The standardized bone marrow exam: pearls for residents in transition to practice

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Disclosures

- None

- I am not affiliated with the examination committee and am not involved in the exam process

Objectives

- Review of standardized (ICSH, CAP, WHO) guidelines for bone marrow examination
- Review of process and ICSH standardized guidelines for bone marrow IHC
- Discuss key topics important for RCPSC exam preparation and transition to early practice
- Brief overview of QC/QA pertaining to bone marrow

Why standardize bone marrow examination?

- Variation in preparation, processing and reporting of BM specimens
- Lack of uniformity can lead to inconsistencies in disease diagnosis and classification, and thereby affect treatment and clinical outcomes
- In an attempt to standardize the indications for BM examination, the specimens required and report format, the International Council for Standardization in Hematology (ICSH) released consensus guidelines in 2008 for bone marrow examination and in 2015 for bone marrow IHC

Bedtime reading for the exam

ICSH guidelines for the standardization of bone marrow specimens and reports

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Standardization, bone marrow, aspirate, trephine biopsy, report

SUMMARY

The bone marrow examination is an essential investigation for the diagnosis and management of many disorders of the blood and bone marrow. The aspirate and trephine biopsy specimens are complementary and when both are obtained, they provide a comprehensive evaluation of the bone marrow. The final interpretation requires the integration of peripheral blood, bone marrow aspirate and trephine biopsy findings, together with the results of supplementary tests such as immunophenotyping, cytogenetic analysis and molecular genetic studies as appropriate, in the context of clinical and other diagnostic findings. Methods for the preparation, processing and reporting of bone marrow aspirates and trephine biopsy specimens can vary considerably. These differences may result in inconsistencies in disease diagnosis or classification that may affect treatment and clinical outcomes. In recognition of the need for standardization in this area, an international Working Party for the Standardization of Bone Marrow Specimens and Reports was formed by the International Council for Standardization in Hematology (ICSH) to prepare a set of guidelines based on preferred best practices. The guidelines were discussed at the ICSH General Assemblies and reviewed by an international panel of experts to achieve further consensus.

ICSH guidelines for the standardization of bone marrow immunohistochemistry

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SUMMARY

Bone marrow (BM) tissue biopsy evaluation, including trephine biopsy and clot section, is an integral part of BM investigation and is often followed by ancillary studies, in particular immunohistochemistry (IHC). IHC provides *in situ* coupling of morphological assessment and immunophenotype. The number of different IHC tests that can be applied to BM trephine biopsies and the number of indications for IHC testing is increasing concurrently with the development of flow cytometry and molecular diagnostic methods. An international Working Party for the Standardization of Bone Marrow IHC was formed by the International Council for Standardization in Hematology (ICSH) to prepare a set of guidelines for the standardization of BM IHC based on currently available published evidence and modern understanding of quality assurance principles as applied to IHC in general. The guidelines were discussed at the ICSH General Assemblies and reviewed by an international panel of experts to achieve further consensus and represent further development of the previously published ICSH guidelines for the standardization of BM specimens handling and reports.

COLLEGE of AMERICAN PATHOLOGISTS

Protocol for the Examination of Specimens From Patients With Hematopoietic Neoplasms Involving the Bone Marrow

Based on AJCC/UICC TNM, 7th Edition
Protocol web posting date: June 2012

Procedures
- Bone marrow aspiration
- Bone marrow core (trephine) biopsy

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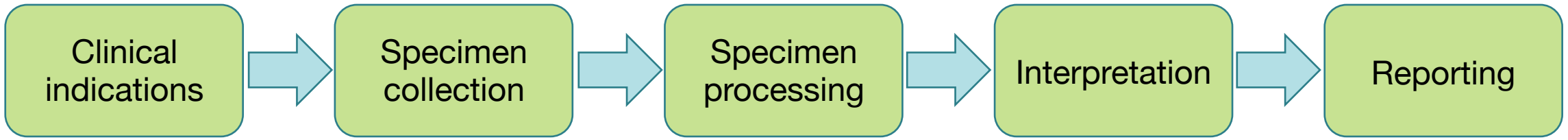
Conceptual view of bone marrow exam

- Think of variables at each point in process
- What variables could be standardized at each step?
- Senior year: how do the details fit into the big picture?



Conceptual approach in senior year

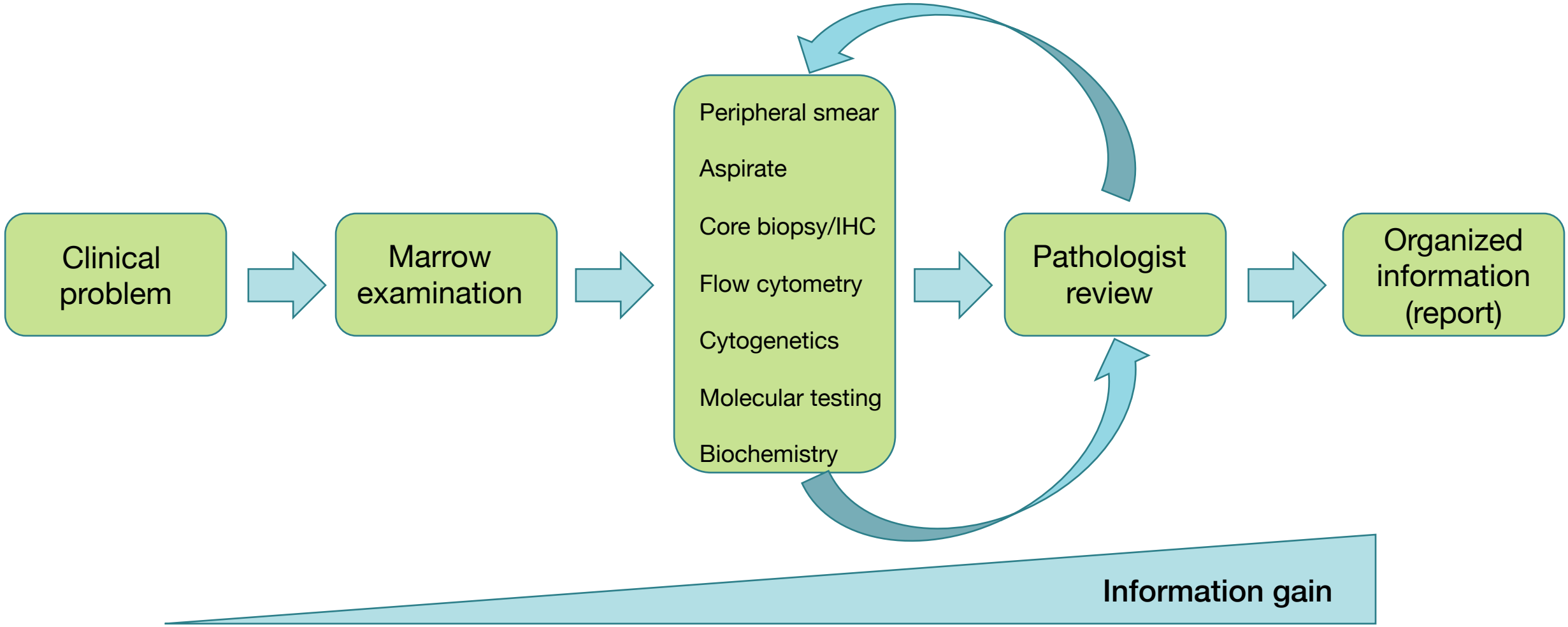
1) Understand basic laboratory setup and workflow as it pertains to hematopathology

2) Conceptual understanding of each step in bone marrow exam, how validate, maintain, troubleshoot

3) Basics of quality control (QC) and quality assurance (EQA) as it pertains to hematopathology

4) Ask **WHY** at each step… then ask **WHY** again…

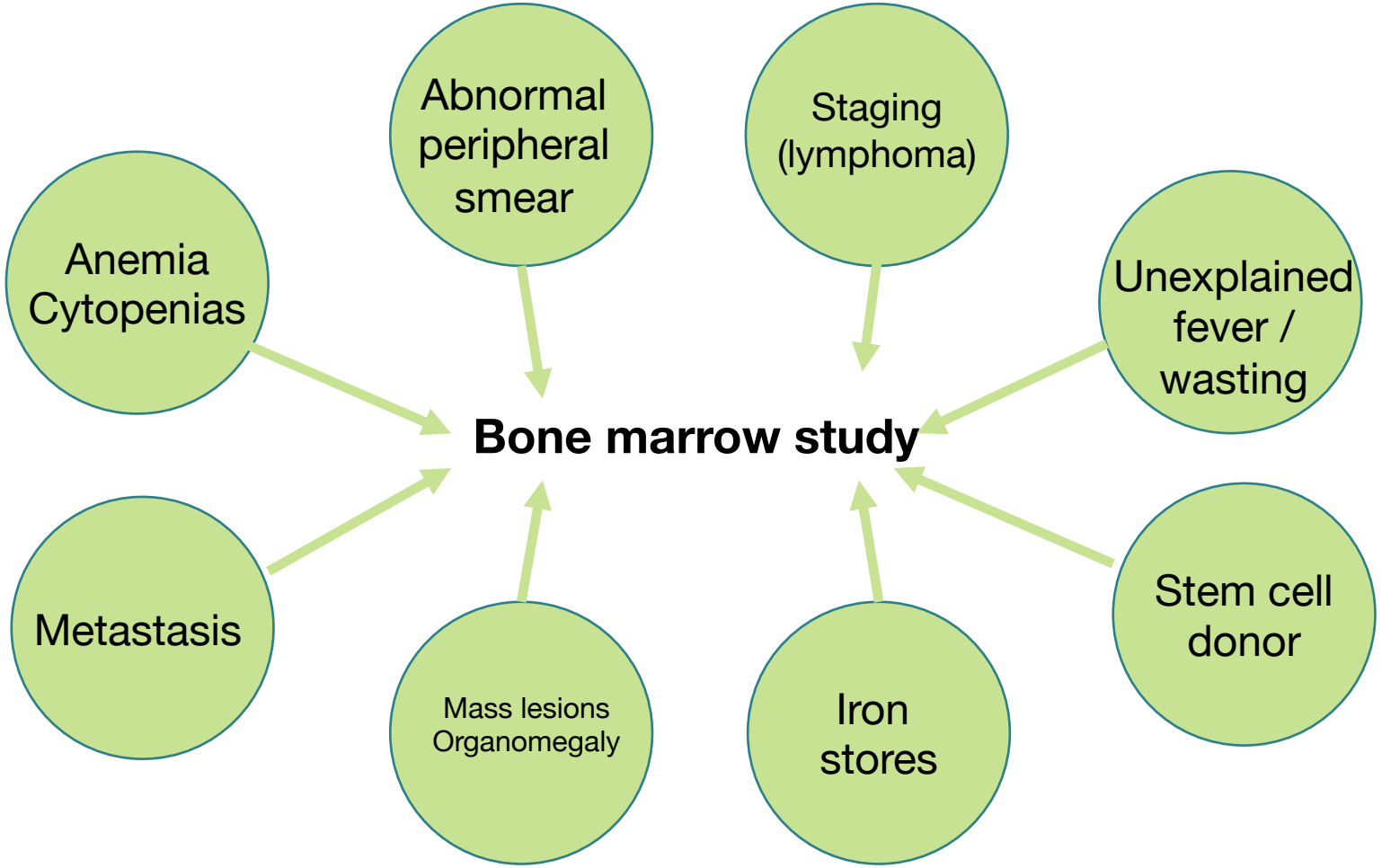
Organizing information in pathology



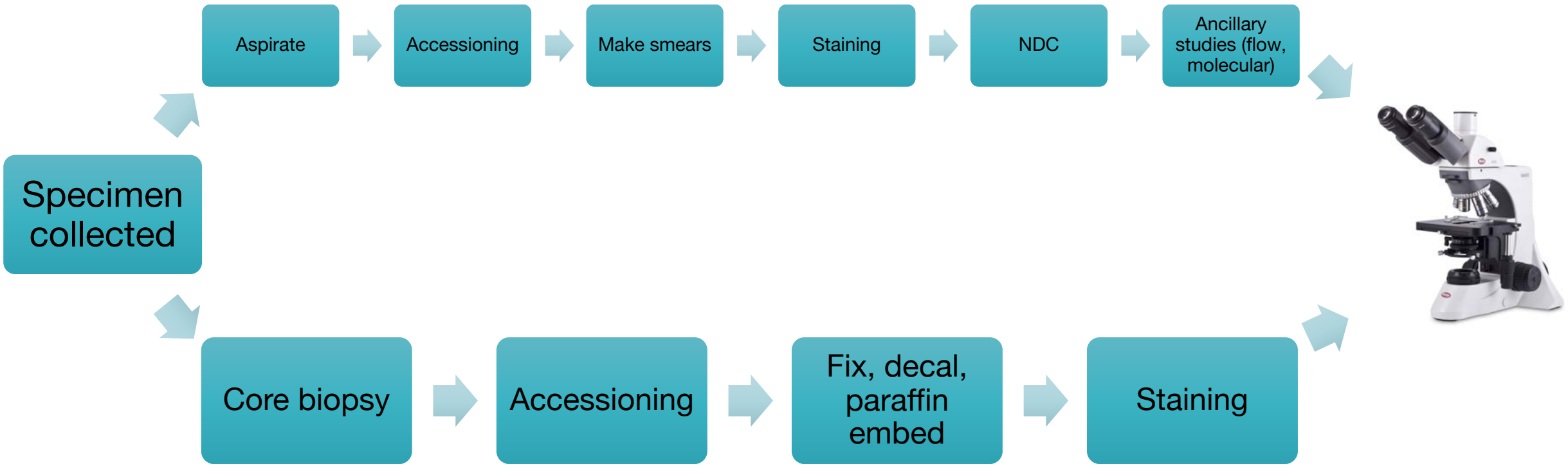
Integration of information

- Bone marrow reporting requires integration of a significant amount of information beyond morphology
- Often done in “real time” with clinical input
- Standardization of methods is essential for a robust and universal interpretation

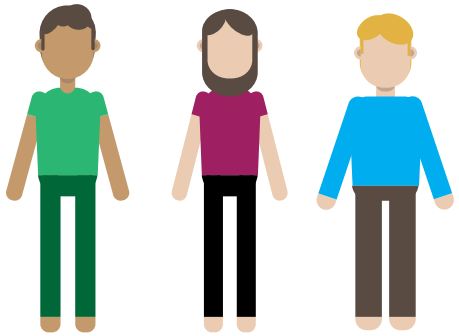
Why is a bone marrow study performed?



