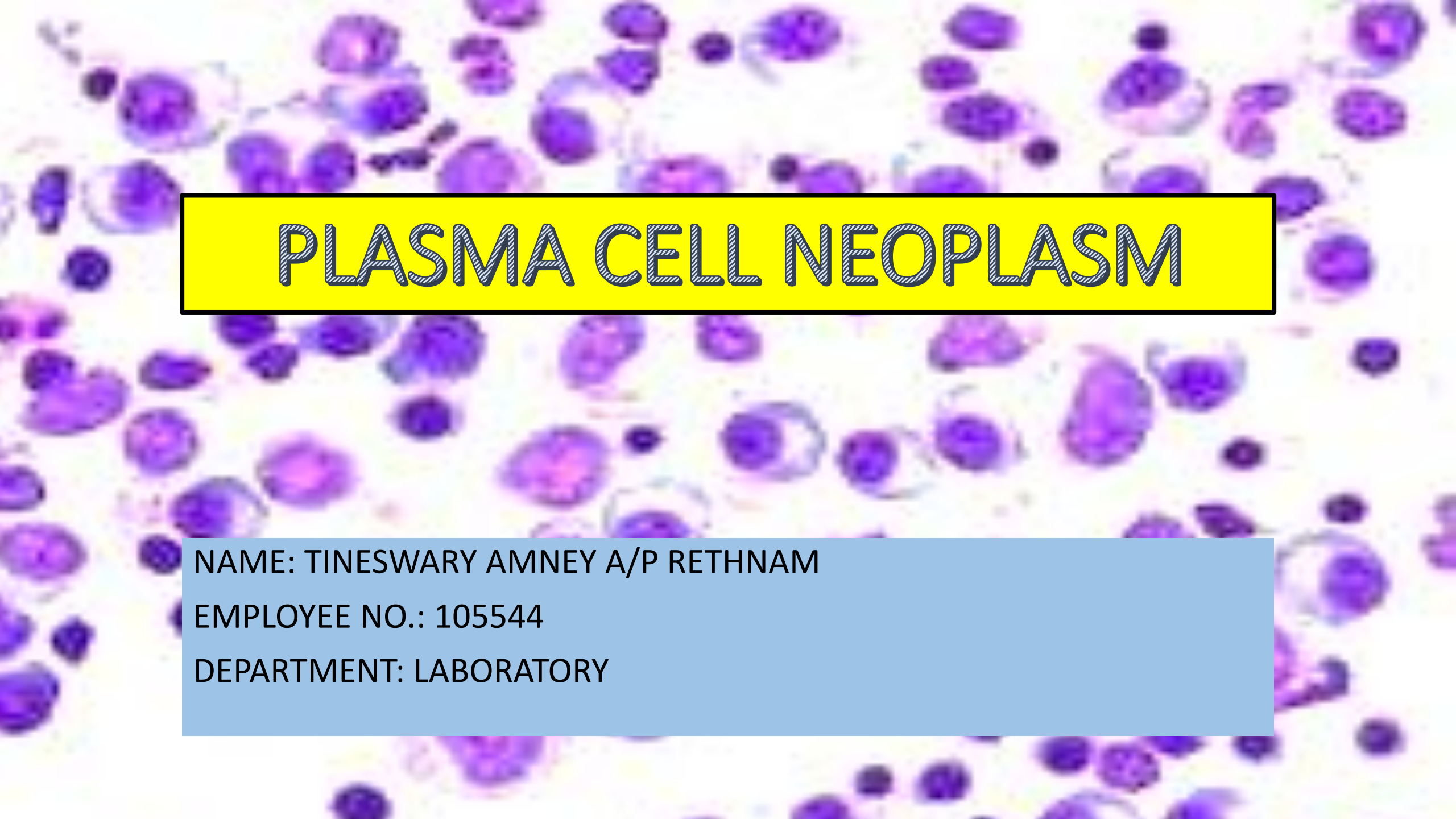


PLASMA CELL NEOPLASM

NAME: TINESWARY AMNEY A/P RETHNAM

EMPLOYEE NO.: 105544

DEPARTMENT: LABORATORY

Types of plasma cell neoplasm (PCM)

1. Monoclonal gammopathy of undetermined significance (MGUS)
2. Plasma cell myeloma
3. Plasmacytoma
4. Immunoglobulin deposition diseases
5. Osteosclerotic myeloma (POEMS syndrome) (R.W. McKenna, 2008)

