

## Contents

<b>S.No.</b>	<b>Topic</b>	<b>Page No.</b>
1	ANTI RETEROVIRAL THERAPY	2
2	ENCEPHALITIS	29
3	INFECTIOUS DIARRHOEA	33
4	HEPATITIS B	66
5	PRE-OPERATIVE HAEMOSTASIS SCREEN:	87
6	ACTIVATED PARTIAL THROMBOPLASTIN TIME:	93
7	BLEEDING TIME:	95

# ANTI RETEROVIRAL THERAPY

## 1. When to suspect/ recognize HIV?

a) Introduction: There is no clinical description; the diagnosis is based on lab criteria

### **Laboratory criteria for diagnosis:**

1. HIV positive serology (ELISA)
2. Confirmation should be a second ELISA

b) Case definition: WHO clinical case definition for AIDS in an adult or adolescents (>12 years of age) is considered to have AIDS if at least 2 of the following major signs are present in combination with at least 1 of the minor signs listed below, and if these signs are not known to be related to a condition unrelated to HIV infection

### **Major signs (2 signs or more):**

- a. Weight loss >\_10% of body weight
- b. Chronic diarrhoea for >1 month
- c. Prolonged fever for >1 month (intermittent or constant)

### **Minor signs (1 or more):**

- a. Persistent cough for >1 month
- b. Generalized pruritic dermatitis
- c. History of herpes zoster
- d. Oropharyngeal candidiasis

**Incidence of the condition in our country:** As per the provisional HIV estimate of 2008-09, there are an estimated 22.7 lakh people living with HIV/AIDS in India. The HIV prevalence rate in the country is percent (2008-09) and most infections occur through heterosexual route of transmission. The absolute number of HIV infections in the country is expected to be close to 2.5 million.

### **Differential Diagnosis:**

- ☐ Flu - the very early HIV infection symptoms are like flu.
- ☐ Immunodeficiency syndromes
- ☐ Epstein -Barr virus
- ☐ Dementia
- ☐ Hypogammaglobulinemia
- ☐ Severe combined immunodeficiency disease
- ☐ Depression
- ☐ B-cell deficiency
- ☐ T-cell deficiency
- ☐ Non-Hodgkin's Lymphoma
- ☐ Pelvic Inflammatory Disease
- ☐ Pyoderma Gangrenosum

**Prevention and counseling:** the primary focus of NACP. III is to halt and reverse the spread of the HIV epidemic in India by 2012. The programme plans to cover 80 percent of HRGs with primary prevention services, including:

- ☐ Treatment for sexually transmitted infections

- ☐ Condom provision
- ☐ Behaviour change communication

- ☒ Creating an enabling environment with community involvement and participation
- ☒ Linkages to care and support services

**Optimal diagnostic criteria, investigations, treatment and referral criteria**

**Situation 1: At secondary hospital/ non-metro situation: Optimal standards of treatment in situations where technology and resources are limited.**

- a) Diagnosis: the diagnosis is based on lab criteria
- b) Investigations: 1.HIV positive serology (ELISA)  
2.Confirmation should be a second ELISA
- c) Treatment: after counseling patient is referred to super speciality centre

**Situation 2: At super specialty facility in Metro location where higher-end technology is available.**

- a) Clinical Diagnosis:

**Physical Examination:** Record vital signs, body weight, height and body mass index (BMI), temperature, blood pressure, pulse rate, respiratory rate

# Physical examination checklist

<b>Appearance</b>	<ul style="list-style-type: none"> <li>• Unexplained moderate or severe weight loss, HIV wasting</li> <li>• Rapid weight loss is suggestive of active OI, especially if associated with fever</li> <li>• Gradual weight loss (not caused by malnutrition or other obvious illness) is suggestive of HIV infection</li> <li>• "Track marks" and soft tissue infections which are common among IDUs</li> </ul>
<b>Consider conditions other than HIV</b>	<ul style="list-style-type: none"> <li>• Malaria, tuberculosis, syphilis, gastrointestinal infections, bacterial pneumonia, pelvic inflammatory disease, viral hepatitis</li> </ul>
<b>Skin</b>	<ul style="list-style-type: none"> <li>• Look for signs of HIV-related and other skin problems. These include diffuse dry skin, typical lesions of PPE, especially on the legs, seborrhoeic dermatitis on face and scalp</li> <li>• Look for herpes simplex and herpes zoster or scarring of previous herpes zoster (especially multi-dermatome)</li> </ul>
<b>Lymph nodes</b>	<ul style="list-style-type: none"> <li>• Start with posterior cervical nodes</li> <li>• PGL (persistent glandular lymphadenopathy) typically presents as multiple bilateral, soft, non-tender, mobile cervical nodes. Similar nodes may be found in the armpits and groins</li> <li>• Tuberculous lymph nodes typically present as unilateral, painful, hard, enlarging nodes, with constitutional symptoms such as fever, night sweats and weight loss</li> </ul>
<b>Mouth</b>	<ul style="list-style-type: none"> <li>• Look for signs suggestive of HIV infection including white plaques on tongue, cheeks and roof of mouth (oral candida), white stripped lesions on the side of the tongue (OHL) and cracking at the corners of the mouth (angular cheilitis)</li> <li>• Difficulty in swallowing is commonly caused by oesophageal candida</li> </ul>
<b>Chest</b>	<ul style="list-style-type: none"> <li>• The most common problems will be PCP and TB</li> <li>• Signs and symptoms are cough, shortness of breath, haemoptysis, weight loss, fever, congestion or consolidation</li> <li>• Perform a chest X-ray, if symptomatic</li> </ul>
<b>Abdomen</b>	<ul style="list-style-type: none"> <li>• Hepatosplenomegaly, masses and local tenderness</li> <li>• Jaundice may indicate viral hepatitis</li> </ul>
<b>Ano-genital</b>	<ul style="list-style-type: none"> <li>• Herpes simplex and other genital sores/lesions, vaginal or penile discharge</li> <li>• Perform PAP smear, if possible</li> </ul>
<b>Neurological examination</b>	<ul style="list-style-type: none"> <li>• Focus on visual fields and the signs of neuropathy (bilateral peripheral or localized mono-neuropathies)</li> <li>• Assess focal neurological deficit</li> </ul>

*Note:* During each consultation, patient is to be clinically screened for TB (history and physical examination).

## Clinical considerations

### WHO clinical staging of HIV disease in adults and adolescents

<b>Clinical stage 1</b>
Asymptomatic Persistent generalized lymphadenopathy
<b>Clinical stage 2</b>
Moderate unexplained weight loss (under 10% of presumed or measured body weight) Recurrent respiratory tract infections (sinusitis, tonsillitis, otitis media, pharyngitis) Herpes zoster Angular cheilitis Recurrent oral ulcerations Papular pruritic eruptions Seborrhoeic dermatitis Fungal nail infections
<b>Clinical stage 3</b>
Unexplained severe weight loss (over 10% of presumed or measured body weight) Unexplained chronic diarrhoea for longer than 1 month Unexplained persistent fever (intermittent or constant for longer than 1 month) Persistent oral candidiasis Oral hairy leukoplakia Pulmonary tuberculosis Severe bacterial infections (e.g. pneumonia, empyema, meningitis, pyomyositis, bone or joint infection, bacteraemia, severe pelvic inflammatory disease) Acute necrotizing ulcerative stomatitis, gingivitis or periodontitis Unexplained anaemia (below 8 g/dl ), neutropenia (below $0.5 \times 10^9/l$ ) and/or chronic thrombocytopenia (below $50 \times 10^9/l$ )

## Clinical stage 4

HIV wasting syndrome

*Pneumocystis jiroveci* pneumonia

Recurrent severe bacterial pneumonia

Chronic herpes simplex infection (orolabial, genital or anorectal of more than 1 month's duration or visceral at any site)

Oesophageal candidiasis (or candidiasis of trachea, bronchi or lungs)

Extrapulmonary tuberculosis

Kaposi sarcoma

Cytomegalovirus disease (retinitis or infection of other organs, excluding liver, spleen and lymph nodes)

Central nervous system toxoplasmosis

HIV encephalopathy

Extrapulmonary cryptococcosis including meningitis

Disseminated nontuberculous mycobacteria infection

Progressive multifocal leukoencephalopathy

Chronic cryptosporidiosis

Chronic isosporiasis

Disseminated mycosis (histoplasmosis, coccidiomycosis)

Recurrent septicaemia (including nontyphoidal *Salmonella*)

Lymphoma (cerebral or B cell non-Hodgkin)

Invasive cervical carcinoma

Atypical disseminated leishmaniasis

Symptomatic HIV-associated nephropathy or HIV-associated cardiomyopathy

*Source:* Revised WHO clinical staging and immunological classification of HIV and case definition of HIV for surveillance. 2006.

### **b) Investigations:**

Baseline laboratory tests:

- ☐ Baseline screening of CD4 to determine eligibility for starting ART
- ☐ Baseline laboratory assessment, including CBC, ALT/AST, ALP, urinalysis
- ☐ For women: Annual PAP smear screening or acetic acid cervical screening at district health care facilities
- ☐ HBsAg and HCV screening for IDUs/those with transfusion-associated infections or elevated liver enzyme levels
- ☐ Any other relevant investigations (symptom-driven) and screening for TB at every visit
- ☐ Where routinely available, use VL every 6 months to detect viral replication.

**Table 6: CD4 monitoring and follow-up schedule**

CD4 count	Repeat at
< 350 and not on ART	3 months
> 350 and not on ART	6 months
on ART(any value)	6 months
> 500	Annual screening

*Note:* If the CD4 count is between 200 to 250 cells/mm<sup>3</sup> and the patient is not on ART; repeat CD4 assessment after 4 weeks and consider treatment in asymptomatic patients. See Table 13 for more details p19.

**c) Treatment:**



# RECOMMENDATIONS AT A GLANCE

<b>When to start</b>	All adolescents and adults including pregnant women with HIV infection and CD4 counts of $\leq 350$ cells/mm <sup>3</sup> , should start ART, regardless of the presence or absence of clinical symptoms. Those with severe or advanced clinical disease (WHO clinical stage 3 or 4) should start ART irrespective of their CD4 cell count.
<b>What to use in first-line therapy</b>	First-line therapy should consist of an NNRTI + two NRTIs, one of which should be zidovudine (AZT) or tenofovir (TDF). Countries should take steps to progressively reduce the use of stavudine (d4T) in first-line regimens because of its well-recognized toxicities.
<b>What to use in second-line therapy</b>	Second-line ART should consist of a ritonavir-boosted protease inhibitor (PI) plus two NRTIs, one of which should be AZT or TDF, based on what was used in first-line therapy. Ritonavir-boosted atazanavir (ATV/r) or lopinavir/ritonavir (LPV/r) are the preferred PIs.
<b>Laboratory monitoring</b>	All patients should have access to CD4 cell-count testing to optimize pre-ART care and ART management. HIVRNA (viral-load) testing is recommended to confirm suspected treatment failure. Drug toxicity monitoring should be symptom-directed.
<b>HIV/TB coinfection</b>	Irrespective of CD4 cell counts, patients coinfecting with HIV and TB should be started on ART as soon as possible after starting TB treatment.
<b>HIV/HBV coinfection</b>	Irrespective of CD4 cell counts or WHO clinical stage, patients who require treatment for HBV infection should start ART. First-line and second-line regimens for these individuals should contain TDF and either emtricitabine (FTC) or lamivudine (3TC).

## When to start antiretroviral therapy

Target population	2010 ART guideline	2006 ART guideline
HIV+ asymptomatic ARV-naive individuals	CD4 $\leq$ 350 cells/mm <sup>3</sup>	CD4 $\leq$ 200 cells/mm <sup>3</sup>
HIV+ symptomatic ARV-naive individuals	WHO clinical stage 2 if CD4 $\leq$ 350 cells/mm <sup>3</sup> <b>OR</b> WHO clinical stage 3 or 4 irrespective of CD4 cell count	WHO stage 2 or 3 and CD4 $\leq$ 200 cells/mm <sup>3</sup> WHO stage 3 if CD4 not available WHO stage 4 irrespective of CD4 cell count Consider treatment for WHO clinical stage 3 and CD4 cell count between 200 and 350 cells/mm <sup>3</sup>
HIV+ pregnant women	CD4 $\leq$ 350 cells/mm <sup>3</sup> irrespective of clinical symptoms <b>OR</b> WHO clinical stage 3 or 4 irrespective of CD4 cell count	WHO stage 1 or 2 and CD4 $\leq$ 200 cells/mm <sup>3</sup> WHO stage 3 and CD4 $\leq$ 350 cells/mm <sup>3</sup> WHO stage 4 irrespective of CD4 count
HIV/TB coinfection ARV-naive individuals	Presence of active TB disease, irrespective of CD4 cell count	Presence of active TB disease and CD4 $\leq$ 350 cells/mm <sup>3</sup> ART Initiation can be delayed if CD4 $\geq$ 200 cells/mm <sup>3</sup>
HIV/HBV coinfection ARV-naive individuals	Individuals who require treatment for their HBV infection*, irrespective of CD4 cell count	No specific recommendation

## What antiretroviral therapy to start

<b>Target population</b>	<b>2010 ART guideline</b>	<b>2006 ART guideline</b>
<b>HIV+ ARV-naive adults and adolescents</b>	No change, but in settings where d4T regimens are used as the principal option for starting ART a progressive plan to move towards AZT-based or TDF-based first-line regimens should be developed, based on an assessment of cost and feasibility	AZT or TDF + 3TC (or FTC) + EFV or NVP
<b>HIV+ pregnant women</b>	AZT preferred but TDF acceptable  EFV included as a NNRTI option (but do not initiate EFV during first trimester)  Benefits of NVP outweigh risks where CD4 count is 250–350 cells/mm <sup>3</sup>  In HIV+ women with prior exposure to MTCT regimens, see ART recommendations in section 13.2	AZT + 3TC + NVP
<b>HIV/TB coinfection</b>	No change  ART should be initiated as soon as possible in all HIV/TB-coinfected patients with active TB (within 8 weeks after the start of TB treatment)	AZT or TDF + 3TC (or FTC) + EFV
<b>HIV/HBV coinfection</b>	NNRTI regimens that contain both TDF + 3TC (or FTC) are required	TDF + 3TC (or FTC) + EFV

## Criteria for ART Initiation in specific populations

Target population	Clinical condition	Recommendation
Asymptomatic individuals (including pregnant women)	WHO clinical stage 1	Start ART if CD4 $\leq$ 350
Symptomatic individuals (including pregnant women)	WHO clinical stage 2	Start ART if CD4 $\leq$ 350
	WHO clinical stage 3 or 4	Start ART irrespective of CD4 cell count
TB and hepatitis B coinfections	Active TB disease	Start ART irrespective of CD4 cell count
	HBV infection requiring treatment*	Start ART irrespective of CD4 cell count

## Recommended second-line antiretroviral therapy

Target population	2010 ART guideline*		2006 ART guideline
HIV+ adults and adolescents	If d4T or AZT used in first-line therapy	TDF + 3TC (or FTC) + ATV/r or LPV/r	ABC + ddl or TDF+ ABC or ddl +3TC or TDF + 3TC (± AZT) plus ATV/r or FPV/r or IDV/r or LPV/r or SQV/r
	If TDF used in first-line therapy	AZT + 3TC (or FTC) + ATV/r or LPV/r	
HIV+ pregnant women	Same regimens as recommended for adults and adolescents		ABC + ddl or TDF+ ABC or ddl +3TC or TDF + 3TC (± AZT) plus LPV/r or NFV or SQV/r
HIV/TB coinfection	If rifabutin available (150 mg 3 times/ week)	Same regimens as recommended for adults	ABC + ddl or TDF+ ABC or ddl +3TC or TDF + 3TC (± AZT) plus LPV/r or SQV/r with adjusted dose of RTV (LPV/r 400 mg/400 mg twice a day or LPV/r 800 mg/200 mg twice a day or SQV/r 400 mg/400 mg twice a day)
	If rifabutin not available	Same NRTI backbones recommended for adults plus LPV/r or SQV/r with adjusted dose of RTV (LPV/r 400 mg/400 mg twice a day or LPV/r 800 mg/200 mg twice a day or SQV/r 400 mg/400 mg twice a day)	
HIV/HBV coinfection	AZT + TDF + 3TC (or FTC) + ATV/r or LPV/r		3TC- and/or TDF-containing regimens

\* ABC and ddl can be considered as backup options in case of AZT or TDF toxicity or if AZT or TDF are contraindicated.

## Preferred first-line ART in treatment-naive adults and adolescents

Target population	Preferred options	Comments
Adults and adolescents	AZT or TDF + 3TC or FTC + EFV or NVP	Select the preferred regimens applicable to the majority of PLHIV Use fixed-dose combinations
Pregnant women	AZT + 3TC + EFV or NVP	Do not initiate EFV during first trimester TDF acceptable option In HIV women with prior exposure to PMTCT regimens, see ART recommendations in Table 11
HIV/TB coinfection	AZT or TDF + 3TC or FTC + EFV	Initiate ART as soon as possible (within the first 8 weeks) after starting TB treatment NVP or triple NRTIs are acceptable options if EFV cannot be used
HIV/HBV coinfection	TDF + 3TC or FTC + EFV or NVP	Consider HBsAg screening before starting ART, especially when TDF is not the preferred first-line NRTI Use of two ARVs with anti-HBV activity required

### Triple NRTI option:

It is recommended that the triple nucleoside regimens AZT + 3TC + ABC or AZT + 3TC + TDF should be used for individuals who are unable to tolerate or have contraindications to NNRTI-based regimens, particularly in the following situations:

- ~ HIV/TB coinfection;
- ~ pregnant women;
- ~ chronic viral hepatitis B;
- ~ HIV-2 infection.

## WHEN TO SWITCH ART

Recommendations:

1. Where available, use viral load (VL) to confirm treatment failure.  
(Strong recommendation, low quality of evidence)
2. Where routinely available, use VL every 6 months to detect viral replication.  
(Conditional recommendation, low quality of evidence)
3. A persistent VL of >5000 copies/ml confirms treatment failure.  
(Conditional recommendation, low quality of evidence)
4. When VL is not available, use immunological criteria to confirm clinical failure.  
(Strong recommendation, moderate quality of evidence)

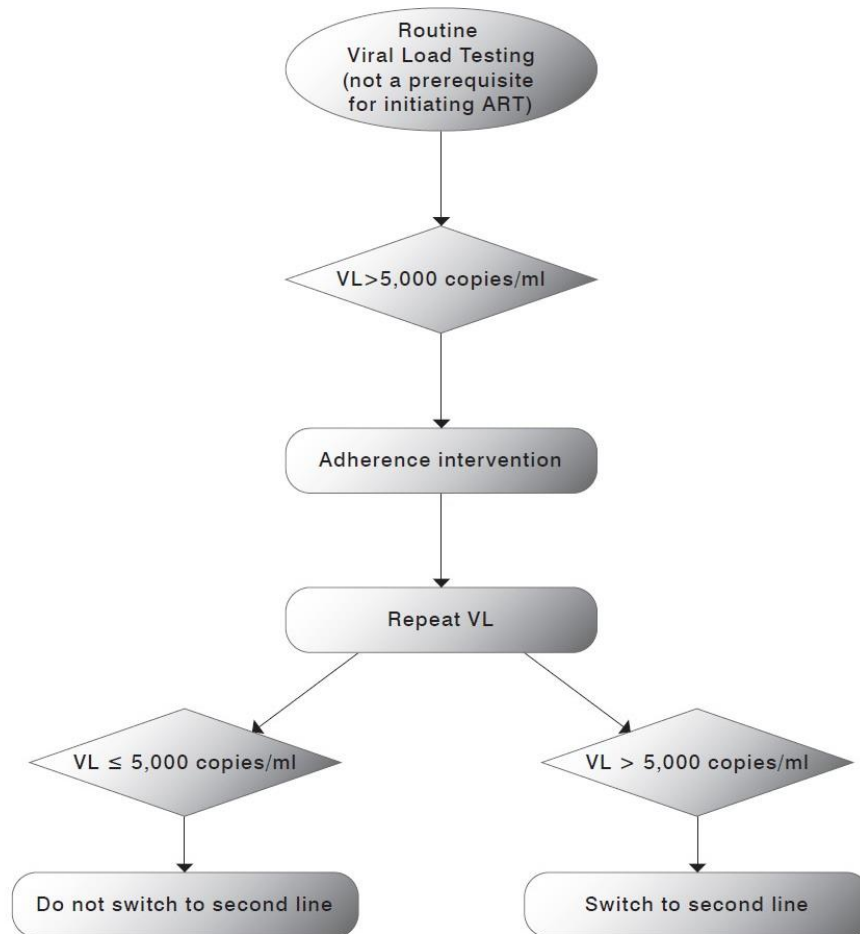
### ART switching criteria

Failure	Definition	Comments
<b>Clinical failure</b>	New or recurrent WHO stage 4 condition	Condition must be differentiated from immune reconstitution inflammatory syndrome (IRIS)  Certain WHO clinical stage 3 conditions (e.g. pulmonary TB, severe bacterial infections), may be an indication of treatment failure

<b>Immunological failure</b>	Fall of CD4 count to baseline (or below) OR 50% fall from on-treatment peak value OR Persistent CD4 levels below 100 cells/mm <sup>3</sup>	Without concomitant infection to cause transient CD4 cell decrease
<b>Virological failure</b>	Plasma viral load above 5000 copies/ml	The optimal viral load threshold for defining virological failure has not been determined. Values of >5 000 copies/ml are associated with clinical progression and a decline in the CD4 cell count

## Routine viral load strategy for failure and switching





*NOTE:* This algorithm also applies to the recommendation to check viral load 6 months after initiation of ART in women who have been exposed to sd-NVP for PMTCT.

### WHO does what? And Timelines

- Doctor: proper monitoring, evaluation and guiding ART
- Nurse: proper care support and treatment
- Technician:
  - ✓ Encourage use of standard reagents/ methodology and trained personnel
  - ✓ Monitor laboratory performance and evaluate quality control measures
  - ✓ Promote high standards of good laboratory practices
  - ✓ Stimulate performance improvement
  - ✓ Identify common error

### Further Reading / References:

1. WHO. Antiretroviral treatment working group treatment white paper. 2010.
2. Siegfried NL, Uthman OA, Rutherford GW. Optimal time for initiation of antiretroviral

therapy for asymptomatic, HIV-infected, treatment-naive adults. *Cochrane Database of Systematic Reviews 2010*.

3. Severe P, Pape J, Fitzgerald D, et al. *A randomized clinical trial of early versus standard antiretroviral therapy for HIV-infected patients with a CD4 cell count of 200-350 cells/mm<sup>3</sup> (CIPRATH-001)*. 49th Interscience Conference on Antimicrobial Agents and Chemotherapy, San Francisco, 2009

4. Sterne JA, May M, Costagliola D, de Wolf F, Phillips AN, Harris R, et al. Timing of initiation of antiretroviral therapy in AIDS-free HIV-1-infected patients: a collaborative analysis of 18 HIV Edathodu J, Ali B, Alrajhi AA. CD4 validation for the World Health Organization classification and clinical staging of HIV/AIDS in a developing country. *Int J Infect Dis* 2009;13(2):243-6.cohort studies. *Lancet* 2009;373(9672):1352-63.

5. Department of AIDS Control, Ministry of Health and Family Welfare. Annual report 2009-2010.

## **Abbreviations:**

3TC lamivudine

ABC abacavir

ATV atazanavir

AZT zidovudine (also known as ZDV)

d4T stavudine

ddl didanosine

DRV darunavir

EFV efavirenz

ETV etravirine

FTC emtricitabine

HRG high risk group

LPV lopinavir

LPV/r lopinavir/ritonavir

NFV nelfinavir

NVP nevirapine

RAL raltegravir

RBV ribavirin

RTV ritonavir

SQV saquinavir

# **IV catheter related blood stream infections ( CR-BSI)**

## **2. When to suspect/ recognize?**

Situation 1: Catheter-related blood stream infection (CRBSI): CRI is suspected when there is unexplained fever in a catheterized patient especially if fever can be linked to manipulation of the device, or if there are local inflammatory signs.

Situation 2: Catheter-related blood stream infection (CRBSI): Isolation of the same organism from a semiquantitative or quantitative culture of a catheter segment and from the blood (preferably drawn from a peripheral vein) of a patient with accompanying clinical symptoms of bloodstream infection without any other apparent source of infection.

2. **Incidence of the condition in our country:** Catheter-related bacteremia is the third most frequent cause of nosocomial bacteremia, with an incidence of 20 to 30%. The emergence of CRB is directly related to the duration of catheterization and ranges from 0.02 to 0.66 episodes of bacteremia per 100 days, according to the series and the type of catheter used.

## **3. Differential Diagnosis.**

Enteric fever

Endocarditis

Thrombophlebitis

Lymphangitis

Brucellosis

## **4. Prevention and counseling;**

1. Limit duration of IV catheter
2. Dedicated IV line team
3. Aseptic precautions
4. Glycemic control
5. Health education

**5. Optimal diagnostic criteria, investigations, treatment and referral criteria**

**Situation 1: At secondary hospital/ non-metro situation: Optimal standards of treatment in situations where technology and resources are limited.**

a) **Clinical Diagnosis:**

Fever with or without chills and inflammation or purulence around the intravascular device.

b) **Investigations:** 1. **Blood culture:** Isolation of pathogenic microorganisms with corresponding clinical features of blood stream infections.

2. **Rapid Diagnostic Technique:** Gram staining of catheter segments may be helpful for the diagnosis of the local infections.

c) **Treatment:**

a- In Patient: It is recommended to treat empirically with vancomycin and change to semisynthetic penicillin if the isolate is susceptible and third generation cephalosporins especially that active against pseudomonas should also be added. 14 days of treatment is recommended.

b- Out Patient: 1<sup>st</sup> generation cephalosporins ( Cefazolin) and Fluoroquinolones

**d) Referral criteria:**

1. Associated complications like thrombosis, endocarditis, osteomyelitis, metastatic seeding.
2. CR-BSI infections could not be ascertained.
3. No improvement after antimicrobial therapy.

**Situation 2: At super specialty facility in Metro location where higher-end technology is available.**

a) **Clinical Diagnosis:** Fever with or without chills and inflammation or purulence around the intravascular device.

b) **Investigations:**

***Cultures of Samples of IV Catheters:***

1. The most widely used laboratory technique for the diagnosis of Catheter related infections is the semi quantitative method in which the catheter segment is rolled across the surface of an agar plate and colony forming units (CFU) are counted after overnight incubation.
2. To obtain the quantitative culture of a catheter, the catheter segment either flushed with and then immersed in broth or placed in broth and vortexed/sonicated; quantitative cultures are done on the broth recovered from these procedures. The limitation of the quantitative and semi quantitative catheter methods is that they require removal of the catheter to aid in the diagnosis of CR-BSIs.

