6. Plan of Action

The participants recommended that surveillance should be established for: 1) Disease syndromes; 2) Specific pathologies and 3) Antimicrobial resistance.

6.1. Disease Syndromes

The participants selected **seven** disease syndromes to be included in the proposed surveillance program. A definition of each syndrome is given below:

1. *Undifferentiated febrile syndrome*: Fever (axillary temperature ≥38° C) that has n obvious etiology and has no more than 7 days evolution in a previously healthy person 5

years of age or older.

2 *Hemorrhagic fever syndrome*: An acute febrile (axillary temperature ≥ 38° C) hemorrhagic illness with or whitout. evidence of capillary fragility in a person of any age.

3 *Febrile icteric syndrome*: Febrile patients (axillary temperature ≥38°C) more than one year of age with acute or insidious onset of icterus in whom there is no detectable cholelitiasis or biliary obstruction or malignancy

4 *Acute respiratory distress syndrome*: A febrile illness (temp. ≥38°C) more than 5 years of age characterized by bilateral diffuse interstitial edema, with respiratory compromise requiring supplemental oxygen, developing within 72 hours of hospitalization, and occurring in a previously healthy person.

5 *Unexpected death syndrome*: Previously healthy persons, 5-49 years of age, who are hospitalized (or admitted to an emergency room) with a life threatening illness with hallmarks of an infectious disease for which no cause is identified.

6 *Infectious neurologic syndrome*: Febrile neurologic symptoms with clear CSF in a non-immunodepressed patient.

7 *Enteric syndrome*: Bloody or non bloody, acute diarrhea, with fever or not, in children or adults.

Excluded from this study will be patients with pre-existing chronic medical conditions such as malignancy, HIV infection, chronic cardiac, pulmonary, renal or rheumatologic disease, diabetes mellitus, immunosuppressive therapy, trauma, toxic ingestion or exposure, or nosocomial infection.

6.2. Infectious Agents for Disease Syndromes

A list of possible diseases that will be tested for was prepared for each of the disease syndromes. Convalescent sera from patients enrolled in the study will be screened for antibodies against the agents causing the diseases listed below. If the serum is negative in the initial screen, it then will be tested (in a second or third screen) against other less common agents**.** Attempts can also be made to culture/isolate infectious agents from samples such as blood, serum, stools, pharyngeal washes, spinal fluid, autopsy tissues. **The order of the agents to be tested may vary from one country/area to the others.**

Undifferentiated febrile syndrome

(a) Initial screen
Dengue (types 1, 2, 3, and 4)
Brucellosis
Leptospirosis
Influenza
Malaria (*P. falciparum* and *P. vivax*)
Yellow fever
Q-fever

(b) Secondary screen:
Ehrlichoses
Rickettsioses
Parvovirus B-19 infection
Measles
Rubella
Hepatitis A
Hepatitis B
Hepatitis C
Ilheus virus infection
Phlebovirus infection (phelobotomus fever)
Vesiculovirus infection
Cache Valley & related bunyavirus infections
Typhoid fever
St. Louis encephalitis virus infection

Hemorrhagic fever syndrome

(a) Initial screen: (in order of probability at each site)
Leptospirosis
Arenavirus infections (Junin, Machupo or others, depending on region)
Dengue (types 1, 2, 3, and 4)
Yellow fever

(b) Secondary screen:
Ehrlichiosis
Rickettsioses
Hepatitis B
Hepatitis C
Hantavirus infection (hemorrhagic fever with renal syndrome)

Febrile icteric syndrome

(a) Initial Screen: (in order of probability at each site)
Leptospirosis
Hepatitis A
Hepatitis B
Hepatitis C
Yellow fever

(b) Secondary Screen:
Hepatitis D (only if patients is positive for hepatitis B virus infection)
Hepatitis E

Acute (noncardiogenic) respiratory distress syndrome (ARDS)

Leptospirosis
Influenza
Hantavirus infection (Hantavirus pulmonary syndrome)
Legionellosis
Q fever
Psittacosis

Unexpected death syndrome

(a) Initial Screen (done locally):
Culture
Histopathology

(b) Secondary Screen (done at a reference laboratory):
immunohistopathology
nucleic acid probes with PCR
electron microscopy

