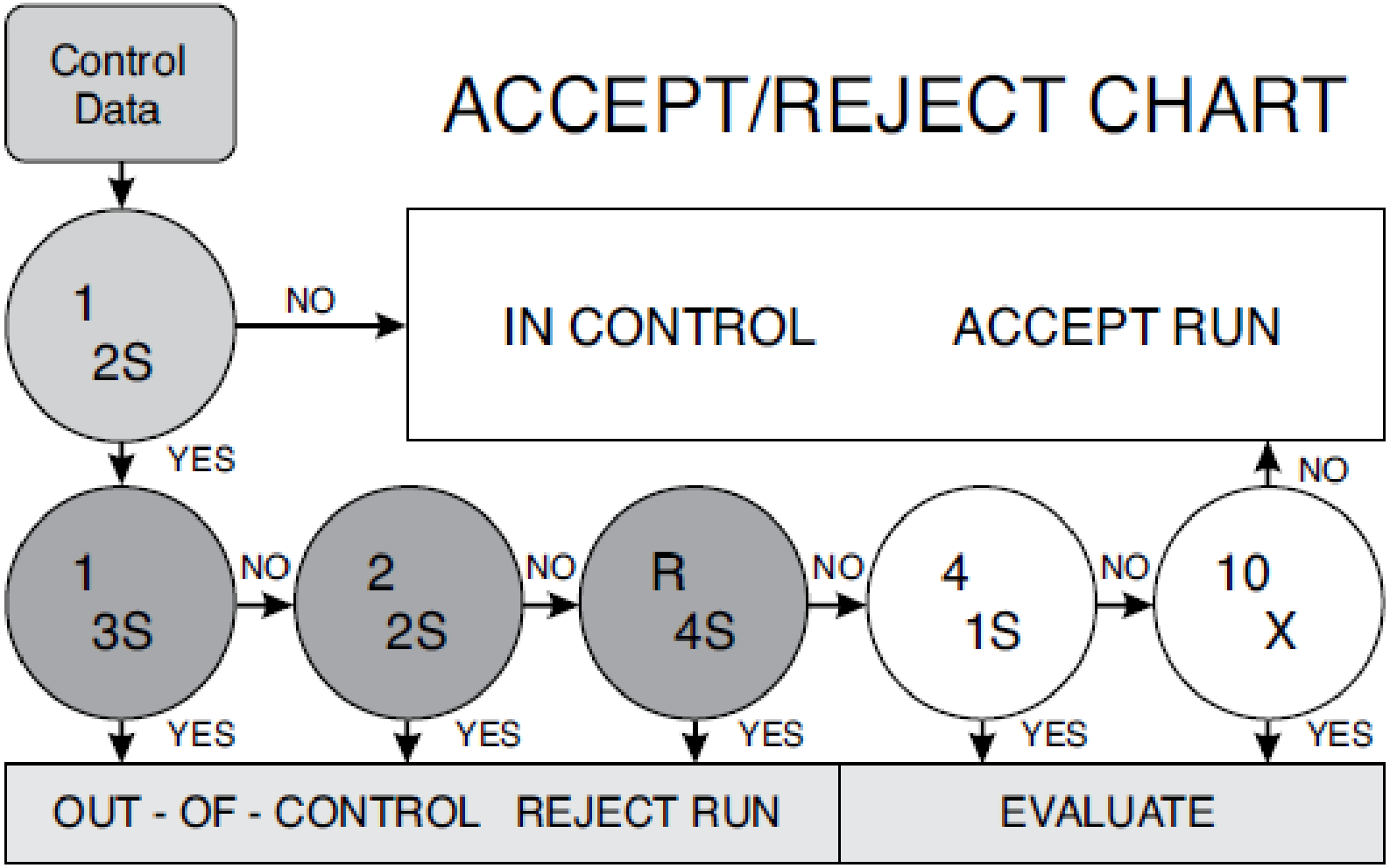


Westgard -12_x Rule

- reject when 12 consecutive control measurements fall on one side of the mean.



Westgard Multirule QC



In situations where 3 different control materials are being analyzed, some other control rules fit better and are easier to apply, such as:

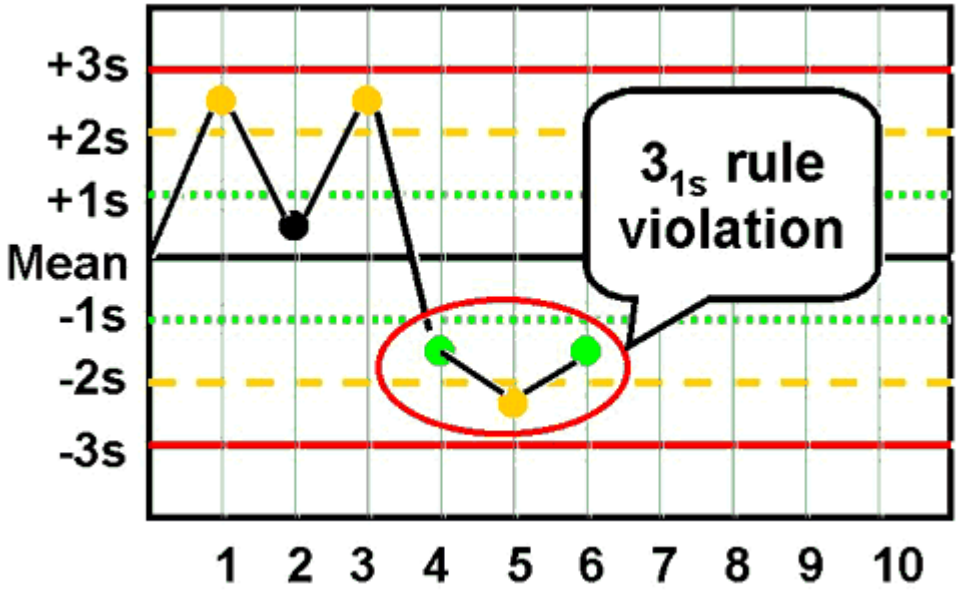
- **$2of3_{2s}$**
- **3_{1s}**
- **6_{x}**
- **9_{x}**

Westgard - 2 of 3_{2s} Rule

- reject when 2 out of 3 control measurements exceed the same mean plus 2s or mean minus 2s control limit;

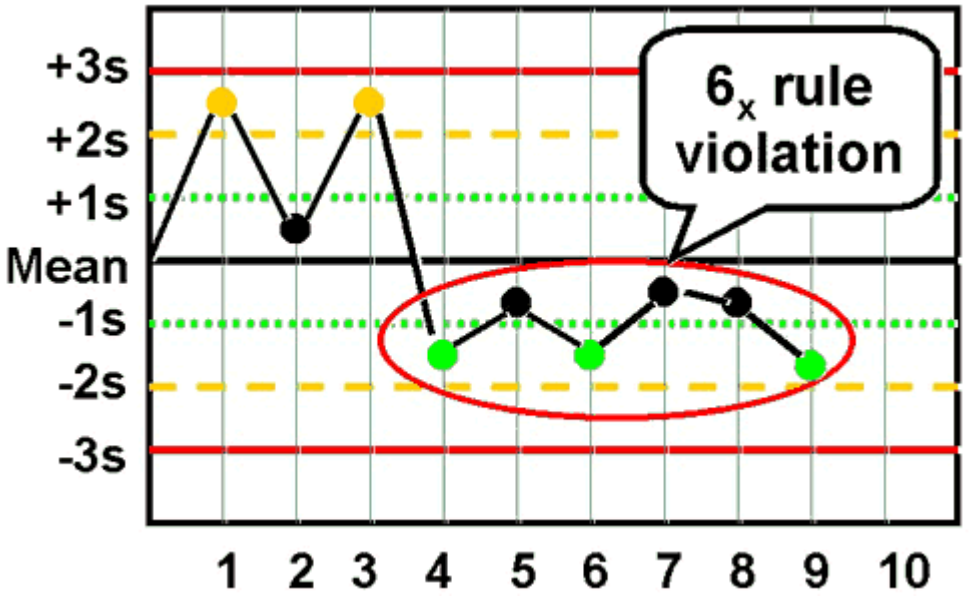
Westgard -3_{1s} Rule

- reject when 3 consecutive control measurements exceed the same mean plus 1s or mean minus 1s control limit.



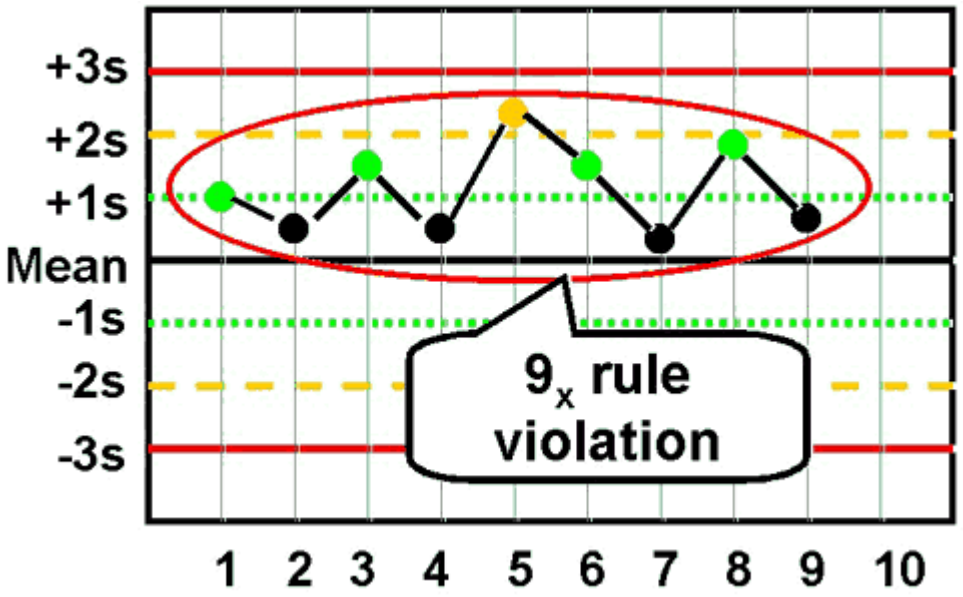
Westgard - 6_x Rule

- reject when 6 consecutive control measurements fall on one side of the mean.



Westgard - 9_x Rule

- reject when 9 consecutive control measurements fall on one side of the mean.



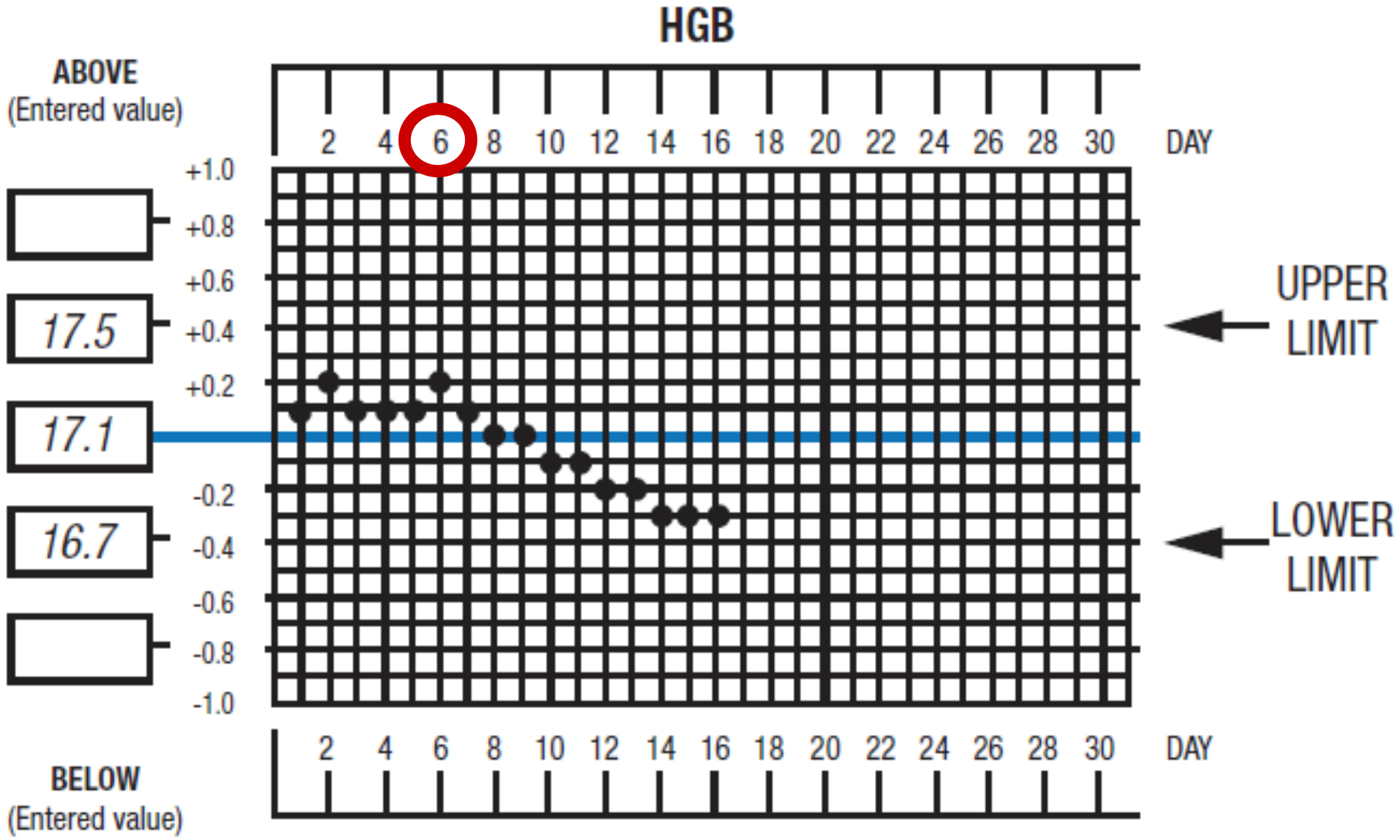
Westgard - 7_T Rule

- A related control rule that is sometimes used, particularly in Europe, looks for a "trend" where several control measurements in a row are increasing or decreasing [note: it is increasingly rare to see this rule in use]:
- reject when seven control measurements trend in the same direction, i.e., get progressively higher or progressively lower.

Trend

ABNORMAL HIGH CONTROL

HGB assigned value = 17.1
Expected range =± 0.4



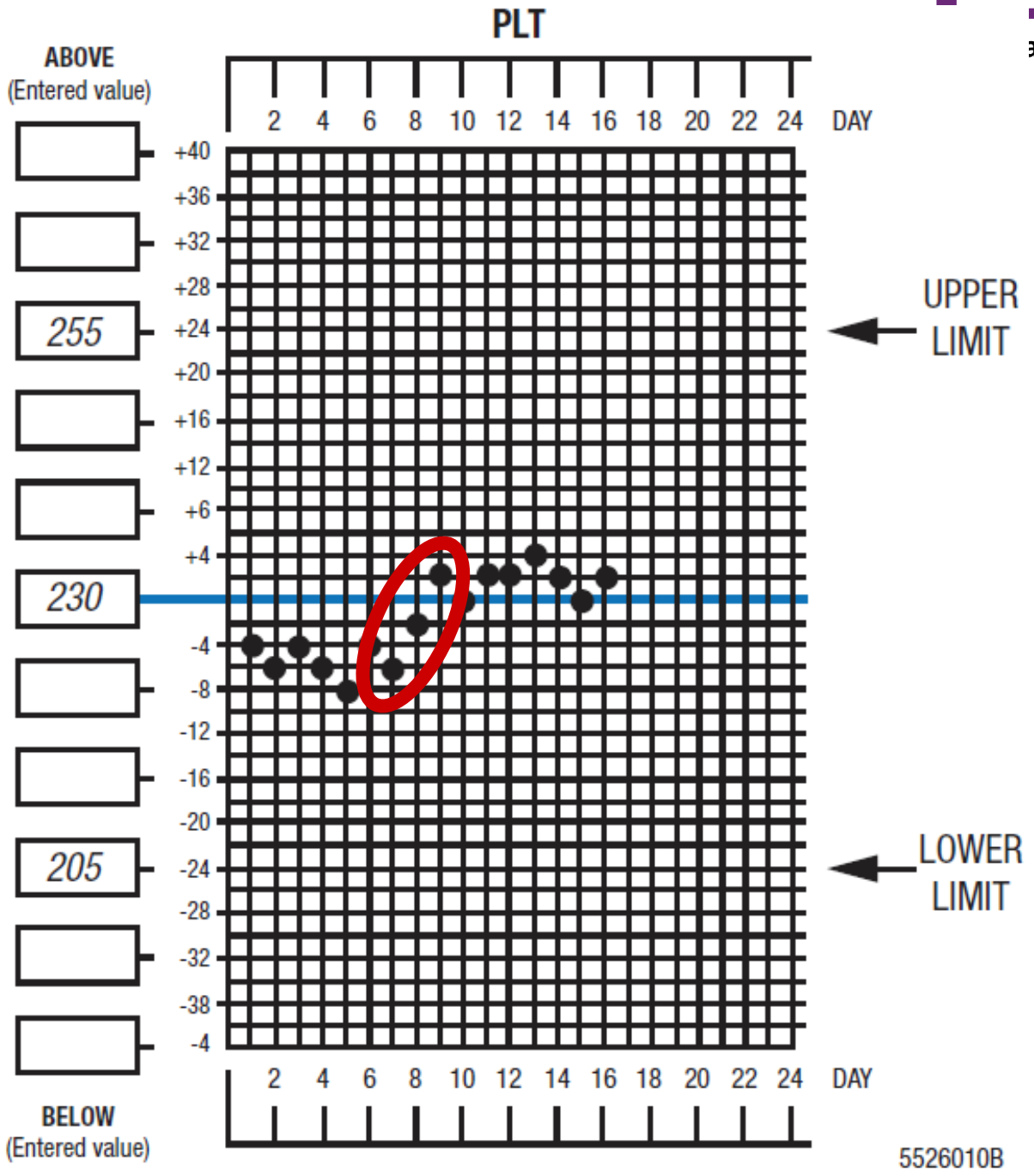
- Because hematology controls are cell-based, some trending in sizing parameters can be expected. As stated in the Storage and Stability section of the control product insert, “The MCV, RDW and/or RDW-SD parameters may trend up through the product’s shelf-life. This is inherent to the product and should not be considered an indicator of product instability. Recovered values for these parameters should remain within the Expected Range.”
- If an unexpected trend is noticed:

1. Record the information on your corrective action log.

2. Troubleshoot the problem(s) and remedy the situation prior to running patient samples.

Shift

- A shift occurs when there is a one run or day to the next.



NORMAL CONTROL

PLT assigned value = 230
Expected range =± 25

- A shift does not always mean that a problem exists. If the system was calibrated or some troubleshooting was performed before running the control, a shift could occur.
- If a shift is noticed:

1. Record the information on your corrective action log.

2. Troubleshoot the problem(s) and remedy the situation prior to running patient samples.

When a rule is violated

- Warning rule = use other rules to inspect the control points
- Rejection rule = “out of control”
  - Stop testing
  - Identify and correct problem
  - Repeat testing on patient samples and controls
  - Do not report patient results until problem is solved and controls indicate proper performance
  - After troubleshooting, record the corrective action in the comments section of your control files, or in a correction action log sheet.

- To record a Corrective Action, enter:

1. The date of the occurrence.
2. The condition (such as: WBC low on 4C PLUS Normal).
3. The lot number and expiration date.
5. The action performed, such as “repeated-back in range.”
6. The initials of the person who performed the corrective action.

Errors

- Systema… &trends) – affect the accuracy of system
  - Usua…
  - Can …
    - …dequate storage o…
    - deterioration of reagent or calib… …e in incubator unit,
    - deterioration of photometric light source

Systemic errors are most often related to reagent or calibration problems

Errors

- Random errors-these errors affect the the reproducibility of precision of a test system.
  - Usually 1_{2S} , 1_{3S} , R_{4S} Rules
  - Can be due to:
    - bubbles in reagents& reagent lines ,
    - Inadequately mixed reagents
    - unstable Tem. &incubation,
    - individual operator variation in pipetting….

Duplicate tests on patients' specimens

- Duplicate tests on patients' specimens provide another way of checking the precision of routine work.
- To start the process, test 5 consecutive specimens in duplicate under careful conditions.
- Calculate the differences between the pairs of results and derive the SD Subsequent duplicate measurements on any specimen in the same batch of tests should not differ from each other by more than ±2SD.
- This method will detect random errors but not incorrect calibration.

Duplicate test

Specimen	1st count	2st count	d	d2
1	5.4	5.8	0.4	0.16
2	8.3	10.5	2.2	4.84
3	17.2	18	0 .8	0.64
4	5.4	5.4	0	0
5	12.2	11.8	0.4	0.16

∑d2= 5.8

SD= √ d2/2n

SD= 0.76 **2SD=1.5**

✓none of the duplicate tests should differ from each other more than 2SD

✓**d>2SD → Random Error**

Check test

- Similar to duplicate test but use specimen with have been measure originally in an earlier batch
- The test should agree with each other with ±2SD
- Detection deterioration of apparatus and reagent between tests
- Suitable for Hb &RBC less for WBC &PLT

Delta test

- Delta checking can also be used as a quality control method. It is a means of checking to see if certain parameters are within a user-defined range when the parameter results of two samples from the same patient are compared.
- Comparison of current results with a recent previous result on the same patient

Parameter	Difference
Hb	2 g/dL
PCV	0.05 L/L
MCV	> 6 fL
MCH	> 5 pg
WBC	Change normal to abnormal
PLT	Increase or Decrease > 50 %

Calibration (adjust the accuracy)

- Calibration is the process used to adjust the accuracy of the instrument being used. It requires the use of a calibrator, such as Sysmex -CAL. Calibration is an essential part of the hematology laboratory's quality assurance program. Before performing a calibration, know what to expect as an outcome.
- به مجموعه فعالیت هایی اطلاق می شود که ارتباط میان مقادیر اندازه گیری شده یک کمیت توسط یک دستگاه یا روش آزمایشگاهی را با مقادیر واقعی آن ماده که توسط روش های مرجع اندازه گیری شده است مشخص نماید.

Calibration Verification

- با توجه به پایداری پارامتر ها و اندکس های خون محیطی به مدت ۲۴ ساعت در دمای ۴ درجه می توان از آزمون آماری T-Brittin جهت کنترل کیفی استفاده نمود.
- حداقل ۵ نمونه (T=2.78) و ترجیحا ۱۰ نمونه (T=2.26)

$$SD = \sqrt{\sum(d^2) - (\Sigma d)^2/n \,/n-1}$$

$$tn = \frac{d\sqrt{n}}{SD}$$

When to Calibrate

1-At installation

2-Complete changes of reagents (i.e., change in type of reagent from same vendor, or change to a different vendor)

3. After major maintenance or service

4. When recommended by the manufacturer

5. At least every six months

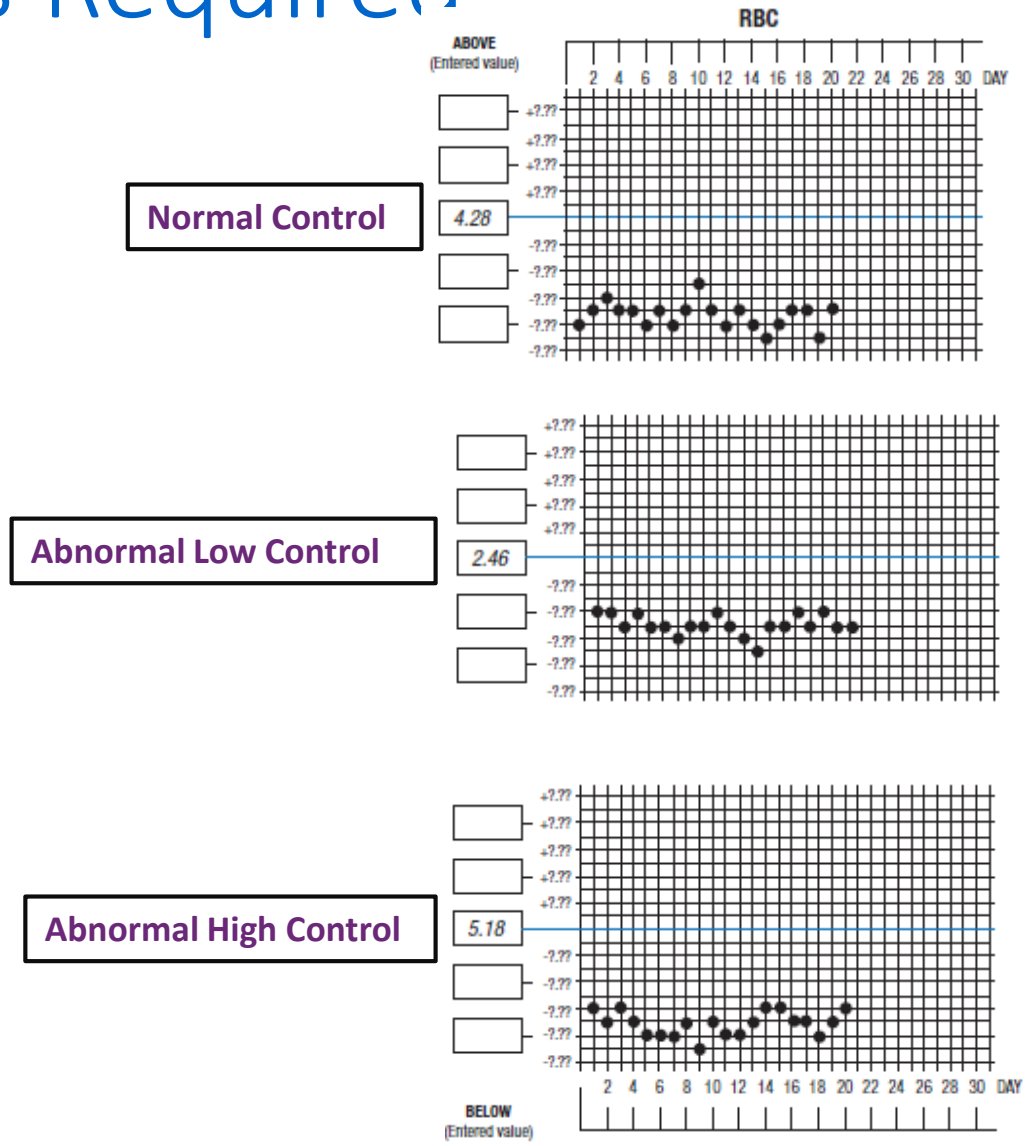
6. When indicated by quality control data

- **Calibration with Reference Standards**
  - Instruments: 6-month intervals or more frequently if control chart or EQA indicates bias or fluctuation in results and after any repair/service

Determining if Calibration is Required

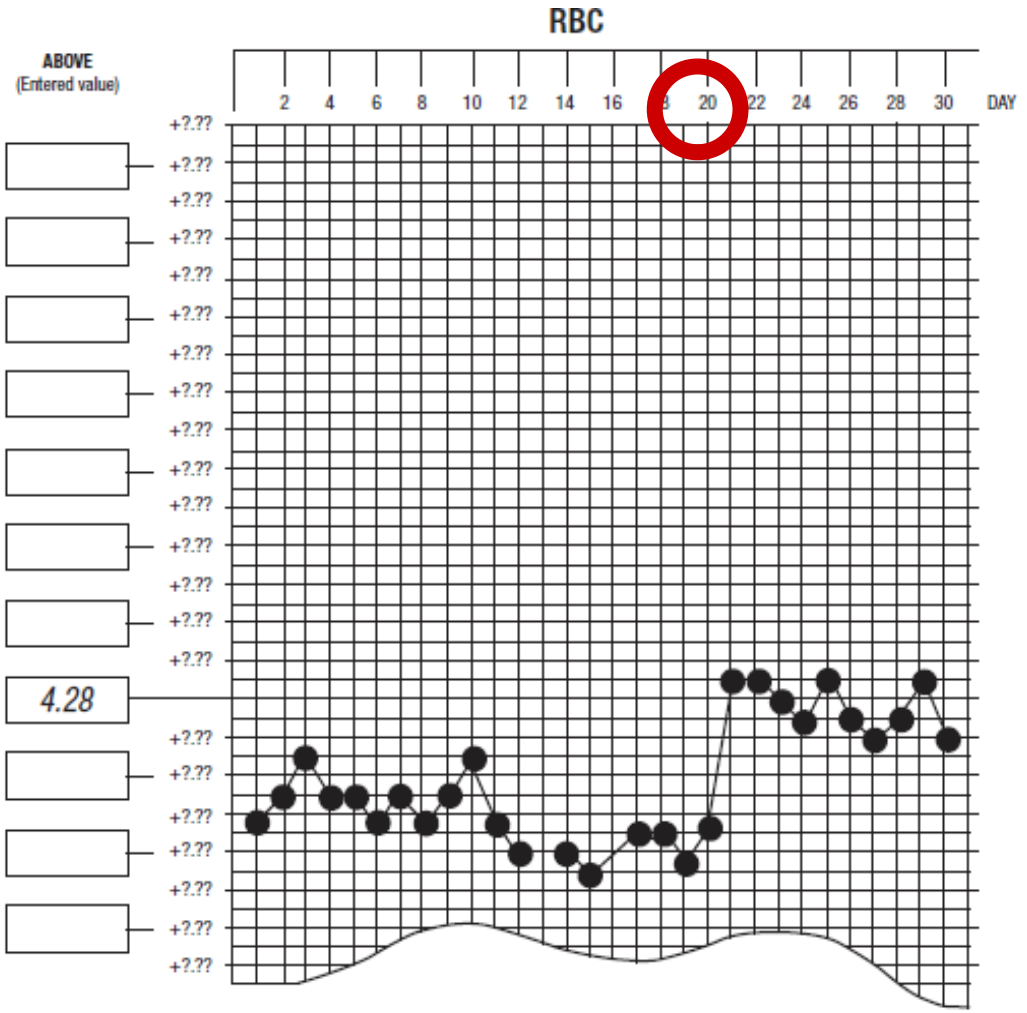
Control graphs can help determine if calibration is required.

- Figures show the graphs of all three levels of control for the RBC parameter.
- All three graphs show the same issue. The instrument is providing precise (reproducible) results; however, the results are not as accurate as they could be.



Calibration Procedure

After calibrating the instrument, you may see a shift in the control results. Calibration is a normal cause for a shift.



How

- Calibrator
- Fresh blood sample: minimum 3 sample, each duplicate with reference (manual) and current method

$$CF = \frac{\text{میانگین روش دستگاهي - میانگین روش دستي}}{\text{میانگین روش دستگاهی}} \times 100$$

- For best performance, calibrate all the CBC parameters. The WBC differential is calibrated at the factory. They do not require calibration in the laboratory

Manual method

- اندازه گیری هموگلوبین

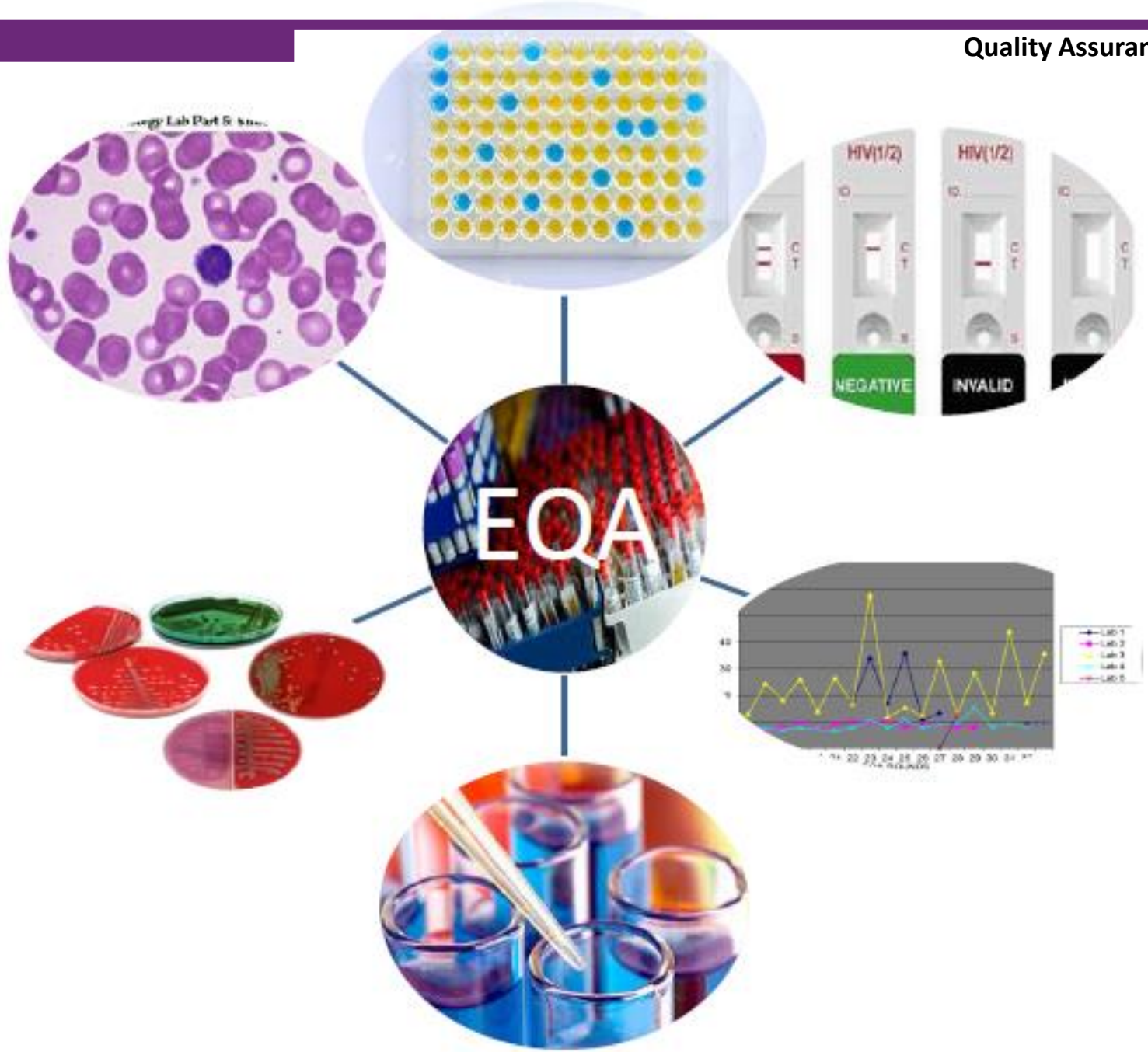
-استفاده از استاندارد تایید شده با تاریخ انقضای معتبر
-تهیه منحنی استاندارد با حداقل ۴ رقت
-استفاده از وسایل شیشه ای کلاس A ، ابزار وتجهیزات کالیبره ضروری می باشد .
- ماکرودیلوشن (۴۰ لاندا در ۱۰ میلی لیتر)

- هماتوکریت

دور سانتریفوژ، عملکرد تایمر، حداکثر توان فشردگی سلولها

- شمارش گلبول های سفید و پلاکت

استفاده از لام نئوبار


HIV(1/2)
HIV(1/2)
NEGATIVE
INVALID
EQA

Internal quality control

- Repeat measurements on routine specimens
- Measurements on specially prepared samples
- Daily statistical analysis of data - continual evaluation of the reliability of the work of the laboratory

External quality assessment

- Retrospective analysis of performance
- http://www.eqas.ir/
- http://www.eqap.ir/

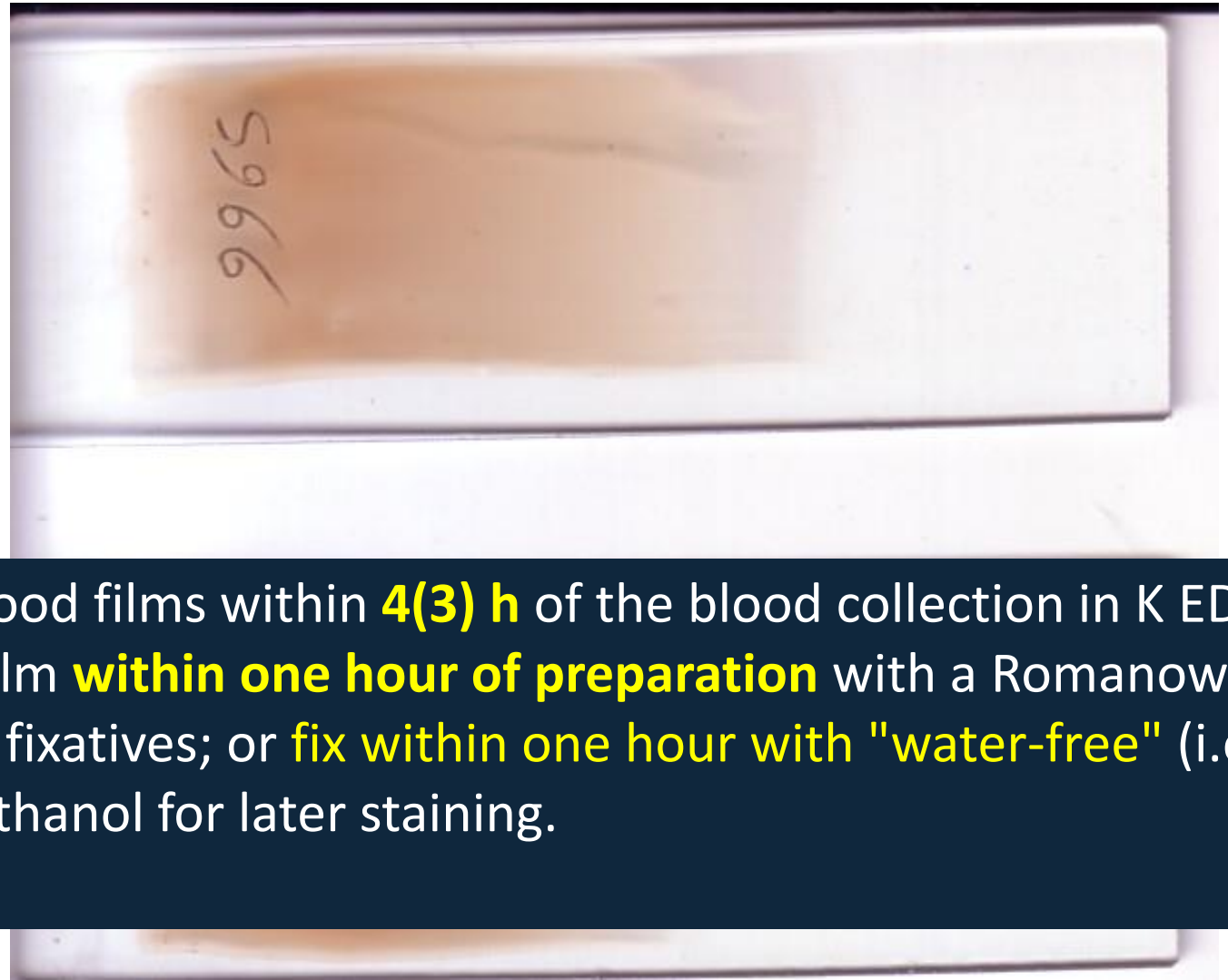
External quality assessment

- Analysis of EQA data
  - Deviation Index – same as the z score (i.e. = (result – mean)/SD) – if non-Gaussian distribution then can be calculated from the median

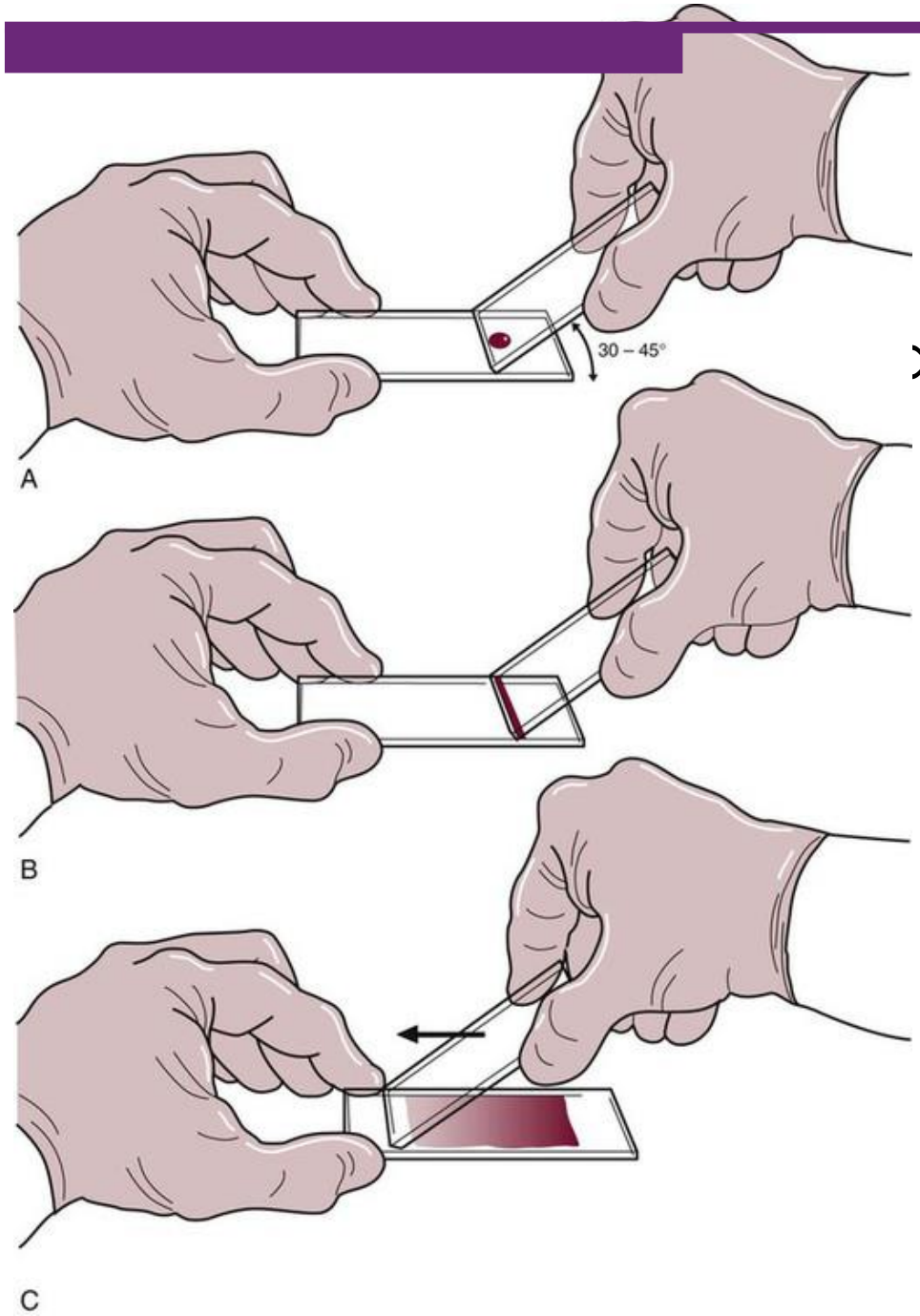
DI score	
<0.5	Excellent
0.5-1	Satisfactory
1-2	Acceptable
2-3	Calibration needs to be checked
>3	Serious error

Break Time

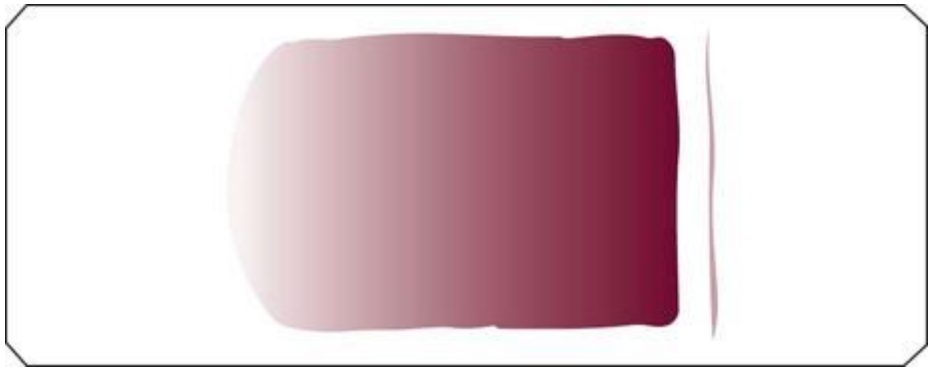
Good Peripheral Blood Smear



Prepare blood films within **4(3) h** of the blood collection in K EDTA. Stain the film **within one hour of preparation** with a Romanowsky stain, containing fixatives; or fix within one hour with "water-free" (i.e., **<3% water**) methanol for later staining.

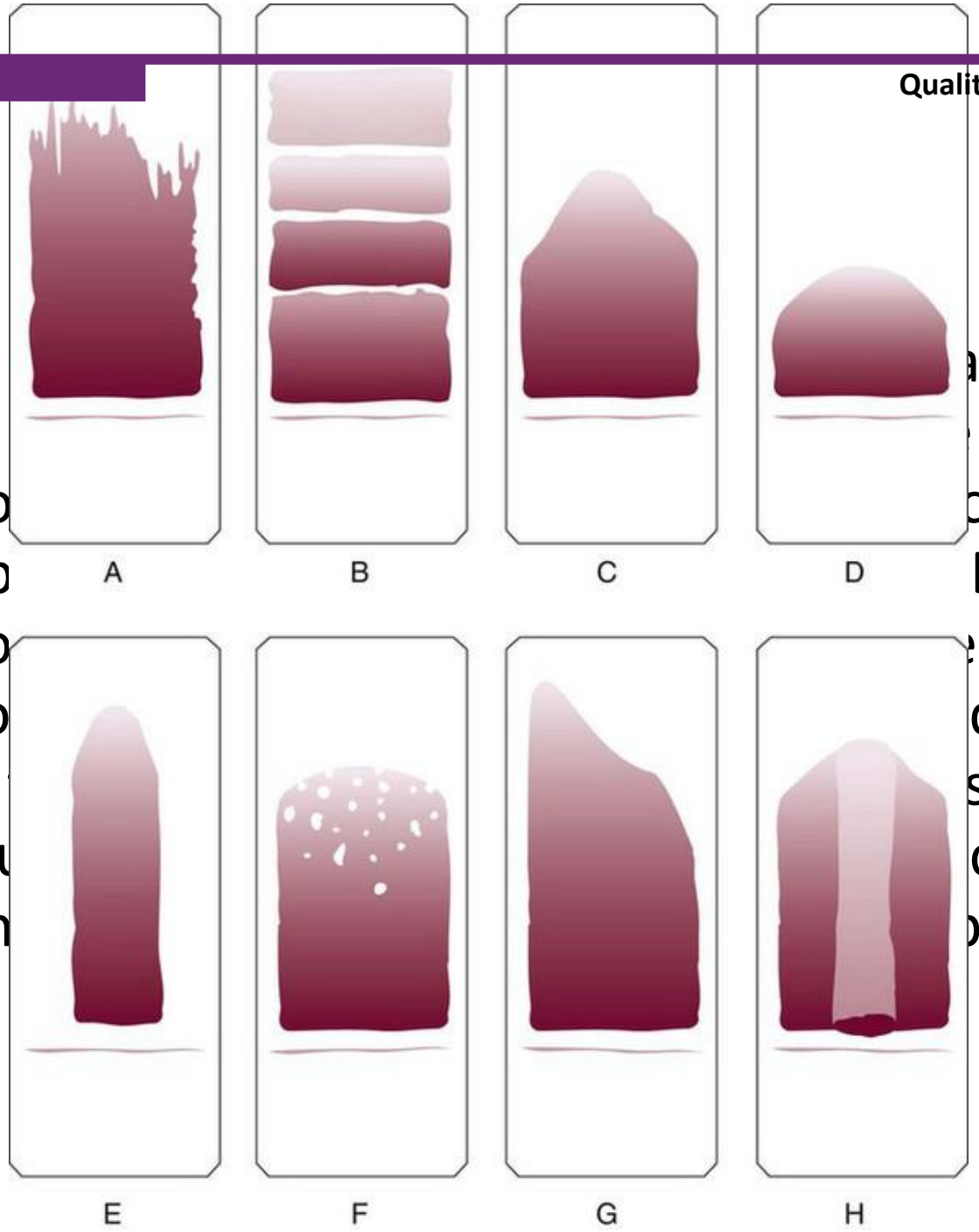


d film.



Spreader of PBS preparation

- Unacceptable ances associated with the most that a combination o cceptable films. **A,** Chipp **B,**Hesitation in forward motio pushed too quickly. **D,** Dro d not allowed to spread across se on the slide; may also be du cimen. **G,** Uneven pressure on th of blood began to dry.