***Paired cultures of blood drawn percutaneously and through the IV catheter:***

An alternative for diagnosis of CR-BSI in patients where catheter removal is undesirable because of limited vascular access. These techniques rely on quantitative culture of paired blood samples, one obtained through the central catheter and other from peripheral venipuncture site. A colony count from the blood obtained from

the catheter that is five to ten folds greater than the colony count from the blood obtained from a peripheral vein has been predictive of CR-BSI.

**Differential time to positivity for CVC versus Peripheral blood culture:** This new method, which correlates well with quantitative blood cultures, makes use of continuous blood culture monitoring for positivity (radiometric method) and compares the differential time to positivity (DTP) for qualitative cultures of blood samples drawn from the catheter and a peripheral vein. **Infusate related blood stream infection:** Samples are taken from both infusate and separate percutaneous blood culture for diagnosis of infusate related blood stream infection.

b) **Treatment:** Management of intravascular device associated infection depends on several variables including; the type of infection, local or bacteremic; the organisms involved; the type of device, (peripheral or central catheters, totally implanted devices); and the severity of the illness of the patient.

**General guidelines:**

For nontunneled catheters

- Catheter should be removed in patients with CRBSIs if the infection is complicated with septic thrombosis, endocarditis, osteomyelitis. Systemic antibiotic therapy is to be continued for 4-6 weeks.
- In patients with uncomplicated CR-BSIs, catheter should be removed and systemic antibiotic therapy should be given for 5-7 days with coagulase negative staphylococcus infection and for 10-14 days in case of staphylococcus aureus and gram-negative bacilli.
- In case of fungal infection, catheter should be removed and antifungal therapy is to be continued for 14 days.

For tunneled catheters :

- Catheter should be removed if the infection is complicated with tunnel infection, port abscess, septic thrombosis, endocarditis and osteomyelitis.



- Catheter can be retained with uncomplicated *Staphylococcus aureus* and gram-negative bacilli infection under cover of 10-14 days of systemic antimicrobial therapy with antibiotic lock therapy. These devices should be removed if there is clinical deterioration, persisting or relapsing bacteremia.
- Salvage therapy for infected tunneled CVCs or IDs is not recommended for routine use in case of fungal infection because salvage rates with systemic fungal therapy and ALT for *Candida* species have been in the 30% range.

**Empirical therapy:**

- ☐ It is recommended to treat empirically with vancomycin and change to semisynthetic penicillin if the isolate is susceptible and third generation cephalosporins especially that active against *Pseudomonas* should also be added

**Specific therapy:**

Microorganism	Preferred antibacterial agents	Out Patient	In Patient
S.aureus ( MSSA)	Penicillinase resistant penicillins	Cloxacillin 25-100 mg/kg/day in 4 divided doses	Nafcillin or Oxacilin 2gm q4h
S. aureus ( MRSA)	Vancomycin or Quinopristine/Dalfopristine	Linezolid 600mg q12h	Linezolid, Vancomycin 1gm IV q12h
S.aureus ( VRSA)	Linezolid or Quinopristine/Dalfopristine	Linezolid 600mgq12h	Linezolid, Quinopristine/ Dalfopristine 7.5 mg/kg q8h
CoNS ( MSSE)	Penicillinase resistant penicillins	Cotrimoxazole 960 mg q 12h	Nafcillin or Oxacilin 2 gm q12h or Cotrimoxazole 960 mg q 12h
CoNS ( MRSE)	Vancomycin	Linezolid 600 mg q12h	Vancomycin 1gm q 12h

Enterococcus ( Amp Susceptible)	Ampicillin+ Gentamicin		Ampicillin 2g q4h -6h + Gentamicin 1mg/kg q 8h
Enterococcus ( Amp	Vancomycin or Vancomycin +	Linezolid 600 mg q	Vancomycin 1 gm IV

resistant)	Aminoglycoside	12h	q12 h+ Gentamicin 1mg/kg q 8h
Enterococcus( Vancomycin resistant)	Linezolid or Quinopristine+ Dalbapristine	Linezolid 600 mg q 12h	Linezolid 600 mg q 12h or Quinopristine+ Dalbapristine 7.5 mg/kg q8h
Gram Negative Bacilli ( non ESBL)	Third generation cephalosporins	Ciprofloxacin 750 mg q12h	Ceftriaxone 1-2g qd
Candida	Amphotericin B or Fluconazole	Fluconazole 400-600 mg qd	Amphotericin B 0.3mg/kg/d or Fluconazole 400-600 mg qd

**Further reading:**

1. Singhal A K, Mishra S and Bhatnagar S. Recent advances in Management of Intravascular Catheter Related Infections. Indian Journal Of Medical and Paediatric Oncology, Vol. 26 No.1, 2005.
2. Mandell G L, Benett J E, Dolin R. Principles and Practice of infectious diseases. 6<sup>th</sup> edition. Vol 1.

**Encephalitis**

3. **When to suspect/ recognize?** : Severe headache, high grade fever, altered consciousness, stiff neck & back
4. a) Introduction: Encephalitis is an acute inflammation of the brain. Encephalitis with meningitis is known as meningo encephalitis.

b) Case definition

For both situations of care (mentioned below\*)

Situation 1 Any person of any age admitted to hospital with encephalopathy (altered consciousness that persisted for longer than 24hr, including lethargy, irritability, or a change in personality and behavior) and with fever or history of fever ( $\geq 38^{\circ}\text{C}$ ) during the presenting illness; seizures and/or focal neurological findings (with evidence of brain parenchyma involvement); CSF pleocytosis (more than 4 four white blood cells per  $\mu\text{l}$ ).

Situation 2 Any person of any age admitted to hospital with encephalopathy (altered consciousness that persisted for longer than 24hr, including lethargy, irritability, or a change in personality and behavior) and with fever or history of fever ( $\geq 38^{\circ}\text{C}$ ) during the presenting illness; seizures and/or focal neurological findings (with evidence of brain parenchyma involvement); CSF pleocytosis (more than four white blood cells per  $\mu\text{l}$ ), electroencephalographic (EEG) findings indicative of encephalitis; and abnormal results of neuroimaging (CT or MRI) suggestive of encephalitis.

6. **Incidence of the condition in our country:** About 20 to 30% of all cases presented with fever
7. **Differential Diagnosis:** Malaria, Enteric fever, Dengue, Chikungunya, Leptospira, Urinary tract infection, PID, Tuberculosis, Meningitis or Encephalitis .
8. **Prevention and counseling:** Prompt distinction between causes of acute encephalitis is essential to direct appropriate management.
9. **Optimal diagnostic criteria, investigations, treatment and referral criteria**  
**Persistent fever in spite of treatment given for Malaria Enteric fever & tuberculosis, Dengue and Chikungunya**

**Situation 1: At secondary hospital/ non-metro situation: Optimal standards of treatment in situations where technology and resources are limited.**

a) **Diagnosis:** Adult patients have acute onset of fever, headache, confusion, sometimes seizures. Pediatric cases present with fever, irritability or loss of appetite. Stiff neck indicates that patient has either meningitis or meningoencephalitis.

b) **Investigations:** Whole Hemogram,

Peripheral smear (Malaria) and other blood parasite

Blood culture and widal for enteric fever

Sputum examination for Tuberculosis or other infection

X-ray chest (Lung infection or patches)

c) **Treatment:** It is usually symptomatic. Keep airway patent. Turn patient to side to avoid aspiration. Seizures can be controlled with diazepam. Acyclovir can be given for herpes simplex virus. In very sick patients mechanical ventilation may be done. Corticosteroids reduce brain inflammation and swelling. Sedatives are used to reduce irritability or restlessness.

Standard Operating procedure

a- In Patient: Day & night temperature recording chart

b- Out Patient

c- Day Care

d) Referral criteria: Refer as early as possible

**Situation 2: At super specialty facility in Metro location where higher-end technology is available.**

a) Clinical Diagnosis: Neurological examinations usually reveal a drowsy or confused patient. Stiff neck, due to the irritation of the meninges covering the brain, indicates that the patient has either meningitis or meningo-encephalitis

Examination of the [cerebrospinal fluid](#) obtained by a [lumbar puncture](#) procedure usually reveals increased amounts of protein and white blood cells with normal glucose, though in a significant percentage of patients, the cerebrospinal fluid may be normal. [CT scan](#) often is not helpful, as cerebral abscess is uncommon. Cerebral abscess is more common in patients with meningitis than encephalitis. Bleeding is also uncommon except in patients with [herpes simplex](#) type 1

encephalitis. [Magnetic resonance imaging](#) offers better resolution. In patients with herpes simplex encephalitis, electroencephalograph may show sharp waves in one or both of the temporal lobes. Lumbar puncture procedure is performed only after the possibility of prominent brain swelling is excluded by a CT scan examination. Diagnosis is often made with detection of antibodies in the cerebrospinal fluid against a specific viral agent (such as herpes simplex virus) or by [polymerase chain reaction](#) that amplifies the [RNA](#) or [DNA](#) of the virus responsible (such as [varicella zoster virus](#)).

b) Investigations: test for malaria, blood culture for Salmonella, Widal test, CSF examination, PCR for antigen detection

c) Treatment: Establish adequate airway, mechanical ventilation may be needed

Administer oxygen,

Maintain blood pressure,

Avoid fluid overload,

Avoid hypoglycemia,

Manage seizures,

Reduce raised intracranial tension,

Do urinary catheterization,

Standard Operating procedure

- a- In Patient
- b- Out Patient
- c- Day Care
- d) Referral criteria

**6. WHO does what? And Timelines**

- a- Doctor- ensure patency of airway, manage symptomatically
- b- Nurse- do suction regularly
- c- Technician

**10. Further Reading / References**

1. Granerod et al ,Lancet Infect Dis 2010; 10:835-44
2. Khetsuriani N et al, Clin Infect Dis 2002;35:175-82

**Resources required**

**For one patient**

**Procedure (Patient weight 60 KGS)**

**(Units to be specified for human resources, investigations, drugs and consumables and equipment. Quantity to also be specified)**

<b>Situation</b>	<b>Human Resources</b>	<b>Investigations</b>	<b>Drugs &amp; Consumables</b>	<b>Equipment</b>
1	One doctor, one nurse	Tests for malaria,typhoid,s erology		
2	One doctor, one nurse,	Above with		
	one technician	Serology and PCR		



# Infectious Diarrhoea

## Case Definition

### ACUTE INFECTIOUS DIARRHOEA

#### Clinical Case Description:

Acute watery diarrhoea (passage of 3 or more loose or watery stools in the past 24 hours) due to an infectious etiology, often accompanied by symptoms of nausea, vomiting, or abdominal cramps presenting with or without dehydration.

“Acute diarrhea” is an episode of diarrhea of <14 days in duration.

“Persistent diarrhea” is diarrhea of 14 days in duration.

### ACUTE INFECTIOUS DYSENTERY

#### Clinical case description:

Acute infective diarrhoea with visible blood in the stool.

#### 11. Incidence of the condition in our country

Number of episodes of diarrhoea in each year - About 2 million.

Death due to diarrhoea among children < 5 years - 1.87 million.

No. of episodes of diarrhoea in a child less than 5 years of age - 2-3.

## 12. Differential Diagnosis

1. Irritable bowel syndrome,
2. Inflammatory bowel diseases
3. Ischemic bowel disease
4. Partial bowel obstruction
5. Pelvic abscess in the area of the rectosigmoid colon
6. Pernicious anemia
7. Pellagra
8. Malaria
9. Whipple's disease
10. Diabetes mellitus
11. Small bowel scleroderma
12. Small bowel diverticulosis
13. Malabsorption syndrome

## 13. Prevention and counseling

Water, sanitation, and hygiene:

É Safe water

É Sanitation: houseflies can transfer bacterial pathogens

É Hygiene: hand washing

Safe food:

É Cooking eliminates most pathogens from foods

É Exclusive breastfeeding for infants

É Weaning foods are vehicles of enteric infection

Micronutrient supplementation: the effectiveness of this depends on the child's overall immunologic and nutritional state; further research is needed.

Vaccines:

É Measles immunization can substantially reduce the incidence and severity of diarrheal diseases. Every infant should be immunized against measles at the recommended age.

#### 14. Optimal diagnostic criteria, investigations, treatment and referral criteria

Situation 1: At secondary hospital/ non-metro situation: Optimal standards of treatment in situations where technology and resources are limited.

### Classifications of diarrhea episodes

Acute diarrhea	<ul style="list-style-type: none"><li>• Presence of three or more loose, watery stools within 24-hours</li></ul>
Dysentery	<ul style="list-style-type: none"><li>• Bloody diarrhea, visible blood and mucous present</li></ul>
Persistent diarrhea	<ul style="list-style-type: none"><li>• Episodes of diarrhea lasting more than 14 days</li></ul>

### Linking the main symptoms to the causes of acute diarrhea.

Fever	<ul style="list-style-type: none"><li>• Common and associated with invasive pathogens.</li></ul>
Bloody stools	<ul style="list-style-type: none"><li>• Invasive and cytotoxin releasing pathogens</li><li>• Suspect EHEC infection in the absence of fecal leukocytes</li><li>• Not with viral agents and enterotoxins releasing bacteria</li></ul>
Vomiting	<ul style="list-style-type: none"><li>• Frequently in viral diarrhea and illness caused by ingestion of bacterial toxins (eg, <i>S. aureus</i>)</li></ul>

## Evaluation of the acute diarrhea patient.

History	Physical examination	Assess dehydration
<ul style="list-style-type: none"> <li>•onset, frequency, quantity</li> <li>•character - bile/blood/mucus</li> <li>•vomiting</li> <li>•past medical history, underlying medical conditions</li> <li>•epidemiological clues</li> </ul>	<ul style="list-style-type: none"> <li>•body weight</li> <li>•temperature</li> <li>•heart &amp; respiratory rate</li> <li>•blood pressure</li> </ul>	<ul style="list-style-type: none"> <li>•general appearance, alertness</li> <li>•pulse and blood pressure</li> <li>•postural hypotension</li> <li>•mucous membranes and tears</li> <li>•sunken eyes, skin turgor</li> <li>•capillary refill, jugular venous pressure</li> <li>•sunken fontanelle</li> </ul>

## Levels of dehydration in children with acute diarrhea

No dehydration	Mild dehydration ( <u>≥2</u> signs)	Severe dehydration ( <u>&gt; 2</u> signs)
alertness normal	restless or irritable	abnormally sleepy or lethargic
no sunken eyes	sunken eyes	sunken eyes
normal drinking	drinks eagerly	drinking poorly or not at all
immediate skin pinch	slow skin pinch (<2 seconds)	very slow skin pinch (>2 seconds)

**Signs of dehydration in adults:**

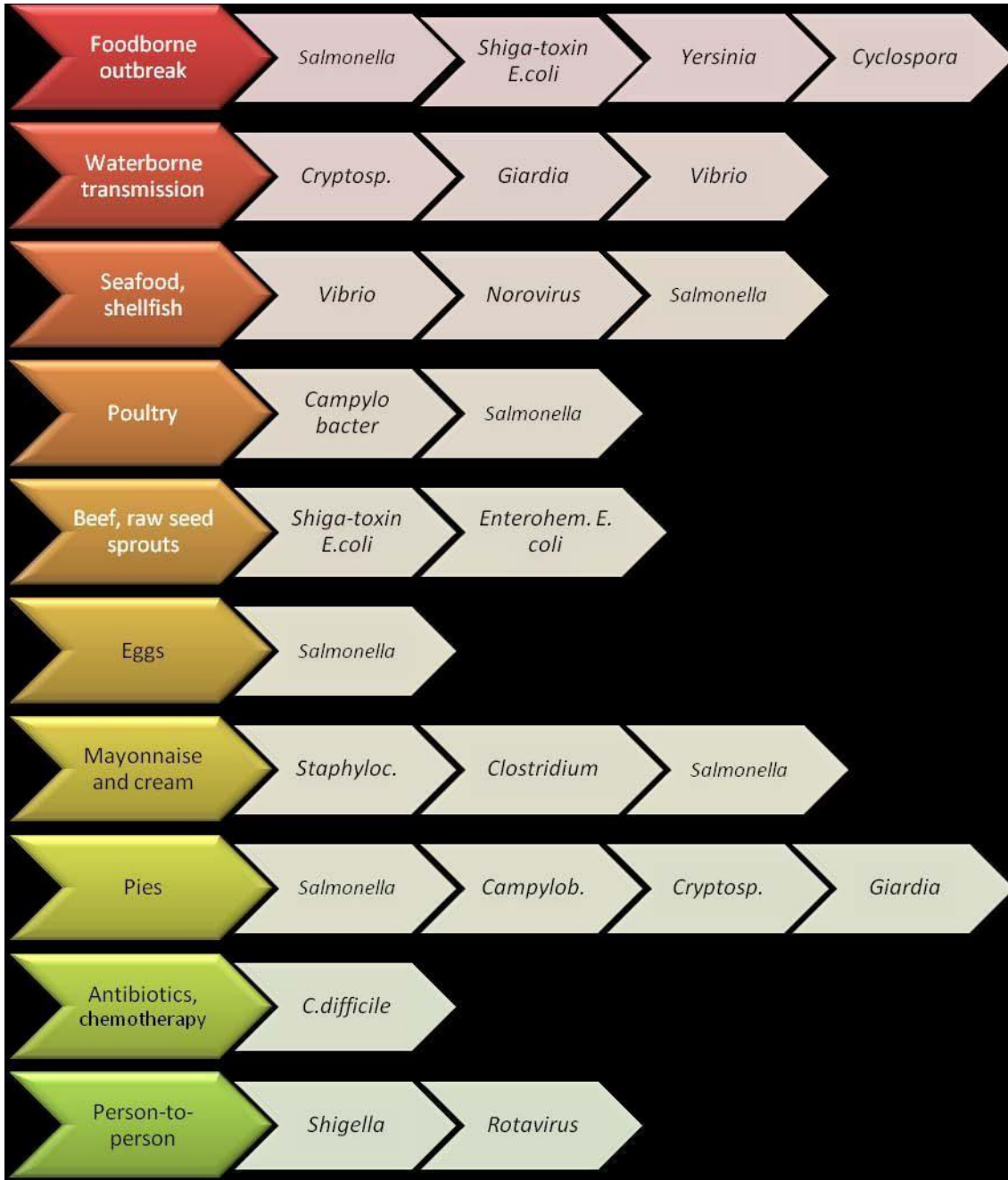
ÉPulse rate > 90/ min

ÉPostural hypotension

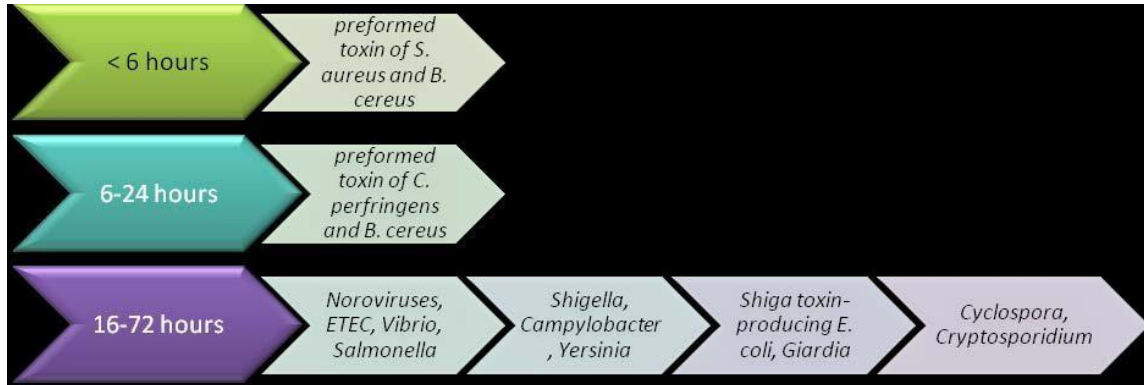
ÉSupine hypotension and absence of palpable pulse

- ÉDry tongue
- ÉSunken eyeballs
- ÉSkin pinch

**Patient history details and causes of acute diarrhea**



## The incubation period and likely causes of diarrhea.



## Investigations

1. Fecal leucocyte examination
2. Wet mount for parasitic infection

# TREATMENT

## Rehydration

**Oral rehydration therapy (ORT)** is the administration of fluid by mouth to prevent or correct dehydration that is a consequence of diarrhea. ORT is the standard for efficacious and cost-effective management of acute gastroenteritis.

**Oral rehydration solution (ORS)** is the fluid specifically developed for ORT.

ORT consists of:

- “ Rehydration - water and electrolytes are administered to replace losses.
- “ Maintenance fluid therapy (along with appropriate nutrition).

Contraindication of ORT

Children with hemodynamic shock



with abdominal ileus

Nasogastric administration of ORS is an alternative for children who are unable to tolerate ORS via the oral route (with persistent vomiting),

<b>Oral rehydration solution(ORS) constituents</b>	<b>mmol/L</b>
Sodium	75
Chloride	65
Glucose (Anhydrous)	75
Potassium	20
Citrate	10
-----	- -
Total osmolarity	245

### **Supplemental zinc therapy, multivitamins, and minerals**

Micronutrient supplementation · Supplementation treatment with zinc (20 mg per day until the diarrhea ceases)

Children with persistent diarrhea should receive supplementary multivitamins and minerals each day for 2 weeks at least two recommended daily allowances (RDAs) of folate, vitamin A, zinc, magnesium, and copper.

As a guide, one RDA for a child aged 1 year is

Folate	50	g
Zinc	20	mg
Vitamin A	400	g
Copper	1	mg
Magnesium	80	mg

## Diet

The practice of withholding food for > 4 hours is inappropriate.

Food should be started 4 hours after starting ORT or intravenous fluid. The notes below apply to adults and children unless age is specified.

Give:

- “ An age-appropriate diet · regardless of the fluid used for ORT/maintenance
- “ Infants require more frequent breastfeedings or bottle feedings · special formulas or dilutions unnecessary
- “ Older children should be given appropriately more fluids
- “ Frequent, small meals throughout the day (six meals/day)
- “ Energy and micronutrient-rich foods (grains, meats, fruits, and vegetables)
- “ Increasing energy intake as tolerated following the diarrheal episode

Avoid:

- “ Canned fruit juices · these are hyperosmolar and can aggravate diarrhea.

## Antimicrobials

### INDICATIONS

- Children with bloody diarrhea (most likely shigellosis)
- Suspected cholera with severe dehydration
- Serious nonintestinal infections (e.g., pneumonia)
- Diarrhea due to *Giardia*, *Entamoeba histolytica*.

In adults, the clinical benefit should be weighed against the cost, the risk of adverse reactions, harmful eradication of normal intestinal flora, the induction of Shiga toxin production, and the increase of antimicrobial resistance.

Antimicrobials are the drugs of choice for empirical treatment of traveler's diarrhea and of community-acquired secretory diarrhea when the pathogen is known.

Considerations with regard to antimicrobial treatment:

“ Consider antimicrobial treatment for:

- Persistent *Shigella*, *Salmonella*, *Campylobacter*, or parasitic infections. · Infections in the aged, immunocompromised patients, and patients with impaired resistance, sepsis, or with prostheses.
- Moderate/severe traveler’s diarrhea or diarrhea with fever and/or with bloody stools · quinolones (co-trimoxazole second choice).

“ Nitazoxanide is an antiprotozoal and may be appropriate for diarrhea due to *Cryptosporidium*.

### Antimicrobial agents for the treatment of specific causes of diarrhea.

<b>SYNDROME</b>	<b>PREFERRED ANTIBIOTICS</b>	<b>ALTERNATIVE ANTIBIOTICS</b>
<i>cholera</i>	Doxycycline Adult: 300 mg OD  or  Tetracycline: 500 mg 4 times a day for 3 days	Azithromycin Ciprofloxacin

<i>Shigellosis</i>	Ciprofloxacin Children: 15 mg/kg 2 times a day for 3 days Adults: 500 mg 2 times a day for 3 days	Ceftriaxone Children: 50 – 100 mg/kg once a day IM f 2 to 5 days
--------------------	---	---

<i>Amoebiasis</i>	Metronidazole Children: 10 mg/kg 3 times a day for 5 days* Adults: 750 mg 3 times a day for 5 days* *10 days for severe disease	
<i>Giardiasis</i>	Metronidazole ** Children: 5 mg/kg 3 times a day for 5 days Adults: 250 mg 3 times a day for 5 days	
<i>Campylobacter</i>	Quinolones	Azithromycin

\*\* Tinidazole can also be given in a single dose (50 mg/kg orally; maximum dose 2g). Ornidazole can be used in accordance with the manufacturers recommendations.

- Azithromycin
  - Adult - 250 mg or 500 mg once daily for 3. 5 days.
  - Children - 5 mg to 20 mg per kilogram of body weight per day, once daily for 3. 5 days.
- Treatment for amebiasis should, ideally, include diloxanide furoate following the metronidazole, to get rid of the cysts that may remain after the metronidazole treatment.
- Selection of an antimicrobial should be based on the sensitivity patterns of strains of *Vibrio cholerae* O1 or O139, or *Shigella* recently isolated in the area.
- An antimicrobial is recommended for patients older than 2 years with suspected cholera and severe dehydration.
- Alternative antimicrobials for treating cholera in children are TMP-SMX (5 mg/kg TMP + 25 mg/kg SMX, b.i.d. for 3 days), furazolidone (1.25 mg/kg, q.i.d. for 3 days), and norfloxacin. The actual selection of an antimicrobial will depend on the known resistance/sensitivity pattern of *V. cholerae* in the region.

