PERIPHERAL BLOOD EXAMINATION

Dr. Prasanna N Kumar
Head of Department of Pathology
BLOOD SMEAR – A CRUCIAL DIAGNOSTIC AID

- Automated cell counters – ↓ need for PS
- Fallacies in automated reports – machines not immune to deception!!
- Examination of smear – labour intensive – expensive but... can cell counters do morphology???
NO MORE HAEMATOLOGY TECHNOLOGISTS?

- Peripheral blood morphology for hematological disorders remains a very important diagnostic tool
- Give vital clues for further work up
- Essential in situations where automated cell counter fails due to interferences
- Peripheral blood smear examination – a reflex test
TYPES OF REQUESTS

- Physician initiated requests – clinical indications/abnormalities in a past blood count
- Laboratory initiated requests – abnormality in counts/flags
- Pathologist’s curiosity
- Laboratory policy – eg: smear for all lymphadenopathies/splenomegaly
- International Society for Laboratory Haematology – consensus criteria eg: failed delta check

INITIATE A BLOOD SMEAR WHEN.....

- Highly improbable results from cell counter
- False results due to hyperlipidaemia, cryoglobulinaemia, bacteria, fungi
- Pseudoneutropenia due to myeloperoxidase deficiency (when cell counter uses a peroxidase reaction)
- False low results – neutrophil/platelet clumping/platelet satellitism
SPECIMEN COLLECTION - EDTA

Advantages
- Many smears can be done in just a single draw
- Immediate preparation of the smear is not necessary
- Prevents platelet clumping on the glass slide

Disadvantages:
- Platelet satellitosis
- Pseudothrombocytopenia - platelet specific auto antibodies that react best at room temperature
SLIDE PREPARATION

- Always have a good spreader in the lab
- Too slow a slide push – poor WBC distribution, larger cells are pushed at the end of the slide
- Polycythemia – lower angle
- Severe anaemia – raise angle
IN A WELL
STAINED PBS....

- Rose-pink smear
- RBCs – orange to salmon-pink
- Neutrophils – deep purple nuclei, lilac cytoplasm freckled with fine, barely visible granules
- Eosinophils – 2-3 thick lobes, refractile bright orange granules, eosinophils – very good pH indicator
- Basophils: dark blue to black granules
IMPERFECTLY STAINED SMEARS

SMEARS TOO PINK
- Understained
- Overrinsing
- Water/stain too acidic
- Very thin smears

SMEARS TOO BLUE
- Overstained
- Underrinsing
- Water/stain too alkaline
- Very thick smears
- Heparinized sample

Stain debris – especially in tropical climes
- filter regularly, always cover
PERIPHERAL SMEAR – COMMONLY ENCOUNTERED PROBLEMS

- Stain precipitate
- Uneven WBC distribution – at tail end
- Refractile RBCs – water
- Nuclear degenerative changes – pyknosis, rupture
THE POWER OF OBSERVATION

- You are the tech on duty and you wake up the doctor on call reporting an “odd” appearance of the sample that you have received from a 60-year-old man
- You are unable to make a smear from this sample
Autoagglutination – grainy appearing peripheral smear
A 50-year-old male presents with H/O
His fingers, toes, ears, or nose becoming blue when he is exposed to cold
He also states that his fingers get numb
And are occasionally painful
A 55-year-old male with gradual onset of confusion and lethargy is admitted for severe back pain. Radiographs demonstrate a vertebral compression fracture as well as multiple osteolytic lesions.

