WSLH WISCONSIN STATE LABORATORY OF HYGIENE

EVALUATION OF DIAGNOSTIC TESTING FOR OUTBREAK RESPONSE IN A PUBLIC HEALTH LABORATORY

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ABSTRACT

Public health laboratory response to emerging and reemerging disease threats is evolving from serological and culture-based methods to molecular based methods. In the past 5 years, the Wisconsin State Laboratory of Hygiene (WSLH) has been repeatedly called upon to respond to outbreaks of non-routine infectious disease including monkeypox, norovirus, *B. pertussis*, and mumps virus. Traditional "gold standard" tests for these agents are either unavailable or unreliable. However, prompt development and deployment of rapid, sensitive, and high-throughput real-time PCR has proven to be essential for public health response to these outbreaks. A case in point is the recent mumps outbreak. The lack of availability of a standardized, FDA approved test method, lack of formal proficiency testing and the inexperience of microbiologists and clinicians in recognizing and identifying mumps infections created a major challenge.

Specifically, while the "gold standard" diagnostic method for mumps was considered to be serology, concerns with false positive results arose due to the low prevalence of disease and the large volume of specimens received from individuals with non-specific clinical findings. Consequently, it was necessary to provide an alternative testing method (PCR) that had high sensitivity and specificity and rapid turn around time so that appropriate public health measures could be implemented. WSLH developed a real-time PCR assay for mumps virus within two weeks and evaluated it concurrently with specimens received by comparison to virus culture as well as serology. Subsequently, serology was discontinued and mumps PCR became the preferred testing mechanism.

Lessons can be learned from events such as these. Molecular assays can be developed and evaluated quickly and they provide both good sensitivity and high throughput capabilities. However, there are drawbacks including lack of a reliable "gold standard" method with which to compare, lack of control materials, unfamiliarity with basic virological characteristics due to very few positives and lack of residual specimens, to name a few. There can also be debate as to the best testing method to use. Often more than one is chosen. WSLH is now more experienced using molecular assays for outbreak response. We have learned how to deal with the increased sample load of an outbreak efficiently while being able to accomplish routine testing. Due to recent resurgence of vaccine preventable diseases in Wisconsin, steps have been taken to establish molecular assays for measles, rubella virus, and others in the event that they too reemerge.

OBJECTIVE

- To describe the development and implementation of a Mumps real-time PCR assay.
- To describe public health laboratory outbreak response evolving from traditional serological and culture-based methods to molecular methods.

METHODS

- **Serology:** Serum specimens were tested for IgM using Bion® mumps virus antigen substrate IFA slides according to conventional methods.
- **Virus Culture:** Buccal swabs and urine specimens were tested by culture using primary Rhesus monkey kidney tubes according to standardized methods. Tubes were observed for 10 days for CPE, hemadsorbed at days 5 and 10, and stained with Chemicon Mumps MAB, if necessary.
- **Real-time PCR:** 10 ml of each urine specimen was centrifuged at 400 *x g* for 10 minutes. 8.5 ml of the supernatant was removed and the sediment resuspended in the remaining 1.5 ml. 0.5 ml of urine and 1.0ml of each buccal swab specimen was centrifuged at 5000 *x g* for 10 minutes to concentrate further. 0.2ml of the concentrated specimen was used for viral RNA extraction. Viral RNA was extracted using the Total Nucleic Acid kit with the Roche MagNA Pure® LC and eluted in 50µl of buffer. 5µl of RNA was tested using primers designed by Uchida, et al. Data analysis was performed using the fit points method with the LightCycler® software. A positive mumps specimen was defined as having a crossing threshold (CT) value of $\leq$40 cycles.

