Conventional Cytogenetics in Hematopathology

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Definition

- Cytogenetics is the science that combines the methods and findings of cytology and genetics in the study of abnormal chromosomal structure and numbers in evaluating particular disease states.

Utility of Cytogenetics in Hematopathology

- Used along with morphology, flow cytometry, and immunohistochemistry in the evaluation of particular hematologic and lymphoid neoplasms.
- Many studies over time have established that specific recurring cytogenetic abnormalities occur with a variety of hematologic and lymphoid neoplasms.

General Indications

- NHLs with known recurring cytogenetic abnormalities.
- Acute leukemias for dx and px.
- CML and other CMPDs at dx.
- MDS
- Progression of CML or MDS.
- Lymphoproliferative disorders were specific subtype is difficult to determine.
- CLL for prognosis.

Overall Incidence

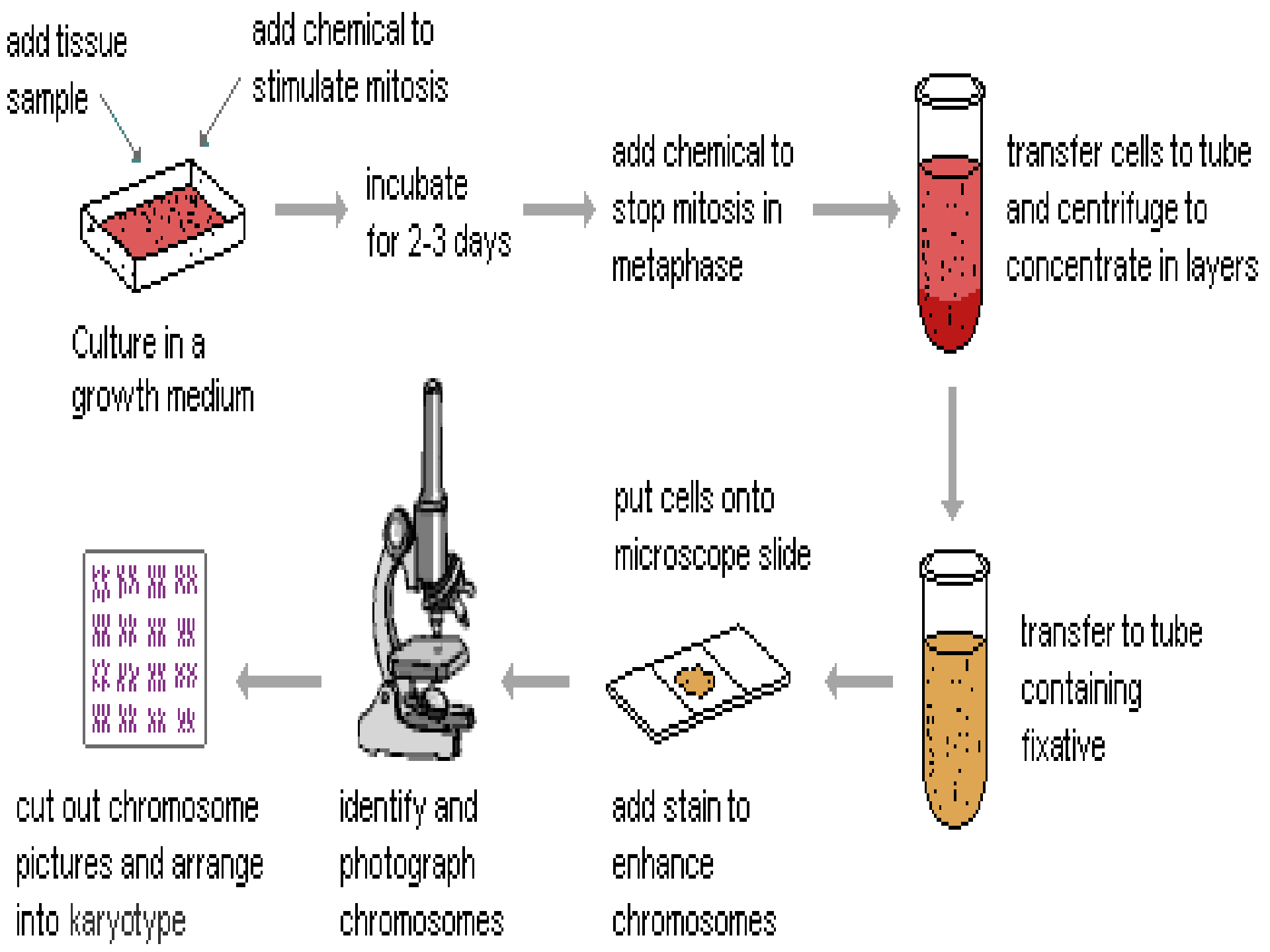
- 50-60% of AML
- 60-85% of ALL
- 30-90% of Myelodysplastic Syndrome(MDS)
- 30% of CMPDs (excluding CML)
- 70% of CLL
- Variable in NHLs

Appropriate Specimens

- Specimens must contain viable cells capable of mitotic activity.
- Bone marrow aspirate, peripheral blood, lymph node, effusion fluid, or other tissue that contains viable cells may be used.
- Dry tap, consider submitting bone marrow core biopsy in sterile transport media for subsequent disaggregation.

