Rapid detection of viruses of concern with Blazar® Platform - In line with ICH Q5A(R2)

CMC Strategy Forum Japan 2022
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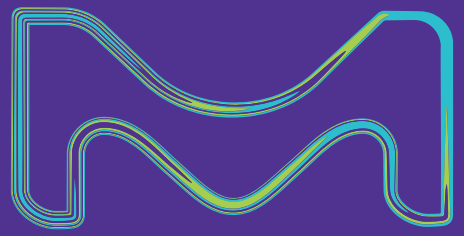

The life science business of Merck operates as MilliporeSigma in the U.S. and Canada.

BioReliance®
Pharma & Biopharma Manufacturing & Testing Services

The life science business of Merck KGaA, Darmstadt, Germany operates as MilliporeSigma in the U.S. and Canada

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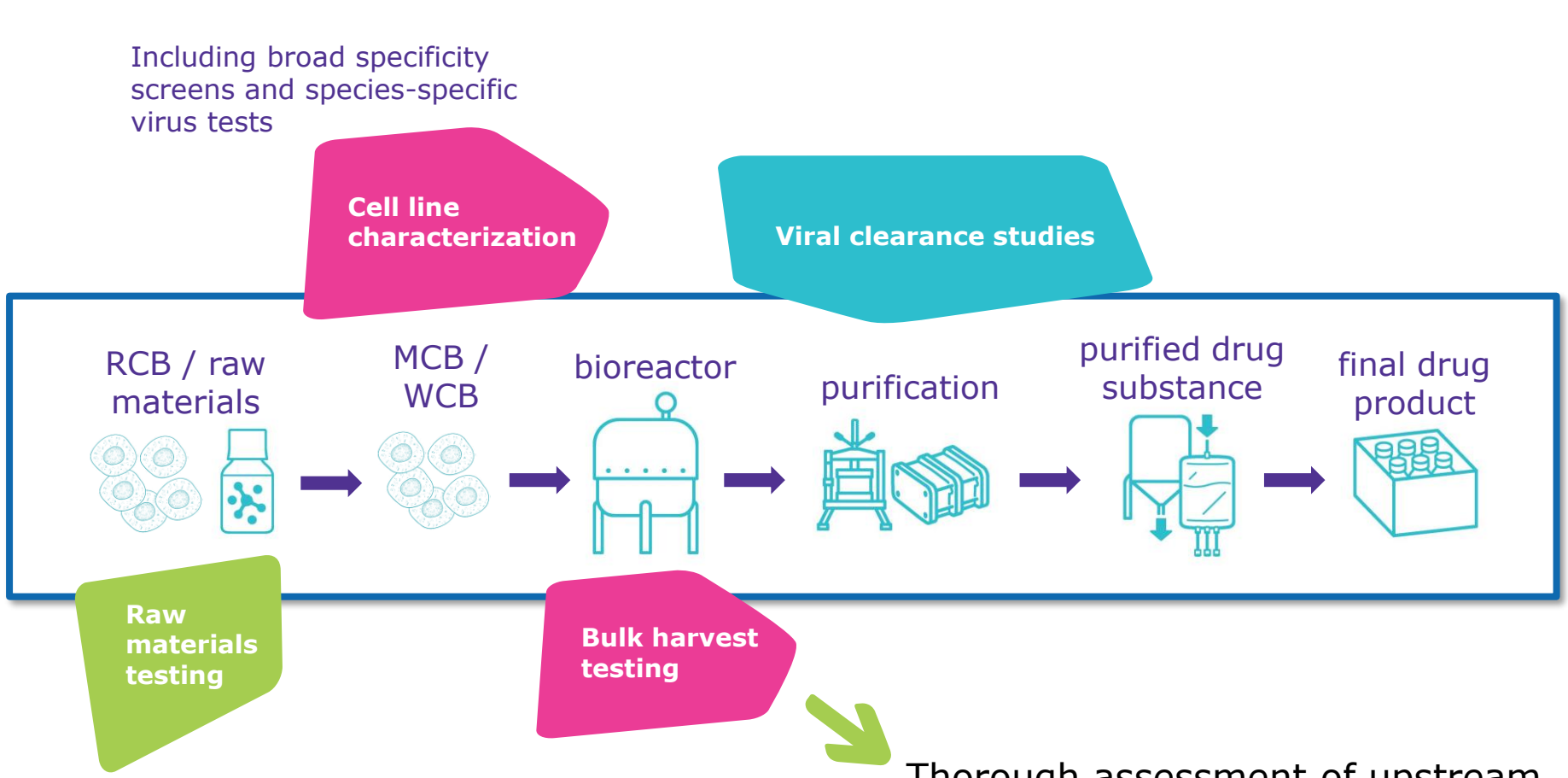
Pharma & Biopharma Manufacturing & Testing Services

Viral safety assurance requires risk mitigation at a number of stages



Thorough assessment of upstream components (raw materials/cell lines) allows bulk harvest testing to focus on immediate process risks and emerging threats