Infectious neurologic syndrome

(a) enterovirus
herpes simplex

Enteric syndrome

(a) Initial Screen

V. cholerae 01,0139, no-01
V. parahaemolytius and other vibrio
Rotavirus
E. coli 0157 and other Shiga toxin-producing *E. coli*
Salmonella
Shigella
Diarrheigenic *E. Coli*

(b) Secondary Screen

Adenovirus 40 and 41
Norwalk virus

6.3. Specific Pathologies

In addition to the above mentioned syndromes the participants suggested to include the following disease/syndromes to be under surveillance:

6.3.1 Hemolytic uremic syndrome (HUS). Previously healthy children who developed acute renal injury thrombocytopenia and microangiopathic hemolytic anemia, following an acute diarrhoeal prodromal illness.
6.3.2 Muco-cutaneous and visceral leishmaniasis
6.3.3 Plague

6.4. Surveillance for Antimicrobial Resistance

Indiscriminate use of antimicrobial agents in the last decade has generated the appearance of multiresistant strains, thus complicating treatment and, allowing for the re-emergence of some diseases such as tuberculosis that were almost under control. Similar situation is shared with other diseases:

a. Resistance of *Streptococcus pneumoniae* and *Haemophilus influenzae* to penicillin
b. Resistance of *Salmonella* and *Shigella* to one or more drugs.
c. Vancomycin resistant Enterococcus.
d. Yeast and filamentous fungi.

This situation requires urgent implementation of surveillance to establish strategies and control measure.

Pathogen agents proposed to enter under surveillance of antimicrobial resistance

1. *Mycobacterium*
2. *Plasmodium*
3. *Salmonella,*

4. *Shigella*
5. *Vibrio cholerae*
6. *Klebsiella, Enterococcus*
7. *Staphylococcus aureus*
8. *Streptococcus pneumoniae*
9. *Haemophilus influenzae*
10. *Neisseria. gonorrheae*
11. *Candida albicans*
12. *Cryptococcus neoformans*

6.5. Syndromes/Diseases for Initial Surveillance

The following syndrome/diseases were selected for initial surveillance:

- Influenza
- Antimicrobial resistance, including tuberculosis
- Acute diarrhea including *E coli* O157 (HUS)
- HPS/Hantavirus disease

6.6. Specimens

Acute and convalescent serum specimens will be obtained from all patients when possible. To insure that convalescent samples are obtained from most of the people enrolled in the study, home visits are recommended. In cases where specific diseases are suspected (i.e. influenza, leptospirosis, rickettsiosis), additional samples (i.e. nasopharyngeal swab, urine or blood clot, respectively) may be collected. (**See table 4**).

Acute serum: During interepidemic periods, acute blood (serum) samples will be collected from patients within the first five days of their illness. During epidemics, acute blood samples should be taken within the first three days of illness. If appropriate, the acute phase blood clot may also be saved. Acute phase samples will be stored at –70 C.

Acute phase respiratory samples: In illness with respiratory symptoms, nasopharyngeal swabs or pharyngeal washes will be collected. These samples should be processed immediately or frozen at –20 C.

Convalescent serum samples: An attempt will be made to obtain a second (convalescent) serum sample from all surviving patients within 2-3 weeks after the onset of their illness. In cases of hemorrhagic fever, where arenavirus infection is suspected, a third serum sample will be obtained 5 or 6 weeks after onset of illness.

Autopsy sample: In fatal cases, an attempt will be made to obtain permission for an autopsy (or viscerotomy) in order to obtain tissue samples. Ideally fresh tissue samples should be saved (untreated and frozen at –70 C) for culture and fixed in 10% buffered formalin for histopathology.

6.7. Reagents

All participants emphasized the importance of having available adequate amounts of standardized and high quality diagnostic reagents. After discussing alternative strategies for this goal, it was decided that most reagents would be purchased from commercial vendors or obtained from an international reference laboratory such as CDC. If not available from a commercial or reference source, then one or more of the network laboratories will prepare and share the reagents with the other laboratories. Training of laboratory staff in reagent preparation is proposed to ensure adequate amounts of reagents.

