Plants are gaining momentum as an alternative for use in biopharmaceutical manufacture of therapeutic proteins. Some of the more notable species are tobacco, carrot, and chia seed. Tobacco plants (Nicotiana benthamiana) are able to produce large quantities of human proteins rapidly and economically in a process commonly referred to as Plant Molecular Farming (PMF). PMF has grown and advanced considerably over the past two decades, with at least 13 companies currently using PMF platforms as well as nine drugs that are presently in National Institutes of Health clinical trials.

As in more commonly used manufacturing host systems, recombinant proteins and antibodies produced in PMF platforms also require monitoring of host cell protein (HCP) contaminants using immunoassays and other orthogonal methods. HCP antibody reagent generation specific for plant systems is not yet well established as its mammalian or E. coli counterparts. As a HCP antibody generation host species, plants provide a unique opportunity to generate broad coverage HCP specific antibodies. This is due to the lack of homology between host and common host-cell models (1).

Here we present the successful use of a chicken host species for the generation of a process-specific HCP reagent antibody to the PMF system. A chicken-in-process sample was used to immunize chicken host species for the generation of antisera and purified IgY antibody reagent. Data are presented that show rapid development of a broad coverage polyclonal antibody reagent in the chicken. The polyclonal antibody was screened by immunoblot and ELISA, with overall coverage being assessed using 2D western blot. This study supports a strong alternative species to rabbit for the generation of HCP reagents. Further effort to produce a tobacco-process specific immunoassay is underway.

The use of recombinant therapeutic proteins is an increasingly common approach in the treatment of a wide range of human diseases. Biopharmaceuticals employ host organisms such as bacteria, yeast, plants, insects and mammalian cells as the manufacturing platform to produce these recombinant therapeutic proteins (1-4). In the current study, tobacco plant (Nicotiana benthamiana) was used for the expression of recombinant vaccine proteins (3,4).

Endogenous host cell proteins (HCPs) co-expressed with the therapeutic product during production may constitute a safety issue and must be closely monitored and adequately removed in order to prevent potential adverse effects in patients.

Several analytical methods for detecting and monitoring HCPs in process and in the final product are available. Well-established immunoassays that use process or platform specific polyclonal antibody reagents as well as mass spectrometry are some of the methods employed for HCP detection. HCP immunoassays are more cost effective, but rely on the ability of anti-HCP antibodies to successfully detect a broad range of HCP impurities with a high-level of sensitivity. Thus, the performance of the assay is tightly linked to the quality of the antibody reagent used. Historically, rabbits (most common) and goats (less common) have been used as the host organism for the generation of broad coverage polyclonal antibodies. In the current anti-HCP antibody generation study we focus on the generation of an anti-tobacco HCP antibody reagent. Prior to the present chicken-based study, a rabbit antibody generation system was attempted (data are not reported here). These initial attempts to generate tobacco-specific antibodies using rabbit models failed to generate antibodies with sufficient specific activity in the final qualification. Chicken hosts are becoming increasingly popular as a model for large-scale polyclonal antibody generation. There are several distinct advantages to using rabbits over rabbits, or other host animals, for the generation of polyclonal antibodies. Chicken IgY is functionally similar to IgG and therefore can be used in already established immunoassays protocols. The phynogamous difference of rabbits also allows for a more rapid and general coverage from the immune response to mammalian and other eukaryotic cell lines such as plant. IgY is naturally highly concentrated in the egg yolk of immunized chickens, which can result in extremely high yields of purified antibodies. As IgY is purified from harvested eggs instead of animal sera, the antibody generation process is also more humane and economical.

In this work chickens are immunized following a similar strategy to those currently used with mammalian hosts. In the current anti-HCP antibody generation study we focus on the generation of an anti-tobacco HCP antibody reagent. Prior to the present chicken-based study, a rabbit antibody generation system was attempted (data are not reported here). These initial attempts to generate tobacco-specific antibodies using rabbit

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