Product Testing & Release

PACT Workshop: Design & Operation
of GMP Cell Therapy Facilities

April 10th/11th, 2007

Molecular&Cellular Therapeutics
University of Minnesota

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Product Testing

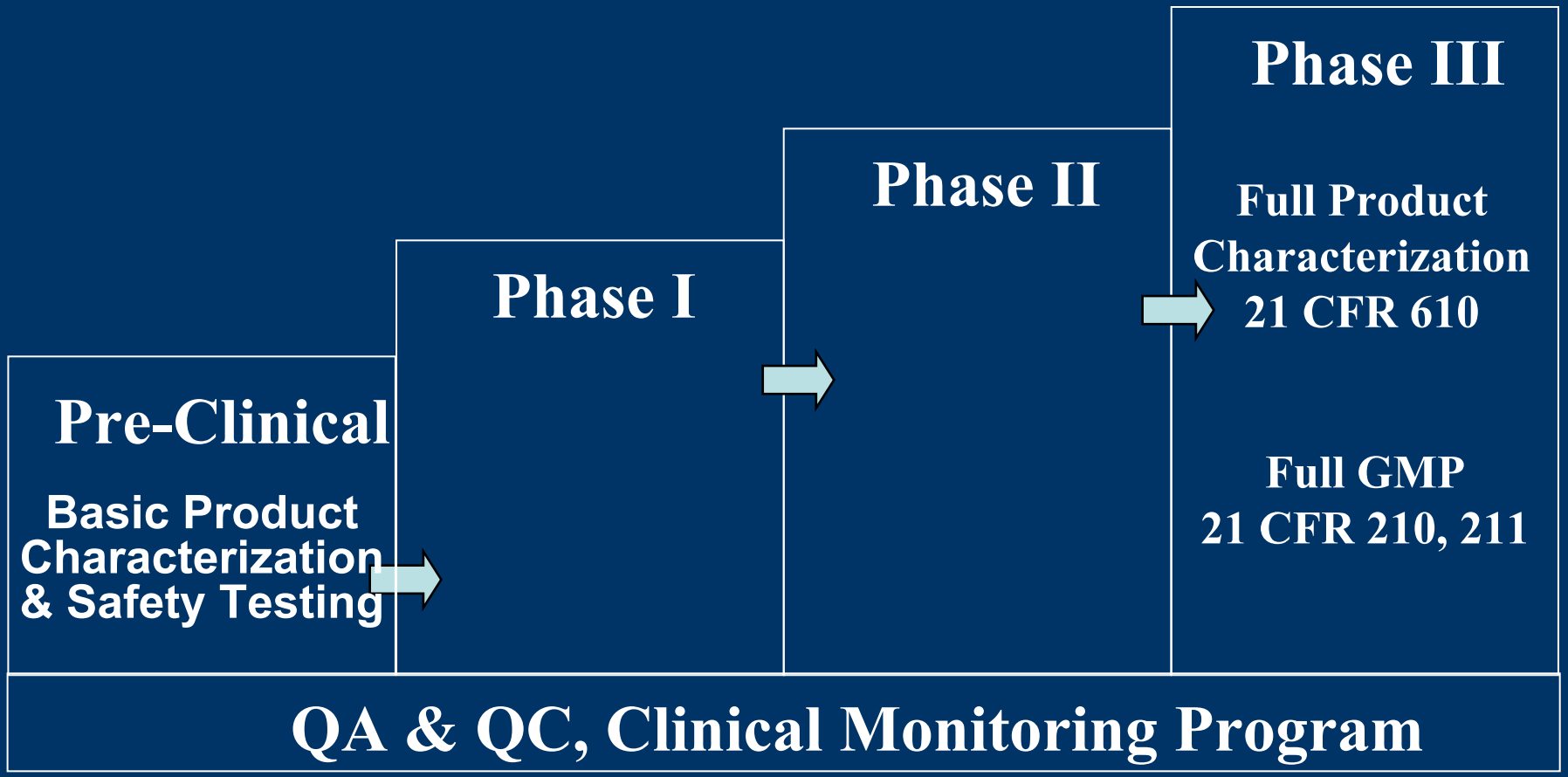
- Used to determine…
  - Safety, Purity, Identity, Potency, Quality
    - Suitability of the product for the individual
    - Adequacy of laboratory practices

