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Avaliação da aplicação clínica de um analisador automatizado de partículas urinárias por citometria de fluxo na triagem de amostras de urina e como preditor de provável contaminação/colonização do trato urinário

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“O sucesso nasce do querer, da determinação e persistência em se chegar a um objetivo. Mesmo não atingindo o alvo, quem busca e vence obstáculos, no mínimo fará coisas admiráveis."

José de Alen

RESUMO

Introdução: Infecções do trato urinário (ITUs) estão entre as infecções mais comuns, afetando aproximadamente 150 milhões de pacientes em todo o mundo a cada ano, sendo as mulheres as mais afetadas. Embora a cultura de urina seja considerada o padrão-ouro para o diagnóstico, tem como aspecto negativo o tempo de pelo menos 24h para a emissão dos resultados. Diante disso, o desenvolvimento de ferramentas mais rápidas e precisas para triagem e diagnóstico de ITUs tornou-se fundamental. O Exame Qualitativo de Urina consiste em uma análise simples, rápida e econômica que, em combinação com analisadores automatizados, pode otimizar a produtividade em laboratórios clínicos, mantendo a qualidade e reduzindo custos e tempo de resposta. **Objetivo:** Avaliar a aplicação clínica do equipamento Sysmex UF-5000 na detecção de ITUs e como preditor de possível contaminação ou colonização do trato urinário. **Métodos:** Um estudo transversal, observacional e retrospectivo foi conduzido em janeiro e fevereiro de 2021. As partículas urinárias foram avaliadas usando o equipamento Sysmex UF-5000. A cultura de urina foi realizada em placa de ágar de cultura cromogênica, usando MALDI-TOF para identificar o agente etiológico. Todas as avaliações foram realizadas na mesma amostra de urina, obtida de pacientes na Santa Casa de Porto Alegre. **Resultados:** O crescimento de microrganismos foi negativo em 1761 (69,7%) amostras e positivo em 766 (30,3%). Dentre as amostras positivas, 488 (19,3%) apresentaram agentes etiológicos bacterianos, 240 (9.5%) foram classificadas como culturas mistas e 38 (1,5%) apresentaram crescimento fúngico. O equipamento Sysmex UF-5000 identificou corretamente 1008 (57,2%) amostras negativas e 270 amostras positivas (65,5%), das quais 193/195 (99,0%) e 77/105 (73,3%) foram classificadas com precisão como Gram-negativas e Gram-positivas, respectivamente, com 90% de concordância [coeficiente kappa de Cohen de 0,765 (IC 95% 0,652-0,878)]. Através da análise da curva ROC, uma AUC de 0,851 (IC 95%: 0,831-0,871) foi selecionada, com um ponto de corte de 123,8 bactérias/μL (Sensibilidade: 90,0%; Especificidade: 53,6%; Valor preditivo positivo: 31,7%; Valor preditivo negativo: 95,7%; Acurácia: 60,6%). Contagens de leucócitos >141,45/μL combinadas com contagens de bactérias >123,8/μL indicaram 28,9 vezes mais chances de ITUs ao comparar casos com ambos os marcadores abaixo dos pontos de corte. Amostras de urina com contagens de bactérias e células epiteliais (/μL) superiores a 123,8 e 59,05, respectivamente, tinham 9,29 vezes mais chances de serem classificadas como culturas mistas do que amostras com contagens abaixo desses valores. Células epiteliais foram encontradas com mais frequência em amostras de urina com bactérias Gram-positivas do que Gram-negativas, sugerindo que a presença de bactérias Gram-positivas na urina está mais intimamente associada à contaminação ou colonização. **Conclusões:** Considerando a baixa taxa de falsos negativos (4,3%) e o valor preditivo negativo relativamente alto (95,7%) no ponto de corte de 123,8 bactérias/μL, concluímos que o analisador pode ser usado como uma ferramenta de triagem rápida e apropriada para amostras negativas de urina em nossa população. Bactérias Gram-positivas estão mais frequentemente associadas à contaminação/colonização do trato urinário. Associando contagens de células epiteliais e bactérias na urina, casos como esses podem ser avaliados com maior precisão e rapidez.

Palavras-chave: Infecção do trato urinário, bactérias, urocultura, analisador automatizado.

ABSTRACT

Introduction: Urinary tract infections (UTIs) are among the most common infections, affecting approximately 150 million patients worldwide each year, with women being the most affected. Although urine culture is considered the gold standard for diagnosis, it is time-consuming to obtain results, typically taking at least 24 hours. Given this, developing faster and more accurate tools for screening and diagnosing UTIs has become crucial. Urinalysis is a simple, rapid, and cost-effective analysis. When combined with automated analyzers, it can optimize productivity in clinical laboratories while maintaining quality and reducing costs and response time. This study aims to evaluate the clinical application of the Sysmex UF-5000 equipment in detecting UTIs and as a predictor of possible contamination or colonization of the urinary tract. **Methods:** A cross-sectional, observational, retrospective study was conducted in January and February 2021. The urinary particles were evaluated using the Sysmex UF-5000 equipment. Urine culture was performed in chromogenic culture agar plate, using MALDI-TOF to identify the etiological agent. All evaluations were conducted on the same urine sample, obtained from patients at Santa Casa de Porto Alegre. **Results:** The growth of microorganisms was negative in 1761 (69.7%) samples and positive in 766 (30.3%). Among the positive samples, 488 showed bacterial growth (19.3%), 240 showed mixed cultures (9.5%), and 38 urine cultures had yeast growth (1.5%). The Sysmex UF-5000 equipment correctly identified 1,008 (57.2%) negative samples and 270 positive samples (65.5%), of which 193/195 (99.0%) and 77/105 (73.3%) were accurately classified as Gram-negative and Gram-positive, respectively, with 90% concordance [Cohen's kappa coefficient of 0.765 (95% CI 0.652-0.878)]. Through ROC curve analysis, an AUC of 0.851 (95% CI: 0.831-0.871) was selected, with a cutoff of 123.8 bacteria/µL (Sensitivity: 90.0%; Specificity: 53.6%; Positive predictive value: 31.7%; Negative predictive value: 95.7%; Accuracy: 60.6%). White blood cell counts (WBC) >141.45/µL combined with bacteria counts >123.8/µL indicated 28.9 times higher odds of UTIs when comparing cases with both markers below the cutoffs. Urine samples with bacteria and epithelial cell counts (/µL) higher than 123.8 and 59.05, respectively, had 9.29 times higher odds of being classified as mixed cultures than samples with values below these thresholds. Epithelial cells were found more frequently in urine samples with Gram-positive bacteria than Gram-negative, suggesting that the presence of Gram-positive bacteria in urine is more

closely associated with contamination or colonization. **Conclusions**: Considering the low false-negative rate (4.3%) and the relatively high negative predictive value (95.7%) at the cutoff of 123.8 bacteria/μL, we conclude that, the analyzer can be used as a rapid and appropriate screening tool for negative urine samples in our population. Gram-positive bacteria are more often associated with urinary tract contamination/colonization. Integrating counts of epithelial cells and bacteria in urine, cases like these can be assessed with greater accuracy and speed.
Keywords: Urinary tract infection, bacteria, urine culture, automated analyzer.

LISTA DE ABREVIATURAS E SIGLAS

DNA	Ácido desoxirribonucleico
DSS	Luz despolarizada lateral
EQU	Exame qualitativo de urina
FSC	Luz dispersa frontal
SCPA	Santa Casa de Porto Alegre
ITU	Infecção de trato urinário
MALDI-TOF MS	Matrix Assisted Laser Desorption Ionization Time of Flight Mass Spectrometry
RNA	Ácido ribonucleico
SSC	Luz dispersa lateral
SFL	Luz fluorescente lateral
UFC	Unidades formadoras de colônia
UPEC	*Escherichia coli* uropatogênica

SUMÁRIO

1) INTRODUÇÃO

As infecções do trato urinário (ITUs) estão entre as mais frequentes infecções e acometem, aproximadamente, 150 milhões de pacientes por ano em todo o mundo, causando custos para a economia global de mais de 6 bilhões de dólares. As mulheres entre 18 e 39 anos são as principais acometidas, com uma incidência notoriamente maior (cerca de 6 vezes) em comparação ao sexo masculino [1,2,3,4]. Cerca de 50% das mulheres provavelmente serão afetadas pelo menos uma vez na vida e até 30% sofrerão recorrência de ITU [2,4,5].

Em geral, no início da infecção observa-se a uretra como porta de entrada de agentes etiológicos ao trato urinário, sendo que os principais responsáveis pelas ITUs são os microrganismos colonizantes da uretra ou os pertencentes à microbiota do trato gastrointestinal, tendo em vista a proximidade anatômica da abertura retal e da região periuretral, especialmente nas mulheres. Em condições fisiológicas a urina e o trato urinário são estéreis, porém a exceção é a porção distal da uretra que contém uma microbiota bacteriana normal. Com a micção da urina, essa microbiota é lavada e eliminada, impedindo o movimento ascendente de microrganismos e, consequentemente, o acesso a porções estéreis do trato urinário. Quando um microrganismo consegue ascender na uretra e chegar até a bexiga, por meio de mecanismos ainda não completamente elucidados, específicos para cada patógeno e seus fatores de virulência, tem-se o início da ITU [2,5,6].