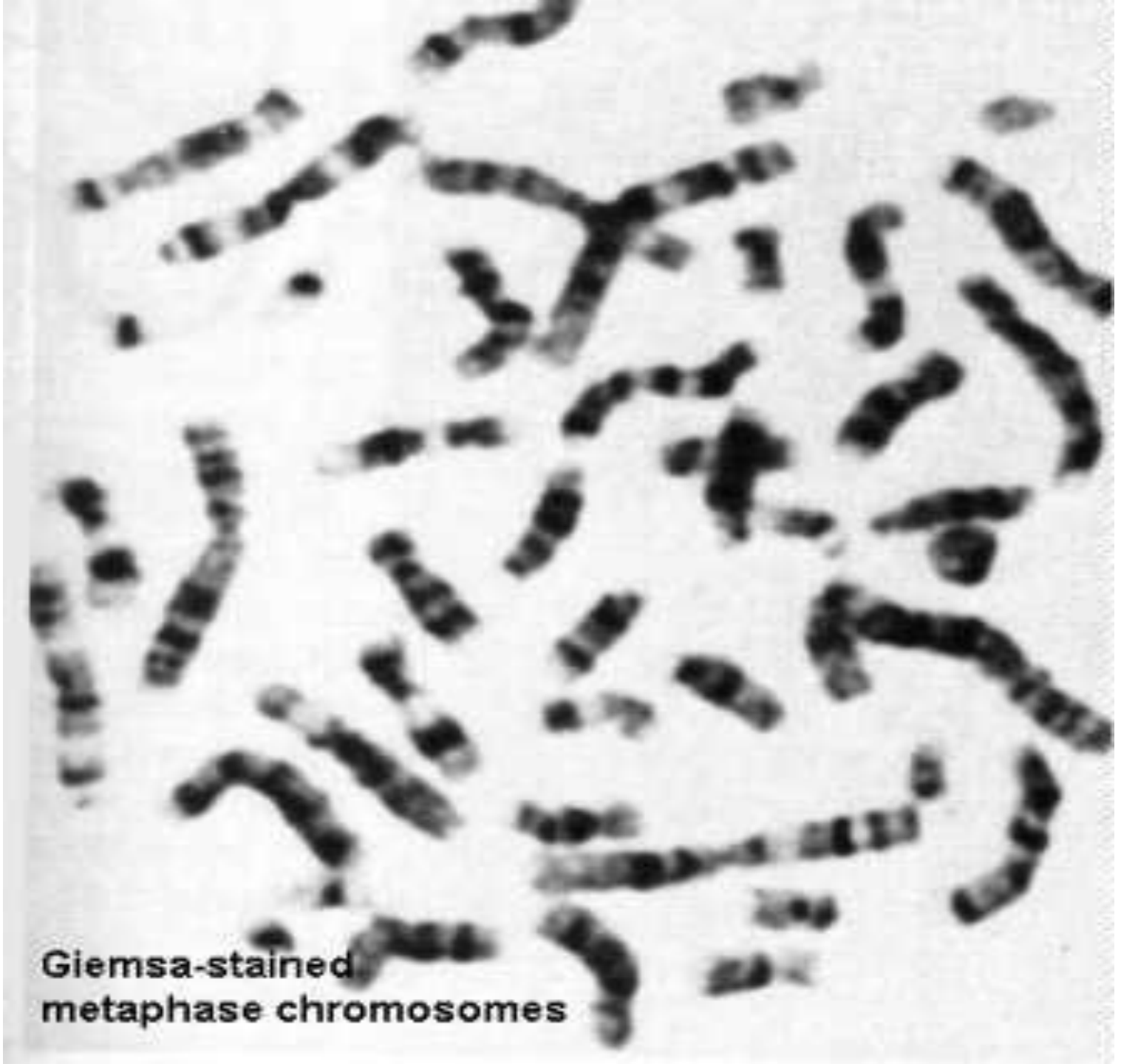
Factors affecting specimen processing and results

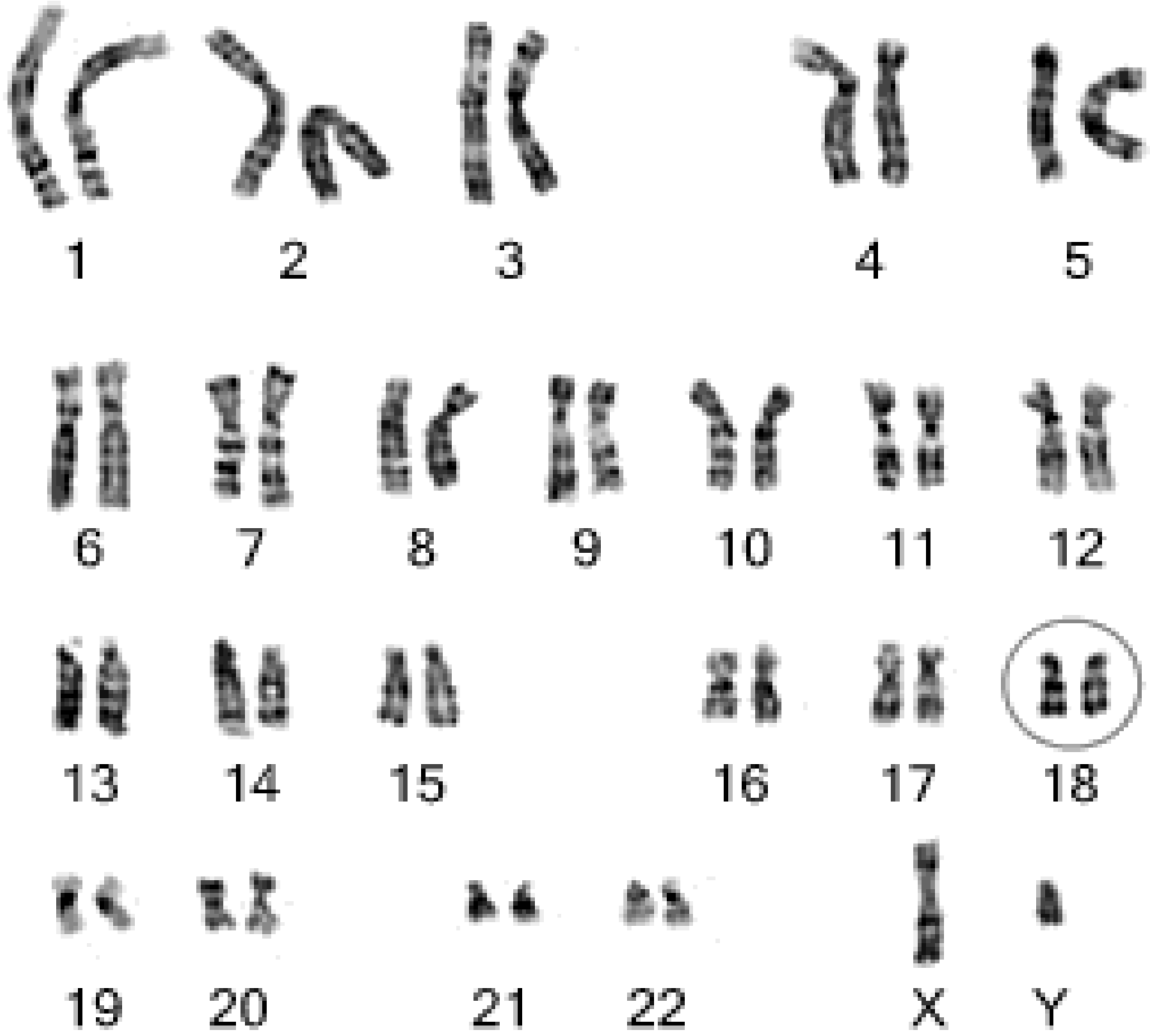
- Time between sampling and processing.
- Transport
- Cellularity of the sample.
- Composition of sample.
- Diagnostic suspicion mature neoplastic cells may require mitogen stimulation.