Testing and Release for Distribution

21CFR 211.165

- For each batch there shall be determination of conformance to final specifications (identity and strength)
- Testing for objectionable microorganisms
- Sampling and testing plans
  - Include test method and number of units tested
  - Defined acceptance criteria
  - Accuracy, sensitivity, specificity & reproducibility established

Fulfillment of Regulatory Requirements…



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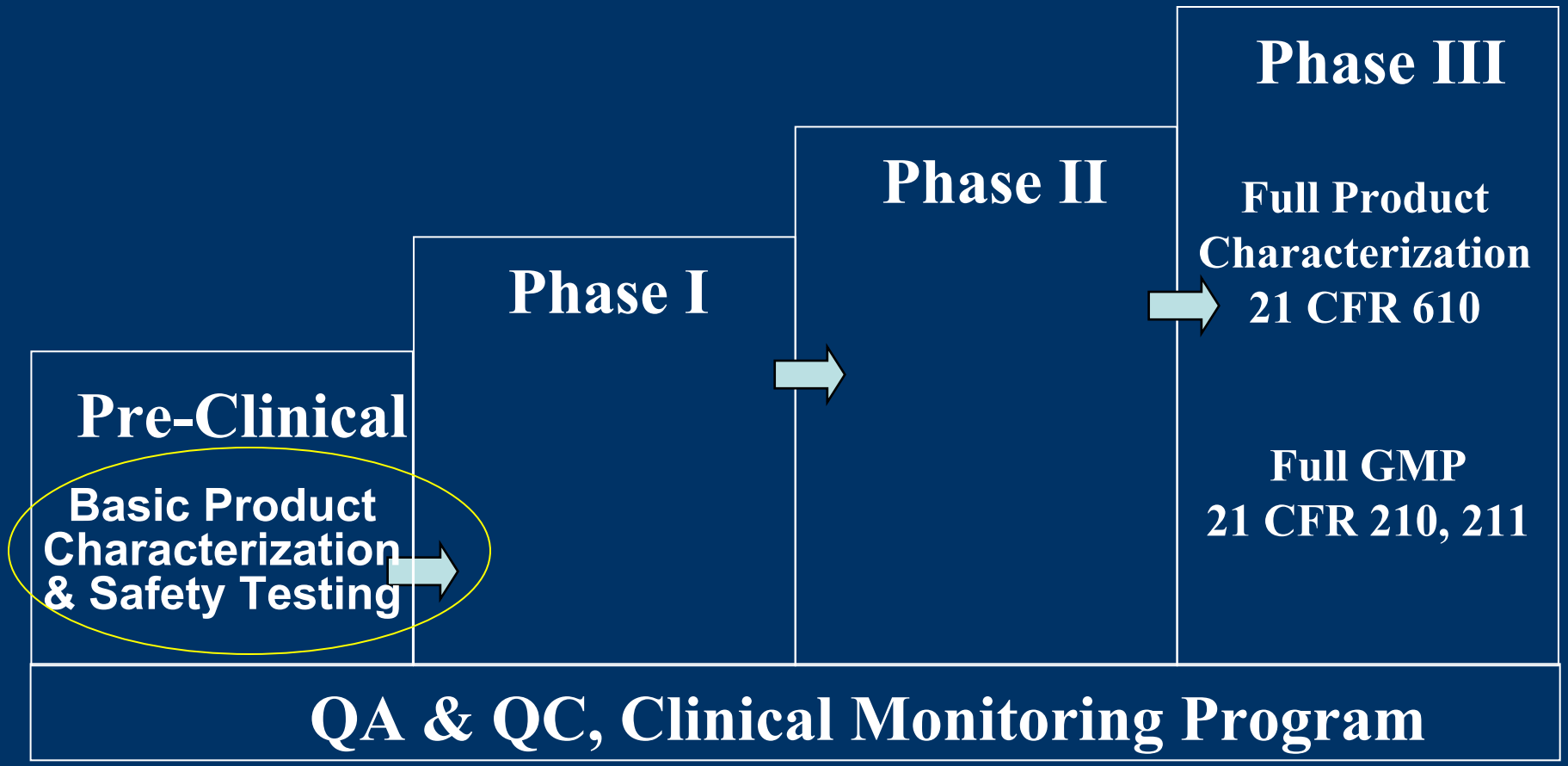

