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PESQUISA DE INTERLEUCINAS 10, 12, 17 e 23 NO FLUIDO PERITONEAL E SANGUE DE MULHERES COM ENDOMETRIOSE

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Sumário

Lista de abreviaturas

EDT – endometriose

IL – interleucina(s)

FIV – fertilização in vitro

FSH – *follicle-stimulating hormone* (hormônio folículo-estimulante)

IMC – índice de massa corpórea

ELISA – *Enzyme-linked immunosorbent assay* (teste de imunoabsorbância ligado a enzima)

NK – células *Natural Killers*

FP – fluido peritoneal

PCR – *polymerase chain reaction* (reação em cadeia da polimerase)

Resumo

A endometriose (EDT) é uma doença prevalente e impactante quando tratamos de casais inférteis. Devido às constantes pesquisas, melhorou a compreensão da doença. Muitos autores têm focado seus estudos na área da Imunologia. De fato, sabe-se que mulheres com EDT apresentam resposta imune aberrante, tanto humoral como celular, refletindo-se em um padrão alterado de secreção de citocinas ou interleucinas (IL). Demonstrou-se em estudos prévios um predomínio da citocinas e outros fatores regulatórios secretados por linfócitos auxiliares Th2, o que explicaria o implante dos focos ectópicos endometrióticos e a gênese da doença. Um novo subtipo de células recentemente descobertas, chamadas Th17, parece estar associado à IL-23, o que abre uma nova rota de resposta imune.

Nesse estudo, optamos por pesquisar as interleucinas 10, 12, 17 e 23 no fluido peritoneal e sangue de mulheres com EDT mínima e leve. Foi realizado um estudo transversal. O grupo em estudo foi constituído por 40 mulheres inférteis com endometriose mínima ou leve e o grupo controle por 40 mulheres férteis sem a doença. Todas as pacientes foram submetidas à laparoscopia, momento no qual foram coletados sangue periférico e fluido peritoneal. Foram dosadas as concentrações das interleucinas em questão no sangue e fluido peritoneal utilizando kits comerciais com metodologia ELISA. Os grupos não diferiram significativamente em idade, índice de massa corpórea (IMC), dosagem do hormônio folículo-estimulante (FSH). Não houve diferença significativa entre pacientes com e sem EDT nas IL 10, 12 e 17, tanto no sangue quanto no fluido peritoneal. A IL-23 mostrou-se aumentada significativamente no fluido peritoneal da pacientes com EDT (P = 0,003). No sangue, as concentrações foram semelhantes.

Foi a primeira vez que se demonstrou um aumento da IL-23 no fluido peritoneal de pacientes com endometriose. As demais citocinas não apresentaram diferenças entre os grupos, o que indica que pode existir uma rota alternativa ligada à IL-23 e às células Th17 para o desequilíbrio imunológico Th1/Th2 das pacientes inférteis com endometriose mínima e leve.

Introdução

A endometriose é uma doença caracterizada por tecido endometrial fora da cavidade uterina e está associada à infertilidade e à dor pélvica crônica. Essas patologias estão associadas de inúmeras formas: alterações imunológicas, anatômicas, uterinas, endometriais, entre outras (Shaw 1995; Speroff and Fritz 2005).

Nosso grupo já demonstrou que mulheres com endometriose têm secreção e controle da prolactina alterada (Cunha-Filho, Gross et al. 2001; Cunha-Filho, Gross et al. 2002) assim como anormalidades da fase lútea (Cunha-Filho, Gross et al. 2003) evidenciada pela disfunção na secreção de esteróides ovarianos, concentração folicular de fatores de crescimento modulada de forma anômala (Cunha-Filho, Lemos et al. 2003). Já investigamos a presença de interleucina 18 no líquido peritoneal de mulheres inférteis com endometriose, não encontrando diferença entre os controles (Glitz, Souza et al. 2009).

Desde a primeira descrição da doença por Rokitansky em 1860, a teoria mais provável ainda que incerta data de 1927 (Sampson 1927). Podemos perceber que, apesar de há muito conhecermos a doença, e muito sabermos a seu respeito já que é uma das doenças mais investigadas na Ginecologia (Guarnaccia and Olive 1997), temos certeza de muito menos do que gostaríamos. Após décadas de estudos, a etiopatogenia dessa doença tão relevante ainda permanece obscura (Antsiferova, Sotnikova et al. 2005; Fairbanks, Abrao et al. 2009).

Entretanto, uma série de questões e hipóteses foi levantada a partir destes estudos, para melhor entendimento dos mecanismos envolvidos na infertilidade das mulheres com endometriose. É no mínimo intrigante a dificuldade para gestar nos casos de endometriose mínima e leve, onde não se evidencia uma alteração

anatômica significativa que a justifique. Em busca de respostas para esse enigma, vários pesquisadores voltaram seus olhares para o sistema imunológico. Já possuímos evidências de alterações na resposta imune celular e humoral de pacientes com endometriose (Senturk and Arici 1999; Berkkanoglu and Arici 2003).

Em se falando de alterações no sistema imunológico das pacientes com endometriose, diversos autores já evidenciaram alterações na regulação das diversas células, especialmente linfócitos e células *Natural Killers* (NK). A comunicação entre as diversas células efetoras e reguladoras se faz através da secreção de diversas substâncias, em especial as citocinas, também chamadas interleucinas (IL). Essas moléculas desempenham papel crucial na iniciação e propagação da resposta imunológica, e um desequilíbrio entre elas pode gerar um desequilíbrio capaz de explicar a dificuldade de gestar neste grupo de pacientes.

Estudiosos já detectaram um predomínio da resposta linfocitária Th2 através de diferenças no perfil de citocinas dessas pacientes. Tal alteração poderia justificar a razão pela qual as células endometriais regurgitadas durante a menstruação conseguiriam se implantar na pelve, de acordo com a teoria de Sampson (Sampson 1927; Berkkanoglu and Arici 2003; Antsiferova, Sotnikova et al. 2005). Além de contribuir para a gênese da doença, essa mudança no perfil de células e citocinas do fluido peritoneal tornaria o mesmo um ambiente hostil, dificultando a fertilização e, conseqüentemente, poderia contribuir para a dificuldade de engravidar dessas pacientes (Gupta, Goldberg et al. 2008).

É justamente para elucidar estas questões que foi realizado este trabalho. Esta pesquisa tem como objetivo inédito o estudo de uma série de interleucinas procurando alguma alteração imunológica nas pacientes com endometriose mínima e leve e infertilidade.

Revisão da literatura

Endometriose

A endometriose, tecido glandular e estroma endometrial fora da cavidade uterina (Olive and Schwartz 1993; Koninckx 1994; Nisolle and Donnez 1997) foi descrita pela primeira vez em 1860 por Rokitansky. Em 1927, Sampson foi o primeiro a caracterizar a endometriose como uma patologia responsável por alterações na pelve feminina (Sampson 1927). Leyendecker et al. discute a endometriose como doença da unidade endometrial-subendometrial (Leyendecker, Kunz et al. 1998). A etiologia da endometriose, ocorrência de tecido endometrial ectópico ainda é enigmática e está baseada nas teorias mais conhecidas: a metaplasia celômica, os implantes ectópicos e a indução de células multipotenciais.

Estima-se que 10% a 15% da população feminina apresentam endometriose (Muse and Wilson 1982; Bancroft, Vaughan Williams et al. 1989; Olive and Schwartz 1993). A endometriose pode ser assintomática, ocorrendo diagnóstico, muitas vezes, somente por ocasião da incapacidade de gestar ou na presença de desconforto pélvico grave. É uma patologia de importância clínica ginecológica e na medicina reprodutiva, sendo foco de constante pesquisa. A associação entre endometriose e infertilidade é bem estabelecida (Mahutte and Arici 2002; 2004). Mulheres com endometriose têm 20 vezes mais chances de serem inférteis (Muse and Wilson 1982; Koninckx 1994; Lima and Baracat 1995). Trinta a sessenta por cento das pacientes com endometriose apresentam infertilidade associada (Kistner 1975; Bancroft, Vaughan Williams et al. 1989; Olive and Schwartz 1993; Koninckx 1994). Em 1993,

Wardle&Hull apontaram a endometriose como fator de infertilidade em 80% dos casos (Wardle and Hull 1993).

De acordo com a teoria de Sampson, ocorre regurgitação de células endometriais através das trompas durante a menstruação, com posterior implantação das mesmas na cavidade peritoneal. Apesar de ser a mais aceita, essa teoria não consegue explicar adequadamente o surgimento da EDT. Embora a menstruação retrógrada ocorra em torno de 76 a 90% das mulheres submetidas à diálise peritoneal (Blumenkrantz, Gallagher et al. 1981) e à laparoscopia (Halme, Hammond et al. 1984), uma porcentagem bem inferior, ao redor de 10%, desenvolve a doença. Essa divergência nos leva a crer que outros fatores estão envolvidos no desenvolvimento dos implantes ectópicos (Berkkanoglu and Arici 2003; Kyama, Debrock et al. 2003).

As pacientes com infertilidade e endometriose moderada ou grave, segundo a classificação da Sociedade Americana de Medicina Reprodutiva (ASRM), apresentam um substrato anatômico para sua incapacidade de gestar, tendo uma apresentação mais evidente desta patologia (1985; 1997). Entretanto, na ausência deste substrato anatômico, são propostos vários mecanismos para a etiologia da infertilidade de pacientes com endometriose mínima ou leve, sem ter, até o momento, uma definição clara a respeito (Muse, Wilson et al. 1982; Olive and Haney 1986; Candiani, Vercellini et al. 1991; Inoue, Kobayashi et al. 1992; Olive and Schwartz 1993; Shaw 1995).

Aspectos hormonais como anormalidades na secreção de progesterona, estradiol, prolactina e na função lútea das pacientes inférteis com endometriose, anovulação, síndrome do folículo não roto e uma diversidade de anormalidades endócrinas vem sendo relacionadas à etiologia da endometriose que podem contribuir para dificuldade de gestar nestas pacientes (Muse, Wilson et al. 1982).

A qualidade do ambiente folicular também tem sido foco de investigação nestas pacientes. Estudos sugerem alterações no fluido folicular de pacientes com

endometriose que são capazes de causar infertilidade. Estes autores também evidenciaram alterações oocitárias e embrionárias com conseqüente diminuição na capacidade de implantação (Garrido, Navarro et al. 2000).

Nosso grupo de pesquisa já evidenciou uma série de anormalidades neste grupo de mulheres tais como: secreção de prolactina alterada (Cunha-Filho, Gross et al. 2002), insuficiência lútea (Cunha-Filho, Gross et al. 2001), anormalidade no sistema IGF-IGFBP-1 no fluido folicular (Cunha-Filho, Lemos et al. 2003) e, mais recentemente, diminuição da reserva ovariana com coorte folicular heterogênea nestas pacientes (Lemos, Arbo et al. 2008). Por outro lado, refutamos a hipótese de anormalidade gênica do polimorfismo do FSH neste grupo de mulheres (Rodini 2010).

Aspectos imunológicos

Diversos estudos têm associado endometriose com mudanças na imunidade humoral e celular. Portanto, tais observações sugerem que uma resposta imune inadequada pode explicar a falha em remover células endometriais e debris da cavidade peritoneal, permitindo o implante de focos ectópicos de endométrio (Speroff and Fritz 2005). Em mulheres com endometriose, essas células parecem estar ativadas para secreção de fatores que contribuem para proliferação de endométrio ectópico.

As células que compõem o sistema imune se dividem em: linfócitos (células B, T e *Natural Killers*), fagócitos (incluindo monócitos, neutrófilos e eosinófilos) e células auxiliares (basófilos, mastócitos e plaquetas). Os linfócitos T têm como tarefa reconhecer os antígenos, auxiliar os linfócitos B na diferenciação celular e na

produção de anticorpos e auxiliar fagócitos na destruição de patógenos (Roitt, Brostoff et al. 1998).

As citocinas ou interleucinas (IL) são proteínas de baixo peso molecular ou glicoproteínas que podem ser sintetizadas pelos macrófagos peritoneais, linfócitos, implantes endometriais ectópicos ou células mesoteliais do peritônio (Kyama, Debrock et al. 2003). Desempenham papel importante na regulação da proliferação, ativação, motilidade, adesão, quimiotaxia e morfogênese celular. São fundamentais na iniciação, propagação e regulação da resposta inflamatória e imunológica (Bedaiwy, Falcone et al. 2002).

O fluido peritoneal normalmente contém inúmeras células, incluindo macrófagos, células mesoteliais, linfócitos, eosinófilos e mastócitos. Linfócitos são células também presentes na cavidade peritoneal. Subpopulações de linfócitos T helper podem ser classificados em T helper 1 (Th1) e T helper 2 (Th2) dependendo do perfil de citocinas secretadas. Linfócitos Th2 produzem apenas as seguintes citocinas: IL4, IL5, IL6, IL9, IL10, IL13 e estão envolvidos no desenvolvimento de imunidade humoral (secreção de anticorpos) contra patógenos. Células Th1 produzem interferon (IFN-γ), IL2, IL18 e fator de necrose tumoral (TNF -α) e estão envolvidos em imunidade mediada por células. (Harada, Iwabe et al. 2001; Kwak-Kim, Chung-Bang et al. 2003).

Estudos têm encontrado níveis elevados de inúmeras citocinas no fluido peritoneal de mulheres com endometriose, implicando então essas citocinas no desenvolvimento e progressão da endometriose e da infertilidade (Harada, Iwabe et al. 2001; Kyama, Debrock et al. 2003). As citocinas encontradas em mulheres com endometriose incluem, até o presente momento: IL-1, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12, IL-13, IL-17, interferon-γ, fator de necrose tumoral (TNF) entre outros (Harada, Iwabe et al. 2001; Bedaiwy, Falcone et al. 2002; Kalu, Sumar et al. 2007). As citocinas podem regular a

ação de leucócitos no fluido peritoneal ou agir diretamente no endométrio ectópico, onde podem exercer vários papéis na patogênese e fisiopatologia da endometriose.

O desequilíbrio entre a produção de citocinas Th1 e Th2 tem sido aventada como contribuinte para a implantação e proliferação de endométrio ectópico em mulheres com endometriose e também associada com dificuldades de gestar. O endométrio nas pacientes com EDT também se mostra alterado, com maior proliferação e habilidade em se implantar e sobreviver em focos ectópicos (Vinatier, Cosson et al. 2000). Um estudo com modelo animal identificou que injeção de tecido endometrial na cavidade peritoneal causa diminuição na população de linfócitos T citotóxicos e aumento de IL-4, sugerindo modulação da resposta imunológica Th2 (Henandez-Guerrero, Vadilllo-Ortega et al. 2001). Outro estudo demonstrou ativação da resposta imune Th2 sistêmica e peritoneal em mulheres com endometriose através do aumento da produção de IL4 e IL10 (Antsiferova, Sotnikova et al. 2005).

Apesar de parecerem histologicamente semelhantes, o endométrio de pacientes com e sem EDT apresentam inúmeras diferenças de acordo com estudos publicados (Vinatier, Cosson et al. 2000; Antsiferova, Sotnikova et al. 2005; Ulukus, Cakmak et al. 2006; Gupta, Goldberg et al. 2008). Propriedades de invasão, redução da apoptose, alterações na expressão de genes e proteínas específicas e aumento na produção de esteróides e citocinas específicas foram identificados no endométrio de pacientes com EDT (Sharpe-Timms 2001; Ulukus, Cakmak et al. 2006).

Uma base imunocelular tem sido aventada para explicar o surgimento da EDT. Foi demonstrada uma redução na atividade das células NK no soro e fluido peritoneal de mulheres com endometriose (Oosterlynck, Meuleman et al. 1993). Aumento da concentração de macrófagos e linfócitos ativados no fluido peritoneal (Haney, Muscato et al. 1981), bem como aumento na produção de citocinas específicas e fatores de crescimento, como IL-1b, TNF α, IL-6, IL-8, fator de crescimento endotelial vascular,

fator de crescimento endotelial (Harada, Iwabe et al. 2001; Gazvani and Templeton 2002) também já foram há muito tempo demonstrados em pacientes com EDT.

Diversos estudos têm demonstrado que um desequilíbrio imunológico pode estar associado com gestações que terminam em abortamento. Kwak-Kim et al. estudou o perfil imunológico do líquido peritoneal em mulheres com abortamentos de repetição ou múltiplas falhas de implantação após FIV e verificou um viés importante Th1 (Kwak-Kim, Chung-Bang et al. 2003). Yokoo et al. estudou pacientes com abortamento de repetição que receberam terapia com células do marido. Essa terapia induziu um estado imunológico predominantemente Th2 que parece ter sido benéfico para evolução da gestação (Yokoo, Takakuwa et al. 2006). Esses achados indicam a importância destas citocinas para a fertilidade e progressão adequada da gestação.

Como já citado anteriormente, um dos mecanismos na tolerância imune é a mudança no padrão de secreção de citocinas. A resposta inflamatória é controlada por mediadores antiinflamatórios que são secretados durante este processo regulatório. A habilidade de controlar o estado antiinflamatório depende do equilíbrio local entre fatores pró e anti-inflamatórios. A diferenciação de células T, que expressam citocinas inflamatórias Th2 e inibem a geração de células auto-reativas Th1 que produzem citocinas, também contribui para esse processo de modulação do sistema imunológico. A diferenciação em células efetoras Th1 ou Th2 é controlada pelo ambiente de citocinas e pelas células apresentadoras de antígenos (Gonzalez-Rey, Chorny et al. 2007).

A teoria de um balanço Th1/Th2 ser responsável pela gênese de doenças autoimunes ou associadas à inflamação, apesar de ser a mais conhecida e estudada, falha em responder muitos dos questionamentos científicos, inclusive no que tange a EDT (Podgaec, Abrao et al. 2007). Com a descoberta de um novo tipo de célula imunológica, novas perspectivas surgiram para tentar sanar tantas dúvidas. A célula

Th17 é um subtipo de linfócito T CD4+ que, assim como as células Th1 e Th2, sofre diferenciação a partir de células T nativas (Hirata, Osuga et al. 2008). Essas células produzem IL-17A, IL-17F, IL-21 e IL-22. As células Th17 e suas citocinas efetoras modulam mecanismos de defesa contra várias infecções, especialmente infecções por bactérias extracelulares, e estão involvidas na patogênese de doenças autoimunes, como, por exemplo, a doença inflamatória intestinal (Liu, Yadav et al. 2009). Interleukin-17A é uma das principais citocinas secretadas pelas células Th17 e está associada a vários tipos de resposta imune e inflamação (Ouyang, Kolls et al. 2008). Um estudo recente mostrou que aumentos nos níveis dessa citocina no fluido peritoneal correlacionam-se com a severidade da doença e infertilidade associada (Zhang, Xu et al. 2005).