add tissue sample
add chemical to stimulate mitosis
Culture in a growth medium
incubate for 2-3 days
add chemical to stop mitosis in metaphase
transfer cells to tube and centrifuge to concentrate in layers
transfer to tube containing fixative
put cells onto microscope slide
add stain to enhance chromosomes
identify and photograph chromosomes
cut out chromosome pictures and arrange into karyotype

How it Works

- Cells from a sample are cultured (feeding and maintaining cells).
- Once enough cells are present in culture, a mitotic inhibitor is added to arrest cells at metaphase.
- Arrested cells are harvested and exposed to a hypotonic solution followed by a series of fixative solutions. This causes the cells to expand so the chromosomes will spread out and can be individually examined.
- The chromosome preparations are stained (G banding technique) to detect possible numerical and structural changes.
- The banding pattern can be used along with shape and length of arms to identify genetic translocations, deletions or inversions.


Giemsa-stained
metaphase chromosomes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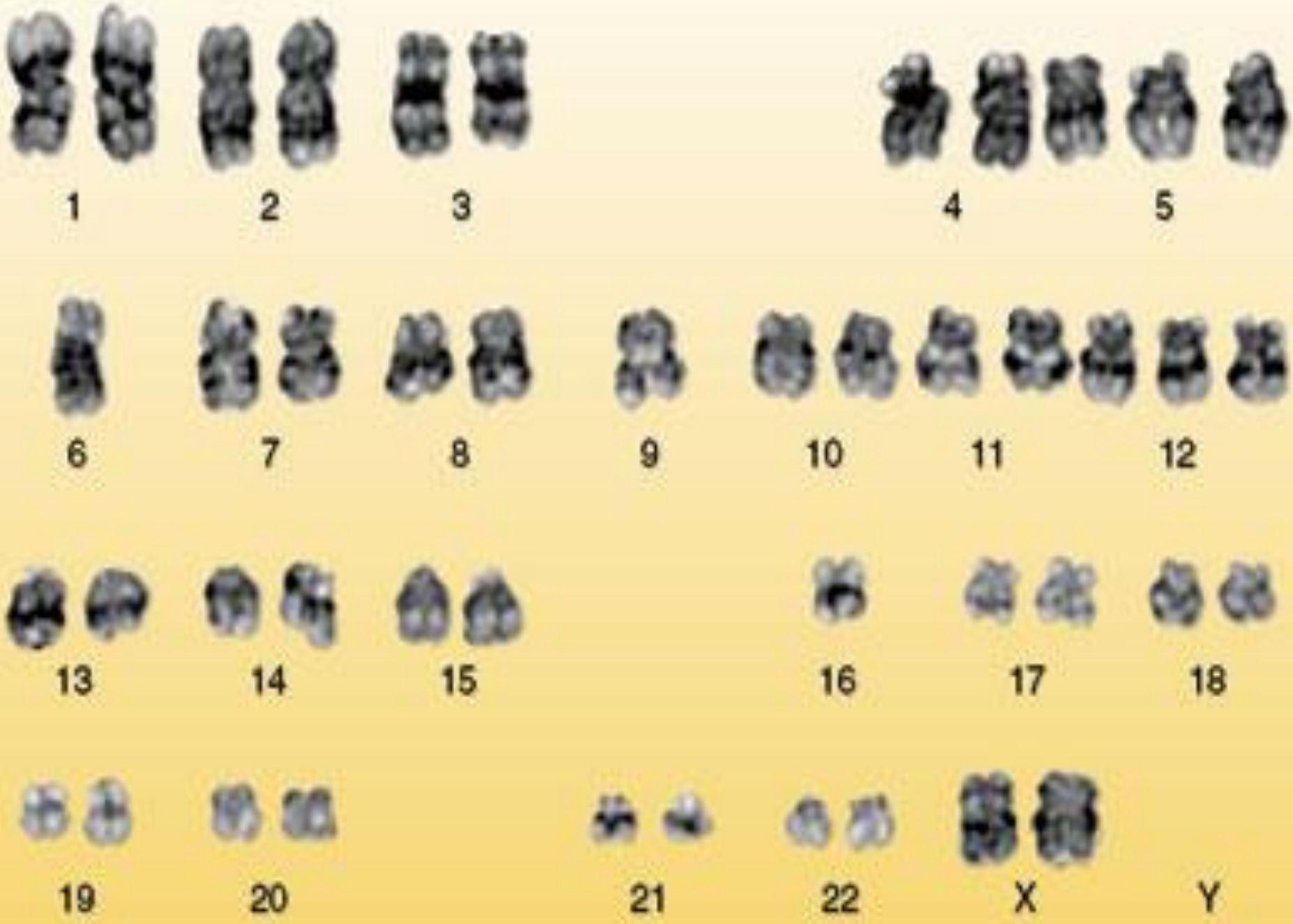
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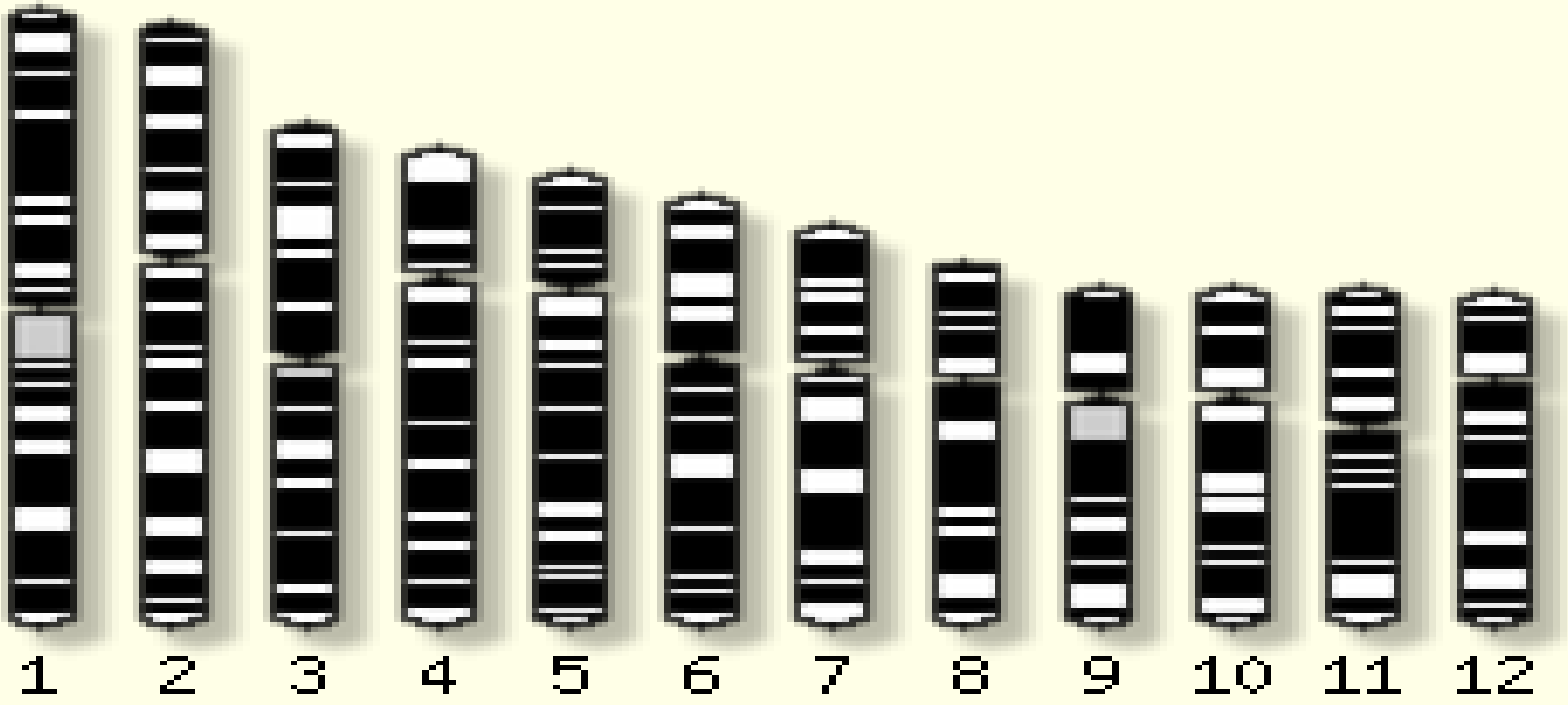
Analysis