Differentiate rouleaux from agglutination – saline replacement test.
APPROACH TO A PERIPHERAL SMEAR - 1

- Good look at slide with naked eye – quality of preparation and staining
- 10 X – distribution of cells, select area (atleast 10 “good” LP fields), rouleaux, agglutination, platelets, parasites. Fibrin strands - reject
- 40 X – RBC morphology, WBC DC
- 100 X – DC, WBC and platelet morphology, RBC inclusions, haemoparasites
FACTITIOUS THROMBOCYTOPENIA

Ptl. aggregates

Ptl. and leukocyte aggregates
MICROSCOPIC APPROACH TO A PERIPHERAL SMEAR
APPROACH TO A PERIPHERAL SMEAR - 2

- Examine where red cells are just without overlapping
- “Battlement” pattern for performing a WBC differential count
RBC PARAMETERS

- RBC Count
- Hb
- Hct
- Mean Cell Volume
- Mean Cell Hb
- Mean Cell Hb Concentration
- RBC distribution – RDW – useful parameter – raised in IDA, N or mildly increased – Beta thal trait
- Morphology
**GRADING OF ANISOCYTOYSIS, MICROCYTOYSIS, MACROCYTOYSIS**

<table>
<thead>
<tr>
<th>Grade</th>
<th>Anisocytosis</th>
<th>Microcytosis</th>
<th>Macrocytosis</th>
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<tbody>
<tr>
<td>1+</td>
<td>2- 4%</td>
<td>5-20%</td>
<td>2-10%</td>
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<td>2+</td>
<td>5-7%</td>
<td>11-25%</td>
<td>21-50%</td>
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<tr>
<td>3+</td>
<td>8-12%</td>
<td>26-50%</td>
<td>51-75%</td>
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<tr>
<td>4+</td>
<td>13-25%</td>
<td>51-100%</td>
<td>76-100%</td>
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</table>

(When both microcytes and macrocytes are seen, the grading of anisocytosis is increased by 1+)**
MICROCYTIC RBCs

MACROCYTIC RBCs
IRON DEFICIENCY ANAEMIA

- Commonest disease
- Commonest anaemia
- Commonest deficiency
- ↓ Hb  ↓ HCT  ↓ MCV
- ↑ RDW
<table>
<thead>
<tr>
<th></th>
<th>Range (1)</th>
<th>Range (1)</th>
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<tbody>
<tr>
<td>WBC</td>
<td>11.3</td>
<td>H</td>
</tr>
<tr>
<td>RBC</td>
<td>3.15</td>
<td>L</td>
</tr>
<tr>
<td>HGB</td>
<td>7.6</td>
<td>LL</td>
</tr>
</tbody>
</table>
| MCV        | 71        | L         | fL        | 55.0 /
| MCH        | 24.2      | LL        | pg        | 34.0 /
| RDW        | 12.6      | %         | 10.0 / 20.0 |
| PLT        | 561       | HH        | 10^3/μL   | 150 / 400 |
| MPV        | 6.5       | fL        | 6.0 / 10.0 |
| PCT        | 0.366     | %         | 0.200 / 0.500 |
| PDW        | 8.5       | %         | 8.0 / 18.0 |

Flags:
- WBC: MN
- RBC:
- PLT:

Lymphopenia: NE: 10^3/μL 2.00 / 8.00

Anemia: LY: 10^5/μL 1.00 / 5.00

Thrombocytosis: MO: 10^3/μL 0.10 / 1.00

In USA, Pct., PDW, ATL and IMM are for Research Use Only.
β THALASSAEMIA TRAIT
<table>
<thead>
<tr>
<th>Metric</th>
<th>Value</th>
<th>Unit</th>
<th>Reference Range</th>
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<tr>
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<td>6.2</td>
<td>/μL</td>
<td>4.0 / 11.0</td>
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<tr>
<td>RBC</td>
<td>2.79</td>
<td>/μL</td>
<td>4.00 / 6.20</td>
</tr>
<tr>
<td>HGB</td>
<td>10.4</td>
<td>/dL</td>
<td>11.0 / 18.8</td>
</tr>
<tr>
<td>HCT</td>
<td>30.1</td>
<td>%</td>
<td>35.0 / 55.0</td>
</tr>
<tr>
<td>MCV</td>
<td>108</td>
<td>/fL</td>
<td>80 / 100</td>
</tr>
<tr>
<td>MCH</td>
<td>37.2</td>
<td>/pg</td>
<td>26.0 / 34.0</td>
</tr>
<tr>
<td>MCHC</td>
<td>34.4</td>
<td>/dL</td>
<td>31.0 / 35.0</td>
</tr>
<tr>
<td>RDW</td>
<td>11.6</td>
<td>%</td>
<td>10.0 / 20.0</td>
</tr>
</tbody>
</table>

