Mission

Partners for Health Reformplus is USAID’s flagship project for health policy and health system strengthening in developing and transitional countries. The five-year project (2000-2005) builds on the predecessor Partnerships for Health Reform Project, continuing PHR’s focus on health policy, financing, and organization, with new emphasis on community participation, infectious disease surveillance, and information systems that support the management and delivery of appropriate health services. PHRplus will focus on the following results:

- Implementation of appropriate health system reform.
- Generation of new financing for health care, as well as more effective use of existing funds.
- Design and implementation of health information systems for disease surveillance.
- Delivery of quality services by health workers.
- Availability and appropriate use of health commodities.

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Abstract

A well-functioning laboratory service is an important component of the vaccine-preventable disease (VPD) surveillance system in Georgia.

The second edition of the laboratory reference manual for VPD surveillance has been developed by a group of the Georgian specialists under the guidance of a PHRplus consultant from the World Health Organization’s Laboratory Surveillance Strengthening Unit in Lyon on a basis of needs identified during the laboratory system assessment carried out by partners in 2002, and feedback received during the piloting of the VPD surveillance reforms in the Imereti region of Georgia in 2003.

The manual incorporates the standards for sample collection, storage and transportation, laboratory protocols and procedures for VPDs, and guidelines on biosafety and quality control. It is recommended for use by health workers nationwide.
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<th>Description</th>
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<td>AFP</td>
<td>Acute Flaccid Paralysis</td>
</tr>
<tr>
<td>BAP</td>
<td>Blood Agar Plate</td>
</tr>
<tr>
<td>BG</td>
<td>Bordet–Gengou</td>
</tr>
<tr>
<td>CIF</td>
<td>Curatio International Foundation</td>
</tr>
<tr>
<td>CPH</td>
<td>Center of Public Health</td>
</tr>
<tr>
<td>CRS</td>
<td>Congenital Rubella Syndrome</td>
</tr>
<tr>
<td>CSF</td>
<td>Cerebral Spinal Fluid</td>
</tr>
<tr>
<td>CTBA</td>
<td>Cystine tellurite blood agar</td>
</tr>
<tr>
<td>EIA</td>
<td>Enzyme Immunoassay</td>
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<td>ELISA</td>
<td>Enzyme-Linked Immunosorbent Assay</td>
</tr>
<tr>
<td>EQC</td>
<td>External Quality Control</td>
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<td>HBsAg</td>
<td>Hepatitis B Surface Antigen</td>
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<td>HBV</td>
<td>Hepatitis B Virus</td>
</tr>
<tr>
<td>IgG</td>
<td>Immunoglobulin G</td>
</tr>
<tr>
<td>IgM</td>
<td>Immunoglobulin M</td>
</tr>
<tr>
<td>IQC</td>
<td>Internal Quality Control</td>
</tr>
<tr>
<td>MoLHSA</td>
<td>Ministry of Labor Health and Social Affairs</td>
</tr>
<tr>
<td>MV</td>
<td>Measles virus</td>
</tr>
<tr>
<td>NCDC</td>
<td>National Center for Disease Control and Medical Statistics</td>
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<td>PHRplus</td>
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<tr>
<td>RL</td>
<td>Regan–Lowe</td>
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<tr>
<td>USAID</td>
<td>United States Agency for International Development</td>
</tr>
<tr>
<td>VPD</td>
<td>Vaccine Preventable Diseases</td>
</tr>
<tr>
<td>VTM</td>
<td>Viral Transport Medium/a</td>
</tr>
</tbody>
</table>
This manual has been prepared by the Ministry of Labor, Health and Social Affairs expanded Working Group headed by P. Imnadze, Director of the National Center for Disease Control (NCDC), with technical assistance received from Partners for Health Reformplus, in particular Antoine Pierson, PHRplus consultant from the WHO/Lyon Laboratory Surveillance Strengthening Unit, and Curatio International Foundation.

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The “Georgian National Health Policy” adopted in 1999 declares the improvement of maternal and child health and the reduction of communicable and socially dangerous diseases among the main priorities for maintaining and improving the health of the population of Georgia over the next decade. Improved coverage of target populations with immunizations and increased effectiveness of epidemiological surveillance are viewed as important strategies to achieve these objectives. The policy links these strategies with the need for improvements of the Georgian Health Information System in order to provide managers, stakeholders, and the public with appropriate information to make correct strategic, tactical and operational decisions.

These guidelines are focused on laboratory procedures with regard to eight vaccine-preventable diseases (VPDs), most of which are targeted for elimination or considerable reduction as outlined in the National Health Policy, namely:

- Diphtheria
- Measles
- Rubella
- Tetanus
- Poliomyelitis
- Mumps
- Pertussis
- Hepatitis B

Effective communicable disease control relies on functioning disease surveillance, which considers the systematic and regular collection of information on the occurrence infectious diseases. Thus, core functions of disease surveillance system include disease detection and verification, which is based on laboratory confirmation of infectious disease.

In a well-functioning surveillance system, disease confirmation rates are expected to exceed 70-80%. For diseases in the elimination phase (polio, measles) this rate is expected to approach 100%.

In the case definition/case classification section of the general guidelines on Vaccine Preventable Disease Surveillance, a confirmed case is one which has been confirmed by disease-specific laboratory tests and/or where an epidemiological link to other confirmed case(s) has been established. In those instances where lab confirmation of a disease is not widely accessible, cases can be confirmed if they meet a clinical description of the disease and are epidemiologically linked to a probable (clinical) case (e.g., mumps). For some diseases, (e.g., tetanus) definition of a confirmed case is not applicable, because there is no lab test and no epidemiological link – they are confirmed based purely on clinical presentation.

Using case confirmation rates, it is possible to assess the maturity of the surveillance system in various regions and in various settings, assess the success of disease elimination efforts, plan surveillance/laboratory system strengthening activities, develop disease elimination strategies, suggest policy changes, and plan other long-term interventions such as mass immunization campaigns. These are decisions for which health managers cannot rely on reports that include unconfirmed cases. Monitoring of the proportion of cases that have been laboratory tested is important, first and foremost, for diseases targeted for elimination or considerable reduction (polio, measles, diphtheria).
Table 1 presents a summary of epidemiological conditions triggering mandatory laboratory case confirmation and guidelines for collecting, storing, and transporting specimens.

Table 1. Epidemiologic conditions under which VPDs trigger mandatory laboratory case confirmation

<table>
<thead>
<tr>
<th>Disease</th>
<th>Epidemiologic Conditions under which Laboratory Confirmation Is Mandatory</th>
<th>Where to Send Specimens¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diphtheria</td>
<td>Any probable case</td>
<td>Contact regional centers for public health (CPH) for the most current list of National Center for Disease Control (NCDC)-recognized and - recommended laboratories in your area</td>
</tr>
<tr>
<td>AFP/Polio</td>
<td>Any probable case</td>
<td>NCDC</td>
</tr>
<tr>
<td>Congenital rubella syndrome</td>
<td>Any probable case</td>
<td>NCDC</td>
</tr>
<tr>
<td>Measles</td>
<td>Three or more cases² during the same period consistent with measles incubation period in a given geographic territory</td>
<td>NCDC</td>
</tr>
<tr>
<td>Rubella</td>
<td>Three or more cases² during the same period consistent with rubella incubation period in a given geographic territory</td>
<td>NCDC</td>
</tr>
<tr>
<td>Pertussis</td>
<td>Three or more probable cases² during the same period, consistent with pertussis incubation period in a given geographic territory</td>
<td>NCDC</td>
</tr>
<tr>
<td>Acute viral hepatitis</td>
<td>All clinical (probable) cases of hepatitis B. Where an outbreak of hepatitis A is suspected, it is required to confirm at least one case (where all cases are epidemiologically linked) or every other case where such link can not be established</td>
<td>Contact regional CPH for the most current list of NCDC-recognized or - recommended laboratories in your area</td>
</tr>
<tr>
<td>Tetanus</td>
<td>No lab confirmation for this disease</td>
<td></td>
</tr>
<tr>
<td>Mumps</td>
<td>Lab confirmation is not mandatory</td>
<td></td>
</tr>
</tbody>
</table>

¹ Specimen collection should take place at the facility where a patient has come to seek care if such facility is equipped with a specimen collection kit. Referring sick patients (instead of specimens) to a laboratory is discouraged.
² Try to obtain specimens from three patients; one positive test result in a case that is epidemiologically linked to others will be sufficient to confirm an outbreak.
1. Premises and Generic Procedures

Sections 1.1, 1.2, 1.3 and 2.1 contain recommended guidelines on premises and general procedure based on international standards. Section 1.4 presents list of current Decrees and Laws that regulate laboratory activities.

1.1 Signage/access to Laboratory

- The laboratory is a restricted area.
- "No entrance to outside staff" sign should be posted immediately after the reception room. Area where samples are received should be strictly separated from the other rooms of the laboratory.
- A bell is recommended at the entrance. The best way to accept the sample would be through a small opening built in a wall or in a door, so only staff would have to ring to enter the laboratory.
- A separate room for coffee and smoking should be also clearly defined and labeled.
- The international biohazard sign (see 3-3) should be displayed on the doors of rooms where Risk Group 2 micro-organisms are handled.

1.2 Thermal Area Procedures

- **Air conditioning:** Air conditioning is recommended for rooms containing incubators, spectrophotometer (including ELISA reader), and all other temperature-sensitive equipment.
- **Ventilation and illumination:** In the planning of new facilities, however, consideration should be given to the provision of mechanical ventilation systems that provide an inward flow of air without recirculation. If there is no mechanical ventilation, windows should be openable and preferably fitted with arthropod-proof screens. Skylights should be avoided.
- **Cold rooms:** Refrigerators and freezers (especially minus-80 freezers) should not be placed in corridors; such placement can dramatically decrease their lifespan.

1.3 Cold Chain Procedures

- All reagents must be stored according to manufacturer recommendations.
- For instances of electrical power outage, some cooling devices (refrigerators, freezers) have to be protected with a generator. The main critical reagents have to be stored in these protected devices. An automated system should allow the launch of the generator in case of power outage.
Problems that might happen at night or on weekends should be planned for in advance; clearly written standardized procedures to deal with such problems must be developed.

For cases of major electrical problems that affect generators, an alternative strategy for transport of goods and reagents to an other location also must be planned for.

Daily temperature monitoring should be available through a recording system using weekly disks (one week recorded on one disk).

Alarms should be installed on the main refrigerators, to warn the technical staff temperature that rise above the predetermined threshold.

When reagents are received at night or on weekends, they should be directly transported to the main cold chamber available at NCDC.

### 1.4 Decrees and Official Texts that Regulate Laboratories

Decrees of the Minister of Labor, Health and Social Affairs of Georgia

**Note:** All decrees are available at NCDC and in all regional CPHs.

- A Decree provides indicators for effectiveness of laboratory performance, sample passport (form) for laboratory testing, and instructions for sample collection and transportation. "Concerning additional activities for poliomyelitis eradication and improvement of AFP surveillance" – Decree No 243/o July 2, 1997, Ministry of Labor, Health and Social Affairs (MoLHSA).

- Confirmation of diphtheria cases by laboratory testing (identification of *C. diphtheria* oxygenic strain) is mentioned in "Concerning Diphtheria cases, treatment of contacts and bacteria-carriers and additional preventive activities against diphtheria in Georgia" – Decree No. 51/o, February 6, 1998, Tbilisi, MoLHSA;

- All technical staff (doctors and nurses) and auxiliary personnel in contact with patients must be certified by the State Commission according to "Concerning Conferring State Certificates and State Licenses to High- and Medium-Level Medical Personnel” – Decree No. 425/o, October 27, 1998, Tbilisi, MoLHSA;

- A Decree classifies laboratory services as being in extremely poor conditions, and orders establishment of laboratories in those medical institutions where there are no labs and improving performance of existing laboratories. "Concerning Improvement of Measures to Combat Communicable Diseases – Decree No. 467/o, December 1, 1998, Tbilisi, MoLHSA;


- Importance of laboratory isolation of antibiotic-resistant pathogens is underlined in "Concerning Establishing the System of Monitoring on Spread of Antibiotic –Resistant Microorganisms” – Decree No. 22/o February 25, 2000, Tbilisi, MoLHSA;

- Requirements for handling materials suspected or containing poliovirus are described in "Concerning Containment of Poliomyelitis Virus in Georgia” – Decree No. 97/o June 6, 2000, Tbilisi, MoLHSA;
Instructions for handling medical waste, their storage, decontamination, and disposal are in “Concerning storage and disposal of medical waste of health care facilities” – Decree No. 300/n August 16, 2001, Tbilisi, MoLHSA;

Bylaws and composition of the entire state sanitary-hygienic norms expert commission are in “Concerning approval of the bylaw and composition of the entire state sanitary commission on sanitary-hygienic norms” – Decree No 39/o, February 19, 2002;

Vaccination of personnel who work with live cultures is recommended in “Concerning approval of the Preventive Vaccination Calendar” – Decree No 122/N June 4, 2003;

Activities required for improved epidemiological control of VPDs and hydrophobia in Georgia, specific enforcement recommendations on case investigation and analysis, case classification and coding requirements, specification of VPDs that require laboratory investigation for confirmation are described in “Concerning Communicable Disease cases/outbreak investigation, coding, prevention and control of VPDs (Diphtheria, Pertussis, Tetanus, Measles, Rubella, Mumps, Hepatitis B) and Hydrophobia in Georgia” – Decree No. 55/o February 2, 2005, Tbilisi, MoLHSA;

Rules of urgent notification of communicable diseases are established in this decree. All institutions and providers rendering health care services regardless of their subordination and forms of ownership including laboratories must notify the local public health service whenever they diagnose, suspect, or even receive positive lab results. The decree endorses lab notification form “Management and submitting of Medical Statistical Information” – Decree No. 101/n April 5, 2005, Tbilisi, MoLHSA;

Reagents and diagnostics for laboratory testing of infectious diseases in limited amounts are provided by the “State Program of Coordination of Epidemiological Surveillance, Control of Quarantined (Plague, Cholera, Hemorrhagic Contagious Viral Fevers), Particularly Dangerous and Equated to them Infectious Disease and Prevention of Other Communicable Diseases”, 2003, MoLHSA and DPH. The goal of the program is gradual reduction of spread of infectious diseases by means of their liquidation, elimination, and control. The program is financed by the State budget.

Laws of Georgia

Sanitary Code of Georgia – May 5, 2003. The Law regulates legal contacts related to providing healthy environment, also implementation of state supervision on implementation of sanitary norms and regulations.

Concerning Medical and Pharmaceutical Activity Licensing – May 8, 2003. According to this Law, all medical institutions in Georgia must be licensed by the MoLHSA.


Concerning Health Care – December 12, 1997. The Law regulates relations between state and legal institutions and physical bodies in the field of health care in the whole territory of Georgia.
2. **Sample Procedures**

2.1 **Sampling Rooms and Material**

Improper collection, internal transport, and receipt of specimens in the laboratory carry a risk of infection to the personnel involved. Specimens have to be collected in separate room. In laboratories, it is strictly forbidden to:

- Eat
- Drink
- Chew gum
- Use cosmetics
- Smoke
- Put hands into the mouth (e.g., bite nails!)

**Requirements for sampling**

Sample has to be taken by trained staff.

Material needed:

**General material**

- Gloves
- Labcoat
- Glasses
- Labels, pens
- Waste bin, including container for sharps

**Blood sampling**

- Disposable syringes and needles or all-in-one systems such as vaccutainer
- Tubes, with and without anticoagulant
- Tourniquet
- Cotton
- Disinfectants (alcohol, iodine, etc.)
Swab sampling
- Swab
- Transport media

Stool sampling
- Sterile container
- Transport media for viruses

Note: a sampling room is not a reception room: a sampling room contains a “dirty” area, a reception room is ONLY administrative. Samples received from outside cannot be opened at the reception.

2.2 Sample Criteria for Rejection or Acceptance, Sample Identification

Specimen reception

Maintain registration book:
- Document the time the specimen was received.
- Verify that the patient identification on the request form matches the one on the specimen.
- Assign an accession number to be used as specimen identification in the laboratory.
- Examine the specimen visually.
- Carefully review and evaluate the specimen request for appropriateness of orders.
- Determine the appropriateness of the container, including the following:
  - Holding medium or preservative
  - Intact transport void of leaks and cracks

Specimen acceptance and rejection

Unlabeled or mislabeled specimens:
- An unlabeled, mislabeled, or mismatched specimen is unacceptable.
- If the unacceptable specimen can be replaced, notify the ward and request another specimen.
- Do not discard the specimen until the patient’s physician or nurse has confirmed that a repeat specimen can be collected.
- Document the reason for the specimen unacceptability and request for a repeat specimen.
- If the patient has already been started on an antimicrobial therapy or if a repeat specimen cannot be collected, this must also be documented.
Duplicate specimens:

- Most duplicate specimens received on the same day should not be processed; exceptions include blood cultures, CSF (cerebral spinal fluid), tissue, and sterile body fluid excluding urine.
- If it has been verified by the person collecting the specimen that two specimens received at the same time are the same, these specimen may be combined and processed as one specimen.
- If duplicate specimens are received at different times on the same day, notify the patient’s physician or nurse, and document. If it is acceptable not to process the specimen, report “Duplicate specimen: test not performed,” and refer to the number of the specimen that was processed for smear and/or culture results.
- Do not process more than three duplicate specimens on consecutive days.

Leaky containers:

- A specimen is unacceptable when the outside of the container is grossly contaminated with the specimen. Follow the steps outlined above.
- If the container is leaking, set up the specimen only if it is possible to process it without contaminating the processor.

Contaminated specimens:

- Do not contaminate the specimen with another type of specimen. For example, a urine specimen should not contain stool, and vice versa.

Unacceptable specimen sources:

- Do not process saliva in place of sputum.
- 24-hour urine specimens are unacceptable for routine bacterial cultures.
- Request a proper specimen for the test requested.

Delayed transport time and specimen processing:

- Ideally, all other specimens should be less than 2 hours old when received. Appropriate transport media and detailed instructions should be available for specimens transported to reference laboratories (see 3.1).
- Request a new specimen as outlined above.
- Note that the specimen was received after prolonged delay.

Test request or order, sample identification:

The test request or order must include the following:

- Complete name of patient
- Hospital number
- Hospital location
- Complete and specific description of specimen source
- Name of requesting physician
2.3 Blood Sampling

From a VPD perspective, blood sampling collection is done mostly for serology. These procedures are applicable to rubella, hepatitis B, and measles.

1. Collect a minimum of 5 ml (at least 3 ml in newborns) of blood in any collection tube without anticoagulant.
2. Allow blood to clot.
3. Centrifuge blood at low speed long enough to obtain cell-free serum (10 minutes at 1000 rpm).
4. Remove the serum by pipette to a sterile vial or small test tube. Whole blood may be sent if specimen is shipped and received on day of collection.

Note: special vacutainer tubes containing a gel (yellow cap) may be used for serological purpose. After centrifugation, no separation of the serum is needed; the gel making an interface between cells and serum.

Never freeze whole blood.

Serum or plasma may be used in the test. The common anticoagulants are acceptable for blood collection. Always ensure that serum is aliquoted from completely clotted blood. Store separated serum or plasma for up to 5 days at 4°C; and thereafter, freeze it at –20°C if testing is delayed. If specimen shows evidence of precipitation or particulate matter, clarify the sample by centrifugation at 100 x g for 10 to 15 minutes prior to testing. Avoid repeated freezing and thawing.

Note: Universal precautions should be used when collecting any blood specimen, and all blood products (e.g., serum and plasma) should be considered potentially infectious in this testing procedure. Refer to Chapter 4, especially 4.10 and 4.13.

Whole blood can be sent on the same day that it is collected.

2.4 Stool Sampling

Location of the collection: preferably in the laboratory, otherwise at home. Bring the collection to the laboratory as soon as possible, preferably immediately after obtaining.

Collection of fecal specimens for bacterial cultures

1. General considerations:

- Keep stool specimen cool; do not incubate it.
- If a stool specimen cannot be plated within 1 hour of collection, it should be mixed with transport medium (for example, Cary-Blair transport or buffered glycerol saline).
Do not use toilet paper to collect stool. Toilet paper may be impregnated with barium salts, which are inhibitory for some fecal pathogens.

**2. Have patient obtain stool specimen by one of the following methods:**

- Pass stool directly into a sterile, wide-mouth, leak-proof container with tight-fitting lid.
- Pass stool into a clean, dry bedpan, and transfer stool into a sterile, leak-proof container with a tight-fitting lid.

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**Collection of fecal specimens for virology**

1. Place 2–4 g of the stool into a sterile container (e.g., penicillin vial, washing of which is not necessary).
2. Add 8–10 ml of VTM (Viral Transport Medium) to prevent drying if transport to laboratory is not immediate. Order, quality control, management, and pre-positioning of the VTM at peripheral level are done by the World Health Organization (WHO) polio eradication program.
3. Remember to label and regularly pack the stool for shipment.
4. Ship the parcel to NCDC’s enteric viruses department.

---

**2.5 Nasopharyngeal Sampling**

1. Collect specimen with as little contamination from indigenous microbiota as possible to ensure that the sample will be representative of the infected site.
2. Select the correct anatomical site from which to obtain the specimen, and use the proper technique and supplies (calcium alginate swab, Dacron swabs), to collect specimens.
3. Utilize appropriate collection devices. Use sterile equipment and aseptic technique to collect specimen to prevent introduction of micro-organisms during invasive procedures.
4. Clearly label the specimen container with the patient’s name and identification number, source (i.e., specific site), date, time of collection, and initials of collector. Place the specimen in a container designated to promote survival of suspected agents and to eliminate leakage and potential safety hazards.