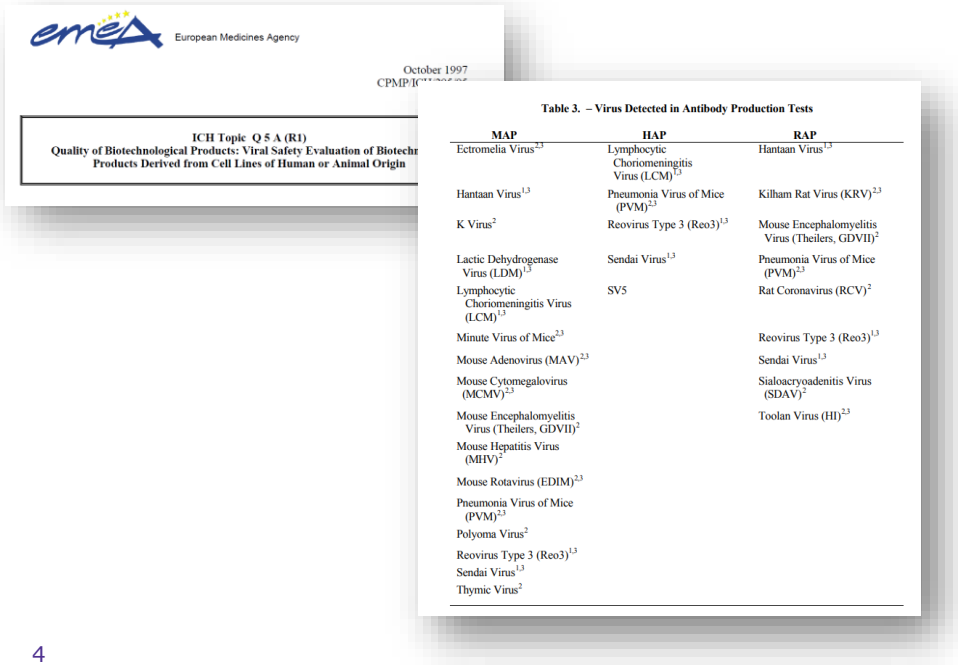
Traditional cell line characterization tests for ~20 specific viruses and takes 45+ days

Cell characterization assay for rodent cells – e.g. CHO

Inoculation

Sampling

~45 days



European Medicines Agency

October 1997

ICH Topic Q 5 A (R1)
Quality of Biotechnological Products: Viral Safety Evaluation of Biotechn
Products Derived from Cell Lines of Human or Animal Origin

Table 3. – Virus Detected in Antibody Production Tests

MAP	HAP	RAP
Ectromelia Virus[2,3]	Lymphocytic Choriomeningitis Virus (LCM)[1,3]	Hantaan Virus[1,3]
Hantaan Virus[1,3]	Pneumonia Virus of Mice (PVM)[2,3]	Kilham Rat Virus (KRV)[2,3]
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Lactic Dehydrogenase Virus (LDM)[1,3]	Sendai Virus[1,3]	Pneumonia Virus of Mice (PVM)[2,3]
Lymphocytic Choriomeningitis Virus (LCM)[1,3]	SV5	Rat Coronavirus (RCV)[2]
Minute Virus of Mice[2,3]		Reovirus Type 3 (Reo3)[1,3]
Mouse Adenovirus (MAV)[2,3]		Sendai Virus[1,3]
Mouse Cytomegalovirus (MCMV)[2,3]		Sialoacryoadenitis Virus (SDAV)[2]
Mouse Encephalomyelitis Virus (Theilers, GDVII)[2]		Toolan Virus (HI)[2,3]
Mouse Hepatitis Virus (MHV)[2]		
Mouse Rotavirus (EDIM)[2,3]		
Pneumonia Virus of Mice (PVM)[2,3]		
Polyoma Virus[2]		
Reovirus Type 3 (Reo3)[1,3]		
Sendai Virus[1,3]		
Thymic Virus[2]		

ICH Q5A: recommends testing for ~ 20 viruses (depending on the cell type and history)

- Mice, hamsters and/or rats inoculated
- Animal observations for ~28 days
- LCMV challenge
- Animals euthanized and samples taken
- Antibody production measured immunologically – e.g. ELISA

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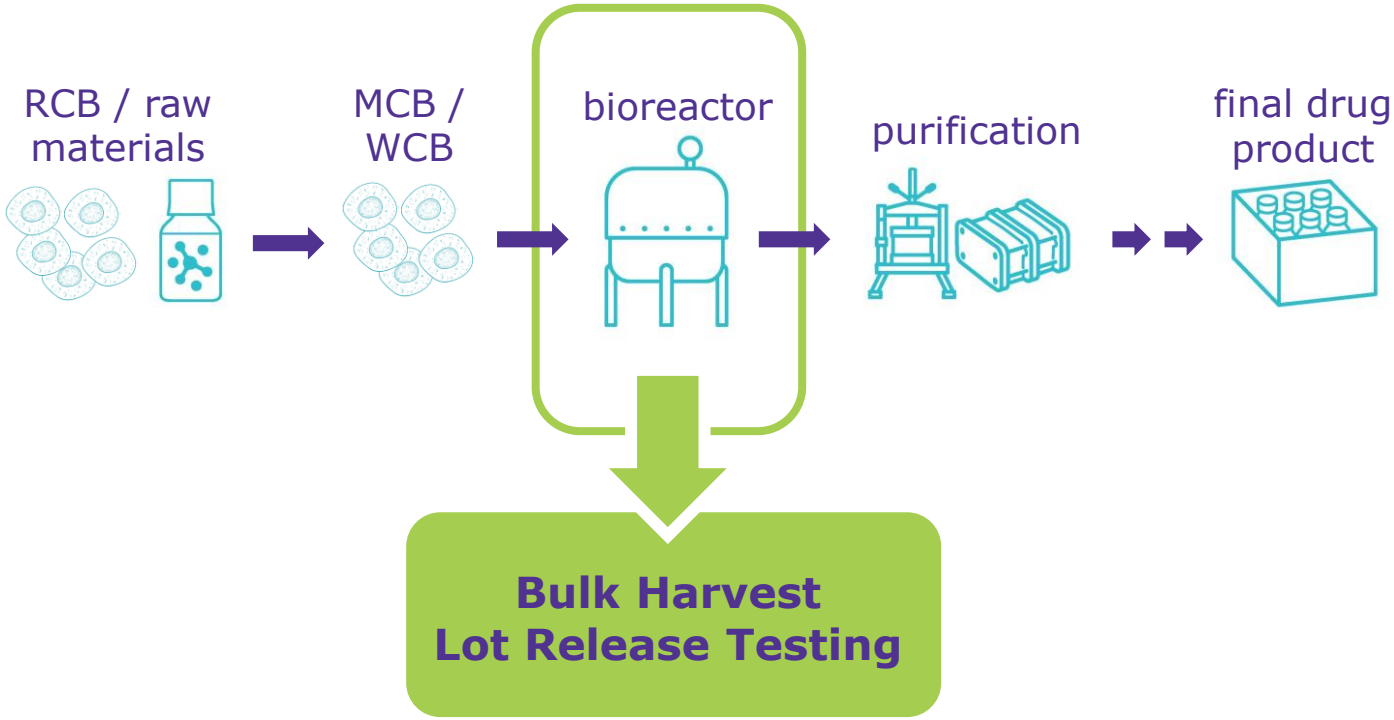
Adventitious agent testing is often a rate-limiting step in downstream processing



