

CONFERENCE ABSTRACTS

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Pharmaceutical Practice: Clinical Biology, Military & Emergency Pharmacy

Rapid biosensor for differentiation of types of bacteria as point-of-care testing

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Background: Detection of the bacterial cell type is an essential process in determining the treatment protocol. Analysis of detecting the type of bacteria, such as bacterial culture, takes a lot of time or is high cost such as PCR test.

Purpose: Development of a biometric sensor to detect the type of bacterial cells within 45 to 90 minutes with high accuracy.

Method: The idea of the sensor depends on a distinctive fingerprint for each type of bacteria. Its concentration is estimated to be 0.5 McFarland after exposing it to an amount of oxygen of 0.5 ml/dl for a period of 30 minutes, then exposing it to an increase in osmotic pressure to stimulate the osmo-regulation mechanism, which leads to speeding up the process by accelerating the absorption of oxygen by the bacteria, and thus Speed of obtaining the distinctive fingerprint in a time ranging from 45 to 90 minutes and the code is analysed by a computer. Experiments were conducted on 15 different types of aerobic bacteria and ten types of anaerobic bacteria at the University of Misr for Science and Technology, Faculty of Pharmacy and Cairo University and Faculty of Veterinary Medicine.

Results: The results showed a distinct and clear fingerprint for each type of bacteria; and the test can be relied upon to distinguish between the different types of bacteria in lab in a period ranging from 45 to 90 minutes.

Conclusion: This method is the fastest and least costly method globally for clinical uses, which is also appropriate for the capabilities of developing countries and can be applied as point of care testing.

Infertility factors and success in conjugal artificial inseminations

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Background: In clinical practice the authors observed that, when complying with the WHO (2010) requirements proposed as a candidate for artificial conjugal insemination (ACI), that this may not increase the possibility of pregnancy compared to couples who do not use ACI.

Purpose: To investigate a possible relationship between the number of inseminated sperm (IS) and female sterilisation-causing (SC) pathologies with the success of insemination.

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Method: Retrospective study of ICAs in our hospital (249 couples: 643 ICAs). Data collected: IS, female SC and whether or not pregnancy occurred. We selected patients who became pregnant and those with four or more ICAs without pregnancy. Classification based on the origin of the pathology: ovarian, tubal, uterine and sterility of unknown origin (SUO). Uterine and tubal pathologies were excluded due to low sample size.

Results:

-645 cycles, 72 pregnancies were obtained, calculating the relative frequencies for the different SI ranges. Similar results (approx. 20%) obtained in all ranges.

- x² statistic: p=0.16

- 249 couples, 89 with ovarian pathology and 52 with SUO were selected. ODDs ratio>1: higher probability of pregnancy in ovarian pathology.

Conclusion: There is no IS range in which the pregnancy rate is significantly higher. Probably because the sperms are previously selected by capacitation techniques, recovering the fittest. In ACI sperm are deposited directly at the bottom of the uterus, avoiding the physiological barrier of the cervix, losing SI importance.

SC ovarian pathologies are mainly related to hormonal imbalances, so the drugs used in ovarian stimulation prior to IAC can improve the chances of pregnancy. Patients with ovarian pathology are more than twice as likely to achieve pregnancy (OR=2.68) than the rest of the patients.

Results of Prevecolon programme in a sanitary district for a year

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Background: Prevecolon programme is aimed to asymptomatic population between 50-69 years old. This study is applied to faecal occult blood test (FOBT). A positive FOBT test indicates an intestinal bleeding, whose etiology must be determined performing a colonoscopy.

Purpose: To carry out a study of the results of FOBT from the Prevecolon programme in the authors' sanitary district for a year and associate it with the result of the colonoscopies performed to find out its usefulness in the early diagnosis of colon cancer.

Method: This test is based in antigen-antibody agglutination between human haemoglobin in the sample and polystyrene particles coated with human anti-haemoglobin antibodies.

It is a retrospective study with 14,285 samples included: 13,667 processed and 618 rejected.

Results: Thirteen thousand, six hundred and sixty-seven (13,667) samples were processed, with 948 positives; 863 colonoscopies were performed and 85 not performed. The results of the colonoscopies were:

- 43 adenocarcinomas; 53.0% corresponded to patients >68 years: 21 samples with results >1000ngHb/mL. Only three had results in the critical range (117-130 ngHb/mL)

- 49 adenomas with high grade dysplasia; 55.0% corresponded to patients >68 years: 21 samples with values >1000 ngHb/mL and four with values in the in the critical range.