- Once stained metaphase chromosome preparations have been obtained they can be examined under the microscope.
- Typically 15-20 cells are scanned and counted with at least 5 cells being fully analyzed.
- During a full analysis each chromosome is critically compared band-for-band with it's homolog. It is necessary to examine this many cells in order to detect clinically significant mosaicism or clonality.
- Following microscopic analysis, either photographic or computerized digital images of the best quality metaphase cells are made.
- Each chromosome can then be arranged in pairs according to size and banding pattern into a karyotype.
- The karyotype allows the cytogeneticist to even more closely examine each chromosome for structural changes. A written description of the karyotype which defines the chromosome analysis is then made.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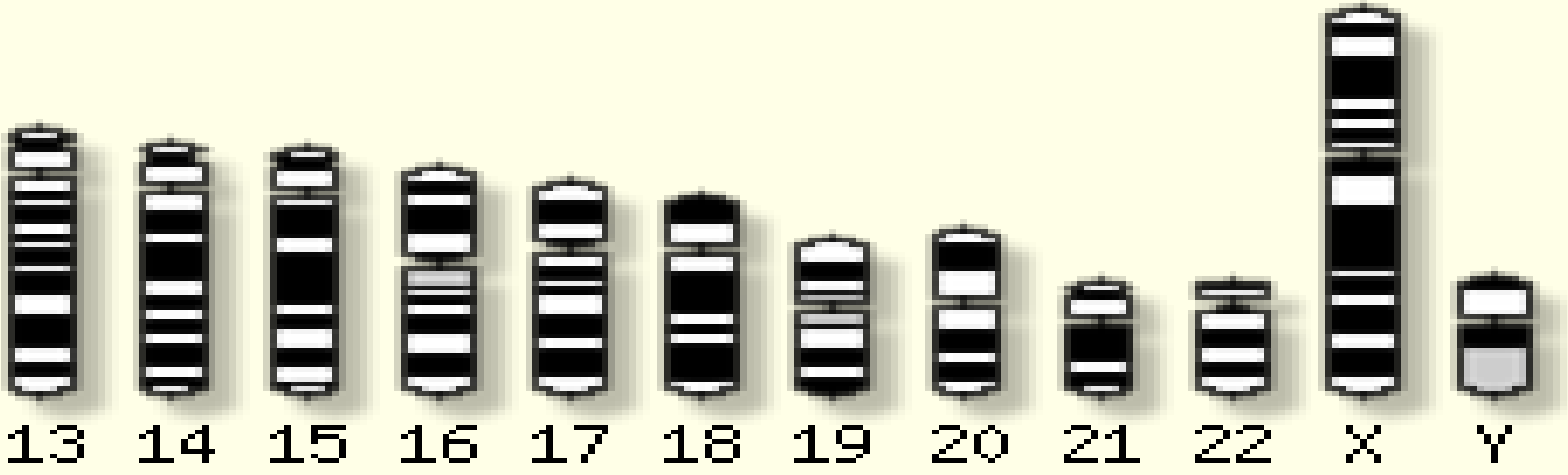
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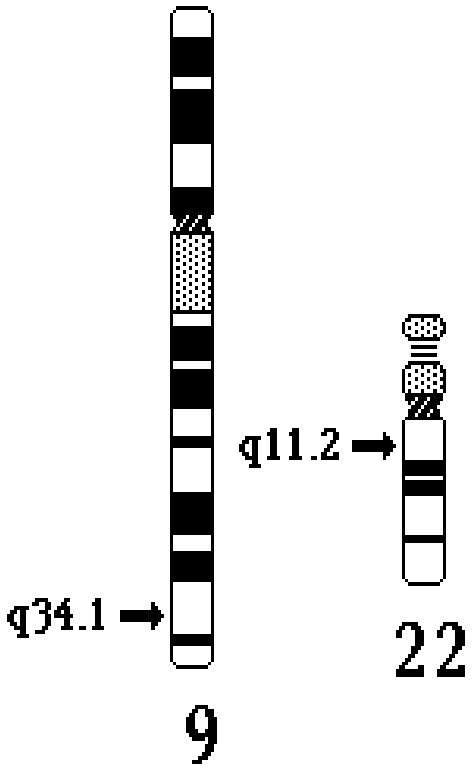
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q11.2
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Limitations of Conventional Cytogenetic Studies

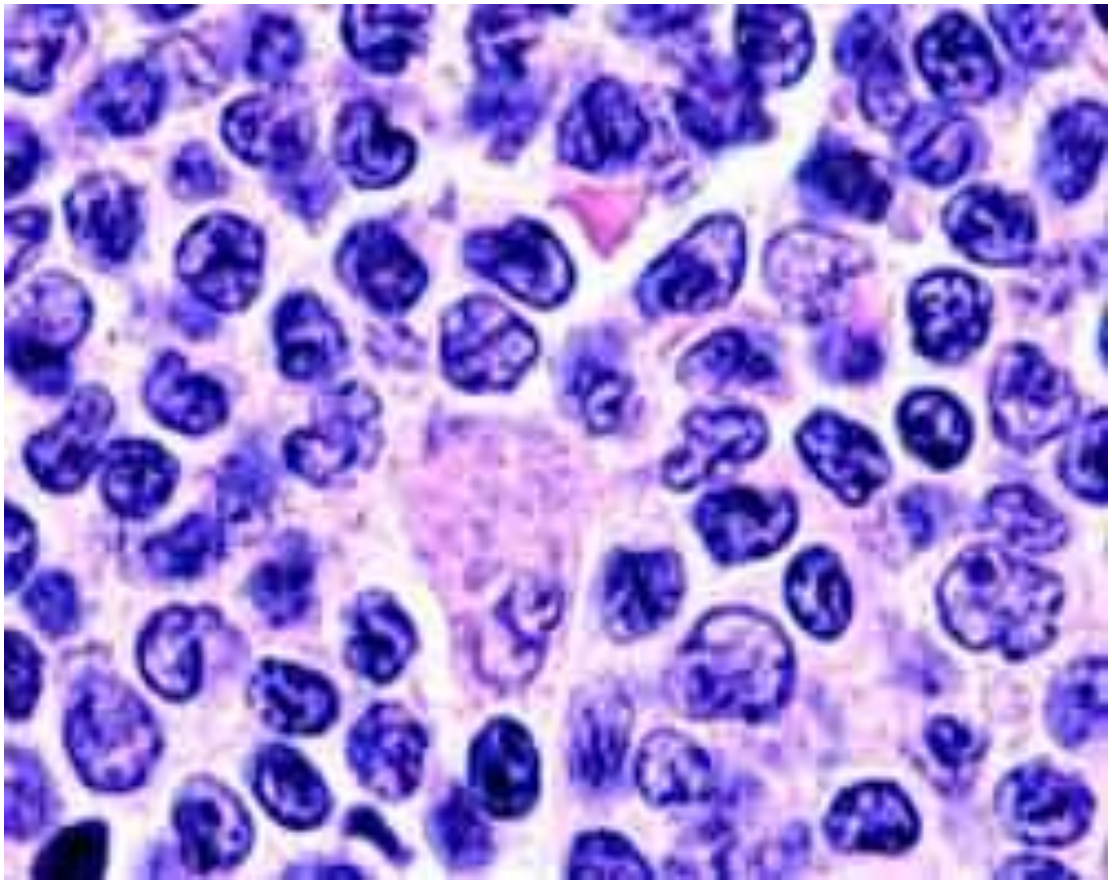
- Low sensitivity compared to FISH and PCR
- At least 2 of 20 metaphases must have same chromosomal anomaly to call clonal.
- Results may be misleading if cells of interest did not proliferate.
- Malignant cells sometimes grow less well in culture than normal cells.
- The cell type for each metaphase is unknown
- Cryptic chromosomal defects are not detected: t(15;17), t(9;22) in 5% of CML, and t(12;21) in B cell ALL.

