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Verification of Real-time PCR Qualitative Methods

Measure twice, cut once accuracy.

Flu Season 2011 – 2012

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report

CONTENTS WINTER 2012

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COVER STORY 3



Verification
of Qualitative
Real-time PCR
Methods

INSIDE 8



Flu Season
2011–2012

Cepheid's ON-DEMAND Report is distributed four times a year. We welcome communication from users of Cepheid systems and tests and invite suggestions for articles in future issues. Send correspondence to:

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**David Persing
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Chief Medical and
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From the Editor

During the production of this issue of On Demand, the CDC declared the official start to the flu season, the latest start in 25 years. Drs. Ellen Jo Baron and Fred Tenover have written an up-to-date review of molecular diagnostic methods for respiratory infections, including the Xpert Flu test which provides on-demand results for FluA/B and H1N1 novel 2009, all of which are circulating this flu season. As the science and practice of Precision Medicine evolves toward the ultimate goal — providing the right patients with the right doses of the right medication at the right time — the impact of molecular diagnostics has never been greater. In the article by Donna Wolk and Daniel Olsen, you will find an excellent overview of the Verification and Validation processes that underpin the basic credibility and reliability of the diagnostic results we furnish on a daily basis. We hope you will benefit from reading both articles in this issue.

David A. Persing

Verification of Qualitative Real-time PCR Methods

MEASURE TWICE, CUT ONCE ACCURACY.



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Clinical laboratories rely on the FDA clearance/approval process to ensure that commercially available molecular tests have been evaluated and found to be accurate and medically useful. However, the FDA-approval process is only the first step towards ensuring diagnostic accuracy in your laboratory. The process of in-house verification is governed by the Clinical Laboratory Improvement Amendments (CLIA) regulations⁵, and begins with the laboratory undertaking studies to reproduce the manufacturer's claimed performance characteristics^{4,5,9}. Verification of an FDA-cleared assay by a laboratory performing the test exactly as described in the product insert is less stringent than the validation process needed for a modified test. If a test is not performed according to the published manufacturer guidelines, for instance, if the sample transport matrix is different from that in the package insert, then a more extensive method validation (not simply a verification) must occur and the test is then considered "off-label use" or a laboratory developed test (LDT), depending on the extent of deviation from approved protocols. This makes the user legally responsible for complete validation of the assay⁵. This article focuses on the process for implementing an FDA-cleared assay in your laboratory. The parameters of a commercial test that a laboratory must document in their own performance of the test are accuracy (analytical sensitivity and specificity), precision (reproducibility), reference range (for qualitative assays), and reportable range (for quantitative assays).

The first step of the in-house verification process is to reproduce the analytical sensitivity (% positive for spiked specimens) and limit of detection (LOD), which addresses the reference range requirement, reported in the package insert. The LOD is the lowest density of the target organism that can be detected [i.e., statistically different from a blank, typically determined to be in the region where the analytical signal can reliably be distinguished from “analytical noise,” which is the signal produced in the absence of analyte²]. The 95% LOD is the density at which the target microorganism is detected 95% of the time. Limits of detection are matrix, method, and analyte specific. There are two common ways to confirm these values: replicate testing and probit analysis.

1) REPLICATE TESTING: In replicate testing, multiple aliquots of the genetic target are prepared and tested. It is best to use whole organisms spiked into the same matrix as the samples to determine the LOD to ensure adequacy of the extraction process prior to real-time PCR. Plasmid or genomic DNA of a known organism load (available from commercial sources, such as Zeptomatrix, Acromatrix, ATCC, and others) may be used in cases when it is difficult or impossible to grow the microorganism in question. Many laboratories are able to grow bacteria and make dilutions in a liquid for spiking into the matrix but they may need to purchase viral DNA or RNA. One way to prepare the sample matrix for spiking experiments is to pool negative specimens to a sufficient volume to allow aliquoting of at least 20 replicate samples for testing. To begin, one first prepares a fresh microbial suspension to the appropriate density (colony forming units [cfu]/ml). Log phase cultures are optimal to avoid including too many dead bacteria. The suspension, when added to the negative matrix and mixed to homogeneity, should yield an organism load at the LOD stated in the product insert. You can then aliquot the appropriate number of samples to be tested (**Table 1**). It is a good idea to confirm the organism concentration (cfu/ml) by direct plate counts. To determine an accurate measurement of the 95% LOD, it is optimal to test ≥ 20 replicates. If only one of 20 samples is a false negative, then you have re-verified the assay's stated 95% LOD. If no false negatives are observed, you can document a 100% LOD, while 2 negative results = 90% LOD, and so on. You may consider asking several technologists to perform the replicate testing stage of this assay over a series of days. Thus, you can complete training, reproducibility studies, and verification of the LOD at the same time.

