

**The National Toxicology Program Interagency Center for the
Evaluation of Alternative Toxicological Methods (NICEATM)
Prescreen Evaluation on Five *In Vitro* Pyrogenicity Assays
(PBMC/IL-6; WB/IL-1; cryo WB/IL-1; WB/IL-6; MM6/IL-6)
Submitted for Evaluation to the Interagency Coordinating Committee
on the Validation of Alternative Methods (ICCVAM) by the European
Centre for the Validation of Alternative Methods (ECVAM)**

November 1, 2005

TABLE OF CONTENTS

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20

SUMMARY1

1.0 INTRODUCTION.....2

1.1 Test Method Validation Database2

2.0 NICEATM PRESCREEN EVALUATION OF THE FIVE *IN VITRO*

PYROGENICITY TEST METHODS4

2.1 Applicability to Current U.S. and European Union (EU) Regulatory

Testing Needs.....5

2.2 Applicability to Multiple Agencies or Programs.....8

2.3 Extent of Expected Use or Application and Impact on Human Health.....9

2.4 The Potential for the Proposed Test Method, Compared to Current

Accepted Test Methods, to Refine, Reduce, or Replace Animal Use9

2.5 The Potential for the Proposed Test Method to Provide Improved

Prediction of Adverse Health Effects, Compared to Current Accepted

Test Methods9

2.6 The Extent to Which the Test Method Provides Advantages (e.g.,

Reduced Cost and Time to Perform) Compared to Current Methods10

2.7 Conclusion10

3.0 KEY REFERENCES11

21 **SUMMARY**

22

23 In June 2005, Background Review Documents (BRDs) detailing five *in vitro* human
24 blood cell pyrogenicity tests were submitted by the European Centre for the Validation of
25 Alternative Methods (ECVAM) as replacement tests for the currently required tests (i.e.,
26 rabbit pyrogen test and the bacterial endotoxin test; BET). These test methods are similar
27 to each other in that they involve the measurement of cytokine levels from human blood
28 cells or a human monocytoid cell line. The validation database for each test method
29 consisted of the same 13 pyrogen-free, marketed, parenteral pharmaceuticals (10 for
30 accuracy evaluations and 3 for reliability evaluations), each spiked with multiple
31 concentrations of a bacterial endotoxin standard. Accuracy was determined by
32 comparison of the results generated using a prediction model to the “true status” of the
33 samples. The adequacy of each submission was evaluated based on 1) the extent to
34 which the submissions provide the information requested in the ICCVAM Guidelines for
35 the Nomination and Submission of New, Revised, and Alternative Test Methods (NIH
36 Pub. No. 03-4508); and 2) the extent to which the submissions address the Interagency
37 Coordinating Committee on the Validation of Alternative Methods (ICCVAM)
38 prioritization criteria. With the exception of specific monetary cost, the BRDs addressed
39 the ICCVAM prioritization criteria, and it appears that there are sufficient data to warrant
40 an independent evaluation of the relevance and reliability of each of the five *in vitro*
41 pyrogenicity test methods. However, minor deficiencies in the organization and content
42 of the BRDs and supporting information were noted that should be corrected prior to a
43 formal review by an expert peer review panel.

44

45 1.0 INTRODUCTION

46

47 In June 2005, the European Centre for the Validation of Alternative Methods (ECVAM)
48 submitted five *in vitro* human blood cell pyrogenicity tests to the National Toxicology
49 Program Interagency Center for the Evaluation of Alternative Toxicological Methods
50 (NICEATM) for consideration by the Interagency Coordinating Committee on the
51 Validation of Alternative Methods (ICCVAM) as replacement tests for the currently
52 required *in vivo* rabbit test or an *in vitro* test that requires the use of horseshoe crabs
53 (bacterial endotoxin test; BET). A list of key references is included in **Section 3.0**.

