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Validation and assessment of mepacrine testing in delta storage pool disease: A 3-centre study

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neutrophil erythrophagocytosis) allowing the identification of this cytogenetic abnormality with high sensitivity (70%) and specificity (85.7%). Suspected ider(20q) by morphology should therefore support targeted FISH tests in case of non-informative karyotype. This combined approach will allow a better estimation of the prevalence of this underdiagnosed entity.

Conclusion.— The overall survival and progression free survival did not statistically differ in both groups. However, hypogranulated and vacuolized neutrophils were significantly associated with survival.

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Thrombin generation, flow cytometry and electron microscopy is a useful combination to study tissue-factor bearing microparticles in thrombosis associated with breast cancer

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Introduction.— Patients with cancer have a 7- to 10-fold overall increased risk of developing venous thromboembolism (VTE). This risk has been notably associated with circulation of active tissue factor (TF) bearing microparticles (TF-MPs) shedding from tumors cells.

Aims.— The primary objectives of this study were to characterize structure, size and PCA of tumor-cell derived MPs released by breast cancer cells MDA-MB-231 (MDA) using thrombin generation assay (TGA), flow cytometry (FCM) and transmission electron microscopy (TEM).

Methods.— Serial dilutions of cell suspension (MDA) were incubated for 45 min at 37 °C with/without stirring condition. Samples were then centrifuged to remove cells according to three different protocols and the supernatants (Sup), containing MPs, were used for EM, FCM and TGA. Alternatively, MP fractions were filtered through 0.1, 0.22, 0.45 or 0.65 μm membranes and subjected to activity assays.

Results.—

— At the highest concentration (600,000 MDA cells/mL), neither the stirring nor the type of centrifugation significantly influenced TGA results.

— MDA and MDA Sup significantly increased the active thrombin in comparison to Normal Pool Plasma (NPP) alone. MPs fraction derived from 500 MDA cells/ml is sufficient to significantly increase the thrombin generation of NPP. The highly significant difference between MDA and its Sup was due to a loss of MPs by centrifugation as shown by FCM.

— Both PL and TF contributed to PCA of MPs, but at different stages of the coagulation cascade. Active TF is mainly present on the MPs.

— EM showed that MPs derived from MDA-MB-231 are comprised between 30 and 200 nm and that the vast majority were under 100 nm. Such results were in agreement with FCM and TGA.

Conclusions.— TGA, FCM and TEM are very interesting methods that should be combined to adequately determine the phenotype of tumor-cell derived MPs whatever their size. More sensitive techniques are currently under investigation to measure and quantify MPs.

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Separation index: A useful tool to follow up and compare instrument performance for microparticle analysis by flow cytometry

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Background.— Microparticles (MP) have high potential as diagnostic biomarkers. Standardization of their analysis by flow cytometry is limited by size-related issues. Submicron beads (Megamix [Mgx]) were proposed to help qualifying instruments and measure a reproducible part of the largest MP.

Objectives.— Based on this initial approach, the aim of this study was to develop a formula to determine the ability of a flow cytometer (FCM) to study the “large MP” subset, to monitor the instrument, to study possible optimization via technical service or optics modification and to compare instrument performance. Resolution in scatter parameters was compared on 14 FCMs of different types and brands (at least 1 of each), using a separation index (SI) based on the FSC or SSC peaks of Mgx and calculated as follows: $SI = (\text{median } 0.9 \mu\text{m} - \text{median } 0.5 \mu\text{m}) / (S.D. \ 0.9 \mu\text{m} + S.D. \ 0.5 \mu\text{m})$. The relative positions of platelet MP and Mgx were also compared.