Apesar do grande espectro de possíveis agentes etiológicos, como bactérias Gram-negativas, Gram-positivas e leveduras, na maior parte das vezes as ITUs são causadas por bactérias pertencentes à ordem *Enterobacterales*, sendo a *Escherichia coli* responsável por até 80% dos casos. Diferentemente das cepas de *E. coli* que normalmente habitam o trato gastrointestinal, as cepas responsáveis por esse grande número de casos de ITUs são cepas mais adaptadas para viver no trato urinário e que também conseguem evadir a resposta imune do hospedeiro por meio de mecanismos como a adesão e invasão da células uroteliais. Essas cepas são conhecidas como *Escherichia coli* uropatogênicas (UPEC) e, seguido delas, os outros principais causadores de ITUs são outros bacilos Gram-negativos, como *Klebsiella pneumoniae* e *Proteus* sp., e cocos Gram-positivos, como *Enterococcus* sp. e *Staphylococcus saprophyticus* [4,5,7,8].

As ITUs são infecções que atingem tanto pacientes da comunidade quanto os hospitalizados e são classificadas em infecções do trato urinário superior ou inferior. Essa classificação é realizada de acordo com o local em que acontecem, sendo a pielonefrite uma infecção do trato urinário superior e a uretrite, a cistite e a prostatite exemplos de infecções do trato urinário inferior. Também, clinicamente, as ITUs são classificadas em complicadas ou não complicadas, conforme a presença de fatores de risco associados [1,2,9,10]. Tradicionalmente, as ITUs não complicadas são aquelas que acometem hospedeiros saudáveis e que não apresentam nenhuma anormalidade funcional ou estrutural no trato urinário e, no caso das mulheres, aquelas que acometem as não gestantes. Já as infecções complicadas são aquelas que, além das gestantes, acometem indivíduos que apresentam alguma particularidade no trato urinário como, por exemplo, obstrução, presença de corpos estranhos (como cálculos), retenção urinária causada por doença, imunossupressão, insuficiência renal, transplante renal, inserção de cateteres, entre outros [1,2,10].

Além dos fatores funcionais e estruturais do trato urinário, outros fatores como idade, atividade sexual, histórico familiar, presença de outras comorbidades e histórico individual de ITU são também determinantes para o desenvolvimento da infecção [5].

O padrão ouro para diagnosticar uma ITU é a cultura de urina (urocultura), para a qual a urina é geralmente obtida por micção espontânea pela coleta do jato médio urinário após a higiene da região urogenital, com posterior avaliação microbiológica. O diagnóstico é realizado de acordo com a combinação de sintomas urinários e a cultura da urina, que demonstra quantificações do patógeno acima de um limite estabelecido [2]. Sintomas clínicos como disúria, urgência urinária, hematúria e dor lombar são sintomas frequentemente relatados, porém, não são sintomas exclusivos apenas de ITU [11]. Quantificações do agente etiológico superiores a 10^3 unidades formadoras de colônia (UFC) por mililitro (mL) são consideradas significativas, embora possam estar também associadas à colonização ou contaminação urinária. Além disso, em alguns casos, limites tão baixos quanto 100 UFC/mL são também considerados e, da mesma forma, quantificações maiores ou iguais a 10^5 UFC/mL são normalmente as mais utilizadas e aceitas como ponto de corte no diagnóstico de ITU [2,12].

Sintomas clínicos e presença de bacteriúria também ocorrem, frequentemente, de modo independente, uma vez que aproximadamente 20% das mulheres que apresentam sintomas clássicos de ITU têm uroculturas negativas. Desse modo, a indicação de tratamento com base exclusivamente nos sintomas pode superestimar a presença de ITU em mais de 33% [13,14]. Adicionalmente, indivíduos saudáveis e/ou assintomáticos também podem apresentar contagens significativas de bactérias na urina. A presença de bacteriúria, de forma assintomática, pode estar relacionada com idade avançada e com atividade sexual recente e, ainda que aumente o risco de desenvolvimento de ITU sintomática, não deve ser tratada farmacologicamente, exceto para o caso de mulheres grávidas ou submetidas a procedimentos invasivos no trato urinário. Além disso, o tratamento desses casos, muitas vezes, gera sobrediagnósticos e afeta a microbiota do paciente, trazendo possíveis danos à saúde, visto que possibilitam a seleção de patógenos e o desenvolvimento de resistência aos antimicrobianos [2].

A cultura de urina, além de permitir a estimativa da concentração de microrganismos isolados, também auxilia na escolha da melhor terapia com antimicrobianos, quando realizados os testes de sensibilidade. Todavia, além da prevalência de resultados negativos ser significativamente elevada (em torno de 60% a 80% dos casos), o tempo para que os resultados das culturas sejam disponibilizados é de, pelo menos, 24 horas, sendo necessário, muitas vezes, iniciar tratamentos empíricos com antimicrobianos sem o apoio de evidências laboratoriais, provocando o tratamento excessivo e desnecessário dos sintomas em muitos casos [4, 7, 15, 16, 17].

As ITUs são a razão mais comum para a prescrição de antibióticos a nível mundial, representando, aproximadamente, 15% de todos os antibióticos prescritos para humanos no Estados Unidos, com um custo anual estimado de US$ 1 bilhão e um o custo anual indireto estimado em aproximadamente US$ 1,6 bilhão [18,19]. Como consequência disso, muitas das bactérias causadoras de ITUs tornaram-se capazes de desenvolver resistência aos antimicrobianos, de modo que a capacidade de as eliminar com o uso de terapias antimicrobianas diminuiu significativamente com o tempo. Estudos publicados entre 2009 e 2014 apresentaram taxas de resistência a antibióticos encontradas em bacilos Gram-negativos em todo o mundo, que variavam de 10 a 80% para fluoroquinolonas, de 10 a 70% para cefalosporinas de terceira geração e de 5 a 35% para carbapenêmicos, sendo essa variação associada e dependente da localização geográfica em que o estudo foi realizado [10]. Dessa forma, além de se observar cada vez mais a necessidade da pesquisa e do desenvolvimento de novas drogas capazes de combater a resistência antimicrobiana, o diagnóstico preciso das ITUs é fundamental e imprescindível [4].

As culturas de urina são realizadas a partir da semeadura quantitativa de 1 µL da amostra, com alça bacteriológica estéril, em ágar cromogênico para urocultura. Após 18 a 24h de incubação aeróbica a 36°C, o número de colônias é contado e multiplicado por 1.000 para calcular o(s) microrganismo(s) potencialmente patogênico(s), expressos por mililitro de urina. As culturas com crescimento bacteriano inferior a 10 colônias na placa (10^4 UFC/mL) são consideradas negativas. Amostras puras, com crescimento igual ou superior a 100 colônias, isto é 10^5 UFC/mL, são automaticamente consideradas positivas e enviadas para identificação. Amostras com crescimento de dois ou mais microrganismos, sem que um deles seja predominante, são classificadas como “crescimento bacteriano misto” e não são enviadas para a identificação. Já as culturas com dois microrganismos, ambos com quantificação superior a 10^5 UFC/mL, bem como as amostras com crescimento de um único microrganismo em quantificação de 10 a 90 colônias em placa (10 a 90.000 UFC/mL), são também consideradas positivas e enviadas para identificação, porém, com indicação de provável colonização de trato urinário e/ou contaminação da amostra.

Após a avaliação do crescimento do patógeno na cultura urinária, a correta identificação do microrganismo também é de extrema importância, sendo essa etapa fundamental para verificar a necessidade de submeter o microrganismo presente na amostra de urina ao teste de suscetibilidade a antimicrobianos. A tecnologia MALDI-TOF MS (*Matrix Assisted Laser Desorption Ionization Time of Flight Mass Spectrometry*) é a tecnologia padrão-ouro para a identificação de microrganismos e nos últimos anos tem-se expandido significativamente, sendo amplamente utilizada para os mais variados tipos de amostras clínicas, como urina, sangue, escarro, líquor, etc [17,19,20,21].

Para a identificação do microrganismo, uma colônia pura é transferida para a placa de identificação específica do equipamento, sendo posteriormente adicionada uma matriz também específica, composta por ácido α-ciano-4-hidroxicinâmico. Após secagem, a placa é colocada dentro do equipamento e a análise é iniciada. Sobre a placa contendo o microrganismo e a matriz, ocorre a irradiação de um laser e as proteínas bacterianas, principalmente as ribossomais, são ionizadas. O tempo necessário para essas proteínas ionizadas se deslocarem até o detector, após formação de vácuo, determina a relação massa-carga (m/z) das proteínas componentes e a intensidade do sinal fornece um espectro de massas padrão. O espectro de massa obtido é então comparado com os disponíveis em um banco de dados de referência para a identificação dos microrganismos, a nível de gênero e espécie [20]. Além de ser um método robusto, preciso, fácil, rápido e de baixo custo quando comparado a outros métodos convencionais de identificação, o emprego do MALDI-TOF MS possibilita à equipe médica escolhas mais rápidas e assertivas para a terapia de pacientes, sendo especialmente importante no diagnóstico e tratamento de doenças infecciosas que requerem uma resposta rápida [19, 20].

A avaliação do crescimento bacteriano, após a realização da cultura urinária, associada a avaliação de outros parâmetros urinários, por meio da realização do Exame Qualitativo de Urina (EQU) é de grande valia na distinção entre ITU real e contaminação/colonização. Através do EQU, é possível observar uma grande variedade de partículas e elementos como cilindros, albumina, muco, cristais, leucócitos, células epiteliais, hemácias, que auxiliam na contextualização dos prováveis cenários [12,18]. Na presença de ITU, por exemplo, a resposta inflamatória contra os microrganismos patogênicos é frequentemente observada, com a presença de leucocitúria e hematúria [18, 22].