A IL-23 é uma citocina da família da IL-12, composta por uma sub-unidade p19 e outra p40. A sub-unidade p40 é comum à IL-12 e, portanto, a IL-23 pode exercer uma função de desencadear uma resposta Th1. Evidências atuais sugerem que a IL-23 pode estimular a diferenciação de linfócitos T nativos em células Th17 (Iwakura and Ishigame 2006). Vários estudos têm associado IL-23, células Th17 e doenças autoimunes (McGeachy and Cua 2007).

Diversos estudos mostraram um aumento na prevalência de doenças autoimunes em pacientes com endometriose, entre elas lúpus eritematoso sistêmico, artrite reumatóide, síndrome de Sjögren, hipotireoidismo, entre outras (Nothnick 2001; Sinaii, Cleary et al. 2002; Pasoto, Abrao et al. 2005; Seery 2006; Petta, Arruda et al. 2007). Os mecanismos associados não estão totalmente esclarecidos, mas um dos principais parece ser uma maior prevalência de anticorpos anti-nucleares em mulheres com endometriose, o que aumenta o risco de desenvolvimento de algumas doenças autoimunes (Seery 2006).

Métodos laboratoriais de detecção

As citocinas são proteínas produzidas e secretadas pelas células do sistema imune, podendo ser detectadas basicamente de duas formas: intracelular ou extracelular. Como essas substâncias estão presentes em diversos fluidos e tecidos, a maneira mais utilizada é a detecção extracelular das mesmas.

O teste de imunoabsorbância ligado a enzima (ELISA – *Enzyme-linked immunosorbent assay*) é uma das mais utilizadas na pesquisa de citocinas (Vignali 2000). De fato, essa técnica laboratorial é utilizada quase que exclusivamente na área de Imunologia, tanto na pesquisa de antígenos como de anticorpos. De uma maneira resumida, o antígeno em estudo, no caso a citocina, é colocado em uma placa contendo anticorpo específico. Quanto maior a concentração da citocina, maior será a ligação antígeno-anticorpo. Um segundo anticorpo se liga ao complexo, e uma enzima acoplada ao mesmo converte o substrato produzindo determinada cor. É feita uma leitura da intensidade dessa cor em equipamento específico, obtendo então as concentrações desejadas (Lequin 2005). Diversos fabricantes oferecem *kits* comerciais para inúmeras citocinas, o que justifica a ampla aceitação e utilização desse método diagnóstico em pesquisas.

Outra técnica baseada em ELISA é o teste *Enzyme-linked immunosorbent spot* (ELISPOT). É usado para identificar e enumerar células produtoras de citocinas, no nível individual de cada célula. Esse teste permite visualizar o produto da secreção de cada célula ativada, dessa forma permitindo análise qualitativa (tipo de proteína) e quantitativa (número de células reativas). A sensibilidade do ELISPOT é muito superior à técnica ELISA (Czerkinsky, Nilsson et al. 1983).

A citometria de fluxo é outra técnica que pode ser utilizada na detecção de citocinas. Basicamente, uma fonte de luz, geralmente *laser*, é direcionada contra uma

corrente de fluido. Múltiplos detectores interpretam os sinais luminosos para fornecer os resultados desejados, podendo utilizar fluorescência (Loken 1990). Uma modernização da citometria de fluxo é a *Cytometric Bead Array* (CBA), que permite em um único teste a determinação simultânea de inúmeras citocinas. Com isso, é possível economizar tempo e amostra, já que um volume pequeno permite detectar até 30 citocinas, dependendo do fabricante (Morgan, Varro et al. 2004). Dentro do grupo de testes baseados no fluxo de partículas, estão sendo desenvolvidos testes para múltiplas citocinas, também chamados de *multiplex*. O princípio é o mesmo da citometria de fluxo, porém com mais de uma cor fluorescente e possibilitando análise simultânea de inúmeras citocinas. O teste mais conhecido é o Luminex®, e permite a análise simultânea de múltiplas citocinas com maior rapidez e menor consumo de amostras. O principal empecilho ao uso dessa técnica ainda é o custo elevado (Vignali 2000).

Outra maneira de estudar o comportamento das células do sistema imunológico é através da pesquisa de RNA mensageiro (mRNA) de diferentes citocinas após estimulação celular utilizando a técnica de *real time* PCR. Essa técnica permite avaliar a secreção das citocinas, porém é um exame mais complexo para ser realizado e seu custo também dificulta a utilização (Vignali 2000).

Vignali (Vignali 2000) faz uma análise comparativa dos diversos métodos disponíveis para avaliação de citocinas, conforme descrito a seguir. ELISA possui a vantagem de medir a proteína com sensibilidade e especificidade, porém permite apenas a análise de uma citocina por teste e demanda tempo considerável para sua realização. A técnica ELISPOT permite a análise de cada célula e fenótipo simultâneo e mede a proteína, porém não é muito sensível e pode não se correlacionar com a secreção. A técnica de PCR é muito sensível e específica, pode ser multiplex (até 10 citocinas), mas é difícil de ser estabelecida e não mede a proteína. Por fim, os testes baseados no fluxo de partículas (citometria de fluxo, CBA, Luminex®) são sensíveis,

específicos e permitem análises de múltiplas citocinas (mais de 10). Além disso, o uso de testes comerciais já estabelecidos, com grupos (perfis) de citocinas, é custo-efetivo, e permite ainda a análise de proteínas e/ou DNA. A desvantagem desses testes é que eles não estão completamente estabelecidos e sua montagem inicial é cara e demorada.

Fica claro o motivo pelo qual a maioria dos autores utiliza ELISA para analisar citocinas, pois trata-se de uma técnica conhecida, com custo acessível e amplo acesso aos pesquisadores, mas também possui limitações (Vignali 2000). Os avanços nas técnicas laboratoriais de detecção de citocinas apontam para o desenvolvimento e aprimoramento de tecnologias *multiplex*, fazendo que no futuro próximo os pesquisadores utilizem tais técnicas modernas (Vignali 2000).

Justificativa

Apesar de surgirem progressivamente estudos tentando identificar um responsável por desencadear o surgimento da EDT, ainda não é possível estabelecer uma relação causa-efeito livre de questionamentos. Buscamos então, baseados na teoria principal de que mulheres com EDT têm uma resposta imune inadequada com desequilíbrio Th1/Th2, pesquisar as interleucinas 10, 12, 17 e 23. Pretendemos primeiramente detectá-las no sangue e especialmente no fluido peritoneal, e com os resultados, determinar um perfil de citocinas. Queremos também avaliar a rota Th17 e verificar uma possível associação com endometriose. Não encontramos na literatura nenhum estudo que tenha pesquisado essas quatro citocinas no mesmo momento. Além disso, não há publicação com a IL-23. Nenhum estudo foi feito com um grupo composto apenas por pacientes inférteis com endometriose mínima e leve, o que torna o presente estudo original.

Hipótese nula

Nossa hipótese nula é de que a concentração de IL 10, 12, 17 e 23 no sangue e fluido peritoneal das mulheres inférteis com EDT mínima e leve é semelhante à de mulheres férteis sem endometriose.

Objetivos

1. Comparar as concentrações séricas de IL-10 em mulheres com e sem endometriose
2. Comparar as concentrações no fluido peritoneal de IL-10 em mulheres com e sem endometriose
3. Comparar as concentrações séricas de IL-12 em mulheres com e sem endometriose
4. Comparar as concentrações no fluido peritoneal de IL-12 em mulheres com e sem endometriose
5. Comparar as concentrações séricas de IL-17 em mulheres com e sem endometriose
6. Comparar as concentrações no fluido peritoneal de IL-17 em mulheres com e sem endometriose
7. Comparar as concentrações séricas de IL-23 em mulheres com e sem endometriose
8. Comparar as concentrações no fluido peritoneal de IL-23 em mulheres com e sem endometriose

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Artigo

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Th1, Th2 and Th17 interleukin pathway in infertile patients with minimal/mild endometriosis

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Abstract

Objective: To evaluate interleukin (IL)-10, IL-12, IL-17 and IL-23 levels in the serum and peritoneal fluid of women with and without endometriosis.

Design: Cross-sectional survey.

Setting: University hospital.

Patients: Interleukin-10, IL-12, IL-17 and IL-23 levels were measured in 40 patients submitted to laparoscopy as part of an investigation on infertility (study group), as well as in 40 fertile patients submitted to lapararoscopy for tubal ligation (control group). Minimal or mild endometriosis was confirmed in the study group and endometriosis was excluded from the control group.

Intervention(s): Blood sample and peritoneal fluid were obtained from patients during video laparoscopy.

Main Outcome Measure(s): The levels of IL-10, IL-12, IL-17 and IL-23 in peripheral blood and peritoneal fluid were determined by enzyme immunoassay and compared between groups.

Result(s): IL-23 levels measured in peritoneal fluid were higher in patients with endometriosis compared with the control group. This difference was not demonstrated in the serum for IL-23. No statistically significant differences were found in IL-10, IL-12 and IL-17 levels, either in serum or in peritoneal fluid samples.

Conclusion(s): Patients with minimal or mild endometriosis and infertility have higher peritoneal IL-23 levels irrespective of the other cytokines measured, suggesting that in this disease an alternative pathway is involved in induction of the Th2 immune response, possibly trough Th17 cells. This imbalance could contribute to these patients' infertility.

Key Words: Endometriosis, immunology, infertility, interleukin-10, interleukin-12, interleukin-17, interleukin-23

Introduction

Endometriosis is characterized by the presence of endometrial tissue outside the uterine cavity (Olive and Schwartz 1993; D'Hooghe, Debrock et al. 2003). Its prevalence varies according different studies, but it is believed to be around 10% of the female population in the reproductive age (Bancroft, Vaughan Williams et al. 1989; Olive and Schwartz 1993; Missmer and Cramer 2003). Since 1927 when Sampson described his theory on the implantation of ectopic endometrial cells (Sampson 1927), large amounts of papers have been published. Although many researchers have tried

to explain the pathogenic mechanisms of this disease, several aspects remain unclear (Antsiferova, Sotnikova et al. 2005; Fairbanks, Abrao et al. 2009).

The association between endometriosis and infertility is long known and many authors have tried to dissect this association (Mahutte and Arici 2002; 2004). Numerous mechanisms have been proposed to account for fertility impairment. These include altered folliculogenesis (Doody, Gibbons et al. 1988), leading to ovulatory dysfunction (Cunha-Filho, Gross et al. 2002) and poor oocyte quality, as well as luteal phase defects (Cunha-Filho, Gross et al. 2001; Cunha-Filho, Gross et al. 2003), diminished ovarian reserve (Lemos, Arbo et al. 2008), reduced fertilization (Wardle, Mitchell et al. 1985), abnormal embryogenesis (Garrido, Navarro et al. 2002), endometrial alterations (Vinatier, Cosson et al. 2000; Ulukus, Cakmak et al. 2006), and immunological disturbances (Senturk and Arici 1999; Berkkanoglu and Arici 2003). The theory of an altered immune system and endometriosis suggests that changes in cell-mediated immunity and humoral immunity may contribute to the development of the disease (Berkkanoglu and Arici 2003).

It is known that effector lymphocytes are divided into two types based on the pattern of cytokine secretion produced by these cells following stimulation. Th1 cells are secretors of cytokines denominated pro-inflammatory, principally interleukin (IL)-2 and interferon-gamma (IFN-gamma), whereas Th2 cells produce cytokines referred to as antiinflammatory, especially IL-4 and IL-10. The Th1 immune response pattern triggers an immune cell process, while Th2 response is characterized by its activation of B-lymphocytes, triggering a process that involves humoral immunity. The two responses coexist and rarely fall into exclusive pro-Th1 or -Th2 patterns, but there is a predominance of one over the other depending on a complex balance (Podgaec, Abrao et al. 2007).

The recent discovery of another type of T cell substantially revised the conventional Th1/Th2 hypothesis of T cell immunology. The Th17 cells is a novel CD4+ subset that, along with Th1 and Th2 cells, also differentiate from naive T cells (Hirata, Osuga et al. 2008). They produce IL-17A, IL-17F, IL-21, and IL-22. Th17 cells and their effector cytokines mediate host defensive mechanisms to various infections, especially extracellular bacterial infections, and are involved in the pathogenesis of many autoimmune diseases, such as inflammatory bowel disease (Liu, Yadav et al. 2009). IL-17A is a representative cytokine secreted from Th17 cells and has been described in various immune responses and inflammation (Ouyang, Kolls et al. 2008). The existence of elevated levels of inflammatory substances and cells in the peritoneal fluid (PF) of women with endometriosis is highly indicative of pelvic cavity inflammation (Hirata, Osuga et al. 2008). A recent study demonstrated that increases in the level of IL-17A in PF correlate with the severity of endometriosis and infertility associated with this disorder (Zhang, Xu et al. 2005).

Several studies tried to determine, trough the analysis of cytokines, if a Th1 or a Th2 response would be present in endometriosis patients, and conflicting data were published. The most accepted theory seems to be that the refluxed endometrial cells should be swept out of the peritoneal cavity, but an altered immune response with a preponderance of Th2 pattern would allow their implantation and growth (Podgaec, Abrao et al. 2007).

It is currently accepted that an increase occurs in interleukins 1, 4, 5, 6, 8, and 10, tumor necrosis factor-alpha (TNF-a), and in the vascular endothelial growth factor in endometriosis (Harada, Iwabe et al. 2001). Only one study found higher levels of IL-17 in the peritoneal fluid of women with minimal or mild endometriosis, especially when infertility coexisted (Zhang, Xu et al. 2005). Our group studied IL-18 and we found no correlation with endometriosis (Glitz, Souza et al. 2009). However, there is no data on IL-23 and endometriosis until present days and, moreover, no paper studied a

subgroup of minimal/mild endometriosis with infertility and the Th1/Th2/Th17 pathways profile.

Current evidence suggests that IL-23 is responsible for the differentiation and expansion of Th17/ThIL-17 cells from naive CD4+ T cells (Iwakura and Ishigame 2006). Several studies revealed the association between IL-23, Th17 cells and autoimmune diseases (McGeachy and Cua 2007).

Only one study found higher levels of IL-17 in the peritoneal fluid of women with minimal or mild endometriosis when compared to moderate or severe disease, especially when infertility coexisted (Zhang, Xu et al. 2005).

In view of the emerging significance of Th17 cells in a novel paradigm in immunology, we investigated, for the first time, the role of IL-10, IL-12, IL-17 and IL-23 in infertile patients with minimal/mild endometriosis.

Patients and methods

Design

A cross-sectional study was performed on 80 patients who were enrolled from March 2007 to December 2008 at Hospital de Clínicas de Porto Alegre when seeking care for the investigation of infertility or tubal ligation.

Subjects

Forty patients were submitted to laparoscopy to investigate infertility. Infertility was defined as the inability of a couple to achieve pregnancy after one year of regular unprotected sexual intercourse (1997). Endometriosis was diagnosed during laparoscopy and categorized according to the classification proposed by the American Society for Reproductive Medicine (1997). All patients had peritoneal implants and met

criteria for minimal or mild endometriosis. The same surgical staff performed all endoscopic procedures.

Women aged 18 or more who would be submitted to laparoscopic surgery for infertility investigation or tubal ligation were included after giving their consent. Exclusion criteria comprised: the presence of any autoimmune disease, absence of peritoneal liquid during laparoscopy, the coexistence of any other causes of infertility, and having used any hormonal medication in the 3 months before surgery. Other causes of infertility were excluded by hysterosalpingraphy, sperm evaluation, and measurements of serum follicle-stimulating hormone, prolactin and thyroid-stimulating hormone levels on the third day of the menstrual cycle. Laparoscopy was done in the first phase of menstrual cycle in all patients (cases and controls).

The control group consisted of 40 patients who underwent laparoscopy for tubal ligation. None of these patients were diagnosed with endometriosis, all of them were fertile and none had a significant past medical history. Once again, none of the patients had been on hormonal medications for at least 3 months prior to surgery.

Study protocol

The samples of peritoneal fluid (3 to 6 mL) were collected during the laparoscopic procedure under general anesthesia, immediately after the introduction of the second trocar, and the fluid was aspirated from the anterior or posterior peritoneal deflection. Samples were not collected when the presence of bleeding in the cavity was detected and the peritoneal cavity was not washed. The blood samples were collected at the time of the laparoscopic procedure in all patients. All samples were centrifuged and serum and peritoneal fluid were stored at -80°C until the time for measurement.

Clinical data regarding menstrual cycle, obstetrical history, previous surgical procedures, and history of hormone use were also obtained by means of an appropriate questionnaire. All patients and control subjects who participated in this

study signed an informed consent form. The study was approved by the Ethics Committee of Hospital de Clínicas de Porto Alegre.

Serum and peritoneal cytokines were measured by enzyme immunoassay (ELISA) applying Human Ready-SET-Go! commercial kits (eBioscience, San Diego, CA), including: IL-10, IL-12 (p70), IL-17A and IL-23 (p19/p40). The sensitivity was 2 pg/ml, 4 pg/ml, 4 pg/ml and 15 pg/ml, respectively.

For statistical analysis, data with nonparametric distribution are reported as median values with the 95% confidence interval and those with parametric distribution as means ± SD. The Student t-test was used to compare means, the Mann-Whitney test to compare median values. When $P < 0.05$, the samples were considered to be statistically different.

Results

The clinical and demographic characteristics of the two groups did not differ, as shown in Table 1. Of the 40 patients with endometriosis, 26 showed minimal and 14 mild disease.

Mean IL-23 levels measured in the peritoneal fluid were higher in patients with endometriosis compared with the control group (*P*=0,003). No statistically significant differences were found in the serum concentrations of IL-23 in the overall evaluation between cases and controls. No statistically significant differences were found in the levels of IL-10, IL-12 and IL-17 in blood or in peritoneal fluid between endometriosis and control group, as shown in Table 2.

