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Comparison of a new transport medium with universal transport medium at a tropical field site☆,☆☆,☆☆☆,★,★★

Elizabeth P. Schlaudecker [a,*], Joan P. Heck [b], Elizabeth T. MacIntyre [b], Ruben Martinez [b], Caitlin N. Dodd [c], Monica M. McNeal [d], Mary A. Staat [d], Jeffery E. Heck [e], Mark C. Steinhoff [a]

[a] Division of Infectious Diseases, Global Health Center, Cincinnati Children's Hospital and Medical Center, Cincinnati, OH, USA
[b] Shoulder to Shoulder, Santa Lucia, Intibucá, Honduras
[c] Global Health Center, Cincinnati Children's Hospital and Medical Center, Cincinnati, OH, USA
[d] Division of Infectious Diseases, Cincinnati Children's Hospital and Medical Center, Cincinnati, OH, USA
[e] Department of Family Medicine, University of North Carolina, Asheville, NC, USA

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ABSTRACT

Limited data are available in rural Honduran settings describing the etiology of respiratory infections, partially due to limited specimen transport. A new molecular transport media (MTM) preserves released nucleic acid at ambient temperature for later detection. Prospective surveillance was conducted in a Honduran clinic to identify 233 children less than 5 years of age presenting with respiratory symptoms. We obtained 2 nasopharyngeal samples and stored 1 in PrimeStore® MTM at room temperature and 1 in universal transport media (UTM) at −80 °C. The specimens were then transported to Cincinnati Children's Hospital and tested for 16 respiratory viruses using a multiplex PCR panel. The 2 specimen collection systems were similar for detecting the 4 most common viruses: influenza (Kappa = 0.7676, $P < 0.0001$), human metapneumovirus (Kappa = 0.8770, $P < 0.0001$), respiratory syncytial virus (Kappa = 0.6849, $P < 0.0001$), and parainfluenza (Kappa = 0.8796, $P < 0.0001$). These results suggest that clinical specimens transported via PrimeStore® MTM and UTM yield similar viral multiplex PCR results.

1. Introduction

Acute respiratory infections (ARIs), including pneumonia, are the leading cause of death among children less than 5 years of age (Black et al., 2010; Rudan et al., 2008). Recent data from the World Health Organization (WHO) suggest that 18% of global deaths in children less than 5 are due to pneumonia (Black et al., 2010; Rudan et al., 2008). Throughout the world, the etiologies of ARIs and pneumonia are largely unknown. It is estimated that 18%–65% of global pediatric patients admitted to the hospital for ARIs and pneumonia are infected with viruses (Arnold et al., 2008; Bonzel et al., 2008; Juven et al., 2000; Tajima et al., 2006; Tsolia et al., 2004), including influenza. Some data from the tropics and subtropics demonstrate incidence and hospitalization rates for influenza that exceed those reported for temperate regions (Brooks and Steinhoff, 2011; Chiu et al., 2002; Henkle et al., 2011; Nascimento-Carvalho et al., 2008; Zaman et al., 2008). Several sites in sub-Saharan Africa, Latin America, and Asia have recently added influenza surveillance programs (Higgs et al., 2008; Nair et al., 2011; Yazdanbakhsh and Kremsner, 2009) but new technologies, including reverse transcription-polymerase chain reaction (RT-PCR) for virus detection, are often unavailable in these settings.

Honduras is a resource-limited country in Central America with a population of approximately 7.5 million people and gross national income per capita of US$ 1869.8 (United Nations Statistics Division, 2009). Honduras is classified as a lower middle income country by the World Bank (World Bank, 2013). The under-five mortality rate per 1000 births is 42.6, and acute respiratory infections are the leading cause of death in this age group (United Nations Department of Economic and Social Affairs, 2007). Previous reports of viral etiology of respiratory

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* Corresponding author. Tel.: +1-513-803-5187; fax: +1-513-636-8055.
E-mail address: Elizabeth.Schlaudecker@cchmc.org (E.P. Schlaudecker).