PLT 155 /μL 150 / 400

MPV 8.1 /fL 6.0 / 10.0

PCT 0.125 /L 0.200 / 0.500

PDW 12.9 % 8.0 / 18.0

In USA, Pct, PDW, ATL, and IMM are for Research Use Only.
<table>
<thead>
<tr>
<th>Grade</th>
<th>Appearance</th>
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<tbody>
<tr>
<td>1+</td>
<td>Slight increase in central pallor</td>
</tr>
<tr>
<td>2+</td>
<td>Equal amount of haemoglobin and central pallor</td>
</tr>
<tr>
<td>3+</td>
<td>Central pallor greater than rim of haemoglobin</td>
</tr>
<tr>
<td>4+</td>
<td>Cells appear as ghost cells</td>
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</table>
POIKILOCYTOSIS

Poikilocytes - variation in shapes of RBCs

- OVALOCYTES OR ELLIPTOCYTES
- PENCIL SHAPED CELLS
- SPHEROCYTES
- TARGET CLLS OR LEPTOCYTES
- SICKLE CELLS
- SCHISTOCYTES
- CRENATED CELLS
- ACANTHOCYTES
- BURR CELLS
- STOMATOCYTES
A 12-year-old boy presents to the gastroenterologist with the complaints of intermittent jaundice and pain in the right hypochondrium. O/E – anemia +, jaundice +, splenomegaly +
- Labs - Hb 9 g/dL hematocrit 27%
- White blood cell count – 6.2x10^9/L
- Platelet count of 201x10^9/L
- MCV 75 fl (N: 80-97), MCHC 39 g/dl (N:33-37).
Hereditary spherocytosis
Autoimmune hemolytic anaemia
HDN – ABO incompatibility
Post –transfusion – stored RBCs
G6PD DEFICIENCY

- Oxidant damage – “bite” cells
  - “blisters cells”
- Commonly seen with G6PD deficiency
- Smear examination may be critical in G6PD def – more rapid than assay, suggests diagnosis even with normal G6PD levels
- Naphthalene balls
A 56-year-old woman presented in an obtunded state, febrile, disoriented and had difficulty with speech. Petechiae + Scleral icterus + Thrombocytopenia+ Serum creatinine - ↑
Basophilic stippling - lead poisoning, thalassaemia, macrocytic anaemias.

Pappenheimer bodies – iron containing – hemolytic anaemias, MDS, sideroblastic anaemias.

Howell-Jolly bodies – nuclear fragments, post splenectomy, macrocytic, haemolytic anaemias.
WBCs

- Buffy coat preparations for leukopenic samples, immature cells, bacteria, fungi, parasites
- Look at edges of smears for immature cells
- Abnormal neutrophil morphologies, abnormal WBC inclusions
- Nucleated RBCs which give spurious increases in automated WBC counts
ASSESSMENT OF WBCs

40x objective - number of (WBCs) in 5 to 10 fields

<table>
<thead>
<tr>
<th>No. of cells/hpf</th>
<th>WBC count (approx)</th>
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<tr>
<td>2-4</td>
<td>4-7 x 10^9/L</td>
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<tr>
<td>5-6</td>
<td>7-10 x 10^9/L</td>
</tr>
<tr>
<td>7-10</td>
<td>10-13 x 10^9/L</td>
</tr>
<tr>
<td>11-20</td>
<td>13-18 x 10^9/L</td>
</tr>
</tbody>
</table>
CORRECTION FOR NUCLEATED RBCs