Comparing all IL-10, 12, 17 and 23 regarding the endometriosis stage (minimal versus mild), those cytokines were not significantly different between the groups (*P*>0.05)

Within the control group, we could not detect IL-10, IL-12, IL-17 and IL-23 in the serum in 1, 33, 27 and 18 patients, respectively. Within the endometriosis group, we could not detect these cytokines in 3, 34, 27 and 26 patients.

Furthermore, in the peritoneal fluid of the control group, we could not detect IL-12, IL-17 and IL-23 in 19, 36 and 5, respectively.

Moreover, in the endometriosis group, we could not detect the same cytokines in the peritoneal fluid in 26, 34 and 1 patient. IL-10 was detectable in all the samples of peritoneal fluid.

TABLE 1
Demographic characteristics (mean±SD, *t* test or qui-square utilized)

Parameter	Endometriosis group *N* = 40	Control group *N* = 40	*P* value
Age (years)	32.48±4.99	33.63±6.51	*NS*
BMI (kg/m²)	23.75±4.37	25.31±3.95	*NS*
Regular cycles *N* (%)	35 (87.5%)	32 (80%)	*NS*

TABLE 2
Comparison of interleukin levels in serum and peritoneal fluid between endometriosis (N=40) and control group (N=40)

Cytokine levels (pg/ml)	Endometriosis group[a]	Control group[a]	P value[b]
IL-10 serum	7.25 (2.30~185.60)	8.05 (1.4~44.2)	0.130
IL-10 peritoneal fluid	35.50 (12.29~365.42)	34.98 (19.01~227.46)	0.956
IL-12 serum	6.87 (4.40~12.10)	9.21 (2.40~34.40)	0.315
IL-12 peritoneal fluid	1.73 (0.30~12.38)	2.03 (0.57~47.74)	0.115
IL-17 serum	1.33 (0.10~29.80)	1.33 (0.10~8.00)	1.000
IL-17 peritoneal fluid	5.41 (1.00~161.10)	3.70 (1.00~6.30)	0.584
IL-23 serum	5.16 (1.4~17.4)	9.64 (1.40~45.30)	0.301
IL-23 peritoneal fluid	13.60 (2.20~25.00)	9.81 (0.30~30.70)	0.003

a Median (range)

b Non-parametric test (Mann-Withney)

Discussion

The present study evaluated the levels of a group of cytokines, namely IL-10, IL-12, IL-17 and IL-23, in the serum and peritoneal fluid of 40 infertile patients with endometriosis compared to 40 fertile patients without the disease (control group).

Interleukin-23 levels in peritoneal fluid were found to be significantly higher in patients with endometriosis compared with the control group; in serum, the same difference was not detected. We could demonstrate for the first time the presence of IL-23 in the peritoneal fluid of endometriosis patients.

Previous studies have found increased levels of IL-10 in the peritoneal fluid of endometriosis patients (Harada, Iwabe et al. 2001). However, there are still conflicting data. Pogdaec et al. (Podgaec, Abrao et al. 2007) found significantly higher levels of IFN-gamma and IL-10 in the peritoneal fluid of endometriosis patients when compared to controls using flow citometry to measure free cytokines. In this study, all patients had clinical signs and symptoms suggesting the disease, and the control group was composed by women without detectable endometriosis during laparoscopy. In a recent study, Hassa et al. (Hassa, Tanir et al. 2009) found similar levels of IL-2, IL-4, IL-10 and IFN-gamma both in serum and peritoneal fluid of control and endometriosis group, and also no difference between early and late-stage disease, although the assays where made using ELISA technique.

Fairbanks et al. (Fairbanks, Abrao et al. 2009) demonstrated higher levels of IL-12 in the peritoneal fluid of endometriosis patients using ELISA assay. Again, the control group was composed by symptomatic patients without evidence of the disease during surgery, and none was infertile. They found higher levels in late-stage disease when compared to early-disease. Galinelli et al. (Gallinelli, Chiossi et al. 2004) found significantly higher peritoneal fluid IL-12 levels in women with moderate or severe endometriosis (stages III and IV) than in healthy controls using ELISA assays, but the endometriosis group was composed by patients with pain or infertility.

Moreover, Bedaiwy et al. (Bedaiwy, Falcone et al. 2002) found similar levels of IL-12 in serum and peritoneal fluid of women with and without endometriosis using ELISA assays. The study group was composed by patients with pain or infertility and confirmed endometriosis (any grade), and the control group by asymptomatic women without endometriosis. In addition, even when comparing the three groups among each one, there was still no difference in IL-12 levels. Gazvani et al. (Gazvani, Bates et al. 2001) found no correlation between the presence of endometriosis, grade of the disease or menstrual cycle phase and IL-12. Furthermore, they could not detect the

cytokine in 67% of the patients with endometriosis using ELISA assay. The study group was also composed by pain and infertility complaints and all grades of the disease.

The literature on IL-17 and endometriosis is nearly absent. Zhang et al. (Zhang, Xu et al. 2005) showed no difference in IL-17 levels between endometriosis and control group. However, the concentrations of IL-17 in peritoneal fluid were correlated with the severity of endometriosis. The mean concentrations of IL-17 in peritoneal fluid were significantly higher in the patients with minimal/mild endometriosis than those with moderate/severe endometriosis and those without endometriosis. The menstrual cycle phase upon collection of the sample did not influence the results. The study group was formed by patients undergoing laparoscopy because of pain, infertility or pelvic mass, and all grades of the disease where included. We found similar levels between endometriosis and controls.

Interleukin-23 has been implicated in the differentiation on naïve T cells into Th17 cells, a novel route for immune response. Several researches were made and published data have associated this cytokine with many autoimmune and immune-mediated diseases. A link between IL-23, Th17 response and inflammatory bowel disease was reported by various authors (Liu, Yadav et al. 2009). There is a significant body of evidence that IL-23 is involved in rheumatoid arthritis pathogenesis (Kunz and Ibrahim 2009). There are also publications linking IL-23/Th17 axis with psoriasis, multiple sclerosis, lupus, asthma, vasculitis and many others (McGeachy and Cua 2007; Kunz and Ibrahim 2009). A link between endometriosis and autoimmune diseases is probable, since many authors described higher prevalence of diseases such as systemic lupus erythematosus, Sjögren's syndrome, rheumatoid arthritis, hypothyroidism and others (Nothnick 2001; Sinaii, Cleary et al. 2002; Pasoto, Abrao et al. 2005; Seery 2006; Petta, Arruda et al. 2007). Until now, IL-23 was not studied in endometriosis or even detected in the peritoneal fluid, which make our result unique.

For the first time we demonstrated higher IL-23 levels in the peritoneal fluid of infertile endometriotic patients.

The composition of our groups make our study original, since we included only infertile patients with minimal or mild endometriosis and compared them with fertile asymptomatic patients without the disease. For this reason, we were not able to make associations between cytokines and endometriosis grade. As our patients had early-staged disease, the levels of the cytokines may actually be lower and therefore we were not able to detect them. Since the indication for laparoscopy in our study was infertility and not pain, the same issue may influence the results.

Most of the authors used ELISA assays due to its availability and costs when compared to others refined techniques. However, ELISA has several limitations, like the sensibility not as low as we may need, the consumption of a relatively large amount of sample, and a very complex protocol. Other methods, like flow citometry, Luminex, real time PCR for mRNA, may provide more accurate results and reduce the conflicting data reported previously.

The limitation of our study is the fact that several patients (study and control) did not reach the cutoff for IL detection using ELISA. We could analize this data considering the low importance/relevance of these IL on mild/minimal endometriosis physiology. Moreover, our conclusion is valid only for infertile patients with minimal/mild endometriosis. In contrast with the majority of papers that included pain endometriosis patients with an obvious more inflammatory component.

The discovery of Th17 provides us another path to follow, since the long known Th1/Th2 immune responses cannot give us all the answers to such an enigmatic disease as endometriosis. We are introducing a new research trend based on a novel concept. Evidences linking IL-23, Th17 cells and autoimmune and immune-mediated diseases provide us suspicious of a role also on the pathogenesis of endometriosis.

In conclusion, we found similar levels of IL-10, IL-12 and IL-17 in the serum and peritoneal fluid of infertile women with endometriosis compared to fertile healthy controls. Interleukin-23 levels were similar in the serum, but significantly higher in the peritoneal fluid of infertile women with minimal/mild endometriosis and may be implicated in the sub-fertility in this group of patients

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Considerações gerais

1. Não houve diferença significativa na concentração de IL-10 no soro entre pacientes com endometriose e sem endometriose
2. Não houve diferença significativa na concentração de IL-10 no fluido peritoneal entre pacientes com endometriose e sem endometriose
3. Não houve diferença significativa na concentração de IL-12 no soro entre pacientes com endometriose e sem endometriose
4. Não houve diferença significativa na concentração de IL-12 no fluido peritoneal entre pacientes com endometriose e sem endometriose
5. Não houve diferença significativa na concentração de IL-17 no soro entre pacientes com endometriose e sem endometriose
6. Não houve diferença significativa na concentração de IL-17 no fluido peritoneal entre pacientes com endometriose e sem endometriose
7. Não houve diferença significativa na concentração de IL-23 no soro entre pacientes com endometriose e sem endometriose
8. A concentração de IL-23 no fluido peritoneal de pacientes com endometriose foi significativamente maior que a de pacientes sem endometriose

Perspectivas

A descoberta de uma nova rota na resposta imunológica, a chamada rota Th17, surgiu como forte candidata na patogênese da endometriose, principalmente pela sua associação com diversas doenças autoimunes, as quais compartilham aspectos semelhantes com a EDT conforme já comentado (Nothnick 2001; Sinaii, Cleary et al. 2002; Pasoto, Abrao et al. 2005; Seery 2006; Petta, Arruda et al. 2007). O achado de aumento nos níveis de IL-23 no fluido peritoneal corrobora essa teoria. Acreditamos que essa via deve estar associada com a endometriose, e estudos futuros devem avaliar melhor essa associação. A avaliação de grupos contendo graus mais severos da doença e pacientes com queixa de dor pode acrescentar novos dados nesse sentido. Além disso, a determinação das citocinas utilizando técnicas diagnósticas mais refinadas também pode contribuir para uma maior identificação das mesmas e esclarecer dúvidas.

O presente estudo levanta uma questão importante para o planejamento de estudos futuros. Muitos resultados obtidos em outros estudos utilizaram, para a determinação da concentração de citocinas, a metodologia ELISA. Pudemos perceber as dificuldades técnicas inerentes ao método, tais como um limite de detecção inferior insuficientes para determinar algumas citocinas presente na maioria dos kits comerciais, propriedades próprias das moléculas que dificultam sua determinação no compartimento extracelular, uma necessidade de amostras relativamente volumosas, entre outras. Muitas conclusões na literatura foram feitas baseadas nessa metodologia, e esse é um motivo pelo qual encontramos resultados contraditórios.

Como pudemos perceber, as alterações imunológicas nas pacientes com endometriose vão muito além de concentrações diferentes de citocinas. As citocinas

servem como reguladoras da função celular e, portanto, encontrar diferentes concentrações da substância indica que ela foi secretada de maneira anormal, mas também que ela, por estar atuando de maneira mais intensa, também exercerá uma ação regulatória diferente sobre os linfócitos, macrófagos e outras células do sistema imunológico.

Tendo em vista essas características das citocinas, temos duas possibilidades. Uma delas é pesquisar as citocinas nos valendo de métodos mais sensíveis que nos possibilitem um limite de detecção mais baixo e um consumo de menor volume amostral. Destacam-se a citometria de fluxo e as técnicas para avaliação de múltipas citocinas simultaneamente, entre elas a técnica de Luminex, que é uma das técnicas de citometria de fluxo *multiplex*..

Outra alternativa é avaliar a secreção das citocinas pelos linfócitos após estimulação in vitro, pesquisando o RNA mensageiro através da técnica de PCR, o que fornece uma visão mais fidedigna da resposta celular. O empecilho ao uso dessa técnica, além do custo, é a complexidade do teste e necessidade de equipamentos robustos com rotinas laboratoriais bem estabelecidas.

Nosso estudo, como já mencionado, é inédito pela composição dos grupos, visto que incluímos apenas pacientes inférteis com endometriose mínima e leve. Isso impediu uma possíveis interferência de pacientes com dor, onde mediadores inflamatórios diferentes poderiam estar expressos, e também uma falta de achados em pacientes com endometriose assintomática. A utilização de um grupo controle com pacientes férteis sem endometriose ao invés de pacientes sintomáticas (dor ou infertilidade) permite ligar os resultados à endometriose e/ou infertilidade dessas pacientes, excluindo-se outras para alterações nas citocinas. O achado original de aumento nas concentrações de IL-23 no fluido peritoneal das pacientes inférteis com endometriose abre caminho para estudos futuros nessa nova via Th17, e esperamos

que isso possibilite ampliar cada vez mais nosso conhecimentos na Imunologia da endometriose.

Anexos

Instrumento de coleta de dados

Protocolos dos kits ELISA

Leituras das placas ELISA

Curva-padrão das citocinas

Tabelas do artigo

Instrumento de coleta de dados

PROTOCOLO DE COLETA DE LIQUIDO PERITONEAL E SANGUE

Data___/___/___

CÓDIGO

GRUPO: ( ) INFERTILIDADE (tempo _____ anos)

( ) LIGADURA TUBÁRIA

NOME:

IDADE: PRONTUÁRIO:

PESO: ALTURA: IMC (Kg/m2):

DUM: PARIDADE: G____P___C___A___

CICLOS: ( ) REGULARES ( ) IRREGULARES

ESPEMOGRAMA: milhões/mL _____ A+B _____ normais _____

DOSAGENS HORMONAIS:

LH _____ FSH _____ prolactina _____ TSH _____

SDHEA_____ 17-OH-P _____ testosterona total _____

ACHADOS:

( ) ENDOMETRIOSE: I ( ) II ( ) III ( ) IV ( )

( ) ADERÊNCIAS PELVICAS

( ) FATOR TUBÁRIO

( ) OUTROS

( ) PELVE NORMAL

COLETADO POR:

CENTRIFUGADO POR:

OUTRAS OBSERVAÇÕES:

Protocolos dos kits ELISA

IL-10

IL-12

IL-17

IL-23

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Product Information

Contents: Human IL-10 (Interleukin-10) ELISA Ready-SET-Go!
Catalog Number: 88-7106
Sensitivity: 2 pg/ml
Standard Curve Range: 2 pg/ml - 300 pg/ml

Four parameter fit of human IL-10 ELISA Ready-SET-Go! Recombinant standard concentration is in pg/ml.

Description

This Human IL-10 ELISA Ready-SET-Go! reagent set (with or without high-affinity binding microwell plates) contains the necessary reagents, buffers and diluents for performing quantitative enzyme linked immunosorbent assays (ELISA). This ELISA reagent set is specifically engineered for accurate and precise measurement of human IL-10 protein levels from samples including serum, plasma, and supernatants from cell cultures.

Components

1. Capture Antibody: Pre-titrated, purified antibody
2. Detection Antibody: Pre-titrated, biotin-conjugated antibody
3. Standard: Recombinant cytokine for generating standard curve and calibrating samples
4. ELISA/ELISPOT Coating Buffer Powder. *Reconstitute to 1L with dH20 and filter (0.22 uM).*
5. Assay Diluent. *5X concentrated*
6. Detection enzyme. *Pre-titrated Avidin-HRP*
7. Substrate Solution. *Tetramethylbenzidine (TMB) Substrate Solution*
8. Certificate of Analysis. *Lot-specific instructions for dilution of antibodies and standards*
9. 96 Well Plate. *Corning Costar 9018 (included with product Cat. #'s ending in suffixes -22, -44, -76, -86)*

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TDS Protocol

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Other Materials Needed

- Buffers
  - Wash Buffer: 1 x PBS, 0.05% Tween-20 (or eBioscience ELISA Wash Buffer Powder, cat 00-0400)
  - Stop Solution: 1M H_3PO_4 or 2N H_2SO_4
- Pipettes and pipettors
- Refrigerator
- 96-well plate (Corning Costar 9018)

  NOTE: The use of ELISA plates which are not high affinity protein binding plates will result in suboptimal performance, e.g., low signal or inconsistent data. Do not use tissue culture plates or low protein absorption plates. Use only the Corning Costar 9018 or NUNC Maxisorp 96 well plates provided or suggested.
- 96-well ELISA plate reader (microplate spectrophotometer)
- ELISA plate washer

NOTE: To ensure optimal results from this ELISA Ready-SET-Go! set, please only use the components included in the set. Exchanging of components is not recommended as a change in signal may occur.

Stability

This ELISA set is guaranteed to perform as specified at least 12 months from date of receipt if stored and handled as instructed according to this datasheet and the Certificate of Analysis, which is included with the reagents.

Storage Instructions for Cytokine Standards

The frozen cytokine standard is already aliquoted at 20 µl per vial. Upon receipt, frozen cytokine standard should be immediately stored at -80°C; stable for at least 12 months. After thawing, quick-spin vial prior to opening. Do not re-aliquot into smaller fractions. These are single use vials. Use one time and discard. For dilution of the standard, please see instructions on the Certificate of Analysis and follow these as written.

Storage Instructions for Other Set Reagents

Store at 4°C.

