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சுகாதார அமைச்சு
Ministry of Health

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Provincial/Regional Directors of Health Services,
Directors of Teaching Hospitals/Specialized Campaigns,
MSS/DMOO of Provincial/Base Hospitals,
Heads of Decentralized Units,
Regional Epidemiologists, Medical Officers (MCH)
Medical Officers of Health.

Guidelines for paediatric autopsies on deaths following Immunization

Surveillance of Adverse Events Following Immunization (AEFI) is an important part of the National Immunization Programme (NIP) to ensure vaccine safety. It is of utmost importance to identify and establish causes of all serious AEFI including deaths to carry out timely follow-up and corrective action to safeguard the vaccine recipients and also to maintain public trust on the NIP.

On a recommendation by the national expert committee on vaccine safety and AEFI, it is advised to carry out a comprehensive autopsy for the establishment of cause of death whenever the cause of death is suspected with a vaccination. Investigation of suspected infant deaths following immunization is an issue of great national importance with regard to the Immunization programme, as the proper causality assessment would enable to differentiate vaccine related deaths from deaths due to other causes. The assistance that could be provided by a comprehensive autopsy in the establishment of the cause of death is immense, and adherence to a standard protocol would enable obtaining that assistance to the optimum. The College of Forensic Pathologists in Sri Lanka has drafted a detailed protocol on paediatric autopsy and this guideline will function as an interim guideline based on the detailed protocol and until it is available for implementation.

Considering the importance of autopsy in the investigation of infant/child deaths following immunization, head of the institution needs to make arrangements for a death inquest and conducting an autopsy. In an event of infant/child death following immunization at home, conducting an autopsy is important and the respective authority shall be aware of this guideline and followed the same. Where possible, autopsies of all deaths following immunization should be carried out by a specialist in forensic medicine. Where a specialist is not available the autopsy should be performed by a medical officer qualified with Diploma in Legal Medicine, who is also trained in performing such post mortems. Where both are not available the hospital authorities should make arrangements with

the closest specialist in forensic medicine regarding conducting the post-mortem. It is the responsibility of the hospital authority to make transport arrangements.

This guideline provides measures to be followed and adhered to in a standard autopsy protocol which would enable conduct of a comprehensive causality assessment of a reported death following immunization.

In an event of all such deaths a detailed autopsy should be performed including the following;

- review of detailed preclinical and clinical history including laboratory and radiological findings,
- if necessary visit to the death scene for additional evidence,
- radiological, histopathological, toxicological and microbiological investigations.

Microbiologist and Histopathologist of the local institution will be the focal point for microbiology and histopathology examinations respectively. Where a specialist Microbiologist and/or Histopathologist is not available at the local level, or when virological / immunological investigations need to be carried out focal point for such examinations will be the MRI (Tel: 112693532-4).

Also, in situations where the cause of death is inconclusive or not straightforward, obtaining a second opinion on histopathological examinations from another pathologist is recommended. Department of Government Analyst (Tel: 2694786, 2694787, 2695881, 2699753) will be the focal point for toxicological examinations. It is advisable to contact the respective institutions on detailed information on collection, storage and transport of specimens.

Where possible samples of the organs should be preserved for further evaluation at the institution where the postmortem is conducted.

The epidemiology Unit should be informed (Tel: 112695112, Fax: 112696583) by the head of the institution and the Regional Epidemiologist of the district to be contacted to coordinate assistance. In situations where local facilities are insufficient to perform such examinations, assistance from the Epidemiology Unit could be obtained to seek external assistance. The Medical Officer of Health (MOH) or MOH staff who carried out the vaccination shall provide all necessary information to carry out the autopsy, but their presence during the autopsy is not mandatory.

1. Basic Information

Information about the identity of the deceased and the details of authorization should be obtained and included in the report.

2. Preliminaries

Antemortem samples should be taken over and investigated in cases such as alleged intoxications, allergy related incidents etc. Therefore, as soon as information is received the clinical staff should be advised to preserve the antemortem samples such as blood in a plain bottle, urine, CSF, vomitus, faeces, sputum, swabs etc that have already been obtained and hand them over to the forensic specialist at the earliest. In an anaphylactic reaction, ante-mortem samples collected between 30 min to preferably 3 hours after reaction (up to 6 hours) and 24 hour baseline samples (if available) are to sent for immunological investigations. If there is a delay in transport centrifuge the sample and refrigerate at 4⁰ C.

Ward staff should be advised to document all therapeutic interventions. The area MOH, where child is vaccinated need to be informed by the head of the institution to preserve and handover empty vials, diluents, syringes, needles, with control samples of same batch. Regional Epidemiologist should coordinate this activity.

- Ensure maintenance of the chain of custody throughout the investigation (collected by, custody of, dispatched to).
- Record date and time of collection and type of preservative for each and every ante-mortem sample collected
- Document reports of clinical investigations and medical records related to the incident such as microbiology, biochemistry, immunology, histopathology, haematology, radiology etc.