---

**Material**

Small-tipped calcium alginate or Dacron swabs are suitable for collection of nasopharyngeal specimens. Rayon or cotton swabs should be avoided for pertussis cases, since they contain fatty acids that are toxic to *B. pertussis*.

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**Technique**

Nasopharyngeal specimens are obtained under direct vision using over-the-shoulder illumination.

1. With the thumb of one hand, gently elevate the tip of the nose.
2. Moisten the tip of a small flexible wire nasopharyngeal swab with sterile water or saline and gently insert it into the one of the nares.

3. Guide the swab backward and upward along the nasal septum until a distinct give of resistance indicates that the posterior pharynx has been reached.

4. Gently remove the swab.

5. If while guiding the swab undue resistance is met, attempt the procedure through the opposite nares (pay attention when one teardrop appears, you are at the right place!).

Note: Nasopharyngeal specimens for children are easier to obtain guiding the swab to posterior pharynx not from nasal septum but from behind the uvula to posterior pharynx.

2.6 Throat Sampling

Important note

Do not obtain throat samples if epiglottis is inflamed, as sampling may cause serious respiratory obstruction.

Introduction

1. Collect specimen with as little contamination from indigenous microbiota as possible to ensure that the sample will be representative of the infected site.

2. Select the correct anatomical site from which to obtain the specimen, and use the proper technique and supplies (calcium alginate swab, Dacron swabs), to collect specimens.

3. Utilize appropriate collection devices. Use sterile equipment and aseptic technique to collect specimen to prevent introduction of micro-organisms during invasive procedures.

4. Clearly label the specimen container with the patient’s name and identification number, source (i.e., specific site), date, time of collection, and initials of collector. Place the specimen in a container designated to promote survival of suspected agents and to eliminate leakage and potential safety hazards.

Technique

1. A bright light from over the shoulder of the specimen collector should be focused into the oral cavity so that the swab can be guided to the posterior pharynx.

2. The patient is instructed to tilt his or her head back and breathe deeply.

3. The tongue is gently depressed with a tongue blade to visualize the tonsillar fossae and posterior pharynx.

4. The swab is extended between the tonsillar pillars and behind the uvula. Care should be taken not to touch the lateral walls of the buccal cavity or the tongue to minimize contamination with commensal bacteria.

5. Having the patient phonate a long “aaah” serves to lift the uvula and helps prevent gagging.

6. The tonsillar areas and the posterior pharynx should be firmly rubbed with the swab.
7. Any purulent exudate should also be sampled. After collection, the swab should be placed immediately in a sterile tube or other suitable container for transport to the laboratory.

### 2.7 Skin Sampling

1. Lesions should be cleansed with normal saline and crusted material removed
2. Press the swab firmly into the lesion.

**Note:** in case of skin or eye diphtheria, throat and nasal specimens should be taken as well.
3. Sample Transportation Procedures

3.1 General Procedures for Transport Media

All specimens must be promptly transported to the laboratory, preferably within 2 hours. If processing is delayed, store specimens for bacterial agents under the specific conditions shown in Table 2. In general, do not store bacterial agents for more than 24 hours. Viruses, however, usually remain stable for 2 to 3 days at 4°C.

Table 2. Storage conditions for bacterial transport systems

<table>
<thead>
<tr>
<th>Transport system</th>
<th>Specimen to hold at storage temp of:</th>
<th>Transport system</th>
<th>Specimen to hold at storage temp of:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4C</td>
<td>25C</td>
<td>Directly inoculated medium</td>
</tr>
</tbody>
</table>

* BG=Bordet–Gengou medium, RL=Regan–Lowe medium
** Transport media:
1. Stuart’s medium, charcoal impregnated swabs originally formulated for *N. gonorrhoeae* transport.
2. Amies medium, modified Stuart’s, the most frequently used medium.
3. Cary–Blair medium, similar to Stuart’s medium be modified for fecal specimens with pH from 7.4 to 8.4.

In order to keep the sample compatible with a further analysis, the sample may be prepared and/or a sample transportation media has to be used. A transport media also requires following minimal biosafety rules.
Main transport media used for a prospective VPD

- Viral transport media
- Amies & Stewart 's media
- Regan–Lowe: pertussis-specific

Transport media to be avoided

Do not use Petri dishes and other primary containers that cannot be securely closed. In addition, a pure culture is considered as a dangerous specimen and not a diagnostic specimen.

Do not send swabs just after sampling. It is not possible to keep micro-organisms alive in these conditions.

Once the transport media are correctly used with the sample, ship the sample according to national or international regulations, depending on the recipient. (See 3.3.)

3.2 Safe Transportation Rules

Safe transportation procedures

Safe transportation procedures are universal for local or international ground/air transportation – in normal circumstances materials should not leak out of a container. The following rules should be followed:

- The specimen container should be waterproof and airproof.
- If specimen is placed in the tube it should be firmly closed and placed vertically.
- Specimen container should be placed in metal or plastic, firm, hermetically sealed transport container with tightly closed cap.
- Transport container should be carefully treated during transportation.
- Each transport container should have a label describing its content.
- Each transport container should be accompanied with forms indicating specimen details and identification data.
- The transport device should be equipped with emergency kit consisting of absorbent material, disinfectant, wastage container, and gloves.

Emergency response procedures

- Isolate spill or leak area immediately in all directions.
- Keep unauthorized personnel away.
- Obtain identity of substance involved if possible and report the spill to the appropriate authorities.
- Do not touch or walk through spilled material.
3. Sample Transportation Procedures

▲ Do not touch damaged containers or spilled material unless wearing appropriate protective clothing.
▲ Be particularly careful to avoid contact with broken glass or sharp objects that may cause cuts or abrasions that could significantly increase the risk of exposure.
▲ Damaged packages containing solid CO2 as a refrigerant may produce water or frost due to condensation. Do not touch this liquid as it could be contaminated by the contents of the parcel.
▲ Liquid nitrogen may be present and can cause severe burns.
▲ Absorb spilled materials with earth, sand, or other non-combustible material while avoiding direct contact.
▲ Cover damaged package or spilled material with damp towel or rag and keep wet with liquid bleach or other disinfectant. Liquid bleach will generally effectively inactivate the released substance.

**DO NOT CLEAN UP OR DISPOSE OF, EXCEPT UNDER SUPERVISION OF A SPECIALIST.**

First aid

Move exposed person(s) to a safe isolated area.

**CAUTION: Exposed person(s) may be a source of contamination.**

1. Call emergency medical services.
2. Remove and isolate contaminated clothing and shoes.
3. In case of contact with substance, immediately flush skin or eyes with running water for at least 20 minutes.
4. Effects of exposure (inhalation, ingestion, or skin contact) to substance may be delayed.
5. For further assistance, contact the appropriate public health authority.
6. Ensure that medical personnel are aware of the substances involved, and take precautions to protect themselves

3.3 Sending the Sample

After preparing the sample with an adequate transport media (see 3.1, 3.5, 3.6), transportation rules have to be followed. These rules have two major aims:

1. Protection of the sample
2. Protection of the transporters, the environment, and the person receiving the sample
International rules for dangerous goods transportation

The main rules are organized around the “triple package” concept; three different layers are used

1. **Primary layer**: leak-proof specimen container. The container must be wrapped in sufficient absorbent material to absorb the entire contents of the primary container.

2. **Secondary layer**: leak-proof secondary container, usually plastic or metal. The primary or secondary container must withstand, without leakage, an internal pressure producing a pressure differential of 95 kPa between -40°C and +55°C.

3. **Third layer**: an outer shipping container. The smallest overall external dimension must be 10 mm.

* For “diagnostic specimens,” use IATA Packing Instruction 650; for “Infectious materials,” use IATA Packing Instruction 602. Please refer to current shipping guidelines for more details about definitions, packaging requirements, marking and labeling, accompanying documentation, and refrigerants (see 3.2.)

Other guidelines for packing and shipping

- Do not contaminate primary container (tube, transport media, or other)
- The secondary container, i.e., the container that surrounds the primary container, must be sealable and break resistant. Tape the secondary container lid to prevent loosening during transport.
- All labels must be legible, indelible, and completely visible. (Do not let them overlap). Label all material following labeling rules (above).
- Make sure the patient's name and date of birth exactly match what is written on the transport tubes.
- Place absorbent material between the primary and secondary containers to absorb the entire contents of the primary container in the event of breakage or leakage.
- Include the names, addresses, and telephone numbers of the sender and recipient laboratories on both the containers and the accompanying documents.

Example of a biohazard label

Do not forget not to use it if only diagnostic specimen.
Example of regular international packages

650 package for diagnostic specimens (including quality control material depending on the levels of danger)
No biohazard label is required for diagnostic specimens

602 package for infectious specimens
Biohazard label is required
Conditions of shipment are changing
Shipments price is multiplied by 4 (at least)

### 3.4 Biosafety Measures When Receiving Specimen

The reception of samples is not only an administrative duty. Samples may have been broken or may have leaked during transportation. Biosafety rules should be applied when taking receipt of specimens.

**Requirements**

- Act in a “dirty” area (not on the reception desk!)
- Wear gloves, glasses, and labcoat
- Manipulate parcels behind a screen if possible
When checking the integrity of the samples, the rules for sample rejection or acceptance (see 2.2) have to be considered.

Only after these activities may administrative registration begin.

### 3.5 Diphtheria Transport Media

In case of diphtheria suspicion, all specimens must be promptly transported to the laboratory in a transport medium (compulsory), preferably within 2 hours. If processing is delayed, store throat and nasopharyngeal specimens at 25°C. In general, do not store bacterial agents for more than 24 hours.

**Note:** if sampling is processed in the recipient laboratory, a transport media is not required.

Transport media that can be used with diphtheria:

- Amies or Stewart’s transport medium
- Blood agar slant (see formula in 6.3)

#### Formula and preparation

**Amies transport medium (modified Stuart’s)**

- Distilled water to equal 1.0L
- Agar 4.0g

Heat until dissolved by boiling, and add while hot but not boiling, the following ingredients:

- NaCl 3g
- KCl 0.2g
- Na<sub>2</sub>PO<sub>4</sub> 1.15g
- KH<sub>2</sub>PO<sub>4</sub> 0.2g
- Sodium thioglycollate 1.0g
- CaCl<sub>2</sub> - 1% aqueous 10.0ml
- MgCl<sub>2</sub>·6H<sub>2</sub>O - 1% aqueous 10.0ml
- pH = 7.3

1. Stir until dissolved and add 10g charcoal (pharmaceutical neutral grade).
2. Mix thoroughly and distribute in 7 ml bottles. Apply screw cap and screw down tightly.
3. Autoclave at 121°C for 15 minutes. Invert the bottles during cooling to distribute the charcoal evenly, making sure that the caps are tightened securely. Final pH 7.2. store in the dark in a cool place.

If it is not possible to use such media, the swab should be sent to the reference laboratory, shipped dry in a sterile tube or in a special packet containing a desiccant such as silica gel.
3.6 Pertussis Transport Media

*B. pertussis* is very susceptible to drying and a very slow grower, so transport has to be done extremely quickly.

If pertussis is suspected, all specimens must be promptly transported to the laboratory in a transport media (compulsory), to keep the organism moist and prevent overgrowth of normal flora.

Ideally, culture medium should be inoculated at the bedside and delivered to the laboratory immediately.

When such practice is not feasible, use RL semisolid transport/enrichment medium. In addition to being a transport medium, it is also a culture medium. (See 6.5.)

**Note:** Stuart and Amies transport medium formulations are not suitable for maintaining the viability of *B. pertussis*. 
4. Sterilization, Hygiene, and Security Procedures

4.1 Dry Heat Sterilization

Dry heat sterilization is the most common among the physical agents used for the decontamination of pathogens. “Dry” heat, which is totally non-corrosive, is used to process many items of laboratory ware which can withstand temperatures of 160°C or higher, for 2–4 hours. Burning or incineration is also a form of dry heat.

Sterilization

An oven is used to sterilize. Usually, a table of time and temperature is provided by the manufacturer in order to help the practical implementation (160°C for two hours is usually recommended).

Material usually dry-heated:

- All water-sensitive devices
- Glassware
- Large parts (not fitting into autoclave)

Control of the sterilization

Temperature sensitive strips have to be used during each sterilization process. The change in their color (time and temperature dependant) signifies the quality of the sterilization. All indicators have to be put into a dry heat sterilization logbook, in order to track each sterilization activity.

Incineration

Incineration is useful for disposing of animal carcasses as well as anatomical pieces and other laboratory waste with (recommended) or without prior decontamination. Incineration of infectious materials is an alternative to autoclaving only if the incinerator is under laboratory control, and it is provided with an efficient means of temperature control and a secondary burning chamber. Many incinerators, especially those with a single combustion chamber, are unsatisfactory for dealing with infectious materials, animal carcasses, and plastics. Such materials may not be completely destroyed and the effluent from the chimney may pollute the atmosphere with microorganisms, toxic chemicals,
and smoke. However, there are many satisfactory configurations for combustion chambers. Ideally the temperature in the primary chamber should be at least 800°C and that in the secondary chamber at least 1000°C. Materials for incineration, even with prior decontamination, should be transported to the incinerator in bags, preferably plastic. Incinerator attendants should receive proper instructions about loading and temperature control. It should also be noted that the efficient operation of an incinerator depends heavily on the right mix of materials in the waste being treated.

4.2 Wet Heat Sterilization

Introduction

“Wet” or “moist” heat is most effective when used in the form of autoclaving.

Boiling does not necessarily kill all microorganisms and/or pathogens, but it may be used as the minimum processing for disinfections where other methods (chemical disinfections or decontamination, autoclaving) are not applicable or available.

Sterilized items must be handled and stored such that they remain uncontaminated until used.

Autoclaving

Saturated steam under pressure (autoclaving) is the most effective and reliable means of sterilizing laboratory materials. For most purposes, the following cycles will ensure sterilization of correctly loaded autoclaves:

- 3 min holding time at 134 °C
- 10 min holding time at 126 °C
- 15 min holding time at 121 °C
- 25 min holding time at 115 °C.

Each autoclave in technical manual has its best regime of sterilization. Examples of different autoclaves include the following.

Gravity displacement autoclaves: In gravity displacement autoclaves, steam enters the chamber under pressure and displaces the heavier air downwards and through the valve in the chamber drain, fitted with a HEPA filter.

Pre-vacuum autoclaves: Pre-vacuum autoclaves allow the removal of air from the chamber before steam is admitted. The exhaust air is evacuated through a valve fitted with a HEPA filter. At the end of the cycle, the steam is automatically exhausted. These autoclaves can operate at 134 °C and the sterilization cycle can therefore be reduced to 3 minutes. They are ideal for porous loads, but cannot be used to process liquids because of the vacuum.

Fuel-heated pressure cooker autoclaves: Fuel-heated pressure cooker autoclaves should be used only if a gravity displacement autoclave is not available. They are loaded from the top and heated by gas, electricity, or other types of fuels. Steam is generated by heating water in the base of the vessel and air is displaced upwards through a relief vent. When all the air has been removed, the valve on
the relief vent is closed and the heat reduced. The pressure and temperature rise until the safety valve operates at a preset level. This is the start of the holding time. At the end of the cycle the heat is turned off and the temperature allowed to fall to 80°C or below before the lid is opened.

*Loading autoclaves:* Materials should be loosely packed in the chamber of a loading autoclave for easy steam penetration and air removal. Bags should allow the steam to reach their contents.

**Precautions for the use of autoclaves**

The following rules can minimize the hazards inherent in operating pressurized vessels:

- Responsibility for operation and routine care should be assigned to trained individuals and a preventive maintenance program should include yearly inspection of the chamber, door seals, and all gauges and controls by qualified personnel.
- The steam should be saturated and free from corrosion inhibitors or other chemicals, which could contaminate the items being sterilized.
- All materials to be autoclaved should be in containers that allow ready removal of air and permit good heat penetration; the chamber should not be tightly packed or steam will not reach the load evenly.
- For autoclaves without an interlocking safety device that prevents the door being opened when the chamber is pressurized, the main steam valve should be closed and the temperature allowed to fall below 80°C before the door is opened.
- Operators should wear suitable gloves and visors for protection when opening the autoclave, even when the temperature has fallen below 80°C.
- In any routine monitoring of autoclave performance, biological indicators or thermocouples should be placed at the center of each load. Regular monitoring with thermocouples and recording devices in a “worst case” load is highly desirable to determine proper operating cycles.
- The drain screen filter of the chamber (if available) should be removed and cleaned daily.
- Care should be taken to ensure that the relief valves of pressure cooker autoclaves do not become blocked by paper, etc. in the load.
Control of the process

As for dry heat, temperature sensitive strips have to be used during each sterilization process. Change in their color (time and temperature dependant) signs the quality of the sterilization. All indicators have to be put into a wet heat sterilization logbook in order to track each sterilization activity.

4.3 Chemical Cold Sterilization

Chemical germicides

Many types of chemicals can be used as disinfectants and antiseptics and there is an ever-increasing number and variety of commercial products. Formulations must therefore be carefully selected for specific needs, and stored, used, and disposed of as directed by the manufacturer. The germicidal activity of many chemicals is faster and better at higher temperatures. At the same time, higher temperatures can accelerate their evaporation and also degrade them faster. Particular care is needed in the use and storage of such chemicals in tropical regions, where their shelf-life may be reduced because of high ambient temperatures.

Many germicides can be harmful to humans and the environment. They should therefore be selected, handled, and disposed of with care. For personal safety, gloves, aprons, and eye protection are recommended when preparing use-dilutions of chemical germicides. Chemical germicides are therefore not required for regular and general cleaning of floors, walls, equipment, and furniture except in cases of outbreak control.

Equipment

Temperature sensitive
Control process

Outside real bacteriological control (incubation in a nutritive broth and subculture on agar), no real internal quality control process is available.

4.4 Disinfectants, Disinfection

Important note

Before any disinfection activities, materials and equipment have to be regularly “precleaned,” for two main reasons:

- Decreasing the number of micro-organisms
- Avoiding the precipitation of proteins by highly effective disinfectant agents

In conclusion, only “clean” material will be disinfected.

Pre-cleaning and cleaning laboratory materials

In practical terms, cleaning is the removal of visible dirt and stains. This is generally achieved either by (a) brushing, vacuuming, or dry dusting; or (b) washing or damp mopping with water containing a soap or detergent. Where the risk of human or animal contact with pathogen-contaminated materials is high and subsequent decontamination is needed, precleaning is routinely carried out. This is necessary because dirt and soil can shield microorganisms and can also interfere with the killing action of chemical germicides. In such cases, precleaning is essential to achieve proper disinfections or sterilization. Also, many germicidal products claim activity only on precleaned items. Pre-cleaning must be carried out with care to avoid exposure to infectious agents, and materials chemically compatible with the germicides to be applied later must be used. It is quite common to use the same chemical germicide for precleaning and disinfections.

Main chemical disinfectants

- Chlorine
- Sodium dichloroisocyanurate
- Chloramines
- Chlorine dioxide
- Formaldehyde
- Glutaraldehyde
- Phenolic compounds
- Quaternary ammonium compounds
- Alcohols
- Iodine
- Hydrogen peroxide and peracids
Chlorine (sodium hypochlorite)

Chlorine, a fast-acting oxidant, is a widely available and broad-spectrum germicide. It is normally sold as bleach, an aqueous solution of sodium hypochlorite (NaOCl), which can be diluted with water to provide various concentrations of available chlorine. Chlorine, especially as bleach, is highly alkaline and can be corrosive to metal. Its activity is considerably reduced by organic matter (protein). Storage of stock or working solutions of bleach in open containers, particularly at high temperatures, releases chlorine gas, thus weakening their germicidal potential. The frequency with which working solutions of bleach should be changed depends on their starting strength, the type (e.g., with or without a lid) and size of their containers, the frequency and nature of use, and ambient conditions. As a general guide, solutions receiving materials with high levels of organic matter several times a day should be changed at least daily, while those with less frequent use may last for as long as a week.

A general all-purpose laboratory disinfectant should have a concentration of 1g/l available chlorine. A stronger solution, containing 5g/l available chlorine, is recommended for dealing with biohazardous spillage and in the presence of large amounts of organic matter. Sodium hypochlorite containing 5g/l available chlorine is recommended as the disinfectant of choice in emergency situations involving viruses such as Hantavirus, and Lassa and Ebola viruses.

Sodium hypochlorite solutions such as domestic bleach contain 50g/l available chlorine and should therefore be diluted 1:50 or 1:10 to obtain final concentrations of 1g/l and 5g/l, respectively. Industrial solutions of bleach have a sodium hypochlorite concentration of nearly 120g/l and must be diluted accordingly to obtain the necessary levels.

Granules or tablets of calcium hypochlorite (Ca(ClO)2) generally contain about 70% available chlorine. Solutions prepared with granules or tablets, containing 1.4g/l and 7.0g/l, will then contain 1.0g/l and 5g/l available chlorine, respectively.

Bleach is not recommended as an antiseptic, but may be used as a general-purpose disinfectant and for soaking contaminated metal-free materials. In emergencies, bleach can also be used to disinfect water for drinking with a final concentration of 1–2 mg/l available chlorine.

