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Lyophilized combination pools of enterovirus equine antisera: new LBM pools prepared from reserves of antisera stored frozen for two decades

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This paper describes the preparation and test procedures for a second batch of lyophilized LBM combination antiserum pools, A through H, used for identifying 42 enteroviruses. Each pool is selectively composed of 10 or 11 of 42 individual enterovirus equine sera so that it contains 500 antibody units of each serum component per 0.1 ml. The new pools have been constituted from equine monovalent antisera that were prepared during the period 1962–67 and then evaluated and standardized in a series of collaborative international studies. An essential aspect of preparing the new pools was ensuring that the individual sera had retained high antibody titres through the long period of storage. At the time of retesting, the original stocks of these monovalent sera had been stored frozen at −20 °C for periods ranging from 16 to 20 years.

The first set of LBM (Lim, Benyesh-Melnick) antiserum pools, A–H, composed of 42 enteroviral types in the desired combinations, became available in 1973 as standard reference reagents for worldwide use in identifying isolates of 42 enteroviruses (*8*). Our laboratory, at that time designated as the WHO International Reference Centre for Enteroviruses, initially coordinated and participated in the standardization of the monovalent sera and prepared the combination pools. This was truly an international effort in which 14 virus laboratories in 10 countries cooperated (*7*). The original pools were recently exhausted. We therefore prepared new lots of pools, A through H, from reserve stocks of the individual sera which, fortunately, had been held in long-term storage at −20 °C, under the sponsorship of the Research Resources Branch, NIAID, National Institutes of Health, Bethesda, MD.

Various sets of standard monovalent enterovirus antisera had been prepared over a period many years previously in monkeys and rabbits, beginning with poliovirus standard reagents in the prevaccine era. As the field of enterovirology expanded, not only in numbers of known enteroviruses and their involvement in disease, but also in numbers of enterovirus laboratories around the world, it became desirable to have greater volumes of antisera that could be obtained best from large domestic animals. In an initial series of studies (*2*), the horse was shown to be the large animal of choice for preparing such antisera. Large stocks of standard prototype viruses were prepared and certified, and a series of monovalent enterovirus equine antisera were prepared, and were then evaluated for specificity and potency by extensive international collaborative studies (*1, 2, 5–7*).

With so many enteroviruses becoming known, an almost prohibitive number of tests were required to identify an enterovirus isolate by use of monovalent antisera. Methods for combining the antisera into typing pools were needed. Practicable and feasible patterns for preparing satisfactory combinations of antisera for use as typing reagents had been designed by Lim & Benyesh-Melnick (*3*) and by Schmidt et al. (*11*). The usefulness of the two patterns was compared in collaborative tests involving hundreds of field isolates, and the LBM design was selected (*12*). Both schemes were equally satisfactory for identifying the field strains, but only 8 LBM pools—as against 13 for the intersecting pool scheme—were required to identify 42 enteroviral serotypes. Subsequently, another set of standardized antisera was prepared and combined into pools J–P, covering all

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of the remaining coxsackievirus types — 19 "mouse-grown" coxsackieviruses of group A (*9*). The original stock of the J-P pools is still sufficient for anticipated needs.

MATERIALS AND METHODS

After the original 5-litre pools were prepared (*8*), the remaining bulk stocks of the equine antisera, which had been diligently prepared at Tuskegee Institute, Alabama (*2*), were stored frozen at − 20 °C. To initiate this new study, the antisera were shipped on dry ice from Washington to our laboratory, where they were immediately placed in a − 20 °C freezer room and held frozen until they were prepared for testing. Exceptions were antisera to poliovirus type 3 and echovirus types 4 and 11, which were available only in lyophilized form.

The virus strains used for verification of the monovalent sera and of the composition of the pools were the prototype strains — which had been stabilized with $MgCl_2$ in the early 1960s — and some additional strains as indicated. Serially propagated African green monkey kidney cells (BGM or Vero) were used for the propagation and titration of virus and for performing neutralization tests of monovalent sera and combination pools. Vero cells were employed in the neutralization tests that were done by the plaque reduction method. Melnick's medium B, used for diluting the virus or serum and for maintaining the cells during the test, consists of 0.5% lactalbumin hydrolysate in Earle's salt solution. The details of cell culturing, virus titrations, and neutralization have been described (*10*). The serum titres are all reported as neutralizing antibody units per 0.1 ml of serum, when tested against 100 TCD_{50} of virus.