Result.— As a result, BD FACSCantoll, Arial or LSRII showed significant inter-instrument variability on FSC with highly variable SI (< 3 to 23) but more homogeneous and higher SI based on SSC (4.5 to 25), suggesting a preferential use of SSC for sizing MP. BC FC500 and Gallios showed rather homogeneous FSC resolution with SI from 5 to 15.5 and 25 to 35, respectively. AccuriC6 showed FSC SI from 8.5 to 11; Attune, FACSArial, LSR-Fortessa, BD Influx, Partec Cy-Flow showed encouraging resolution ($SI \geq 10$); Levy-Jennings charts of FSC SI allowed raising the need for maintenance; optics optimization trials led to improved FSC resolution on LSR-Fortessa and Apogee A50. Thus, FS/FSC resolution (SI value with Mgx) is a useful Q.C. parameter for MP analysis.

Conclusion.— Comparing multiple FCMs' behavior may lead us to propose some modifications to the current ISTH MP protocol specifically for BD instruments, with emphasis on the use of SSC. This may require new standardization tools since relative positions of biological MP as compared to beads are different in SSC and FSC.

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Validation and assessment of mepacrine testing in delta storage pool disease: A 3-centre study

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Introduction.— Delta storage pool disease (dSPD) is probably the most frequent platelet disorder but is still underdiagnosed. Several methods exist to document dSPD among which flow cytometry (FCM) using mepacrine (fluorescent acridine derivative, which rapidly binds with high affinity and specificity to adenine nucleotides in platelet dense granules). However, neither standardization nor international recommendations exist.

Methods.—

— In center 1, to assess a defined mepacrine FCM protocol (BD FACSCalibur) when dSPD is suspected, 99 patients with bleeding symptoms were studied. Comparison with BC Navios (centre 1) and BD FACSCantoll (centre 3) were performed;

— Preanalytical, analytical and postanalytical variables were assessed on Cantoll (centre 2);

— After definition of a consensus revised protocol, normal values ($n=20$) of the two protocols were compared on the same samples from healthy subjects. Within assay variation ($n=10$) was established;

— The new protocol was applied to 14 samples from 12 patients suspected of dSPD and compared with the former ($n=3$);

— Two samples from healthy subjects were run in two centers with a 3-hour time delay.

Results.—

— We noted that 37 patients showed a pattern in LTA, ATP secretion or mepacrine testing, suggestive of dSPD. Analysis of mepacrine capture and release, TRAP-induced CD63 platelet membrane expression may allow a mechanistic classification of storage pool defects;

— The major changes in the consensus revised protocol are: whole blood instead of PRP, optimization of mepacrine concentration and inclusion of red blood cells as an internal control;

— Within assay variation for mepacrine capture and release were 3.0 and 6.6%, respectively;

— The same laboratory conclusion was obtained for the three samples suspected of dSPD run with both protocols in one centre and for the two same samples run in both centres.

Conclusions.— We propose a validated consensus rapid protocol for mepacrine testing by FCM which could be widely used to evidence dSPD.

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Évaluation du réactif lamotrigine QMS[®] automatisé sur CDx90[®] (Microgenics ThermoFisher Scientific)

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Introduction.— Disponible depuis 2002 (AMM) en France, la lamotrigine (Lamictal[®]) est un antiépileptique ayant des propriétés thymorégulatrices. Habituellement dosée par CLHP, l'étude porte ici sur l'évaluation du réactif QMS[®] par immunoturbidimétrie sur l'automate CDx90[®] (Microgenics ThermoFisher Scientific).

Méthodes.— Le principe de la technique est un phénomène de compétition, en présence de lamotrigine, du développement d'une turbidité avec une détection à 700 nm ; le calibrage est effectué en 6 points (de 0 à 40 mg/L). Les performances analytiques et la stabilité du réactif ont été évaluées à l'aide des solutions de contrôle et de calibrage du fournisseur. Les échantillons sériques de quatre patients ont été dosés et comparés avec une technique de CLHP (Biomnis, Lyon). Les statistiques ont été réalisées à l'aide du logiciel Methval[®].