2) PROBIT ANALYSIS: This process, i.e., determining “probability units” (or “probits”), is only necessary for quantitative tests for which there is a binary (positive or negative; yes or no) result. Samples are prepared in a similar manner as for replicate testing, except that several dilutions of the organism suspensions (organism counts/ml) are prepared, much like the process of creating a standard curve with 3-4 targets of different density. One can think of the basis of the process as a dose-response curve, since the probit was originally created to assess dose-response³. Commonly, 3-12 replicates are prepared for each density, avoiding 0% positive and 100% positive densities, as they will skew the creation of the slope of your regression line. The suspensions can encompass low (3-4 x LOD), medium (5-6 x LOD) and high densities (7-8 x LOD). Following this process will enable the user to plot the graph and calculate the 95% LOD. The probit analysis transforms the proportions of positive responses detected into a “probability unit” (or “probit”). A table or software program is used to convert the proportions of positive response (response, y axis) to a probit, which is then plotted against the logarithm of the density (dose, x axis) and thereby, obtain a series of probabilities of positive responses associated with different concentrations. If you have access to cycle threshold values from a real-time PCR assay, the assignment of a cut-off value for the assay can be based on the 95% confidence interval (CI) of the Ct observed at the density associated with the corresponding 95% LOD value obtained from the probit analysis. Note that if using an LDT, the assay cutoff value must be revalidated periodically in accordance with clinical guidelines. Every six months is a commonly used metric.

Next, analytical specificity (% accuracy for negative specimens) is determined by challenging the assay with genetically similar or closely related organisms to assess cross-reactivity. Spiked specimens containing organisms commonly found in the sample type can be used for this assessment. This should include organisms that are genetically similar to those targeted in the assay. You can enhance your assay specificity process by using a variety of known negative patient samples, which were used for your % agreement assessment (see below). Reviewing the results from these samples may yield identifications of other isolated microorganisms that can provide additional evidence that no cross reaction with non-target microorganisms occurred during testing. Finally, if primers and probes are known (which is uncommon for commercial assays), it is prudent to perform virtual specificity studies (i.e., using computer-aided technology) to compare the oligonucleotide sequences to all known genetic sequences, testing for any potential cross-reactivity. This process is done by manufacturers of commercial assays and checked during the FDA clearance process, so laboratories are not obligated to repeat the exercise. Although this approach is able to identify potential cross-reactions, it cannot predict the evolution of microorganisms or the emergence of newly identified microorganisms that could cross-react with primers and probes in the future. For these reasons, continual assessment of specificity is essential throughout the life of any molecular assay.

Another and often parallel step in the verification process is documentation of assay reproducibility (also known as variability) in order to determine acceptable value ranges for the important assay parameters. For qualitative assays, reliability of positive and negative results over time with different operators is sufficient. For quantitative assays, intra-assay variability (within assay) can be obtained by using the same 12-20 repetitions performed to determine LOD. One can calculate the mean Ct values for both target and internal controls, along with the standard deviation (SD), coefficient of variation (% CV), and other variance measures. Reproducibility should be defined at several analyte densities (i.e., low, medium, and high) and multiple operators should be included in the testing. Although it is a commonly used metric, the % CV is not an optimal measure of variability because it will vary by organism density; therefore, the % CV must be calculated at each density. Inter-assay variability (between assays) is determined by calculating the same variance measures as intra-assay variability; however, the data used are those obtained by replicate testing across many days and the

TABLE 1. Examples of colony counts to determine 95% LOD, n = 20. Replicate sample #3 is the only negative sample. Note: The 90% LOD would be calculated if n were equal to 10 replicates.