Smoldering Multiple Myeloma (SMM)

- Both criteria must be met:

1. Serum monoclonal protein (IgG or IgA) ≥ 30g/L or urinary monoclonal protein ≥ 500mg per 24h and/or clonal bone marrow plasma cells 10-60%
2. Absence of myeloma-defining events or amyloidosis

Rajkumar et.al.,(2014),The lancet oncology,e538-48

IgM MGUS

- Serum IgM monoclonal protein <30 g/L
- Bone marrow lymphoplasmacytic infiltration <10%
- No evidence of anaemia, constitutional symptoms, hyperviscosity, lymphadenopathy, hepatosplenomegaly, or other end-organ damage that can be attributed to the underlying lymphoproliferative disorder

Rajkumar et.al.,(2014),The lancet oncology,e538-48

Light chain MGUS

- Abnormal FLC ratio (<0.26 or >1.65)
- Increased level of the appropriate free light chain (increased κ FLC in patients with ratio >1.65 and increased κ FLC in patients with ratio <0.26)
- No immunoglobulin heavy chain expression on immunofixation
- Absence of end-organ damage such as hypercalcemia, renal insufficiency, anemia, and bone lesions (CRAB) or amyloidosis that can be attributed to the plasma cell proliferative disorder
- Clonal bone marrow plasma cells <10%
- Urinary monoclonal protein <500mg/24h

Rajkumar et.al.,(2014),The lancet oncology,e538-48

Solitary plasmacytoma

- Biopsy-proven solitary lesion of bone or soft tissue with evidence of clonal plasma cells
- Normal bone marrow with no evidence of clonal plasma cells
- Normal skeletal survey and MRI (or CT) of spine and pelvis (except for the primary solitary lesion)
- Absence of end-organ damage such as hypercalcemia, renal insufficiency, anemia, and bone lesions (CRAB) or amyloidosis that can be attributed to the plasma cell proliferative disorder

Rajkumar et.al.,(2014),The lancet oncology,e538-48

Solitary plasmacytoma with minimal marrow involvement

- Biopsy-proven solitary lesion of bone or soft tissue with evidence of clonal plasma cells
- Clonal bone marrow plasma cells <10%
- Normal skeletal survey and MRI (or CT) of spine and pelvis (except for the primary solitary lesion)
- Absence of end-organ damage such as hypercalcemia, renal insufficiency, anemia, and bone lesions (CRAB) or amyloidosis that can be attributed to the plasma cell proliferative disorder

Rajkumar et.al.,(2014),The lancet oncology,e538-48

POEMS syndrome

- Polyneuropathy
- Monoclonal plasma cell proliferative disorder
- Any one of the 3 other major criteria: sclerotic bone lesions, Castleman's disease, elevated levels of VEGFA
- Any one of the following 6 minor criteria:
- Organomegaly (splenomegaly, hepatomegaly, or lymphadenopathy)
- Extravascular volume overload (edema, pleurl effusion, or ascites)
- Endocrinopathy (adrenal, thyroid,pituitary, gonadal, parathyroid, pancreatic)
- Skin changes (hyperpigmentation, hypertrichosis, glomeruloid hemangiomata, plethora, acrocyanosis, flushing, white nails)
- Papilloedema
- Thrombocytosis/polycythemia

Rajkumar et.al.,(2014),The lancet oncology,e538-48

Systemic AL amyloidosis

- Presence of an amyloid-related systemic syndrome (e.g., renal, liver, heart, gastrointestinal tract, or peripheral nerve involvement)
- Positive amyloid staining by Congo red in any tissue (e.g., fat aspirate, bone marrow, or organ biopsy)
- Evidence that amyloid is light-chain-related established by direct exmination of the amyloid using mass spectrometry-based proteomic analysis or immunoeletronmicroscopy
- Evidence of a monoclonal plasma cell proliferative disorder (serum monoclonal protein, abnormal free light chain ratio, or clonal plasma cells in the bone marrow)

Rajkumar et.al.,(2014),The lancet oncology,e538-48

Non-IgM monoclonal gammopathy of undetermined significance (MGUS)

- Serum monoclonal protein <30g/L
- Clonal bone marrow plasma cells <10%
- Absence of end-organ damage such as hypercalcemia, renal insufficiency, anemia, and bone lesions (CRAB) or amyloidosis that can be attributed to the plasma cell proliferative disorder

Rajkumar et.al.,(2014),The lancet oncology,e538-48

MULTIPLE MYELOMA

- Definition by WHO classification :

Plasma cell myeloma (PCM) is a bone marrow based, multifocal plasma cell neoplasm associated with an M-protein in serum and/or urine.