Process flow in the bone marrow exam



Bone marrow aspirate



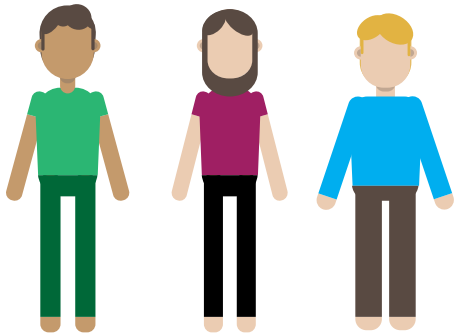
Patient consent

Aspirate and biopsy needles

Aspirate
Aseptic technique
posterior superior iliac spine (PSIS)

- 10 – 20 mL syringe
- Usually no anticoagulant
- Smears may be made at bedside (first draw)
- Aspirate may be placed into EDTA tubes for making smears in lab
- Clot ideally made and placed into fixative at the bedside
- Second draw more diluted (used for ancillary studies)

Bone marrow trephine core biopsy

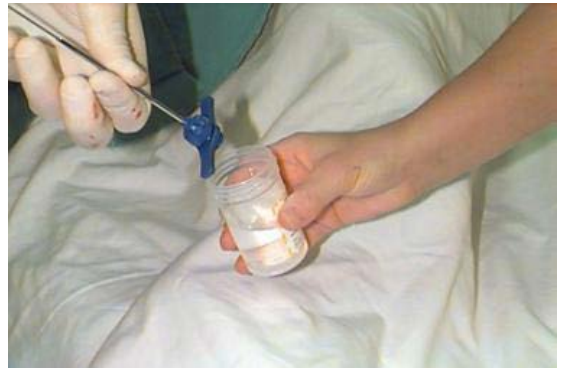


Patient consent

Aspirate and biopsy needles

Core biopsy
Aseptic technique
posterior superior iliac spine (PSIS)

- Core usually done after aspirate
- Separate needle
- Same incision
- Placed directly into fixative <u>at bedside</u>



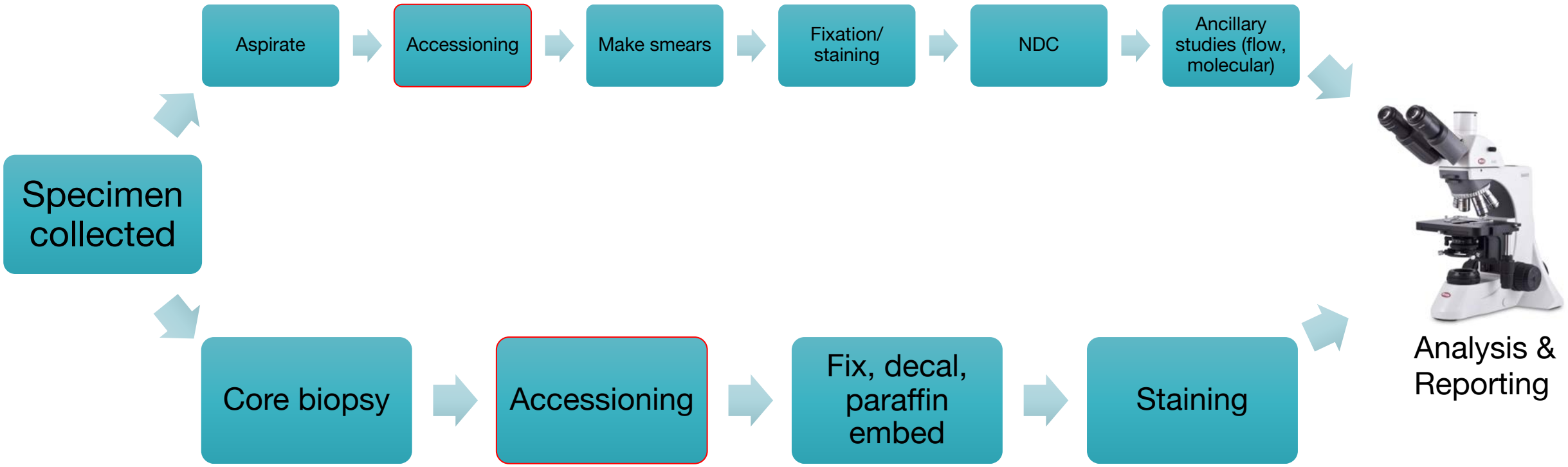
Q: What the components of a bone marrow exam?

Components of bone marrow exam (ICSH 2008):

- Aspirate
  - Smear (6 slides) (EDTA)
  - Squash or crush preparation (≥ 2 slides) (EDTA)
  - Iron stain (on smear or squash preparation)
  - Particle clot
  - Flow cytometry (Heparin)
  - Molecular Biology (EDTA)
  - Cytogenetics (in tissue culture media or heparin)
- Peripheral blood smear (within 2 days of marrow)
- Core biopsy Histology (Fixative, Staining)
  - Touch preparation (≥ 2 slides)

Q: What the components of a bone marrow exam?

Process flow for bone marrow specimen



Q: What is specimen accessioning?

What is accessioning?

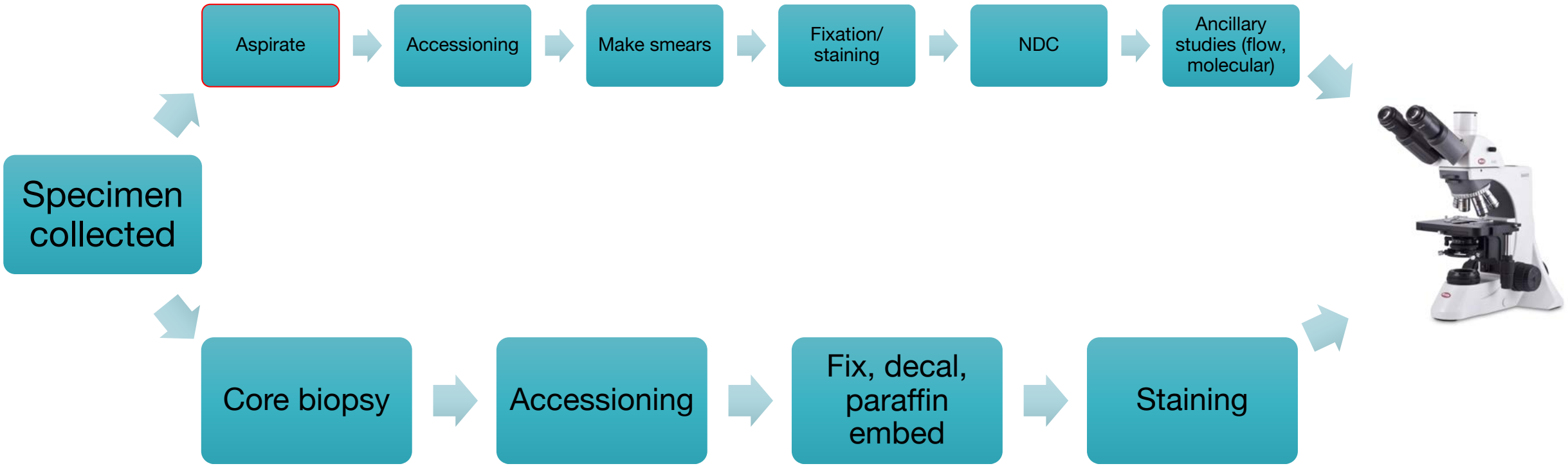
- Specimen Accessioning and Processing (Laboratory Receiving) is the process by which specimens are:
- received
- sorted
- and entered into the Laboratory Information System (LIS)
- labelled with barcoded labels and processed…

Generating accessioning label

The bone marrow aspirate and clot: accessioning

- Be familiar with your LIS; how it works, communicates with instruments, how to validate it etc.
- Use 2 patient identifiers to open a new specimen accession, plus time collected and time received
- Each part of the case (clot, core, aspirate, flow, molecular etc.) usually separately accessioned
- Single piece workflow (only one specimen at a time)

Process flow for bone marrow specimen



The bone marrow aspirate: collection

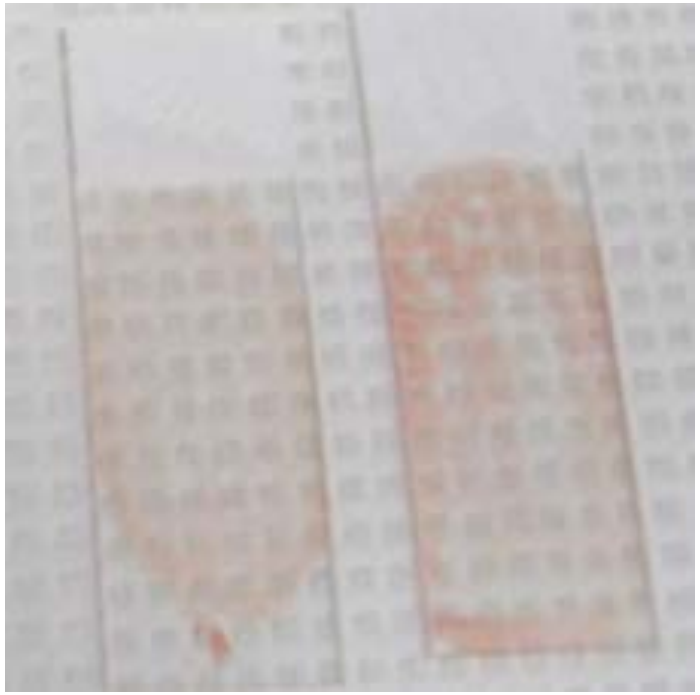
Collect into 10-20 mL syringe

Transfer remaining to EDTA tube

Collect into second syringe

Transfer remaining to EDTA and heparin tubes

- EDTA for molecular
- Heparin for flow cytometry
- and cytogenetics



Unstained bedside smear

- Ideal method (less artifact)
- Takes operator time
- Must be done ASAP (CLOTTING)

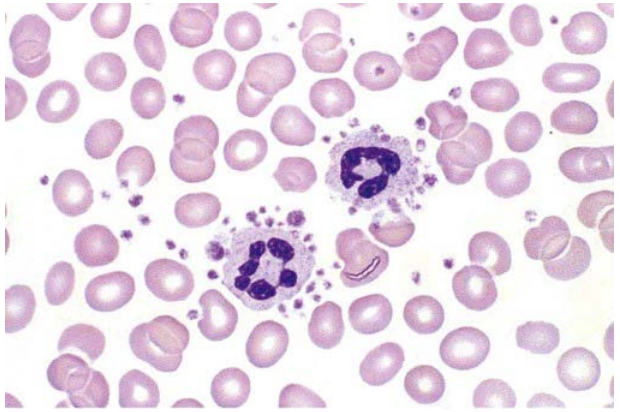
The bone marrow aspirate: collection

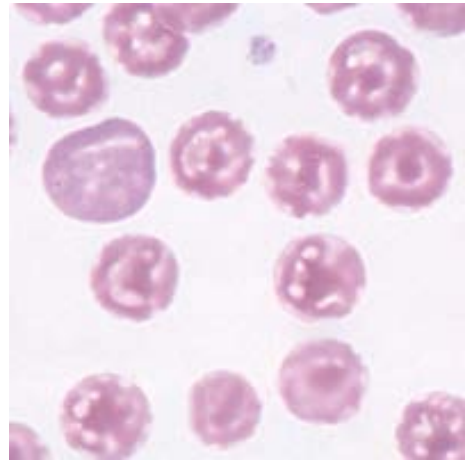
Collect into 10-20 mL syringe

Transfer remaining to EDTA tube

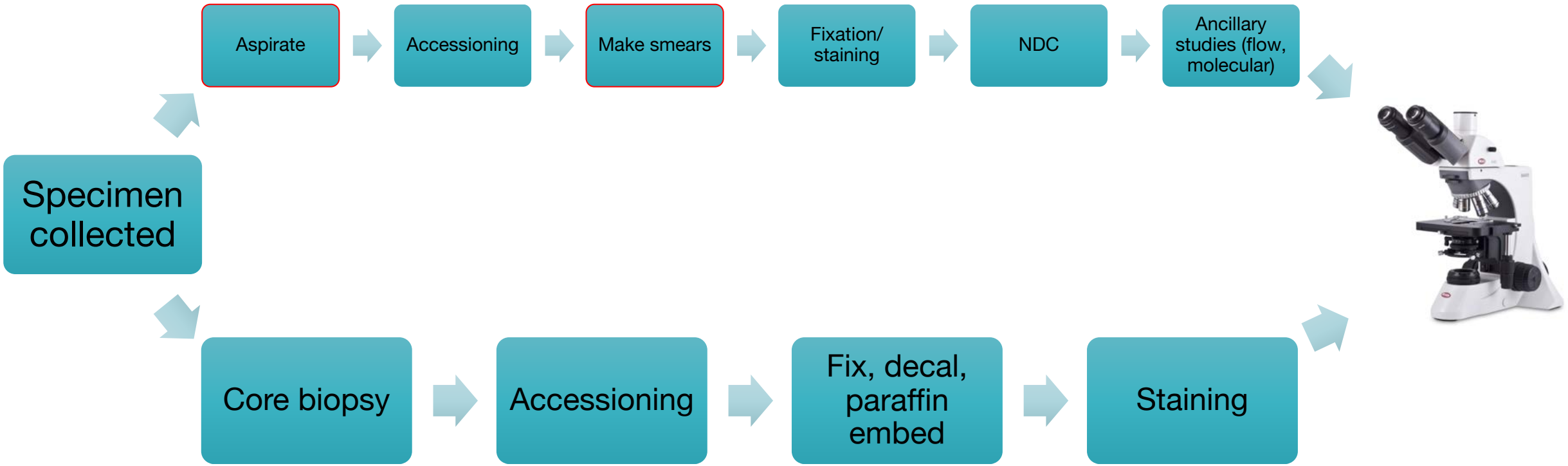
Collect into second syringe

Transfer remaining to EDTA and heparin tubes





Process flow for bone marrow specimen



Q: What are some advantages/ disadvantages of a push prep versus a squash prep for morphology?

