Budget Planning Guidance for the Influenza Incidence Surveillance Project FOA

May 6, 2010

For the funding opportunity titled "Influenza Incidence Surveillance Project", we would like to provide some guidance in budgetary planning for the cost of laboratory testing and the number of specimens to expect.

Specimen Estimation

It is difficult to predict the volume of ILI that providers will in a given season. Below are some calculations based on historic ILINet data and the previous year's experience by Influenza Incidence Surveillance Project participating sites.

- 5-6 providers per site x 10 specimens per provider per week= 50-60 specimens received at each state lab per week maximum
- Providers with a weekly patient volume of 100-150 patients will average 6 ILI patients per week (CDC ILINet data).
    - 6 specimens x 6 providers x 52 weeks = 1872
    - During the 1st year of this surveillance project, sites with excellent provider compliance and 5 or 6 providers enrolled collected ~500 specimens. Since our project began in October and the influenza season began in August, doubling this number gives 1000 specimens expected for the coming year.
- Additional strategies can also minimize the volume of testing specimens by multi-pathogen testing methods, such as pre-screening for influenza and batching a few weeks of specimens during the non-influenza season weeks.

Multi-pathogen Testing

The project requires PCR methods for identifying multiple respiratory pathogens which have a high cost and are rather labor intensive. The PCR methodology may vary by site – please note that we have no preference since each method has advantages and disadvantages. Below is some estimated pricing information on a couple of options for testing. Please note that these costs may vary depending on the availability of different reagents in the laboratories, different protocols, etc. In addition, extraction costs are not included.

General comparison of methodologies

	Real-time PCR	Multiplex PCR Bead System†	Comment
Cost*	$	$$	*May need to supplement multiplex PCR-bead system with real-time PCR assays for some targets requiring investment in both platforms.
Flexibility	Yes	No	
Throughput**	5-6 samples per plate	92-94 samples per plate	**Although real-time PCR is much faster than bead-based systems (< 3hrs vs up to 9hrs), more time will be necessary to assemble multiple monoplex real-time PCR assays, so overall throughput would be greater for multiplex bead assays.
Sensitivity/Specificity***	Very high	High	***The Luminex x-TAG RVP assay cannot adequately detect adenovirus species C, or serotypes 7a and 41.
Risk of assay error	High	Low	
Includes pH1N1 test	Yes	No	

†Luminex-based: Luminex x-TAG RVP, Qiagen ResPlex I & II, EraGen MultiCode PLx RVP

1. **<u>CDC Real-Time PCR/RT-PCR Assays for Respiratory Pathogens</u>**: These assays utilize Taqman® probes to provide cost-effective, robust, sensitive and specific detection of non-flu respiratory pathogens in "single-plex" reactions. These assays are designed to work along with Influenza Real-time RT-PCR assays using a single chemistry and the same platforms. The cost is approximately $2 per target per specimen. Thus for 12 targets, the cost per specimen would be approximately $24. On a 96 well plate, 12 targets can be run for 5 or 6 specimens. Note there is flexibility in the actual targets chosen for the assay. Given that a smaller number of specimens can be run at a time, batching for several weeks before running would not be needed for testing in low volume weeks (ie, most of the year).
    a. <u>Laboratorian time</u>: approximately 30 minutes to set up and 1 ½ hours to run for each plate, if only 1 plate is run at a time.
    b. <u>Time comparison estimation</u>: in 8 hrs, Luminex will run 92 specimens. In 8 hours, the CDC assay would run 20 specimens if only 1 PCR machine is being used.
    c. <u>Reporting test results</u>: if CDC provides the sequences and protocol, and the site creates their own assay, this could be CLIA approved and used as a diagnostic test. If CDC provides the reagents as an assay, this is not FDA approved and cannot be used for clinical treatment decisions.

