product (mcg/mL) levels limit the analytical tool kits for vaccines and can challenge the limit of detection increasing the challenge in establishing the necessary stability profile required to achieve the QTPP requirements. To emphasize the challenges with analytical development, two different examples are presented below.

The first example where characterization of the degradation mechanisms is truly challenging is associated with live-attenuated vaccines. These vaccines usually consist of crude cellular extracts that have multiple proteins and components. This can often lead to significant matrix effects in the characterization and stability assays. Furthermore, in live-attenuated combination vaccines (e.g., ProQuad®), stability assays must be developed carefully to ensure there is no interference across vaccine components [13]. In many cases, it may be necessary to inactivate with anti-sera to get a true measure of one virus' stability profile [13].

Another example where analytics can be challenged is in the case of Prevnar®13. Here, there are 13 different serotype-specific conjugates combined into the vaccine. The dose level associated with 12 of the serotypes is at 2.2 mcg while the 13th is 4.4 mcg [29]. Having the analytics to tease each serotype stability independent of the others is difficult and ensuring that the sensitivity within the assays is met places a significant burden on the analytical method. Additionally, with the low-dose levels, there can be a potential for surface adsorption of the antigens during routine testing. As a result, the analytical scientist may need to add surfactants into their assay buffers to mitigate the loss of antigen in the actual assays. This could impact the assays that can be utilized for determining stability indication and need to be carefully considered.

Each stage of the formulation development is further described in greater detail below. This is usually developed in a staged approach and may include: preformulation, early-stage formulation development, drug product optimization, establishment of design space, and late-stage formulation development. For clarity and alignment with this book regarding lyophilization development, the discussion around vaccine development and the use of lyophilization have been specifically mentioned in a stand-alone format. Additionally, guidelines to achieve these stages and relevant case-studies are presented below.

Stage 1

Preformulation

One of the main goals during preformulation development is for the formulation scientist to initiate understanding and characterization of the respective antigen [19, 22–24]. Preformulation allows the formulation scientist an ability to overcome any inherent physical or chemical instabilities associated with the respective antigen and improve the antigen prior to moving into full preclinical development. At this stage of development, the availability of robust stability-indicating assays are lim-