Note: Chlorine gas is highly toxic. Bleach must therefore be stored and used in well-ventilated areas only. Also, bleach must not be mixed with acids in order to avoid the rapid release of chlorine gas. Many by-products of chlorine can be harmful to humans and the environment, so that indiscriminate use of chlorine-based disinfectants, and in particular bleach, should be avoided.

Sodium dichloroisocyanurate

Sodium dichloroisocyanurate (NaDCC) in powder form contains 60% available chlorine. Solutions prepared with NaDCC powder at 1.7g/l and 8.5g/l will contain 1g/l or 5g/l available chlorine, respectively. Tablets of NaDCC generally contain the equivalent of 1.5g available chlorine per tablet. One or four tablets dissolved in 1 liter of water will give the required concentrations of 1 g/l or 5 g/l, respectively. NaDCC as powder or tablets is easy and safe to store. Solid NaDCC can be applied on spills of blood or other biohazardous liquids and left for at least 10 min before removal. Further cleaning of the affected area can then take place.
**Chloramines**

Chloramines are available as powders containing about 25% available chlorine. Chloramines release chlorine at a slower rate than hypochlorites. Higher initial concentrations are therefore required for efficiencies equivalent to those of hypochlorites. On the other hand, chloramine solutions are not inactivated by organic matter to the same extent as hypochlorite solutions, and concentrations of 20g/l are recommended for both “clean” and “dirty” situations. Chloramine solutions are virtually odor-free. However, items soaked in them must be thoroughly rinsed to remove any residue of the bulking agents added to chloramine-T (sodium tosylchloramide) powders. Chloramines can also be used to disinfect water for drinking when used at a final concentration of 1–2 mg/l available chlorine.

**Chlorine dioxide**

Chlorine dioxide is a strong and fast-acting germicide, often reported to be active at levels lower than those needed by chlorine as bleach. To obtain an active solution for laboratory use it is generally necessary to mix two separate components, hydrochloric acid (HCl) and sodium chlorite (NaClO2). Stability can be an important issue with this germicide, and materials compatibility and corrosiveness must also be considered when selecting products based on it.

**Formaldehyde**

Formaldehyde (HCHO) is a gas that kills all microorganisms and their spores at temperatures above 20°C. Formaldehyde is not active against prions. It is relatively slow-acting and needs a relative humidity level of about 70%. It is marketed as the solid polymer, paraformaldehyde, in flakes or tablets, or as formalin, a solution of the gas in water of about 370g/l (37%), containing methanol (100ml/l) as a stabilizer. Both formulations are heated to liberate the gas, which is used for decontamination and disinfection of enclosed volumes such as safety cabinets and rooms. Formaldehyde (5% formalin in water) may be used as a liquid disinfectant.

**Note:** Formaldehyde is a suspected carcinogen. It has a pungent smell and its fumes can irritate eyes and mucous membranes. It must therefore be stored and used in a fume-hood or well-ventilated areas. Applicable regulations on chemical safety must be consulted prior to its use.

**Glutaraldehyde**

Like formaldehyde, glutaraldehyde (OHC(CH2)3CHO) is also active against vegetative bacteria, spores, fungi and lipid- and non lipid-containing viruses. It is non-corrosive and faster acting than formaldehyde. However, it takes several hours to kill bacterial spores. It is generally supplied as a solution with a concentration of about 20g/l (2%) and most products need to be “activated” (made alkaline) before use by the addition of a bicarbonate compound supplied with the product. The activated solution can be reused for 1–4 weeks depending on the formulation and type and frequency of its use. Dipsticks supplied with some products give only a rough indication of the levels of active glutaraldehyde available in solutions under use. Glutaraldehyde solutions should be discarded if they become turbid.

**Note:** Glutaraldehyde is toxic and an irritant to skin and mucous membranes, and contact with it must be avoided. It must be used in a fume-hood or in well-ventilated areas. It is not recommended as a spray or solution for the decontamination of environmental surfaces. Applicable regulations on chemical safety must be consulted prior to its use.
**Phenolic compounds**

Phenolic compounds, a broad group of agents, were among the earliest germicides. However, results of more recent safety concerns restrict their use. They are active against vegetative bacteria and lipid-containing viruses and, when properly formulated, also show activity against mycobacteria. They are not active against spores and their activity against non-lipid viruses is variable. Many phenolic products are used for the decontamination of environmental surfaces, and some (e.g., triclosan and chloroxylenol) are among the more commonly used antiseptics. Triclosan is common in products for hand-washing. It is active mainly against vegetative bacteria and safe for skin and mucous membranes. However, in laboratory-based studies, bacteria made resistant to low concentrations of triclosan also show resistance to certain types of antibiotics. The significance of this finding in the field remains unknown.

**Note:** Phenolic compounds are not recommended for use on food contact surfaces and in areas with young children. They may be absorbed by rubber and can also penetrate the skin.

**Quaternary ammonium compounds**

Many types of quaternary ammonium compounds are used as mixtures and often in combination with other germicides, such as alcohols. They have good activity against vegetative bacteria and lipid-containing viruses. Certain types (e.g. benzalkonium chloride) are used as antiseptics.

**Note:** The germicidal activity of certain types of quaternary ammonium compounds is considerably reduced by organic matter, water hardness, and anionic detergents. Care is therefore needed in selecting agents for precleaning when quaternary ammonium compounds are to be used for disinfection. Potentially harmful bacteria can grow in quaternary ammonium compound solutions. Owing to low biodegradability, these compounds may also accumulate in the environment.

**Alcohols**

Ethanol (ethyl alcohol, C2H5OH) and 2-propanol (isopropyl alcohol, (CH3)2CHOH) have similar disinfectant properties. They are active against vegetative bacteria, fungi and lipid-containing viruses but not against spores. Their action on non lipid viruses is variable. For highest effectiveness they should be used at concentrations of approximately 70% (v/v) in water: higher or lower concentrations may not be as germicidal. A major advantage of aqueous solutions of alcohols is that they do not leave any residue on treated items. Mixtures with other agents are more effective than alcohol alone, e.g., 70% (v/v) alcohol with 100g/l formaldehyde, and alcohol containing 2g/l available chlorine. A 70% (v/v) aqueous solution of ethanol can be used on skin, work surfaces of laboratory benches and biosafety cabinets, and to soak small pieces of surgical instruments. The contact time on skin should be no less than 10 seconds and that on environmental surfaces no less than 3 minutes. Since ethanol can dry the skin, it is often mixed with emollients. Alcohol-based hand-rubs are recommended for the decontamination of lightly soiled hands in situations where proper hand-washing is inconvenient or not possible. However, it must be remembered that ethanol is ineffective against spores and may not kill all types of non-lipid viruses.

**Note:** Alcohols are volatile and flammable and must not be used near open flames. Working solutions should be stored in proper containers to avoid the evaporation of alcohols. Alcohols may harden rubber and dissolve certain types of glue. Proper inventory and storage of ethanol in the laboratory is very important to avoid its use for purposes other than disinfection. The availability and use of ethanol in certain regions may be restricted owing to religious reasons. Bottles with alcohol-containing solutions must be clearly labeled to avoid their accidental autoclaving.
Iodine and iodophors

The action of these disinfectants is similar to that of chlorine, although they may be slightly less inhibited by organic matter. Iodine can stain fabrics and environmental surfaces and is generally unsuitable for use as a disinfectant. On the other hand, iodophors and tinctures of iodine are good antiseptics. Polyvidone-iodine is a reliable and safe surgical scrub and preoperative skin antiseptic. Antiseptics based on iodine are generally unsuitable for use on medical/dental devices. Iodine should not be used on aluminum or copper.

Note: Iodine can be toxic. Organic iodine-based products must be stored at 4–10 °C to avoid the growth of potentially harmful bacteria in them.

Hydrogen peroxide and peracids

Like chlorine, hydrogen peroxide (H2O2) and peracids are strong oxidants and can be potent broad-spectrum germicides. They are also safer than chlorine to humans and the environment. Hydrogen peroxide is supplied either as a ready-to-use 3% solution or as a 30% aqueous solution to be diluted to 5–10 times its volume with sterilized water. However, such 3–6% solutions of hydrogen peroxide alone are relatively slow and limited as germicides. Products now available have other ingredients to stabilize the hydrogen peroxide content, to accelerate its germicidal action and to make it less corrosive. Hydrogen peroxide can be used for the decontamination of work surfaces of laboratory benches and biosafety cabinets, and stronger solutions may be suitable for disinfecting heat-sensitive medical/dental devices. The use of vaporized hydrogen peroxide or peracetic acid (CH3COOOH) for the decontamination of heat-sensitive medical/surgical devices requires specialized equipment.

Note: Hydrogen peroxide and peracids can be corrosive to metals such as aluminum, copper, brass and zinc, and can also decolorize fabrics, hair, skin, and mucous membranes. Articles treated with them must be thoroughly rinsed before contact with eyes and mucous membranes. They should always be stored away from heat and protected from light.

4.5 Vaccination Required for Laboratory Staff

According to “Laboratory Biosafety Manual” (WHO/CDS/CSR/LYO/2003.4):

1. It is recommended that all laboratory personnel receive protective immunization against the following diseases:
   △ Diphtheria
   △ Hepatitis B
   △ Measles
   △ Mumps
   △ Poliomyelitis
   △ Rubella
   △ Tetanus
   △ Tuberculosis
   △ Typhoid fever
Some workers may have been immunized during childhood but documentary evidence of current protection should be obtained.

Note. BCG immunization does not appear to give as much protection against TB.

2. All persons who work with or who handle animals infected with the following agents should be given the appropriate vaccine or toxoid, and facilities for specific clinical case management following accidental infections should be available:

- Bacillus anthracis
- Clostridium botulinum
- Francisella tularensis type A
- Haemophilus influenzae
- Japanese B encephalitis virus
- Mycobacterium leprae
- Neisseria meningitidis
- Yersinia pestis
- Hepatitis A virus
- Influenza virus
- Louping ill virus 3
- Rabies virus
- Rift Valley fever virus
- Tick-borne encephalitis viruses 3 (Absettarov virus, Hanzalova virus, and Omsk hemorrhagic fever virus)
- Varicella-zoster virus
- Venezuelan equine encephalomyelitis virus
- Yellow fever virus

Vaccinia vaccine is recommended for persons who work with orthopoxviruses. Other vaccines are available and may be indicated in specific circumstances for laboratory workers at high risk.

The national Georgian policy on laboratory staff vaccine is defined by the Decree # 122/N on “Preventive Immunization Calendar.” Personnel who work with the following live cultures should be given appropriate vaccine:

- Tularemia
- Plaque
- Brucellosis
- Anthrax
- Rabies
- Leptospirosis
- Tick-born encephalitis
4. Sterilization, Hygiene, and Security Procedures

- Q fever
- Yellow fever
- Typhoid fever
- Hepatitis B (refers to the personnel working with the blood)

4.6 Clothing Required in the Laboratory

Personal protective clothing and equipment may act as a barrier to minimize the risk of exposure to aerosols, splashes, and accidental inoculation. Clothing and equipment selected is dependent on the nature of the work performed. Protective clothing should be worn when working in the laboratory. Before leaving the laboratory, protective clothing should be removed, and hands should be washed. Table 2 summarizes some personal protective equipment used in laboratories and the protection afforded.

### Table 3. Personal protective equipment

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<td>▲ Hand protection</td>
</tr>
</tbody>
</table>

**Laboratory coats, gowns, coveralls, aprons**

Laboratory coats should preferably be fully buttoned. However, long-sleeved, back-opening gowns or coveralls give better protection than laboratory coats and are preferred in microbiology laboratories and when working in the biological safety cabinet. Aprons should be worn over laboratory coats or gowns where necessary to give further protection against spillage of chemicals or biological materials such as blood or culture fluids.
Goggles, safety spectacles, face shields

The choice of equipment to protect the eyes and face from splashes and impacting objects will depend on the activity performed. Prescription or plain eye glasses can be manufactured with special frames that allow lenses to be placed in frame from the front, using shatterproof material either curved or fitted with side shields (safety glasses). Goggles should be worn over normal prescription eye glasses and contact lenses, which do not provide protection against biological hazards. Face shields (visors) are made of shatterproof plastic, fit over the face, and are held in place by head straps or caps.

Respirators

Respiratory protection may be used when carrying out high-hazard procedures (e.g., cleaning up a spill of infectious material). The choice between mask and respirator, and type of respirator will depend on the type of hazard. Respirators are available with interchangeable filters for protection against gases, vapors, particulates, and microorganisms. Note that no filter other than a HEPA filter will provide protection against microorganisms, and it is imperative that the filter be fitted in the correct type of respirator. To achieve optimal protection, respirators should be individually fitted to the operator’s face and tested. Fully self-contained respirators with an integral air supply provide full protection. Advice should be sought from a suitably qualified person, e.g., an occupational hygienist, for selection of the correct respirator.

Gloves

Contamination of hands may occur when laboratory procedures are performed. Hands are also vulnerable to “sharps” injuries. Disposable latex or vinyl surgical-type gloves are used widely for general laboratory work, and for handling infectious agents and blood and body fluids. Reusable gloves may also be used but attention must be given to their correct washing, removal, cleaning, and disinfection. Gloves should be removed and hands thoroughly washed after handling infectious materials and working in a biological safety cabinet, and before leaving the laboratory. Used disposable gloves should be discarded with infected laboratory wastes. Allergic reactions such as dermatitis and immediate hypersensitivity have been reported in laboratory and other workers wearing latex gloves, particularly those with powder. Alternatives such as powder-free latex or vinyl gloves should be used if problems occur. Stainless steel mesh gloves should be worn when there is a potential exposure to sharp instruments e.g. during postmortem examinations. However, such gloves protect against slicing motion but do not protect against needle injury.

4.7 Labcoat, Napkin, and Tissue Washing

Reusable laboratory clothes cannot be washed as regular clothes. Some rules apply:

- Pre-disinfections of clothes used in dirty area is recommended, by immersing them in disinfectants (see 4.4)
- Contaminated areas of clothing also must be processed this way before washing
- Laboratory clothes cannot be washed at home
- Laboratory clothes must be washed only with other laboratory clothes
Frequency of washing:

- Napkins and tissues: daily
- Labcoat: weekly

Once contaminated, clothes have to be washed immediately.

### 4.8 Hand-washing

Whenever possible, suitable gloves should be worn when handling biohazardous materials. However, this does not replace the need for regular and proper hand-washing by laboratory personnel.

**Hands must be washed**

- Before and after sampling
- After microbiological manipulation
- Before and after lunch and coffee breaks
- Before and after going to the restroom
- At the end of the day, after washing the laboratory, just before leaving

Usually, a liquid soap is recommended. It should contain an antiseptic such as chlorhexidine or iodine.

**Note**: take care not to mix several different antiseptics, such as hypochlorite and chlorhexidine, or iodine and mercurochrome

**Procedure for hand-washing**

1. Open the tap.
2. Wet hands and wrist.
3. Take a few milliliters of soap.
4. Carefully wash both hands for 2–3 minutes. Focus on areas between fingers, nails, and palm. Never use a brush.
5. Rinse hands with clean water. This operation is very important, because it will flush the dirt away. If needed, soap and rinse a second time.
6. Dry hands preferably using paper tissue. Close the tap with a tissue in the hands.
   If no paper tissue is available, use a napkin that will be changed and disinfected daily

### 4.9 Washing the Laboratory, Including Floor and Benches

See Section 4.4 for materials to use in precleaning and cleaning the laboratory.
4.10 Safe Manipulation Procedures

In many laboratories and national laboratory programs, a code of Safe Manipulation Procedure may be given the status of “rules” for laboratory operation.

Good microbiological technique is fundamental to laboratory safety and cannot be replaced by specialized equipment, which can only supplement it. Some requirements are the same as sampling requirements:

1. The international biohazard sign (see 3.3) should be displayed on the doors of rooms where Risk Group 2 micro-organisms are handled.
2. Pipetting by mouth should be prohibited.
3. Eating, drinking, smoking, storing food, and applying cosmetics must not be permitted in the laboratory work areas.
4. Labels must not be licked but stucked; materials must not be placed in the mouth.
5. The laboratory should be kept neat, clean, and free of materials that are not pertinent to the work.
6. Work surfaces must be decontaminated after any spill of potentially dangerous material and at the end of the working day.
7. Members of the staff must wash their hands after handling infectious materials and animals, and before they leave the laboratory.
8. All technical procedures should be performed in a way that minimizes the formation of aerosols and droplets.
9. All contaminated materials, specimens, and cultures must be decontaminated before disposal or cleaning for reuse. They should be placed in leak-proof, color-coded plastic bags for autoclaving or incineration on the premises. These bags should be supported in rigid containers. If it is necessary to move the bags to another site for decontamination, they should be placed in leak-proof (i.e., solid-bottomed) containers which can be closed before they are removed from the laboratory.
10. Laboratory coveralls, gowns, or uniforms must be worn for work in the laboratory. This clothing should not be worn in non-laboratory areas such as offices, libraries, staff rooms, and canteens. Contaminated clothing must be decontaminated by appropriate methods.
11. Open-toed footwear should not be worn.
12. Protective laboratory clothing should not be stored in the same lockers or cupboards as street clothing.
13. Safety glasses, face shields (visors), or other protective devices must be worn when it is necessary to protect the eyes and face from splashes and impacting objects.
14. Only persons who have been advised of the potential hazards and who meet specific entry requirements (e.g., immunization) should be allowed to enter the laboratory working areas. Laboratory doors should be kept closed when work is in progress; access to animal cages/kennels should be restricted to authorized persons; children should be excluded from laboratory working areas.
15. There should be an arthropod and rodent control program.
16. Animals not involved in the work of the laboratory should not be permitted in or near the lab.
17. The use of hypodermic needles and syringes should be restricted to parenteral injection and aspiration of fluids from laboratory animals. Their use for removing the contents of diaphragm bottles should be limited (opening devices are available, permitting the use of pipettes). Hypodermic needles and syringes should not be used as substitutes for pipetting devices in the manipulation of infectious fluids. Cannulas should be used instead of needles whenever possible.

18. Gloves appropriate for the work must be worn for all procedures that may involve accidental direct contact with blood, infectious materials, or infected animals. After use, gloves should be removed aseptically and autoclaved with other laboratory wastes before disposal. Hands must then be washed. Reusable gloves must be washed while on the hands and, after the gloves are removed, they must be cleaned and disinfected before reuse.

19. All spills, accidents, and overt or potential exposures to infectious materials must be reported immediately to the laboratory supervisor. A written record of such accidents and incidents should be maintained.

20. Appropriate medical evaluation, surveillance, and treatment should be provided.

21. Baseline serum samples may be collected from laboratory staff and other persons at risk. These should be stored as appropriate. Additional specimens should be collected periodically depending on the organisms handled and the function of the laboratory.

22. The laboratory supervisor should ensure that training in laboratory safety is provided. A safety or operations manual that identifies known and potential hazards and that specifies practices and procedures to minimize or eliminate such hazards should be adopted. Personnel should be advised of special hazards and required to read and follow standard practices and procedures. The supervisor should make sure that personnel understand these.

4.11 Procedures in Case of Emergency

Fire, flood, and natural disaster

Fire and other services should be involved in the development of emergency plans. They should be told in advance which rooms contain potentially infectious materials. It is beneficial to arrange for these services to visit the laboratory to become acquainted with its layout and contents if possible.

After a flood or other natural disaster (including earthquake), local or national emergency services should be warned of the potential hazards within and/or near laboratory buildings. They should enter only when accompanied by a trained laboratory worker. Cultures and infectious materials should be collected in leak-proof boxes or strong disposable bags. Salvage or final disposal should be determined by safety staff on the basis of local knowledge.

Vandalism

Vandalism is usually selective (e.g., aimed at animal cages/kennels). Suitable defenses are strong, heavy doors, good locks, and restricted entry. Screened windows and intruder alarms are desirable. Action after vandalism is the same as that for other emergencies.
Emergency services: whom to contact

The telephone numbers and addresses of the following should be prominently displayed near all telephones:

- Institution or laboratory itself (the address and location may not be known in detail by the caller or the services called)
- Director of the institution or laboratory
- Laboratory supervisor
- Biosafety officer
- Fire services
- Hospital/ambulance service (if a particular hospital has arranged to accept casualties, e.g. high-risk personnel, the names of individual departments and doctors)
- Police
- Medical officer
- Responsible technician
- Water, gas, and electricity services.

Emergency equipment

The following emergency equipment must be available:

- First-aid kit, including universal and special antidotes
- Stretcher
- Appropriate fire extinguishers, fire blankets

The following are also suggested but may be varied according to local circumstances:

- Full-face respirators with appropriate chemical and particulate filter canisters
- Room disinfections apparatus, e.g., sprays and formaldehyde vaporizers
- Tools, e.g., hammers, axes, spanners, screwdrivers, ladders, ropes
- Hazard area demarcation equipment and notices

4.12 Procedures for Dealing with Biohazard Injury (chemical, wound, burn)

Accidental injection, cuts, and abrasions

The affected individual should remove protective clothing, wash the hands and the affected part, apply an appropriate skin disinfectant, go to the first-aid room, and inform the person in charge about the cause of the wound and the organisms involved. If considered necessary, a physician should be consulted and his or her advice followed. Appropriate and complete medical records should be kept.

Note: at NCDC, the physician in charge of injuries is Dr Barnabishvili.
Accidental ingestion of potentially hazardous material

Protective clothing should be removed and the individual taken to the first-aid room. A physician should be informed of the material ingested and his or her advice followed. Appropriate and complete medical records should be kept.

