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SINGLE RESPIRATORY COLLECTION SYSTEM, STARPLEX S-160 NASO, FOR MULTIPLE INVESTIGATIONS

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Abstract

Objective: The usual route for bacterial and viral pathogen identification relies on the collection of separate patient samples. Depending on the clinical symptoms there is an opportunity to lessen the invasiveness of the sample collection process. In the early stages of a *Bordetella pertussis* infection, the symptoms resemble those of a common cold and if the patient is in the first two to three years of life, multiple viral pathogens may also be the causative agent. In this facility the only diagnostic test utilized to identify Whooping cough is a Real-Time PCR assay and is run in conjunction with viral DFA and culture for routine samples, and may also include multiplex PCR detection of viruses. A single sample collection and transport system (S-160 NASO) has been validated to give optimal patient results.

Method: Nasopharyngeal swabs are collected for the investigation of *B. pertussis* alone, a panel of viruses or a combination of the two, depending on the presentation. A real-time assay targeting the IS *481* region of *B. pertussis* is utilized to diagnose Whooping cough, while DFA testing with culture back-up is utilized to identify eight major viral pathogens on a routine basis. Multiplex viral PCR investigation is performed for institution outbreaks and special requests where standard tests do not identify a pathogen.

Results: Samples collected in the transport media showed no inhibitory effects on the PCR assays either for the real-time *B. pertussis* assay or in the viral multiplex assay developed by Luminex Molecular Diagnostics. A total of 140 samples were received over a period of 8 months for identification of *B. pertussis* alone or in combination with viral investigation. In the patient population ranging from 4–17 years of age, 24% of the samples were for *B. pertussis* alone. In the <= 3 years age group *B. pertussis* was ordered in conjunction with viral investigation (76%) with the majority (85%) of those being under 2 years of age. Despite the literature reflecting a rise in detection of whooping cough (for multiple reasons) this facility showed only a 2% positivity rate, while viral pathogens were detected at a rate of 57%. Of the viruses identified 43% were Respiratory Syncytial Virus (RSV), 9% were Parainfluenza and 3% each for Influenza B and Metapneumovirus. Dual viral infections were also detected at a rate of 17 % while viral and bacterial (*B. pertussis* and RSV) infections at 1%. These percentages fluctuate year to year based on circulating pockets of infectious agents.

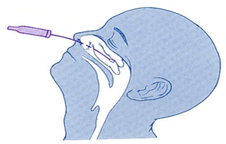
Conclusions: Sample collection can be a challenge and sub-optimal samples can result in anything from useless to misleading information. When multiple diagnostic tests can be run reliably on a single sample the outcome will be beneficial to the laboratory, care provider and patient. The S-160 NASO has proven to be a reliable component of the diagnostic process. A collection procedure that is well tolerated leads to a high quality sample for a number of testing algorithms that may include direct antigen testing, culture amplification (selected pathogens) and molecular based assays without any demonstrated interference.

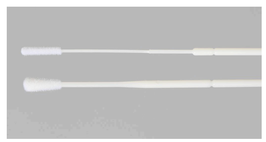
Introduction

While the testing policy and process for *Bordetella pertussis* was being investigated, cited literature indicated that direct testing and culture lacked the required sensitivity. As a result we decided to rely on a molecular based assay for identification of this reportable disease. Due to its presentation concurrent testing for viral pathogens is often requested, requiring a separate swab. Simultaneously the collection systems for viral respiratory illnesses were in transition due to the Post-SARS experience and need to achieve high quality specimens that were easily collected. The result was the implementation of a single Starplex Scientific S-160 NASO for all viral and *B. pertussis* diagnostics.

Materials and Methods

Respiratory sample collection along with the extent of investigation falls to the clinical teams in each area of the hospitals and long-term care facilities. After the emergence of the SARS virus, steps were taken to improve infection control practices when patients exhibiting respiratory symptoms were examined. Our facilities adopted the use of swab collection systems for both children and adults, replacing the often used aspirates. On-line educational material was made available for all staff including a training video for proper collection technique. http://www.lhsc.on.ca/lab/MICRO/virology/virology_videos.htm





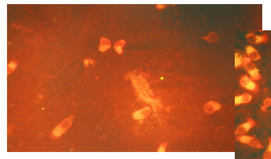
A published protocol for Real-Time PCR detection[1] was adopted by the lab replacing a previous published protocol utilizing conventional PCR and gel detection. The assay utilizes the Roche LightCycler Fast Start DNA Master PLUS Hybridization Probe kit and primers and probes are directed at the IS *481* region of *B. pertussis*. Extractions are performed using the Roche MagNA Pure Compact instrument and Total Nucleic Acid Isolation Kit reagents.

