MDCK as a substrate for influenza vaccine production

Solvay Pharmaceuticals
Presentation to VRBPAC
November 16, 2005

Agenda

- Background Solvay and influenza
- Background Solvay's MDCK project
- Safety analysis of MDCK-based vaccine
- Conclusions

Solvay Pharmaceuticals

- Division of Solvay Group
- Global – top 40 pharma
- 12,800 employees
- 2004 sales: $2.8 billion
- Major R&D sites
  - Marietta, GA
  - Dijon and Paris, France
  - Hanover, Germany
  - Weesp, The Netherlands

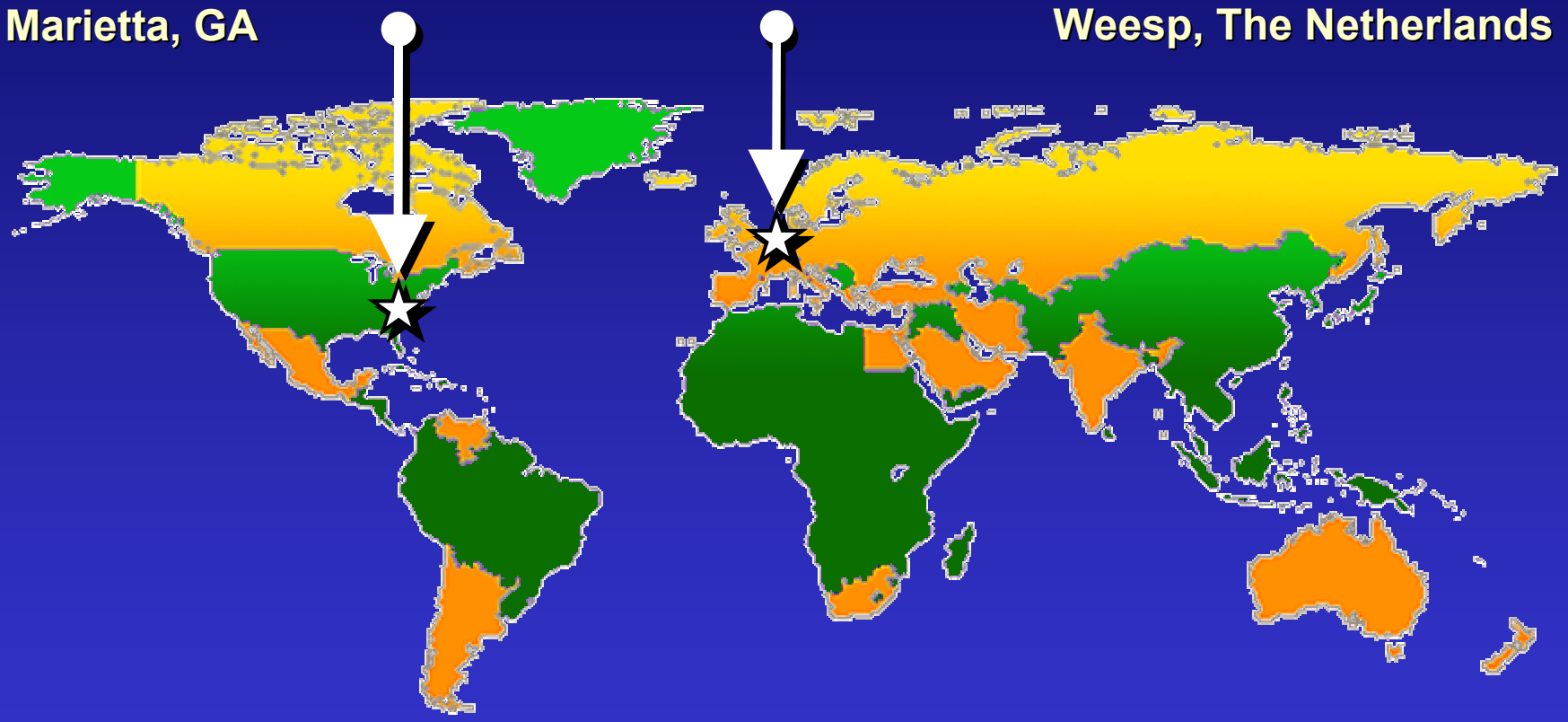
Solvay Pharmaceuticals & Influenza

- Egg-based vaccine
  - First influenza vaccine in Europe (1950)
  - Uninterrupted supply for 55 years
  - Total > 250 million doses
  - Fourth supplier worldwide; over 50 countries