As hemácias são células anucleadas em formato de disco bicôncavo e que apresentam um diâmetro médio de cerca de 7,0 µm. São células encontradas na urina de indivíduos saudáveis, todavia, em baixa quantificação: de 3 a 12 células por microlitro de urina. Normalmente, na análise microscópica do sedimento urinário, não excedem a quantidade de 0 a 3 células por campo de grande aumento (400x). Uma vasta quantidade de doenças pode causar hematúria, isto é, a presença de números anormais de eritrócitos na urina. As causas mais frequentes de hematúria, macroscópica ou microscópica são ITUs, doenças urológicas (como urolitíase, câncer de rim e de bexiga) e doenças glomerulares. Na presença de processos inflamatórios, como uma ITU, alterações na integridade da barreira vascular normal dos órgãos do trato urinário frequentemente acontecem, de modo que as hemácias podem ser extravasadas para fora dos capilares, chegando na urina [22, 23].

Os leucócitos também são células normalmente encontradas em urina de indivíduos saudáveis. Na análise microscópica do sedimento urinário as contagens normais variam de 0 a 8 leucócitos por campo de grande aumento ou, aproximadamente, 10 por microlitro de urina. Tendo em vista que são células de defesa, em resposta a processos inflamatórios como uma ITU, os leucócitos se movem por quimiotaxia através dos tecidos, saindo dos capilares peritubulares em direção ao tecido renal afetado. Na urina, os leucócitos mais encontrados são os neutrófilos, os quais, em especial em ITUs, tendem a se apresentar na forma de aglomerados [22, 23]. Visto que os leucócitos comumente sofrem lise na urina, discrepâncias podem ocorrer entre o número de células vistas microscopicamente e o número real de células. Desse modo, na análise físico-química da urina, a presença de esterase leucocitária é verificada, enzima produzida por neutrófilos e que pode sinalizar leucocitúria associada à ITU [24]. Outro aspecto importante está relacionado ao fato de os leucócitos serem células também encontradas em urinas contaminadas por secreções genitais, em especial em mulheres. Nesses casos, normalmente são observados em conjunto com células epiteliais escamosas, com ou sem a presença de bactérias ou outros microrganismos [23].

As células epiteliais podem ser encontradas na urina em decorrência da renovação celular normal das células envelhecidas do trato urinário ou em resposta a danos e descamação causadas por lesões ou processos inflamatórios renais. Três tipos principais são observados na urina: células epiteliais escamosas, células epiteliais transicionais e células epiteliais tubulares renais, sendo as escamosas as mais largamente encontradas e relatadas. Em geral, a presença de um grande número de células epiteliais escamosas no sedimento urinário está associada a contaminação da região genital do paciente, de forma que em poucos casos têm real significado clínico, indicando contaminação da amostra e/ou coleta inadequada na maior parte dos casos. Em contrapartida, o aparecimento de células epiteliais tubulares renais na urina indica dano tubular renal. Por serem células provenientes do epitélio que reveste os diferentes segmentos dos túbulos renais, as células epiteliais tubulares renais são um importante marcador de condições graves, como necrose tubular aguda, nefrite intersticial aguda e doenças glomerulares proliferativas [23,24].

Tendo em vista o cenário atual de aumento de resistência a antimicrobianos, o desenvolvimento de novas ferramentas mais rápidas e precisas de triagem e diagnóstico de ITUs se tornou imprescindível, visto que possibilitam o tratamento mais direcionado ao paciente e somente quando, de fato, necessário [7, 8, 25].

O EQU é um dos exames laboratoriais mais solicitados pelos médicos de praticamente todas as especialidades, para pacientes com as mais diversas queixas clínicas, ocupando a terceira colocação, após o perfil bioquímico sérico e o hemograma [26]. Por ser um exame simples, rápido e de baixo custo, frequentemente é empregado como teste de triagem de ampla aplicação, fornecendo informações extremamente relevantes para o diagnóstico de doenças que afetam os rins e as vias urinárias, como processos inflamatórios e infecciosos, bem como alguns distúrbios extra-renais, metabólicos como diabetes e acidose metabólica, entre outros [25,26,27]. Na maior parte dos casos, o EQU é realizado por meio de tiras reagentes com testes físico-químicos que avaliam pH, gravidade específica (densidade), urobilinogênio, bilirrubina, nitrito, esterase leucocitária, albumina e hemoglobina, adicionado da análise microscópica do sedimento urinário para detecção de elementos como células e microrganismos. Todavia, ainda que largamente presentes nas rotinas de urianálise, esses métodos são subjetivos e frequentemente apresentam baixa sensibilidade e baixo valor preditivo negativo, sendo também, na maioria das vezes, dependentes de um analista tecnicamente capacitado e com recursos operacionais adequados [25]. Como alternativa para aprimorar essas limitações e com o objetivo de maximizar a produtividade, manter a qualidade e ainda reduzir os custos e o tempo de resposta, os analisadores automatizados estão sendo muito empregados nas rotinas de análise de urina.

Nesse contexto, como alternativa para melhorar os parâmetros apresentados anteriormente e também para aumentar a confiabilidade da inspeção visual, já que esta é totalmente subjetiva e dependente da interpretação do profissional, a leitura instrumental das tiras reativas por analisadores automatizados é uma realidade cada vez mais presente nos laboratórios clínicos. O equipamento Sysmex UC-3500 (Sysmex, Kobe, Japão) é um analisador totalmente automatizado para análise química da urina, avaliando semi-quantitativamente onze parâmetros urinários (pH, esterase leucocitária, nitrito, proteínas, hemoglobina, glicose, corpos cetônicos, urobilinogênio, bilirrubina, creatinina e albumina), além de reportar a densidade urinária, cor, turvação e, ainda, fornecer a relação entre albumina/creatinina e proteína/creatinina, que muito auxiliam na triagem de um possível distúrbio renal [28].

Além dos analisadores de química de urina, os citômetros de fluxo também estão sendo amplamente aplicados como ferramenta rápida e objetiva na triagem para ITUs. Por realizarem a análise do sedimento urinário de forma totalmente automatizada, aumentam a produtividade do laboratório e auxiliam na redução de custos de mão de obra. Como a urina não centrifugada é utilizada, é eliminado o tempo gasto no manuseio e preparo do sedimento urinário concentrado por um analista. Assim, além de reduzir a exposição do profissional a potenciais riscos biológicos, tem-se a padronização da parte do exame originalmente feita no microscópio, com aumento da precisão e da reprodutibilidade dos resultados [22].

A citometria de fluxo é uma tecnologia que analisa rapidamente células e demais elementos, caracterizando-os. Após a adição de um corante fluorescente, partículas em suspensão fluem em uma célula de fluxo, individualmente, sendo atingidas por um feixe de luz de laser. Uma vez que a luz de laser incide sobre a partícula, observa-se a dispersão da luz e fluorescência, as quais, ao serem medidas, caracterizam cada uma das partículas com base em seu tamanho, forma, volume e coloração [22, 29, 30, 31]. Apesar de utilizarem uma tecnologia cujo foco inicial era a imunologia e a hematologia, os citômetros de fluxo são equipamentos rotineiramente empregados há mais de 20 anos para a realização dos exames de urina. Além disso, estes equipamentos, por terem capacidade de detectar e quantificar microrganismos, também auxiliam de forma rápida na verificação da necessidade de encaminhar ou não as amostras de urina para cultura, especialmente ao considerar a alta taxa de resultados negativos observada na prática clínica [7, 8, 30, 32]. Tendo em vista que são capazes de, entre outras partículas e células, identificar e quantificar bactérias, leucócitos, leveduras, eritrócitos e células epiteliais, esses equipamentos poderiam ser empregados para descartar amostras negativas antes de processá-las, evitando testes culturais desnecessários e que oneram pacientes, hospitais e laboratórios, além de possibilitar que os resultados negativos sejam rapidamente disponibilizados (no mesmo dia da coleta da amostra). Ainda, no caso de amostras positivas, a informação já poderia ser adiantada ao corpo clínico, antes que os resultados da cultura estejam disponíveis, possibilitando a escolha de tratamento direcionado com antibióticos mais específicos [7, 25].

O primeiro equipamento lançado utilizando a tecnologia de citometria de fluxo para a análise de amostras de urina foi o UF-100, da empresa Sysmex (Sysmex, Kobe, Japão), no ano de 1998. Por ser um citômetro de fluxo com laser de argônio, esse analisador trouxe grande avanço para análises urinárias, tendo em vista sua capacidade de identificar hemácias, leucócitos, células epiteliais, bactérias, cilindros, leveduras, cristais e espermatozoides [25]. Posteriormente, a linha evoluiu com o lançamento dos analisadores Sysmex UF-500*i e* UF-1000*i*. Esses equipamentos empregavam também a tecnologia de citometria de fluxo fluorescente, com laser semicondutor. Uma das principais melhorias advindas com o lançamento do UF-1000i foi o refinamento na detecção de bactérias, já que o analisador apresenta um canal separado para análise bacteriana [22]. Atualmente, o último lançamento da empresa é o equipamento Sysmex UF-5000 [31].

O Sysmex UF-5000 é um analisador de partículas urinárias que une a citometria de fluxo fluorescente a um sistema óptico formado por um laser semicondutor azul, permitindo a identificação e classificação de células e partículas. Seu princípio de funcionamento está na combinação de dois canais e quatro sinais de luz diferentes, que avaliam as características internas, de membrana e de superfície dos elementos presentes na urina [31]. As partículas urinárias são marcadas com fluorocromos específicos para ácidos nucléicos e para estruturas de superfície e então submetidas ao feixe do laser. A contagem e a classificação são baseadas em sinais de luz dispersa frontal (FSC), que indica o tamanho e o comprimento das partículas; luz dispersa lateral (SSC), que fornece informações sobre o conteúdo celular como núcleo e grânulos; luz fluorescente lateral (SFL), que indica a quantidade de DNA e RNA presente nas células; e a luz despolarizada lateral (DSS), que otimiza a discriminação entre hemácias. Desse modo, o padrão de sinais individuais de luz é traduzido por algoritmos específicos em "impressões digitais" capazes de quantificar, identificar e classificar as partículas presentes na amostra de urina [7, 31].