Common Recurring Translocations of Diagnostic Significance in NHLs

Translocation	Lymphoma	Genes
t(8;14)(q14;132)	Burkitt Lymphoma	C-myc and IgH
t(2;8)(p12;q24)		IgKappa and Cmyc
t(8;22)(q24;q11)		C-myc and IgLambda
t(11;14)(q13;q32)	Mantle cell Lymphoma	Cyclin-1 and IgH
t(14;18)(q32;q21)	Follicular lymphoma	IgH and bcl-2
t(3;14)(q27;q32)	Diffuse large B cell lymphoma	Bcl-6 and IgH
t(11;18)(q21;q21)	Low grade B-cell MALT lymphoma	Api-2 and MALT
t(9;14)	Primary nodal LPL	PAX5 and IgH
t(2;5)(p23;q35)	Anaplastic large cell lymphoma	Alk and NPM

Mechanism

- Immunoglobulin promoter on ch14q is placed under the control of a proto-oncogene: t(11;14), t(14;18), and t(8;14) resulting in over expression of Cyclin D1, Bcl-2, and C-myc, respectively.



Mantle Cell Lymphoma t(11;14)(q13;q32)

FAB Classification of AML(1985)

- M0 – Undifferentiated
- M1 – Myeloblastic without maturation
- M2 – Myeloblastic with maturation
- M3 – Promyelocytic
- M4 – Myelomonocytic
- M4Eo – Myelomonocytic with eosinophilia
- M5 – Monocytic
- M6 – Erythroid
- M7 - Megakaryoblastic

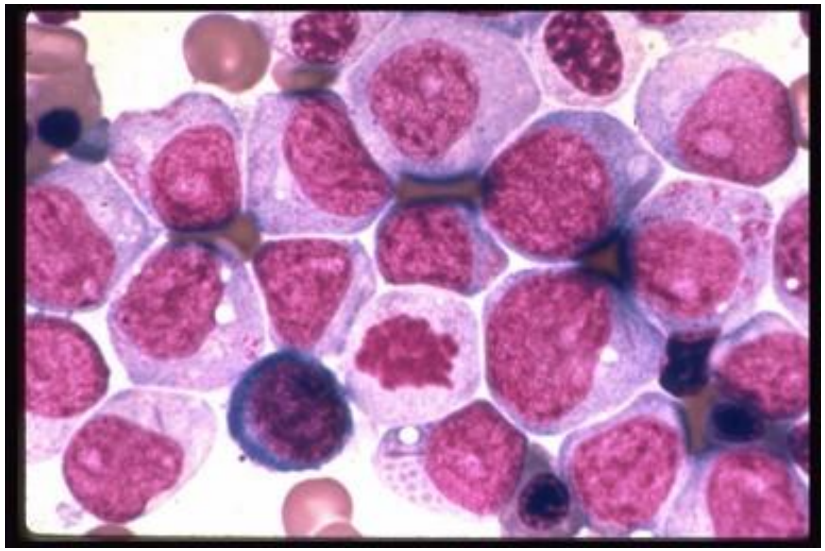
WHO Classification of AML (2001)

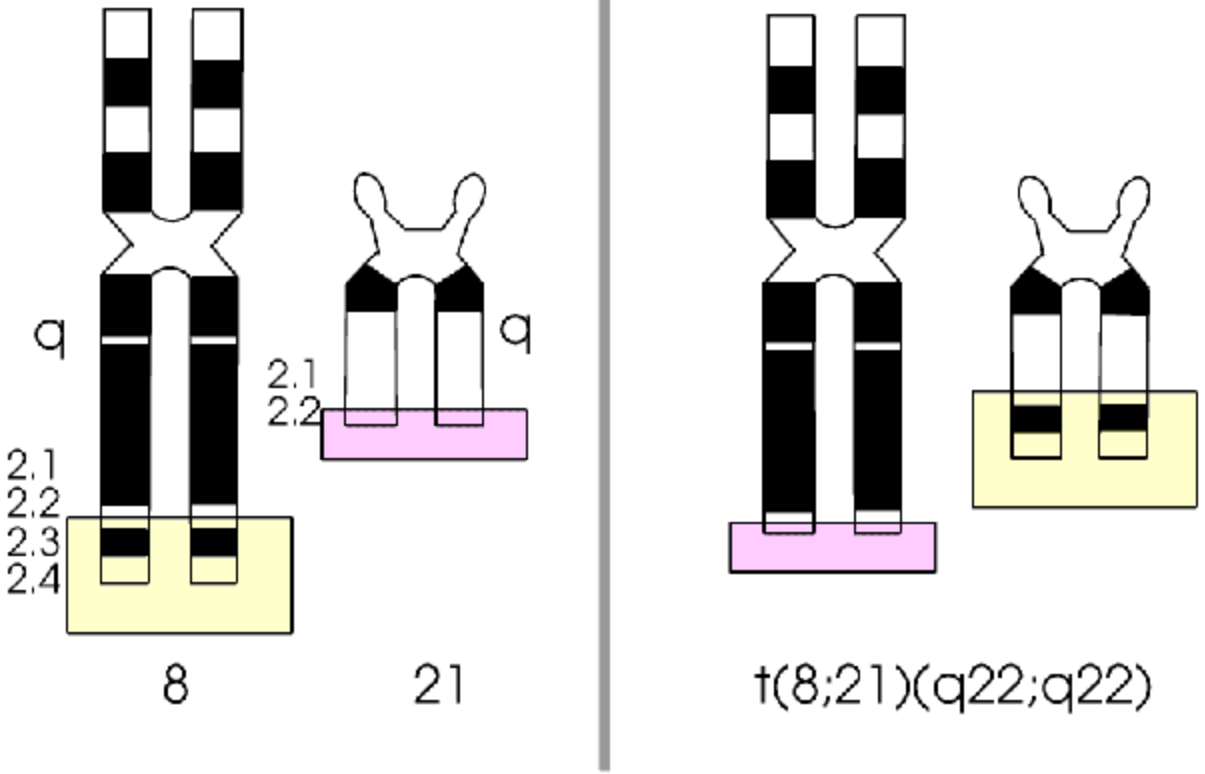
- AML with recurrent genetic abnormalities
  - AML with t(8;21)(q22;q22)
  - AML with inv(16)(q13;q22)
  - AML with t(15;17)(q22;q21)
  - AML with 11q23 abnormalities.
- AML with multilineage dysplasia.
- AML/MDS therapy-related.
- AML NOS
  - FAB categories
  - Acute basophilic leukemia
  - Acute panmyelosis with myelofibrosis
  - Myeloid Sarcoma

