UNIT 7 LABORATORY PROCEDURES

Structure

7.0 OBJECTIVES

After going through this unit you will learn some neonatal procedures and other laboratory support.

7.1 INTRODUCTION

There is an increasing trend to provide bedside testing of important parameters to enable rapid decision making in the management of sick child. It is therefore essential that you should be able to perform some of the essential laboratory work.

7.2 NEONATAL PRACTICAL PROCEDURES

We will discuss three common neonatal procedures below.

7.2.1 Capillary Blood Sampling

Capillary blood sampling is used for collecting samples for hematocrit, micor-ESR, blood suger, sepesis screen, biliruni estimation and for blood chemistry. It is not indicated for blood culture, or determation of partial pressure of oxygen/carbon dioxide in blood.

Technique

- Hold the infant in a position to decrease pain, and physiological compromise.
- Choose the site for skin puncture,
- Warm the skin puncture site with cotton wool/cloth soaked in Luke-warm water to increase the peripheral blood circulation. Hold the cloth on your wrist for 5 seconds to determine correct temperature for infant's foot.
- Clean the puncture site with povidone iodine and then clean with spirit/alcohol and allow drying.
- Perform puncture in the most medial or lateral portion of the plantar surface.
- Following the puncture, the first drop of blood should be wiped off with a gauge as it contains tissue fluid, which may contaminate the specimen.

- Hold the puncture site downward to increase the blood flow. Avoid strong repetitive pressure (milking) as it causes haemolysis and increases the amount of tissue fluid in the specimen.
- Collect the blood in the container or on to the slide appropriate for the individual test.
- Stop the bleeding by holding a gauge pad against the puncture site.

7.2.2 Peripheral Blood Smear

Examination of peripheral blood smear is important tool in the diagnosis of suspected neonatal sepsis.

- Place one drop of blood on one end of a clean glass slide.
- Hold another clean slide at an angle of 45^0 on the previous slide with its edge touching the drop of blood.
- Draw the edge towards another end of previous slide dragging the drop of blood.
- A good film will be made.

7.2.3 Micro-ESR Estimation

It is one of the parameter in the 'sepsis screen' in a baby suspected to have neonatal sepsis.

Technique:

- Clean the heel and prick it by needle/lancet,
- Fill completely, standard heparinized micro-hematocrit tube of 75 mm length with internal diameter of 1.1 to 1.2 mm,
- Close one end of the tube with plasticine,
- Fix the tube vertically by means of sticking plaster (leucoplast),
- Measure the fall of dark red part in one hour to the nearest mm,
- A value more than 15 mm is considered as suggestive of infection.

7.3 HOW TO CHECK BLOOD SUGAR BY DEXTROSTICKS

Technique of dextrostic estimation of blood sugar

Equipment needed

a) soap to wash hands, b) alcohol for skin preparation, c) Test strips d) Glucometer and e) 26 gauge needle or lancets.

Procedure:

- Heel is the commonly used site for performing dextrostix.
- Make sure heel is not cold. Warm by rubbing, if required.
- Prepare the stie with 70% isopropyl alcohol/spirit, using a scrubbing/circular motion.
- Do NOT use povidine/betadine, as specimen contamination may elevate some results.
- Allow spirit to dry. Failure to allow spirit to dry may contaminate the specimen and give fallacious results.
- Make a needle stick puncture on the postero-lateral aspect of heel. Avoid the middle portion of heel and avoid making deep punctures.
- Allow a drop of blood to form and to fall on the strip.
- Do not rub the strip against the skin.
- Follow the instruction on the dextrostix bottle or glucometer.
- A blood sugar/dextrostix value of less than 40 mg/dl is defined as hypoglycemia.
- Delay in lab analysis may result in fall of blood glucose level by 14-18 mg/dl/hour.

7.4 TAKING AND TRANSPORT OF BODY FLUID SAMPLES.

Venepuncture

Check the identity of the patient and explain the procedure to the parents or other attendants of the patient. The taking of blood sample from patient of non-consenting parents might be construed in law as battery. Use the non-dominant hand if possible. Apply a tourniquet around the upper arm over the middle of biceps so as to impede the venous but not arterial flow. Wash or use an alcohol gel to decontaminate your hands. Wear gloves to avoid contamination. Clean the skin over the vein using alcohol. The skin is rendered tense by the operator's left hand; the hub of the needle, attached to a blood collection device (such as a vacuum tube holder), is held parallel to the patient's arm; the needle with bevel upwards is inserted into a prominent vein. The required amount of blood is collected into the appropriate container for the test(s) requested, and the tourniquet is removed before the needle is withdrawn, as otherwise a haematoma may form. As soon as the needle is withdrawn a gauze swab is placed on the puncture site and the attendant is asked to press this against site for 1 – 2 minutes. The needle must be disposed as per guidelines of the hospital waste management protocol.