Replicate	Microbial Target Density (cfu/mL)*	Actual Microbe Density (cfu/mL)	Detected? (Y/N)
1	100	101	Y
2	100	101	Y
3	100	99	N
4	100	103	Y
5	100	100	Y
6	100	102	Y
7	100	99	Y
8	100	101	Y
9	100	101	Y
10	100	103	Y
11	100	98	Y
12	100	104	Y
13	100	103	Y
14	100	105	Y
15	100	100	Y
16	100	98	Y
17	100	100	Y
18	100	100	Y
19	100	97	Y
20	100	102	Y
		100.85 Avg cfu/mL	19/20 = 95% % positive



Candi Grado, B.S. at the microscope in the Microbiology Laboratory, with Dr. Wolk in the background.

TABLE 2. Westgard Rules (<http://www.westgard.com/westgard-rules-and-multirules.htm#westgard>) are a short hand notation used to abbreviate different decision criteria or control rules, e.g., 1_{2s} to indicate 1 control measurement exceeding 2s (aka 2 SD) control limits. Subscripts are used to indicate the control limits and combinations of rules are generally indicated by using a "slash" mark (/) between control rules, e.g. 1_{3s}/2_{2s}.

1_{3s}	Refers to a control rule that is commonly used with a Levey-Jennings chart when the control limits are set as the mean plus 3s and the mean minus 3s. A run is rejected when a single control measurement exceeds the mean plus 3s or the mean minus 3s control limit.
1_{2s}	Refers to the control rule that is commonly used with a Levey-Jennings chart when the control limits are set as the mean plus/minus 2s. In the original Westgard multirule QC procedure, this rule is used as a warning rule to trigger careful inspection of the control data by the following rejection rules.
2_{2s}	Reject when 2 consecutive control measurements exceed the same mean plus 2s or the same mean minus 2s control limit.
R_{4s}	Reject when 1 control measurement in a group exceeds the mean plus 2s and another exceeds the mean minus 2s.
4_{1s}	Reject when 4 consecutive control measurements exceed the same mean plus 1s or the same mean minus 1s control limit.
8_x	Reject when 8 consecutive control measurements fall on one side of the mean.

FIGURE 1. Levey-Jennings Plot depicting Cycle threshold values for a positive control strain of *S. aureus*. The outside dotted lines depict the mean and upper and lower bounds of 3 Standard Deviations from the mean, followed by 2SD and 1SD as they move toward the mean.

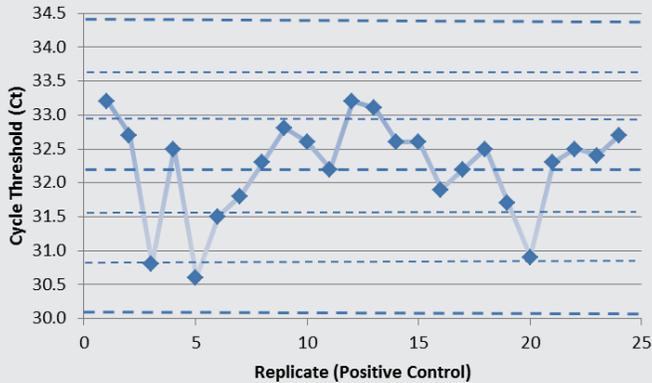


FIGURE 2. Truth (2x2) table reflecting layout to determine diagnostic method performance characteristics, where TP represents true (verified) positive results, TN represents true negatives, FP is false positives, and FN is false negatives.

2x2 Table

		Reference Method Test 2		
New; Test 1		Positive	Negative	
Positive		+/+ TP	-/+ FP	N
Negative		+/- FN	-/- TN	N
		N	N	Total N

FIGURE 3. Truth Table calculations include the following: multiple all equations by 100 for percent calculations.