- Haemolytic anaemias, sepsis, leucoerythroblastic blood picture – eg: bone marrow mets
- 10 or > NRBCs - Make correction

\[
\text{uncorrected WBC count} \times 100 \\
\text{number of nRBC’s per 100 WBC’s + 100}
\]

Exercise:
Total WBC count = 45 \times 10^9/L
Nucleated RBCs = 50/100 WBCs
Corrected TWBC = ?
NUCLEATED RBCs VS LYMPHOCYTES
WBC MORPHOLOGIES

Dohle bodies

Monocyte with toxic granules & vacuoles

Neutrophil with bacteria in a case of sepsis

Hypersegmented neutrophil
WBC MORPHOLOGIES

Dysplastic hypogranular neutrophil

Pseudo-pelgeroid cell in MDS
LEFT SHIFT

- Normal
- Mild-moderate left shift
- Severe left shift

Increasing Neutrophil Maturity
WBC MORPHOLOGIES

- Toxic granules
- Atypical mononuclear cells
- Plasma cells
LEUCOERYTHROBLASTIC BLOOD FILM
(Myelophthisic Anaemia)

- presence of both NRBC and immature myeloid precursors with teardrop poikilocytes – should stimulate a bone marrow examination
  - idiopathic myelofibrosis or bone marrow infiltration
Different normal and abnormal cell populations
A 33-year-old lady seen in the pulmonology out-patients for the diagnosis of bronchial asthma

<table>
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**Clinical Pathology Lab**

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<td>8.8</td>
<td>4.0 - 11.0</td>
</tr>
<tr>
<td>RBC</td>
<td>3.04 L</td>
<td>4.0 - 6.20</td>
</tr>
<tr>
<td>HGB</td>
<td>8.7 L</td>
<td>11.0 - 18.0</td>
</tr>
<tr>
<td>HCT</td>
<td>26.0 %</td>
<td>35.0 - 55.0</td>
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<tr>
<td>MCV</td>
<td>85 FL</td>
<td>80 - 100</td>
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<tr>
<td>MCH</td>
<td>28.6 pg</td>
<td>26.0 - 34.0</td>
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<tr>
<td>MCHC</td>
<td>33.6 g/dL</td>
<td>31.0 - 35.0</td>
</tr>
<tr>
<td>RDW</td>
<td>11.8 %</td>
<td>10.0 - 20.0</td>
</tr>
<tr>
<td>PLT</td>
<td>333</td>
<td>150 - 400</td>
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<tr>
<td>MPV</td>
<td>7.6 FL</td>
<td>6.0 - 10.0</td>
</tr>
<tr>
<td>PCT</td>
<td>0.251 %</td>
<td>0.200 - 0.500</td>
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<tr>
<td>PDW</td>
<td>11.5 %</td>
<td>8.0 - 18.0</td>
</tr>
</tbody>
</table>

**Cells**

<table>
<thead>
<tr>
<th>Cell Type</th>
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<th>Reference Range</th>
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<tr>
<td>NE</td>
<td>42.9 %</td>
<td>50.0 - 80.0</td>
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<tr>
<td>LY</td>
<td>23.3 %</td>
<td>25.0 - 50.0</td>
</tr>
<tr>
<td>MO</td>
<td>5.0 %</td>
<td>10.0 - 20.0</td>
</tr>
<tr>
<td>EDH</td>
<td>27.9 %</td>
<td>0.0 - 5.0</td>
</tr>
<tr>
<td>BA</td>
<td>0.9 %</td>
<td>0.0 - 2.0</td>
</tr>
<tr>
<td>ATL</td>
<td>0.3 %</td>
<td>0.0 - 2.0</td>
</tr>
<tr>
<td>IMM</td>
<td>2.0 %</td>
<td>0.0 - 4.0</td>
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</tbody>
</table>