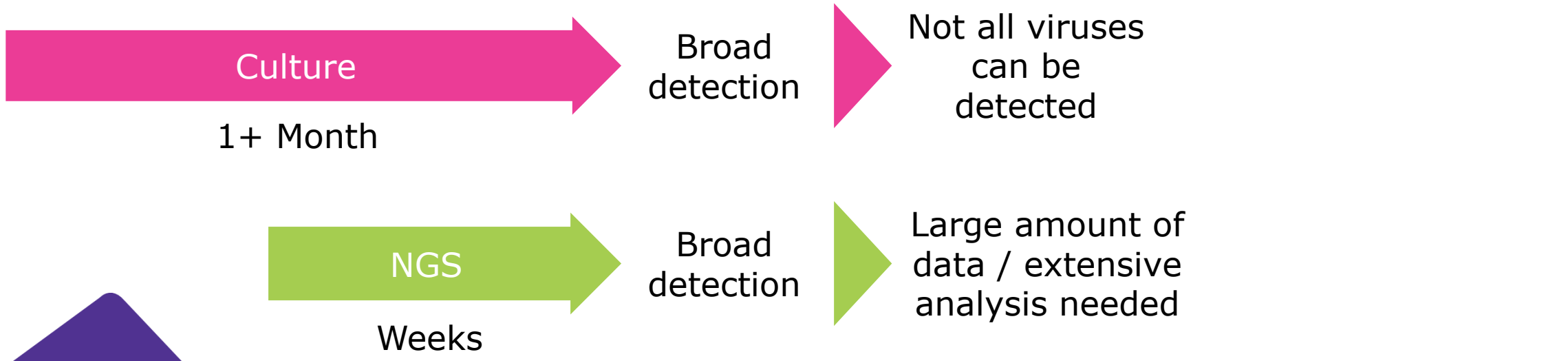
For CHO-based processes, the industry shift towards intensified and continuous manufacturing increases the need for rapid testing methods to detect:

- Bacteria/fungi
- Mycoplasma
- Adventitious viruses
- Endogenous retrovirus-like particles

Alternatives to culture-based testing approaches are required

- Traditionally, most testing at the bulk harvest stage is performed using culture-based methods
- Alternative methods should provide breadth of detection and sensitivity, as well as speed

Molecular methods can accelerate virus testing



The **Blazar® platform** combines speed, sensitivity and breadth of detection

Ideally suited for bulk harvest testing

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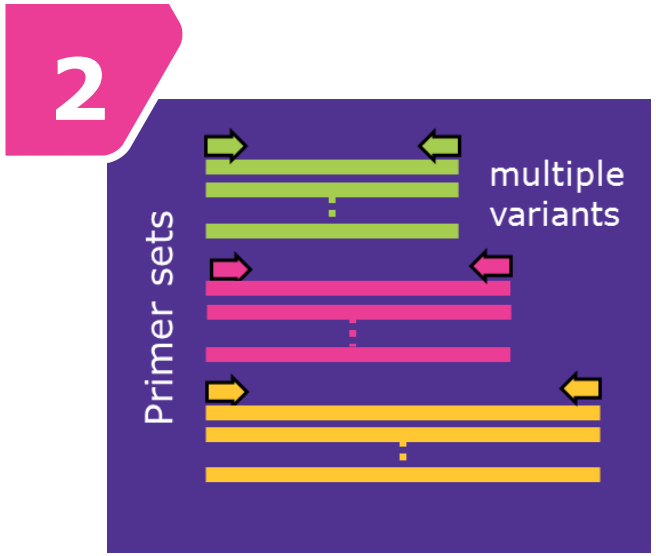
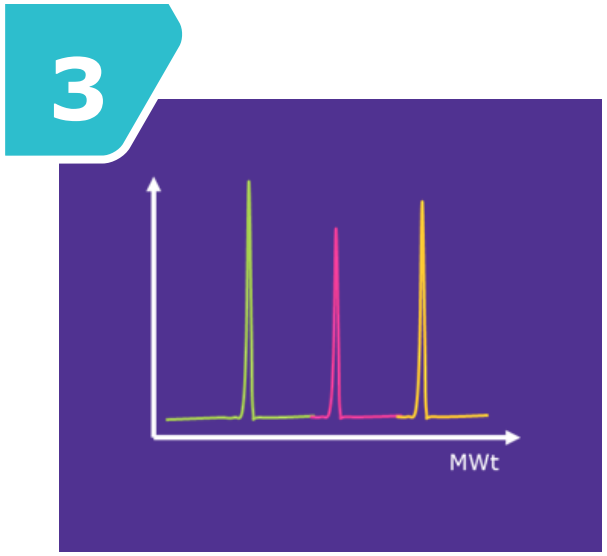
The Blazar® platform workflow uses a 3-step method for virus detection