In most cases, there is disseminated BM involvement. The disease spans a clinical spectrum forms and disorders due to deposition of abnormal immunoglobulin chains in tissue. The diagnosis is based on a combination of pathological and clinical features. (R.W. McKenna, 2008)

- PCM comprises about 1% of malignant tumours & 10-15% of haematopoietic neoplasms.
- More common in men than women (1:4:1).
- Caused by chronic antigenic stimulation from infection & exposure to specific toxic substances/ radiation
- Variants:

    -Asymptomatic (smoldering) myeloma

    -Non-secretory myeloma

    -Plasma cell leukaemia

Clinical features/symptoms (CRAB):

C - Hypercalcemia (70% of cases)

R - Renal insufficiency due to tubular damage results from monoclonal light chain proteinuria

A- Anaemia (67%)

B- Bone lesions (R.W. McKenna, 2008)

Cytogenetics specifc test -**KARYOTYPING**

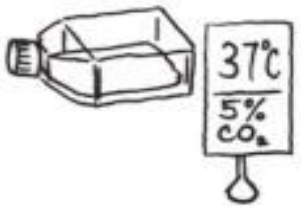
1) Sample collection
2) Sample culturing
3) Cell harvesting
4) Dropping and staining of metaphases
5) Cut and karyotype metaphases (Chauhan, 2020)

MARROW
BLOOD

Sample collected in Sodium Heparin tube at room temperature.

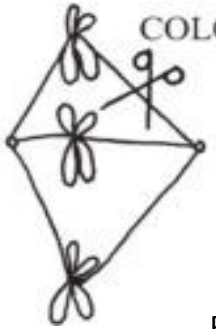
CELL
CULTURE

Cells cultured according to the diagnosis and maintained at 37 degree celcius with 5% CO2 supply in incubator.

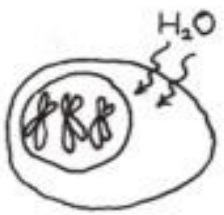


METAPHASE
ARREST

Upon harvesting, ethidium bromide (except 72hr stimulated culture) and colcemid will be added and will be incubated accordingly.



HYPOTONIC
TREATMENT

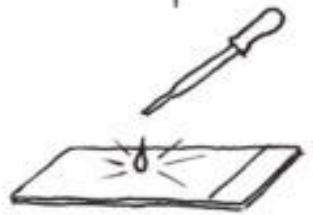


Potassium chloride will be added during the harvesting process and fixation process will take place right after that.

FIXATION

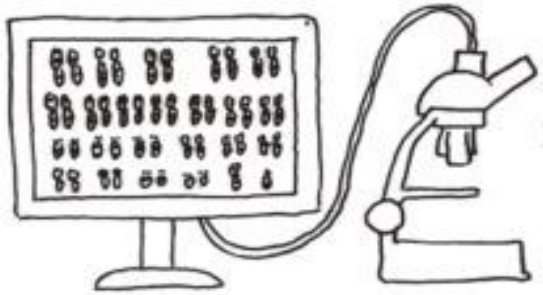
SPREADING

Harvested cells will be dropped and examined under microscope for enough metaphases.



BANDING

The metaphases then will be G-banded using Wright's stain and will be cover slipped.