**Neutropenia**

- **WBC** x 10^9/μL
- **LY** x 10^9/μL
- **MO** x 10^9/μL
- **EDH** x 10^9/μL
- **BA** x 10^9/μL
- **ATL** x 10^9/μL
- **IMM** x 10^9/μL

**Research Use Only**

- **Platelet Aggregates**
- **Neutropenia**
- **Eosinophilia**
- **Neutrophilia**

**Diagrams**

- WBC
- RBC
- PLT
A 15-year-old boy admitted with the diagnosis of acute appendicitis
- A 4-year-old female patient admitted with enlarged lymph nodes
A 43-year-old lorry driver admitted to the intensive medical care unit for very high fever, with chills and rigors. He was in a semi-comatose state.
- Typical picture of a highly infected malaria patient
- Falsely elevated WBC count – high numbers of gametocytes, counted as WBCs
- Interference with the lymphocytes (flags) – manual lymphocyte count – 7%
ACUTE LYMPHOBLASTIC LEUKAEMIA

<table>
<thead>
<tr>
<th>REL NO</th>
<th>WBC</th>
<th>LY</th>
<th>MO</th>
<th>GR</th>
<th>LY#</th>
<th>MO#</th>
<th>GR#</th>
<th>RBC</th>
<th>Hgb</th>
<th>Hct</th>
<th>MCV</th>
<th>MCH</th>
<th>MCHC</th>
<th>MCHC</th>
<th>RDW</th>
<th>Plt</th>
<th>MPV</th>
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<tr>
<td></td>
<td>65.5</td>
<td></td>
<td>16.3</td>
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<td>10.7</td>
<td>19.7</td>
<td>12.5</td>
<td>2.83</td>
<td>8.4</td>
<td>23.8</td>
<td>84.1</td>
<td>29.7</td>
<td>35.3</td>
<td>19.8</td>
<td>52</td>
<td>7.2</td>
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</table>
ACUTE LEUKAEMIA

- Multiple flags with the WBCs
- WBC histogram – unimodal peak at 120fL – blasts likely
- Increased RDW – post-transfusion
- Reduced platelets

<table>
<thead>
<tr>
<th>WBC</th>
<th>RBC</th>
<th>HGB</th>
<th>HCT</th>
<th>MCV</th>
<th>MCH</th>
<th>MCHC</th>
<th>RDW</th>
<th>PLT</th>
<th>MPV</th>
</tr>
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<tbody>
<tr>
<td>8.6</td>
<td>3.99</td>
<td>11.1</td>
<td>33.9</td>
<td>85</td>
<td>27.9</td>
<td>32.9</td>
<td>13.9</td>
<td>18</td>
<td>8.4</td>
</tr>
</tbody>
</table>

Range (1)

- WBC: 5.0 × 10^9/L
- RBC: 4.5 × 10^12/L
- HGB: 13.5 g/dL
- HCT: 40%
- MCV: 80 fL
- MCH: 27 pg
- MCHC: 32.5 g/dL
- RDW: 15

Range (1)

- NE: 4.0 × 10^9/L
- LY: 50.0 × 10^9/L
- MO: 33.6 × 10^9/L
- EO: 0.5 × 10^9/L
- BA: 1.8 × 10^9/L

Range (1)

- NE#: 2.0 × 10^9/L
- LY#: 5.0 × 10^9/L
- MO#: 2.9 × 10^9/L
- EO#: 0.04 × 10^9/L
- BA#: 0.16 × 10^9/L

- Neutrophilia
- Lymphocytosis
- Atypical Cells
- Monocytosis
- Platelet interpretation impossible
ACUTE MYELOID LEUKAEMIA

- Intense population of cells from lymphocyte region till upper monocyte area
- Immature myeloid cells
- Platelet histogram – interference from schistocytes