Potentially hazardous aerosol release (other than in a biological safety cabinet)

All persons should immediately vacate the affected area and any exposed persons should be referred for medical advice. The laboratory supervisor and the biosafety officer should be informed at once. No one should enter the room for at least 1 hour, to allow aerosols to disperse and heavier particles to settle. If the laboratory does not have a central air exhaust system, entrance should be delayed for 24 hours. Signs should be posted indicating that entry is forbidden. After the appropriate time, decontamination should proceed, supervised by the biosafety officer. Appropriate protective clothing and respiratory protection should be worn for this.

Broken and spilled infectious substances, including cultures

Broken objects contaminated with infectious substances, including vials or containers, or spilled infectious substances, including cultures, should be covered with a cloth or paper towels. Disinfectant should then be poured over these and left for at least 30 minutes. The cloth or paper towels and the broken material may then be cleared away; glass fragments should be handled with forceps. The contaminated area should then be swabbed with disinfectant. If dustpans are used to clear away the broken material, they should be autoclaved or placed in an effective disinfectant for 24 hours. Cloths, paper towels, and swabs used for cleaning up should be placed in a contaminated-waste container. Gloves should be worn for all these procedures. If laboratory forms or other printed or written matter are contaminated, the information should be copied onto another form and the original discarded into the contaminated-waste container.

4.13 Waster Disposal (biohazard and chemical)

The disposal of laboratory and medical waste must be carried out according to "Sanitary Norms of Waste Gathering, Keeping and Decontamination in Medical and Prophylactic Institutions" (Sanitary Rules and Norms from 2.1.7.000-00, Ministry of Labor, Health and Social Affairs of Georgia, Tbilisi, 2000).

Waste handling and disposal procedures

An identification and separation system for infectious materials and their containers should be adopted.

Categories should include the following:

1. Non-contaminated (non-infectious) waste that can be reused or recycled or disposed of as general, “household” waste.
2. Contaminated (infectious) “sharps” – hypodermic needles, scalpels, knives and broken glass; these should always be collected in puncture-proof containers fitted with covers and treated as infectious

3. Contaminated material for decontamination by autoclaving and thereafter washing and reuse or recycling

4. Contaminated material for autoclaving and disposal

5. Contaminated material for direct incineration

**Sharps**

After use, hypodermic needles should not be recapped, clipped or removed from disposable syringes. The complete assembly should be placed in a sharps container. Sharps containers must be puncture proof and not be filled to capacity. When they are three-quarters full they should be placed in “infectious waste” containers and incinerated, with prior autoclaving if laboratory practice requires it. Sharps containers must not be disposed of in landfills.

Disposable syringes, used alone or with needles, should be placed in containers and incinerated, with prior autoclaving if required.

**Contaminated (infectious) materials for autoclaving and reuse**

No precleaning should be attempted of any contaminated (infectious) materials to be autoclaved and reused. Any necessary cleaning or repair must be done only after autoclaving or disinfection.

**Contaminated (infectious) materials for disposal**

Apart from sharps, which are dealt with above, all contaminated (potentially infectious) materials should be autoclaved in leak-proof containers, e.g., autoclavable, color-coded plastic bags, before disposal. After autoclaving, the material may be placed in transfer containers for transport to the incinerator. If possible, materials deriving from health care activities should not be discarded in landfills even after decontamination. If an incinerator is available on the laboratory site, autoclaving may be omitted: the contaminated waste should be placed in designated containers (e.g., color-coded bags) and transported directly to the autoclave or incinerator. Reusable transfer containers should be leak proof and have tight-fitting covers. They should be disinfected and cleaned before they are returned to the laboratory for further use.

Discard pots, pans or jars, preferably unbreakable (e.g., plastic), and containing a suitable disinfectant (see 4.4), freshly prepared each day, should be placed at every work station. Waste materials should remain in intimate contact with the disinfectant (i.e., not protected by air bubbles) for the appropriate time, according to the disinfectant used. The disinfectant should then be poured into a container for autoclaving or incineration. The discard pots should be autoclaved and washed before reuse.

Incineration is the method of choice for the final disposal of contaminated waste, including carcasses of laboratory animals. Incineration of contaminated waste must meet with the approval of the public health and air pollution authorities, as well as that of the laboratory biosafety officer.
5. Staining Procedures

5.1 Preparing Smears, Films, and Slides

Labeling of slides

Frosted ends are desirable for accurate labeling and convenient handling. If not, the edge of another slide can be used for labeling. Special markers can be used.

Smear preparation

Proper preparation should produce a monolayer of organisms sufficiently dense for easy visualization but sparse enough to reveal characteristic arrangements. Use clean glass slides. Best results are obtained with slides kept in an alcohol container and removed just prior to use. They may be air dried or flamed.

Colonies from solid media

1. Place a drop of a sterile saline on slide. Distilled water may distort cellular morphology of fragile organisms and has to be avoided.
2. Transfer a small portion of colony with a sterile applicator stick, wire needle, or loop (preferably).
3. Gently mix to emulsify. The resulting smear should be slightly cloudy and homogenous (adjust saline drop size, area of smear, and/or inoculum to achieve optimum results). If swirl lines are evident as the smear dries, the inoculum was too heavy, the drop of saline too small, and/or the smear spread over too small an area.

Note: Never mix vigorously. Avoid creation of aerosols.

Smear fixation

Smears may be fixed with heat.

1. Air-dry smears on a flat surface, or place them on a 60°C electric slide warmer until dry (photo).
2. If smears are air-dried, pass them two or three times through a flame. To avoid distortions do not overheat. Allow slide to cool before staining.
5.2 Quality Control of Staining Methods

In order to control the stains and staining procedure (differential staining of gram-positive and gram-negative organisms) use local strains of

*E. coli* for *C. diptheriae* stain procedure and for *B. pertussis* stain procedure.

- When to performed such control:
- When preparing new Gram-stain reagents
- When changing batch number or stain kits
- In case of problems while staining
- In any case, regularly on a weekly basis

Some examples of staining:

![Corynebacteria, Gram positive](image1.jpg) ![E. coli, Gram negative](image2.jpg)

5.3 Methylene Blue Staining

Principle

Methylene blue is a simple stain that is particularly useful in the identification of Corynebacterium species.

The metachromatic granules of *C. diptheriae* readily take up Methylene blue dye and appear deep blue. Although some authors have stated that the cytoplasmic granule formation characteristic of *C. diptheriae* is rarely seen with saprophytic species of Corynebacterium, this criterion is unreliable and cannot be used for definitive identification of *C. diptheriae* without further studies.
Procedure

1. Heat-fix the smear. Flood the surface of the smear with the Methylene blue staining solution for 1 minute. Wash the slide with water and blot dry.

2. In the past it was necessary to add alkali to the above solution before use. However, Methylene blue dyes prepared in recent years do not require this additional step because acid impurities found in older stains have been removed.

Interpretation

The Corynebacteria are pleomorphic bacilli that range in size from 0.5 to 1.0 µm in width and from 2 to 6 µm in length and appear as straight, curved, or club-shaped rods. Characteristic for the micro-organisms are metachromatic granules that take up Methylene blue stain and appear dark blue. Although this finding is characteristic of the Corynebacteria, species of the Propionibacterium, some of the actinomycetes, pleomorphic strains of streptococci, and other bacteria may also morphologically resemble the Corynebacteria and must be differentiated by other cultural and biochemical characteristics.

Preparation of reagent

Dissolve 0.3 g of Methylene blue in 30 ml of 95% ethanol, and then add 100 ml of 0.01 % potassium hydroxide. If some precipitation occurs, just filtrate the stain.

To get a better staining, let the stain reagent with opened lid at the air during at least 2 weeks (ideally 6 months), the metachromatic side of the stain will become much better.

5.4 Gram staining

This differential stain divides the majority of bacteria to into two groups: gram-positive and gram-negative bacteria.

Hucker’s modification of the Gram stain is widely used for routine work.

In general, do not apply stains, water or decolorizer directly to specimen area. Drops may be applied to the frosted end of the slide, allowing reagent to flow over the remaining surface.

Technique

1. Flood the fixed smear in the crystal violet solution. Allow the stain to remain for 30 seconds.

2. Decant crystal violet, and rinse slide gently with running tap water. **Caution: excessive rinsing in this step could cause crystal violet to be washed from gram-positive cells.** The flow of water may be applied to the underside of the angled slide to ensure a gentle flow across the smeared side. Some recommend immersing the slide in a beaker of water under running water for 5 s.

3. Rinse off excess water with iodine solution, and then flood the slide with fresh iodine solution. Allow iodine to remain for 30 seconds.
4. Rinse gently with flowing tap water.
5. Decolorize by letting the reagent flow over the smear while the slide is held at an angle. Stop when the runoff becomes clear. Adjust decolorization time to thickness of smear and type of decolorizer used.
6. Remove excess decolorizer with gentle flow of tap water. *Caution: Excessive rinsing in this step could cause dye-iodine complex to be washed from gram-positive cells.* See recommendations in step 2 above.
7. Flood the slide with safranin, and allow counterstain to remain for 30 seconds. Some laboratories may prefer to use 0.1% to 0.2% basic fuchsine as a counterstain.
8. Remove excess counterstain with a gentle flow of tap water.
9. Drain slide, and air-dry it in an upright position, or use a commercial slide drier.
10. Examine the smear microscopically.

**Variant with Carbo-fuchsine counterstain**

Carbo–fuchsine is recommended for detecting faintly staining gram-negative organisms. The procedure is similar to that for Hucker’s modification except that the recommended decolorizer is 95% ethanol and the counterstain is carbo fuchsine or 0.8% basic fuchsine

- Crystal violet – 30 s
- Gram ‘s iodine – 30 s
- 95% ethanol – 30 s
- Carbo-fuchsine or 0.8% basic fuchsine > 1 m

**Results interpretation:**

In the Gram stain procedure, cells are:

- Stained with crystal violet
- Treated with iodine to form a crystal violet / iodine complex within the cell
- Washed with an organic solvent (acetone–alcohol)
- Stained again with the red counterstain safranin.

In gram-positive bacteria the purple crystal violet/iodine complex is retained within the cell after washing with acid–alcohol because the thick peptidoglican layer doesn’t allow the crystal violet/iodine complex to be washed out of the cell.

In gram-negative bacteria, the crystal violet/iodine complex is leached from the cell (i.e., cell become colorless) due to disruption of the lipid-rich outer membrane by the acetone–alcohol organic solvent. These colorless cells must be counterstained to be seen under the light microscope; this counterstain is provided by safranin or carbo-fuchsine or 0.8% basic fuchsine

- Gram-positive bacteria appear blue–purple
- Gram-negative bacteria are stained red
Gram stain reagents

Crystal violet, Gram’s iodine, acetone–alcohol, Safranin

Gram stain for general bacteriology

▲ Crystal violet

1. Crystal violet stock solution
   △ crystal violet (90–95% dye content) – 40g
   △ ethanol, 95% – 400ml
   Dissolve and mix in glass bottle, label with 1-year expiration date, and store at room temperature. Caution: Ethanol is highly flammable.

2. Ammonium oxalate solution (1%)
   △ ammonium oxalate (reagent grade) – 16 g
   △ distilled water – 1, 600 ml
   Dissolve and mix in a brown – glass bottle., label with a 1 – year expiration date, and store in a room temperature.

3. Crystal violet working solution
   △ crystal violet stock solution – 40ml
   △ ammonium oxalate solution (1 %) – 160ml
   Filter crystal violet stock solution into glass bottle. Allow to filter completely, and then filter ammonium oxalate solution. Label with earliest expiration date of stock solution.

▲ Gram’s iodine

1. Stock Lugol’s iodine solution
   △ iodine crystals (reagent grade) – 25g
   △ potassium iodide (reagent grade) – 50
   △ distilled water – 500ml
   Mix or let stand until dissolved in brown glass bottle, label with a 6–month expiration date, and store at room temperature.

2. Sodium bicarbonate, 5% (wt/vol.)
   △ sodium bicarbonate (NaHCO₃), reagent grade – 25g
   △ distilled water – 1, 000ml
   Dissolve in glass bottle, label with 1-year expiration date, and store at room temperature.

3. Gram’s iodine
   △ Stock Lugol’s iodine solution – 60 ml
   △ distilled water – 220 ml
sodium bicarbonate, 5% – 60ml

Mix in a brown glass bottle. Label with a 6-month expiration date, and store at room temperature. Caution: Iodine and potassium iodide are corrosive. Avoid inhalation, ingestion, or skin contact.

Decolorizer

- Slowest: ethanol, 95%
- Intermediate: acetone–alcohol
  - Ethanol, 95% – 100ml
  - Acetone (reagent grade) – 100ml

Combine in brown glass bottle, label with 1-year expiration date, and store at room temperature.

- Fastest: acetone (reagent grade). Caution: Ethanol and acetone are flammable.

Counterstain

1. Safranin

   - Safranin stock solution
   - safranin O (certified) – 5.0
   - ethanol, 95% – 200 ml

Dissolve in glass bottle, label with 1-year expiration date, and store at room temperature. Caution: ethanol is flammable.

   - Safranin working solution
   - safranin stock solution – 20 ml
   - distilled water – 180 ml

Combine in glass bottle, label with 1-year expiration date, and store at room temperature.

2. Alternatively: basic fuchsine, 0.1 or 0.2 % (wt/vol)

   - basic fuchsine (reagent grade) – 0.1g or 0.2 g
   - distilled water – 100ml

Add basic fuchsine in brown glass bottle. Slowly add distilled water, thoroughly mix in to dissolve. Label with 1-year expiration date, and store at room temperature.
6. Media Procedure

6.1 Media Ordering, Validation, and Importation Procedures

The transport media for the microbiology laboratories will be ordered at the central level through the governmental program by the NCDC.

Transport media will be distributed to the microbiology laboratories upon request.

All other media will be ordered by the microbiology laboratories.

6.2 General Procedure on Media Preparation, Conservation, and Control

Storage of reagents and raw material

Store dehydrated medium, reagent, commercial kit, or ingredient as recommended by manufacturer.

Media preparation and control

Test each new lot number of dehydrated medium, reagent, or ingredient in parallel with an approved lot before it is released for use. This means that orders have to be done enough in advance to allow a little overlapping period between old and new reagents.

Maintain a logbook containing:

- Preparation date
- Name of preparer
- Amount of media prepared
- Source(s) of raw media
- Lot number
- Sterilization method and number of sterilization logbook
- Expiration date
- If needed, other information such as pH, osmolarity, resistivity

Note on each Petri dish/tube/slants the name of the media (or a code), the date of expiration, and the lot number.
Quality control of home-made media

Note aspect, color, and sterility in the same logbook as the one used for preparation.

Check each lot for contamination by incubating a 5% sample for batches of 100 or less or 10 randomly selected units from larger batches.

Incubate medium for 48 hours at the temperature at which it will be used and then for another 48 hours at room temperature.

A weekly growth test can be performed using a regular registered strain.

Storage of prepared media

Store media at 4°C. Note that media can only be used after minimum 2 days (waiting for sterilization results).

6.3 Blood Agar Culture Media

Five percent horse blood or sheep blood agar is the most commonly used nonselective medium and is included in the battery of primary isolation media for virtually every clinical specimens.

Blood agar also is used for the diagnosis of diphtheria, to examine the presence of group A hemolytic streptococci because the patient may have streptococcal pharyngitis or a mixed streptococcal-diphtherial infection. Blood agar also serves as a valuable backup to recover strains of C. diphteriae that do not grow on tellurite-containing media.

Formula

<table>
<thead>
<tr>
<th>Oxoid agar</th>
<th>40g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water</td>
<td>2 litres</td>
</tr>
</tbody>
</table>

Media preparation

1. Boil to dissolve agar.
2. Check and note pH.
3. Autoclave 15 minutes at 121°C, or melt down bottles from stock.
4. Cool to 40°C.
5. Aseptically add 50 ml defibrinated horse (or sheep) blood per liter.
6. Pour the medium into sterile Petri dishes or in tubes.

6.4 Diphtheria-specific Culture Media

Cystine–tellurite agar is a medium used for the primary isolation of Corynebacterium diphteriae. It has advantages over Tinsdale medium in being easier to prepare and having a longer shelf-life.
The potassium tellurite in this medium serves to inhibit growth of most normal bacterial inhabitants of the upper respiratory tract, including most species of *Streptococcus* and *Staphylococcus*. *C. diphteriae* grows well, producing grayish or black colonies after 24 to 48 hours of incubation.

**Media formula**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart infusion agar, 2 % solution</td>
<td>100ml</td>
</tr>
<tr>
<td>Potassium tellurite, 0.3 % solution</td>
<td>15 ml</td>
</tr>
<tr>
<td>L–Cystine</td>
<td>5 mg</td>
</tr>
<tr>
<td>Sheep blood</td>
<td>5 ml</td>
</tr>
</tbody>
</table>

**Media preparation**

1. Melt the sterile heart infusion agar solution.
2. Cool it to 45°C or 50°C. Carefully maintain this temperature while performing subsequent steps.
3. Aseptically add the sterile potassium tellurite solution (previously sterilized by autoclaving) and the sheep blood.
4. Mix thoroughly.
5. Add the cystine powder, mix well.
6. Pour the medium into sterile Petri dishes.
7. Rotate the flask frequently while pouring into plates because the cystine does not go entirely into solution.

**Quality control**

Control your media as specified in 6.2.

### 6.5 Pertussis-specific Culture Media

Special media are required for the isolation of *B. pertussis*. The classic media for this organism are Regan–Lowe and Bordet–Gengou agar.

**Note:** RL is also a transport medium, but the transportation one is a semi-solid agar and only has 50% of the agar amount that the culture one does (see 3.6).

**Regan–Lowe isolation medium**

Per 1L distilled water:

- Charcoal agar (Oxoid Cm 119) – 51g
- Horse blood defibrinated – 100ml
Media preparation

1. Suspend charcoal agar in distilled water, and bring to a boil to dissolve completely.
2. Sterilize by autoclaving at 121°C for 15 min.
3. Cool to 50°C, and add 100ml of defibrinated horse blood.
4. Mix well, and dispense into sterile Petri dishes.
5. For a more selective medium, add cephalaxin to a concentration of 40 µg/mL.

Bordet–Gengou agar

Per 1L distilled water, ready to use media:

- Melted potato – glycerol agar base – 4.5 g
- Protein peptone – 10 g
- Sodium chloride – 5.5g
- Agar – 16g

Final pH 6.7

Defibrinated sheep (or horse) blood – 15-20 % should be added to ready to use media. Methicillin – 2.5µg/mL, or cephalaxin 40 µg/mL should be added to some of the medium to inhibit contaminant gram - positive organisms that may be present in the specimen ). Media may be prepared with or without cephalaxin ( or methicillin ).

Media preparation

1. Bordet–Gengou sgar is ready to use media, except it needs to :
2. Heat ready to use BG media to + 45–50°C.
3. Add 15–20% sheep (or horse) blood, cephalaxin and methicillin.
4. Mix well, avoid formation of air bubbles and dispense into sterile Petri dishes.
   If there is a delay of more than 2 hours between collection and shipment, refrigerate specimens.
7. Reagents and Test Procedures

7.1 Reagent Ordering, Validation, and Importation Procedures

All reagents will be ordered by the microbiology laboratories.

7.2 Reagent Global Use, Quality Control, and Storage

Note: laboratories have to follow the national procedures for reagents ordering, as specified in 7.1.

Policy

Quality control (QC) will be performed on each new lot of reagents, received in, or made up in the microbiology department using suitable reference materials. This QC will be performed before any use of the above-mentioned reagents, and at regular intervals to ensure continued performance.

All internal quality control (IQC) controls on reagents will consist of testing known samples, strains, sera and slides, and must be tested in the same conditions as patient specimens.

No patient results will be reported unless specified QC controls are validated.

Definitions

- **Initial QC**: Quality control testing performed when reagents are first made up or first received in the laboratory. This control can also be made by the MoLHSA, and/or by the manufacturer (official certificate).
- **Interim QC**: Quality control testing performed at specified intervals (after initial QC) to ensure continued quality.

Process

- All reagents will be registered upon arrival, and all further QC activities will be documented in the appropriate section of the QC logbook.
- All reagents will be labeled with their date of reception and/or date of preparation.
- Further, the date of opening or reconstitution (if needed) will be also specified along with the initials of the manipulator.
- Follow directions for individual reagent as described in the Microbiology procedure manual for tests and reagents.
The initial QC has to be specified by one of the qualified body:
- Manufacturer (if the ability was given to them by the MoLHSA).
- MoLHSA, or one of its official designated bodies
- Laboratory end-user

QC will be monitored weekly by the QC responsible

The following list will define who performs initial and interim QC and when.

<table>
<thead>
<tr>
<th>QC material</th>
<th>Procedure</th>
</tr>
</thead>
</table>
| Reagents    | Prepared reagents – initial QC, see also 6-2 for culture media
|             | Commercial reagents – initial QC or designee upon receipt
|             | Interim QC will be performed daily, weekly, or monthly on all types of reagents, as determined by requirements. Expired reagents will be discarded. |

**Principle**

Reagents to be quality controlled are inoculated with stock organisms or known patient isolates which will produce a predetermined positive or negative reaction indicated by a specific color reaction or stain or agglutination reaction. If expected results are not achieved, the reagent is unable to be used until this problem is resolved.

**Clinical Significance**

Proper identification of bacteria from patient specimens is dependent upon characteristic reactions of selected reagents and tests. If the reagent does not produce the expected result, the patient results may be incorrect or delayed. Quality control is performed on each reagent to ensure reactions will be correct when used with patient organisms.