Solvay Pharmaceuticals & Influenza

US Headquarters
Marietta, GA

Influenza vaccine production facilities –
Weesp, The Netherlands



Countries supplied with Solvay's egg-based influenza vaccine

Why a cell-based vaccine project?

- Eggs are open production system prone to contamination
- Availability of eggs is vulnerable to avian diseases

Why MDCK?

- Broad susceptibility to influenza viruses
- Substantial experience in influenza research and surveillance
- High virus yields
  - Economically feasible
  - Favorable ratio of virus to impurities

Solvay Pharmaceuticals' MDCK-based influenza vaccine project

- More than 10 years experience
- Microcarrier, serum-free
- Preclinical and clinical development program
- License granted in The Netherlands
- Commercial scale facility
- License to be updated to commercial scale product, followed by worldwide licensing, including US

Solvay Pharmaceuticals' MDCK-based vaccine facility

Solvay Pharmaceuticals, Weesp, The Netherlands

MDCK safety assessment

- Identify and quantify potential risks of cell line
- Quantify elimination of these potential risks by downstream processing (DSP)
- Quantify potential risks of cell substrate remaining for the vaccine recipient

CBER's Defined-Risks Approach

Lewis AM Jr, Krause P, Peden K. Dev Biol. 2001, vol 106, pp 513-535

MDCK safety assessment

1. Cell line characterization
2. Downstream processing
3. Final product safety

MDCK safety assessment

1. Cell line characterization
   a. Passage history
   b. Adventitious agents
   c. Tumorigenicity

1a. Cell characterization - History

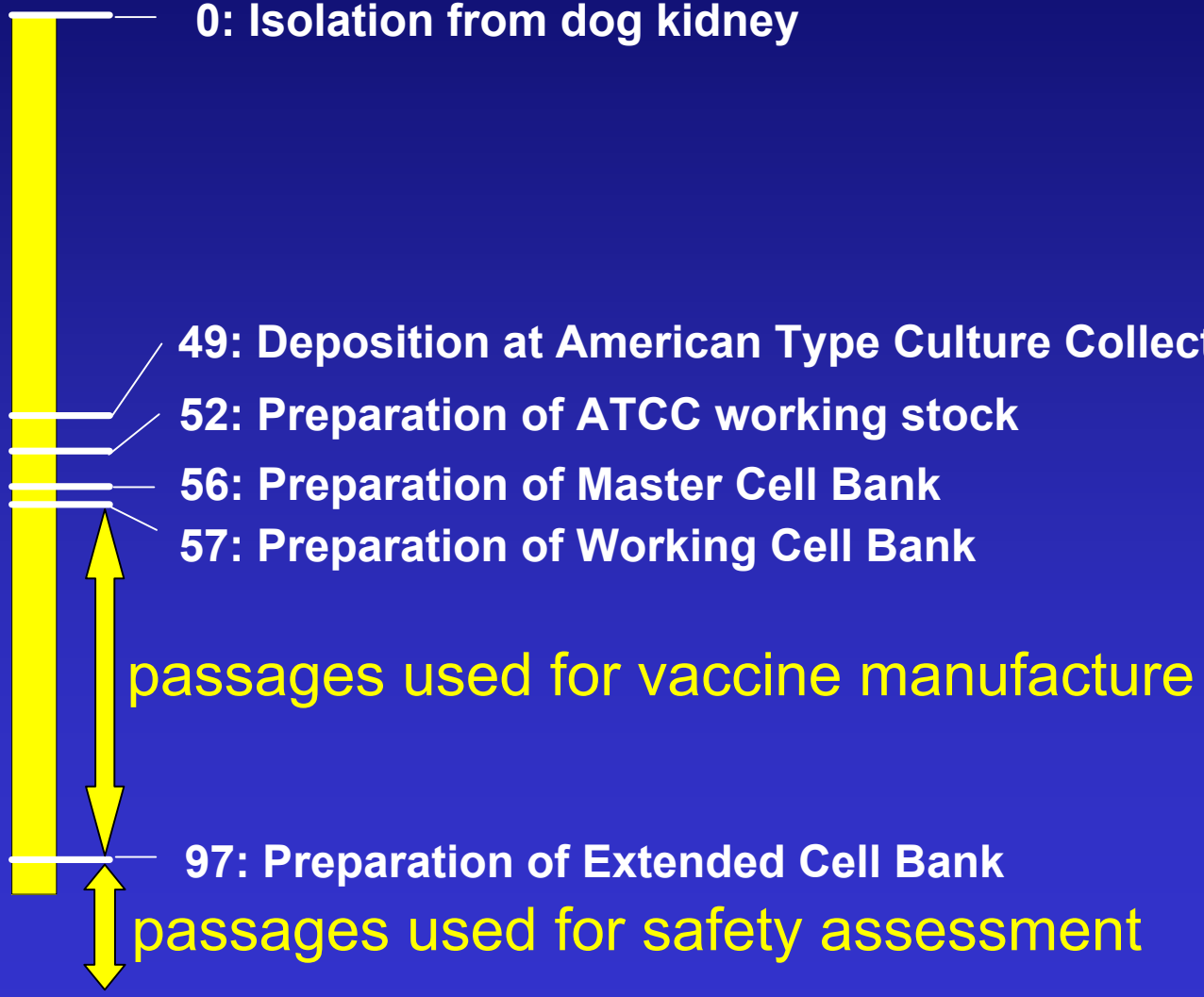
1958: Isolation from dog kidney

1964: Deposition at American Type Culture Collection (ATCC)

No introduction of