54

55 Although the same cells are used for more than one test method, each procedure is
56 considered to be a separate test method. However, these test methods are similar in that
57 each assay involves the measurement of cytokine levels from either human blood,
58 peripheral blood mononuclear cells (PBMC), or a human monocytoid cell line, as a
59 biomarker of a pyrogenic response. In each assay, cytokine levels are measured with an
60 enzyme-linked immunosorbent assay (ELISA). The five *in vitro* pyrogenicity test
61 methods are identified as follows:

- 62 • PBMC/IL-6 (The Human PBMC/IL-6 *In Vitro* Pyrogen Test)
- 63 • WB/IL-1 (The Human Whole Blood/IL-1 *In Vitro* Pyrogen Test)
- 64 • cryo WB/IL-1 (The Human Whole Blood/IL-1 *In Vitro* Pyrogen Test:
65 Application of cryopreserved human whole blood)
- 66 • WB/IL-6 (The Human Whole Blood/IL-6 *In Vitro* Pyrogen Test)
- 67 • MM6/IL6 (An Alternative *In Vitro* Pyrogen Test Using the Human
68 Monocytoid Cell Line MONO MAC-6 [MM6])

69

70 1.1 Test Method Validation Database

71

72 A total of 13 test substances were used in the validation study made up of currently
73 marketed parenteral drugs that have been determined to contain no detectable pyrogens.
74 The positive control was the 2nd International World Health Organization (WHO)
75 Standard for endotoxin (i.e., from *Escherichia coli* 0113:H10:K- [94/580]), 0.5 endotoxin

76 units [EU]/mL in clinical stock saline solution, while the negative control was 0.9%
 77 clinical stock saline solution. For the accuracy evaluation, 10 test substances (**Table 1**)
 78 were spiked with five spike solutions (0, 0.25, 0.5, 0.5, and 1.0 EU/mL) and tested once
 79 in three different laboratories. The spike solutions were made with the same endotoxin
 80 standard used in the positive control. Accuracy was determined by comparison of the
 81 results generated using the prediction model to the “true status” of the samples. The
 82 absorbance of each “unknown” sample was compared to that of an endotoxin standard
 83 curve. The samples were classified as either negative or positive based on the assigned
 84 pyrogen threshold value (0.5 EU/mL). For the reliability analysis, 3 test substances
 85 (**Table 2**) were spiked with four different spike solutions (0, 0, 0.5, 1.0 EU/mL) and
 86 tested 3 times in 3 different laboratories.

87

88 **Table 1¹. Substances used for evaluating test method accuracy.**

Drug Name	Source	Agent	Indication
Glucose 5% (w/v)	Eifel	Glucose	Nutrition
Ethanol 13% (w/w)	B. Braun	Ethanol	Diluent
MCP®	Hexal	Metoclopramid	Antiemetic
Orasthin®	Aventis	Oxytocin	Initiation of delivery
Binotal®	Aventis	Ampicillin	Antibiotic
Fenistril®	Novartis	Dimetindenmaleat	Anti-allergy
Sostril®	GlaxoSmithKline	Ranitidine	Anti-acidic
Beloc®	Astra Zeneca	Metoprolol tartrate	Heart dysfunction
Drug A ²	-	0.9% NaCl	-
Drug B ²	-	0.9% NaCl	-

89 ¹Table 1 modified from Table 3.3.1 of each BRD.90 ²The BRDs indicate that Drugs A and B were included as saline controls using “notional
 91 ELCs”

92

93 **Table 2¹. Substances used for evaluating test method reliability.**

Drug Name	Source	Agent	Indication
Gelafundin®	Braun melsungen	Gelatin	Transfusion
Jonosteril®	Fresenius	Electrolytes	Infusion
Haemate®	Aventis	Factor VIII	Hemophilia

94 ¹Table 2 modified from Table 3.3.2 of each BRD.

95

96 There are no direct comparisons of the proposed *in vitro* test methods to either the rabbit
 97 pyrogen test or the bacterial endotoxin test. Historical data from 171 rabbits tested with

98 endotoxin (0, 5, 10, 15, 20 EU/kg in 1 mL/kg) were obtained. The endotoxin was
99 obtained from 2 sources: 1) *E. coli* EC5; 2) *E. coli* EC6 (reportedly identical to the WHO
100 standard used in the validation studies). From these data, it was established that 50% of
101 the rabbits got fever within 180 minutes of injection with 5 EU/kg. Based on the largest
102 allowable volume for injection in rabbits (10 mL/kg), the limit of detection that
103 alternative pyrogen tests must meet was defined as 0.5 EU/mL. A “theoretical” measure
104 of performance of the rabbit pyrogen test was established for comparison to the *in vitro*
105 test methods. Taking into account the prevalence of the 5 spike solutions and calculating
106 the probabilities of misclassification using the defined threshold of pyrogenicity (i.e., 0.5
107 EU/mL), the theoretical sensitivity was calculated as 75%, and the theoretical specificity
108 was 96%.