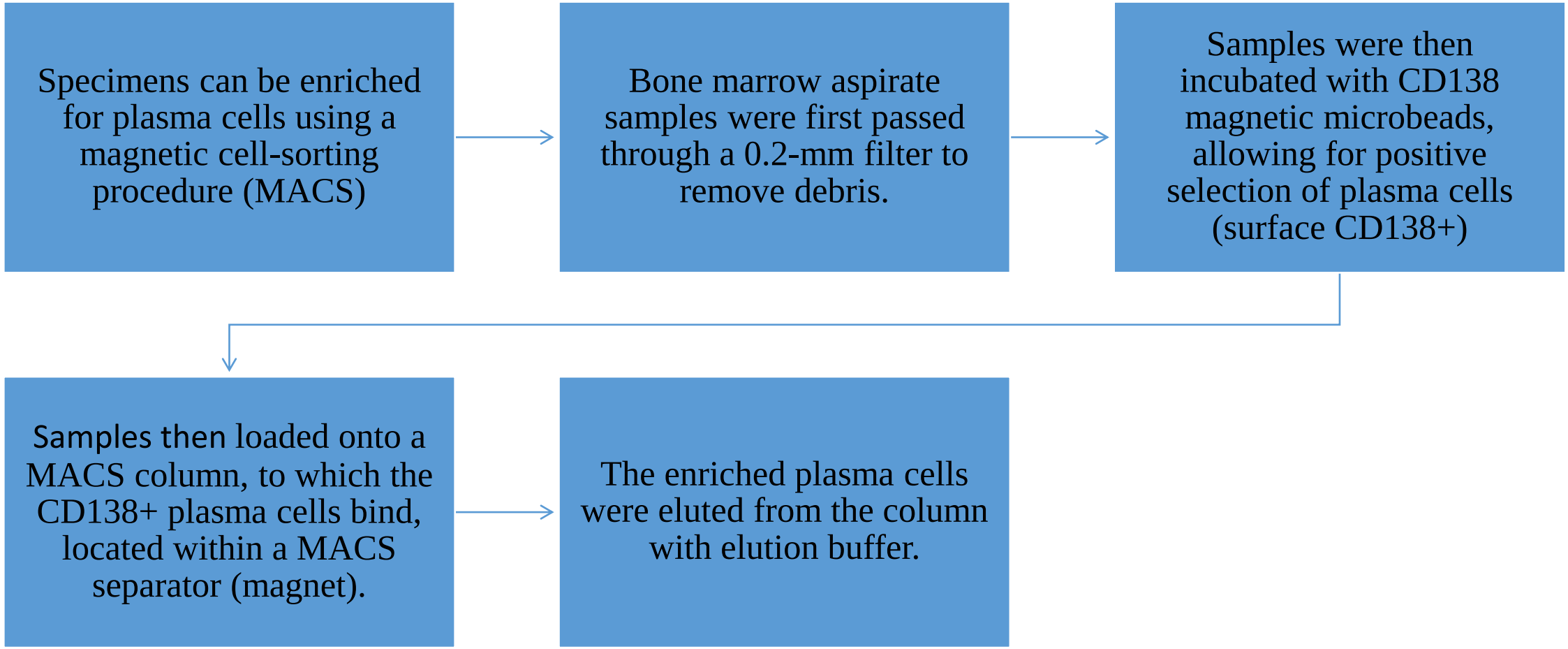


KARYOTYPING

The metaphases will be cut and karyotyped using Cytovision app.