Blood films made on slides

Good vs. Bad Smears

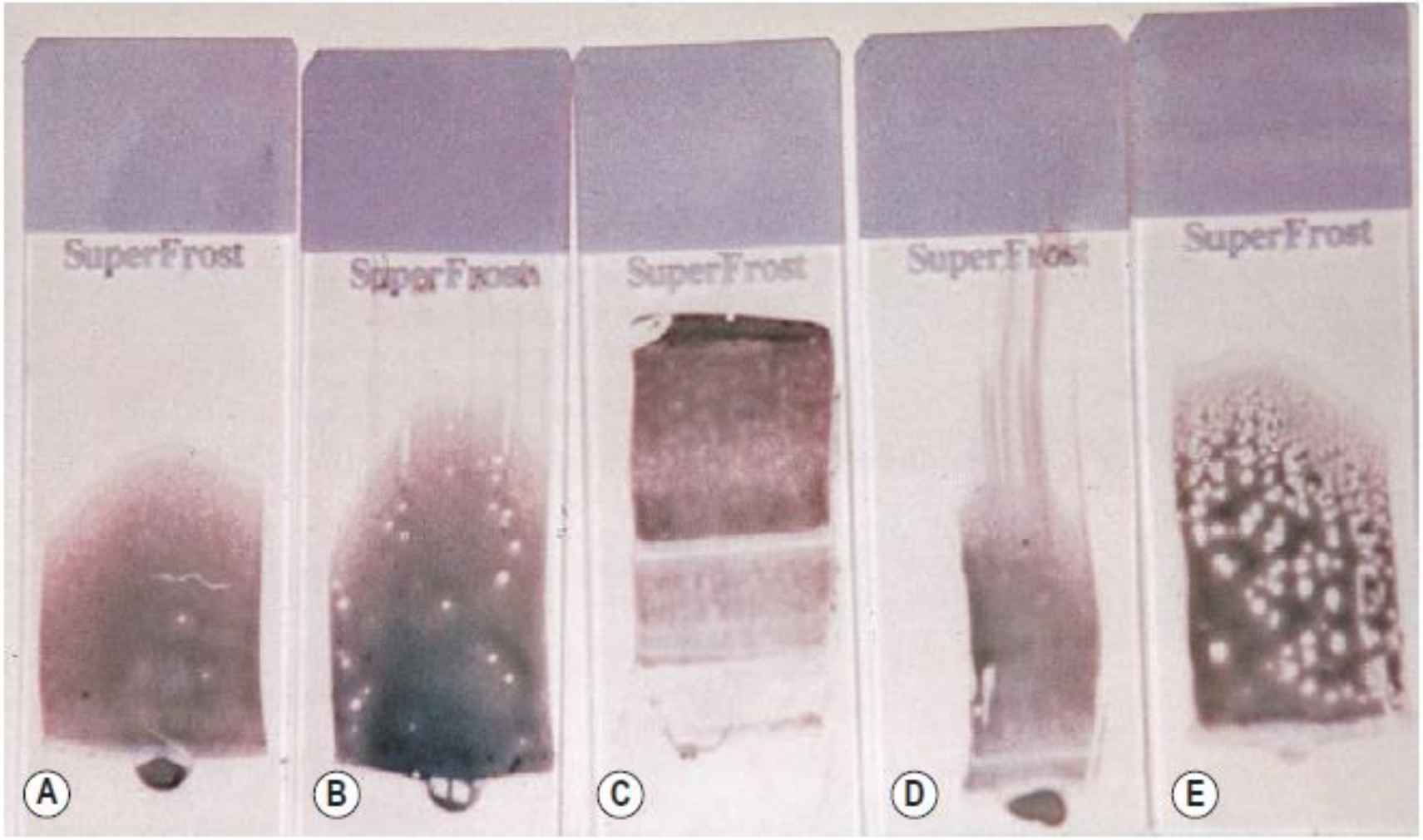
(A) A well-made film.

(B) An irregular patchy film on a dusty slide.

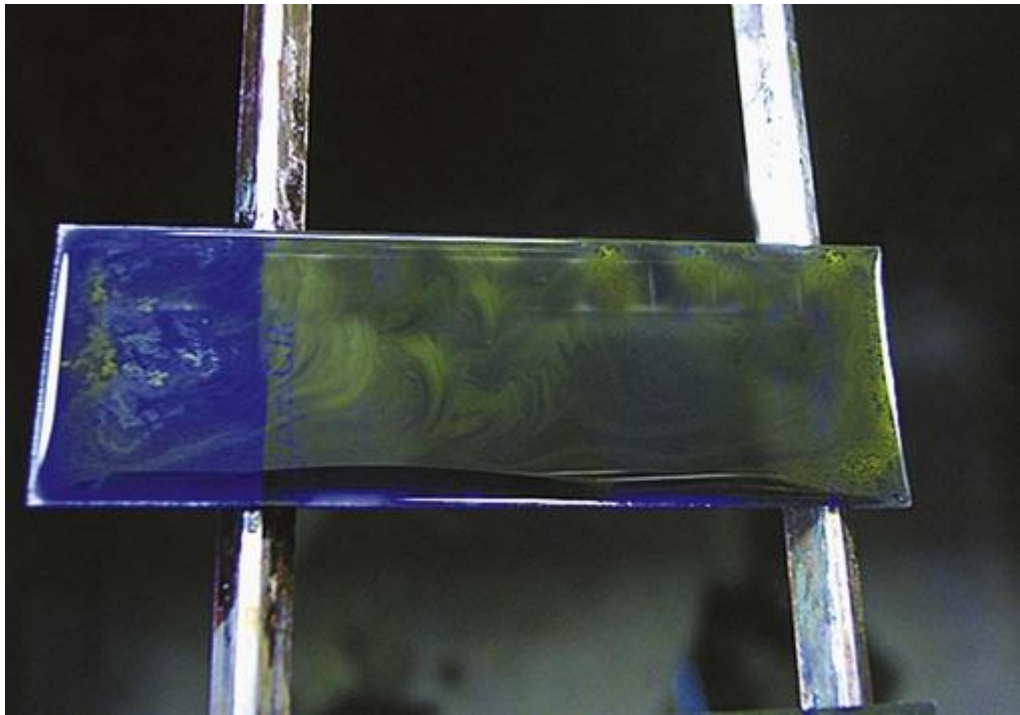
(C) A film that is too thick.

(D) A film that has been spread with inconsistent pressure and using an irregularly edged spreader, resulting in long tails.

(E) A film made ona very greasy slide.



- Manual Wright staining of slides. Note metallic sheen of stain indicating proper mixing.



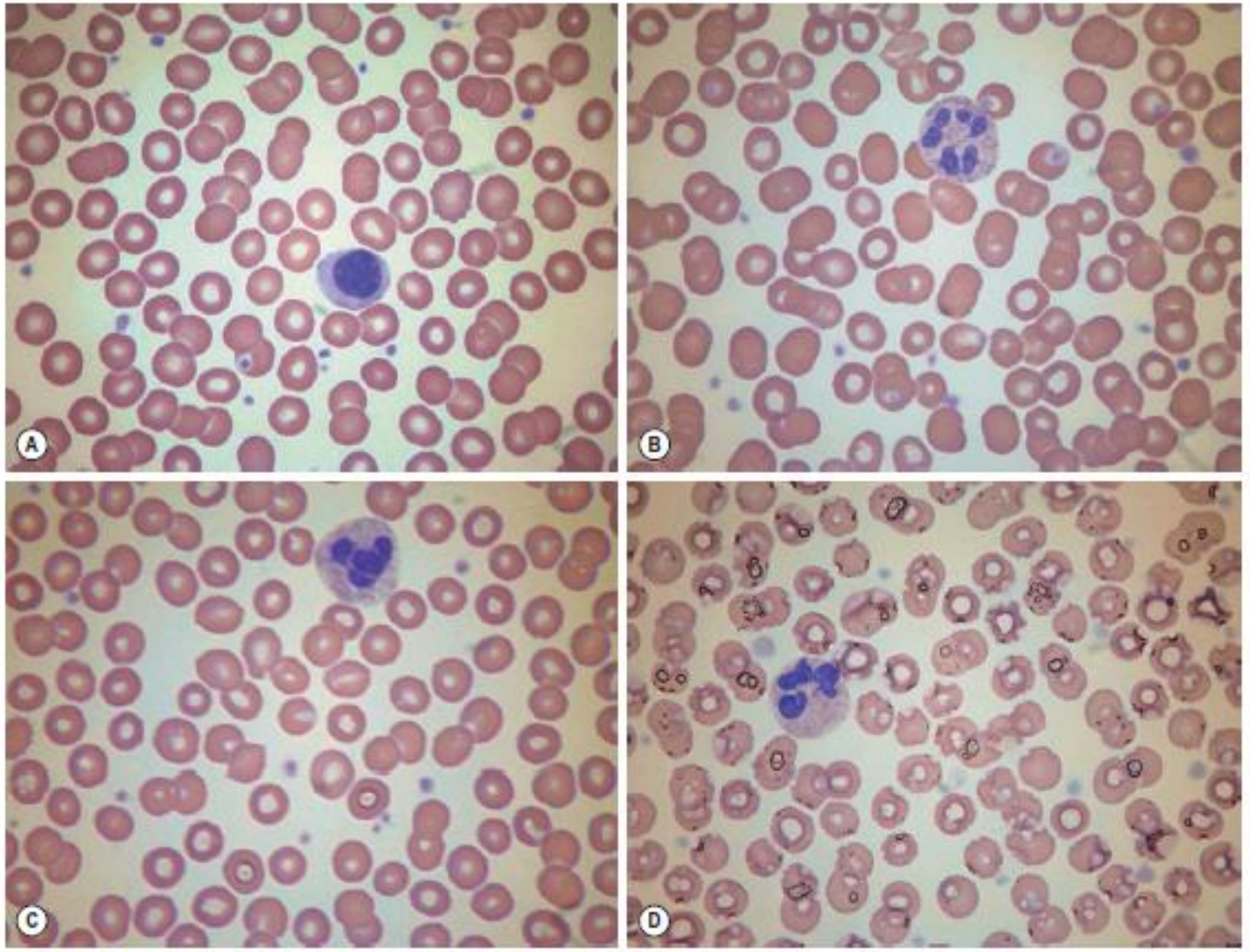
Factors Causing Variation In Staining

- **Too blue:** Incorrect preparation of stock, eosin concentration too low Stock stain exposed to bright daylight, Batch of stain solution overused, Impure dyes, Staining time too short, Staining solution too acidic, Film too thick, Inadequate time in buffer solution
- **Too pink:** Incorrect azure B:eosin Y ratio, Impure dyes, Buffer pH too low, Excessive washing in buffer solution
- **Pale staining:** Old staining solution, Overused staining solution, Incorrect preparation of stock, Impure dyes, especially azure A and/or C, High ambient temperature
- **Neutrophil granules not stained:** Insufficient azure B
- **Neutrophil granules dark blue/black (pseudotoxic):** Excess azure B
- **Stain deposit on film:** Stain solution left in uncovered jar, Stain solution not filtered
- **Blue background:** Inadequate fixation or prolonged, storage before fixation, Blood collected into heparin as anticoagulant

Staining Methods May–grünwald–giemsa Stain

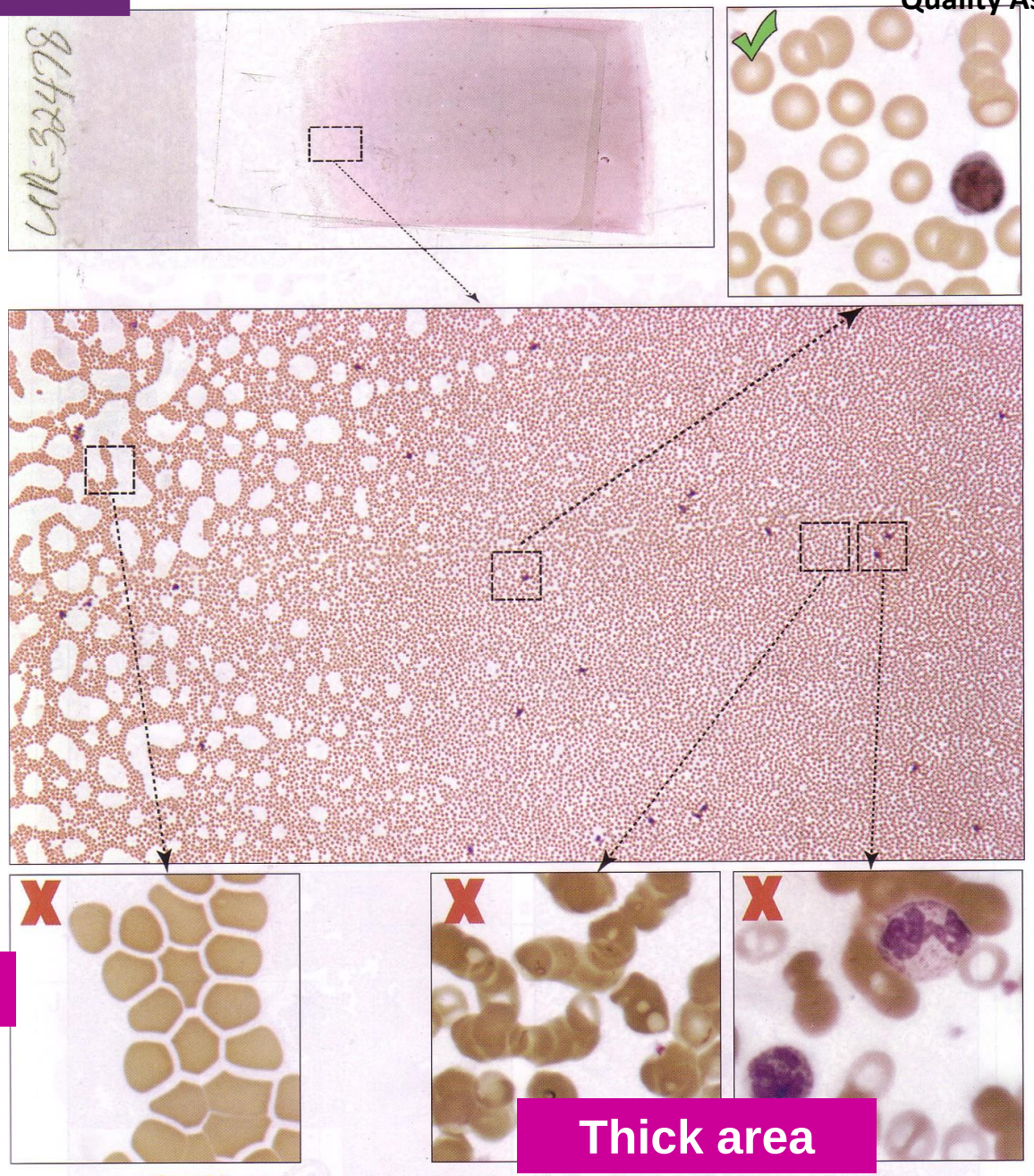
- To prevent the alcohol from bec
  stored in a bottle with a tightly-
  especially in humid climates

- **Blood film appearances follow**
  Romanowsky-stained blood film
  - **(A)** 1% water;
  - **(B)** 3% water;
  - **(C)** 4% water; and
  - **(D)** 10% water.
- The red cells and leucocytes are
  badly fixed in **(C)** and **(D)**.



Blood Cell Morphology in Health and Disease

Subfeathery area



feathery end

Thick area

Peripheral Blood Smear

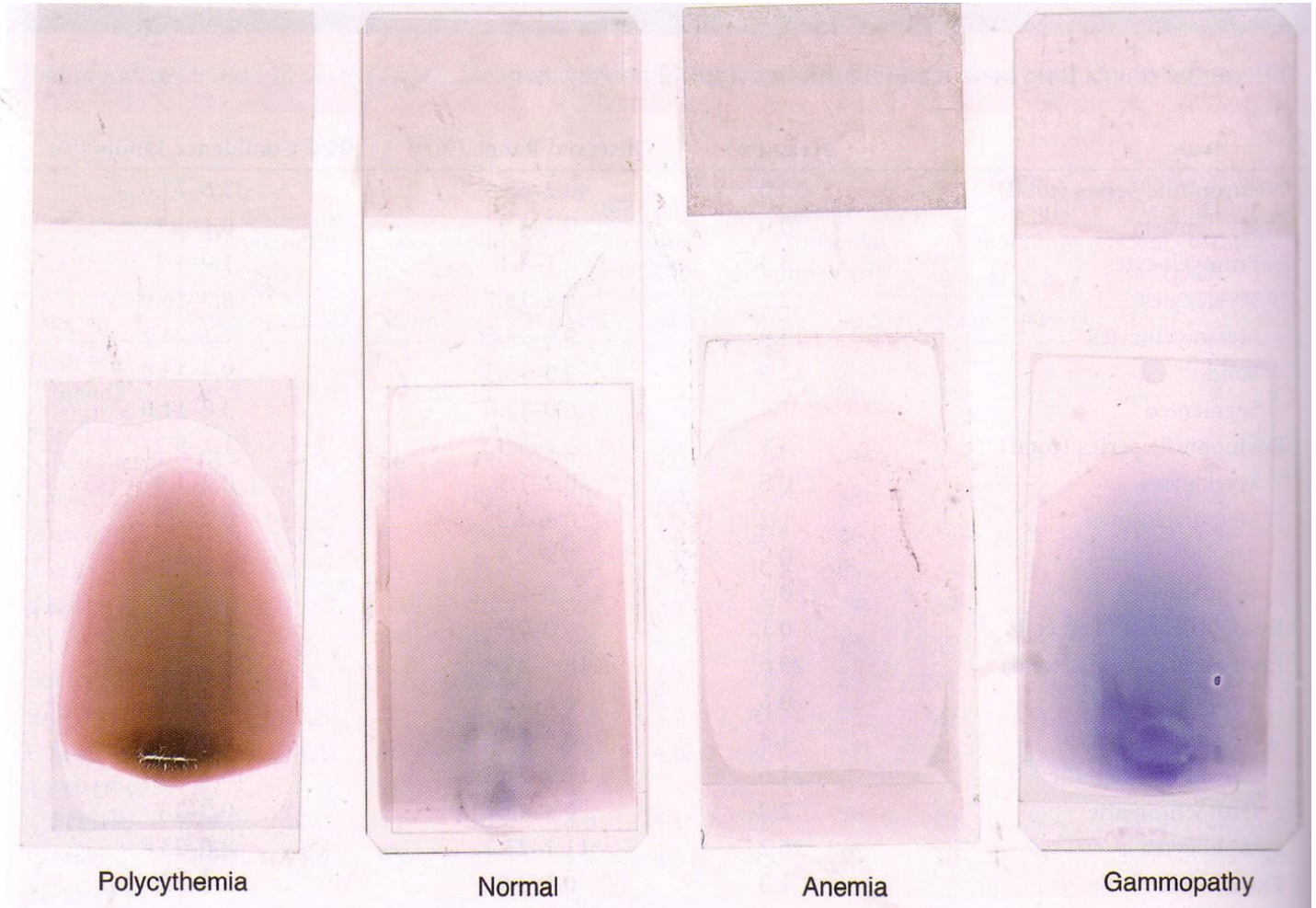
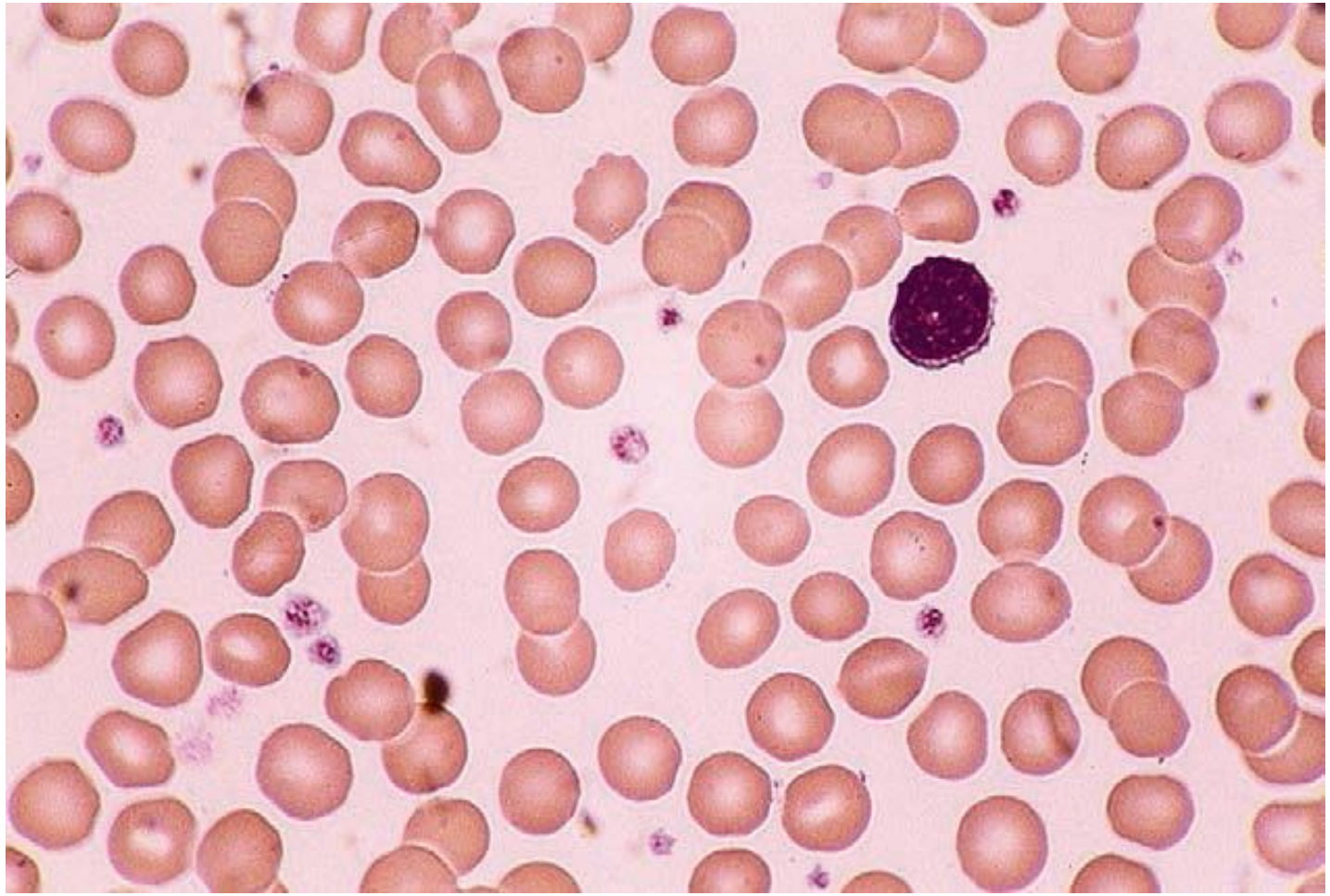


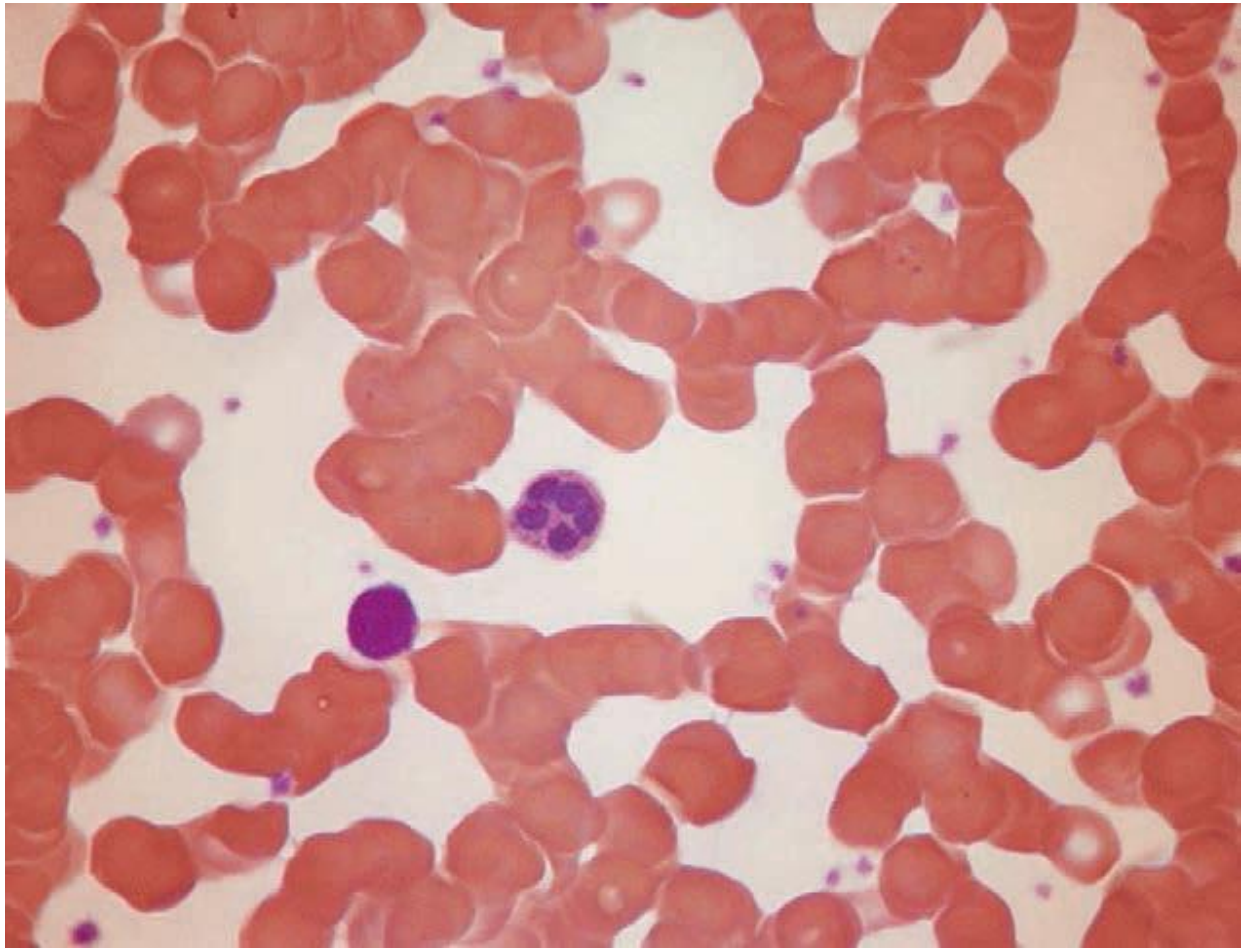
Figure 8.1. Macroscopic appearance of blood films. The color of blood smears can reflect severe underlying abnormalities in hematocrit and the presence of abnormal circulating immunoglobins. The smear on the left, from a patient with polycythemia vera and a hemoglobin of 20 g/dL,

Blood film from a healthy adult



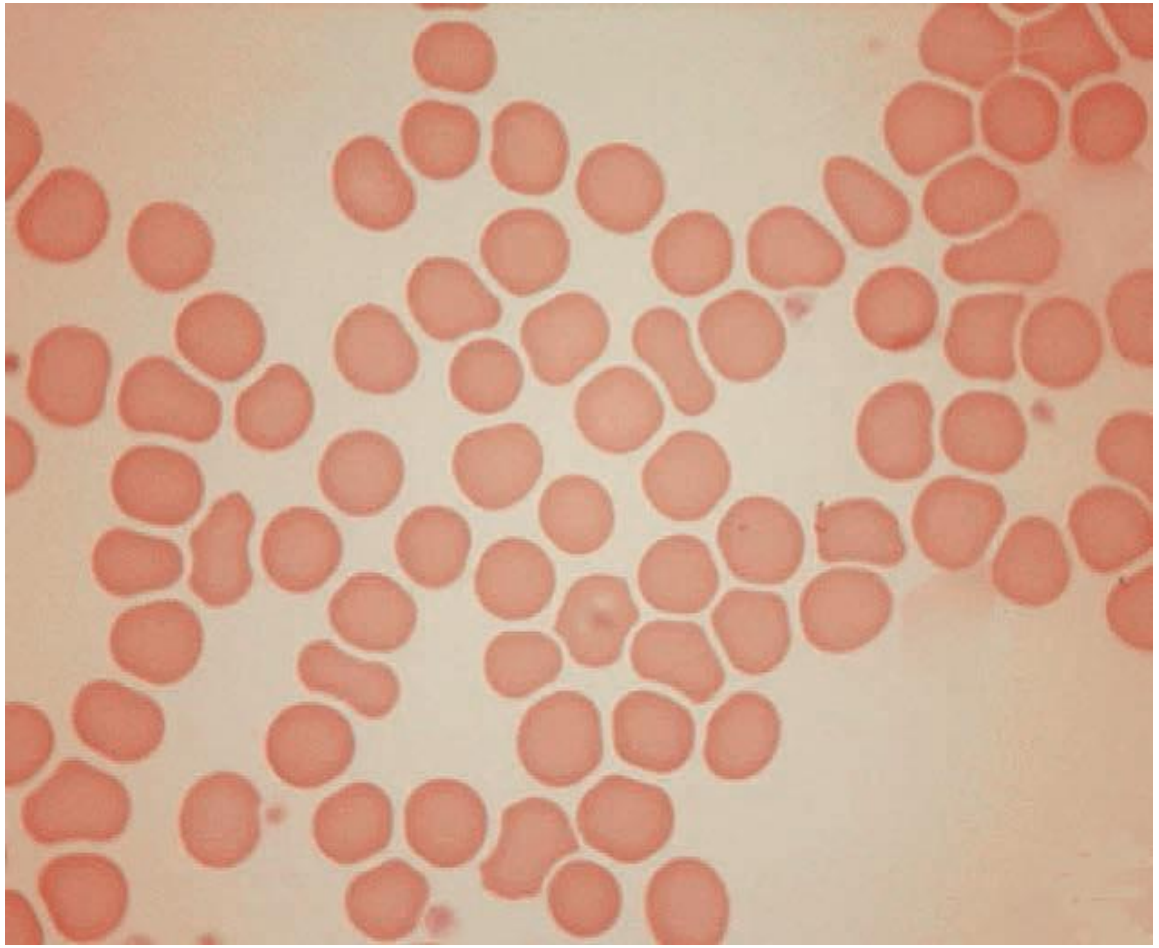
blood film showing an area that is too thick for examination.

No cellular detail can be discerned

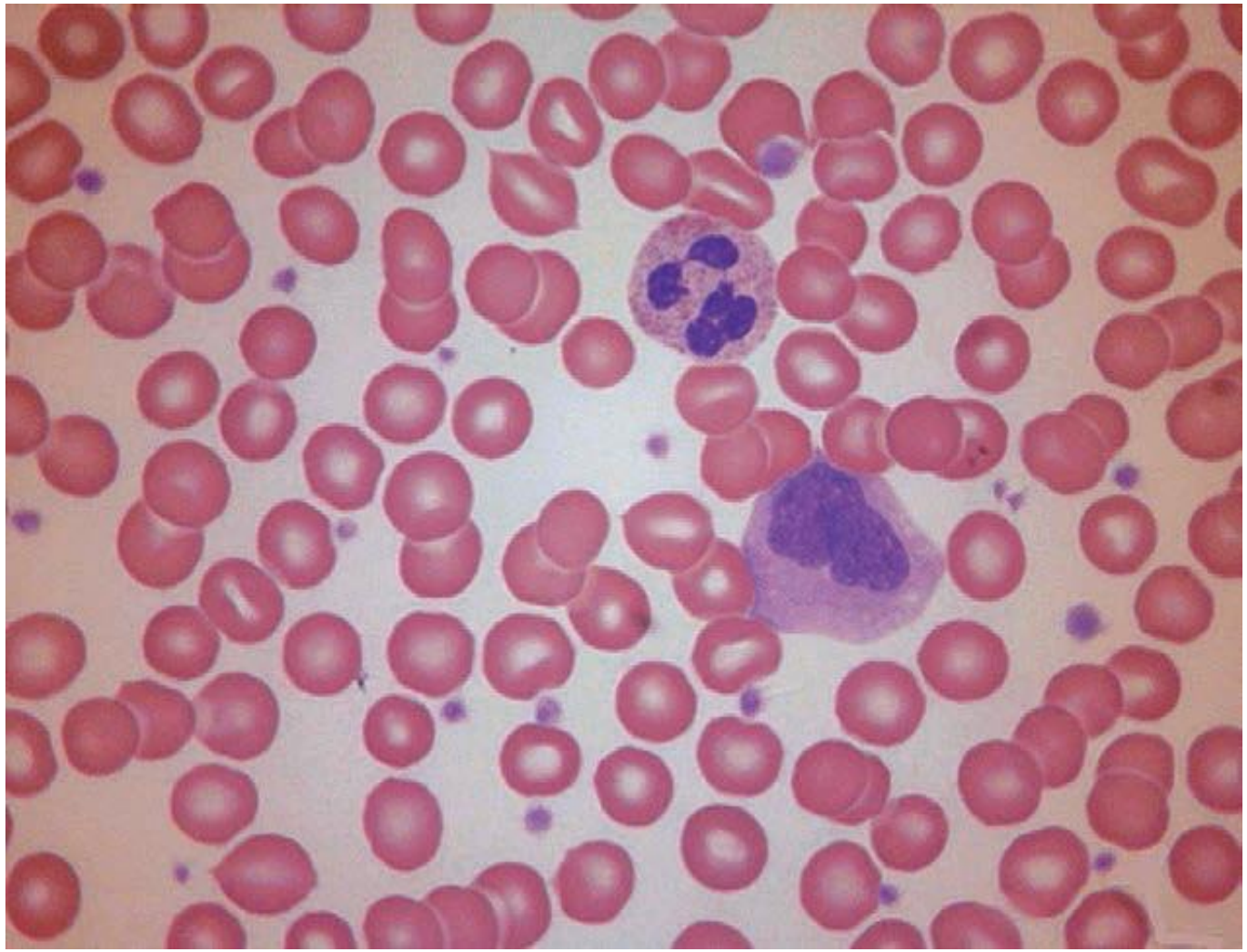


blood film showing an area that is too thin for examination.

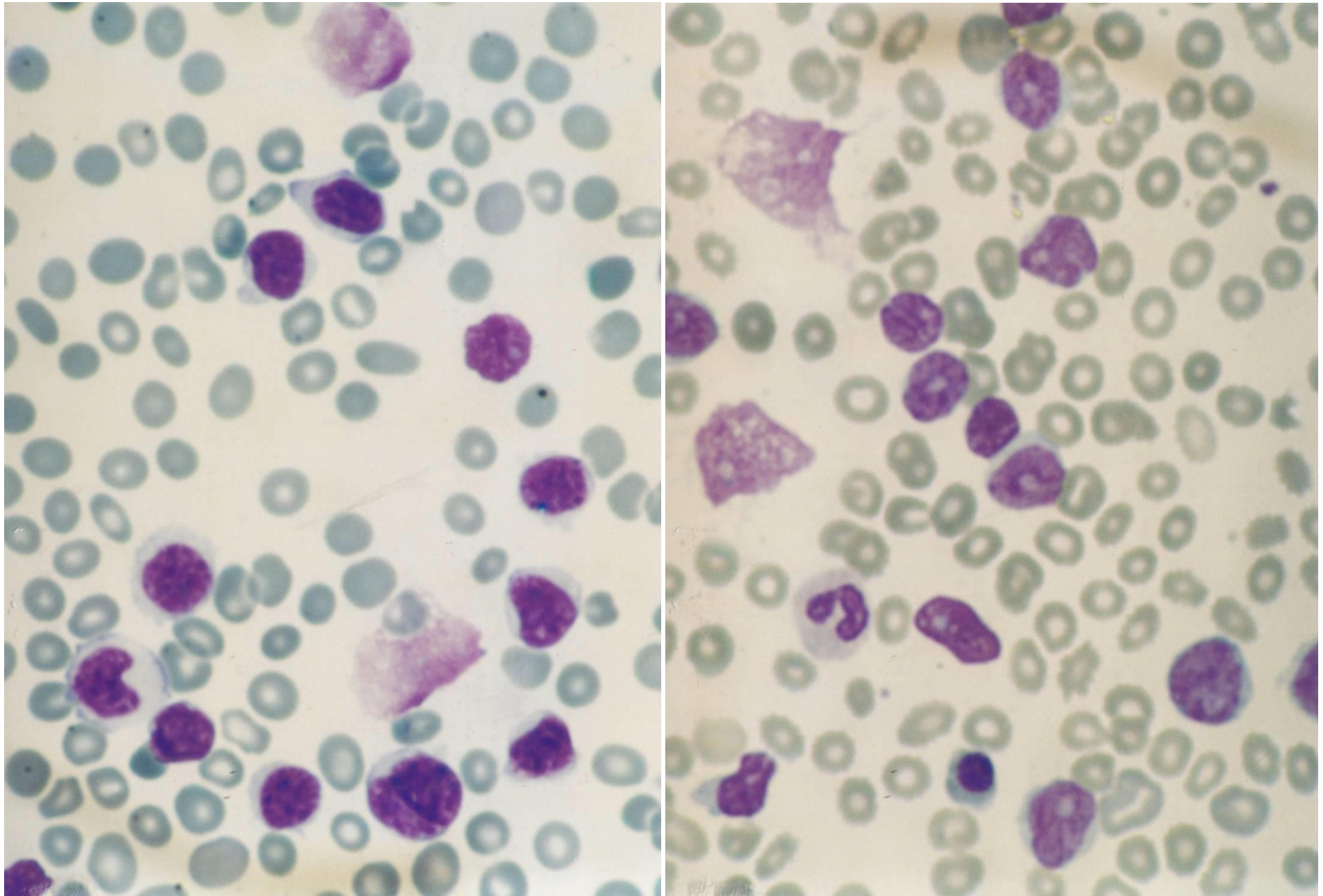
The loss of central pallor can give a false impression of spherocytosis



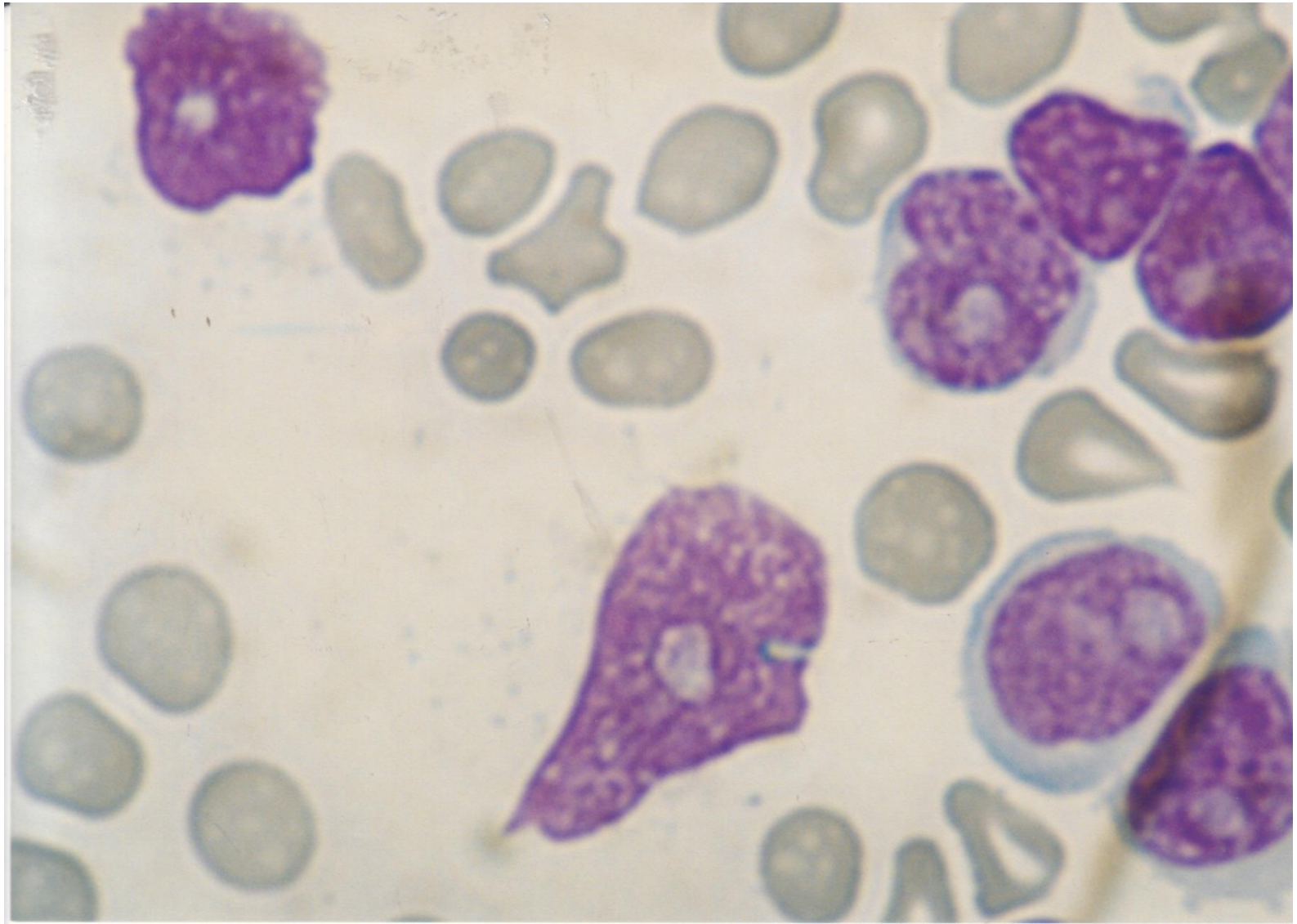
Ideal thickness for examination



What are your diagnosis for these two films?



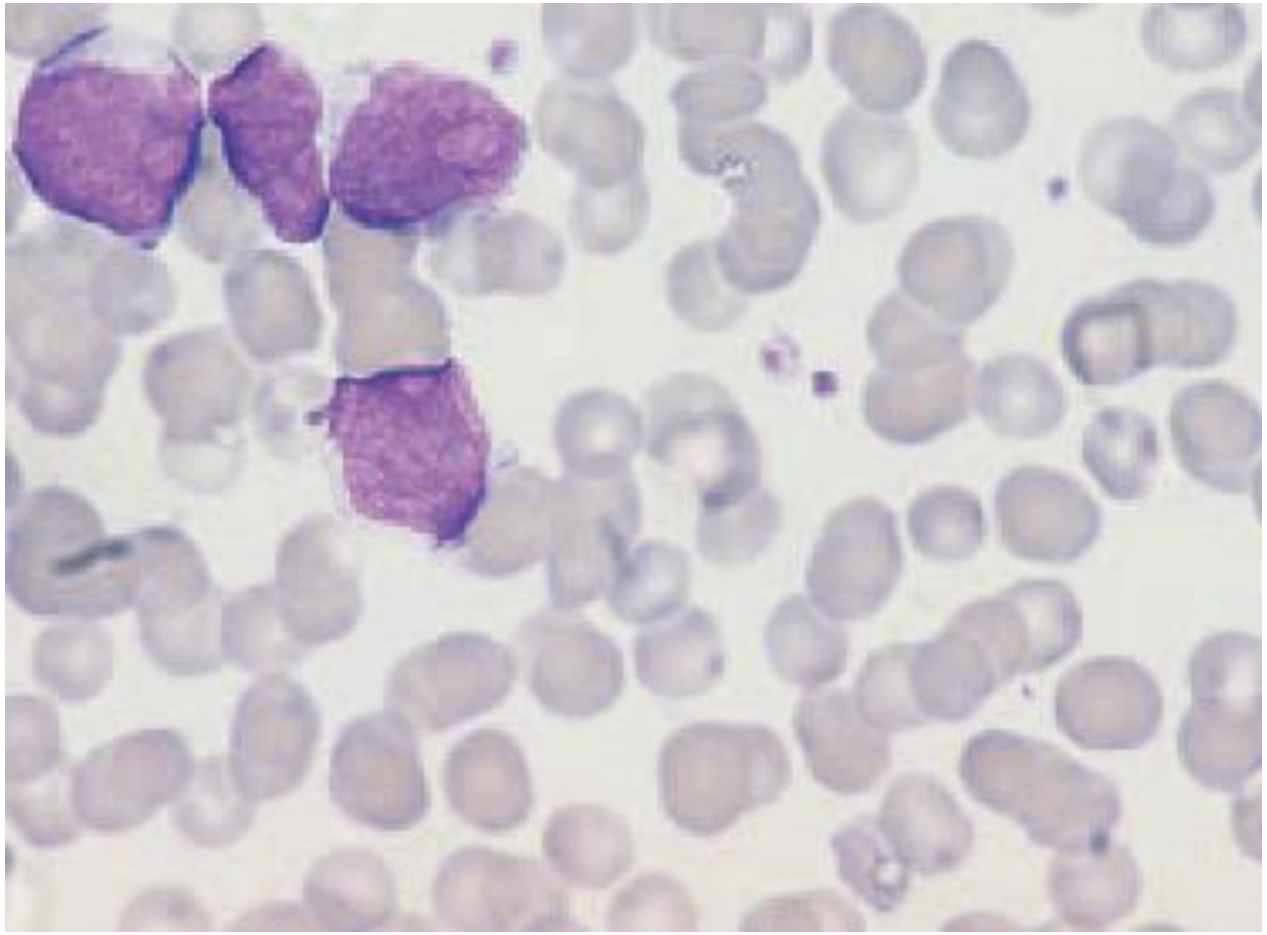
Higher magnification of previous film



CBC of a 45y/o female who has come to lab. for annual check up.

- WBC: 5500/µl
- RBC:4.58 x 10^{12}/µl
- Hb:12.8 g/dl
- Hct: 38.2%
- Plt: 195000/µl
- PBS???

What do you see in PBS?



Interpretation

- **It is important that the spreader is wiped clean with a dry tissue or gauze square after each use since it is otherwise possible to transfer abnormal cells from one blood film to another**

Microscopic Examination of the Blood Film

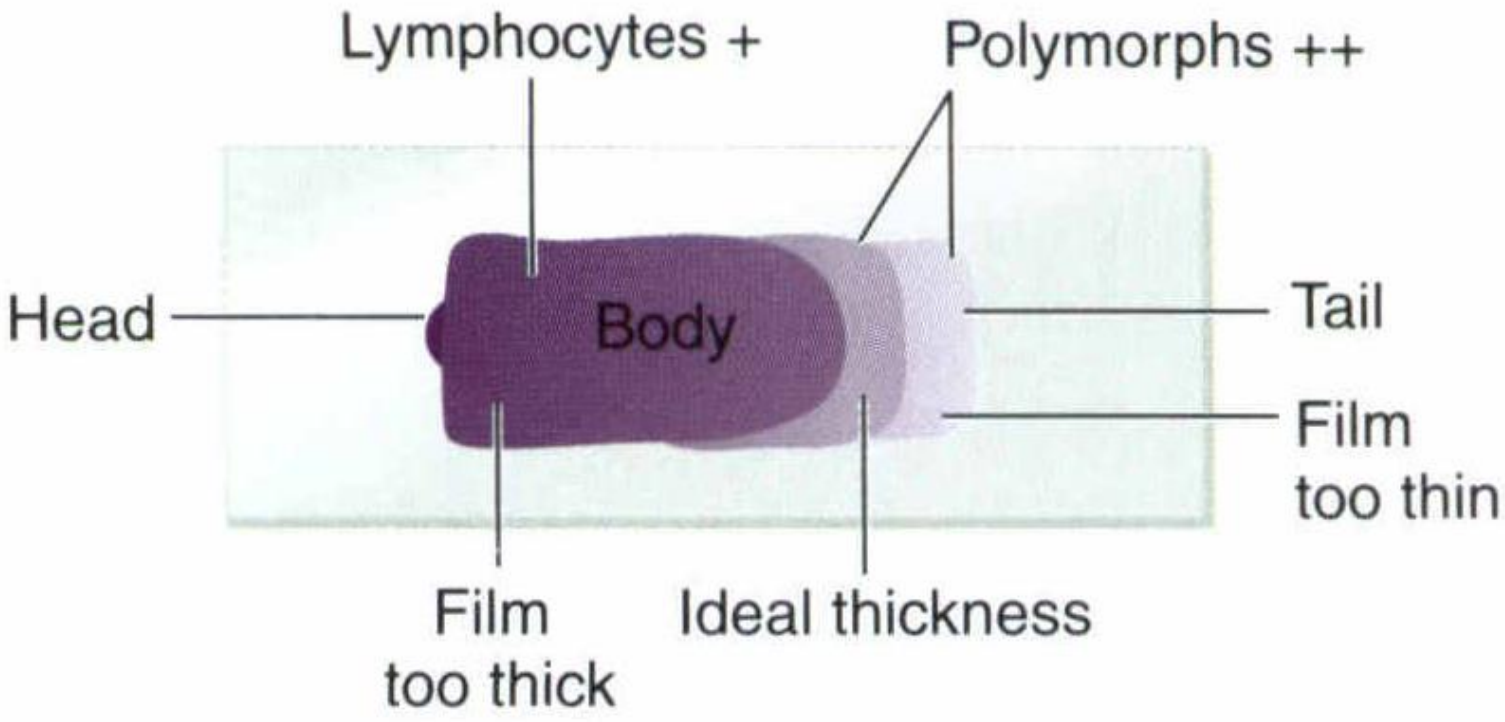
- The blood film should always be ***scanned under low power (10x to 40x objective)*** for unusual or abnormal cells and an acceptable cell distribution.
- Extend the examination from the area where approximately **50%** of the erythrocytes overlap to the region where erythrocytes show a strong tendency to linear orientation.

