TT32 Dundas, Nicola E.

Operational Simplicity of the xTAG RVP Using Lean Analysis – Diagnostics on a Diet

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In today's environment of increased demand and decreased supply of medical technologists, reducing labor time while maintaining and improving quality diagnostic medicine is more important than ever. Lean principals have been applied to other industries and processes with great success. Only recently have these principles been applied to the healthcare industry. The virology laboratory at Children's Medical Center Dallas (CMCD) processes approx. 7000 respiratory samples annually. Turn around time ranges from 2 hours for a direct fluorescent antibody (DFA) assay, to ten days for tube cultures. Not only are these traditional methods limited by a slow turn around time, but they also require a significant amount of hands on time from the technologist. The Luminex xTAG Respiratory viral panel (RVP) offers a faster and more efficient manner to diagnose viral pathogens. To critically evaluate the potential advantages of the RVP in terms of work flow, Lean analysis techniques (operator analysis) were used to compare the RVP workflow and the current respiratory viral identification workflow. An operator analysis includes videotaping the entire work process and doing a very detailed analysis of each step the operator performs. Also included in the analysis is amount of operator hands on time, number of touches necessary to complete the workflow and turn around time. The processes for RVP, routine DFA alone, and routine DFA followed by culture were analyzed. The average time to a result for the RVP was 27 hours including overnight incubation, whereas the average time to result for a DFA followed by an R-mix culture was 54 hours. Minimally, a DFA result is available within 2 ½ hours of the beginning of the process, however the time to a result could be as long as 13 days, including 3 days for confirmation of rhinovirus. The time to a result for the RVP is constant at 27 hours, regardless of the outcome. The number of steps required to produce a result for the RVP was 52. In contrast, the number of steps to result a DFA was 34 per sample, and the maximum number of steps to produce a culture result, including final identification, was 310. **Conclusion**: The LMD xTAG RVP improves turnaround time and decreases required technologist hands on time compared to culture. Additionally, the RVP requires substantially less manipulation by technologists than traditional culture methods, resulting in a simpler, more efficient methodology.

ST50 Harter, Angela

Detection of Novel and Common BRAF Mutations in Thyroid Fine Needle Aspiration (FNA) Samples

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Introduction: Papillary thyroid carcinoma (PTC) is the most common type of thyroid malignancy. BRAF V600E point mutation is associated with PTC and serves as an unfavorable prognostic marker. Detection of BRAF mutation in thyroid fine needle aspiration (FNA) is important for preoperative evaluation of thyroid nodules. In this study we report a method that detects the most common BRAF V600E mutation as well as novel BRAF mutations preoperatively in thyroid FNA samples. **Methods**: Portions of FNA samples were collected directly into preservative solution and DNA was isolated using MagNA Pure Compact (Roche). The method for detection of BRAF mutations at codon 600 and in the adjacent codons was developed and validated by the Molecular Anatomic Pathology laboratory, UPMC and consists of real-time PCR and post-PCR melting curve analysis on LightCycler (Roche). Direct nucleotide sequencing was used for confirmation of mutation type on ABI 3730 (Applied Biosystems). **Results**: BRAF mutations were identified in 32 thyroid FNA samples. In 30/32 (94%) cases, a common BRAF V600E mutation was detected. In addition, two rare types of BRAF mutation were found: one case had a novel complex mutation BRAF T599I, I599_V600insL and one case had a rare BRAF K601E mutation. The patient diagnosed with the novel BRAF T599I, I599_V600insL mutation in the FNA sample subsequently underwent surgical treatment which revealed papillary carcinoma. The same mutation type was confirmed in the resected tumor material. **Conclusions**: The method we developed allows detection of a common BRAF V600E mutation as well as a rare BRAF K601E mutation and other mutations involving this critical region of BRAF in preoperative thyroid FNA samples. The novel BRAF T599I, I599_V600insL mutation discovered with this method is associated with papillary thyroid carcinoma.

TT26 Jama, Mohamed

Direct PCR from Whole Blood

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The advent of the PCR technology has transformed the utility of this diagnostic tool in the clinical laboratory. One of the major limitations in the routine use of PCR in the clinical laboratory diagnosis has been the inability to apply PCR directly to clinical whole blood samples. Typically DNA used in PCR assays is usually extracted according to several procedures that may include, phenol-chloroform, 'salting out', or recently by use of magnetic beads. The procedures are time consuming and increase the overall cost and the turn around time of the clinical assays. To overcome this handicap, we test a commercially available buffer "BloodDirect PCR buffers" on three assays performed at ARUP laboratory, two of which are commercial available kits (CF kit from Abbott diagnostics, AmpFlSTR Identifiler PCR Amplification Kit from ABI) with some minor modifications and an in-house base assay (UGT1A1) on 5 previously tested samples from each assay. All the 5 samples on the three assays performed very well from whole blood using BloodDirect PCR buffers, giving a 100 % concordance with robust results as from extracted DNA. We found this buffer to be compatible with fresh, stored, and dried blood samples on Guthrie cards. The process requires very little human blood and was compatible with a variety of commercially available DNA polymerases, (except for AmpliTaq DNA polymerases from ABI). The BloodDirect PCR buffers neutralize any inhibitory substances that might bind to DNA polymerase or template and ensure a successful PCR amplification directly from blood. More work is needed to test a couple of high volume assay to see the performance of this buffer. But initial results suggest that using BloodDirect PCR buffers with whole blood is as sensitive and specific as using purified DNA as starting material for PCR assays. This procedure provides an alternative to the tedious DNA purifications processes and may prevent possible errors inherent to the DNA extraction procedure such as samples switches or contamination.