6.8. Laboratory Tests

The basic plan will be to begin testing as soon as possible by screening the convalescent serum for antibodies to the probable etiologic agent, and the acute sample(s) (stored at -70C) by appropriate tests (i.e. culture, PCR). The group agreed that the same tests and reagents will be used in each of the network laboratories.

6.9. Provision of Reagents

A suggested list of specific diagnostic tests and source of reagents is given below. This list could change depending on cost, funds available, and/or the development of newer more sensitive diagnostic techniques. New reagents will be developed for new pathologies to be shared between laboratories, which will have the added value of their standardization and validation.

- Arboviruses and arenaviruses - IgM ELISA (antigens to be prepared in one of the network laboratories).
- Hantaviruses - IgM ELISA (antigen from CDC or from ANLIS)
- Influenza - WHO/CDC kits
- Hepatitis A, B, C, D, & E - commercial kits
- Leptospirosis, Q-fever, typhoid fever and brucellosis - IgM ELISA (commercial kits).
- Rickettsioses and ehrlichioses -IFAT or commercial kits (antigen from CDC).
- Malaria - thick smears initially, followed by QBC for confirmation; then commercial tests (Paraslide or OptiMAL) to differentiate *P. vivax* from *P. falciparum*.
- Psittacosis and legionellosis -CF, ELISA or IFAT (antigen from CDC Biomanguinhos, Malbran, or commercial source).
- Enterics – *Salmonella, Shigella, Eicoli, V. cholerae;* Serotype identification (INPB/Malbran).

6.10. Quality Control

It was recognized by the group that it will be important for all collaborating laboratories within the network to maintain the highest quality of diagnostic tests to ensure that results obtained will be accurate and that all participants will have confidence in the observations made and results obtained. Formal quality control may be difficult since some of the tests to be used are not available commercially and the reagents to be used must be individually prepared. To overcome possible variation in test results, as described above, whenever possible, standardized test protocols and a single source of diagnostic reagents will be used. In cases where results of standardized tests are inconclusive or questioned, specimens will be examined with other partners in the network for clarification, confirmation or additional testing, or referred to external reference laboratories such as the CDC. Finally, whenever possible, a battery of well validated positive and negative control specimens will be distributed under code to all laboratory partners for routine proficiency testing. If significant discrepancies are found through the routine quality control and proficiency testing procedures, specialized training will be implemented to correct the problem.

The network should have the support of the regional ongoing quality control program in order to assure the good practice in the laboratories, and a team of expert should be formed to develop the program. Topics for consideration may include:

- Callibration of equipment and validation of installation.
- Validation procedures for new methodologies and new reagents.
- To establish the specifications of sensitivity, specificity and stability of diagnostic reagents and
- Laboratory Biosafety

6.11. Equipment

Provided adequate resources become available, each national surveillance site will be supported with a -70^{o}C and a -20^{o}C freezer and an ELISA reader and washer for dedicated specimen storage and testing of samples, respectively. Also, a minimum of at least one liquid nitrogen shipping container (dry shipper) will be provided to each laboratory for temporary storage and transportation of specimens. Biosafety Cabinet Class II and centrifuges with covers cups should also be available.

Table 4: Syndrome Study: Specimen and Laboratory Methodologies

Syndrome	Specimens	Serology	Culture or Isolation	Molecular techniques	Histopathology
Undifferentiated Febril and Influenza	1. Sera 2. Nasopharyngeal swab	+ 	+ +		
Hemorrhagic Fever	Sera	+	+	+	
Febrile Icteric	Sera	+	+	+	
Acute Respiratory Distress	1. Sera 2. Blood clot 3. Nasopharyngeal swab	+ 	 +	 + 	
Unexpected death syndrome	1. Blood 2. Autopsy tissues	+	+ +	+ +	 +
Infectious Neurologic	1. Sera 2. Cerebrospinal Fluid	+ +	+ +	+ +	
Enteric	3. Sera 4. Stool	+	 +	 +	
HUS	1. Sera 2. Stool	+	 +	 +	

6.12. Project Management

Management of the collaborative project will, by necessity, involve two separate levels. Agreement was reached among all of the laboratories that external sources of funding should be sought to provide resources needed to maintain the network and conduct the proposed investigations. This will require that a single principal investigator with his/her parent organization submitting a specific proposal and assuming responsibility for overall management of funds, including disbursement and accounting. The principal investigator will also be responsible for obtaining progress reports from national laboratories participating in the network and completing formal progress summaries for the funding agency, as required. At least two potential funding sources were discussed, the United States National Institutes of Health (NIH) and the United States Agency for International Development (USAID). If

different individuals and organizations prepare separate proposals for these or other donors, then each principal investigator would have management responsibilities for his/her own grant or contract.