Most biochemical and immunological tests require serum samples and these sample tubes do not contain anticoagulant. Plasma samples require anticoagulants, usually with heparin, citrate or EDTA. For glucose estimation, bottles containing sodium fluoride are necessary.

Special care is necessary both in the collection and the transport of specimens for microbiological examination. Careless collection techniques can lead to cross-contamination with organisms present on the patient's or operator's skin, or in the environment.

Tissue Discharges, PUS, CSF and Other Fluids

It is important always to send a sufficient quantity of material to the laboratory. If possible it is better to send fluid and tissue rather than swabs. Thermolabile organisms or anaerobes do not survive well on swabs, for example bacteria are killed by oxygen (anaerobes) because of the large air-fluid interface of a swab. The use of swabs with a charcoal-containing transport medium improves the chances of pathogen recovery on culture. Swab samples for viral studies require special viral transport media.

URINE

Interpretation of the results of urine culture depends on the quantification of colony forming units per ml (CFU/ml). It is most important that samples are appropriately collected and transported to the laboratory so that bacteria do not proliferate or die off in the sample. Urine specimen should reach the laboratory within 4 hrs of voiding, unless specific precautions are taken to prevent bacterial multiplication before cultures are set up. Urine samples can be stored overnight at 4^0.

Samples collected through the urethra, such as an appropriately collected midstream specimen of urine (MSU) always contain bacteria, but usually in numbers < 10^3 CFU/ml. Urinary Tract Infection (UTI) can only be diagnosed when the numbers exceed a threshold value (10^5 CFU/ml). Any colony count in suprapubic sample is significant. In the absence of UTI, suprapubic samples should be sterile. For tuberculosis three early morning urine (EMU) specimens should be submitted to the laboratory.

Urine Collection Methods

- **Catheter specimen of urine (CSU):** Aspirate the urine, via a 21-gauze needle and syringe, from the rubberized part of tubing connecting the catheter to the collecting bag. Do not collect from the tap outlet of the bag.
- **Early morning urine (EMU):** Send the entire first voided specimen to the laboratory in a large sterile container provided. Three consecutive morning specimens should be taken.
- **Midstream urine (MSU):** A urine specimen is collected by the patient/parents mid-micturition, after instruction or with the assistance of a nurse, after labia or penile orifice has been cleaned with water.

7.5 EXAMINATION OF CSF

Lumbar Puncture (LP)

It is the aspiration of cerebrospinal fluid (CSF) form the spinal sub-arachnoid space.

Technique:

- Proper positioning and restraint are critical for a successful tap.
- Neonate and young infants: perform LP in sitting position without flexing the head and neck (Fig. 7.1).
- Older infant and children: lateral recumbent position (Fig. 7.2).
- The operator's line of vision should be on the same horizontal and vertical planes as the LP site.
- The lumbar spine must be flexed but avoid pressure on neck. Hold the shoulders rather than head and neck.
- Ensure all items (needles, stylets, collecting vials, spirit, betadine, tincture benzoin) are available.
- Site of puncture: interspace between L-3 and L-4 or L4 and L5. A line joining the highest point of the two iliac crests passes just above the fourth lumbar spine.
- Clean and sterilize the site with iodine and spirit, after thorough hand washing.
- Anaesthetize the skin and tissue down to the laminae with 0.5% lignocaine and then insert a short bevel needle with stylet in the midline in the space selected. Keep the bevel in the long axis of the dura as not to cut across the fibres. As you pierce the dura, you will the resistance gives way and you have reached the subarachnoid space.
- The distance between the skin and subarachnoid space is 1.5 to 2.5 cm in infants and 5 cm in children between 3 to 5 years of age while it is 6 – 8 cm in adolescents.
- Remove the stylet and collect the CSF in 3 sterile vials/bottles.
- If there is no fluid rotate the needle. If it is dry, you may advance the needle little further cautiously. Check again for fluid.
- Do not aspirate CSF with syringe.

After collecting the sample withdraw the needle and seal the site with tincture benzoin and put the child in prone position or mother's lap for at least 15 minutes.