## CLINICAL PRACTICE

### Approach in adults with acute diarrhea

### Perform initial assessment

- Dehydration
- Duration (>1 day)
- Inflammation (indicated by fever, bloody stool, tenesmus)

### Provide symptomatic treatment

- Rehydration
- Treatment of symptoms (if necessary consider bismuth subsalicylate or loperamide if diarrhea is not inflammatory or bloody)

### Stratify subsequent management

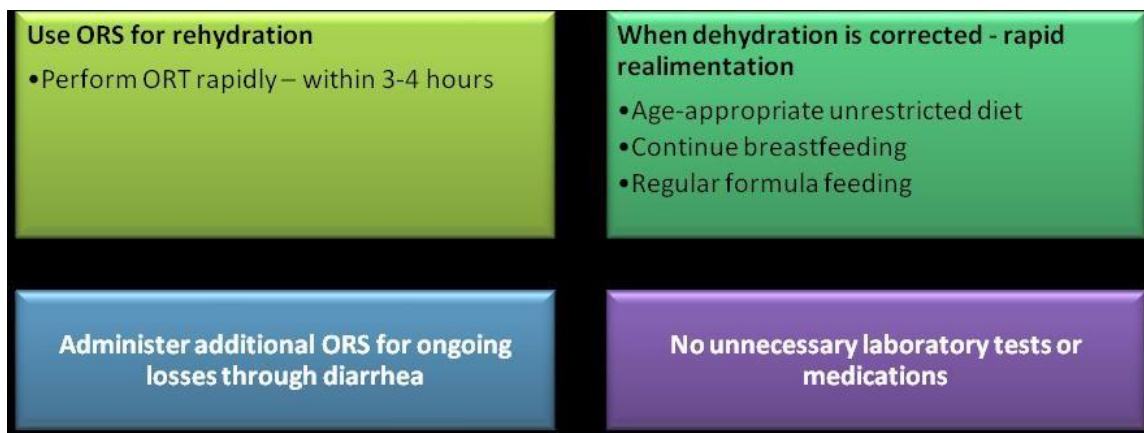
- Epidemiological clues: food, antibiotics, sexual activity, travel, day-care attendance, other illness, outbreaks, season
- Clinical clues: bloody diarrhea, abdominal pain, dysentery, wasting, fecal inflammation

### Obtain fecal specimen for analysis

- If severe, bloody, inflammatory, or persistent diarrhea or if outbreak suspected

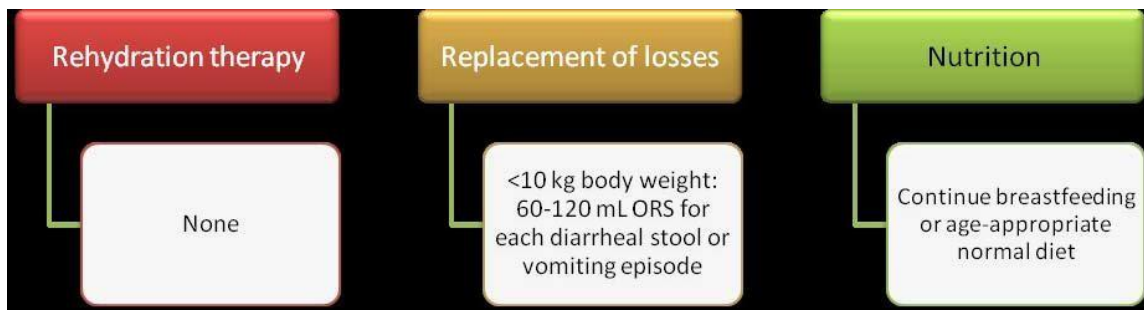
Consider antimicrobial therapy for specific pathogens

**Principles of appropriate treatment for children with diarrhea and dehydration.**



**Treatment for children based on the degree of dehydration.**

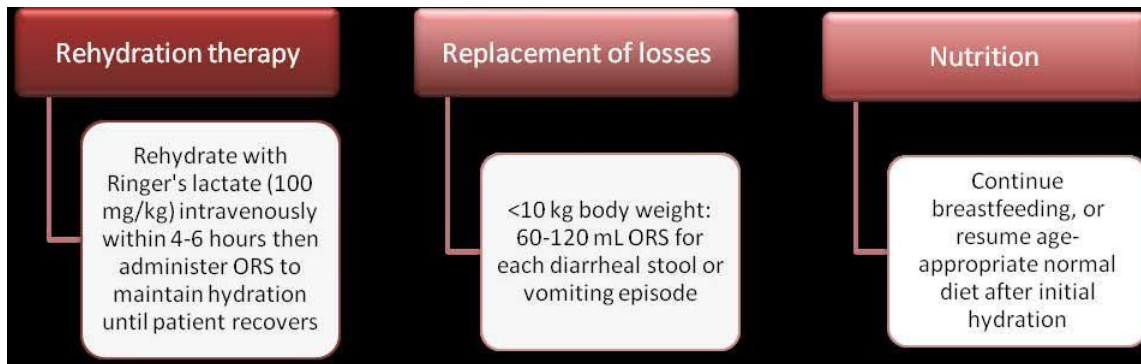
**Minimal or no dehydration.**



**Treatment for children based on the degree of dehydration (Mild to moderate dehydration). Note: if vomiting is persistent, the patient (child or adult) will not take ORS and is likely to need intravenous fluids.**

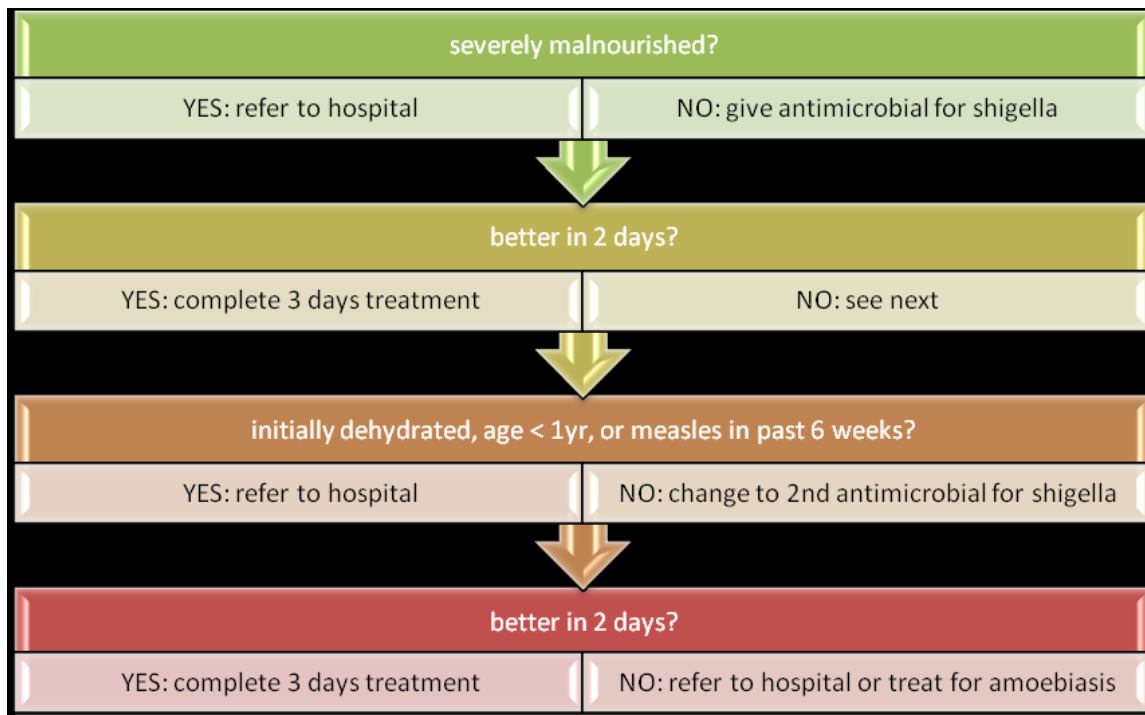


**Treatment for children based on the degree of dehydration Severe dehydration.**



**Therapeutic approach to acute bloody diarrhea (dysentery) in children**





## Referral criteria:

1. Persistent diarrhea
2. Irritable bowel syndrome
3. Inflammatory bowel disease
4. Malabsorption syndrome
5. Reiter's syndrome (reactive arthritis)
6. Hemolytic-uremic syndrome
7. Child with dysentery who is initially dehydrated, age <1 year or measles in past 6 weeks.



Situation 2: At super specialty facility in Metro location where higher-end technology is available.

a) Clinical Diagnosis:

## Classifications of diarrhea episodes

Acute diarrhea	<ul style="list-style-type: none"><li>• Presence of three or more loose, watery stools within 24-hours</li></ul>
Dysentery	<ul style="list-style-type: none"><li>• Bloody diarrhea, visible blood and mucous present</li></ul>
Persistent diarrhea	<ul style="list-style-type: none"><li>• Episodes of diarrhea lasting more than 14 days</li></ul>

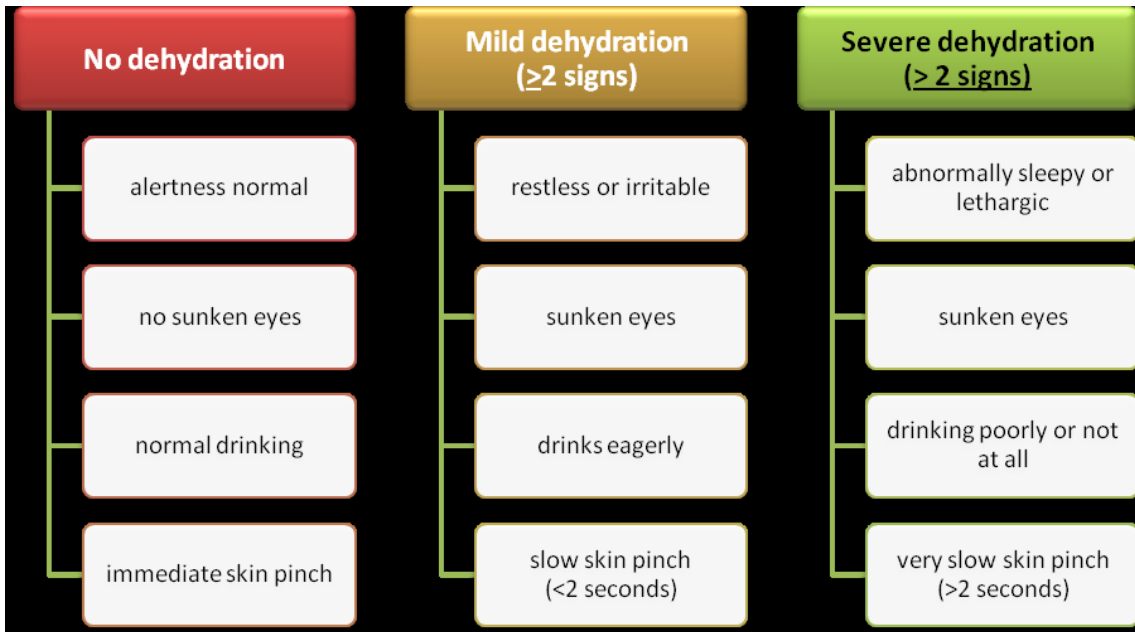
**Linking the main symptoms to the causes of acute diarrhea.**

Fever	<ul style="list-style-type: none"> <li>•Common and associated with invasive pathogens.</li> </ul>
Bloody stools	<ul style="list-style-type: none"> <li>•Invasive and cytotoxin releasing pathogens</li> <li>•Suspect EHEC infection in the absence of fecal leukocytes</li> <li>•Not with viral agents and enterotoxins releasing bacteria</li> </ul>
Vomiting	<ul style="list-style-type: none"> <li>•Frequently in viral diarrhea and illness caused by ingestion of bacterial toxins (eg, <i>S. aureus</i>)</li> </ul>

### Evaluation of the acute diarrhea patient.

History	Physical examination	Assess dehydration
<ul style="list-style-type: none"> <li>•onset, frequency, quantity</li> <li>•character - bile/blood/mucus</li> <li>•vomiting</li> <li>•past medical history, underlying medical conditions</li> <li>•epidemiological clues</li> </ul>	<ul style="list-style-type: none"> <li>•body weight</li> <li>•temperature</li> <li>•heart &amp; respiratory rate</li> <li>•blood pressure</li> </ul>	<ul style="list-style-type: none"> <li>•general appearance, alertness</li> <li>•pulse and blood pressure</li> <li>•postural hypotension</li> <li>•mucous membranes and tears</li> <li>•sunken eyes, skin turgor</li> <li>•capillary refill, jugular venous pressure</li> <li>•sunken fontanelle</li> </ul>

### Levels of dehydration in children with acute diarrhea



**Signs of dehydration in adults:**

ÉPulse rate > 90

ÉPostural hypotension

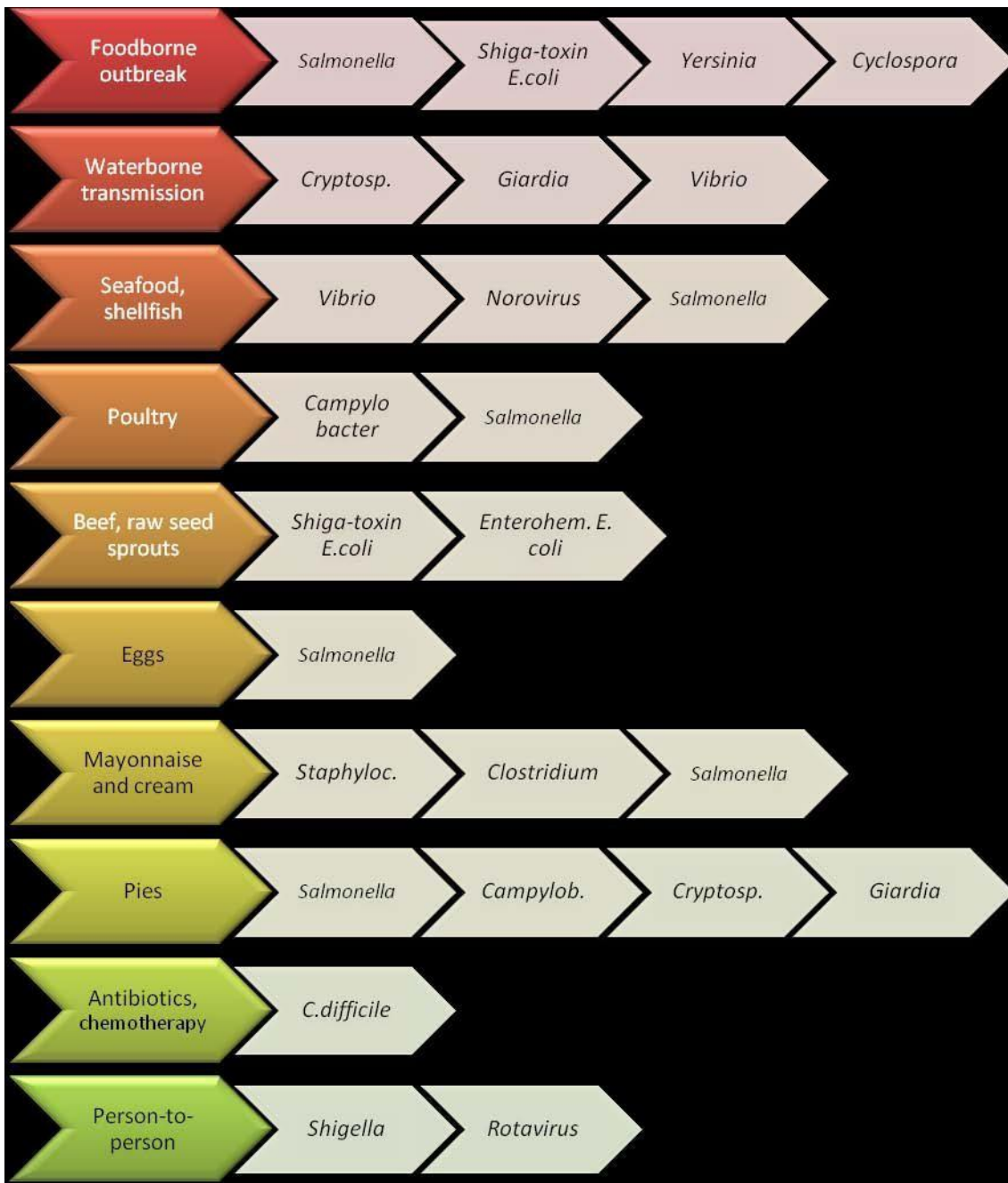
ÉSupine hypotension and absence of palpable pulse

ÉDry tongue

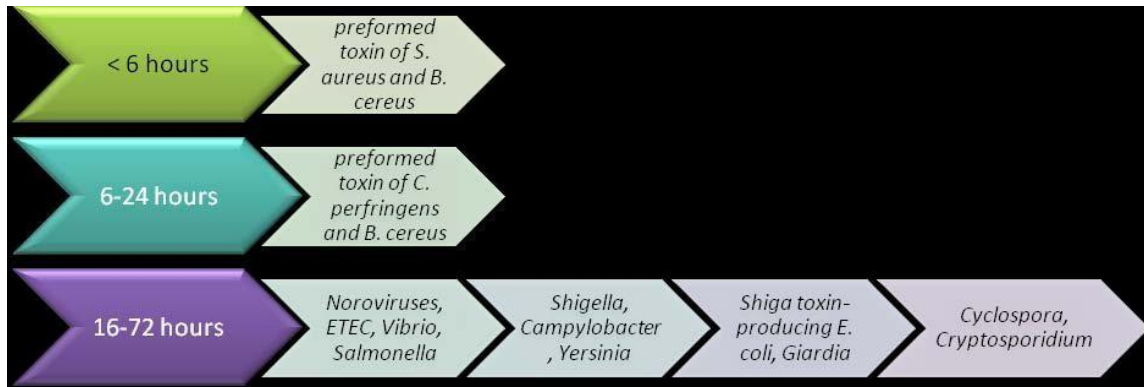
ÉSunken eyeballs

ÉSkin pinch

**Patient history details and causes of acute diarrhea**



## The incubation period and likely causes of diarrhea.



## Investigations:

1. Routine stool culture
2. Detection of E. coli enterotoxin
3. Detection of Rota virus antigen(Latex agglutination)
4. RT- PCR and specific antigen detection ELISA for Noro virus.
5. Special staining to detect Cryptosporidium
6. C.difficile toxin detection by rapid immunoassay or Latex agglutination test.
7. Flexible sigmoidoscopy with biopsies and upper endoscopy with duodenal aspirates and biopsies
8. Sigmoidoscopy
9. Colonoscopy
10. Abdominal CT scanning

# Treatment

## Rehydration

**Oral rehydration therapy (ORT)** is the administration of fluid by mouth to prevent or correct dehydration that is a consequence of diarrhea. ORT is the standard for efficacious and cost-effective management of acute gastroenteritis.

**Oral rehydration solution (ORS)** is the fluid specifically developed for ORT.

ORT consists of:

“ Rehydration - water and electrolytes are administered to replace losses.

“ Maintenance fluid therapy (along with appropriate nutrition).

Contraindication of ORT

Children with hemodynamic shock

with abdominal ileus

Nasogastric administration of ORS is an alternative for children who are unable to tolerate ORS via the oral route (with persistent vomiting),

**Oral rehydration solution(ORS) constituents mmol/L**



Sodium	75
Chloride	65
Glucose (Anhydrous)	75
Potassium	20
Citrate	10
-----	-----
Total osmolarity	245

### Supplemental zinc therapy, multivitamins, and minerals

Micronutrient supplementation · Supplementation treatment with zinc (20 mg per day until the diarrhea ceases)

Children with persistent diarrhea should receive supplementary multivitamins and minerals each day for 2 weeks at least two recommended daily allowances (RDAs) of folate, vitamin A, zinc, magnesium, and copper.

As a guide, one RDA for a child aged 1 year is

Folate	50	g
Zinc	20	mg
Vitamin A	400	g
Copper	1	mg
Magnesium	80	mg

### Diet

The practice of withholding food for > 4 hours is inappropriate.

Food should be started 4 hours after starting ORT or intravenous fluid. The notes below apply to adults and children unless age is specified.

Give:

- " An age-appropriate diet · regardless of the fluid used for ORT/maintenance
- " Infants require more frequent breastfeedings or bottle feedings · special formulas or dilutions unnecessary
- " Older children should be given appropriately more fluids
- " Frequent, small meals throughout the day (six meals/day)
- " Energy and micronutrient-rich foods (grains, meats, fruits, and vegetables)
- " Increasing energy intake as tolerated following the diarrheal episode

Avoid:

- " Canned fruit juices · these are hyperosmolar and can aggravate diarrhea.

## Antimicrobials

### INDICATIONS

- Children with bloody diarrhea (most likely shigellosis)
- Suspected cholera with severe dehydration
- Serious nonintestinal infections (e.g., pneumonia)
- Diarrhea due to *Giardia*, *Entamoeba histolytica* and *Cryptosporidium*

In adults, the clinical benefit should be weighed against the cost, the risk of adverse reactions, harmful eradication of normal intestinal flora, the induction of Shiga toxin production, and the increase of antimicrobial resistance.

Antimicrobials are to be considered the drugs of choice for empirical treatment of traveler's diarrhea and of community-acquired secretory diarrhea when the pathogen is known.

Considerations with regard to antimicrobial treatment:

“ Consider antimicrobial treatment for:

- Persistent *Shigella*, *Salmonella*, *Campylobacter*, or parasitic infections. · Infections in the aged, immunocompromised patients, and patients with impaired resistance, sepsis, or with prostheses.
- Moderate/severe traveler’s diarrhea or diarrhea with fever and/or with bloody stools · quinolones (co-trimoxazole second choice).

“ Nitazoxanide is an antiprotozoal and may be appropriate for diarrhea due to *Cryptosporidium*

### Antimicrobial agents for the treatment of specific causes of diarrhea.

SYNDROME	PREFERRED ANTIBIOTICS	ALTERNATIVE ANTIBIOTICS
<i>cholera</i>	Doxycycline Adult: 300 mg OD  or  Tetracycline: 500 mg 4 times a day for 3 days	Azithromycin Ciprofloxacin
<i>Shigellosis</i>	Ciprofloxacin Children: 15 mg/kg 2 times a day for 3 days Adults: 500 mg 2 times a day for 3 days	Ceftriaxone Children: 50 – 100 mg/kg once a day IM f 2 to 5 days
<i>Amoebiasis</i>	Metronidazole Children: 10 mg/kg 3 times a day for 5 days*	

	Adults: 750 mg 3 times a day for 5 days* *10 days for severe disease	
<i>Giardiasis</i>	Metronidazole ** Children: 5 mg/kg 3 times a day for 5 days Adults: 250 mg 3 times a day for 5 days	
<i>Campylobacter</i>	Quinolones	Azithromycin

\*\* Tinidazole can also be given in a single dose (50 mg/kg orally; maximum dose 2g).

- Azithromycin
  - Adult - 250 mg or 500 mg once daily for 3. 5 days.
  - Children - 5 mg to 20 mg per kilogram of body weight per day, once daily for 3. 5 days.
- Treatment for amebiasis should, ideally, include diloxanide furoate following the metronidazole, to get rid of the cysts that may remain after the metronidazole treatment.
- Selection of an antimicrobial should be based on the sensitivity patterns of strains of *Vibrio cholerae* O1 or O139, or *Shigella* recently isolated in the area.
- An antimicrobial is recommended for patients older than 2 years with suspected cholera and severe dehydration.
- Alternative antimicrobials for treating cholera in children are TMP-SMX (5 mg/kg TMP + 25 mg/kg SMX, b.i.d. for 3 days), furazolidone (1.25 mg/kg, q.i.d. for 3 days), and norfloxacin. The actual selection of an antimicrobial will depend on the known resistance/sensitivity pattern of *V. cholerae* in the region.