<table>
<thead>
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<th>Range (l)</th>
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<th>Range (l)</th>
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<td>6.0 / 11.0</td>
<td>NE</td>
</tr>
<tr>
<td>RBC</td>
<td>3.99 L 10⁶/µL</td>
<td>4.00 / 6.20</td>
<td>LY</td>
</tr>
<tr>
<td>HGB</td>
<td>11.1 g/dL</td>
<td>11.0 / 18.8</td>
<td>MO</td>
</tr>
<tr>
<td>HCT</td>
<td>33.9 L %</td>
<td>35.0 / 55.0</td>
<td>EO</td>
</tr>
<tr>
<td>MCV</td>
<td>85 fL</td>
<td>80.0 / 100.0</td>
<td>BA</td>
</tr>
<tr>
<td>MCH</td>
<td>27.9 pg</td>
<td>26.0 / 34.0</td>
<td></td>
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<tr>
<td>MCHC</td>
<td>32.9 g/dL</td>
<td>31.0 / 35.0</td>
<td></td>
</tr>
<tr>
<td>RDW</td>
<td>13.9 %</td>
<td>10.0 / 20.0</td>
<td></td>
</tr>
<tr>
<td>PLT</td>
<td>18 RLL 10³/µL</td>
<td>150 / 400</td>
<td>NE#</td>
</tr>
<tr>
<td>MPV</td>
<td>8.4 R fL</td>
<td>6.0 / 10.0</td>
<td>LY#</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>MO#</td>
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Lymphocytosis
Neutropenia
Immature Cells
Atypical Lymp, Monocytosis
Plt interpretation impossible
An albino child with recurrent infections looking for a diagnosis
– Blood film
PLATELETS

- Blood smear for thrombocytopenia – confirm counts, cause

- Platelet count – 150-400 $X 10^9/L$ – about 7 – 20 per oil immersion field or number of platelets in 10 oil.imm. fields $X 20,000$ – select area where RBCs barely touch

- Falsely low platelet ct – small clots, platelet clumping, platelet satellitism, abnormally large platelets
Increased Platelets in a case of ET

Platelet aggregation

Platelet satellitosis
PLATELETS

- Blood smear for high platelet counts – confirm counts, cause (eg: MPS)
- Spurious thrombocytosis – red cell fragments, fragments of leukaemic cells, fungi, bacteria

*J Clin Pathol 2004:57;1096-97*
• A 45-year-old lady admitted for surgery detected to have severe thrombocytopenia using the cell counter

• Peripheral smear examination – platelet aggregates
- Repeat platelet counts – similar results
- Blood collected in citrate and reassessed - similar results
- Blood collected without anticoagulant
- Anticoagulant induced platelet aggregation – pseudothrombocytopenia due to EDTA
A 35-year-old male admitted with gram-negative septicaemia and Disseminated Intravascular Coagulation
ITP – LARGE YOUNG PLATELETS
ITP – LARGE YOUNG PLATELETS
BLOOD SMEAR – PART OF MEDICAL RECORD

- Blood smear – primary/only evidence of a disease eg. MDS, leukaemia, lymphoma, haemolytic anaemia
- Store smears/digital images
- Modern investigations of haematologic disorders + peripheral blood features + clinical findings
INTERNATIONAL CONSENSUS GROUP – SUGGESTED CRITERIA FOR SLIDE REVIEW

- Individual labs follow different criteria – depends on patient population, type of analyser etc
- MCV <7f fl, > 105 fl, RDK > 22
- Platelets < 100,000, > 10,00,000
- First sample in a neonate
- No WBC differential or incomplete diff.
PERIPHERAL SMEAR WITH ??
RETICULOCYTE COUNT

- Most useful lab test for differentiating underproduction from haemolysis
- Appropriate supravital stain
- Reticulocytopenia – hypoplastic / aplastic marrow
- Reticulocytosis – haemolysis
- Early inexpensive indicator of response to iron/B₁₂ therapy
Systematic examination of blood film gives the best diagnostic support and all the other tests are either complimenting or confirming it and not on many occasions make you wiser.

Thank you