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illness in Honduras were limited to specimens from urban regions (Laguna-Torres et al., 2011; Reyes et al., 1996). There is currently 1 WHO National Influenza Center located in the capital of Tegucigalpa, Honduras (Pan-American Health Organization, 2012). One of the most significant difficulties of providing access to advanced diagnostic technologies in a low-resource setting is transport of specimens under adverse conditions. Previous reports show that transport time must be kept to a minimum (less than 1 day) to allow subsequent culture isolation of respiratory syncytial virus (RSV) and other viruses (Jensen and Johnson, 1994). Very little is known about the impact of transit time and temperature on nucleic acid detection by PCR (Druce et al., 2012). As researchers and clinicians in remote locations begin to utilize RT-PCR and other molecular diagnostic procedures, specimens must be transported in hot, humid climates without freezers or dry ice that are necessary for safe shipment of specimens in universal transport media. A new molecular transport medium (MTM) blends cell lysing and nucleic acid stabilizing reagents that can inactivate nucleases and preserve released nucleic acid at ambient temperature for later nucleic acid detection procedures. A recent study found this transport medium to effectively kill viral pathogens, including highly pathogenic H5 influenza virus, and to preserve the nucleic acid at ambient temperatures (Daum et al., 2010). It is therefore well-suited for potentially infectious biological pathogens that need to be transported with minimal risk or for clinical specimens that require field collection in remote areas such as Honduras. Because mortality rates for acute respiratory infections in resource-limited countries far exceed those of economically developed countries (Cashat-Cruz et al., 2005; Nair et al., 2011; Williams et al., 2002), information about burden of these pathogens in the resource-limited countries is crucial for the development of effective prevention, surveillance, and treatment strategies. Effective technologies for transporting infectious agents from remote locations to advanced diagnostic laboratories are therefore necessary. The objective of our study was to compare PrimeStore® MTM at room temperature to universal transport media (UTM) shipped on dry ice in the detection of respiratory viruses in rural Honduran children less than 5 years of age.

2. Materials and methods

2.1. Study design

As part of a clinic surveillance study to describe the spectrum of viral etiologies of acute respiratory infections in a rural outpatient population of Honduran children less than 5 years of age (Schlaudecker et al., 2012), we prospectively compared the detection of multiple respiratory viruses in clinical specimens stored in PrimeStore® MTM versus UTM.

2.2. Surveillance sites

We recruited participants in 2 villages, Santa Lucia and Magdalena, located in Intibucá, 1 of the poorest of the 18 departments of Honduras. Santa Lucia is located in a remote, mountainous region at 13°N and 88°W. The average monthly temperature ranges from 17° to 22 °C, and the rainy season extends from May through October. Santa Lucia is located approximately 5 kilometers from the El Salvador border, and Magdalena is approximately 3 kilometers north of Santa Lucia. The capital of Intibucá, La Esperanza, is approximately 3 hours by car from each town. The mountainous terrain and limited transportation hinder access to care for Hondurans in this region, and most laboratory tests require transport of specimens to remote locations.

We recruited participants at the Clinica Hombro a Hombro in Santa Lucia and Centro de Salud in Magdalena. The 2 clinics provide primary medical care, health education, and community resources for more than 20,000 rural Hondurans. Both clinics are operated by Shoulder to Shoulder and Hombro a Hombro, 2 partnering, private, non-profit, non-governmental organizations formed in the United States and Honduras, respectively. No other access to primary care is available in this region of Honduras.

2.3. Subjects

All children less than 5 years of age who presented at the 2 clinics from February 2010 through June 2011 and met the study criteria were eligible for enrollment. Children were eligible for recruitment if they presented with an acute respiratory illness defined as a maternal report of illness, with any one respiratory symptom including runny nose, nasal congestion, cough, difficulty swallowing, or difficulty breathing, occurring more than 7 days after a previous illness. For children greater than 1 year of age, fever was defined as a temperature >37.8 °C. This definition of respiratory illness with fever was modified for infants and young children from the Centers for Disease Control and Prevention (CDC) definition of influenza-like illness (Zaman et al., 2008). Patients were excluded if they were >5 years of age, previously enrolled within 7 days, or symptomatic for greater than 5 days.