- 480 adenomas with low grade dysplasia; 52.5% corresponded to patients > 68 years, 96 samples with values >1000ngHb/mL and 95 with values in the critical range.

Conclusion: A higher incidence of malignant processes has been observed with increasing age of the patients. The severity of the pathology is correlated with the concentration of FOBT. Results >1000ngHb/mL must be prioritised. Malignant processes are really low in the critical range.

Measurement of glomerular filtration rate (GFR) in live renal donors

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Background: Renogram in live renal donors is routinely performed using 99Tc-labeled diethylene-triamino-pentaacetic acid (99mTc-DTPA). Correlation and concordance with other measures of GFR is not already cleared.

Purpose: Comparison of GFR measurement methods in live renal donors.

Method: Retrospective study of 22 patients over 14 months. Measurement of GFR using 99mTc-DTPA, creatinine clearance (CrCl) and calculated GFR (MDRD6, MDRD4-IDMS and CKD-EPI). GFR (99mTc-DTPA). Intravenous dose of 150 μ Ci of DTPA. Blood sampling in contralateral limb two, three and four hours post-administration. Serum drug activity is counted in solid scintillation counter (Packard Cobra I). Mistry method with three extractions is used to calculate GFR. Serum and urinary creatinine. Spectophotometry (Jaffe method) in Abbott Architect c16000. Correlation between variables was analysed using the Spearman coefficient and concordance with the Bland Altman method. Data were managed with SPSS 15.0 (Chicago, SPSS Inc.) and Epidat 4.2 (Consellería de Sanidade, Xunta de Galicia).

Results: Correlation with GFR (99mTc-DTPA). Significant correlation (p<0.05) with CrCl (p=0.551, p=0.008) but not with calculated GFR. Concordance. GFR (99mTc-DTPA) did not show concordance with calculated GFR. Bland Altman analysis between CrCland GFR (99mTc-DTPA) showed an average of the differences of 3.92 (IC95 (-11.03 to 18.87)) with a standard deviation of 33.74.

Conclusion: GFR (99mTc-DTPA) is widely used in live renal donors due to its high correlation with the gold standard (inulin). Our results show positive correlation and lack of bias between CrCl and GFR (99mTc-DTPA) suggesting both methods could be exchangeable.

Comparative analysis of two methods of sperm cryopreservation

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Background: Assisted reproductive techniques with frozen semen are dependent on sperm quality post-thaw. There are several sperm cryopreservation methods and every laboratory must use the most optimal

Purpose: To test the performance of two cryopreservation methods through sperm survival estimated with motility (MES) or vitality (VES).

Method: A prospective study was carried out over 29 semen samples. Every sample was divided in two aliquots in order to test both cryopreservation methods. All aliquots had 0.7 mL of a glycerol-based cryoprotectant (SpermFreeze from FertiPro) added per mL of sample.

Cryopreservation methods:

<u>Method 1:</u> Aliquots are at room temperature for 10 minutes, then they are frozen in nitrogen vapours for 15 minutes.

<u>Method 2:</u> Aliquots are left for one hour at 4°C and they are frozen in nitrogen vapours for 15 minutes. Both are stored in liquid nitrogen for at least 24 hours.

Samples were thawed in cold water bath for five minutes. MES and VES were calculated as the percentual difference between the fresh sample (unfrozen) and after thawing. Aliquots assigned to each method were thawed on different days and were evaluated by the same observer. Statistical analysis of comparison of means was performed using Student's *t*-test on paired samples in the SPSS 13.0.1. Results were expressed as percentual average of the difference of MES or VES between methods.

Results: MES1- MES2. 8.6% (IC95(-0.47 to 17.71) *p*=0.062. VES1- VES2. 11.34% (IC95(4.59 to 18.09) *p*=0.002.

Conclusion: The results indicated that method one is better than method two. Using method two there is a significant decrease in VES and almost significant difference (p=0.062) in MES suggesting the lowest survival is caused by mortality of nonmotile sperm.