Time Requirements

- 1 overnight incubation
- 4½-hour incubations
- 1 hour washing and analyzing samples

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TDS Protocol

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Experimental Procedure

1. Coat Corning Costar 9018 ELISA plate with 100 µl/well of capture antibody in Coating Buffer (dilute as noted on Certificate of Analysis, which is included with the reagent set). Seal the plate and incubate overnight at 4°C.
2. Aspirate wells and wash 5 times with >250 µl/well Wash Buffer*. Allowing time for soaking (~ 1 minute) during each wash step increases the effectiveness of the washes. Blot plate on absorbent paper to remove any residual buffer.
3. Dilute 1 part 5X concentrated Assay Diluent with 4 parts DI water.* Block wells with 200 µl/well of 1X Assay Diluent. Incubate at room temperature for 1 hour.
4. Aspirate/wash as in step 2. Repeat for a total of 5 washes.
5. Using 1X Assay Diluent*, dilute standards as noted on the Certificate of Analysis (C of A). Add 100 µl/well of standard to the appropriate wells. Perform 2-fold serial dilutions of the top standards to make the standard curve. Add 100 µl/well of your samples to the appropriate wells. Cover or seal the plate and incubate at room temperature for 2 hours (or overnight at 4°C for maximal sensitivity).
6. Aspirate/wash as in step 2. Repeat for a total of 5 washes.
7. Add 100 µl/well of detection antibody diluted in 1X Assay Diluent* (dilute as noted on C of A). Seal the plate and incubate at room temperature for 1 hour.
8. Aspirate/wash as in step 2. Repeat for a total of 5 washes.
9. Add 100 µl/well of Avidin-HRP* diluted in 1X Assay Diluent (dilute as noted on C of A). Seal the plate and incubate at room temperature for 30 minutes.
10. Aspirate and wash as in step 2. In this wash step, soak wells in Wash Buffer* for 1 to 2 minutes prior to aspiration. Repeat for a total of 7 washes.
11. Add 100 µl/well of Substrate Solution to each well. Incubate plate at room temperature for 15 minutes.
12. Add 50 µl of Stop Solution to each well.
13. Read plate at 450 nm. If wavelength subtraction is available, subtract the values of 570 nm from those of 450 nm and analyze data.

*NOTE: Be certain that no sodium azide is present in the solutions used in this assay, as this inhibits HRP enzyme activity.

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Standard Calibration

The standard of the Ready-SET-Go! is calibrated against NIBSC standards:

Table of Standard Calibration				
Cytokine	ng of eB standard	ng of NIBSC standard	U of NIBSC standard	NIBSC Lot #
hIL-2	1	1.1	14.6	86/564
hIL-4	1	2.2	22	88/656
hIL-5	1	2.2	22	90/586
hIL-6	1	1.7	170	89/548
hIL-10	1	0.8	4	93/722
hIL-12	1	0.8	8	95/544
hIFN-g	1	1.1	22	87/586
hTNF-a	1	0.9	36	87/650
mIL-2	1	3.1	310	93/566
mIL-4	1	3	30	91/656
mIL-6	1	8.5	850	93/730
mIFN-g*	1		4.5	Gg02-901-533
mTNF-a	1	1.7	340	88/532
* Mouse IFN-g is calibrated using NIH standard (Lot Gg02-901-533) and is measured in Units (U)				

ELISA Troubleshooting Guide		
Problem	Possibility	Solution
A. High Background	1. Improper and inefficient washing	1. Improve efficiency of washing. Fill plates completely, soak for 1 minute per wash, as directed
	2. Cross contamination from other specimens or positive control	2. Repeat ELISA, be careful when washing and pipetting
	3. Contaminated substrate	3. Substrate should be colorless
	4. Incorrect dilutions, e.g., conjugate concentration was too high	4. Repeat test using correct dilutions; check with the recommendations of the antibody manufacturer
B. No signal	1. Improper, low protein binding capacity plates were used	1. Repeat ELISA, using recommended high binding capacity plates
	2. Wrong substrate was used	2. Repeat ELISA, use the correct substrate
	3. Enzyme inhibitor present in buffers; e.g., sodium azide in the washing buffer and Assay Diluent inhibits peroxidase activity	3. Repeat ELISA, make sure your system contains no enzyme inhibitor

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C. Very weak signal	1. Improper and inefficient washing	1. Make sure washing procedure is done correctly
	2. Incorrect dilutions of standard	2. Follow recommendations of standard handling exactly as written on the certificate of analysis
	3. Insufficient incubation time	3. Repeat ELISA, follow the protocol carefully for each stepEs incubation time
	4. Incorrect storage of reagents	4. Store reagents in the correct temperature, avoid freeze and thaw, avoid using the Efrost freeE freezer
	5. Wrong filter in ELISA reader was used	5. Use the correct wavelength setting
	6. Wrong plate used	6. Use the recommended Corning Costar 9018 or NUNC Maxisorp flat bottom 96 well plates
D. Variation amongst replicates	1. Improper and inefficient washing	1. Make sure washing procedure is done correctly; see certificate of analysis
	2. Poor mixing of samples	2. Mix samples and reagents gently and equilibrate to proper temperature
	3. Plates not clean	3. Plates should be wiped on bottom before measuring absorbance
	4. Improper, low binding capacity plates were used	4. Use recommended high binding capacity plates
	5. Reagents have expired	5. Do not use if past expiration date

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Product Information

Contents: Human IL-12 (Interleukin-12) p70 ELISA Ready-SET-Go!
Catalog Number: 88-7126
Sensitivity: 4 pg/ml
Standard Curve Range: 4 - 500 pg/ml

Standard curve of human IL-12 p70 ELISA Ready-SET-Go!
Recombinant concentration in pg/ml.

Description

This Human IL-12 p70 ELISA Ready-SET-Go! reagent set (with or without high-affinity binding microwell plates) contains the necessary reagents, buffers and diluents for performing quantitative enzyme linked immunosorbent assays (ELISA). This Human IL-12 p70 ELISA Ready-SET-Go! reagent set specifically measures the bioactive, heterodimeric form of IL-12, p70, without interference by p40 monomer, homodimer, or IL-23 (p19/p40). This ELISA reagent set is specifically engineered for accurate and precise measurement of human IL-12 p70 protein levels from samples including serum, plasma, and supernatants from cell cultures.

Components

1. Capture Antibody: Pre-titrated, purified antibody
2. Detection Antibody: Pre-titrated, biotin-conjugated antibody
3. Standard: Recombinant cytokine for generating standard curve and calibrating samples
4. ELISA/ELISPOT Coating Buffer Powder. *Reconstitute to 1L with dH20 and filter (0.22 uM).*
5. Assay Diluent. *5X concentrated*
6. Detection enzyme. *Pre-titrated Avidin-HRP*
7. Substrate Solution. *Tetramethylbenzidine (TMB) Substrate Solution*
8. Certificate of Analysis. *Lot-specific instructions for dilution of antibodies and standards*
9. 96 Well Plate. *Corning Costar 9018 (included with product Cat. #'s ending in suffixes -22, -44, -76, -86)*

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Related Products

Cat. 88-7234 Mouse IL-23 (p19/p40, IL23) ELISA Ready-SET-Go!
Cat. 34-8239 Carrier-Free Recombinant Human IL-23 (Interleukin-23, IL23)

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TDS Protocol

Research Use Only

Other Materials Needed

- Buffers
  - Wash Buffer: 1 x PBS, 0.05% Tween-20 (or eBioscience ELISA Wash Buffer Powder, cat 00-0400)
  - Stop Solution: 1M H_3PO_4 or 2N H_2SO_4
- Pipettes and pipettors
- Refrigerator
- 96-well plate (Corning Costar 9018)

  NOTE: The use of ELISA plates which are not high affinity protein binding plates will result in suboptimal performance, e.g., low signal or inconsistent data. Do not use tissue culture plates or low protein absorption plates. Use only the Corning Costar 9018 or NUNC Maxisorp 96 well plates provided or suggested.
- 96-well ELISA plate reader (microplate spectrophotometer)
- ELISA plate washer

NOTE: To ensure optimal results from this ELISA Ready-SET-Go! set, please only use the components included in the set. Exchanging of components is not recommended as a change in signal may occur.

Stability

This ELISA set is guaranteed to perform as specified at least 12 months from date of receipt if stored and handled as instructed according to this datasheet and the Certificate of Analysis, which is included with the reagents.

Storage Instructions for Cytokine Standards

The frozen cytokine standard is already aliquoted at 20 µl per vial. Upon receipt, frozen cytokine standard should be immediately stored at -80°C; stable for at least 12 months. After thawing, quick-spin vial prior to opening. Do not re-aliquot into smaller fractions. These are single use vials. Use one time and discard. For dilution of the standard, please see instructions on the Certificate of Analysis and follow these as written.

Storage Instructions for Other Set Reagents

Store at 4°C.

Time Requirements

- 1 overnight incubation
- 4½-hour incubations
- 1 hour washing and analyzing samples

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TDS Protocol

Research Use Only
Experimental Procedure

1. Coat Corning Costar 9018 ELISA plate with 100 µl/well of capture antibody in Coating Buffer (dilute as noted on Certificate of Analysis, which is included with the reagent set). Seal the plate and incubate overnight at 4°C.
2. Aspirate wells and wash 3 times with >250 µl/well Wash Buffer*. Allowing time for soaking (~ 1 minute) during each wash step increases the effectiveness of the washes. Blot plate on absorbent paper to remove any residual buffer.
3. Dilute 1 part 5X concentrated Assay Diluent with 4 parts DI water.* Block wells with 200 µl/well of 1X Assay Diluent. Incubate at room temperature for 1 hour.
4. Aspirate/wash as in step 2. Repeat for a total of 3 washes.
5. Using 1X Assay Diluent*, dilute standards as noted on the Certificate of Analysis (C of A). Add 100 µl/well of standard to the appropriate wells. Perform 2-fold serial dilutions of the top standards to make the standard curve. Add 100 µl/well of your samples to the appropriate wells. Cover or seal the plate and incubate at room temperature for 2 hours (or overnight at 4°C for maximal sensitivity).
6. Aspirate/wash as in step 2. Repeat for a total of 3 washes.
7. Add 100 µl/well of detection antibody diluted in 1X Assay Diluent* (dilute as noted on C of A). Seal the plate and incubate at room temperature for 1 hour.
8. Aspirate/wash as in step 2. Repeat for a total of 3 washes.
9. Add 100 µl/well of Avidin-HRP* diluted in 1X Assay Diluent (dilute as noted on C of A). Seal the plate and incubate at room temperature for 30 minutes.
10. Aspirate and wash as in step 2. In this wash step, soak wells in Wash Buffer* for 1 to 2 minutes prior to aspiration. Repeat for a total of 7 washes.
11. Add 100 µl/well of Substrate Solution to each well. Incubate plate at room temperature for 15 minutes.
12. Add 50 µl of Stop Solution to each well.
13. Read plate at 450 nm. If wavelength subtraction is available, subtract the values of 570 nm from those of 450 nm and analyze data.

*NOTE: Be certain that no sodium azide is present in the solutions used in this assay, as this inhibits HRP enzyme activity.

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TDS Protocol

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Standard Calibration

The standard of the Ready-SET-Go! is calibrated against NIBSC standards:

Table of Standard Calibration				
Cytokine	ng of eB standard	ng of NIBSC standard	U of NIBSC standard	NIBSC Lot #
hIL-2	1	1.1	14.6	86/564
hIL-4	1	2.2	22	88/656
hIL-5	1	2.2	22	90/586
hIL-6	1	1.7	170	89/548
hIL-10	1	0.8	4	93/722
hIL-12	1	0.8	8	95/544
hIFN-g	1	1.1	22	87/586
hTNF-a	1	0.9	36	87/650
mIL-2	1	3.1	310	93/566
mIL-4	1	3	30	91/656
mIL-6	1	8.5	850	93/730
mIFN-g*	1		4.5	Gg02-901-533
mTNF-a	1	1.7	340	88/532
* Mouse IFN-g is calibrated using NIH standard (Lot Gg02-901-533) and is measured in Units (U)				

ELISA Troubleshooting Guide		
Problem	Possibility	Solution
A. High Background	1. Improper and inefficient washing	1. Improve efficiency of washing. Fill plates completely, soak for 1 minute per wash, as directed
	2. Cross contamination from other specimens or positive control	2. Repeat ELISA, be careful when washing and pipetting
	3. Contaminated substrate	3. Substrate should be colorless
	4. Incorrect dilutions, e.g., conjugate concentration was too high	4. Repeat test using correct dilutions; check with the recommendations of the antibody manufacturer
B. No signal	1. Improper, low protein binding capacity plates were used	1. Repeat ELISA, using recommended high binding capacity plates
	2. Wrong substrate was used	2. Repeat ELISA, use the correct substrate
	3. Enzyme inhibitor present in buffers; e.g., sodium azide in the washing buffer and Assay Diluent inhibits peroxidase activity	3. Repeat ELISA, make sure your system contains no enzyme inhibitor

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TDS Protocol

Research Use Only

C. Very weak signal	1. Improper and inefficient washing	1. Make sure washing procedure is done correctly
	2. Incorrect dilutions of standard	2. Follow recommendations of standard handling exactly as written on the certificate of analysis
	3. Insufficient incubation time	3. Repeat ELISA, follow the protocol carefully for each stepEs incubation time
	4. Incorrect storage of reagents	4. Store reagents in the correct temperature, avoid freeze and thaw, avoid using the Efrost freeE freezer
	5. Wrong filter in ELISA reader was used	5. Use the correct wavelength setting
	6. Wrong plate used	6. Use the recommended Corning Costar 9018 or NUNC Maxisorp flat bottom 96 well plates
D. Variation amongst replicates	1. Improper and inefficient washing	1. Make sure washing procedure is done correctly; see certificate of analysis
	2. Poor mixing of samples	2. Mix samples and reagents gently and equilibrate to proper temperature
	3. Plates not clean	3. Plates should be wiped on bottom before measuring absorbance
	4. Improper, low binding capacity plates were used	4. Use recommended high binding capacity plates
	5. Reagents have expired	5. Do not use if past expiration date

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Product Information

Contents: Human IL-17A (homodimer) ELISA Ready-SET-Go!™
Catalog Number: 88-7176
Sensitivity: 4 pg/ml
Standard Curve Range: 500 pg/ml - 4 pg/ml

Description

This Human IL-17A ELISA Ready-SET-Go! reagent set (with or without high-affinity binding microwell plates) contains the necessary reagents, buffers and diluents for performing quantitative enzyme linked immunosorbent assays (ELISA). This ELISA reagent set is specifically engineered for accurate and precise measurement of human IL-17A homodimer protein levels from samples including serum, plasma, and supernatants from cell cultures. Minimal crossreactivity to the recombinant human Il-17AF heterodimer is observed at 0.4% when spiked in excess (100ng/ml).

Components

1. Capture Antibody: Pre-titrated, purified antibody
2. Detection Antibody: Pre-titrated, biotin-conjugated antibody
3. Standard: Recombinant cytokine for generating standard curve and calibrating samples
4. ELISA/ELISPOT Coating Buffer Powder. *Reconstitute to 1L with dH20 and filter (0.22 uM).*
5. Assay Diluent. *5X concentrated*
6. Detection enzyme. *Pre-titrated Avidin-HRP*
7. Substrate Solution. *Tetramethylbenzidine (TMB) Substrate Solution*
8. Certificate of Analysis. *Lot-specific instructions for dilution of antibodies and standards*
9. 96 Well Plate. *Corning Costar 9018 (included with product Cat. #'s ending in suffixes -22, -44, -76, -86)*

Applications Reported

For research use only, not for diagnostic or therapeutic use. Human IL-17A (homodimer) ELISA RSGI has been reported for use in ELISAset.

References

Gu Y, Hu X, Liu C, Qv X, Xu C. Interleukin (IL)-17 promotes macrophages to produce IL-8, IL-6 and tumour necrosis factor-alpha in aplastic anaemia. Br J Haematol. 2008 Jul;142(1):109-14. (RSG ELISA kit, BM and PB plasma, PubMed)

Hirota K, Yoshitomi H, Hashimoto M, Maeda S, Teradaira S, Sugimoto N, Yamaguchi T, Nomura T, Ito H, Nakamura T, Sakaguchi N, Sakaguchi S. Preferential recruitment of CCR6-expressing Th17 cells to inflamed joints via CCL20 in rheumatoid arthritis and its animal model. J Exp Med. 2007 Nov 26;204(12):2803-12. (RSG ELISA kit, synovial fluid, PubMed)

Saruta M, Yu QT, Avanesyan A, Fleshner PR, Targan SR, Papadakis KA. Phenotype and effector function of CC chemokine receptor 9-expressing lymphocytes in small intestinal Crohn's disease. J Immunol. 2007 Mar 1;178(5):3293-300. (RSG ELISA kit, TC supernatant, PubMed)

Related Products

Cat. 14-8178 Recombinant Human IL-17A/F (Interleukin-17A/F, IL17A/F)
Cat. 14-8179 Recombinant Human IL-17A (Interleukin-17A, IL17A)
Cat. 14-8479 Recombinant Human IL-17F (Interleukin-17F, IL17F)

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TDS Protocol

Research Use Only

Other Materials Needed

- Buffers
  - Wash Buffer: 1 x PBS, 0.05% Tween-20 (or eBioscience ELISA Wash Buffer Powder, cat 00-0400)
  - Stop Solution: 1M H_3PO_4 or 2N H_2SO_4
- Pipettes and pipettors
- Refrigerator
- 96-well plate (Corning Costar 9018)

  NOTE: The use of ELISA plates which are not high affinity protein binding plates will result in suboptimal performance, e.g., low signal or inconsistent data. Do not use tissue culture plates or low protein absorption plates. Use only the Corning Costar 9018 or NUNC Maxisorp 96 well plates provided or suggested.
- 96-well ELISA plate reader (microplate spectrophotometer)
- ELISA plate washer

NOTE: To ensure optimal results from this ELISA Ready-SET-Go! set, please only use the components included in the set. Exchanging of components is not recommended as a change in signal may occur.

Stability

This ELISA set is guaranteed to perform as specified at least 12 months from date of receipt if stored and handled as instructed according to this datasheet and the Certificate of Analysis, which is included with the reagents.

Storage Instructions for Cytokine Standards

The frozen cytokine standard is already aliquoted at 20 µl per vial. Upon receipt, frozen cytokine standard should be immediately stored at -80°C; stable for at least 12 months. After thawing, quick-spin vial prior to opening. Do not re-aliquot into smaller fractions. These are single use vials. Use one time and discard. For dilution of the standard, please see instructions on the Certificate of Analysis and follow these as written.

Storage Instructions for Other Set Reagents

Store at 4°C.