Bovine Spongiform Encephalopathy-like agents

1991: Preparation of ATCC working stock
1992: Preparation of Master Cell Bank and Working Cell Bank

1a. Cell characterization - Passage history



1b. Cell characterization - Adventitious agents

- Mycoplasmas
- Bacterial and fungal sterility
- *In vitro* co-cultivation in detector cell lines
- *In vivo* testing in various species
- Electron microscopy
- Retrovirus testing

No adventitious agents found in Solvay's MDCK

1b. Cell characterization - Adventitious agents

- Specific testing for naturally-occurring canine viruses
- Specific testing for viruses to which MDCK is susceptible

No adventitious agents found in Solvay's MDCK

1c. Cell characterization – Tumorigenicity

- Phenotypic characteristic; tumor formation in animal models
- Concern of exposure of vaccine recipient to
  - Intact cells
  - Cellular components
  - Residual cellular DNA
- Unlikely in vaccine recipient due to allograft rejection

1c. Cell characterization – Tumorigenicity

- Tumorigenic potential of intact cells
  - 4 week and 6 month study in adult immune-deficient nude mice
- Tumorigenic potential of cell lysates
  - 6 month study in adult and newborn nude mice, newborn rats and newborn hamsters
- Oncogenic potential of DNA
  - 6 month study in adult and newborn nude mice, newborn rats and newborn hamsters

1c. Cell characterization – Tumorigenicity

- Intact cells: 6 month study in 4 week nude mice

	Incidence	Nodule size (mm^2)		Complete regression
		40 days	6 mos	
Negative control	0/26	-	-	-
10^1 MDCK, p98	0/26	-	-	-
10^3 MDCK, p98	0/26	-	-	-
10^5 MDCK, p98	18/26	2	6	5
10^7 MDCK, p98	30/30	28	27	4
10^7 MDCK, p56 (ATCC)	23/26	2	0	23
Positive control (10^7 HeLa)	25/26	109	sac	sac