Pilot study of reference intervals for different parameters in a Spanish paediatric population

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Background: The reference intervals of the biochemical parameters allow interpreting the clinical status in patients, and they are established according to the values in 95% of the healthy population. Scientific societies recommend each laboratory defines its own intervals.

Purpose: To define the reference intervals for different parameters in a Spanish paediatric population.

Method: A retrospective study has been carried out on 2,611 healthy children, whose serum samples were processed during 2018 in automated analysers for the determination of glucose, LDH, GOT, GPT, uric acid, potassium, sodium and chlorine.

The results were classified by sex and age groups (<1, 1-5, 6-10, 11-14, 15-18), and were subjected to a statistical analysis in Excel to divide the sample into percentiles and obtain the lower and upper limits (p2.5-p97.5), according to the methodology used in the CALIPER programme.

Results: The intervals for glucose, GPT, uric acid and ions have been defined without significant differences between sexes. Regarding the age group, it is observed that ions have very stable concentrations, while glucose and uric acid increase their levels with age.

However, patients under one year of age, as well as the LDH and GOT parameters, cannot be considered because a sufficient sample size was not been obtained.

Conclusion: The reference intervals in clinical laboratory tests are a fundamental tool for decision making, so it is necessary to define them based on the evidence, test method and recommendations of clinical guidelines.

In the paediatric population, they are highly important because small variations can be decisive in the diagnosis.

Comparison of two methods for the evaluation of sperm vitality

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Background: The sperm vitality evaluated in a seminogram determines the percentage of live and dead sperm, based on the membrane integrity. This index correlates with mobility and is important in samples that have more than 40% of immobile sperm, allowing to identify if these sperm are really dead.

Purpose: To compare two methods for the evaluation of sperm vitality.

Method: The correlation study was carried out on 50 semen samples, with a sperm mobility of less than 60%.

Sperm vitality was quantified by the following methods:

- The staining method uses eosin that can cross damaged membranes, so that dead sperm appear with the head dyed pink.
- The hypo-osmotic swelling method uses a sodium citrate and fructose solution, that crosses the functional membranes, so that live sperm appear with the swollen tail.

Results: The correlation was evaluated using a linear and Passing-Bablok regression. The linear regression showed a good correlation (r=0.965), while the Passing-Bablok regression proved the absence of systematic differences, with a Spearman correlation coefficient of 0.909 and 95% CI. A linearity test was also applied obtaining a *p*-value of 0.243, so there were no significant differences. A contingency table has been prepared by dividing the samples according to vitality intervals, and it was obtained a concordance index of 88%.

Conclusion: The two methods for the assessment of vitality do not present statistically significant differences, so they are considered interchangeable.

In addition, the hypo-osmotic solution method may be a useful alternative when staining should be avoided, such as in the selection of sperm for in vitro fertilisation. Therefore, the choice of a particular method may be conditioned by the clinical situation.

Clinical and molecular review of congenital adrenal hyperplasia from a laboratory point of view

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Background: Congenital adrenal hyperplasia (CAH) is a disorder in steroidogenesis caused by an enzymatic deficiency, such as 21hydroxylase due to mutations in the CYP21A2 gene. This deficiency produces a decrease in mineralocorticoids and glucocorticoids, and an accumulation of 17 hydroxyprogesterone (17-OHP) and androgens. But clinical forms can be classified into classic (salt-wasting or simple virilising) and non-classic forms.

Purpose: To correlate different forms of CAH with laboratory data.

Method: A retrospective observational study of CAH has been carried out using data from a hospital from between 2013 to 2018. In all patients, baseline 17-OHP was measured by radioimmunoassay, and some subjects also underwent an ACTH stimulation test, considering that baseline and stimulated 17-OHP levels >10ng/ml were the cutoffs. But the confirmation diagnosis is based on the genetic study by PCR-ASO.

Results: A total of 37 cases with 21hydroxylase deficiency have been identified:

- five salt-wasting forms were detected in the first days of life, presenting severe mutations such as p.Q318X and p.R356W.

- one simple virilising form corresponding to a 32 years old man with p.1172N mutation.

- 31 non-classic forms (28 females and three males) resulting the average age at diagnosis of 18.65 years, and average levels of baseline and stimulated 17-OHP of 13 and 35.82ng/ml respectively.

Twenty-nine patients presented a mild p.V281L mutation in one of the alleles, and 19 are homozygous.

