WORLD HEALTH ORGANIZATION ORGANISATION MONDIALE DE LA SANTE

ENGLISH ONLY

THE DEVELOPMENT OF SMALLPOX LABORATORY DIAGNOSTIC SERVICES

CONCLUSIONS AND RECOMMENDATIONS

1 Introduction

Since the clinical diagnosis of smallpox can be readily made in 80-90% of smallpox cases, surveillance activities in highly endemic areas require minimal or no laboratory services. However, as eradication activities progress and there is a reduction in smallpox incidence in the country as a whole and in different areas of the country, the laboratory diagnosis of suspected cases becomes increasingly important. There is a need for these diagnostic tests to be performed quickly and reliably for in many instances, they may serve to indicate the need for more extensive containment measures than have been immediately applied. Laboratory services, therefore, must be established within reasonable proximity to the areas in question. However, the development of a great many laboratories is neither feasible economically nor workable in practice. Experience has clearly shown that unless a laboratory is regularly performing certain specific tests, its results are highly unreliable. A balance must be struck between the demands of **accessability** and the practicalities of cost and consistency of results.

Today, there are comparatively few laboratories in the endemic countries which are capable of performing diagnostic studies on smallpox specimens. In fact, in many countries there are few microbiological laboratories at all and many are severely limited in terms of competent staff.

Bearing in mind these various considerations, plans have been formulated for the development of a network of diagnostic laboratories within the present limitations of personnel and budget.

As an important cautionary note, it was recognized by the Regional Advisers that the presence of a diagnostic laboratory may induce some not to report cases until confirmed by the laboratory. It was recognized that strong efforts would have to be made to discourage this approach, for past

experience has shown that outbreaks of significant size have developed unknown to responsible public health officials while clinicians have awaited laboratory confirmation of suspect cases.

2 Testing methods

The Scientific Group on Smallpox Eradication (October 1967) recommended that at least three laboratory techniques should be readily available for the diagnosis of smallpox in all countries which are smallpox-free or which have reached the consolidation phase of an eradication programme.

i. Microscopic examination of stained smears.

ii. Precipitation in gel

iii. Virus isolation on chorioallantoic membrane of chick embryo (CAM).

These tests are relatively easy to carry out, provide reliable results quickly and do not require elaborate laboratory equipment.

However, considering the difficulties in interpretation of the microscopic smears and recognizing that this method is not significantly faster than the usually more accurate precipitation-in-gel technique, it was decided by the Regional Advisers to omit the microscopic examination from further consideration.

Given a modest amount of equipment, it is believed that a technician with a comparativelylimited education and a 10-14 day intensive training course could perform these tests with a high degree of reliability.

More complex methods such as electron microscopy, tissue culture isolation of virus and serology obviously require considerably more skill and more extensive facilities and equipment. Since these would be possible in a comparatively few laboratories with existing high-level competence, such tests would be expected to play a secondary or minor role among the diagnostic procedures.

3. Types of laboratories

The principal need is the development of national laboratories equipped to carry out the two tests noted above. Few problems should be encountered in making provision for and training personnel to carry out the precipitation-in-gel test. Virus isolation on CAM, however, poses special problems. For virus isolation, 11-13 day old embryonated eggs must be inoculated. It is not practicable for laboratories to arrange for a continuous supply of such eggs

if they are to be used for smallpox virus isolation alone. However, such eggs are required by smallpox vaccine production centres for routine testing of their vaccine; in endemic countries, there are also a few laboratories carrying out investigations of the arboviruses, etc. Those laboratories already employing embryonated eggs for other purposes would, therefore, be suitable as sites where virus isolation could be performed.

In some few instances, patients are seen after the conclusion of the crusting stage and there are no additional cases from whom fresh material can be obtained. It may be important, as was recently the case in Burma, to determine whether or not these cases were smallpox or some other disease. Serological study may permit this question to be answered. In addition, there undoubtedly will be a periodic need for special characterization of puzzling or difficult specimens. For this purpose, Regional Reference Laboratories will be designated which will be capable of carrying out such responsibilities. Such laboratories, necessarily competently staffed and equipped, would further be in a position to provide training to technicians of national laboratories, provide support for research activities, store prototype strains from different areas and conduct other studies as appropriate.

4. Training Manual; Provision of reagents

a. Manual: A step-by-step illustrated Manual describing the two types of tests will be prepared and printed by November 1968, which will permit an ordinary laboratory technician, with minimal training, to carry out the tests. This Manual will be made available in English and French through HQ. The draft of the final text should reach AMRO no later than September to permit translation into Spanish.

A less detailed addendum to the Manual describing other possible examinations such as hemagglutination-inhibition, electron microscopy, microscopic examination of stained smears and differential heat test for identification of strains will be prepared later and made available to those laboratories desiring to employ such techniques.

b. Reagents for examination of specimens by precipitation in gel and virus isolation on CAM (Annex I): Provision by WHO of the requisite reagents and equipment (as necessary) to perform each of these tests will facilitate the establishment of laboratory services and simplify the training procedures. Depending on the stability of the various reagents, sufficient will be provided initially for perhaps 50 to 100 examinations. It is expected that most laboratories will have the equipment and reagents described in the second column in Annex II. If they do not, WHO should be prepared to provide requisite items of equipment. Expendable reagents will be stockpiled at HQ, Geneva.

c. Kits for Specimen Collection: A simple, inexpensive (purchased in quantity) kit for specimen collection will be designed as soon as possible. The kit will include all necessary tools and containers to facilitate the collection of specimens such as material from skin lesions, vesicular and pustular fluid and scabs. The kits will contain a small test tube with needle or scalpel, a box containing slides, a bottle containing capillary tubes, a small bottle for scab collection, a set of instructions regarding specimen collection and a simple form requesting the essential clinical and epidemiological information. These materials will be packed in a metal container and cardboard box conforming with Article 120 of the International Postal Convention (Vienna 1964).

