restrained within a particular batch, and gives a more challenging sensitivity than that seen with internal controls. Monitored over time and across different assay batches, these controls provide reassurance that the assay is as sensitive as it can be, which ultimately leads to accurate and consistent patient diagnosis and treatment.

Inter and intra laboratory variation can lead to erroneous results. It is recognised that a laboratory may achieve relatively consistent results from run to run but there can still be differences between operators, local equipment, pipetting technique and even calibration and training. Different methods exist to gather intra laboratory data from complex excel sheets to hand written lab note books. However it is harder and requires laboratory collaboration to gather inter-lab data. This too should be monitored overtime and if a deviation is seen it is acted upon and investigated to maintain sensitivity and accuracy. Similarly comparing one laboratories data against another is insightful. With this information a laboratory can assess why their data may differ from the consensus and investigate local procedures to rectify such anomalies.

Many laboratories rely on EQA schemes to enable this comparison, whilst an excellent way of comparing performance across a large range of laboratories and assays, such schemes only provide a periodic snap shot of performance.

The National Institute for Biological Standards and Control (NIBSC) has developed a web-based Result Reporting System (RRS), for the data monitoring of its serology and NAT quality control (QC) reagents. Through the provision of Intra-lab charts and Inter box plots. It allows real time intra and inter-laboratory comparison and monitoring and by applying Westgard Rules to the data any deviations from the norm are flagged, thus this software can provide an early warning sign that a laboratories assay or equipment is failing.

Aided by a demonstration of RRS, this presentation will outline the need for the use of external control material over solely using internal controls and will importantly highlight the necessary monitoring needed in order to ensure reproducibility and consistency of assay results.

http://dx.doi.org/10.1016/j.jcv.2016.08.047

Abstract no: 124
Presentation at ESCV 2016: Poster 8

CMV Run control r-gene® (ARGENE® range, bioMérieux): A tool to ensure the reliability of human cytomegalovirus nucleic acid amplification technique results

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The primary aim of any laboratory is the timely delivery of reliable results with a minimum of errors, maintaining confidence in the results for all stakeholders. The use of independent quality control (IQC) provides constant and consistent monitoring of an assay results in a systematic manner so that variation in the assay system can be monitored over time (day-to-day test variation, lot-to-lot performance of test kits, operator variation, etc.). An IQC material must be robust, stable and well characterized. Its properties should be as close as practically possible to patient specimens and should be processed throughout the analysis in the same way as the clinical sample.bioMérieux has developed an IQC named CMV Run Control r-gene® (ARGENE® range)*. Its routine use enables monitoring run to run performance for human cytomegalovirus nucleic acid amplification technique (NAT) assays for human clinical samples. CMV Run Control r-gene® is intended for health care professional and for *in vitro* use only.

This CMV Run Control r-gene® (ARGENE® range) consists in a non-inactivated whole CMV strain (AD169) spiked in pooled human plasma tested negative for CMV, HIV, HCV, HBV, Parvovirus B19, and EBV. This formulation allows to mimic naturally occurring specimens containing CMV DNA. The CMV Run Control r-gene® (ARGENE® range) has no assigned concentration value but it is defined in order to be within the dynamic range of most molecular assays. This control should therefore be validated for use as a run control and the expected results determined by the end user for their particular CMV NAT assay, extraction and instrument combination.

Performance results obtained in-house (precision and stability studies) of CMV Run control r-gene® (ARGENE® range) established with the CMV R-gene® kit (ARGENE® range, bioMérieux) on the platform combination NucliSENS® easyMAG® (bioMérieux)/ABI 7500 Fast (Life Technologies™) will be presented.

* Not yet commercialized.

http://dx.doi.org/10.1016/j.jcv.2016.08.048

Abstract no: 126
Presentation at ESCV 2016: Poster 9

RNA internal control, a new tool for the rapid development of detection tools by real time PCR in outbreak situation. Application to the detection of Middle East Respiratory Syndrome human coronavirus

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Since the emergence of Middle East Respiratory Syndrome Coronavirus (MERS-CoV) in 2012 in the Arabian Peninsula, many questions remain unanswered on modes of transmission and reservoirs of virus. The MERS-CoV causes severe respiratory illness. The epidemic origins are uncertain but probably linked to a zoonosis. The bat or the camel are discussed as reservoirs of the virus. Globally, since September 2012, WHO has been notified of 1,714 laboratory-confirmed cases of infection with MERS-CoV, including at least 618 related deaths (WHO report – 14 April 2016).

In such outbreak situations, especially with emerging organisms causing severe human diseases, it is important to quickly develop a test for the detection the virus involved.bioMérieux developed a generic kit, RNA internal control r-gene® (bioMerieux), combining an internal control and a core kit to be used in combination with either proprietary or commercial primers and probes. This tool associated with specific primers and probes constitute a ready-to-use duplex premix for the detection of targeted RNA in a sample.

On this principle, bioMérieux developed a real-time PCR assay for the rapid detection of MERS-CoV. A set of primers and probe and a transcript used as positive control (MERS-HCoV primers r-gene® – RUO #20-010 and MERS-HCoV probe r-gene® – RUO #20-011, MERS-HCoV transcript – RUO #68-010, bioMérieux) were designed on the S gene, coding for the spike structural protein.

The internal control, added before the extraction step, allows to check simultaneously extraction efficiency and presence of inhibitors. Extractions were performed on NucliSENS® easyMAG®

(bioMérieux) followed by amplification on 7500 Fast Real-Time PCR System Dx (Applied Biosystems®).

Results of analytical sensitivity, exclusivity and inclusivity studies are presented below.