109

110 **2.0 NICEATM PRESCREEN EVALUATION OF THE FIVE *IN VITRO*** 111 **PYROGENICITY TEST METHODS**

112

113 A Background Review Document (BRD) was submitted for each *in vitro* pyrogenicity
114 test method. The five individual BRDs were reviewed for completeness and to identify
115 aspects or omissions that could impede an expert peer review. The BRDs were not
116 reviewed with respect to data quality or presentation, or validation study conclusions.
117 Rather, the adequacy of each submission was evaluated based on the following criteria:

- 118 1) The extent to which the submissions provide the information requested in the
119 ICCVAM Guidelines for the Nomination and Submission of New, Revised, and
120 Alternative Test Methods (NIH Pub. No. 03-4508).
- 121 2) The extent to which the submissions address the following ICCVAM prioritization
122 criteria:
- 123 • The extent to which the proposed test methods are:
 - 124 ○ Applicable to regulatory testing needs
 - 125 ○ Applicable to multiple agencies/programs
 - 126 ○ Warranted, based on the extent of expected use or application and
127 impact on human, animal, or ecological health
 - 128 • The potential for the proposed test methods, compared to current test methods

- 129 accepted by regulatory agencies, to:
- 130 ○ Refine animal use (decrease or eliminate pain and distress)
 - 131 ○ Reduce animal use
 - 132 ○ Replace animal use
 - 133 • The potential for the proposed test methods to provide improved prediction of
134 adverse health or environmental effects, compared to current test methods
135 accepted by regulatory agencies
 - 136 • The extent to which the test methods provide other advantages (e.g., reduced
137 cost and time to perform) compared to current methods

138

139 Due to the similarities among the five test methods, much of the information contained in
140 each BRD relevant to the ICCVAM prioritization criteria is duplicative. For this reason,
141 unless otherwise indicated, the responses included below are relevant to all five test
142 methods.

143

144 **2.1 Applicability to Current U.S. and European Union (EU) Regulatory Testing** 145 **Needs**

146

147 There are current regulatory requirements to test pharmaceuticals and other products
148 (e.g., medical devices) for pyrogenicity (**Tables 3 and 4**). The pyrogenicity assays that
149 are currently acceptable to regulatory authorities require intact animals (rabbits) or an *in*
150 *vitro* test that requires the use of horseshoe crabs (BET). According to the BRDs,
151 “dependent on the product and the presence of relevant clinical data on unexpected
152 pyrogenicity of clinical lots, the proposed test method[s] may be an alternative method
153 for pyrogen testing, thus substituting [for] the rabbit pyrogen test or the BET. In certain
154 cases, the proposed test method may function as a supplementary test method to assess
155 compliance to the licensing dossier. In case the proposed test method [s] is an alternative
156 for pyrogenicity testing, a thorough cross-validation between the proposed test method
157 and the original method for the specific medicinal product is warranted. In case the
158 proposed test method[s] is an adjunctive test to screen for (unexpected) pyrogenic lots,

159 **Table 3. Regulations/Guidance Documents on the Requirements for Pyrogenicity**
 160 **Testing**
 161