As soon as the design is completed, the Regions will be notified and Purchase Authorizations solicited for common purchase.

5. Selection of national laboratories: training courses

As mentioned in the introduction, the number of diagnostic laboratories must be limited in number for practical reasons. However, arrangements should be made for all endemic and immediately neighbouring countries to have immediate access to a diagnostic laboratory. These laboratories must be selected with care for certain prerequisites are necessary. It is believed that each laboratory should have at least one experienced microbiologist with overall responsibility for the work of the laboratory as a whole (i.e. including diagnostic work, etc. for a variety of diseases), and one laboratory technician specifically trained in the diagnostic procedures for smallpox.

Since the work involves the handling of infectious material, a special room for smallpox diagnostic work should be provided and arrangements made for particular precautions to be taken in the laboratory as well as for the staff, including sterilization of test material, vaccination of personnel, etc. It is anticipated that most laboratories will be associated with a vaccine production facility because of the need for a continuing supply of fertile eggs. This poses special problems since diagnostic facilities which would be handling variola virus would need to be strictly separated from the production plant. Also, incubators and sterilizers would have to be separate for each function.

A provisional listing of laboratories is noted in Annex II. In all, 14 laboratories in South America, SEARO, EMRO and WPRO are proposed.

Special courses should be developed by SE Advisers in all Regions by the end of 1969. Experience has shown that two working weeks is a suitable length; the size of the course shall be no more than 12. Courses announced are as follows:

AMRO - December, 1968 - working seminar for those already having participated in courses

EMRO - December, 1968 - as part of broader course in virology

SEARO - 1969

AFRO - ?

WPRO - 1969

Possible consultants include:

Dr N. Maltseva, Institute of Virus Preparations, Moscow, USSR
Dr A.W. Downie University of Colorado Medical Center, Denver, Colorado, USA
Dr K.R. Dumbell St Mary's Hospital Medical School, Paddington, London, U.K.
Dr A.S. Benenson The Jefferson Medical College of Philadelphia, USA
Dr J. Noble (AMRO only) National Communicable Disease Center, Atlanta, USA

6. Regional Reference Laboratories

Two Regional Smallpox Reference Centres have been designated thus far: National Communicable Disease Center, Atlanta, and the Research Institute of Virus Preparations, Moscow. The term "Regional", of course, does not imply a WHO Regional coverage but serves only to indicate that their frame of reference is broader than "national". Only rarely would these laboratories be directly concerned with diagnostic specimens sent from the

field. Their main functions would be as described. It is obvious that a high level of competency and extensive facilities are required if a laboratory is to serve effectively as a Regional Centre. Additional Centres would be useful. Subsequent Centres should be located with consideration given to geographical dispersion and preferably sited in endemic areas. Centres will be considered in 1969 in East Africa (Nairobi), Eastern Mediterranean Region (Cairo, Dacca), South-East Asia (Calcutta) and South America (Sao Paulo). Monetary support of $1 000 to $2 000 annually may be provided to enable them to cary out their functions effectively.

7. Development and quality control - Proposed WHO "certification" of diagnostic laboratories

At the end of each training course, the respective participant laboratories would be designated as "WHO certified smallpox diagnostic laboratories". However, because of the difficulties in maintaining competency, even in otherwise excellent laboratories, when specimens are not continually being tested and, further, because of the problems with respect to turnover in personnel, 5 or 6 "unknown" specimens will be sent to each of the laboratories every 6 months. This will be arranged by HQ with the co-operation of the laboratories in the USSR and USA. Results will be required to be submitted within a specified period of time, and, unless a high degree of accuracy is achieved, certification will be terminated. At regular intervals, a list of "certified" laboratories will be distributed. Laboratories will, of course, have to agree to this form of participation and certification as a prerequisite to receiving assistance.

Reagents and Equipment for Diagnostic Laboratories

	Provided by WHO	Provided by WHO if necessary	Provided by laboratory
Precipitation in gel	Normal serum Agar Anti-vaccinia serum Reference antigen Square pieces of glass 1 mm thick Plastic template with holes Cutting instrument Pipettes - assorted sizes (1, 5, 10 ml)	Buffer solution Xylol Alcohol Vaseline Microscope slides Pasteur pipettes Petri dishes Rubber bands Rubber teats Mortars and pestles Graduated cylinders (100, 500 ml) Test tubes Sharp knife Cotton Clip Alcohol lamp	Water bath
Virus isolation on CAM	Antibiotics Candler Hole punch Rubber cap	Alcohol Buffer solution Needle and syringe Scotch tape Scissors Forceps Petri dishes Alcohol lamp Cotton Glass pencil	Embryonated eggs Incubators

Distribution of Laboratories by country

African Region *

East - South	Congo Democratic Republic: Vaccine Production Laboratory (Lumbumbashi)
	Kenya
	Uganda
West - Central	Senegal
	Nigeria
	Ghana

* Excluding Union of South Africa, Southern Rhodesia, Portuguese areas

American Region (South)	Argentina (2)
	Brazil (4)
	Colombia
	Ecuador
	Peru
	Venezuela

Eastern Mediterranean Region	Israel
	Ethiopia
	Iran
	Pakistan East West
	U.A.R.
	Tunisia

South-East Asia Region	Afghanistan
	Burma
	Ceylon
	India (7)
	Indonesia (3)
	Thailand
Western Pacific Region	Cambodia
	Philippines
	Singapore
	Taiwan
	Australia
	Japan
	New Zealand