## CLINICAL PRACTICE

### Approach in adults with acute diarrhea

### Perform initial assessment

- Dehydration
- Duration (>1 day)
- Inflammation (indicated by fever, bloody stool, tenesmus)

### Provide symptomatic treatment

- Rehydration
- Treatment of symptoms (if necessary consider bismuth subsalicylate or loperamide if diarrhea is not inflammatory or bloody)

### Stratify subsequent management

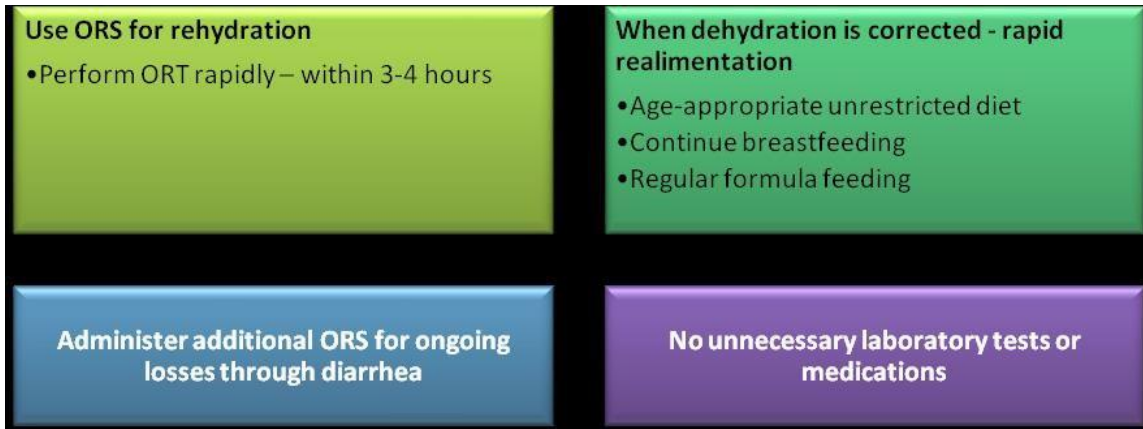
- Epidemiological clues: food, antibiotics, sexual activity, travel, day-care attendance, other illness, outbreaks, season
- Clinical clues: bloody diarrhea, abdominal pain, dysentery, wasting, fecal inflammation

### Obtain fecal specimen for analysis

- If severe, bloody, inflammatory, or persistent diarrhea or if outbreak suspected

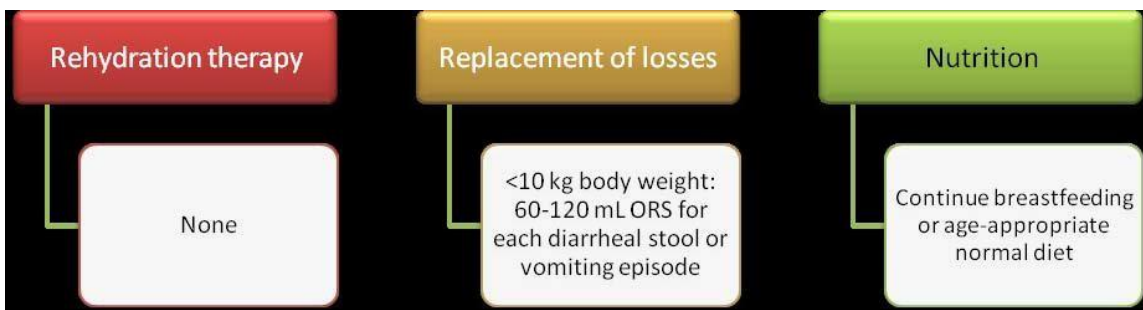
Consider antimicrobial therapy for specific pathogens

**Principles of appropriate treatment for children with diarrhea and dehydration.**



**Treatment for children based on the degree of dehydration.**

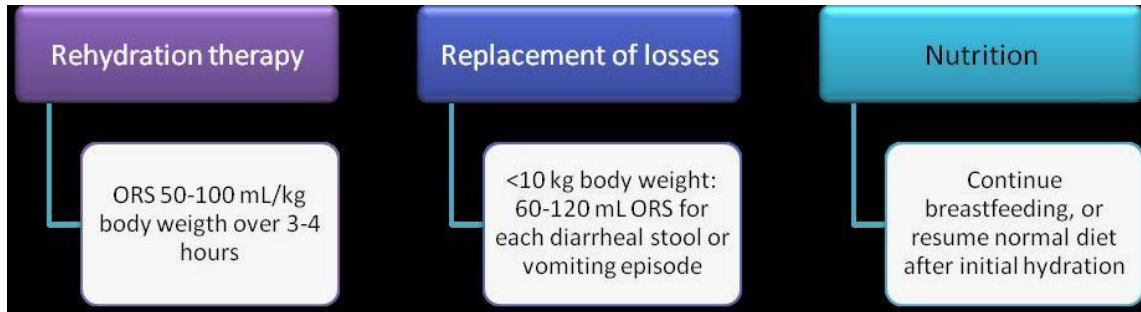
**Minimal or no dehydration.**



**Treatment for children based on the degree of dehydration**

## Mild to moderate dehydration

**Note: if vomiting is persistent, the patient (child or adult) will not take ORS and is likely to need intravenous fluids.**



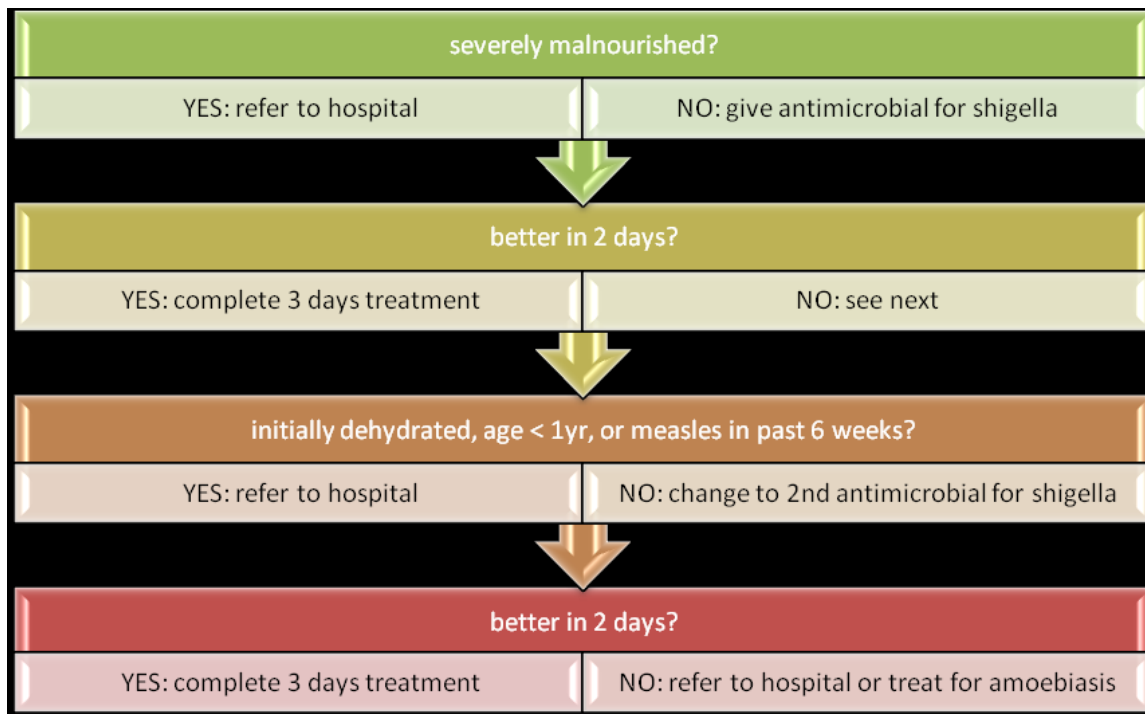
### Treatment for children based on the degree of dehydration

**Severe dehydration.**



### Therapeutic approach to acute bloody diarrhea (dysentery) in children





## Further Reading / References

1. World Gastroenterology Organisation practice guideline, Acute diarrhea, 2008
2. *Harrison's Principles of Internal Medicine*, 17th Edition, Chapter 40
3. *Harrison's Principles of Internal Medicine*, 17th Edition, Chapter 122
4. Guerrant RL, Gilder TV, Steiner TS, Thielman NM, Slutsker L, Tauxe RV, Hennessy T, Griffin PM, DuPont H, Sack RB, Tarr P, Neill M, Nachamkin I, Reller LB, Osterholm MT, Bennish ML, Pickering LK: IDSA Practice Guidelines for the Management of Infectious Diarrhea, *Clinical Infectious Diseases* 2001; 32:331. 50
5. Park K, Park S text book of preventive and social medicine, 20<sup>th</sup> ed, M/s Banarsidas Bhanot, India, 2009

## **Name of the condition: Hepatitis B**

### **Introduction**

Hepatitis B infection is common and potentially fatal problem and is common all over the world, particularly the developing countries where routine vaccination against this disease is not done in the childhood. It can lead to acute and chronic liver disease, fulminant hepatic failure and cirrhosis is liver, which may be complicated by liver cancer in the long-run. Hepatitis B is a preventable disease. If detected early, it can be treated also.

### **Case definition**

A clinical case of acute viral hepatitis is an acute illness that includes the discrete onset of symptoms and jaundice or elevated serum aminotransferase levels (>2.5 times the upper limit of normal)

A confirmed case of hepatitis B is a suspected case that is laboratory confirmed: HBsAg positive or anti-HBc-IgM positive.

Chronic HBV infection is defined as HBsAg positive irrespective of presence or absence of any symptom.

### **Prevalence of hepatitis B in our country**

India is in intermediate endemicity zone for hepatitis B. Frequency varies from 4-8% of population studied in various reports.

### **Differential diagnosis**

#### Acute hepatitis

Acute hepatitis due to other hepatotropic viral infection such as hepatitis A and hepatitis E may mimic hepatitis B before tests for the later in undertaken.

#### Chronic liver disease

Chronic liver disease due to other causes such as that due to alcohol, hepatitis C virus, non-alcoholic steatohepatitis and auto-immune liver disease. In fact, hepatitis B infection may co-exist with some of the other etiologies.

## Prevention and counseling

1. Mass vaccination of new-born and children is useful to prevent the infection. Though some authorities advocate selective vaccination of offspring born to infected mother, this strategy is unlikely to control the infection as some of the carrier get the infection later in life. Also, universal vaccination of all new-born is found to be more cost-effective.
2. Vaccination of family members and contacts of infected persons.
3. Vaccination of individuals at high risk to acquire hepatitis B such as those on dialysis, health care workers, intravenous drug addicts, those requiring multiple transfusions of blood and blood products such as hemophiliacs, thalasseemics, persons with multiple sexual partners etc.

## Optimal diagnostic criteria

Optimal tests required to decide on management decision includes

1. HBsAg
2. HBeAg
3. Anti-HBe
4. IgM anti-HBc
5. HBV DNA
6. Serum alanine aminotransferase and aspartate aminotransferase

## Criteria

1. HBsAg +ve, IgM anti-HBc +ve, transaminase raised (>3 times upper limit of normal): Acute hepatitis

There is no need for referral unless the patient is in altered state of consciousness, has kidney failure, dehydration or severe vomiting

7. HBsAg +ve, HBeAg +ve or -ve, Anti-HBe óve or positive, HBV DNA high, Serum alanine aminotransferase and aspartate aminotransferase high: chronic hepatitis, requires referral and treatment.
8. HBsAg +ve, HBeAg -ve, Anti-HBe positive, HBV DNA negative, Serum alanine aminotransferase and aspartate aminotransferase normal, inactive carrier state, does not require any treatment.

### **Investigation:**

- ☐ HBsAg
- ☐ HBeAg
- ☐ Anti-HBe
- ☐ IgM anti-HBc
- ☐ HBV DNA
- ☐ Serum alanine aminotransferase and aspartate aminotransferase

### **Treatment:**

- ☐ Interferon
- ☐ PEG interferon
- ☐ Antiviral drugs: Lamivudine, Adefovir, Tenofovir, Entecavir, Telbivudine.

### **Referral criteria**

- ☐ Acute hepatitis

Patient with altered state of consciousness, those with kidney failure, dehydration or severe vomiting

- ☐ Chronic hepatitis

Patients with positive HBsAg +ve, HBeAg +ve or -ve, Anti-HBe +ve or positive, HBV DNA high, serum alanine aminotransferase and aspartate aminotransferase high: requires referral and treatment.

### **Situation 1: At secondary hospital /non-metro situation: Optimal standards of treatment in situations where technology resource is limited**

- ☐ Diagnosis: Test for HBsAg to screen for the infection. Test for serum alanine aminotransferase and aspartate aminotransferase. If facilities are available, tests for HBeAg, anti-HBe, IgM anti-HBc and HBV DNA should also be done.
- ☐ Investigations: As above
- ☐ Treatment: For prevention, subjects at high risk for HBV infection should be vaccinated against HBV infection.

## Standard Operating procedure

- a. *In patients*: One has to get the above investigations done and decide on management as outlined above. In patients with decompensated cirrhosis of liver, management of ascites, liver failure, hepatorenal syndrome and variceal bleeding may be needed.
- b. *Out patients*: Screen patients with liver disease and other risk factors for HBV for serum HBsAg test. Those patients found positive to this test, should be further investigated and managed as outlined above.
- c. *Day care*: Screen patients with risk factors for HBV for serum HBsAg test. Those patients found positive to this test, should be further investigated and managed as outlined above.
- d. **Referral criteria**
  - ☐ Acute hepatitis: Patient with altered state of consciousness, those with kidney failure, dehydration or severe vomiting
  - Chronic hepatitis: Patients with positive HBsAg +ve, HBeAg +ve or -ve, Anti-HBe óve or positive, HBV DNA high, serum alanine aminotransferase and aspartate aminotransferase high: requires referral and treatment.

### **Situation 2: At super speciality facility in Metro location where higher-end technology is available:**

- a. Clinical diagnosis: For acute hepatitis B: Diagnose complications including fulminant liver failure, acute renal failure using standard criteria and investigations

Diagnose chronic liver disease by ascites, pedal edema, jaundice, encephalopathy, varices on upper gastrointestinal endoscopy and findings at ultrasonography. Look for complications such as hepato-renal syndrome and hepato-pulmonary syndrome.

- b. Investigations

#### **Investigation:**

- ☐ HBsAg
- ☐ HBeAg
- ☐ Anti-HBe
- ☐ IgM anti-HBc
- ☐ HBV DNA
- ☐ Serum alanine aminotransferase and aspartate aminotransferase
- ☐ Ultrasonography
- ☐ Upper GI endoscopy

#### **Treatment:**

- ☐ Interferon
- ☐ PEG interferon
- ☐ Antiviral drugs: Lamivudine, Adefovir, Tenofovir, Entecavir, Telivudine.
- ☐ Treatment for liver failure
- ☐ Treatment for hepato-renal syndrome
- ☐ Terminal liver failure patients may require liver transplantation

## Standard Operating procedure

- a. *In patients*: One has to get the above investigations done and decide on management as outlined above. In patients with decompensated cirrhosis of liver, management of ascites, liver failure, hepatorenal syndrome and variceal bleeding may be needed. Most of such patients may need liver transplantation.
- b. *Out patients*: Screen patients with liver disease and other risk factors for HBV for serum HBsAg test. Those patients found positive to this test, should be further investigated and managed as outlined above.
- c. *Day care*: Screen patients with risk factors for HBV for serum HBsAg test. Those patients found positive to this test, should be further investigated and managed as outlined above.
- d. *Referral criteria*: Patients with terminal liver disease would require referral to centers active in liver transplantation

Chronic Leukaemias:

### Myeloid:-

The MPN are clonal haematopoietic stem cell disorders characterized by proliferation of one or more of the myeloid lineages. (ie granulocytic, erythroid, megakaryocytic and mast cell). They are primary neoplasms of adults. The MPNs are predisposed to a significant risk of thrombohemorrhagic complications.

The prerequisites for WHO classification of myeloid neoplasms relies on the morphologic, cytochemical and immunophenotypic features of the neoplastic cells to establish the lineage and degree of maturation and to decide whether cellular proliferation is cytologically normal or dysplastic or effective or ineffective.

The classification is based on criteria applied to initial specimens obtained prior to any definite therapy to judge their progression. Cytogenetic and molecular genetic studies are required at the time of diagnosis not only for recognition of specific genetical entities, but for establishing a baseline against which future studies can be judged to assess disease progression.

### Classification

- ☐ The classic MPNs include polycythemia vera (PV), essential thrombocythemia (ET), chronic myeloid leukemia (CML), primary myelofibrosis (PMF), chronic neutrophilic leukemia, chronic

eosinophilic leukemia, and mast cell disease.

- ▣ Atypical MPNs include those chronic myeloid disorders which are currently not classifiable as belonging to either the myelodysplastic syndrome or classical MPN. These include chronic myelomonocytic leukemia, juvenile myelomonocytic leukemia, atypical CML, and unclassifiable myelodysplastic syndrome (MDS)/MPN.

## Diagnosis

- ▣ CML is characterized by a reciprocal chromosomal translocation between chromosomes 9 and 22 (ie, t(9;22)). The diagnosis of chronic myelogenous leukemia requires the presence of BCR-ABL1, while its absence is required for all other MPN.
- ▣ PV is diagnosed when an otherwise unexplained increased hematocrit/red blood cell mass is accompanied by the presence of a JAK2 mutation along with a decreased erythropoietin level. JAK2 V617F is found in most patients with polycythemia vera, essential thrombocythemia, or primary myelofibrosis and is, therefore, useful as a clonal marker in those settings
- ▣ PMF is characterized by a leukoerythroblastic blood picture, splenomegaly, and bone marrow fibrosis that cannot be attributed to another myeloid

**Chronic myelogenous leukemia(CML):** is a myeloproliferative neoplasm that originates in an abnormal pluripotent stem cell.

The natural history of CML is bi- or tri phasic.: an initial indolent chronic phase(CP) is followed by an accelerated phase (AP) . a blast phase(BP) or both.

Clinical features: Most patients are diagnosed in CP which usually has an insidious onset.

Common findings at presentation include: fatigue, weight loss, anemia and splenomegaly.

Transformed phase(AP and BP) are generally accompanied by worsened performance status and symptoms related to anemia, thrombocytopenia and marked splenomegaly.

Diagnostic criteria:

High total leukocyte count ( $>10 \times 10^9/l$ )

Different stages of maturation in the neutrophils with peaks in the myelocyte and segmented neutrophils. No leukemic hiatus seen.

No significant dysplasia

Splenomegaly

Thrombocytosis

Thrombocytopenia refractory to treatment

20% or more basophils in the peripheral blood

$<2\%$ - 19% blasts in blood or bone marrow.

Cytochemistry: SBB nad MPO positive- all stages of maturation of neutrophils are positive.

Immunophenotype:



The neutrophils in CP have markedly decreased neutrophil alkaline phosphatase.

In myeloid BP the blasts may have strong, weak or no MPO and with antigens associated with granulocytic, monocytic and megakaryocytic lineage. Lymphoid BP phase shows B or T cell antigens.

Genetics:

90- 95% CML have the characteristic t(9;22)(q34;q11.2) reciprocal translocation that results in ph chromosome.

BCR ABL 1 fusion gene detection by FISH analysis, RT- PCR or southern blot technique.

Prognosis and predictive factors:

Median survival in CML ranged between 2-3 years. Prognosis worsens in transformed phases.

### **CMML**

Is a neoplasm characterized by features of both myeloproliferative and myelodysplastic syndrome.

Diagnostic criteria: persistent monocytosis

No philadelphia chromosome.

No rearrangement of PDGFRA/PDGFRB.

Fewer than 20% blasts

Dysplasia in one or more myeloid lineage.

Presentation: hepatosplenomegaly

Lymphadenopathy

Total leukocyte count elevated

Cytochemistry: NSE positive

IPT:

Myelomonocytic markers: CD 33 ,13 14, 68 and 64.

Genetics:

+8, -7/del(7q) and structural abnormalities of 12p.

### **JMML:**

Is a clonal hamatopoietic neoplasm of childhood characterized by proliferation of principally of the granulocytic an monocytic lineages.

Diagnostic criteria:

Persistnet monocytosis

Blasts<20%

No ph chromosome.

Plus two or more of the following

HbF

Immature granlocytes

WBC >10X10<sup>9</sup>/L

Monosomy 7

GMô CSF hypersensitivity of myeloid progeintors.

Presentation;

Organomegaly

Recurrent infection

Signs of bleeding.

Cytochemistry;NSE;Positive

IPT: no specifc markers

Genetics:

Monosomy 7

## **Lymphoid**

### **CHRONIC LYMPHOID LEUKEMIAS:**

CLL / SLL is a disease of advancing age, the mean age at diagnosis is 65 years. it is a neoplasm of lymphoid origin with a an equal male:female ratio.

Clinical presentation:

Most patients are asymptomatic but some present with fatigue, autoimmune hemolytic anemia, infections, oraganomegaly( spleen and liver).

Diagnostic criteria:

Persistent lymphocytosis >3 months.

Absolute lymphocyte count of  $> 10 \times 10^9/l$ .

In absence of extramedullary involvement there must be  $>- 5 \times 10^9/l$  monoclonal lymphocyte with a CLL phenotype.

Morphology: small mature lymphoid cells along with smear/ smudge cells and  $<55\%$  prolymphocytes.

Immunophenotype: B cell markers, weak expression of clonality and co express CD5 and CD 23.  
ZAP- 70.

Progression and transformation to High grade lymphomas: CLL may show an transformation to B-PLL though extremely rare.

genetics:

IG genes are rearranged with 40-50 % cases unmutated and 50-60% showing somatic hypermutation.

## B PLL

is a neoplasm of B prolymphocytes affecting the peripheral blood, bone marrow and spleen.

It is an extremely rare disease most patients being  $>60$  years.

Diagnostic criteria:

Rising total leukocyte.

$>55\%$  of prolymphocyte and usually  $>90\%$ .

massive splenomegaly.

rapidly rising lymphocyte count usually over  $100 \times 10^9/l$ .

anemia and thrombocytopenia.

IPT: Strongly express Ig M+/-IgD as well B cell antigens (CD 19,20,22,79a and b,FMC 7).

genetics:

Ig genes are clonally rearranged with unmutated heavy chain in 50% cases.

## T PLL:

TPLL is an aggressive T cell neoplasm characterized by the proliferation of small to medium sized prolymphocytes with a mature post thymic T cell phenotype involving the peripheral blood, bone marrow, lymph node, liver, spleen and skin.

it is rare approximately 2% of cases of mature lymphocytic neoplasm with a median age of 65 years.

Presentation;

Hepatosplenomegaly.

Generalised lymphadenopathy.

Anemia.

Thrombocytopenia.

Lymphocyte count  $>100 \times 10^9/l$ .

Morphology: small to medium sized lymphoid cells with non granular basophilic cytoplasm, cytoplasmic blebs, irregular nucleus with well circumscribed nucleoli .

IPT:

Peripheral T cell markers: CD 2, 3, 7 and CD 52 positive.

Cytogenetics: inversion of chromosome 14 with break points in the long arm at q11 and q32.

Abnormalities of chromosome 8, idic(8p11), t(p11-12;q12) and trisomy 8q.

Abnormalities of TP 53 gene seen.

## **Acute Leukaemias**

### **Acute myeloid leukemias:**

#### ***Clinical Diagnostic Features of AML***

- Adults with AML generally present with a vague history of chronic progressive lethargy; and symptoms of granulocytopenia and thrombocytopenia are common.
- As many as 1/3 of patients with AML will be acutely ill at presentation because of a significant skin, soft tissue, or respiratory infection.
- Petechiae with or without bleeding may be present. Patients with acute promyelocytic (M3) leukemia may have severe hemorrhaging secondary to a clotting factor deficiency, which results in an intravascular coagulopathy.
- Hyperuricemia is frequent; splenomegaly is present in about 1/3 of patients.
- Leukemias with a monocytic component (M4 & M5) may be associated with gingival hypertrophy from leukemia infiltration. Patients with these types of AML are most likely to present with or develop meningeal leukemia, retinal infiltration, leukemia cutis, or other localized leukemia infiltrations. In the absence of meningeal signs, a lumbar puncture is not indicated.
- Chloromas (granulocytic sarcomas, myeloblastomas) are present in about 5% of patients. They may take on a dull green color due to the high peroxidase content in the leukemia cell, may precede other diagnostic evidence, may occur after diagnosis, or may indicate relapse. They commonly occur in the skin but can be present in other body organs. They are most often associated with M4 and M5 leukemias.
- Lymphadenopathy and hepatomegaly are uncommon.

#### **Peripheral Blood Features of AML**

- The peripheral white blood cell count may be increased, decreased, or normal with approximately equal frequency.

- Granulocytopenia is very common. Approximately 1/2 of all patients will have granulocyte

counts < 1,000/uL.

- Thrombocytopenia is frequently observed and platelet counts <20,000/uL are common.
- The hematocrit is generally low but severe anemia is uncommon.
- Circulating blast cells are absent from the peripheral blood in approximately 15% of AML patients initially, and in 1/2 of patients presenting with leukopenia.

### ***Diagnostically Important AML Markers***

- Morphologic
  - Auer bodies
  - "malignant" primary granules
- Cytoplasmic organelles
  - Sudan B
  - myeloperoxidase
- Cytogenetic
  - t(8;21)
  - t(15;17)
  - -5,-7
- Immunologic
  - cell surface markers
- A variety of morphologic, cytoplasmic, cytochemical, and biochemical features are associated with AML that can aid in diagnosis.
- No available marker is either totally specific or sensitive enough to be used in all cases.
- Cytogenetic analyses should be performed at diagnosis in all patients with AML. Approximately 65% of patients will have abnormal karyotypes.
- Cytogenetic abnormalities may help determine prognosis.

<b>Favorable</b>	
young age	reactivity with CD2(T1)
FAB types M2, M3, M4	t(8;21) and t(15;17)
inversion of chromosome 16	
<b>Unfavorable</b>	
older age	aneuploidy
FAB type M7	trisomy 8
hyperleukocytosis	deletion of all or part of chromosomes 5 and/or 7
prior treatment	abnormalities of chromosome 11 at band q23
prior hematologic disorder	
infection	

### ***Prognostic Factors in AML***



Acute Leukemias	IPT	Cytogenetics
AML t(8:21)	CD13,CD33,CD34,MPO,HLADR	CD56,KIT mutation ó bad prognosis
AML with inv (16)	CD34,CD117,CD13,CD33,MPO,CD14,CD11b	Good prognosis
APML t(15:17)	HLD DR(-),CD33(+++)	PML RARA fusion gene ó good prognosis
AML with t(9:11)	CD33,CD65,CD4,HLADR(+)	MLLT3MLL ó Intermediate survival
AML with t(6:9)	MPO	t(6:9) - Poor prognosis
AML with inv(3)	CD13,CD33,HLADR	Poor prognosis

### ***Laboratory Factors Correlating With Response to AML Therapy***

- The following factors have been found to correlate with achievement of a complete response:
  - younger age and good performance status
  - prior bone marrow disorder
  - Auer rods marrow eosinophilia
  - rapid clearance of blast cells from the peripheral blood and a low percentage in the bone marrow aspirate ~ days post-treatment

- The following factors have had a negative influence on the achievement of a complete response:
  - older age
  - co-morbid diseases
  - specific karyotypic abnormalities
  - specific cell surface antigens
  - abnormalities of the erythroid series

### ***Prognostic Significance of MDRI Expression in AML***

- Drug resistance may be due to several mechanisms. These include increased expression of multidrug-resistant (MDR1 or MDR3~ gene, alterations in topoisomerase 11 activity, or enhanced glutathione activity.
- In patients with AML, MDR1 messenger RNA is increased in the minority of *de novo* cases, but is increased in those with refractory, relapsed, and secondary leukemia.
- The MDR1 messenger RNA levels correlate with response to therapy.
- Older patients have a higher incidence of MDR1 messenger RNA than younger patients.

AML with minimal differentiation: is an AML with no evidence of differentiation.

Presentation

Anemia

Thrombocytopenia

Neutropenia

Leukocytosis with high blast count.

Cytochemistry:MPO, SBB :Negative.

IPT:

Early CD 34, 38 and HLADR

AML without maturation: Auer rod seen

MPO: POSITIVE.

IPT:

MPO,HLA DR CD 34, 13, 33 and 117.

NO SPECIFIC CYTOGENETIC abnormality

AML with maturation:

High leukocyte count

>20% blasts with evidence of maturation.

>10% maturation of the myeloids .

IPT: as above CD 65, 11b, 15.

No cytogenetic abnormality.

AML with myelomonocytic: proliferation of both neutrophils and monocytes each comprising of at least 20% of the cells

Presentation: anemia thrombocytopenia with an elevated TLC and a high blast count .

IPT: CD 14,4, 11b and 11c and 64 .

CYTO: At least 3% blasts- MPO.

Dual positive blasts in NSE and MPO.

AML with monocytic and monoblastic :

Presenting feature:

Young individuals

Bleeding.

Extramedullary lesions

Gingival infiltration

CNS

>80% or more of the leukemic cells are of monocytic lineage( monocytes, promonocytes and monoblasts) and a minor neutrophilic component.(<20%). This further can be distinguished based on the relative proportion of monocytes and blasts. No auer rods seen.

IPT: MONOCYTIC MARKERS

Acute erythroid leukemia: presenting symptom anemia

Total count variable with circulating blasts.

Cytochemistry: PAS positive

IPT: Hb A and glycophorin.

Complex karyotype.

Acute megakaryoblastic:

Cytopenia.

Hepatosplenomegaly

## **Fibrosis**

### **Acute lymphoid leukemias:**

Acute lymphoid leukemias /lymphoblastic lymphomas is a neoplasm of B or T cell lineage.

B cell lineage (Good prognosis):

ALL is primarily a disease of children (under 6 years of age).

Presentation:

Bone marrow failure: anemia, thrombocytopenia with a variable leukocyte count.