Within each of the collaborating laboratories, a single individual will be identified to serve as the primary point of contact and responsible party to oversee the activities of his/her laboratory in implementing the plans of the network. This individual will also serve as a member of the network executive committee. Responsibilities of the executive committee will include design and agreement upon collaborative studies to be undertaken, management and accounting of funds provided to support these efforts, and timely preparation of semi-annual and annual reports as required by funding agencies. The executive committee will meet at least annually to review progress and define future priorities, and to discuss current and future efforts with the principal investigator(s) of supporting grant(s).

6.13. Financial Support

Funding must be utilized efficiently and surveillance goals must be achieved in a timely manner. Funding is limited and must lead to well defined accomplishments. This will require an action plan with well defined goals and an infrastructure capable of supporting the execution of the plan in a timely manner.

The CDC has and will continue to provide support to PAHO to fund the Southern Cone and the Amazon network meetings. USAID (Global Bureau) has provided $250K to CDC to implement the Amazon and South Cone surveillance networks. Also, there is limited funding for laboratory diagnostic and epidemiology training at CDC. Other possible sources of funding are the Rockefeller Foundation, Gates Foundation and the R.J. Williams Foundation.

A separate TCC to support Southern Cone is also underway. Also, PAHO has limited funds for diagnostic reagents.

The U.S. Department of Defense (DoD) Emerging Infectious Disease Detection and Response System has funding to increase the capabilities of DoD overseas laboratories over the next 6 years. DoD plans are similar to those of the Amazon Basin and Southern Cone networks, with interest in surveillance for influenza, antibiotic resistance among enteric pathogens, fever of undetermined etiology, and anti-malaria drug resistance.

Another possible source of funding is from the DOD humanitarian assistance program, some of which is targeted for surveillance infrastructure building projects. In the Caribbean, funding was provided to purchase computers to establish a surveillance communication system. A similar project will be carried out in Peru using a new version of the PHLIS system. Humanitarian Funds can be applied for through the local Ministry of Health to the United States Ambassador.

There is also the possibility for industrial funding, large corporations such as MRL in Virginia, work with 100 hospitals in the U.S., downloading information every 24 hours and

allow you to search into this database, if you are a subscriber.

World Bank loans are being provided to Argentina (VIGIA) and Brazil (VIGISUS). Goals and objectives of these loans are consisent with many of those for the Southern Cone Network and these funds will help to achieve several specific requirements for these countries.

6.14. Associated Institutions

Centers for Disease Control and Prevention (CDC)

The CDC serves as the national public health reference laboratory for the United States, and as such, maintains technical expertise in virtually all infectious diseases of public health importance. Within the CDC, the National Center for Infectious Diseases (NCID) houses laboratory facilities using state-of-the-art procedures to isolate, cultivate and identify infectious pathogens. Other centers within CDC maintain programs in immunization against vaccine preventable diseases, training in epidemiology, toxicology expertise, and others. Virtually all centers within CDC have an interest in global health and disease prevention or control. The CDC, and especially NCID, are capable and willing to assist the Southern Cone Region Emerging Diseases Laboratory Network by providing assistance in characterizing isolated agents, helping to resolve difficult clinical diagnoses, providing pathological analysis of clinical specimens, and offering training in specialized laboratory techniques. The CDC also houses several World Health Organization Collaborating Centers, and through the activities of these Centers, assists WHO and PAHO in implementing global and regional activities. For example, the CDC contributes significantly to the global monitoring of influenza viruses, leading to the annual recommendations for influenza virus composition. Finally, various countries to assist in outbreak investigations often call upon the CDC.