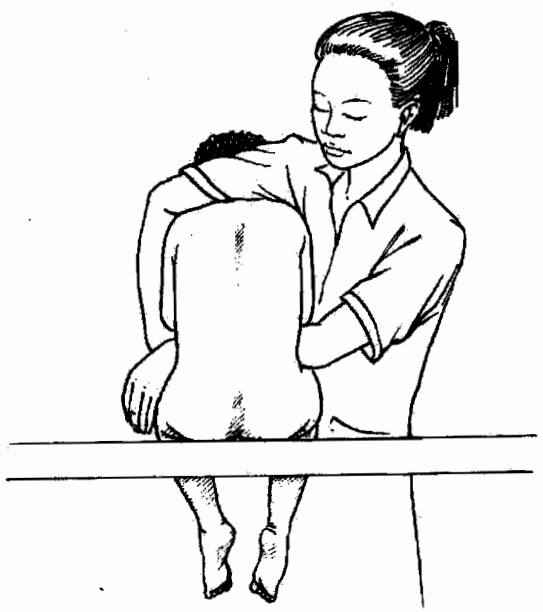


Fig. 7.1: Sitting Position

Fig. 7.2: Lateral Recumbent Position

7.6 EXAMINATION OF URINE

Early morning sample is usually preferred but not must.

Microscopic Examination

- Decant 5 ml of urine into a conical bottomed test tube.
- Centrifuge at 1500 rpm for 3 minutes. Pour off the supernatant and resuspend the deposit.
- Place a drop on glass slide, cover with a cover slip and see under the microscope.

Chemical Examination

Urine specimens can be tested using a commercially available stick for the presence of leucocytes esterase and/or nitrites. Urinary tract infection can be reliably excluded in most outpatient and some inpatient groups when the urine is visually clear and such tests are negative.

7.7 TUBERCULIN SKIN TEST

Skin reactivity with tuberculin indicates infection with the tubercle bacilli, with or without disease, vaccination with BCG or infection with other mycobacteria. The test may be negative in severe malnutrition, immunocompromised conditions and in fulminant tuberculosis.

Mantoux Test

- The test is performed on the volar aspect of forearm on left side.
- The purified protein derivative of tuberculin with RT 23 as preservative is used for this test.
- Inject 0.1 ml intradermally with bevel of needle facing upward.
- A Skin induration is noted 48 – 72 hours after the injection.
- Measure the transverse diameter of palpable induration only.

Negative = 0 – 4 mm Doubtful = 5 – 9 mm

Positive = 10 – 14 mm Strongly positive = 15 mm or more.

7.8 UMBILICAL VEIN CATHETERIZATION

A correctly positioned umbilical venous catheter (UVC) passes through the ductus venosus to lie at the junction of inferior vena cava/right atrium. UVC provides secure vascular access during the first week of life, preserving peripheral veins.

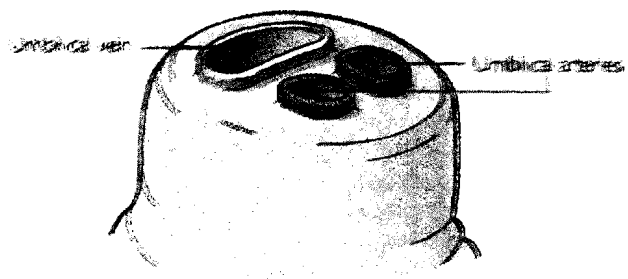


Fig. 7.3: The appearance of the cut surface of the umbilical cord

Indications

- Emergency vascular access in delivery room during resuscitation.
- Vascular access for administration of drugs, parenteral nutrition, vasoactive and hyper-osmolar infusions and blood products.
- Exchange Transfusion.

Equipment

- Catheterization packs containing sterile gown, drapes, artery clamps, fine forceps, dilating probes.
- Umbilical catheters, typically 3.5 Fr for babies < 1500 g and 5.0 Fr for those > 1500 g. Catheters should have Luer-lock attachments. In emergencies, nasogastric tubes have been inserted, but there is greater risk of accidental disconnection and blood loss.
- Sterile cord ligature, three way tap, Luer-lock extension sets, syringes, sutures.
- Heparinised saline or saline (1 U/ml).