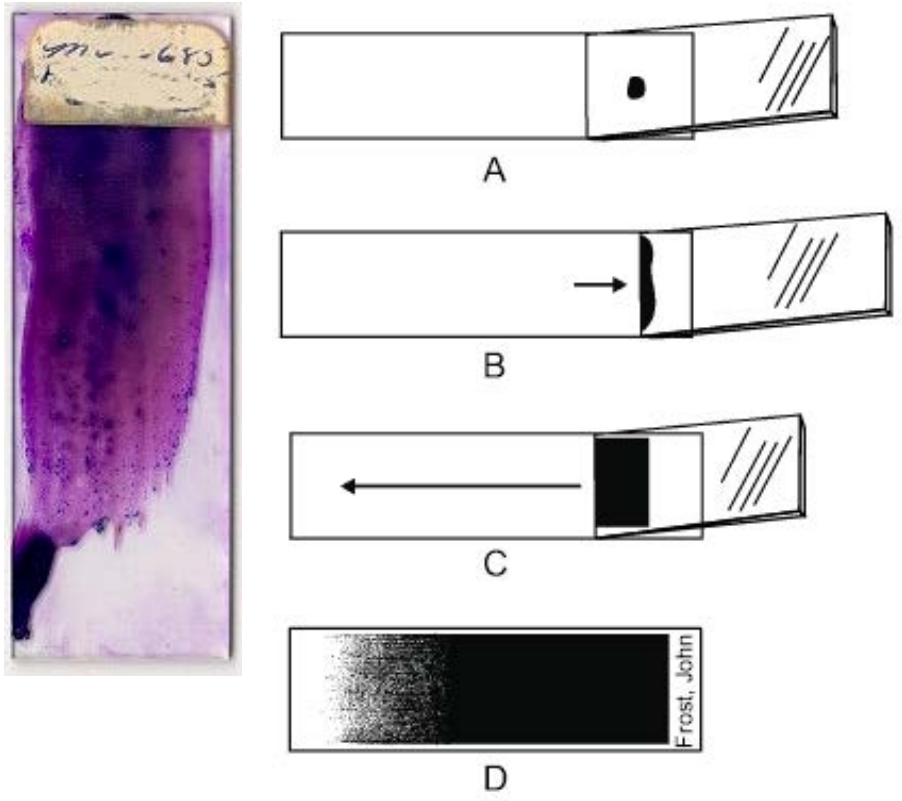
The bone marrow aspirate: push preparation

Advantages

- Arguably better morphological details (specificity)
- Accurate cell counts in particle trails

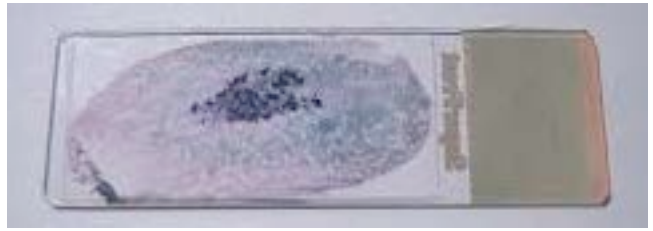
Disadvantages

- Reduced cell release from particles compared to squash prep (i.e., reduced sensitivity)



Push preparation smear

The bone marrow aspirate crush preparation

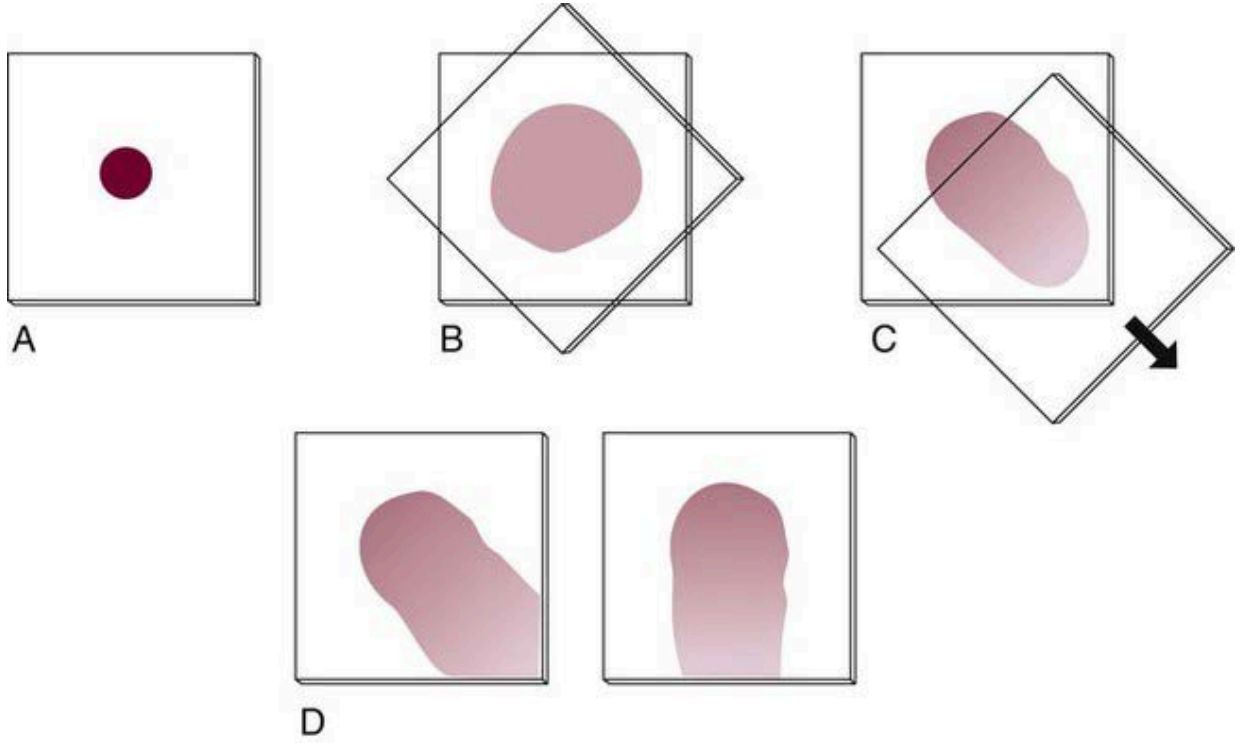


Advantages

- Pelatively better cell release
- Enhances sensitivity for rare cells (ex. megakaryocytes)
- Fibrotic marrows

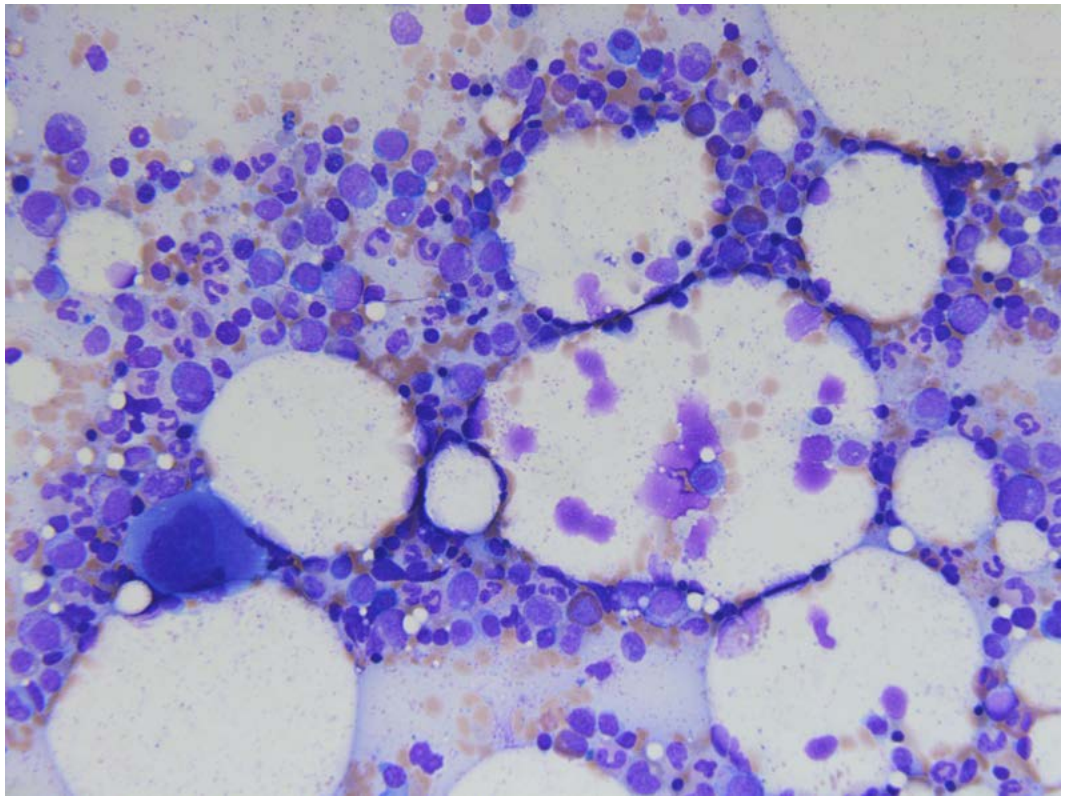
Disadvantages

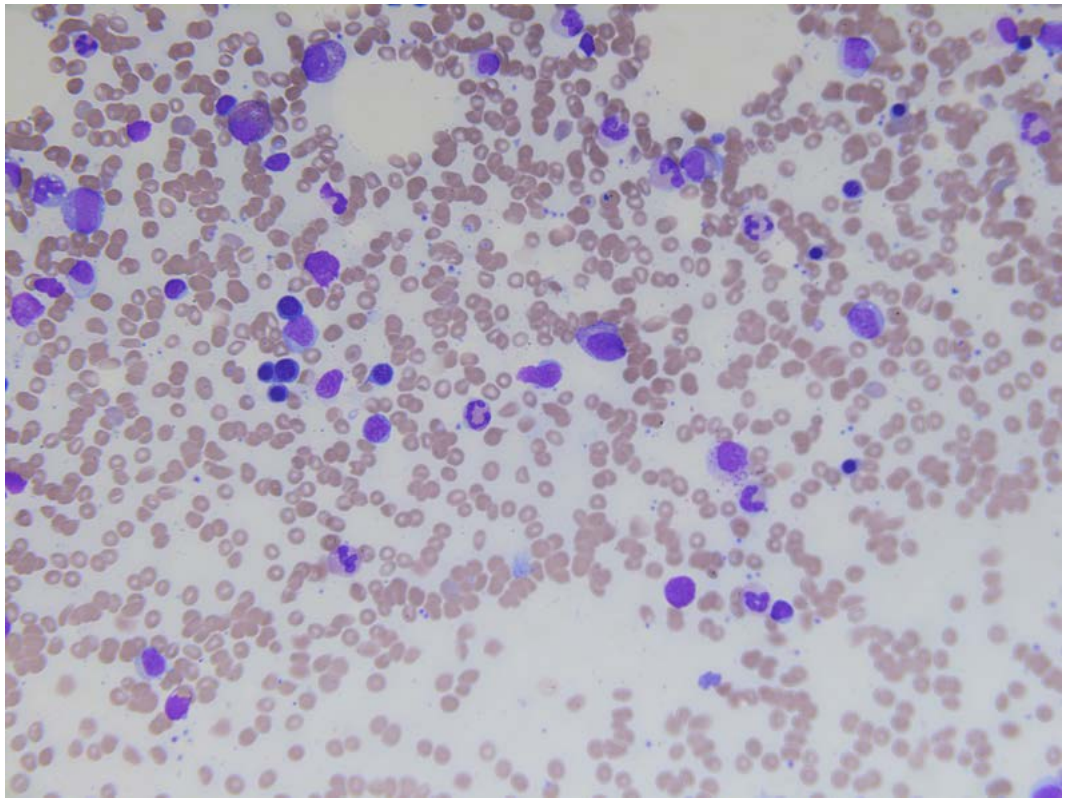
- Some would say loss of morphological details (specificity)



Squash or crush preparation smear

Smear versus crush preparation





Q: What EDTA concentration is recommended by ICSH for morphology?

Q: What is a disadvantage of using heparinized specimens for bone marrow morphology?