**Special note: use of outdated reagents**

Some laboratories regularly complain about costly reagents that often expire before being used, or after partial use. These reagents should normally be discarded. However, due to cost and logistic constrain, these reagents may be used under certain condition.

- The laboratory must have a written policy specifying which reagents can be used, in which circumstances.
- This only applies when those reagents (or kits) are expensive and/or hard to obtain.
- Special QC procedures have to be implemented: QC testing is done each time the reagent(s) or kit is used.
- The final registration in the logbook should clearly specify that the reagent used was expired.
- In case of any doubt, the final report will not be issued and the person who ordered the expired reagent to be used will be contacted directly.
7.3 General Procedure for Enzyme Immunoassay

Principle

The general principle of any enzyme immunoassay (EIA) used for serology is that purified virus antigen is attached to a solid surface (such as wells of a 96-well microplate). When a standard dilution (predetermined for optimal range) of patient serum is added, any virus-specific antibody binds to the antigen after any unbound serum is washed away, an antibody to human globulin is added to the wells; this antiglobulin has been previously conjugated with an enzyme and is called the conjugate. After further washes, a substrate for the enzyme is added, and the bound enzyme conjugate begins a hydrolytic reaction to produce a colored product. After a predetermined time, the reaction is stopped by addition of a strong acid or base. The intensity of the color generated is proportional to the amount of virus-specific antibody present in the patient’s serum and can be read spectrophotometrically on a microplate reader. Quantitative results are obtained by the use of internal standards and control sera; assays commercially available today usually provide software that calculates the curve from the absorbance values and analyses the data from patient sera. The assay is made specific for Ig G antibody by choosing the conjugate.

Note: An increase in the sensitivity of immunoassays can sometimes be achieved by use of indirect instead of direct assays. In the direct assay, the antibody when optimally labeled usually contains 2–3 enzyme molecules per antibody molecule. In the indirect test, which utilizes enzyme-labelled anti-immunoglobulin, approximately five anti-immunoglobulin molecules can combine with one antibody molecule, which results in a greater number of enzyme molecules in the overall reaction.

Reagents

Antigen-coated solid phase, conjugate, substrate, stop reagent, diluents, wash buffers, standards, and controls must be stored and prepared in accordance with the manufacturers’ instructions. Usually these reagents are provided in kit form, with instructions not to mix components from different kit lots. If a laboratory prepares its own reagents, it must be carefully evaluated with appropriate controls to verify that its reagents provide reproducible results when used together, and the protocol must be standardized. It is unadvisable for even an experienced user to combine homemade reagents and commercially supplied kits, because the latter frequently contain components that are proprietary and the composition is therefore not fully specified. Expiration dates must be strictly observed; therefore, it is important to consider both volume and the batch size required and compare this with kit size and availability of separate components (such as extra control serum) when selecting the kit.

Instruments

Typically required are a micromixer, washing system for plates or tubes, and spectrophotometric reader, usually with software for data analysis.

Procedure

Enzyme Immuno-Assay procedures vary in their details depending on the solid phase and format selected. Any detailed protocol is based on the manufacturer’s instructions and the needs of the laboratory performing the test. One advantage of EIA is that a variety of tests may be performed with
a standard protocol. In order to obtain consistent results, such protocols usually contain details of technique that are not difficult but that must be followed exactly. The following is general outline of the steps involved.

1. Prepare working dilution of reagents as required, and warm it to room temperature.
2. Assign numbers to specimens, controls, and worksheets.
3. Dispense diluent and predilute sera as required (typically a single dilution, such as 1:20, may be prepared in a microdilution plate). Mix adequately.
4. Number the solid phase (microplates, microwell strips, beads, tubes, etc.) and prewashed as directed by the manufacturer. Observe storage conditions carefully for remaining solid-phase components.
5. Add diluted sera, standards, and controls to solid phase (typically, use a multichannel micropipette).
6. Incubate and wash the solid phase as directed. Incubation periods for EIA currently available may be as short as 15 minutes, often at room temperature. Washing has always been one of the most critical steps in any EIA procedure and must be done consistently, with or without automated instrumentation.
7. Add conjugate to solid phase.
8. Incubate and wash as in step 6.
10. Incubate as directed for color development. Time and temperature for the enzymatic reaction must be observed.
11. Add stop reagent and mix.
12. Read colour spectrophotometrically within specified time (usually 1 hour).
13. Analyse data. Usually, data reduction is done automatically by instrumentation provided with the kit. While some assays permit visuals reading to determine a qualitative result, it is important to consider whether the patient population needs to be monitored over time for possible changes in antibody level. If so, select a method that provides quantitative results based on internal standards to permit some comparability between runs.

Calculations

The presence or absence of antigen (virus) is determined by comparing the absorbance value of the specimen with the cut-off value. The cut-off value is calculated from the negative and positive controls. Each kit contains the calculations procedure to be used.

Interpretation of results

a. Antibody status is determined with reference to a standard containing the minimal detectable level of antibody (the cut-off value). Intelligent definition of the cut-off value can adjust the sensitivity and specificity of an EIA to reflect the consequences of false-positive versus false-negative results. Usually and equivocal zone, such as within 20% of the cut-
of, is defined to acknowledge the existence of borderline sera that cannot be reproducibly characterized. Testing of a repeat sample sometimes clarifies these results.

b. Significant changes in antibody level must be determined with paired sera in the same run, although they may be suspected by monitoring a patient over time in different runs.

Note: Different commercial test-systems are available. Attached instruction should be used for each system.

### 7.4 Rapid Immunocomb Test

#### Analysis procedures

This is a test intended for the qualitative detection of IgM and IgG antibodies in human serum or plasma.

#### Principle

The immunocomb test is an indirect solid-phase enzyme immunoassay (EIA). The solid phase is a comb with 12 projections (‘teeth’). Each tooth is sensitized at two positions:

- **Upper spot** – human IgM (Internal control)
- **Lower spot** – inactivated rubella antigens.

The developing plate has 6 rows (A-F) of 12 wells, each row containing reagent solution ready to use at a different step in the assay. The test is performed stepwise, by moving the comb from row to row, with incubation at each step.

1. At the outset of the test, serum or plasma specimens are pretreated with anti-human IgG (stripping), in order to prevent interferences as a result of competition by anti IgG, and by rheumatoid factor.

2. Pretreated specimens are further incubated with the solution in the wells of row A of the developing plate.

3. The comb is than inserted in the wells of row A. Anti-IgM M, if present in the specimens, will specifically bind to the antigens on the lower spot on the teeth of the comb.

4. Unbound components are washed away in row B. In row C, anti-IgM captured on the lower spots of the teeth, and the human IgM on the upper spots (internal control), will react with alkaline phosphatase (AP)-labeled anti-human IgM antibodies.

5. In the next two rows, unbound components are removed by washing. In row F, the bound (AP) will react with chromogenic components.

6. The results are visible as gray-blue spots on the surface of the teeth of the comb.

The kit includes a Positive Control (anti-rubella IgM) and a Negative Control to be included in each assay run. Upon completion of the test, the tooth used with the **Positive Control** should **show 2 gray-blue spots**. The tooth used with the **Negative Control** should **show the upper spot and either no lower spot or a faint lower spot**. The upper spot should also appear on all other teeth, to confirm that the kit functions properly and that the test was performed correctly.
Contents

Combs

The kit contains 3 plastic combs. Each comb has 12 teeth, one tooth for each test. Each tooth is sensitized with two reactive areas.

1. **Upper spot** – human IgM, IgG (internal control)
2. **Lower spot** – inactivated antigens

Developing plates

The kit contains 3 developing plates covered by aluminum foil. Each developing plate contains all reagents needed for the test. The developing plate consists of 6 rows (A-F) of 12 wells each. The contents of each row are as follows:

- **Row A** – specimen diluent, containing goat antibodies to human IgG
- **Row B** – washing solution
- **Row C** – AP-labeled goat anti-human IgM antibodies
- **Row D** – washing solution
- **Row E** – washing solution
- **Row F** – chromogenic substrate solution containing 5 bromo-4-chloro-3-indolyl phosphate (BCIP) and nitro blue tetrazolium (NBT) Positive Control – 1 vial (red-colored cap) of 0.5 ml heat-inactivated human plasma, containing IgM, IgG antibodies to virus. Negative Control – 1 vial (green-colored cap) of 0.5 ml heat-inactivated human plasma. Negative for antibodies to virus.

- Stripping solution 1 vial (transparent cap) containing 4 ml diluted goat antibodies to human IgG.
- **Perforator** (for perforation of aluminum foil) covering the wells of the developing plate.

Procedure

Preparing the developing plate

- Bring all components, developing plates, combs, reagents and specimens to room temperature and perform the test at room temperature (22°–26°C).
- Incubate the developing plate in an incubator at 37°C for 45 minutes.
- Cover the worktable with absorbent tissue to be discarded as biohazardous waste at the end of the test.
- Mix the reagents by shaking the developing plate.

**Note:** Do not remove the foil cover of the developing plate. Break the foil cover by using the disposable tip of the pipette or the perforator only when instructed to do so by the test instructions.
Preparing the comb:

**Caution:** To ensure the proper functioning of the test, do not touch the teeth of the comb.

- Tear the aluminum pouch of the comb and developing plate or only a part.
- You may use the entire comb and developing plate or only a part.

To use part of a comb:

- Determine how many teeth you need for testing the specimen and controls. You need one tooth for each test. each tooth displays the code number ‘01’ of the kit, to enable identification of detached teeth.
- Bend and break the comb vertically or cut with scissors to detach the required number of teeth (no. of tests including 2 controls).
- Return the unused portion of the comb to the aluminum pouch (with desiccant bag).
  **Close pouch tightly**, e.g., with a paper clip, to maintain dryness. Store the comb in the original kit box at 2°–8°C for later use.

Test Instructions

**Pretreatment of Specimens and Controls**

1. For each specimen and control, dispense 100 µl of stripping solution into a microtube or microtitre well.
2. To each microtube or well, add 25 µl of a specimen, or of the Positive Control or Negative Control supplied with the kit. Mix by repeatedly refilling and ejecting the solution.
3. Set the timer and incubate for 10 minutes at room temperature (22°–26°C).

**Adding Pretreated Specimens to Developing Plate**

_**Note:** Perform **incubations at 37° C!** [Washings should be carried out at room temperature (22°–26°C)]._

4. Pipette 25 µl of a pretreated specimen. Perforate the foil cover of one well of row A of the developing plate with the pipette tip or perforator and dispense the specimen at the bottom of the well. Mix by repeatedly refilling and ejecting the well. Discard pipette tip.
5. Repeat step 4 for the other pretreated specimens and the two pretreated controls. Use a new well in row A and change pipette tip for each specimen or control.
6. Set the timer and incubate for 10 minutes at 37 C.

**Antigen – Antibody Reaction (Row A of the developing plate)**

7. a. Insert the comb (**printed side** facing you) into the wells of row A containing specimens and controls.
   **Mix:** Withdraw and insert the comb in the wells several times.
   b. Leave the comb in row A and incubate for 30 minutes at 37°C. Set the timer. Near the end of 30 minutes, perforate the foil of row B using the perforator. Do not open more wells than needed.
   c. At the end of 30 minutes, take the comb out of row A.
Absorb adhering liquid from the pointed tips of the teeth on clean absorbent paper. Do not touch the front surface of the teeth.

First Wash (Row B)

8. Insert the comb into wells of row B. Agitate: Vigorously withdraw and insert the comb in the wells for at least 10 seconds to achieve proper washing. Repeat agitation several times during the course of 2 minutes; meanwhile perforate the foil of row C. After 2 minutes, withdraw the comb and absorb adhering liquid as in step 7c.

Binding of Conjugate (Row C)

9. Insert the comb into the wells of row C. Mix as in step 7a. Incubate developing plate with comb for 20 minutes (set timer) at 37°C. Perforate the foil of row D. After 20 minutes, withdraw the comb and absorb adhering liquid.

Second Wash (Row D)

10. Insert the comb into the wells of row D. Repeatedly agitate for 2 minutes, as in step 8. Meanwhile, perforate the foil of row E. After 2 minutes, withdraw the comb and absorb adhering liquid.

Third Wash (Row E)

11. Insert the comb into the wells of row E. Repeatedly agitate for 2 minutes. Meanwhile, perforate the foil of row F. After 2 minutes, withdraw the comb and absorb adhering liquid.

Color Reaction (Row F)

12. Insert the comb again into row F. Mix. Incubate the developing plate with the comb for 10 minutes (set timer) at 37°C. After 10 minutes, withdraw the comb.

Stop Reaction (Row E)

13. Insert the comb again into row E. After 1 minute, withdraw the comb and allow it to dry in the air.

Waste disposal

Dispose of used developing plates, pipette tips, microtubes or wells, absorbent paper, and gloves as biohazardous waste.

Storing unused part of kit

Developing plate

If you have not used all the wells of the developing plate, you may store it for future use:

▲ Seal used wells with wide adhesive tape so that nothing can spill out of the wells, even if the developing plate is tipped over.

Other kit materials

▲ Return remaining developing plate(s), comb(s), perforator, controls, stripping solution, and instructions to the original kit box. Store at 2°–8°C.
Test Results

Validation

In order to confirm that the test functions properly and to demonstrate that the results are valid, the following four conditions must be fulfilled.

1. The Positive Control must produce two spots on the comb tooth.
2. The signal of the lower spot of the Positive Control should be at least 35 when measured using program 91 of the Combscan refractometer (approximately equal to the second color frame starting from the left, if assessed using a Combiscale).
3. The Negative Control must produce an upper spot (internal control). The lower spot will either not appear or appear faintly, without affecting the interpretation of the results.
4. Each specimen tested must produce an upper spot (internal control).

If any of the four conditions are not fulfilled, the results are invalid, and the specimens and controls should be retested.

Quantitative interpretation of results

Visual Interpretation

Compare the intensity of the lower spot of each specimen tooth with that of the lower spot of the Positive Control tooth.

- A spot with an intensity higher than or equal to that of the Positive Control indicates the presence of IgM or IgG antibodies to virus.
- A spot with an intensity slightly lower than that of the Positive Control should be considered an indeterminate result, and the specimen should be retested.
- A faint spot or no spot should be considered a negative result.

Instrumental reading

Combscan refractometer (see the Combscan User Manual for detailed instructions) enables rapid and objective measurement of color intensity of the spots on the Immunocomb. Read test results as relative absorbance using program # 92 of the Combscan, designating reading of the lower spot by entering ‘1’.

When the message ‘spots: 1,2,3’ appear on the screen:

- A specimen reading equal to or higher than 700 is considered positive for the presence of IgM antibodies to rubella.
- A specimen reading between 600 and 700 should be considered an indeterminate result for the presence of IgM antibodies to rubella and should be retested.
- A specimen reading lower than 600 indicates a negative result.
Documentation of results

As the color developed on the comb is stable, the comb may be stored for documentation.

Limitations

As with other tests intended for in vitro diagnostic use, the results of this test should be evaluated in relation to all symptoms, clinical history and other laboratory findings for the patient.
8. General Equipment Procedures

8.1 Distribution of Guidelines and Procedures

Once finalized, the guideline will be distributed to all laboratory technical staff. The practical implementation of this guideline will be implemented through training activities.

The responsible of each laboratory will be in charge of:

- Verification of procedure distribution
- Verification of procedure implementation
- Eventually propose to the guideline working group the main improvements to be included.

Staff in charge of cleaning, disinfections, and washing with be provided with biosafety and hygiene security procedures.

8.2 Update of QA Manual

A committee gathering experts will be in charge of updating this manual.

Initial author of this guideline should be included in this committee:

- Nino Macharashvili
- Giorgi Kurtsikashvili
- Lela Bakanidze
- Tamar Zardiashvili
- Tamar Ghaniaishvili

The following persons should also be represented:

- NCDC director
- NCDC deputy Director in charge of laboratories
- NCDC responsible of unit (biosafety, respiratory diseases, serology, enteric diseases, virology)
- One representative of Curatio International Foundation

This committee will have to hold regular meetings minimum twice a year, more if necessary.
Responsibilities of the group

- Gathering comments and improvement from laboratories
- Follow-up of the modifications
- Update of the guidelines
- Distribution list update
- Participation in the training and practical implementation of the guideline

9.1 General Consideration about Equipment

The laboratory should have the necessary equipment and instruments for the correct performance of the tests. New instruments and equipment should be installed and calibrated and have a written record of all actions performed on it. The system is established to ensure correct functioning and to maintain the service.

The laboratory should have several logbooks, one for each piece of equipment in the laboratory. This logbook should include:

- Name of the equipment
- Brand
- Inventory number
- Serial number
- Model and year
- Location
- Cost
- Date of purchase
- Date of first use
- Type of maintenance
- Regular preventive maintenance to be performed, and timing of this activity
- Calibration activities
- Record of preventive maintenance activities
- Record of repair activities
- Record of parts of the equipment that have been changed or repaired

A summary in Georgian language of the general user manual should also be available in order to allow any person to light on, calibrate, control, and use the equipment. In addition, the original operating manual of each part of the equipment should be available in the language on which it was issued.

If the equipment is not functioning anymore and cannot be repaired, it should be discarded following a specific procedure.
9.2 Links with Metrology

The calibration of equipment and other subjects related with metrology will be linked to the appropriate service approved by the Georgian official agencies. (currently this could be "Medservice").

9.3 Centrifuge Use, Control, and Maintenance

General

- Satisfactory mechanical performance is a prerequisite of microbiological safety in the use of laboratory centrifuges.
- Centrifuges should be operated according to the manufacturer’s instructions.
- Full user manual should be readily available. A Georgian-language summary of this manual must be available at bench level.
- Centrifuges should be placed at a level allowing all users to see into the bowl to place trunnions and buckets correctly.
- Centrifuge tubes and specimen containers for use in the centrifuge should be made of thick-walled glass or preferably of plastic and should be inspected for defects before use.
- When using angle head centrifuge rotors, care must be taken to ensure that the tube is not overloaded as it might leak.
- After use, buckets should be stored in an inverted position to drain the balancing fluid.
- Never try to open a centrifuge while it is running and/or stop the rotor in order to save some time. The centrifuge produces aerosols of potentially contaminated liquids that may injure hands and fingers.

Biosafety

- Tubes and specimen containers should always be securely capped (screw-capped if possible) for centrifugation.
- Buckets and trunnions should be paired by weight and, with tubes in place, correctly balanced. Distilled water or alcohol (propanol, 70%) should be used for balancing empty buckets. Saline or hypochlorite solutions should not be used, as they corrode metals.
- A sealed bucket is recommended to avoid manipulators contamination. If manipulating micro-organisms of Risk Groups 3 and 4, sealable centrifuge buckets must absolutely be used.
- If possible, the manipulation of tubes and buckets has to be done in a biological safety cabinet.
- Infectious airborne particles may be ejected when centrifuges are used. These particles travel at speeds too high to be retained by the eventual safety cabinet air flow, in the case that the centrifuge is placed in a traditional open-fronted Class I or Class II biological safety cabinet.
- Enclosing centrifuges in Class III safety cabinets prevents emitted aerosols from
dispersing widely. However, good centrifuge technique and securely capped tubes offer adequate protection against infectious aerosols and dispersed particles.

**Preventive maintenance**

- The interior of the centrifuge bowl should be inspected daily for staining or soiling at the level of the rotor. If staining or soiling are evident then the centrifugation protocols should be re-evaluated.
- Centrifuge rotors and buckets should be inspected daily for signs of corrosion and for hair-line cracks.
- Buckets, rotors, and centrifuge bowls should be decontaminated weekly or after any hazardous manipulation.
- Charbons have to be checked every 3 months, and the dust should be cleaned. They should be changed yearly.

**Breakage of tubes containing potentially hazardous material in centrifuges not having sealable buckets**

If a breakage occurs or is suspected while the machine is running, the motor should be switched off and the machine left closed for 30 minutes. If a breakage is discovered after the machine has stopped, the lid should be replaced immediately and left closed for 30 minutes. In both instances, the biosafety officer should be informed. Strong (e.g., thick rubber) gloves, covered if necessary with suitable disposable gloves, should be worn for all subsequent operations. Forceps, or cotton held in the forceps, should be used to retrieve glass debris. All broken tubes, glass fragments, buckets, trunnions, and the rotor should be placed in non-corrosive disinfectant known to be active against the organisms concerned and left for 24 hours and/or autoclaved. Unbroken, capped tubes may be placed in disinfectant in a separate container and recovered after 60 minutes.

The centrifuge bowl should be swabbed with the same disinfectant, at the appropriate dilution, left overnight, and then swabbed again, washed with water and dried. All materials used in the clean-up should be treated as infectious waste.

**Breakage of tubes inside sealable buckets (safety cups)**

All sealed centrifuge buckets should be loaded and unloaded in a biological safety cabinet. If a breakage is suspected, the cap should be opened and left loose and the bucket autoclaved.

**9.4 Microscope Use, Control, and Maintenance**

First, define who is in charge of each microscope

The person assigned to a bench is responsible for daily cleaning and inspection of the microscope on that bench.

Annually, microscopes are cleaned and inspected by an outside contractor, and repaired and calibrated as needed.
General

- Always keep microscopes covered when they are not in use.
- Do not leave oil magnification lens in the down position when not in use.
- Turn off light when not in use.
- Always cover lense plugs with the correct cap. If no cap is available, cover the plug with adhesive, in order to keep dust from accumulating.
- Don’t change lenses from one microscope to the other. They may not be compatible, even if they are from the same trademark.

