AUTOMATED DATA INTERPRETATION
of H1N1/09 ('swine') influenza Real-time PCR data

AUTOMATED ANALYSIS OF REAL-TIME PCR DATA CAN REPLACE USER BASED INTERPRETATIVE METHODS

azure pcr Value in Knowledge

Health Protection Agency

St George's University of London

St George's Healthcare NHS NHS Trust

OBJECTIVE

To optimise a process enabling automated analysis of influenza real-time PCR data, requiring no input from the user.

Introduction

Real-time PCR is presently the gold standard for influenza virus detection from clinical specimens.

The technique possesses high sensitivity, specificity and reduced turnaround times when compared to the culture and serological methods it has replaced in the diagnostic laboratory. More recently there has been a shift to automate the processes of nucleic acid extraction and assay preparation and consequently these steps can readily be performed by staff members with limited training.

The raw data generated by real-time PCR assays consists of multiple fluorescence readings in a time slice derived from excitation spectra of specific dyes. This change in fluorescence can be used to infer the increase in the presence of an amplicon. In a diagnostic setting, data analysis and interpretation is often based on arbitrary thresholds. Sample variability and poor assay performance can lead to ambiguous and subjective sample calling that is dependant upon the expertise and experience of the healthcare scientist interpreting the assay. The raw data output, produced by thermal cyclers in digital form, is never interpreted manually, but instead processed using data smoothing and curve-fitting in order to make them more easily interpretable by the human eye. This can lead to a loss of data and therefore contribute to the error prone calling.

Commercial assays for pathogen detection by real-time PCR are frequently packaged with standalone analysis software to enable data interpretation. This assay specific analysis is inflexible and not suitable for application to other assays and is therefore not readily applied to non-commercial and bespoke assays required for an outbreak response by the healthcare system.

In this study we retrospectively examined data from H1N1/09 ('swine') influenza real-time PCR testing performed at St. George's Hospital, in order to assess if manual data analysis could be successfully replaced by an automated methodology resulting in both a time saving and more consistent sample calling protocol. Additionally, we have examined the potential impact of the automated analysis on the H1N1/09 ('swine') influenza real-time PCR's analytical sensitivity.

Method

St George's NHS Trust used a customised assay for the detection of influenza.

Samples where defined as positive for Influenza A and H1N1/09 ('swine') influenza if there was a detection of nucleic acid amplification product after manual user analysis. This was achieved by visual confirmation of an amplification plot using the RotorGene software (Rotor-Gene 6000 series software 1.7). If there was a four cycle difference between the threshold cycle derived from the RNA spike of the extraction control and that of the respective sample, it was deemed inhibited. If the K-Ras channel was negative this was taken as indicating the absence of human cellular material in the sample.

Samples were anonymised and re-analyzed retrospectively using the established analysis and interpretation methodology defined in HPA vSOP25 giving specificity of 99.47%, a threshold delta Rn of 0.05 was applied after curve smoothing and fitting by the RotoGene software and a corresponding Ct-value was assigned.

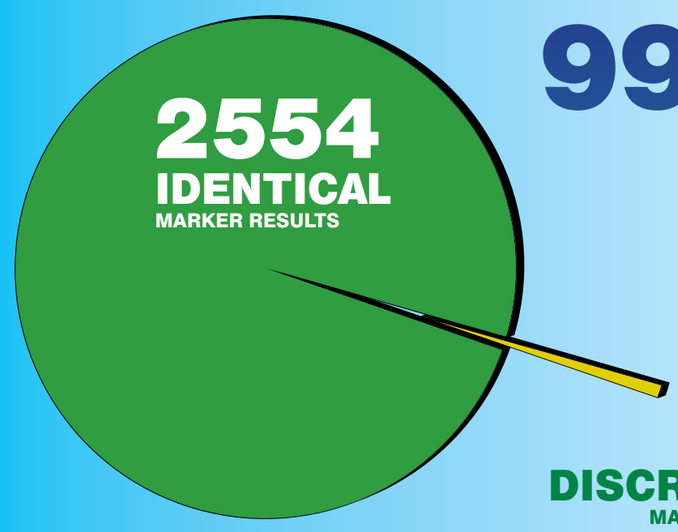
Following this, raw, unprocessed data was exported from the RotorGene for automated analysis using the **AzurePCR** methodology. The **AzurePCR** system is based upon automated machine learning in which the software evolves in an iterative process allowing greater accuracy of calling, based solely on the empirical data provided. This process is performed in an automated fashion which is self-calibrated in three stages.

The first of these allow for the optimization of the parameters used to extract the data for further analysis. Secondly a training dataset is generated from internal controls for the test data supplied and a database is built forming a basis for subsequent machine learning. Thirdly, individual samples are automatically assessed in the light of the training data set and the database is expanded and improved by this in an iterative process until a steady state is achieved and no further adjustment of the database results.

For the purposes of this study, 644 samples were subject to automated data analysis and these results were then compared with previously obtained manual interpretation as carried-out at source. Discrepant samples underwent a second manual interpretation, if this proved inconclusive the samples were re-run where possible.