1c. Cell characterization – Tumorigenicity

- Intact cells: 6 month study in 4 week nude mice

	Nodule at injection site	**Tumor at injection site** (MDCK adeno-carcinoma)	**Tumor in spleen** (hystiocytic tumor)	**Tumor in lung** (adenoma)
Negative control	0/26	-	-	-
10^1 MDCK, p98	0/26	-	1*	-
10^3 MDCK, p98	0/26	-	-	-
10^5 MDCK, p98	18/26	6	-	-
10^7 MDCK, p98	30/30	16	-	1
10^7 MDCK, p56 (ATCC)	23/26	-	-	1

* Non-MDCK

1c. Cell characterization – Tumorigenicity

- Lysate of 10^7 cells: 6 month oncogenicity study

	Test group	N	Nodules at injection site	Tumors in other tissues
4w + 0-4d nude mice	cell lysate	68	0	0
	neg. controls	50	0	0
0-7d hamsters	cell lysate	98	0	0
	neg. controls	54	0	0
0-7d rats	cell lysate	91	0	0
	neg. controls	58	0	0

1c. Cell characterization – Tumorigenicity

- 0.1mg MDCK-DNA: 6 month oncogenicity study

	Test group	N	Nodules at injection site	Tumors in other tissues
4w + 0-4d nude mice	0.1mg DNA	48	0	2
	neg. controls	48	0	0
0-7d hamsters	0.1mg DNA	64	0	0
	neg. controls	48	0	0
0-7d rats	0.1mg DNA	69	0	0
	neg. controls	39	0	0

1c. Cell characterization – Tumorigenicity

- Characterization of tumors in DNA study
  - Histopathology
    - One mouse: histiocytic tumor in liver
    - One mouse: lymphoma in thoracic cavity
  - Spontaneous tumors not unexpected in immune-deficient nude mice

1c. Cell characterization – Tumorigenicity

Follow-up studies with MDCK cell DNA

- Larger study in nude mice
- Fetal and neonatal safety study in rats

1c. Cell characterization – Tumorigenicity summary

- Moderate tumorigenic potential in immune-deficient animals shown at dose levels $\geq 10^5$
- Majority of nodules partially regress; complete regression in 5/25 at 10^5 and in 4/28 at 10^7
- Increases with passage level and/or adaptation to serum-free growth
- Histopathology at passage 98 is in agreement with the literature for MDCK cells