		TRUTH	
		Positive	Negative
Test Result	Positive	A	B
Test Result	Negative	C	D

- SENSITIVITY: $A / (A+C)$
- SPECIFICITY: $D / (B+D)$
- FALSE NEGATIVE %: $C / (A+C)$
- FALSE POSITIVE %: $B / (B+D)$
- POSITIVE PREDICTIVE VALUE: $A / (A+B)$
- NEGATIVE PREDICTIVE VALUE: $D / (C+D)$
- PREVALENCE NEW METHOD = $(A+B) / (A+B+C+D)$
- PREVALENCE OLD METHOD = $(A+C) / (A+B+C+D)$
- % TOTAL AGREEMENT: $A+D / (A+B+C+D)$

mean and three Standard Deviations (3SD) can be used to monitor lot-to-lot performance and operator competency via trend analysis, the assessment of Ct values across time, reagent lots, shifts, and operators. Alternately, the 95% confidence interval may be used for trend analysis; however, 3SD is more commonly used. To obtain the most accurate measurement, 28 measurements are optimal; however, 6-12 replicates will often provide a reasonable mean and SD from which you can launch your assay and begin trend analysis. Mean and 3SD can then be adjusted after obtaining 20-28 runs. In this instance, the upper and lower confidence limits are determined. Performing and characterizing 28 separate test runs provides the clinical laboratory scientist sound results from which new lots can be assessed over time⁹. Often, results obtained from the external positive controls can be charted over time and graphed against the mean in order to describe the inter-assay variability. The Levey-Jennings Plot⁶ is a useful way to depict these results (Figure 1).

Trend analysis is part of the qualitative assay characterization that can be utilized as an ongoing process to provide quality assurance for the verified assay. Some investigators have used Westgard rules for assessment of trend analysis and have adapted those rules, or created their own rules, for monitoring the trend results; examples are listed in Table 2^{7,8}. For example, if the percent of positive patient results appears to be slowly getting higher over time and the patient characteristics have not changed, you may need to investigate if something has changed with regards to the test. In this case, the Ct values should be graphed to spot trends.

Clinical percent agreement or qualitative accuracy.

These two parameters can be established by comparing the performance of your new assay to a reference (gold) standard method. Ideally, this would be accomplished by using known positive specimens over a known range of target densities (low, medium, and high-signal positives), including enough samples of weakly positive specimens to establish accuracy at the lower end of the spectrum.



Paulette J. Zovistoski B.S., MT(ASCP), running assays on the GeneXpert® System in Dr. Wolk's laboratory.

In order to obtain enough statistical power to compare the assay performances and give the laboratory director sufficient information on whether or not to replace an existing assay, a power calculation should be performed; however, for the sake of cost and expediency, the ASM Cumitech¹ recommends a minimum of 50 positives and 100 negatives for testing. Depending on the performance of the assay, and the criticality of test results, more specimens may need to be tested. Laboratories should allow for random testing of the specimens in order to imitate a real testing environment; that is, do not test all the positives in one run and all the negatives in another run. Assessment of the % agreement as well as performance parameters such as clinical sensitivity, clinical specificity, positive predictive value (PPV), and negative predictive value (NPV), allow laboratorians and clinicians to calculate test performance relevant to their particular locale and disease prevalence. Clinical sensitivity and specificity should be calculated in a scenario as close as possible to the real-life population the laboratory will serve since PPV and NPV will vary by disease prevalence. The PPV is the proportion of persons who are truly diseased and are identified as diseased by the test under investigation. The NPV is the proportion of truly non-diseased persons who are classified as negative by the assay. Often a 2x2 table is used to determine clinical performance characteristics of the assay (Figures 2-3). With newer molecular assays, it is possible that the previous “gold standard” is not as accurate as the new test. This can lead to discrepant results that may require a third method for resolution. In some cases, clinical information from symptomatic patients may be used, and in other cases a different molecular method or sequencing the amplified product of the new assay must be performed. The results of the comparator methods, sometimes combined into a final disease assessment, are considered the clinical “truth” (Figure 3).