Extraction

- Nucleic acid extraction from total bulk harvest material (cells and supernatant)
- Samples are pre-spiked with control viruses to confirm that extraction and PCR perform as expected

Degenerate PCR

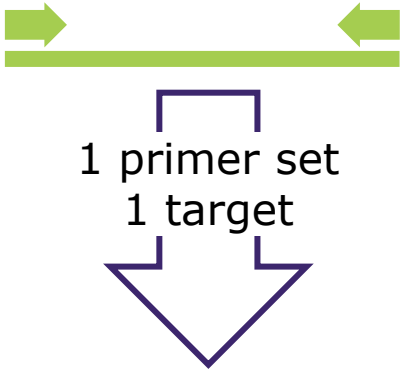
- Two rounds of PCR (first round and nested PCR) to increase sensitivity and specificity
- Separate assays for DNA and RNA viruses (with preceding reverse transcriptase step for RNA viruses)

Fragment analysis

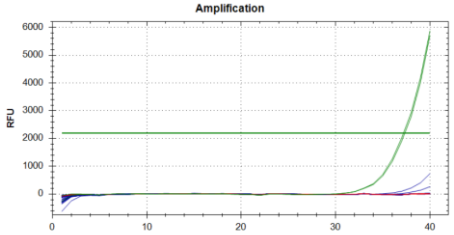
- Separation by capillary electrophoresis and automated analysis of peaks in relation to expected size ranges

Use of degenerate primers provides broad specificity

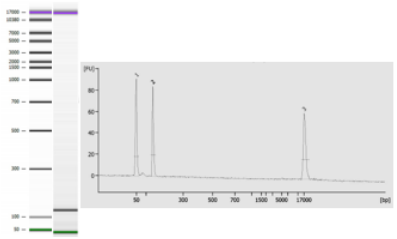
Single-Plex PCR



1 primer set
1 target



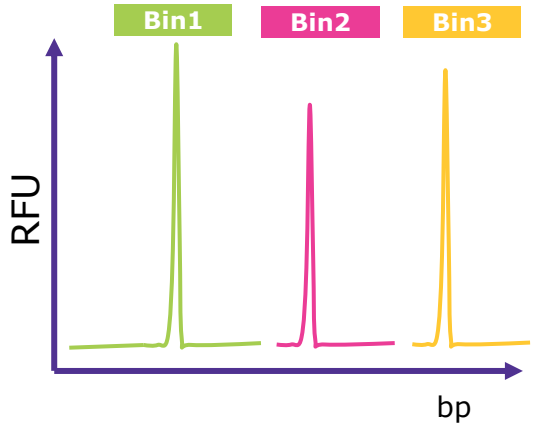
Real-Time PCR



End point detection

Multiplex Degenerate PCR

1 primer set
multiple targets



Fragment detection
(followed by sequencing of positives if needed)

A **bioinformatics approach** to primer design, coupled with experimental optimization of reaction conditions, enables a **high degree of multiplexing**