Organomegaly.

Lymphadenopathy

Arthralgia.

Morphology: peripheral blood and bone marrow involved (>20% blasts)-

Cyto:

Blasts are MPO and SBB negative

PAS:Block positive.

IPT:

B CELL MARKERS: CD 19, cytoplasmic CD 79a and cytoplasmic CD 22

GENETICS:

Clonal rearrangement of IgH and T cell receptor.

FISH analysis detects t(9;22) translocations.

T ALL(bad prognosis):is a neoplasm committed to T cell lineage.

Presentation: mediastinal involvement.( usually respiratory embarrassment)

Lymphadenopathy

Hepatosplenomegaly

High leukocyte count

>20% blasts in PB and BM.

Cytochemistry: MPO, SBB negative. Few cases show PAS positivity.

IPT: TdT positive

CD1a, 2,3,3,5,7 and 8.

Cytoplasmic CD 7 and CD3 are specific.

Genetics:

Clonal rearrangement of T cell receptor(TCR).

CYTOGENETICS;

Alpha and Delta TCR loci.

Transcription factor HOX11 and MYC.

## **PRE-OPERATIVE HAEMOSTASIS SCREEN:**

**By. Dr. Sukesh C Nair**

Professor, Head, Div of Laboratory Haematology, Head, Dept of Transfusion Medicine and Immuno-haematology, Christian Medical College, Vellore, Tamil Nadu - 632004

**Introduction:** Preoperative screen should comprise the following:

1. Good clinical history especially history of bleeding tendency
2. Platelet count
3. Prothrombin time (PT)
4. Activated partial thromboplastin time (aPTT)
5. Bleeding time

### **Clinical History:**

A thorough and structured history of bleeding tendency (including previous surgery, family history and trauma) and physical examination are the most important approaches to assess risk of excessive bleeding during surgery. The British Committee for Standards in Haematology published 'Guidelines on the assessment of bleeding risk prior to surgery or invasive procedure'<sup>1</sup> and summarised to say, 'The positive predictive value (0.03 to 0.22) and likelihood ratio (0.94 to 5.1) for coagulation tests indicate that they are poor predictors of bleeding. Patients undergoing surgery should have a bleeding history taken. This should include details of previous surgery and trauma, a family history and details

of antithrombotic medication. Patients with a negative bleeding history do not require routine



coagulation screen prior to surgery. However, there have been reports to suggest that in an occasional patient excessive bleeding had occurred that would have been prevented if haemostatic screen had been done. The check list for clinical history is given in table 1.

**Table 1 - History of Bleeding Tendency – Check List**

Symptom	Score			
	0	1	2	3
Epistaxis	No or trivial	Present	Packing, cauterization	Blood transfusion or replacement therapy
Cutaneous	No or trivial	Petechiae or bruises	Haematomas	Consultation
Bleeding from minor wounds	No or trivial	Present (1–5 episodes per year)	Consultation	Surgical haemostasis
Oral cavity	No or trivial	Present	Consultation only	Surgical haemostasis/blood transfusion
GI bleeding	No or trivial	Present	Consultation only	Surgery/blood transfusion
Tooth extraction	No or trivial	Present	Suturing or packing	Blood transfusion
Surgery	No or trivial	Present	Suturing or resurgery	Blood transfusion
Menorrhagia	No or trivial	Present	Consultation, pill use, iron therapy	Blood transfusion, hysterectomy, dilatation and curettage
Postpartum haemorrhage	No or trivial	Present, iron therapy	Blood transfusion, dilatation and curettage, suturing	Hysterectomy
Muscle haematomas	No or trivial	Present	Consultation only	Blood transfusion, surgery
Haemarthrosis	No or trivial	Present	Consultation only	Blood transfusion, surgery

**Notes:**

Bleeding score below 3 in males and below 5 in females always allows exclusion of a bleeding disorder.

Ref: A. TOSETTO, G. CASTAMAN and F. RODEGHIERO: Bleeding scores in inherited bleeding disorders: clinical or research tools? *Haemophilia* (2008), 14, 4156422

Laboratory tests that are to be performed depend on the type of surgery (table 2).

**GUIDELINES FOR PREOPERATIVE HAEMOSTASIS EVALUATION**

Level	Bleeding History	Surgical Procedure	Recommended Haemostasis Evaluation
<b>I</b>	Negative	Minor	None
<b>II</b>	Negative	Major	Platelet Count, APTT, PT

<b>III</b>	Equivocal	Major, involving haemostatic impatient	Platelet count, APTT, PT and factor XIII screening assay
<b>IV</b>	Positive	Major or Minor	Consult haematologist

**Platelet count:**

A platelet count must be performed prior to surgery. Low platelet counts reported on the automated cell counters must be confirmed by microscopic examination of blood film to exclude psuedothrombocytopenia.

**PT & aPTT**

Recommendations of Sample collection and processing:

1. Avoid prolonged venous stasis
2. Use a 21g needle for adults.
3. Avoid indwelling catheters or lines.
4. Mix immediately with 0.105-0.109mol/L tri-sodium citrate at 1:9 ratio with Blood..
5. Discard sample if any delay or difficulty in collection.
6. Discard if marked hemolysis or evidence of clotting
7. Underfilling (<80-90% of target volume) prolongs some screening tests.
8. If hemotocrit is > 55% adjust anticoagulant: blood ratio. More blood to be added to the same volume of anticoagulant. { 60/(100-Hct) x standard volume of blood that is added }
9. Sample collection system can affect results by upto 10%
10. For plasma tests centrifuge at 2000g for atleast 10min at room temperature.
11. Test within 4hours.
12. Freezing may affect results depending on temperature and time of storage.
13. Any deep frozen plasma should be thawed rapidly at 37deg C.

**Prothrombin Time:**

**Principle:**

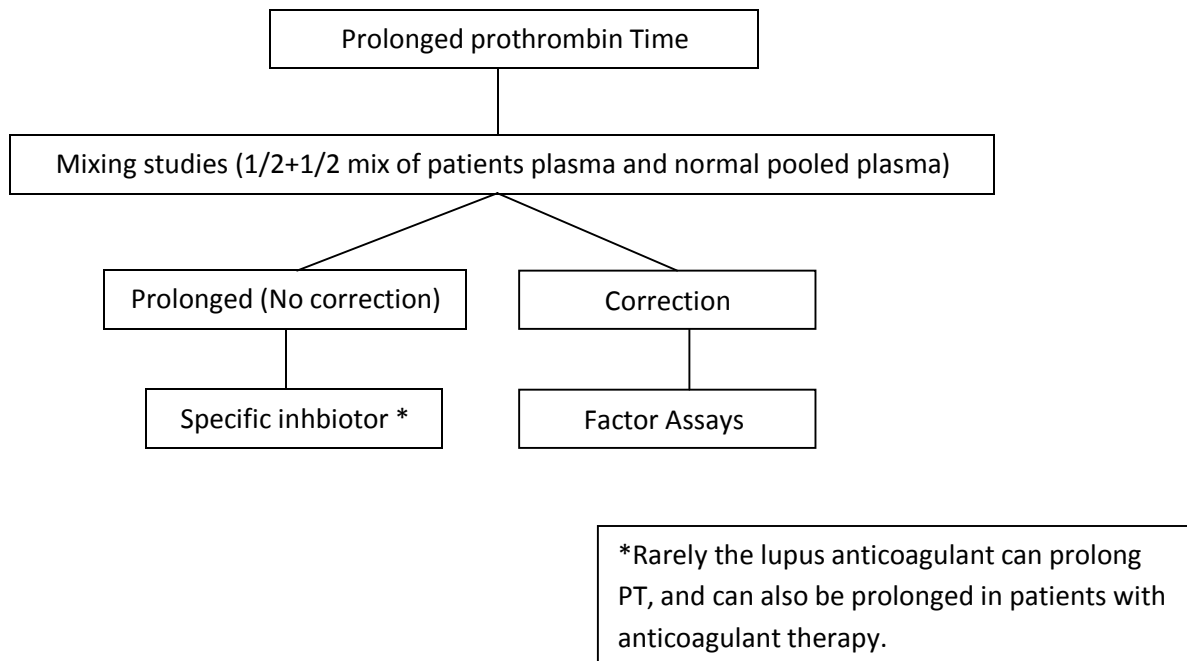
Time taken by recalcified citrated plasma to clot after Tissue factor (in the form of tromboplastin) is added to plasma. Tissue factor reacts with FVIIa to activate the ðextrinsicöpathway and thus form a clot.

**Use of the prothrombin time test:**

- The PT is sensitive to and thus prolonged in patients with deficiencies of Factors VII, X, V, II and fibrinogen.

- It is particularly useful in monitoring anticoagulation in patients on warfarin.

### Investigation of a prolonged Prothrombin Time:



### Procedure:

#### REAGENTS AND MATERIALS:

1. PT Reagent: Reagent needs to be reconstituted as per product insert/instruction sheet, and prewarmed to 37°C. This reagent already contains CaCl<sub>2</sub>.
2. Patient plasma, control plasmas as required. (NB: test normal plus abnormal QC plasma whenever a fresh vial of recombiplastin is reconstituted).
3. Waterbath, 12 x 75 test tubes, stop watches, yellow tips, and 100 and 200ul automatic pipettes and light source.

#### METHOD:

1. Add 0.1ml plasma into duplicate small glass tubes placed in a 37°C water bath, and leave 3 min to equilibrate to 37°C. Add 0.2ml prewarmed PT reagent and simultaneously start stop watches.
2. Mix and tilt tubes to nearly horizontal position at one second intervals, otherwise maintaining in 37°C water bath.
3. Depress stop watch mechanism on first appearance of a clot. The time taken for the clot to form (i.e. between addition of PT reagent and the appearance of the clot) is the 'prothrombin time' (PT).
4. Take the mean of the duplicate readings (providing that they don't differ by more than two seconds; otherwise repeat the procedure and check the reagent; replace if necessary).

#### Quality Control Procedures:

A normal control (e.g. pooled normal plasma [PNP]) and an abnormal control plasma (eg Accuclot II (sigma) control) should also be included with every batch of patient plasma tested, or every few hours if testing a large number of plasmas throughout the day.

Run normal control and abnormal control plasma for 10 days and calculate the mean and +/-2SD. The control values should fall within +/-2SD.

**Interferences:**

Do not test plasma from badly haemolysed blood . Badly haemolysed blood may give an artifactual coagulation results that do not accurately represent the coagulation status of the patient under investigation. Haemolysed blood may suggest a traumatic blood collection and you may need to request a repeat sample.

**Expression of the Results**

The results are expressed as a mean of the duplicate reading in seconds, both mean of the patient time and mean of the normal control time, either as raw data or in the form of a rati (prothrombin ratio). Patients on OAT the results are always interpreted with INR (International Normalized Ratio).

**Normal Ranges**

11 ó 16Secs.

(Normal values varies depending on the Thromboplastin used the exact technique under visual or automated end point reading is used)

$$INR = \frac{\text{Prothrombin Time (PT) of test plasma (sec)}}{\text{Mean Normal Prothrombin Time (MNPT) (sec)}} \times ISI$$

ISI = International Sensitivity Index.

INR= International normalized ratio.

Ideally, each laboratory should establish its own Mean Normal Prothrombin Time (MNPT). Classically, for the MNPT: this is obtained by testing plasmas from at least 20 normal individual in that laboratoryø PT assay, and taking the mean PT. Selected instrument specific ISI values are usually provided for each batch of Thromboplastin.

## Activated Partial thromboplastin time:

### Principle:

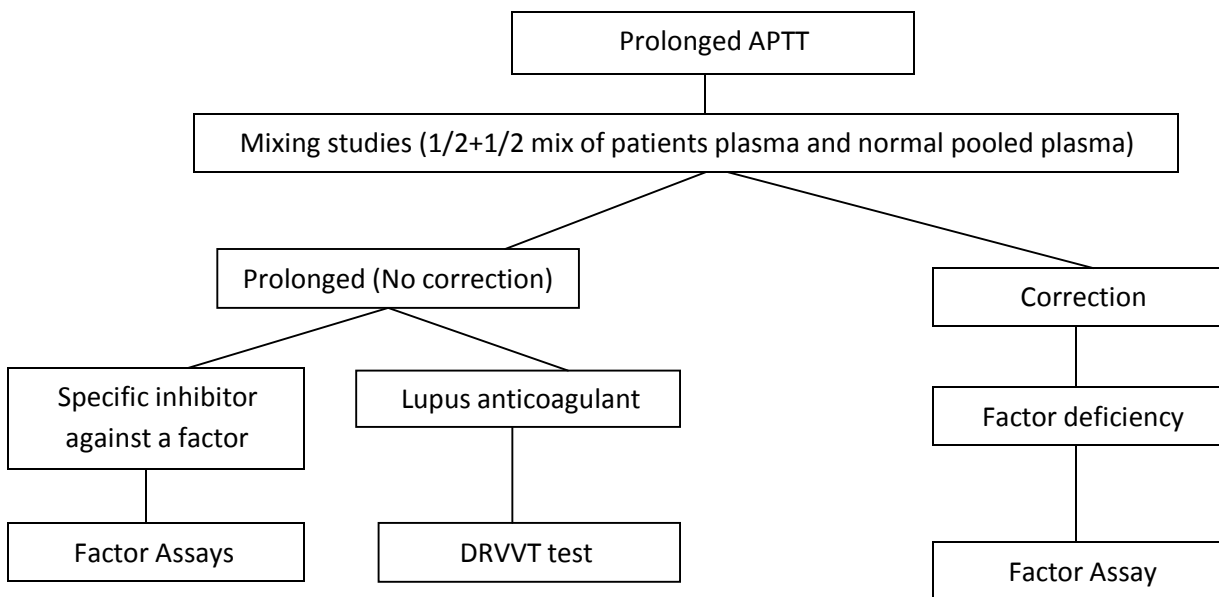
Time taken by recalcified citrated plasma to clot after Phospholipid (Lacking tissue factor, hence the term "partial" thromboplastin) and particulate matter (such as kaolin, silica & elagic acid) are added to plasma. Abnormalities in the "intrinsic" and "common" pathway will result in prolongation of the APTT.

### USE OF APTT:

This test is abnormal in patients:

1. With deficiencies of factors XII, XI, X, IX, VIII, V, II ad fibrinogen.
2. On heparin therapy; or
3. Who have the lupus anticoagulant.

### Investigations of prolonged APTT:



**REAGENTS AND MATERIALS:**

1. APTT Reagent (SynthAsil)
2. CaCl<sub>2</sub> 0.025M pre-warmed to 37°C
3. Patient plasma and control plasma as required
4. Glass tubes and stopwatches.
5. Water bath
6. Automated Pipettes/glass pipettes and tips.

**METHOD:**

1. Add 0.1 ml of plasma and 0.1 ml APTT reagent in a glass tube (12 x 75) and place in a 37°C water bath.
2. Mix, and leave for 5 minutes to equilibrate to 37 deg C and to provide suitable activation of plasma with contact factor.
3. Add 0.1 ml of warmed 0.025M CaCl<sub>2</sub>, and simultaneously start stopwatch.
4. Mix and tilt tubes to nearly horizontal position at one-second intervals, otherwise maintaining in 37 deg C water bath.
5. Depress stopwatch mechanism on first appearance of a clot. The time taken for the clot to form (i.e., between addition of CaCl<sub>2</sub> and the appearance of the clot) is the APTT.
6. Always do in duplicates.
7. Take the mean of the duplicate reading (provided that they don't differ by more than 2 seconds otherwise repeat procedure and check reagents).

**Interferences:**

Do not test plasma from badly haemolysed blood. Badly haemolysed blood may give an artifactual coagulation results that do not accurately represent the coagulation status of the patient under investigation. Haemolysed blood may suggest a traumatic blood collection and you may need to request a repeat sample

**RESULTS:**

Report APTT results in seconds for example control 30 sec, patient 36 sec.

**REFERENCE RANGE:**

Each laboratory has to establish their own range.

As a rough guide the aPTT of normal plasma should be 25 to 35 sec.

Specific assays like FVIII:C and VWF:Ristocetin Co-factor assays needs to be done if the screening tests are normal but History is significant due to high prevalence of Mild bleeding disorders like VWD Type1 and few Type2 and Mild inherited/ acquired Haemophilia A.

Caution with :- Alternative methods to perform PT and APTT.

Automated and semi-automated methods:- Validation required but if using semi-automated methods all the tests are to be done in Duplicate.

## **BLEEDING TIME:**

### **Principle**

The bleeding time is the time taken for a standardized skin cut of fixed depth and length to stop bleeding.

Prolongation of the bleeding time occurs in patients with thrombocytopenia, von Willebrand's disease, Glanzmann's thrombasthenia, Bernard-Soulier disease, storage pool disease and other platelet disorders. Fibrinogen is required and a role for factor V has been suggested. The bleeding time can therefore be prolonged in patients deficient in fibrinogen or factor V. Prolongation also occurs in some patients with renal disease, dysproteinemias and vascular disorders.

### **Materials**

Sphygmomanometer.

Cleansing swabs.

Template bleeding time device.

Filter paper 1 mm thick.

Stopwatch.

### **Method**

- The sphygmomanometer cuff is placed around the upper arm and inflated to 40mm of mercury. This pressure is maintained throughout the test.
- The dorsal surface of the forearm is cleaned and the bleeding time device placed firmly against the skin without pressing. The trigger is depressed and the stopwatch started.
- Superficial veins, scars and bruises should be avoided.
- At 30 second intervals, blot the flow of blood with filter paper - bring the filter paper close to the incisions without touching the edge of the wound.
- Record the time from puncture to cessation of bleeding.

### **Interpretation**

- The normal range in adults is up to 6 minutes but may vary according to method used.
- *Note:*

- At the time of writing there are two commercially available disposable devices for performing the bleeding time both compare very well with each other. Whichever device is used a normal range should be established locally.
- The incision should be made in a direction parallel to the length of the arm. Cuts made perpendicular bleed for longer.
- It is not necessary to record endpoints if bleeding continues beyond 15 minutes.
- The effect of drugs interfering with platelet function should be considered. For example, drugs containing aspirin can cause prolongation and, where possible, should not be taken for 7 days prior to testing.
- There is a possibility of scarring at the site of bleeding time incisions more with the  $\text{Simplatex}$  method. This should be brought to the attention of patients prior to performing the incision.

Test to be performed before doing Bleeding time:

- Platelet count.

Advantage:

- Simple test of natural hemostasis
- Avoids potential anticoagulation artefacts.

Disadvantages:

- Poorly reproducible
- Poor correlation between the risk of bleeding at surgery and the bleeding time. Hence, some authors do not recommend it as a preoperative screening test in patients without any positive bleeding history.
- Insensitive to milder forms of platelet dysfunction.
- Interpersonal variations.

Suggestions to overcome:-

- Have 1-2 technologists only doing BT.
- To do specific PFT if BT is normal and History is significant.\*
- Devices like PFA-100 too have limitations in relation to sensitivity to detect Mild PFdefects or Milder forms of VWD.

### **Whole blood clotting time (Clotting Time /CT)**

CT is abnormal when the deficiency of the coagulation factors is gross such as a factor VIII or factor IX of less than 10%. Thus, the test lacks sensitivity. Further, it has poor reproducibility and individuals with such gross deficiency of clotting factors are symptomatic. The test was deleted from textbooks of practical haematology about 20 years back.

References for BT



1. Y.L.Chee, J.C. Crawford, H.G. Watson and M.Greaves, 2008, Guidelines on the assessment of bleeding risk prior to surgery or invasive procedures, British Committee for Standards in Haematology, British Journal of Haematology, 140,496-504
2. Ivy AC, Nelson D, Bucher G. The standardization of certain factors in the cutaneous venostasis bleeding time technique. J Lab Clin Med 1941;26:1812-1822.
3. Koster T, Caekebeke ó Peerlinck KMJ, Briet E. A randomized and blinded comparison of the sensitivity and the reproducibility of the Ivy and Simplate II bleeding time techniques. Am J Clin Pathol 1989;92:3156320.

## QUALITY ASSURANCE IN A CLINICAL LABORATORY

### Introduction:

The laboratory tests are required for several purposes:

1. Arriving at diagnosis, prognostication and monitoring progress
2. Health check
3. Medical check up for employment, insurance and others
4. Disease surveillance for health planning

For all these situations, reliability of test results (precision and accuracy) is essential.

*“Accuracy is the closeness of agreement between the measurement that is obtained and the true value”.*

*“Precision is the closeness of the agreement when the test is repeated number of times <sup>1</sup>”.*

Each analytical procedure has inherent probability of error and uncertainty of measurement (UoM). Quality assurance aims at minimising these errors, narrowing the UoM and reducing the turnaround time (TAT). It is important that the procedures are developed, documented, implemented and monitored. Documentation system should be on the lines of ISO 1900. There is a common saying “What is not documented is a rumour”.

### Components of quality assurance:

Control of pre-examination (pre-analytical) phase

Control of examination (analytical) phase

Control of post-examination (post-analytical) phase

### Control of pre-examination phase:

Several factors have improved precision and accuracy. These include robust and user-friendly automation and hence precision, accurate calibration with reference materials (calibrators), regular use of controls with each assay and availability of inter-laboratory comparison as national and international 'External Quality Assessment Schemes' (EQAS) or 'Proficiency Testing' (PT). Consequently in a modern laboratory with established quality assurance programme, examination errors are few and over 90% errors are attributable to the pre-examination phase<sup>2,3</sup>.

Pre-examination phase includes 'controllable' and 'non-controllable' variables. The controllable variables relate to patient preparation and sampling (time, proper site and method, labelling to ensure unique identification, transport, storage and preparation). Each one of these is important.

Control of pre-examination phase and patient-related variables, including interference from administration of drugs, (which may grossly affect test results), is discussed in the 'Specimen Collection Manual' annex-1 – table 2.

**Control of Examination Phase (Analytical Phase):** This includes use of calibrated equipment, application of standard analytical procedures, appropriate training of staff, internal quality control, external quality assessment and standardization.

#### **Calibration of Equipment:**

Procure the equipment, which is suitable for the intended use. Give it a unique identification number. It is a good practice to maintain history sheet of the equipment that records the agreement (including AMC Terms) and date of purchase, addresses and contact details of suppliers, down time and repair details.

Calibrate equipment, with the frequency defined by the manufacturers and/or by National Accreditation Board for Testing and Calibration Laboratories (NABL) vide the document number 112.

The calibration standard should be traceable to National Physical Laboratory (NPL) or equivalent. Calibration should be performed by a laboratory accredited for ISO Standard 17025 by an accreditation body such as NABL.

In case of automated equipment, the suppliers calibrate the system as their engineers are especially trained for maintenance and repair of such equipment. The calibration certificate should contain raw data: just a certificate that the apparatus has been calibrated is not sufficient.

Most automated systems in haematology, are calibrated twice a year generally at the time of preventive maintenance. However, the calibration is required whenever the instrument has major repair or there is change in lot. In biochemistry analysers there is a more frequent need for calibration when the instrument flags a shift in control values. This may occur in days or months. Some biochemistry instruments require daily calibration and in some others the re-calibration occurs automatically.

The calibrators are provided by the manufacturers.

Some equipment can be calibrated in-house using **calibration standards**: It is important to develop capability of in-house calibration of such equipment. The apparatuses that can be calibrated in house include micro-pipettes, glass pipettes<sup>4</sup>, weighing scales, centrifuges<sup>5</sup>, spectrophotometers and colorimeters<sup>4</sup> (annex 2). A sticker should be placed on the machine showing calibration status (date calibrated and next due date). Calibration status of small equipment such as pipettes can be maintained in a file.

#### **Standard analytical procedures:**

All analytical procedures should be internationally acceptable – taken from standard books or journals. Any in-house deviation must be validated employing standard statistical methods<sup>5</sup> – Annex 3.

#### **Staff Training:**

Training of the staff before introduction to new procedure is vital to ensure quality of test results. It is important to keep records of training and to check the effectiveness of training

#### **Internal Quality Control:**

Most modern laboratories perform tests using commercially available kits. Practically all these procedures include use of stable controls with each run. The controls are part of the kit or can be purchased from another vendor such as Bio-Rad. The controls ensure precision and determine uncertainty of measurement (imprecision). To check precision/imprecision the exact value of the control specimen is not required. However, if the value has been determined using a standard technique, reference material and a calibrated instrument this can be taken as exact value. Generally, 2 to 3 levels of controls are used. If the laboratory is processing only a small number of samples a single level control may be used per batch. The values of controls can be maintained as a table. It however, is preferable to plot the values as Levy-Jennings (LJ) charts. The mean value of control is determined and standard deviation (SD) and coefficient of variation (CV %) are calculated. The CV is preferred as it describes the significance of SD irrespective of the measured value. The degree of precision required for most of the analytes has been described<sup>6</sup>. The LJ chart plots the actual value of the control/s obtained with each batch. The following rules are applied to accept or reject the values (run) of the analyte:

Following procedures are used for IQC

Control Charts

Replicate testing of patient samples

Delta Check

Daily means

Correlation with other values

Clinical correlation

Control Charts:

The term *Control* is applied to a stable material that is used with every test run. It can be prepared in-house for some analytes. However, current practice is to purchase the material from commercial sources. *Controls* should be stable, homogeneous and have the same matrix as that of the specimens to be analysed. These are procured from the manufacturers of the *kits* or from alternative vendors.

Commercially supplied control specimens (reagents) are assigned a target value (mean and  $\pm 2$  SD). The lab mean may differ from the assigned mean. The lab should determine its own mean and 2 SD values. Coefficient of variation (CV %) is calculated from the SD achieved and it should be within the levels described by (Reference)

For complete blood counts (CBC) the control material is artificial and does not have the same matrix as blood specimen. Further, it has a short shelf life 14 – 30 days to 1 month creates problem of determining lab mean of the control.

Large laboratories must use 2 or 3 level controls. The values obtained on the controls are plotted on Levy and Jennings Charts to detect deviation from the acceptable precision suggesting a fault with the reagents, individuals performing the test and/or the machine. Westgard’s multi-rules are applied to take action on the deviations (Reference). However, it may not be necessary to apply all the rules. We suggest the following actions to be taken on violations of the multi-rules.

**Table: Westgard’s Multirules**

Sr. #	Control Value	Interpretation	Action to be taken
1	1 value outside 2s	Random error Warning sign	If other control values are within 2 s accept run. Re-run the previous control to verify deterioration of material
2	1 value outside 3s	Random error Gross error	If other control values are within 2 s accept run. If not reject run. Re-run the previous control to verify deterioration of material. Possibilities are deterioration of reagents or fault analyser. Call engineer; get it repaired and recalibrated
	2 consecutive values exceed 2s on either side of mean	Systematic error Gross error	If other control values are within 2 s accept run. If not reject run. Re-run the previous control to verify deterioration of material.

