

CLAIMS

1. A method of aseptic processing and production of cells in a nearly aseptic enclosure apparatus without biocides comprising
 - a. at least one chamber in an enclosure apparatus having a plurality of chambers providing a nearly aseptic environment adapted for optimal *ex vivo* cultivation, growth, processing or transport of prokaryotic or eukaryotic cells, wherein atmospheric gases, relative humidity (RH), temperature, and gas circulation can be precisely controlled;
 - b. adjusting the RH of the at least one chamber to 20% or less;
 - c. adjusting the temperature of the at least one chamber to 37°C or other elevated temperature not suboptimal for cells;
 - d. wherein a nearly continuously flowing atmosphere not suboptimal for cells mixes and homogenizes RH throughout the at least one chamber;
 - e. wherein the RH, temperature and flowing atmosphere within the at least one chamber elicits a physical antimicrobial effect by rapid desiccation of any microbes inside the at least one chamber, resulting in the death or immobilization by adhesion of all microbes on any surfaces in the at least one chamber, whereby microbial reproduction is prevented and no floating or airborne viable undesirable microbes can be detected;
 - f. wherein any higher RH in the at least one chamber required for the cultivation, growth, processing or transport of cells is controlled to the RH level no higher than necessary for the shortest duration necessary, and then immediately returning the RH to 20% or less;
 - g. wherein any lower temperature in the at least one chamber required for the cultivation, growth, processing, or transport of cells is controlled to the temperature no lower than necessary for the shortest duration necessary, and then immediately returning to the elevated temperature of about 37°C not suboptimal for cells;
 - h. wherein (a) upon initial closure and after each periodic opening and re-closure of the enclosure apparatus, all areas, atmosphere, and surfaces within the

enclosure are rapidly dried; or (b~~ii~~) any material or apparatus, when moved into the enclosure from the external environment, is rapidly dried.

2. The method of claim 1, wherein the relative humidity is controlled to 15% or less, or to 10% or less.
3. The method of claim 1, wherein the enclosure apparatus comprises two or more interconnected chambers, co-chambers, and sub-chambers, compartmentalized by internal doors between chambers, permanently connected or temporarily connected, monolithic or modular.
4. The method of claim ~~1~~3, wherein the RH, temperature, and flowing atmosphere is controlled for each chamber independently of any other chamber in the enclosure apparatus.
5. The method of claim 1, wherein the enclosure apparatus is at least one of the following:
 - (i) stationary or mobile; or
 - (ii) made of rigid or flexible walls, metal or plastic walls, or any combinations thereof; or
 - (iii) is fitted with functional or physical inputs/outputs through walls, either permanent or temporary, either sealed or semi-sealed in passageways not compromising control inside enclosure.
- ~~6. The method of claim 1, wherein the enclosure apparatus is made of rigid or flexible walls, metal or plastic walls, or any combinations thereof.~~
- ~~7~~6. The method of claim 1, wherein the interior surfaces are hydrophobic or hydrophilic.
- ~~8~~7. The method of claim 1, further comprising interior surfaces in the contained environment made from a material selected from polyethylene, polypropylene, stainless steel, and glass.
- ~~9. The method of claim 1, wherein the enclosure apparatus is fitted with functional or physical inputs/outputs through walls, either permanent or temporary, either sealed or semi-sealed in passageways not compromising control inside enclosure.~~
- ~~10~~8. The method of claim 1, wherein the atmospheric gases within the enclosure apparatus that can be precisely controlled comprise oxygen, nitrogen, carbon dioxide, nitric oxide,

carbon monoxide; and wherein volatile organic compounds (VOC's), particulates, and pressure can be precisely controlled.

~~119.~~ The method of claim 1, further comprising an atmosphere in the contained environment having an oxygen level at 0.1% to 35% v/v, and carbon dioxide at 0.1% to 20% v/v.

~~1210.~~ The method of claim 1, wherein cells are processed and produced inside the enclosure manually by gloves or telemanipulators through sealed ports or by integrated process and analytic machines or by full or partial automation.

~~1311.~~ The method of claim 1, wherein the cells are eukaryotic cells.

~~1412.~~ The method of claim ~~13-11,~~ wherein the eukaryotic cells are mammalian cells.

~~1513.~~ The method of claim ~~1412,~~ wherein the mammalian cells are human cells.

~~1614.~~ The method of claim 1, wherein no measurable CFU's (colony forming units) of desiccation resistant microbes are detected as measured by environmental monitoring with settle plates or active air sampling plates inside the enclosure.

~~1715.~~ The method of claim 1, wherein no measurable CFU's (colony forming units) of microbes susceptible to desiccation are detected as measured by environmental monitoring with settle plates, active air sampling plates, or contract plates.