Passage and propagation of prototype enterovirus strains

The frozen virus stocks were thawed, diluted 100 times, and passed once before use. Each diluted stock was inoculated in 0.2-ml quantities into two 30-ml drained culture flasks containing complete cellular monolayers. The inoculated bottles were incubated for 1 h at 37 °C, with mild shaking at 15-minute intervals. Then, 3 ml of medium B were added to each flask to cover the monolayer which contained adsorbed virus, and the flasks were returned to the 37 °C incubator. The inoculated bottles were examined microscopically each day for cytopathic effect (CPE). As soon as virus showed CPE to a degree of 3+ or more (i.e., CPE involving 75% or more of the cells in the culture), the cultures were frozen and thawed 3 times, the suspension was harvested and centrifuged (1000 rpm for 10 min). The supernatant fluid was then collected and the virus stabilized by adding $MgCl_2$ to make a final concentration of 1 mol/l. Five of the old frozen virus stocks, when first thawed, failed to pass on BGM cells, but all grew readily on human RD cells. Each of the new virus stocks was distributed in 0.5-ml portions, labelled, and stored at − 70 °C.

Each stock virus was titrated in culture tubes containing monolayers of BGM cells, to determine the dilution needed to contain the challenge CPE dose of 100 TCD_{50} units to be used in subsequent neutralization tests. Titres were calculated on day 7, and expressed as TCD_{50} per 0.1 ml.

Titration of monovalent antisera to determine their relative stability after up to 20 years of storage at − 20 °C

Before preparing the pools, we felt it necessary to determine whether the antibody titres had remained stable. Eight representative sera were chosen on the basis of their previous titres — some with low titres, some intermediate, and some with high titres. The sera chosen for retesting were those against poliovirus type 3, coxsackieviruses B1 and B5, and echoviruses 2, 3, 4, 6, and 19. Seven sera had been kept in the frozen state; one (E4) had been held in the lyophilized form. These sera also represented some of the earliest groups prepared in our previous series of studies, and had therefore been stored for longer periods. All those retested had been stored since 1965 or earlier. Because of the known difficulty of echovirus 4 antiserum in neutralizing the prototype echovirus 4 (Pesascek strain), tests were also done with the echovirus 4 serum against the more easily neutralized DuToit strain of echovirus 4 (*2, 13*).

The bulk bottles of serum were partially thawed at room temperature and then placed at 4 °C, in order to avoid subjecting the liquid serum to temperatures above 5-6 °C. Each vial containing lyophilized serum was rehydrated to its original concentration with 5 ml of distilled water, and a number of vials were then pooled.

A 1:10 dilution of each serum to be tested was made, and the sample inactivated at 56 °C for 30 min. Fourfold dilutions of each serum were made and challenged with an equal volume of the homologous virus at a dilution that had been calculated to contain 100 TCD_{50} or more per 0.1 ml. The mixtures were incubated for 2 h in a 37 °C water bath. A sample of the challenge virus used was similarly incubated and subsequently titrated to determine the exact virus dose that had been present in the test.

After incubation, two cell culture tubes were inoculated with each serum-virus mixture (0.2 ml/tube). Samples of the challenge virus were then seeded into cell cultures (4 tubes per dilution). The inoculated

tubes were incubated at 37 °C for 7 days. Microscopic readings for CPE were made from the second day of incubation. Titres were expressed as the highest dilution of serum showing 50% neutralization of the challenge virus per 0.1 ml.

Parallel titration of monovalent antisera by the plaque reduction method

Five of the antisera selected for retesting (those against poliovirus 3 and echoviruses 2, 3, 4, and 19) were also tested by the plaque reduction method, using the same serum dilutions and virus control dilutions as were used for the tube test. Again, tests were done with echovirus 4 antiserum against the DuToit strain as well as against the prototype Pesascek strain. Plaque assays were conducted on Vero cell cultures grown in 60-mm Petri plates. After inoculation, a layer of methylcellulose was added and the cultures were incubated at 37 °C in a 5% CO_2 atmosphere.