Regulation/Guideline	Pyrogenicity Testing Requirements
21CFR610.13 – Purity. (April 1, 2005)	“Each lot of final containers of any product intended for use by injection shall be tested for pyrogenic substances by intravenous injection into rabbits as provided in paragraphs (b) (1) and (2) of this section: <i>Provided</i> , That notwithstanding any other provision of Subchapter F of this chapter, the test for pyrogenic substances is not required for the following products: Products containing formed blood elements; Cryoprecipitate; Plasma; Source Plasma; Normal Horse Serum; bacterial, viral, and rickettsial vaccines and antigens; toxoids; toxins; allergenic extracts; venoms; diagnostic substances and trivalent organic arsenicals.”
FDA - Guideline on Validation of the Limulus Amebocyte Lysate Test as an End-Product Endotoxin Test for Human and Animal Parenteral Drugs, Biological Products, and Medical Devices (December, 1987)	<p>“This guideline sets forth acceptable conditions for use of the Limulus Amebocyte Lysate test. It also describes procedures for using this methodology as an end-product endotoxin test for human injectable drugs (including biological products), animal injectable drugs, and medical devices. The procedures may be used in lieu of the rabbit pyrogen test.”</p> <p>“On the basis of extensive experience in review of LAL data on devices since November 1977, CDRH believes that the LAL test, when validated according to this guideline, is at least equivalent to the rabbit pyrogen test as an end-product test for medical devices. A manufacturer labeling a device as non-pyrogenic must validate the LAL test for that device in the test laboratory to be used for end-product testing before using the LAL test as an end-product endotoxin test for any device.”</p> <p>IV. Human and Animal Drugs and Biological Products “A batch which fails a validated LAL release test should not be retested by the rabbit test and released if it passes. Due to the high variability and lack of reproducibility of the rabbit test as an endotoxin assay procedure, we do not consider it an appropriate retest procedure for LAL failures.”</p> <p>V. Medical Devices “Manufacturers may retest LAL test failures with the LAL test or a USP rabbit pyrogen test. If the endotoxin level in a device eluate has been quantitated by LAL at 0.5 EU/mL endotoxin or greater, then retest in rabbits is not appropriate.”</p>
FDA - Guidance for Reviewers: Instructions and Template for Chemistry, Manufacturing, and Control (CMC) Reviewers of Human Somatic Cell Therapy Investigational New Drug Applications (INDs) (August 2003)	“Endotoxin testing using the Limulus Amebocyte Lysate (LAL) assay method is typically done as an alternative to pyrogenicity testing (see 21 CFR 610.13(b)) for early-phase trials. If the sponsor is using the LAL endotoxin method, you should inform the sponsor that, for licensure, the LAL endotoxin test must be shown, as explained in 21 CFR 610.9, to be equivalent to that of the pyrogenicity test described in 21 CFR 610.13(b).”
FDA - Guidance for Industry: Considerations for Plasmid DNA Vaccines for Infectious Disease Indications (February 2005)	“We recommend that you perform a test for pyrogenic substances and that you include the test results with the bulk release documentation. The Limulus Amebocyte Lysate (LAL) test is a sensitive indicator of the presence of bacterial endotoxins and endotoxin contamination should not exceed 5.0 EU/kg body weight for the intended recipients.”

Regulation/Guideline	Pyrogenicity Testing Requirements
<p>FDA - Guidance for FDA Review Staff and Sponsors: Content and Review of Chemistry, Manufacturing, and Control (CMC) Information for Human Gene Therapy Investigational New Drug Applications (INDs) (November 2004)</p>	<p>“Endotoxin testing using the Limulus Amebocyte Lysate (LAL) assay method is typically done to detect pyrogens (endotoxin) for products in early-phase clinical trials, and for marketed products. If you are using the LAL endotoxin method, the process for manufacture may also need to be evaluated for production of intrinsic pyrogenic substances other than endotoxin using the pyrogenicity test described in 21 CFR 610.13 (b).”</p>
<p>FDA - Guidance for Industry and/or for FDA Reviewers/Staff and/or Compliance: Preparation of a Premarket Notification Application for a Surgical Mesh (March 1999)</p>	<p>In accordance with the Blue Book Guidance G95-1 (“Use of International Standard ISO-10993, ‘Biological Evaluation of Medical Devices Part 1: Evaluation and Testing’”), acceptable test results should be supplied for ... pyrogenicity. If the [device] is to be labeled “pyrogen free” or “nonpyrogenic,” satisfactory results from the USP pyrogen test (rabbit) or an equivalent test, performed on the final end product, should be provided and lot release criterion for pyrogenicity need to be identified.</p>
<p>FDA- Center for Veterinary Medicine Program Policy and Procedures Manual (Guide 1240.4122, 4/25/00)</p>	<p>“The United States Pharmacopeia (USP) has recognized the Limulus Amebocyte Lysate (LAL) method as the official method for assaying drug products for lipopolysaccharides produced by gram negative microorganisms (bacterial endotoxins). The rabbit pyrogen test may be used only if a product is incompatible with the LAL test. The CVM endorses this position. However, during the development of a product and the manufacturing process validation (the first 3 commercial batches manufactured), the product should be assayed by both the LAL test and the rabbit pyrogen test. This is because there is the possibility of the presence of pyrogenic materials in the product that are not lipopolysaccharides. Testing the first 3 commercial batches would demonstrate if pyrogen contamination other than lipopolysaccharides is present in the final drug product. After the first 3 commercial lots, provided the rabbit pyrogen testing is negative, the LAL test should be utilized for release testing.”</p>
<p>USP XXII <1041> Biologics (1990)</p>	<p>“No lot of any licensed biological product is to be distributed by the manufacturer prior to the completion of the specified tests. Provisions generally applicable to biologic products include tests for potency, general safety, sterility, purity, water (residual moisture), pyrogens, identity, and constituent materials (see <i>Safety Tests-General</i> under <i>Biological Reactivity Tests, In vivo</i> <88>, <i>Sterility Tests</i> <71>, <i>Water Determination</i> <921>, and <i>Pyrogen Test</i> <151>, as well as <i>Bacterial Endotoxins Test</i> <85>).”</p>