4	1 value exceeds +2s other value exceed -2s	Radom error Gross error	Repeat test. If value acceptable no further action required. If other control values also outside limit reject run. Recalibrate the machine.
5	4 consecutive values exceed 1s on the same side of mean	Possibly systematic error	Check values of two other controls if values acceptable change the first control
6	Ten consecutive control values on one side of the mean but within 1s	Systematic error or the mean on your machine differs from the assigned mean	Redraw the chart with your mean as the target value

s denotes standard deviation

The laboratory can choose the rules to be applied. Rule number 6 is generally not applied. Rule number 2 must be used. Any other 2 rules may be added. The laboratory must document which rules are going to be applied.

***Replicate testing of patient samples:***

Replicate testing of patient samples is another way of ensuring precision. It does not check accuracy. This procedure is applicable to very small laboratories where the workload does not justify purchase of 'controls'. The CV % cannot be determined or monitored from the data derived by this procedure. Initially, analyse 10 samples in duplicate. Determine the differences between the paired results. Calculate the standard deviation (SD) of the differences. Every day perform duplicate tests on 2 to 3 specimens. The difference between the paired results should not be more than 2SD derived earlier from 10 paired test results.

***Delta check***

A homeostatic mechanism sets the values of analytes in an individual, which is considered normal for that individual. In the hospital setting a sudden change in the value of analyte does not occur without change in clinical condition or without therapeutic intervention. For example a significant rise in haemoglobin concentration over 1 or 2 days may be due to blood transfusion that the patient may have received. Delta check is not applicable in stand-alone laboratories. It however, is an excellent method of internal quality control and should be applied in the hospital setting.

***Daily means***

Mean values of an analyte do not significantly change in a laboratory performing over 300 tests of an analyte per day. This is particularly applicable to high volume tests in biochemistry and for Complete Blood Counts (CBC) in haematology. Procedure is as follows. Determine the mean and 2 SD values. If subsequently the mean is outside 2 SD values, investigate especially with another run of 'Controls'.

#### **Correlation with other parameters:**

For example in a patient with acute myocardial infarction several cardiac markers will show abnormality. If there is dissociation, check the control data and repeat the tests. The CBC values correlate extremely well with the microscopic examination of blood film. Abnormal CBC values particularly severe thrombocytopenia must be confirmed on examination of blood film. Some individuals develop platelet antibodies against cryptic antigens, which are exposed when blood sample is taken in EDTA tube. This is an in-vitro phenomenon and causes no morbidity. This leads to formation of platelet clumps, which are counted by the automated systems as leucocytes and platelet counts are erroneously reported very low. This has led to many unnecessary platelet transfusions.

#### **Clinical Correlation:**

The clinical diagnosis depends on the test results given by the laboratory. More often than not the laboratory is right and the treating doctor must respect the laboratory observations. The same time the laboratory physicians should never dismiss the lack of correlation with clinical profile. If discrepancies are observed the laboratory must review its SOPs and other analytical systems including reagent kits.

#### **Inter-laboratory comparison:**

Internal Quality Control ensures precision and not necessarily accuracy. Accuracy is ensured by proper technique, good quality reagents, reliable equipment, suitable IQC and most importantly by accurate calibration and concordance with other laboratories through a formal '*External Quality Assessment Scheme (EQAS) or 'Proficiency Testing' (PT)*'. The participation in EQAS is mandatory requirement. Many national and international programmes are available (references). The analysis is provided by the nodal agency that provides the EQAS. The action taken on the EQAS results should be documented.



## Alternative assessment procedures when no formal EQAS is available:

For some analytes no national programme may exist and small laboratories cannot afford participation in international programmes. In such situations the alternative approach is described below. Source: Guidelines approved by the Clinical Laboratory Standards Institute (CLSI) Document HIS – volume 22 GP29-A

The document suggests many approaches & the lab to choose from those. The following method is preferred:  
annex

### SPLIT-SAMPLE TESTING

- É A single biologic sample divided into aliquots, wherein 1 aliquot is tested on a particular assay system (or by a particular analyst) other aliquot(s) are tested on other instrument(s) (or by other analyst[s]) and the results are compared

Note: It is often done in different labs. Frequency: At least every 3 months

### REASONS FOR THIS CHOICE

- É Other lab(s) to which the sample is sent for ILC may not have the same analytical system with differences in instrumentation reagents / reference intervals and hence with interpretation problems
- É The statistical validity of test being done in less than 20 labs is questionable
- É Hence intra-laboratory split-sample testing is recommended
- É It is a **Mandatory** requirement for **all** analytes where no formal EQAS is available

### Prerequisites for split-sample testing

- É Lab must follow IQC apply methods traceable to reference methods
- É The comparability between 2 instruments / techniques should be shown through Bland-Altman Plot
- É Define acceptable differences in 2 values by 2 analysts or 2 instruments and take corrective action if differences are outside those limits

### Calculations

- É Calculate difference (d) between each paired result and the mean of differences
- É Determine 1 and 2 SD values for the differences
- É Run is unsatisfactory if  $d > 2 \text{ SD}$

### References

1. Dacie and Lewis Practical Hematology 2006 Ed S Mitchell Lewis, Barbara J. Bain, Imelda Bates, 10/e Churchill Livingstone, Philadelphia
2. Lippi G, Guidi GC, Mattiuzzi C and Plebani M (2006); Preanalytical Variability: the dark side of the moon in laboratory testing. Clin Chem Lab Med, 44: 358-365.
3. Bonini Pierangelo, Plebani Mario, Ceriotti Ferruccio and Rubboli Francesca (2002); Error in Laboratory Medicine, 48: 691-698.
4. Burtis Carl A., Ashwood Edward R. and Bruns, David E.: 2006 Tietz Text Book of Clinical Chemistry and Molecular Diagnostics Saunders St. Louis Missouri, USA
5. Ricos C, Baadenhuijsen h, Libeer JC, Htlytoft Peterson P, Stockl D, Eur J Clin Chem Clin Biochem 1996;34:159-65 and Scan J Clin Lab Invest 1988;48:757-64:

## **ANNEX - 1 SPECIMEN COLLECTION MANUAL**

**PURPOSE:** To obtain an adequate sample for various laboratory tests

Results of the laboratory depend upon the quality of the specimen received. Poorly collected or transported specimen can result in inaccurate analysis and to improper treatment of the patient.

### **Specimen Collection:**

Specimens are of several types such as blood (collected in a plain vial//tube or containing EDTA, oxalate, fluoride, culture media, heparin, serum separator gel), urine, CSF, fluids (pleural, peritoneal), feces, tissue, BAL, semen, throat swab, pus and tissue, Any of these samples may be required for microbiological examination which very often is collected in a culture bottle.

### **General Principles:**

- ☐ Match the request form with the patient to confirm identity of the patient correctly
- ☐ Reassure the patient to avoid stress, which can cause a rise or fall in levels of many analytes
  - Obtain patient's or guardian's consent for specified tests where required
  - Information required in the request forms includes patient's data (name, age (DOB), gender, location (address/ phone/ ward or clinic); tests requested; date & time specimen collected, requester's data (name, address, phone) and pertinent clinical information.
- ☐ Generate Bar code (when applicable) depicting name, age, sex of the patient and name of the test and fix on the appropriate collection tubes

## Collection of Blood Specimen:

- ☒ Site for withdrawal of blood specimen:
  - Ante-cubital vein: the most preferred site
  - Dorsum of the hand: Veins on the dorsum of the hand have a tendency to bleed, however, are chosen in an obese subject when it may be difficult to access an ante-cubital vein.
  - Skin puncture: is used for obtaining a small amount of blood or when it is not possible to obtain venous blood(e.g. in infants <1 yr, in cases of gross obesity)
- ☒ When serum or plasma is required for analysis, the specimen volume is 2 to 2.5 times the volume of serum or plasma required for the investigations
- ☒ Maintain the ratio of anti-coagulant and blood
- ☒ Seat the patient in a chair or request him to lie down on the couch. *Venipuncture is never performed while a patient is standing*
- ☒ Examine both arms. Select best accessible antecubital vein. Apply tourniquet 3-4 inches above the venipuncture site. Palpate vein to determine its size, depth and direction. Release tourniquet
- ☒ Wear gloves and reapply tourniquet
- ☒ Re-palpate the vein
- ☒ Patient is asked to close his fist
- ☒ Clean the area with 70% ethyl alcohol or 0.5% chlorhexidine using sterile gauze swab in a circular motion, moving clockwise from inside to out. Allow to air-dry
- Keep patient's arm in a downward position to prevent backflow or reflux
- ☒ Use new needle holder for each patient. Thread needle snugly into holder but do not make it over tight. Insert the required collection tube into the tube holder until it makes contact with the backside of the needle. Do not push the collection tube onto the needle
- ☒ Remove the needle cover and perform venipuncture with bevel pointing up
- ☒ Push the tube to the end of the holder, puncturing the diaphragm of the stopper. At this point, blood should be visible in tube.
- ☒ Release tourniquet.
- ☒ Pull out collection tube only after the vacuum is exhausted & blood flow has stopped (especially applicable to tubes with additive). Fill tubes in the recommended order of draw. Mix the tubes with additive immediately by **gently** inverting eight to ten times. **Do not shake**. Partial mixing may result in formation of clot, which interferes with many tests especially platelet counts and clotting tests
- After the last tube is withdrawn, place sterile gauze over the puncture site remove needle from the arm, press at the site, remove the gauze, check that there is no oozing and stick sterile adhesive such as 'Bandaid' or 'Handyplast'.
- *Do not recap needle*. Dispose the needle in the "sharps container".
- Label the collection tubes with patient's name, date & time and the identity of the phlebotomist or place the bar code on the tubes as per the practice
- ☒ Withdrawing blood with a **syringe** is avoided for safety reasons. The procedure is essentially similar as for the evacuated tubes except the following::Check that the sterility seal is intact. As there is no vacuum, blood does not flow automatically and plunger of the syringe is gently & slowly drawn back (allowing blood to flow 1~2 mm behind plunger). Continue until the required amount of blood is obtained. Carefully puncture the stopper of the evacuated tube and allow the blood to be sucked into the tube/s. *Avoid needle stick injury*

**ORDER OF DRAW:** The following order of draw is recommended when drawing multiple specimens for clinical laboratory testing during a single venepuncture. Its purpose is to avoid possible test result error due to cross contamination from tube additives.

Tube	Additive	Determination
Blood Culture container	Sterile media	For microbiological tests
Light blue	3.2 % Sodium citrate	Maintain the ratio of blood with that of anticoagulant
Gold	Clot activator and gel	Serum separation (SST)
Red	Clot activator	For serum
Green	Heparin	For plasma
Lavender	K <sub>2</sub> EDTA	For haematology
Gray	Sodium Fluoride with K <sub>2</sub> EDTA or Potassium Oxalate	For glucose

#### Timed specimens:

Postprandial glucose - 2 hours after meal

Blood glucose at specified time - to check the effect of insulin therapy

#### Multiple Specimens:

- ☐ The most common timed procedures are oral glucose challenge and tolerance tests. Draw blood specimen in a fasting state. For **GCT**, give 50 g glucose orally and collect blood after one hour and for **GTT**, give 75 g glucose orally and draw samples every half h for 2<sup>1/2</sup> h or 100 g glucose and take sample every 1 h for 3 h. Collect urine to test for presence of glucose and acetone with every blood sample.
- ☐ To test the effect of a certain medication, determination of glucose level is requested on consecutive days, before, during, &/or after the patient has received a medication.
- ☐ Collection of an acute & convalescent serum to aid in the diagnosis of a viral infection when culturing is not feasible.
- ☐ Other examples include tests such as stool for occult blood, ova & parasites & blood cultures.

#### Sequential Sampling:

- ☐ Diagnosis of many endocrine diseases requires sequential sampling of blood &/or urine. All sequential specimens are from the same patient & are sent to the laboratory in the same order as they are collected.
- ☐ The specimens are clearly labeled in the chronological sequence (1 of 6, 2 of 6, or time drawn) & with the patient's name & date of collection.

Only one test request form accompanies the serial samples, & it is completed with all patient information, including any medications administered & the number of samples sent.

#### Precautions:

- Strictly follow safety precautions e.g. (a) use sterile disposable syringes & needles (b) wear gloves, (c) avoid injuries from sharps (do not capped needles destroy those by mechanical / electric devices), (d) dispose sharps into puncture resistant containers.
- Avoid stress to the patient significantly affects analytes values such as blood glucose & total leukocyte count..
- Avoid excessive negative pressure when syringe is used

- Dispatch samples to the laboratory without delay
- Obtaining blood from an indwelling line or catheter is a potential source of error. Because it is a common practice to flush line with heparin, they are flushed free from heparin before any blood is collected for laboratory tests.
- If intravenous fluids are being transfused into an arm, the blood sample is collected from opposite arm. If an intravenous infusion is running in both arms, sample is drawn after the intravenous infusion is turned off for at least two minutes before venipuncture & applying the tourniquet below the intravenous infusion site.

**Special precautions for collection of blood for clotting tests:**

- ☒ Strictly maintain the ratio of blood to the additive
- ☒ Puncture must be clean as clotting affects the test results
- ☒ If the sample has to be transported to another city or there is likely to be delay in processing; prepare platelet-poor plasma at the collection centre

**Special considerations for microbiological samples:**

**Blood culture:** Routine blood cultures are indicated inpatient suspected to have bacteremia or candidemia.

**Timing**

- ☒ Blood for cultures is drawn prior to the institution of antibiotics.
- ☒ If antibiotic/s are given empirically in an emergency blood cultures if required, should be performed as soon as possible before the next scheduled dose.

**Volume of blood per set**

- ☒ 5-10 ml for adults and 2-3 ml of blood for children
- ☒ There is a direct relationship between the volume of blood obtained and the yield of a blood culture set.

**Number of Sets of Blood Cultures**

- The optimal yield for patients suspected to have bacteremia or candidemia is obtained with two or three sets of blood cultures.
- No more than three blood cultures

**Blood Culture Bottles:**

Separate blood culture bottles are used for children and adults

**Site of blood culture**

- ☒ Blood is obtained from peripheral venous sites
- ☒ A paired set of culture is recommended when sample is taken from central venous catheters-one from the CVC line and another from the periphery
- ☒ Blood for culture is not drawn from catheters or the groin when a peripheral (i.e., non-catheterized) site is available.

**Labeling**

- ☐ The site of each set of blood cultures is labeled, particularly regarding whether a set was drawn from a catheter, the groin, or not. It is of utmost importance in helping to distinguish pathogens from contaminants in those cases in which no peripheral access can be found

**Preparation of the site for culture**

- ☐ Disinfect a 5 cm area of skin by swabbing concentrically with 70% ethyl alcohol, from the venipuncture site outward. Allow the site to dry
- ☐ Remove the plastic cap covering of the blood culture bottle. Disinfect the rubber stopper with 70% alcohol. (Iodine solutions will disintegrate the rubber and should not be used.)

**NOTE:** Needles are not changed between venipuncture and inoculation of the bottles, or between bottles. The risk of needle stick is increased, while the chance of contamination is not significantly lessened

**Patient Variables** Many variables related to patient can affect analyte values

- ☐ **Basal State:** Generally, the specimen to determine concentration of body constituents is collected in the morning around 8.00 AM when the patient is in a basal state {i.e. early in the morning after overnight fasting (about 12-14 h after ingestion of food)}. Reference intervals, most often are derived from the analyses on specimens collected during this period.
- ☐ **Exercise:** Moderate exercise can cause an increase in blood glucose, lactic acid, serum proteins and creatine kinase (CK)
- ☐ **Emotional or Physical Stress:** The clinical status of the patient can cause variations in test results.
- ☐ **Circadian rhythm:** Levels of many analytes significantly change due to circadian rhythm. For example, growth hormone level peaks in the morning before waking and falls throughout the day. Serum iron level may fall by 30% to 50% from morning until evening.

**Test Interference:**

Certain foods & drugs, investigations involving injection/ingestion of dyes etc. may interfere in correct analysis of analytes. Such interfering items are discontinued for at least 6 to 7 days; if there is a doubt, the clinician is made aware either to avoid the test or to correlate the results with limitations.

Bananas contain 5 hydroxy indole acetic acid and may increase its level. Ingestion of green vegetables increase vitamin K level and may decrease the prothrombin time and hence the INR.

Administration of dyes and contrast media for investigations interfere with many tests

**Table-2 Effect of time of sampling and patient management on analyte values**

Analyte/Therapy	Remarks/Special instructions/Interferences
Default rule	As a rule blood specimen should be taken early morning while the patient is fasting for 10 h
Thiazide administration	Produces hyperuricaemia & hyperglycaemia

Catecholamines	Catcholamine (epinephrine, nor-epinephrine and dopamine) show variable rise in pheochromocytoma, neuroblastoma and carcinoid. Tricyclic antidepressants and phenoxybenzamine, levodopa and methyl dopa administration can raise levels of catecholamines in blood and
----------------	---

	urine. If possible these should be withdrawn before these tests
Oral contraceptives	Mild to marked fall in levels of vitamin B <sub>12</sub> , increase in thyroxine-binding globulin (hence rise in thyroxin & unsaturated thyroxin-binding globulin), but with no significant change in unbound (free) thyroxin
Serum iron	Marked diurnal variation (32 to 50% fall from level at 8.00 a.m. to a level at 2.00 p.m. (Collect fasting sample). Stop iron therapy 6 days before. Reference intervals differ with regard to gender, age and physiological status (pregnancy, lactation and menstruation.
Bleeding time	Prolonged after aspirin ingestion
Lipid profile	Fasting sample
Hormonal studies,	Specimens are collected at specific times e.g. for Cortisol at 8.00 A.M. & 4.00 P.M. for LH, FSH, Prolactin (because of the episodic, circadian & cyclic variations in secretion of gonadotropin), three sample drawn 20 minute intervals are pooled. Prolactin sample 3 to 4 hours after the subject has awakened
Vitamin B12/ Vitamin D	Cover the tube with aluminum foil
PTH	.Collect in pre-chilled SST evacuated tube & send immediately to lab where it is centrifuged & serum is frozen till it is out sourced
24 hour urine collection	<ul style="list-style-type: none"> <li>• For safety purposes, instruct the patient to urinate in a clean, dry container, then transfer the urine to the collection bottle. The patient should not urinate into the collection bottle.</li> <li>• Avoid alcoholic beverages &amp; vitamins for at least 24 hours before starting to collect urine, &amp; during the collection period</li> <li>• Do not exceed normal intake of liquids or change dietary habits during the day before &amp; the day of collection unless physician gives specific instructions to do so.</li> <li>• Empty bladder (void) into the toilet on the morning of the collection day. Do not include the first urine specimen of the day.</li> </ul>
Oral Glucose Tolerance Test (OGTT)	Medications known to affect GTT, diet (give unrestricted for 3 days containing at least 150 g of carbohydrate), bed rest impairs with the test (perform test on ambulatory patients)
<b>Effect of Circadian variation</b>	
Serum iron	Level may fall by 50% from 8 am level to 2 pm
Cortisone	Level may fall by 50% from 8 am level to 4 pm
Serum potassium	Level may fall from 5.4 mmol/L 50% from 8 am level to 4 pm
Serum TSH	Level may fall by 50% from 2 am- 4 am level to 6 pm- 10pm
Serum Phosphate	Level are as much as 30% higher at night than during morning



<b>Effect of Prolonged Bed Rest</b>	
Haematocrit	Levels can increase by 10% within 4 days
Plasma protein	Levels may fall by 0.5g/dl
Serum Potassium	Levels may fall by 0.5mmol/l
<b>Effect of Exercise</b>	
Plasma Lactate	Levels may increase by 2-10 folds
Plasma rennin activity	Plasma rennin activity by 400% after strenuous exercise for 10 mins.
Serum CK	Increased CK activity seen after intramuscular injection of certain drugs
<b>Effect of Ingestion of specific foods</b>	
Caffeine	
Serum Cortisol	Normal diurnal variation may be suppressed.
Serum Gastrin	Levels may increase by as much as 5 times.
<b>Effect of Smoking</b>	
Serum growth hormone	Levels may increase upto 10 folds within 30 mins of smoking a cigarette
Serum Cortisol	Levels may increase by 40% within 5 mins of start of smoking
TLC	Increased by as much as 30% in smokers
Vit B12	Levels are notably reduced in smokers
<b>Effect of Alcohol</b>	
Serum triglycerides	There is marked hypertriglyceridemia
Serum HDL	Increased HDL levels are seen with prolonged moderate ingestion
GGT	Increased GGT activity ( marker of persistent drinking)
MCV	Increased MCV
<b>Antoconvulsants (Phenytoin)</b>	<p>Reduced serum and red cell folate levels.</p> <p>Reduced serum calcium and folate levels.</p> <p>Increased ALP activity.</p>

	T3 levels are reduced but TSH are normal.
<b>Fever</b>	Increased blood glucose levels. Plasma cortisol levels are increased.
<b>Effect of Shock and Trauma</b>	
Serum Cortisol	Levels may increase by 3-5 fold.
Plasma fibrinogen	Levels may double in 2-8 days after surgery.
Serum CK	Increased CK activity seen after trauma and surgery.
T3	Levels reduce by 50% after surgery.

### Collection of Urine Specimen

- ☒ Urine for routine urinalysis is collected in clean container and for culture in a sterile container. Perform urinalysis within 2 hours of collection. Specimens held at room temperature for more than 2 hours may show changes in pH and chemical constituents. If the specimen cannot be analyzed within 2 hours, it is refrigerated to prevent bacterial growth
- ☒ Ensure that the date and time of collection is returned in the request form or on the container. If the sample has been refrigerated it is indicated on the request form
- ☒ If both urinalysis and culture are requested, 2 separate specimens are collected
- The barcode label should withstand refrigeration and should include the patient's identification and date and time of specimen collection. The labels are placed on the container, not on the lid.

### Urine for microbial culture

- ☒ Urine for microbial culture is collected in sterile screw cap container.
- ☒ Morning voided urine , mid stream specimen is preferred
- ☒ The clean catch technique must be followed as mentioned below:
  - Urine collection (female): The patient is given instructions to wash her hands with soap and water, rinse and dry. Then cleanse the urethral opening and vaginal vestibule area with soapy water, rinse the area with water dry and hold the labia apart during voiding. Allow a few ml of urine to pass and then collect the midstream portion of urine in a sterile container.
  - Urine collection (male): The patient is given instructions to wash his hands with soap and water, rinse and dry. Then cleanse the penis, retract the foreskin (if not circumcised) and wash with soapy water, and then rinse the area well with water and dry. Keeping foreskin retracted, allow a few ml of urine to pass and then collect the midstream portion of urine in a sterile container.
  - For neonates a suprapubic collected urine is preferred
- ☒ **Foleys catheter tips are not recommended for culture**
- ☒ A minimum of 3 ml urine sample is sent for culture.
- ☒ Cultured within 2 hours after collection or refrigerated at 4<sup>0</sup>C and then cultured within 8 hours.

### Urine: 24h Urine Collection

- ☒ Take a clean container of 2 litre capacity and add preservative as below:

TEST	PRESERVATIVE	QUANTITY
Urinary Urea	Boric Acid	10 gms
Urinary Phosphorus	Boric Acid	10 gms
Urinary Uric Acid	Sodium Carbonate	05 gms
Urinary Calcium	6 N HCL	10 ml.
Urinary Magnesium	6 N HCL	24 ml.
17 OH Corticosteroid	6N HCL	25 ml.
17 Ketosteriod	6 N HCL	25 ml.
Catecholamines	6 N HCL	25 ml.
Metanephrines	6 N HCL	25 ml.
Urinary Protein	Boric Acid	05 gms
Urinary Cortisol	6 N HCL	25 ml.
5 Hydroxyindole Acetic Acid	6 N HCL	25 ml.
Vanilmandelic acid	6N HCL	25 ml

**Note:** No preservative is added for the following tests; Urinary Sodium, Urinary Potassium, Urinary Chloride, Urinary creatinine and Urinary glucose.

- ☐ To start the collection, the patient is instructed to discard the first morning urine specimen and note the exact time.e.g.7.00 A.M
- ☐ The subsequent urine samples are collected in the container provided. The collection is continued till the same time next day e.g. up to 7.00A.M. That is the 24 hours urine sample
- ☐ The 24 hours urine sample is submitted to the lab within 1.00 hour
- ☐ Total urine output in 24 hours is measured and recorded on the request form and 20 ml well mixed aliquot from the total volume is sent for the analysis. In case of Creatinine Clearance Test the height, weight, and age of the patient is specified.
- ☐ Preservative to be added as per the test requirement:

**Possible interferences:**

- Stop intake of alcohol and following foods or drugs that may affect analyte values 72 hours prior to the start of collection. Consult the treating physician before stopping any medication.

TEST	FOODS	MEDICATIONS
Metanephrines	Coffee, tea, soda, chocolate	None
VMA	None	Labetolol, methyldopa
5HIAA	Bananas, pineapple, plums, tomatoes, walnuts	Acetaminophen, Aspirin, Mephenesin, Methocarbamol, Imipramine, Isoniazid, Phenothiazines, Phenacetin, cough syrup containing glyceryl guaiacolate

### Collection of C.S.F

- The collection of specimen is performed under strict aseptic conditions by the clinician and is sent to the laboratory in sterile screw-capped containers. Usually two specimens are collected from the arachnoids space by inserting a sterile wide bore needle between the 4<sup>th</sup> and 5<sup>th</sup> lumbar vertebrae. Minimum suggested volume is 1-2 ml for routine, fungal/ mycobacterial cultures respectively. Maximum sample is sent to microbiology laboratory. The specimen is transported to the lab without any delay.
- CSF is analyzed as early as possible but within 2h of collection
- CSF samples for different laboratories are sent in different containers.
- If CSF sample is sent in 2 containers for same laboratory, the sample is pooled before analysis.
- CSF specimens not refrigerated unless viral studies alone are requested.
- CSF always kept at 37<sup>o</sup>C for bacteriological investigation.

### Collection of Sputum

- ☐ Sputum is collected in a wide mouthed disposable universal container
- ☐ If organism like *Corynebacterium diphtheriae*, cryptosporidium, *Bordetella pertussis* a suspected, the concerned doctor contacts the laboratory before sending the specimen because it may require special technique to collect the specimen or media for isolation of these agents.
- ☐ Expecterated sputum: if possible ask the patient to rinse mouth and gargle with water prior to sputum collection. Instruct the patient not to expectorate saliva or postnasal discharge into the container. Collect specimen resulting from deep cough in sterile screw- cap container.
- ☐ Induced sputum is sent when the patient is unable to produce sputum on his own.
- ☐ 24 h sputum collections are not recommended for culture.