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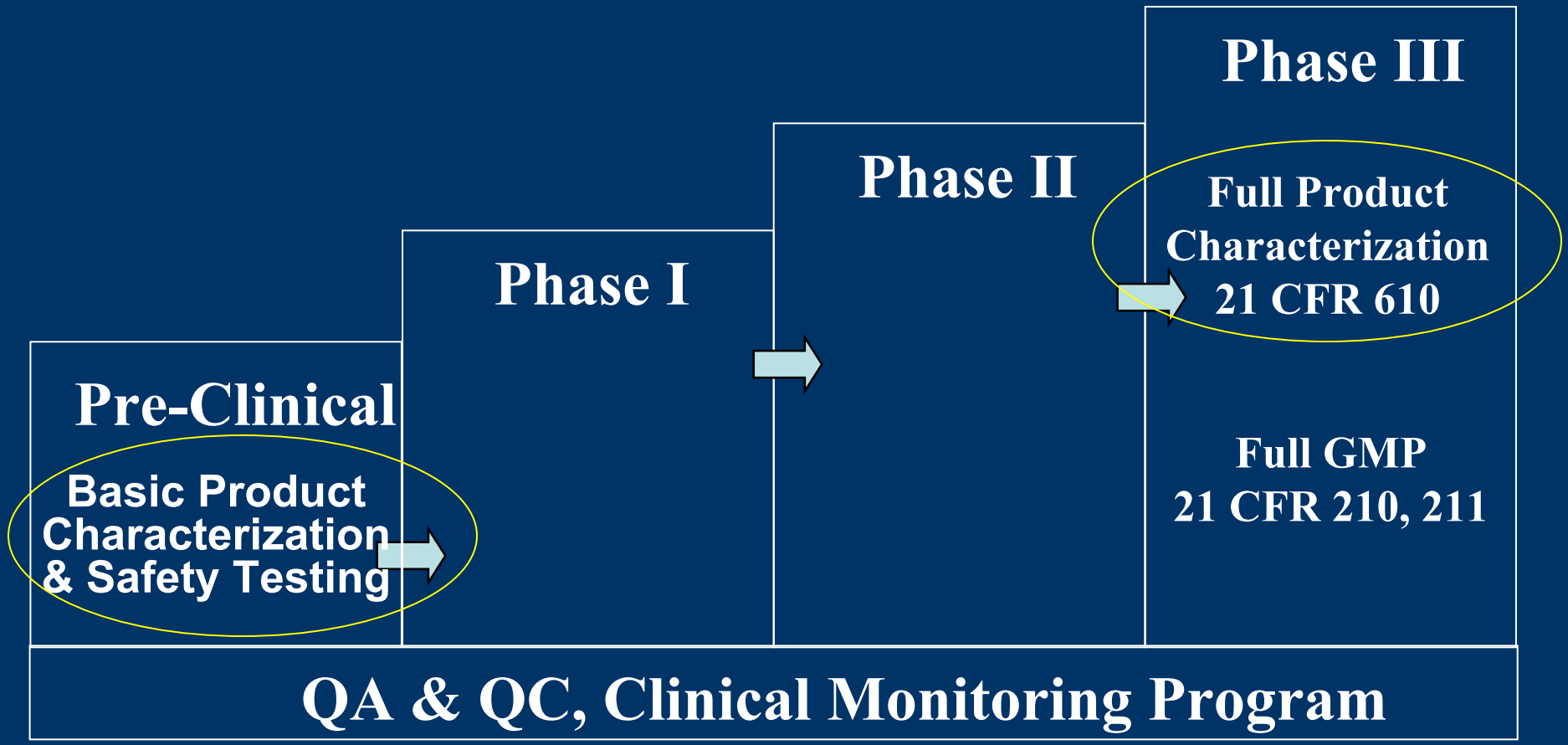

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Guidance for Industry

INDs – Approaches to Complying with CGMP during Phase I

"For known safety-related concerns, specifications should be established and met. For some product attributes, all relevant acceptance criteria may not be known at this stage of product development. This information will be reviewed in the IND submission."

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Part 610 – General Biological Products Standards

§610.1 Tests prior to release required for each lot.

"No lot of any licensed product shall be released by the manufacturer prior to the completion of tests for conformity with standards applicable to such product."