Preparation of LBM combination pools

Combination pools were prepared in five-litre quantities containing 500 antibody units of each antiserum type per 0.1 ml of the pool. For example, the assigned titre for echovirus type 1 antiserum is 1:11 000 (the dilution that contains 1 antibody unit per 0.1 ml). Therefore, a 1:22 dilution (11 000/500) would contain 500 antibody units per 0.1 ml; for a 5-litre pool, 227 ml (5000/22) of undiluted echovirus-1 serum is required. As a precautionary measure, only one pool per day was prepared, and the individual components were checked and verified by both authors. The required volumes were measured to the nearest millilitre. After each pool was prepared, a sample was taken for testing and verification, and the bulk pool was labelled and stored at −20 °C. Ultimately they were dispatched by air to the State Serum Institute, Copenhagen, where a part of each pool was distributed into 1-ml ampoules, lyophilized, and labelled as LBM pools A through H. Before use, the contents of each ampoule should be diluted to 10 ml to make a working stock containing 50 antibody units per 0.1 ml.

Verification and testing of combination serum pools

The test samples of the individual pools, A–H, were diluted 1:10 to give a final working concentration of 50 antibody units per 0.1 ml. The diluted pools were inactivated by heating at 56 °C for 30 minutes. To each serum pool, there was added an equal volume of the virus dilution calculated to contain from 100 to 300 TCD_{50} per 0.1 ml. The serum-virus mixtures were then incubated in a 37 °C water bath for 2 h. A sample of the virus dilution used was similarly incubated and subsequently titrated to determine the virus dose that was actually present in the test. After incubation, each of two cell culture tubes was inoculated with 0.2 ml of the serum-virus mixture. Samples of the challenge virus dilutions were then titrated using 4 tubes per dilution. The inoculated tubes were incubated at 37 °C for 7 days. Microscopic readings for cytopathic effect were made from the second day of incubation. All 42 viruses were tested against the A–H pools.

Table 1. Stability of monovalent antisera after storage at −20 °C for 20 years

		Homologous titre determined after storage for 20 years	
Antiserum[a]	Serum antibody titre at time of storage	Test tube method	Plaque reduction method
P3	16 000	16 000	80 000
CB1	38 000	50 000	–
CB5	22 000	24 000	–
E2	35 000	40 000	80 000
E3	4 700	6 000	20 000
E4:			
(Pesascek)	6 000	80	10 240
(DuToit)	6 000	10 240	20 480
E6	8 000	12 000	–
E19	35 000	40 000	80 000

[a] Letters P, CB and E refer to poliovirus, coxsackievirus group B and echovirus, respectively.

Table 2. Composition of serum pools A–H and titres of the sera used (each pool contains 500 antibody units per 0.1 ml of each of its serum components)

Antiserum[a]	Antibody titre per 0.1 ml (1 antibody unit)	Pools containing the antiserum								Combination pools for virus identification
		A	B	C	D	E	F	G	H	
P1	8 000			X			X			CF
P2	19 000		X			X				BE
P3	16 000				X				X	DH
CA7	9 200	X	X							AB
CA9	8 000		X					X		BG
CA16	1 500								X	H
CB1	38 000	X		X						AC
CB2	22 000		X		X					BD
CB3	4 000			X				X		CG
CB4	20 000	X				X				AE
CB5	22 000			X		X				CE
CB6	32 000						X		X	FH
E1	11 000	X							X	AH
E2	35 000		X	X						BC
E3	4 700		X						X	BH
E4	6 000	X						X		AG
E5	13 000	X				X		X		AEG
E6	8 000			X	X					CD
E7	15 000	X					X			AF
E9	7 500		X				X		X	BFH
E11	2 000					X				E
E12	26 000			X					X	CH
E13	17 000				X	X				DE
E14	7 300				X		X			DF
E15	2 000	X								A
E16	12 500				X			X		DG
E17	5 000					X		X		EG
E18	15 000					X	X			EF
E19	35 000		X				X			BF
E20	13 000						X	X		FG
E21	2 000		X							B
E22	13 000					X			X	EH
E23	16 000							X	X	GH
E24	3 400			X						C
E25	2 000				X					D
E26	19 000		X		X		X			BDF
E27	2 000						X			F
E29	8 000	X		X			X			ACF
E30	5 500			X		X		X		CEG
E31	9 600							X		G
E32	16 000				X	X			X	DEH
E33	8 000	X			X					AD

[a] Letters P, CA, CB and E refer to poliovirus, coxsackievirus groups A and B, and echovirus, respectively.

