

Sero-survey under Yaws Eradication Programme

Guidelines for Medical Officers

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1. Introduction

Yaws is a disfiguring, debilitating non-venereal treponemal infection. It is a contagious disease transmitted by direct (person-to-person) contact with an infectious yaws lesion.

The organism responsible for Yaws is *Treponema pallidum* sub-species *pertenue*. It is identical in appearance to *T.pallidum pallidum* (the organism that causes venereal syphilis). Early yaws is primarily a disease of children and adolescents in the endemic situation. Man is the only reservoir of infection. The cases of early yaws serve as the source of infection. Incubation period is 9-90 days (average 21 days). Yaws also exhibits latency. After initial yaws which lasts for few months, the infection may remain for many months to years. However, lesions which reappear within 5 years may be infectious.

Early lesions of this disease manifest in the form of skin lesions, which on healing show little scarring. The disease can be progressive wherein bone and cartilage are affected leading to disability. The disease can be cured and prevented by a single injection of long acting (benzathine benzyl) penicillin.

Yaws is amenable to eradication as it does not have any extra human reservoir of infection, organism is sensitive to a single dose of long acting penicillin and yaws infection is limited to a small pocket.

As per available records, the disease has been reported from 10 states of the country viz., Andhra Pradesh, Assam, Chhattishgarh, Gujarat, Jharkhand, Madhya Pradesh, Maharashtra, Orissa, Tamil Nadu, and Uttar Pradesh. The problem perpetuated in remote, inaccessible, hilly and forest tribal areas.

Govt. of India approved Yaws Eradication Programme as a central sector health scheme as a Pilot Project for undivided Koraput district, Orissa during the year 1996-97. Subsequently, in March 1999, the Standing Finance Committee of Government of India approved extension of the scheme to cover all the endemic states.

2. Programme objectives and strategy:

The objectives include:

2.1. Yaws Elimination: Nil reporting of early yaws cases on the basis of good quality search, supported by laboratory investigations in all endemic areas of the country and validated by independent appraisals; and

2.2 Yaws Eradication: Absence of new cases for a continuous period of three years, supported by absence of evidence of transmission with sero-survey among 1-5 years children (i.e. no sero reactivity to RPR/VDRL in <5 yr children).

2.3. The Programme Strategy includes:

- Manpower development,
- Case finding & treatment of cases and contacts simultaneously; &
- IEC activities harnessing multisectoral approach.

Once elimination is achieved, sero-surveillance would be initiated.

2.4. Justification for sero-survey

- Elimination by definition means absence of new/early cases, likewise, eradication means absence of evidence of transmission of infection
- Thus validation of eradication rests i.e. demonstration of absence of transmission in the community would be possible by undertaking sero-survey in the community.
- Once infected the serological positivity persist for long time. Hence, it is possible that, due to old infection, some adult persons may be found positive for yaws infection by serological test.
- On the other hand sero-positivity in young children will reasonably indicate a recent infection as they are new entrants in the potential pool of infection. Hence, no sero-reactivity to RPR test among 1-5 years children will be indicative of absence of transmission i.e., Eradication.

3. Sero-survey under YEP:

3.1. Methodology: Selection of villages and Planning

- Cover all children of 1-5 years age-group from yaws and non yaws* villages as per list provided by NICD (Annexure-1)

* In case of non-yaws big villages (defined as having a population of >500 persons), cover two randomly selected natural segments traditionally known as tola, pada, falia, etc from the village and collect blood samples from all children of 1-5 years in these two segments only.

- Information to the villagers about the activity specifying time of visit should be given in advance, in particular to Panchayat Raj Institutions (PRI).
- Village Health sanitation & nutrition committee should also be informed.
- Repeat the sero-surveys annually for three consecutive years.
- Sero-surveys should be carried out once a year coinciding with the two active case searches. However, any areas left un-surveyed due to any unavoidable reason/operational problems should be covered promptly at the earliest within the same year.

3.2. Sample collection and transportation

3.2.1. Collection of blood samples & processing

- Prepare the field kit as per check list(Annexure-II)
- Record details of the child as per sero- survey proforma (annexure-III)
- Collect blood sample by finger/heel prick ensuring all aseptic measures using a lancet or hypodermic needle.
- Press the finger below the pricked area by applying adequate pressure to express the blood.
- Take 0.2 ml clean micro centrifuge (MC) tube (Eppendorf) containing a pinch of EDTA as anticoagulant (for carrying out RPR test, plasma sample obtained from anti-coagulated blood is required).
- Collect blood by touching the rim of the vial against the drop of blood from the punctured finger and applying pressure intermittently, till the tube is at least 3/4th full. (in case full face of the vial is put against drop of blood, it may lead to air bubble formation causing blockage of blood flow inside the vial)
- The tube should be labeled with patient's identification number (corresponding with the entry in the proforma).
- Ensure thorough mixing of the blood with EDTA by rolling/turning the tube upside down several times. This is essential failing which

blood is liable to clot thereby rendering the sample unfit for testing.