Pan American Health Organization (PAHO)

PAHO has a mandate to help all countries in this hemisphere to improve their health structure; it has offices in all six countries involved in this project. PAHO can provide assistance to the network in several ways, such as direct technical cooperation or mobilizing consultants, particularly on epidemiological surveillance. PAHO can also purchase and deliver equipment and reagents, and help transfer funds to the network. Another area of cooperation can be the organization of proficiency programs of teams for external evaluations and technical meetings. Interaction between PAHO task force on surveillance of emerging and re-emerging infectious diseases and the network will also be encouraged.

World Health Organization (WHO)

The WHO was directed by the World Health Assembly in 1995 by formal resolution to address emerging infectious diseases. In response to this resolution, the Division of Communicable Diseases was reorganized into a new structure, the Division of Emerging and

other Communicable Disease Surveillance and Control (EMC). Among other activities, this division assists region and member states in developing and implementing disease surveillance activities, responding to outbreaks, and developing national capacity through training activities and workshops. WHO/EMC also attempts to provide accurate, timely information on disease outbreaks around the world through both formal publications in the Weekly Epidemiological Record and electronically through a dedicated Web site and various electronic distribution lists. Other divisions within WHO provide regions and member states with technical assistance and training opportunities on specific diseases, conditions or programs. These include the Division for Control of Tropical Diseases (CTD), the program for Tropical Diseases Research (TDR), the Global Program for Vaccines (GPV), and others.

Pan American Institute for Food Protection and Zoonosis (INPPAZ)

The INPPAZ is a specialized center of the Pan American Health Organization/World Health Organization (PAHO/WHO) in charge of the execution of the Plan of Action of PAHO in food safety. Among its tasks is included the technical cooperation in the epidemiological surveillance of food-borne diseases to the member countries of the organization. Several pathogens transmitted by food like *E. coli 0157 H7, Salmonella enteritidis, Listeria monocytogenes, Yersinia enterocolitica,* among others, are considered emergent diseases. The INPPAZ is prepared to organize the monitoring of these pathogens through traditional methods and through bio-molecular techniques. In the other hand the INPPAZ has started a training program for laboratory analysts of the Region in techniques for isolation and identification of emerging food-borne pathogens and in microbiological diagnosis through rapid and automatic methods. This capability can be applied in the surveillance of the emerging infection diseases in the regional network that is being implemented. INPPAZ, in coordination with the United Nations Organization for Food and Agriculture (FAO), is the *ex-officio* Secretariat of the Inter-American Network of Food Analysis Laboratories (INFAL) and collaborates with the countries in the organization of their national networks. This initiative complements the effort for developing the surveillance network of emerging diseases. INPPAZ has experience in studies of resistance on anti-microbial drugs, particularly on anti-tuberculosis drugs, which could be applied to the surveillance of the drug resistant pathogens proposed by the network.

6.15. Study Sites and Populations

Individual countries will develop specific plans for the surveillance network, taking into consideration the following factors:

1. Areas of disease risk
2. Existing laboratory capacity
3. Existing epidemiological capacity
4. Diversity of environment (climate and/or socioeconomic)

Every effort should be made to strengthen broadly the capacity of existing laboratories. In defining areas at risk, the participating sites should consider not only areas where a disease is

known to occur, but also the surrounding areas to which the disease might spread, including areas in neighboring countries.

6.16. Number of Subjects

The ideal number of subjects will vary depending on the specificity of the syndrome, how widely the syndrome occurs with respect to geography and time, and other special circumstance, such as a need to monitor for antimicrobial resistance. Countries will develop testing quotas based on the preceived prevalence of the syndrome and the availability of resources.

6.17. Follow-up of Patients

Countries will collectively develop a plan for follow-up based on the needs for individual diseases and syndromes. The group felt that testing of the acute specimen would be adequate for most diseases, although attempts should be made to obtain follow-up specimens whenever possible.

6.18. Outbreak Investigations

The capacity for conducting outbreak investigations must be an integral part of the surveillance system. To assist in the identification of outbreaks, sentinel sites should tally weekly the number of cases of each syndrome and, if an excess is noted, report that information to the epidemiology unit. Outbreak investigations should involve a task force that includes laboratory, epidemiology and local personnel. Network countries agree to provide advice to one another as needed to respond to outbreaks.

6.19. Questionnaire

Countries will develop questionnaires based on their individual needs. Summary information to be shared among countries includes the following core data: patient age and sex, rural or urban setting, clinical syndrome, date of illness onset, final diagnosis, basis of final diagnosis (clinical vs. laboratory confirmed), clinical outcome, geographic region of case.