Figure 1. Mumps Oligos and TaqMan Probe

Primer/probe ID	Sequence (5'-3')	Forward/reverse	Tag
Mumps F	tct cac cca tag cag gga gtt ata t	Forward	---
Mumps R	gtt aga ctt cga cag ttt gca aca a	Reverse	---
Mumps TM	agg cga ttt gta gca ctg gat gga aca	---	6FAM/BHQ1

Figure 2. Mumps LightCycler® PCR master mix

Reagent	Final Concentration
Mumps F primer	0.5µM
Mumps R primer	0.5µM
Mumps TM Probe	0.2µM
$MgCl_2$	4.75mM
RT-PCR Enzyme mix (Vial #1)	0.4µL
RT-PCR Reaction Mix HybProbe (Vial #2)	4.0µL
Uracil-DNA Glycosylase-heat labile	0.5U
Water	to 15µL

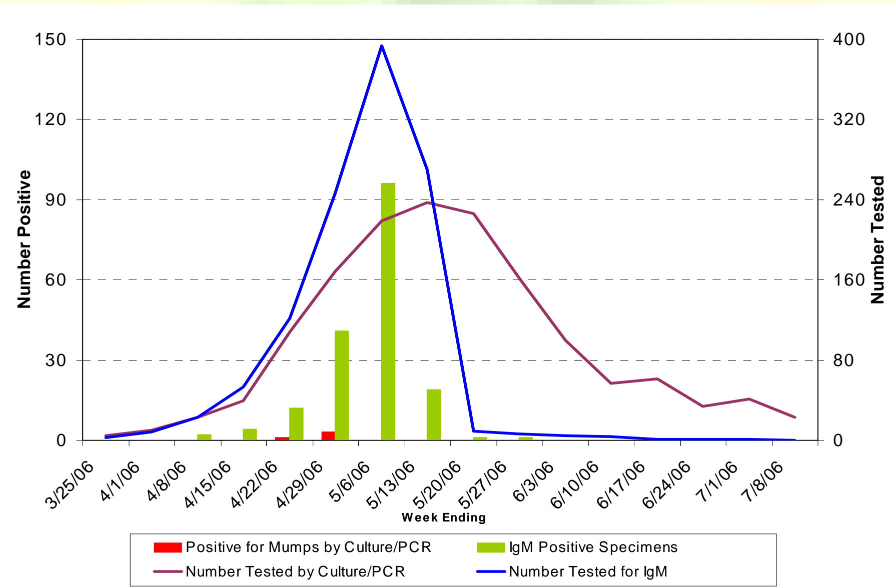
Figure 3. Mumps LightCycler® PCR cycling conditions

Program	Temp (°C)	Hold Time (s)	Slope (°C/s)	Acquisition mode	cycles
RT	55	1800	20	None	1
Denaturation	95	30	20	None	1
Amplification	95	5	20	None	45
	59	30	20	Single	
Cooling	40	30	20	None	1

RESULTS

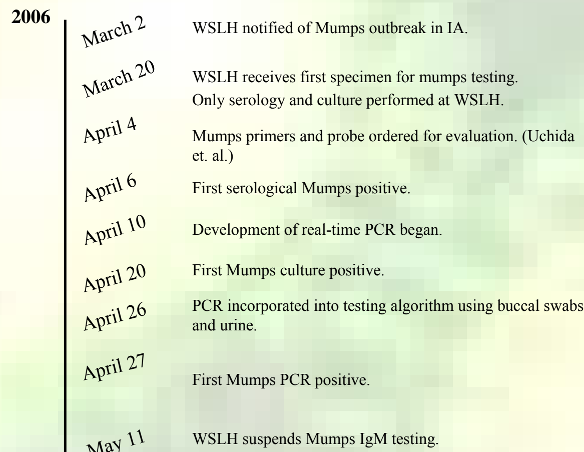
- Pathogens known to cause respiratory disease and viruses in the paramyxovirus family were selected to determine any cross-reactivity with the Mumps PCR assay. Influenza A H1, H3; Influenza B; RSV A, B; Parainfluenza 1, 2, 3, 4a; Measles; Rubella; *Chlamydia pneumoniae; Mycomplasma pneumoniae; B. pertussis; B. bronchiseptica;* Adenovirus 35; Metapneumovirus, Legionella, Cocksackie B1; Rhinovirus 16, 49; Parechovirus1; SARS; group A Streptococcus; and human DNA were tested with 100% specificity.
- 17 Mumps tissue culture isolates and CDC wild type Mumps RNA were all tested with 100% being positive.
- 176 of 1146 patient specimens were positive for Mumps IgM.
- Of the 1513 patient specimens tested by Mumps PCR and culture, only 4 were positive. All 4 were positive by both methods.