Always have an extra bulb and extra fuse in order to be able to perform any change without any delay. To replace a light bulb, refer to the manual for that individual microscope for bulb specifications and directions.

Table 4 summarizes microscope maintenance.

Table 4. Microscope maintenance

<table>
<thead>
<tr>
<th>Frequency</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>After each use Clean objective lenses and eyepieces with lens cleaner and lens paper.</td>
</tr>
<tr>
<td>2</td>
<td>Daily Clean slide stage with alcohol to remove dust and oil. If a certain amount has gotten on stage, remove with a small drop of xylene. Be careful: xylene is carcinogen.</td>
</tr>
<tr>
<td>3</td>
<td>Monthly Check the Kohler centering of the lenses/diaphragm/condenser; if not correct, call the maintenance service.</td>
</tr>
<tr>
<td>4</td>
<td>Yearly General inspection and cleaning of the microscope</td>
</tr>
</tbody>
</table>
9.5 Incubator Use, Control, and Maintenance

General

▲ Full user manual should be readily available. A Georgian-language summary of this manual must be available at bench level.
▲ Always keep the incubators doors closed, even when a double door system is available.
▲ Incubator has to be filled with specific devices in order to hold tubes (racks) and Petri dishes (metal holders).
▲ The space between the trays should let a candle jar to be inserted.
▲ Each incubator has to be equipped with a thermometer.
▲ Incubator temperature is monitored twice a day, once in the morning (basal temperature) and once in the afternoon (working temperature), and temperature chart is filled in. This action is the main IQC activity for the incubator.

▲ Linkage with metrology unit (see 9.2) must be carried out, in order to check thermometer on a yearly basis.

Biosafety

▲ Incubator has to be cleaned inside and outside once a week and fully disinfected once a month. Usually, a specific anionic disinfectant is used (see 4.4 for more details).
▲ If leakage or breakage occurs inside the incubator, it should be cleaned immediately after the problem occurs.

9.6 Refrigerator and Freezer Use, Control, and Maintenance

General

▲ No food, drink, or other personal good should be placed in laboratory refrigerators or freezers.
▲ Refrigerator and freezer temperature is monitored twice a day, once in the morning (basal temperature) and once in the afternoon (working temperature), and temperature chart is filled in. This action is the main IQC activity for the incubator.
An inventory of the contents of the device must be maintained and printed. It is then posted to the door.

Flammable solutions must not be stored in a refrigerator unless they are explosion-proof. Notices to this effect should be placed on refrigerator doors.

**Maintenance of the cooling devices**

- Refrigerators, deep-freezers, and solid carbon dioxide (dry-ice) packs should be defrosted and cleaned periodically, and any ampoules, tubes, etc. that have broken during storage removed. Face protection and heavy-duty rubber gloves should be worn during cleaning. After cleaning, the inner surfaces of the cabinet should be disinfected.

- All containers stored in freezers and eventually in refrigerators should be clearly labeled with the scientific name of the contents, the date stored, and the name of the individual who stored them. Unlabelled and obsolete materials should be autoclaved and discarded.

<table>
<thead>
<tr>
<th>Refrigerators</th>
<th>Clean, defrost (if appropriate), and disinfect every 3 months, more often if needed, and check for expired reagents every 3 months. Monitor twice a day.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Freezers</td>
<td>Monitor twice a day. Remove excess frost at -70°C and clean out and organize at -70° and -20° every 6 months. Disinfect each time you defrost. Do not completely defrost at -70° more than 1 time/year.</td>
</tr>
</tbody>
</table>

### 9.7 Micropipette Use, Control, Calibration, and Maintenance

Different classes of micropipettes are available and vary by accuracy, reliability, precisions, and price. Table 5 shows differences and the frequency with which they should be checked/monitored.

**Table 5. Characteristics of micropipettes**

<table>
<thead>
<tr>
<th>Pipette volume µl</th>
<th>Class</th>
<th>Check freq. in month</th>
<th>Accuracy in µl</th>
<th>Reliability in %</th>
<th>Precision in µl</th>
</tr>
</thead>
<tbody>
<tr>
<td>1000</td>
<td>A</td>
<td>3</td>
<td>20</td>
<td>2</td>
<td>+/-20</td>
</tr>
<tr>
<td>1000</td>
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How to correctly use the micropipette

**Adjustment of the volume** (if not a fixed volume pipette)

**Adjustment of the tip**
- Quality of the tip (different quality are available)

**Aspiration**
- Pre-rinse the tip
- Press till the first step (A)
- Hold pipette vertically
- Immerse the end of the tip in the solution (2–4 mm for blue tips)
- Let gently and regularly the pump go back to initial position (B)
- Wait a second
- Gently absorb extra liquid outside the tip without touching the end

**Distribution**
- Hold pipette at a 10° to 40° angle to the tube
- Push the pump till the first step (C)
- Wait a second
- Push till the second step (D)
- Take the pipette back
- Let the pump go back (E)
- Eject tip
Control of the pipette

Water-tightness

△ Aspirate the maximum volume of water the pipette can hold
△ Hold the pipette vertically during one minute
△ No drop should appear

Accuracy and reliability

A 1/10 mg precise scale is needed.

Local conditions of atmospheric pressure, temperature, and hygrometry may affect accuracy.

To check accuracy:

1. Place a container on the scale
2. Set at the zero position of the scale
3. Aspirate the chosen volume (use volume, minimum volume, maximum volume) of distilled water
4. Dispense in the bin
5. Aspirate
6. Dispense in the container
7. Note the measure
8. Set at the zero position of the scale
9. Aspirate
10. Dispense in the container
11. Note the measure
12. … Repeat till you have 10 measures
13. Report all measures on the control form
14. Calculate
15. Conclude
- External cleaning of the tip-carrier
  - With a paper wet with water or alcohol
- Unscrew the tip-ejector and the tip-carrier (see picture)
- Clean all parts
- Clean and eventually change the pump
- Clean and eventually change the toric rubber

In any case, see also the user manual of the pipette.

9.8 Scale Use, Control, and Maintenance

Control operation should be performed by the metrology unit.

Manipulator has to refer to the user manual and to a summary written in Georgian language.

9.9 LISA Equipment Use, Control, and Maintenance

Use of ELISA equipment

See 7.5.
Control of ELISA chain

Two types of control are available:

1. Control of the equipment itself

This control is usually done by a specialist, with a contract or a subcontract planned by the laboratory. Metrology unit can also be involved (see 9.2). These operations can be summarized:

For the reader

- Control of the precision of the wavelength
- Control of the repeatability of the wavelength
- Control of the precision of the absorbance
- Control of the repeatability of the absorbance
- Control of the linearity of the response
- Control of the presence of parasitic light
- Control of the stabilization time
- Control of decreasing of the light with the time

For the washer

- Check the tubes (water tightness, formation of angles, porosity)
- Check the eventual precipitation of minerals in the circuit

For the incubator

- Check the temperature

2. Control of the biological performance

- Positive and negative control with each series
- External quality control (EQC) on a regular basis (at least twice a year)

Maintenance

- Daily rinsing of the washer (including tanks) with distilled water
- Change the tubes as specified by the manufacturer (usually yearly)
- Change the lamp of the reader when its performances are under the level

9.10 Autoclave and Oven Use, Control, and Maintenance

Refer to procedures that specify how to use and control these pieces of equipment, discussed above in:

- 4.1: Sterilization by the dry heat
- 4.2: Sterilization by the wet heat
In addition, for the autoclave, all rubber parts should be checked monthly, as they ensure to the water-tightness of the autoclave.

### 9.11 Water Distiller Use, Control, and Maintenance

#### Use of the water distiller equipment

Follow the user manual.

**Note:** a summary in the Georgian language should be available at bench level

#### Control of the obtained water

- **Physico-chemical control**
  - A pH-meter (2 decimals) and a conductimeter (units in mS/cm) should be available
  - Check pH, should be at 7.00 +/- 0.01
  - Check conductance, should be at +/- 0.001 mS/cm

  **Note:** before checking both, the final container and the container used for the measure should be rinsed with HCl 0.1 N, in order to decrease the amount of Ca++ and HCO3- ions, which may interfere with the measure. A final rinse with distilled water will be done before the measures

- **Sterilization control**
  - Pour 10 ml of the water into a nutritive broth, incubate 24 hours at 37°C, then subculture the mix on a normal agar, let stand 48 hours at 37°C.
  - No micro-organism should grow

- **Flow control**
  - Measure the volume of water produced in one hour and compare to the volume specified by the manufacturer
  - Conclude

#### Maintenance of water distiller

- Follow the instruction specified by the manufacturer.
- Check the quality of the rubber parts and change them if you detect water leaks.
- Rinse the distiller monthly with first HCl 0.1 N, and with pure distilled water
10. Internal Quality Control Procedures

10.1 IQC to be Performed

1. Quality control of staining methods
2. Quality control of media
3. Quality control of reagents
4. Centrifuge use, control, and maintenance
5. Microscope use, control, and maintenance
6. Incubator use, control, and maintenance
7. Refrigerator and freezer use, control, and maintenance
8. Micropipettes use, control, calibration, and maintenance
9. Scale use, control, and maintenance
10. ELISA chain use, control, and maintenance
11. Autoclave and oven use, control, and maintenance
12. Water distiller use, control, and maintenance

10.2 Archiving IQC Results

All IQC logbooks and protocols should be placed on the special folders or binders and archive every 5 years. All documentation should be labeled for the date of archiving.
10.3 Logbooks Used for IQC

Pipette control form

Trade mark:

Model:

Identification N°                Date:

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11. External Quality Control Procedures

11.1 Policies and Procedures for a National EQC Program

Responsibilities

According to the Law on “Health Care,” all medical and pharmaceutical facilities, including laboratories, are obliged to meet EQC requirements as defined by the legislation regulating medical and pharmaceutical activities.

Purpose

The purpose of the national EQC is:

▲ Identify and document capabilities of participant laboratories for standard diagnostic methods for targeted communicable diseases.
▲ Determine appropriate action to increase competency of testing laboratories.
▲ Assist in the establishment of a communications network among testing laboratories.
▲ Provide continuing education to testing laboratories on standard diagnostic methods for targeted communicable diseases.
▲ Strengthen vertical, pathogen-specific programs, including VPD programs, and their interaction.

Scope

▲ Participating bodies:
  ▲ Organizer – juridical entity that ensures operation of EQC system
  ▲ Reference laboratory/ies – expertise/technological basis for investigations
  ▲ Laboratories

EQC organizers usually are linked to the reference laboratories. These laboratories should be already have one or several EQC programmers participating in EQC before becoming an organizer.

▲ Survey:
  ▲ Surveys are organized by session, with each session containing at least 2 (optimally 3) surveys. The time between 2 surveys is maximum 60 days.
  ▲ At the beginning, one session per year will be organized.
Each survey is designed to assess a variety of pre- and post-analytic steps as well as the actual analytic technique, including:
- The procedures by which test items are obtained, processed, and stored.
- Communication mechanisms for sending results.

Organization

The responsibilities of the organizer include:
- Determining content of surveys and assignments
- Preparing test items, ensuring growth and other characteristics, and dispensing the specimens
- Pack specimens for shipment
- Prepare paperwork (report forms, summary reports, feedback questionnaires)
- Coordinate communication with participating laboratories
- Develop evaluation criteria for acceptable performance
- Analyze data, prepare summaries of results
- Maintain database of results of participating laboratories
- Provide feedback in case of poor performance
- Oversee the program

Collaboration

Reference laboratory collaborates with an international reference laboratory in order to get an international feedback on its program, and be helped in improving it. The Microbiological laboratory of the Croix-Rousse Hospital in Lyon, France, whose staff already collaborates with WHO/Lyon on a program involving Georgian microbiologists, or other internationally recognized laboratories seem to be the ideal partner for such a program.

The role of collaborating laboratories is:
- Review procedures
- Receive surveys and provide feedback
- Advise/consult, interpret performance of testing labs by reviewing NCDC evaluations of sample results
- Advise on assessing technical competence of testing labs
- Provide technical advice
- Develop monographs on specialized areas for use as educational material
- Participate in resolving disputes between organizing and testing laboratories, if necessary.
Information

All information will be sent to all laboratories in the Georgian language, except for the collaborating laboratories, to which it will be sent in English.

Initial information sent to laboratories

Introductory information is sent to the participating laboratories. The introductory information includes:

△ The purpose of the external quality assessment program
△ The need for regular participation
△ The methods and criteria that will be used in assessing the results of each survey
△ The standard operating procedures for performing the diagnostic tests
△ Contact information for reaching the organizer.

Information to accompany each shipment

△ A description of the test items
△ Recommended procedures to use for handling test items
△ A report form and how to record results
△ Where and how to return the report form, and the shipment closing date
△ Relevant information about any tests for which specimens have been screened, hazard warnings, storage conditions, reconstitution method, disposal recommendations, etc.

Packaging and shipping of the control material

Packaging and shipping all materials are done according to general procedures as outlined above (Chapter 3).

Results, evaluations, and reports

△ Results

Participating laboratories record the results of each survey on the results form included in the shipment.

The timeframe for results communication is as follows:

△ Participating laboratories are requested to send results to the organizers by email (preferably), mail, fax, or telephone, within 2 weeks of the receipt of the shipment.

△ Data entry and survey evaluation

The organizing laboratory records the results of each survey in an electronic database. The results are analyzed and evaluated according to a standard operating procedure.

△ Reports on survey evaluations
The organizers prepare reports of the evaluation data for each survey and distribute them as follows:

- Individualized evaluations of each survey are sent to each testing laboratory within 1 month of the closing date for results by fax or email. Hard copies of results are sent to each testing laboratory with the subsequent survey.

- A preliminary global commentary, containing statistics without identifiers, is sent to all participating laboratories within 1 month of the closing date for results by fax or email. A hard copy of the final global commentary is sent to each testing laboratory with the subsequent survey.

- Seasonal reports of survey evaluations

At the end of each season, all survey evaluation data are summarized to produce a summary report, without identifiers. This report is sent to participating laboratories.

- Report keeping

The survey results, data analysis, evaluations, and reports are kept by the organizing laboratory in an electronic file and are backed-up regularly. Previous reports are readily accessible by the organizers.

- Confidentiality, ethical issues, complaints

The confidentiality policy of the EQA Program states the following: “EQA results are available only to officials responsible for laboratories. No identifiable data are made available to managers of laboratories outside of their area of authority. All EQA results are highly confidential and may not be disclosed to a third party”.

**Recognition**

- Participation

At the end of each year of the program, the organizers will send a certificate of participation to laboratories that submitted results from at least 2 of the 3 surveys conducted during the previous season.

- Good performance

At the end of each year of the program, the organizer will send a formal certificate to laboratories that achieved “good performance” on at least 1 survey; good performance means meeting the following criteria:

- Having submitted results from at least 2 of the 3 surveys in one discipline
- Having submitted results of each survey within 2 weeks of its receipt in the laboratory.
- Having received a score of at least 3/4 in 75% of the grading areas in each survey

The certificate, for each laboratory and for each survey, will describe the criteria for, and recognize achievement of “good performance.” Certificates will be sent to the head of the participating laboratory.
11.2 VPD-specific Program

**Diphtheria**

The reference laboratory selected by the EQA Program should undertake international quality control on a bi- or tri-annual basis in order to be able to perform EQA for other labs.

**Pertussis**

No EQC is performed today for pertussis diagnosis in Georgia.

An international bi-annual subscription should be carried out as soon as possible.

**Serology**

In terms of VPD, serology concerns applies to:

- Hepatitis B
- Measles
- Rubella

All laboratories performing serologies for the purpose of surveillance should receive at least twice a year EQC material for each serology performed.

This EQC in serology could be organized under certain conditions:

- Policies and procedures should be fully and carefully respected (see 11.1).
- Non-infectious positive samples for the targeted disease have to be bought to international providers (manufacturer, QC organizers); the use of sera from infected patient is not recommended due to the risks of contamination.
- If reference laboratory unit in charge of these diseases is participating, the confidentiality and the separation between organizer and participant has to be very effective; a QC provider cannot be also a participant to its own QC program.

11.3 Corrective Actions to be Taken after Bad EQC Results

**Corrective plan of action**

After each survey, the organizer will send a letter/email to any laboratory that obtains a score less than 2 for any section of the survey (i.e., an unacceptable result). The communication will include:

- A description of the deficiency(ies) identified by the EQA results.
- A request for a Corrective Plan of Action related to the deficiencies identified.
- A standardized Corrective Plan of Action form for the laboratory to complete.
- A sample Corrective Plan of Action that addresses a relevant laboratory deficiency.
Response to request for assistance

In response to requests for technical advice from participating laboratories, the organizing laboratory will provide informal follow-up via telephone or email to individual laboratories. The organizer may call upon reference laboratories to assist with provision of procedural documents or literature reprints.

On-site visits

It is recommended that the organizer conduct site visits to laboratories whose EQA results show unacceptable performance. The purpose of the visit will be to identify problems that may not be apparent otherwise and guide in any corrective actions to be taken for improvement.

Documentation and evaluation of follow-up

A detailed file is maintained on each testing laboratory that includes criteria for follow-up, data collected on significant needs, follow-up methods implemented, corrective plans of action, and the survey evaluations after follow-up. The impact of the follow-up on improving performance on the EQA surveys is evaluated by comparing before and after results.
12. Specific VPDs

12.1 Pertussis

Causative organism

Pertussis is reemerging worldwide. A clinical diagnosis may be elusive, since the typical cough with whoop is frequently lacking. Instead, symptoms are often non-specific and virus-like, especially in older children and adults. In young children, the disease may be severe, with choking, apnea or cyanosis, and causes significant morbidity and mortality. Treatment in the early stages of the infection reduces the severity of the illness and further spread of the disease. Thus, an accurate early laboratory diagnosis remains a key tool in the control and prevention of this disease. A specimen should be taken 1-2 weeks after initiation of the cough and before antibiotic therapy. Pertussis is caused primarily by *B. pertussis*, occasionally by *Bordetella parapertussis*, and very rarely by *Bordetella bronchiseptica*.

Specimen

The preferred specimen is a nasopharyngeal swab. Cough plates and throat swabs by themselves are unsatisfactory. Aspirates of the nasopharynx or bronchus are ideal but are feasible only in a hospital setting.

Generally, two nasopharyngeal swab specimens are collected by passing the swabs through the nares as far as possible into the posterior pharynx. See Sections 2.5 for sampling procedures, 3.5 for transport media procedure, and 6.5 for culture media procedures.

Material needed

**Supplies**

- Sterile Petri dishes
- Sterile Pasteur pipettes
- Sterile test tubes
- Sterile swabs
- Inoculating loops and calibration loops

**Equipment**

- Biological safety cabinet
- Incubator (35° to 37°C)
Fluorescent microscope with filter system for fluorescein isothiocyanate and microscope slides

Gram stain reagents

See 5.1 and 5.4

Analysis procedures

One of the following primary isolation media may be selected: Bordet–Gengou, Regan–Lowe charcoal agar.

Culture procedures

Firmly roll the swab over one-third of the agar surface and streak carefully for isolation, because overcrowding of breakthrough organisms (namely, *S. aureus*) may allow inhibitory substances to suppress growth of the bordetellae.

Incubate at 35°C for 5–7 days. Normal air is preferable to CO₂ for incubation. Keeping plates moist during incubation is critical for this organism. Use a thick pour for the agar plates, and tape the plates to seal them, or incubate the plates in a plastic bag or moist chamber.

After 48 hours, inspect the culture daily with the aid of magnification. Colonial appearance is described below.

Results on BG agar

B. pertussis

Colonies are smooth, transparent, glistening, and convex with an entire edge; may have a tiny zone of hazy beta–hemolysis; often are not visible until after 3 or more days; and measure approximately 1 mm in diameter.

B. parapertussis

Colonies are similar to those of *B. pertussis* but are larger and duller, may be slightly brownish, and become visible sooner.

B. bronchiseptica

Tiny colonies with rough pitted surfaces like beaten metal may appear in 24 hours.

Results on R–L agar

B. pertussis

Colonies are convex, gray, smooth, and very shiny, like drops of mercury. Although tiny, they may be variable in size and may run together. They require 3 or more days to appear.

B. parapertussis

Colonies are similar to those of *B. pertussis* but grayer and less domed, and they grow more rapidly than *B. pertussis* colonies.

B. bronchiseptica

Colonies are easily visible in a day or two, vary little in size, and have a non-homogenous appearance and a putrid odor.
Pick typical colonies for Gram stain. Bordetellae are gram-negative coccobacilli, although they may become more elongated when grown on media containing cephalixin.

Serodiagnosis and other immunological tests

Non-cultural diagnosis of pertussis using direct fluorescent antibody testing.

**Note:** this is not a serodiagnosis (indirect) but an immunological direct diagnosis

Direct fluorescent–antibody testing of properly procured pharyngeal specimens for the presence of *B. pertussis* and *B. parapertussis* is an important tool in early diagnosis. It is too insensitive to be done instead of culture; however, it is an important adjunct to culture for this disease, since a positive result offers an early presumptive diagnosis.