6.20. Specimen Banking

Banking of all specimens will be impractical for most countries. Depending on the available resources in each country, specimens for which demographic and clinical data are available will be banked with the priority:

1. Specimens that are part of an outbreak
2. Specimens for which both acute and convalescent serum are available.
3. Sera without diagnosis.

6.21. Communications/Banking

Participants reported that e-mail was available to all and agreed that this would be used for most communications.

6.22. Training

It was recognized that training and technology transfer will be important components in the development of the Southern Cone Region Emerging Disease Network. Although all of the collaborating laboratories are well established and employ highly competent professionals and staff, recent discoveries such as hantavirus pulmonary syndrome and technological advances, such as the widespread diagnostic application of polymerase chain reaction, necessitate the availability of periodic, specialized training opportunities. CDC has volunteered to provide training for the appropriate staff of collaborating centers in newly developed techniques to diagnose hantavirus pulmonary syndrome. Likewise, the CDC has recently offered a sub-Regional workshop in Chile for the isolation and characterization of influenza viruses, and future workshops in the Region may be appropriate. The need for hands-on training for the use of specialized diagnostic reagents (primarily antigens) to be prepared and distributed among the collaborating laboratories was discussed and agreed upon. Likewise, a need was expressed for training opportunities in techniques of vector (mosquitoes and culicoides primarily) identification and control, and rodent identification and control. Finally, all participating laboratories were requested to itemize their anticipated training requirements so that targeted training opportunities will become a significant component of the network.

6.23. Evaluations

It was agreed that evaluation is an important component of this project. An evaluation model based on the successful example of the Southern Cone subregional project to eliminate *Triatoma infestans* was chosen. Participants agreed to meet annually to discuss progress in implementing common projects and protocols, to present results, and to identify strategies to address regional problems. It was noted that the evaluation will be a dynamic process. Early meetings will inevitably focus on implementation issues, while later ones will emphasize results. Participants also agreed that site visits to the laboratories conducted approximately

every two years by other participants and perhaps one or two outside consultants would be useful.

6.24. Integration of the Network to the National Surveillance Systems

It is anticipated that the proposed network of laboratories for surveillance of EID in the Southern Cone Region will be fully integrated into their respective national surveillance systems; thus, they will complement the national surveillance activities, particularly regarding EID. The information generated by the network should be readily available to the local, state and federal health systems for the implementation of appropriate control actions. The network will also contribute to the identification and control of risk factors that can affect the health of populations of the Southern Cone Region. In addition the epidemiologic studies conducted by the network will improve the knowledge about health problems of national and international importance. The international cooperation between the regional laboratories will require governmental coordination, which will involve mainly the Ministries of Health and Foreign Affairs, taking into account presently existing agreements among the nations and, if necessary, establishing new ones.

In the case of Brazil, the National Center of Epidemiology (CENEPI), Ministry of Health will participate in this initiative through its National System of Public Health Laboratories (COLAB), particularly interacting with FIOCRUZ and the Instituto Adolfo Lutz. The strengthening of these two institutes will in turn improve the Brazilian network of public health laboratories. A similar approach exists in some of the other countries and should be considered by all countries, involving their national respective laboratory agencies.

6.25. National Technical Committee

A National Technical Committee should be established in countries that do not have one, in order to organize, strengthen, define, and execute policies related to activities aimed at the creation and maintenance of a EID surveillance network.

6.26. Surveillance of HUS and Influenza

Working groups were formed during the meeting to discuss the surveillance of HUS and Influenza. The reports of their discussions are presented in Annexes 1 (HUS) and 2 (Influenza). These reports provide useful information for guidance of countries of the Southern Cone network on how to initiate or improve the surveillance of HUS and Influenza, including protocol designs, list of activities to be performed and their costs. Training activities on HUS surveillance to take place in Argentina during the current year are being organized by ANLIS Malbran in collaboration with the CDC, for epidemiologists, clinicians and laboratory personnel from countries of the network. Subsequently a similar workshop will be held in Bolivia for personnel from three Bolivian cities. A workshop on Influenza has also been proposed to be conducted by the ANLIS Malbran.