Analytical sensitivity was determined on the whole system using *in vitro* transcript spiked in respiratory samples. This study showed a 95% limit of detection at 2.89 $\log_{10}$ cp/mL of sample [IC 95%: 2.65–3.29] i.e. 780 cp/mL of sample [IC 95%: 450–1950].

Exclusivity was confirmed with the major human respiratory viruses including other human coronaviruses. No cross-reaction was observed. The QCMD Panel MERS-CoV 2015 was tested and results are as expected, Core and educational samples were detected. Among the 19 other commercial kits, all but one gave also the correct identification. The bioMerieux's solution targeting S gene gave equivalent results than the kits targeting upE or N gene.

The combination of RNA internal control r-gene® ready-to-use premix with the MERS-CoV primers r-gene®, MERS-CoV probe r-gene® and MERS-CoV transcript r-gene® (research use Only) is a good candidate solution for the detection of MERS-CoV virus and demonstrate the interest and reliability of the RNA internal control for the rapid development of detection tool, in outbreak situation.

http://dx.doi.org/10.1016/j.jcv.2016.08.049

Abstract no: 131
Presentation at ESCV 2016: Poster 10

Multicentre evaluation of the variability of adenovirus quantification by PCR

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Background: Viral load measurements using nucleic acid amplification techniques (NAT) are critical for the diagnosis and management of human adenovirus (HAdV) infections. A variety of laboratory-developed tests (LDT) and commercial assays are used. The aim of this study was to evaluate variability in the quantification of HAdV by NAT, and the effectiveness of candidate HAdV reference materials to harmonise viral load measurements.

Methods: HAdV positive patient samples, including; whole blood, plasma, serum, urine, stool, eye swab, nasal lavage and sputum, were sourced from clinical laboratories and typed by sequencing. Virus stocks representing the 9 HAdV types identified in the clinical samples (Types 1, 2, 4, 5, 7, 14, 31, 40, 41), were grown in Hep2C cells. The HAdV DNA concentration in the clinical and cultured virus samples was determined at NIBSC using a commercial and LDT.

Study samples comprised cultured virus representing 9 HAdV types in 10 mM Tris–HCl (pH7.4) containing 0.5% human serum albumin (TCS1-9), clinical samples diluted in HAdV-negative sample matrix, and a dilution series of cultured virus prepared in HAdV-negative sample matrix (TCS-matrix samples, these represented the same HAdV type and matrix as the clinical samples). Twelve laboratories from 6 European countries took part in the study. Each laboratory tested TCS1-9, and the clinical and TCS-matrix samples relevant to their quantitative HAdV NAT assay.

Results: In total, 16 datasets were received. The SD of the overall laboratory mean HAdV concentrations for TCS1-9 ranged from 0.40 to 1.03 Log_{10} copies/mL. The SDs were highest for Types 7, 31 and 41. The SD of the overall laboratory means for clinical samples (representing the same HAdV types) ranged from 0.33 to 1.09 Log_{10} copies/mL. For all TCS and clinical samples inter-laboratory variation in HAdV quantification was higher than the intra-laboratory variation.

The effectiveness of candidate HAdV reference materials to harmonise viral load measurements by NAT was evaluated by 'relative potency'. For all clinical samples, apart from one of the stool samples, the SD of the overall laboratory mean was reduced when the results were expressed relative to the corresponding TCS-matrix sample. The effect of diluting the cultured virus in different sample matrices was determined by plotting the individual laboratory results for the TCS-matrix dilution series. The mean slope for each TCS-matrix sample ranged from 0.76 to 1.19.

Conclusions: The results suggest that there is variability in the quantification of different HAdV types by pan-HAdV NAT, but this would reduce through standardisation to a common reference material. A proposal to develop the 1st WHO International Standard for HAdV for NAT has been endorsed. The results of this study will be used to determine the most appropriate source material and formulation for the candidate standard. The availability of a WHO International Standard for HAdV for NAT will help to standardise these assays and enable comparison of measurements within and between different laboratories, thereby improving patient management.

http://dx.doi.org/10.1016/j.jcv.2016.08.050

Abstract no: 132
Presentation at ESCV 2016: Poster 11

Evaluation of two algorithms for diagnosis of Epstein-Barr virus infection

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Introduction: Epstein-Barr virus (EBV) serology is mainly used to identify primary infections. Two algorithms have been proposed for diagnostic approach: the anti-VCA approach, in which the study continues if at least one of the markers, IgG or IgM, is reactive; and the anti-EBNA approach, in which the study continues only if the anti-EBNA-1 marker is negative. In our hospital we have used the full panel until now.

Since the EBV seroprevalence rate is as high as 95% amongst adults and the majority is reactive to anti-VCA IgG and anti-EBNA-1 antibodies, it is expected that the anti-VCA approach would trigger more sequels than the anti-EBNA approach, with more costs.

Objective: To evaluate if the use of the anti-EBNA and anti-VCA approaches prevent the identification of important clinical situations.

Material and methods: We have retrospectively applied both screening algorithms to all EBV serology results done in our hospital between January 2013 and December 2015 ($n = 3090$). Diagnoses obtained with both algorithms were compared with those obtained with the full panel. We have analysed the distribution of serological patterns and the clinical relevance of those patterns that would be lost with the algorithms' implementation.

Results: The anti-VCA approach would prevent the use of anti-EBNA-1 test in 3.9% of all cases. The anti-EBNA approach would prevent the use of anti-VCA IgG and IgM tests in 95.7% of all cases.

Regarding the anti-VCA approach, when we have an isolated anti-EBNA-1 pattern (suggesting a past infection), it would be classified as negative (2.94% of all cases). It will not be a problem in the investigation of a primary infection but it may be relevant in the study of diseases that we know can be associated with EBV infection (autoimmune diseases, post-transplantation lymphoproliferative disease, transplant rejection, lymphomas in the context