FIGURE 4. Verification sections included for each new assay/procedure.

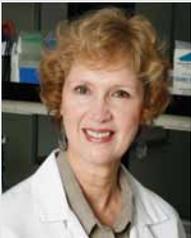
Title Page	1	Verification summary (statistical analysis & summary, consultant authorization recommendation, site publications or abstracts)
Assay name	2	Client communications, fact sheets, announcements, web information
Consultant	3	SOPs, control material, forms, training/competency, initial proficiency testing
Section	4	Pre-analytical and post-analytical information, specimen transport, physician test results computer printouts, etc.
Company	5	Product information and literature review
Product Name	6	Contact list and relevant correspondence
Product Numbers	7	Raw data and test results
	8	Computer entry information and Laboratory Information Systems
	9	Post-implementation assessment (if applicable)
	10	Cost analysis and test reimbursement information (HCPCS or CPT)*
	11	Instrument and other manuals (if applicable)
	12	Miscellaneous genetic sequence information (if applicable), safety, human subjects (if applicable)

Summarizing Results:

Only after you perform in-house method verification, can you begin patient testing. Typically a summary of the entire method verification is created and signed by the Medical Director prior to testing and bundled with raw data and other information in the form of a verification folder or binder. Examples of the Table of Contents headings and subheadings used in the University of Arizona Medical Center Verification Binder is shown in Figure 4.

Flu Season 2011-2012

Laboratories performing tests for respiratory viruses can attest to the changing epidemiology of this formerly reliable season. For many years, like clockwork, new influenza strains, having mixed and matched up genetic material from pigs, birds, and humans, originated in Southeast Asia. In the past, the first influenza cases of the year appeared in Australia in June, peaked in late July, and petered out around December.¹



Ellen Jo Baron
Ph.D., D(ABMM)

Prof. Emerita, Stanford University
Director of Medical Affairs, Cepheid

In Europe, influenza season typically started in late August, peaked in October, and ended before Christmas.^A Flu cases usually appeared on the East Coast of the U.S. in October and spread westward, reaching California in late October or early November, peaking in February, and finally disappearing by end of March.^{1,B} But something totally unexpected happened in 2009. Both the United States, possibly in one of the U.S.'s large factory pig farms, and Asia (particularly South Korea and Thailand, where people and pigs live in close proximity) are thought to be areas where the genetic reassortment leading to the most recent pandemic influenza recombinant virus, novel H1N1 2009 strain, originated.² And the source of the new strain was not the only surprise; this strain swept through Mexico, moved up to the Southwest U.S. and from there spread to the rest of the world starting in April, after northern hemisphere laboratories had

traditionally finished their flu season and were putting their reagents away for another year (Figures 1 and 2).

What has happened with regard to influenza epidemiology since then? Although the new strain, H1N1 novel 2009, dominated the population in its first year, it has since dropped its prevalence back to become just another contributor to the circulating strains of influenza in the world. In fact, it may be only a minor agent of influenza disease this year; approximately 5% of all subtyped influenza A strains as of the first week of 2012.^C Is it still important to identify this strain in a particular patient? Most authorities would say "yes," partly because it has a predilection to quickly move from its initial colonization site in the upper respiratory epithelium into deeper lung sites, causing more severe disease, especially in some vulnerable patient populations.³ The

influenza A H1N1 novel strain infected younger people more vehemently than the influenza’s usual victim, the elderly, and in greater numbers than previously circulating influenza A strains (**Figure 3**).⁴ And some novel populations, such as diabetic patients and pregnant women, also seemed to be at increased risk.⁵ Equally important was the differential susceptibility of various influenza virus strains to the commonly used antiviral treatments (**Table 1**). This year, in contrast, all 53 strains (including only 2 influenza B) tested so far have been susceptible to both of the neuraminidase inhibitors, oseltamavir and zanamivir.