Time Requirements

- 1 overnight incubation
- 4½-hour incubations
- 1 hour washing and analyzing samples

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TDS Protocol

Research Use Only
Experimental Procedure

1. Coat Corning Costar 9018 ELISA plate with 100 µl/well of capture antibody in Coating Buffer (dilute as noted on Certificate of Analysis, which is included with the reagent set). Seal the plate and incubate overnight at 4°C.
2. Aspirate wells and wash 5 times with >250 µl/well Wash Buffer*. Allowing time for soaking (~ 1 minute) during each wash step increases the effectiveness of the washes. Blot plate on absorbent paper to remove any residual buffer.
3. Dilute 1 part 5X concentrated Assay Diluent with 4 parts DI water.* Block wells with 200 µl/well of 1X Assay Diluent. Incubate at room temperature for 1 hour.
4. Aspirate/wash as in step 2. Repeat for a total of 5 washes.
5. Using 1X Assay Diluent*, dilute standards as noted on the Certificate of Analysis (C of A). Add 100 µl/well of standard to the appropriate wells. Perform 2-fold serial dilutions of the top standards to make the standard curve. Add 100 µl/well of your samples to the appropriate wells. Cover or seal the plate and incubate at room temperature for 2 hours (or overnight at 4°C for maximal sensitivity).
6. Aspirate/wash as in step 2. Repeat for a total of 5 washes.
7. Add 100 µl/well of detection antibody diluted in 1X Assay Diluent* (dilute as noted on C of A). Seal the plate and incubate at room temperature for 1 hour.
8. Aspirate/wash as in step 2. Repeat for a total of 5 washes.
9. Add 100 µl/well of Avidin-HRP* diluted in 1X Assay Diluent (dilute as noted on C of A). Seal the plate and incubate at room temperature for 30 minutes.
10. Aspirate and wash as in step 2. In this wash step, soak wells in Wash Buffer* for 1 to 2 minutes prior to aspiration. Repeat for a total of 7 washes.
11. Add 100 µl/well of Substrate Solution to each well. Incubate plate at room temperature for 15 minutes.
12. Add 50 µl of Stop Solution to each well.
13. Read plate at 450 nm. If wavelength subtraction is available, subtract the values of 570 nm from those of 450 nm and analyze data.

*NOTE: Be certain that no sodium azide is present in the solutions used in this assay, as this inhibits HRP enzyme activity.

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TDS Protocol

Research Use Only

Standard Calibration

The standard of the Ready-SET-Go! is calibrated against NIBSC standards:

Table of Standard Calibration				
Cytokine	ng of eB standard	ng of NIBSC standard	U of NIBSC standard	NIBSC Lot #
hIL-2	1	1.1	14.6	86/564
hIL-4	1	2.2	22	88/656
hIL-5	1	2.2	22	90/586
hIL-6	1	1.7	170	89/548
hIL-10	1	0.8	4	93/722
hIL-12	1	0.8	8	95/544
hIFN-g	1	1.1	22	87/586
hTNF-a	1	0.9	36	87/650
mIL-2	1	3.1	310	93/566
mIL-4	1	3	30	91/656
mIL-6	1	8.5	850	93/730
mIFN-g*	1		4.5	Gg02-901-533
mTNF-a	1	1.7	340	88/532
* Mouse IFN-g is calibrated using NIH standard (Lot Gg02-901-533) and is measured in Units (U)				

ELISA Troubleshooting Guide		
Problem	Possibility	Solution
A. High Background	1. Improper and inefficient washing	1. Improve efficiency of washing. Fill plates completely, soak for 1 minute per wash, as directed
	2. Cross contamination from other specimens or positive control	2. Repeat ELISA, be careful when washing and pipetting
	3. Contaminated substrate	3. Substrate should be colorless
	4. Incorrect dilutions, e.g., conjugate concentration was too high	4. Repeat test using correct dilutions; check with the recommendations of the antibody manufacturer
B. No signal	1. Improper, low protein binding capacity plates were used	1. Repeat ELISA, using recommended high binding capacity plates
	2. Wrong substrate was used	2. Repeat ELISA, use the correct substrate
	3. Enzyme inhibitor present in buffers; e.g., sodium azide in the washing buffer and Assay Diluent inhibits peroxidase activity	3. Repeat ELISA, make sure your system contains no enzyme inhibitor

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TDS Protocol

Research Use Only

C. Very weak signal	1. Improper and inefficient washing	1. Make sure washing procedure is done correctly
	2. Incorrect dilutions of standard	2. Follow recommendations of standard handling exactly as written on the certificate of analysis
	3. Insufficient incubation time	3. Repeat ELISA, follow the protocol carefully for each stepEs incubation time
	4. Incorrect storage of reagents	4. Store reagents in the correct temperature, avoid freeze and thaw, avoid using the Efrost freeE freezer
	5. Wrong filter in ELISA reader was used	5. Use the correct wavelength setting
	6. Wrong plate used	6. Use the recommended Corning Costar 9018 or NUNC Maxisorp flat bottom 96 well plates
D. Variation amongst replicates	1. Improper and inefficient washing	1. Make sure washing procedure is done correctly; see certificate of analysis
	2. Poor mixing of samples	2. Mix samples and reagents gently and equilibrate to proper temperature
	3. Plates not clean	3. Plates should be wiped on bottom before measuring absorbance
	4. Improper, low binding capacity plates were used	4. Use recommended high binding capacity plates
	5. Reagents have expired	5. Do not use if past expiration date

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Product Information

Contents: Human IL-23 (p19/p40) ELISA Ready-SET-Go!
Catalog Number: 88-7237
Sensitivity: 15 pg/ml
Standard Curve Range: 15 pg/ml - 2.0 ng/ml

Four-parameter fit of human IL-23 ELISA Ready-SET-Go! Recombinant cytokine standard concentration in pg/ml.

Description

This Human IL-23 Ready-SET-Go! ELISA Set contains the necessary reagents, standards, buffers and diluents for performing quantitative enzyme-linked immunosorbent assays (ELISA). This ELISA set is specifically engineered for accurate and precise measurement of human IL-23 protein levels from samples including serum, plasma, and supernatants from cell cultures. Interference with CpG has been observed; therefore controls must be added if using this compoound in the assay. The assay demonstrates parallelism in measuring recombinant and native human IL-23 proteins with a standard curve range of 15 pg/ml to 2,000 pg/ml, and assay sensitivity below 15 pg/ml. The assay has been validated by specific detection of significant levels of native human IL-23 protein in supernatants from a variety of different activated dendritic cell populations. The use of a p19-specific capture antibody and a p40-specific detection antibody renders this sandwich ELISA exquisitely specific for human IL-23. IL-12 p40 monomer and IL-12 p70 were run in the assay at 200 ng/ml with no interference or cross-reactivity observed. A panel of 20 unrelated cytokines was also run in the IL-23 ELISA at 100 ng/ml with no cross reactivity observed.

IL-23 is a heterodimeric cytokine composed of the p40 subunit of IL-12 disulfide-linked with a protein p19. p19, like p35 of IL-12, is biologically inactive by itself. IL-23 interacts with IL-12Rbeta1 and an additional, novel beta2-like receptor subunit with STAT4 binding domain, termed IL-23R. IL-23 is secreted by activated mouse and human dendritic cells. Biological activities of mouse IL-23 are distinct from those of mouse IL-12. House IL-23 was found not to induce significant amounts of IFN-g. Mouse IL-23 does induce strong proliferation of memory T cells (but not naive T cells), whereas IL-12 has no effect on memory cells. Additionally, mouse IL-23 (but not IL-12) can activate mouse memory T cells to produce the proinflammatory cytokine IL-17. Human IL-23 has biological properties which are less distinct from human IL-12; human IL-23 induces proliferation of memory T cells and induces moderate levels of IFN-g production by naive and memory T cells, as compared to IL-12. IL-23-dependent, IL-17-producing CD4+ T cells (Th-17 cells) have been identified as a unique subset of Th cells that develops along a pathway that is distinct from the Th1- and Th2- cell differentiation pathways. The hallmark effector molecules of Th1 and Th2 cells, e.g., IFN-g and IL-4, have each been found to negatively regulate the generation of these Th-17 cells. More recently, de novo differentiation of Th-17 cells in the absence of IL-23 has been demonstrated by treatment of naive CD4 cells with TGF-β1 and IL-6.

Components

1. Capture Antibody: Pre-titrated, purified antibody
2. Detection Antibody: Pre-titrated, biotin-conjugated antibody
3. Standard: Recombinant cytokine for generating standard curve and calibrating samples
4. ELISA/ELISPOT Coating Buffer Powder. *Reconstitute to 1L with dH20 and filter (0.22 uM).*
5. Assay Diluent. *5X concentrated*
6. Detection enzyme. *Pre-titrated Avidin-HRP*
7. Substrate Solution. *Tetramethylbenzidine (TMB) Substrate Solution*
8. Certificate of Analysis. *Lot-specific instructions for dilution of antibodies and standards*
9. 96 Well Plate. *Corning Costar 9018 (included with product Cat. #'s ending in suffixes -22, -44, -76, -86)*

Applications Reported

For research use only, not for diagnostic or therapeutic use.

References

Goyarts E, Matsui H, Mammone T, Bender AM, Wagner JA, Maes D, Granstein RD. Norepinephrine modulates human dendritic cell activation by altering cytokine release. Exp Dermatol. 2008 Mar;17(3):188-96. (RSG ELISA kit, TC supernatant, PubMed)

Enstrom A, Onore C, Hertz-Picciotto I, Hansen R, Croen L, Van de Water J, Ashwood P. Detection of IL-17 and IL-23 in Plasma Samples of Children with Autism. American Journal of Biochemistry and Biotechnology 4 (2): 114-120, 2008. (RSG ELISA kit, plasma)

Iwakura Y, Ishigame H. The IL-23/IL-17 axis in inflammation. J Clin Invest. 2006 May;116(5):1218-22.

Veldhoen M, Hocking RJ, Atkins CJ, Locksley RM, Stockinger B. TGFbeta in the context of an inflammatory cytokine milieu supports de novo differentiation of IL-17-producing T cells. Immunity. 2006 Feb;24(2):179-89.

Harrington LE, Hatton RD, Mangan PR, Turner H, Murphy TL, Murphy KM, Weaver CT. Interleukin 17-producing CD4+ effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages. Nat Immunol. 2005 Nov;6(11):1123-32.

Brombacher F, Kastelein RA, Alber G. Novel IL-12 family members shed light on the orchestration of Th1 responses. Trends Immunol. 2003 Apr;24(4):207-12.

Aggarwal S, Ghilardi N, Xie MH, de Sauvage FJ, Gurney AL. IL-23 promotes a distinct CD4 T cell activation state characterized by the production of IL-17. J. J Biol Chem. 2003 Jan 17;278(3):1910-4.

Oppmann B, Lesley R, Blom B, Timans JC, Xu Y, Hunte B, Vega F, Yu N, Wang J, Singh K, Zonin F, Vaisberg E, Churakova T, Liu M, Gorman D, Wagner J, Zurawski S, Liu Y, Abrams JS, Moore KW, Rennick D, de Waal-Malefyt R, Hannum C, Bazan JF, Kastelein RA. Novel p19 protein engages IL-12p40 to form a cytokine, IL-23, with biological activities similar as well as distinct from IL-12. Immunity. 2000 Nov;13(5):715-25.

Related Products

Cat. 88-7126 Human IL-12 (Interleukin-12, IL12) p70 ELISA Ready-SET-Go!
Cat. 88-7176 Human IL-17A (homodimer) (Interleukin-17A, IL17A) ELISA Ready-SET-Go!
Cat. 16-7232 Functional Grade Purified anti-mouse IL-23 (Interleukin-23, IL23) p19 (clone G23-8)
Cat. 88-7234 Mouse IL-23 (p19/p40, IL23) ELISA Ready-SET-Go!
Cat. 88-7239 Human IL-23 (p19/p40) (Interleukin-23, IL23) ELISA Ready-SET-Go! Kit (with Pre-Coated Plates)
Cat. 88-7274 Mouse IL-27 (p28/EBI3, IL27) ELISA Ready-SET-Go!
Cat. 88-7876 Human IL-17A (Interleukin-17A, IL17A) ELISPOT Ready-Set-Go!
Cat. 88-7879 Human IL-12 (Interleukin-12, IL12) p70 ELISPOT Ready-Set-Go!
Cat. 34-8129 Carrier-Free Recombinant Human IL-12 (Interleukin-12, IL12) p70
Cat. 34-8179 Carrier-Free Recombinant Human IL-17A (Interleukin-17A, IL17A)
Cat. 34-8239 Carrier-Free Recombinant Human IL-23 (Interleukin-23, IL23)
Cat. 39-8239 Single-Use ELISA RSG Standard Recombinant Human IL-23 (Interleukin-23, IL23)
Cat. 14-8348 Recombinant Human TGFb1 (Transforming Growth Factor beta 1, TGF-beta1, TGF-b1)

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TDS Protocol

Research Use Only

Other Materials Needed

- Buffers
  - Wash Buffer: 1 x PBS, 0.05% Tween-20 (or eBioscience ELISA Wash Buffer Powder, cat 00-0400)
  - Stop Solution: 1M H_3PO_4 or 2N H_2SO_4
- Pipettes and pipettors
- Refrigerator
- 96-well plate (Corning Costar 9018)

  NOTE: The use of ELISA plates which are not high affinity protein binding plates will result in suboptimal performance, e.g., low signal or inconsistent data. Do not use tissue culture plates or low protein absorption plates. Use only the Corning Costar 9018 or NUNC Maxisorp 96 well plates provided or suggested.
- 96-well ELISA plate reader (microplate spectrophotometer)
- ELISA plate washer

NOTE: To ensure optimal results from this ELISA Ready-SET-Go! set, please only use the components included in the set. Exchanging of components is not recommended as a change in signal may occur.

Stability

This ELISA set is guaranteed to perform as specified at least 12 months from date of receipt if stored and handled as instructed according to this datasheet and the Certificate of Analysis, which is included with the reagents.

Storage Instructions for Cytokine Standards

The frozen cytokine standard is already aliquoted at 20 µl per vial. Upon receipt, frozen cytokine standard should be immediately stored at -80°C; stable for at least 12 months. After thawing, quick-spin vial prior to opening. Do not re-aliquot into smaller fractions. These are single use vials. Use one time and discard. For dilution of the standard, please see instructions on the Certificate of Analysis and follow these as written.

Storage Instructions for Other Set Reagents

Store at 4°C.

Time Requirements

- 1 overnight incubation
- 4½-hour incubations
- 1 hour washing and analyzing samples

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TDS Protocol

Research Use Only
Experimental Procedure

1. Coat Corning Costar 9018 ELISA plate with 100 µl/well of capture antibody in Coating Buffer (dilute as noted on Certificate of Analysis, which is included with the reagent set). Seal the plate and incubate overnight at 4°C.
2. Aspirate wells and wash 5 times with >250 µl/well Wash Buffer*. Allowing time for soaking (~ 1 minute) during each wash step increases the effectiveness of the washes. Blot plate on absorbent paper to remove any residual buffer.
3. Dilute 1 part 5X concentrated Assay Diluent with 4 parts DI water.* Block wells with 200 µl/well of 1X Assay Diluent. Incubate at room temperature for 1 hour.
4. Aspirate/wash as in step 2. Repeat for a total of 5 washes.
5. Using 1X Assay Diluent*, dilute standards as noted on the Certificate of Analysis (C of A). Add 100 µl/well of standard to the appropriate wells. Perform 2-fold serial dilutions of the top standards to make the standard curve. Add 100 µl/well of your samples to the appropriate wells. Cover or seal the plate and incubate at room temperature for 2 hours (or overnight at 4°C for maximal sensitivity).
6. Aspirate/wash as in step 2. Repeat for a total of 5 washes.
7. Add 100 µl/well of detection antibody diluted in 1X Assay Diluent* (dilute as noted on C of A). Seal the plate and incubate at room temperature for 1 hour.
8. Aspirate/wash as in step 2. Repeat for a total of 5 washes.
9. Add 100 µl/well of Avidin-HRP* diluted in 1X Assay Diluent (dilute as noted on C of A). Seal the plate and incubate at room temperature for 30 minutes.
10. Aspirate and wash as in step 2. In this wash step, soak wells in Wash Buffer* for 1 to 2 minutes prior to aspiration. Repeat for a total of 7 washes.
11. Add 100 µl/well of Substrate Solution to each well. Incubate plate at room temperature for 15 minutes.
12. Add 50 µl of Stop Solution to each well.
13. Read plate at 450 nm. If wavelength subtraction is available, subtract the values of 570 nm from those of 450 nm and analyze data.

*NOTE: Be certain that no sodium azide is present in the solutions used in this assay, as this inhibits HRP enzyme activity.