Procedure

1. Catheters must be inserted with aseptic technique with the infant draped and closely monitored.
2. The umbilical cord and skin surrounding the stump must be cleaned with antibacterial solution. Alcohol-based solutions can burn the skin; spillage around the flanks, buttocks and back must be avoided. The solution should be allowed to dry then wiped off with sterile water.
3. The catheter and 3-way tap must be flushed with saline.
4. The insertion distance (in centimeters) for catheter placement in the inferior vena cava is calculated by the formula (2 x weight [in kg]) + 5 + stump length (in cm). Shoulder tip to umbilicus measurement can also be used.
5. The cord should be cut horizontally with a scalpel 1 –2 cm above the abdominal wall; a cord ligature placed around the base of the stump minimizes blood loss. The cut cord is held with artery forceps gripping the Wharton's jelly, and the two thick walled arteries and a single thin walled gaping umbilical vein identified (see photograph above).
6. The vein may need little or no dilatation. The catheter should be inserted into the vein and advanced with gentle pressure to the calculated distance. Blood cannot always be aspirated.
7. The Catheter can be secured using a zinc oxide tape fitted around the catheter and two sutures carefully inserted into the stump avoiding occlusion of vein and arteries.
8. Once catheter has been inserted, a purse string suture should be placed around the top of the stump to prevent bleeding. H-tape bridges or commercial devices can be attached to the abdominal wall.
9. The catheterization site must not be covered.

10. The UVC placement should be confirmed with chest/abdominal X-ray.
11. The procedure must be documented in the case-notes, detailing blood loss, state of peripheral perfusion and haemostasis, presence of femoral pulses and position of UVC tip on X-ray.
12. Fluids are infused at higher rates through UVCs and routine heparinisation is not universally used.
13. UVCs should be removed when not required, with care taken to achieve haemostasis.

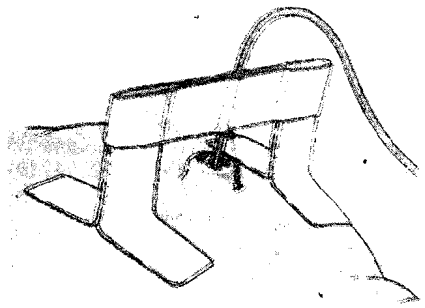


Fig. 7.4: 'H' Technique for immobilisation of the umbilical catheter

Complications

The complications arise at or during insertion, secondary to the presence of the catheter itself or due to infusate.

One of the principal complications remains the sepsis. Use of antibiotics, duration of catheterization and infusate type all are likely to influence catheter-related infection rates. Catheter removal in the face of sepsis is not always an option in the central venous line-dependant preterm, but serially positive blood culture with coagulase-negative staphylococci or growth of Gram-negative organism or Staph. aureus are indications for catheter removal.

The risk of thrombosis can be reduced by heparinisation of the infusate, and if hepatic vein thrombosis does occur, it can be relieved with streptokinase.

Hepatic abscesses can be managed conservatively or with percutaneous drainage.

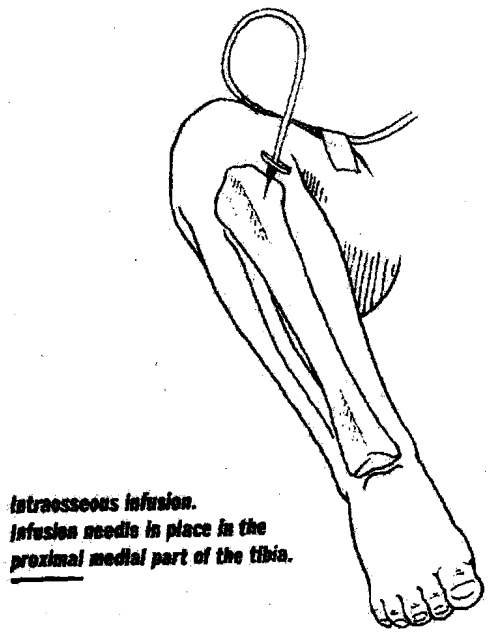
Table: Complications of umbilical venous catheterisation

Complication	Site of UVC tip (intended or after migration
Air embolism	Supradiaphragmatic
Cardiac arrhythmias	Intracardiac
Sepsis	Any site
Biliary-venous fistula	
Hepatic abscess	
Hepatic vein thrombosis and portal hypertension	
Hepatic infarction and necrosis	Hepatic venous Tree
NEC	IVC, portal venous system
Peritoneal perforation and Ascites	Any site (during insertion)
Pericardial temponade	Right atrium
Pulmonary haemorrhage, hydrothorax	Left atrium.

7.9 INTRAOSSEOUS INFUSION.

When carried out by a well trained and experienced physician, intraosseous infusion is a safe, simple and reliable method of giving fluid and drugs in an emergency. Almost all parental fluids and drugs recommended in these guidelines can be given by this route.