Conclusion: Neonatal screening for the 21hydroxylase deficit is essential because it is an alteration with serious consequences. It is important to consider that mutations of the 21hydroxylase enzyme gene are frequent. However, non-classic forms remain under-diagnosed in patients who consult for hyperandrogenism.

Microfluidic device for detection of COVID-19 detection in hospitals and medical labs

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Background: COVID-19 is the current most prominent global health problem. Rapid and accurate diagnosis of disease is one of the most important factors in eliminating the spread of the virus; developing countries are currently facing many problems related to the high cost of PCR tests for COVID-19.

Purpose: To develop a fast, accurate and low-cost method for making a PCR test for COVID-19.

Method: The method was based on the use of the RPA (Recombinase Polymerase Amplification) method. By making a microfluidic device including restored (RPA) Mixture and immobilised probes designed for the RPA reaction to take place inside. The experiments were conducted on 20 clinical samples, and conducted at the Faculty of Pharmacy, Tanta University.

Results: The results were identical in approximately 90% of the samples used and results were available after 30 minutes at normal room temperature. The results were read by measuring the level of the precipitate of the RPA reaction products resulting from the interaction of the reaction mixture with the Viral RNA.

Conclusion: This method is considered one of the fastest ways to detect COVID19 infection and it is the least expensive and can be used in developing countries and as point-of-care testing.

Background: Disaster management in hospital pharmacies is poorly studied and trained for.

Purpose: To assess the benefit of full-scale simulations to improve hospitals pharmacists' disaster preparedness in Switzerland.

Method: Successive full-scale simulations were realised in four hospital pharmacies. The full-scale simulations were approximatively six months apart. Three scenarios were created by an inter-professional team. Each scenario represented credible regional disasters with approximatively 50 casualties (multiple-vehicle collision, terrorist attacks and internal technical failures, respectively). Four evaluators used evaluation grids to judge participants during the simulation (rating on a scale of 1-5).

Results: All hospitals performed the initial simulation, two completed the second run and a last one realised a third exercise. The mean duration of simulations was 3.3 hours. On average, the four hospitals responded to 69% (\pm 6%) of the expected actions. Differences between exercise one and two were observed. The average rate of action achieved increased from 64% to 79% (p<0.005). Moreover, the quality of these actions improved from 3.9/5 to 4.2/5 for these two hospitals (p<0.005). The first simulation resulted in both hospital pharmacies to create a disaster plan and train their staff on it.

Conclusion: This study highlights the value of full-scale disaster simulations for hospital pharmacies. The number of correct actions increased significantly. Globally, the full-scale simulations have improved the preparedness of the hospital pharmacies involved and promoted staff awareness. Results of further simulations in the four hospitals and others are warranted to confirm these preliminary observations.

Multi-centre full-scale simulations in hospital pharmacies to improve disaster preparedness

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Militia pharmacy officers' roles in the Swiss Armed Forces during the COVID-19 pandemic

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Background: On March 2020, because of the COVID-19 pandemic, the Swiss Federal Council mobilised conscript formations of the Swiss Armed Forces. This was the largest military mobilisation since the Second World War.

Purpose: To assess the roles of the militia pharmacy officers deployed throughout the country to assist the healthcare system.

Method: All missions performed by militia pharmacy officers were systematically collected and evaluated. They were also compared to the official duties of pharmacists in the Swiss Armed Forces.

Results: Ten pharmacy officers were enlisted in two out of four hospital battalions deployed, as well as in the medical logistic battalion and in the staff of the logistic brigade that embedded them. Their missions were mainly planning, conduct and control of medical logistics, as well as hygiene and drug manufacturing activities. In the hospital battalions, they especially managed:

1) supply of medical material dedicated to mission-related training, civilian health facilities assistance and medical transportation;

2) establishment and application of hygiene procedures;

3) provision of conscripts' own medication. In the medical logistic battalion, the support of both military and civilian pharmaceutical production facilities was the most important activity (e.g. disinfectants and anaesthetics manufacturing).

Conclusion: Thanks to their civilian and military background, militia pharmacy officers have been quickly and effectively deployed throughout the country. The role of pharmacists within their respective battalions has emerged as especially crucial in the pandemic context and some of the performed missions were beyond their traditional duties. Their basic training has to be further developed accordingly.