Example of plate set up for 12 targets

	1	2	3	4	5	6	7	8	9	10	11	12
A	NTC	NTC	NTC	NTC	NTC	NTC	NTC	NTC	NTC	NTC	NTC	NTC
B	MOCK*	MOCK	MOCK	MOCK	MOCK	MOCK	MOCK	MOCK	MOCK	MOCK	MOCK	MOCK
C	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1
D	S2	S2	S2	S2	S2	S2	S2	S2	S2	S2	S2	S2
E	S3	S3	S3	S3	S3	S3	S3	S3	S3	S3	S3	S3
F	S4	S4	S4	S4	S4	S4	S4	S4	S4	S4	S4	S4
G	S5	S5	S5	S5	S5	S5	S5	S5	S5	S5	S5	S5
H	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS
	Target1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T12

*MOCK may not be required with each run, thus 6 specimens could be tested.

2. **Luminex xTAG RVP assay (**http://www.luminexcorp.com/rvp/overview.html): Luminex xTAG RVP kit is the only FDA approved assay that may be used for the reporting of results for clinical use; however, your site may determine if this is a requirement. We are working with two distributors of the Luminex xTAG RVP assays to get the best possible price for the kits (assuming that the assays are ordered in bulk). It looks like we can get a discounted "Public Health" price of *approximately* $5000 per kit, or ~$53-54 per specimen. This estimate includes the cost of additional reagents required, but not included in the xTAG kit (listed below). The kit runs 96 tests, and there is some discussion about whether 2 to 4 controls are needed per run. Right now, it would be safe to plan for 92 specimens to be tested per kit.
    a. Additional reagents required but not included in xTAG kit:
        i. TaKaRa Taq™ Hot Start Version (10X PCR Buffer and 2.5 mM TaKaRa dNTPS). This particular reagent should be purchased in the same lot number as the xTAG RVP kit for which it will be used, otherwise the assay is not considered FDA approved.
        ii. Luminex sheath fluid, 20L 1x container (should only need to purchase this about 2x during the study period)
        iii. Luminex calibrator beads 1 & 2 (purchased once to twice during study period)
        iv. Luminex control beads 1 & 2 (purchased once to twice during study period)
    b. Laboratorian time: 6-8 hours, start to finish, but does not have to be hands-on during that time.
    c. Training: The training at each state location is provided at No Charge. The state will receive 2 kits(96) reactions in each kit , all ancillaries need to run the kits and 3 days of onsite training that is complimentary.
    d. Software compatibility: depending upon when a site has purchased their Luminex instrument, there may be an issue with compatibility of the analysis software that is included in the xTAG kit with the Luminex machine. Software status checks on exisitng

Luminex instrument done complimentary and upgrades provided at no charge as needed. Complimentary instrument checks done by a field service engineer and service provided as needed to bring instruments into working condition (some of the systems purchased through Bio-rad as long as 5 years have never been used and need to be brought into condition to perform assays)

e. Instrumentation: Complimentary assessment of PCR capability done to insure thermal cyclers on site will optimize assay performance.

3. **Other possible assays**:
   a. Qiagen ResPLEX II: Luminex bead-based, includes bacterial targets (http://www1.qiagen.com/Products/ResPlexIPanel.aspx)
   b. Fast-Track FTD RP: Multiplex real-time PCR suited for use with the ABI 7500, Rotor-Gene 6000, Bio-Rad CFX96 and the LightCycler480 (http://www.fast-trackdiagnostics.com/pages/order_cat/catalogue.cgi?state=11&cust=0&cat_no=FTD-2)
   c. EraGen MultiCode PLx RVP: Luminex bead-based (http://www.eragen.com/contentPage.cfm?ID=430)
   d. Seegene Seeplex RPA: Capillary amplicon analysis (http://www.seegene.com/en/diagnosis/d_seeplex.php)
   e. AutoGenomics INFINITI RVP+: Biofilm array (http://www.autogenomics.com/1/infectious_respiratory.php)
   f. Idaho Technology Film Array: Biofilm array (pouch) (http://www.idahotech.com/FilmArray)