3. Pre autopsy information

Obtain a detailed history which includes past medical history, drug history, immunization history, history of allergies and findings of medical records etc.

4. Pre autopsy preparation

Preserve autopsy blood – untreated and spun – in case required for later analysis. Make arrangements to take photographs at the autopsy. Be prepared to take X-rays if and when necessary while dissection (specially in cases of suspected air embolism). Be prepared to preserve the heart after careful dissection if the cause of death cannot be ascertained at the end of the autopsy.

5. External examination

In addition to routine external examination, record features of allergic reaction, anthropometric parameters when relevant (Weight, Height/Crown-heel length, Crown-rump length, Foot length, Occipito-frontal circumference, Chest circumference at nipple level, Abdominal circumference at umbilicus), Congenital abnormalities, nutritional and developmental state, features of neglect, therapeutic interventions, and paraphernalia, features of complications of therapy such as presence of subcutaneous emphysema etc. It is important to examine vaccination/ injection site regarding local reaction.

6. Internal examination

In addition to routine internal examination, record features of allergy and anaphylaxis such as laryngeal oedema, petechial haemorrhages, froth / secretions in upper airway, mucus plugging of the bronchi, effusions in body cavities, tissue oedma and the degree of local reaction at the vaccination/ injection site etc.

Look for complications of therapy such as pneumothorax, degree of haemorrhage in relation to puncture sites. Look for congenital abnormalities

Whenever possible record the weights of internal organs including thymus (children).

7. Post-mortem samples

Samples for microbiology, immunology, histopathology and virology, should be collected according to the instructions given by the relevant laboratories. (Details of instruction on sending samples to MRI is available on laboratory manual issued by the SLCM or could be obtained from the respective departments at the MRI by telephone 112693532-4). All samples should be clearly labelled and dated. The request form should have the patient's information, a short history including clinical presentation, duration of illness and date of death. Indicate the necessary tests (if known) to be performed. In case special investigations are needed, contact the laboratory for instructions prior to sending samples. If possible mention the tentative /provisional diagnosis. MRI will accept the samples for special investigations 24 hours / day, 7 days of the week.

7.1 Histopathology

Samples for histopathology obtained from all major organs are as follows: any macroscopically visible lesions should be described in detail and sample extensively. Sample should be taken in 10% formal saline or in dry ice for frozen section. Specify the required special stains when necessary.

- *Central nervous system:* Brain (If the brain is to be examined after fixation suspend in 20% formal saline for two weeks). Sections from Middle frontal gyrus, hippocampus, basal ganglia (putamen+globus pallidus)+ insular cortex, mamillary bodies, thalamus, left cerebellum, dentate nucleus, mid brain, pons, and medulla should be obtained.
- *Cardio-vascular system:* Myocardium (LV, RV, RA, LA, septum , other areas where relevant), cardiac valves, coronary arteries, conduction system and others
- *Respiratory system :* Epiglottis, Tonsils, Larynx, Trachea, Bronchi and Lungs- (at least one sample from each lobe including hilum and periphery) Hilar lymph nodes
- *Digestive system:* liver, pancreas,
- *Genitourinary system:* Kidneys including cortex and medulla
- *Mononuclear Phagocyte system:* Spleen, thymus, bone marrow
- *Endocrine system:* Adrenal gland, pituitary, thyroid gland
- Other: eg. Injection sites, including control, injuries and others

7.2 Microbiology

7.2.1 Bacteriological Investigation

Type of specimens and tests: Blood for culture, CSF and body fluids for culture, pus for culture, tissues for culture and blood for serology

Collection and transport :

- Blood and Body- fluids for Culture after death should be collected as early as possible and preferably before the body is sent to the morgue.
- If the body is already at post-mortem, following guidelines should be followed: Blood, CSF and Body- fluids should be collected before the dissection is started. Follow Standard Precautions for collection of samples. Clean the over-lying skin with 70% alcohol. Draw the sample using a sterile disposable needle and syringe. For Blood –culture 3-5 ml blood (heart or venous) should be added into a blood-culture bottle with 30-40 ml BHI and mix carefully.
- CSF and other Body- fluids also should be sent in sterile screw-capped containers. (Send these samples as soon as possible at room- temperature).
- Tissue samples should be sent in sterile N. Saline in screw capped containers.
- Pus samples / swabs in sterile screw capped containers.
- Blood for serology and bacterial testing: Plain blood in sterile containers.

7.2.2 Immunology

A post mortem sample for serum tryptase should be taken from femoral vessels, and not heart blood. Serum should be separated and stored at 4⁰C, or frozen if the assay is delayed.