Standardized published sample handling procedures are utilized for producing DFA slide material for rapid testing, followed by a culture confirmation backup of all samples, possibly excluding institutional outbreaks. The laboratory currently examines both direct smears and tissue culture for Influenza A/B, RSV, Parainfluenza 1/2/3, Adeno and Metapneumovirus. Shell vial culture from Diagnostic Hybrids (R-Mix) and antibody reagent material from Chemicon and DHI are used in the viral identification.

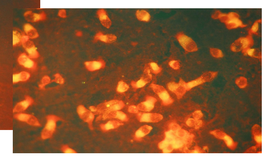
When institutional outbreaks go undiagnosed or in special cases cleared by the Medical Microbiologist, the xTAG™ RVP from Luminex Molecular Diagnostics is employed. Extraction procedures are the same for *B. pertussis* and this assay normally requires 6.5 hours of technologist time to complete.

Results

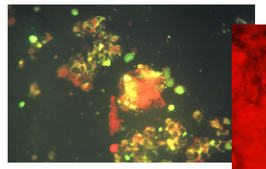
One of the main advantages of switching to a single recovery sample collection system for multiple investigations has been the reduced invasiveness and higher comfort level to the patient. The move away from aspirate collection has improved Infection Control measures in the hospital and almost eliminated the labs reception of leaking samples. It has improved upon cellular material recovery with the elimination of samples labeled as NP aspirates, but resembling a sputum collection.



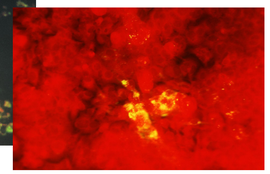
Conventional Swab



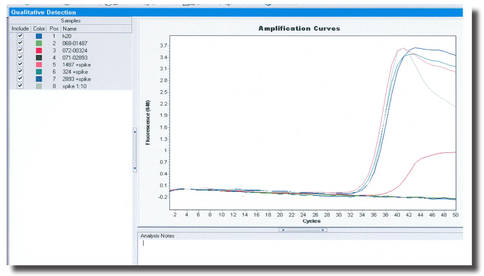
Flocked Swab



DFA



Culture Confirmation



RT - PCR

Although *B. pertussis* has seen resurgence in the last few years world wide, the prevalence in this institution remains quite low. The ruling out of this respiratory agent along with similarly presenting viral etiologies does continue to be the focus of the clinical units throughout our In-patient, Out-patient and long-term care areas.

Respiratory Samples Received: November 2006 – June 2007

	Negatives	*B. pertussis* Positives	Viral Agent Positives	Total
***B. pertussis* Requests**	34	2	N/A	36
***B. pertussis* + Viral Requests**	44	1*	59*	104
TOTAL	78	3	59	140

- Single patient sample(*) dual infection with *B. pertussis* and RSV
- Note: Recent single respiratory sample received tested positive for *B. pertussis*, Influenza A and RSV (April 2008)

Molecular analysis with xTAG™ RVP from Luminex Molecular Diagnostics was the primary method for detection of dual infections. PCR inhibition did not present a problem with the use of this collection system in either molecular amplification assay. All of the dual infections that did not involve Entero/Rhino viruses were confirmed by culture isolation. Current sample protocols do not include extended incubation times or the use of screening antibodies to detect members of the *Picornaviridae* family. As expected in the younger patient population the predominant virus identified was Respiratory Syncytial Virus (43%) and with the help of RVP almost equal numbers of Type A and B were detected. The remainder of the viruses identified in our geographic area included Parainfluenza (Type 1 and 3), Influenza B and Metapneumovirus.

Conclusion

Providing a patient with the least taxing hospital encounter coupled with useful diagnostic information should be the goal of all health care facilities. Due to the testing algorithm of this laboratory we are allowed to take advantage of innovative products in order to provide a high quality service. The S-160 NASO has enabled us to provide rapid turn-around-time on high quality samples and supply clinically relevant and reliable diagnostic information. A majority of sample processed are collected in Emergency departments where clinical decisions are being made related to severity of disease and admission to the facility. The ability to utilize a single patient sample for a series of clinical investigations has proven to be of great benefit to the testing lab and has also contributed to a higher level of safety for health care professionals that are involved in patient contact.

Reference

1. Udo et. al. Real-Time PCR Assay Targeting IS *481* of *Bordetella pertussis* and Molecular Basis for Detecting *Bordetella holmesii*. JCM, Vol. 39, No.5, pgs 1963-1965.