Good PBS must be well prepared , fixed, stained & has acceptable cellular distribution

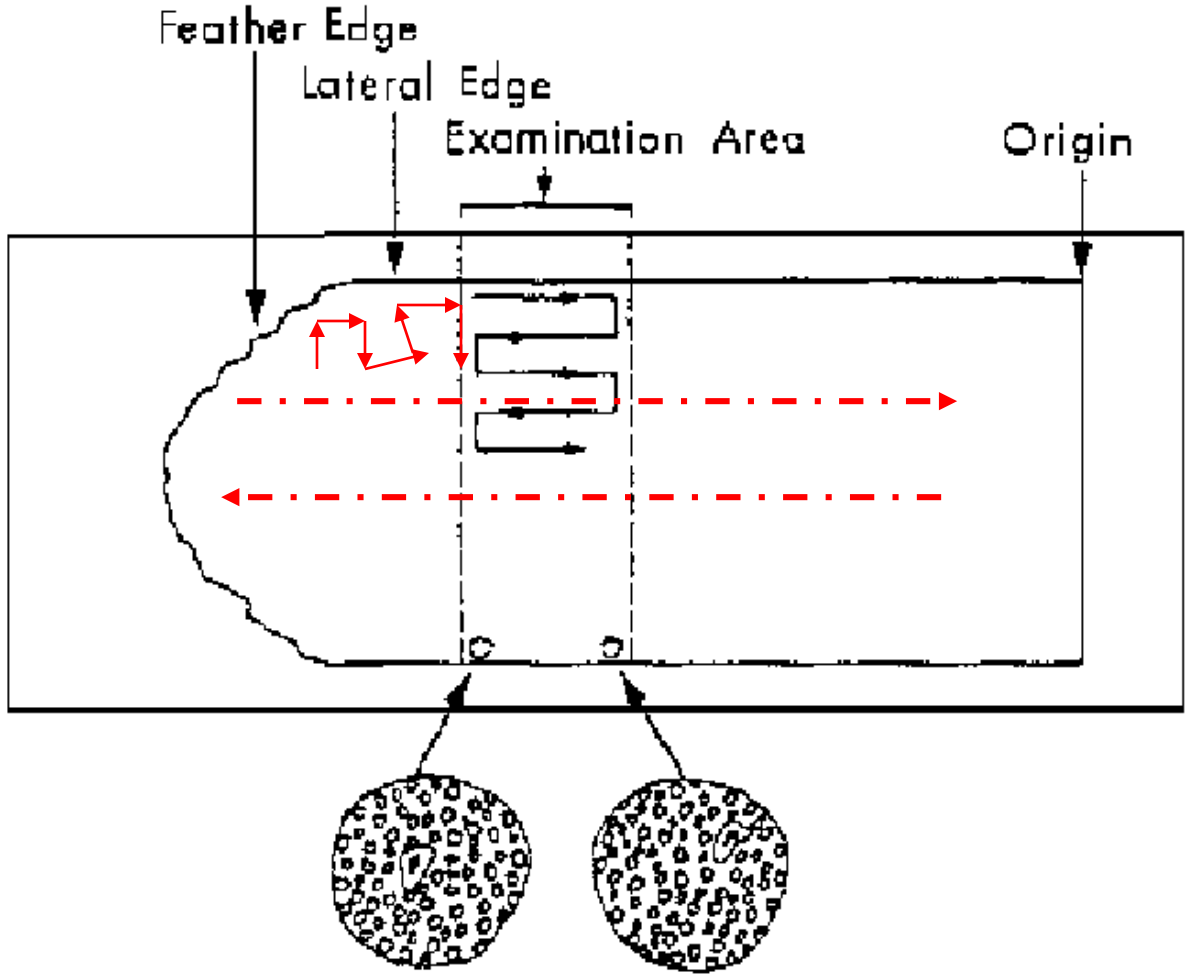
Parameters which evaluated in PB Films

- Leukocyte Differential Count
- RBC abnormal Morphology
- Platelet Count Estimation
- Abnormal Morphology of WBC & Platelets
- Spurious instrument results
- Neoplastic Cells
- Blood Parasites
- Others(eg.,Endothelial cells)

Distribution of White Blood Cells



Leukocyte Differential Count

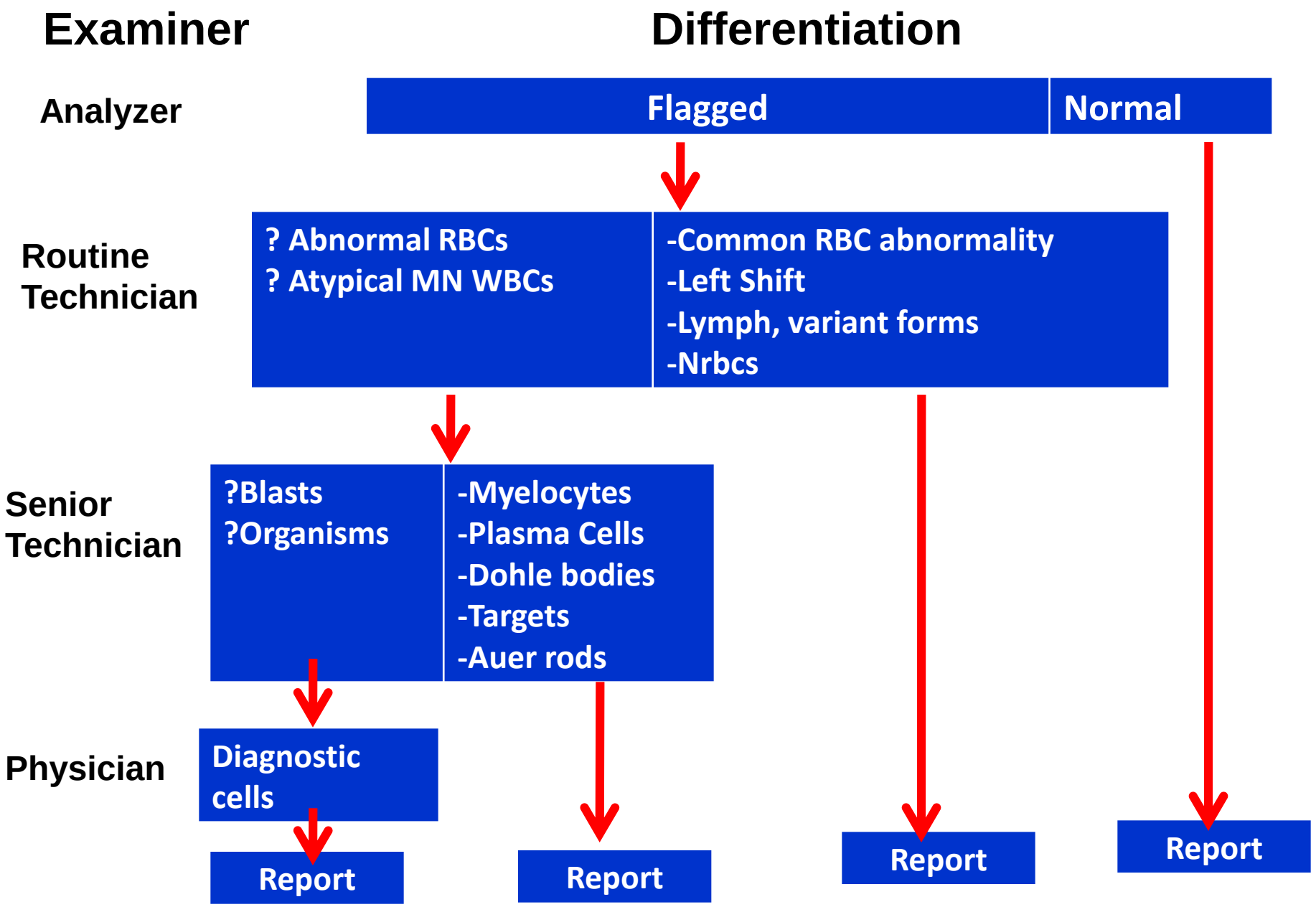


"battlement" track for this examination

The differential white cell count

- ***ICSH*** recommends that the differential leukocyte count be expressed in ***absolute numbers***
- ***Absolute no.: Relative no.(%) x WBC***
- ***10000/µl x 0.50= 5000/µl***

- Manual differential counts are generally fairly accurate, but their **precision is poor**, whereas automated counts are generally **fairly precise** but are sometimes inaccurate.


Examiner
Differentiation
Analyzer
Flagged
Normal
Routine Technician
? Abnormal RBCs
? Atypical MN WBCs
-Common RBC abnormality
-Left Shift
-Lymph, variant forms
-Nrbcs
Senior Technician
?Blasts
?Organisms
-Myelocytes
-Plasma Cells
-Dohle bodies
-Targets
-Auer rods
Physician
Diagnostic cells
Report
Report
Report
Report

How can you estimate the WBC from PBS?

Estimation of WBC

$$\frac{\text{Total number of leukocytes counted}^{*}}{5} \bullet 0.2 \times 10^{9}/L^{**} = \text{leukocytes} \times 10^{9}/L$$

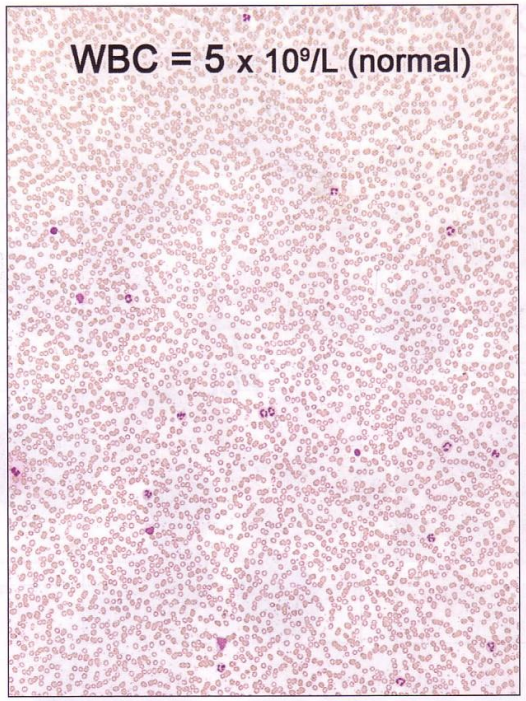
* Total number of leukocytes counted in 5 fields at 100x (10x objective) magnification

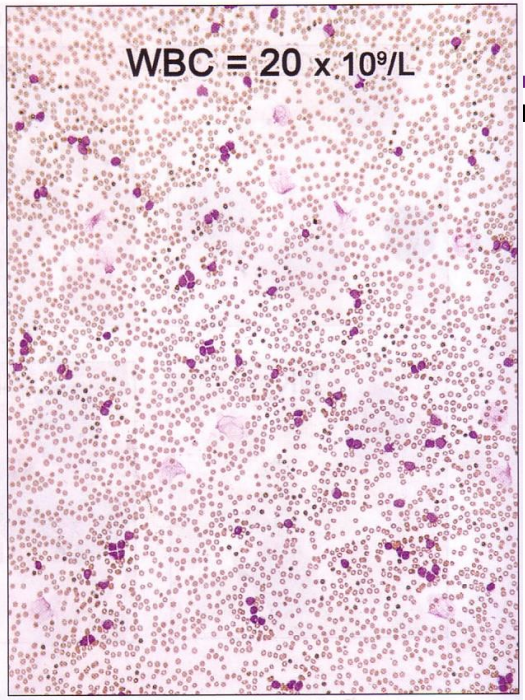
** 1 leukocyte = $0.2 \times 10^{9}/L$

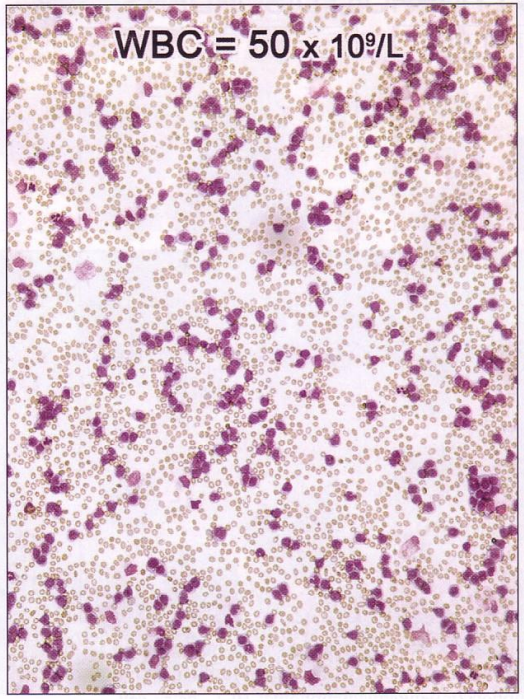
Example: If total number of leukocytes counted = 150

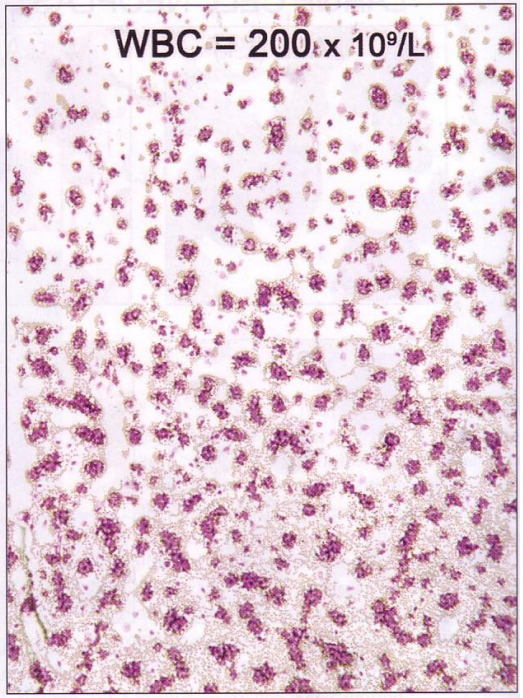
$150/5 \bullet 0.2 \times 10^{9}/L = 6.0 \times 10^{9}/L$

The leukocyte estimate should correlate with the leukocyte count **+/- 25%.**


WBC = 5 x 10⁹/L (normal)


WBC = 20 x 10⁹/L


WBC = 50 x 10⁹/L


WBC = 200 x 10⁹/L

Causes of Spurious Increase in WBC

- Cryoglobulin,cryofibrinogen
- Heparin
- Monoclonal proteins
- Nucleated red blood cells
- Corrected WBC= WBC x 100/ Nrbc + 100
- Platelet clumping / Giant platelets
- Unlysed red blood cells / reticulocytosis

Causes of Spurious Decrease in WBC

- Clotting
- Smudge cells
- Uremia plus immunosuppressant
- Leukocyte clumping

Corrected WBC

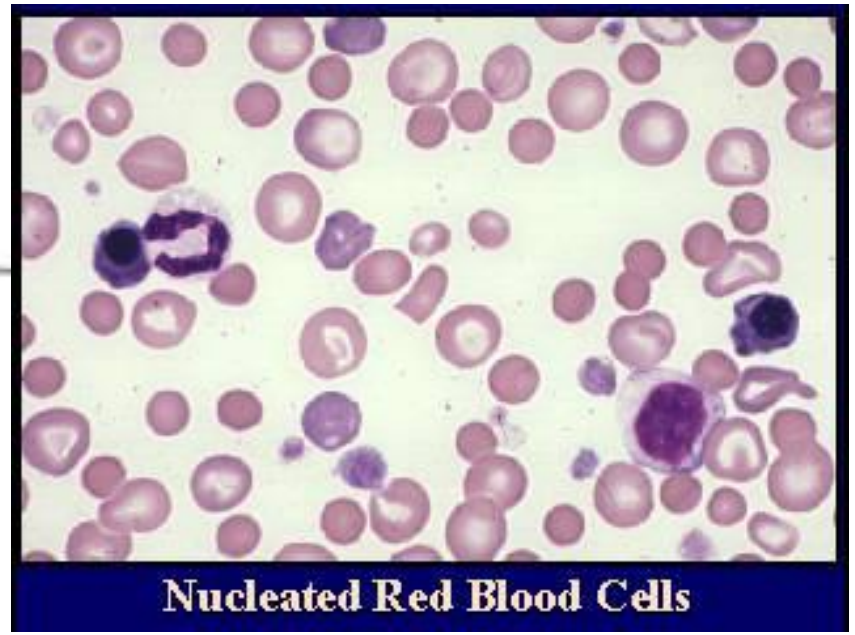
$$\frac{\text{Leukocyte count} \bullet 100}{100 + \text{number of nucleated erythrocytes}^{*}} = \text{Corrected Leukocyte Count}$$

*Number of nucleated erythrocytes counted per 100 leukocytes at 1000x (100x objective) magnification

Example: Leukocyte count = 20.0×10^9/L
Nucleated erythrocytes/100 leukocytes = 10

$$\frac{20.0 \times 10^9/L \bullet 100}{100 + 10} = 18.2 \times 10^9/L$$

Nrbc/100 wbc


Nucleated Red Blood Cells

If 10 (5) or more Nrbc are observed , corrected WBC must be calculated

International Journal of Laboratory Hematology
The Official Journal of the International Society for Laboratory Hematology
ORIGINAL ARTICLE INTERNATIONAL JOURNAL OF LABORATORY HEMATOLOGY

ICSH recommendations for the standardization of nomenclature and grading of peripheral blood cell morphological features

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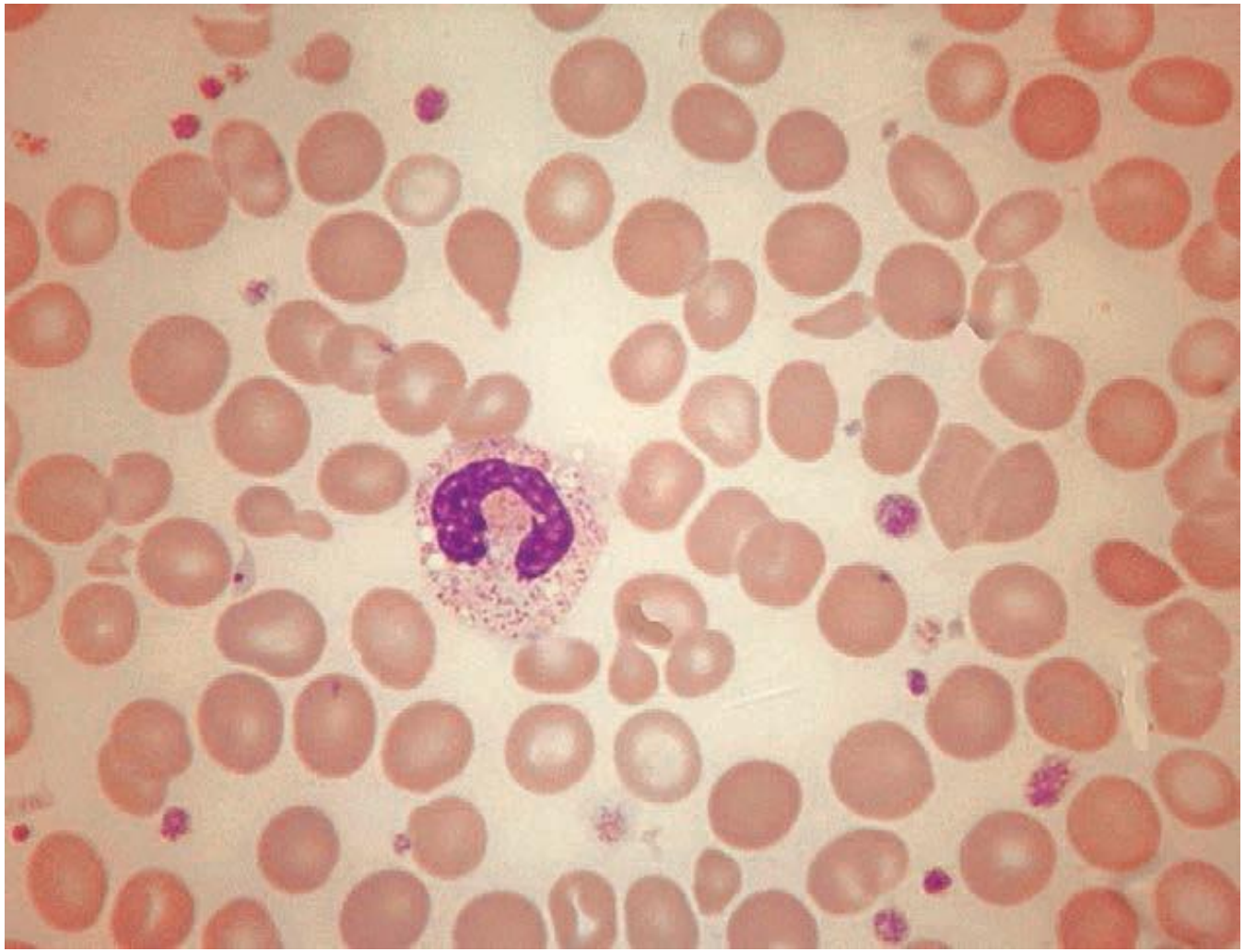
Received 26 August 2014;
accepted for publication 10 December 2014

Grading System

Cell Name	Few/1+	%	%
RBC			
Anisocytosis	N/A	11–20	>20
Macrocytes	N/A	11–20	>20
Oval macrocytes	N/A	2–5	>5
Microcytes	N/A	11–20	>20
Hypochromic cells	N/A	11–20	>20
Polychromasia	N/A	5–20	>20
Acanthocytes	N/A	5–20	>20
Bite cells	N/A	1–2	>2
Blister cells	N/A	1–2	>2
Echinocytes	N/A	5–20	>20
Elliptocytes	N/A	5–20	>20
Irregularly contracted cells	N/A	1–2	>2
Ovalocytes	N/A	5–20	>20
Schistocytes	<1 %	1–2	>2
Sickle cells	N/A	1–2	>2
Spherocytes	N/A	5–20	>20
Stomatocytes	N/A	5–20	>20
Target cells	N/A	5–20	>20
Teardrop cells	N/A	5–20	>20
Basophilic stippling	N/A	5–20	>20
Howell-Jolly bodies	N/A	2–3	>3
Pappenheimer bodies	N/A	2–3	>3
WBC			
Döhle bodies	N/A	2–4	>4
Vacuolation (neutrophil)	N/A	4–8	>8
Hypogranulation (neutrophil)	N/A	4–8	>8
Hypergranulation (neutrophil)	N/A	4–8	>8
Platelets			
Giant Platelets	N/A	11–20	>20

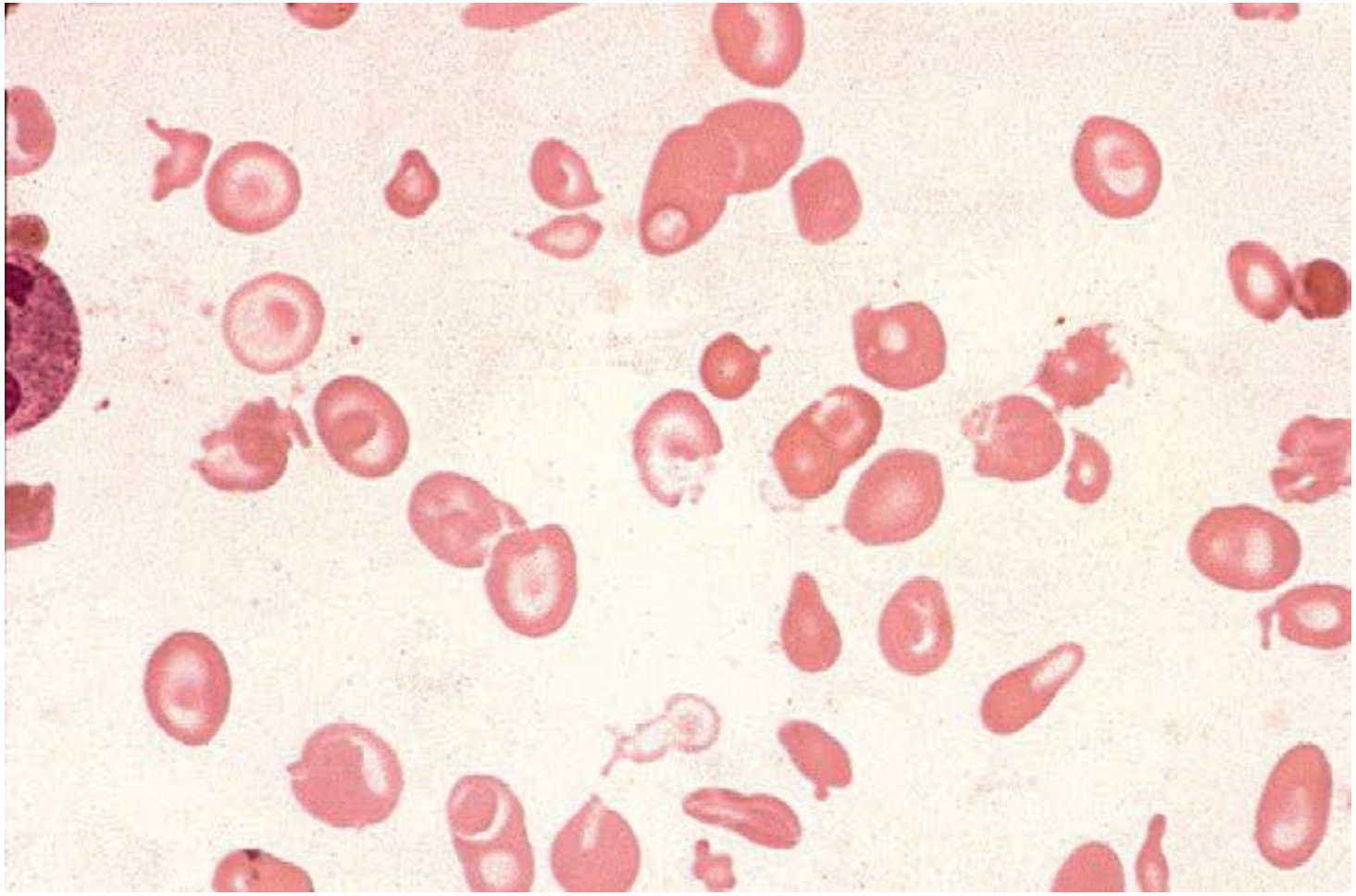
blood film from a patient with a myelodysplastic/myeloproliferative neoplasm, unclassified.

- **Shows moderate anisocytosis, anisochromasia and poikilocytosis.**
- **There are several hypochromic microcytes and one neutrophil band form, which is vacuolated.**



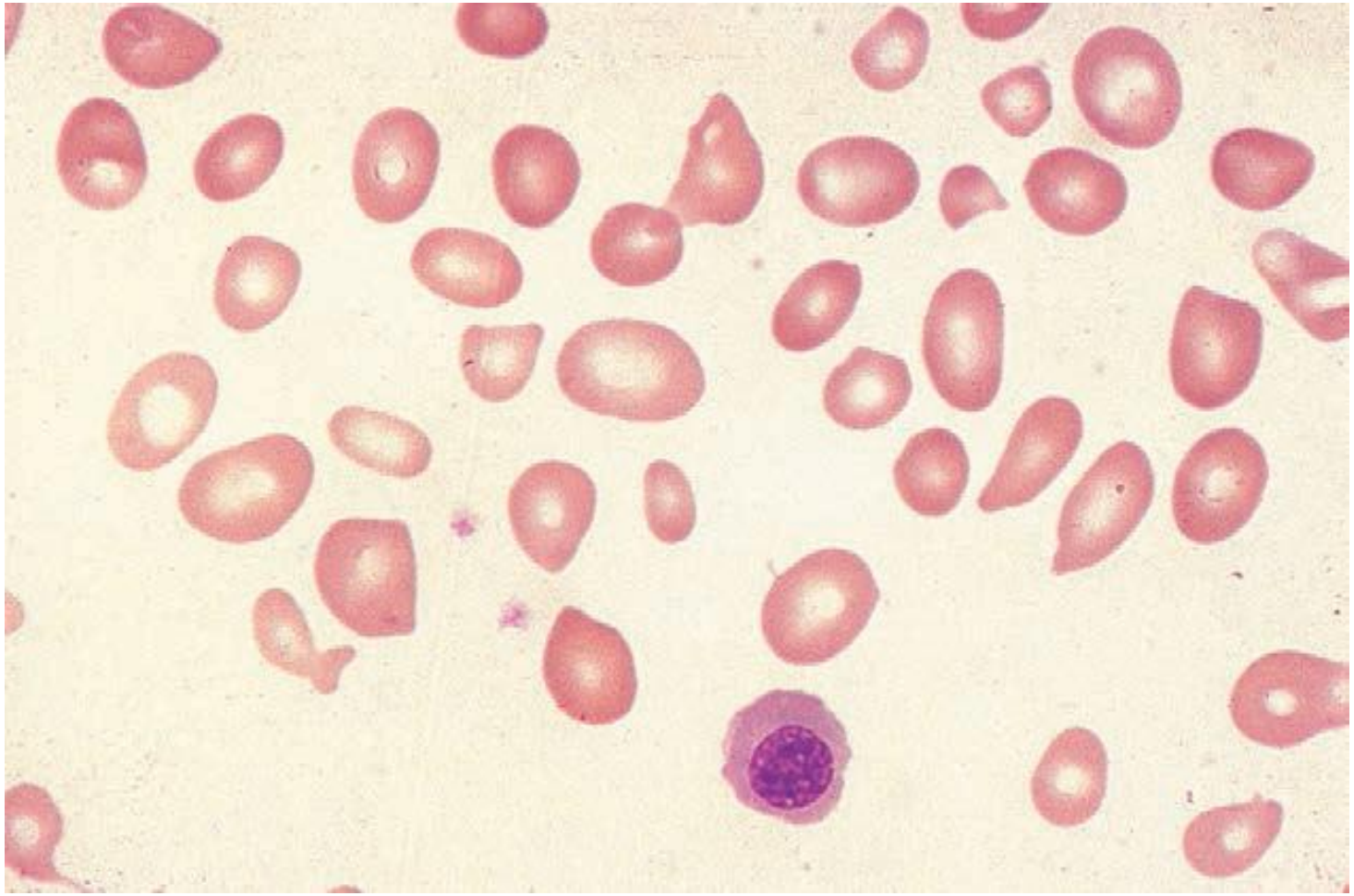
blood film from a patient with compound heterozygosity for haemoglobin E and β0 thalassaemia.

- **Shows marked anisocytosis and poikilocytosis.**



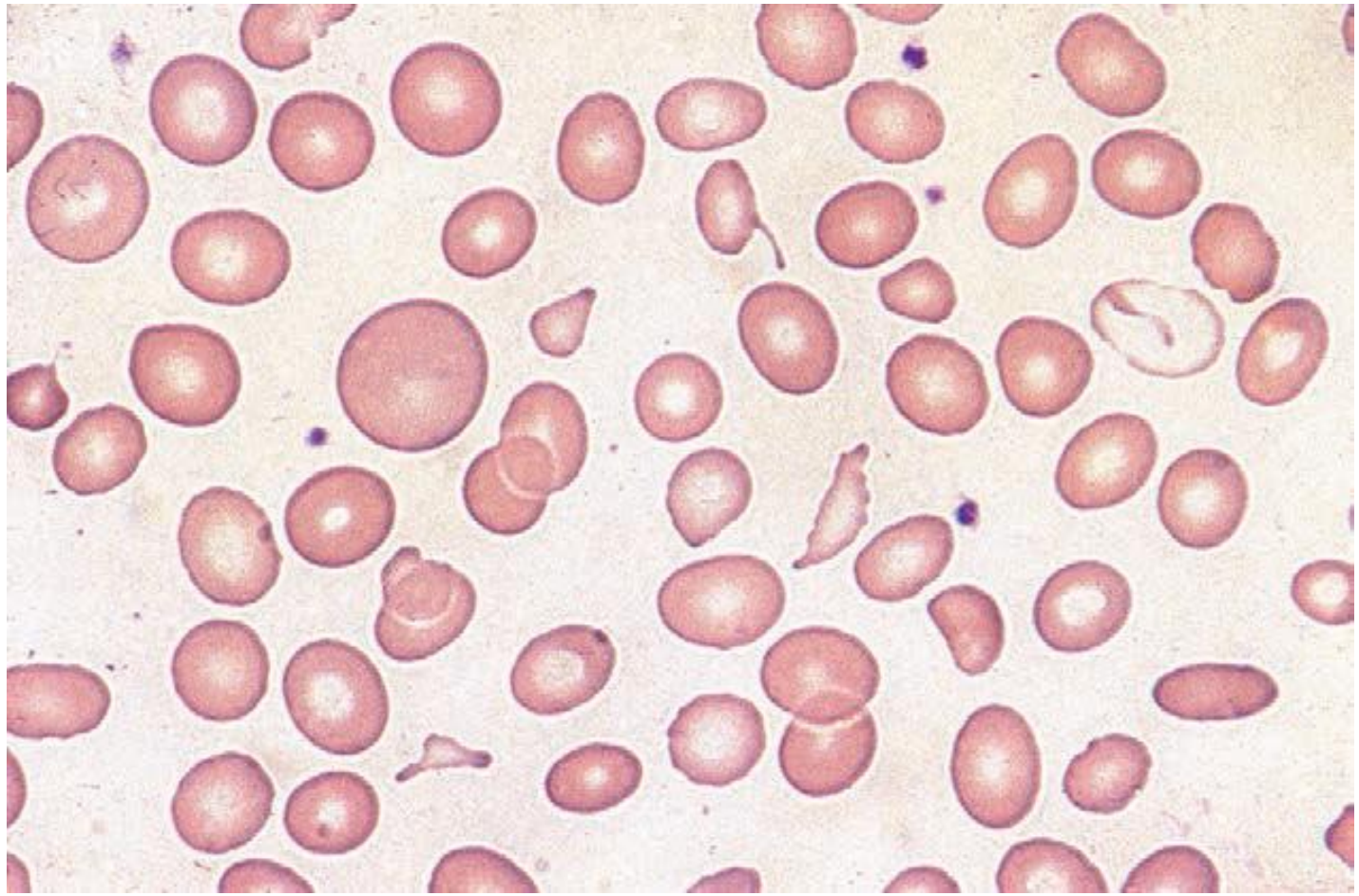
Pernicious anemia

- **Shows marked anisocytosis, moderate poikilocytosis (including oval macrocytes and teardrop cells) and a megaloblast.**



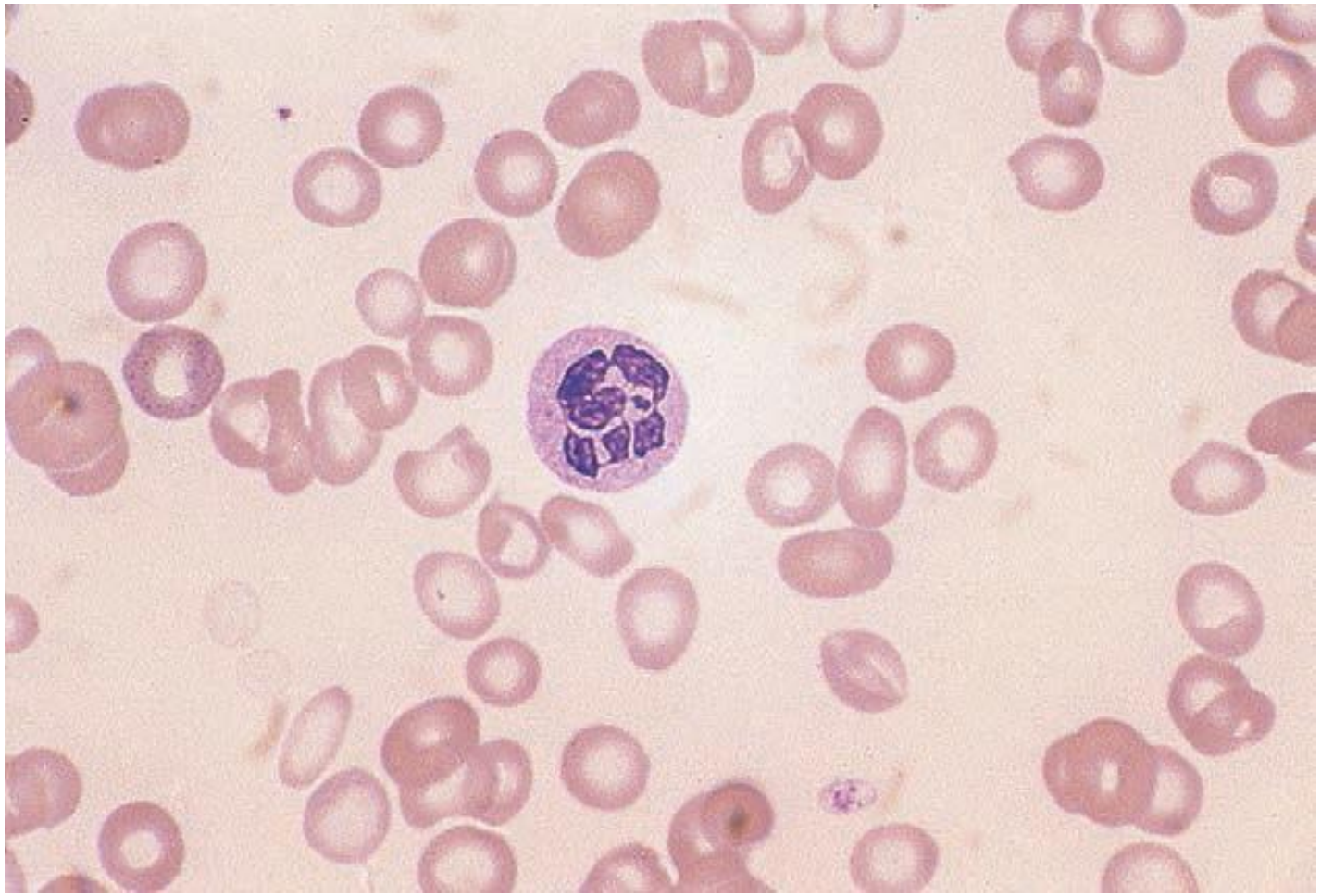
Congenital dyserythropoietic anaemia type II

Shows marked anisocytosis, marked poikilocytosis, one unusually large macrocyte and one severely hypochromic cell.



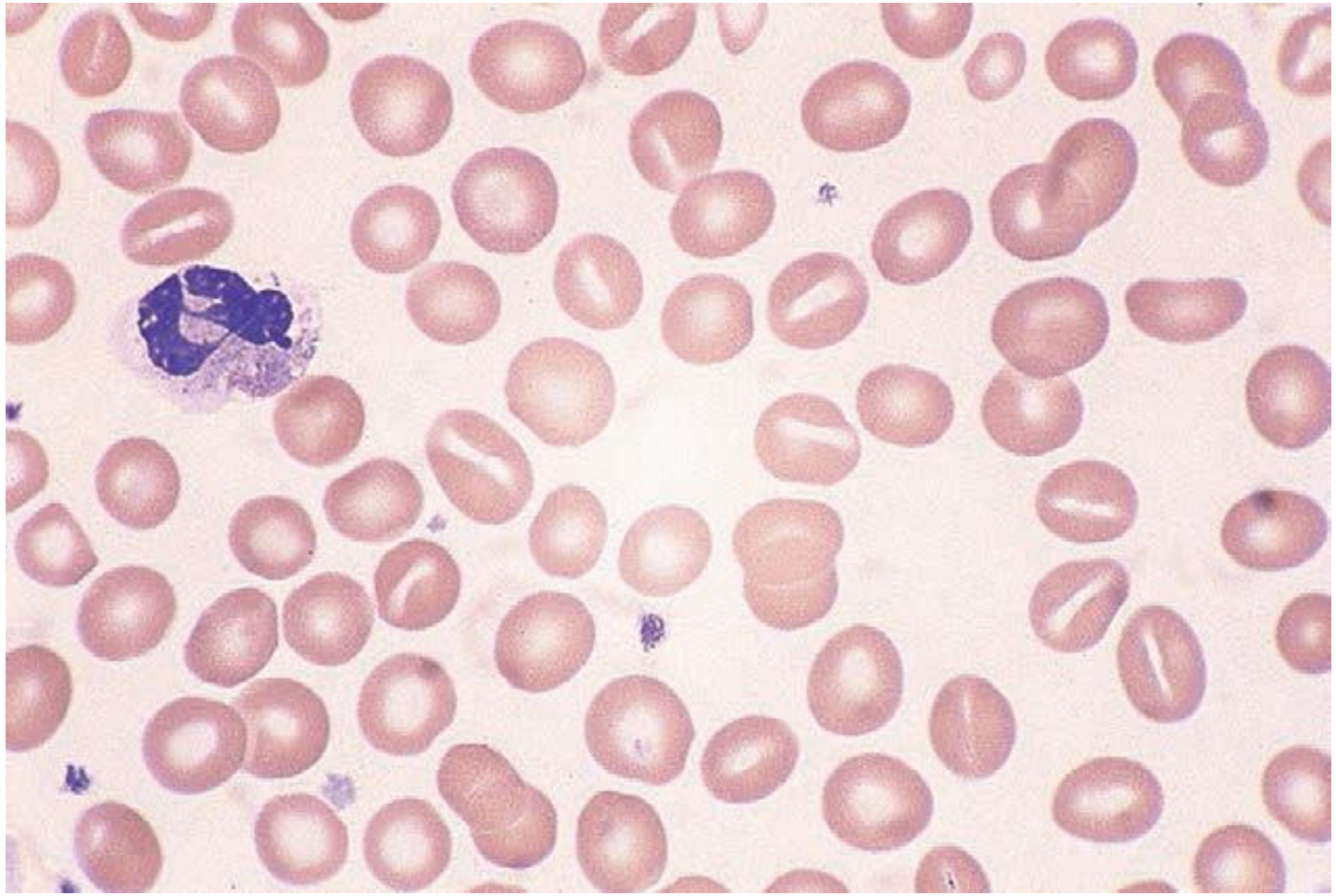
Megaloblastic anaemia

Shows macrocytes, oval macrocytes and a hypersegmented neutrophil. There are also hypochromic cells and a mixed vitamin B12 and iron deficiency might be suspected.



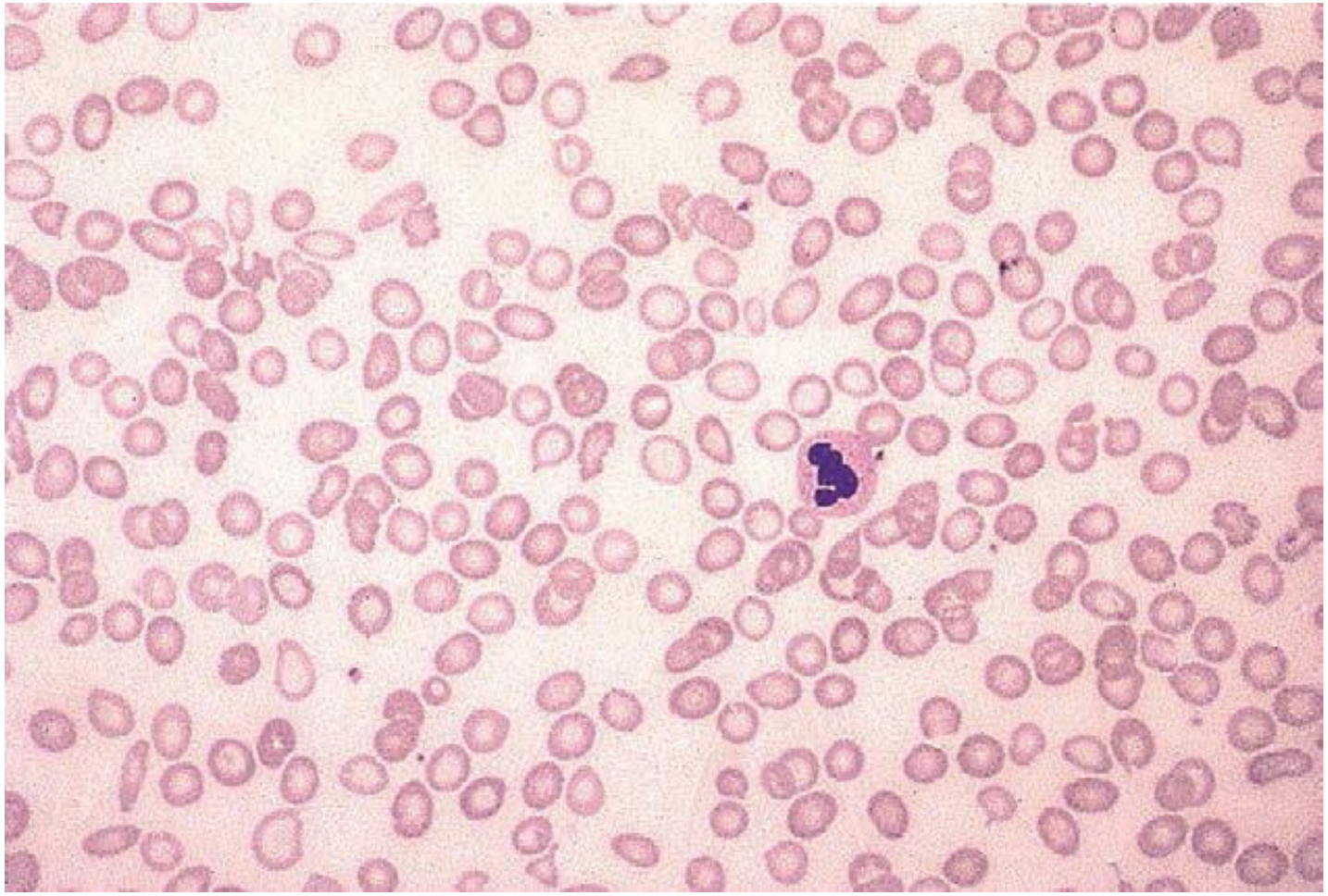
Liver disease

- **Shows macrocytosis and stomatocytosis.**



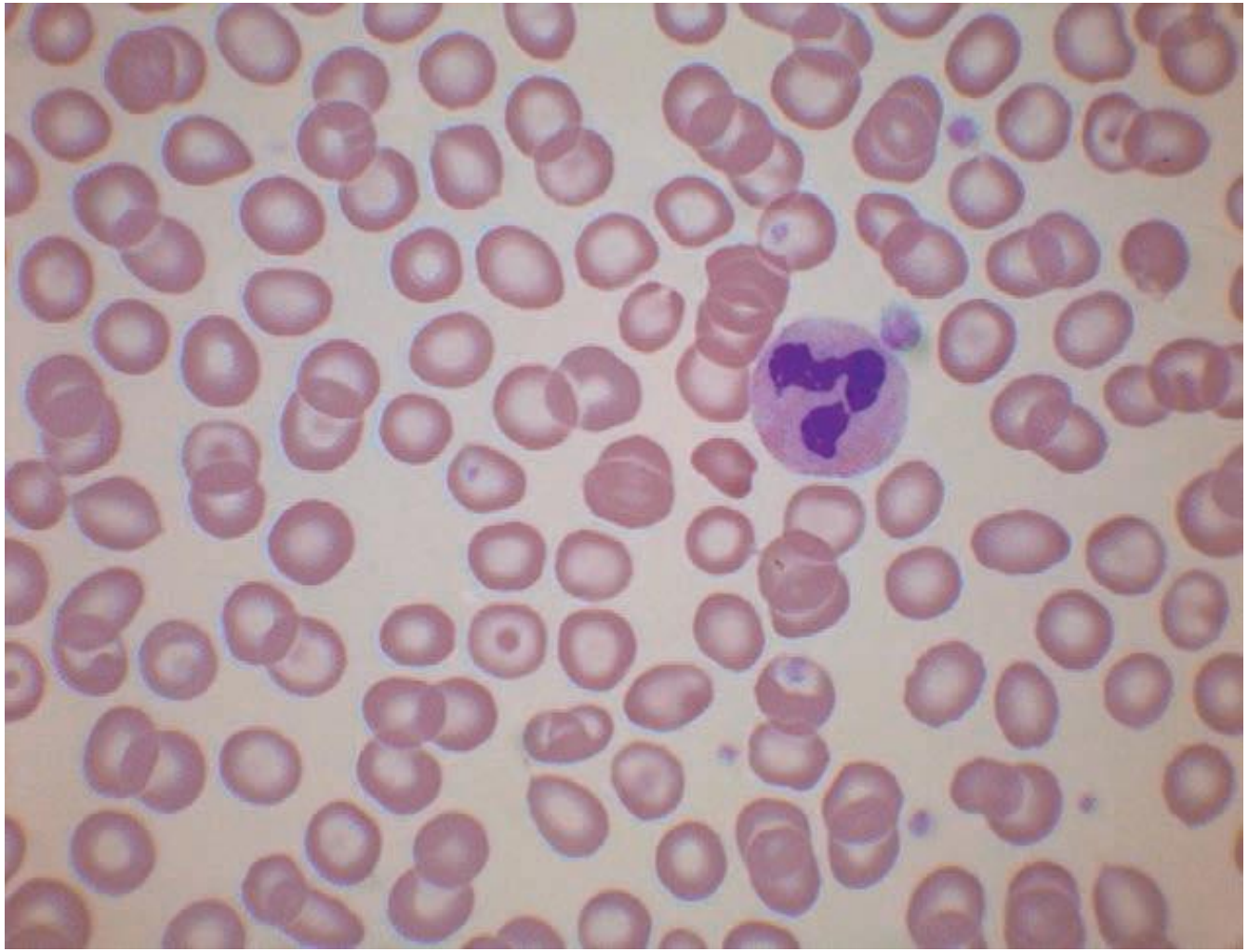
Iron deficiency anemia

Shows hypochromia, microcytosis and poikilocytosis.