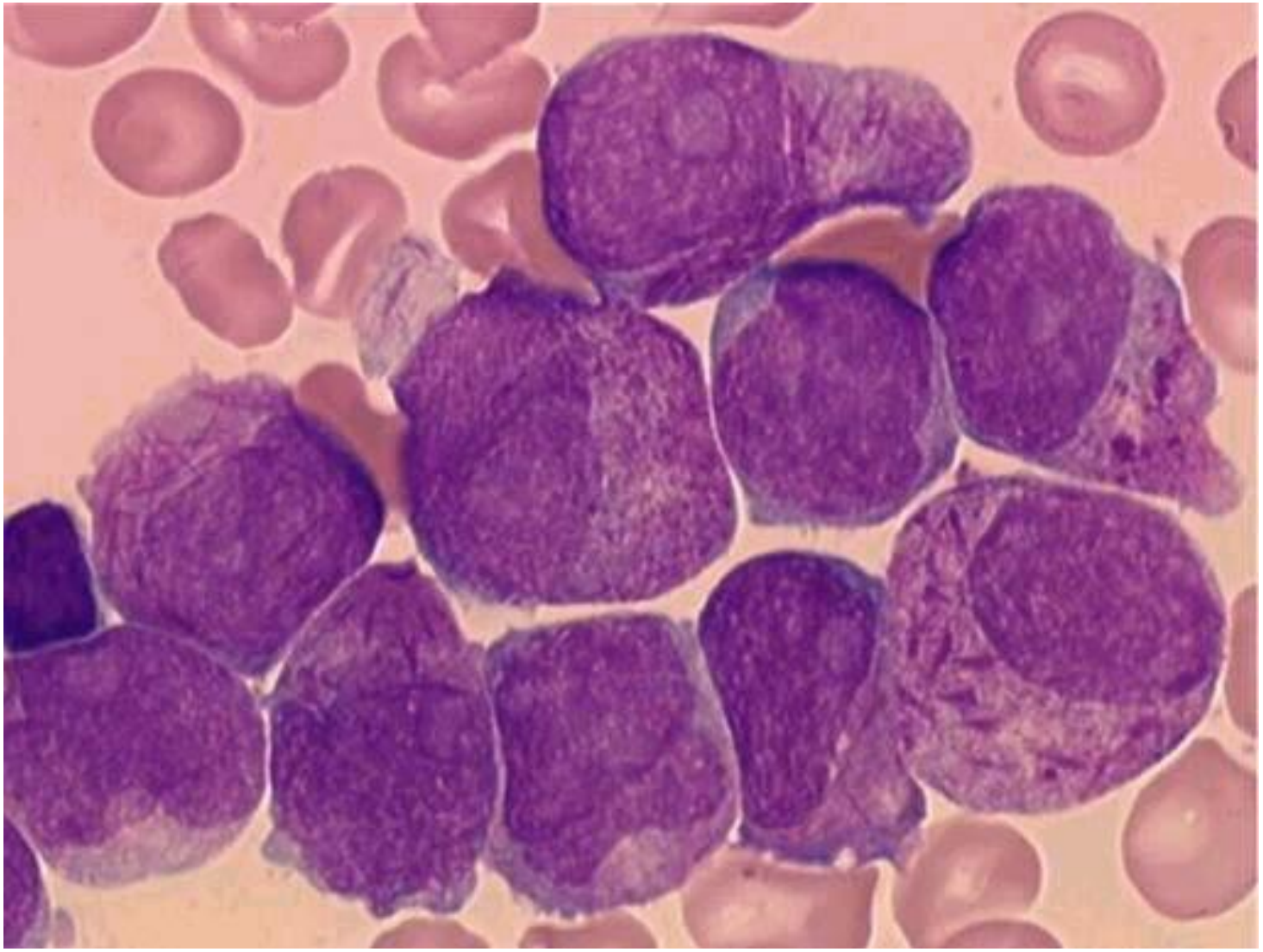
Chromosomal Anomalies in AML

Ch Aberration	Genes Involved	Associated Morphology	Comments
t(8;21)(q22;q22)	ETO/AML1	M2	Good Px
t(15;17)(q22;q11)	PML/RARA	M3	Response to ATRA
Abnormal 11q23	MLL	Monocytic	Topoisomerase II inhibitor, Poor px
Inv(16)(p13q22)	MYH11/CBF	M4Eo	Good px
t(16;16)(p13;q22)			
Del 16q22			
t(8;16)(p11;p13)	MOZ/CBP	Monocytic with erythrophagocytosis	poor px, M4, may be seen in M2
t(1;22)(p13;q13)		M7	Pedi only
t(6;9)(p23;q34)	DEK/CAN	Basophilia and dysplasia	1% of AML,M2
Inv 3(q21;q26) t(3;3)(q21;q26)	EVI1	Thrombocytosis; micromegakaryocytes	
-5 or del 5q, -7 or del 7q, t(1;7)(p11;p11)			Secondary leukemia
t(4;11)(q21;q23)	AF4/MLL	Bi-phenotypic	Poor px