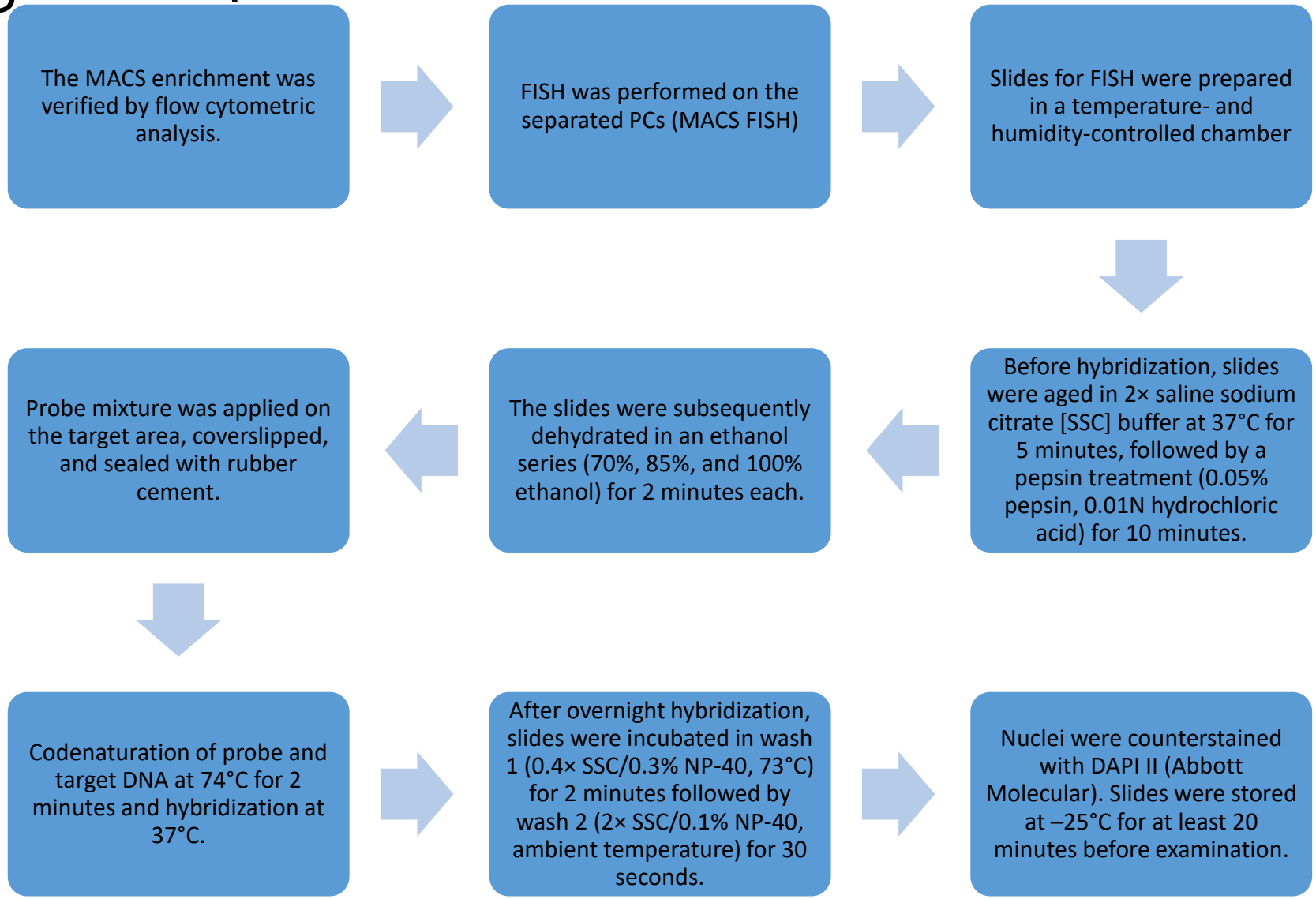
(Chauhan, 2020)

Cytogenetics specific tests- Magnetic cell-sorting FISH procedure



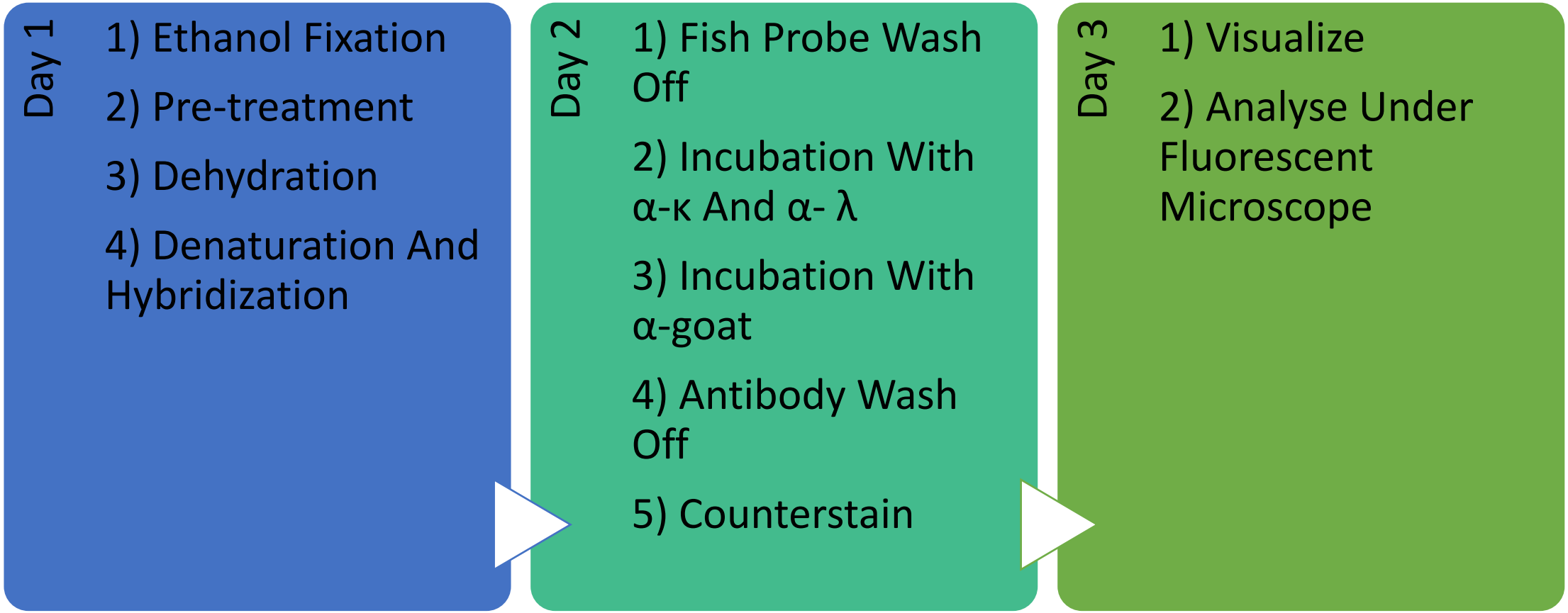
Lu et.al.,(2013), Archives of pathology & laboratory medicine,625-631

