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# Cell substrates for the production of viral vaccines

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ABSTRACT

Vaccines have been used for centuries to protect people and animals against infectious diseases. For vaccine production, it has become evident that cell culture technology can be considered as a key milestone and has been the result of decades of progress. The development and implementation of cell substrates have permitted massive and safe production of viral vaccines. The demand in new vaccines against emerging viral diseases, the increasing vaccine production volumes, and the stringent safety rules for manufacturing have made cell substrates mandatory viral vaccine producer factories. In this review, we focus on cell substrates for the production of vaccines against human viral diseases. Depending on the nature of the vaccine, choice of the cell substrate is critical. Each manufacturer intending to develop a new vaccine candidate should assess several cell substrates during the early development phase in order to select the most convenient for the application. First, as vaccine safety is quite naturally a central concern of Regulatory Agencies, the cell substrate has to answer the regulatory rules stringency. In addition, the cell substrate has to be competitive in terms of viral-specific production yields and manufacturing costs. No cell substrate, even the so-called "designer" cell lines, is able to fulfil all the requested criteria for all viral vaccines. Therefore, the availability of a variety of cell substrates for vaccine production is essential because it improves the chance to successfully respond to the current and future needs of vaccines linked to new emerging or re-emerging infectious diseases (e.g. pandemic flu, Ebola, and Chikungunya outbreaks).



## 1. History of cell substrates in vaccine development

Q2 The lifesaving potential of vaccines, for humans and animals, has been largely proven by the comparison of the infectious disease burden before and after the introduction of national vaccination programs [1,2]. Over time, there have been significant advances in vaccine manufacturing technologies, leading to achievement of greater productivity as well as production of safer and more immunogenic vaccines.

This review will focus on cell substrates used for the manufacturing of viral vaccines based on replication–competent viruses (e.g. live attenuated, recombinant, chimeric, and inactivated vaccines) intended for human use. Cell substrates have been the result of active research for decades and have been implemented to improve the product safety, answer the increasing demand of vaccine production and decrease the associated manufacturing costs.

### 1.1. Evolution of vaccine production techniques

First vaccines were derived from sick people and infected animals. Jenner's first inoculation was made by injecting an 8-year old boy with the pus from a cowpox lesion on a milkmaid's hand [3]. From that point, and through the early 20th century, vaccines were mostly produced by employing animal tissues, such as nervous tissues extracted from rabbit, sheep or goat, suckling animal brain (mouse, rat, or rabbit), or using blood serum of infected animals.

*Abbreviations:* BSE, bovine spongiform encephalopathy; CAP, CEVEC'samniocyte production; CEF, chicken embryo fibroblast; DNA, deoxyribonucleic acid; EP, European Pharmacopeia; FBS, fetal bovine serum; GCCP, good cell culture practice; GMP, good manufacturing practices; HCD, host cell DNA; HEK, human embryonic kidney; MCB, master cell bank; MDCK, Madin Darby canine kidney; MRC, Medical Research Council; NRA, national regulatory authority; PERT, product-enhanced reverse transcriptase; RA, regulatory agency; RMK, rhesus monkey kidney; SCID, severe combined immunodeficiency; SV40, simian virus 40; TEM, transmission electron microscopy; $TPD_{50}$, tumour-producing dose in 50 per cent of animals; TSE, transmissible spongiform encephalopathy; WCB, working cell bank; WHO, World Health Organisation; WI, Wistar Institute.

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Since the 1930s, methods for growing viruses in the laboratory using embryonated hen eggs have been developed and used to produce and manufacture human and veterinary vaccines [4]. Eggs are still largely used, particularly in the seasonal flu vaccine manufacturing [5]. However, they pose a number of limitations including risk of insufficient supply, time-consuming processes with inconsistent yields, high costs of manufacture, and the potential for allergic responses to egg-components [6–8].

To overcome egg limitations, cell culture technology has been introduced, offering higher flexibility than the traditional manufacturing procedures. The first important step in the vaccine field was the production of polio vaccine using monkey kidney cells by Jonas Salk in 1954 [9,10]. Since then, the use of primary cell substrates, such as primary chicken embryo fibroblasts (CEFs), has greatly facilitated vaccine manufacturing (e.g. measles, and mumps) [11]. Primary cells were derived directly from an animal source and were not stored—or to a limited extent—as cell banks. However, their use raised concerns due to their limited self-renewal capacity and to the risk of contamination of primary cultures, as cells needed to be freshly prepared for each vaccine production lot.