**Technical procedure**

a. Use nasopharyngeal swabs to inoculate 0.5 ml of Casamino Acid solution, and make two smears from the solution. Stain one smear with *B. pertussis* fluorescent–antibody conjugate for 30 minutes, and stain the other with *B. parapertussis* fluorescent–antibody conjugate for 30 minutes.

b. Titrate antibody conjugates prior to use to determine the highest dilution that will still give a 4 + fluorescence when stock strains of organisms are used. Aliquots of diluted conjugate may be stored at minus 20°C for several months. The expiration date must appear on the label.

c. After staining the smears, wash them in phosphate-buffered saline (pH 7.2), air-dry them, and use a coverslip with fluorescent–antibody mounting fluid. Examine the smears with a fluorescence microscope.

d. Positive reactions are identified by intensity of fluorescence and characteristic plump coccobacillary morphology of organisms.

e. Some experience is required to grade the intensity of the fluorescence and to ignore the occasional non-specific staining of gram-negative diplococci, gram-positive cocci, and diphtheroid-like rods.
f. Each time tests are performed, stain a smear of each organism from stock strains with each conjugate as a QC check.

**Note:** Smears can be sent to NCDC in addition to the normal sample. They can be frozen or kept at 4°C.

**Further analysis to be sent out of the laboratory: Identification procedure**

If colony appearance on BG agar is similar as mentioned above send culture to NCDC for further biochemical and serological identification procedure.

See 3.1, 3.3, and 3.6.

**Reporting of the results, units of the results**

Notification and reporting requirement are in Section 13.

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### 12.2 Diphtheria

**Causative organism**

Diphtheria is an acute infectious disease primarily of the upper respiratory tract but occasionally of the skin. It is caused by the toxigenic strains of the Corynebacterium diphteriae, in which the presence of a grey pseudo-membrane covering the mucous membrane of oropharynx is an initial clue to diagnosis. The classic pseudo-membrane of the pharynx may be lacking in mild cases, which usually mimic streptococcal pharyngitis. There are four predominant biotypes of the Corynebacterium. diphteriae: mitis, intermedium, gravis and belfanti. A less severe diphtheria-like disease caused by non-toxigenic strains is also seen. Diphtheria exacts a high mortality rate, approximating 10%.

Laboratories must be prepared to handle emergency requests to recover and identify bacterial species from throat cultures for less commonly encountered diseases. Antibiotics have little or no effect on the clinical outcome, and laboratory confirmation of diphtheria requires several days, yet diagnosis and antibiotic treatment are essential in limiting further spread.

**Specimen**

Both throat and nasal, or nasopharyngeal specimens are necessary. Swabs should be taken from the nose, throat, membrane, and behind the membrane, if possible. If any membrane is present, swab from the edge of membrane One swab is needed from each anatomical site. Any purulent exudate should also be sampled. Swab should be taken before administration of antibiotics at the first contact with patients. Refer to 2.5 and 2.6.
Material needed

**Culture media:**
- See 6.4.
- Cystine tellurite blood agar (CTBA): a heart infusion agar supplemented with rabbit blood and L-cystine
- Blood agar plate (BAP)

**Supplies**
- Sterile Petri dishes
- Sterile Pasteur pipettes
- Sterile test tubes
- Sterile swabs
- Inoculating loops and calibration loops

**Equipment**
- Biological safety cabinet
- Incubator (35° to 37°C)
- Microscope and microscope slides.
- Gram stain reagents See 5.1 and 5.4.

Analysis procedures

**Culture procedure**
- Inoculate the specimen to CTBA and BAP, streak for isolation on an LAS agar slant, and leave the swab on the slant during incubation.
- Inoculate media at 35°C aerobically for 18-48 hours.
- Examine all agar plates at 24 and 48 hours for colonies typical of *C. diphteriae*. Then subculture colonies that are catalase positive (see 7.4) and exhibit typical morphology on gram stain to BAP to provide growth for identification procedures.

**Morphology of C. diphteriae**
- Cellular morphology
- Gram stain: pleomorphic gram-positive rods that occur in angular arrangements (commonly referred to as palisades or Chinese letters); possibly coccobacillary forms, most notably in older cultures.
- See a Gram stain picture of *C. diphteriae* in 5.4.
- Colonial morphology
- CTBA: grayish black (gunmetal grey) and 1–3 mm in size, with a garlic-like odor
- Biotype intermedius: smallest (0.5-1 mm in size), flat, round, “soft,” grayish colonies which do not mix.
Biotypes mitis: larger (1-2 mm in size), convex, smooth
Biotypes gravis: larger (1-2 mm in size), convex, wrinkled
BAP
Biotype intermedius: smallest, flat, creamy, transparent, no hemolysis
Biotypes gravis: larger, convex, weak beta–hemolysis

**Identification procedures**

- If in gram stain pleomorphic gram-positive rods in angular arrangements: pleomorphic beaded rods whose ends may be swollen, producing a club shape; angular arrangements
- Inoculate the culture on Amies or Stuart transport media and
- send it to NCDC respiratory laboratory for biochemical identification and toxigenicity testing. The isolate must be toxigenic to confirm a clinical diagnosis of diphtheria. See 3.1, 3.3, and 3.5 for transportation procedures.

**Reporting of the results**

The culture report should include one of the following:

- “Negative for *C. diphteriae*”, or
- “Presumptive *Corynebacterium diphteriae* isolated”

Notification and reporting requirement are in Section 13.

### 12.3 Tetanus

**Causative organism**

Tetanus is a disease of global incidence produced by the toxin of Clostridium tetani. The risk of acquiring the disease increases in people > 60 years of age and in neonates, especially when poor sanitary conditions predispose the neonates to umbilical stump contamination.

The pathogenesis of tetanus involves the absorption of performed toxin, or, less commonly, invasion of toxin-producing organisms from contaminated wounds; it may complicate surgical wounds colonized with *C. tetani*.

Incubation periods vary depending on the portal of entry. The toxin, named tetanospasmin, blocks the transmission of inhibitory neurons, which results in flexor and extensor muscle spasms that are triggered by sensory stimuli.

Diagnosis of tetanus is made clinically.
Laboratory confirmation:

None

12.4 Rubella

Causative organism

Rubella virus produces an acute febrile illness with a maculopapular rash (German measles) and may cause arthralgia/arthritis, lymphadenopathy, or conjunctivitis. Fifty percent of infected persons do not have symptoms. If a pregnant woman is infected, especially in the first trimester, infection of the fetus may occur; the resultant infection in the infant may be asymptomatic but may result in severe congenital abnormalities or death of the fetus. Diagnosis of rubella infections is almost always serologic.

**Congenital rubella syndrome**

Non-immune pregnant women infected with rubella are at risk for having a fetus with congenital rubella syndrome (CRS). The risk and severity of the defects are higher earlier in the pregnancy. Roughly 85% of fetuses infected in the first trimester will have some anomalies.

Hallmarks of CRS include sensorineural deafness, intrauterine growth retardation, cataracts, retinopathy, and cardiac defects. Some infections will result in spontaneous abortion. Many of these infants will grow to have mental retardation, developmental delay, and behavioral disorders. Some will also have late-onset endocrinopathies such as insulin-dependent diabetes mellitus.

**Direct diagnosis**

Virus can be recovered from pharynx 1 week to two weeks after rash onset. Specimen can be collected by a mucus extractor with a mucus trap of the type commonly used in infectious disease wards. The collected mucus is refrigerated and sent immediately to the reference laboratory for direct immuno-fluorescence or antigen detection by immunoassay.

Specimens should be obtained early in the course of illness, when virus titre is highest.

**Indirect diagnosis**

Sero logic diagnosis is generally based on demonstration of a significant increase in antibody titre in paired serum specimens. The first specimen should be taken as soon as possible after the onset the disease. The second specimen can be taken as early as 7 days but preferably 10–14 days after the first specimen.

A single serum specimen is sufficient for the demonstration of rubella virus-specific IgM antibodies, proving that it has been taken within 4–5 weeks of the onset of illness.

The immunity of an individual is monitored by an IgG ELISA testing. Increasing IgG titres are helpful in the determination of the causative agent. In the early stage of the disease, significant IgM titres may be monitored.

**Specimen**

- Routinely, serum or plasma specimens is used. See 2.3.
Initial diagnosis, IgM: Single specimen collected within 4–28 days following onset of symptoms.

Disease onset, IgG: Acute and convalescent samples. Collect early sample in the course of illness and convalescent sample 10–14 days later.

Material needed

- Rapid Immunocomb Test and ELISA Kit. See 7.4.

ELISA (Enzyme Linked Immunosorbent Assay) for rubella IgM and IgG

See ELISA for measles IgM and IgG.

12.5 Mumps

Mumps virus produces infectious parotidis, an illness with acute onset of unilateral or bilateral tender, self-limited swelling of the parotid or other salivary gland, lasting >2 days, and without other apparent cause. It frequently causes aseptic meningitis, which is usually mild, and may also affect pancreas and gonads. The introduction of effective vaccines has greatly reduced the incidence of mumps virus infection.

Laboratory confirmation is not required

Notification and reporting requirements are in Section 13.

12.6 Measles

Causative organism

Measles virus (MV) causes an acute, generalized infection that until recently has been one of the common viral diseases of childhood worldwide. Measles is rarely fatal, but mortality after MV infection can be as high as 20% in developing countries. Measles is traditionally a disease of early childhood, but a vaccination program may change the epidemiological pattern in the future.

Measles is spread from a MV-infected person to a susceptible person, mainly via aerosol. The prodromal symptoms of measles are similar to those of other upper respiratory tract infections: running nose, sneezing, cough, and fever. Redness of eyes and photophobia are also early symptoms. Koplik’s spots on the mouth epithelium, which usually appear after the prodromal symptoms, are the hallmark of clinical signs of measles. At the time of the viremia, a typical macular or maculopapular exanthema appears. High fever at the time of rash onset is characteristic.

Two types of diagnoses are available:

Direct diagnosis

- Virus can be recovered from nasopharyngeal secretion collected by a mucus extractor with a mucus trap of the type commonly used in infectious disease wards.
The collected mucus is refrigerated and sent immediately to the reference laboratory for direct immuno-fluorescence or antigen detection by immunoassay.

- Specimens should be obtained early in the course of illness, when virus titre is highest.

**Indirect diagnosis**

- Serologic diagnosis is generally based on demonstration of a significant increase in antibody titre in paired serum specimens. The first specimen should be taken as soon as possible after the onset the disease. The second specimen can be taken as early as 7 days but preferably 10–14 days after the first specimen.

- A single serum specimen is sufficient for the demonstration of MV-specific IgM antibodies, proving that it has been taken within 4–5 weeks of the onset of measles.

- The immunity of an individual is monitored by an IgG ELISA testing. Increasing IgG titres are helpful in the determination of the causative agent. In the early stage of the disease, significant IgM titres may be monitored.

**Specimen**

Routinely, serum or plasma specimen is used. See 2.3.

- **Initial diagnosis, IgM:** Single specimen collected > 3 days following onset of symptoms but can be collected up to 30 days after onset.

- **Disease onset, IgG:** Acute and convalescent samples. Collect early sample in the course of illness and convalescent sample 10–14 days later.

**Timing of single blood specimens for IgM serology**

**First blood sample**

- The correct timing of specimens with respect to the clinical signs is important for interpreting results and arriving at an accurate conclusion. IgM ELISA tests are most sensitive between day 4 and 28 after rash onset.

**Second blood sample**

- These may occasionally be required under the following circumstances: the first blood sample submitted for IgM was collected within 4 days of rash onset and is negative by ELISA.

The laboratory may request a second sample for repeat IgM testing given the probability of false negatives on early samples A second sample for IgM testing may be collected any time 4–28 days after rash onset. Collection of a second sample 10–20 days after the first will allow the laboratory to retest IgM and/or, if a quantitative method is available, test for an increase in IgG antibody level. But this is not recommended on a regular basis because additional information obtained will be limited.

- Rapid immunocomb test and general procedures for immunoferment assay (see Section 7).
Material needed

- ELISA kit
- ELISA washer or comparable washing devices;
- ELISA reader or spectrophotometer with 450 nm wavelength (450/470nm)
- Automatic micropipettes, as needed
- Pocket calculator with exponential and logarithmic functions is required for the quantitative evaluation of the test.

Analysis procedures

**Measles-specific IgM antibodies:**

Measles-specific IgM antibodies appear within the first few days of the rash and decline rapidly after one month. Their presence provides strong evidence of current or recent measles infection. IgM is also produced on primary vaccination, and, although it may decline more rapidly than IgM produced in response to the wild virus, vaccine and wild virus IgM cannot be distinguished by serological tests. A vaccination history is therefore essential for interpretation of test results. The following methods are commonly used to detect measles-specific IgM.

- IgM ELISA requires only one blood sample for case confirmation. The test can be done with minimal training and results may be available within 2–2.5 hours of starting the assay.

- IgM indirect ELISA also requires only one blood sample for case confirmation. The test can be done with minimal training and results can be available within 3–3.5 hours of starting the assay. Indirect ELISA assays are the most widely used. However, this type of assay requires a specific step to remove IgG antibodies. Problems with the incomplete removal of IgG can lead to inaccurate results.

Timing of single blood specimens for IgM serology

**First blood sample**

- The correct timing of specimens with respect to the clinical signs is important for interpreting results and arriving at an accurate conclusion. While IgM ELISA tests are most sensitive days 4–28 after rash onset.

**Second blood sample**

- These may occasionally be required under the following circumstances: the first blood sample submitted for IgM was collected within 4 days of rash onset and is negative by ELISA.

- The laboratory may request a second sample for repeat IgM testing given the probability of false negatives on early samples. A second sample for IgM testing may be collected any time 4–28 days after rash onset. Collection of a second sample 10–20 days after the first will allow the laboratory to retest IgM and/or, if a quantitative method is available, test for an increase in IgG antibody level. But this
is not recommended on a regular basis because additional information obtained will be limited.

Test principle of ELISA for measles IgM:

- **Test principle**
  - In the antibody-capture ELISA technique, IgM antibodies in the patient’s serum are bound to anti-human IgM antibody absorbed onto a solid phase. This step is non virus-specific.
  - The plate is then washed, removing other immuno-globulins and serum proteins.
  - Measles antigen is then added and allowed to bind to any measles-specific IgM present.
  - After washing, bound measles antigen is detected using anti-measles monoclonal antibody, following which a detector system with chromogenic substrate reveals the presence or absence of measles IgM in the test sample.

- **Test requirements**
  - This test is available commercially in kit form, such as the Chemicon Measles IgM EIA Procedure. The Chemicon kits contain:
    - Anti-human IgM coated microtitre plates
    - Positive and negative control sera
    - Measles recombinant nucleoprotein antigen and control antigen
    - Enzyme-conjugated anti-nucleoprotein and
    - Substrate, buffers, and diluents.

Indirect ELISA for measles IgM

- **Test principle**
  - In the indirect ELISA for IgM, a rheumatoid factor absorbent is used for the removal of IgG antibodies from test sera in a pre-treatment step.
  - The first step is the absorption of measles antigen onto the solid phase.
  - The patient’s serum is then added and any measles-specific antibody (IgM and non-removed IgG) binds to the antigen.
  - IgM antibody is detected either directly, by means of an enzyme-labeled anti-human IgM monoclonal antibody or indirectly by means of anti-human IgM monoclonal antibody plus enzyme-labeled anti-mouse antibody. A chromogenic substrate is added to reveal the presence of specific measles IgM in the test sample.

- **Test requirements**
  - Several commercial kits are available. Basically, assay kit contains:
    - Test plates coated with measles antigen and control antigen
    - Positive and negative control sera
    - Anti-human IgM conjugate, buffers, and diluents
    - The chromogenic detector reagents are separately supplied
An RF absorbent is supplied for the removal of IgG antibodies from test sera in a pre-treatment step.

**Indirect ELISA for measles IgG**

- **Test principle**
  - Measles antigen are absorbed onto a solid phase;
  - The patient’s serum is added and any measles-specific antibody binds to the antigen; measles-specific IgG is detected using anti-human IgG;
  - The reaction is revealed by a direct or indirect detector system using a chromogenic substrate.

**Interpreting laboratory results**

- Only patients who have a positive result with a validated IgM ELISA assay or an epidemiological link to such a case are considered to be laboratory confirmed measles cases.
- Patients with assay results obtained by other methods are considered as suspected pending final laboratory testing.
- If, for any reason, an approved IgM ELISA is not performed on samples positive by other methods, these cases, for surveillance purposes, are considered as “clinically confirmed” measles cases.

**Reporting of the results**

Notification and reporting requirement are in Section 13.

### 12.7 Hepatitis B

**Causative organism**

Viral hepatitis is primarily a disease of the liver that is caused by a number of well-characterized viruses, including hepatitis B virus. The clinical features of hepatitis B virus (HBV) are extremely variable. In most acutely infected patients, unapparent (subclinical) hepatitis develops without symptoms or jaundice. Other patients acquire symptoms without jaundice (anicteric hepatitis), while jaundice with symptoms (icteric hepatitis) occurs in approximately 25–35% of those infected with HBV. Symptoms may range from mild and transient to severe and prolonged. Patients may recover completely, be overwhelmed by fulminant hepatitis and die, or become chronically infected.

HBV is a DNA hepdnavirus. It produces acute and chronic hepatitis and is a etiologic agent of hepatocellular carcinoma. HBV has a long incubation period (45–120 days) and is transmitted primarily by parenteral means.

- **Acute:** An cute onset of symptoms and jaundice or elevated serum aminotransferase levels. Clinical signs and symptoms of acute HBV infection include anorexia, nausea, malaise, vomiting, jaundice, dark urine, clay-colored or light stools, and abdominal pain. Occasionally, extrahepatic manifestations occur and include skin rashes, arthralgia, and arthritis.
▲ Chronic: A person who is HBsAg-positive for 6 months or who is IgM anti-HBc-negative and HBsAg-positive.

▲ Non-specific diagnosis: nonspecific findings in blood are
  ▲ Elevations of hepatic enzymes, globulin, and prothrombin time
  ▲ Decrease of albumin and blood leukocytes
  ▲ Timing of appearance of hepatitis B antigen and antibodies is shown in Table 6.

Markers used for diagnosis

<table>
<thead>
<tr>
<th>Marker</th>
<th>Acronym</th>
<th>Use</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatitis B surface antigen</td>
<td>HBsAg</td>
<td>Detection of acutely or chronically infected persons; antigen used in hepatitis B vaccine</td>
</tr>
<tr>
<td>M class immunoglobulin antibody to hepatitis B core antigen</td>
<td>IgM Anti-HBc</td>
<td>Identification of acute or recent HBV infections (including those in HBsAg-negative persons during the &quot;window&quot; phase of infection)</td>
</tr>
<tr>
<td>Antibody to hepatitis B core antigen</td>
<td>Anti-HBc</td>
<td>Identification of persons with acute, resolved, or chronic HBV infection (not present after vaccination)</td>
</tr>
<tr>
<td>Antibody to Hepatitis B surface antigen</td>
<td>Anti-HbS</td>
<td>Identification of persons who have resolved infection with HBV; determination of immunity after immunization</td>
</tr>
<tr>
<td>Hepatitis B e antigen</td>
<td>HBeAg</td>
<td>Identification of infected persons at increased risk for transmitting HBV</td>
</tr>
<tr>
<td>Antibody to Hepatitis B e antigen</td>
<td>Anti-Hbe</td>
<td>Identification of infected person with lower risk for transmitting HBV</td>
</tr>
</tbody>
</table>

Figure 1. Timing of Markers

![Figure 1. Timing of Markers](image)
Sequence of appearance of viral antigens and antibodies in acute self-limiting cases of hepatitis B:

- HBsAg, hepatitis B surface antigen
- HbeAg, hepatitis Be antigen; anti-HBc
- antibody to hepatitis B core antigen, anti-Hbe
- antibody to HbeAg, anti-HbS
- antibody to HBsAg

Specimen collection

Serum or plasma specimen is used. Collect acute and convalescent samples. Collect acute early in course of illness and convalescent 10-14 days later (see 2.3). Each specimen should be handled with care and treated as is potentially infectious.

Material needed

Hexagon HBsAg and ELISA Kit (see analysis procedure)

Analysis procedures

Example of rapid test for HBsAg: Hexagon HBsAg Immuno-chromatographic Method for the Detection of for HBsAg in Human Serum or Plasma.

Principle

The Human Hexagon HbS Ag test is a 1-step test for the qualitative detection of Hepatitis B surface Antigen (HBsAg) and human serum or plasma. The test is based on an immunochromatographic method. HBsAg present in serum or plasma reacts with colloidal gold particles which have been coated with monoclonal antibodies (mouse) against HBsAg. The resulting immunocomplexes migrate along the membrane and are bound in the test zone by a second monoclonal anti-HBsAg antibody (mouse) which is fixed there in form of a horizontal line (test line). Excessive immunocomplex and/or unreacted colloidal gold particles migrate further and are bound in a second line by anti-mouse IgG G antibodies (rabbit), forming the control line. Two visible lines will appear in the presence of HBsAg in the sample. If no HBsAg is present in the sample, or the concentration of HBsAg is below 1 ng/ml, only the control line will appear. This second line serves as a control for proper handling and intact reagents. No visible lines in the reaction zone indicate either improper performance or deterioration of the reagents.

Contents

- 3x20 test strips in aluminum containers with stoppers including desiccant. Test strip layers comprise colloidal gold particles coated with monoclonal antibodies (mouse) against HBsAg. Immobilized monoclonal antibodies (mouse) against HBsAg and immobilized antibodies (rabbit) against mouse IgG.
Storage and stability: The Human Hexagon HBsAg test is stable up to the given expiry date when stored at 2-30°C in a dry place. The canisters should always be closed immediately after removal of the test strips.