## Respiratory Specimens

- ☐ **Tracheostomy and endotracheal aspirations:** The clinician aspirates the specimen into a sterile sputum trap. **Tracheostomy tube tip or endotracheal tube tip are not recommended for culture, hence are not accepted.**
- ☐ **Bronchoscopy specimens:** These include bronchoalveolar lavage, bronchial washing, bronchial brushing and transbronchial biopsy specimens. These samples are collected by the clinicians and sent to the laboratory in sterile containers.
  - **Throat swabs:** Throat samples are not obtained if epiglottitis is inflamed, as sampling may cause serious respiratory obstruction. Tongue is gently pressed with a tongue depressor and sterile swab is rotated between the tonsillar pillars and behind the uvula and around the pharynx, tonsillar areas and any inflamed or ulcerated areas for obtaining the specimen.
  - **Post nasal swabs:** These are used to sample nasopharyngeal secretion for the diagnosis of meningococcal carriage. Nasopharyngeal suction when introduced through the mouth, it carries the swab up behind the soft palate into the nasopharynx. Nasal swabs are used for the detection of Staphylococcal infections.
  - **Nasopharyngeal suction:** Submitted for the detection of carriers of *S. pyogenes*, *N. meningitidis*, *C. diphtheriae* and *B. pertussis*
  - **Fungal cultures:** Recommended volume of the samples is 3-5 ml. Collect 3 early morning specimens by deep coughing or sputum induction. Lung biopsy or lung aspirates are also used for fungal culture.
  - **Anaerobic cultures:** Sinus aspirates, tympanocentesis fluid, transtracheal aspirate and lung aspirates or biopsy specimens are appropriate.
  - **Mycobacterium cultures:** Recommended volume of the samples is 5-10 ml. Collect 3 early morning specimens by deep coughing or sputum induction. Lung biopsy or lung aspirates are also used for culture.

## Collection of Faeces

The universal container for fecal specimen should be wide-mouthed and disposable and should contain a small wooden or metal spatula-shaped stick.

Instructions to the patient: "Unscrew the container; hold the stick and pick up a spoonful of faeces. Place it into the universal container. Replace the cap tightly. Deliver the specimen in the laboratory as early as possible. When there is likely to be delay under warm conditions add neutral glycerol saline to the container.

## Superficial/ Deep Wounds, Aspirates and Tissue Specimens

- ☐ Syringe aspirates or biopsy specimens are preferable to swab specimens
- ☐ Superficial fungal lesions; Clean the surface with alcohol, Scrape periphery (border) of the lesion with a scalpel. Selectively include hair from scalp lesions for examination. Collect debris or material beneath the nail plate when nail is involved. Transport in folded filter paper. If material is very scanty place directly on a glass slide. Put a drop of KOH solution over it, cover with a cover slip, and send it to the laboratory.

## Ocular Specimen Collection

- Prepared smears (KOH mounts) and inoculated media (obtained from the microbiology laboratory after prior consultation) are sent to the laboratory immediately.
- Wet mounts are made for detection of *Acanthamoeba* sp, or the sample (intraocular fluid) is transported to the laboratory immediately in a capped syringe with bubbles expelled.

**Genital Tract Specimen Collection**

- ☐ Vaginal swabs: High vaginal swabs are preferred.
- ☐ Urethral swab (male): the clinician collects specimens at least 2 h after the patient has urinated.

**CAPD (continuous ambulatory peritoneal dialysis) Fluid Collection**

At least 100 ml of fluid is transferred from the bag aseptically and sent to the laboratory in a sterile container. Alternatively whole dialysate bag is sent to the laboratory.

**Intravascular Devices Tips**

The clinicians remove the cannula in an aseptic manner after skin disinfection and send 4cm of the intravascular segment of the catheter in a sterile container. If purulence of the catheter exit site is evident, pus is sent for culture and gram stain.

**Specimen Collection for Semen Analysis**

- ☐ The sample is collected after a 2- 3 day period of sexual abstinence.
- ☐ Specimen is collected by masturbation into a sterile plastic container
- ☐ Specimen is kept as close to body temperature as possible until delivery is made to the laboratory. Extreme heat or cold will ruin the specimen.
- ☐ Specimen is delivered to the laboratory within half an hour after collection

**Anaerobic Culture**

ACCEPTABLE SPECIMENS	UNACCEPTABLE SPECIMENS
----------------------	------------------------

**Sample  
Collecti  
on and  
Transp  
ort  
Details  
for  
Polyme  
rase  
Chain**

<ul style="list-style-type: none"> <li>❖ Normal sterile body fluids</li> <li>❖ Bile, pleural, sinus, joint, pericardial and peritoneal fluid, blood.</li> <li>❖ Surgical specimens from normally sterile sites:</li> <li>❖ Abscess contents, deep aspiration tissue from wounds, sinus.</li> <li>❖ Suprapubic urine, transtracheal and culdoscopy aspirates</li> <li>❖ Bone marrow</li> <li>❖ Uterine contents, if collected using a protected swab</li> </ul>	<ul style="list-style-type: none"> <li>❖ Saliva, throat swab</li> <li>❖ Expecterated sputum, endotracheal aspirates</li> <li>❖ Unprotected BAL</li> <li>❖ Vaginal/ cervical swab</li> <li>❖ Faeces (except for <i>C. difficile</i>)</li> <li>❖ Colostomy or ileo-stomy effluent</li> <li>❖ Skin swabs</li> <li>❖ Superficial wound swabs</li> <li>❖ Rectal swab</li> <li>❖ Urethral swab</li> <li>❖ Voided/ catheterized urine</li> <li>❖ Semen/ prostatic secretions</li> </ul>
--	--

**Reaction (PCR), Genotyping and Viral Load Monitoring**

Specimen type	Minimal quantity	Optimal quantity temperature (optimal)	Storage and transport (optimal)	Collection tubes (additives)
<b>Mycobacterium Tuberculosis</b>				
Sputum (3 consecutive days first morning expectorate)	1 ml	As much as practical	Refrigerated	Sterile vial
Pleural/ body fluids other than synovial and Ocular fluid/ aspirate	1 ml	As much as practical	Refrigerated	Sterile vial
Synovial/ ocular fluid	As much as practical	0.5 ml	Refrigerated	Sterile vial
Endometrial tissue	As much as practical	As much as practical	Refrigerated	Sterile vial
Urine (first morning single day/ 3 consecutive days)	1 ml	10 – 100ml	Refrigerated	Sterile vial
CSF	0.5 ml	2 ml	Refrigerated	Sterile vial
Formalin fixed/ paraffin embedded tissue	-	As much as practical	Room temperature	Sterile vial

Culture isolate	1 ml	5 ml	Refrigerated	Sterile vial
-----------------	------	------	--------------	--------------



Whole blood (tasing)	4 ml	4 ml	Refrigerated	EDTA/ACD
Bone marrow		2 ml	Refrigerated	EDTA/ACD
Menstrual blood, (first day)	1 ml	5 ml	Refrigerated	Sterile vial
<b>HBV/PCR</b>				
SST serum/ EDTA or ACD	1 ml	3 ml	Refrigerated/frozen	Sterile vial plasma
Paraffin block of liver biopsy	-	As much as possible	Room temperature	Sterile vial
<b>HCV PCR</b>				
SST serum/ EDTA or ACD	1 ml	3 ml	Refrigerated/frozen	Sterile vial
Paraffin block of liver biopsy	-	-	-	-
<b>CMV</b>				
Amniotic fluid/ CSF	1 ml	As much as possible	Refrigerated	Sterile vial
Bal/ Saliva/ Urine	5 ml	As much as possible	Refrigerated	Sterile vial
EDTA plasma/ SST serum	1 ml	2 ml	Refrigerated	Sterile vial

#### Sample Collection for Mycobacteriological Investigation\*

Urine	3 consecutive early morning urine whole samples (minimum 40 ml each).
Sputum	At least 2 consecutive early morning samples
Gastric lavage	At least 2 consecutive early morning sample to be neutralized (sodium bicarbonate) soon after collection.
Endometrial curettage	(preferably pre menstrual)
Pus/ tissue/ aspirate and other samples	As for other investigations

Quantiferon Tb Gold **	Specialized vial (2 vials) for collection of blood is available at sample collection area (OPD) and microbiology lab (IPD). Blood is to be collected till the given mark in each vial.
------------------------	--

- ☒ **\*All samples are to be refrigerated till processed**

## Transport of specimens

Transport of specimens to the laboratory is as important a step as any other to ensure accuracy. The following considerations apply to transport of specimens

- ☒ Prepare guideline for transport of specimens
- ☒ Maintained the identity of the specimen during transport
- ☒ Avoid mild haemolysis, leakage and deterioration of the specimens
- ☒ The specimens can be transported manually or through pneumatic chutes. The pneumatic chutes system can cause mild haemolysis which may not be visible on naked eye examination but may be enough to significantly raise plasma haemoglobin concentration and activity of red cell enzymes such as LDH.
- ☒ The following specimens are not sent by pneumatic chutes: Histopathology and Cytopathology samples, Open vials/ vials with loose caps, Urine, faeces & Blood culture bottles
- ☒ If there is delay in sending the samples, unstable constituents significantly fall to give erroneous result. When samples are sent to referral laboratory by courier to another city plasma should be separated and frozen to ensure stability of several analyte (table 2-7 page 54 Tietz Text Book of Clinical Chemistry and Molecular Diagnostics, Fourth Edition 2006 Saunders, USA)
- ☒ Blood collected in capillary tubes is placed in a larger tube, which can be labeled with all the information required.
- ☒ Specimen from patients with the infectious disease should not be labeled as infectious. Every specimen should be treated as potentially infectious
- ☒ Samples for determination of ammonia, blood gases, acid phosphatase, lactate, pyruvate, gastrin, renin must be transported at 4° C even if these are sent to the laboratory with in the hospital

## Histopathology & Cytology Specimen Collection

### Procedure-

- Request prepared and specimens labeled in the Operating Room or other location where the biopsy or surgical procedure was performed. Requisitions include patient identification, source of organ/tissue, name of submitting physician(s), pertinent clinical information, pre- and/or postoperative diagnosis, and date of specimen obtained.
- Specimens are normally submitted in plastic cups (small specimens can be submitted in plastic sputum cups) in 10% buffered formalin, and labeled with the name of the fixative. The required minimum ratio of formalin-to-tissue that permits adequate fixation is 10:1 (i.e., 10 times more formalin than tissue). Specimens are thus submitted in containers at least ten times the volume of the specimen to accompany a sufficient amount of formalin.

- In addition, formalin cannot penetrate through very thick, dense tissue regardless of the amount of formalin present, and large tissues whose delivery to the histology section may be delayed is refrigerated and/or cut to allow sufficient penetration of formalin to tissue surfaces.
- Specimen containers have a patient label, barcode attached if applicable. Specimen containers are always submitted with a completed histopathology request form. A physician's signature and a short history are required. A description of the lesion is useful, especially when only a small portion of the lesion is biopsied. Duration of the lesion, age of the patient and clinical differential for the lesion are always appreciated and many times necessary. Specimens arriving with incomplete forms are not accessioned (and not processed) until the forms are completed. The department makes reasonable attempts to contact the responsible physician. We appreciate prompt compliance when we request additional information.

**The above instructions are applicable to most submissions to the histopathology department. The exceptions are as follows:**

- Specimens needing procedures not offered in our department.
  - Occasionally, physicians will prefer that a specialized outside laboratory perform a specialized diagnostic test on tissues from patients. Before making such arrangements, the clinician notifies the histopathologist. In all such cases, the clinician is responsible for supplying a copy of the report produced by the outside laboratory. The department making these arrangements must pay for the special services,
  - Occasionally, the pathologist who receives a specimen may determine that special studies need to be performed at an outside laboratory, in which case the pathologist will make arrangements with the outside laboratory to perform the studies, and will make prior arrangements for funding of the tests.
- ❏ **Frozen Sections:** See FROZEN SECTION PROCEDURES (INTRAOPERATIVE CONSULTATIONS).
- ❏ **Aspirations.** Aspirations are always considered cytology specimens, and are only submitted to the cytology laboratory.
- ❏ **Large Specimens.** Large specimens (such as amputation stumps) are submitted refrigerated, rather than formalin-fixed. When submitted to histology, requisition form is left on the accession desk. The actual specimen (wrapped and clearly labeled) is placed in the refrigerator.
  - All specimens are submitted for microscopic examination, except for amputations due to peripheral vascular disease; degenerative joint disease; bunions; and hernia sacs; and metal or plastic prosthetic devices.

#### **Container:**

- Volume of sample collected should be as minimum as possible. However, the evacuated tubes collect sample according to the size of the tube. Hence, the evacuated tubes should be of minimum volume required
- Use proper specimen vials/tubes/containers
- Mentioned name of the test on the container
- When applicable fix Bar code on the container and the request form
- Record the name of the person collecting the specimen

#### **Procedure for sending Histopathology specimens**

- Specimen can be hand-delivered or sent to the laboratory in a proper fixative (buffered formalin).

Specimen should be placed in a white mouth container. The ratio of volume of formalin to the

specimen size is normally 1:5 to 1: 10. In case of small endoscopic biopsies and needle biopsies the containers may be smaller with the narrow mouth. A detailed note on the patient's clinical history and all relevant investigations is detailed in the request form. The specimens for frozen section are sent fresh without delay in saline soaked gauze. Specimens for Immunofluorescence are sent in normal saline. Specimen container must be labeled with the details of the name, age, sex of the patient and the date of surgery and the doctor identity.

- The laboratory matches the details of patient and doctor identity on the specimens with those on the request form. It assigns the specimens a lab reference number (unique identity) and process it further.

### **Cytopathology**

- Cytopathology specimens comprise gynecology cytology smears received in 95% ethanol fixative, non-gynecology cytology FNAC smears, which are air-dried before put in 95% ethanol fixative and exfoliative cytology (body fluids, BAL, brushings) smears in 95% ethanol fixative. Note: The specimens collected by the pathologist are generally superior to those collected by others. A request form with details as specified in the previous section should be sent along with the specimen.

### **Specimen Rejection Criteria**

Each laboratory must defined its specimen rejection criteria and communicate to the same to the users of the laboratory

- Generally specimens are rejected if there are incorrectly labeled, collected in a wrong tube, are leaking, inadequate in volume, outdated or have inadequate clinical information. However some laboratories except specimens, particularly those, which are critical such as tissues or CSF and are vital for diagnosis. The specimens are processed but no report is issued tin the identity of the specimens is established by the referring physician, clinical details are made available or viability of the specimen is ensured

### **Safe Disposal of Materials Used In Sample Collection**

- Regulatory requirements must be followed by each laboratory for disposal of waste generated in the lab. Please refer to Bio-Medical Waste Management Rules 1998 of Govt. of India and the state rules if any. Modifications made after the publication of rules must be adhered to.

Validation of modified/new method:

Perform tests on at least 20 samples with the standard method and the modified method

Perform regression analysis and Bland-Altman plot to show agreement. T test may also be done; though not necessary

The coefficient of correlation ( $R^2$ ) should be more than 0.97.

Perform 20 tests on low and high controls to calculate imprecision; it should not be significantly different from that for the Standard method

Perform linearity check to show the linearity for the modified method is the same as for the standard method

**NOTE: It is better that the modification should not compromise the principle of the method**

**Annex-2**

**In-house calibration of equipments in clinical laboratory**

**Source: 1998, WHO/LAB/98.4, World Health Organization, Quality Assurance in Haematology by Dr.S.M. Lewis**

**Calibration of volumetric pipettes**

Weigh a beaker in calibrated balance using calibrated mass (weight box). Fill the pipette to the calibration mark and deliver it a weighed beaker. Reweigh the beaker. Note the ambient temperature and calculate the volume of the pipette (in mL) by dividing the weight of the water (in mg) by one of the following factors depending on temperature:

Temperature(°C)	Factor
18	0.9986
19	0.9984
20	0.9982
21	0.9980
22	0.9978
23	0.9976
24	0.9973
25	0.9971
26	0.9968
27	0.9965
28	0.9963
29	0.9960
30	0.9957

m

The calibration must be performed in duplicate for each pipette

#### **Calibration of micropipettes:**

The weighing scale must be calibrated using calibrated mass.

Volume of the micropipettes varies from 1 to 500  $\mu\text{L}$ . The procedure described above can be applied. However, 1  $\mu\text{L}$  of water weighs 1 mg the balance should have sensitivity of 0.1 mg to ensure a measurement error less than 2%. As this type of balance is not commonly available, the following modified procedure is recommended.



The example given is for a 20  $\mu\text{L}$  micropipette and can be adapted to suit other volumes.

Calibrate a 0.2 mL pipette, a 5 mL pipette and a 50 mL volumetric flask by the procedure described above. Mix and lyse (e.g. by adding a few drops of saponin solution), 2-3 mL of EDTA specimen of whole blood. Deliver with a calibrated pipette, 0.2 ml of the specimen in 50 mL haemiglobincyanide reagent in the volumetric flask to reach a dilution in 1/251.

Fill the 20 µL pipette to be calibrated, up to the mark with blood and deliver into a test tube containing 5 mL haemiglobincyanide reagent. (The reagent should be delivered with the previously calibrated 5 mL pipette. The dilutions are carried out in duplicate and the absorbance (A) read at 540 nm on a spectrophotometer.

A<sub>1</sub> – Absorbance using the previously calibrated (0.2 ml) pipette

A<sub>2</sub> – Absorbance using the micropipette (20 µL)

Ratio A<sub>1</sub> and A<sub>2</sub> should be 1. If ratio is different use this figure as a division factor to arrive at correct volume of the pipette under calibration. Suppose the ratio is 0.95 the volume delivered by 20 µL divided by 0.95 = 21 µL

**Control of calibration of photometer:**

To ensure that a photometer is functioning correctly a calibration graph should be prepared when it is first put into use in the laboratory, and thereafter at intervals- usually every six months, but every one to two weeks if there is any doubt about its performance. The following example illustrates the preparation of a calibration graph for use in haemoglobinometry, but the same principles also apply to other tests.

Take a specimen of blood with apparently normal values. Make a concentrated HICN preparation by delivering approximately 0.1 ml blood in approximately 20 ml of haemiglobincyanide reagent (Drabkin’s Reagent). Determine its concentration against a haemiglobincyanide reference and express it as g/dl or g/L depending on the unit adopted by your laboratory. Make dilutions as follows using calibrated pipettes and measure absorbance at 540 nm wavelength.

Tube	Volume of concentrated preparation (ml)	Volume of Drabkin’s solution (ml)	Expected Absorbance

1	5 (Approximately)	Nil	'A'
---	-------------------	-----	-----

2	4 (Accurately)	1 (Accurately)	'A' x 4/5
3	3 (Accurately)	2 (Accurately)	'A' x 3/5
4	2 (Accurately)	3 (Accurately)	'A' x 2/5
5	1 (Accurately)	4 (Accurately)	'A' x 1/5
6 (Blank)	Nil	5 ((Approximately)	0

The values can be plotted on linear-linear graph (concentration on the abscissa and absorbance on the ordinate). If accurately performed, the line joining the points passes through zero point and the absorbance of each dilution is very close to the expected value.

If the line of the calibration graph is not linear throughout its length, only the linear portion can be used for deriving measurements. If there is no linearity at all, the instrument requires attention by the manufacturer.

Calibration using other standard solutions and / or filters is also described <sup>1</sup>

1. Burtis Carl A., Ashwood Edward R. and Bruns, David E.: 2006 Tietz Text Book of Clinical Chemistry and Molecular Diagnostics Saunders St. Louis Missouri, USA

### **Testing for Thrombophilia**

Thrombophilia is used to describe disorders of haemostatic system which leads to thrombosis.(1990 BCSH).The term thrombophilic is frequently used by clinicians to describe patients who have developed venous thrombosis either spontaneously or of a severity out of proportion to any recognized stimulus, patients who have recurrent venous thrombotic events and patients who develop venous thrombosis at an early age. Individuals who have a thrombophilic defect identified on laboratory testing and who have a family history of proven venous thrombosis are at greater risk of thrombosis than individuals who have a thrombophilic defect without family history of venous thrombosis (Lensen et al, 1996).<sup>1</sup>

**Prothrombotic states may be genetic or acquired, it includes**

Antithrombin deficiency

Protein C and Protein S

Activated Protein C resistance(APCR)

FV Leiden mutation

Prothrombin 20210A

Dysfibrinogenemia

Lupus Anticoagulant

Elevated FVIII levels

Hyperhomocysteinemia

**Conditions associated with acquired thrombophilic laboratory abnormalities<sup>3</sup>.**

**Acute thrombosis**

Low protein S

Low protein C

Low antithrombin

**Infection**

Antiphospholipid antibodies

**Inflammation**

Elevated factor VIII

Low free protein S

Elevated Lp(a)

**Nephrotic syndrome**

Low protein C

Low protein S

Elevated Lp(a)

**Complex congenital heart disease (single ventricle)**

Low protein S

Low protein C

Low antithrombin

**Asparaginase (acute lymphoblastic leukemia)**

Low antithrombin

**Liver disease**

Low protein S

Low protein C

Low antithrombin

**Warfarin therapy**

Low protein S

Low protein C

**Heparin therapy**

Low antithrombin

**Nutritional deficiency**

Elevated homocysteine

**Pregnancy**

Low protein S

**The Reason for testing:**

Identification of risk factors either as single or multifactorial helps decide secondary prophylaxis or ? prevention of rethrombosis.

**When should Thrombophilia testing be performed?**

Testing done during acute phase of thrombus may result in incorrect results especially in case of inherited deficiency. There is a transient decrease in the levels of AT, protein C and S during acute thrombosis. Any abnormal results during this phase might be repeated at a later date for confirmation along with devoid of anticoagulation.<sup>1,2,3</sup> Molecular testing can be tested and reported without any caution since there is no interference by these test results during acute episode. The acute thrombosis period is not appropriate since activation of coagulation due to the thromboembolic event may induce perturbation of coagulation with false positive or negative results. However at certain circumstances some test can be performed at the time of the event diagnosis<sup>4</sup>,

LA,aCL,coagulation factors (prolongation of APTT)

Protein C,protein S and APCR(skin necrosis)

AT (thrombosis at unusual site/young age).

**Who to test/screen?**

1. Evidence of heritable thrombophilia testing is only appropriate in the presence of family history, especially with the history of idiopathic venous thrombosis<sup>3,4</sup>.
2. All patients with venous thromboembolism, independently of the age of onset (before or after 45 years), the circumstances of thrombosis (provoked or unprovoked), and the severity of the clinical manifestations.
3. Patients developing skin necrosis at the initiation of VKA.

4. All women with complications of a pregnancy other than venous thromboembolism:



- One or more episodes of late fetal loss
- Two or more episodes of early fetal loss

5. Women with pre-eclampsia, fetal growth retardation or abruptio placentae are potential candidates.

6. All asymptomatic individuals who are first-degree relatives of a diagnosed carrier of a thrombophilic trait. This should be accompanied by accurate information and counseling.

### Recommendations regarding thrombophilia testing in children<sup>3</sup>

Who	Recommendation	Why	Comments
Adolescents with spontaneous thrombosis	Testing should be strongly considered	Identify combined defects Counsel regarding risk of recurrence Counsel/test other family members	This group has the highest prevalence of inherited thrombophilia
Neonates/children with non-catheter related venous thrombosis or stroke	Testing should be considered	Identify combined defects Counsel regarding risk of recurrence Counsel/test other family members	—
Neonates/children with symptomatic catheter-related thrombosis	Not enough data to make a recommendation	Reports vary regarding the role of thrombophilia in catheter-related thrombosis	—
Neonates/children with asymptomatic catheter-related thrombosis	Testing is not recommended	Thrombosis in the setting of catheter-related thrombosis is extremely common No data to suggest thrombophilia is increased	Consider testing if there are recurrent events
Asymptomatic children with a positive family history	Decision to test should be made on an individual basis only after counseling	Counsel adolescent females on risk of estrogen Thromboprophylaxis in high-risk situations	Be careful about false reassurance Test parent first, if possible Encourage waiting until child is older
Asymptomatic children-routine screening (prior to catheter placement, leukemia therapy or oral contraceptives)	Testing is not recommended	Not cost effective Many patients with risk factor will not have an event Catheter-related thrombosis not necessarily increased with inherited thrombophilia and there is no effective prophylaxis	—
Neonates/children participating in thrombosis research	Testing is recommended	More data on long term outcomes are needed to definitively determine the role of genetic risk factors and optimal therapies]	—

Antithrombin deficiency	Chromogenic or clotting
Protein C	Chromogenic or clotting assay
Protein S	Immunologic assay of free and total protein S antigen -ELISA
Activated Protein C resistance(APCR)	Clotting assay

Factor V Leiden mutation	PCR
Elevated factor VIII	One-stage clotting assay, chromogenic assay
Antiphospholipid antibodies	Phospholipid-based clotting assays, (PTT and DRVVT) with confirmatory assay using exogenous phospholipid, ELISA assays for IgG and IgM antibodies directed against cardiolipin and 2 glycoprotein
Elevated lipoprotein (a)	ELISA
Hyperhomocystenemia	Fasting homocysteine

#### Laboratory Tests:

It includes phenotypic measurement of natural anticoagulant levels and genetic analysis of gain of function polymorphism<sup>1,2,3</sup>.

**Antithrombin deficiency:** Antithrombin (previously called antithrombin III) is synthesized by the liver. Its inhibitory effect is not confined to thrombin. It also inhibits the activated proteolytic clotting factors IXa, Xa, XIa, and tissue factor-bound factor VIIa. Heparin also accelerates this effect. There are two major types of AT deficiency, Type I is characterized by a quantitative reduction of qualitatively (functionally) normal antithrombin protein. Type II deficiency is due to the production of a qualitatively abnormal protein. In both types of antithrombin deficiency, antithrombin activity is reduced to a variable extent.

**Prevalence:** The prevalence of type I antithrombin gene mutations in the general population is around 0.2% (Tait et al, 1994). Family studies suggest that antithrombin deficiency is a more severe disorder than deficiencies of protein C or protein S with the majority of patients suffering thrombosis before the age of 25 years (Thaler & Lechner, 1981; Hirsh et al, 1989; Demers et al, 1992). The relative risk of venous thromboembolism is around 25-50 fold for individuals with type I antithrombin deficiency (Rosendaal, 1999).