RESULTS

Titrations of monovalent antisera to determine whether adequate antibody titres had been retained after storage

The results of neutralizing antibody titrations are shown in Table 1 for the sera selected as representative samples to be tested for stability. The serum antibody titres before storage are shown in the left-hand column. These are the titres that were assigned to the materials stored in 1963-65, on the basis of the international collaborative tests using the conventional tube method (*8*). In the two right-hand columns are shown the results of our current tests using the tube method and the plaque reduction method. All of the samples yielded antibody titres equal to or better than the initial titres prior to storage some 20 years ago. It should be noted that the echovirus-4 antiserum showed plaque reduction activity to satisfactory levels against the prototype Pesascek strain, but that — as in the initial testing (*2, 13*) — this antiserum showed very low neutralizing activity when tested against the Pesaseck virus in the conventional tube test; however, it showed satisfactory antibody activity against the DuToit strain of echovirus 4. For this reason, as in the past, the DuToit strain was used in the subsequent testing and verification. When tested by the plaque reduction method, all the antisera demonstrated titres twofold higher than by the tube method. The higher neutralizing antibody titres obtained with the plaque reduction method are not surprising. Only a few unneutralized virus particles would be sufficient to cause complete CPE in a tube culture, yielding a reading of "no neutralization" in that tube. The same condition by the plaque reduction method could result in a plaque reduction of 95-98%, indicating almost complete neutralization.

Verification and testing of combination pools

The composition of the pools and the neutralization pattern that identifies each of the 42 enteroviruses are shown in Table 2. For constructing the pools, we used the original antibody titres obtained in cooperative studies involving 14 laboratories in 10

Table 3. Identification of viruses on the basis of neutralization test results with A-H combination antiserum pools

If neutralized by pool(s):	Virus is:[a]	If neutralized by pool(s):	Virus is:[a]
A	E15	CD	E6
B	E21	CE	CB5
C	E24	CF	P1
D	E25	CG	CB3
E	E11	CH	E12
F	E27	DE	E13
G	E31	DF	E14
H	CA16	DG	E16
		DH	P3
AB	CA7		
AC	CB1	EF	E18
AD	E33	EG	E17
AE	CB4	EH	E22
AF	E7	FG	E20
AG	E4	FH	CB6
AH	E1	GH	E23
BC	E2	ACF	E29
BD	CB2	AEG	E5
BE	P2	BDF	E26
BF	E19	BFH	E9
BG	CA9	CEG	E30
BH	E3	DEH	E32

[a] Letters E, CA, CB and P refer to echovirus, coxsackievirus groups A and B, and poliovirus, respectively.

countries. Then, using 100 to 300 TCD_{50} of the different viruses, we verified that the combination of pools listed in the last column of the Table correctly neutralized only the one virus type listed in the first column of the Table.

In Table 3, we present a simple code for reading the results. For example, if a virus is neutralized by pools F and H, the agent is identified as coxsackievirus B6.

All of the prototype strains checked were correctly identified by the use of the pools. Often a firm identification could be made after 3 to 4 days. This was especially true of the coxsackieviruses and the other fast-growing enteroviruses, or when the virus dose was in excess of 100 TCD_{50}; otherwise, the test was read through the full 7-day observation period. The mishaps that occurred in preparation of the first set of pools were now avoided. In the first set, because of faulty labelling of some of the serum containers by a commercial processor, coxsackievirus B3 antiserum failed to be included and had to be supplied separately, along with monovalent E11 and E12 antisera (*8*). This is no longer required.

Some comments and recommendations

With some enterovirus isolates there may be complete neutralization for a few days followed by a slow breakthrough on the 4th to 7th days. This may be the result of small aggregates of the virus (*14*), or the appearance in nature of new antigenic variants. We found the latter to be true with recent isolates of coxsackievirus B3. It has long been recognized that field strains of group B coxsackieviruses may show a considerable amount of antigenic variation from the parent strain (*4*). However, in our verification tests the prototype strain of coxsackievirus B3 obtained from the American Type Culture Collection was correctly neutralized. If difficulties are encountered in identifying new isolates suspected to be coxsackievirus B strains, one should be alert to the possible occurrence of early breakthroughs, and should consider performing further tests with a lower dose of virus. In general, early neutralization followed by a slow breakthrough is considered adequate for typing, although in special circumstances it may be advisable to confirm the identification by the use of type-specific monovalent antiserum.