- Separate plasma immediately by centrifuging at 1500-2000 rpm for 5-10 minutes to avoid haemolysis. In case of non-availability of centrifuge, allow the blood sample to stand at room temperature for 3-4 hours, the time needed for separation of plasma. Ensure that plasma is separated at the earliest.
- Separate the overlying plasma with Pasteur/ micro pipette and carry out the RPR test.
- In case it is not feasible to test the sample on the same day, store the plasma sample at 4-8^oC if proposed to be tested within a week or else keep at -20^oC or in the freezer compartment of the ordinary refrigerator for longer storage.

3.2.2. Sample Transportation:

3.2.2.1. From collection site to PHC/CHC/district laboratory:

- The MC tube containing the samples should be placed in tightly fitting tube racks/thermocool sheets.
- Ensure that specimen tube does not have cracks/leakage.
- Place some cotton or absorbent material between the tubes to ensure that they don't move or rattle during transport and put inside the vaccine carrier/thermocool box. Arrange for an adequate amount of refrigeration (minimum 3-4 ice packs will maintain refrigeration for 2-3 days) in case of delay in transportation.
- Securely fasten transport boxes in the transport vehicle; avoid excessive vibration of samples during transport as this can haemolyse samples, rendering them useless.
- The requisition slip should be placed in a plastic zip lock bag inside the vaccine carrier.

3.2.2.2. From District lab to NICD/Central Lab:

- Cover the rack containing plasma samples with a layer of cotton or any other absorbent material to ensure that there is no spillage. Place the rack in another container/plastic bag and then place in the vaccine carrier/thermocool box. Ensure triple packaging for biosafety purposes. Place ice packs below and on sides of the sample box and seal/secure the lid of the cool box. The requisition slip should be kept inside the zip locker in the transport box.
- A biohazard mark should be pasted on the visible outer surface of the cool box with 'This side up mark (↑)' clearly mentioned on it.
- Samples should be transported to the testing laboratory with prior intimation to the laboratory in-charge, ensuring that the transit period is minimal.

Note:

RPR test kits should be stocked at district and distributed in such a way that the concerned PHC/CHC at any point of time has an adequate number of test kits available with them.

3.3. Sample testing by RPR test:

The RPR (Rapid Plasma Reagin) is a slide agglutination test to detect antibodies against syphilis/yaws. The test is reliable, economical, reproducible, rapid and easy to perform even under field conditions and is easily readable with naked eye. The specificity and sensitivity of the test is similar to that of VDRL test.

3.3.1. Principle of test:

RPR antigen suspension is a carbon coated non-treponemal cardiolipid antigen which detects reaginic antibodies present in serum/plasma of patients suffering from treponemal infections. Flocculation occurs in specimens containing antibodies against treponemal antigen due to co-agglutination of the carbon particles of the RPR antigen, which appear as black clumps against the white background of the card. False positive reactions (around 1%) can be seen occasionally in sera of persons with other non-treponemal conditions like malaria, kala-azar, rheumatoid arthritis, tuberculosis, etc.

3.3.2. Test procedure:

Follow the instruction manual provided with the test kits. Briefly, the procedure is as follows:

- Bring all reagents as well as samples to room temperature before performing the test.
- Wear gloves before performing the test.
- The test card provided with the kit should be appropriately labeled using permanent marker.
- Positive and negative controls should be tested with each run.
- Place one drop of serum or plasma (50 µl) on the card with the help of a dropper provided with the kit for this purpose.
- After thoroughly mixing RPR antigen suspension, place one drop (15-20 µl) of the same alongside the drop of plasma on the card, using another dropper provided with the kit.
- Mix these drops well, and spread out the pool of liquid uniformly within the entire area of the circle by using the applicator stick provided with the kit. Use separate stick for each sample.
- Shake the card gently using a rotatory movement up to 5 minutes and observe for clumping under a good light source. In case a VDRL shaker is available, it can be used for shaking of the cards.
- Results should be read in comparison with those of +ve and -ve controls provided with the kit.

3.3.3. Interpretation of test results:

Positive result

Black aggregates/clumps are formed within 5 minutes.