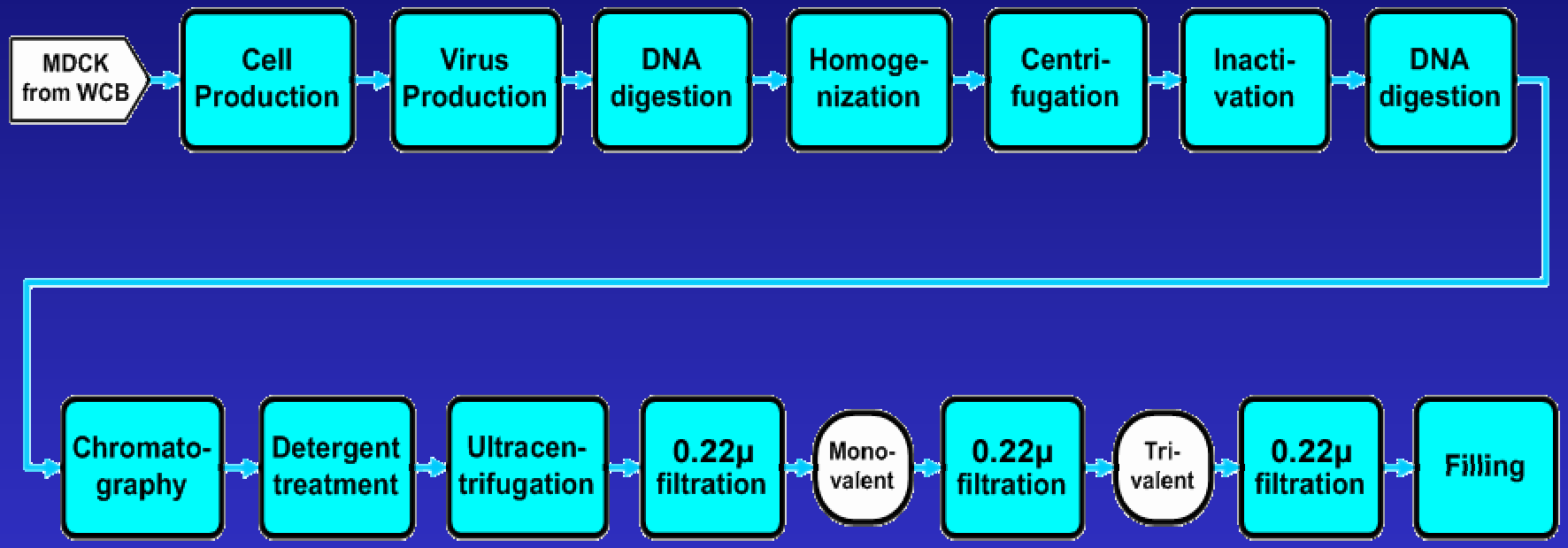
1c. Cell characterization – Tumorigenicity summary

- Tumorigenic potential not observed in immune-competent animals
- Lysates of 10^7 MDCK cells not tumorigenic
- 0.1 mg MDCK-DNA not considered oncogenic; confirmative studies initiated