Product Testing	Example Method(s)
Microbiological Testing	
-Sterility (Bacterial & Fungal)	USP<71>, 21 CFR 610.12, Bactec*
-Gram Stain	Routine
-Mycoplasma	21 CFR 610.30, PCR-based*
-Adventitious Viral Agents	*In vitro* (indicator cell lines), *In vivo* (animals), PCR
Identity	HLA, flow cytometry, ABO/Rh, genetic polymorphisms
Purity	Endotoxin (e.g., LAL*), assays for residual extraneous material (e.g., cells, cytokines, antibodies)
Potency	Assays for biological function (in vitro, in vivo)
Other	
-Viability	Trypan blue, AO/PI, flow cytometry
-Cell Number/Dose	Cell counter, hematology analyzer
Stability	As noted above after short- and long-term storage

*May be used for early phase IND products; equivalency to 21 CFR +/or demonstration of adequate sensitivity/specificity performed by phase III/licensure.

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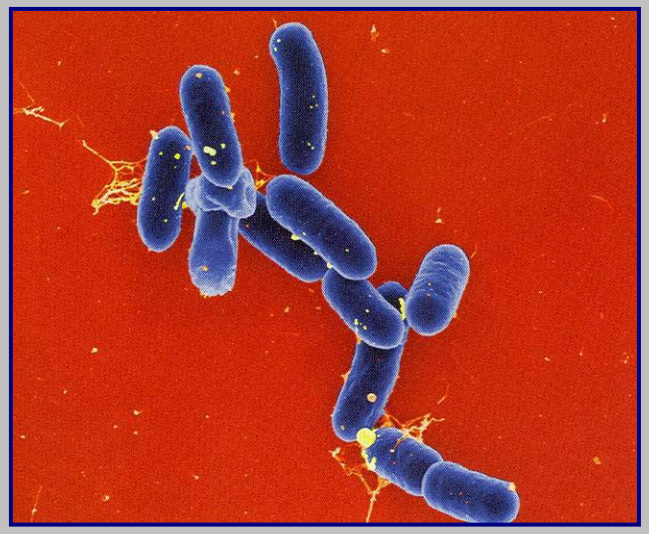
Microbiological Testing - 1

- Primary safety testing



<u>Sterility</u>:

  - Test time
  - In process and final product, as appropriate
  - 21CFR, USP, Bactec* (or other clinical lab method); 14 days (read @ 7)
  - *Equivalency to 21 CFR 610.12 required prior to licensure
  - Khuu H, et al.¶

¶Comparison of automated culture systems with a CFR/USP-compliant method for sterility testing of cell-therapy products. Cytotherapy (2004); 6 (3): 183-195.

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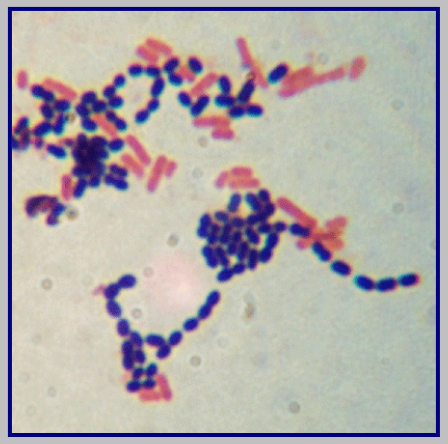
Microbiological Testing - 2

Gram Stain:

- Questionable sensitivity with cell therapy products
- Still expected by FDA as a rapid turn–around test



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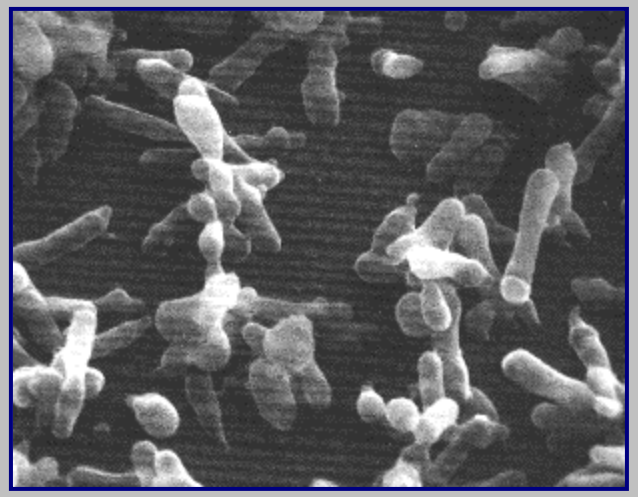
Microbiological Testing - 3