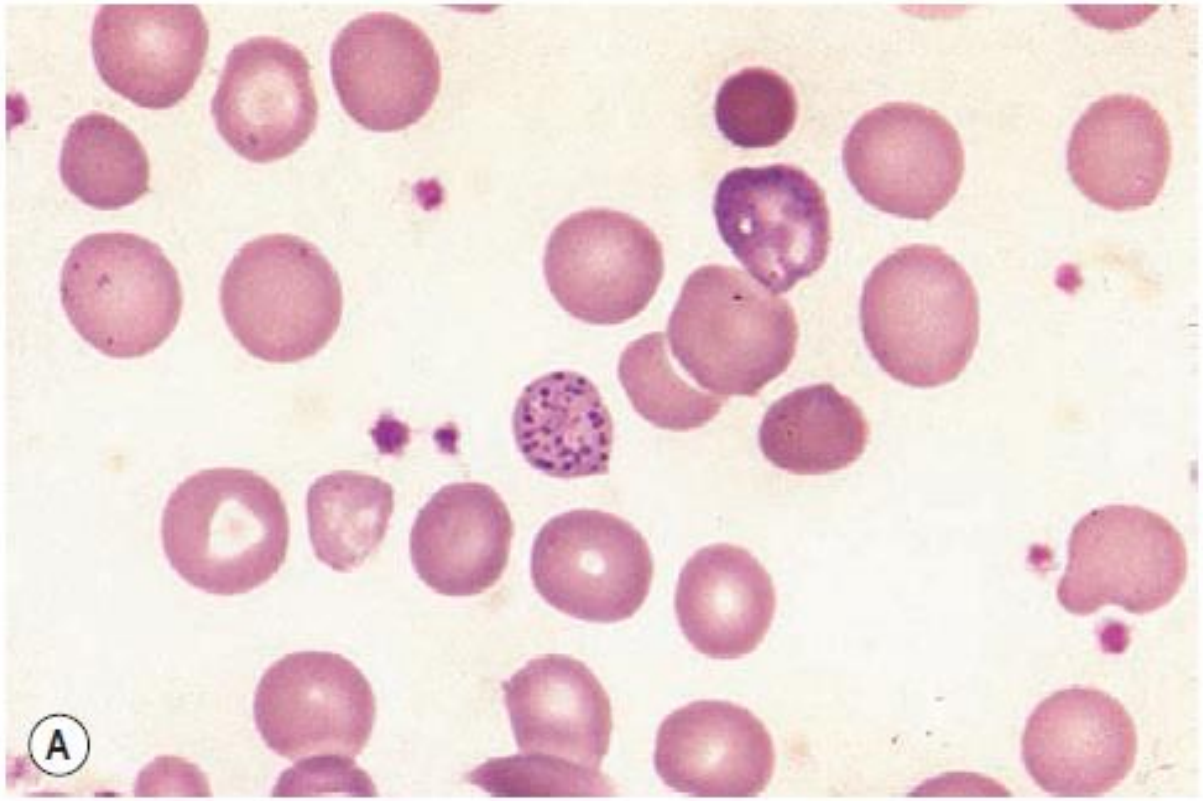
β thalassaemia heterozygosity

Shows hypochromia, microcytosis and several target cells.



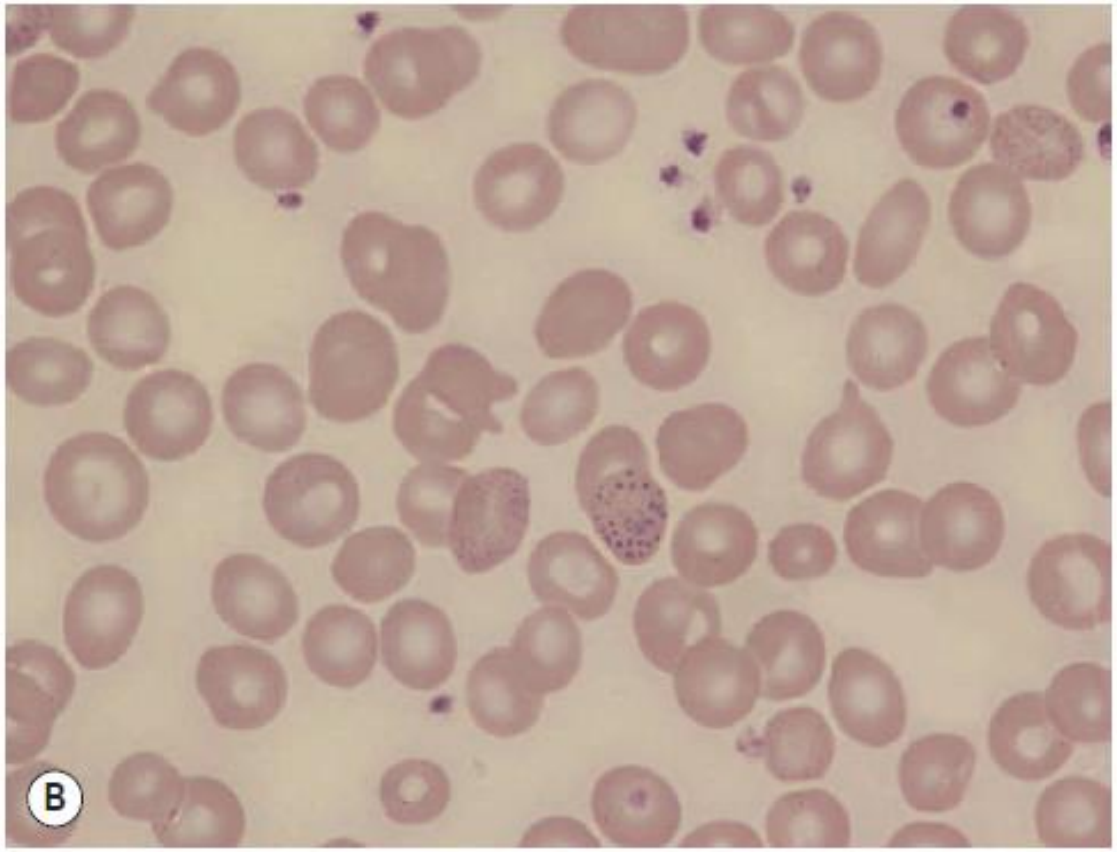
(A) β thalassaemia trait

- shows hypochromia, microcytosis and basophilic stippling.



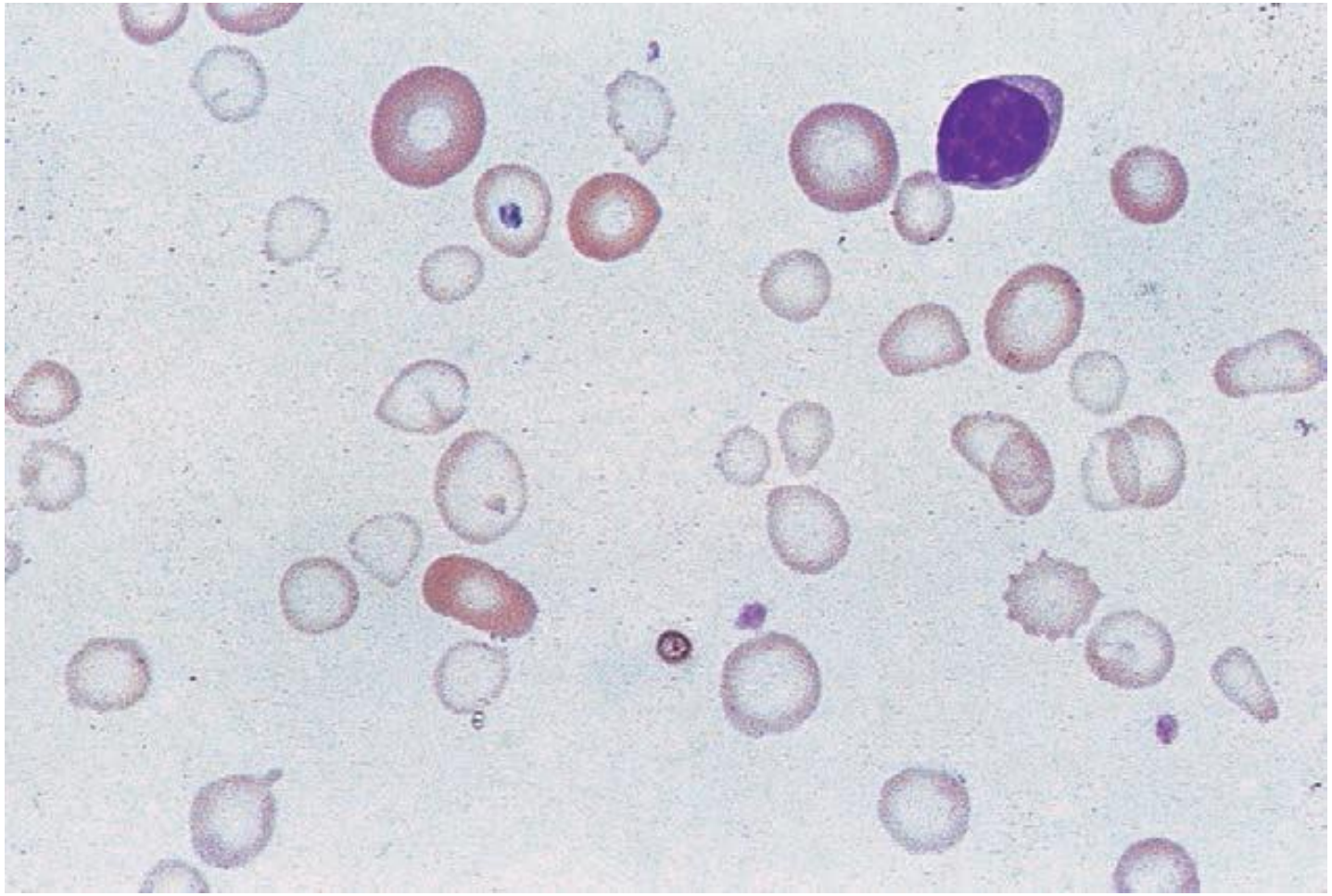
(B) Lead poisoning

- One erythrocyte shows coarse basophilic stippling, while several others show fine basophilic stippling.



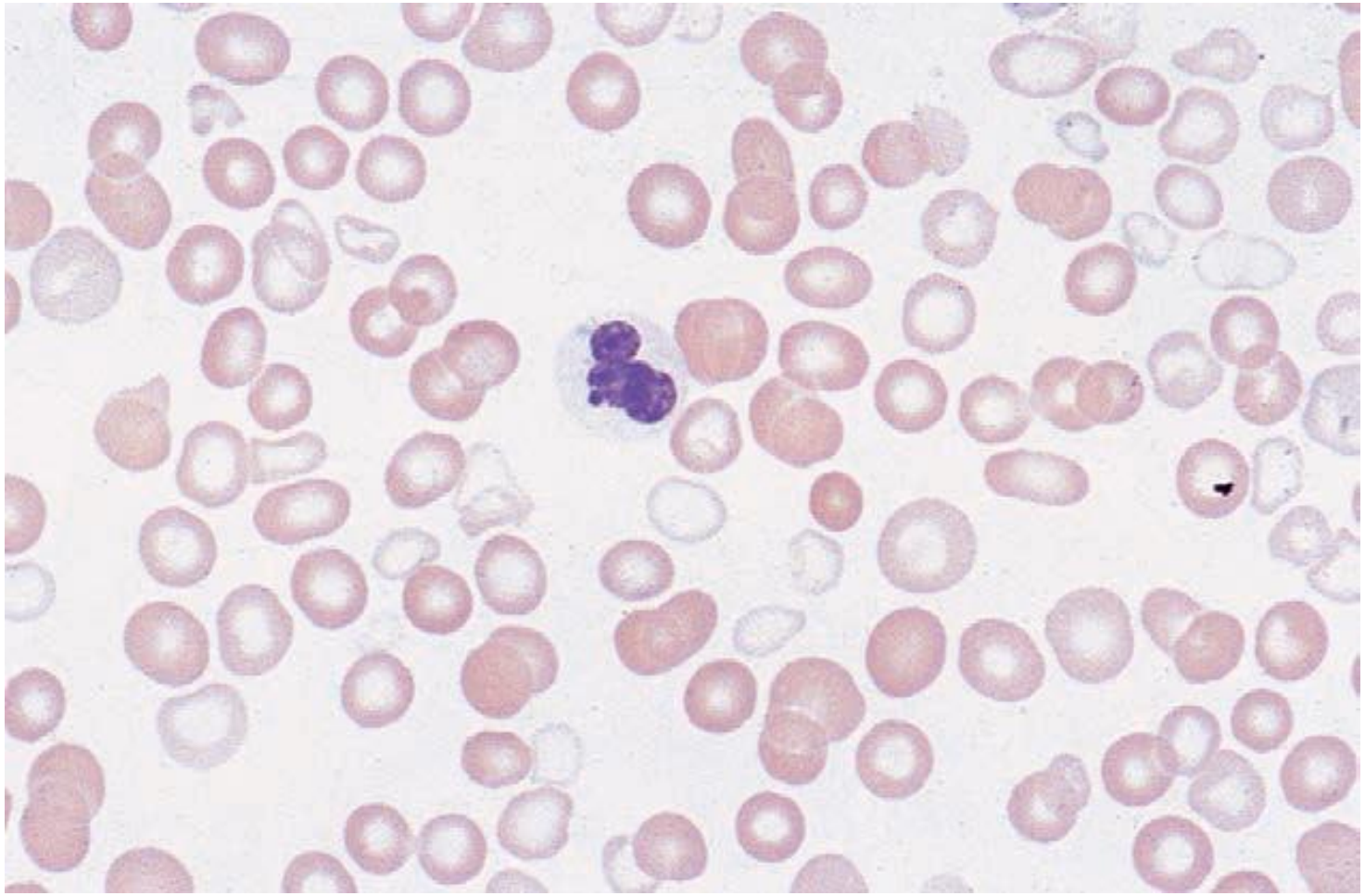
Severe iron deficiency anaemia

Shows a marked degree of hypochromia, microcytosis,marked anisocytosis and mild poikilocytosis; there are some normally haemoglobinised cells.



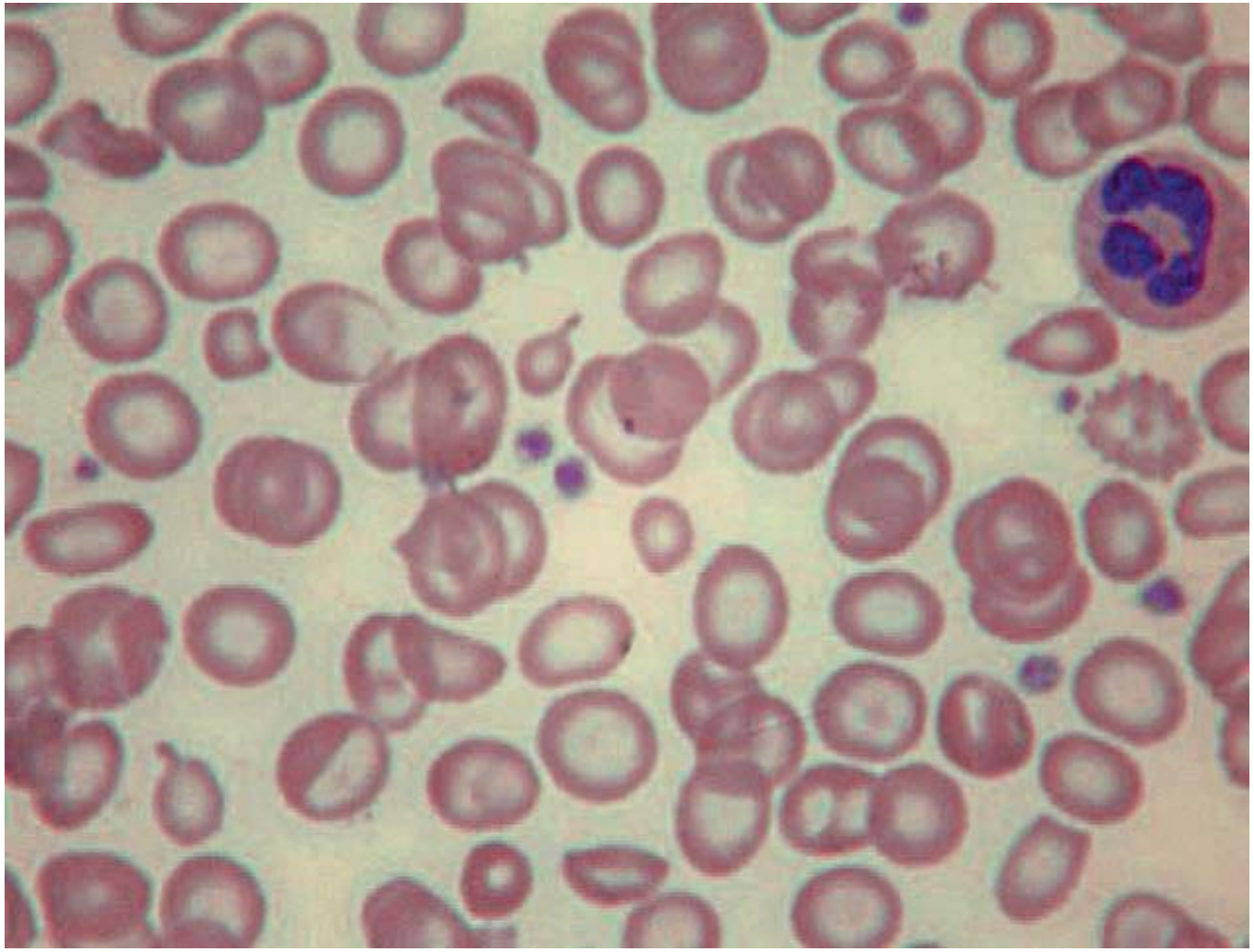
Acquired sideroblastic anaemia (refractory anaemia with ring sideroblasts).

- **Shows a dimorphic blood film with a mixture of normochromic normocytic cells and hypochromic microcytes; there are also several polychromatic macrocytes.**



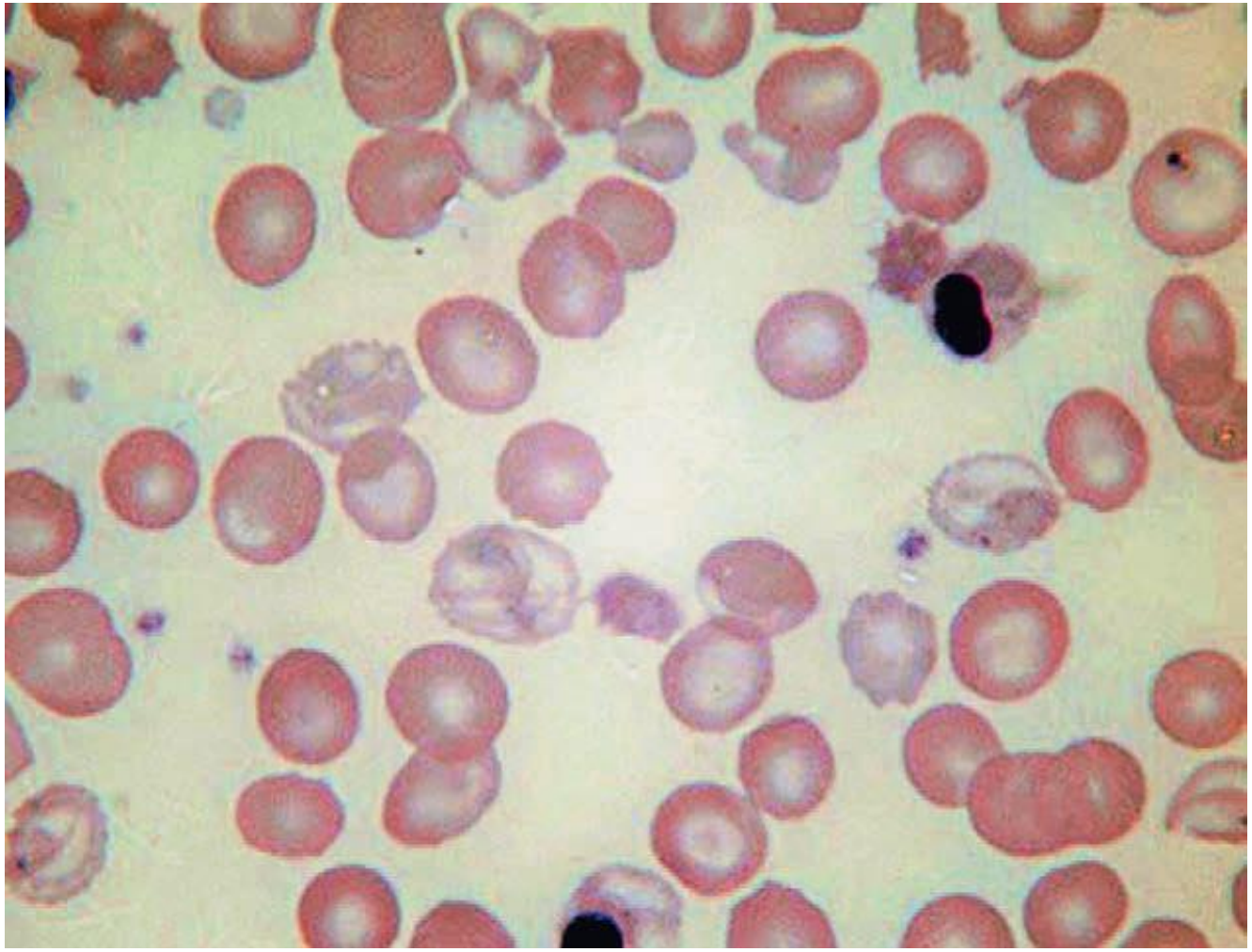
Hemoglobin H disease

- **Shows microcytosis, moderate hypochromia, moderate anisocytosis and some poikilocytes (including teardrop poikilocytes and red cell fragments).**



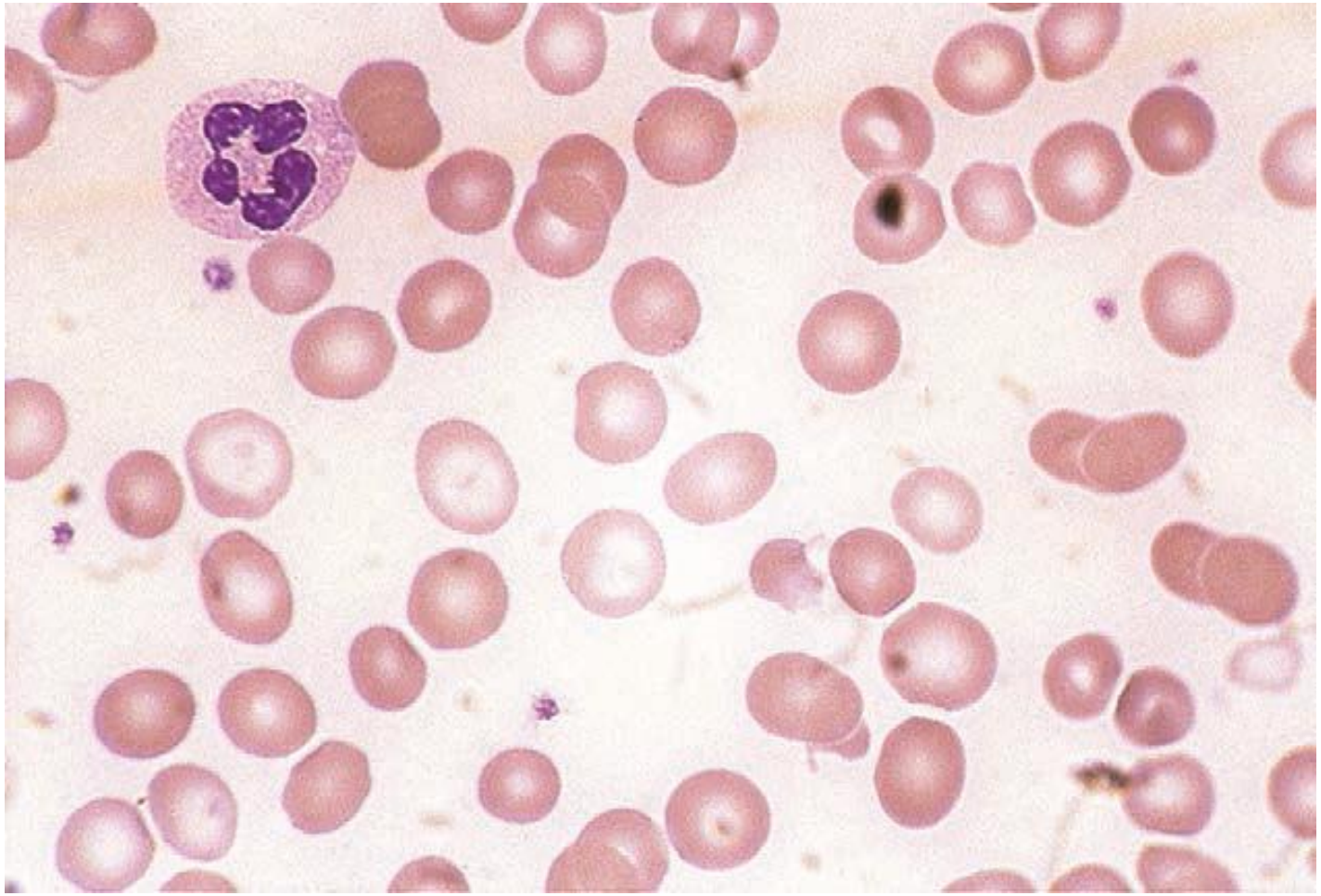
β thalassaemia major

Shows a dimorphic blood film. The normal cells are transfused cells. The patient's own cells show severe hypochromia. There are two nucleated red blood cells.



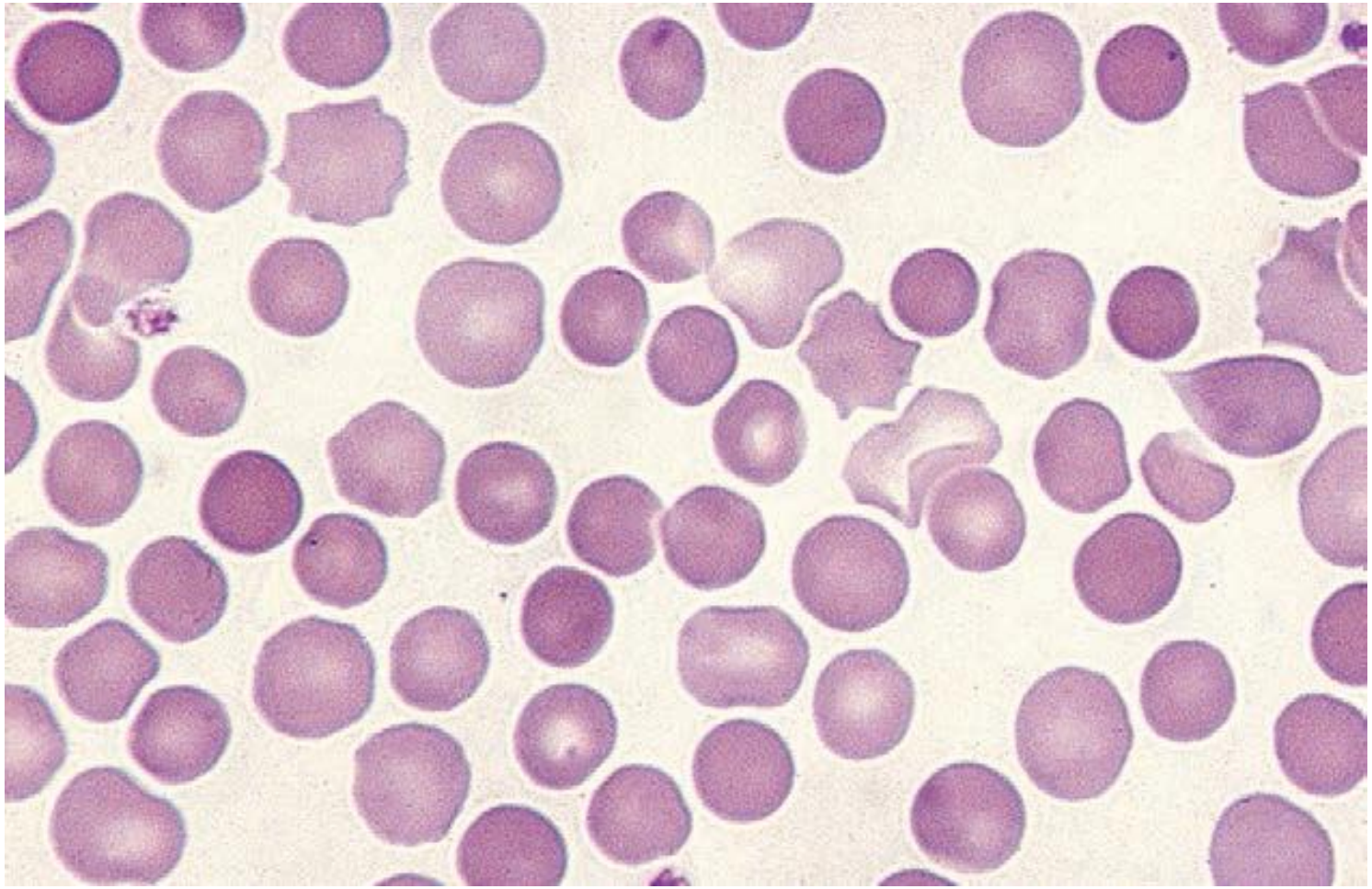
Acquired sideroblastic anemia

- **Shows two distinct populations of cells: hypochromic cells, which also tend to be microcytic, and normocytic normochromic cells.**



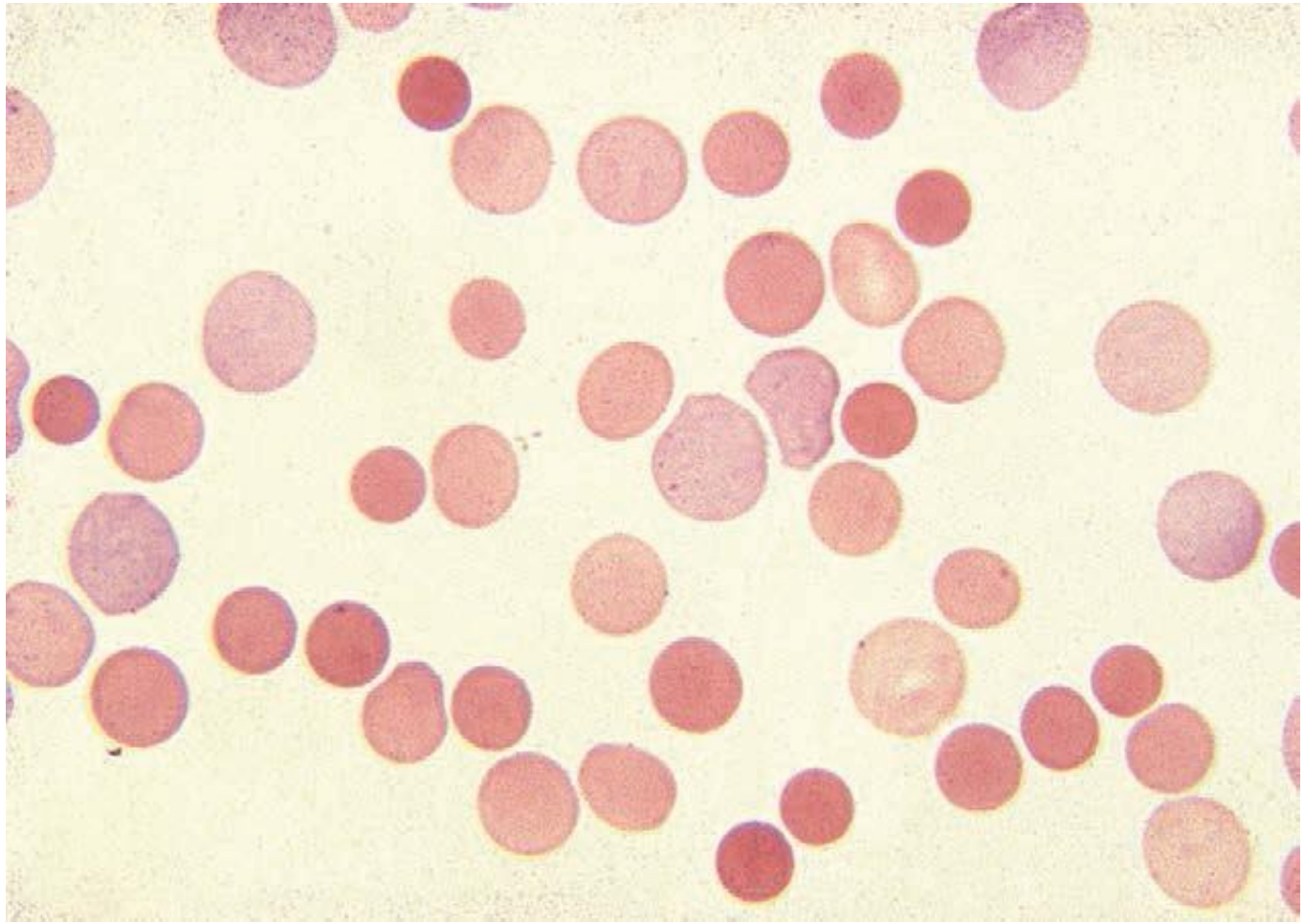
Hereditary spherocytosis

- **Shows marked spherocytosis and some anisocytosis.**
- **Note the round contour of the spherocytes. The larger cells have a faint blue tinge, indicating that the reticulocyte count is increased.**



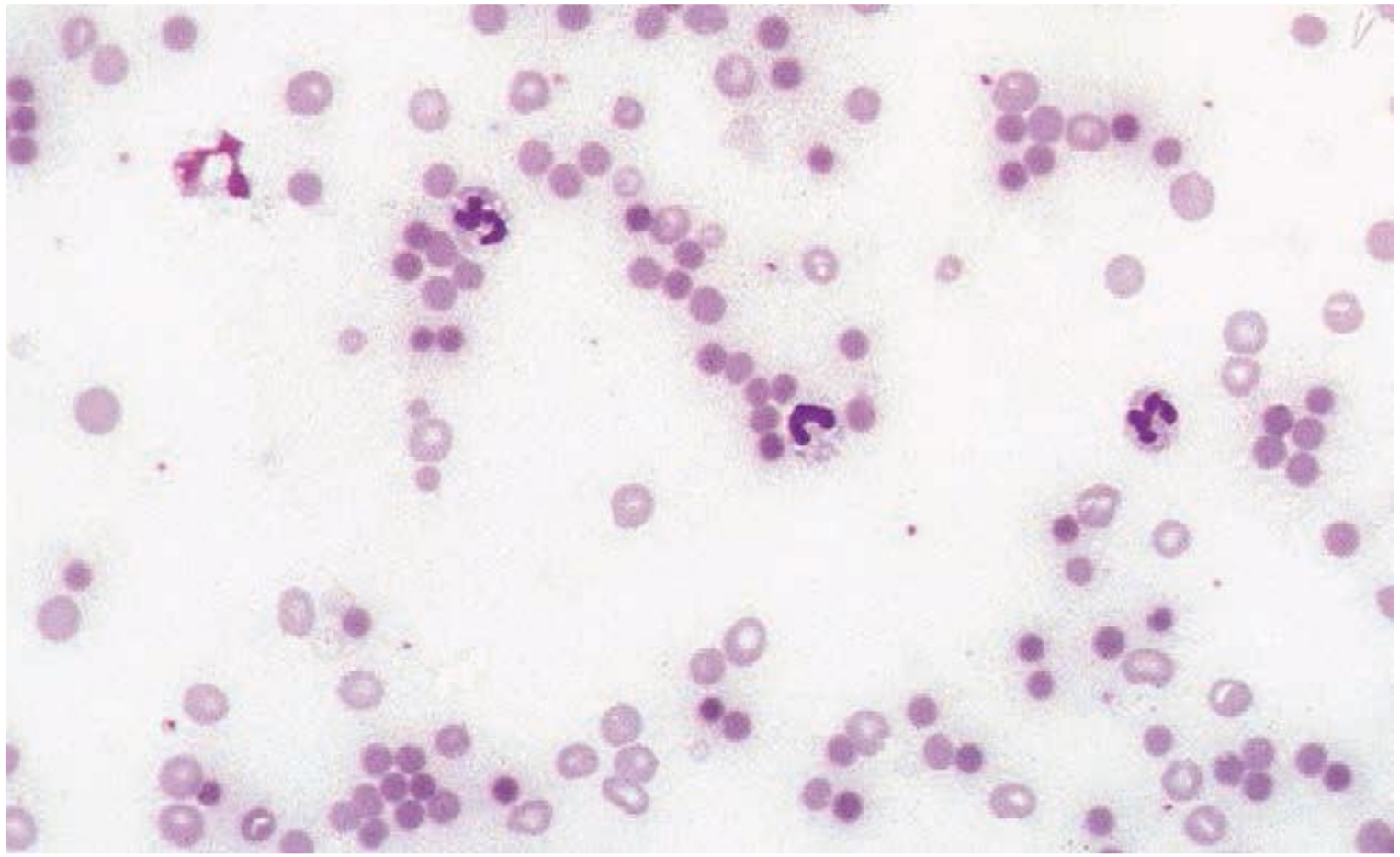
Autoimmune hemolytic anemia

- **Shows marked spherocytosis and anisocytosis.**
- **There are numerous polychromatic macrocytes.**



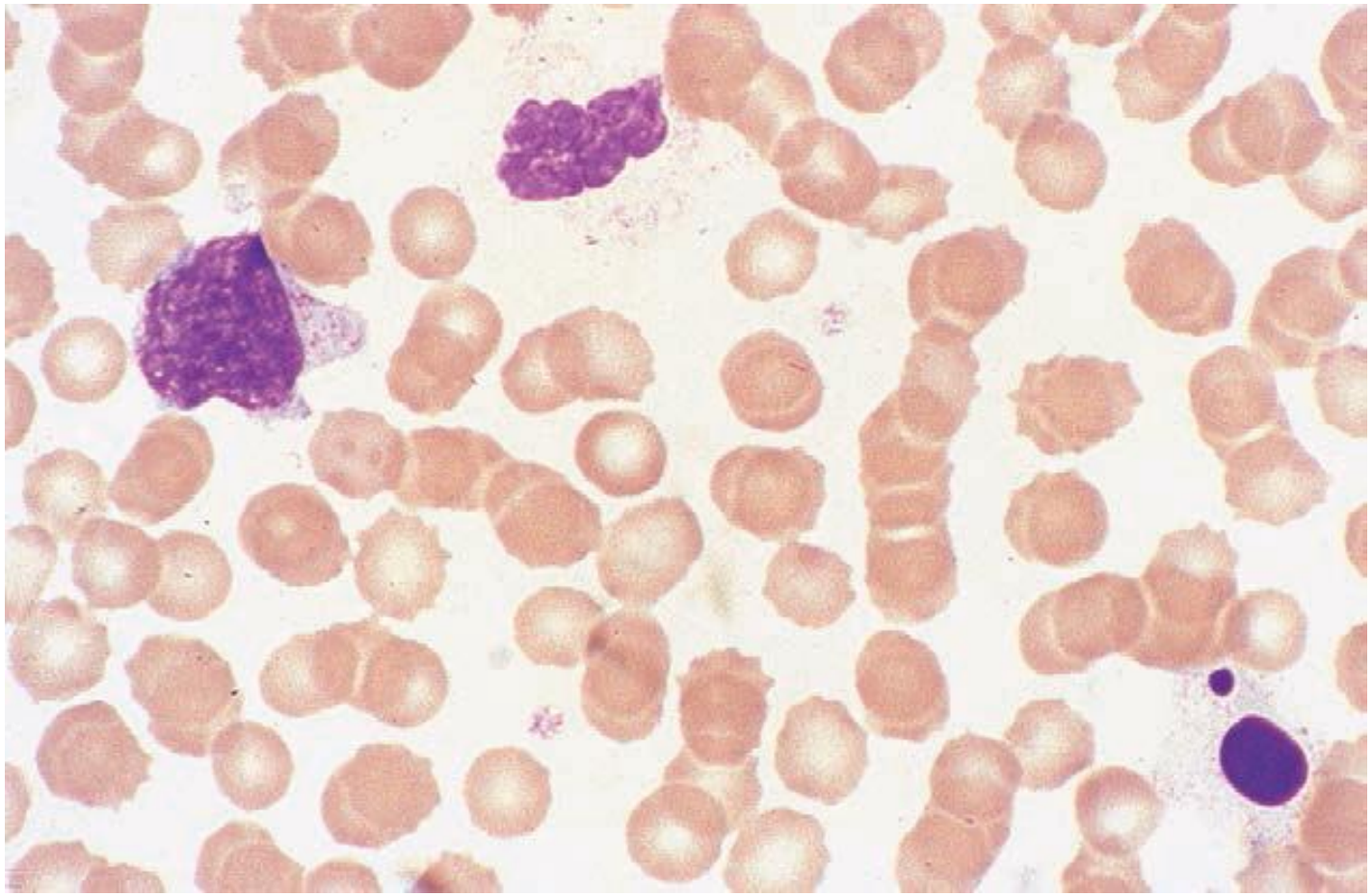
Clostridium perfringens septicaemia

- **Shows an extreme degree of spherocytosis;note the round contour of the spherocytes. A markedly dimorphic picture.**

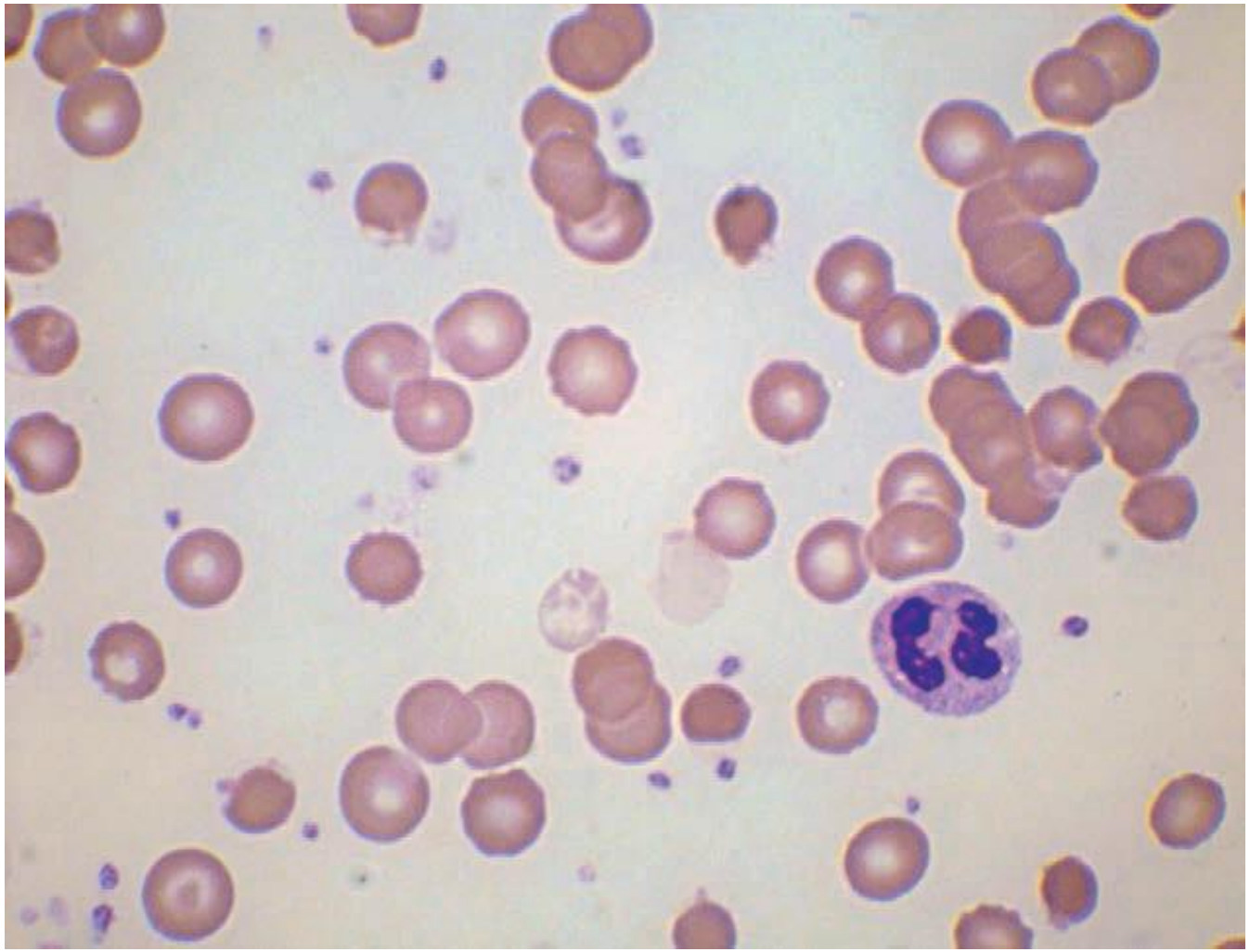


Normal blood

- **after 24 h at 20 °C. Shows a marked degree of crenation; also degenerative changes in white cells.**

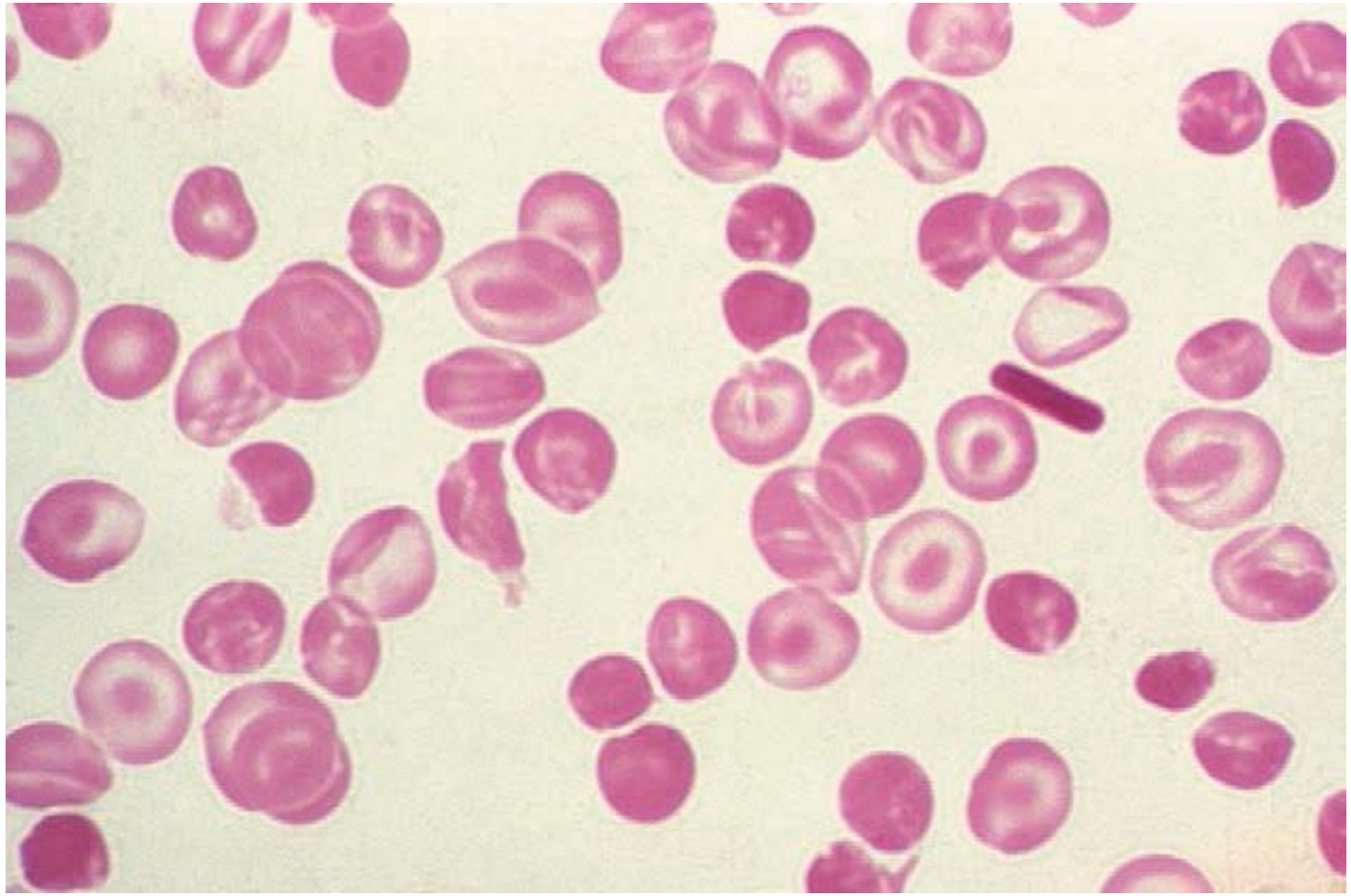


Acute drug induced haemolysis in a patient with glucose-6 phosphate dehydrogenase (G6PD) deficiency. There are irregularly contracted cells and ghost cells *(black arrows).* Heinz bodies can be seen protruding from erythrocytes *(blue arrows).*