The Blazar® Rodent virus panel covers a wide range of viruses

1997 ICH Q5(A) guidelines

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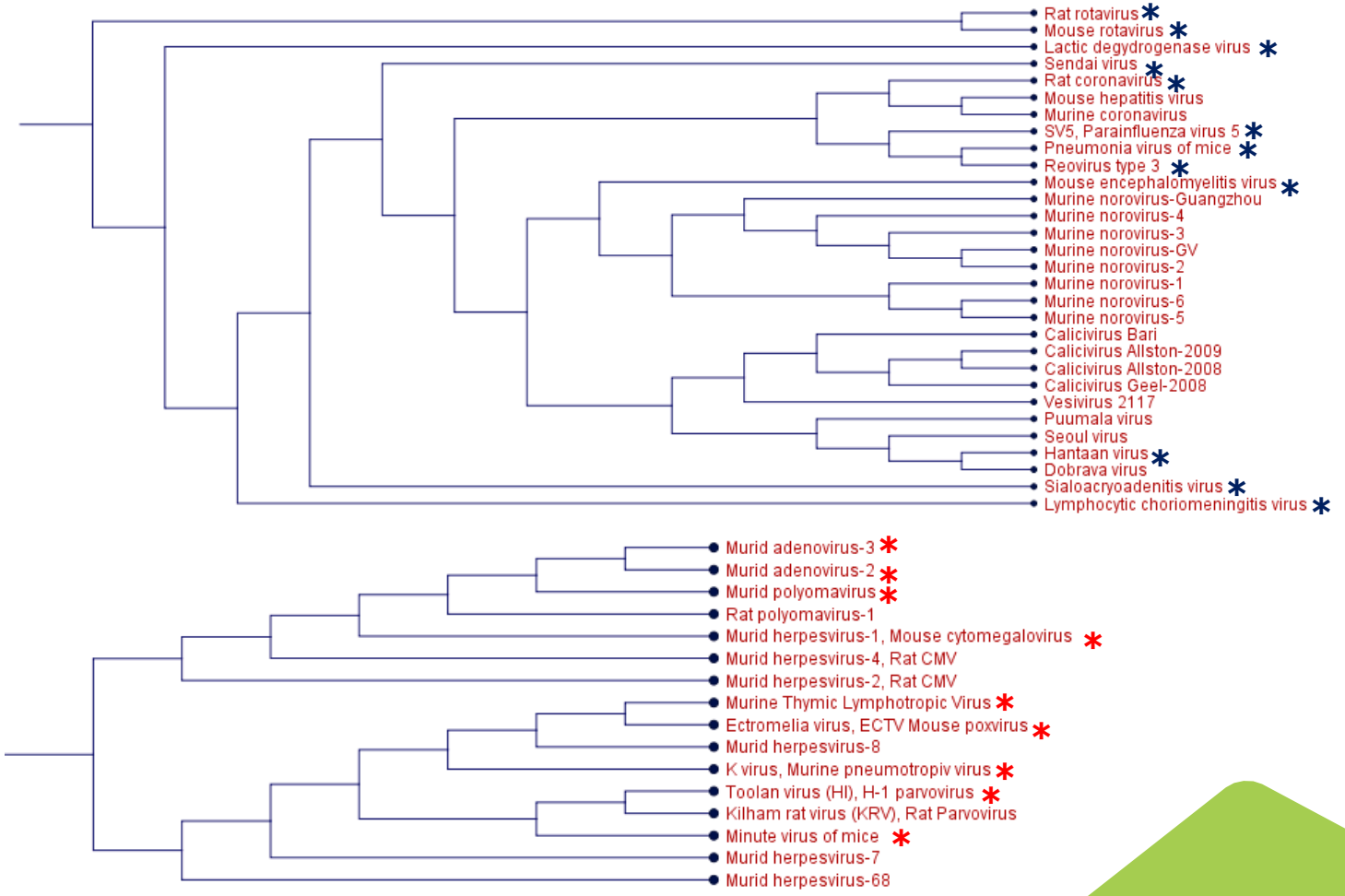
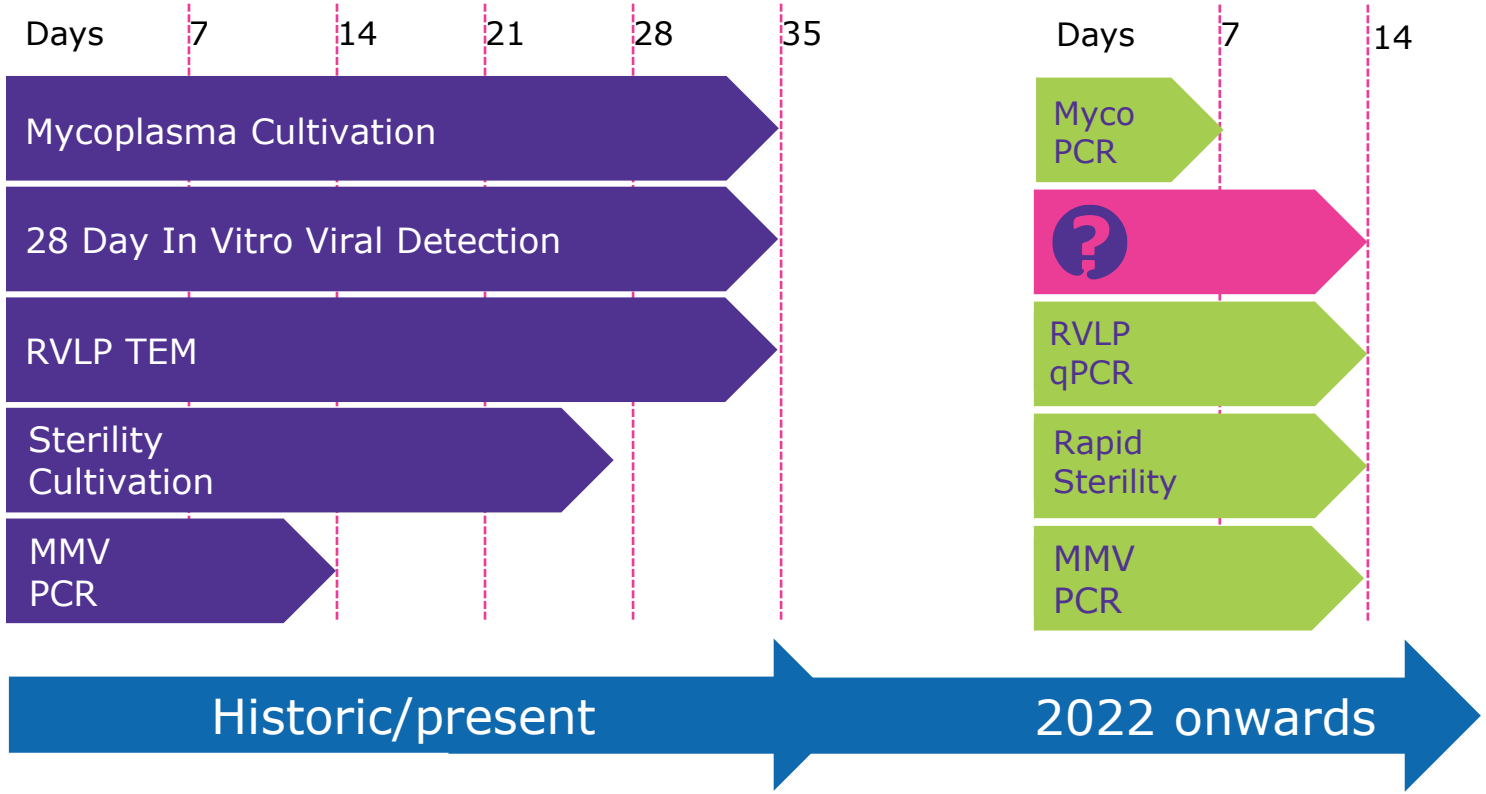
Blazar® Rodent virus panel