O Sysmex UF-5000 é capaz de diferenciar células epiteliais em escamosas, não escamosas, transicionais e células epiteliais tubulares renais. Além disso, detecta automaticamente e quantifica hemácias, hemácias lisadas, leucócitos, aglomerados de leucócitos, bactérias, leveduras, cristais, esperma, muco, cilindros hialinos e cilindros patológicos (não realizando a distinção entre estes). As partículas urinárias encontradas que não podem ser classificadas em uma das categorias anteriormente citadas são classificadas como "outras células", totalizando dezessete parâmetros reportáveis. Assim, por apresentarem alta capacidade de identificação, com o emprego dos citômetros de fluxo nas rotinas de uroanálise, percebe-se um aumento na sensibilidade e na especificidade do diagnóstico das ITUs. No entanto, para se determinar a identidade específica de elementos sinalizados pelo equipamento, isto é, para classificar o tipo de cilindro - que não hialino -, identificar o tipo de cristal, ou algum outro componente classificado como "outras células", a análise microscópica manual do sedimento urinário por um profissional especialista, ainda se faz necessária [7, 8, 22, 25, 31].

Estudos prévios apontam que, com o emprego da citometria de fluxo e, consequentemente, com a identificação e quantificação de células e partículas, é possível reduzir em 28-60% o número de amostras urinárias que requerem revisão microscópica convencional em um laboratório clínico. No entanto, observa-se que há uma grande variação nos pontos de corte utilizados para células, partículas e microrganismos, bem como na sensibilidade e especificidade dos resultados obtidos. Essas variações devem-se principalmente às características clínicas das populações de pacientes incluídas nos diferentes estudos e dos critérios adotados para a classificação das ITUs [7, 25].

Em relação ao Sysmex UF-5000, estudos anteriores avaliaram a aplicação do equipamento como ferramenta de triagem de amostras de urina e verificaram que o analisador pode, potencialmente, reduzir 19-32% das culturas de urina, a partir da exclusão de amostras clinicamente irrelevantes. Assim, em comparação com a urocultura, é possível disponibilizar o resultado de forma muito mais rápida, diminuindo a prescrição de antimicrobianos e os custos para as instituições e pacientes [8, 14].

A alta precisão diagnóstica do equipamento na triagem de ITU também foi evidenciada previamente, uma vez que o equipamento apresenta baixos índices de falsos negativos. Em comparação com a urocultura, foi verificada a capacidade do equipamento em classificar corretamente bactérias Gram-negativas, com taxas de sensibilidade e especificidade superiores a 80 e 90%, respectivamente [7, 8]. Embora o equipamento tenha demonstrado melhor desempenho na caracterização de bactérias Gram-negativas do que Gram-positivas, as taxas de concordância não foram suficientemente altas para justificar a substituição do método padrão-ouro, isto é, a cultura urinária das amostras de rotina [8].

Por isso, apesar dos resultados promissores, sugere-se a realização de mais estudos de avaliação do desempenho do UF-5000, visto que seus achados são extremamente dependentes da população analisada e das características dos pacientes, sendo necessário estabelecer e aprimorar pontos de corte para populações específicas [8].

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3) JUSTIFICATIVA

Dada a alta demanda de exames de urina em grandes centros laboratoriais e hospitalares como o Laboratório de Análises Clínicas Carlos Franco Voegeli do Hospital Santa Casa de Porto Alegre, o emprego de ferramentas que otimizem o processo, a identificação de agentes etiológicos e a liberação de laudos é fundamental. Isso porquê, além de reduzir a realização de etapas desnecessárias, é possível proporcionar à equipe médica a tomada de decisões mais precoces, assertivas e adequadas para o tratamento do paciente, evitando a prescrição de tratamentos desnecessários e, também, o risco de desenvolvimento de resistência bacteriana. Nesse contexto, a avaliação do desempenho diagnóstico de um analisador automatizado de partículas urinárias por citometria de fluxo surge como uma excelente alternativa para otimizar essa demanda. Por possuir a capacidade de detectar e quantificar microrganismos, elementos e células, o equipamento Sysmex UF-5000, já utilizado para realizar o Exame Qualitativo de Urina no laboratório da instituição, surge como uma ferramenta alternativa capaz de analisar e descartar amostras negativas antes de processá-las, possibilitando que os resultados negativos sejam mais rapidamente disponibilizados aos médicos e pacientes, muitas vezes até no mesmo dia da coleta da amostra. Ademais, além do ganho de agilidade proporcionado pelo equipamento, se aplicado para este fim, é possível evitar testes culturais desnecessários e que oneram pacientes, hospitais e laboratórios.

4) OBJETIVOS

4.1) Objetivo geral

Analisar a aplicação clínica de um analisador automatizado de partículas urinárias por citometria de fluxo como ferramenta de triagem de infecção e de colonização/contaminação do trato urinário.

4.2) Objetivos específicos

- Avaliar a sensibilidade e especificidade e um analisador automatizado de partículas urinárias por citometria de fluxo para amostras de urina, em comparação com a urocultura.
- Identificar o ponto de corte (*cutoff)* para a bacteriúria avaliada no analisador e que se relaciona ao crescimento bacteriano em urocultura.
- Verificar a adequação do reporte de bactérias Gram-positivas/Gram-negativas do equipamento em comparação com os resultados de urocultura.
- Verificar a associação entre a identificação de parâmetros sugestivos de colonização/contaminação (como leucócitos e células epiteliais) do analisador automatizado, em comparação com os resultados de urocultura.

5) ARTIGO ORIGINAL

Evaluation of the clinical application of an automated urine particle analyzer using flow cytometry in screening urine samples and as a predictor of likely contamination/colonization of the urinary tract.

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ABSTRACT

Background: We evaluated the clinical application of a flow cytometer analyzer as a tool for urinary tract infection screening and to identify the colonization/contamination of the urinary tract.

Methods: A retrospective observational cross-sectional study was conducted with 2527 urine samples from patients at Santa Casa de Porto Alegre, RS, Brazil. Urinalysis results from Sysmex UC-3500 and UF-5000 analyzers were included, related to samples processed for urinalysis and urine culture.

Results: A total of 1008 negative culture samples and 270 positive samples were correctly classified. ROC curve analysis indicated a cutoff of 123.8 bacteria/µL. WBC count >141.45/µL combined with bacteria counts >123.8/µL indicated 28.9 higher odds of ITU when comparing cases with both markers below the chosen values. Urine samples with bacteria and epithelial cell counts (/µL) higher than 123.8 and 59.05, respectively, had 9.29 higher odds of being classified as mixed cultures than samples with values below these thresholds. Epithelial cells are most often found in urine samples with Gram-positive bacteria rather than Gram-negative.

Conclusion: We concluded that the analyzer is suitable technology for discriminating culture-negative urine specimens in our population. Furthermore, it was possible to highlight that Gram-positive bacteria are more frequently indicative of urinary tract contamination/colonization.

1. INTRODUCTION

Urinary tract infections (UTIs) are among the most common infections, affecting approximately 150 million patients worldwide each year, incurring a global economic cost of over 6 billion dollars. Women between 18 and 39 years old are the most affected population, with a significantly higher incidence (about 6 times) than males [1,2,3,4]. Despite a wide range of potential etiological agents such as Gram-negative bacteria, Gram-positive bacteria, and yeast, UTIs are most often caused by bacteria belonging to the *Enterobacterales* order, with *Escherichia coli* responsible for over 80% of cases [5,6,7].

In combination with the presence of symptoms such as dysuria, urinary urgency, hematuria, and lower back pain, the urine culture is the gold-standard method for UTI diagnosis. The sample is usually obtained through spontaneous midstream urine collection after urogenital tract hygiene and through microbiological assessment. [2, 5,8, 9,10].

In addition to the significantly high prevalence of urine samples with no microbial growth (approximately 60% to 80% of cases), urine culture also is time-consuming, as the culture results typically take at least 24 hours to be available. Consequently, empirical antimicrobial treatments are often initiated without the support of laboratory evidence, resulting in unnecessary over-treatments in many cases, which can collaborate to resistance to antibiotics [4, 5, 8, 11, 12]. Clinical symptoms and bacteriuria often occur independently, as approximately 20% of women with classic UTI symptoms have negative urine cultures. Furthermore, healthy and/or asymptomatic individuals may also exhibit urine with significant bacterial counts. The presence of asymptomatic bacteriuria may be associated with advanced age and recent sexual activity and, although it increases the risk of symptomatic UTI development, and in most cases, it should not be pharmacologically treated as it raises the likelihood of overdiagnosis and affects the patient's microbiota [2].

Flow cytometers are equipment with the ability to detect and measure cells and particles such as bacteria, leukocytes, yeast, erythrocytes, and epithelial cells, and they can be employed to rule out negative samples before processing, avoiding unnecessary culture tests that burden patients, hospitals and laboratories. This approach allows the rapid release of negative results, often on the same day as the sample collection. Moreover, in the presence of samples with parameters associated with an infectious/inflammatory urinary profile, the clinical staff can be informed before culture results are available, making easier and faster the selection of the correct antibiotic treatment [5, 13]. Given that the assessment of some urinary parameters obtained through urinalysis can improve the accurate identification of a UTI [10,14], this study aimed to analyze the clinical application of a flow cytometer analyzer (Sysmex UF-5000) as a tool of UTI screening, and to signalize urinary tract contamination or colonization, corroborating in evaluating the sample's quality.