Bone marrow aspirate: anticoagulants

EDTA

- ICSH recommends the dipotassium EDTA salt at a concentration of 1.50 ± 0.25 mg/ml
- Can distort cellular morphology
- Smears must be made and fixed within 24 h of collection (less time ideal)
- Should not be used for cell culture (cytogenetics)

Lavender top tube (EDTA)

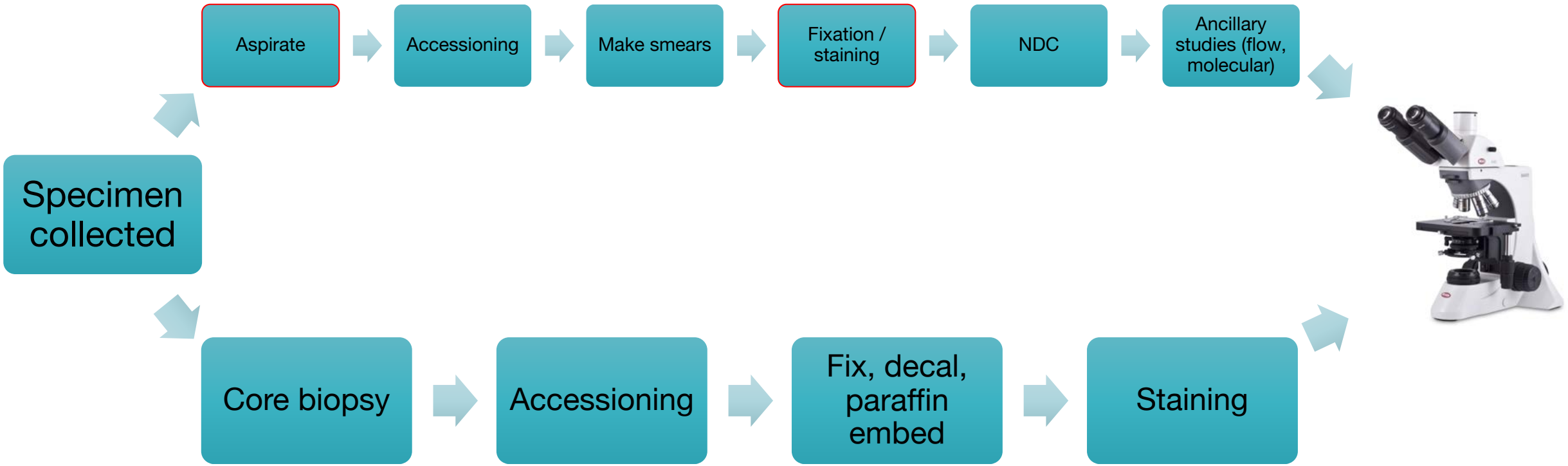
Bone marrow aspirate: anticoagulants

Heparin

- ICSH recommends sodium heparin for flow cytometry and cytogenetics
- Heparinized samples should not be used for morphology (staining artifact)
- Heparin should not be used for molecular biology (may interfere with PCR reactions)

Green top tube (heparin)

Process flow for bone marrow specimen

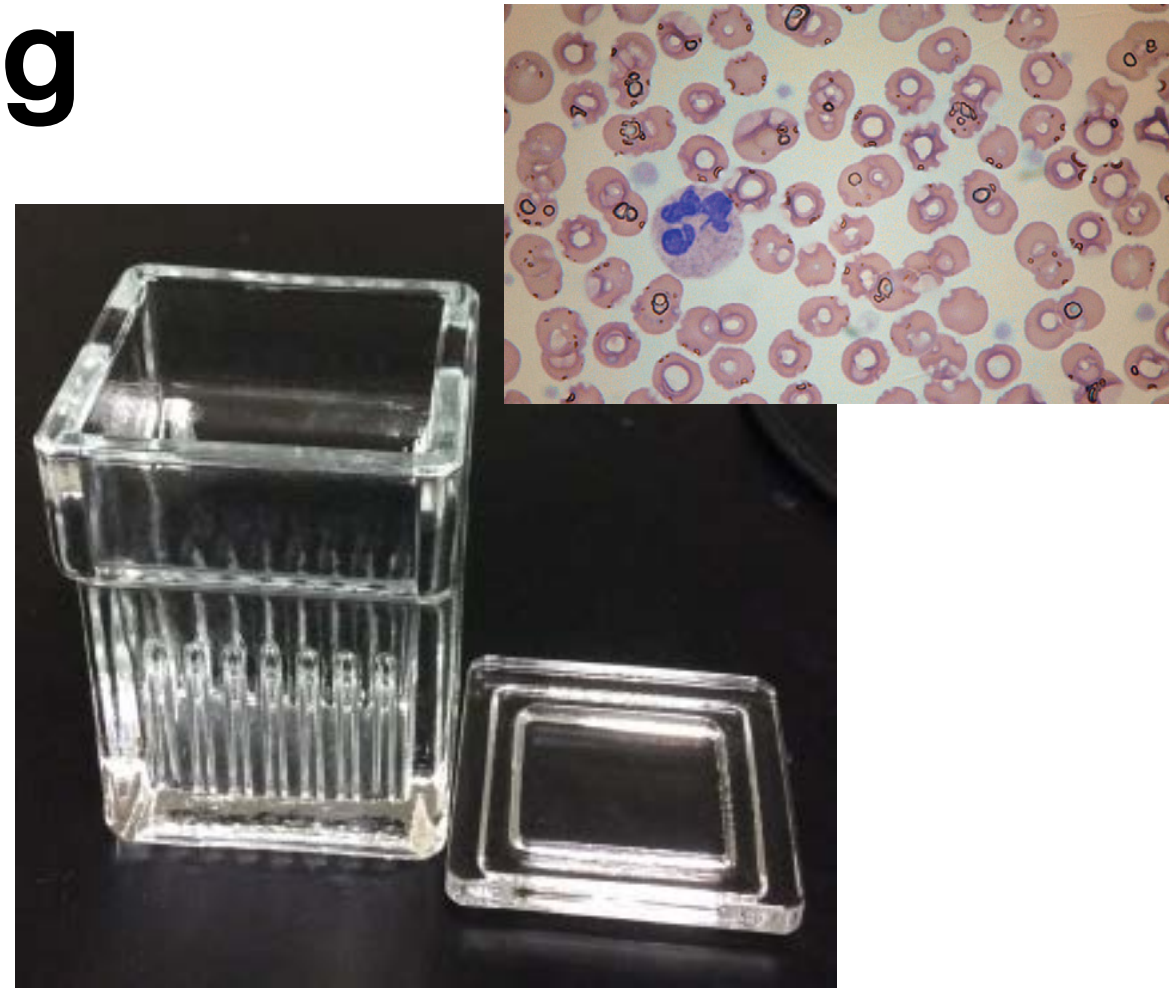


Q: What is the ICSH recommended fixative for bone marrow aspirates?

Bone marrow Aspirate: Fixation and Staining

According to ICSH

- Air dry and fix in absolute methanol
- Dry ~ 20 mins
- Acetone-free absolute methanol (~ 10 min)
- Water in methanol can lead to hydration artifact
- Fixation and staining is automated in most labs now



Medium jar

Q: What is the principle of a romanowsky stain?

Bone marrow Aspirate: Fixation and Staining

Romanowsky staining principle:

- Basic and acid component
- Eosin Y <u>ACIDIC</u>
  - Stains positively charged material (cytoplasmic granules and proteins, ex. hemoglobin).
- Azure B / methylene blue <u>BASIC</u>
  - Stains negatively charged material (nucleic acids).

Bone marrow Aspirate: Fixation and Staining

Staining process now automated

Example process for MGG stain:

- Spread Slide
- Place in methanol ASAP
- May-Grunwald
- Giemsa
- wash in water
- dry
- TAT 45 mins

Bone marrow Aspirate: IRON stain

Perls’ Stain / Perls’ reaction / Prussian blue reaction

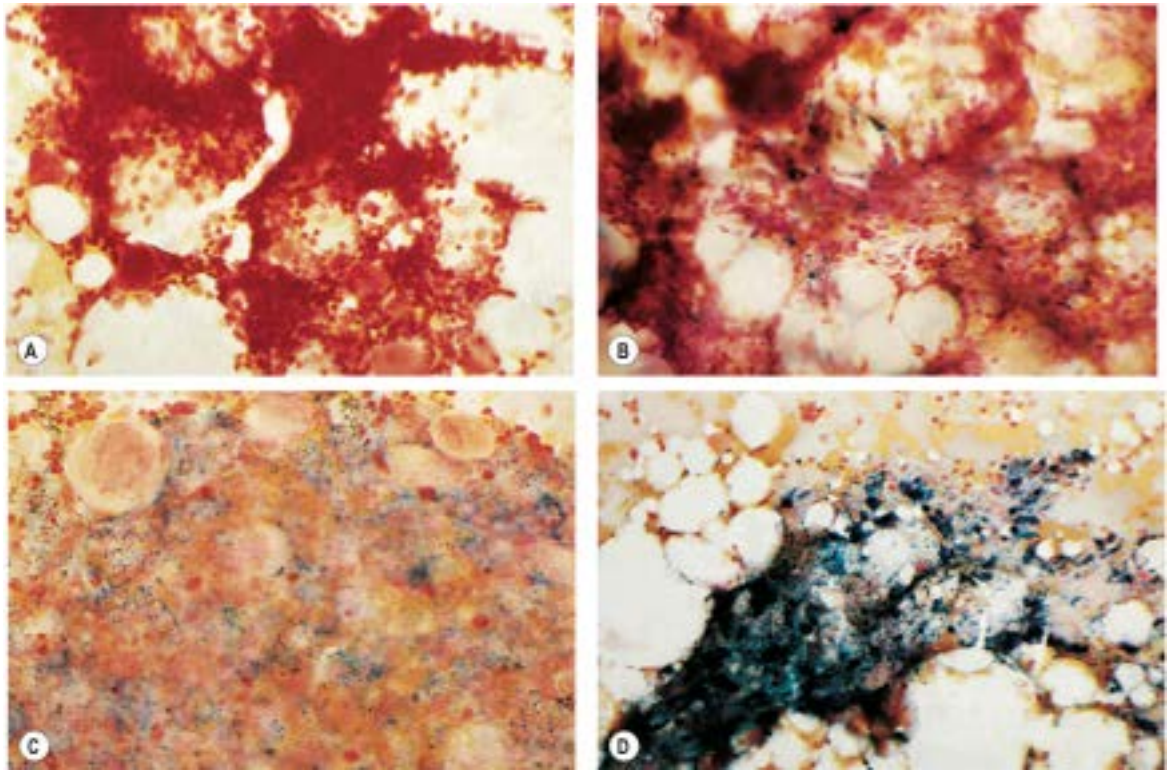
The Perls' reaction occurs in THREE steps, with sequential treatment of marrow aspirate sections:

1) 5% hydrochloric acid solution

2) 5% ferrocyanide

3) counterstain, nuclear fast red or Safranin-O

- Any ferric ion (+3) in the tissue combines with the ferrocyanide and results in the formation of a bright blue pigment called ferric ferrocyanide (Prussian blue).

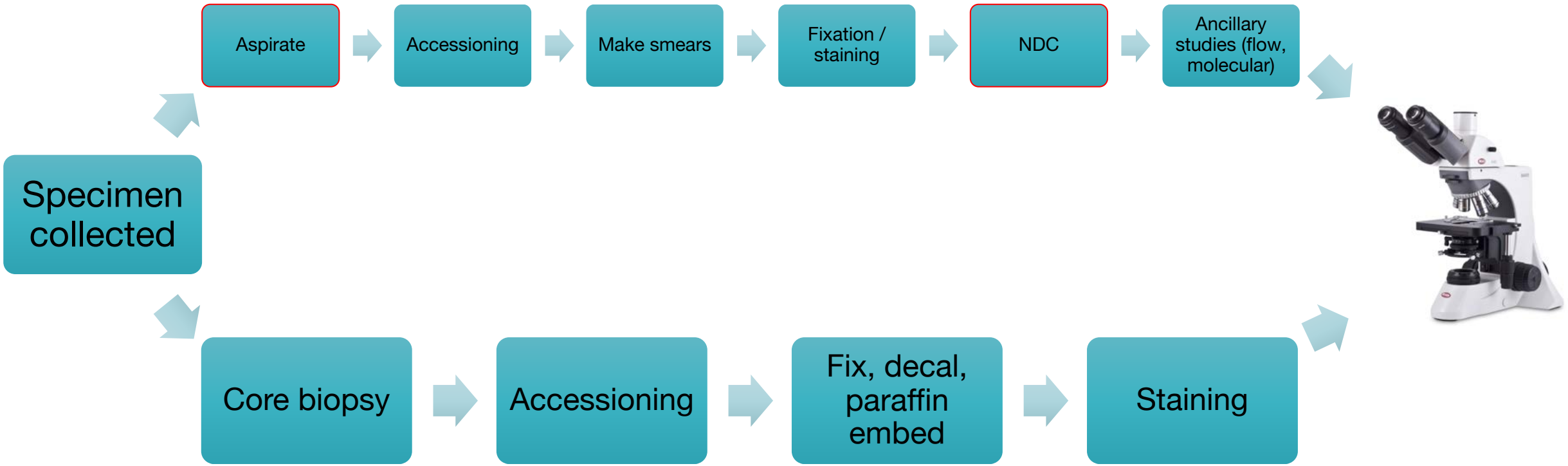


Bone marrow study: other special stains to know

Basic rule: if it's on your lab's hematology staining menu, then you should know HOW it works and at least ONE application…

- Periodic acid schiff (PAS)
- Gomori Methenamine-Silver (GMS)
- Gram
- Ziehl–Neelsen stain
- Reticulin
- Trichrome

Process flow for bone marrow specimen



Q: How many cells does ICSH recommend counting on a marrow NDC?