2.4. Study procedures

If the child was eligible for the study and the parent agreed to participate, informed consent from the parent or guardian of the child was obtained. Following informed consent, we inserted 1 flexible nasopharyngeal nylon flocked swabs (FLOQSwabs™; Copan Diagnostics, Murrieta, CA, USA) into each nares. The distance between the participant's nares and ear lobe was measured to estimate the maximum depth of insertion, and the swabs were gently inserted towards the pharynx until resistance was felt and then rotated 3 times to obtain epithelial cells from the nasopharynx.

2.5. Laboratory procedures

One nasopharyngeal swab was placed in PrimeStore® MTM (Longhorn Vaccines and Diagnostics™, San Antonio, TX, USA), separated into aliquots, and stored at room temperature in an air-conditioned laboratory within the clinic. The MTM samples remained at room temperature during storage and throughout the duration of transport to Cincinnati Children's Hospital Medical Center (CCHMC). The other nasopharyngeal swab was placed in UTM (Copan Diagnostics). The UTM specimens were then separated into aliquots and stored in the laboratory freezer (−80 °C) in the Honduran clinic. Prior to shipment by air on dry ice to CCHMC, all UTM specimens were packed in small coolers with ice packs. Specimens were transported from Santa Lucia to the dry ice facility in San Pedro Sula, Honduras. Specimens were stored in the coolers with fresh ice or within freezers (approximately −18 °C) for 24 hours prior to receipt of dry ice in San Pedro Sula. Specimens were then shipped by air on dry ice to CCHMC with appropriate labeling and permit from the CDC. All UTM specimens were stored on dry ice approximately 12 hours prior to storage in the freezer (−80 °C) at CCHMC. Paired MTM and UTM samples for each subject remained together throughout the transport process, so duration of storage did not differ between samples stored in MTM versus UTM by subject.

Specimens were thawed according to the manufacturer's protocol and tested for respiratory viruses using the commercially available respiratory viral panel (RVP ID-Tag™; Luminex Diagnostics, Austin, TX, USA), a multiplex nucleic acid amplification test. This assay can detect 16 different viruses and subtypes from a single respiratory specimen, including: parainfluenza 1, 2, 3, 4, influenza A (non-H3), influenza A (subtype H3), influenza B, human metapneumovirus (hMPV), RSV A, RSV B, enterovirus/rhinovirus, adenovirus, and 4 human coronaviruses. This method has a reported sensitivity of 98.4% and a specificity of 96.4% for detection of any of these respiratory viruses (Mahony et al., 2007) and is a Federal Drug Administration certified diagnostic test.

Table 1
Detection of viruses.

	Virus detected[a]				
Media	Flu	hMPV	RSV	Paraflu	Total
MTM	33	23	12	18	86
UTM	32	22	22	18	94
					180

[a] Number of children with a virus detected in nasopharyngeal samples in MTM compared to gold standard (UTM).

Table 2
Statistical comparison of collection systems.

	Statistical test[a]			
Virus	Sensitivity	Specificity	PPV	NPV
Flu	81.25%	96.52%	78.79%	97.00%
hMPV	90.91%	98.58%	86.96%	99.05%
RSV	54.55%	100.00%	100.00%	95.48%
Paraflu	88.89%	99.07%	88.89%	99.07%

PPV = positive predictive value; NPV, negative predictive value.
[a] Sensitivity, specificity, positive predictive value, and negative predictive value of each virus detected in MTM compared to gold standard (UTM).

2.6. Statistical analysis

The number of positive viral isolates in PrimeStore® MTM was compared to the number of positive viral isolates in UTM for each of 4 viruses (influenza, RSV, hMPV, and parainfluenza). The Kappa statistic was performed to determine agreement between the 2 storage media. We also calculated sensitivity, specificity, positive predictive value, negative predictive value, and mean specimen storage time with standard statistical tests.

2.7. Ethical considerations

The protocol was approved by the institutional review board (IRB) of both CCHMC and the Hospital Regional del Occidente in Santa Rosa de Copan, Honduras.