2. METODOLOGY

2.1 Study design: A retrospective observational cross-sectional study was carried out with urine samples from patients of a quaternary hospital (Santa Casa de Porto Alegre - SCPA) that performs highly complex surgeries and organ transplants located in Porto Alegre, RS, Brazil, from January to February 2021. The urinalysis results present in the central processing unit of the Urinalysis Section of the Carlos Franco Voegeli Clinical Analysis Laboratory, referring to samples processed for urinalysis and urine culture, simultaneously. The SCPA Research Ethics Committee evaluated the study and approved it under nº 4,577,958.

2.2 Urinalysis: Analysis of urinary parameters was performed using the Sysmex UC-3500 and UF-5000 analyzers (Sysmex Corporation, Kobe, Japan). The parameters pH, specific gravity, hemoglobin, glucose, ketones, leukocyte esterase, nitrite, urobilinogen, bilirubin, creatinine, proteins, color, and turbidity were evaluated using the Sysmex UC-3500 equipment and, red blood cells (lysed and non-lysed), leukocytes, epithelial cells, renal tubular epithelial cells, casts (hyaline and pathological), bacteria, yeast, sperm, mucus, conductivity, and osmolality were evaluated using the Sysmex UF-5000 equipment. Samples with ITU flag release, was realized a microscopic review of the urine sample

2.3 Urine culture: The urine samples were performed according to the institution's standard operating procedure. Before analyzing the samples into the Sysmex UC-3500 and UF-5000, inoculation for urine culture was carried out with aerobic incubation at 36ºC for 18 to 24 hours. Cultures with bacterial growth <10^4 CFU/mL were considered negative and those with a single microorganism growing ≥10^5 CFU/mL were considered positive. Cultures with the growth of two microorganism and quantification <10^5 CFU/mL and cultures with the growth of three or more microorganism, without one of them being predominant, were classified as mixed cultures. Cultures with two microorganisms and both with quantification >10^5 CFU/mL as well as samples with the growth of a single microorganism and growth between 10 to 90,000 CFU/mL were considered positive and sent for identification, however, with the indication of probable colonization of the urinary tract and/or sample contamination. The identification of microorganisms was performed using the MALDI-TOF (Matrix Assisted Laser Desorption Ionization Time of Flight Mass Spectrometry) methodology, in the MALDI Biotyper equipment (Bruker Daltonics, Bremen, Germany) (BrCast, 2021; Manual de Microbiologia Clínica para o Controle de Infecção em Serviços de Saúde, 2004; Procedimentos Básicos em Microbiologia Clínica, 2010).

2.4 Statistical analysis: Data analysis was performed using the Statistical Package for the Social Sciences (SPSS) version 23.0 for Windows®. A p-value of <0.05 was considered statistically significant. To characterize the sample, categorical variables were presented according to their frequency and percentage, and quantitative variables with mean and standard deviation for parametric variables and median for non-parametric ones. The Chi-square, Mann-Whitney, and Kruskal-Wallis tests with Dunn's test were used to assess differences in the distribution of the analyzed categorical variables. For purposes of comparison between the two groups, Student t-test and logistic regression were performed. The ROC curve was used to assess the sensitivity and specificity of the parameters for which cutoffs were established.

3. RESULTS

In our study 2527 urine samples were evaluated, 1593 were from female patients (63.0%). Most patients were between 18 and 60 years old (53.4%), followed by >60 years old patients (38.7%) and only a small sample was from patients <18 years old (7.9%). The urine culture was negative in 1761 (69.7%) samples, while 766 (30.3%) samples were positive for microorganism growth. Out of these, 488 showed bacterial growth (19.3%), 240 showed mixed cultures (9.5%), and 38 urine cultures had yeast growth (1.5%). The urine samples that showed bacteria as etiological agents had a higher number of WBC, WBC clusters, and RBC in comparison to negative samples or urine cultures with yeast growth or mixed cultures (p<0.001). The urines that presented mixed cultures or yeast growth had a higher number of epithelial cells than samples with positive or negative urine cultures (p<0.001). The negative samples showed a smaller number of WBC, RBC, and epithelial cells in comparison with positive samples (bacteria and yeast) and mixed cultures (p<0.001). Indeed, 99.2% of the negative samples show a negative nitrite test result (p<0.001) (Table 1).

Table 1 – Chemical and microscopic parameters of urine samples, according to urine culture.

		Urine Culture								
Parameter	**Result**	**Total (2527)**		**Negative (1761)**		**Positive/Bacteria (488)**		**MC+YLC (278)**		***p-value***
		n	**%**	**n**	**%**	**n**	**%**	**n**	**%**	
WBC Clusters (/µL)	mean±SD	15.6±143.9		2.0±24.9		50.3±184.1		40.5±348.3		**<0.001**
Epithelial cells (/µL)	mean±SD	25 .1±49.5		20.6±45.4		29.2±45.1		45.9±71.4		**<0.001**
Nitrite	Negative	2334	92.4	1747	99.2	321	65.8	266	95.7	**<0.001**
	Positive	193	7.6	14	0.8	167	34.2	12	4.3	
Leukocyte esterase	Negative	1684	66.6	1390	78.9	176	36.1	118	42.4	**<0.001**
	Traces	0	0.0	0	0.0	0	0.0	0	0.0	
	1+	272	10.8	166	9.4	66	13.5	40	14.4	
	2+	262	10.4	116	6.6	89	18.2	57	20.5	
	3+	309	12.2	89	5.1	157	32.2	63	22.7	
WBC (/µL)	0	2	0.1	2	0.1	0	0.0	0	0.0	**<0.001**
	1-12	1242	49.1	1085	61.6	85	17.4	72	25.9	
	13-36	451	17.8	323	18.3	73	15.0	55	19.8	
	≥37	832	32.9	351	19.9	330	67.6	151	54.3	
RBC (/µL)	0	17	0.7	12	0.7	5	1.0	0	0.0	**<0.001**
	1-12	1577	62.4	1186	67.3	260	53.3	131	47.1	
	13-36	423	16.7	291	16.5	71	14.5	61	21.9	
	≥37	510	20.2	272	15.4	152	31.1	86	30.9	

SD: Standard deviation; WBC: White blood cells; RBC: Red blood cells; YLC: Yeast-like cell; MC: Mixed culture. Pearson Chi-Square test.

Table 2 – Bacterial parameters evaluated by the urine flow cytometer associated to urine culture.

		Urine culture								
Parameter	**Result**	**Negative**		**Positive (bacteria)**		**YLC Growth**		**MC**		***p-value***
		n	**%**	**n**	**%**	**n**	**%**	**n**	**%**	
Flag "*Urinary tract infection*?"	No	1008	57.2	76	15.6	3	7.9	57	23.8	**<0.001**
	Yes	753	42.8	412	84.4	35	92.1	183	76.3	
Gram classification	Not reported	227	30.1	12	2.9	7	20.0	10	5.5	**<0.001**
	Gram Positive	427	56.7	105	25.5	14	40.0	139	76.0	
	Gram Negative	12	1.6	195	47.3	2	5.7	10	5.5	
	Gram Pos/Neg	10	1.3	93	22.6	4	11.4	17	9.3	
	Unclassified	77	10.2	7	1.7	8	22.9	7	3.8	

Neg: Negative; Pos: Positive; MC: Mixed culture; YLC: Yeast-like cell; Pearson Chi-Square test, Kruskal-Walli's test and Dunn's post hoc tests.

We evaluated parameters associated with bacterial performance of the analyzer Sysmex UF-5000 and observed that 1008 negative culture samples (57.2%) didn't show the flag "urinary tract infection" (true negative result) and 753 (42.8%) did (false positive result). Indeed, 412 of 488 samples (84.4%) that showed a positive result for bacteria in urine culture showed the flag "urinary tract infection" (true positive result). This flag was also reported for 35 of 38 cases of urines with yeast growth (92.1%) and 183 of 240 mixed cultures (76.3%).

Considering the urine samples that showed the flag "urinary tract infection", we also evaluated the Gram classification reported for Sysmex UF-5000: the samples with mixed culture were classified as Gram-positive and Gram-negative in 139 (76%), and only 10 (5.5%) samples, respectively. About half of the yeast growth couldn't have the Gram classification reported or it was unclassified (20% and 22.9% respectively), but in those that the equipment showed classification, 70% (14/20) were classified as Gram-positive. Indeed, of the 412 positive samples that showed the flag "urinary tract infection" 195 were classified as Gram-negative (47.3%) followed by 105 and 93 of Gram-positive (25.5%) and Gram-positive/negative (22.6%), respectively. Moreover, the samples that showed negative urine culture (427/753) were also classified as Gram-positive for most cases (56.7%) and only 12 (1.6%) were classified as Gram-negative.

Table 3 – Bacterial parameters evaluated by the urine flow cytometer and colony forming unit count.

Parameter	Result	Urinary culture count (CFU/mL) – Total samples								p-value
		<10,000		10,000-90,000		≥100,000		MC		
		n	%	n	%	n	%	n	%	
Flag "*Urinary tract infection?*"	No	1008	57.2	32	32.0	47	11.0	57	23.8	**<0.001**
	Yes	753	42.8	68	68.0	379	89.0	183	76.3	
Bact (/µL)	Mean ±SD	823±3261		1874±8900		20015±24694		4848±12211		**<0.001**

Bact: Bacteria; SD: standard deviation; MC: Mixed culture. Pearson Chi-Square test, Kruskal-Walli's test and Dunn's post hoc tests.

