4.1.3 Considerations to requests for changes to outlines of production for bacterial products

Changes to the manufacturing process of bacterial products can significantly affect the antigenic structure such that the protective antigens are hidden, reduced in number or absent. This is an important issue for inactivated products because they do not have the option of replicating in the host. In contrast, modified live bacterial products are less likely to be affected because they go through one or more replications in the host resulting in expression of target antigens. Bacteria unlike viruses do not express all or most of the possible genes under most *in vitro* growth conditions. Many of the genes are inducible by various signals and remain repressed until those signals are present. For example certain outer membrane proteins in *Mannheimia haemolytica* are induced under iron limiting conditions but not under iron replete conditions (1). There are many changes in manufacturing that may affect antigen expression. This guide will list many of these but be aware there may be other considerations.

Below are listed factors that may have an effect on the final product. These have been broken into modifications that affect antigen expression, recovery and modification of the expressed antigen(s) and formulation.

EXPRESSION

This section refers to the portion of the manufacturing process that may have an effect on the expression of the antigens of interest. Changes in the nutrients, atmosphere (3, 4), pH, *etc.* can affect a change in the presence or amount of an antigen. Sometimes expression may not change but availability changes (the antigen of interest is hidden by other structures). Regardless, proposals to change these conditions must be supported by evidence the antigen(s) of interest is/are expressed at levels and in a form similar to the original, licensed manufacturing process. This is a data driven process that must include analytical information comparing the current process to the proposed.

1) Medium (3)
    a) Change in source (manufacture)-all sources are not equivalent and need to be justified.
    b) Change in composition-changing the medium is a major change
        i) mammal to fish or plant
        ii) Complex to synthetic, chemically defined or vice versa
        iii) Ratio of components
2) Change in buffer composition-salts, trace minerals may significantly change expression conditions
    a) Vessel used for growth-Changes in the vessel can result in significant changes in antigen expression; regulated growth in a fermenter is different than static growth in a flask or carboy as is growth in liquid versus solid medium.
        i) Regulated fermenter vs. flask or carboy

ii) Large scale vs. small
iii) Liquid medium vs. solid or semi-solid
iv) Chemostat (steady state, continuous culture) vs. batch
v) Thin film vs. bulk

b) Agitation-this effects the gas exchange and therefore the type of metabolism (aerobic versus anaerobic) and therefore antigen expression (3, 4).
   i) Some vs. none
   ii) Little vs. a lot (low vs high shear)
   iii) Active sparging versus none
      (1) O_2
      (2) Air
      (3) Anaerobic e.g. w/N_2
      (4) CO_2
   iv) Monitoring and adjusting O_2 and CO_2 vs. not
c) pH
   i) regulated vs. not
   ii) change in pH
d) Iron limitation vs. iron replete (limitation of other nutrients may also be a factor)(1)
   i) Low iron medium
   ii) Iron chelators
e) Temperature of incubation (2)
f) Stage of growth (when harvested)
   i) Log phase
   ii) Stationary phase

RECOVERY AND MODIFICATION

This stage does not affect the expression of the antigen but instead the recovery or level of harvest, the level of other stuff that may be present in the harvested fluids and end up in the formulated product, and potential modifications to the antigen. The reviewer should evaluate the effect a change these processes have on the amount of antigen(s) present, the presence or absence of extraneous material (e.g. Endotoxin), and modification of the antigen(s) (e.g. proteolysis, denaturation),

1) Conditions
   a) Centrifugation vs. filtration
2) Washing vs. no washing
   a) Change in Molecular weight cut-off (MWCO) of membranes
   b) Buffer changes
3) Post-harvest processing
   i) Lysis
      (1) Mechanical

(a) French press
(b) Sonication
(c) Microfluidizer
(d) Combination
(2) Chemical
(3) Enzymatic

ii) Chromatography

4) Inactivation
   a) Agent change. Change from one inactivating agent to another is significant.
   b) Procedure
      i) Temperature
      ii) pH
      iii) Medium
      iv) Time
      v) Scale

FORMULATION

1) Change in adjuvant procedure
   a) Bulk or stepwise
   b) Order of addition
2) Change in adjuvant (this is a major change that requires the material be treated as a new product.)

How important are these changes? That depends on the organism and the composition of the product. Centrifugation vs. filtration may be very important if the important antigen is easily removed by washing and the filtration step includes vigorous washing (diafiltration) or of no importance if the antigen of interest is firmly bound to the cell. Some antigens require vigorous oxygenation for expression, others do not.

The burden of proof is on the firm to demonstrate the change has not altered the product. Various analytical tools such as PAGE, Western Blotting, HPLC, IEF, protein sequencing etc. may be of use. The change may require licensing the product as a new product. Simple serological tests are probably of no value unless they measure a functionally relevant parameter such as neutralization, agglutination or other related to the protective mechanism.

Potency tests – For inactivated bacterial products tested by an in vitro test, production changes could alter the relationship between the reference bacterin and current serials of product.

References

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