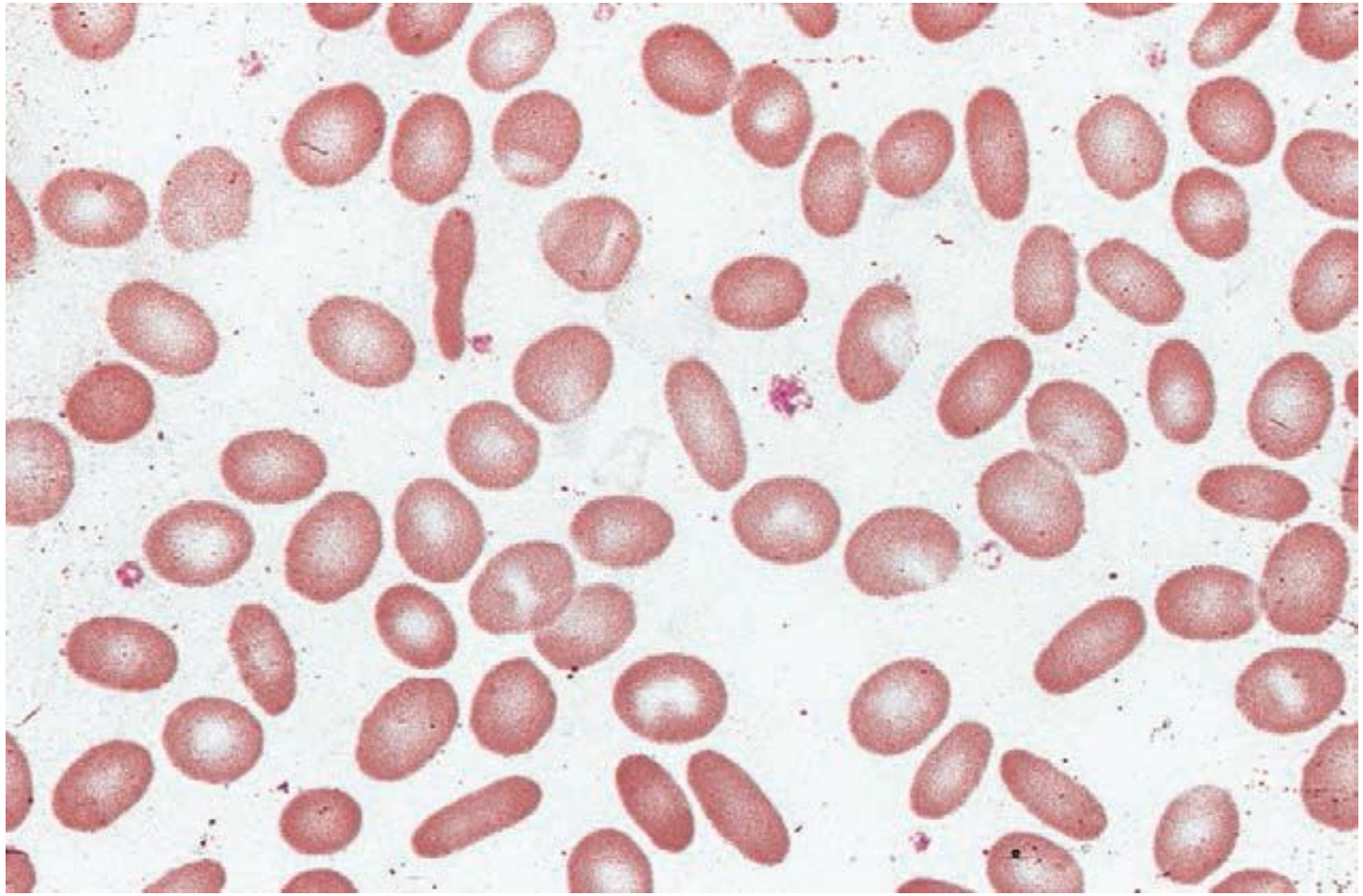
Hemoglobin C disease (homozygosity for hemoglobin C)

- **Shows many target cells, irregularly contracted cells and a crystal of haemoglobin C.**
- **Sometimes it is apparent that haemoglobin C crystals are within otherwise empty red cell membranes.**



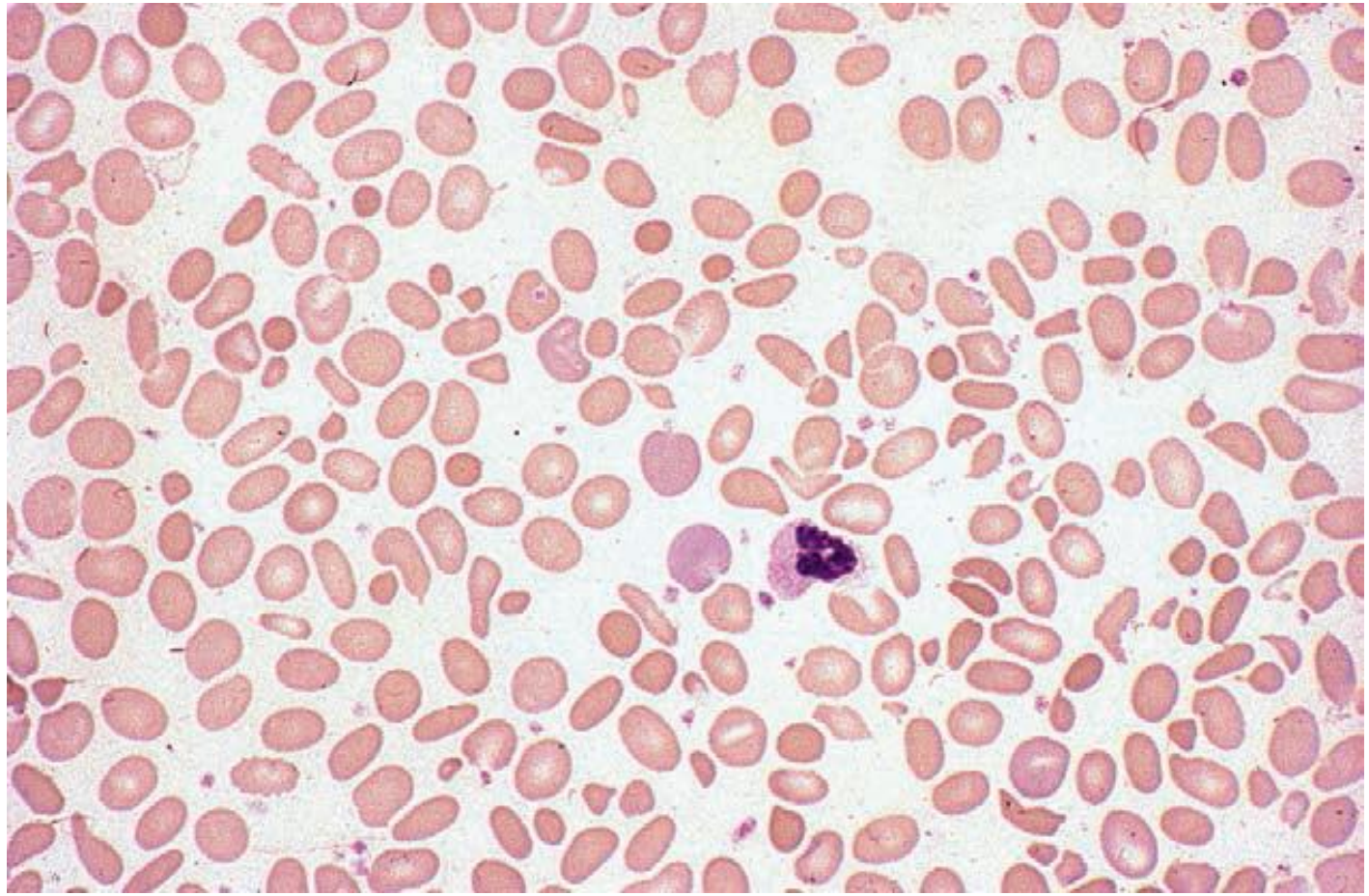
Hereditary elliptocytosis

Many of the cells are elliptical, and others are oval.



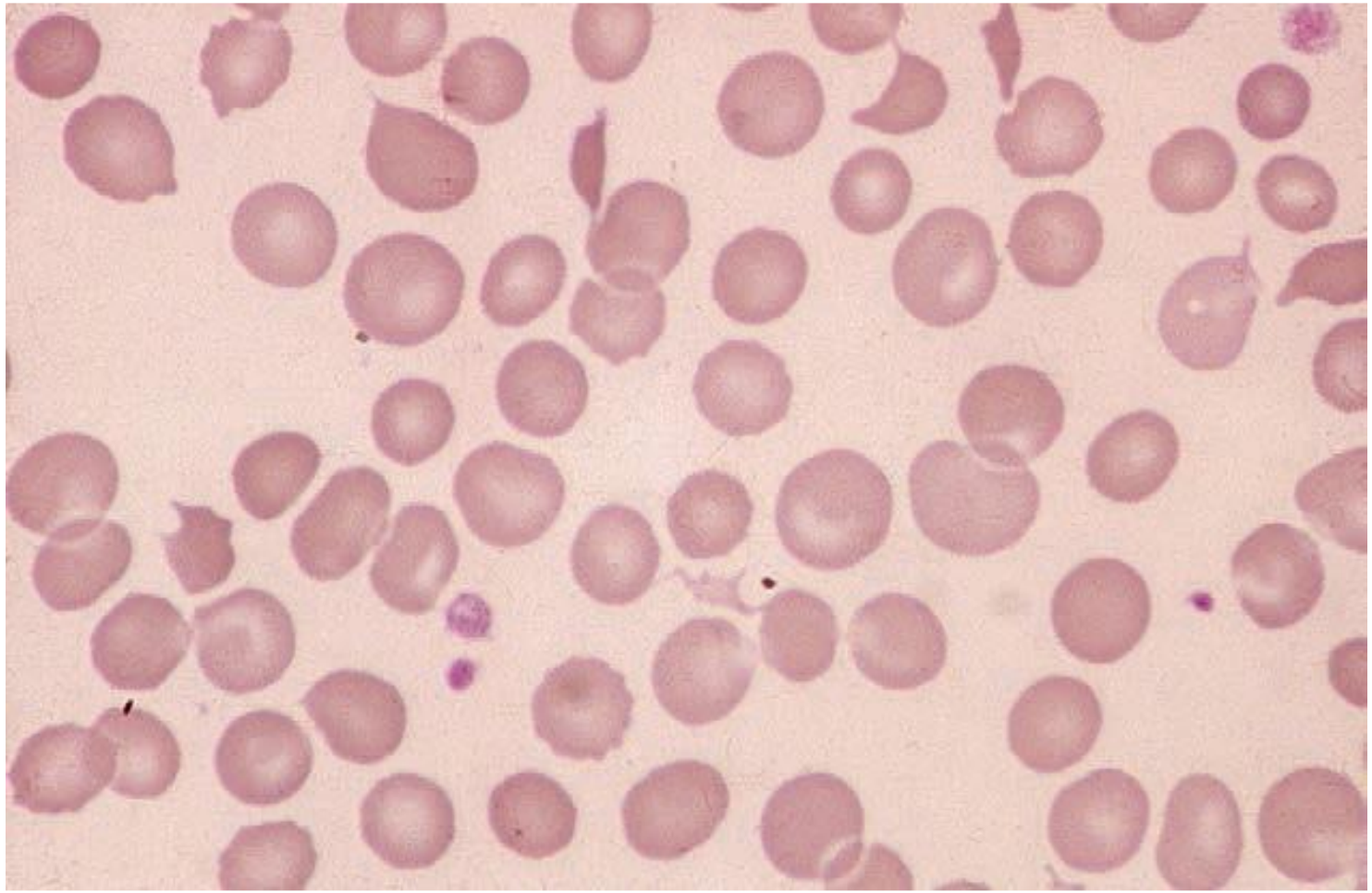
blood film from a child with hereditary pyropoikilocytosis.

- **Shows spherocytes, elliptocytes, ovalocytes, red cell fragments and polychromatic macrocytes.**



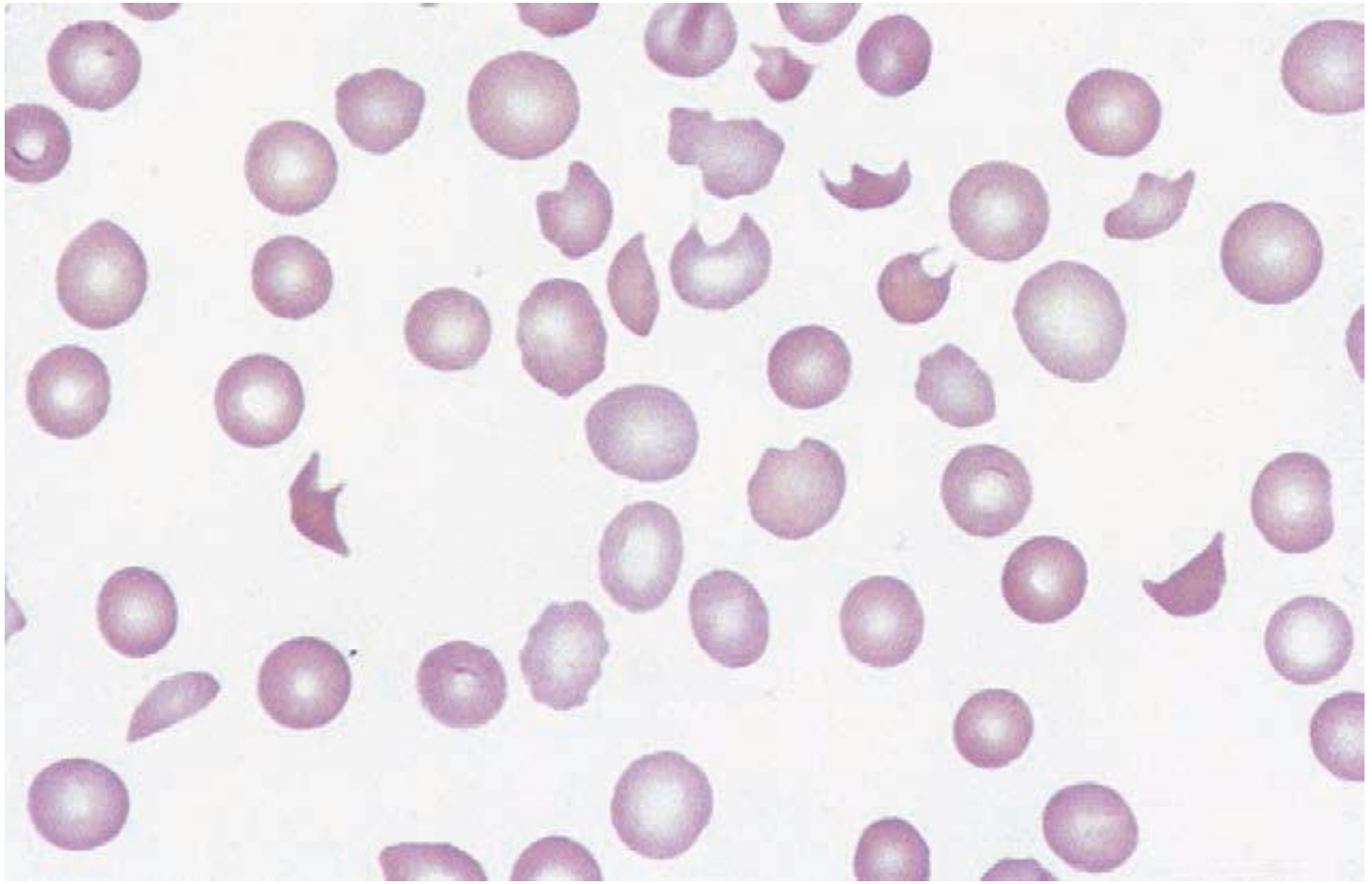
Microangiopathic hemolytic anemia

- **Shows angular red cell fragments.**



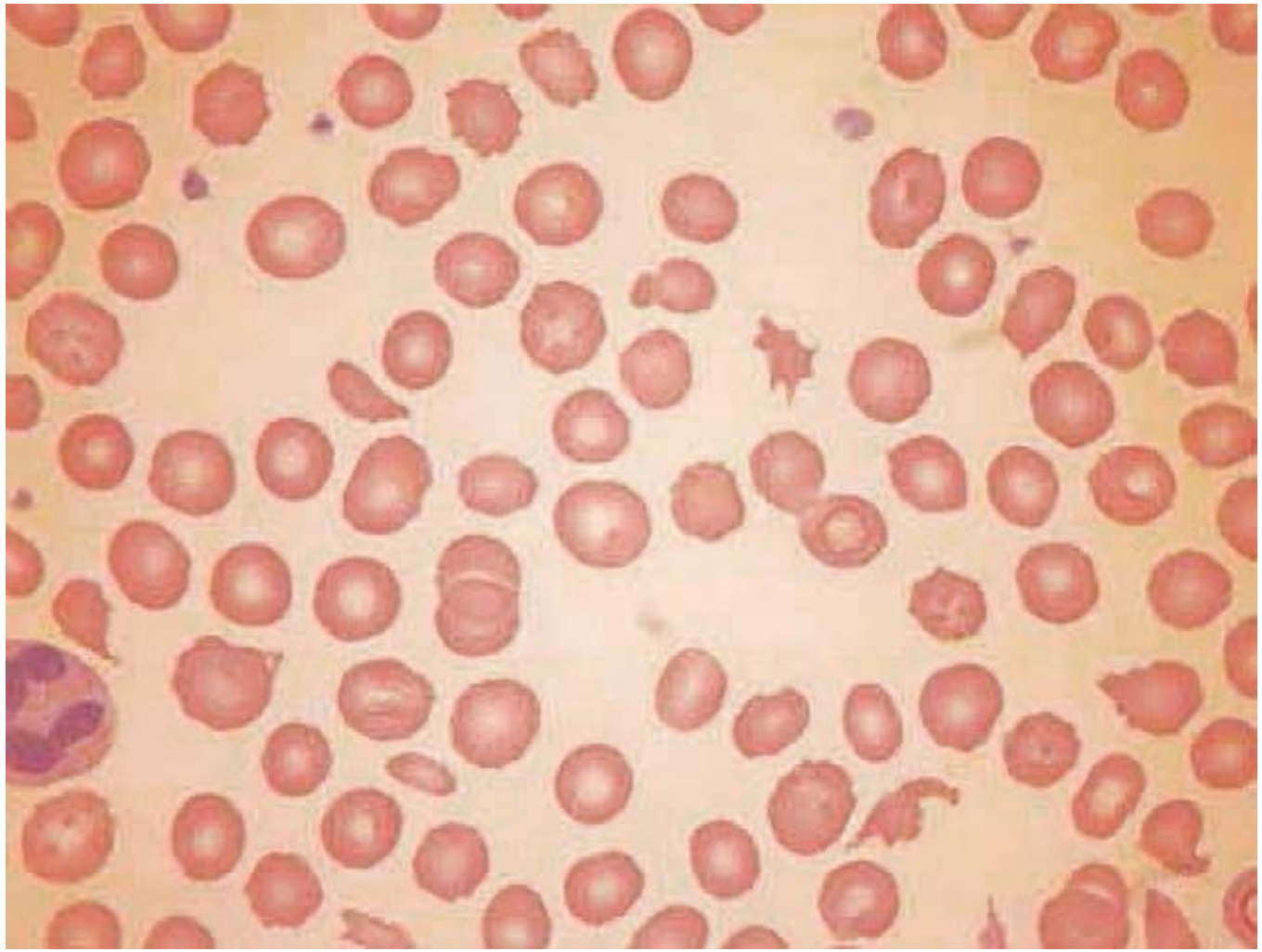
Microangiopathic hemolytic anemia

Shows numerous bizarrely shaped red cell fragments.



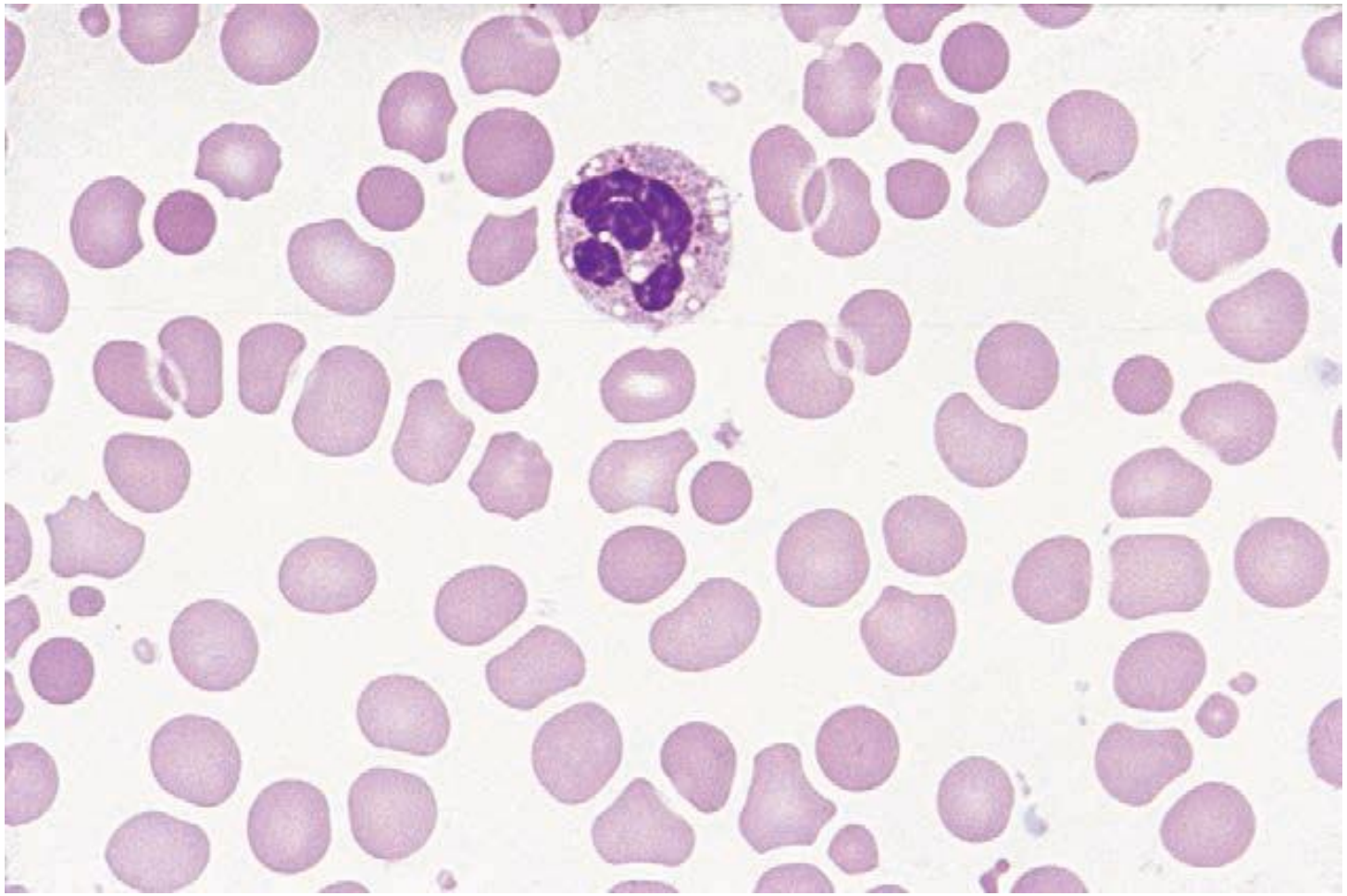
Haemolytic anaemia after previous cardiac surgery

- **Shows numerous irregularly shaped cell fragments.**

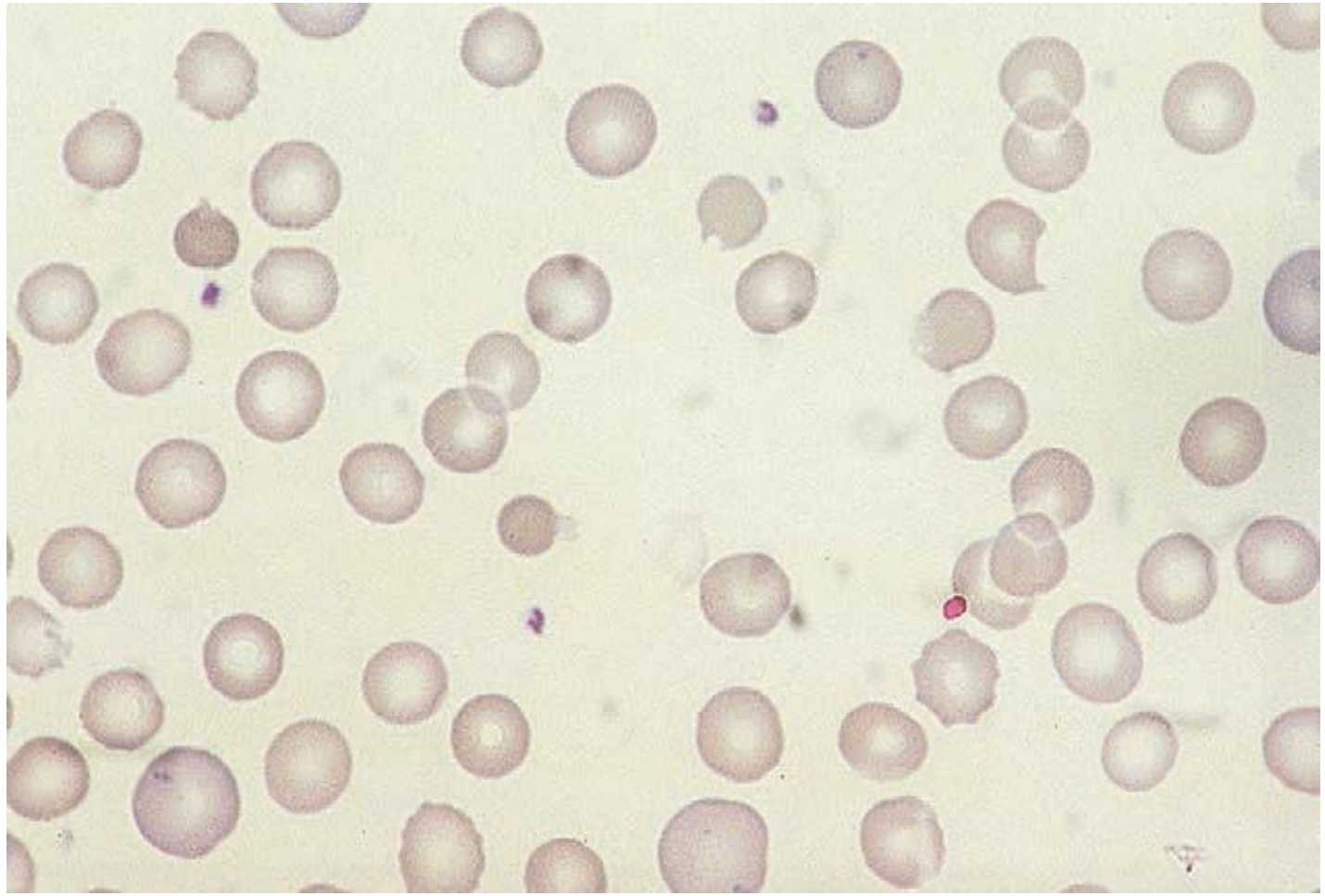


Severe burns

- **Shows many very small, rounded red cell fragments (microspherocytes), a 'microdiscocyte' (bottom right), another characteristic feature of burns, and one vacuolated neutrophil.**

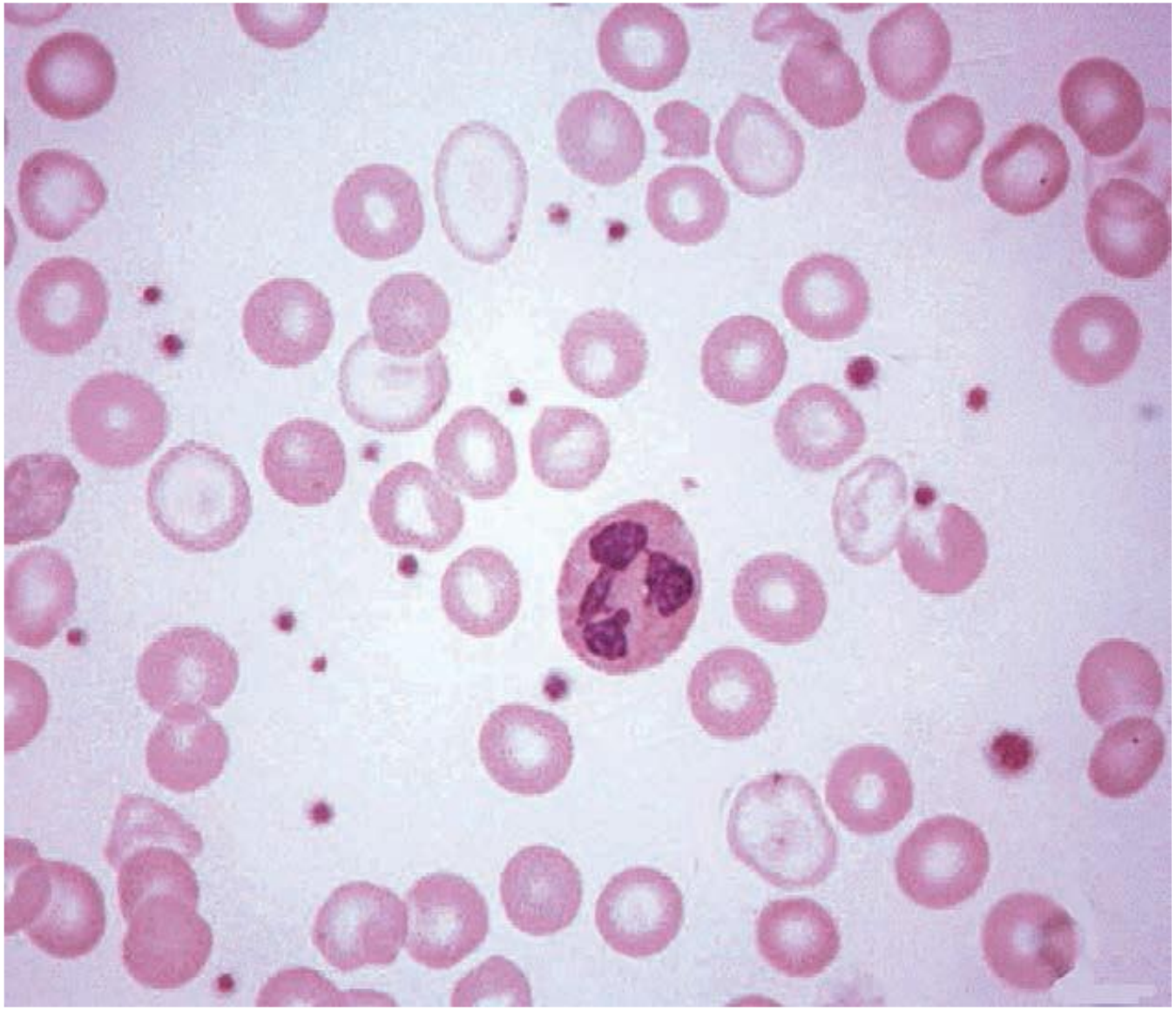


Keratocytes and irregularly contracted cells in a patient with haemolysis caused by G6PD deficiency.



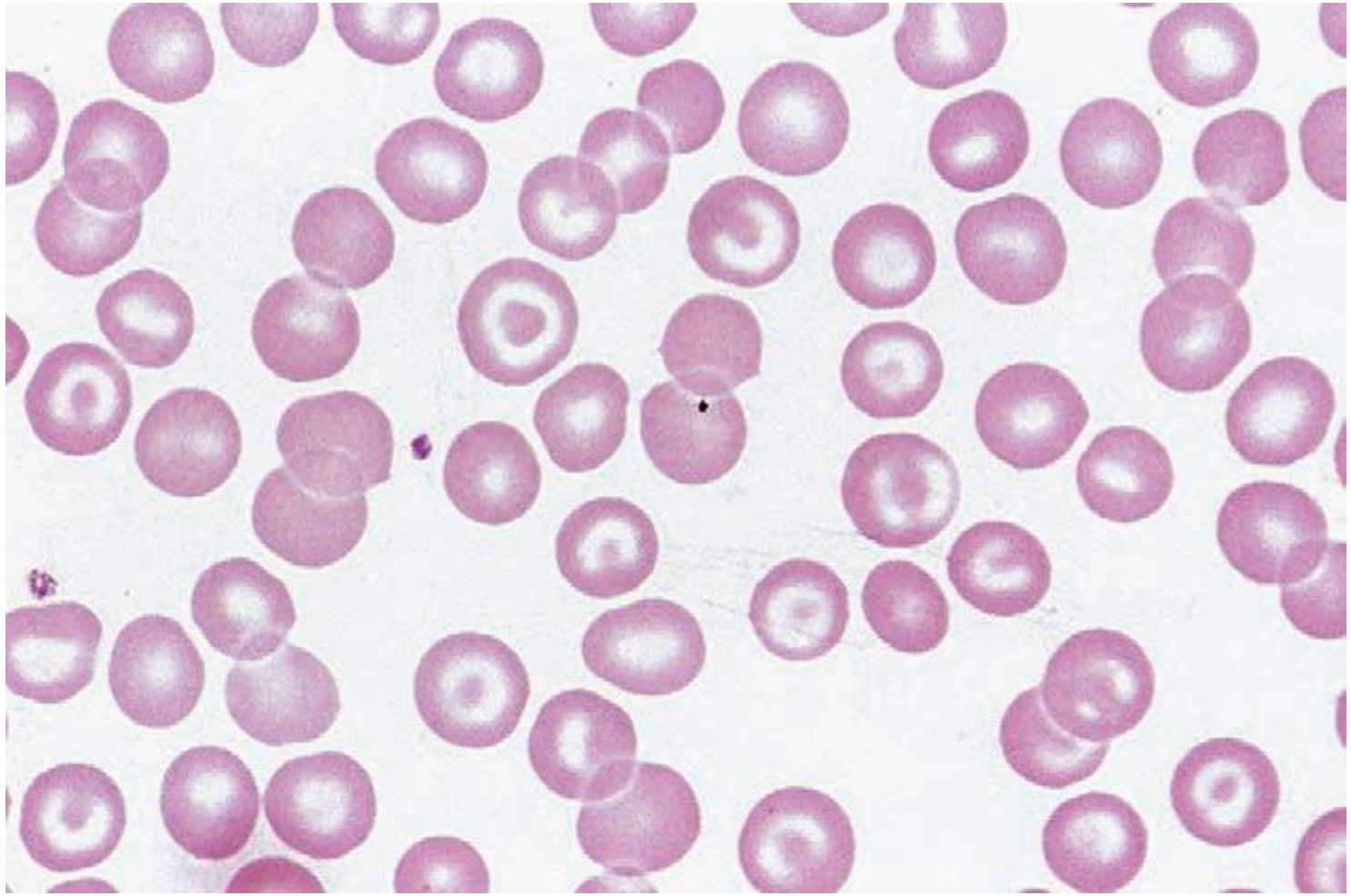
β thalassaemia major, after splenectomy

- **Shows one target cell and cells grossly deficient in haemoglobin, which are leptocytes. There are some transfused cells.**



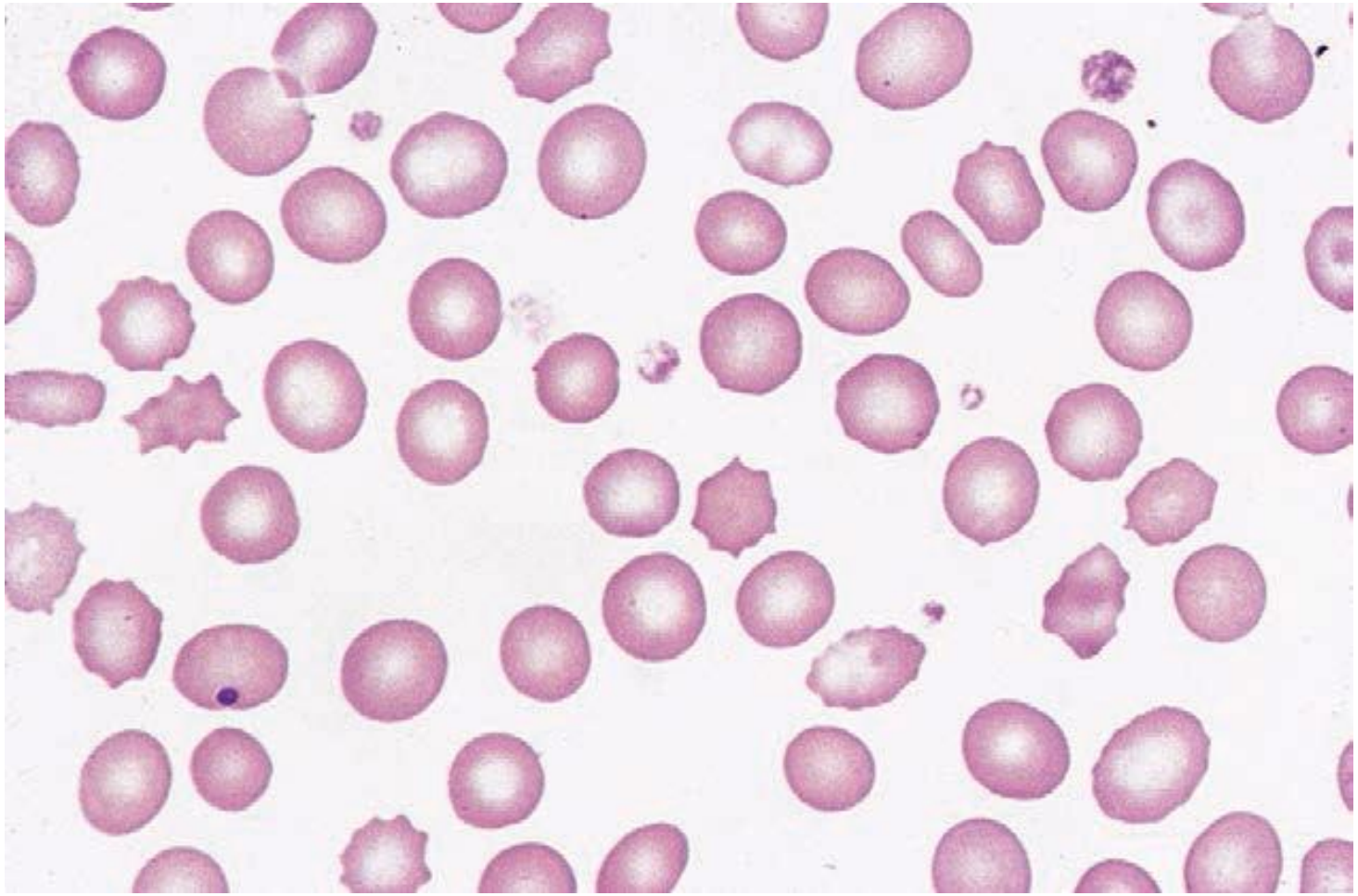
Alcoholic liver disease

- **Shows many target cells.**



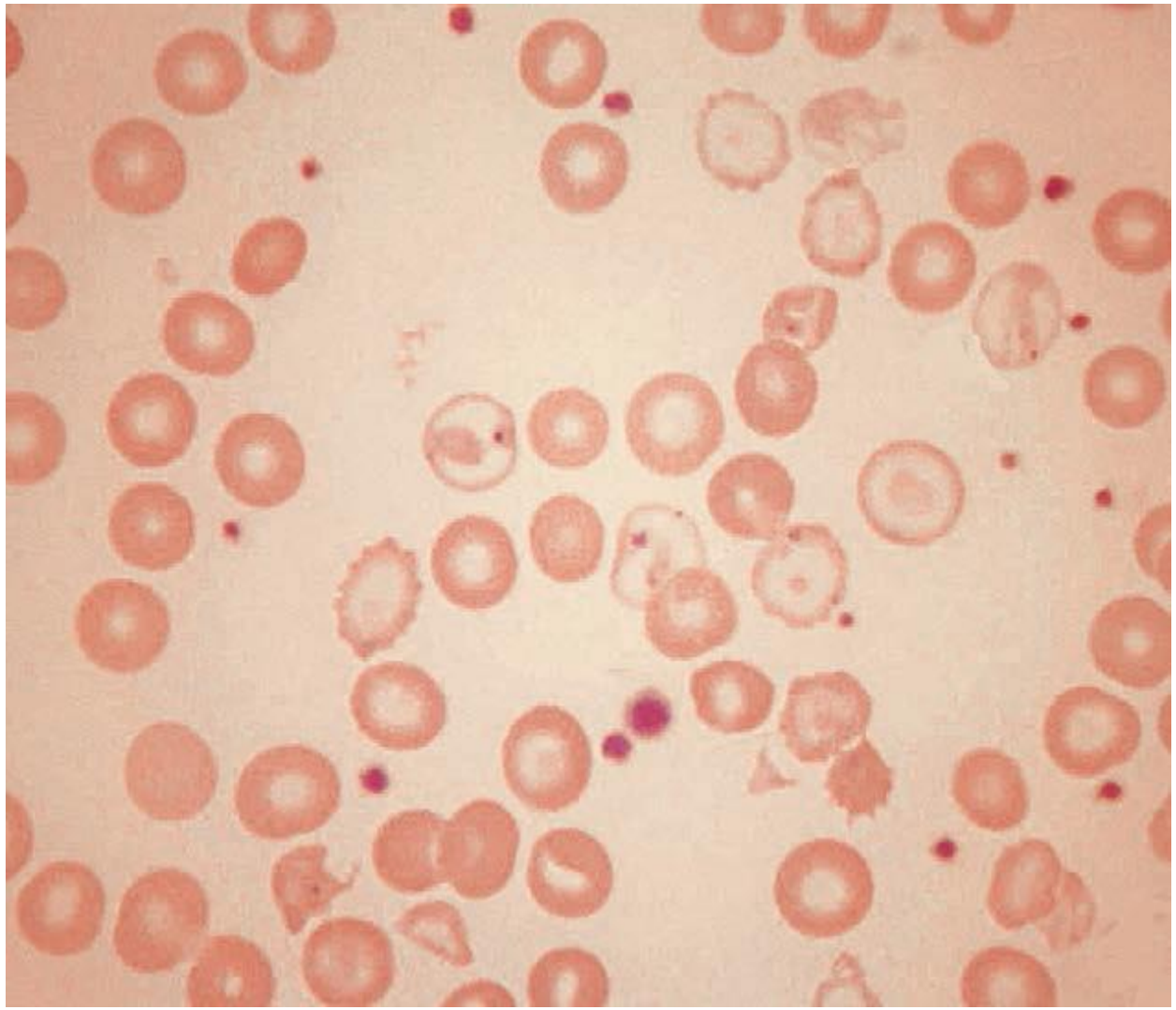
Postsplenectomy

- **Shows acanthocytes, a target cell and a Howell–Jolly body.**

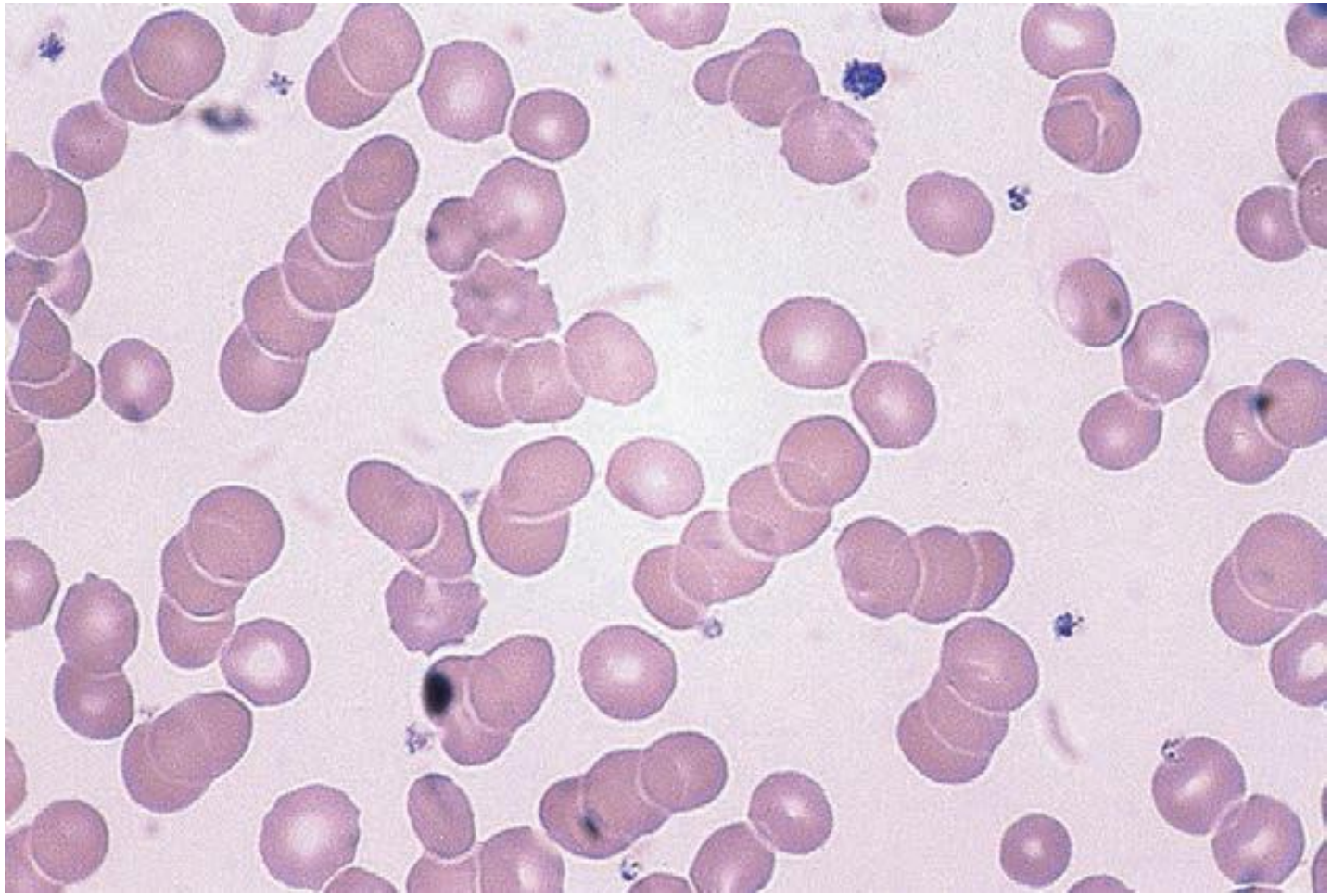


β thalassaemia major

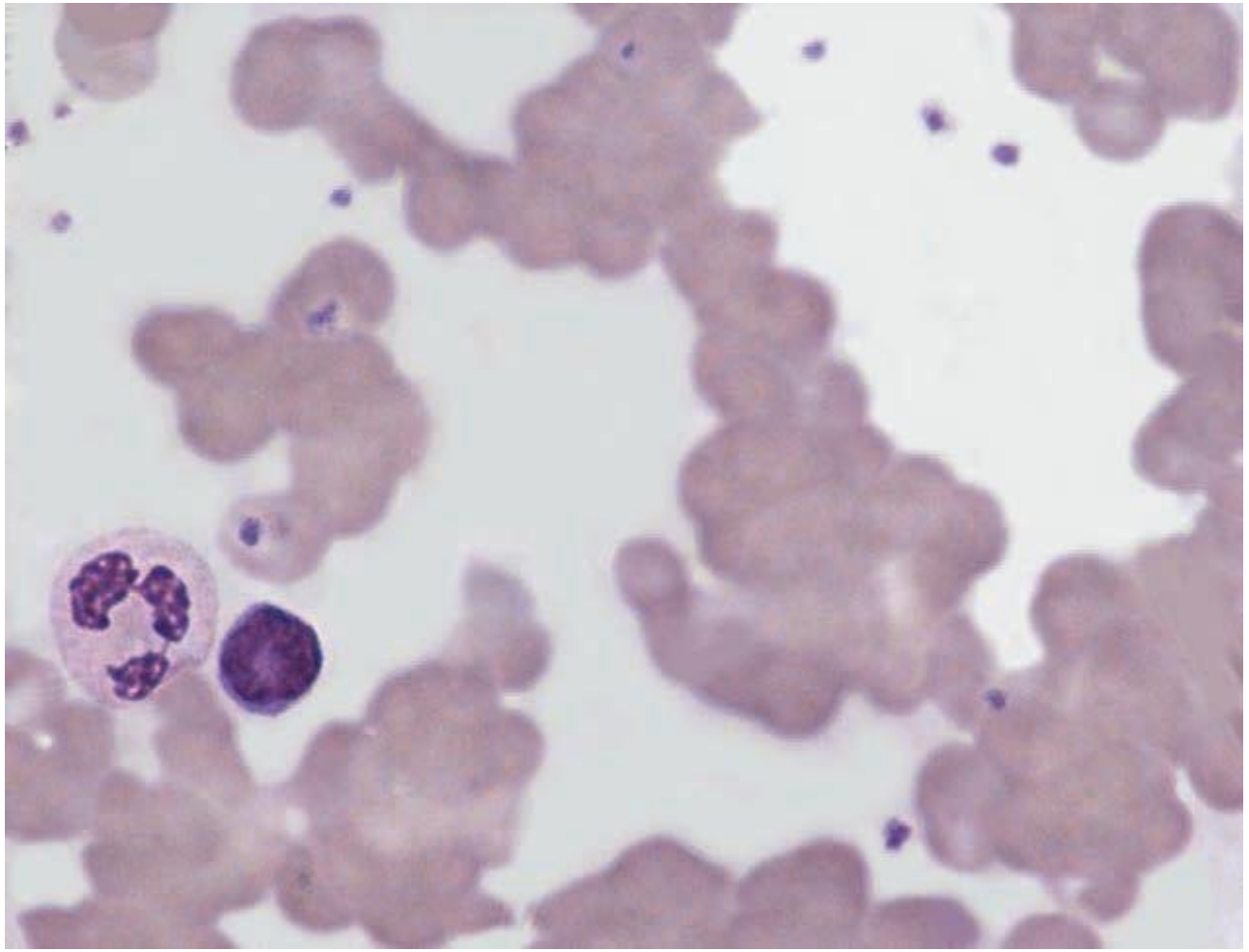
- **patient on regular blood transfusions, showing Pappenheimer bodies in several poorly haemoglobinised cells.**