* DNA Viruses from ICH List
* RNA Viruses from ICH List

Accelerating CHO bulk harvest testing requires a full set of rapid methods

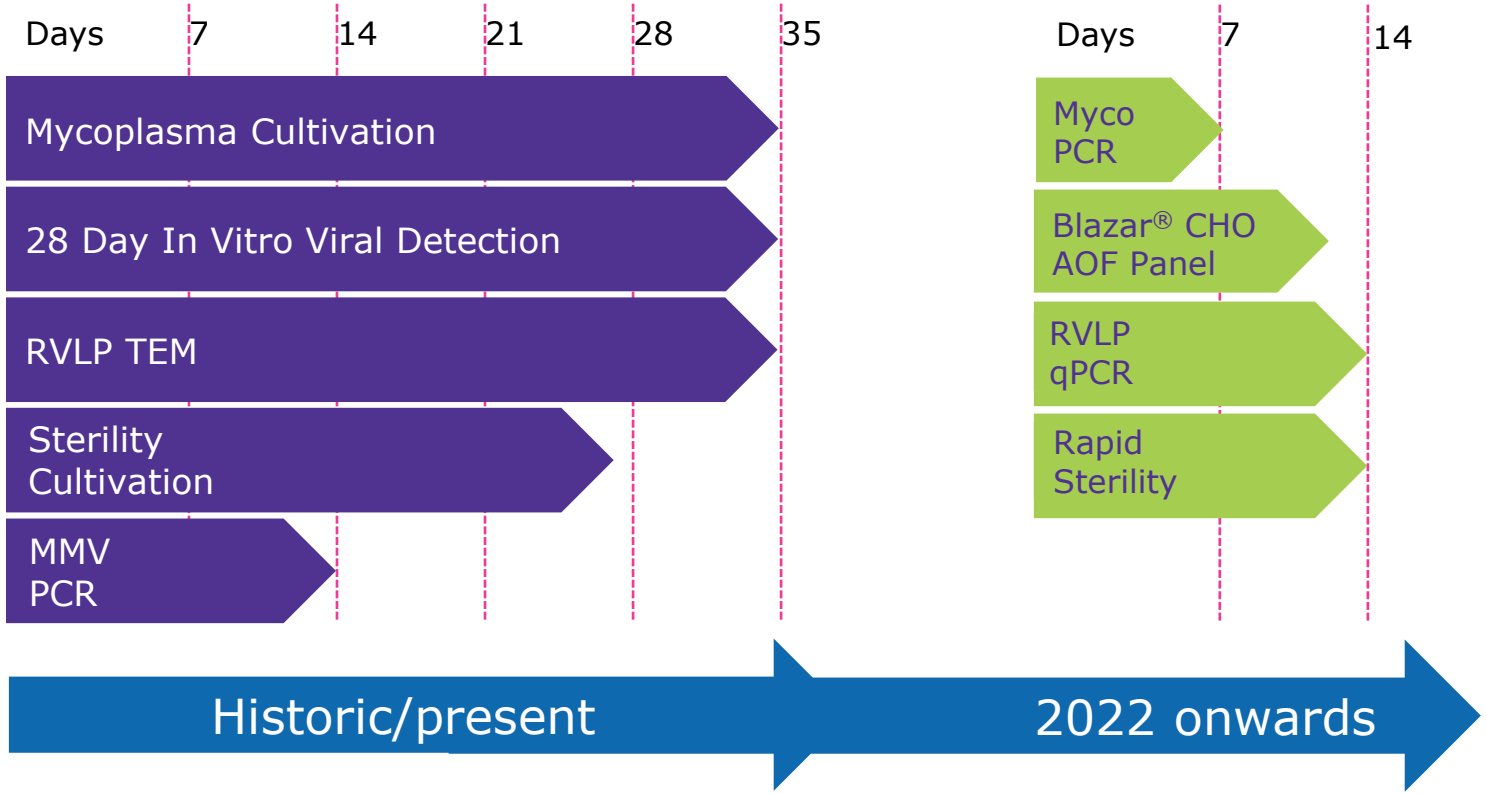


Several rapid methods for bulk harvest testing are already available

A rapid method for broad specificity virus detection is required to replace the *in vitro* virus test at the bulk harvest stage

Timelines are shown as minimum total assay turnaround time

Accelerating CHO bulk harvest testing requires a full set of rapid methods



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A rapid method for broad specificity virus detection is required to replace the *in vitro* virus test at the bulk harvest stage

Blazar® CHO AOF panel provides the solution for **A**nimal **O**rigin-**F**ree processes

Timelines are shown as minimum total assay turnaround time

Blazar® CHO AOF panel targets 15 virus families

Consultation and literature review

Internal experts, industry consultants

Consideration of historical CHO cell contamination events

Focus on viruses for which CHO cells are permissive, and/or which may have potential to infect human cells