- **M2 AML**
- **t(8:21)**







AML3 with t(15;17)(q22;q12)

Chromosomal anomalies in ALL

Phenotype	Ch Aberration	Genes	Prognosis	% of ped cases	% of adult cases
Early B cell CD10 +	Normal		Good	15-20	15
	Hyperdiploid > 50		Good	30	4
	Hyperdiploid 47-50		Intermediate	20	15
	Hypodiploid		Poor	1	8
	46, abn		Intermediate		59
	Del 6q		Intermediate	10	5
	Del or t 12p		Intermediate		
	Del or t 9p	INFA/INFB	Intermediate	5	
	t(12;21)(p12; q22)	TEL/AML1 FISH or PCR	Good	20	3
	t(9;22)(q34;q 911.2)	ABL/BCR	Poor	3-4	29

Chromosomal Aberrations in ALL

Phenotype	Ch Anomalies	Genes Involoved	Prognosis	% of ped	% of adult
Eosinophilia	t(5;14)(q31;q32)	IL3/IgH	Intermediate		
CIg+	t(1;19)(q23;p13)	PBX/E2A(TCF3)	Poor	6	3
Biphenotypic and CALLA -	t(4;11)(q21;q23)	AF4/Mll	Poor	2	4
B cell SIg+	t(8;14)(q24;q32)	MYC/IgH	Poor	2	5
	t(2;8)(12;q24)	IgK/MYC	Poor		
	t(8;22)(q24;q11)	MYC/IgL	Poor		
T cell	t(11;14)(p13;q11)	RBTN2/TCRA	Poor	2	9
	t(8;14)(q24;q11)	MYC/TCRA	Poor		
	inv 14(q11;q32)	TCRA/IgH	Poor		
	t(14;v)(q11;v)	TCRA	Poor		
	t(7;v)(q34-36;v)	TCRB	Poor		

Chromosomal Anomalies in CMPDs

Ch Aberration	Comment
t(9;22)(q34;q11)	Hallmark of CML; 95% detected by CC and 5% by FISH or PCR
Del 20 q11	PCV and CIMF
Del 13 q12-22	CIMF and occasionally in ET
Interstitial del 4q12 (cryptic)	FIP1L1-PDGFRalpha fusion gene CEL or systemic mast cell disease; responsive to Gleevec. Only detected by FISH.
Del 5, del 1, del 3	Occasionally PCV
+8, -7	CIMF or PCV
Various t with 8p11	Proposed new subtype of CMPD (myeloid hyperplasia with eosinophilia that progresses to acute lymphoblastic blast crises
JAK2 kinase gene mutation(9p24)	JAK 2 mutation in majority (65-97%) of PCV and occasionally (35-57%) in CIMF and ET. Only detectable by FISH.

Additional Comments on CMPDs

- Chronic cytogenetic abnormalities are found in virtually all cases of CML, but found by standard cytogenetics in only 30% of the other CMPDs.
- Clonal cytogenetic abnormalities by standard cytogenetics is very uncommon in ET.
- FISH and molecular assessment for specific mutations have increased the detection rate of genetic abnormalities in CMPDs.
- In CML, cytogenetic anomalies additional to t(9;22) are associated with accelerated and blast phases of the disease (+8, 17q10, +19).
- JAK2 kinase gene mutation, promising target for chemotherapy.

Cytogenetic Anomalies in MDSs

- No unique cytogenetic abnormality is specific for MDS
- If present, anomalies are often characterized by deletions of portions or all of chromosomes 5, 7, and 20.
- 5q syndrome – subtype of MDS with isolated del 5q
- 20-30% of primary MDS are associated with cytogenetic anomalies
- 70-90% of MDS secondary to chemo or radiation therapy are associated with cytogenetic anomalies.
- MDS and AML secondary to topoisomerase II inhibitors are associated with aberrations involving 11q23(MLL gene).
- Normal karyotype, del 5q, -Y, del 20q good px
- Complex (>3), or ch 7 anomalies poor px

Common Cytogenetic Anomalies in B-CLL

Ch Anomaly	Comments
13q deletion	15-40%, good px.
Tri 12	10-30%, intermediate px. Associated with progression to CLL/PLL.
11q deletion	5-20%, advanced stage, poor px
17p13 deletion	7-13%, inactivation of p53, advanced stage, resistance to chemotherapy.

FISH or Molecular Testing

- If morphology and/or immunophenotype suggest a particular cytogenetic anomaly not identified by conventional cytogenetics.
- t(15;17) – M3 ATRA
- t(9;22) – CML Gleevec
- Inv 16 – difficult to visualize by conventional banding techniques.
- Identification of minimal residual disease or disease recurrence (PCR is superior to FISH).

Important Points

- Cytogenetics used in conjunction with morphology, flow and immuno.
- Important for tumor dx, px and classification
- Less sensitive than PCR or FISH, but examines all chromosomes at same time.
- If particular cytogenetic defects anticipated are not identified, PCR or FISH should be used.

References

- 1) K. Foucar, MD and K. Reichard. Practical Approach to Bone Marrow examination. Pg 22-106, Sept 2005.
- 2) Nanette Clare MD. Applications of cytogenetics in Hematopathology. Pg 1-8, Jan 2005.
- 3) J.B. Henry, MD. Clinical Diagnosis and Management by Laboratory Methods. Twentieth edition. Pg 1304-1331.