Elevated temperature (> 30°C) over a prolonged period of time should be avoided.

**Procedure**

- Test strips and samples should be brought to room temperature prior to use.
- Remove the appropriate number of test strips from canister.
- Take care to touch the strips only at the upper end with the arrows pointing downwards. Close the canister immediately after removal of the test strips.
- Immerse the test strips with the arrows pointing downwards into a tube with 0.25–0.5 ml sample. The sample must not touch the blue foil of the test strips. Do not move the test strip while the chromatographic process is incomplete.
- Read the result 20 minutes after immersion into the sample.

**Notes:**

1. Though a positive result (2 lines) typically appears within 5 minutes, a negative result may be confirmed only after 20 minutes.
2. Borderline samples (1-2 ng/ml) may show a positive signal only after 30 minutes incubation.
3. The prozone phenomenon, a false negative result obtained with extremely high levels, has been checked and can be excluded up to 5,000,000 ng/ml. Such concentration has never been reported with native serum or plasma samples.

**Interpretation of results**

- **Negative**
  - A negative result is indicated by a signal colored line at the upper end of the reaction zone (< 1 ng/ml HBsAg).
  - This control always appears if the test is carried out correctly and the reagents are active. Even if the control line appears weak or faint, the test has to be considered valid. Absence of this line indicates either improper performance of the test or deterioration of reagents. In this case the test should be repeated using fresh test strips.

- **Positive**
  - Two colored lines in the reaction zone indicate the presence of HBsAg (> 1 ng/ml). The result must be rated as positive, even if only a weak line appears at the lower end of the reaction zone.

**Standardization**

The test was standardized against a reference preparation of the Paul Ehrlich Institute, the German federal health authority. One unit (U) corresponds to 1ng HBsAg protein as it can be found in untreated serum or plasma specimen.
Sensitivity and specificity

The test detects HBsAg concentrations as low as 1 ng/ml. Based on elevation of 540 native serum samples, the test yielded a specificity better than 99.7% and a sensitivity of 100%. Even so a negative result does not necessarily exclude a Hepatitis B infection, as the HBsAg concentration may be below 1 ng/ml initially. Positive results should, as principally claimed, be confirmed by an independent method. The final diagnosis should generally not be based on a single test result, rather the patient’s history together with typical clinical signs and symptoms should be taken into consideration.

Analysis procedure: example of enzyme immunoassay for Hepatitis B

- **Hepatitis B Surface Antigen**
  - **Principle**
  - The detection of HBsAg in human serum or plasma by EIA can provide a rapid and sensitive approach for supporting the diagnosis of acute or chronic HBV infection. See 7.5 for the general procedure about immunoassay.

**Reagents**

- Positive control (human plasma containing human HbsAg)
- Negative control {recalculated human plasma anti-HbS (-) and HbsAg(-)}
- Anti-HbS–HRP
- OPD tablets
- OPD diluent (citrate phosphate buffer containing 0.02 % H2O2)
- 1N sulfuric acid

**Supplies**

- Pipettes and plastic tips with delivery volumes of 50, 200, 300, and 1,000µl
- Disposable gloves
- Cover seals
- Plastic trays (20 or 60 wells)
- Clean, dark, capped bottle or dispenser

**Equipment**

- 40°C water bath or incubator
- Washer
- Spectrophotometer
- Vortex mixer

**Before testing**

1. Remove the test kit from the refrigerator, and allow it to reach room temperature (approximately 20 minutes).
2. Prepare a worksheet, and indicate the following information:
Test date and start and completion times
Kit lot number and expiration date
Patient name
Patient identification number
Tray location number
Technologist’s initials
Place powder-free disposable gloves on both hands.

Testing

Note: There are several variations of this test depending on commercial test system, though the principle of the test is the same. All procedures below are given in the classic way of describing of testing procedures, so give this as appendix instead of core part of the procedure

1. Leaving 1 well blank, dispense 200 µl of each control (negative and positive) or patient serum into predesignated wells of a 20- or 60-well tray (depending on number of samples tested). Dispense this volume at the bottom of the well. Avoid splashing, and visually ensure that the entire 200 µl volume was dispensed. Also avoid coating destruction with the pipette.

2. Using a pipette with the appropriate secured tip, dispense 50 µl of conjugate into each control or patient well. Tap the plate tightly, and avoid splashing.

3. Seal the plate with an adhesive paper cover by gently applying the cover to the plates. Do not press the cover too securely, or it will be hard to remove. Incubate the plates at 38°C for appropriate time (according to the time indicated in the diagnostic test procedure).

4. Retrieve the tray from the water bath (or incubator), and carefully remove the adhesive cover and discard it in a biohazard waste container. Remember, the cover is sticky, and adherence to gloves and loss of samples are not uncommon.

5. Wash the wells five times with wash buffer.

6. Gently turn over the plate to remove liquid by gently pressing it on the blotting paper.

7. Using a pipette with the appropriate secured tip, dispense 200 µl of conjugate into each control or patient well. Tap the plate tightly, and avoid splashing.

8. Seal the plate with an adhesive paper cover by gently applying the cover to the plates. Do not press the cover too securely, or it will be hard to remove. Incubate the plates at 38°C for appropriate time (according to time indicated in the diagnostic test procedure).

9. Retrieve the tray from the water bath (or incubator). Carefully remove the adhesive cover and discard it in a biohazard waste container. Remember, the cover is sticky, and adherence to gloves and loss of samples are not uncommon.

10. Approximately 10 minutes before proceeding with the test, prepare the OPD solution by adding 1 OPD tablet per 5 ml of diluent to a clean dark bottle or dispenser. Use the appropriate number of tablets and volume of diluent. The OPD solution is stable for only 60 minutes at room temperature.

11. Wash the wells five times with wash buffer.
12. Gently turn over the plate to remove liquid by gently pressing it on the blotting paper.

13. Use a pipette dispenser to dispense 300µl of OPD solution into each well. Gently tap the plate to remove any bubbles, and cover it. Incubate the covered plate for 30 minutes at room temperature.

14. Use a 1,000-µl pipette with the appropriate tip or a dispenser to stop the reaction by adding 1.0ml of 1N H₂SO₄ to each reaction well. Gently tap the wells to mix the contents, and read at 492 nm with a spectrophotometer.

Calculations

- Calculate cut-off (CO) according to the commercial test system procedure.
- The other hepatitis B markers follow the same type of analysis procedures.

Interpretation of the results

Table 7. Interpretation of hepatitis B serological tests

<table>
<thead>
<tr>
<th>Tests</th>
<th>Results</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>HbsAg</td>
<td>Negative</td>
<td>Susceptible (Never infected or immunized)</td>
</tr>
<tr>
<td>Anti-Hbc</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>Anti-HbS</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>HbsAg</td>
<td>Negative or Positive</td>
<td>Immune</td>
</tr>
<tr>
<td>Anti-Hbc</td>
<td>Positive</td>
<td></td>
</tr>
<tr>
<td>Anti-HbS</td>
<td>Positive</td>
<td></td>
</tr>
<tr>
<td>HbsAg</td>
<td>Positive</td>
<td>Actively infected</td>
</tr>
<tr>
<td>Anti-Hbc</td>
<td>Positive</td>
<td></td>
</tr>
<tr>
<td>IgM anti-Hbc</td>
<td>Positive</td>
<td></td>
</tr>
<tr>
<td>Anti-HbS</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>HbsAg</td>
<td>Positive</td>
<td>Chronically infected</td>
</tr>
<tr>
<td>Anti-Hbc</td>
<td>Positive</td>
<td></td>
</tr>
<tr>
<td>IgM anti-Hbc</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>Anti-HbS</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>HbsAg</td>
<td>Negative</td>
<td>Four interpretations possible</td>
</tr>
<tr>
<td>Anti-Hbc</td>
<td>Positive</td>
<td></td>
</tr>
<tr>
<td>Anti-HbS</td>
<td>Negative</td>
<td></td>
</tr>
</tbody>
</table>

May be recovering from acute HbV infection
May be distantly immune and test not sensitive enough to detect very low level of anti-HbS in serum
May be susceptible with a false positive anti-Hbc
May be undetectable level of HbsAg present in the serum and the person is actually a carrier

Source: Centers for Disease Control and Prevention

Reporting

Notification and reporting requirement are in Section 13.
12.8 Poliomyelitis

Causative organism

Acute onset of a flaccid paralysis of one or more limbs with decreased or absent tendon reflexes in the affected limbs, without other apparent cause, and without sensory or cognitive loss (as reported by a physician). Humans are the sole reservoir for poliovirus. Wild polioviruses are spread directly or indirectly from person to person. Virus dissemination is facilitated by poor sanitation. In all countries, children under 2 years of age create a microenvironment of less than optimal hygiene within the family and within daycare settings, readily facilitating fecal-oral and oral-oral (mouth-fingers-mouth) transmission. Feces can serve as a source of contamination of water, milk, or food, and houseflies can passively transfer poliovirus from feces to food.

Laboratory confirmation

- Specimen (see 2.4).
- Ship the parcel to NCDC enteric viruses department
- **Note:** Ordering, quality control, management and pre-positioning at peripheral level of the VTM are done by the WHO polio eradication program.
13. Reporting and Notification

13.1 Urgent Notification

“Urgent notification” implies urgent (during the same business day, but under no circumstances more than 24 hours from first identification) submission of information about probable (clinical) or laboratory-revealed cases to the next superior level of the public health service. In such cases, the provider must notify the rayon centers for public health (CPH) about such case with any available means of communication (notification card, phone, fax, e-mail). The rayon CPH in its turn must submit the appropriate information to the central (NCDC, MoLHSA) and regional (regional CPH) institutions.

Every single case of the diseases and conditions listed in Table 8 must be notified as shown in the table:

Table 8. List of urgently notifiable diseases

<table>
<thead>
<tr>
<th></th>
<th>Name</th>
<th>ICD-10 Code</th>
<th>Notification should be sent to the rayon CPH</th>
<th>Notification should be sent from the rayon CPH to the regional CPH and NCDC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>By health care providers</td>
<td>By laboratories</td>
</tr>
<tr>
<td>1.</td>
<td>Diphtheria</td>
<td>A36</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>2.</td>
<td>Pertussis</td>
<td>A37</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>3.</td>
<td>Neonatal tetanus</td>
<td>A33</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>4.</td>
<td>Tetanus</td>
<td>A34 -35</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>5.</td>
<td>AFP / Acute poliomyelitis</td>
<td>A80</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>6.</td>
<td>Measles</td>
<td>B05</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>7.</td>
<td>Rubella</td>
<td>B06</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>8.</td>
<td>Congenital rubella syndrome</td>
<td>P.35.0</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>9.</td>
<td>Mumps</td>
<td>B26</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>10.</td>
<td>Acute viral hepatitis A</td>
<td>B15</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>11.</td>
<td>Acute viral hepatitis B</td>
<td>B16</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>12.</td>
<td>Acute viral hepatitis C</td>
<td>B17.1</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>13.</td>
<td>Acute viral hepatitis E</td>
<td>B17.2</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>14.</td>
<td>Cholera</td>
<td>A00</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>15.</td>
<td>Typhoid fever</td>
<td>A01</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>16.</td>
<td>Paratyphoid fevers A, B, C;</td>
<td>A01.1-4</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>17.</td>
<td>Other salmonella infections</td>
<td>A02</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>18.</td>
<td>Shigellosis</td>
<td>A03</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td></td>
<td>Name</td>
<td>ICD-10 Code</td>
<td>Notification should be sent to the rayon CPH</td>
<td>Notification should be sent from the rayon CPH to the regional CPH and NCDC</td>
</tr>
<tr>
<td>---</td>
<td>----------------------------------------------------------------------</td>
<td>-------------</td>
<td>---------------------------------------------</td>
<td>--------------------------------------------------------------------------</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>By health care providers</td>
<td>By laboratories</td>
</tr>
<tr>
<td>19</td>
<td>Other bacterial intestinal infections among them:</td>
<td>A04</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>20</td>
<td>Esherichiosis</td>
<td>A04.4</td>
<td>X</td>
<td>X</td>
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<tr>
<td>21</td>
<td>Yersinia</td>
<td>A04.6</td>
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<td>X</td>
</tr>
<tr>
<td>22</td>
<td>Food-borne bacterial intoxications among them:</td>
<td>A05</td>
<td>X</td>
<td>X*</td>
</tr>
<tr>
<td>23</td>
<td>Botulism</td>
<td>A05.1</td>
<td>X</td>
<td>X X X</td>
</tr>
<tr>
<td>24</td>
<td>Unspecified infectious diarrheal diseases</td>
<td>A09</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>Tuberculosis meningitis</td>
<td>A17.0</td>
<td>X</td>
<td>X X X</td>
</tr>
<tr>
<td>26</td>
<td>Plague</td>
<td>A20</td>
<td>X</td>
<td>X X X</td>
</tr>
<tr>
<td>27</td>
<td>Tularemia</td>
<td>A21</td>
<td>X</td>
<td>X X X</td>
</tr>
<tr>
<td>28</td>
<td>Anthrax</td>
<td>A22</td>
<td>X</td>
<td>X X X</td>
</tr>
<tr>
<td>29</td>
<td>Brucellosis</td>
<td>A23</td>
<td>X</td>
<td>X X X</td>
</tr>
<tr>
<td>30</td>
<td>Leptospirosis</td>
<td>A27</td>
<td>X</td>
<td>X X X</td>
</tr>
<tr>
<td>31</td>
<td>Listeriosis</td>
<td>A32</td>
<td>X</td>
<td>X X X</td>
</tr>
<tr>
<td>32</td>
<td>Meningococcal infection</td>
<td>A39</td>
<td>X</td>
<td>X X X</td>
</tr>
<tr>
<td>33</td>
<td>Relapsing fever</td>
<td>A68</td>
<td>X</td>
<td>X X X</td>
</tr>
<tr>
<td>34</td>
<td>Lyme disease (Boreliosis)</td>
<td>A69.2</td>
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<td>X X X</td>
</tr>
<tr>
<td>35</td>
<td>Flea-borne typhus</td>
<td>A75</td>
<td>X</td>
<td>X X X</td>
</tr>
<tr>
<td>36</td>
<td>Q fever</td>
<td>A78</td>
<td>X</td>
<td>X X X</td>
</tr>
<tr>
<td>37</td>
<td>Rabies</td>
<td>A82</td>
<td>X</td>
<td>X X X</td>
</tr>
<tr>
<td>38</td>
<td>Unconfirmed viral infections of CNS</td>
<td>A89</td>
<td>X</td>
<td>X X X</td>
</tr>
<tr>
<td>39</td>
<td>Arthropod-transmitted viral fevers and viral hemorrhagic fevers</td>
<td>A90-A99</td>
<td>X</td>
<td>X X X</td>
</tr>
<tr>
<td>40</td>
<td>Yellow fever</td>
<td>A95</td>
<td>X</td>
<td>X X X</td>
</tr>
<tr>
<td>41</td>
<td>Malaria</td>
<td>B50-54</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>42</td>
<td>Trichinosis</td>
<td>B75</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>43</td>
<td>Haemophilus influenza B pneumonia or meningitis</td>
<td>J14; G00.0</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>44</td>
<td>Hospitalized cases of influenza-like illness</td>
<td>J- 06.9; 22; 10; 10.1; 11; 11.1; 12; 12.1; 12.2; 12.8; 18.</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>45</td>
<td>Fever of unknown etiology (&gt;38 and lasts more than 5 days)</td>
<td>R - 50.0; 50.1; 50.9.</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>46</td>
<td>Radiological lesions</td>
<td>W88; 91</td>
<td>X</td>
<td>X X X</td>
</tr>
<tr>
<td>47</td>
<td>Acute occupational poisonings</td>
<td>Z 57.4 -57.5</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Number</td>
<td>Name</td>
<td>ICD-10 Code</td>
<td>Notification should be sent to the rayon CPH</td>
<td>Notification should be sent from the rayon CPH to the regional CPH and NCDC</td>
</tr>
<tr>
<td>--------</td>
<td>----------------------------------------------------------------------</td>
<td>-------------------</td>
<td>-----------------------------------------------</td>
<td>--------------------------------------------------------------------------</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>By health care providers</td>
<td>By laboratories</td>
</tr>
<tr>
<td>48.</td>
<td>Post-vaccination, unusual reactions and complications</td>
<td>Y58–59; 64.1</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>49.</td>
<td>Intrahospital infections</td>
<td>Y95</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>50.</td>
<td>Isolations of vancomycin-resistant staphylococcus</td>
<td></td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>51.</td>
<td>Severe acute respiratory syndrome</td>
<td></td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>52.</td>
<td>Fatal cases of acute infectious diseases</td>
<td></td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>53.</td>
<td>Contact with animals (risk of rabies)</td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>54.</td>
<td>Group cases of infectious diseases</td>
<td></td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>

* Indicating all identified pathogens

Laboratories detecting/conforming a case of a notifiable disease from the list above must urgently notify (during the same business day, but under no circumstances more than 24 hours from identification) the local CPH via any available means of communication.

Notifications should be made by a person responsible for the test result using the standard “Laboratory Confirmation of a Communicable Disease Result/Notification Form.”

In case of a negative result of a test, there is no need to send notification to the CPH. In such case, a response (the same form) should be only sent to the physician requesting the test.

Submission of only one notification to the CPH is required even though during one episode of illness more than one specimen of similar type may be taken from the patient.
<table>
<thead>
<tr>
<th>CONFIDENTIAL</th>
<th>LABORATORY RESULT/NOTIFICATION of a COMMUNICABLE DISEASE FORM #58/2</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Case:</strong></td>
<td>Last Name I First Name I Middle Name I Age I Sex I ID #</td>
</tr>
<tr>
<td>Address [Apt #: Street; City (village); Country]</td>
<td>Tel: I Date specimen was taken [dd / mm / yy] -------/ -------/------</td>
</tr>
<tr>
<td>Referred by</td>
<td>Contact information of the referring physician or institution:</td>
</tr>
<tr>
<td>O Self-referral</td>
<td>Name________________________________________</td>
</tr>
<tr>
<td>O Physician</td>
<td>Address______________________________________</td>
</tr>
<tr>
<td>O Health facility/CPH</td>
<td>Telephone_________________________________</td>
</tr>
<tr>
<td>O Laboratory</td>
<td>Fax_________________________________________</td>
</tr>
<tr>
<td>O Other__________</td>
<td>E-mail_______________________________________</td>
</tr>
<tr>
<td><strong>Result (outcome) (indicate if pathogen is isolated)</strong></td>
<td></td>
</tr>
<tr>
<td>Type of specimen</td>
<td>Type of test performed</td>
</tr>
<tr>
<td>O Blood</td>
<td>O Culture</td>
</tr>
<tr>
<td>O CSF</td>
<td>O Bacteriology</td>
</tr>
<tr>
<td>O Stool</td>
<td>O Virology</td>
</tr>
<tr>
<td>O Urine</td>
<td>O Parasitology</td>
</tr>
<tr>
<td>O Sputum</td>
<td>O Other</td>
</tr>
<tr>
<td>Other__________</td>
<td>O Serology (specify)</td>
</tr>
<tr>
<td>Other__________</td>
<td>O Immunology (specify)</td>
</tr>
<tr>
<td>Other__________</td>
<td>O Microscopy</td>
</tr>
<tr>
<td>Other__________</td>
<td>O Histology</td>
</tr>
<tr>
<td>Other__________</td>
<td>O Molecular identification</td>
</tr>
<tr>
<td>Date and time of the result:</td>
<td>Date and time of notifying CPH:</td>
</tr>
<tr>
<td></td>
<td>Name and address of laboratory</td>
</tr>
<tr>
<td>Notification sent by [name and signature] :</td>
<td>Name and signature of the person responsible for the result:</td>
</tr>
<tr>
<td>Mode of notification:</td>
<td>Notification recipient:</td>
</tr>
<tr>
<td>O Telephone</td>
<td>[name and position]</td>
</tr>
<tr>
<td>O Fax</td>
<td></td>
</tr>
<tr>
<td>O Courier</td>
<td></td>
</tr>
</tbody>
</table>

Chiefs/managers of private sector facilities and laboratories involved in diagnosis and treatment of infectious diseases are responsible for ensuring that their staff is aware and comply with the above case notification requirements.

Confidentiality of all laboratory notifications is regulated by “Law on Health Care”.

Below is a Universal Investigation Form, which should be attached to the specimen and specimen label.
**UNIVERSAL LABORATORY INVESTIGATION REQUEST FORM**

<table>
<thead>
<tr>
<th>Date  /  /</th>
<th>Patient Name</th>
<th>Ref. Number</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
</table>

Address: ________________________________________________________________________________

Age ____ years or Date of birth  /  /  (for children under 5)

Preliminary clinical diagnosis __________________________ (if specimen is taken from a contact, state so here)

Date of disease (rash in case of measles) onset  /  /  

Date of the last dose of vaccine specific for the disease in question  /  /  (for VPDs only)

Type of specimen (e.g., feces, blood) _______ Date collected  /  /  

Date shipped  /  /  

Name of the person to whom laboratory test results should be sent ____________________________

Facility name and address ___________________ ___________________ Tel. ______ Fax ________

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This part should be filled by the Laboratory

Date specimen received by the laboratory  /  /  / 

Name of the person who received specimen __________________________

Is the specimen in good condition Yes No

**ADHESIVE SPECIMEN LABEL**

Patent name __________________________ Identification number ______

Specimen collected on  /  /  (date) at _______ (time)