**Antithrombin assays:** Only functional assays of heparin cofactor activity will detect both type I and type II antithrombin deficiencies. For routine clinical purposes it is recommended that a heparin cofactor activity assay be used in the initial screen.

**Antigenic** ó not adequate for diagnosis especially because of the commoner Type II.

**Functional** ó two types, progressive inhibitory activity and heparin cofactor activity. The later is ideal as it involves Heparin but has two methodologies involved based on the target enzyme used namely thrombin or Xa. The Xa isolates AT as heparin cofactor II interferes with anti IIa effects.

**Protein C deficiency:** Protein C is a vitamin K-dependent glycoprotein that is synthesized in the liver. Before activation by the thrombin-thrombomodulin complex on the endothelial cell surface, it circulates as a two-chain zymogen. By degrading activated clotting factors Va and VIIIa, activated protein C (APC) functions as one of the major inhibitors of the coagulation system. Activated protein C also reduces platelet prothrombinase activity by degrading plateletbound factor Va at the receptor for factor Xa. The inhibitory effects of activated protein C are facilitated through the cofactor activity of protein S. It is classified into two types ,type 1(reduction of functional and immunological protein C),type 2(functional is substantially lower than antigen). Many subtypes in this group based on the functional site affected, either the calcium/phospholipid binding site, proteolytic site or co-factor binding sites.

**Prevalence:** heritable protein C deficiency in the general population is approximately 0.2±0.3% (Miletich et al, 1987; Tait et al, 1995) and in unselected patients with venous thromboembolism is around 3% (Heijboer et al, 1990; Koster et al, 1995a; Mateo et al, 1997). The relative risk of venous thromboembolism is between 10 and 15 fold for individuals with protein C deficiency (Rosendaal, 1999)

**Antigenic:** not popular as it misses Type II defects.

Most functional assays of protein C use the specific activator Protac which is derived from snake venom. The activated protein C formed can be quantified by clotting or chromogenic methods. Both are available in kit form from commercial manufacturers. A standard calibrated against the current International Standard for protein C must be used. Chromogenic assays are simple to perform and will detect all type I defects and the vast majority of type II defects.

**Functional:** These may be based on measurement of the anticoagulant activity of APC exerted against the natural substrates factor VIIIa and Va, or of the amidolytic activity against small synthetic substrates. Both types of assays require activation of PC. This may in turn be achieved by thrombin-thrombomodulin complex or snake venom. Thrombin-thrombomodulin activation mimicks in vivo conditions more closely than any other test. These tests are commercially available and may be easily adapted to automation in many coagulometers. However, they are potentially susceptible to artifacts because they may be affected by other conditions, such as APC resistance or high concentrations of factor VIII, and require considerable experience to interpret the results. All of these problems may be circumvented by use of amidolytic assays with snake venom as the activator. These assays may leave undetected cases of subtle PC dysfunction where the defect is restricted to the active site responsible for inactivation of the natural substrates.

**Protein S deficiency:** Protein S, another vitamin K-dependent protein, is a cofactor for activated protein C. Approximately 65% of the total plasma protein S is complexed with C4b-binding protein (C4bBP) and has no cofactor activity. The remaining 35%, designated free protein S, remains uncomplexed and is the active moiety. The bioavailability of protein S is closely linked to the concentration of C4bBP, which acts as an important regulatory protein in the activated protein C:protein S inhibitory pathway. Type I- Decrease in levels of Free and Total PS (detected by antigenic assays for both Free and Total PS), Type III- Decrease in levels of Free PS but normal total (probably a phenotypic variant of Type I- same assays), Type II- Qualitative defect (will require functional assays in addition to antigenic levels- but extremely rare).

**Prevalence:** Protein S deficiency in the general population remains unknown. It has been suggested that the best way to determine protein S deficiency is by measurement of free protein S antigen (Faioni et al, 1997). In the Leiden Thrombophilia Study (Koster et al, 1995a) and in a subsequent population-based case control study reported from Italy (Faioni et al, 1997), low levels of free protein S antigen were found in around 3% of patients with venous thromboembolism and in 2.1% and 1.3% of the controls, respectively, suggesting that low free protein S levels have a mild effect on the risk of venous thrombosis increasing the risk by only around twofold.

**Protein S assays:** Three main types of assay are available: for functional protein S, and for total immunoreactive protein S and free immunoreactive protein S.

**Functional:** the present day functional assays are not specific and is low for PS in cases of APCR, raised FVIII and LA. Not recommended at all at this stage.

**Antigenic:** both for total (ELISA, using monoclonal antibody to PS) and free PS. Out of the two the Free PS is a must and is the one STRONGLY RECOMMENDED. For antigenic Free PS the method is ELISA but are based on two main principles: use of a monoclonal antibody that recognizes only the free form of PS or utilization of the natural ligand C4BP $\beta$  as "catcher".

Both are comparable and are also widely available on latex beads, which help automation, and the LIA are most reliable since it overcomes the high CV of ELISA especially in low PS cases due to fall out of protein during prolonged incubation between steps of ELISA..

### **Activated Protein C Resistance(APCR)**

APC resistance is defined as an impaired plasma anticoagulant response to APC added in vitro. APC resistance co-segregated with thrombosis in families with familial venous thromboembolism (Dahlback et al, 1993). These cases have the same point mutation in the gene for clotting factor V (1691G-A), the factor V Leiden mutation (Bertina et al, 1994). The factor V Leiden is the most common cause of inherited APC resistance, other changes in haemostasis cause acquired APC resistance, e.g. increased plasma levels of factor VIII or the presence of antiphospholipid antibodies.

**APC resistance tests:** The most commonly used test system is the activated partial thromboplastin time (APTT). Samples are tested with and without added APC and the

resultant clotting times are expressed as a ratio (Dahlback et al, 1993; De Ronde & Bertina, 1994)  $\pm$  the so-called APC sensitivity ratio (APC:SR). When testing for APC resistance it is important to avoid platelet contamination and activation. Another test is the APTT-based methods in which test plasma is prediluted with FV-deficient plasma. This modification is highly (close to 100%) sensitive and specific for FV Leiden in both healthy controls and patients with suspected acute venous thromboembolism. However there is evidence that APCR determined with the original unmodified test correlates with venous thrombosis risk, irrespective of whether or not factor V Leiden is present<sup>1,7</sup>.

**Factor V Leiden mutation:** Detection of the factor V Leiden mutation relies on amplification of the nucleotide region close to the exon- intron boundary in exon 5 of the factor V gene from either genomic DNA or from mRNA followed by a mutation detection step.

**Prevalence of the factor V Leiden mutation and risk of thrombosis:** In Caucasian populations, factor V Leiden is much more common than any of the other genetic thrombophilias having a reported prevalence of between 2% and 15% (Rees et al, 1995).

**Prothrombin G20210A mutation:** The G to A transition at nucleotide 20210 in the 3' untranslated region of the prothrombin gene is associated with elevated plasma levels of FII and increased risk for VT. In the absence of a specific phenotypic test, DNA based procedures are required.

#### **Elevated FVIII:C :**

Role of elevated FVIII:C in VTE have been suggested in literature, the levels of FVIII are persistent, independent of the acute phase reaction and represents as a constitutional risk factor for VTE<sup>6</sup>. Subjects with plasma FVIII above 150IU/dl had a 4.8 fold higher risk of DVT than those with plasma levels of 100IU/dl. Childrens were six times as likely to have poor outcome when FVIII levels exceeded cut off value at diagnosis, elevation of FVIII and D dimer at diagnosis was 91% specific and had a positive likelihood ratio of 6.1 for a poor outcome<sup>9</sup>.

#### **LA testing<sup>11</sup>:**

##### **(A) Blood collection**

1. Blood collection before the start of any anticoagulant drug or a sufficient period after its discontinuation
2. Fresh venous blood in 0.109 M sodium citrate 9:1
3. Double centrifugation
4. Quickly frozen plasma is required if LA detection is postponed
5. Frozen plasma must be thawed at 37 °C

##### **(B) Choice of the test**

1. Two tests based on different principles
2. dRVVT should be the first test considered
3. The second test should be a sensitive aPTT (low phospholipids and silica as activator)
4. LA should be considered as positive if one of the two tests gives a positive result
5. For interpretation see Below

(C) Mixing test

1. PNP for mixing studies should ideally be prepared in house. Adequate commercial lyophilized or frozen PNP can alternatively be used
2. A 1:1 proportion of patient : PNP shall be used, without preincubation within 30 min.
3. LA can not be conclusively determined if the TT of the test plasma is significantly prolonged
4. For interpretation see Below

(D) Confirmatory test

1. Confirmatory test(s) must be performed by increasing the concentration of PL of the screening test(s)
2. Bilayer or hexagonal (II) phase PL should be used to increase the concentration of PL.
3. For interpretation see Below

(E) Expression of results

1. Results should be expressed as ratio of patient-to-PNP for all procedures (screening, mixing and confirm)

(F) Transmission of results

1. A report with an explanation of the results should be given

**CUT-OFFs for Interpretation**

How should this be determined

1. Perform testing on plasmas from healthy donors
2. Take the cut-off as the value above the 99th percentile of the distribution

Interpretation

1. Results of screening tests are potentially suggestive of LA when their clotting times are longer than the local cut-off value

**DDimer:** The potential use of D dimer measurements for the diagnosis of DVT is essentially due to its high sensitivity and negative predictive value and this has become so evident after the development of new commercial assay using a cut-off of 500 mg/mL. However the specificity has been shown to be good since D dimer levels for DVT was much higher in symptomatic than in asymptomatic patients. Repeated D dimer testing especially in the first 3 months after VKA withdrawal for a first episode of unprovoked VTE could identify a subgroup of patients with low risk of recurrence<sup>10</sup>. A positive D-dimer here was the best predictor of re-thrombosis than any of the risk factors identified. A continuingly positive D-Dimer after therapy whether on it or after stopping it should prompt search for any occult malignancy.

**Regulations for testing:**

Testing at the time of acute venous thrombosis is not indicated as the utility and implications of testing need to be considered and the patient needs to be counselled before testing. As treatment of acute venous thrombosis is not influenced by test results, testing can be performed later if indicated<sup>2</sup>.

ÉThe prothrombin time (PT) should be measured to detect the effect of oral vitamin K dependent Anticoagulants, which will cause a reduction in protein C and S levels.

ÉFunctional assays should be used to determine antithrombin and protein C levels.

ÉChromogenic assays of protein C activity are less subject to interference than clotting assays and are preferable.

ÉImmunoreactive assays of free protein S antigen are preferable to functional assays. If a protein S activity assay is used in the initial screen, low results should be further investigated with an immunoreactive assay of free protein S.

ÉIf an APC (Activated protein C) resistance assay is performed to detect the F5G1691A , An APC resistance assay is unnecessary if a direct genetic test for F5G1691A is used initially.

ÉRepeat testing for identification of deficiency of antithrombin, protein C and protein S is indicated and a low level should be confirmed on one or more separate samples. Deficiency should not be diagnosed on a single abnormal result.

ÉRigorous internal quality assurance and satisfactory participation in accredited external quality assessment schemes are mandatory.

ÉThrombophilia testing must be supervised by experienced laboratory staff and the clinical significance of the results must be interpreted by an experienced clinician who is aware of all relevant factors that may influence individual test results in each case.

## References:

1. Guideline, Investigation and Management of Heritable Thrombophilia, *Br J Haematol* 2001,114,512-528.
2. T.Baglin, Elaine Gray, Mike Greaves et al., Clinical guidelines for testing for heritable thrombophilia. *Br J Haematol*, 2010,149,209-220.
3. Leslie Raffini , Thrombophilia in Children: Who to Test, How, When, and Why? *Hematology* 2008,228 ó 235.
4. T.Baglin, Management of Thrombophilia: Who to Screen?, *Pathophysiol Haemost Thromb* 2003/2004:33:401-404.
5. RM.Bertina ,Elevated clotting factor levels and Venous Thrombosis. *Pathophysiol Haemost Thromb* 2003/2004:33:399-400.
6. O Donnell, Mumford et al., Elevation of FVIII:C in venous thromboembolism is persistent and independent of the acute phase response. *Thromb Haemost* 2000 Jan ;83(1):10-3.



7. Legnani C , Palareti G, Biagi R, Bertina RM, Activated protein C resisance : a comparison between two clotting assays and their relationship to the presence of the factor V Leiden mutation. *Br J Haematol* 1996 Jun;93(3):694 ó 9.
8. Francesco Dati, Gerd Hafner, Hide Erbes et al., ProC Global : the first functional screening assay for the complete protein C pathway. *Clinical Chemistry*(1997) 43:9 1719-1723.
9. Neil A. Goldenberg, Knapp ó Clevenger et al., Elevated Plasma FVIII and D ó Dimer Levels as Predictors of Poor Outcomes of Thrombosis in children. *N Eagl J Med* 2004;351:1081-8.
10. Benilde Cosmi , Cristina Legnani,Alberto Tosetto, et al., Usefulness of repeated D ó dimer testing after stopping anticoagulation for a first episode of unprovoked venous thromboembolism: the PROLONG II prospective study. *Blood*.2010;115:481 ó 488.
11. Pengo V, Tripodi A , Reber G et al. Update of the guidelines for detection of Lupus anticoagulant. *J Thromb Haemost* 2009; 7: 1737ó1740

## ***Introduction:***

The hemostatic system consists of platelets, coagulation factors, and the endothelial cells lining the blood vessels. The platelets arise from the fragmentation of the cytoplasm megakaryocytes in the bone marrow and circulate in blood as disc-shaped anucleate particles.

**Thrombocytopenia** may be defined as a subnormal number of platelets in the circulating blood. (which occurs when platelet count is less than 100,000/cumm) and is the most common cause of serious bleeding encountered clinically. (Note: The normal platelet count in an adult is 1.5 to 4 lakhs/cumm)

Pathophysiology:

Thrombocytopenia can be further divided into constitutional, increased destruction or decreased production..

- Constitutional disorders of platelets - Mediteranean Macrothrombocytopenia, Bernard soulier syndrome.
- Causes of thrombocytopenia related to increased destruction include (1) immune thrombocytopenias (eg, autoimmune, alloimmune, drug-induced) and (2) increased consumption (eg, DIC, TTP).
- Causes of thrombocytopenia related to decreased production include bone marrow depression. Production defects result from those diseases that cause , such as aplastic anemia, infiltration by leukemia or another malignancy, fibrosis or bone marrow failure or granulomatous disorders, or tuberculosis

## **Presentation:**

### **Thrombocytopenias from Peripheral Destruction:**

#### Immune thrombocytopenic purpura

Immune thrombocytopenia(ITP) is one of the most common autoimmune disorders. It occurs in 2 distinct clinical types, an acute self-limiting form observed almost exclusively in children (5 cases per 100,000 persons), and a chronic form, observed mostly in adults (3-5 cases per 100,000 persons) and rarely in children.

ITP is caused by autoantibodies to platelets.

ITP occurs commonly in otherwise healthy individuals

#### Acute ITP

Acute ITP is a disease that occurs exclusively in children. It affects both sexes equally and has a peak incidence in children aged 3-5 years. Most patients have a history of an antecedent acute viral

syndrome.

The onset is sudden, with symptoms and signs depending on the platelet count. Bleeding is usually mild, unless the platelet count drops below 20,000/ $\mu$ L. With platelet counts from 20,000/ $\mu$ L to 50,000/ $\mu$ L, petechiae and ecchymoses are observed following mild trauma. With platelet counts less than 10,000/ $\mu$ L, generalized petechiae, ecchymoses, and mucosal bleeding occur. With platelet counts less than 2000/ $\mu$ L, widespread ecchymoses, hemorrhagic bullae, and retinal hemorrhage occur.

Physical examination reveals only the presence of petechiae and ecchymoses. The presence of lymphadenopathy or splenomegaly suggests other secondary causes of thrombocytopenia rather than ITP. The peripheral smear shows a decreased number of platelets. Often, the smear shows large young granular platelets, which is a reflection of increased thrombopoietin-induced stimulation of the bone marrow. These platelets reflect the increased megakaryocytic mass in the marrow.

**At times, the smear may show eosinophilia and lymphocytosis, possibly reflecting hypersensitivity to the inciting viral antigens.**

**This condition is typically observed in adults aged 20-40 years. It has an insidious onset, and a history of an antecedent infection need not be present. Unlike childhood ITP, chronic ITP is more common in females than in males. As in childhood ITP, the bleeding manifestations depend on the platelet count.**

The diagnosis of ITP is established by the exclusion of other causes of thrombocytopenia. The peripheral blood film should be examined to rule out (fragments) or spurious thrombocytopenia resulting from clumping. Bone marrow examination, which is not always necessary, shows increased megakaryocytes.

### **Drug-induced thrombocytopenia**

Drugs can induce thrombocytopenia by a number of mechanisms. Drug-induced thrombocytopenia results from the immunologic destruction of platelets. Drugs can induce antibodies to platelets, either acting as a hapten or as an innocent bystander. Also, drugs such as gold salts and interferon can induce an ITP-like disorder.

Common drugs associated with thrombocytopenia include quinidine, amiodarone, gold, captopril, sulfonamides, glibenclamide, carbamazepine, ibuprofen, cimetidine, tamoxifen, ranitidine, phenytoin, vancomycin, and piperacillin.

The diagnosis of drug-induced thrombocytopenia is often empirical. A temporal relationship must be present between the administration of the drug and the development of thrombocytopenia, with no other explanations for the thrombocytopenia. Recurrent thrombocytopenia following reexposure to the drug confirms the drug as the cause of thrombocytopenia. Identifying the drug that is causing severe thrombocytopenia in an acutely ill patient who is taking multiple drugs is often challenging.

Heparin causes a unique situation among drug-induced thrombocytopenias in that the antibodies also activate platelets and induce a hypercoagulable state called HITTS.

## **Thrombotic thrombocytopenic purpura**

TTP is a rare but serious disorder that was initially described as a pentad of thrombocytopenia (with purpura), red blood cell fragmentation, renal failure, neurologic dysfunction, and fever. Relatively recent evidence indicates that this disorder results from the abnormal presence of unusually large multimers of von Willebrand protein. These ultralarge precursors, normally synthesized in the endothelial cells, are processed by a plasma enzyme to normal-sized multimers. This enzyme is now identified as ADAMTS13, a metalloproteinase synthesized in the liver.

TTP is often associated with an episode of flulike illness 2-3 weeks before presentation. Most patients with TTP do not have the classic pentad. The most common presentation is petechiae and neurologic symptoms.

## **Hemolytic uremic syndrome**

Patients with hemolytic uremic syndrome have vascular lesions indistinguishable from those observed in patients with TTP, but the renal vasculature endures the most lesions, with minimal neurologic dysfunction. This is a catastrophic illness that predominantly affects children aged 4-12 months, sometimes affects older children, and rarely affects adults. The causative organism is *Shigella*-like toxins (secreted by *Escherichia coli* serotype O157:H7 or *Shigella dysenteriae* serotype I).. Diarrhea and abdominal cramps are very prominent symptoms.

Post transfusion purpura typically occurs 10 days following a transfusion. This syndrome can be induced by a small amount of platelets contaminating a red blood cell transfusion or, occasionally, following fresh frozen plasma (FFP) transfusion. The thrombocytopenia responds to intravenous immunoglobulin (IVIG). Other platelet alloantigens are occasionally implicated in posttransfusion purpura.

This alloantibody destroys the transfused platelets and the patient's own platelets, leading to a severe form of thrombocytopenia that lasts for several weeks and, sometimes, several months.

### Neonatal alloimmune thrombocytopenia

It is the most common cause of severe neonatal thrombocytopenia. Maternal antibodies against the fetal platelet antigens, inherited from the father but absent in the mother, cross the placenta and induce severe thrombocytopenia.

Typically, the diagnosis of neonatal alloimmune thrombocytopenia is considered when bleeding or severe thrombocytopenia occurs in a baby after an otherwise uncomplicated pregnancy. The affected infant may have intracranial hemorrhage, and the disorder is associated with a relatively high mortality rate. The platelet count should be checked immediately after delivery and 24 hours later as it continues to fall.

### **Disorders of platelet function**

Functional disorders of platelets associated with low platelet count are relatively rare, and most of these disorders are mild and may not be recognized early in life.

#### von Willebrand disease

In the type IIB variant, the von Willebrand protein has heightened interaction with platelets, even in the absence of stimulation causing mild to moderate thrombocytopenia. With numerous aggregates seen in the peripheral blood.

A disorder of platelet GP Ib has also been described. In this condition, increased affinity for von Willebrand protein in the resting stage leads to the deletion of plasma von Willebrand protein. This disease is called pseudo von Willebrand disease or platelet-type von Willebrand disease.

## Bernard-Soulier syndrome

Bernard - Soulier syndrome results from a deficiency of platelet glycoprotein protein Ib, which mediates the initial interaction of platelets to the subendothelial components via the von Willebrand protein. It is a rare but severe bleeding disorder. The platelet count is low, but, characteristically, the platelets are large, often the size of red blood cells, and may be missed because most automatic counters do not count them as platelets.

Storage pool deficiency: Gray platelet syndrome is due to decreased alpha granules in the platelets, which is most often asymptomatic unless it is associated with mixed storage pool deficiency. The platelet count is mildly decreased with few giant platelets.

## Workup

### *Lab Studies*

Initial detection/screen:-

Cell counter aid in the diagnosis of platelet disorders:

Subnormal platelet count and the abnormal platelet morphology can be easily picked up with the aid of counters, thereby acts as a screening tool in the diagnosis of thrombocytopenia.

#### Platelet data in cell counters

From Platelet histogram

Platelet count

Fitted curve

Mean platelet volume

Wiskot aldrich syndrome the MPV will be low.

BSS/ macrothrombocytopenia: High MPV and fitted line cannot be drawn.

TTP: Platelet histogram appears abnormal with high MPV. However In TTP RBCs morphology too interferes with the platelet histogram as fragments RBCs are counted as platelets. So a analysis of RBC histogram and Platelet histogram is a must in these cases.

Gray platelets: Marked anisocytosis in the platelets which is depicted in terms of a high PDW with abnormal histogram as the agranular platelets may not be picked by the counter.

**In all cases of thrombocytopenia, the peripheral blood smear must be reviewed to confirm the thrombocytopenia. This review is crucial.**

Spurious thrombocytopenia due to platelet clumping or platelets adhering to neutrophils (platelet satellitism) can be seen in the smear.

Examination of the peripheral smears in immune thrombocytopenic purpura often shows large young platelets. These platelets reflect the increased megakaryocytic mass in the marrow. These large young platelets appear uniformly hypergranular (metabolically active) and are often seen in persons with ITP.

Bernard-Soulier syndrome: Peripheral smear of a patient with Bernard-Soulier syndrome showing giant platelets. These platelets are not counted as platelets in most particle counters. The fitted curve cannot be drawn and PDW is very high.

Examination of the smear is essential to exclude TTP and rare instances of acute leukemia.

In TTP, a striking degree of red blood cell fragmentation is seen in addition to thrombocytopenia. Examination of the peripheral smear shows red blood cell fragments, schistocytes, basophilic cells, in addition to thrombocytopenia in thrombotic thrombocytopenic purpura.

In gray platelet syndrome the platelets appear gray (due to decreased granules) with anisocytosis of platelets which is depicted in the platelet histogram (ie fitted line cannot be drawn)

## ***Imaging Studies***

- Imaging studies are not necessary to diagnose uncomplicated ITPs.
- Rarely, platelet survival studies may be necessary to document decreased platelet survival before splenectomy in a patient with possible bone marrow hypofunction. Typically, the platelet half-life is decreased from the normal 5-7 days. A normal platelet survival curve is not consistent with increased splenic destruction.
- In a patient who has relapsed following splenectomy, an indium-labeled platelet imaging study is sometimes useful for localizing an accessory spleen.

## **Other Tests**

- Bone marrow examination is not necessary in most cases of platelet disorders. The isolated presence of large platelets in the peripheral blood, in the absence of any other signs of bone marrow dysfunction, is very suggestive of normal marrow activity
- Bone marrow examination is necessary in patients whose condition has an atypical course, have splenomegaly, or will undergo splenectomy, and in patients over age 60 years, as thrombocytopenia may be the initial manifestation of myelodysplastic syndrome.



- Bone marrow examination in patients with ITP shows megakaryocytic hyperplasia. Quantifying the megakaryocytes in the bone marrow is technically difficult. Usually, 2-3 megakaryocytes are present in each spicule in typical marrow. Clusters of immature megakaryocytes are often observed in patients with ITP.

### ***Prognosis***

- ITP is generally a benign disorder. Severe ITP with a platelet count of less than 5000/ $\mu$ L is occasionally associated with fatal hemorrhages in the brain or internal organs. Patients who are elderly, those whose disease is refractory to treatment, those who have a previous history of hemorrhage, and patients with concomitant bleeding disorders (hemophilia and uremia) are at higher risk for serious life-threatening hemorrhage.
- TTP is a very serious disorder. With the introduction of plasma exchange therapy, the prognosis is better but mortality rates remain approximately 20%. TTP should be distinguished from ITP before platelet transfusions, because a worsening of clinical manifestations occurs in patients with TTP who receive platelet transfusions.
- Among congenital bleeding disorders involving decrease in platelets BSS, type II von Willebrand disease are severe diseases associated with lifelong hemorrhages. Most other platelet disorders are mild bleeding disorders.
- Patients who have undergone splenectomy should be warned about the low risk of severe sepsis following splenectomy. These patients should be immunized with pneumococcal vaccine before splenectomy.

### **Special Concerns:**

- The major causes of thrombocytopenia in pregnant women are immune thrombocytopenia, incidental thrombocytopenia of pregnancy (gestational thrombocytopenia), and hypertensive disorders of pregnancy.
- ITP during pregnancy
- Gestational thrombocytopenia
  - This form of thrombocytopenia is defined as mild thrombocytopenia in an otherwise healthy pregnancy. How this can be distinguished from a mild form of ITP is not clear.
  - This disorder does not result in infants developing thrombocytopenia.
- Hypertensive disorders of pregnancy
  - These disorders of pregnancy (ie, eclampsia/ preeclampsia syndrome) are associated with increased platelet turnover, even when the platelet count is normal. Controlling hypertension and delivering the fetus lead to restoration of the platelet count.
  - Occasionally, thrombocytopenia is associated with hemolysis and elevated liver enzymes (ie, hemolysis, elevated liver enzymes, and low platelet [HELLP] syndrome). This serious disorder often mimics TTP.