We evaluated how the microorganism growth (including bacteria and yeast) was related to the flag "urinary tract infection?" and observed if it matched with the bacteria count (/µL) performed by the equipment. The flag "urinary tract infection" was shown in 89% and 68% of urine cultures with microorganism growth >100,000 CFU/mL and between 10,000-90,000 CFU/mL, respectively. Indeed, 76.3% of mixed cultures showed the same flag and 57.2% of negative cultures didn't show the flag. Bacterial count (/µL) by Sysmex UF-5000 was higher to urine culture with >100.000 CFU/mL in comparison to other samples (negative samples, mixed cultures, and cultures with microorganism growth between 10,000 and 90,000 CFU/mL) (Table 3). Moreover, the mixed cultures showed a higher number of bacteria count (/µL) in comparison with cultures with microorganism growth between 10,000 and 90,000 CFU/mL and negative samples ($p<0.001$). Indeed, we also evaluate how the flag "urinary tract infection?" is related to bacteria growth considering the 488 urine cultures positive for bacteria. Our findings demonstrated that 402 (82.4%) and 86 (17.6%) had growth >100,000 CFU/mL and 10,000-90,000 CFU/mL, respectively. The flag "urinary tract infection" was shown in 412 (84.4%) urines with bacteria growth, of which 356 (86.4%) showed >100,000 CFU/mL and 56 (13.6%) between 10,000-90,000 CFU/mL. As seen previously for all samples, bacteria count (/µL) by Sysmex UF-5000 was also higher in cultures with bacteria growth >100,000 CFU/mL than 10,000-90,000 CFU/mL and the mean values ±SD were 21,273.1±25,026.6 and 2,046.1±9,587.1 bacteria/µL, respectively (Table 3).

Table 4 – Gram classification evaluated by the urine flow cytometer and urine culture.

			Gram classification - Sysmex UF 5000								
Parameter	**Result**	**Total**	**Gram+**		**Gram-**		**Gram+/-**		**Unclassified/ Not reported**		***p-value***
			n	**%**	**n**	**%**	**n**	**%**	**n**	**%**	
Gram classification - urine culture	Neg	314	28	8.9	193	61.5	85	27.1	8	2.5	**<0.001**
	Pos	98	77	78.6	2	2.0	8	8.2	11	11.5	
	Total	412	105		195		93		19		

Neg: Negative; Pos: Positive; Pearson Chi-Square test.

Among our 412 positive samples for bacteria in urine culture which also showed the flag "urinary tract infection?" 314 (76.2%) and 98 (23.2%) had Gram-negative and Gram-positive bacteria in urine culture, respectively. Sysmex UF-5000 showed a correct Gram classification in 270 cases (65.5%), of which 193/195 (99.0%) were correctly classified as Gram-negative and 77/105 (73.3%) were correctly classified as Gram-positive, resulting in a substantial agreement with a Cohen's kappa concordance coefficient of 0.765 (IC95% 0.652-0.878) and 90% of concordance (Table 4). From 93 samples identified as Gram-positive/negative, 8 and 85 were Gram-positive and Gram-negative (79 *Enterobacterales*, 6 non fermenter Gram-negative) respectively, while 11 Gram-positive and 8 Gram-negative were 19 unclassified specimens or not reported cases.

Table 5 – Epithelial cells count evaluated by the urine flow cytometer associated to urine culture results.

	Parameter	**Result**	**Total**	**Epithelial cells (/µL)**	***p-value***
Sysmex UF-5000	Flag Urinary tract infection?#	No	1144	8.3±13.5	**<0.001**
		Yes	1383	39±62.5	
	Gram classification&	Not reported	256	13.4±14.8	**<0.001**
		Gram+	685	51±70.7	
		Gram-	219	24.7±36.9	
		Gram+/-	124	57.9±91.5	
		Unclassified	99	30.1±48.3	
Urine Culture	Urine culture&	Negative	1761	20.6±45.4	**<0.001**
		Positive (Bacteria)	488	29.2±45.1	
		YLC Growth	38	40.8±67.5	
		MC	240	46.8±72.1	
	Gram classification#				

	Negative	364	26.6±42.4	**0.015**
	Positive	124	37±51.7	
CFU/mL – All samples&	0	1761	20.6±45.4	**<0.001**
	10.000 - 90.000	100	28.9±45.2	
	≥ 100,000	426	30.4±47.6	
	MC	240	46.8±72.1	
CFU/mL - Bacteria#	10,000 - 90,000	86	29.6±48	0.345
	≥ 100,000	402	29.2±44.6	

YLC: Yeast-like cell; MC: mixed culture; CFU: colony-forming unit. Results are expressed as mean±S.D. #Mann-whitney test, &Kruskal-Wallis test, and Dunn's post hoc tests.

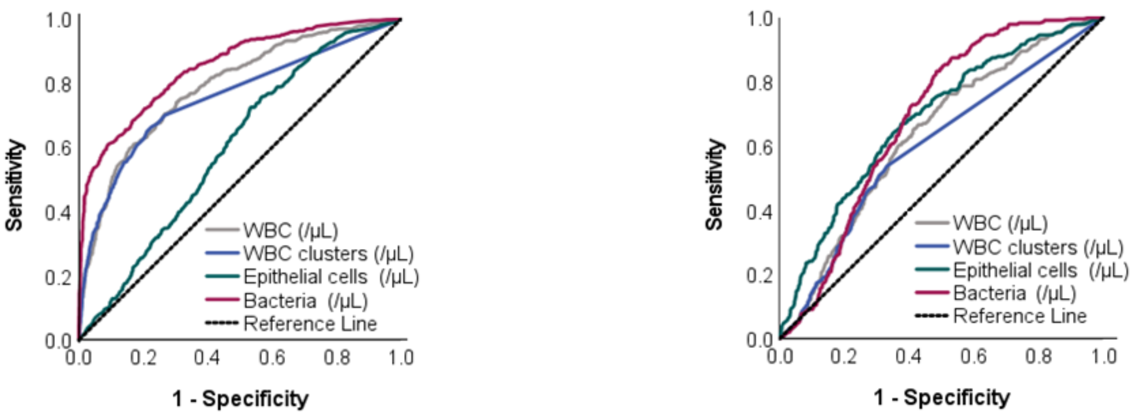
Epithelial cells presented higher counts in samples classified as Gram+/- (Sysmex UF-5000) (p <0.001) and in those that presented mixed cultures or yeast growth (p <0.001). Indeed, urine cultures with Gram-positive bacteria growth showed higher counts of epithelial cells in comparison with Gram-negative (p <0.001).

We evaluated bacteria count (/µL) performed by the equipment Sysmex UF-5000 in comparison with the urine culture results using ROC curve analysis and the area under the curve (AUC) was 0.851 (95% CI: 0.831-0.871) (Figure 1). The performance of Sysmex UF-5000 was examined using different cutoff values of bacteria count according to sensitivity and specificity. In screening tests, sensitivity is the most important criterion, and the optimal bacteria count cutoff selected from the ROC analysis was 123.8 bacteria/µL (Sensitivity: 90.0%; Specificity: 53.6%; Positive predictive value 31.7%; Negative predictive value: 95,7%; Accuracy: 60.6%). Among 2527 urine samples 1142 (45.19%) present bacteria count lower than the cutoff value, from which, 49 samples (4.3%) were positive for bacteria in urine culture (false negative) and 38/49 (77.6%) showed bacteria growth in urine culture (10,000-90,000 CFU/mL) probably being cases of urine contamination/colonization. However, 439/ 488 (90.0%) samples showed bacteria count >123.8 bacteria/µL and showed bacteria growth in culture (true positive), and 391/439 (89.1%) had growth >100,000 CFU/mL. 335/439 (76.3%) samples had Gram-negative and 104/439 (23.7%) Gram-positive bacteria. Indeed, 946 other samples present bacteria count (/µL) higher than the cutoff value, of which 714 and 232 showed negative results and mixed cultures/yeasts growth in urine culture, respectively.

We also evaluated WBC, WBC clusters, and epithelial cell counts (/µL) performed by the equipment Sysmex UF-5000 and the area under the curve (AUC) were 0.788 (95%CI: 0.765-0.811), 0.754 (95%CI: 0.728-0.781) and 0.600 (95% CI: 0.574-0.626), respectively (Figure 1A).

After that, we performed ROC curve analysis, evaluating the same parameters in comparison with mixed cultures samples. The area under the curve (AUC) were 0.686 (95% CI: 0.659-0.713), 0.635 (95% CI: 0.601-0.670), 0.599 (95% CI: 0.561-0.636), 0.683 (95% CI: 0.649-0.718) for bacteria, WBC, WBC clusters and epithelial cells, respectively (Figure 1B).

Figure 1 – ROC for bacteria WBC, WBC clusters and epithelial cells count (Sysmex UF-5000) versus urine culture and mixed cultures.



A **B**

A) ROC for bacteria WBC, WBC clusters, and epithelial cells count versus urine culture. (n = 2,527 samples and 488 positives for bacteria). B) ROC for bacteria, WBC, WBC clusters, and epithelial cells count versus mixed cultures (n = 2,527 samples and 240 mixed cultures).

Finally, we also conducted a logistic regression analysis to determine whether the combination of variables could increase the likelihood of a specific outcome, specifically, positive cultures for bacteria or mixed cultures. We investigated whether a WBC count >141.45 WBC/µL (Sensitivity: 48.4%; Specificity: 90.0%; Positive predictive value: 53.6%; Negative predictive value: 87.9%; Accuracy: 82.0%) combined with bacteria counts >123.8 bacteria/µL (Sensitivity: 90.0%; Specificity: 53.6%; Positive predictive value: 31.7%; Negative predictive value: 95.7%; Accuracy: 60.6%) could indicate a higher likelihood of a UTI. We found an odds ratio (OR) of 28.9 (95% CI: 20.37-41.02; $p < 0.001$) when comparing cases with bacteria and WBC counts below the chosen values. Similarly, regarding cases of mixed cultures, we observed an OR of 9.29 (95% CI: 5.92-14.58; $p < 0.001$) when the epithelial cell counts/μL were higher than 59.05 (Sensitivity: 24.2%; Specificity: 90.9%; Positive predictive value: 21.8%; Negative predictive value: 92.0%; Accuracy: 84.6%) and bacteria >123.8 bacteria/μL (Sensitivity: 90.0%; Specificity: 53.6%; Positive predictive value: 31.7%; Negative predictive value: 95.7%; Accuracy: 60.6%), in comparison to both markers below the chosen cutoffs.