The nucleated differential count (NDC)

- ICSH recommends a NDC on every marrow specimen
- Critical information about quantity and quality of hematopoiesis
- At least **500 cells** counts over 2 or more smears of NDC essential to diagnosis
- At least **300 cells** counted if NDC is not essential to diagnosis

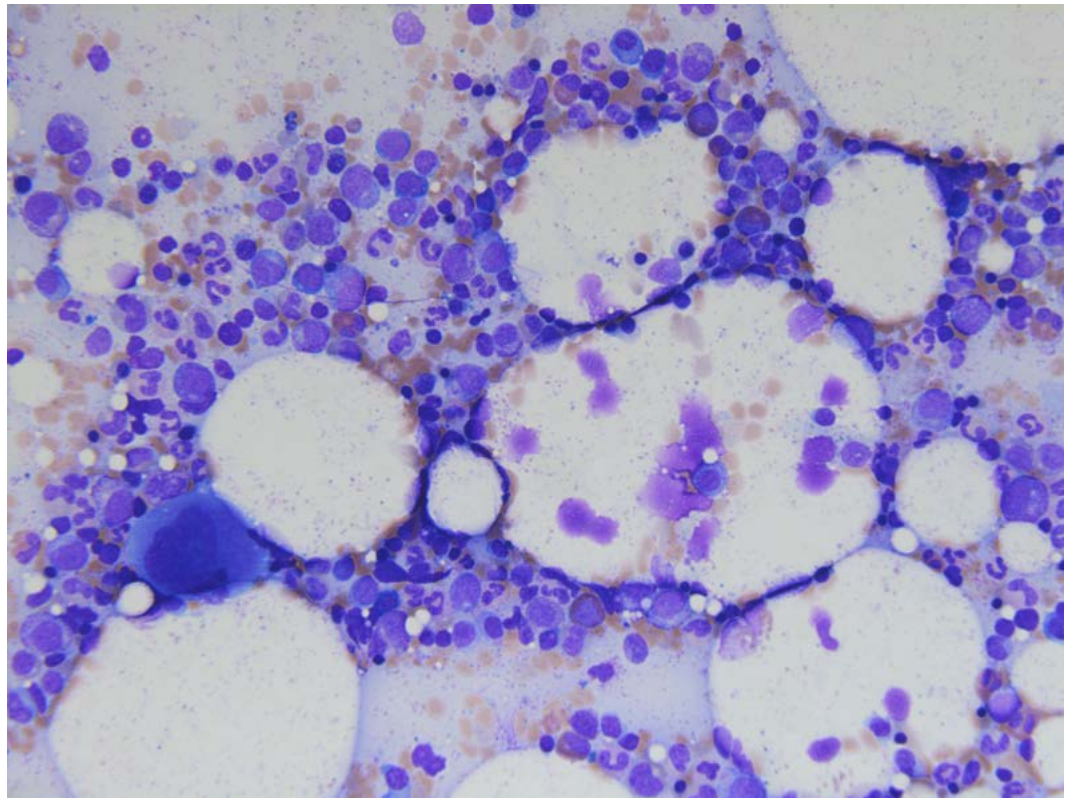
The nucleated differential count (NDC)

Why perform a NDC on every marrow?

1) To assess hematopoietic activity

2) To compare the proportions of the different lineages (M:E ratio)

3) To quantify abnormal cells



Q: What are the components of a NDC?

Included:

Granulopoiesis (mature and precursors)

Erythropoiesis (only precursors)

Monocytes

Lymphocytes

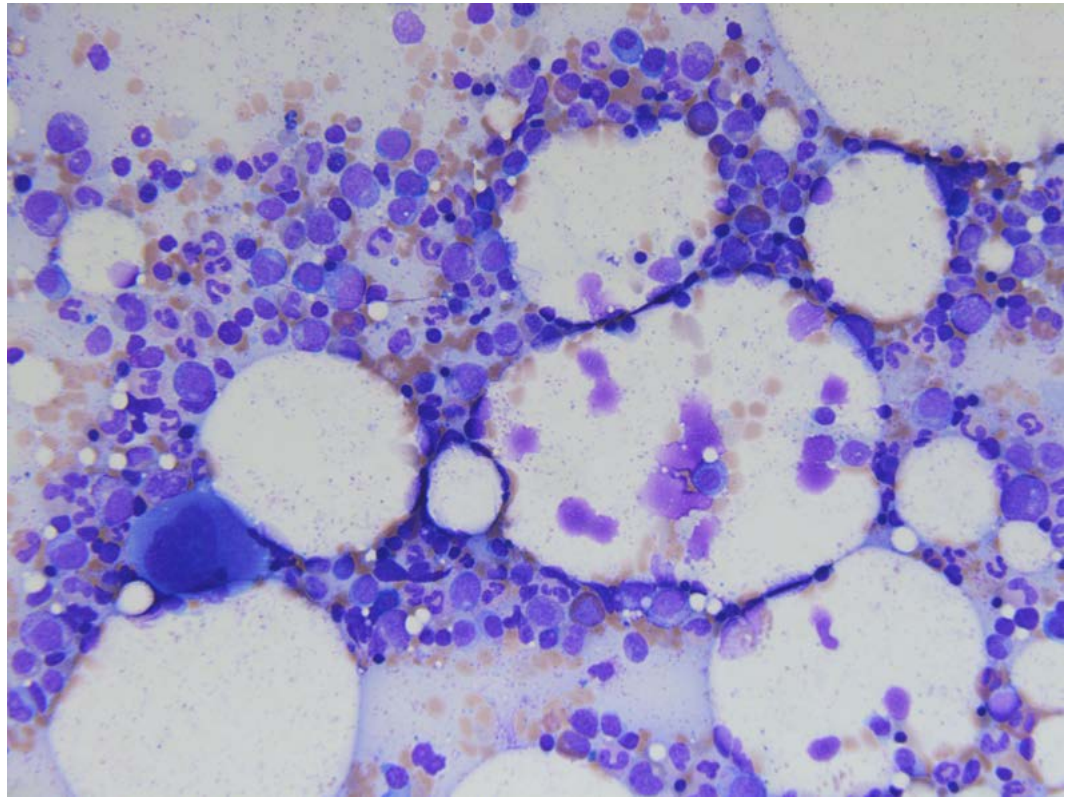
Plasma cells

Mast cells

“Other cells” : may have this category for blasts / blast equivalents or abnormal lymphocytes in some cases

Excluded:

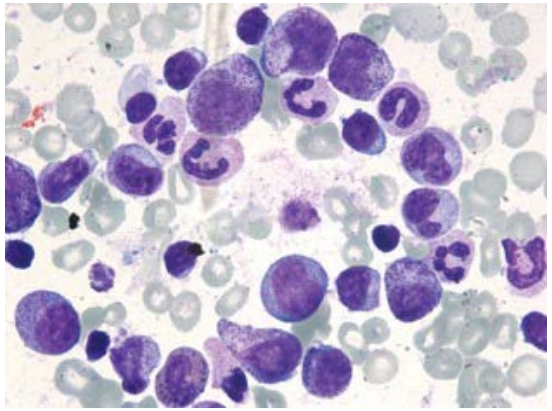
Megakaryocytes, osteoblasts, osteoclasts, non-hematopoietic cells excluded



The nucleated differential count (NDC)

- Performed in the cellular trails of the BM smear behind the particle
- Look for areas that are well dispersed with good cytological detail, and where there is the least number of smudged (lysed) cells
- Should NOT be performed on aparticulate or hemodiluted specimens
- In the presence of particles with absent or very reduced cellularity, only a qualitative description should be provided

Marrow aspirate smear

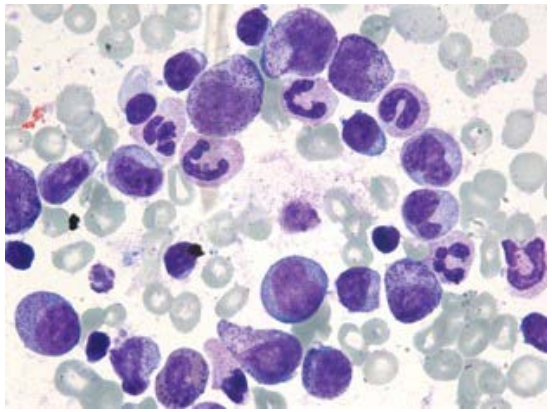


Q: What are the components of the M:E ratio?

Myeloid : Erythroid ratio

- Ratio of all myeloid cells (granulocytes and monocytes and precursors) to all erythroid precursors
- normally **2-4: 1**

Marrow aspirate smear



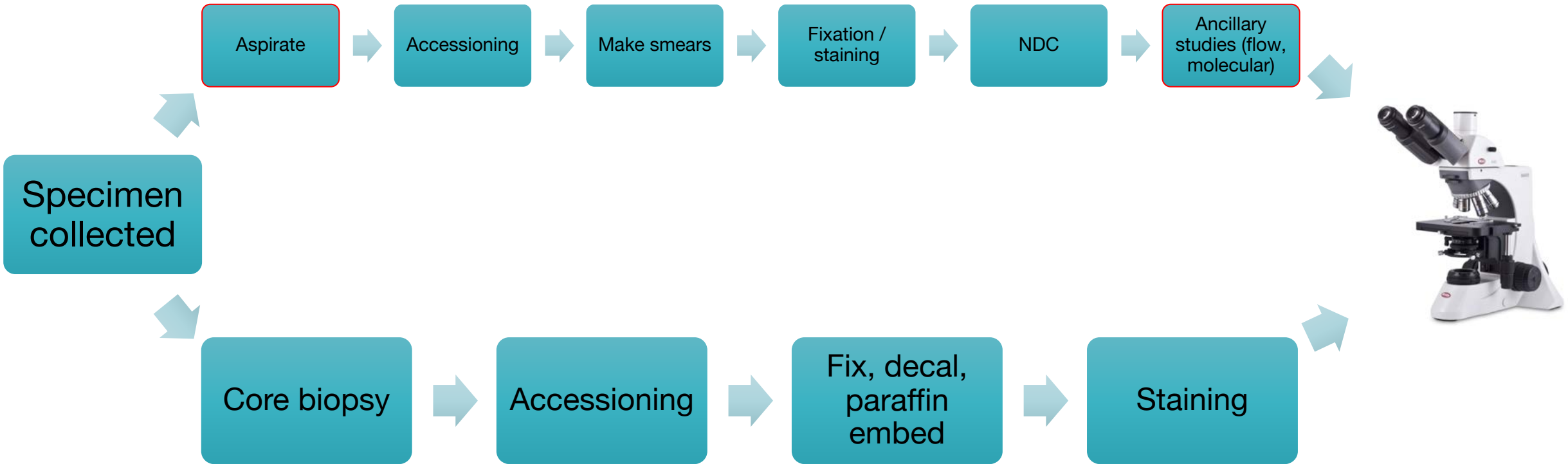
Increased M/E:

- >5:1
- Infection, CML/MPN, erythroid hypoplasia, inflammation,

Decreased M/E:

- <1.2:1
- Decreased leukopoiesis or erythroblast proliferation

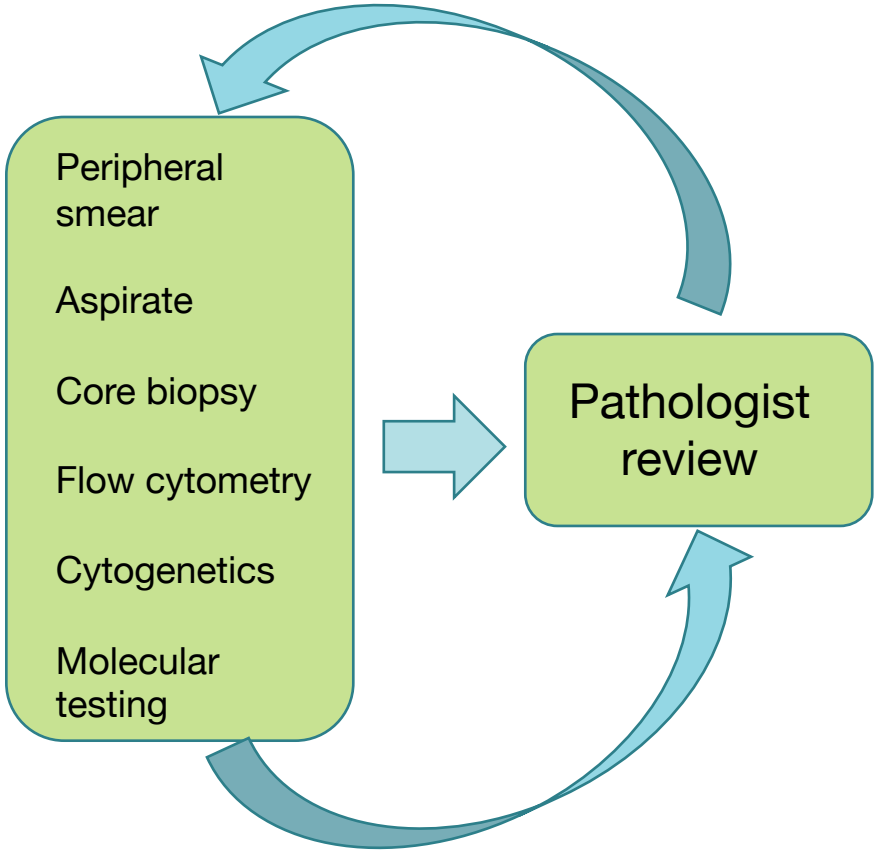
Process flow for bone marrow specimen



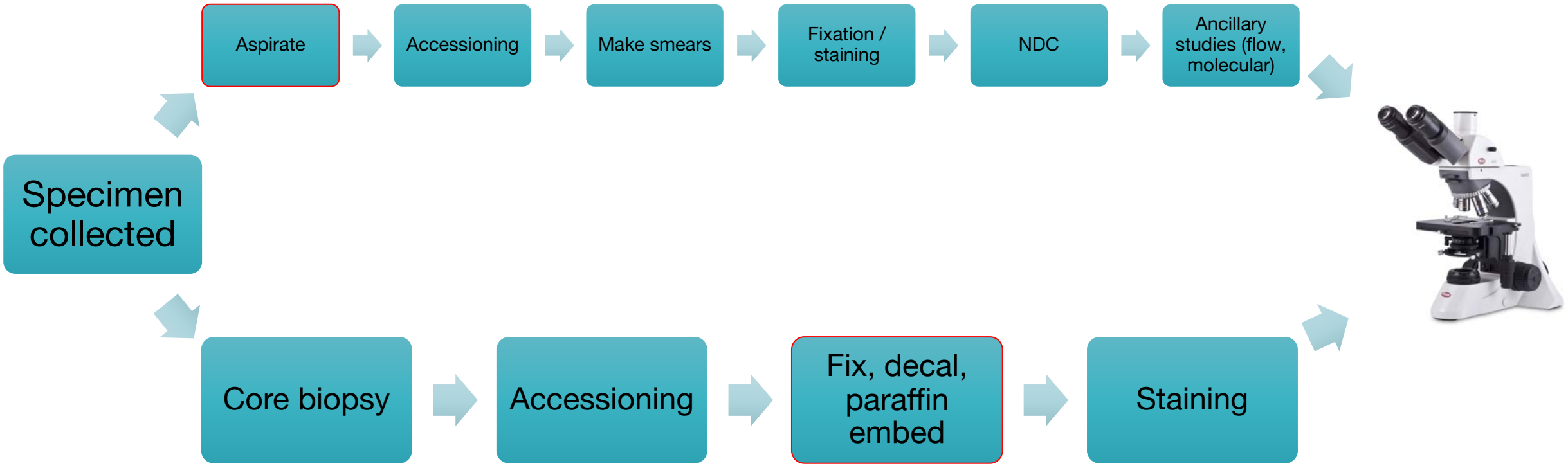
Ancillary studies on marrow aspirate

- Investigations that add additional information beyond morphological findings

- ICSH recommends that following as essential components of any bone marrow examination:
  - Flow cytometry (immunophenotype, maturational stage, lineage assignment)
  - Molecular studies (Clonality, mutations ex. PML-RAR, BCR-ABL1, prognostication)
  - Cytogenetics (Diagnosis, prognostication)

