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LABORATORY DIAGNOSIS OF SMALLPOX

by

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This brief note has been prepared to introduce several important points regarding the laboratory diagnosis of smallpox in non-endemic countries in the Eastern Mediterranean Region. A comprehensive review on the same subject is provided in the attached "Guide to the Laboratory Diagnosis of Smallpox for Smallpox Eradication Programmes"(1) and "Laboratory Aids to the Control of Smallpox in Countries where the Disease is not Endemic"(2).

Laboratory Diagnosis and Disease Containment Measures

In non-endemic countries, a laboratory for the diagnosis of smallpox is an integral part of the surveillance network. However, it is well to recall the following points:

Before laboratory diagnosis can be requested, the possibility of smallpox must first be suspected. This is the most difficult part of smallpox surveillance in non-endemic countries. In countries which have been free of smallpox for a long time, persons knowledgeable of smallpox are few and the level of suspicion is low. Even in countries where smallpox has been eradicated in recent years, the interest of medical personnel in smallpox decreases, which often results in the delayed detection of cases for two or three generations of the disease after the first case was imported. This problem is particularly true in countries which are in close contact with endemic areas. Therefore, every effort should be made to alert the medical personnel of the possible introduction of smallpox into such countries.

2. Once the illness is suspected to be smallpox, necessary action to contain the disease should be carried out immediately, presuming that it is smallpox. Action should not be postponed until the laboratory can confirm the diagnosis.

Establishment of Laboratories

Of the methods available for smallpox diagnosis, as shown in Table 1, virus isolation on the chorioallantoic membrane of chick embryo and precipitation-in-gel testing are the most suitable methods for national laboratories to use. These methods are simple as compared with electron microscopic examination, virus isolation on tissue culture and serological testing. If properly done, these can provide conclusive results quickly: results of precipitation-in-gel within 24-48 hours and results of virus isolation within 6-9 days. However, laboratories should obtain chick embryos incubated for 10-13 days, which are

ready for inoculation of the specimen at any time testing is requested. This is only possible in laboratories where daily work with eggs is under way. In addition, experience has shown that even a competent laboratory has not infrequently encountered equivocal test results and such findings will increase considerably if the laboratory staff do not practise testing techniques at reasonable intervals.

These considerations indicate that, although it may be desirable for each individual country to have a diagnostic laboratory, it is of practical importance to have only a limited number of laboratories for such purposes in the Region. With suitable arrangements, countries where such facilities are not available could send specimens to regional laboratories.

WHO Diagnostic Testing Service

As the eradication programme progresses, our main concern is to avoid the re-establishment of smallpox endemic foci in countries which have already become smallpox free. This implies that, at present, diagnostic services should be immediately available whenever such countries require them. In this respect, WHO will be in a position to arrange testing services for such countries in co-operation with the WHO Reference Centres and Collaborating Laboratories. A list of these Reference Centres and Collaborating Laboratories is attached (Table 2). Specimens can be sent to the Smallpox Eradication Unit, WHO, Geneva either through the WHO Regional Office for the Eastern Mediterranean or directly by the quickest means. WHO will request a Reference Laboratory to perform the tests, taking into consideration the type of testing required for individual specimens. In our experience, preliminary testing results are normally available within 2-3 days after receipt of the specimen in Geneva and the final results are available within 7 days. The results will be cabled immediately to the countries concerned. However, it should be noted that reliable and quick results can only be obtained if the specimen is properly collected, packed and despatched as described in the "Guide to the Laboratory Diagnosis of Smallpox for Smallpox Eradication Programmes"(1). A sufficient amount of fluid or crus and necessary clinical information helps considerably.

In some countries, case detection might be delayed and the suspected case is already in the convalescent stage when detected. From such patients, it is not possible to obtain the usual specimens, such as vesicular or pustular fluid or crusts. In such cases, sera should be obtained for antibody titration. I

it is smallpox, antibody titres such as haemagglutination-inhibiting antibody and neutralizing antibody are usually significantly high to permit a reasonably specific diagnosis with the aid of the additional epidemiological and clinical information on the patient. This point is of particular interest, since we have on several occasions used serological testing to confirm cases recognized during convalescence in countries which have recently become smallpox-free.

WHO Assistance

Some minor instruments, as well as reagents such as anti-vaccinia sera, control antigen, etc., are available for distribution to the national laboratories. The WHO Reference Laboratories will assist national laboratories by conducting confirmatory tests upon request. A plan is needed to increase laboratory accuracy and proficiency by supplying unknown specimens to the national laboratories for testing.

References

(1) "Guide to the Laboratory Diagnosis of Smallpox for Smallpox Eradication Programmes", World Health Organization, Geneva, 1969.

(2) "Laboratory Aids to the Control of Smallpox in Countries where the Disease is not Endemic". Keith R. Dumbell, M.D., Virology Dept., St. Mary's Hospital Medical School, London, W.2.
Progr. med. Virol. vol. 10. pp.288-397 (Karger, Basel/New York 1968).

Table 1. List of Testing Methods*

A. Direct demonstration of virus particles

A.1. Electron microscopy

A.2. Light microscopy of stained smear (silver impregnation).

B. Demonstration of variola/vaccinia antigen

B.1. Precipitation-in-gel test

B.2. Complement fixation

C. Isolation of virus in

C.1. Chorioallantoic membrane of chick embryo

C.2. Tissue culture.

D. Demonstration of specific antibody in serum

D.1. Neutralizing antibody

D.2. Haemagglutination inhibiting antibody

D.3. Complement fixing antibody

D.4. Precipitating antibody.

* Based on Dr Dumbell's paper, reference (2), with some modification

Table 2. List of WHO Reference Centres and Collaborating Laboratories for the Laboratory Diagnosis of Smallpox

	Laboratory		Responsible Officer
1.	Research Institute of Virus Preparations, Dubrovskaya ul. 15, Moscow, USSR.	Reference Centre	Dr S.S. Marennı
2.	National Communicable Disease Center, Atlanta, Georgia 30333, USA.	Reference Centre	Dr Roslyn Q. Robinson
3.	Laboratoire National de la Santé Publique, 25 Boulevard Saint-Jacques, Paris XIVe, France.	Collaborating Laboratory	Dr R. Netter
4.	Department of Virology, The Wright-Fleming Institute of Microbiology, St Mary's Hospital Medical School, Paddington, London, W.2., England.	Collaborating Laboratory	Dr K.R. Dumbell