16. The method of claim 1, wherein

the nearly continuously flowing atmosphere comprises a nearly continuously flowing atmosphere optimal for cells to mix and homogenize the RH throughout the at least one chamber;

in step (b), adjusting the RH of the at least one chamber to 20% or less comprises adjusting the RH of the at least one chamber to 20% or less with the nearly continuously flowing atmosphere optimal for cells to mix and homogenize the RH throughout the at least one chamber;

in step (c), adjusting the temperature of the at least one chamber to 37°C or other elevated temperature not suboptimal for cells comprises adjusting the temperature of the at least one chamber to 37°C or other elevated temperature optimal for cells;

the physical antimicrobial effect is to disinfect and clean the interior of the enclosure apparatus by rapid desiccation of any microbes floating or suspended in the atmosphere

in the interior of the at least one chamber or on the interior walls or other surfaces of the at least one chamber;

the elevated temperature of about 37°C is optimal for cells; and

the material or apparatus is rapidly dried, to create and maintain an aseptic or nearly aseptic environment optimal for the *ex vivo* cultivation of cells.

1817. An environment for aseptic processing and production of cells in non-sterile enclosure apparatus without biocides comprising
- a. at least one chamber in an enclosure apparatus having a plurality of chambers providing a nearly aseptic environment adapted for optimal *ex vivo* cultivation, growth, processing or transport of prokaryotic or eukaryotic cells, wherein atmospheric gases, relative humidity (RH), temperature, and gas circulation can be precisely controlled;
 - b. adjusting the RH of the at least one chamber to 20% or less;
 - c. adjusting the temperature of the at least one chamber to 37°C or other elevated temperature not suboptimal for cells;
 - d. wherein a nearly continuously flowing atmosphere not suboptimal for cells mixes and homogenizes RH throughout the at least one chamber;
 - e. wherein the RH, temperature and flowing atmosphere within the at least one chamber elicits a physical antimicrobial effect by rapid desiccation of all microbes inside, resulting in the death of most, immobilization by adhesion of all those on surfaces to that surface, and prevention of all microbial reproduction, such that no floating or airborne viable microbes can be detected;
 - f. wherein any higher RH in the at least one chamber required for the cultivation, growth, processing or transport of cells is controlled to the RH level no higher than necessary for the shortest duration necessary, and then immediately returning the RH to 20% or less;
 - g. wherein any lower temperature in the at least one chamber required for the cultivation, growth, processing, or transport of cells is controlled to the temperature no lower than necessary for the shortest duration necessary, and then immediately returning to the elevated temperature of about 37°C not suboptimal for cells;

- h. wherein (a_i) upon initial closure and after each periodic opening and re-closure of the enclosure apparatus, all areas, atmosphere, and surfaces within the enclosure are rapidly dried; or (b_{ii}) any material or apparatus, when moved into the enclosure from the external environment, is rapidly dried.
1918. The environment of claim ~~18~~17, wherein no measurable CFU's (colony forming units) of desiccation resistant microbes are detected as measured by environmental monitoring with settle plates or active air sampling plates inside the enclosure.
2019. The environment of claim ~~18~~17, wherein no measurable CFU's (colony forming units) of microbes susceptible to desiccation are detected as measured by environmental monitoring with settle plates, active air sampling plates, or contract plates.
20. The environment of claim 17, wherein
- the nearly continuously flowing atmosphere comprises a nearly continuously flowing atmosphere optimal for cells to mix and homogenize the RH throughout the at least one chamber;
- at (b), adjusting the RH of the at least one chamber to 20% or less comprises adjusting the RH of the at least one chamber to 20% or less with the nearly continuously flowing atmosphere optimal for cells to mix and homogenize the RH throughout the at least one chamber;
- at (c), adjusting the temperature of the at least one chamber to 37°C or other elevated temperature not suboptimal for cells comprises adjusting the temperature of the at least one chamber to 37°C or other elevated temperature optimal for cells;
- the physical antimicrobial effect is to disinfect and clean the interior of the enclosure apparatus by rapid desiccation of any microbes floating or suspended in the atmosphere in the interior of the at least one chamber or on the interior walls or other surfaces of the at least one chamber, resulting in the death or immobilization by adhesion of all microbes on any surfaces in the at least one chamber, whereby microbial reproduction is prevented and no floating or airborne viable undesirable microbes can be detected;
- the elevated temperature of about 37°C is optimal for cells; and
- the material or apparatus is rapidly dried, to provide and maintain an aseptic or nearly aseptic environment optimal for the ex vivo cultivation of cells.