Section 3. Laboratory Capacity
The Contra Costa Public Health Laboratory is a modern, state-of-the-art public health laboratory with capabilities to rapidly identify Influenza A/H5 in specimens from patients with influenza-like illness. The laboratory has the ability to perform direct antigen tests for Influenza A and B, isolation of Influenza viruses in cell culture, detection of Influenza A and B RNA directly from patient specimens by reverse transcriptase polymerase chain reaction (RT-PCR), and subtyping of Influenza A viruses (H1, H3, and H5) by RT-PCR.

The strategy for initial laboratory testing is to rapidly identify an influenza A virus infection and to exclude other common viral respiratory infections. Results of rapid antigen and RT-PCR assays are available within 1-3 hours of receipt of the sample in the Contra Costa Public Health Laboratory. Subtyping of influenza A viruses detected by PCR will require an additional 2 hours.

Identification of Influenza A/H5
An immunochromatographic assay is used to detect influenza A or B viruses directly in the clinical sample. Clinical specimens, obtained as soon as possible after the onset of symptoms, are preferable as the number of virus infected cells decreases during the course of infection. This assay can distinguish between influenza A and B but not between subtypes of influenza A. This assay can also be used to identify influenza A or B virus isolated in cell culture. This assay is rapid, requiring only 15 minutes, but it is less sensitive than isolation of the virus in cell culture or detection of viral RNA by RT-PCR. This assay is performed in the Biosafety Level 2 Virology Laboratory.

Virus isolation is a sensitive technique with the advantage that the virus is available both for identification and further antigenic and genetic characterization including vaccine development and subtyping of influenza A isolates. The clinical specimen is treated with antibiotics and inoculated into a Rhesus monkey cell line for culturing respiratory viruses. Influenza viruses are usually detected in 1 to 7 days. Identification of a viral isolate is then done using an immunofluorescent antibody assay (approximately 2 hours). Subtyping of an influenza A isolate is performed using PCR (approximately 3 hours). Initial culturing and identification (including PCR) are performed in the Biosafety Level 2 Virology Laboratory. Biosafety Level 3 Ag (BSL-3 (Ag)) practices, procedures and facilities are required to work with the Highly Pathogenic Avian Influenza (H5N1). This requires registration as a Select Agent Laboratory and a BSL-3 (Ag) facility. The Contra Costa Public Health Laboratory is registered as a Select Agent Laboratory and has a BSL-3 facility. However, once a virus isolate has been identified as influenza A H5N1, our laboratory will not perform any additional testing on the isolate because our facility does not have a specific permit from
APHIS and is not approved as an agricultural (Ag) BSL-3 laboratory. We will transfer the isolate to the State VRDL for additional characterization.

Polymerase chain reaction (PCR) is a powerful and sensitive technique for the identification of influenza virus genomes. The influenza virus genome is single stranded RNA, and a DNA copy (cDNA) must be synthesized first using a reverse transcriptase (RT) polymerase. The procedure for amplifying the RNA genome (RT-PCR) requires a pair of oligonucleotide primers. These primer pairs are designed on the basis of the known HA sequences of influenza A and B and of A subtypes (H1, H3 and H5). The RT-PCR assay requires approximately 3 hours to perform from the time the specimen is received in the Contra Costa Public Health Laboratory. PCR is performed in the Biosafety Level 2 Virology Laboratory.

No serologic tests for influenza infection are available at the Contra Costa Public Health Laboratory.

The above assays are routinely performed by the Contra Costa Public Health Laboratory during the influenza season, but are available all year long for detection of non-contemporary strains or avian strains of influenza. Avian testing is not performed at the Contra Costa Public Health Laboratory. Contact the University of California, Davis or the California Department of Agriculture regarding animal testing.

**Laboratory Surge Capacity**
Currently there is one Senior Public Health Microbiologist and four Public Health Microbiologists assigned to the Virology/Immunology section of the Contra Costa Public Health Laboratory. All of these individuals have been trained and are experienced in performing the influenza direct immunochromatographic assay and virus isolation in cell culture. Five individuals in the laboratory have been trained and are experienced in performing the influenza RT-PCR assays. Four additional Public Health Microbiologists have been trained in performing virus isolation in cell culture and in performing the influenza direct immunochromatographic assay. All Senior Public Health Microbiologists will be trained in performing the RT-PCR influenza assay by December 31, 2007.

**Collaboration with Respiratory Laboratory Network (CDC/VRDL/CAPHLD)**
The Contra Costa Public Health Laboratory is an active participant in the Respiratory Laboratory Network (RLN). We participated in all teleconferences and training activities provided to date by the RLN. We have received and validated the use of the CDC reagents for influenza A and B and for subtyping of H1, H3, and H5 influenza A viruses. We also have purchased and evaluated two commercial RT-PCR assays for influenza A and H5 viruses. We have shared this evaluation data with the State VRDL and the other local public health laboratories in California through the CAPHLD.
On December 19, 2005 the Contra Costa Public Health Laboratory sent a community update on Avian Influenza Testing to all clinical laboratories in Contra Costa County. This update was based on the State VRDL recommendations and included information on criteria for testing, reporting and consultation, safety, testing at their facility, laboratory specimen collection and shipping.