If anomalous results are obtained (e.g., neutralization patterns not matching any of those described herein or in the instructions given with the reagents, or if the isolate is not neutralized), the user may also want to carry out further tests with only 20 antibody units rather than 50. For many years, enterovirus typing was accomplished by the use of the smaller amount of antibodies.

Another possible reason for failure of a virus to be neutralized is that the isolate may be a mixture of viral types.

DISCUSSION

It has been reassuring to learn that the antibody titres of these enterovirus sera were maintained after storage for two decades at −20 °C. The reason for the somewhat higher titres found in some of the sera tested could be due to ordinary experimental variation since the original assigned titres were at levels based on the results of a number of different laboratories, while ours were obtained in a single series of tests. Finding that the titres had remained as high as those determined in the initial collaborative testing enabled us to prepare the pools with little additional processing or alteration.

Through the collaborative studies of the WHO Centres for Virus Reference and Research, two sets of enterovirus equine antisera were prepared and assayed for homologous and heterotypic reactions. Forty-two different sera were prepared for the enteroviruses that are readily propagated in cell cultures. Nineteen different sera were prepared for those enteroviruses which normally require the use of infant mice for assay. The first set of 42 antisera had been distributed into 8 combination pools, designated as A through H, for the identification of the 42 viruses (*8*). Over a period of 10 years these have been made available for typing new isolates throughout the world. Similarly, the 19 sera of the second set have been distributed into J through P combination pools (*9*).

The stock of the first set of A–H pools has been exhausted and a new set has now been prepared and re-standardized. They are now stored at the WHO Collaborating Centre for Virus Reference and Research, State Serum Institute, Copenhagen, which is also responsible for their distribution. Arrangements for obtaining the sera can be made through the Virus Diseases Unit, WHO, Geneva.[a] In order to obtain a set of reagents, each laboratory is asked to inform WHO of their recent experiences with isolating and typing enteroviruses, and of their planned use of the new pools. Only one set of typing reagents will be sent per year to a single laboratory.

The present set of typing pools was prepared by the same methods that were used for the original pools, and they may be used for typing by the previously recommended methods, namely, reading CPE

[a] Information on how to obtain these reagents is available from the Virus Diseases Unit, World Health Organization, 1211 Geneva 27, Switzerland. The 8 dried serum pools are supplied as a unit package. Instructions for use and for interpretation of results are included in each package.

endpoints in tube cultures, using 0.1 ml of 50-antibody-unit antiserum per culture. However, laboratories experienced in microtitre assays should also apply such reagent-saving techniques when using the new pools.

Another recommendation is to use the pools mainly to determine the cause of an epidemic. Then, as soon as the enterovirus type responsible has been determined, all other isolates from the outbreak should be identified with a locally prepared monovalent antiserum. Furthermore, it is not necessary to type every single enterovirus isolate. These reference sera, which are of limited supply, should be reserved for identifying important human pathogens isolated from cases of particular epidemiologic importance or from patients who have severe illnesses, such as paralysis or encephalitis.

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RÉSUMÉ

MÉLANGES LYOPHILISÉS D'IMMUNSÉRUMS DE CHEVAL ANTI-ENTÉROVIRUS: NOUVEAUX MÉLANGES LBM PRÉPARÉS À PARTIR DE STOCKS D'IMMUNSÉRUMS CONSERVÉS À L'ÉTAT CONGELÉ PENDANT 20 ANS

Le présent article décrit le mode de préparation d'un nouveau stock de mélanges lyophilisés d'immunsérums LBM (Lim, Benyesh-Melnick) utilisés pour identifier les entérovirus; les essais pratiqués sont également indiqués. Les études comparatives conduites par les Centres OMS de référence et de recherche pour les virus avaient permis de préparer des immunsérums de cheval dirigés contre les entérovirus et de les mettre à l'épreuve en vue de réactions homologues et hétérotypiques. Quarante-deux sérums différents avaient ainsi été préparés pour les entérovirus qui poussent facilement en culture cellulaire. D'après les taux d'anticorps, ils avaient été répartis en huit mélanges, désignés par les lettres A à H, en vue de l'identification des 42 entérovirus. Il y a 10 ans qu'ils étaient disponibles partout dans le monde pour le typage des nouveaux isolements.