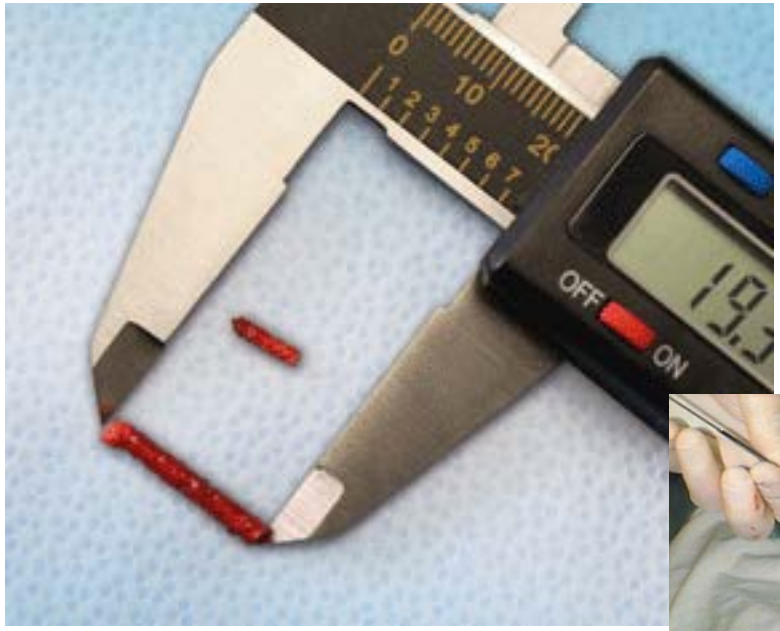


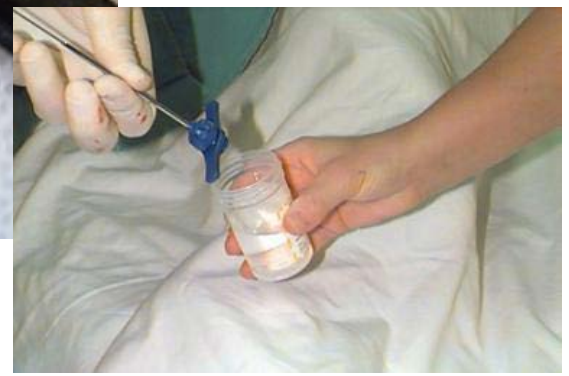
Process flow for bone marrow specimen



The core biopsy specimen: fixation

- Should be at least 2 cm (in reality it rarely is)
- Placed in fixative at the bedside
- Trephine core specimens may be fixed by a number of different methods
- Fixation methods can significantly affect histomorphology, cytological detail and immunoreactivity

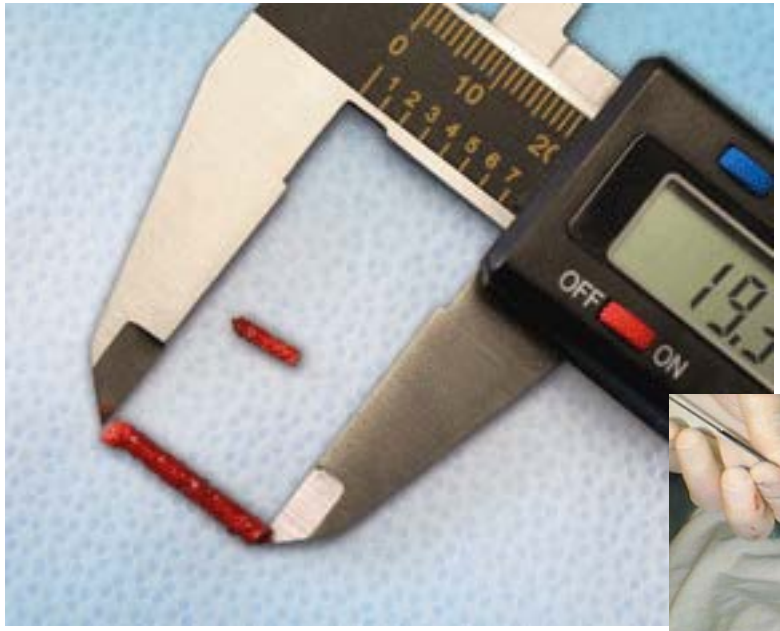


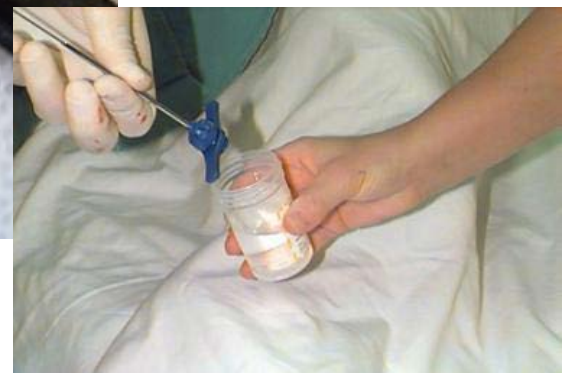


Q: What are three possible bone marrow fixatives?

The core biopsy specimen: fixation

- A standard fixative is neutral buffered formalin (NBF)(10%) for at least 6 h (ICSH recommends overnight)
- Other fixatives commonly used include zinc formaldehyde, AZF (acetic acid-zinc-formalin), IBF (isotonic buffered formalin).
- Fixation time varies depending on the fixative used, from a minimum of 1 h to maximum of >24 h
- Fixative type can affect turnaround time (TAT), morphology, IHC etc.





Know the main fixatives used

Fixative	Advantages	Disadvantages
Neutral buffered formalin (10%) (NBF)	Optimal for staining and nucleic acid recovery	Slower TAT
Acid zinc formalin (AZF)	Suboptimal for staining and nucleic acid recovery	Rapid TAT (~2 h possible)

The core biopsy specimen: decalcification

- Many different decalcification methods are available
- Commonly used solutions are EDTA, formic acid, or commercial decalcifying agents
- Decalcification time varies from 15 min to 72 h, depending both on the type of decalcifying agent as well as on the size of the biopsy specimen

Q: What are at least two decalcification reagents used in bone marrow processing?

The core biopsy specimen: decalcification

Decalcification:

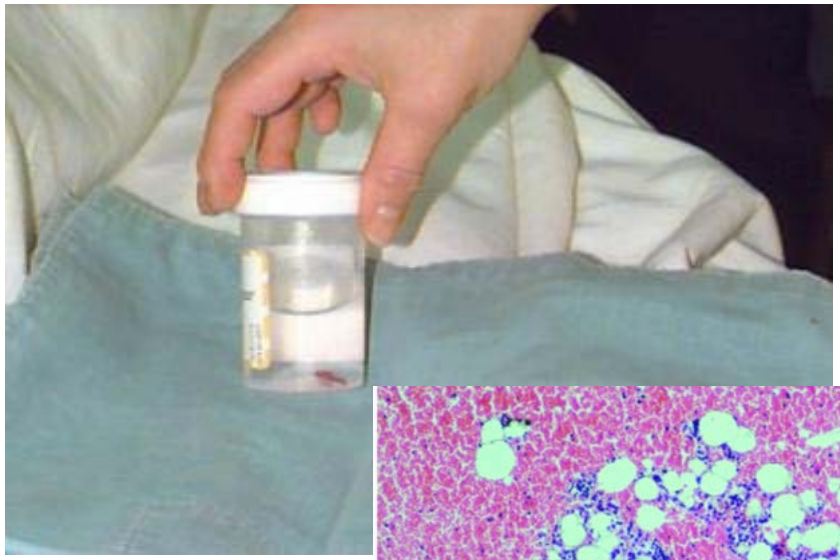
- Chelates storage iron
- Affects histomorphology and cytological detail
- Affects the ability to perform immunohistochemistry
- Affects ability and to retrieve material suitable for molecular analysis and iron staining

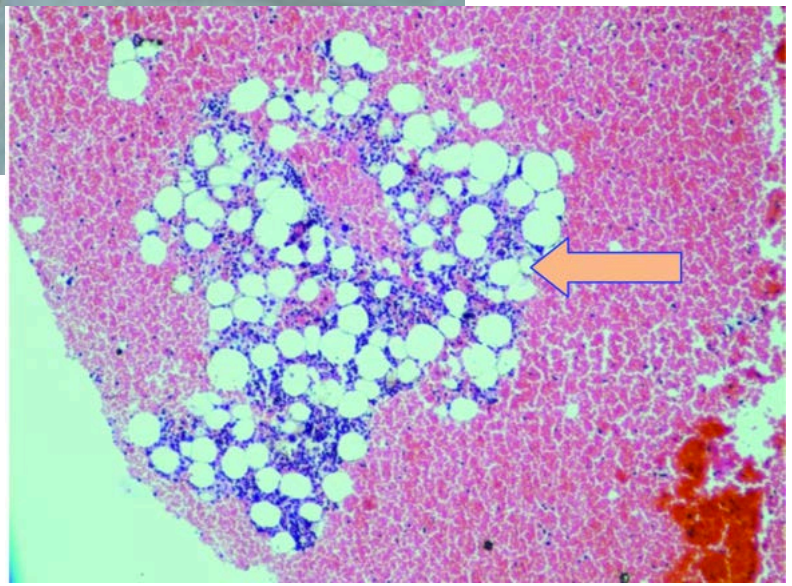
- Decalcification with EDTA is recommended by ICSH as it results in better preservation of nucleic acids, but is slower than other methods (~24 h versus 6 h for formic acid)

Bone marrow clot section

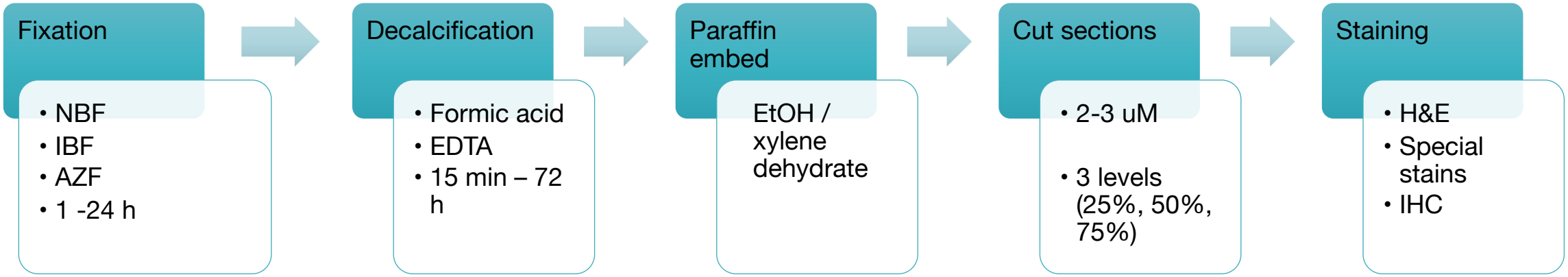
Why do a clot section?