Figure 4. Comparison of the number of specimens tested by serology and culture/PCR and number positive for mumps by serology and culture/PCR.



DISCUSSION

- "Gold standard" diagnostic method for mumps virus was IgM serology.
- It was necessary to provide an alternative testing method, PCR, that had high specificity and sensitivity and rapid turn around time so public health measures could be implemented.
- Real-time PCR was ran concurrently with specimens received by comparison to virus culture.



DISCUSSION (continued)

- Issues of evaluating and validating new tests for immediate use in outbreak response:
  - **Lack of the perfect "gold standard":** Evaluation of a new method is difficult when the gold standard is imperfect.
    - **Unfamiliarity with the basic culture characteristics:** Due to the infrequency of the virus circulating in recent years, few microbiologists were familiar with the characteristics of mumps virus in cell culture.
    - **Concerns with false serological IgM positives:** Concerns arose due to the low positivity rate of the Mumps culture, the low prevalence of disease and the large volume of specimens received from individuals with non-specific clinical findings.
  - **Lack of residual positive specimens for methods evaluation.**
  - **Lack of clinical or epidemiological information to correlate with test results:** Mumps in a highly vaccinated population varies in clinical symptoms ranging from asymptomatic to those with classical parotitis.
  - **Lack of time to perform thorough and complete evaluation before using the test in the outbreak.**
  - **Continued use of virus culture with PCR:** This increased the workload significantly while having to continue routine testing.
- Advantages of molecular assays for outbreak response:
  - **Rapid development and implementation:** Within weeks, the Mumps real-time PCR was evaluated and implemented into the testing algorithm.
  - **High sensitivity and specificity:** Known concentrations of mumps virus and other bacterial and viral pathogens including paramyxoviruses were tested. Archived specimens and stock virus were tested to confirm analytical sensitivity and specificity.
  - **High throughput and rapid turn around time:** WSLH has increased its molecular testing capacity by the addition of 3 MagNA Pure® instruments, 3 LightCycler® instruments, and an ABI 7500 therefore making high volume testing feasible.
  - **Decrease reliance on cell culture:** Increased detection by molecular methods of viruses that are difficult or impossible to isolate using traditional methods can justify decreasing or eliminating cell culture in virology laboratories.
  - **Training can be achieved easily:** Because of standardized methods of molecular assays, cross training can easily be achieved among staff.

CONCLUSIONS

- WSLH has demonstrated that molecular assays can be evaluated and implemented very quickly in response to an outbreak.
- WSLH has demonstrated the value of rapid and sensitive molecular assays for outbreak response.
- WSLH has learned how to deal with the increased sample load of an outbreak efficiently while continuing routine testing.
- Due to recent resurgences of vaccine preventable diseases in Wisconsin and worldwide, steps have been taken to establish molecular assays for measles, rubella virus, and others in the event they too reemerge.
- **The use of unstandardized, infrequently used tests in a low prevalence population with non-specific clinical findings can result in false positive results.**

REFERENCES

Uchida, K., Shinohara, M., Shimada, S., Segawa, Y., Doi, R., Gotoh, A., and Hondo, R. 2005. *Rapid and Sensitive Detection of Mumps Virus RNA Directly From Clinical Samples by Real-Time PCR.* Journal of Medical Virology. 75:470-474.