Negative result:

Absence of black aggregates/clumps at the end of 5 minutes.

3.4. Quality Control:

- All samples positive by RPR and 5% of randomly selected negative sera should be sent to NICD/designated central laboratory for cross checking by RPR and TPHA.
- Details as per sero-survey proforma and RPR test results should accompany the samples.
- Results of cross checking/QA by the central laboratory should be communicated at the earliest to the laboratories that have sent these samples.

- **3.5. Bio-safety measures during sample collection/transportation/testing:**

- i) Use clean and well lighted place for sample collection.
- ii) Avoid crowding and make the child sit comfortably.
- iii) Always use disposable gloves during collection and testing of blood sample and change on tearing /when soiled with blood.
- iv) Wash hands thoroughly before and after the sample collection/testing.

- v) Discard the lancet and hypodermic needles after use in puncture proof containers; autoclave or handover to nearest central treatment facility (if not available, then dispose in burial pit/landfill as per the biosafety manual).
- vi) Discard the used spirit swabs in the waste disposal bag as per guidelines.
- vii) Transport samples following triple packaging system to ensure biosafety; wash and reuse after sterilization.
- viii) Sample vials with residual blood/plasma to be discarded in buckets/waste disposal containers with 1% sodium hypochlorite solution.

Refer to IDSP biosafety manual for details. Also available at www.nicd.nic.in

Annexure II

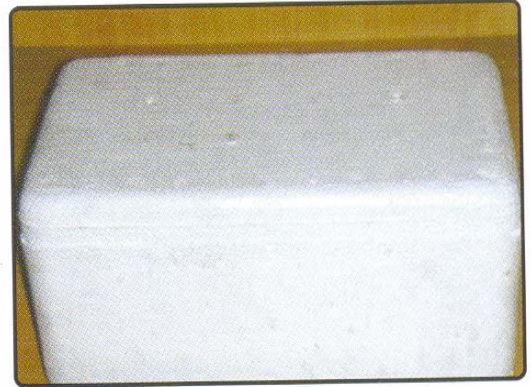
Check List of kit contents during sero-survey:

- 0.2 ml EDTA microcentrifuge/eppendorf vials
- Lancets
- Spirit swabs
- Vial tray /thermocool sheets for placing the sample vials
- Needles & syringes
- 5ml screw cap vials
- Permanent water proof marker
- Vaccine carrier/ Thermocol box along with ice packs for packaging and transportation of samples.
- Waste disposal bags for used swabs
- Puncture proof container with 1% sodium hypochlorite for used needles and sharps.
- Bottle with concentrated sodium hypochlorite
- Disposable gloves
- Leucoplast/stickers
- Scissors/blade
- Medicines :
 - Paracetamol
 - ORS
 - B complex
 - Albendazole
 - Perinorm
 - Cetrizine
 - Iron & Folic acid
 - Dressing material
 - Betadine lotion
 - Benzyl benzoate
- Anti-septic solution/ soap
- Hand towels/Tissue paper roll
- Proforma for sero-survey

Materials for Sample Collection & Transportation



Disposable Sterile Gloves



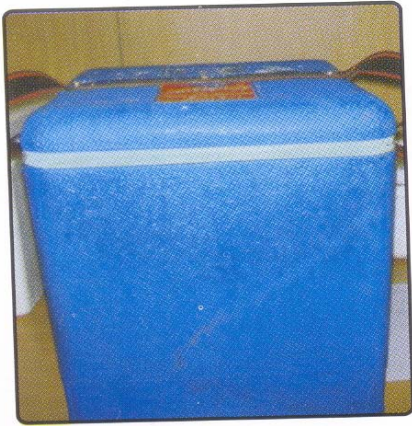
Thermocol Box



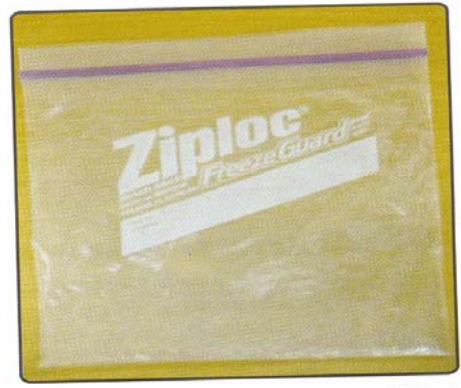
Ice Pack



Microcentrifuge (MC) Tube



Vaccine Carrier



Ziploc Bag



Cryo-vial Box



Lancets



Microcentrifuge vial tray