Two unexpected benefits of the 2009 influenza season were the knowledge gained about the relatively poor performance of commonly used rapid antigen enzyme-immunoassays and the availability of new, accurate molecular tests for respiratory viruses. With the increased need to correctly identify and triage patients with influenza, both for individual patient care needs and for infection control purposes, the reliability of laboratory tests gained importance. Unfortunately, the currently available tests were not up to the job.⁶ The largest concentration of influenza patients in the U.S., centered on Long Island, provided an excellent resource for comparing test performance. By chance, a large group of high school boys had been visiting Mexico City when the influenza hit. They returned to the New York City area and promptly spread the disease throughout the community. Dr. Christine Ginocchio was in the perfect place to study the laboratory diagnosis of influenza. She received >6,000 samples within a few short months and used them for comparison testing.⁷ She found that performance varied widely among test types but that rapid antigen tests performed most poorly (**Table 2**). A retrospective meta-analysis of influenza A point-of-care tests (all rapid antigen format) compared with various high complexity diagnostic assays, primarily molecular assays, for detection of influenza A (novel H1N1 2009) performed

by scientists from Johns Hopkins University showed pooled sensitivity to be 68% and specificity to be 81%. The ranges of results among published studies were great (sensitivities varied from 10 to 88% and specificities from 51 to 100%).⁸

Multiplex molecular platforms are considered the most inclusive and sensitive for diagnosis of respiratory tract infections (**Tables 2 and 3**). The classic xTAG Respiratory Viral Panel by Luminex Molecular Diagnostics can detect 10 types of viruses after initial extraction of the sample, an off-board nucleic acid amplification, and processing in the Luminex platform. The test is highly complex and requires considerable expertise and 2.5-3 hours of hands-on time to perform; results are available in 5-6 hours, which effectively limits testing to once/day, with an actual average turnaround time of approximately 22-47 hours.^{9, 10} A newer version, RVP Fast, showed slightly decreased sensitivity for some viruses but 1.5 hours less of hands-on time with a time to results of 8 hours from specimen receipt.¹¹ This assay has been expanded to detect 18 viruses, including several human coronavirus strains, 8 of which have received FDA clearance. The ProFlu test by Prodesse/Gen-Probe also showed good results for detection of RSV and influenza viruses.¹² A newer multiplex test, which also detects 18 viruses, the FilmArray from Idaho Technologies, has a dramatically decreased hands-on time (3-5 minutes) and yields results from a single sample in about one hour.¹⁰ Each instrument can run a single test packet in an hour, so the current system is geared to medium-to-small volume laboratories.

Whether highly multiplexed tests are appropriate in all situations has not been fully evaluated. In fact, a recent study evaluated the clinical impact of reporting the presence of respiratory viral pathogens in a normal pediatric population within one day of admission versus standard results.¹³ Among 583 patients less than 12 years old presenting with

TABLE 1. Antiviral resistance 2009 and 2011.

	% resistant oseltamavir		% resistant zanamivir		% resistant amantadines	
	2009	2011	2009	2011	2009	2011
A H1N1 seasonal	99.6	NA	0	NA	0.5	
A H1N1 (2009)	0.6	0	0	0	100	
A H3N2	0	0	0	0	100	
B	0	0	0	0	NA	

TABLE 2. Relative sensitivity of various virus laboratory detection assay types during 2009 epidemic (modified from Reference 7).

	Influenza A (all strains)	Influenza A H1N1 novel 2009
Rapid antigen EIAs	21%	18%
Direct fluorescent antibody	49%	47%
Culture	83%	89%
Molecular assay	98%	98%



acute respiratory tract illness, half had results generated using nucleic acid amplification tests (NAATs) for 17 viruses reported to clinicians 12-36 hours after sample collection and the other half had reports of results determined by the usual direct fluorescent antibody (DFA) method. Not unexpectedly, DFA detected fewer pathogens overall. However, there was no statistically significant difference between the groups with regard to hospitalizations, antibiotic administration, or length of stay. Surprisingly, antibiotics were started significantly more often in the group who received rapid reports. One reason given for lack of shorter courses of antibiotics in patients with positive viral NAATs was the fear of bacterial superinfection. As these authors noted, a patient population with significant co-morbidities, including asthma, organ transplantation, and other immunocompromising conditions, would be more likely to benefit from relatively rapid detection of respiratory viruses, including the presence of more than one virus in the sample.