Emerging viruses included to cover future risks

Virus family	Type	Example viruses covered by CHO AOF panel
Adenoviridae	dsDNA	human adenovirus A/B/C/D/E/F/G
Anelloviridae	ssDNA	rat torque teno virus-1/2
Circoviridae	ssDNA	rat circovirus, porcine circovirus-1/2/3
Parvoviridae	ssDNA	minute virus of mice, hamster parvovirus, rodent protoparvovirus, rat parvovirus 2, rat bocavirus
Polyomaviridae	dsDNA	rat polyomavirus 2
Bornaviridae	ssRNA	borna virus
Caliciviridae	ssRNA	vesivirus 2117, rat norovirus, mouse norovirus, Calicivirus-Allston-2008
Coronaviridae	ssRNA	mouse hepatitis virus
Hepeviridae	ssRNA	hepatitis E virus
Orthomyxoviridae	ssRNA	influenza virus A/B
Paramyxoviridae	ssRNA	beilong virus, Human respirovirus 1 strain, Human parainfluenza virus types 1/2/3/4/5, Measles virus, Mumps
Picornaviridae	ssRNA	coxsackie virus B3, Encephalomyocarditis virus
Reoviridae	dsRNA	reovirus-1/2/3, Mammalian orthoreovirus, Epizootic hemorrhagic disease virus
Rhabdoviridae	ssRNA	vesicular stomatitis virus
Togaviridae	ssRNA	semliki forest virus, Chikungunya virus strain, Eastern equine encephalitis virus

Reflecting current understanding of viral risk for animal origin-free processes

Degenerate PCR enables detection of emerging viruses

Multiple variants detected

- For each virus family, degenerate PCR primers are designed against genomic regions representing conserved protein motifs
- This provides broad specificity, allowing multiple family members and related variants to be detected
- The Blazar® CHO AOF RNA panel is predicted to detect >26,900 viral target sequences

Proof of concept

- A coronavirus primer design has the ability to detect the emergent human SARS-CoV-2 virus
- The Blazar® CHO AOF primers were designed in 2018, more than one year before SARS-CoV-2 was described

Nature article

A new coronavirus associated with human respiratory disease in China

https://doi.org/10.1038/s41586-020-2008-3
Received: 7 January 2020
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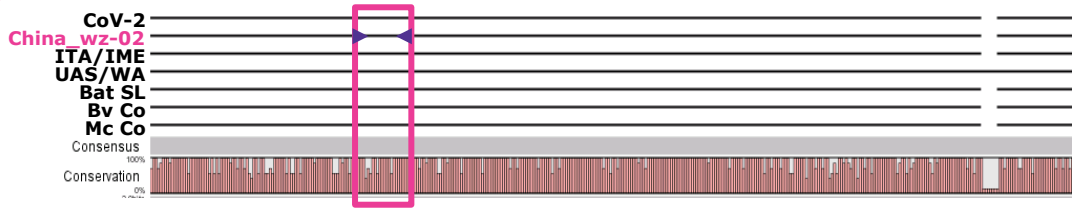
Published online: 3 February 2020
Open access

Fan Wu[1,7], Su Zhao[2,7], Bin Yu[3,7], Yan-Mei Chen[1,7], Wen Wang[4,7], Zhi-Gang Song[1,7], Yi Hu[2,7], Zhao-Wu Tao[2], Jun-Hua Tian[3], Yuan-Yuan Pei[1], Ming-Li Yuan[2], Yu-Ling Zhang[1], Fa-Hui Dai[1], Yi Liu[1], Qi-Min Wang[1], Jiao-Jiao Zheng[1], Lin Xu[1], Edward C. Holmes[1,5] & Yong-Zhen Zhang[1,4,6]✉

Emerging infectious diseases, such as severe acute respiratory syndrome (SARS) and Zika virus disease, present a major threat to public health[1–3]. Despite intense research

Sequence alignment



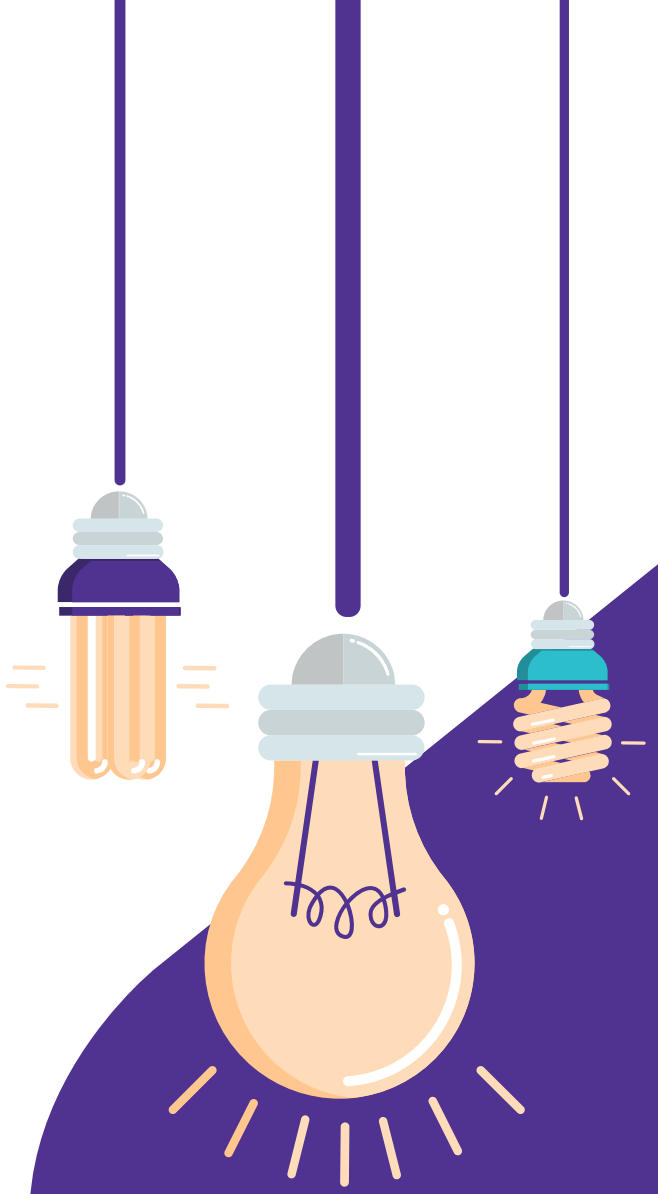
The Blazar® platform mitigates the risk of emerging viruses, which pose a major threat to biologics manufacture