- **Increased rouleaux formation in a patient with bacterial infection.**

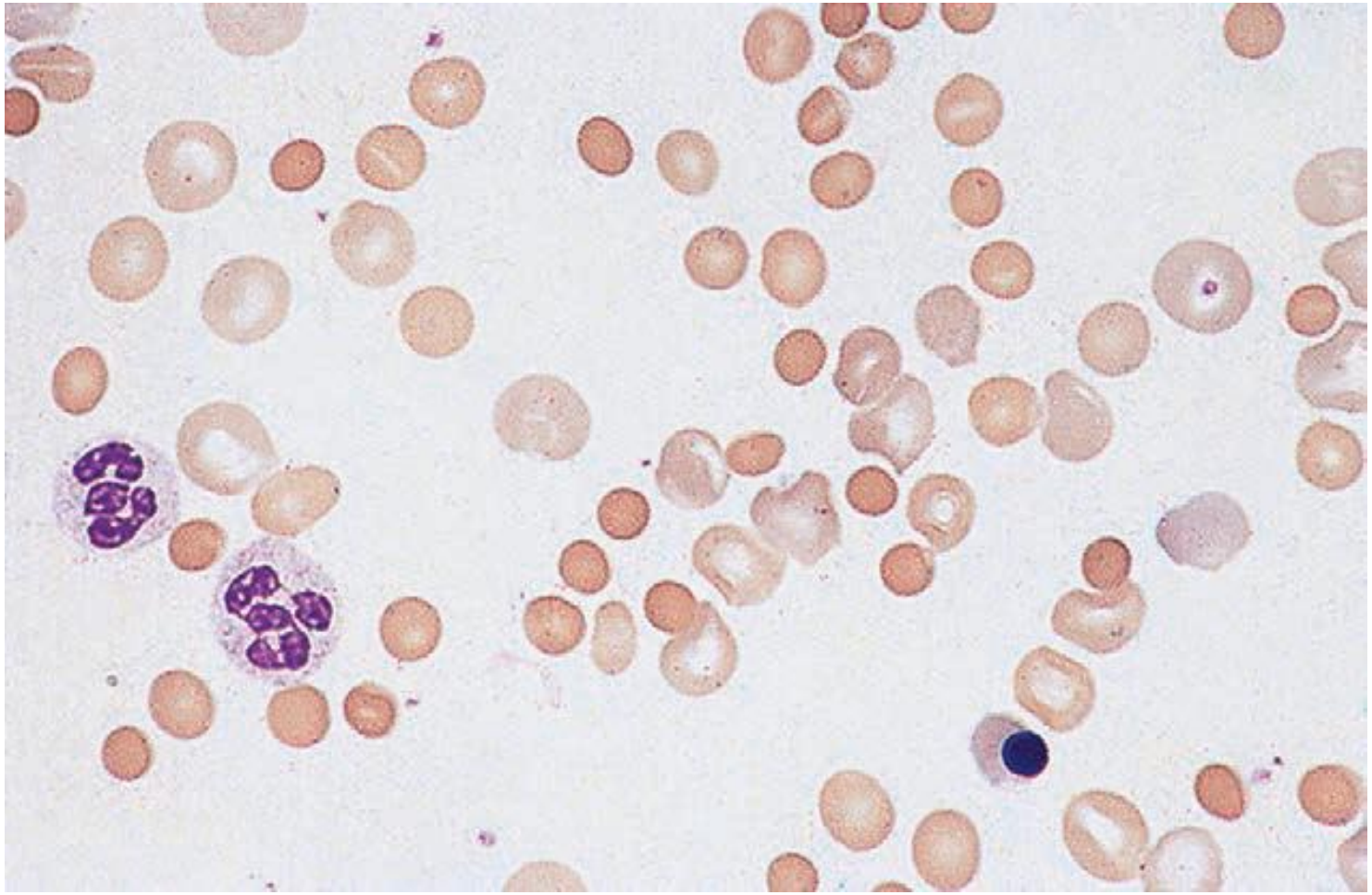


- **Shows agglutination in a patient with chronic cold haemagglutinin disease.**



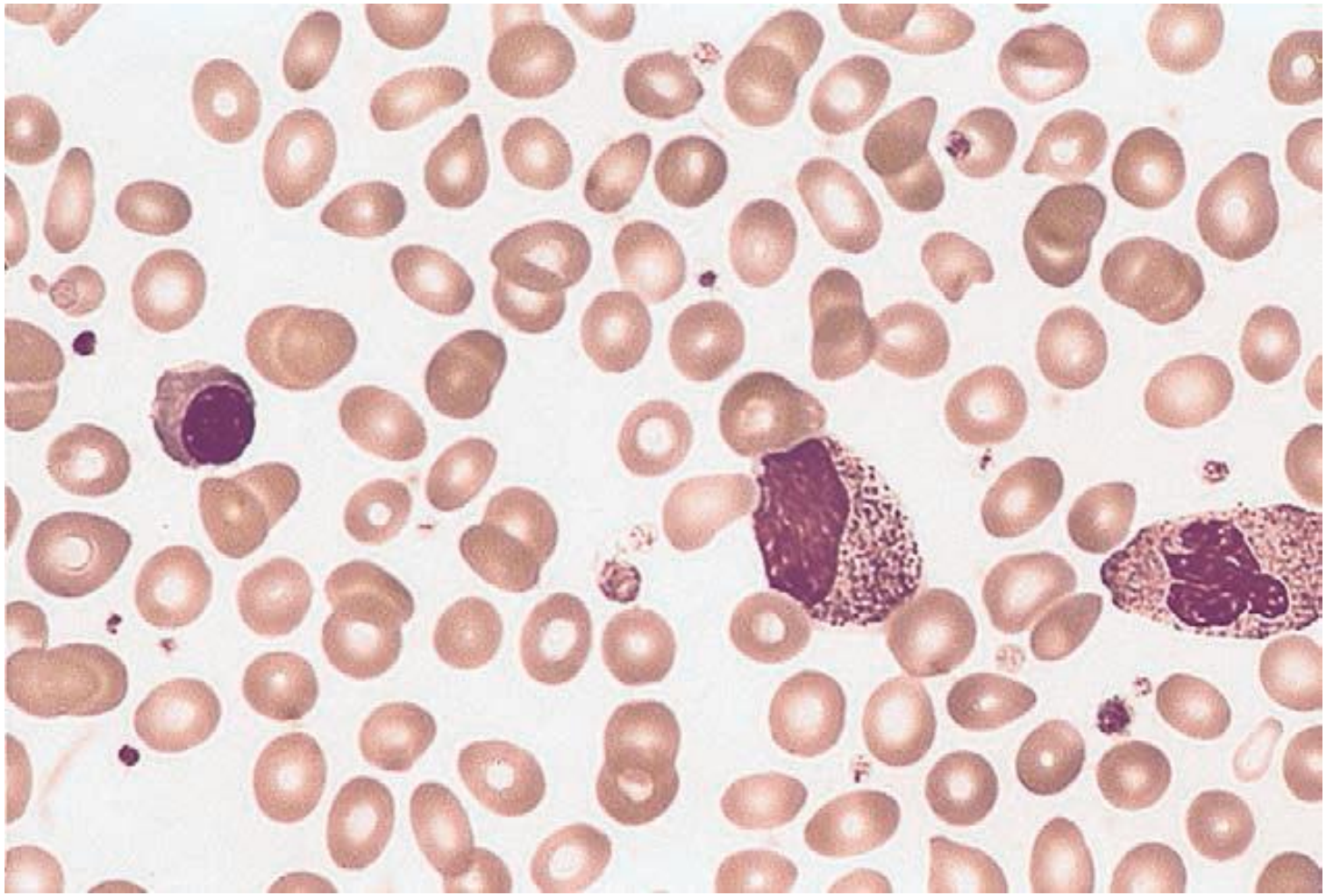
Polychromasia

- **Some red cells stain shades of bluish grey. There are also spherocytes and one nucleated red blood cell.**



Primary myelofibrosis

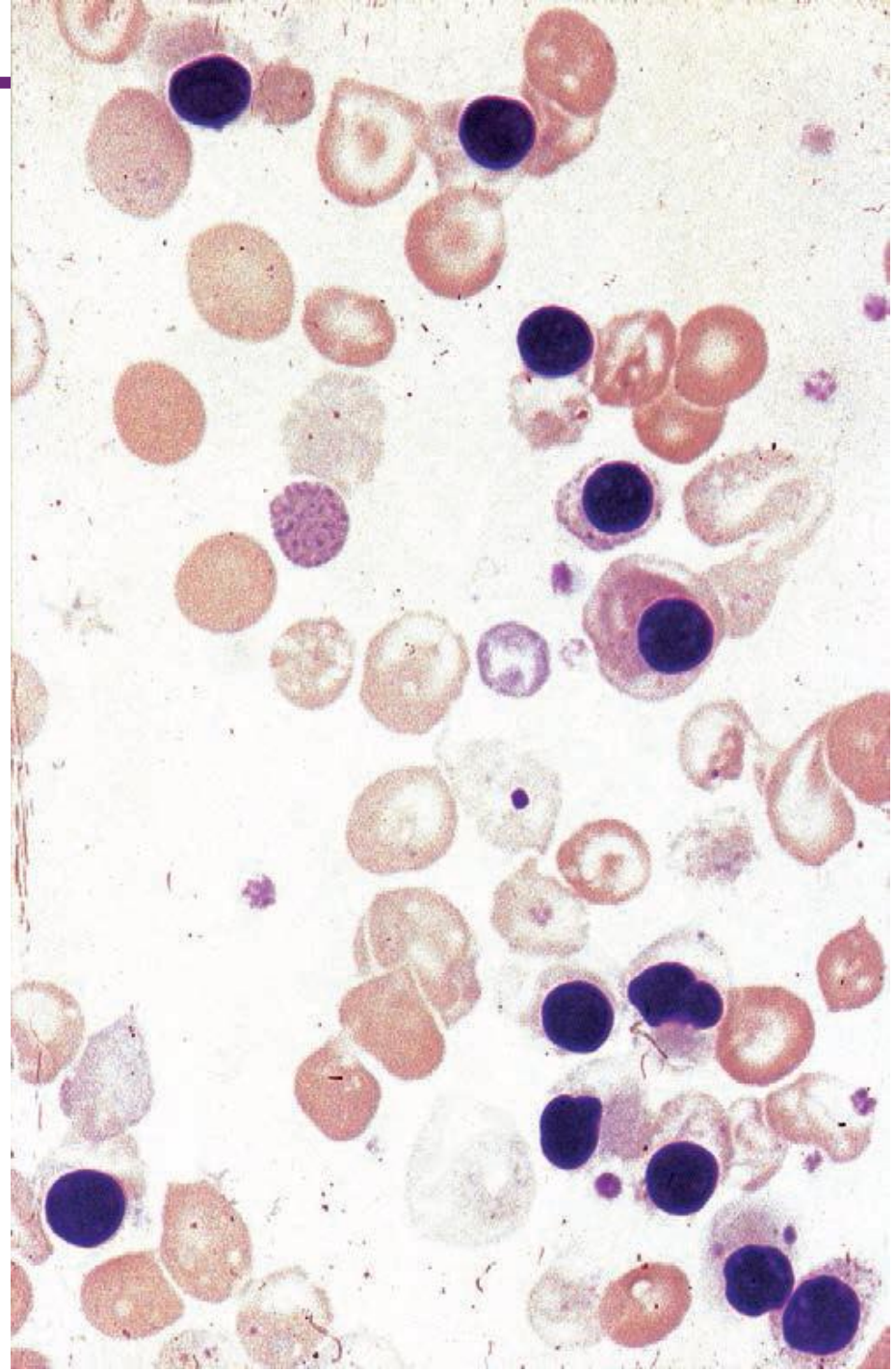
- **Shows a leucoerythroblastic blood film, teardrop poikilocytes and ovalocytes.**



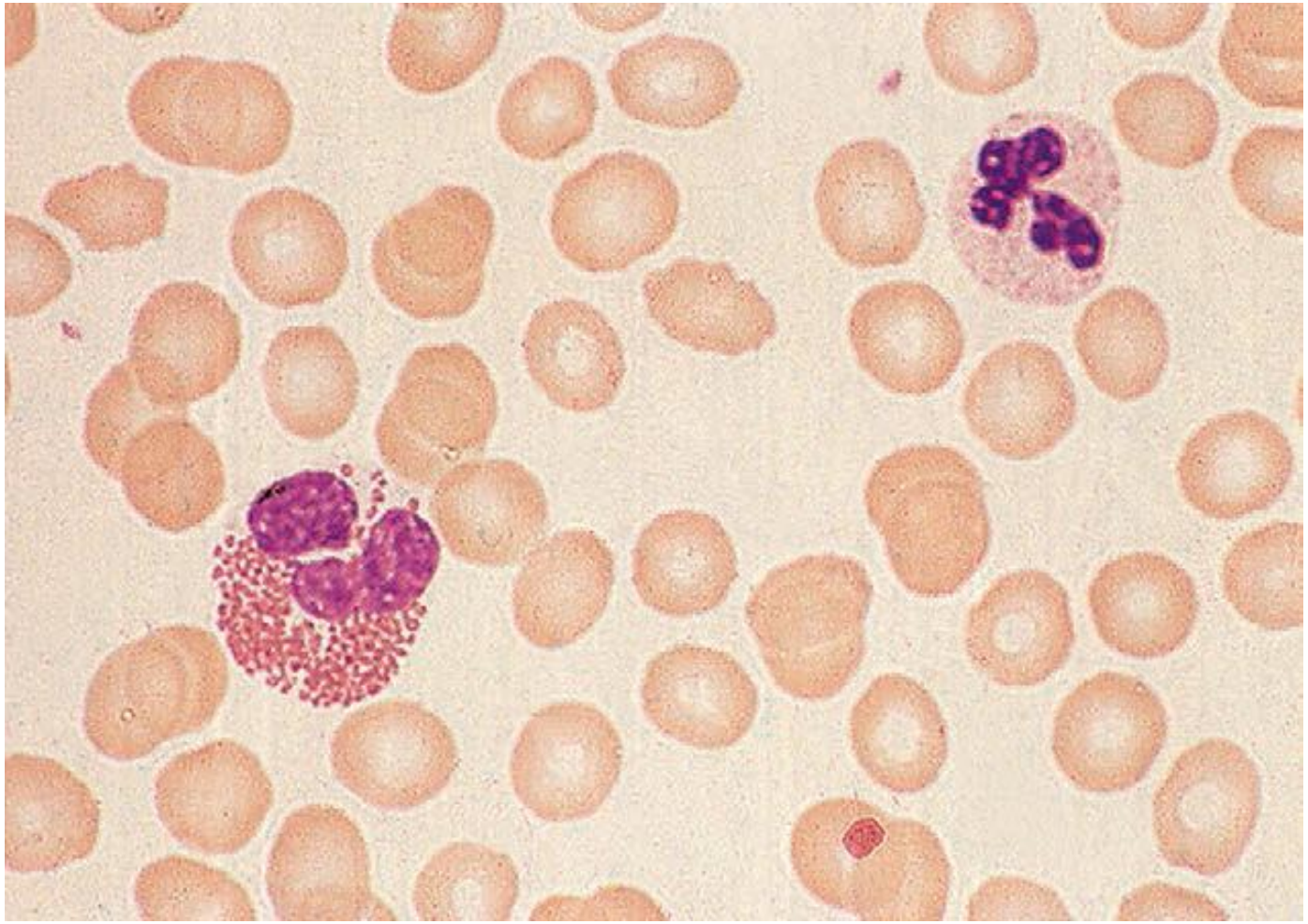
β thalassaemia major

with inadequate blood transfusion support.

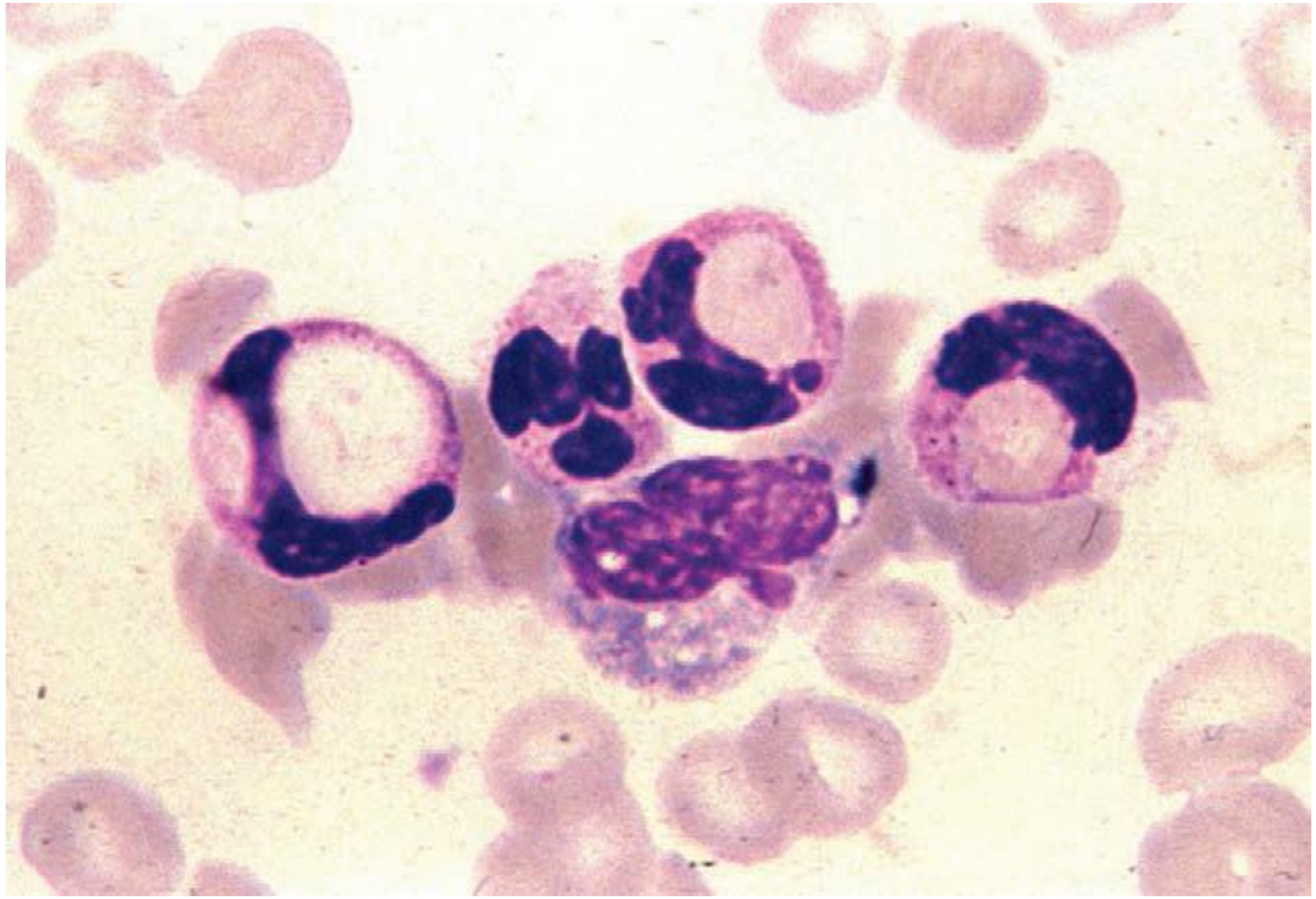
- **There are numerous erythroblasts; there are also hypochromic cells, target cells and a cell containing a Howell–Jolly body.**



Normal polymorphonuclear neutrophil and normal eosinophil.

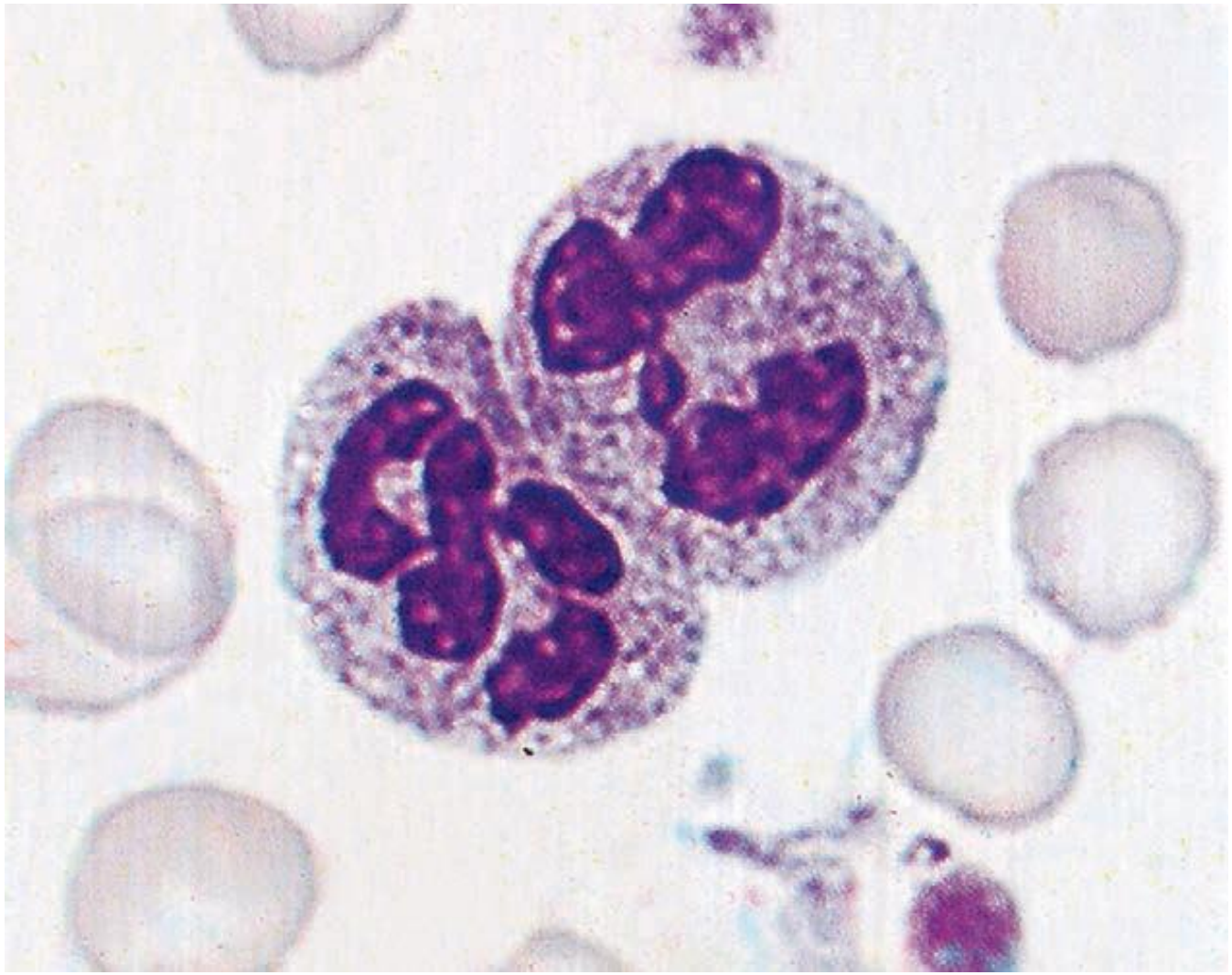


- **Erythrophagocytosis in a patient with a positive direct antiglobulin test.**



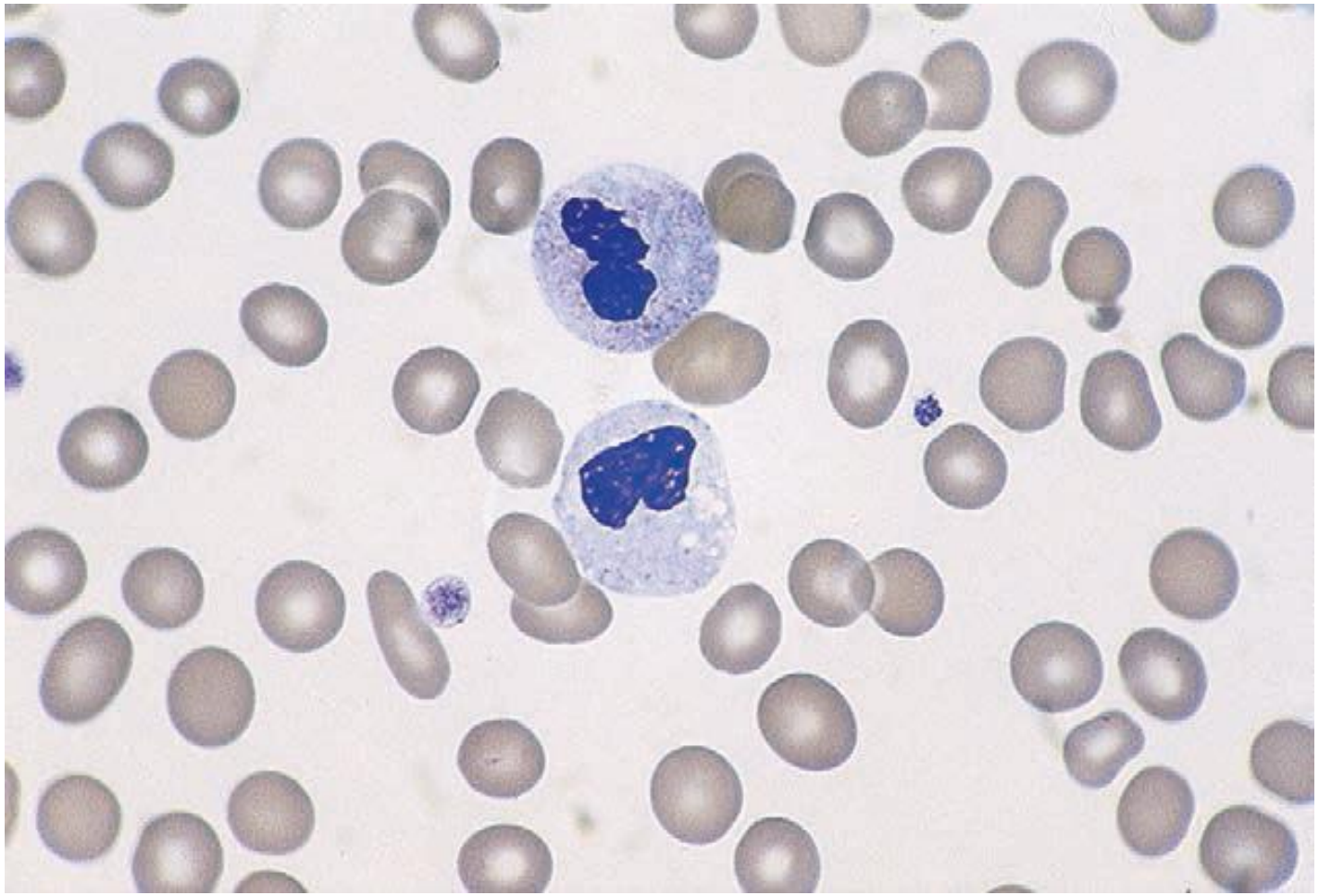
Severe infection

- **Neutrophils show toxic granulation.**



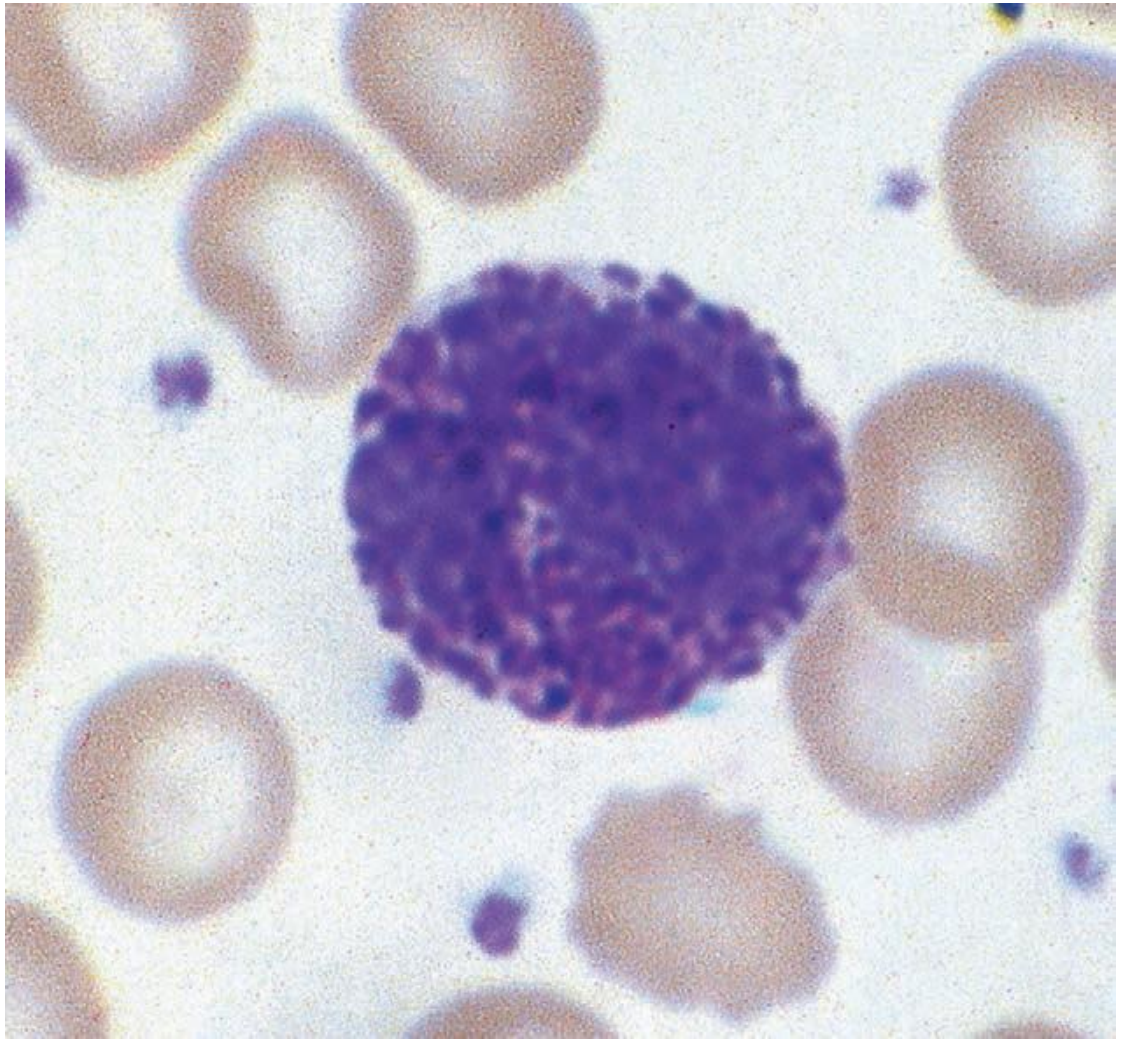
Myelodysplastic syndrome

- **Shows a hypogranular neutrophil and another that is more normally granulated. Both neutrophils have nuclei of abnormal shapes.**



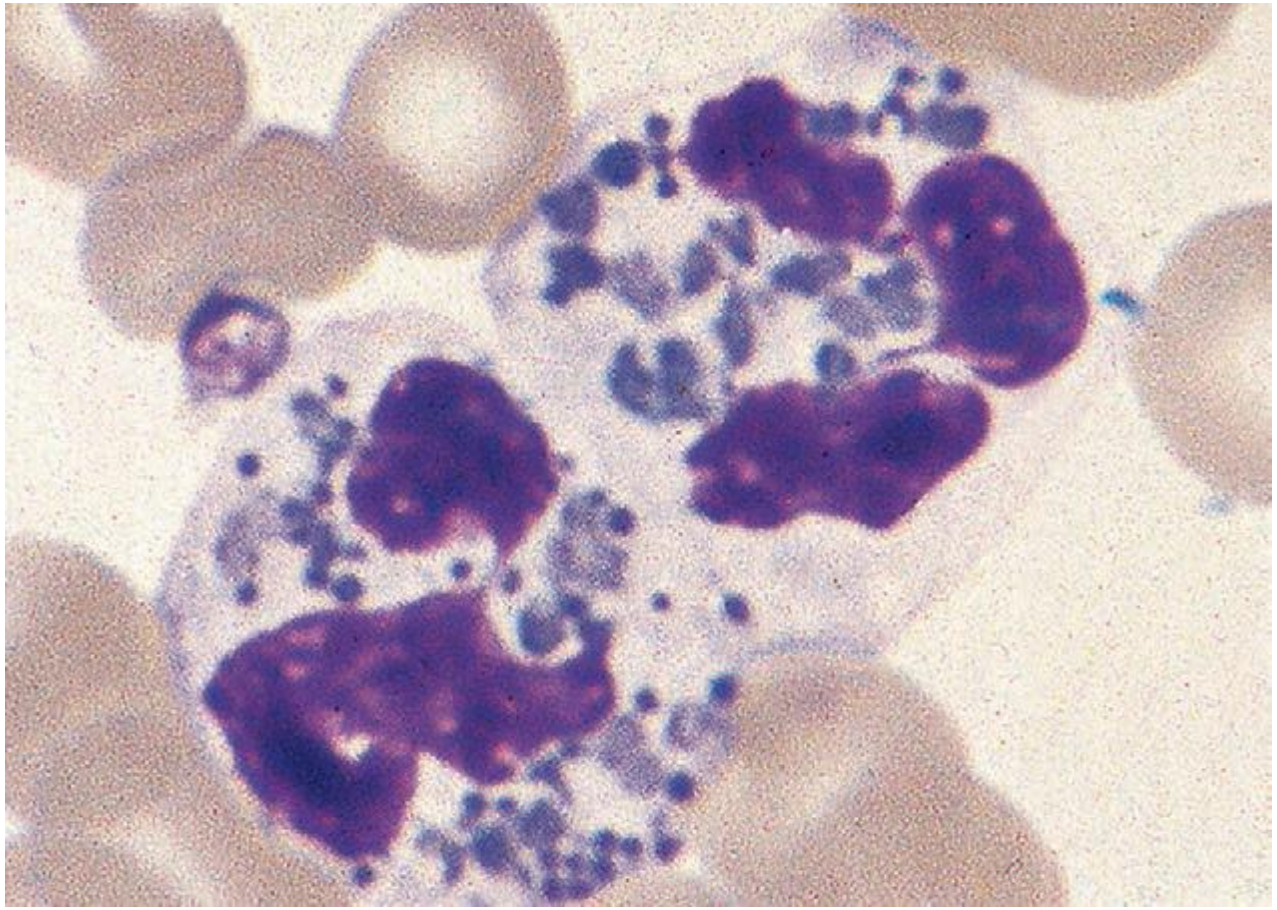
Alder–Reilly anomaly

- **The nucleus is obscured by the cytoplasmic granules.**



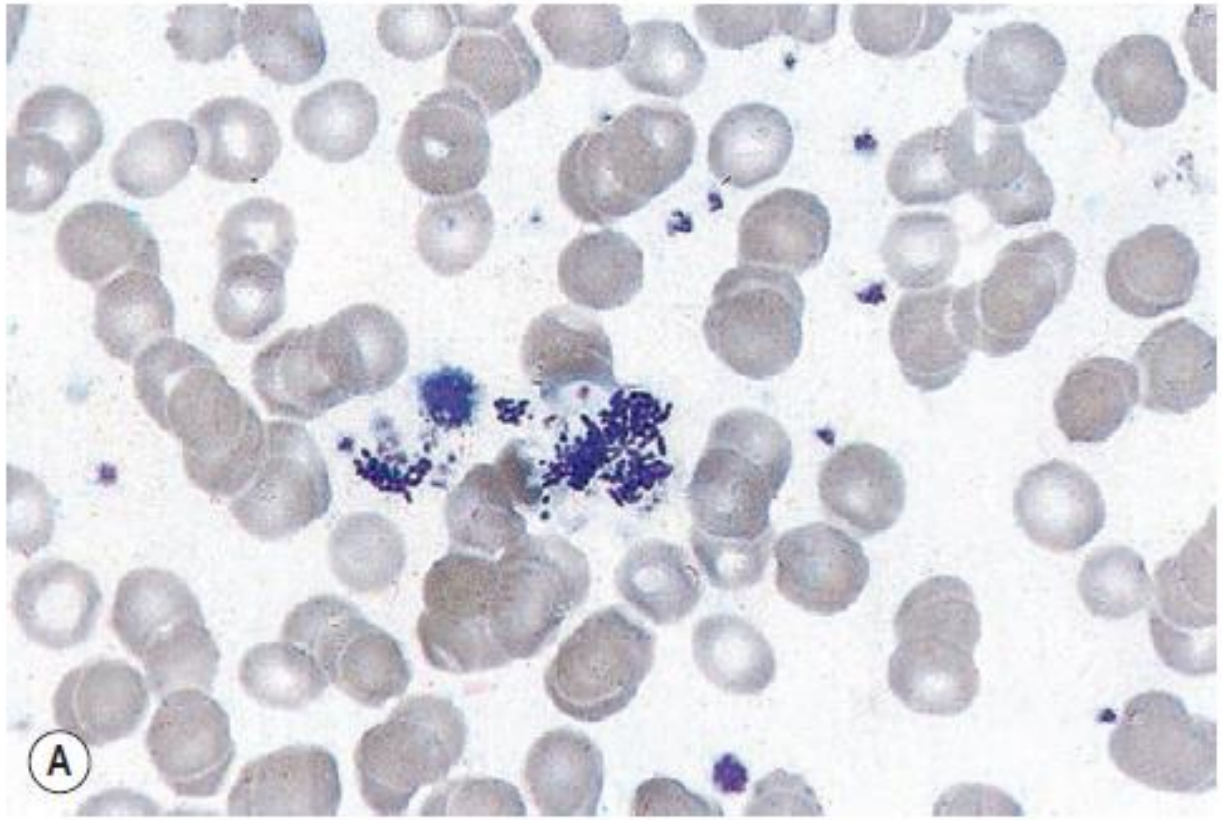
Chédiak–Higashi syndrome

- **Neutrophils show abnormal granules.**

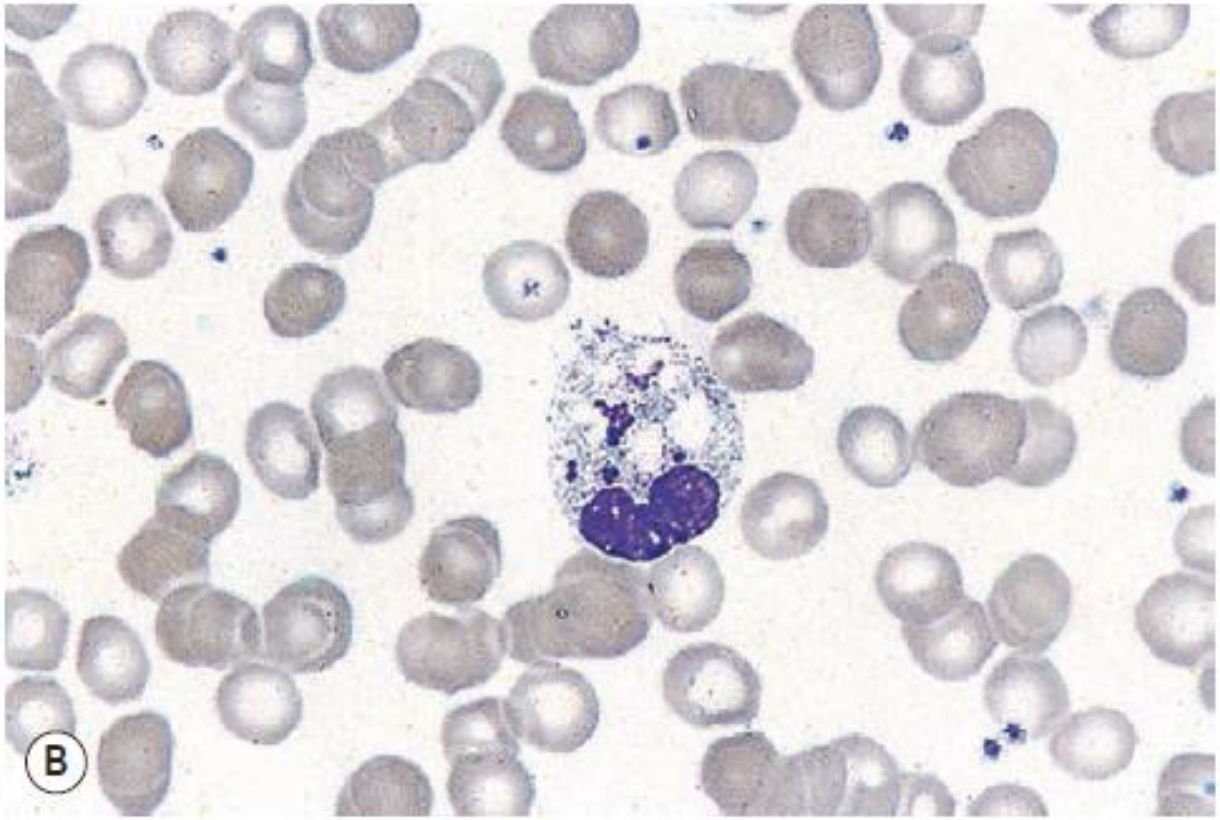


Blood collected from infected site

showing bacteria
in scattered clumps (A)

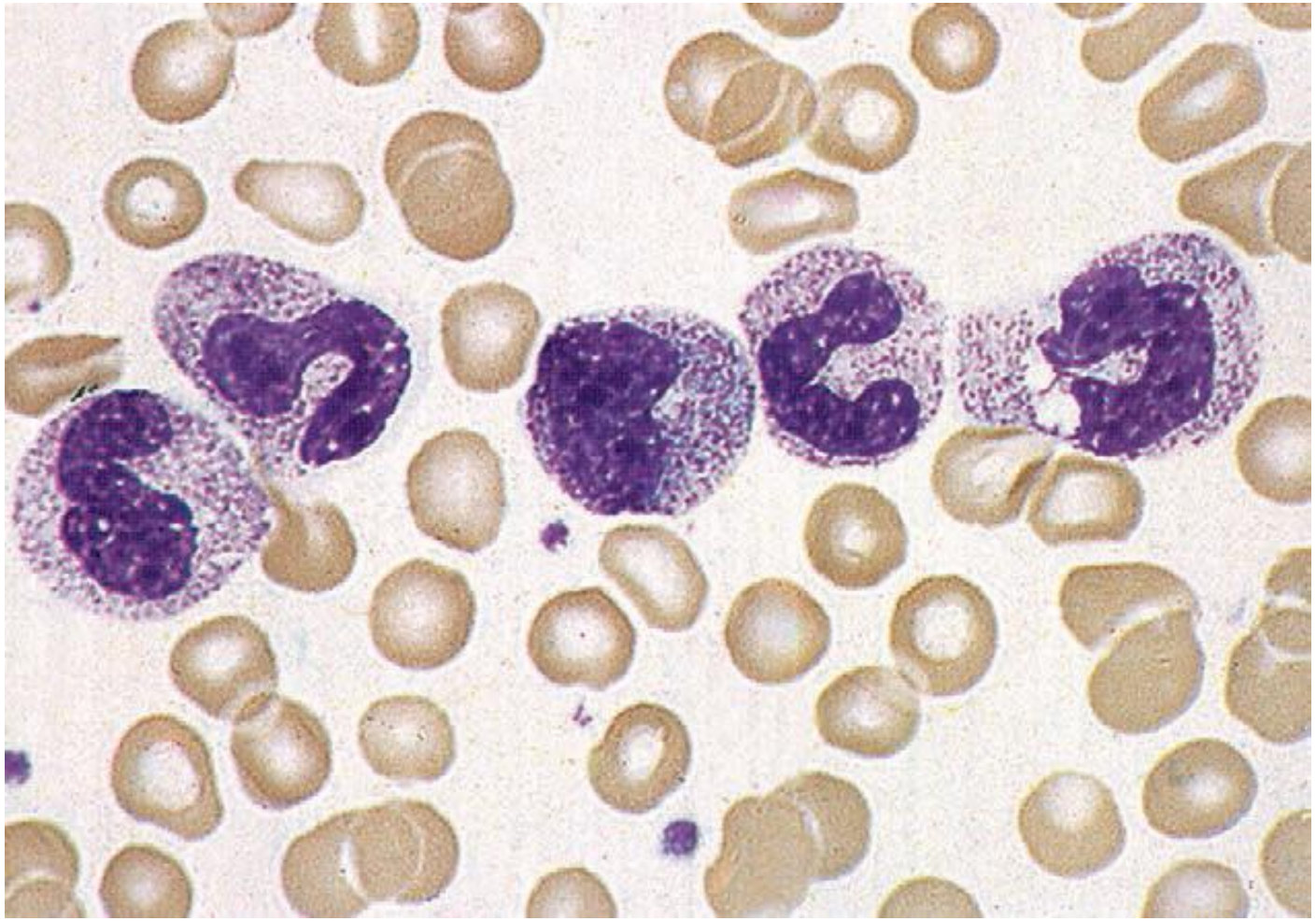


showing bacteria
in a neutrophil (B)