Thus, given the cost, time, and potential reimbursement challenges, it is unclear what the role of the multiplex platforms should be. Two cost analyses, one Canadian and one American, evaluated a multiplex respiratory virus test compared with the cost of a DFA plus culture, i.e., their standard protocols. The Canadian study showed cost-effectiveness when prevalence of disease was >11%.¹⁴ The American study did not make such a connection, but >30% of samples tested were positive.⁹

A recent (September, 2011) update on diagnosis and treatment of upper respiratory tract infections (URI) on Medscape^D suggests “Targeted therapy is not available for most viruses that cause URI. Therefore, viral testing is rarely indicated for uncomplicated viral URIs in the outpatient setting. However, confirmation of a viral condition such as influenza may reduce inappropriate use of antibiotics.”

TABLE 3. Virus strains detected by FDA-cleared multiplex molecular tests

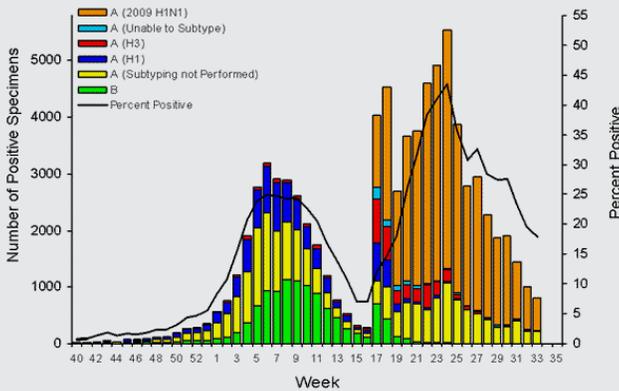
	xTAG RVP			
	xTAG RVP	Fast	ProFlu	FilmArray
Influenza A	x	x*	x	x
Influenza A H1	x	x*		x
Influenza A H1N1 2009				x
Influenza A H3	x	x*		x
Influenza B	x	x*	x	x
RSV	x	x*	x	x
RSV 1		x		
RSV 2		x		
Hu Coronavirus NL 63		x		x
Hu Coronavirus 229E		x		x
Hu Coronavirus OC43		x		x
Hu Coronavirus HKU1		x		x
Parainfluenza 1	x	x		x
Parainfluenza 2	x	x		x
Parainfluenza 3	x	x		x
Parainfluenza 4		x		x
Hu metapneumovirus	x	x*		x
Adenoviruses	x	x*		x
Rhinovirus/ Enteroviruses		x*		x
Hu Bocavirus		x		x

*FDA-cleared

Dr. David Persing, Chief Medical and Technology Officer at Cepheid, suggests that “the real value of these tests will be in the next 5-10 years when new antivirals (for rhinoviruses and others) gain FDA approval, and there is more than one antiviral treatment decision to be made on the basis of the results. In general, the field is moving toward Precision Medicine — giving the right medicines to the right patients at the right time — and Next-Gen molecular methods will play a critical role in the decision support process.”

The Cepheid Xpert[®] Flu assay detects influenza A, the influenza A subtype H1N1 2009 strain, and influenza B with approximately 2-3 minutes of hands-on time; results are available within 75 minutes. Both nasopharyngeal swabs and nasal aspirates/washes can be tested. The current Xpert Flu test is substantially different from the original Emergency Use Authorization (EUA) assay for influenza A H1N1 2009, which was available in 2009 and early 2010 only. Earlier publications that evaluated the performance of the EUA version are no longer relevant to the existing assay. The random access nature of the GeneXpert[®] instrument allows multiple assays to be initiated within the same time frame. Results of comparison studies of the Xpert Flu assay have been presented at national meetings, including the 2011 Clinical Virology Symposium in Daytona, FL. One poster from the University of Virginia Health System (Seaner et al) compared the Xpert Flu and Prodesse’s ProFlu. They found the Xpert assay to be