163
164
165

Table 4. Personal Communications Regarding Regulatory Testing Requirements for Pyrogenicity

Agency/Center	Pyrogenicity Testing Requirements
FDA-CBER	The FDA acknowledges that the rabbit pyrogenicity test and Limulus bacterial endotoxin test (BET) do not measure the same thing. The BET is a test for endotoxin where as the rabbit test will detect any contaminant in a product that is pyrogenic. Generally the BET assay for endotoxin is adequate, but it really depends on the manufacturing process. If the FDA believes that the process introduces impurities/contaminates that have the potential to be pyrogenic or we are uncertain as to whether this will be the case we can then ask the sponsor to do testing according to CFR 610.13 for licensure. During product development (early phase IND) sponsors are asked to test for endotoxin, for which the BET is recommended
FDA-CDER	While the BET is currently accepted, it is not a full replacement for the <i>in vivo</i> rabbit pyrogen test. Rather it is used/accepted whenever considered appropriate. Although it is highly sensitive, the failure of the BET to detect non-endotoxin pyrogens as well as its susceptibility to interference (e.g., high protein levels of test substances) prevents it from being considered a full replacement.
FDA-CDRH	CDRH requires the rabbit test for all new materials before the device is cleared for marketing. However, once the device is approved, the BET can be used as routine test for the presence of endotoxins, which is required for all implants and devices contacting the blood and CSF.
European Commission – Joint Research Centre	There are still circumstances under which the rabbit pyrogenicity test would still be required (e.g., a product that interferes with the BET; when non-endotoxin pyrogens might contaminant the product). Examples of products that currently require the rabbit pyrogen test in the EU include parenteral preparations, Haemophilus B vaccine, Hepatitis B vaccine, Pneumococcal vaccines, rabies for human use, tick-borne encephalitis, human immunoglobulins, human albumin, blood products as coagulation factor VII, VIII, IX, XI, human plasma, and prothrombin.

166

167 alert and alarm limits may be established based on consistency of production lots or
168 (preferably) based on actual clinical data.”

169

170 **2.2 Applicability to Multiple Agencies or Programs**

171

172 These methods will reportedly be applicable to all agencies and programs that require
173 pyrogenicity testing of pharmaceuticals and other products. The U.S. Food and Drug
174 Administration (FDA) Center for Biologic Evaluation and Research (CBER), Center for
175 Drug Evaluation and Research (CDER), Center for Devices and Radiological Health
176 (CDRH), and Center for Veterinary Medicine (CVM) require that human injectable drugs
177 (including biological products), animal injectable drugs, and medical devices be tested
178 for the presence of pyrogenic substances.

179

180

181 **2.3 Extent of Expected Use or Application and Impact on Human Health**

182

183 As detailed in **Section 2.1**, under certain circumstances the proposed tests are intended to
184 replace tests that are used extensively in pharmaceutical development (i.e., *in vivo* rabbit
185 pyrogen test, BET). They are allegedly as good as, if not better than, current test methods
186 for identifying both endotoxin and non-endotoxin pyrogens (see **Section 2.5**). Therefore,
187 they may offer improved prediction of pyrogenicity and subsequently provide greater
188 protection of human health.

189

190 **2.4 The Potential for the Proposed Test Method, Compared to Current Accepted**
191 **Test Methods, to Refine, Reduce, or Replace Animal Use**

192

193 As stated in **Section 2.3**, the proposed test methods are intended to replace tests that are
194 used extensively in pharmaceutical development. The two most common pyrogen tests
195 presently used (i.e., *in vivo* rabbit pyrogen test, BET) require the use of animals. While
196 the BET is most often performed using blood drawn from *Limulus polyphemus* (the
197 horseshoe crab) which are subsequently returned to the wild, a portion of these animals
198 do not survive the procedure (which requires approximately 20% of the total blood
199 volume, according to the BRD). The proposed test methods will reduce and replace
200 animal use because they rely on human blood cells or a human monocytoid cell line that
201 can be isolated and cultured in the test laboratory.