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full spectrum cell analysis
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TDS Protocol

Research Use Only

Standard Calibration

The standard of the Ready-SET-Go! is calibrated against NIBSC standards:

Table of Standard Calibration				
Cytokine	ng of eB standard	ng of NIBSC standard	U of NIBSC standard	NIBSC Lot #
hIL-2	1	1.1	14.6	86/564
hIL-4	1	2.2	22	88/656
hIL-5	1	2.2	22	90/586
hIL-6	1	1.7	170	89/548
hIL-10	1	0.8	4	93/722
hIL-12	1	0.8	8	95/544
hIFN-g	1	1.1	22	87/586
hTNF-a	1	0.9	36	87/650
mIL-2	1	3.1	310	93/566
mIL-4	1	3	30	91/656
mIL-6	1	8.5	850	93/730
mIFN-g*	1		4.5	Gg02-901-533
mTNF-a	1	1.7	340	88/532
* Mouse IFN-g is calibrated using NIH standard (Lot Gg02-901-533) and is measured in Units (U)				

ELISA Troubleshooting Guide		
Problem	Possibility	Solution
A. High Background	1. Improper and inefficient washing	1. Improve efficiency of washing. Fill plates completely, soak for 1 minute per wash, as directed
	2. Cross contamination from other specimens or positive control	2. Repeat ELISA, be careful when washing and pipetting
	3. Contaminated substrate	3. Substrate should be colorless
	4. Incorrect dilutions, e.g., conjugate concentration was too high	4. Repeat test using correct dilutions; check with the recommendations of the antibody manufacturer
B. No signal	1. Improper, low protein binding capacity plates were used	1. Repeat ELISA, using recommended high binding capacity plates
	2. Wrong substrate was used	2. Repeat ELISA, use the correct substrate
	3. Enzyme inhibitor present in buffers; e.g., sodium azide in the washing buffer and Assay Diluent inhibits peroxidase activity	3. Repeat ELISA, make sure your system contains no enzyme inhibitor

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TDS Protocol

Research Use Only

C. Very weak signal	1. Improper and inefficient washing	1. Make sure washing procedure is done correctly
	2. Incorrect dilutions of standard	2. Follow recommendations of standard handling exactly as written on the certificate of analysis
	3. Insufficient incubation time	3. Repeat ELISA, follow the protocol carefully for each stepEs incubation time
	4. Incorrect storage of reagents	4. Store reagents in the correct temperature, avoid freeze and thaw, avoid using the Efrost freeE freezer
	5. Wrong filter in ELISA reader was used	5. Use the correct wavelength setting
	6. Wrong plate used	6. Use the recommended Corning Costar 9018 or NUNC Maxisorp flat bottom 96 well plates
D. Variation amongst replicates	1. Improper and inefficient washing	1. Make sure washing procedure is done correctly; see certificate of analysis
	2. Poor mixing of samples	2. Mix samples and reagents gently and equilibrate to proper temperature
	3. Plates not clean	3. Plates should be wiped on bottom before measuring absorbance
	4. Improper, low binding capacity plates were used	4. Use recommended high binding capacity plates
	5. Reagents have expired	5. Do not use if past expiration date

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Leituras das placas ELISA

IL-10 soro

IL-10 fluido

IL-12 soro

IL-12 fluido

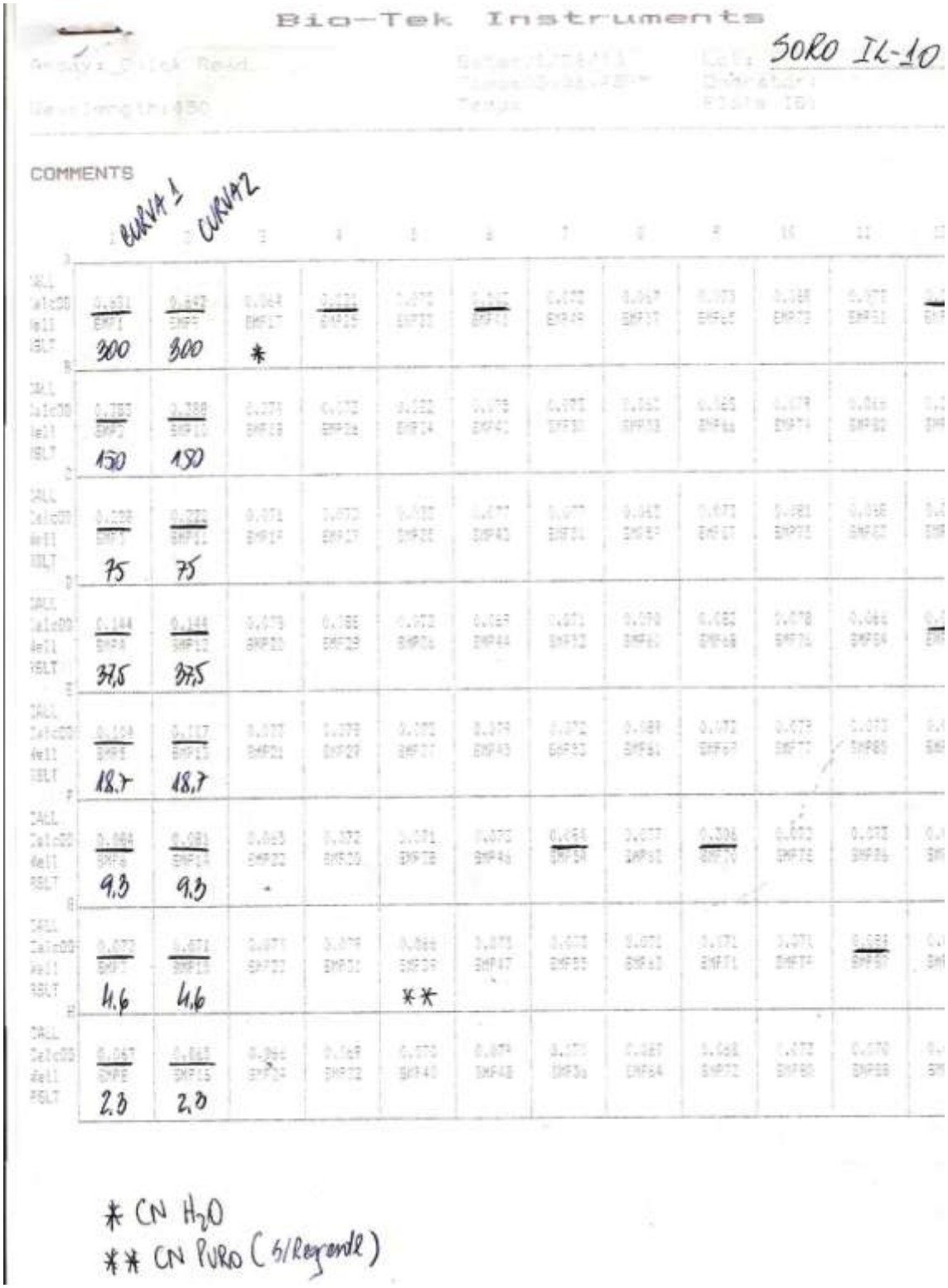
IL-17 soro

IL-17 fluido

IL-23 soro

IL-23 fluido

IL-10 soro



Bio-Tek Instruments

SORO IL-10

COMMENTS

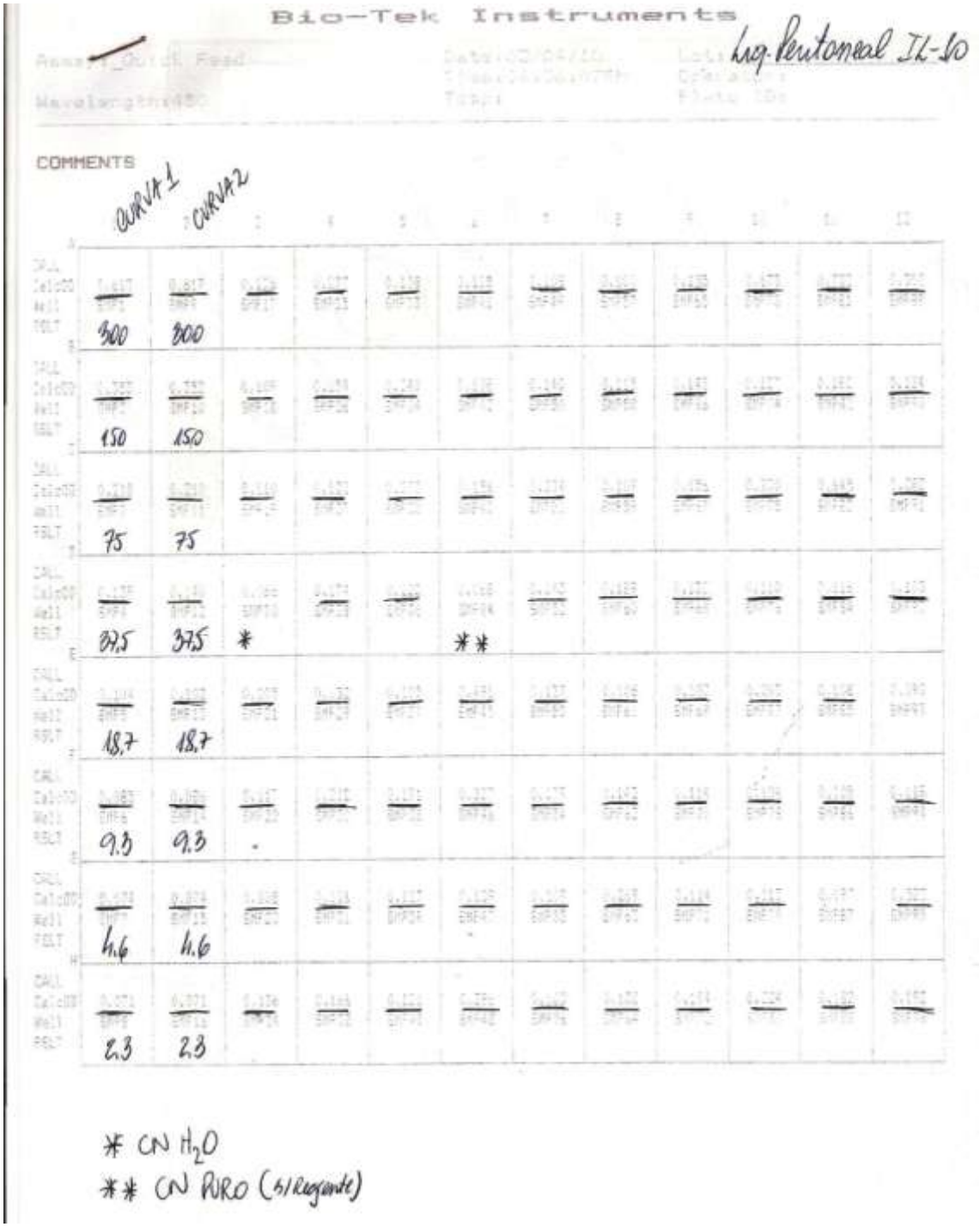
CURVA 1 | CURVA 2

	1	2	3	4	5	6	7	8	9	10	11
A	300	300	*								
B	150	150									
C	75	75									
D	37,5	37,5									
E	18,7	18,7									
F	9,3	9,3									
G	4,6	4,6			**						
H	2,3	2,3									

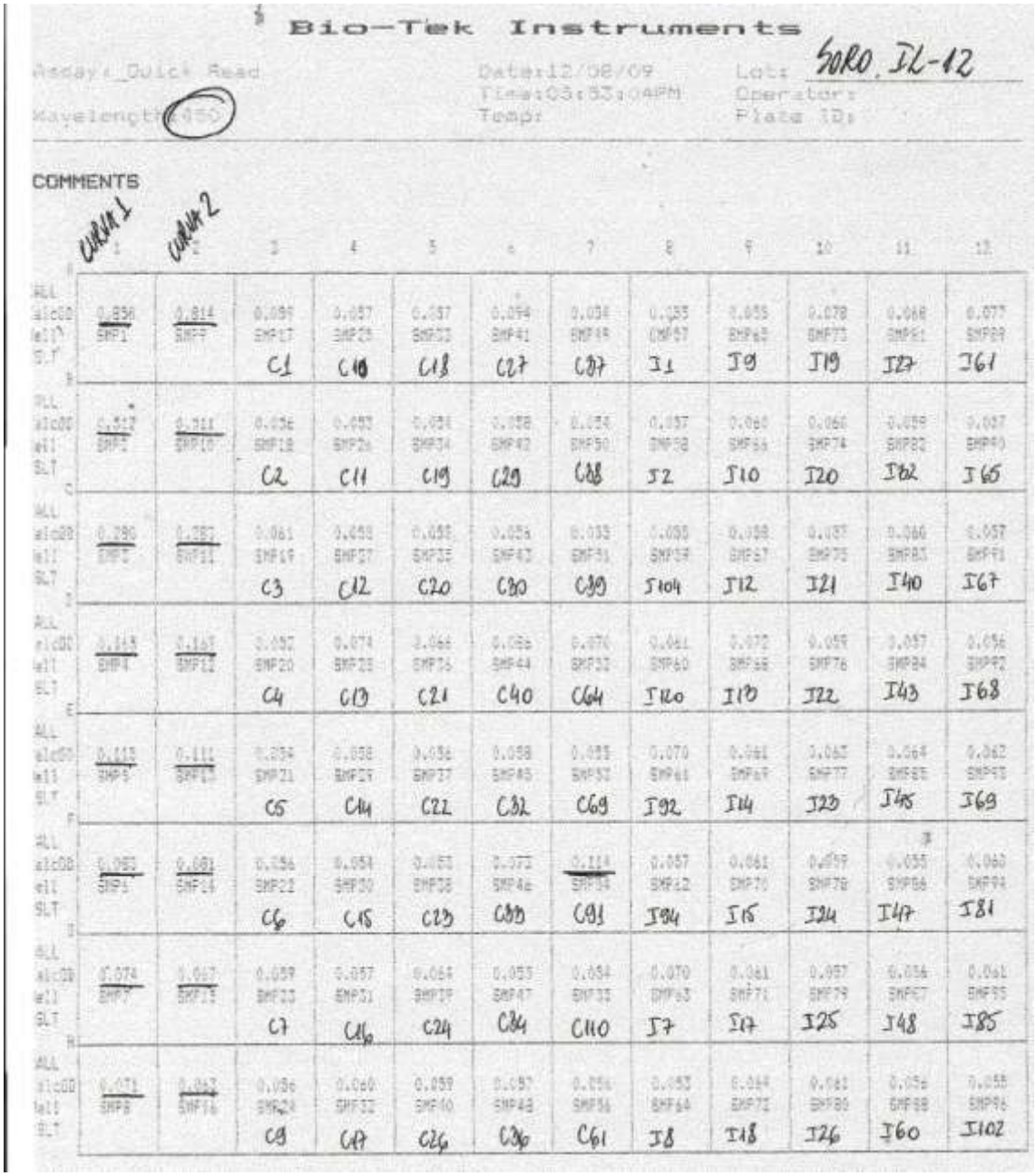
* CN H_2O

** CN PURO (s/Reagente)

IL-10 fluido


Bio-Tek Instruments
Liq. Peritoneal IL-10
COMMENTS
CURVA 1
CURVA 2
300
300
150
150
75
75
37,5
37,5
*
**
18,7
18,7
9,3
9,3
4,6
4,6
2,3
2,3
* CN H_2O
** CN RURO (s/reagente)

IL-12 soro



Bio-Tek Instruments

Readys Quick Read — Date: 12/08/09 — Lot: SORO. IL-12

Wavelength: 450 — Time: 03:53:04PM — Operator:

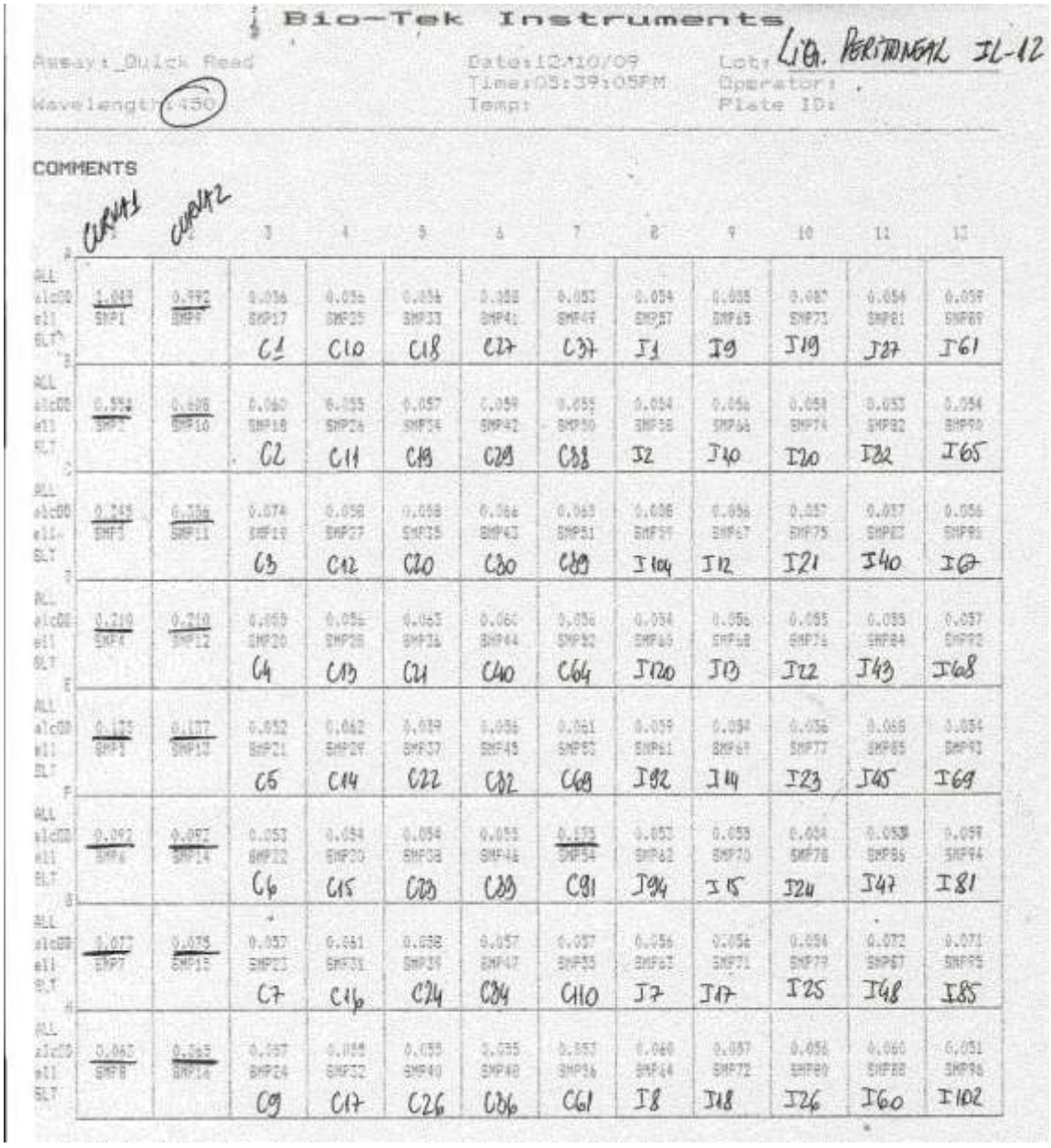
Temp: — Plate ID:

COMMENTS

curva 1 — curva 2

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.856 SMP1	0.814 SMP7	0.059 SMP13 C1	0.057 SMP19 C10	0.057 SMP25 C18	0.054 SMP31 C27	0.054 SMP37 C37	0.055 SMP43 I1	0.055 SMP49 I9	0.058 SMP55 I19	0.058 SMP61 I27	0.057 SMP67 I61
B	0.817 SMP2	0.744 SMP8	0.056 SMP14 C2	0.057 SMP20 C11	0.056 SMP26 C19	0.058 SMP32 C29	0.054 SMP38 C38	0.057 SMP44 I2	0.060 SMP50 I10	0.060 SMP56 I20	0.059 SMP62 I32	0.057 SMP68 I65
C	0.290 SMP3	0.282 SMP9	0.061 SMP15 C3	0.058 SMP21 C12	0.058 SMP27 C20	0.056 SMP33 C30	0.055 SMP39 C39	0.055 SMP45 I104	0.058 SMP51 I12	0.057 SMP57 I21	0.056 SMP63 I40	0.057 SMP69 I67
D	0.149 SMP4	0.147 SMP10	0.057 SMP16 C4	0.054 SMP22 C13	0.056 SMP28 C21	0.056 SMP34 C40	0.056 SMP40 C64	0.061 SMP46 I120	0.057 SMP52 I13	0.059 SMP58 I22	0.057 SMP64 I43	0.056 SMP70 I68
E	0.113 SMP5	0.111 SMP11	0.054 SMP17 C5	0.058 SMP23 C14	0.056 SMP29 C22	0.058 SMP35 C32	0.055 SMP41 C69	0.070 SMP47 I92	0.061 SMP53 I14	0.057 SMP59 I23	0.064 SMP65 I45	0.062 SMP71 I69
F	0.085 SMP6	0.081 SMP12	0.056 SMP18 C6	0.054 SMP24 C15	0.057 SMP30 C23	0.072 SMP36 C33	0.114 SMP42 C81	0.057 SMP48 I94	0.061 SMP54 I15	0.059 SMP60 I24	0.055 SMP66 I47	0.060 SMP72 I81
G	0.074 SMP7	0.067 SMP13	0.059 SMP19 C7	0.057 SMP25 C16	0.064 SMP31 C24	0.055 SMP37 C34	0.054 SMP43 C110	0.070 SMP49 I7	0.061 SMP55 I17	0.057 SMP61 I25	0.056 SMP67 I48	0.061 SMP73 I85
H	0.071 SMP8	0.062 SMP14	0.056 SMP20 C9	0.060 SMP26 C17	0.059 SMP32 C26	0.057 SMP38 C36	0.056 SMP44 C61	0.057 SMP50 I8	0.064 SMP56 I18	0.061 SMP62 I26	0.056 SMP68 I60	0.055 SMP74 I102

IL-12 fluido



Bio-Tek Instruments

Lig. Peritoneal IL-12

Assay: Quick Read
Wavelength: 450

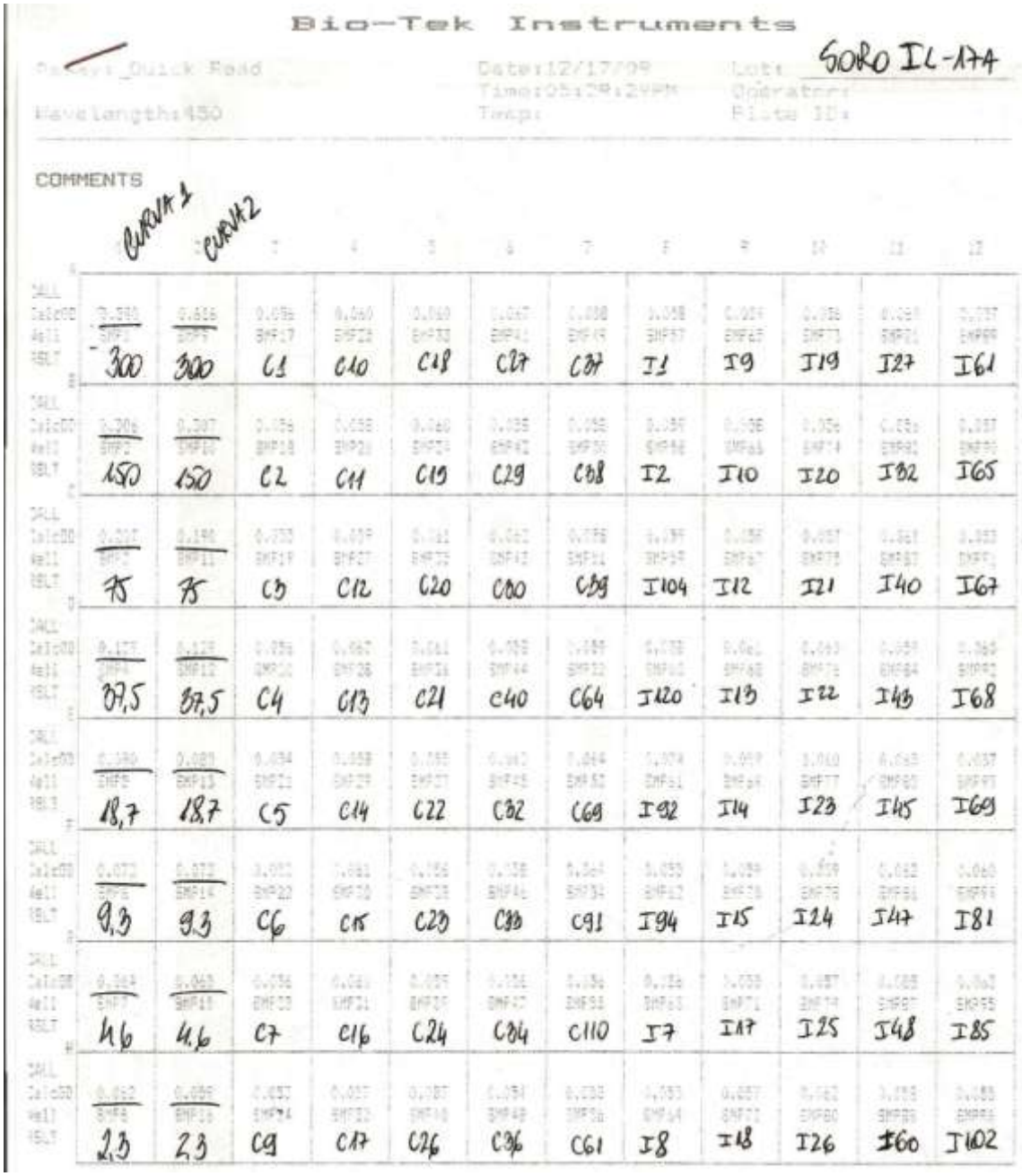
Date: 12-10-09
Time: 03:39:05PM
Temp:

Lot:
Operator:
Plate ID:

COMMENTS

	1	2	3	4	5	6	7	8	9	10	11	12
A	1.843 SNP1	0.992 SNP9	0.056 SNP17 C1	0.056 SNP25 C10	0.056 SNP33 C18	0.058 SNP41 C27	0.057 SNP49 C37	0.054 SNP57 I1	0.055 SNP65 I9	0.057 SNP73 I19	0.056 SNP81 I27	0.059 SNP89 I61
B	0.594 SNP1	0.606 SNP10	0.060 SNP18 C2	0.055 SNP26 C11	0.057 SNP34 C19	0.059 SNP42 C29	0.059 SNP50 C38	0.054 SNP58 I2	0.056 SNP66 I40	0.058 SNP74 I20	0.053 SNP82 I32	0.054 SNP90 I65
C	0.245 SNP3	0.336 SNP11	0.074 SNP19 C3	0.058 SNP27 C12	0.058 SNP35 C20	0.066 SNP43 C30	0.063 SNP51 C39	0.058 SNP59 I104	0.056 SNP67 I12	0.057 SNP75 I21	0.057 SNP82 I40	0.056 SNP91 I67
D	0.210 SNP4	0.210 SNP12	0.059 SNP20 C4	0.056 SNP28 C13	0.063 SNP36 C21	0.060 SNP44 C40	0.056 SNP52 C64	0.054 SNP60 I120	0.056 SNP68 I13	0.055 SNP76 I22	0.055 SNP84 I43	0.057 SNP92 I68
E	0.173 SNP5	0.177 SNP13	0.052 SNP21 C5	0.062 SNP29 C14	0.059 SNP37 C22	0.056 SNP45 C82	0.061 SNP53 C69	0.059 SNP61 I92	0.054 SNP69 I14	0.056 SNP77 I23	0.060 SNP85 I45	0.054 SNP93 I69
F	0.092 SNP6	0.097 SNP14	0.053 SNP22 C6	0.054 SNP30 C15	0.054 SNP38 C23	0.055 SNP46 C89	0.178 SNP54 C91	0.053 SNP62 I94	0.055 SNP70 I15	0.054 SNP78 I24	0.053 SNP86 I47	0.059 SNP94 I81
G	0.077 SNP7	0.075 SNP15	0.057 SNP23 C7	0.061 SNP31 C16	0.058 SNP39 C24	0.057 SNP47 C84	0.057 SNP55 C110	0.056 SNP63 I7	0.056 SNP71 I17	0.054 SNP79 I25	0.072 SNP87 I48	0.071 SNP95 I85
H	0.062 SNP8	0.065 SNP16	0.057 SNP24 C9	0.058 SNP32 C17	0.055 SNP40 C26	0.055 SNP48 C86	0.057 SNP56 C61	0.060 SNP64 I8	0.057 SNP72 I18	0.056 SNP80 I26	0.060 SNP88 I60	0.051 SNP96 I102

IL-17 soro



Bio-Tek Instruments

SORO IL-17A

Wavelength: 450

COMMENTS

CURVA 1 CURVA 2

	1	2	3	4	5	6	7	8	9	10	11	12
A	300	300	C1	C10	C18	C27	C37	I1	I9	I19	I27	I61
B	150	150	C2	C11	C19	C29	C38	I2	I10	I20	I32	I65
C	75	75	C3	C12	C20	C30	C39	I104	I12	I21	I40	I67
D	37,5	37,5	C4	C13	C21	C40	C64	I120	I13	I22	I43	I68
E	18,7	18,7	C5	C14	C22	C32	C69	I92	I14	I23	I45	I69
F	9,3	9,3	C6	C15	C23	C33	C91	I94	I15	I24	I47	I81
G	4,6	4,6	C7	C16	C24	C34	C110	I7	I17	I25	I48	I85
H	2,3	2,3	C9	C17	C26	C36	C61	I8	I18	I26	I60	I102

IL-17 fluido

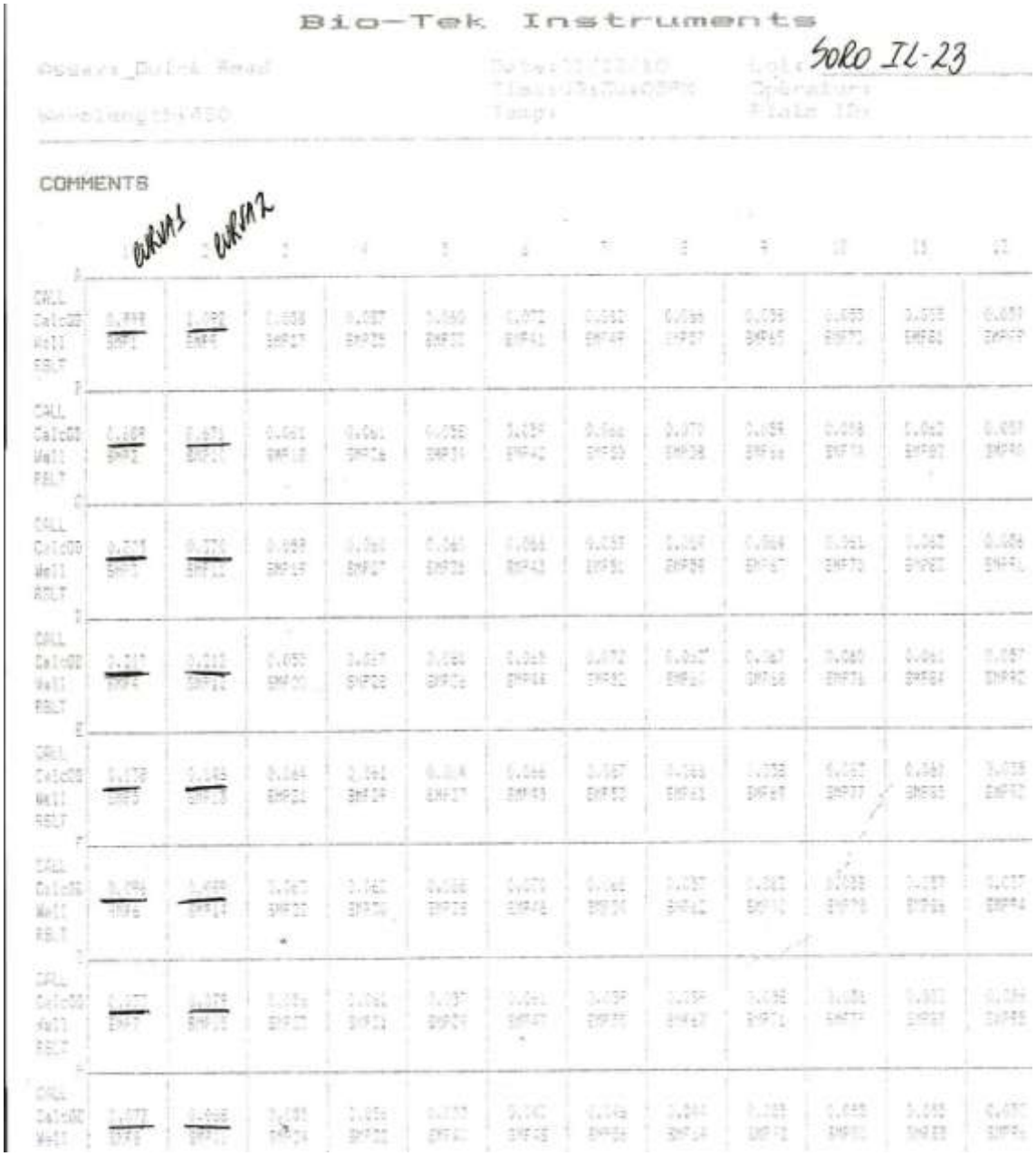
Bio-Tek Instruments

Liq. Peritoneal IL-17A

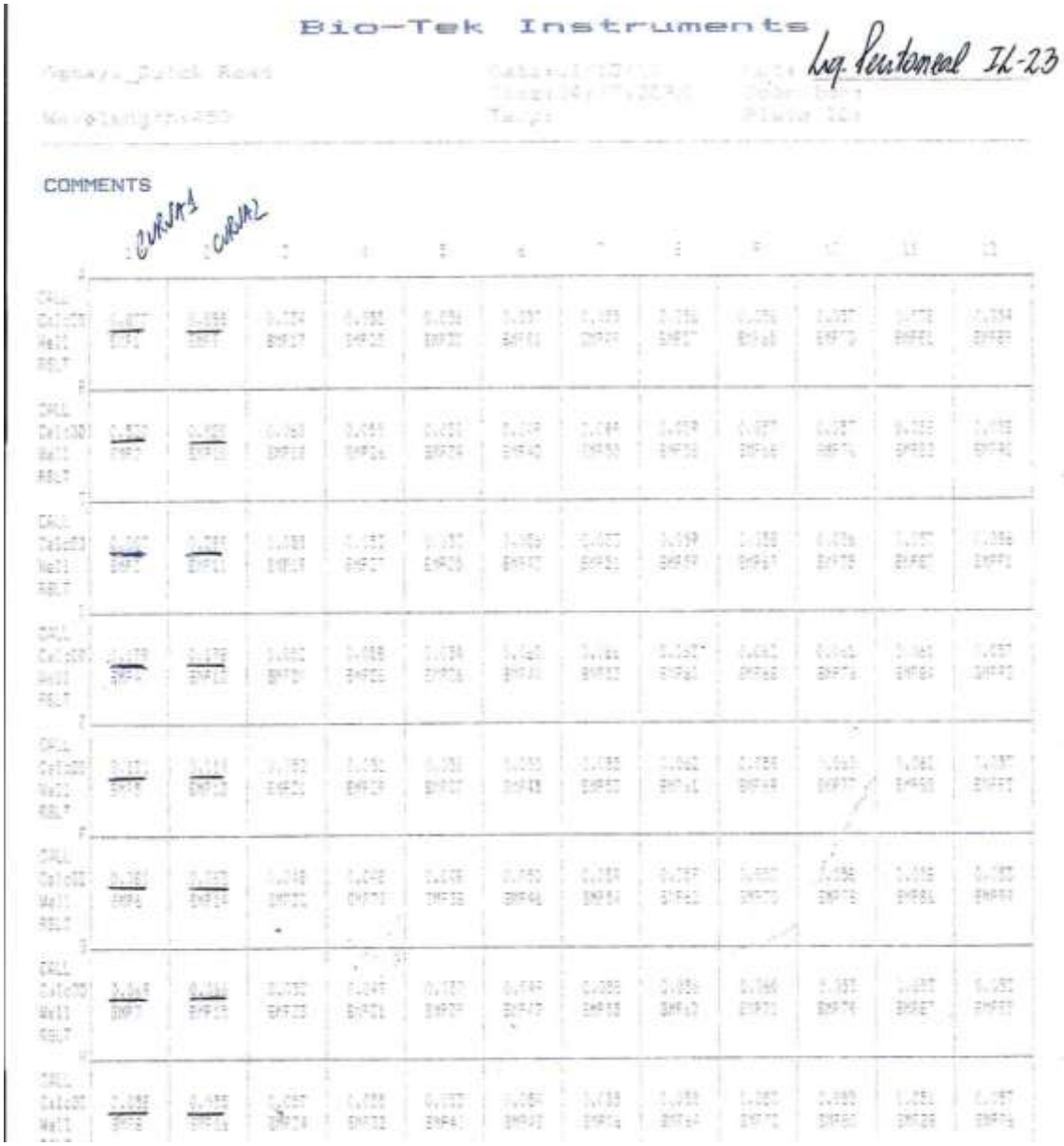
COMMENTS

	1	2	3	4	5	6	7	8	9	10	11	12
	CURVA 1	CURVA 2										
A	300	300	C1	C10	C18	C27	C87	I1	I9	I19	I27	I61
B	150	150	C2	C11	C19	C29	C88	I2	I10	I20	I32	I65
C	75	75	C3	C12	C20	C30	C89	I104	I12	I21	I40	I67
D	37,5	37,5	C4	C13	C21	C40	C64	I120	I13	I22	I43	I68
E	18,7	18,7	C5	C14	C22	C32	C69	I92	I14	I23	I45	I69
F	9,3	9,3	C6	C15	C23	C33	C91	I94	I15	I24	I47	I81
G	4,6	4,6	C7	C16	C24	C34	C110	I7	I17	I25	I48	I85
H	2,3	2,3	C8	C17	C26	C36	C61	I8	I18	I26	I60	I102