- Direct fixation at bedside
- Can be made by allowing aspirate to clot in the syringe or “watchglass method”
- Some methods for making a ”clot” from an EDTA specimen
- Less blood = better fixative penetration
- Valuable information if biopsy is subcortical (only bone) or if no biopsy
- Can stain for iron because it is not decalcified
- Better recovery of nucleic acids for molecular studies (FFPE)

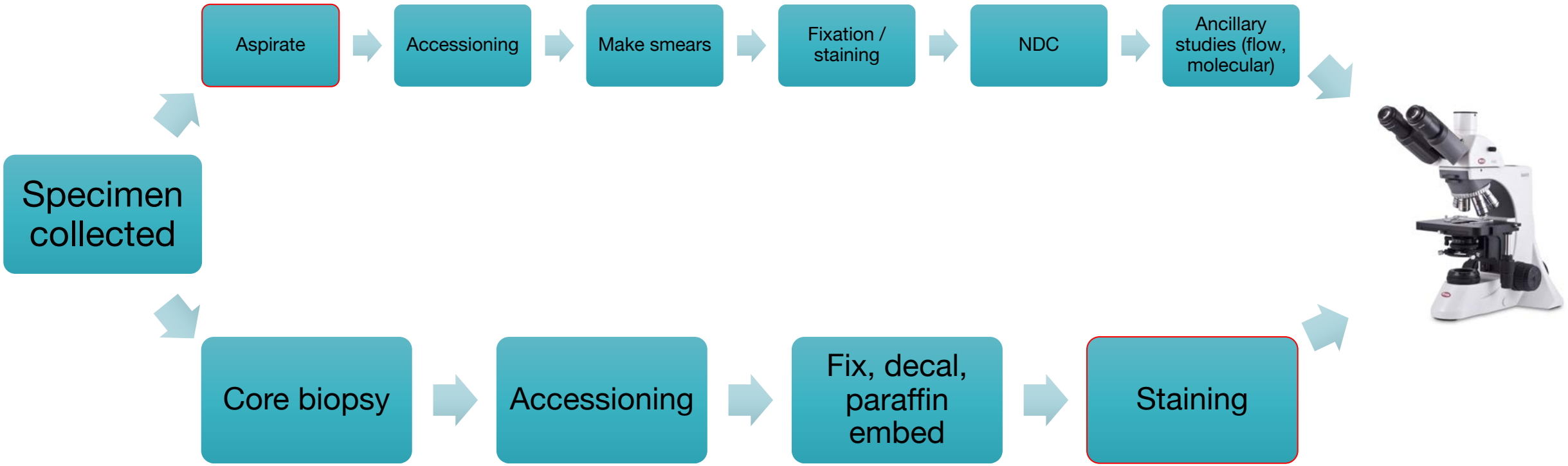




Bone marrow core biopsy: process flowchart

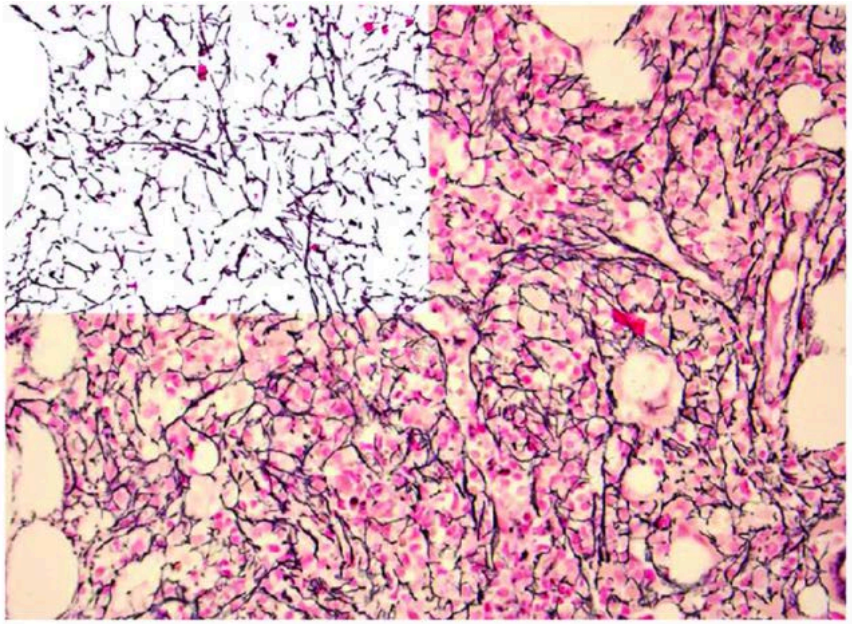


Process flow for bone marrow specimen



The core biopsy specimen: staining

- All cases should have at least three levels staining with H&E
- Optimal length of core biopsy at least 2 cm
- Special stains and immunohistochemistry requested by pathologist's review



The core biopsy specimen: immunohistochemistry

In hematopathology immunohistochemistry (IHC) is mainly important for:

- Detection of disease (lymphoma)
- Lineage assignment (leukemia and lymphoma) +/- flow cytometry
- Maturational stage
- Clonality (plasma cells)
- Not used for prognostication (BM)

- Immunohistochemistry is not usually standardized across labs
- ICSH guidelines for standardization (2015)

REVIEW INTERNATIONAL JOURNAL OF LABORATORY HEMATOLOGY

ICSH guidelines for the standardization of bone marrow immunohistochemistry

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SUMMARY

Bone marrow (BM) tissue biopsy evaluation, including trephine biopsy and clot section, is an integral part of BM investigation and is often followed by ancillary studies, in particular immunohistochemistry (IHC). IHC provides *in situ* coupling of morphological assessment and immunophenotype. The number of different IHC tests that can be applied to BM trephine biopsies and the number of indications for IHC testing is increasing concurrently with the development of flow cytometry and molecular diagnostic methods. An international Working Party for the Standardization of Bone Marrow IHC was formed by the International Council for Standardization in Hematology (ICSH) to prepare a set of guidelines for the standardization of BM IHC based on currently available published evidence and modern understanding of quality assurance principles as applied to IHC in general. The guidelines were discussed at the ICSH General Assemblies and reviewed by an international panel of experts to achieve further consensus and represent further development of the previously published ICSH guidelines for the standardization of BM specimens handling and reports.

IHC test classification

CAP (Canadian Association of Pathologists) guidelines (2015):

Class I IHC tests:

- IHC test results are both interpreted and used by pathologists
- Qualitative with descriptive performance characteristics (ex., positive or negative)

Class II IHC tests:

- IHC test results are interpreted by pathologists; however, these tests have either prognostic or predictive nature and their results are used by treating physician for clinical decision making (used by clinicians).
- Very few, if any in everyday hematopathology practice

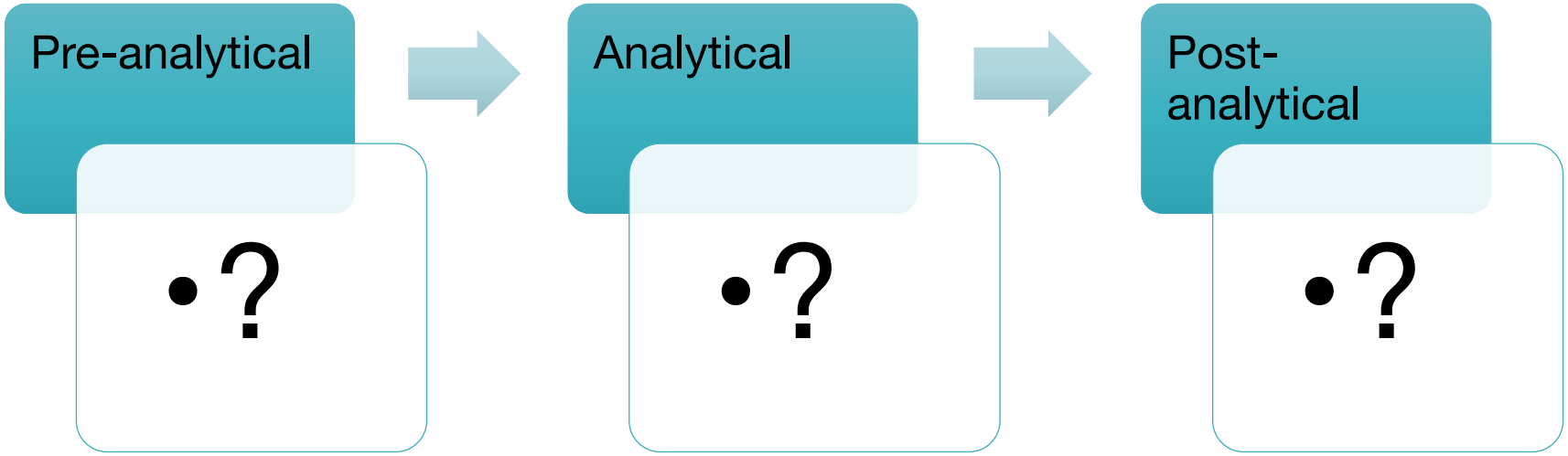
Immunohistochemistry

Why standardize IHC?

- IHC testing may provide critical information important for diagnosis, prognosis and therapy

- Required for building EQA/PT programs (currently not widespread in bone marrow IHC)

Bone marrow IHC standardization



Q: Define the pre-analytical phase for bone marrow IHC. What are the components of this phase?

Bone marrow IHC standardization

Pre-analytical

- ischemic time
- fixative and fixation time
- type of decalcifying reagent and time of decalcification
- clot sample preparation
- embedding media

Define the pre-analytical phase for bone marrow IHC?

- The pre-analytical phase starts at the time of procurement of the BM trephine and aspirate samples and ends by cutting the paraffin-embedded (or plastic-embedded) tissue onto glass slides

Bone marrow IHC standardization

Analytical

- antibody clone
- antigen retrieval
- colour visualization
- counterstain
- validation
- controls
- SOPs
- QC and EQA

Analytical phase IHC recommendations (ICSH):

1) Stain validation

2) Monitor performance characteristics using appropriate controls for each IHC test

3) Separate SOP for each IHC test

Bone marrow IHC standardization

Analytical

- antibody clone
- antigen retrieval
- colour visualization
- counterstain
- validation
- controls
- SOPs
- QC and EQA

Table 2. SOPs components required to be specified for each bone marrow immunohistochemistry tests

SOP component	Descriptors
Primary antibody (Ab) type	Monoclonal *vs*. polyclonal, clone or lot name/number, source, concentrated *vs*. prediluted, and dilution (if concentrated Ab), incubation time, temperature
Antigen retrieval method	Type, pH, concentration (for enzyme-based methods), temperature, time, and source
Detection system	Type, name, temperature, time, source
Amplification	Type, temperature, time, source
Chromogen	Type, time
Enhancement	Type, time
Automated stainer platform	Name, source

Thinking questions…

Q: What are the components of a method or procedure validation?

Q: What are performance characteristics?

Q: How would you validate a new IHC stain for bone marrow?

Q: What is a common cause of “background” or non-specific binding in bone marrow IHC, and what can be done to mitigate this effect?

Bone marrow IHC standardization

Post-analytical

- Ancillary studies
- Integrated core and aspirate report
- Synoptic reporting

Postanalytical standards pertain to the interpretation and reporting of the IHC results by the hematopathologist.

1) Integrated aspirate and biopsy reported by same person
2) Sample must be adequate
3) Proper internal and external controls (positive and negative)
4) Synoptic reporting

Controls in bone marrow IHC

- Controls should be treated the same way as your specimen…
- External controls should ideally have two levels of staining intensity (weak and strong)
- External controls should ideally be mounted on the same slide as the specimen
- Negative controls if there are internal pigments, necrosis, fibrosis etc. that could interfere with interpretation or when only one one IHC test has been ordered

Bone marrow reporting

- Integrated report for bone marrow aspirate and biopsy review is recommended by ICSH
- It is recommended that the aspirate and core biopsy be read by the same individual
- A final integrated consensus conclusion that includes all test results (e.g. flow cytometry, cytogenetics and molecular genetics) if possible

Bone marrow reporting

Table 3. The bone marrow aspirate report

Name of institution
Unique specimen identifier (laboratory accession number)
Details of patient: surname, first name(s), identification number, age or date of birth, gender, contact details (e.g. address, hospital location)
Name of responsible physician
Name of requesting doctor
Date of procedure
Significant clinical history including physical findings, recent chemo/radiotherapy, cytokine therapy and pertinent lab results.
Indication for bone marrow examination
Procedure performed (aspirate/trephine biopsy)
Anatomic site of aspirate/biopsy
Ease/difficulty of aspiration
Blood count: Haemoglobin concentration, total and differential white cell count (neutrophils, eosinophils, basophils, monocytes, lymphocytes) and platelet count
Blood smear description and diagnostic conclusion
Cellularity of particles and cell trails
Nucleated differential cell count
Total number of cells counted
Myeloid:erythroid ratio
Erythropoiesis
Myelopoiesis
Megakaryocytes
Lymphocytes
Plasma cells
Other haemopoietic cells
Abnormal cells (e.g. blast cells, metastatic infiltrates)
Iron stain
Cytochemistry
Other investigations (e.g. cytogenetics, PCR, FISH, microbiology)
Summary of flow cytometry findings, if available
Conclusion
WHO classification (if relevant)
Disease code
Signature and date of report