Blazar® CHO AOF panel specifications

Assay code	399003GMP.BSV **(Rockville, US) Available** 399003GMP.BUK **(Glasgow, UK)** ***Coming soon!***
Sample format	2x1ml of test article
Total turnaround time	**12 days**
Virus coverage	DNA and RNA viruses from 15 virus families
Internal controls	Spike recovery: DNA and RNA virus each spiked at detection limit in the test article prior to extraction, to demonstrate extraction and PCR efficiency
Sensitivity	10 genomic copies per reaction*
False positive rate	<1%
True positive rate	>99%
Specificity (pass criteria)	No target peaks observed in 3/3 test article wells Spike recovery control is detected in ≥1/3 wells
System suitability	No Template Control (NTC) signal detected in 0/3 wells Spike recovery must fall within specified sizing window

* 100 genomic copies per reaction for polyomaviruses

Suitable for CHO bulk harvest from animal origin-free processes, where the MCB has been fully characterized



Building on our success…

Our established Blazar® rodent panel, which uses the same award-winning technology as the CHO AOF panel, is now familiar to regulators through IND submissions and is used by the majority of our clients for cell line characterization

… in line with updated regulatory guidance

The ICH Q5A revision will promote molecular methods for virus detection

ICH Q5A Revision Addresses Important regulatory and industry Advances

Emerging product types

Virus-like particles (VLPs), subunit proteins, and viral-vectored vaccines and gene therapies using novel mammalian and insect-based vector/cell expression systems

Analytical technologies

Nucleic acid-based assays such as **PCR and NGS** may provide rapid and sensitive detection of adventitious and endogenous viruses in the starting and harvest materials

Virus clearance validation strategies

Flexibility in validation approaches should be allowed in order to effectively leverage knowledge gained during development

Manufacturing

Emerging or advanced manufacturing approaches beyond traditional unit and batch process operations

MERCK

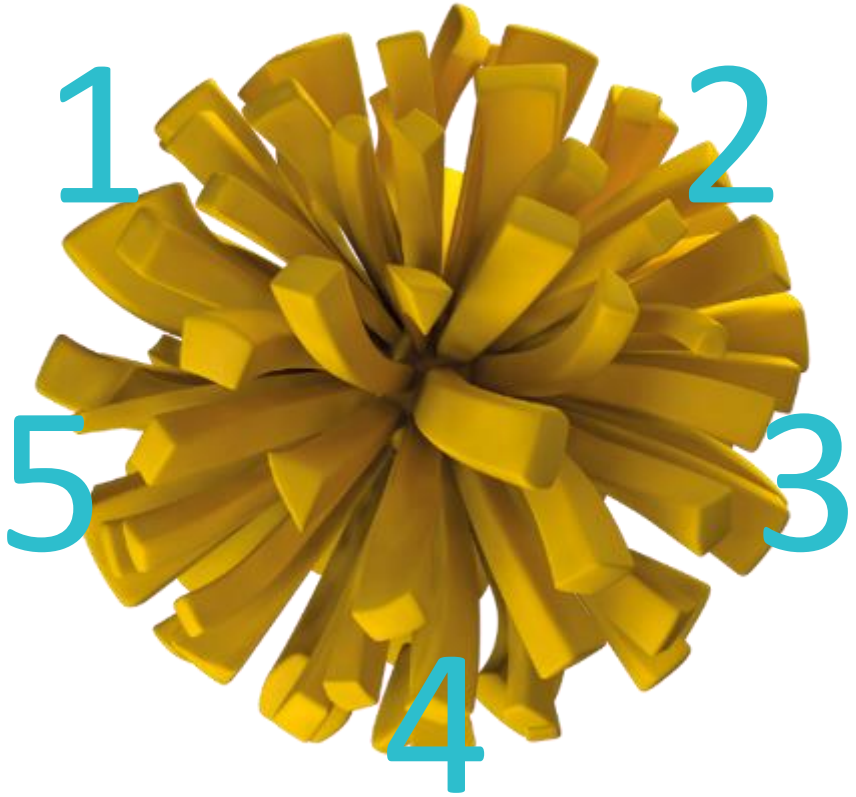
General guidance on the use of molecular methods
ICH Q5A R2 draft (Oct 2022)

3Rs

Virus-specific PCR, targeted molecular methods or NGS can be used as replacement assays for animal-based methods, No head-to-head comparison

Infectious agents

Positive results should be investigated to determine infectivity



Assay quality

Assays should be appropriately qualified or validated for their intended use.

Types

NGS and Nucleic Acid Amplification Techniques such as PCR may be appropriate for broad and specific virus detection, respectively

Comparability

These tests may be introduced without a systematic head-to-head comparison with the currently recommended in vivo assays

Summary: Blazar® CHO AOF panel

Rapid and sensitive virus detection for effective risk mitigation in CHO bulk harvest

and other applications

Shorten timelines to release

- **12-day** turnaround for CHO bulk harvest samples to replace and/or supplement *in vitro* assay results
- Combine with other rapid methods for an accelerated testing package for animal origin-free processes

Meet regulatory compliance

- Molecular assays are acceptable to regulators and reflect industry trends towards more robust and sensitive methods
- Established Blazar® technology provides the expected sensitivity and breadth of detection
- Reduce need for animal-derived assay components

Reduce sample requirements

- Assay requires only 2 x 1 ml bulk harvest

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