4. DISCUSSION

It is widely accepted that the presence of UTI is often accompanied by an inflammatory response, leading to an elevation of red blood cells (RBCs), white blood cells (WBCs), and bacteria counts (number/μL) in the urine and commonly observed alongside the positivity of nitrite and leukocyte esterase. Furthermore, urinary WBCs may also originate from potential contamination of the genital tract, especially in female patients, who are indeed the population most affected by UTI, particularly in the 18 to 39 age group. Consequently, the clinical diagnosis of UTI primarily relies on the quantitative assessment of bacterial growth in urine, which is possible through urine culture, the gold-standard test for diagnosing urinary tract infection [2, 4, 9, 10, 14]

Our study's findings correspond with this information considering, firstly, the study population: women were affected in 2/3 of the cases, with more than half of the cases in the age range between 18 and 60 years old. Furthermore, it was found that urine samples that showed positive urine culture with bacteria as an etiological agent (19.3%) had a higher number of WBC, WBC clusters, and RBC in comparison to negative samples or urine cultures with yeast growth or mixed cultures ($p<0.001$) (Table 1). In 2018, Chaudhari et al. demonstrated that patients with pyuria have an increased likelihood of having a positive urine culture when bacteriuria is present, while this likelihood decreases in the absence of bacteriuria. The study also showed that the presence of bacteria in urine, as analyzed by automated urinalysis, is diagnostically inferior to WBC counts and dipstick leukocyte esterase for presumptive diagnoses of UTIs in infants and young children, the specific population under investigation. However, the diagnostic value of urinalysis is significantly enhanced when combining WBC counts with the presence of bacteriuria [15]. Moreover, nitrite in urine is a frequently used parameter to guide early diagnosis and treatment of UTI infection [9]. In our study, 99.2% of the negative samples had a nitrite negative result ($p<0.001$) and

34.2% of positive cultures for bacteria had a nitrite positive result. These findings corroborate with Gu et al (2020). According to them, conventional UTI markers such as nitrite (dipstick), demonstrate a notable level of specificity but limited sensitivity in UTI diagnosis, resulting in unsatisfactory outcomes when used as an independent diagnostic tool. This may be associated with the fact that not all bacteria can reduce nitrate to nitrite, a characteristic more frequently observed in Gram-negative bacteria. Additionally, dipstick requires a certain quantity of microorganisms for nitrite production to be sufficient for a positive result [9].

According to our findings, 9.5% of all samples were cases of mixed cultures (Table 1) and, of these, the flag "urinary tract infection" was shown in 76.3% of samples (183/240). Indeed, for these flagged cases the analyzer Sysmex UF-5000 was able to classify 139 as Gram-positive bacteria while only 10 were Gram-negative indicating a higher prevalence of Gram-positive microorganisms in mixed cultures and a greater likelihood of them being potential contaminants/colonizers of the urinary tract.

Our study evaluated parameters associated with the bacterial performance of the Sysmex UF-5000 analyzer to test its ability to rapidly discriminate positive urine samples for bacteria and negative specimens in patients with suspected UTIs. It could improve the efficiency of clinical laboratories, reducing unnecessary urine cultures and optimizing patient management while also reducing the overprescription of antibiotics. As shown in Table 2, 84.4% of samples that showed a positive result for bacteria in urine culture showed the flag "urinary tract infection", indicating a true positive result. Besides it, our findings demonstrate a substantial level of agreement, as indicated by a Cohen’s kappa concordance coefficient of 0.765 (95% CI 0.652-0.878) and a 90% concordance rate, emphasizing the Sysmex’s UF-5000 high ability to correctly classify Gram-negative bacteria samples (193/195). However, among the 85 out of 93 samples classified as Gram-positive/negative by the Sysmex UF-5000 analyzer that were samples with Gram-negative bacteria, it was observed that 91.7% were *Enterobacterales* species, and 8.3% were nonfermenting Gram-negative bacteria. Additionally, 96.5% of these samples were urine cultures with microorganism growth >100,000 CFU/mL. Therefore, based on these findings, it’s possible that these samples were cases of urine samples with more than one microorganism, but with only a single predominant one being considered in the urine culture. Consequently, further studies on this topic will be required.

Our ROC analysis demonstrates that the AUC of bacteria was 0.851 (95% CI: 0.831-0.871). Since high sensitivity and a low false negative (FN) rate (<5%) are the priority for a screening method when discriminating culture-negative specimens [12], the optimal bacteria count cutoff selected was 123.8 bacteria/µL. At this cutoff value, we had 1093 true negative samples (95.7%) and 49 false negatives (FN rate = 4.3%). Yet, of these 49 samples, 77.6% showed low bacteria growth in urine culture (between 10,000-90,000 CFU/mL) probably being cases of urine contamination/colonization. As shown by Wingerter and Bachur (2011) in UTI diagnosis, urine culture is widely used as the gold standard. However, there are cases when a culture is positive, but the patient has no infection. One such scenario is when the culture reflects contamination during the collection process, often evidenced by low colony counts (<50,000 CFU/mL). Furthermore, according to the authors, contamination is defined as a urine culture exhibiting any of the following: the presence of multiple microorganisms, non-pathogenic species, or colony counts lower than 10,000 CFU/mL [16]. Therefore, to our population, especially considering the low FN rate and the relatively high NPV (95.7%) we concluded that the cutoff value of 123.8 bacteria/µL is appropriate for discriminating culture-negative urine specimens in our population.

In addition to that, our findings also demonstrate a true positive result for 90.0% of samples with bacteria growth in culture (439/488) and 391 of these had growth >100,000 CFU/mL, which indicates a true UTI [5]. The prevalence of Gram-negative bacteria as etiological agents was 76.3% compared to 23.7% Gram-positive, which supports the findings in the literature [6,7]. Furthermore, despite the suitability of the 123.8 bacteria/µL as the cutoff for discrimination of negative urine cultures, our findings also indicate, on the other hand, that patients with urine samples with WBC counts ≥ 141.45 WBC/µL and bacteria ≥ 123.8 bacteria/µL have 28.9 times higher odds (95% CI: 20.37-41.02; $p < 0.001$) of having a UTI when compared to samples with values lower than those chosen as cutoff for WBC and bacteria.

Regarding microorganism counts, our study also demonstrates that the analyzer Sysmex UF-5000 is more accurate for urine samples with higher counts of microorganisms considering that the flag "urinary tract infection” was shown in 89% of urine culture with microorganism growth >100,000 CFU/mL. These samples also had a bacteria count (/µL) ten times higher than samples with microorganism growth in culture between 10,000 and 90,000 CFU/mL and almost 25 times higher than urines classified as negative cultures (Table 3).

As shown in Table 3, mixed cultures had more than twice the bacteria count found in samples with culture growth between 10,000 and 90,000 CFU/mL. Indeed, we observed that of the 1385 samples with bacteria count higher than our cutoff value (123.8 bacteria/µL) 232 showed mixed cultures or yeast growth in urine culture. Of these, 27 were cultures with yeast growth and 205 were mixed cultures. Considering that of total samples there were 240 classified as mixed cultures, 85.4% had bacteria counts higher than the cutoff value.

According to our ROC curve analysis findings (Figure 1), epithelial cell count is mostly related to mixed cultures as the AUC was 0.683 (95% CI: 0.649-0.718) in these samples compared to 0.600 (95% CI: 0.574-0.626) in urine samples positive for bacteria. Thus, epithelial cells presence may be a better indicator of colonization or contamination rather than a true UTI. Moreover, as seen in Table 5, higher counts of epithelial cells are most often found in urine samples with Gram-positive bacteria growth in comparison with samples with Gram-negative. It also aligns with the findings of this study, which suggest that the presence of Gram-positive bacteria in urine is more closely associated with contamination/colonization.

Considering the 59.05 epithelial cell/µL as the cutoff, we observed that only 58/240 (24.2%) of mixed cultures exhibited epithelial cell counts higher than this threshold. This represents a lower frequency when compared to the bacterial cutoff of 123.8/µL (85.4%), which suggests that bacteria count indeed remains a more reliable indicator for mixed cultures than epithelial cell counts. The Sysmex UF-5000 has a core channel that detects particles with nucleic acid and uses side fluorescent light (SFL) to provide information about particle coloration, ensuring higher sensitivity and specificity for the detection and quantification of bacteria in urine due to the absence of interference [17]. However, when we combined both markers, we observed that urine samples with bacterial and epithelial cell counts (/µL) higher than 123.8 and 59.05, respectively, had nearly 10 times higher odds of being classified as mixed cultures compared to samples with values below these thresholds.