Mycoplasma:

- Source (cells and supernatant)
- Best timing (final product, in-process)
- Culture vs. PCR (prior to licensing, demonstrate adequate sensitivity and specificity)



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Microbiological Testing - 4

Adventitious Viral Agents:

- Variable depending on cell type/source and manipulation (gene transduction, etc.)
- Required for Master Cell Banks
  - In vitro against indicator cells
  - In vivo against live animals
  - Species specific (human CMV, HIV etc…by PCR

Identity - 1

- Identify (physical/chemical characteristics, inspection (macro/micro), specific cultural tests, in vitro/in vivo immunological tests (21 CFR 610.14)
  - HLA (serology/DNA)
  - Flow cytometry
  - ABO/Rh
  - Genetic polymorphisms

- Highly recommended Phase I

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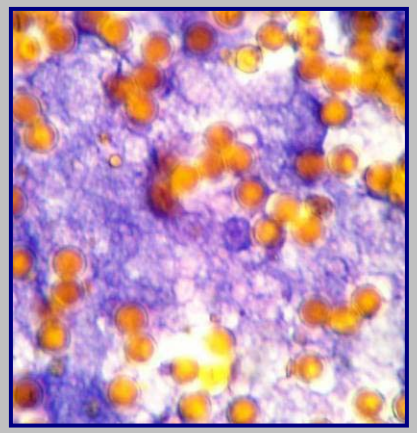
Identity - 2

- Examples:
  - Non-IND: Unrelated Cord Blood without attached segment -
    - Rapid HLA typing (serology)
  - IND: Natural Killer Cells (typically only one such product processed per day) -
    - Immunophenotype (flow cytometry)
    - ABO/RH, rapid HLA type (serology) as back-up

Purity - 1

- “Products shall be free of extraneous material except that which is unavoidable in the manufacturing process described in the approved biologics license application.” (21 CFR 610.13)
  - Includes testing for residuals and endotoxin
- Examples of residual contaminants include:
  - Contaminating cell phenotypes/debris
  - Ex. serum, antibiotics,cytokines



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Purity - 2

- Endotoxin (typically LAL method)
  - Equivalency to Pyrogenicity Testing [21 CFR 610.13(b)] for licensure
  - Upper limit per FDA 5 EU/kg body weight/dose (intra-thecal lower, 0.2 EU)
  - Gel, turbidimetric, kinetic chromogenic
  - PACT Project: EndoSafe®

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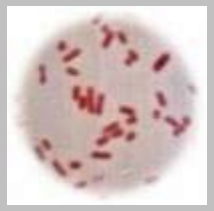
Endotoxin

- Extract derived from circulating amebocytes of horseshoe crab *Limulus polyphemus*
- Amebocyte + LPS (from g-bacteria et al) = clot
- Assays: gel-clot, turbidimetric, chromogenic,



+

Enzymatic Cascade
Serine Proteases

Ac-Ile-Glu-Ala-Arg⋯⋯pNA

Reaction time is inversely proportional to amount endo
Slower rxn time : ↓ Endotoxin
Faster rxn time : ↑ Endotoxin

Clot /
Yellow color
405nm

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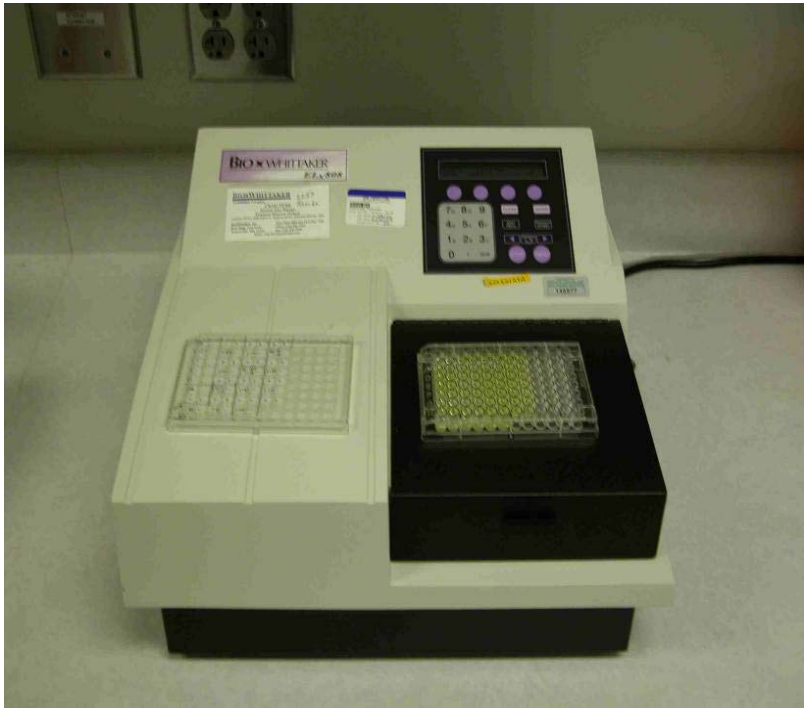
Commercial LAL Detection Assays