**Specific Actions during Pandemic Influenza Stages**

**Interpandemic/Pandemic Alert (WHO Phases 1-5)**

1) Contra Costa Public Health Laboratory will encourage clinical laboratories in Contra Costa County to submit specimens from suspected cases of human infection with novel influenza for viral testing at the Contra Costa Public Health Laboratory.

   a) Contra Costa Public Health Laboratory will continue to distribute as they become available VRDL guidelines describing how to request testing for novel influenza virus.

   b) Contra Costa Public Health Laboratory will continue to distribute as they become available VRDL protocols to ensure clinical laboratories in Contra Costa County notify us that they are requesting testing for novel influenza virus.

   c) Contra Costa Public Health Laboratory will continue to distribute as they become available VRDL guidelines for specimen collection, handling, and shipping, and post them on the Contra Costa Public Health website.

   d) Contra Costa Public Health Laboratory will abide by and distribute to clinical laboratories in Contra Costa County VRDL laboratory biosafety guidelines for handling and processing specimens or isolates of influenza A (H5N1) strains as they become available and to post them on the Contra Costa Public Health website.

2) Contra Costa Public Health Laboratory will continue to provide enhanced laboratory testing protocols in support of and in coordination with enhanced human surveillance protocols. We will maintain the capacity for subtype testing for influenza A (H1, H3, and H5). We will maintain the capacity to test to identify other respiratory pathogens that present with influenza-like illness. We will coordinate with the VRDL transporting to the CDC any influenza A virus that can not be subtyped.

3) Contra Costa Public Health Laboratory will provide detailed guidance to local clinical laboratories on alternative diagnostic testing options, including rapid antigen detection, immunofluorescence assays, and PCR, including required biosafety levels.
4) Contra Costa Public Health Laboratory will work with the VRDL to develop contingency plans for possible nationwide supply and reagent shortages, including performing inventory of our own supplies and equipment and determining trigger points for ordering surge supplies. We will accept and use reagents prepared by the CDC or VRDL for identifying the novel virus strain as a part of our participation in the RLN. We will also continue to evaluate and stock commercially available influenza A H5 reagents and associated supplies for possible surge capacity use.

5) Contra Costa Public Health Laboratory will continue to develop appropriate personnel capacity (including training) to support enhanced laboratory surveillance for influenza at the County level. We will evaluate internally the need for additional personnel surge capacity, including re-certification of non-traditional labor pool and redirection and hiring of additional laboratory employees.

6) Contra Costa Public Health Laboratory will work to develop contingency plans to ensure adequate laboratory capacity for diagnostic testing of bacterial agents and other pathogens associated with infections secondary to influenza.

7) Contra Costa Public Health Laboratory will institute surveillance for influenza-like illness among its own laboratory personnel working with novel influenza viruses, and adopt the VRDL’s protocols for clinical assessment and management of exposed laboratory personnel (both symptomatic and asymptomatic) when the protocols become available.

8) Contra Costa Public Health Laboratory will consider upgrading its BSL-3 laboratory to BSL-3 (Ag) enhanced to provide additional pandemic influenza laboratory capacity in the State’s public health system, if State funding is made available.

9) Contra Costa Public Health Laboratory will review, based on VRDL recommendations, and revise enhanced laboratory diagnostic protocols for influenza and other respiratory pathogens that may mimic influenza and distribute these to local clinical laboratories.

10) Contra Costa Public Health Laboratory will develop contingencies and protocols at the local level to deal with redirecting resources to influenza testing and for rationing influenza testing.

11) Contra Costa Public Health Laboratory will review changes to technical guidance by VRDL and participate in training as needed.

12) Contra Costa Public Health Laboratory will delineate resources needed to maintain expanded critical laboratory testing capacity during a pandemic, including laboratory equipment and supplies, re-certification of non-traditional labor pool, and redirection and hiring of additional laboratory employees.
13) Contra Costa Public Health Laboratory will consider expanded diagnostic testing including antiviral resistance testing, neutralizing antibody assays to test for immunity to the novel virus, and egg-based or other alternate culture methods to isolate novel viruses that are difficult to grow by standard culture methods to provide additional pandemic influenza laboratory capacity in the State’s public health system, if State funding is made available.

14) Contra Costa Public Health Laboratory will ensure capacity to perform/support local special clinical and epidemiological studies.

**Pandemic (WHO Phase 6)**

1) Contra Costa Public Health Laboratory will review recommendations from the VRDL and enhance, as needed, diagnostic capacity for novel strain virus, with particular attention to rationing laboratory testing.

2) Contra Costa Public Health Laboratory will continue other pre-phase 6 activities as appropriate.

**Postpandemic (WHO Postpandemic Period)**

1) Contra Costa Public Health Laboratory will continue to provide laboratory services for more typical wintertime ‘epidemic’ influenza cycle

2) Contra Costa Public Health Laboratory will be prepared for a possible second wave of the pandemic influenza

**Appendix**

1. Community Update: Avian Influenza Testing 12/19/2005
   a. Criteria for testing
   b. Reporting/Consultation
   c. Safety
   d. Testing at your facility
   e. Laboratory specimen collection
   f. Resources/More information

2. Algorithm for submittal of specimens for suspect avian influenza cases – Contra Costa County – 2006

3. Influenza Testing Algorithm at Contra Costa Public Health Laboratory

4. Laboratory Biosafety Guidelines for Handling and Processing Specimens or Isolates of Novel Influenza Strains
5. Laboratory Personnel Available for Response to Pandemic Influenza

6. List of Supplies Needed for 100% Increase in Laboratory Processing