FIGURE 1. CDC data for influenza, 2008-2009 season



dramatically easier to perform, and sensitivities for detection of seasonal influenza A H1/H3, influenza A H1N1 2009, and influenza B to be 97.3%, 100%, and 94.4%, respectively, with specificities all 100% when compared to the Prodesse product. Although the laboratory had previously been running a rapid antigen test, they soon discontinued it in favor of the Xpert Flu assay. Dr. Melinda Poulter, senior author of the study, noted that both physician and patient satisfaction were enhanced due to shorter wait times. In fact, Mendy said

“...almost heaven. I’ve even had docs call to thank me... never had that happen before.”



Dr. Mendy Poulter in her laboratory.

Although actual documentation is now in progress for a publication, it appeared that costs were decreased due to better bed management, and that more targeted drug therapy seems to have reduced both costs and adverse events.

The University of North Carolina also evaluated the Xpert Flu assay.¹⁵ The authors reported the sensitivity of the Xpert Flu assay (compared with their in-house laboratory developed PCR test) was best for influenza A (H1 and H3) and influenza B, with 100% detected from retrospective samples and with 100% of the influenza A strains and >95% of the influenza B strains detected from prospective samples. For H1N1 2009 novel strains, the Xpert showed ≥87% sensitivity overall but better performance on prospective samples and nasopharyngeal (np) samples. Lower sensitivity was observed with non-np swabs, as expected, although only 8 such samples were tested. Subsequent analysis showed that the samples that were not detected by Xpert Flu had very low copy numbers of viruses. The authors noted that their laboratory-developed test, even when performed 3-4 times daily during influenza season, had a turnaround time of 8 to 24 hours, which they deemed “unacceptable,” leading to their conclusion that the GeneXpert Flu, with hands-on time of 2 minutes and results available 75 minutes later was an “attractive approach.”¹⁵

FIGURE 2. Data from European Centres for Disease Prevention and Control

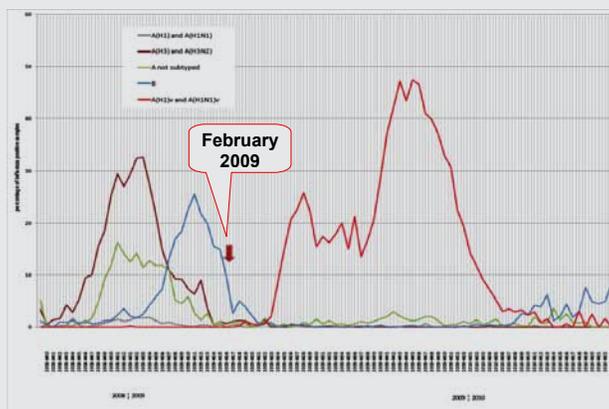
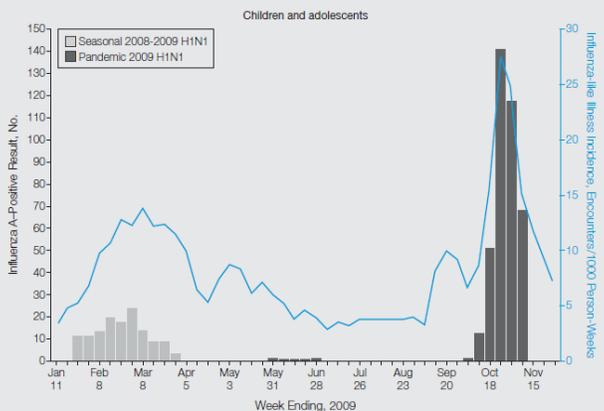


FIGURE 3. Relative numbers of cases of 2009 H1N1 influenza A in children and adolescents compared with previous seasonal influenza⁴



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Verification of Qualitative Real-time PCR Methods

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