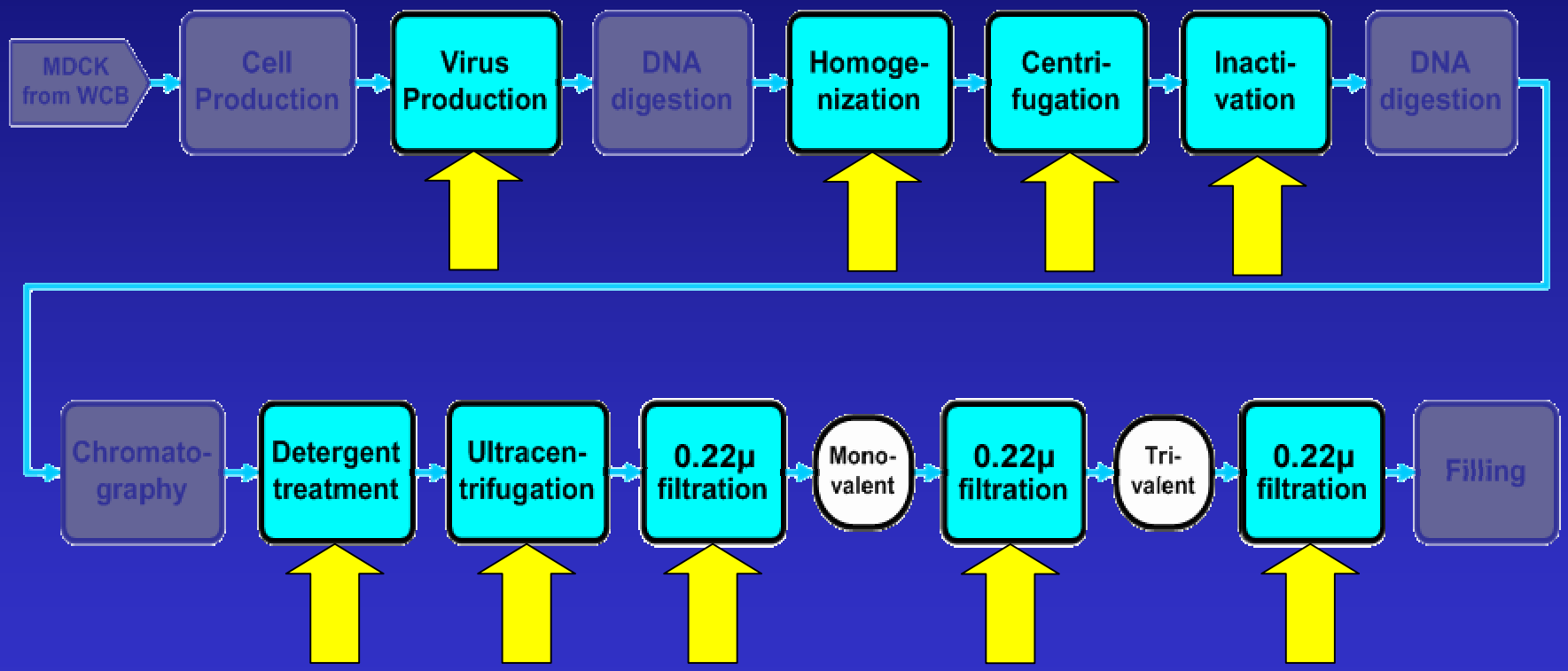
MDCK safety assessment

1. Cell line characterization
2. Downstream processing (DSP)
   - Elimination of intact cells
   - Elimination of cellular DNA

