Science Digest

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Quality assurance in molecular diagnostics

Since the use of molecular diagnostics over the last 30 years the development and performance has become increasingly complex and with it appropriate quality assurance programmes. Despite the rapid development of new or novel molecular techniques and their application to diagnosis, quality assurance and quality control have not necessarily kept-up with the developments. Examples might be automated PCR, next generation sequencing (NGS), and increasingly the use of molecular diagnostics in Point of Care Testing (POCT). A recently published multi-centre article considers correct quality assurance and looks at some future challenges (1). Considering the status-quo the authors discuss the use of ISO 15189 standards and the use of Standard Operating Procedures; validation, adequacy of training, internal QC, laboratory organization and primer design. External quality assessment is discussed in relation to over 20 proficiency providers and there is a substantial section on issues relating to legal frame works. Looking to the future, the authors again highlight the rapid development of technologies in particular multiplex PCR, Digital PCR and next generation sequencing. They discuss the problems of obtaining appropriate controls and the increasing reliance on bioinformatics. In conclusion, the authors highlight the increasing complexity for quality assurance and the necessity for harmonization and standardization of quality measures.

Be wary of autocorrect

Despite the best designed autocorrect programmes there are still issues with their use. Although most everyday applications, such as extra gaps, various versions of "Spell Check" and punctuation etc, generally work well. However, auto correct can lead to distortions of text or data. In a recent publication from Australia, the authors scanned supplementary files associated with publications from 2014 to 2020. Using scanning software developed by the authors 30.9% (3436/11,117) gene error names were identified when supplementary EXCEL lists were analysed. Although this problem had been previously identified it was not as high as the present authors found. Examples identified were auto-conversion of gene names to dates such as *SEP8* and *MARCH3* which would be incorrectly put in to data bases. They point out that typically these errors may occur in large data-sets. Analysing 166,139 genomics articles they identified gene naming errors by year and organism, and in leading high impact factor journals. They tested spreadsheet software and both Excel and Googlesheets had a high gene nomenclature autocorrections. Two other software programmes, LibreOffice and Gnumeric, were very reliable. The authors recommended that to avoid such errors, scripted analysis is preferred, use LibreOffice as the preferred spreadsheet. If using Excel care and importing data is necessary. Consider using "flat-text files" and verify gene names are intact.

Neonatal sepsis at point of care.

Neonatal sepsis is a life-threatening clinical condition, which may occur at two specific periods: within 72 hours of birth or in neonates that are <28 days old. Typically, early onset is usually due to vertical transmission from the birth canal. However, both routes of infection can result in multiple organ failure and death. A review of neonatal sepsis published this year provides an excellent overview of these infections and developments in their rapid diagnosis. (3). The authors discuss the routine methods for detecting pathogen response and the upregulation of various biomarkers during sepsis. There are well written sections relating to routinely used response biomarkers for sepsis and their limitations. Progressing to the development and the use of sensors to detect neonatal sepsis they present a comprehensive overview of what is available and developments with sensors for the rapid diagnosis of neonatal sepsis. The authors conclude that a combination of biomarkers on a single sensor platform will provide rapid, sensitive, and accurate diagnosis of this infection. The article is well-written and well-illustrated and has a comprehensive reference list, which is up to date.

When best to compete for an Olympic medal?

With the Tokyo Olympic Games now history there will be, no doubt, multiple analysis of athlete's performances and what may have contributed to their successes or failures in performance. Although elite athletes' performances are carefully monitored and appraised little attention has been given to the time of day when the athlete undertakes a competition. An international cooperation between European and USA scientists have analysed Olympic swim times from 2004 to 2016 related to the time of day using publicly available Olympic records and reports (4). Swimming was chosen as it had the least number of variables such as equipment, shoes, climate, etc. In total 144 individual swim times were analyzed including 72 female athletes. Times over heats, semi-finals and finals were analysed per stroke and normalised on an individual basis. The authors then analyzed data in three datasets: race type and time of day, time-of-day only, and magnitude of time-of-day i.e., the difference between first and second placings. The overall outcome of this research was that performance was strongly affected by the time-of-day with the fastest swim times in late afternoon (around 1700hr) with a relative improved performance from morning (0800hr). The time-of-day effects were noted as large and exceeded time differences between gold and silver, silver, and bronze, and bronze and no medals. They conclude that physical performance is not only determined by training but also the athlete's endogenous circadian rhythm system and a possible relationship with core body temperature linked to metabolic homeostasis and circadian rhythms.

Is too much exercise detrimental?

Regular exercise is known to be beneficial for both physical and mental health. But when might be too much? Often high-performance athletes comment that they have "nothing left" after extreme exercise or a competition. A recent Swedish publication may well begin to provide some answers to the issue of "nothing left" (6). Exercise training is known to increase mitochondrial oxidative capacity and improve glucose regulation. The Swedish researchers asked the question, is there an upper limit to the beneficial effects relating to the amount of exercise undertaken? To address this question they had six female and five male healthy volunteers who undertook regular exercise by endurance and strength training on a regular basis. They were all pre-tested on a variety of exercise strategies and their physiological and biochemical responses were analysed. Muscle biopsies were also taken for analysis. Diets were, as far as possible. standardised and samples were taken while fasting. The subjects then undertook progressive set exercise programmes starting with light, moderate, to excessive exercise in a controlled environment over a period of 40 days. This was followed by a monitored recovery period. At the end of each training period they had a GTT and a muscle biopsy as well as other biochemical parameters measured both during and at the end of each training period. Overall the testing period took 40 days. Analysis of the data demonstrated that there was an upper limit for the level of intensive exercise that could be undertaken without disrupting metabolic homeostasis. From the muscle biopsies the limitation of intensive exercise was correlated with a partial shutdown of mitochondrial respiratory function and hydrogen peroxide production. Associated with this was glucose intolerance. The authors strongly recommended that those undertaking strenuous exercise should carefully monitor their body responses to reduce the negative effects of exercise, which could be undertaken by monitoring glucose homeostasis.

Biotin interference

Biotin is available as an over-the-counter supplement, which is widely used by members of the public. Previously it has been demonstrated that doses of >10ng/ml of biotin has the potential to interfere with immunoassays using biotin-streptavidin systems and that approximately 60% of popular immunoassay systems use the biotin linked assays. Many of the publications to date have primarily investigated hormone or related assays and biotin interference. However, a publication from Belgium has investigated biotin interference with the serological markers antiHBs, antiHB core total antibody, and antiHBe. The investigators use healthy volunteers who took a 100mg dose of biotin and a series of pre- and post-biotin blood samples were taken. In addition, anti-HIV/24AG and anti-HCV patient samples were 'spiked' with biotin. Overall, the control (non-infected) pre-biotin administration samples were negative. However, after 1.5 hours post-biotin 80 to 90% of assays showed a significant decrease. The biotin-'spiking' demonstrated a dose dependent concentration effect i.e., as the biotin concentration increased the reliability of both assays decreased. The authors concluded that biotin interference may lead to misdiagnosis with undesirable outcomes and that analysers using the streptavidin-biotin system would be most likely to produce false low results.

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