3. Results

From February 15, 2010, to June 14, 2011, we stored nasopharyngeal samples in both PrimeStore® MTM and UTM for 233 children. The 4 most commonly detected viruses were influenza, hMPV, RSV, and parainfluenza (Table 1). The total number of samples positive for 1 of these 4 viruses was 93 for UTM and 85 for MTM. The 2 clinical specimen collection systems were similar for detecting all 4 viruses (Fig. 1). The Kappa values were statistically significant for influenza (Kappa = 0.7676, $P < 0.0001$), hMPV (Kappa = 0.8770, $P < 0.0001$), RSV (Kappa = 0.6849, $P < 0.0001$), and parainfluenza (Kappa = 0.8796, $P < 0.0001$).

The PrimeStore® MTM had a high specificity of detection for all 4 viruses, but the sensitivity was much lower for RSV (Table 2). Out of 22 RSV-positive UTM specimens, only 12 MTM samples tested positive for RSV. The detection of 4 viruses by MTM had positive predictive values ranging from 78 to 100% and negative predictive values ranging from 95 to 99% (Table 2). The samples were tested with nucleic acid detection in CCHMC a minimum of 19 days and a maximum of 196 days after sample collection (mean 116.9 days, SD 44.1). The duration of storage was the same for both MTM and UTM for each subject.

4. Discussion

Our study demonstrated that clinical specimens transported via MTM and UTM yielded similar diagnostic results from multiplex PCR testing for respiratory viruses. There were no statistically significant differences between MTM and UTM for influenza, hMPV, RSV, or parainfluenza. However, despite this statistical similarity, RSV showed the lowest correlation between MTM and UTM (Kappa = 0.6849). Of 233 total specimens, 22 of the UTM samples were positive for RSV compared to only 12 of the MTM specimens. PrimeStore therefore demonstrated a low sensitivity for detection of RSV compared to UTM (54.55%). It is possible that MTM is not optimized for detection of RSV, leading to these lower numbers in specimens stored in MTM. However, larger sample sizes are required to further explore this difference.

The PrimeStore® MTM collection method is ideal for this type of study site. The Clinica Hombro a Hombro is located approximately 6–8 hours away from the nearest dry ice facility in San Pedro Sula. The transport of infectious materials may therefore be compromised during this and other similar travel. However, our study demonstrated comparable results for MTM and UTM. These results remained similar despite lengthy storage time periods for the specimens (mean 116.9 days). We suspect that the statistically significant correlation between results from MTM and UTM reflects the careful monitoring of transport conditions of our specimens. As the principal investigator and other research personnel transported the specimens personally, we were able to guarantee appropriate storage

	UTM		
MTM	Flu	Not flu	Total
Flu	26	7	33
Not Flu	6	194	200
Total	32	201	233

Kappa = 0.7676, $P<0.0001$

	UTM		
MTM	hMPV	Not hMPV	Total
hMPV	20	3	23
Not hMPV	2	208	210
Total	22	211	233

Kappa = 0.8770, $P<0.0001$

	UTM		
MTM	RSV	Not RSV	Total
RSV	12	0	12
Not RSV	10	211	221
Total	22	211	233

Kappa = 0.6849, $P<0.0001$

	UTM		
MTM	Paraflu	Not paraflu	Total
Paraflu	16	2	18
Not paraflu	2	213	215
Total	18	215	233

Kappa = 0.8796, $P<0.0001$

Fig. 1. Comparison of number of detected viruses*.

of the specimens until arrival at CCHMC. This degree of monitoring is not feasible for routine transport of specimens.

There are several limitations to this pilot study. Though it would be helpful to individually characterize and compare the Luminex signals from each storage medium, we were unable to compare these values via Luminex due to differing aliquot sizes. We were also unable to standardize storage times for our specimens, as samples were transported to CCHMC approximately once every 3 months. Our study would benefit from a larger sample size, as there were relatively small numbers of specimens positive for each virus. We also hope to add quantitative viral results to future studies to avoid the possibility that discordant results were due to low copies of virus.

While diagnostic testing is readily available in many high-resource regions, laboratory capabilities are often limited in other parts of the world. These results suggest that the MTM preserves specimens for viral testing as well as universal transport media. MTM effectively lyses and inactivates potentially infectious biological pathogens, such as influenza, reducing infection risk so that samples can be transported with minimal risk in compliance with transport and customs regulations. Data from this study will guide further research into transport of infectious agents, including improved surveillance and diagnostic testing of important respiratory viruses.

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