The circumstances regarding the death are important, as tryptase levels are also increased after myocardial infarction, trauma, amniotic fluid embolism and sudden infant death. Serum tryptase rises in anaphylaxis, if shock is present, or after insect stings, or in circumstances where the allergen enters the body parenterally. Anaphylaxis following ingestion of an allergenic food does not usually lead to an increase in tryptase levels.

Information on allergy to foods (particularly beef, pork, milk, gelatine, previous vaccination) should be obtained. A blood sample should be sent to the Medical Research Institute for testing for allergen specific IgE.

7.2.3 Mycological (fungal) investigations

- Blood: 5-10 ml of venous blood should be collected under strict aseptic precautions. The lid should be wiped with 70% alcohol before inserting the needle to inoculate the blood into a culture bottle containing Brain Heart Infusion (BHI) broth. Mix well and keep at room temperature till dispatched. Smaller volumes of blood from neonates should be collected into paediatric BHI bottles (1-5 ml).(send as soon as possible to the laboratory)
- Bone marrow: 2-3 ml of bone marrow aspirate should be placed in a sterile screw capped container with 0.5 ml of 1:1000 heparin. (Send within 24 hours to the laboratory)
- CSF: 3-5 ml of CSF should be collected into a sterile screw capped bottle.
- Body fluids: Chest, abdominal fluid and any drain fluid should be collected aseptically in to a sterile screw capped bottle
- Respiratory tract: Tissues should be collected into a sterile screw capped bottle containing normal saline. Another sample should be sent in formal saline for histology.
- Blood for serology: 1-2 ml of blood should be collected into a plain bottle.

7.2.4 Virology Investigation

General Considerations: Most antigen / antibody detection assays in virology are compatible with serum / plasma. If the blood is haemolysed as it happens when blood is taken during the post mortem (PM) examination, these tests cannot be performed. Therefore it is recommended to the clinicians, to take a blood sample just before or immediately after death if possible. Similarly other samples like CSF, lung tissue etc, also are recommended to be collected just before or after death. If the facilities are available, serum should be separated before transport.

It is recommended to take multiple specimens including blood, CSF, respiratory secretions, stool, lung tissue etc.

Plural fluid, peritoneal fluid, pericardial fluid etc has limited value as antigen / antibody detection assays cannot be performed using these samples.

Tissue samples, swabs, respiratory secretions are collected into virus transport medium (VTM). VTM can be collected from the Department of Virology, MRI. It can be kept for few weeks at + 4⁰ C (Do not use if the colour has changed from yellowish orange to pink)

All samples, especially the samples intended for virus isolation / molecular assays should be collected with sterile precautions to prevent contamination (If tissue samples are taken, use separate sterile instrument set for each site).

All samples should be transported early to the laboratory at + 4⁰ C. Samples should be stored at + 4⁰ C until transport is arranged. For the transport of specimens, ice packs are preferred than ice cubes, to maintain the temperature. If ice cubes are used, the samples should be packed in polythene bags separately so that there is no leaking once the ice gets melted.

- Blood: Ideally collected just before or just after death or during the autopsy and should be collected into dry, sterile bottle (for PCR collect into lysis buffer). Also blood sample can be collected from heart during the PM. Allowed to clot at room temperature. If facilities are available separate the serum.
- CSF: Ideally collected just before or just after death or during the autopsy, should be collected into dry, sterile bottle and to be sent to the laboratory early.
- Respiratory specimens (Nasopharyngeal swabs, tracheal swabs, bronchial aspirates or swabs): Ideally collected just before or just after death or during the autopsy and should be collected into virus transport medium (VTM). Use sterile throat swabs. Rub the surface with the swab to collect the epithelial cells. Insert the swab with the specimen into the container with VTM.
- Faeces: Collect during the autopsy into dry, bottle. Collect 5 -10 g of faeces (rectal swabs are not satisfactory) in to a leak proof container and sent to the laboratory early. However, anal swabs are not useful in viral diagnosis and not encouraged.
- Vesicular fluid / scrapings / swabs: Collect during the autopsy into virus transport medium (VTM).
- Tissue specimens (lung, spleen, lymph nodes, myocardium, brain): Collect during the autopsy. Use true cut biopsy needle & collect lung necropsy specimens just before or just after death in fatal case of suspected pneumonia. Take 3 specimens one into VTM, one into 70% alcohol, one into formalin. Should collect into container with virus transport medium (VTM). Collect tissue of 1cm x1cm x 1cm size from suspected areas.

7.2.5 Samples for electron microscopy

Mast cell degranulation is an important finding in allergy and anaphylaxis. Presence of this in myocardium specially around coronary arteries can be fatal and has to be differentiated from myocarditis. Therefore a section from the myocardium for this is essential and the sample should be 3mm thick tissue in gluteraldehyde.

Please bring the contents of this circular to the notice of all officers concerned in your Province/ District/ Institution/ Unit.



Dr. U. A. Mendis
Director General of Health Services