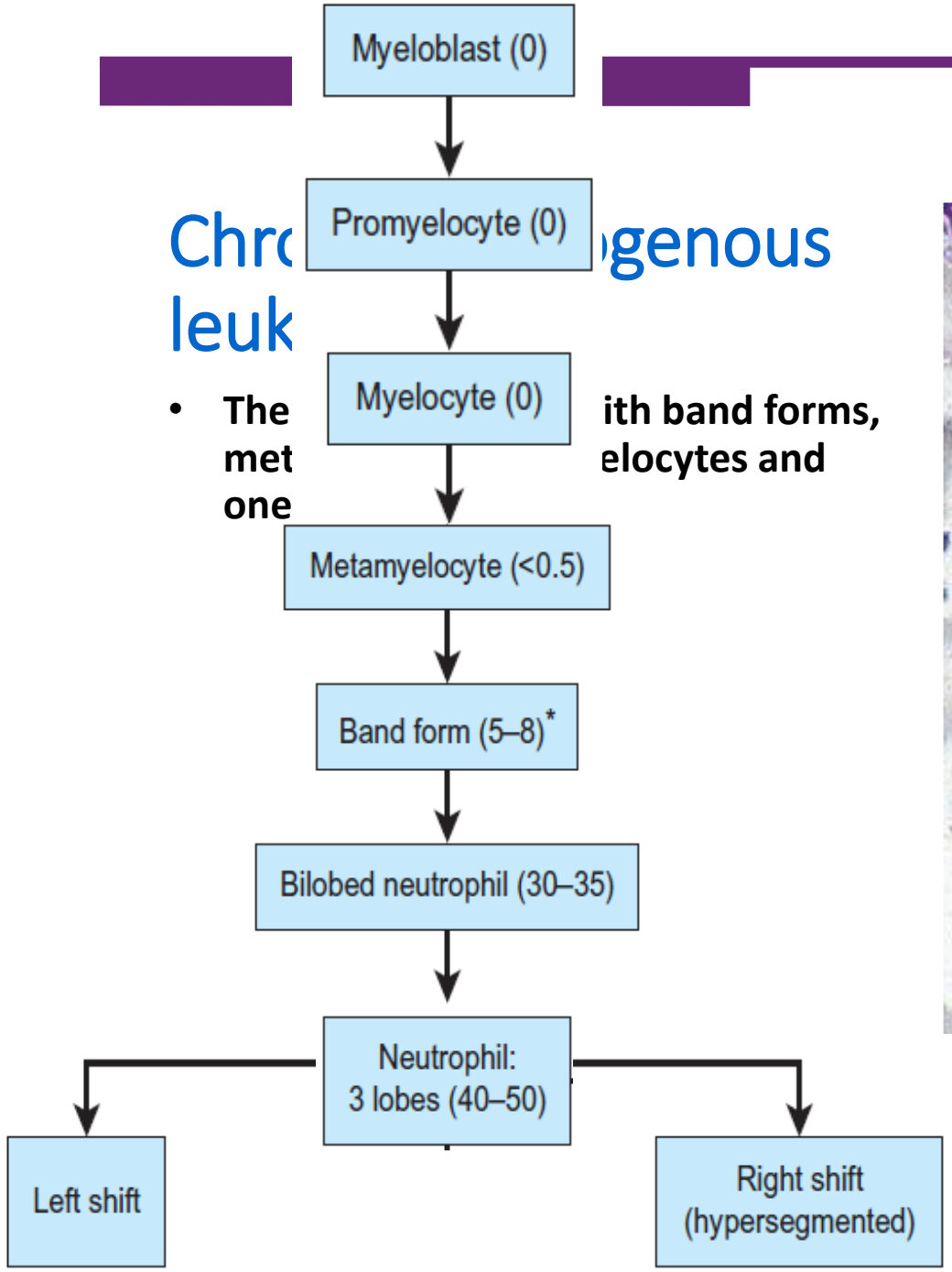
Infection

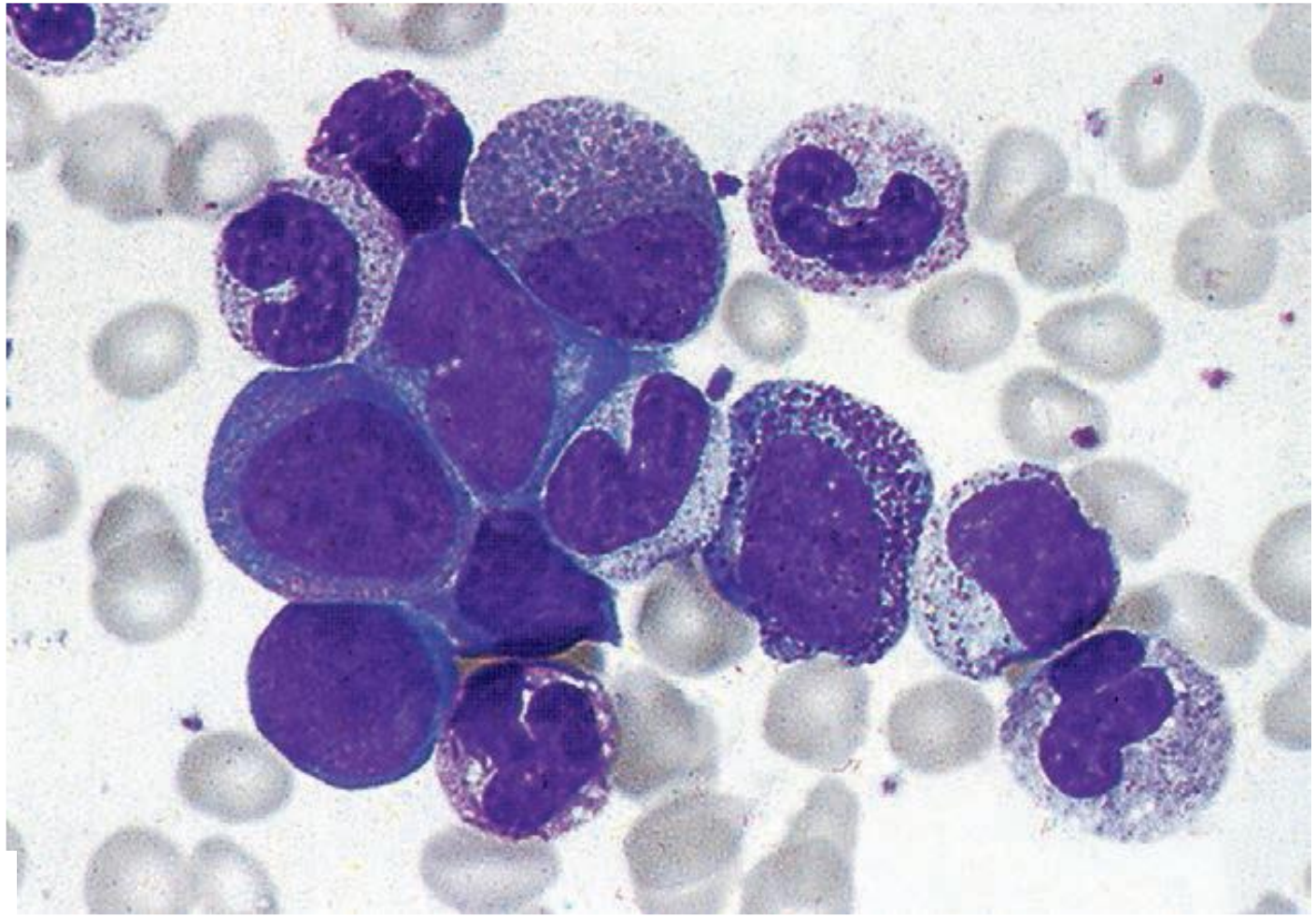
- **Shows left shift of the neutrophils with toxic granulation.**



Chr genous leuk

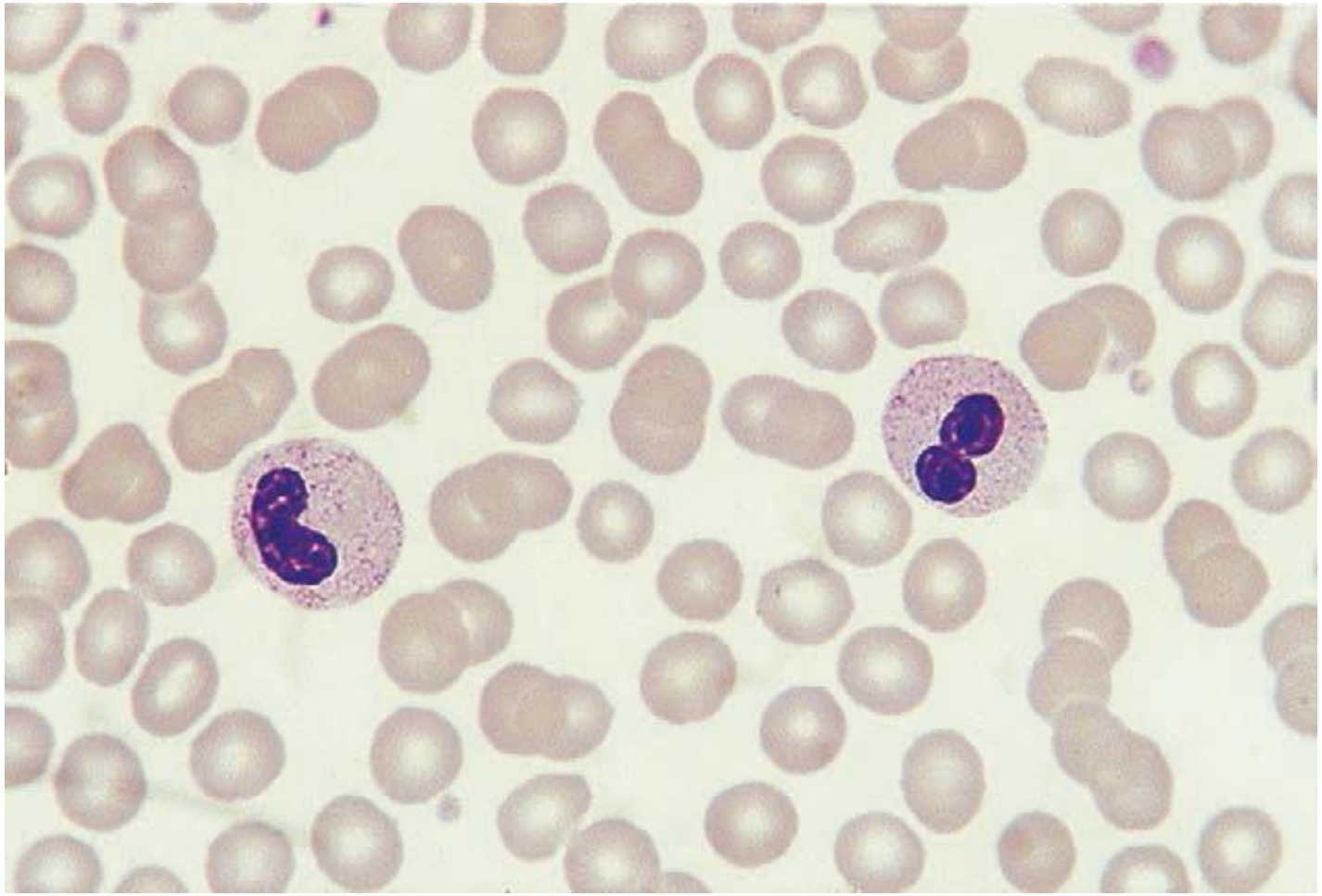
- The ith band forms, met elocytes and one





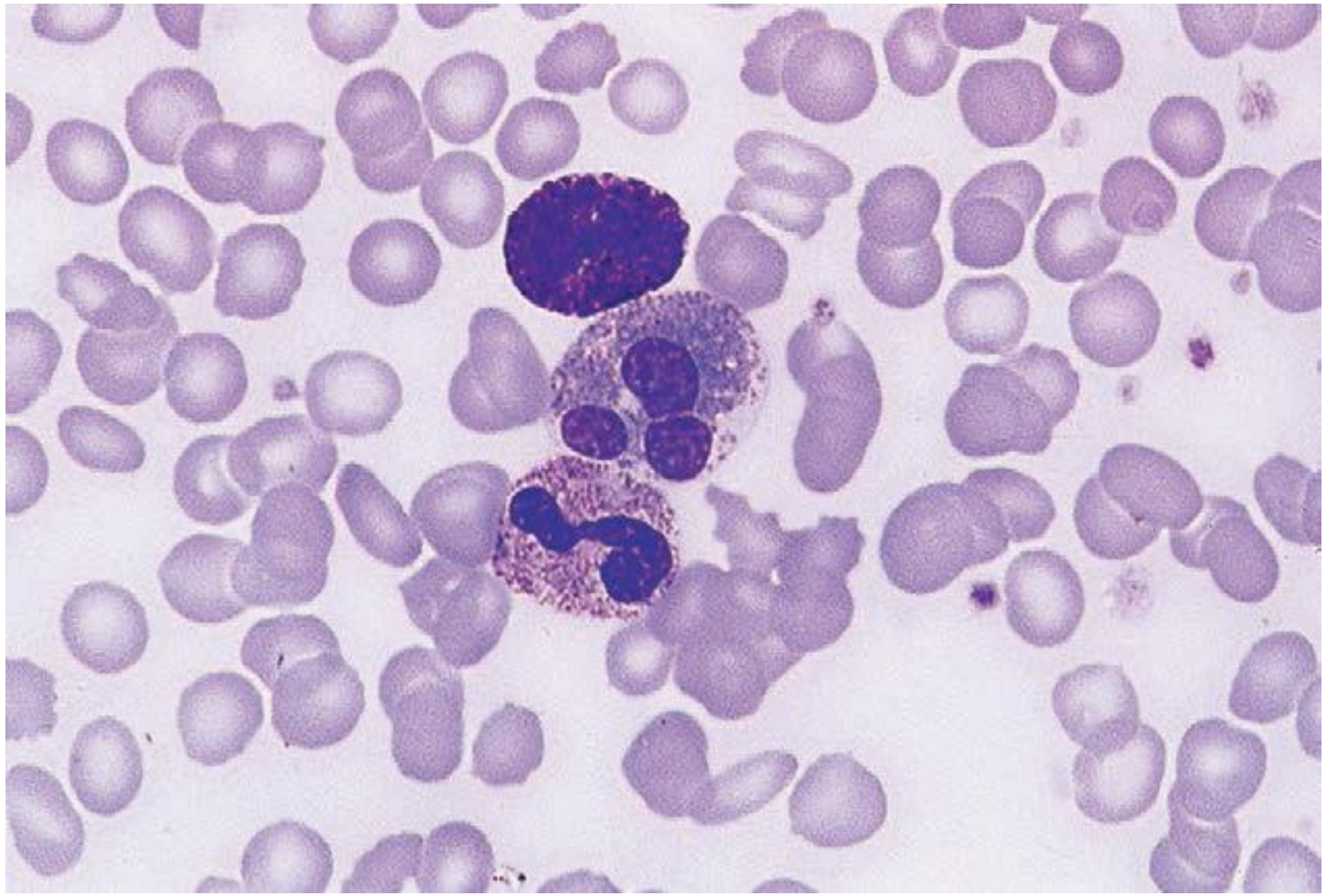
Pelger–Huët anomaly

- **Shows hypolobated neutrophils.**



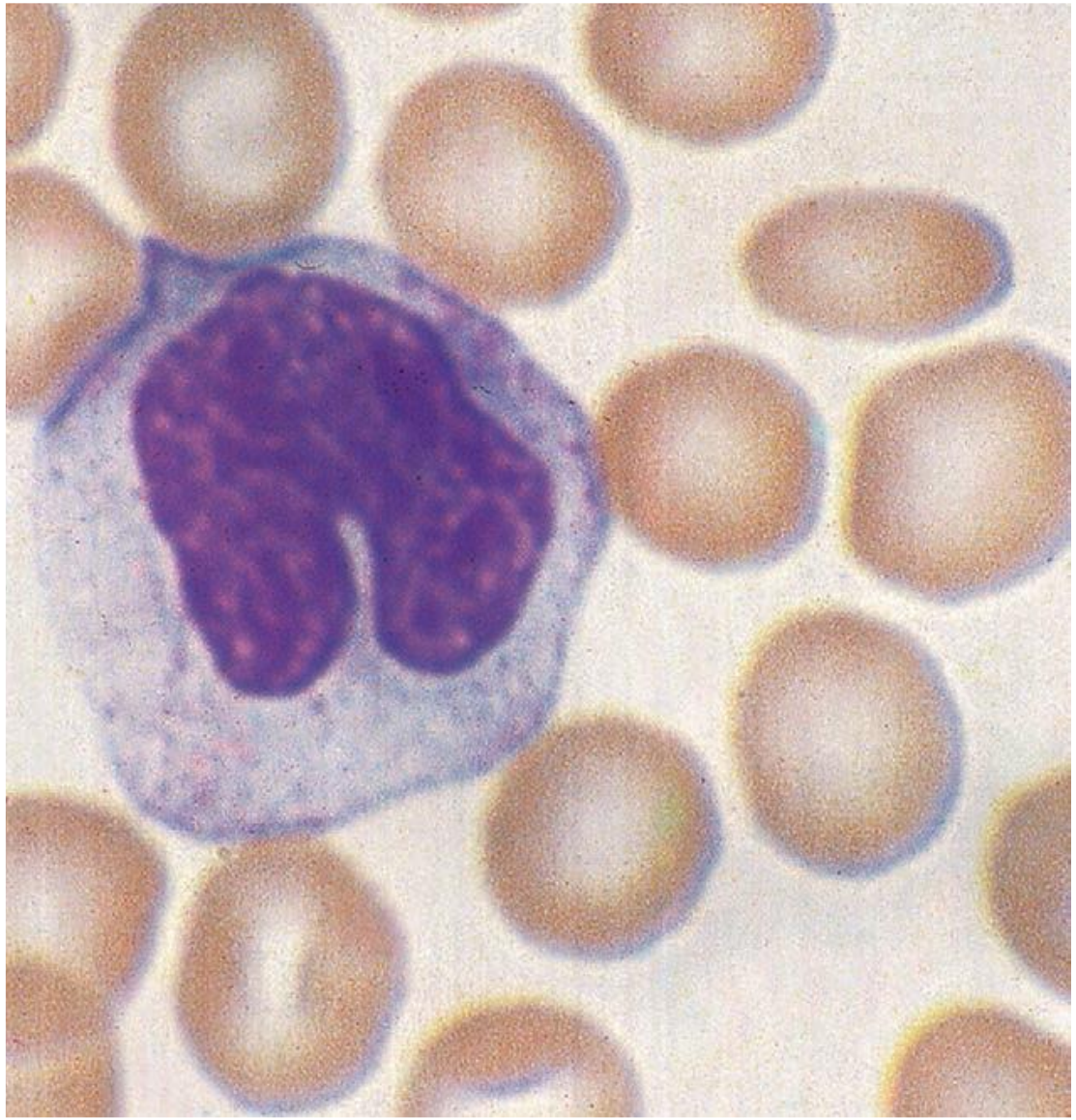
Normal adult

- **Shows a basophil, an eosinophil and a neutrophil.**



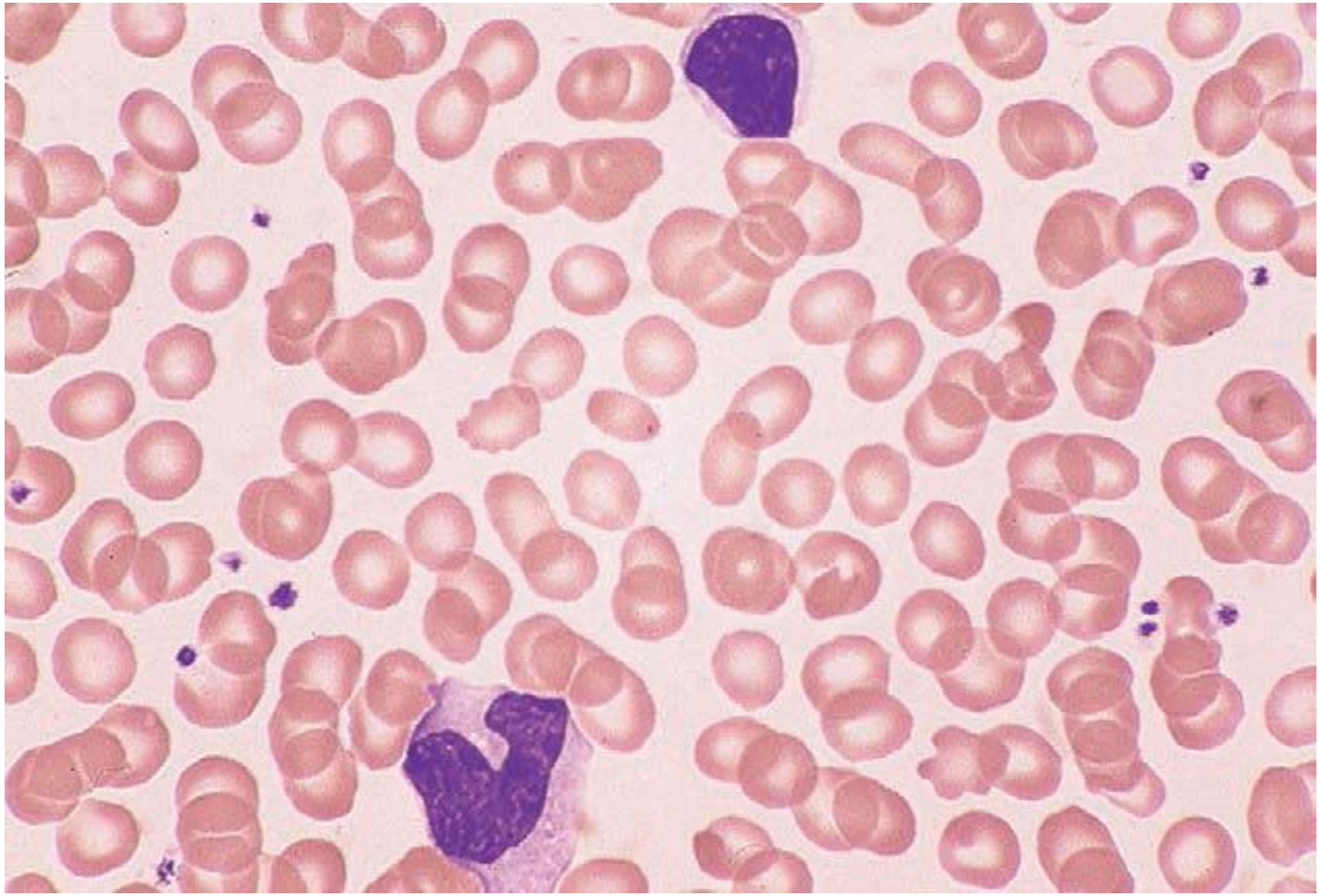
Healthy adult

- **Monocyte.**

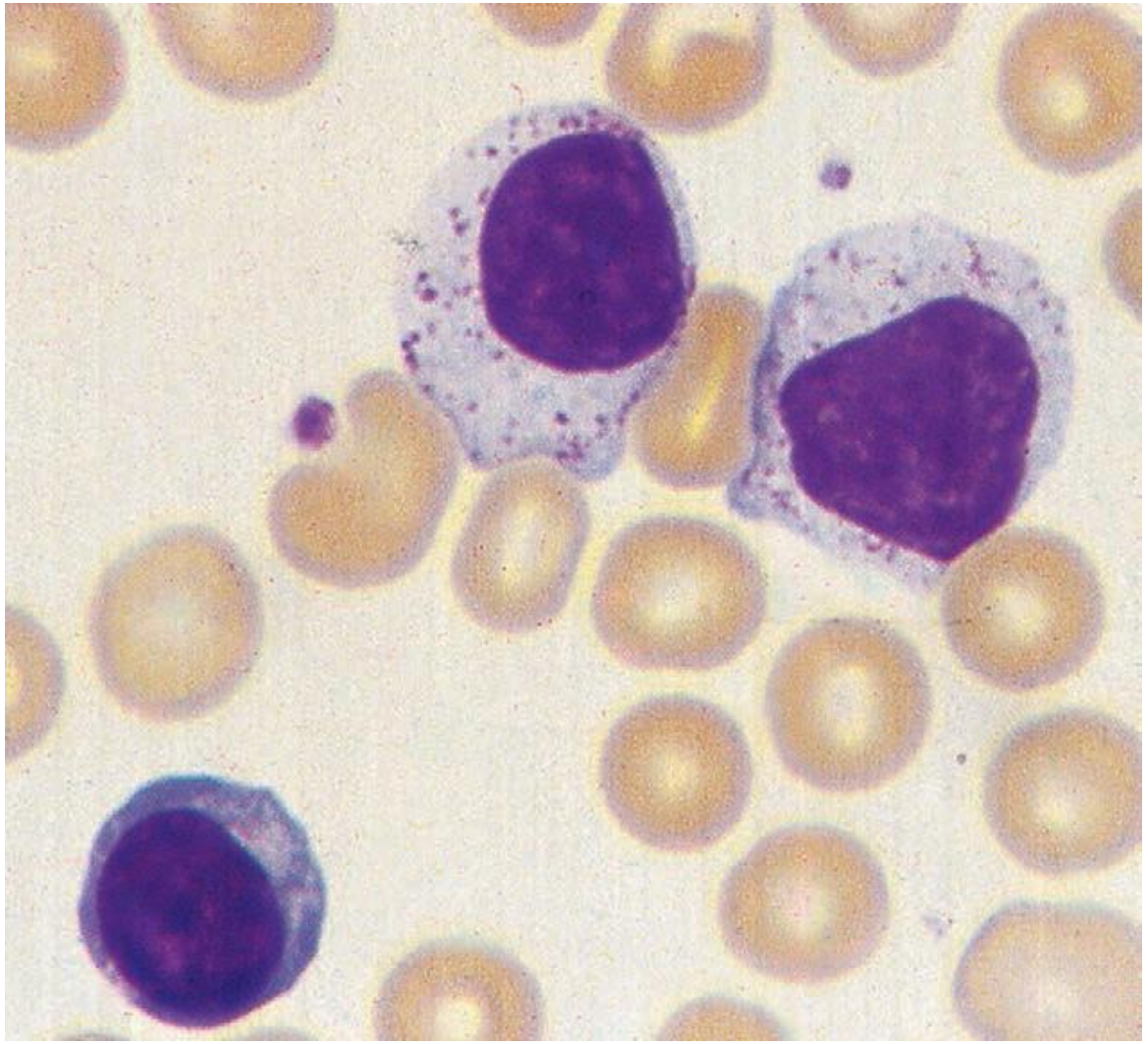


Healthy adult

- **Shows a monocyte and a lymphocyte.**

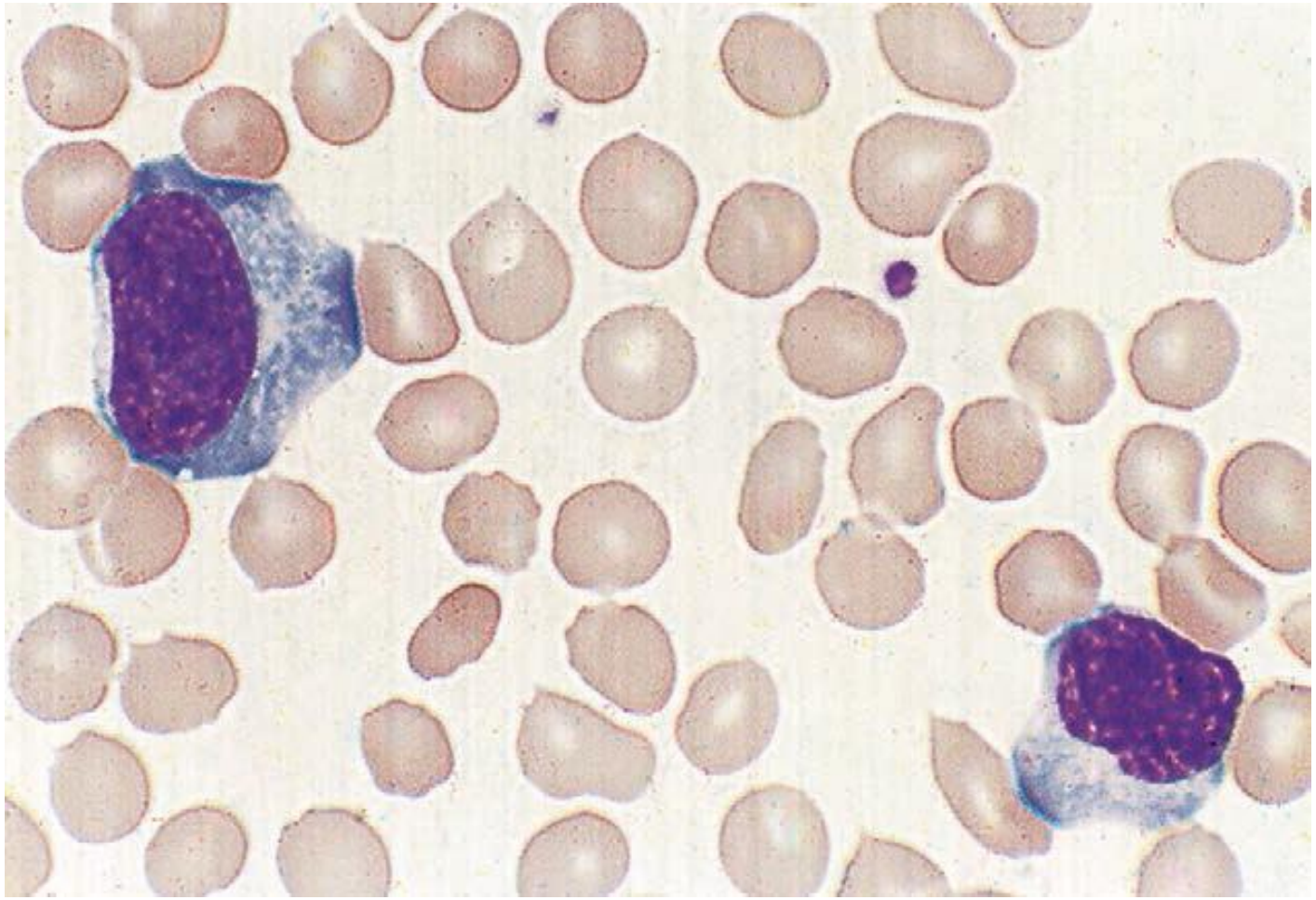


- **Shows a small lymphocyte and two large granular lymphocytes with azurophilic granules.**



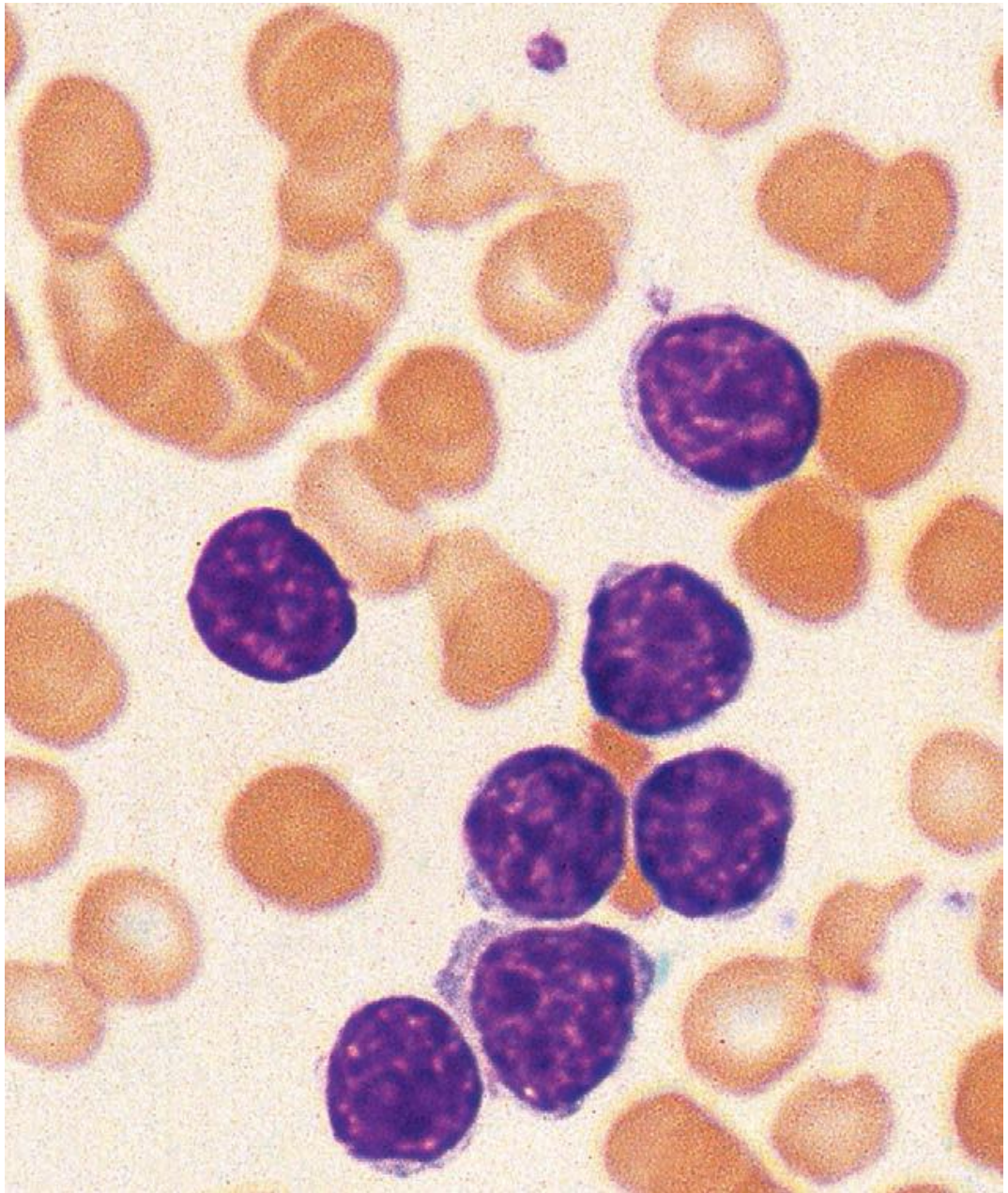
Infectious mononucleosis

- There are two activated lymphocytes (‘atypical mononuclear cells’).
- ICSH recommend that reactive lymphocyte is used to describe lymphocytes with a benign aetiology and abnormal lymphocyte with an accompanying description of the cells is used to describe lymphocytes with a suspected malignant or clonal aetiology.



Chronic lymphocytic leukaemia

- **The cells are small lymphocytes; note that rouleaux formation is increased.**



Laboratory Quality Assurance Program
Hematology QA Committee
College of Physicians and Surgeons

Criteria for Reporting Smudge Cells

Absolute lymphocyte count should be greater than 5.0 x10^9/L.

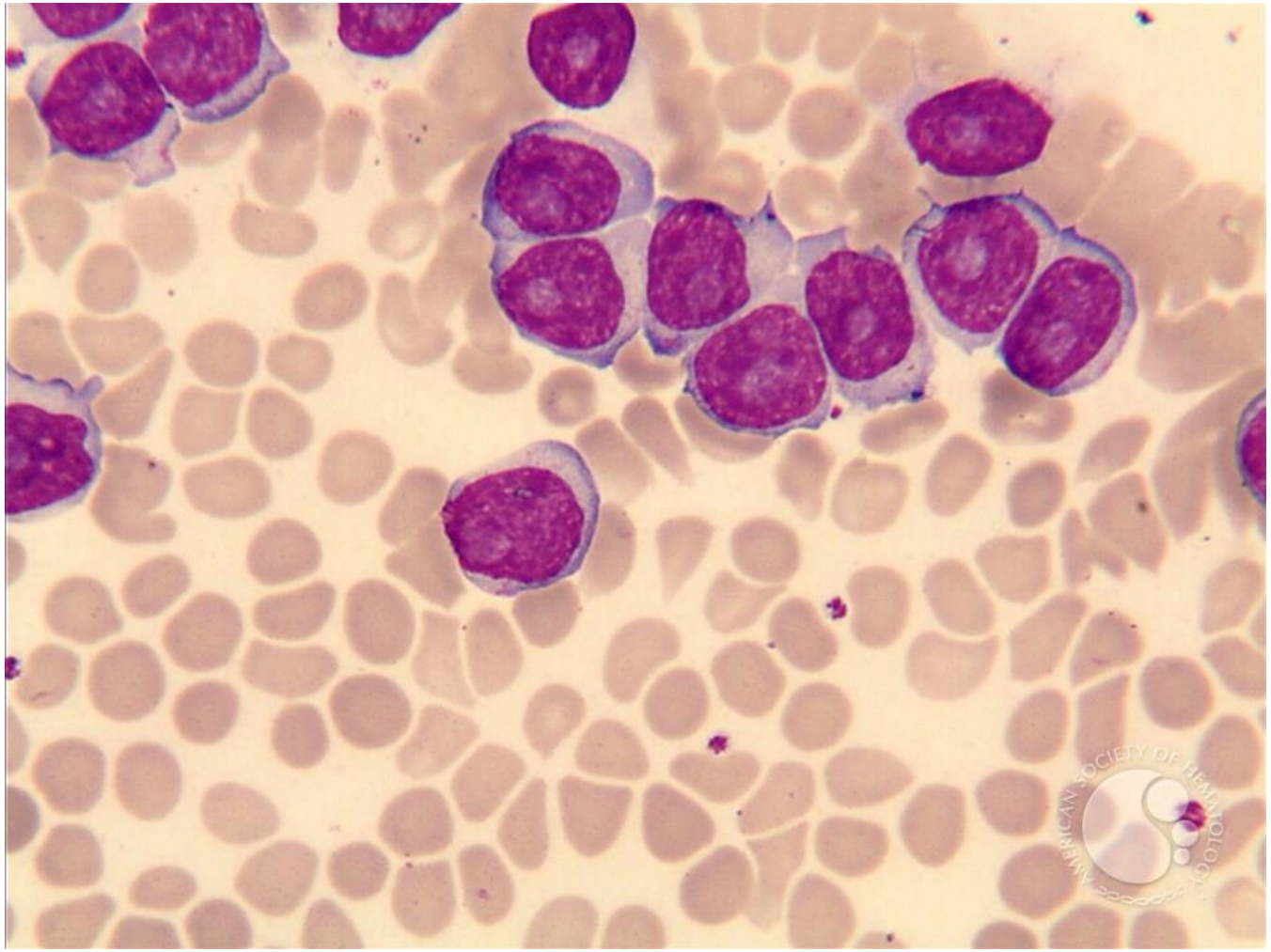
Patient age should be more than 30 years*.

Smudge cells should be reported if greater than 10 per 100 leukocytes. Report smudge cells in absolute numbers.

* Although CLL is not often diagnosed in patients under the age of 40, patients over 30 years of age should be considered potentially at risk. CLL is rare in patients under 30 years of age.

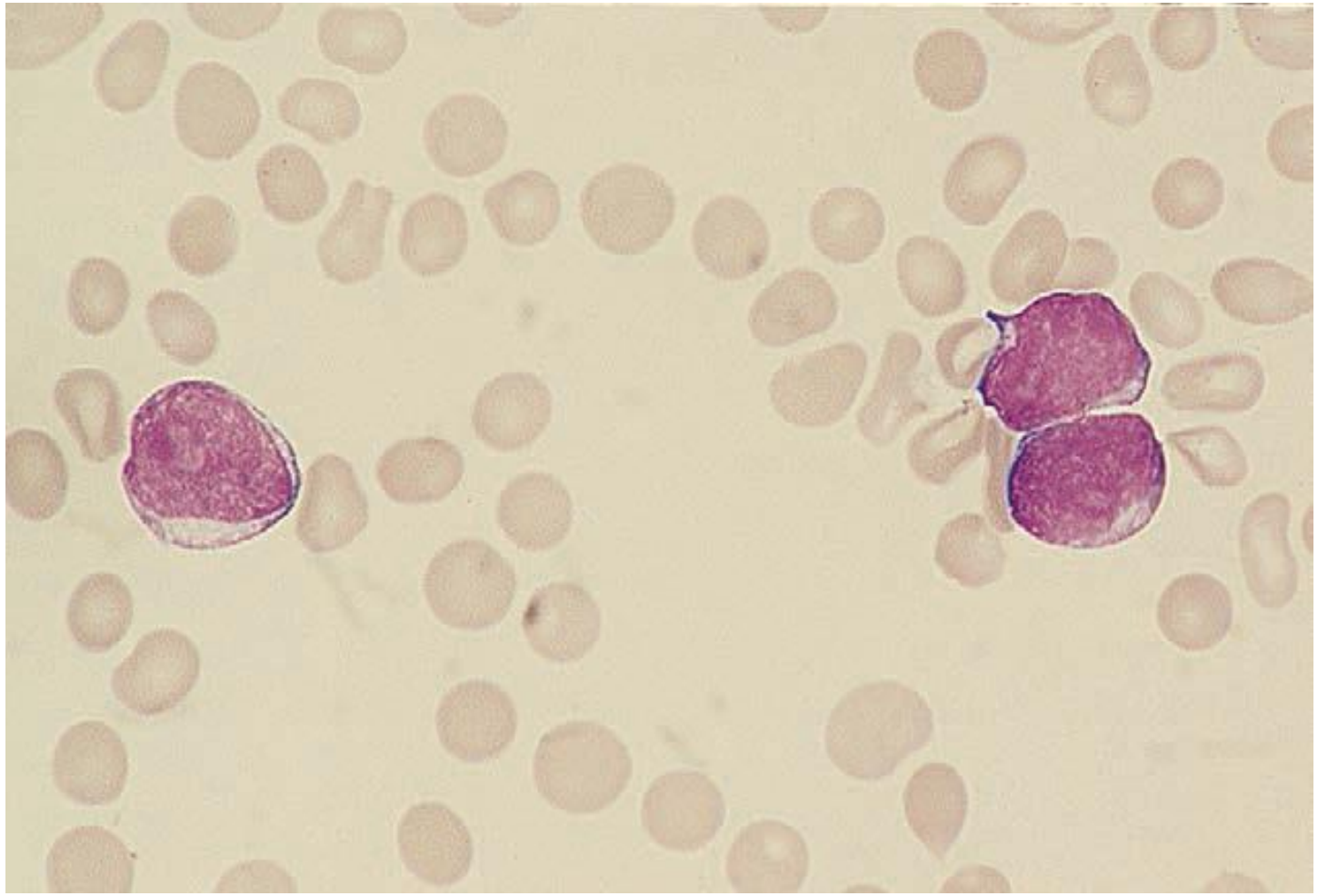
Prolymphocytic leukaemia

- **There is a uniform population of prolymphocytes.**



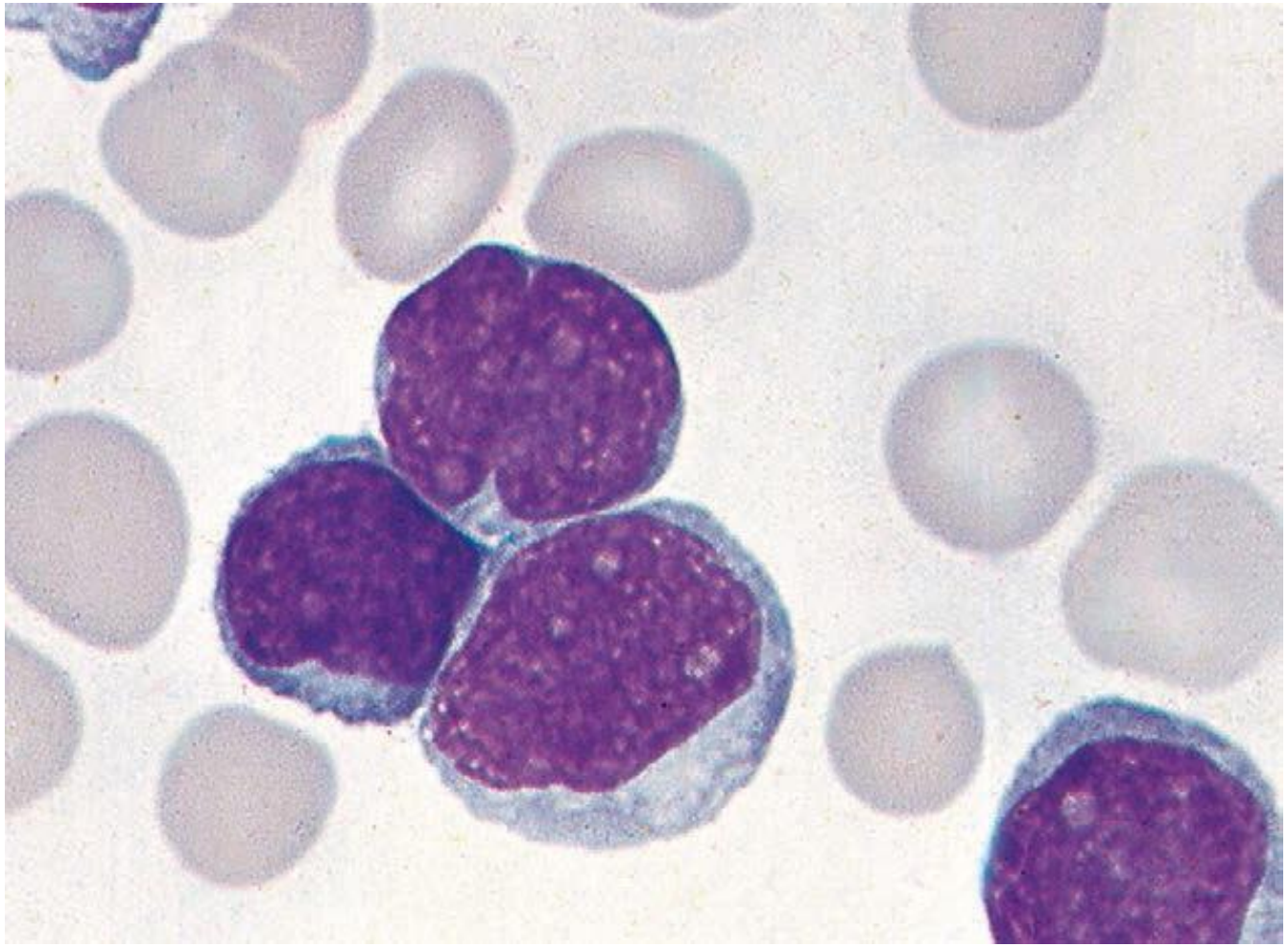
Philadelphiapositive acute lymphoblastic leukaemia (FAB L1 category)

- **Shows three lymphoblasts with a high nucleocytoplasmic ratio, very delicate chromatin and visible nucleoli.**



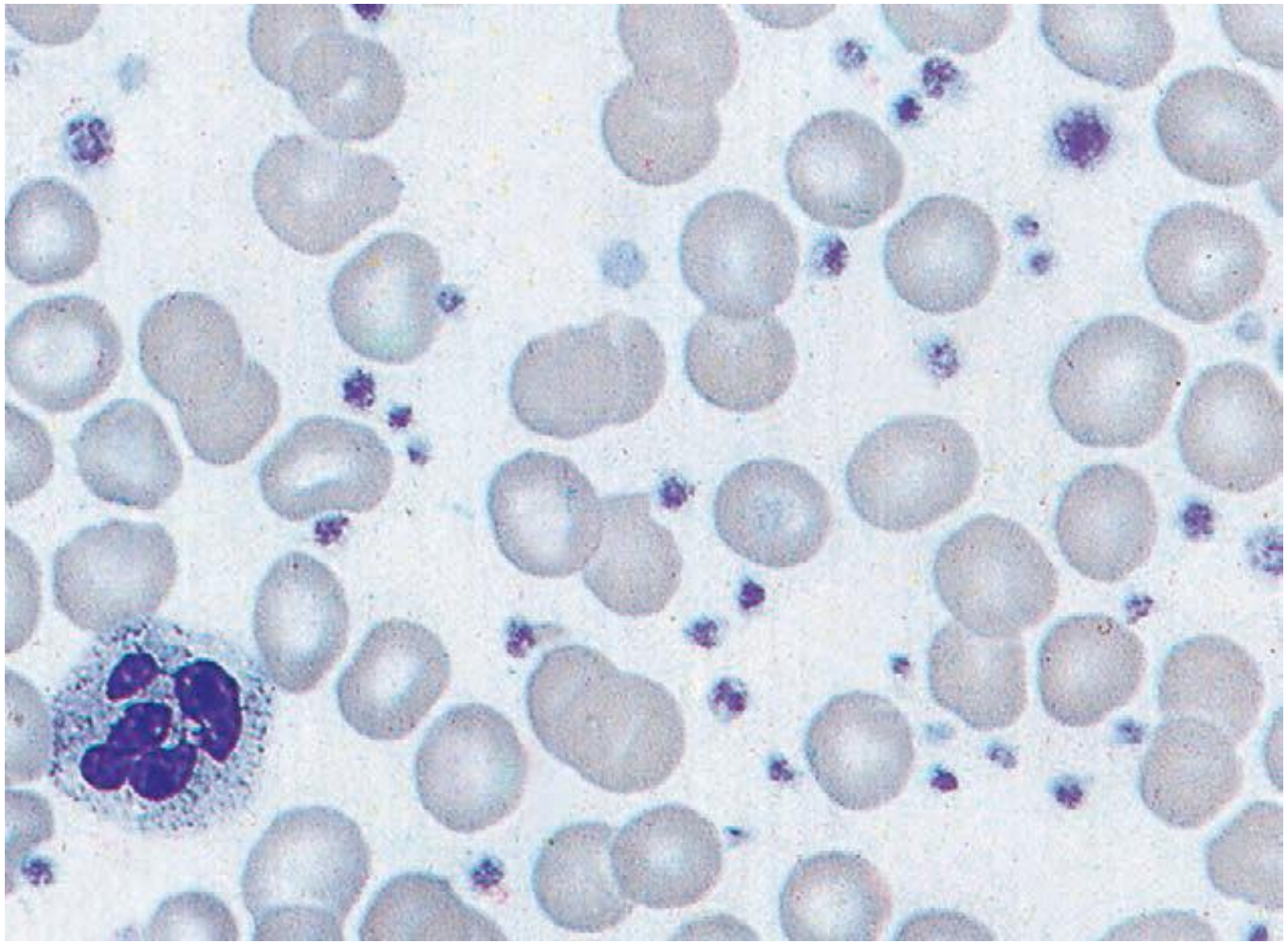
Acute lymphoblastic leukaemia (FAB L1 type)

- **Shows lymphoblasts with some chromatin condensation and small nucleoli.**



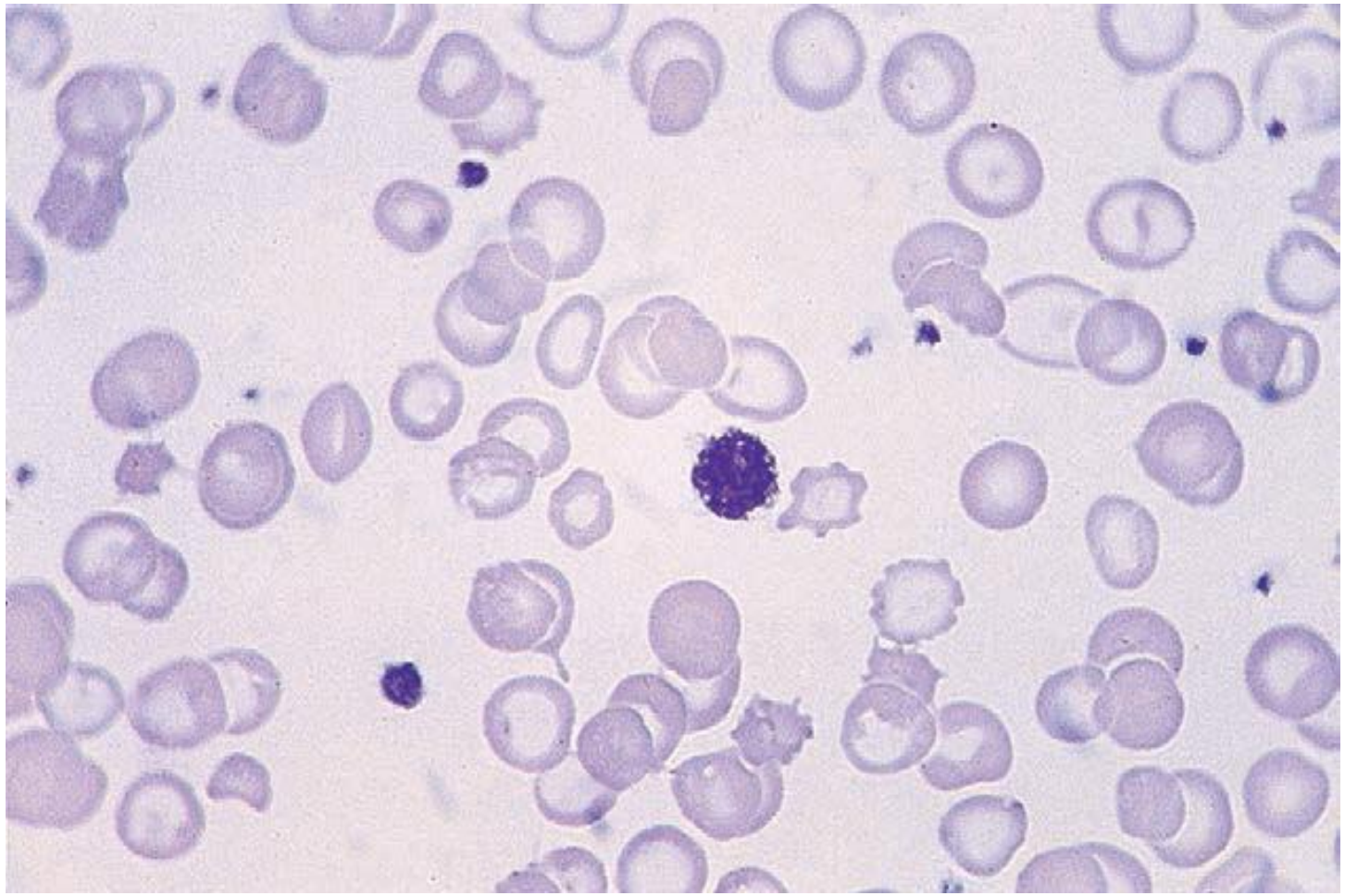
Essential thrombocythemia

- **Shows platelet anisocytosis and increased numbers of platelets.**

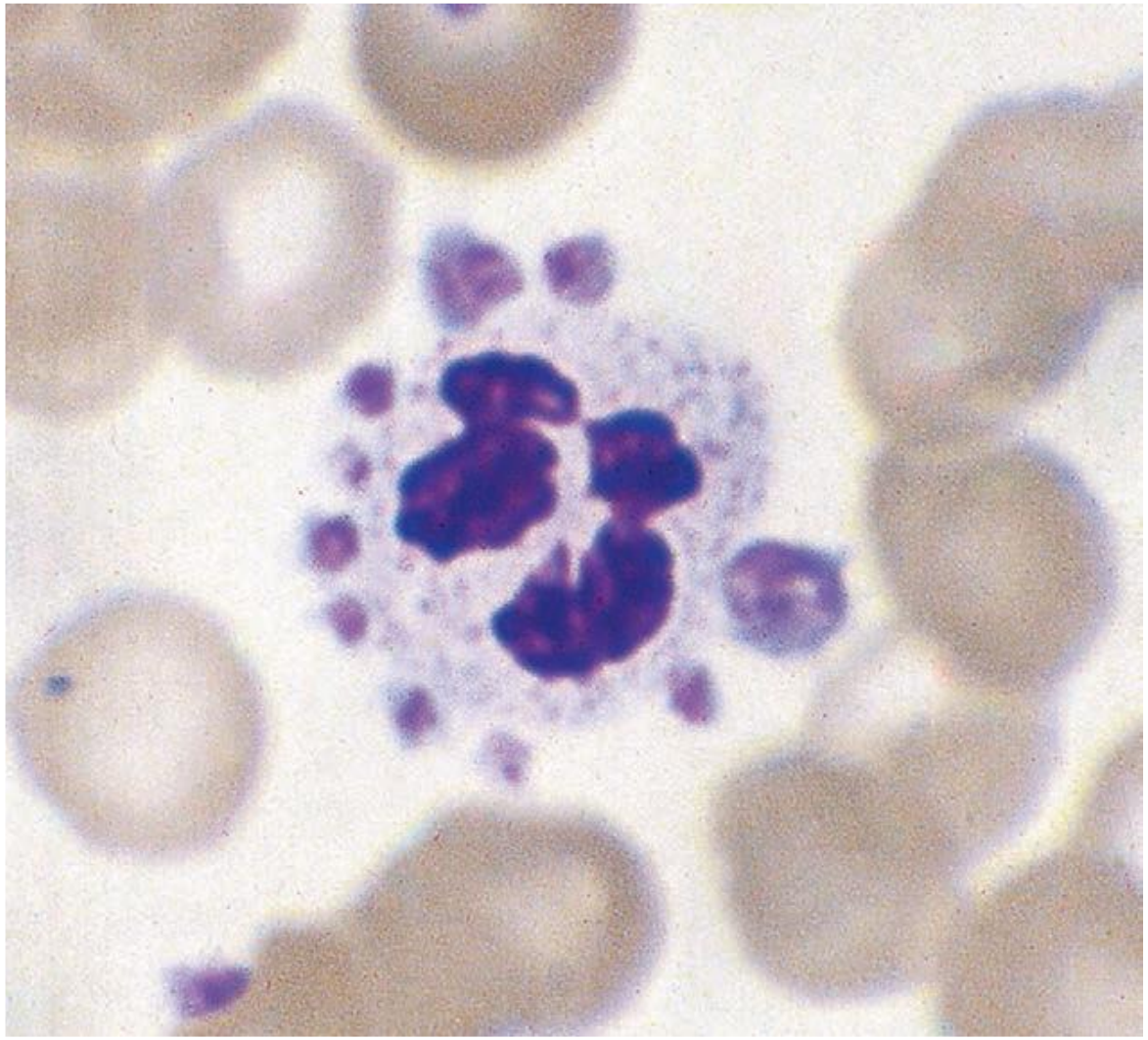


Hyposplenism in coeliac disease

- Shows giant platelet. There is also marked hypochromia, indicative of associated iron deficiency.
- A normal platelet measures 1.5-3 µm in diameter. Large platelets measure 3–7 µm (roughly the diameter of a normal sized red cell), whilst giant platelets, are larger than normal sized red cells at 10–20 µm in diameter and are flagged by automated analysers.
- In a normal person, usually less than 5% of the platelets appear large.
- Platelet size increases gradually during storage in EDTA anticoagulated venipuncture tubes.