In emergency this may be the first choice if access to a peripheral vein does not appear to be obtainable. It takes 1 – 2 minutes to establish intraosseous access. The procedure is painful, but no anaesthetic is required as it should only be used in an emergency (e.g. when a child is in shock).



Intraosseous infusion. Infusion needle in place in the proximal medial part of the tibia.

Fig. 7.5: Intraosseous infusion
(Infusion needle in place in the proximal medial part of the tibia)

Contraindications:

- Infection at the intended puncture site.
- Fracture of bone.

Site for puncture

The first choice for the puncture is the proximal tibia. The site for needle insertion is the middle of the antero-medial surface of tibia, 1 – 2 cm below the tibial tuberosity. An alternative site for needle insertion is the distal femur, 2 cm above the lateral condyle.

Prepare the necessary equipment

- Bone Marrow Aspiration or Intraosseous Needles (15 – 18 gauze or, if not available, large bore hypodermic or butterfly needles can be used in young children).
- Antiseptic solution and sterile gauze to clean the site.
- A sterile 5 ml syringe filled with normal saline.
- A second sterile 5 ml syringe.
- I.V. infusion equipment.
- Sterile gloves.

Procedure

- Place padding under the child's knee so that it is bent 30^0 from the straight (180^0) position, with the heel resting on the table,

- Select the site for cannulation:
  - — First, palpate the tibial tuberosity,
  - — Then, locate one finger's breadth below and medial to the tuberosity (the bone can be felt under the skin at this site)
- Wash the hands and put on sterile gloves,
- Clean the skin over and surrounding the site with an antiseptic solution,
- Stabilize the proximal tibia with the left hand by grasping the thigh and knee above and lateral to the cannulation site, with fingers and thumb wrapped around the knee but not directly behind the insertion site,
- Palpate the landmarks again with the right hand,
- Insert the needle at a 90^0 angle with the bevel pointing towards the foot. Advance the needle using a gentle but firm, twisting or drilling motion,
- Stop advancing the needle when you feel a sudden decrease in resistance. The needle should be fixed in the bone,
- Remove the stylet,
- Aspirate 1 ml of the marrow contents, using 5 ml syringe, to confirm that the needle is in the marrow cavity,
- Attach the second 5 ml syringe filled with normal saline. Stabilize the needle and slowly inject 3 ml while palpating the area for any leakage under the skin. If no such infiltration is seen, start the infusion.
- Apply dressings and secure the needle in place.

Note: While the fluid is being infused, only a slight resistance should be felt, and there should be no visible or palpable infiltration in the area of infusion. Failure to aspirate marrow contents does not mean that the needle is not correctly placed. The fluid infusion should be started.

- Monitor the infusion by the ease with which the fluid flows and by the clinical response of the patient.
- Check that the calf does not swell during the infusion.

Stop the intraosseous infusion as soon as venous access is available. In any case, it should not continue for more than 8 hours.

Complications include:

- Incomplete penetration of the bony cortex
- Sign: The needle is not well fixed; infiltration occurs under the skin.
- Action: The needle must be pushed further.
- Penetration of posterior of the posterior bone cortex (more common)
- Sign: Infiltration occurs (calf becomes tense), with the needle well fixed.
- Action: Remove the needle and repeat at another site. This problem may be avoided by placing the index finger against the skin to prevent the needle from going in too deeply.
- Blockage of the needle by marrow (uncommon)
- Sign: Infusion stops.
- Action: The line must be flushed by 5 ml of normal saline.
- Infection
- Sign: Cellulitis at the site of the infusion (this is rare if the infusion is left for less than 24 hours; osteomyelitis is very rate).

- Action: Remove the intraosseous needle unless it is essential; give local skin care and antibiotic treatment.
- Necrosis and sloughing of the skin at the site of the infusion (this occurs particularly when drugs such as adrenaline, calcium chloride or sodium bicarbonate pass into the tissues).
- Action: Avoid by infusing gently and not under pressure.

7.10 LET US SUM UP

You have learnt about common neonatal laboratory procedures like capillary blood sampling, peripheral blood smear, micro ESR estimation. These tests are generally required in a sick newborn to detect sepsis or infection. This section also dealt with blood sugar testing by hydrostix technique and various laboratory tests e.g. urine, CSF, tuberculin test etc. Most of the tests are simple to perform and interpret. They are important to arrive at a diagnosis and start early treatment.