Résultats.— Les limites de détection et de quantification sont respectivement de 0,48 et 0,66 mg/L ($n=15$). La répétabilité ($n=15$) sur deux niveaux de contrôle (2 et 8,5 mg/L) présente des coefficients de variation (CV) inférieurs à 4%. Les biais moyens sont respectivement de 0,5% et de -1%. Le CV de reproductibilité ($n=15$) est inférieur à 5% à 2 mg/L et égal à 18,6% pour 8,5 mg/L. Il n'existe pas de contamination inter-échantillons entre le calibrant à 2,5 mg/L et celui à 20 mg/L. On observe une relation linéaire significative entre la concentration en lamotrigine et le temps après calibrage pour le contrôle à 2 mg/L ($R^2=0,4720$) et celui à 8,5 mg/L ($R^2=0,9158$) ($p<0,05$). Le biais atteint -10% au bout de 12 jours pour le contrôle à 2 mg/L (sept jours pour le celui à 8,5 mg/L). Par rapport à la technique de CLHP, les résultats sont les suivants : <0,66 mg/L (<0,5), 1,52 mg/L (+26,7%), 2,48 mg/L (3,3%) et 16,97 mg/L (-3,6%) (dilution au dixième).

Conclusion.— Le réactif QMS Lamotrigine sur CDx90 permet un dosage rapide et aisé de ce médicament pour des concentrations sériques proches de 2 mg/L (zone thérapeutique en épilepsie). La stabilité du réactif impose cependant un calibrage tous les 12 jours.

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Dosage des barbituriques totaux par technique Cedia[®] sur CDx90[®] (Microgenics) chez les patients de réanimation traités par thiopental

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Introduction.— Le thiopental est un barbiturique utilisé en réanimation pour induire l'anesthésie et/ou un coma thérapeutique. Son dosage sanguin ou celui des barbituriques totaux est un élément pouvant contribuer au diagnostic de mort cérébrale avant un éventuel prélèvement d'organes. Nous avons évalué une technique d'immunoanalyse automatisée pour le dosage en routine des barbituriques totaux chez les patients traités par du thiopental.

Matériels et méthodes.— Les barbituriques ont été mesurés sur un automate CDx90[®] (Microgenics Thermo Fischer Scientific) avec un réactif CEDIA[®] du fournisseur. La calibration est réalisée en 5 points avec du secobarbital (200 à 3000 ng/mL). La répétabilité et reproductibilité ont été évaluées à partir des contrôles Microgenics (C1 : 225 ng/mL ; C2 : 375 ng/mL). La limite de quantification a été déterminée par dilution dans du plasma du point de calibration 200 ng/mL. Pour le thiopental, le facteur de récupération et le domaine de mesure ont été établis par surcharge de plasma à partir d'une solution mère de Pentothal[®] (Hospira France). Les concentrations de barbituriques ont été mesurées dans le plasma de $n=19$ patients de réanimation traités ($n=4$) ou non ($n=15$) par du thiopental. Les statistiques ont été effectuées à l'aide du logiciel Methval[®].

Résultats.— Les CV (%) de la répétabilité et de la reproductibilité sont pour C1 compris entre 2,4% et 5,2% et pour C2 entre 2,8% et 4,9%. La justesse est de 84% et de 92% respectivement pour C1 et C2. La stabilité de la calibration est de trois semaines, le réactif étant stocké à +4°C. La limite de quantification observée est de 100 ng/mL ; la plage analytique s'étend de 125 à 2500 ng/mL de plasma. Le facteur de récupération du thiopental est de 8,8% pour une concentration allant de 1,4 à 28,4 mg/L (corrélation Passing Bablok, $r=0,965$; $n=6$). Les échantillons des patients non traités par thiopental ont tous été retrouvés négatifs. Les patients traités par du thiopental ont des taux détectables de barbituriques jusqu'à sept jours après l'arrêt du traitement. La stabilité des échantillons