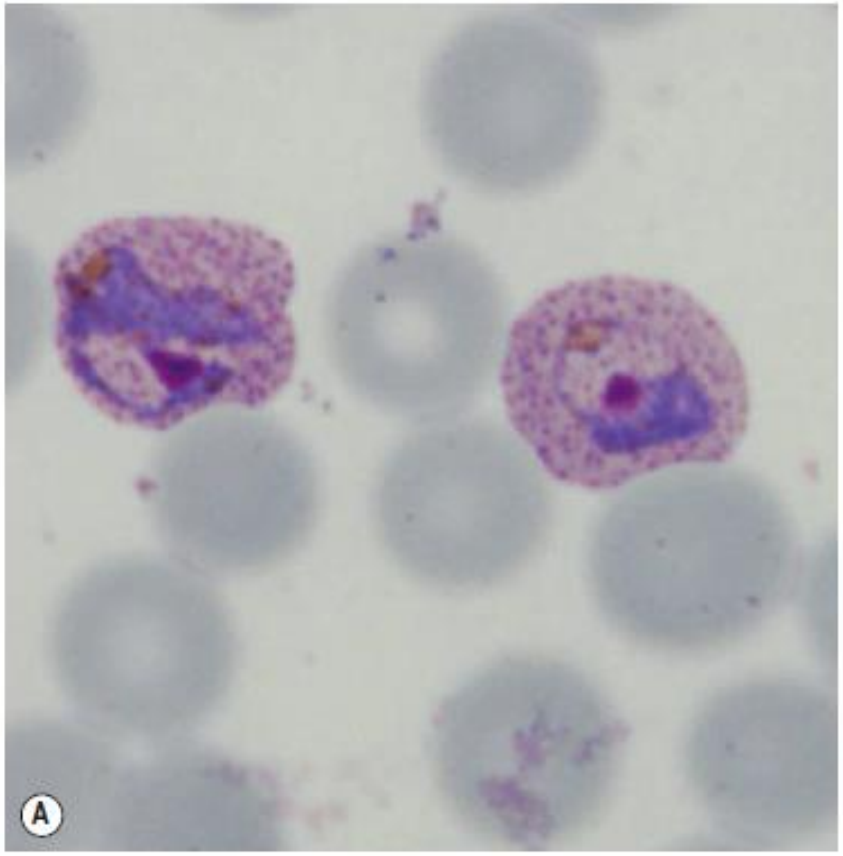


- **Shows adhesion of platelets to a neutrophil (platelet satellitism).**

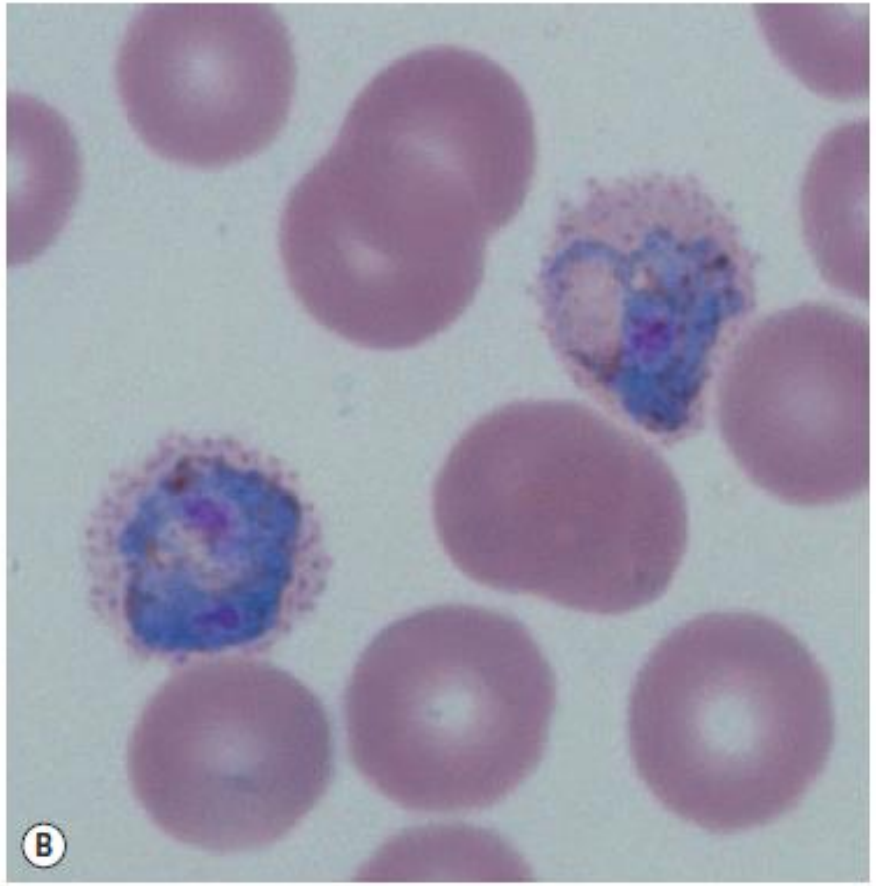


P. vivax: Effects of staining pH on parasite staining characteristics

At pH 7.2 (A) the parasite is readily distinguished from the adjacent blue/grey erythrocytes, chromatin staining is prominent and Schüffner dots are readily distinguished.



At pH 6.9 (B) the parasite remains visible, but there is less contrast from surrounding erythrocyte cytoplasm, relatively weak chromatin staining and poor resolution of Schüffner dots (which may not be visible at this pH).



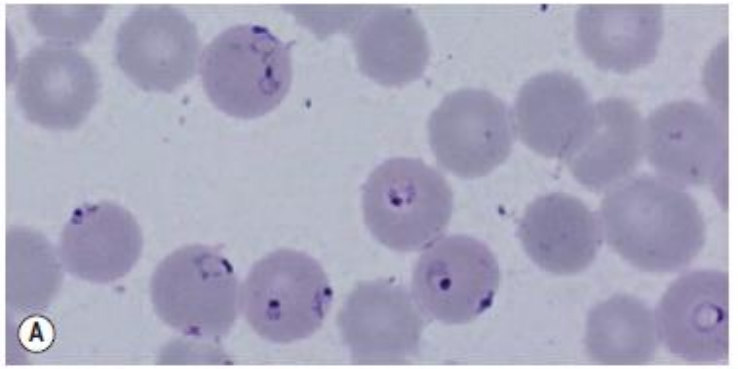
Morphology of *P. falciparum.*

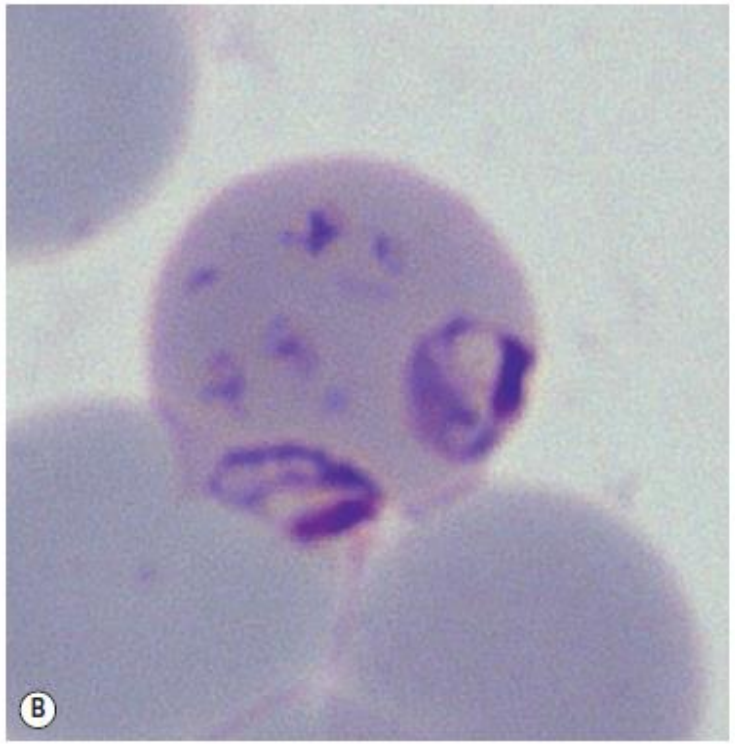
Note that erythrocytes throughout this series are not enlarged or distorted. Early trophozoites

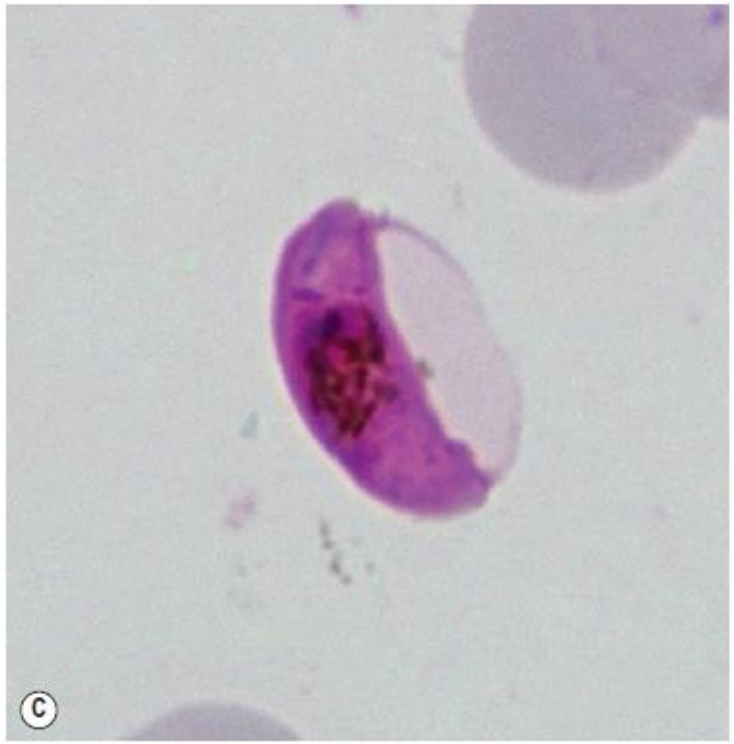
(A) show high parasitaemia, with accolé (appliqué, edge or shoulder) forms, multiply-infected erythrocytes and double chromatin dot forms.

The late trophozoite **(B)** shows two thickened ring forms with characteristic Maurer dots (clefts) in the erythrocyte cytoplasm.

(C) A typical crescent ('banana-shaped') gametocyte is shown. Schizonts are not shown since they rarely circulate in *P. falciparum* infection.





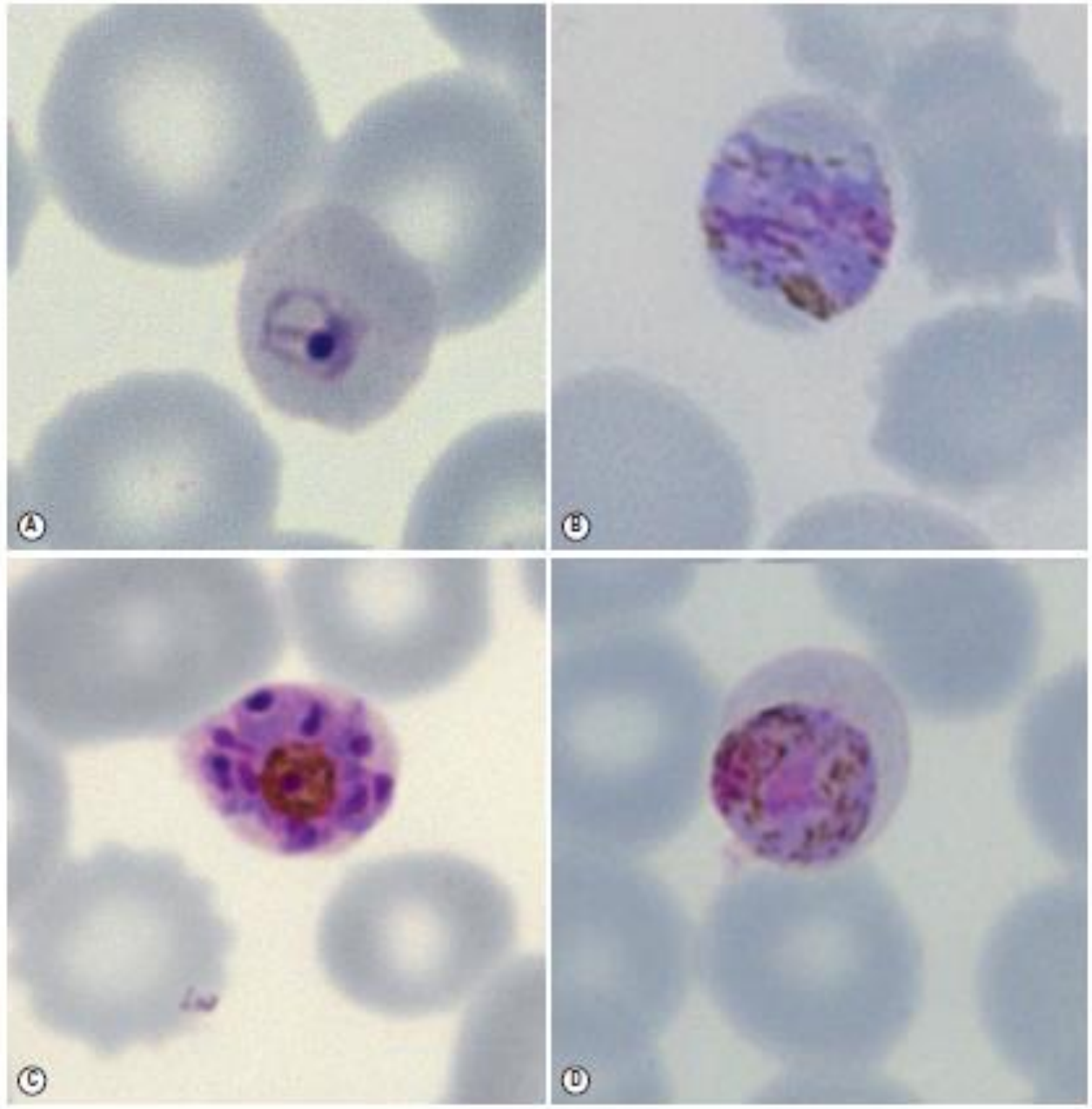


Morphology of *P. malariae.*

- Early trophozoites **(A)** are small but less fine than those of *P. falciparum;* in this case the ring is

irregular and the chromatin dot is within the main parasite cytoplasm.

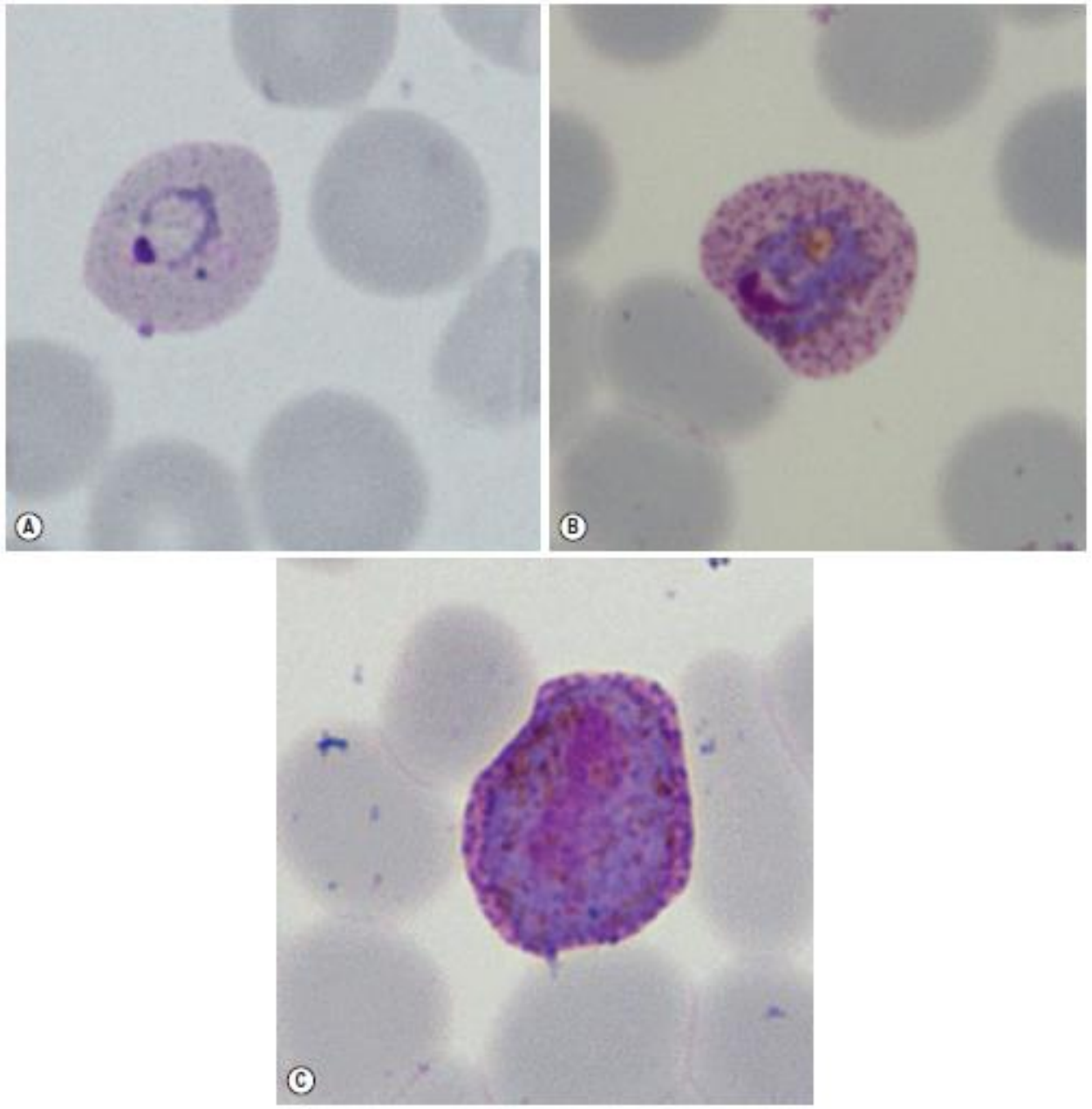
- A late trophozoite **(B)** spans the erythrocyte as a thick ‘band form’.
- A characteristic ‘daisy head’ schizont **(C)** has eight merozoites arrayed around the central pigment.
- The gametocyte **(D)** is typically smalland does not fill the normally sized erythrocyte.



Morphology of *P. vivax.*

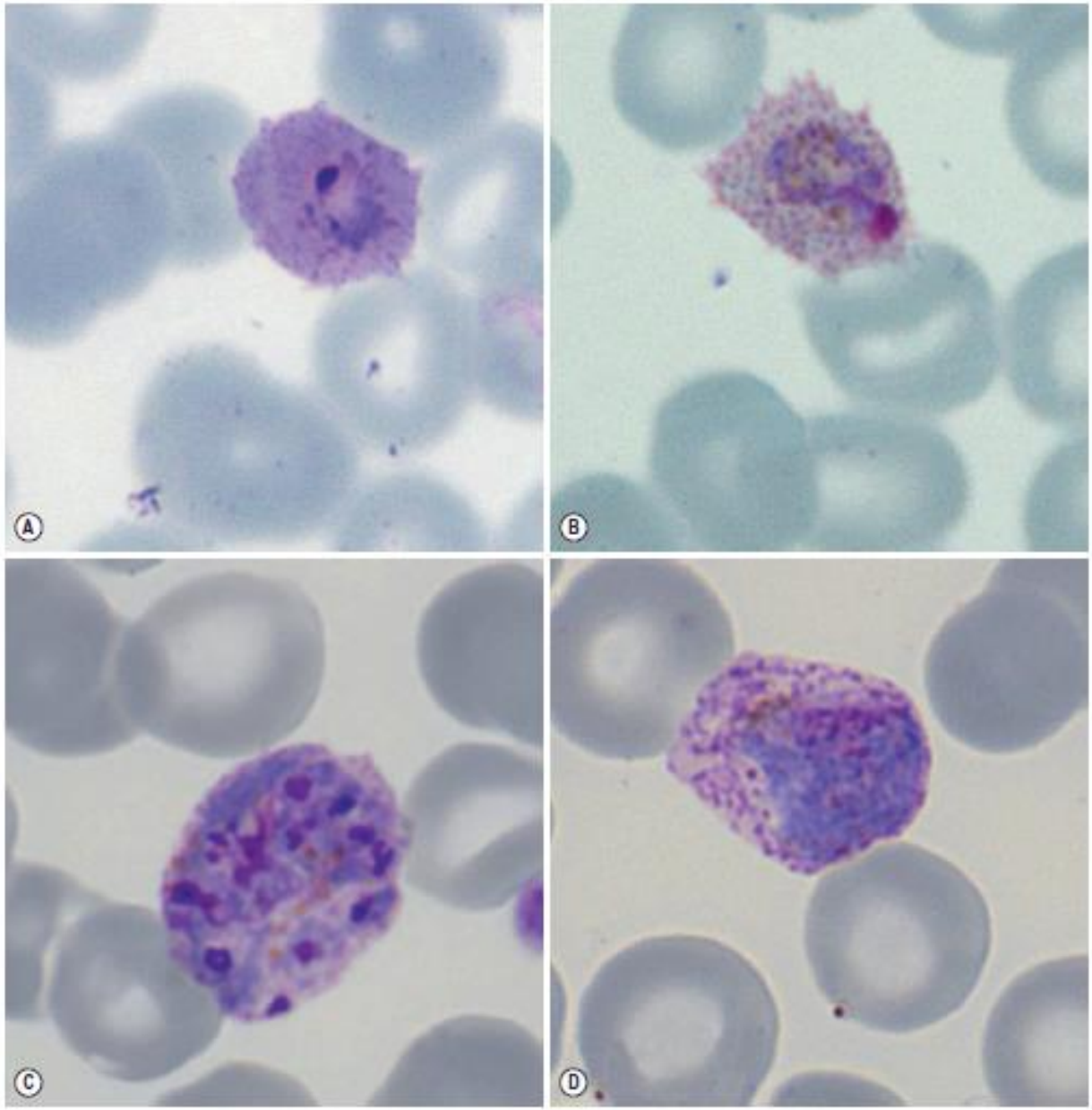
The red cells are enlarged, distorted and contain visible Schüffner dots in all these images. Note that the parasites are larger than in the other malarial forms.

The early trophozoite **(A)** in this figure has the form of a thick ring with a large chromatin dot, but the later trophozoite **(B)** has a distorted 'amoeboid' form. Note that the large macrogametocyte **(C)** fills the enlarged erythrocyte.



Morphology of *P. ovale.*

Distinguishing *P. ovale* from *P. vivax* is perhaps the most difficult morphological distinction in malaria diagnosis. For both species, the red cells are enlarged and distorted and contain visible Schüffner dots. The features that help distinguish these forms are given in Table 6-2. In this figure, note that the parasites and erythrocytes are smaller than for *P. vivax,* and that in some cases the cytoplasm is fimbriated. Both early **(A)** and late trophozoites **(B)** have coarse 'ring' forms. The schizont **(C)** contains fewer than 16 developing merozoites. A microgametocyte **(D)** does not fill the erythrocyte and numerous dots can be seen in the erythrocyte cytoplasm.

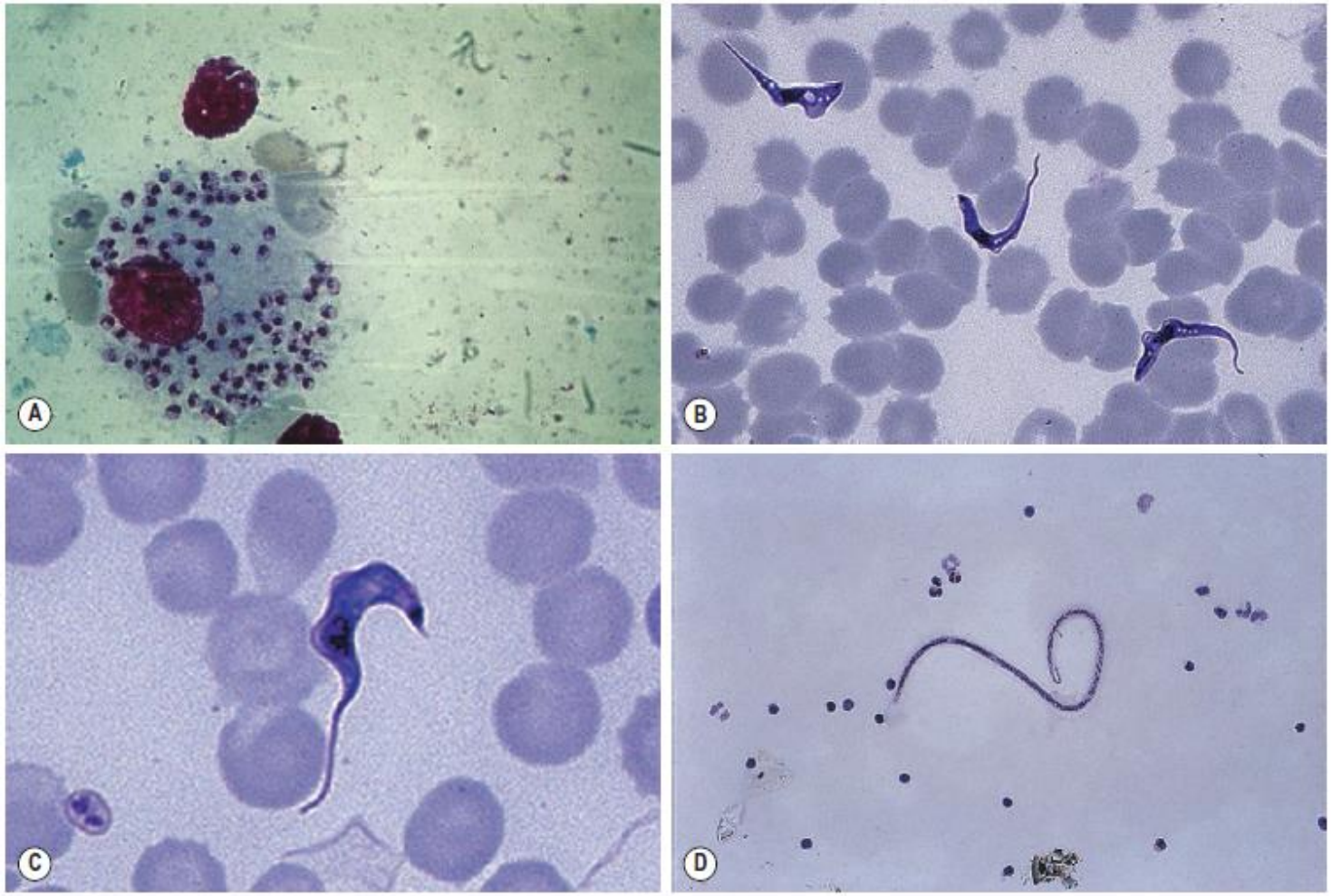


Bone marrow and blood parasites.

(A) Leishmaniasis (Leishman–Donovan bodies);

(B) African trypanosomiasis;

(C) American trypanosomiasis (*T. cruzi*); and
(D) microfilaria.



Quality control for Hb Elechtrophoresis

LABORATORY FINDINGS IN THALASSAEMIA

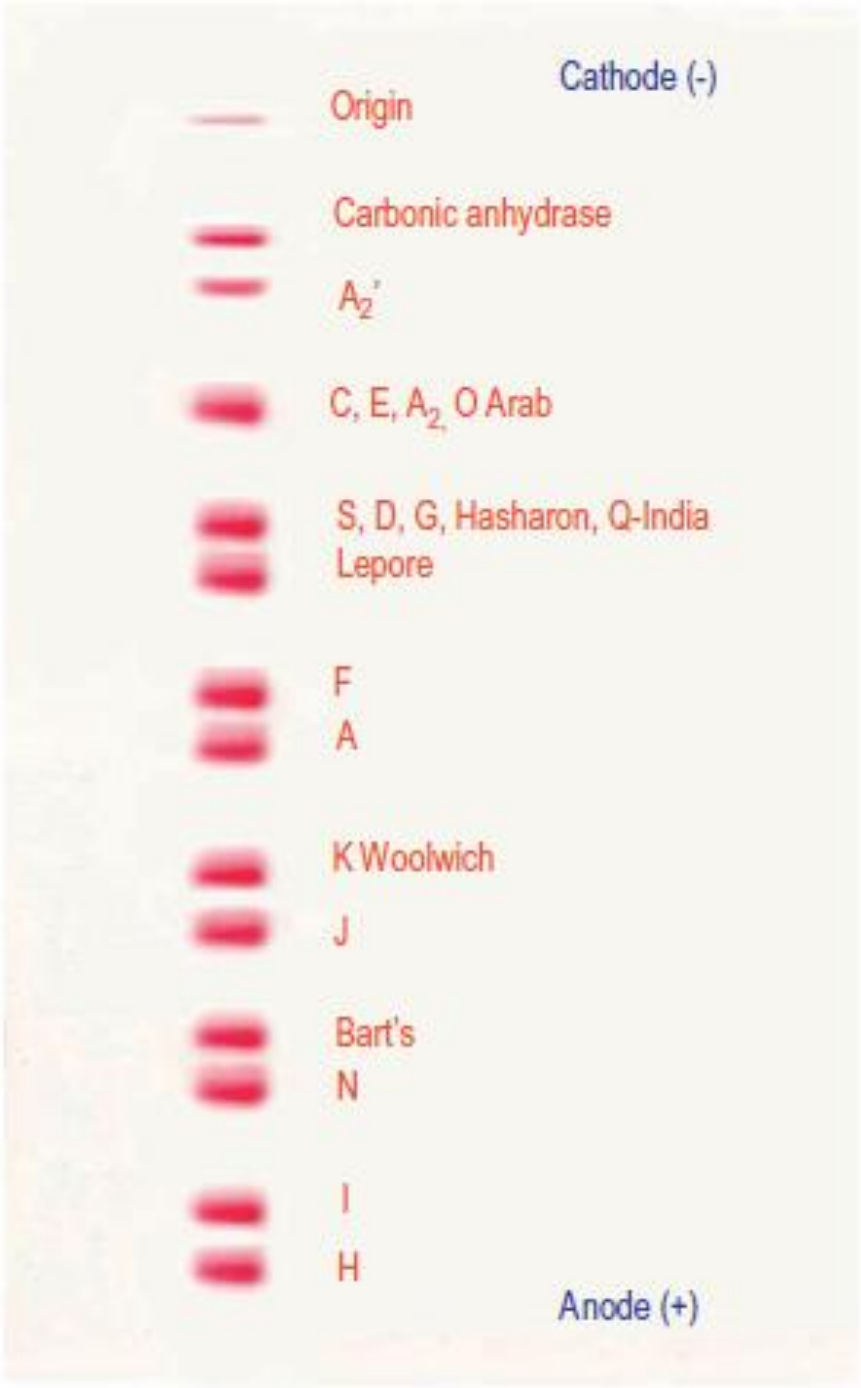
Phenotype	Genotype	Usual MCV	Usual MCH	Haemoglobin A_2	Haemoglobin H Inclusions
α thalassaemia					
α^+ thalassaemia heterozygosity	$-\alpha/\alpha\alpha$	N	N	N or ↓	–
α^+ thalassaemia homozygosity	$-\alpha/-\alpha$	N or ↓	N or ↓	N or ↓	±
α^0 thalassaemia heterozygosity	$--/\alpha\alpha$	↓	↓	N or ↓	+
Haemoglobin H disease					
Mild or moderate	$--/-\alpha$	↓	↓	N or ↓	+++
Severe	$--/\alpha^T\alpha$	↓	↓	N or ↓	+++
Haemoglobin Bart's hydrops fetalis (α thalassaemia major) α^0 homozygosity	$--/--$	↓	↓	–	–
β thalassaemia					
β thalassaemia trait	β^0/β or β^+/β	↓	↓	↑	–
β thalassaemia trait with normal Hb A_2	β^+/β	↓	↓	N	–
δβ thalassaemia trait	$\delta\beta^0/\beta$	↓	↓	N or ↓	–
Hb Lepore trait	$\delta\beta^{Lepore}/\beta$	↓	↓	N or ↓	–
β thalassaemia intermedia	Heterogeneous	↓	↓	↑ or N	–
β thalassaemia major	β^0/β^0, β^0/β^+, β^+/β^+	↓	↓	↑ or N	

Control samples

- Interpretation of migration patterns of test samples is undertaken by comparison with the migration and separation of known variant haemoglobins used as control materials. Ideally, a mixture of haemoglobins A, F, S and C should be included on each electrophoretic separation.
- It is necessary to use reference standards and control materials in each of the analyses undertaken and in some cases to use duplicate analysis to demonstrate precision.
- There are international standards for haemoglobins F and A2, whereas in some countries national reference preparations are also available from national standards institutions. These are extremely valuable because the target values have been established by collaborative studies.
- Control materials can be prepared in-house or obtained commercially.
- Samples stored as whole blood at 4 °C can be used reliably for several weeks.
- All laboratories should confirm the normal range for their particular methods and the normal range obtained should not differ significantly from published data.
- All laboratories undertaking haemoglobin analysis should participate in an appropriate proficiency testing programme.

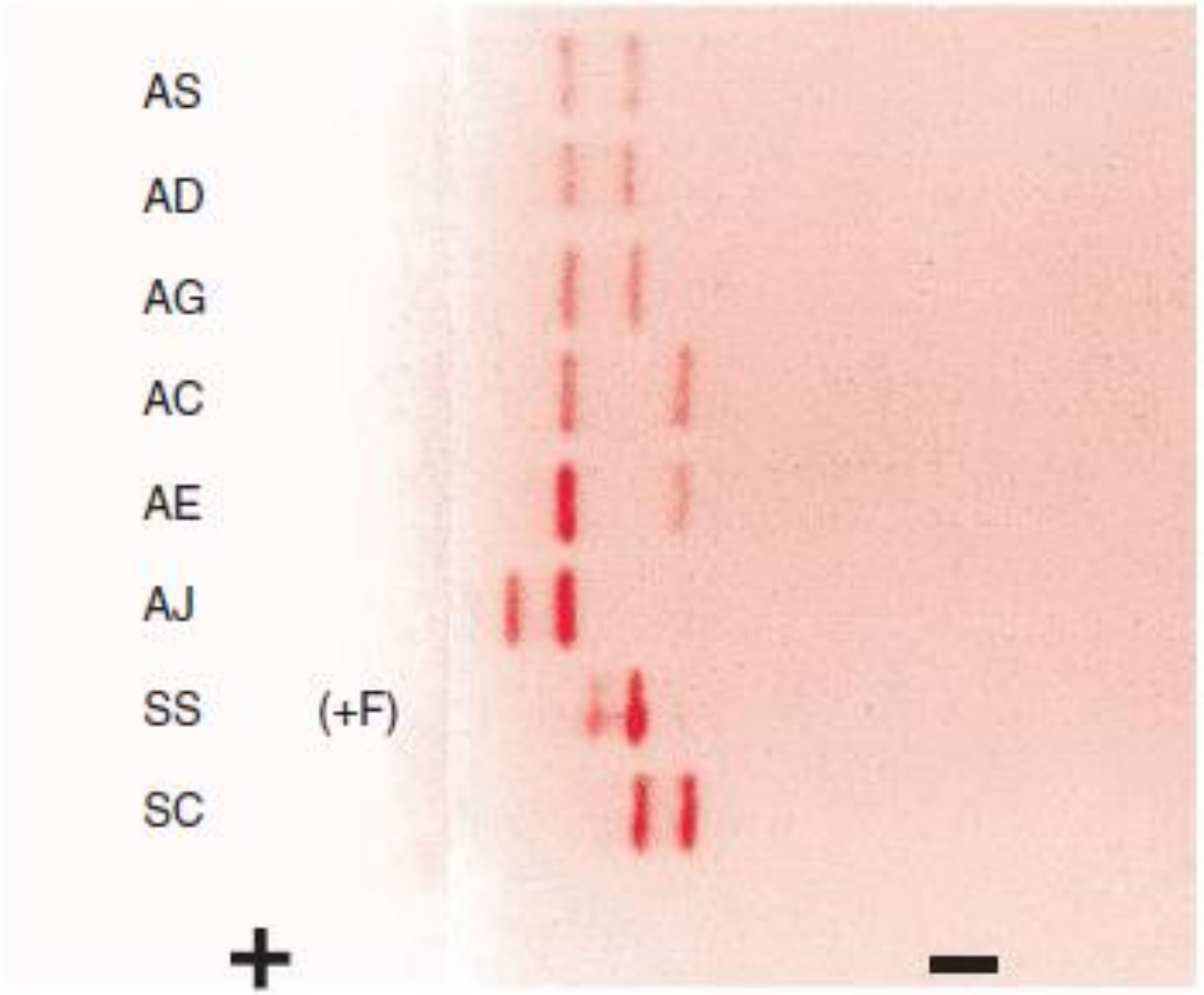
Schematic representation of relative mobilities of some abnormal haemoglobins.

Cellulose acetate electrophoresis, pH 8.5.



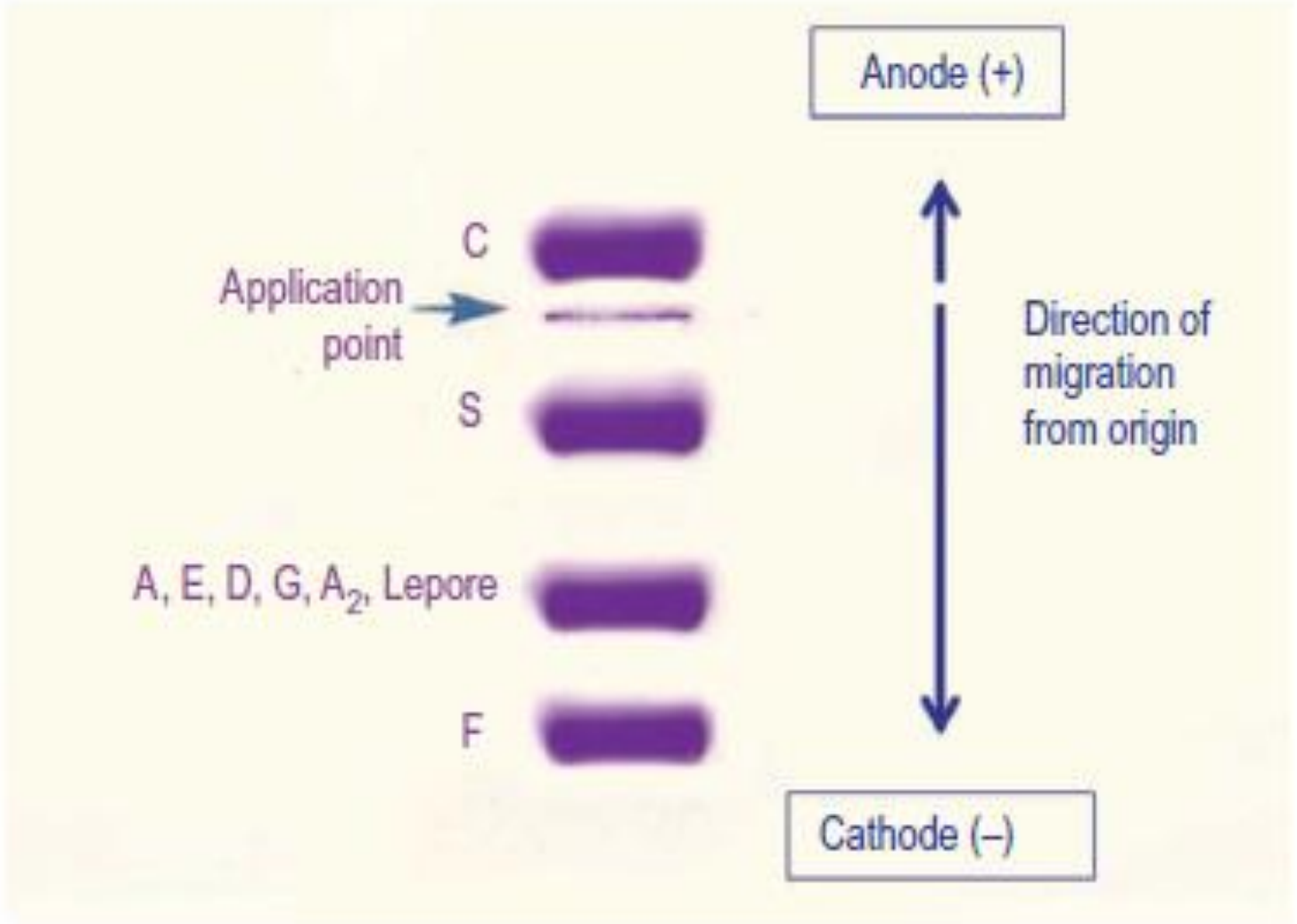
Relative mobilities of some abnormal haemoglobins.

Cellulose acetate electrophoresis, pH 8.5.



Schematic representation of relative mobilities of some abnormal haemoglobins.

Acid agararose gel electrophoresis,pH 6.0.



Quality assurance in Hemostasis

Pre-analytical Variables Including Sample Collection

- To minimise the effects of contact activation, good quality plastic or polypropylene syringes should be used. If glass blood containers are used, they should be evenly and adequately coated with silicone.
- The most commonly used anticoagulant for coagulation samples is trisodium citrate. A 32 g/1 (0.109 M) solution is recommended.
- Other anticoagulants, including oxalate, heparin and ethylenediaminetetra-acetic acid (EDTA) are unacceptable.
- The labile factors (factors V and VIII) are unstable in oxalate, whereas heparin and EDTA directly inhibit the coagulation process and interfere with end-point determinations.

- For routine blood coagulation testing, 9 volumes of blood are added to 1 volume of anticoagulant (i.e. 0.5 ml of anticoagulant for a 5 ml specimen). When the haematocrit is abnormal due to either severe anaemia or polycythaemia, the blood:citrate ratio should be adjusted. For a 5 ml specimen (total), the amount of citrate should be as follows:

Haematocrit	Citrate (ml)
0.20	0.70
0.25	0.65
0.30	0.61
0.55	0.39
0.60	0.35
0.65	0.30
0.70	0.26

Centrifugation: preparation of platelet-poor plasma

- Most routine coagulation investigations are performed on platelet-poor plasma (PPP), which is prepared by centrifugation at 2000 *g* for 15 min at 4 °C (approximately 4000 rev/min in a standard bench cooling centrifuge).
- The sample should be kept at room temperature if it is to be used for PT tests, lupus anticoagulant (LAC) or factor VII assays and it should be kept at 4 °C for other assays.
- The testing should preferably be completed within 2 h of collection. Care must be taken not to disturb the buffy coat layer when removing the PPP.

- **Coagulation**
- All laboratories using *automated coagulation* systems must:
  1. Include two levels of controls for each eight hours of testing; and
  2. Include two levels of controls each time a change in reagents occurs.

- *Each individual* performing coagulation tests using manual coagulation systems must:
- Test two levels of controls before testing patient samples and each time a change in reagents occurs; and
- Perform patient and control testing in duplicate.
- Document all control activities.

Why do we need Quality Assurance Schemes

- There are numerous examples in the literature that highlight the value of internal and external quality assurance schemes. Look at the following [fabricated] case study involving the measurement of the Prothrombin Time [PT] and the subsequent derivation of the INR. Fundamental to this measurement is a knowledge of the ISI of the thromboplastin used in the PT assay. Remember the INR is calculated from the [PT ratio]^{ISI}.

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Thank YOU

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