Les nouveaux mélanges LBM ont été préparés de la même manière que le premier assortiment. Chacun d'eux est composé de 10 ou 11 des 42 sérums de cheval anti-entérovirus, à raison de 500 unités d'anticorps par sérum pour 0,1 ml. Ces mélanges ont été préparés à partir des stocks d'immunsérums de cheval monovalents, internationalement normalisés, qui avaient été constitués de 1962 à 1967 et évalués dans 14 laboratoires répartis dans 10 pays. L'un des points essentiels, lors de la préparation des nouveaux mélanges, a été de s'assurer que chacun des sérums avait conservé un titre élevé d'anticorps tout au long de la période de stockage, de 16 à 20 ans. Dans certains des sérums on a pu observer des titres un peu plus élevés qu'auparavant; ce phénomène s'explique peut-être par la dispersion normale des valeurs expérimentales, vu que les titres attribués initialement l'avaient été en fonction des résultats d'un certain nombre de laboratoires, tandis que les titres actuels reposent sur une unique série d'épreuves.

Comme les titres étaient restés aussi élevés que lors des analyses comparatives initiales, on a pu préparer les nouveaux mélanges sans grande modification ni traitement supplémentaire. La composition des nouveaux mélanges a été déterminée sur la base des titres d'anticorps obtenus initialement lors des études collectives supervisées par l'OMS. On a ensuite vérifié, en utilisant 100 à 300 DCT_{50} des différents virus, que la neutralisation au moyen des mélanges permettait bien d'identifier uniquement le type viral visé lors de la constitution du mélange en cause. On a pu identifier ainsi correctement toutes les souches-types soumises à l'épreuve. Souvent l'identification a été définitive après 3 ou 4 jours, en particulier avec les virus Coxsackie ou avec divers entérovirus à pousse rapide, ou encore lorsque la dose de virus dépassait 100 DCT_{50}. Avec certains isolements, on observe une neutralisation complète pendant quelques jours, suivie d'une recrudescence lente à partir du 4^e au 7^e jour. Ce phénomène peut être la conséquence de la formation de petits agrégats de virus ou de l'apparition à l'état naturel de nouveaux variants antigéniques. Cette dernière hypothèse s'est révélée exacte avec des isolements récents de virus Coxsackie de groupe B3; on sait depuis longtemps que certaines souches de virus Coxsackie B rencontrées sur le terrain manifestent effectivement des variations antigéniques considérables. Il se pourrait aussi que l'isolement soit un amalgame de types viraux. En général, on considère qu'une neutralisation rapide suivie d'une recrudescence lente suffit au typage bien que, dans certains cas, il soit peut-être préférable de confirmer l'identification au moyen d'un immunsérum monovalent, spécifique de type.

L'assortiment actuel de mélanges LBM, préparés par des méthodes identiques à celles qui avaient été employées pour les mélanges initiaux, permet des typages par les méthodes recommandées antérieurement, à savoir la lecture de l'effet cytopathogène par la méthode des dilutions limites, sur culture en tube en présence de 0,1 ml d'immunsérum contenant 50 unités d'anticorps. Cependant, il serait bon que les laboratoires ayant l'expérience des microtitrages appliquent

également ces techniques moins exigeantes en réactifs lorsqu'ils utiliseront les nouveaux mélanges.

Il est en outre recommandé que ces mélanges soient principalement utilisés pour déterminer la cause d'une épidémie. Dès que le type d'entérovirus en cause aura été identifié, on identifiera les autres isolements provenant de la flambée au moyen d'un immunsérum monovalent préparé sur place. De plus, il n'est pas nécessaire de typer chacun des isolements d'entérovirus. Vu l'importance réduite des stocks, ces sérums de référence devront être réservés à l'identification d'agents pathogènes humains importants, isolés chez des cas présentant un intérêt épidémiologique particulier ou chez les patients atteints d'une maladie grave, par exemple de paralysie ou d'encéphalite.

Les nouveaux mélanges LBM sont maintenant conservés au Centre collaborateur OMS de référence et de recherche pour les virus (Statens Seruminstitut, Copenhague) qui est chargé également de leur distribution. Les dispositions nécessaires pour se procurer les sérums peuvent être prises par l'intermédiaire du service des Maladies à Virus, OMS, Genève. Il est demandé à tout laboratoire qui souhaite obtenir un assortiment de réactifs, de faire connaître à l'OMS ses dernières expériences en matière d'isolement et de typage des entérovirus et de l'informer de l'utilisation prévue pour les nouveaux mélanges. Un seul assortiment de réactifs de typage sera envoyé chaque année à un laboratoire donné.

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