5. CONCLUSIONS

Conclusively, our study was able to demonstrate that the Sysmex UF-5000 analyzer is a rapid technology appropriate for discriminating culture-negative urine specimens based on bacterial counts. We were also able to demonstrate that patients with urine samples with WBC counts ≥ 141.45 WBC/µL and bacteria ≥ 123.8 bacteria/µL have 28.9 times higher odds of having a UTI when compared to samples with values lower than those. Furthermore, it was possible to highlight that Gram-positive bacteria are more often indicative of urinary tract contamination or colonization. With the analyzer and observing the counts of epithelial cells (>59.05/µL) and bacteria (>123.8/µL) in urine, mixed cultures can be assessed with greater accuracy and speed. With these findings, we can offer supplementary pathological insights, suggesting Gram identification. Indeed, considering the differences in the source of samples, the cutoff values may vary, and it's recommended that distinct regions establish their cutoff values. Our findings can contribute as another comparison and reference tool to clinical laboratories in different places in the world, especially considering the absence of data in Brazil.

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ANEXOS

ANEXO 1 – Parecer de aprovação do comitê de ética da ISCMPA

IRMANDADE DA SANTA CASA DE MISERICORDIA DE PORTO ALEGRE - ISCMPA

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Avaliação da aplicação clínica de um analisador automatizado de partículas urinárias por citometria de fluxo como ferramenta de triagem de infecção do trato urinário

Pesquisador: GABRIELA BLAUTH WALBER
Área Temática:
Versão: 1
CAAE: 43233421.4.0000.5335
Instituição Proponente: Irmandade da Santa Casa de Misericordia de Porto Alegre - ISCMPA
Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 4.577.958

Apresentação do Projeto:
As infecções do trato urinário (ITU) são infecções com alta prevalência. Os possíveis agentes etiológicos causadores das ITUs, na maior parte dos casos, são bactérias pertencentes à família Enterobacteriaceae, sendo a Escherichia coli a principal responsável. O diagnóstico das ITUs consiste na cultura de urina (urocultura) com vistas à avaliação microbiológica para fins de confirmação do patógeno. Além de permitir a estimativa da
concentração de microrganismos isolados, este método também auxilia na escolha do tratamento mais adequado, a partir de testes de sensibilidade aos antimicrobianos. No entanto, como aspecto negativo, destaca-se o longo tempo para a liberação dos resultados (no mínimo 24 horas) tornandose necessária a realização inicial de tratamentos empíricos, em muitos casos. A fim de otimizar a demanda de trabalho e o tempo na entrega dos resultados, os analisadores automatizados de partículas urinárias, como o Sysmex UF-5000, estão frequentemente inseridos na rotina de laboratórios clínicos, tendo em vista sua capacidade de detectar e quantificar microrganismos, bem como auxiliar na necessidade de encaminhar ou não as amostras de urina para cultura. Este estudo objetiva investigar a utilidade clínica de um analisador urinário automatizado, para fins de triagem de amostras urinárias que seriam submetidas à urocultura. Serão realizadas análises comparativas dos parâmetros urinários (bacteriúria,
leucocitúria, presença de células epiteliais e condutividade) no analisador UF-5000 com os

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resultados da urocultura de pacientes atendidos na Santa Casa de Misericórdia de Porto Alegre.

Objetivo da Pesquisa:

Objetivo Primário:

Analisar a aplicação clínica do analisador Sysmex UF-5000 como ferramenta de triagem de infecção do trato urinário.

Objetivo Secundário:

- Verificar a possibilidade de aplicação do analisador Sysmex UF-5000 como uma ferramenta de triagem para ITU, avaliando sua associação a resultados positivos e negativos de uroculturas.- Verificar a adequação do reporte de resultados negativos e positivos para bactérias do analisador Sysmex UF-5000 em amostras de urina, em comparação com a urocultura.- Identificar o ponto de corte (cut off) para a bacteriúria avaliada no
equipamento e que se relaciona ao crescimento bacteriano em urocultura.6- Verificar a adequação do reporte de bactérias gram positivas/gram negativas do analisador Sysmex UF-5000 em comparação com os resultados de urocultura.- Verificar a associação entre a identificação de parâmetros sugestivos de colonização/contaminação (como leucócitos e células epiteliais) do analisador automatizado, em comparação com osresultados de urocultura.- Verificar a aplicação do parâmetro de condutividade (medido pelo analisador urinário) como ferramenta de avaliação da qualidade da amostra de urina, no que se refere ao período de tempo prescrito para a coleta da amostra.

Avaliação dos Riscos e Benefícios:

Riscos:

Quebra de sigilo.

Benefícios:

Tendo em vista a alta demanda na rotina do Laboratório de Análises Clínicas Carlos Franco Voegeli da Santa Casa de Misericórdia de Porto Alegre
(SCMPA) para a realização de culturas urinárias, o emprego de ferramentas que possam otimizar o processo de identificação do agente etiológico
causador de ITU, bem como reduzir a realização de processos desnecessários é fundamental. Isso traz agilidade para o processo de liberação do
laudo e possibilita à equipe médica escolhas mais precoces, assertivas e adequadas de tratamento ao caso do paciente, evitando tratamentos
desnecessários e, consequentemente, o risco de resistência bacteriana.

IRMANDADE DA SANTA CASA
DE MISERICORDIA DE PORTO
ALEGRE - ISCMPA

Continuação do Parecer: 4.577.958

Comentários e Considerações sobre a Pesquisa:
Pesquisa pertinente tendo em vista que traz a proposta do uso de ferramentas que possam otimizar processos frente à alta demanda na rotina do Laboratório de Análises Clínicas da Instituição.
Como será realizada em banco de dados com o intuito de comparar as informações fornecidas pelo equipamento e os resultados de exames, seus riscos são mínimos.

Considerações sobre os Termos de apresentação obrigatória:
Todos os termos foram apresentados corretamente, segundo Resolução vigente.

Recomendações:
Sem recomendações adicionais.

Conclusões ou Pendências e Lista de Inadequações:
Mesmo sendo esta pesquisa em banco de dados, a mesma encontra-se de acordo com a Norma vigente Resolução 466/12 para pesquisa em seres humanos.

Considerações Finais a critério do CEP:
Após avaliação do protocolo acima descrito, o presente comitê não encontrou óbices quanto ao desenvolvimento do estudo em nossa Instituição e poderá ser iniciado a partir da data deste parecer.

Obs.: 1 - O pesquisador responsável deve encaminhar à este CEP, Relatórios de Andamento dos Projetos desenvolvidos na ISCMPA. Relatórios Parciais (pesquisas com duração superior à 6 meses), Relatórios Finais (ao término da pesquisa) e os Resultados Obtidos (cópia da publicação).

2 – Para o início do projeto de pesquisa, o investigador deverá apresentar a chefia do serviço (onde será realizada a pesquisa), o Parecer Consubstanciado de aprovação do protocolo pelo Comitê de Ética.

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_1698552.pdf	12/02/2021 20:44:30		Aceito
Outros	formulario_de_inscricao_atualizado.	12/02/2021	GABRIELA BLAUTH	Aceito

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Continuação do Parecer: 4.577.958

Outros	pdf	20:43:25	WALBER	Aceito
Folha de Rosto	folhaDeRosto_Gabriela.pdf	10/02/2021 19:39:26	GABRIELA BLAUTH WALBER	Aceito
Outros	RESUMO_E_ABSTRACT.docx	09/02/2021 22:02:10	GABRIELA BLAUTH WALBER	Aceito
Outros	declaracao_onus_instituicao.pdf	09/02/2021 21:59:42	GABRIELA BLAUTH WALBER	Aceito
Outros	declaracao_dados_de_prontuario.pdf	09/02/2021 21:59:00	GABRIELA BLAUTH WALBER	Aceito
Outros	declaracao_confidencialidade.pdf	09/02/2021 21:58:16	GABRIELA BLAUTH WALBER	Aceito
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Projeto Detalhado / Brochura Investigador	Projeto_Gabriela.pdf	09/02/2021 21:54:03	GABRIELA BLAUTH WALBER	Aceito

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

PORTO ALEGRE, 08 de Março de 2021

Assinado por:
JOÃO CARLOS GOLDANI
(Coordenador(a))

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ANEXO 2 - *Guide for authors Clinica Chimica Acta*

INTRODUCTION

Clinica Chimica Acta is a high quality journal which publishes original Research Communications in the field of **clinical chemistry** and **laboratory medicine**, defined as the **diagnostic** application of chemistry, biochemistry, immunochemistry, biochemical aspects of hematology, toxicology, and molecular biology to the study of human disease in body fluids and cells.

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For Europe, Australia and all other territories:
Joris Delanghe, University Hospital Gent joris.delanghe@ugent.be

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Sex generally refers to a set of biological attributes that are associated with physical and physiological features (e.g., chromosomal genotype, hormonal levels, internal and external anatomy). A binary sex categorization (male/female) is usually designated at birth (""sex assigned at birth""), most often based solely on the visible external anatomy of a newborn. Gender generally refers to socially constructed roles, behaviors, and identities of women, men and gender-diverse people that occur in a historical and cultural context and may vary across societies and over time. Gender influences how people view themselves and each other, how they behave and interact and how power is distributed in society. Sex and gender are often incorrectly portrayed as binary (female/male or woman/man) and unchanging whereas these constructs actually exist along a spectrum and include additional sex categorizations and gender identities such as people who are intersex/have differences of sex development (DSD) or identify as non-binary. Moreover, the terms ""sex"" and ""gender"" can be ambiguous—thus it is important for authors to define the manner in which they are used. In addition to this definition guidance and the SAGER guidelines, the resources on this page offer further insight around sex and gender in research studies.

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[7] E. Coon, M. Berndt, A. Jan, D. Svyatsky, A. Atchley, E. Kikinzon, D. Harp, G. Manzini, E. Shelef, K. Lipnikov, R. Garimella, C. Xu, D. Moulton, S. Karra, S. Painter, E. Jafarov, S. Molins, Advanced Terrestrial Simulator (ATS) v0.88 (Version 0.88), Zenodo, March 25, 2020. https://doi.org/10.5281/zenodo.3727209.

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