Cytogenetics specific tests- Magnetic cell-sorting FISH procedure

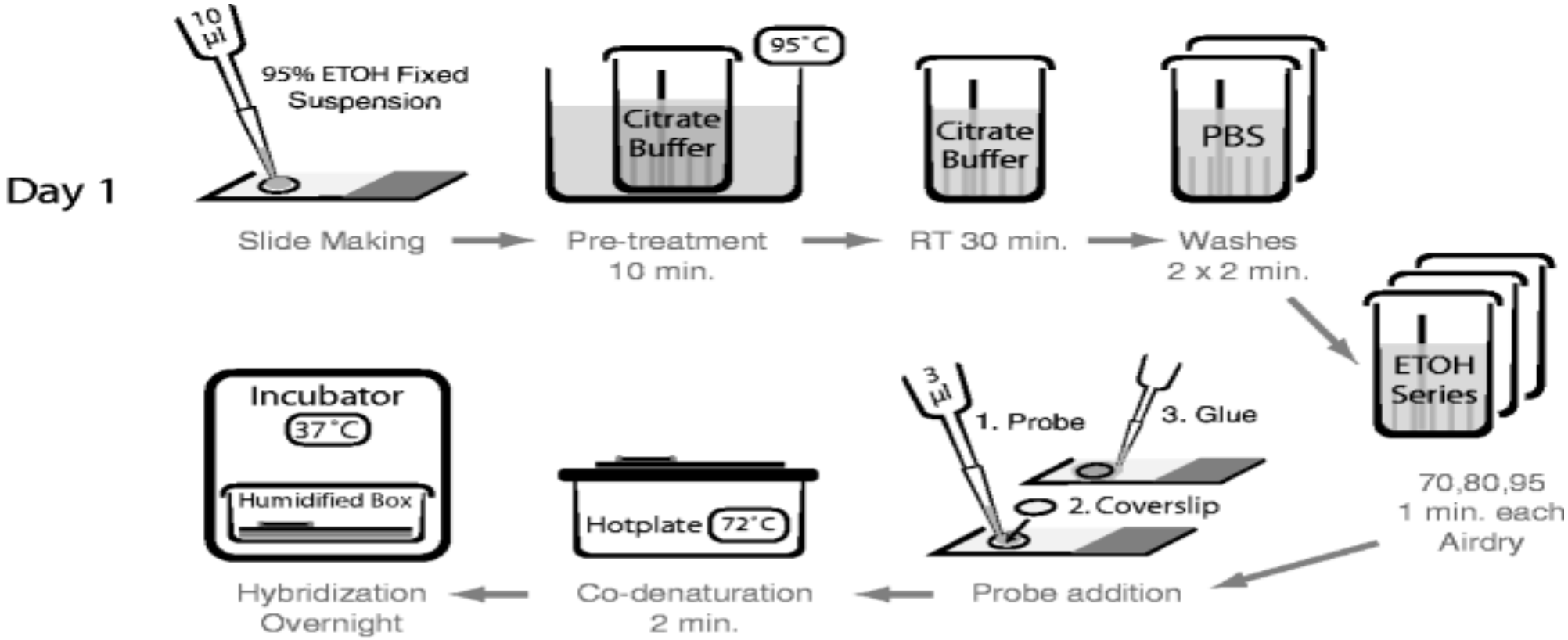


Hartmann et.al.,(2011),American journal of pathology,136

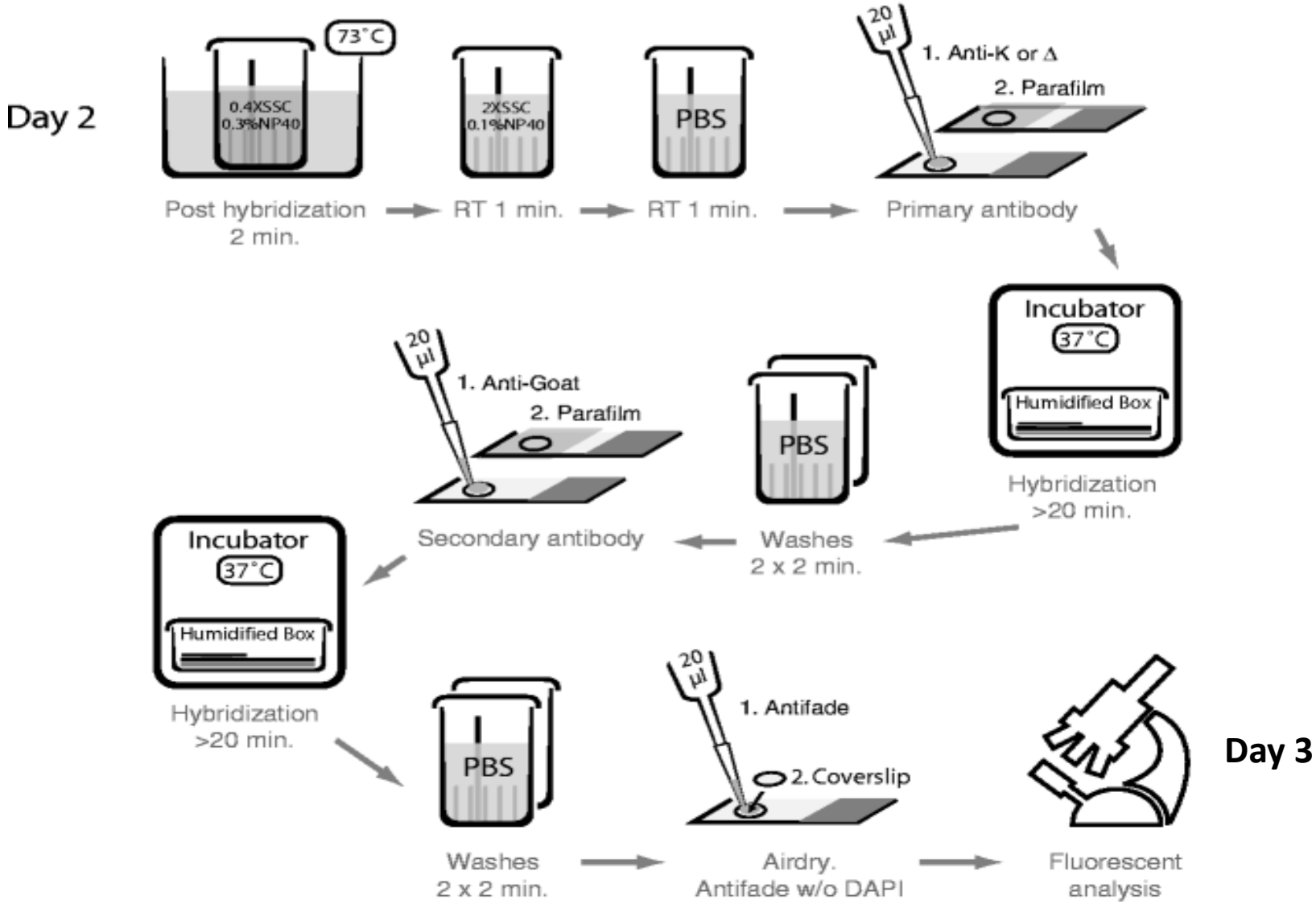
Cytogenetics specific tests- **CYTOPLASMIC Fluorescence in-situ hybridization (FISH)**



Multiple Myeloma – Clg FISH (DAY 1)



Multiple Myeloma – Clg FISH (DAY 2&3)