IL-23 soro

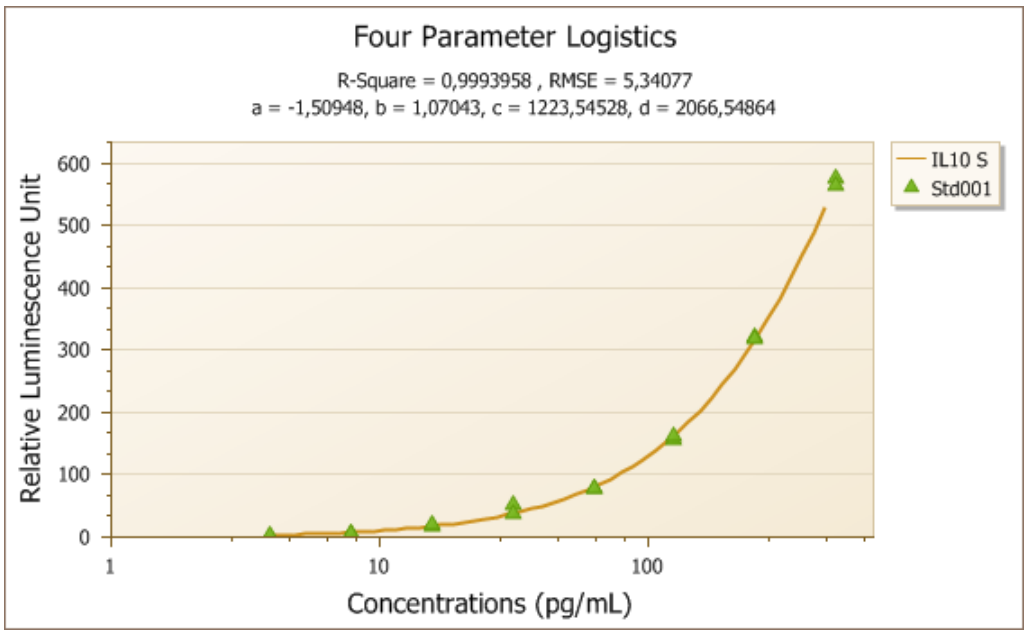


IL-23 fluido

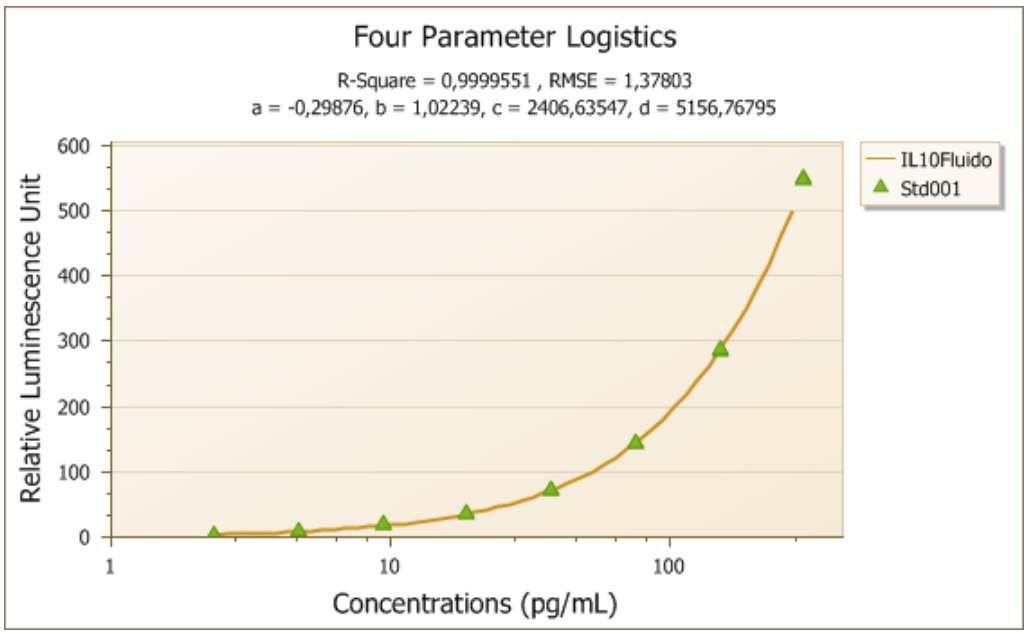


Curva-padrão das citocinas

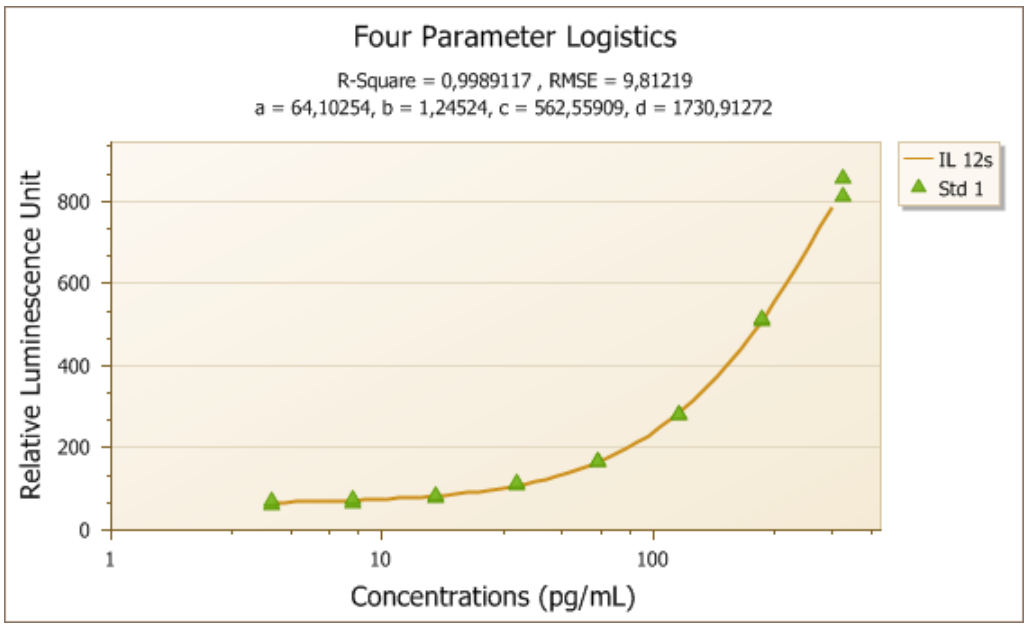
IL-10 soro



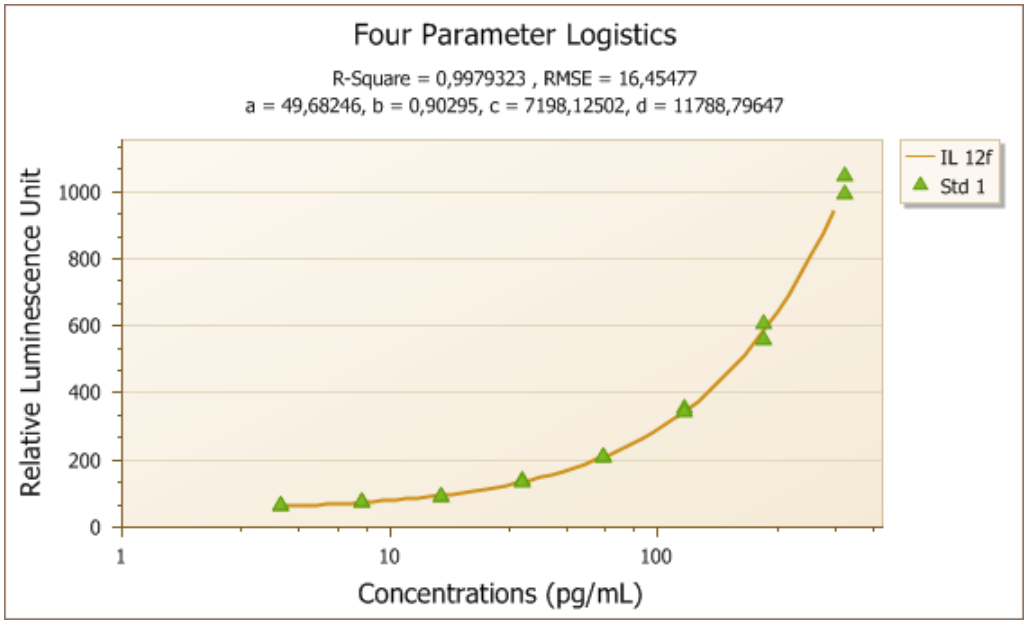
IL-10 fluido



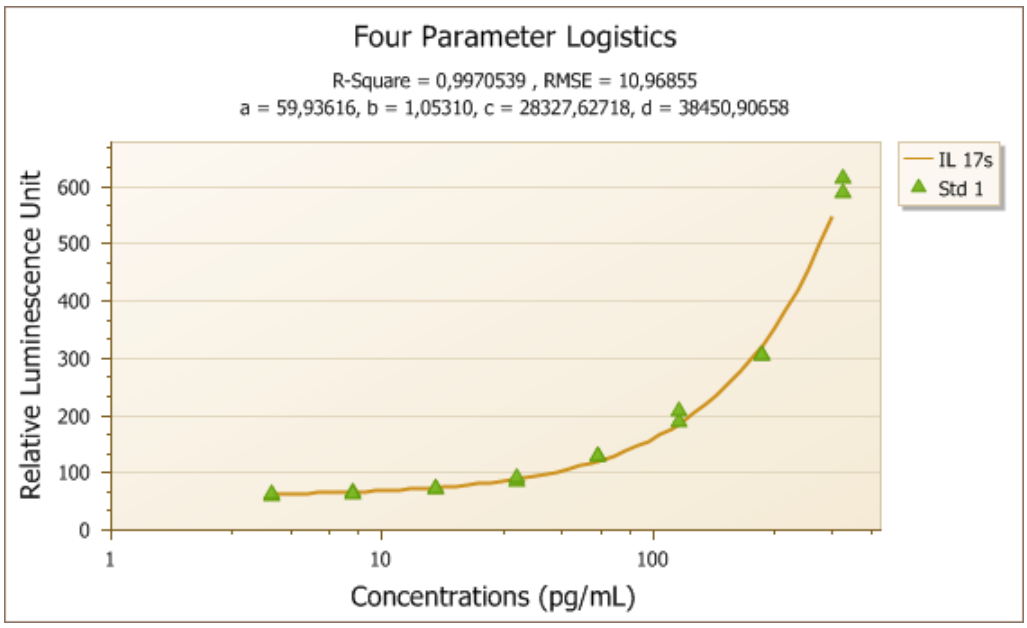
IL-12 soro



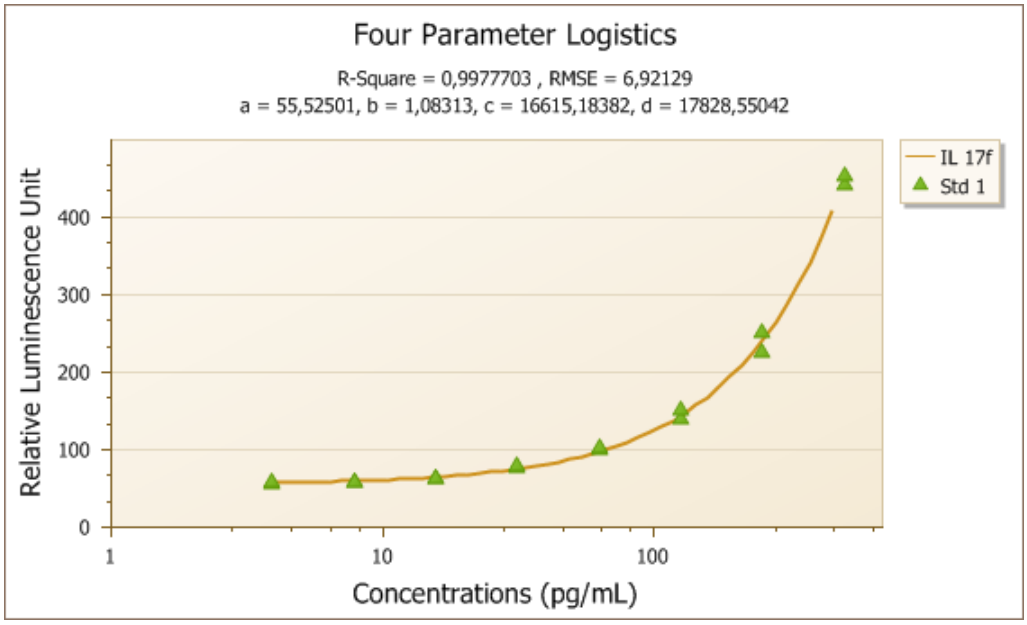
IL-12 fluido



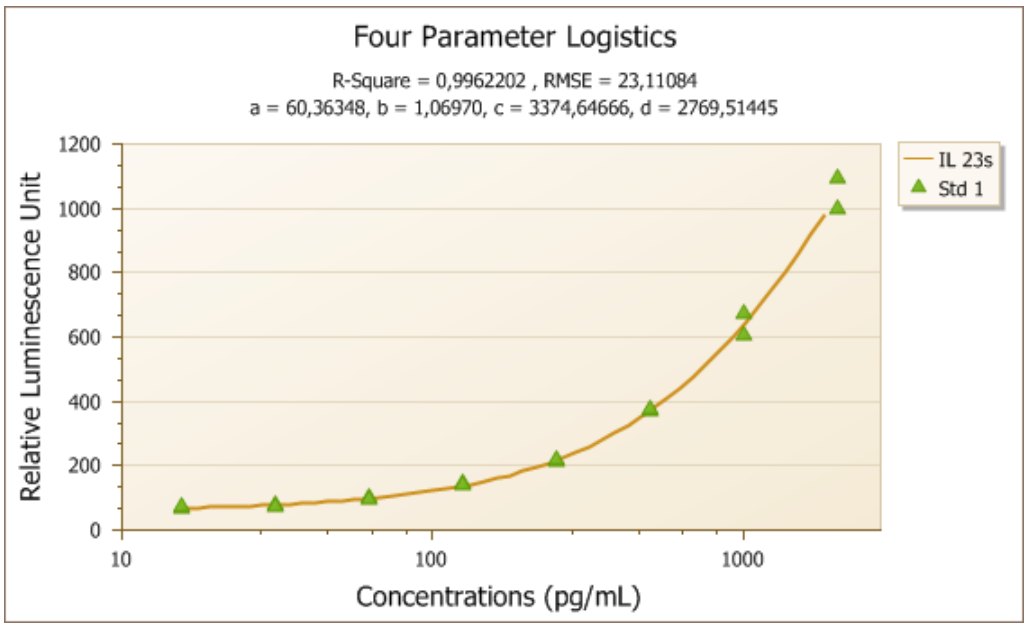
IL-17 soro



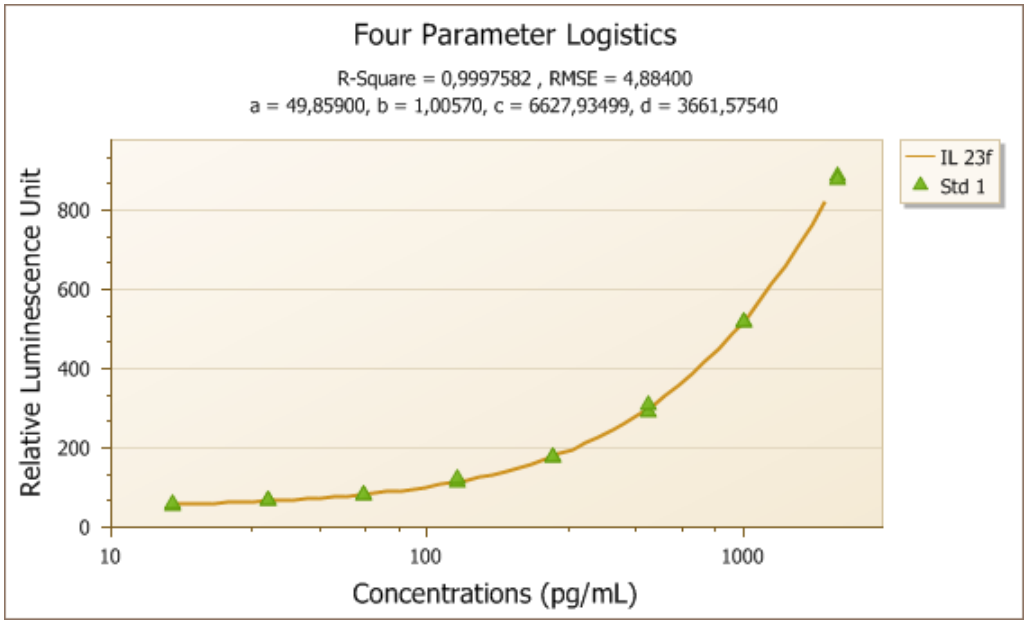
IL-17 fluido



IL-23 soro



IL-23 fluido



Tabelas do artigo

TABLE 1
Demographic characteristics (mean±SD, *t* test or qui-square utilized)

Parameter	Endometriosis group *N* = 40	Control group *N* = 40	*P* value
Age (years)	32.48±4.99	33.63±6.51	*NS*
BMI (kg/m²)	23.75±4.37	25.31±3.95	*NS*
Regular cycles *N* (%)	35 (87.5%)	32 (80%)	*NS*

TABLE 2
Comparison of interleukin levels in serum and peritoneal fluid between endometriosis (N=40) and control group (N=40)

Cytokine levels (pg/ml)	Endometriosis group[a]	Control group[a]	P value[b]
IL-10 serum	7.25 (2.30~185.60)	8.05 (1.4~44.2)	0.130
IL-10 peritoneal fluid	35.50 (12.29~365.42)	34.98 (19.01~227.46)	0.956
IL-12 serum	6.87 (4.40~12.10)	9.21 (2.40~34.40)	0.315
IL-12 peritoneal fluid	1.73 (0.30~12.38)	2.03 (0.57~47.74)	0.115
IL-17 serum	1.33 (0.10~29.80)	1.33 (0.10~8.00)	1.000
IL-17 peritoneal fluid	5.41 (1.00~161.10)	3.70 (1.00~6.30)	0.584
IL-23 serum	5.16 (1.4~17.4)	9.64 (1.40~45.30)	0.301
IL-23 peritoneal fluid	13.60 (2.20~25.00)	9.81 (0.30~30.70)	0.003

a Median (range)

b Non-parametric test (Mann-Withney)