Table 4. The bone marrow trephine report

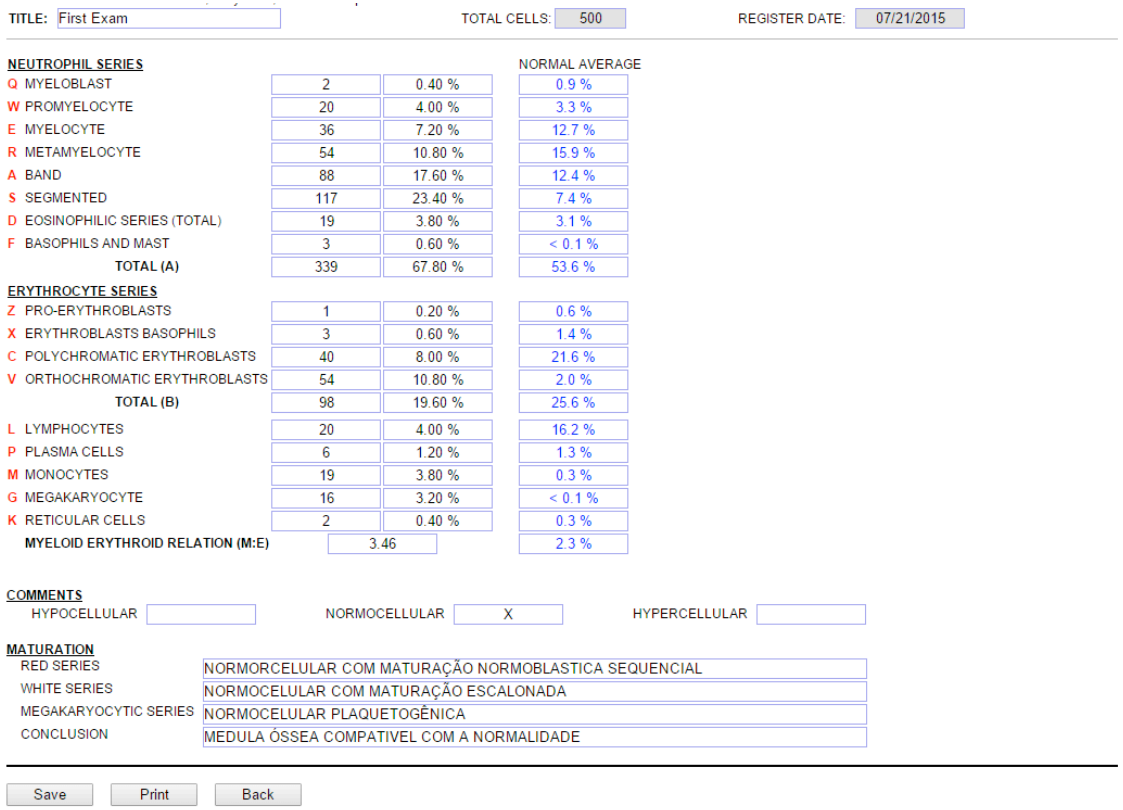
Name of institution
Unique specimen identifier (laboratory accession number)
Details of patient: surname, first name(s), identification number, age or date of birth, gender, contact details (e.g. address, hospital location)
Name of responsible physician
Name of requesting doctor
Date of procedure
Significant clinical history including physical findings, recent chemo/radiotherapy, cytokine therapy and pertinent lab results
Indication for bone marrow examination
Procedure performed (aspirate/trephine biopsy)
Anatomic site of aspirate/trephine biopsy
Aggregate length of biopsy core
Adequacy and macroscopic appearance of core
Percentage and pattern of cellularity
Bone architecture
Location, number, morphology and pattern of differentiation for erythroid, myeloid, megakaryocytic lineages, lymphoid cells, plasma cells and macrophages
Abnormal cells and/or infiltrates
Reticulin stain
Immunohistochemistry
Histochemistry
Other investigations (e.g. FISH, PCR)
Conclusion
Disease code
Signature and date of report

Synoptic reporting

As currently defined by the CAP, synoptic reporting includes the following elements:

- Reporting of scientifically validated data elements that influence clinical outcome and therapeutic decisions
- Display of each data element in a “variable: result” format on a single line
- Display of data elements on separate lines

- Format ensures that critical information is transmitted consistently and succinctly in every report
- Succinct data presentation is free of clutter and irrelevant information
- Supports data mining (ex., machine learning)



TITLE: First Exam TOTAL CELLS: 500 REGISTER DATE: 07/21/2015

NEUTROPHIL SERIES			NORMAL AVERAGE
Q MYELOBLAST	2	0.40 %	0.9 %
W PROMYELOCYTE	20	4.00 %	3.3 %
E MYELOCYTE	36	7.20 %	12.7 %
R METAMYELOCYTE	54	10.80 %	15.9 %
A BAND	88	17.60 %	12.4 %
S SEGMENTED	117	23.40 %	7.4 %
D EOSINOPHILIC SERIES (TOTAL)	19	3.80 %	3.1 %
F BASOPHILS AND MAST	3	0.60 %	< 0.1 %
TOTAL (A)	339	67.80 %	53.6 %
ERYTHROCYTE SERIES			
Z PRO-ERYTHROBLASTS	1	0.20 %	0.6 %
X ERYTHROBLASTS BASOPHILS	3	0.60 %	1.4 %
C POLYCHROMATIC ERYTHROBLASTS	40	8.00 %	21.6 %
V ORTHOCHROMATIC ERYTHROBLASTS	54	10.80 %	2.0 %
TOTAL (B)	98	19.60 %	25.6 %
L LYMPHOCYTES	20	4.00 %	16.2 %
P PLASMA CELLS	6	1.20 %	1.3 %
M MONOCYTES	19	3.80 %	0.3 %
G MEGAKARYOCYTE	16	3.20 %	< 0.1 %
K RETICULAR CELLS	2	0.40 %	0.3 %
MYELOID ERYTHROID RELATION (M:E)	3.46		2.3 %

COMMENTS

HYPOCELLULAR NORMOCELLULAR X HYPERCELLULAR

MATURATION

RED SERIES	NORMORCELULAR COM MATURAÇÃO NORMOBLASTICA SEQUENCIAL
WHITE SERIES	NORMOCELULAR COM MATURAÇÃO ESCALONADA
MEGAKARYOCYTIC SERIES	NORMOCELULAR PLAQUETOGÊNICA
CONCLUSION	MEDULA ÓSSEA COMPATIVEL COM A NORMALIDADE

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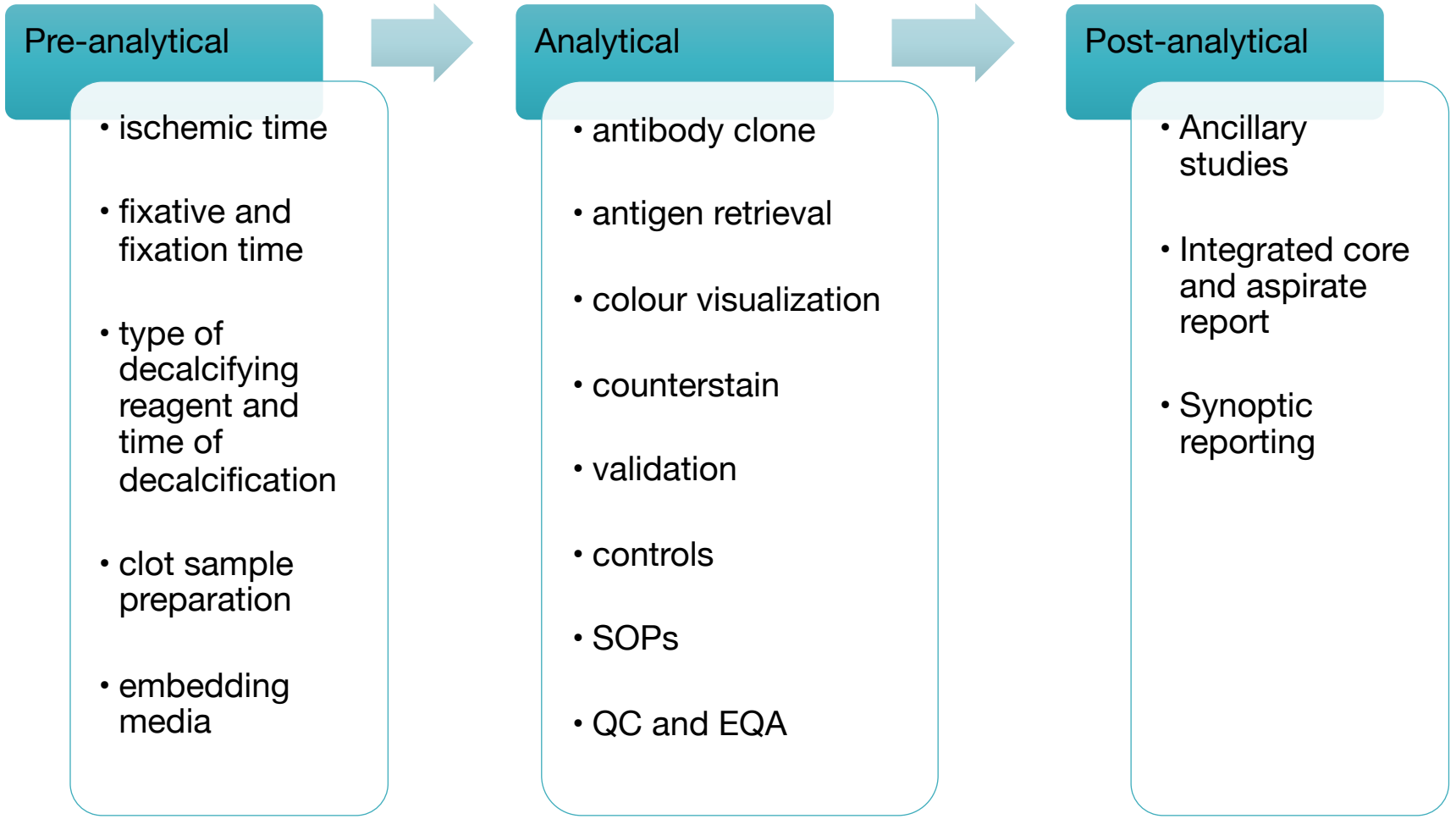
Bone marrow standardization



Q: What is external quality assurance (EQA)?

Quality assurance in BM IHC

- EQA / PT involves comparison of your results to the results of other laboratories
- Is a means of verifying your lab's performance against an external metric, standard or benchmark (part of accreditation)
- It is recommended that laboratories participate in PT for BM IHC only if the EQA program provides samples with identical or nearly identical BM tissue processing

Inside the lab: bone marrow reporting

Turnaround times (TAT)

- Quality metric that is measured and required for lab accreditation
- TAT defined as the time between when at test is ordered or a specimen is submitted to the lab and the time when the results are reported
- It is one of the most clinically apparent/ noticeable signs of laboratory service

TEST ORDER (X) ----------> LAB ----------> RESULT (Y)

TAT = delta Y-X

Inside the lab: bone marrow reporting

Turnaround times (TAT)
ICSH recommendations:

- Reporting TAT for combined aspirate and core biopsy report should be < 5 working days
- < 7 working days if IHC or special stains required
- Varies based on each institution
- Other TAT are time collection, accessioning, processing etc.

TEST ORDER (X) ----------> LAB ----------> RESULT (Y)

TAT = delta Y-X

Inside the lab: bone marrow reporting

Specimen storage and retention:

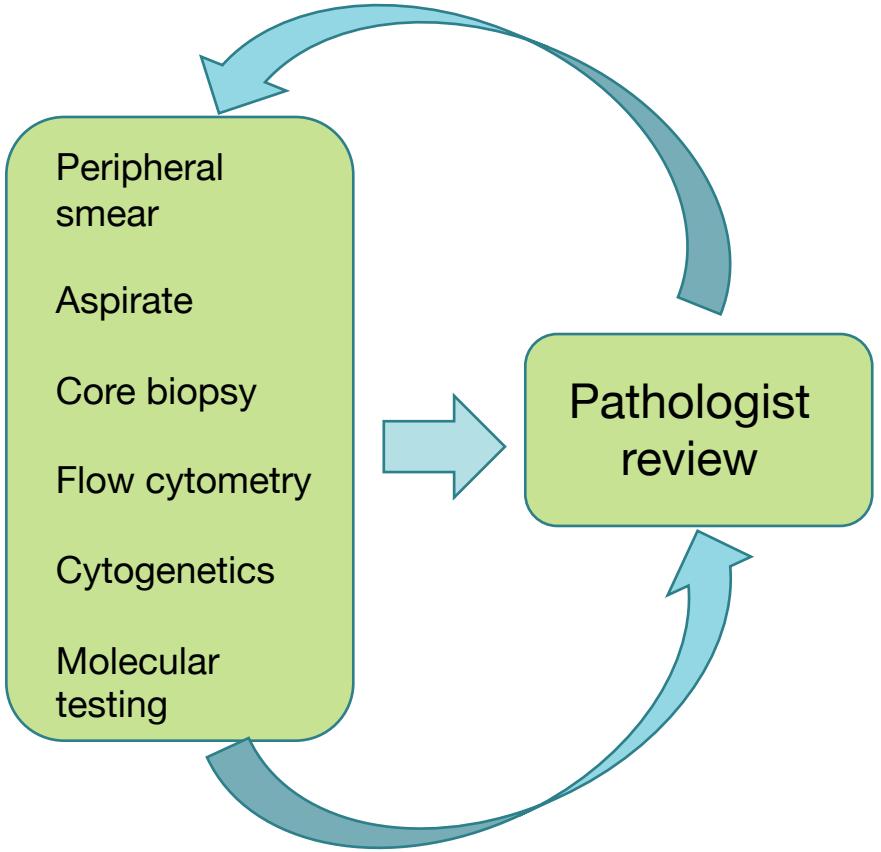
- BM slides should be stored at least 20 years according to most accreditation bodies
- Digital images should be stored indefinitely
- Unstained slides should be stored at - 80°C

Accreditation and EQA:

- Lab should be accredited by recognized body and subscribe to EQA as part of the accreditation

Summary

- In hematopathology, a diagnosis almost always requires integration of ancillary data and clinical information, which are often not immediately available
- Involves a process of continuous assessment and review as information becomes available
- Standardization of methods is essential for a robust and universally applicable interpretation, especially given the large amount of information that needs to be integrated for a final report



For the exam (my advice)

- Spend time with managers and technologists
- Understand every step of the bone marrow process, from bedside to reporting
- Need fine details AND big picture
- Start thinking of yourself as a **laboratory director**: how would you design, validate and troubleshoot a bone marrow program (or any other laboratory process)
- Ask **why** again…and again…and again
- Try to relax, it is not as bad as you think

References

1) LEE, S. H. *et al.* ICSH guidelines for the standardization of bone marrow specimens and reports. *International journal of laboratory hematology* **30,** 349–364 (2008).

2) Torlakovic, E. E. *et al.* ICSH guidelines for the standardization of bone marrow immunohistochemistry. *Int J Lab Hematol* **37,** 431–449 (2015).

3) Protocol for the Examination of Specimens From Patients With Hematopoietic Neoplasms Involving the Bone Marrow (CAP, 2013)