Charles River Laboratories
Endosafe®–Portable Test System

Cambrex Bio Science
Gel clot, Chromogenic, Kinetic
Turbidimetric/Chromogenic



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Potency - 1

- ***"The word potency is interpreted to mean the specific ability or the capacity of the product,*** as indicated by appropriate laboratory tests or by adequately controlled clinical data obtained through the administration of the product in the manner intended, ***to effect a given result."*** [21 CFR 600.3(s)]

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Viability

- Typically acceptable by FDA ≥ 70%
- If lower, provide rationale and support indicating lower viability will not affect safety or efficacy
- Examples:
  - Flow cytometry (e.g., 7-AAD)
  - AO/PI, Trypan Blue

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Cell Number/Dose

- Include minimum # of viable/functional cells
- Document if maximum dose established and how
- Examples:
  - Cell counter +/- flow cytometry
  - Viable cell counter

Stability

- Stability program required to determine storage conditions and expiration dates
- Is product stable for time period required to support study?
- Use previously mentioned testing to establish stability
- Examples:
  - Cryopreserved [pre-freeze vs. post-thaw (immediate and over time)]
  - Fresh [time of final product formulation vs. time of infusion (possibly several add'l time points)]

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Final Product Release Criteria Testing

- Include in CMC
- Final product formulation for patient administration
- Perform on each lot = each product in many (most) cases of cell therapy products
- Final test results available prior to release for administration
- Add'l results not available prior to release (e.g., sterility)
  - Include reporting procedure if post-release testing result not acceptable

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Lot Release- Allo NK Cells

TEST	TEST METHOD	SAMPLING POINT	TEST DATE	SPECIFICATION	RESULT	PASS/ FAIL
CD3+ Cell Dose Section VI, step 11.1 Accn # _____	Flow Cytometry SOP 5.10	**Pre-Incubation** with IL2		< 5 x $\mathbf{10^5}$ CD3+/kg		
Percent CD3-/NK+ Accn # _____	Flow Cytometry SOP 5.11	**Pre-Incubation** with IL2		≥ 20%		
Nucleated Cell Dose Section VI, step 4 or step 6	ACT Diff II MCT3-524	Final Product		≤ 3.0 x $\mathbf{10^7}$ NC/kg		
Viability Accn # _____	7AAD- Flow cytometry SOP 5.12	Final Product		≥ 70%		
Endotoxin (LAL) See attached report	LAL per FDA Guidance MCT3-589	Final Product		< 5 EU/kg		
Gram stain Accn # _____	Microbiology Laboratory SOP 8.25	Final Product		No organisms observed		

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Quality Assurance/Quality Control Unit

GMP 21CFR 211.22

- The Quality Control unit shall have the responsibility and authority for approving or rejecting all procedures or specifications impacting on the identity, strength, quality, and purity of the drug product.

Guidance for INDs – Approaches to Complying with CGMP during Phase I

- FDA recommends that QC function be performed by staff independent of production
  - If not possible, than 2nd person perform final document review

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References

- 21 CFR Part 610 – General Biological Products Standards
- Guidance for Industry INDs – Approaches to Complying with CGMP during Phase I [Jan. 2006]
- Guidance for Reviewers Instructions and Template for Chemistry, Manufacturing, and Control (CMC) Reviewers of Human Somatic Cell Therapy Investigational New Drug Applications (INDs) [Aug. 2003]

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Thank You!

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