RESULTS

Out of 2,576 data points which were interpreted by both St. George's Hospital and **AzurePCR**'s respective methods, 22 results were found as discrepant. From these, 4 were conclusively re-interpreted, and 3 of these were in conclusion with **AzurePCR**.



99.146%
MARKER RESULT SIMILARITY

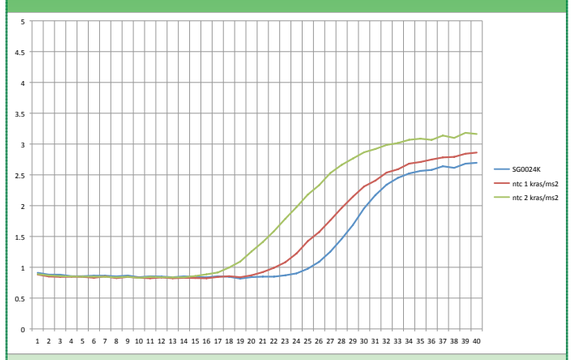
DISCREPANT 22
MARKER RESULTS

Marker	AzurePCR	SGH	Samples
Human DNA Control	FAIL	PASS	7
H1N1 Lineage	– *	+	6
Influenza A	–	+	4
Influenza A	+	–	3
Inhibition Control	FAIL	PASS	2

* InflA positive in all cases

DISCREPANCIES Re-interpreted samples

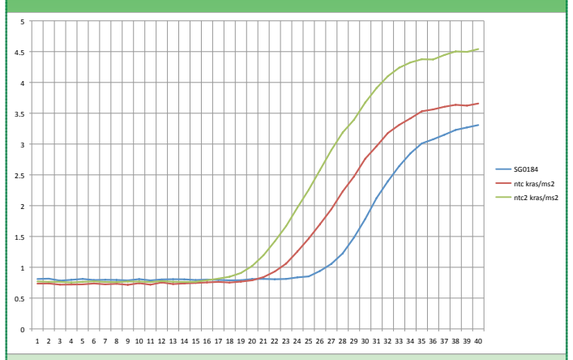
AzurePCR failed inhibition control



Sample 0024
Internal control for inhibition, was manually re-interpreted by St. George's Hospital.

The new result was concluded to be a failed control, identical with **AzurePCR**'s automated method.

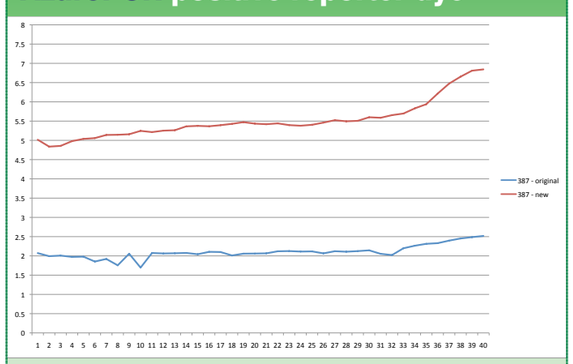
AzurePCR failed inhibition control



Sample 0184
Internal control for inhibition, was manually re-interpreted by St. George's Hospital.

The new result was concluded to be a failed control, identical with **AzurePCR**'s automated method.

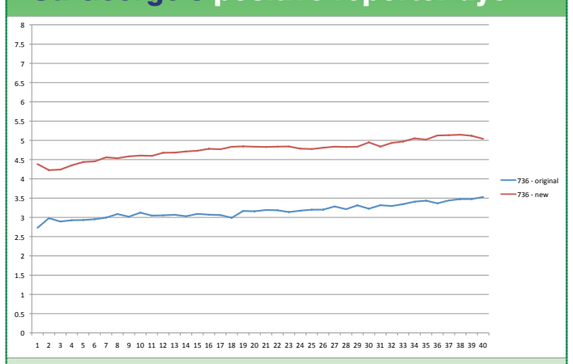
AzurePCR positive reporter dye



Sample 0387
Was re-run and the new run manually interpreted by St. George's Hospital.

The new result was concluded to be positive, identical with **AzurePCR**'s interpretation.

St. George's positive reporter dye



Sample 0736
Was re-run and the new run manually interpreted by St. George's Hospital.

The new result was concluded to be positive, identical with St. George's original interpretation.

CONCLUSION

This retrospective analysis using the AzurePCR method demonstrated similar sensitivity and specificity when compared to manually defined analysis methods.

As a fully automated process, the **AzurePCR** method eliminated the need for expertise and time in interpreting real-time PCR results. As an adaptive process independent of the operator, it did not require user defined intervention such as baseline or threshold setting.

Implementation of this automated process in routine testing would enable healthcare scientists unfamiliar with manual analysis methodology to successfully perform the assay.

The flexible nature of the automated analysis process has the potential for implementation with other formats of real-time PCR detection and therefore could be applied to other non-commercial and outbreak response assays.

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