202

203 **2.5 The Potential for the Proposed Test Method to Provide Improved Prediction**
204 **of Adverse Health Effects, Compared to Current Accepted Test Methods**

205

206 Sufficient data are presented to allow an assessment of the predictivity of the proposed
207 test methods. Because these test methods are conducted using cells of human origin, the
208 submitter contends that they will better reflect the human physiological response than
209 current methods (i.e., *in vivo* rabbit pyrogen test, BET), and thus more effectively predict
210 adverse effects in humans. It is not clear if they would also provide improved
211 predictivity of adverse effects in animals (i.e., when testing veterinary pharmaceuticals).

212

213 **2.6 The Extent to Which the Test Method Provides Advantages (e.g., Reduced** 214 **Cost and Time to Perform) Compared to Current Methods**

215

216 Specific cost requirements are not provided, and therefore a determination of relative
217 costs cannot be made. The BRD cites two factors in contributing to the cost of the
218 proposed test methods: reagent costs and labor costs. Because the proposed test methods
219 are reportedly more labor-intensive than current methods (i.e., *in vivo* rabbit pyrogen test,
220 BET), the costs are anticipated to be greater. However, the proposed methods do appear
221 to be adaptable to higher throughput, which could make them more cost effective.

222

223 The proposed test methods are estimated to require approximately two working days. On
224 day one, test materials are prepared and incubated with the relevant blood cells/cell line.
225 The immunoassay for the appropriate cytokine is conducted on day 2. In comparison,
226 both the BET and the rabbit pyrogen test can be completed in one day. However, prior to
227 a rabbit's first use in a pyrogen test, a sham test (i.e., includes all steps but the injection)
228 must be performed. In addition, positive results in the first three rabbits tested are to be
229 followed by testing in an additional five animals. Such circumstances could cause testing
230 to extend into a second workday.

231

232 **2.7 Conclusion**

233

234 With the exception of specific monetary cost, the BRDs addressed the ICCVAM
235 prioritization criteria, and it appears that there are sufficient data to warrant an
236 independent evaluation of the relevance and reliability of each of the five *in vitro*
237 pyrogenicity test methods. However, minor deficiencies in the organization and content
238 of the BRDs and supporting information were noted that should be corrected prior to a
239 formal review by an expert peer review panel.

240

241 **3.0 Key References**

242

243 Duff GW, Atkins E. 1982. The detection of endotoxin by *in vitro* production of
244 endogenous pyrogen: comparison with amebocyte lysate gelation. J Immunol Methods
245 52: 323-331.

246

247 Hansen, E.W. and Christensen, J.D. 1990. Comparison of cultured human mononuclear
248 cells, Limulus amebocyte lysate and rabbits in the detection of pyrogens. J Clin Pharm
249 Ther. 15: 425-433.

250

251 Hartung T, Aaberge I, Berthold S et al. 2001. Novel pyrogen tests based on the human
252 fever reaction. Altern Lab Anim 29: 99-123.

253

254 Hoffmann S, Pertbauer A, Schindler S, et al. 2005. International validation of novel
255 pyrogen tests based on human monocytoid cell lines. J Immunol Meth 298:161-173.

256

257 Jahnke M, Weigand, Sonntag H-G. 2000. Comparative testing for pyrogens in parenteral
258 drugs using the human whole blood pyrogen test, the rabbit *in vivo* pyrogen test and the
259 LAL test. European Journal of Parenteral Science 5(2):39-44.

260

261 Pool EJ, Johaar G, James S, Petersen I, Bouic P. 1999. Differentiation between endotoxin
262 and non-endotoxin pyrogens in human albumin solutions using an *ex vivo* whole blood
263 culture assay. J Immunoassay 20:79-89.

264

265 Spreitzer I, Fischer M, Hartzsch K, Luederitz-Pueschel U and Montag T. 2000.
266 Comparative Study of Rabbit Pyrogen Test and Human whole Blood Assay on Human
267 Serum Albumin. ALTEX 19. Suppl. 1:73-75