MDCK-based vaccine production



2a. DSP - Elimination of intact cells



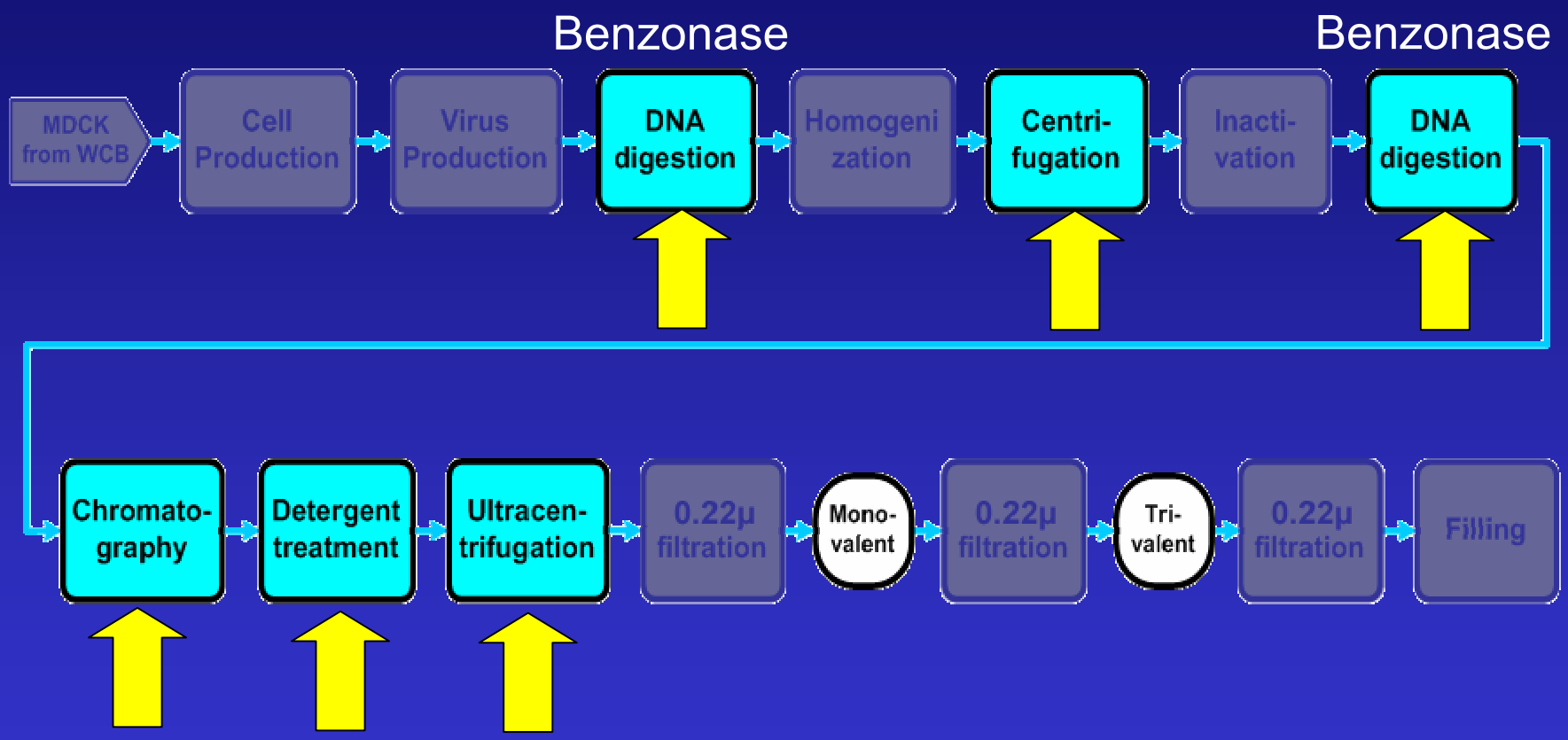
2a. DSP - Elimination of intact cells

Intact cells

^{10} log clearance factor

	Pilot scale	Production scale
Homogenization	-	Validation ongoing
Centrifugation	3.6	
Detergent treatment	≥3.6	
Ultracentrifugation	≥3.6	
0.22µ filtration x3	≥3.6	
Total	**≥ 21.4**	

2b. DSP - Elimination of DNA



2b. DSP - Elimination of DNA

DNA content

clearance factor

	Pilot scale	Production scale
DNA digestion 1	51	Validation ongoing (including content and size)
DNA digestion 2	88	
Detergent treatment/ Ultracentrifugation	168	
Total clearance	**760,000**	

specification: < 10 nanograms per dose

2. DSP - Summary

- Adequate purification and testing warrants vaccine safety
- Solvay is committed to follow the latest scientific insights as well as regulatory guidance

MDCK safety assessment

1. Cell line characterization
2. Downstream processing
3. Final product safety
   - Pre-clinical experience
   - Clinical experience

MDCK-based vaccine – Pre-clinical experience

	Species	Route	Dose (μg HA)
Local tolerance	rat, rabbit	s.c. / i.m. / i.v.	45
Systemic toxicity	rat, rabbit	s.c.	45, 75, 450
Pyrogenicity	rabbit	i.v.	45
Mutagenic potential	mouse	s.c.	45
Active and passive anaphylaxis	guinea pig	s.c. for induction i.v. for challenge	45

MDCK-based vaccine – Pre-clinical experience

- Local tolerance
  - No local irritation
- Systemic toxicity
  - No adverse effects
- Pyrogenicity
  - No distinct increase in body temp
- Mutagenicity
  - No increase in number of micronuclei
- Anaphylaxis
  - No active anaphylaxis
  - Passive anaphylaxis favorable to egg-based

MDCK-based vaccine – Clinical experience

- 14 randomized, double-blind studies
- 1,023 subjects on MDCK-based subunit vaccine
- Population: 18-60, over 60, patients at-risk for complications for influenza, atopic patients and subjects with an egg-allergy
- Major objectives: Show immunogenic non-inferiority and comparable safety to existing egg-based subunit influenza vaccine

MDCK-based vaccine – Clinical experience

- Local and systemic reactogenicity profile is comparable to egg-based vaccine
- Reactions are minor and short-lived
- No unexpected safety findings
- Non-inferior in immunogenicity

MDCK-based vaccine has comparable safety and immunogenicity profile as egg-based vaccine

Conclusions

- Solvay is confident MDCK is a safe substrate for inactivated influenza vaccines
- The use of MDCK will improve reliability of influenza vaccine supply, and enhance pandemic preparedness
- Solvay will pursue licensing worldwide, including US

Participants

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