Cytogenetic abnormalities

Primary cytogenetic abnormalities

- Hyperdiploidy
- Non-hyperdiploidy
- IgH translocations

Secondary cytogenetic abnormalities

- MYC translocation
- 1q gain
- 1p deletion
- 13q deletion
- 17p deletion

Sarah Goldman-Mazur et al. (2021), Acta Haematologica Polonica, 1, 18-28

Karyotype- Common findings & Prognosis

- Gives essential information regarding numerical and structural changes.
  - Numerical:
    - Hyperdiploidy
    - Non-hyperdiploidy
  - Structural
    - Translocations
    - Copy number abberations (deletions /gains)

Sarah Goldman-Mazur et al. (2021), Acta Haematologica Polonica, 1, 18-28

Karyotype- Common findings & Prognosis Hyperdiploidy

- Found in 50% of MM patients
- Gain of odd-numbered chromosome
  - Trisomy 3 & 5 (improve overall survival)
  - Trisomy 21 (impair overall survival)
- In approximately 10% of patients, trisomies and IgH translocation coexist.
- Hyperdiploidy karyotype with ≥2 structural chromosome changes = high risk factor.

Sarah Goldman-Mazur et al. (2021), Acta Haematologica Polonica, 1, 18-28

Karyotype- Common findings & Prognosis Non-hyperdiploidy / hypodiploidy / pseudodiploidy / near-tetraploid karyotypes

- Hypodiploidy - ≤44 chromosomes in a cell
- Pseudodiploidy – 45-46 chromosomes in a cell
- Near-tetradiploidy karyotype – doubling of hypodiploidy & Pseudodiploidy (>75 chromosomes)
- Hypodiploidy
  - independent factor for worse overall survival
  - Associate with higher prevalence of genetic alterations and worse prognosis.

Sarah Goldman-Mazur et al. (2021), Acta Haematologica Polonica, 1, 18-28

Limitations Of Karyotype For MM

- Chromosomal abnormalities are not frequently detected by traditional karyotyping due to the low proliferative rate of malignant plasma cell in multiple myeloma (MM).
- Patients with complex karyotype, some abberations (translocations) are cryptic.
- Low number of plasma cells in an often hemodiluted bone marrow aspirate.
- Any prognostic impact that is seen with a metaphase-detected abnormality is probably not due to that abnormality *per se* but simply a reflection of the fact that the patient has a more proliferative, aggressive form of MM.

Rajkumar et al. (2015), Blood cancer journal, 5,e365
Leena Gole et al. (2014), Cancer genetics,1-3,207
Sarah Goldman-Mazur et al. (2021), Acta Haematologica Polonica, 1, 18-28

FISH – Probes used

1. IGH/CCND1 dual-fusion probe- detect t(11;14)
2. TP53 locus-specific probe - detect deletion of TP53 (17p13.1)
3. MYC break-apart probe - detect disruptions and amplification of the MYC locus (8q24)
4. IGH break-apart probe - detect disruptions of the IGH locus (14q32)
5. CKS1B/CDKN2C probe - detect chromosome 1q gain and/or 1p loss
6. D5S23, D5S721 and CEP15 probes - detect ploidy for chromosomes 5 and 15
7. CEP3 - detect ploidy for chromosome 3 (laboratories, 2016)

SIGNIFICANCE/PROGNOSIS OF FISH

Markers	Characteristics and prognostic value
t(4;14)(p16;q32) IGH-FGFR3/MMSET	Presence: 5% of MM High to intermediate risk Detectable only by FISH (cytogenetically cryptic)
t(11;14)(p13;q32) IGH-CCND1	Good prognosis Presence: 15% of MM Standard risk
t(14;16)(q32;q23) IGH-MAF	Presence: 5% of MM High risk
t(14;20)(q32;q12) IGH-MAFB	Presence: 1-2% of MM High risk

Rajkumar et al. (2015), Blood cancer journal, 5,e365

Markers	Characteristics and prognostic value
t(6;14)(p21;q32)	Good prognosis Standard risk
Gain/amplification of 1q21 (CKS1B)	Presence: 30-70% of MM High risk Confers a poor prognosis in all subtypes May observed in hypodiploid, hyperdipoloid and IGH Translocation-positive MM
Deletion of 17p (TP53)	Presence: 5-10% of MM High risk Confers a poor prognosis in all subtypes May observed in hypodiploid, hyperdipoloid and IGH Translocation-positive MM

Rajkumar et al. (2015), Blood cancer journal, 5,e365

Limitations of FISH

- Only detects aberrations specific to probes used

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