The increasing demands in vaccine production yields and safety have urged the development of safer, cheaper, and more efficient cell substrates. The first cell substrates developed to this aim included both diploid and continuous cell lines (Table 1) [12]. Diploid cell lines, such as human lung-derived MRC5 (Medical Research Council 5) and WI-38 (Wistar Institute) cells [13,14], were obtained from primary cultures. They have a normal or near normal karyotype but show a finite capacity for serial propagation, which ends in senescence and cease of replication. Conversely, continuous cell lines, such as MDCK (Madin Darby canine kidney) cells [15,16] and African green monkey kidney-derived Vero cells [17], display infinite self-renewal capability and can be readily available for production from cell bank systems, allowing extensive characterization and reproducibility of the cell populations for an indefinite period. Thanks to their indefinite lifespan, continuous cell lines can be adapted to modern culture technologies (e.g. culture vessels, large-scale fermenters, micro/macro-carriers, and media). However, depending on the passage number, genetic modifications may occur and lead to a tumorigenic phenotype of the cell substrate. For example, Vero cell line at high passage levels (passage 162) displays genetic instability and develops tumorigenic potential [18]. Thus, only low-passage non-tumorigenic Vero cells can be used for vaccine production, which might raise concerns as the seeds stocks are currently decreasing due to the extensive use of this cell substrate.

Such limitations, combined with the appearance of new technologies for cell line development, have encouraged the establishment of new cell lines, including the so-called "designer" cell lines [19,20]. Several ways have been explored to obtain the currently available new cell substrates, including selection pressure (e.g. suspension MDCK cell line; duck embryonic stem cell-derived EB66® cells [21]) or genetic modification (e.g. human retina-derived PER.C6® cells [22,23], duck retina-derived AGE1.CR® cells [24], and human amniocyte-derived CAP® cells [25]). These cell substrates have been developed for specific applications, such as adenovirus production in gene therapy (PER.C6® cell line) or influenza vaccine production [26] and have been extensively characterized to fulfil regulatory and biosafety requirements. The long and cost-effective derivation process, as well as the high market pressure, hinders the development and the characterization of a new cell substrate for each vaccine indication. Therefore, currently available cell lines are considered as an option to produce new vaccine candidates and are tested for the replication of viruses not efficiently produced by old cell substrates. Table 1 summarizes the most common cell substrates that are currently used in the vaccine field. Their main biological characteristics and properties are displayed in Tables 2 and 3.

### 1.2. Criteria of selection of cell substrate for the manufacturing of viral vaccines

The selection of a cell substrate is an important step for the development and manufacturing of a viral vaccine candidate that relies on several parameters (e.g. cell susceptibility and permissiveness to the viral pathogen, performance in terms of viral antigens quality and production yield, primary versus continuous cells, ethical point of view, tumorigenicity status, anchorage-dependent versus suspension culture, culture medium, manufacturing cost, free of adventitious agents, etc.). The format of the vaccines (e.g. inactivated versus live-attenuated viral vaccines; administration routes; preventive or therapeutic vaccines) has also to be taken into account for the cell substrate selection. Finally, safety and industrial considerations deeply impact the choice of the suitable/optimal cell substrate. They are further detailed in Parts 2 and 3 of this review.

## 2. Regulatory considerations

### 2.1. Evolution of regulatory requirements for vaccine safety

Since the first-generation vaccines produced by employing animal tissues, the main concerns of regulatory agencies (RAs), manufacturers and public health authorities are the possible presence of adventitious agents or cell components, such as deoxyribonucleic acid (DNA) or transforming protein in vaccine products. Indeed, several significant cases of contamination have been evidenced during the last century [27], such as the discovery of simian virus 40 (SV40) in monkey kidney cells (rhesus monkey kidney [RMK] cells) used to produce polio vaccines in the 1960s [28,29], bacterial viruses identified in several live-attenuated viral vaccines manufactured with bovine sera containing bacteriophages, in the early 1970s [30,31] and more recently, the detection of porcine circovirus sequences in rotavirus vaccines (Rotarix® and RotaTeq®) [32].

The advance in science and technology and the use of more powerful analytical methods able to evidence undetectable or previously unknown contaminants has led the RAs to implement new manufacturing and controlling practices edited as guidelines. The basic principle underlying the guidelines is that quality, safety, potency, purity, and efficacy of the vaccine rely on a comprehensive approach based on the risk assessment that impacts the selection and characterization of raw materials and starting materials, the control of intermediate and final product but also the design and validation of the manufacturing process. Specific guidelines are in place and periodically revised to provide manufacturers advices on the selection, characterization, and maintenance of cell substrates used for vaccine production [33–38]. In particular, guidelines have evolved to take into consideration issues related to new cell substrates, including "designer" cell lines, which often exhibit a tumorigenic phenotype [20]. An interesting example is provided by the duck EB66® cell line. As per current version of chapter 5.2.3 of the European Pharmacopeia (EP), the preparation of live vaccines in tumorigenic cell line is prohibited [38]. However, the use of EB66® has been now considered as suitable for the manufacturing of such vaccines according to an anticipated change of the EP that will be harmonized with the World Health Organisation (WHO) recommendations published in 2013 [Personal communication].

### 2.2. Characterization of cell substrates used for the manufacturing of viral vaccines

Regardless of the cell type (e.g. primary, diploid, stem, or continuous cells) and the type of vaccines to be manufactured, the cell



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