SEROLOGICAL AND MOLECULAR DIAGNOSIS Of HEPATITIS B VIRUS

By

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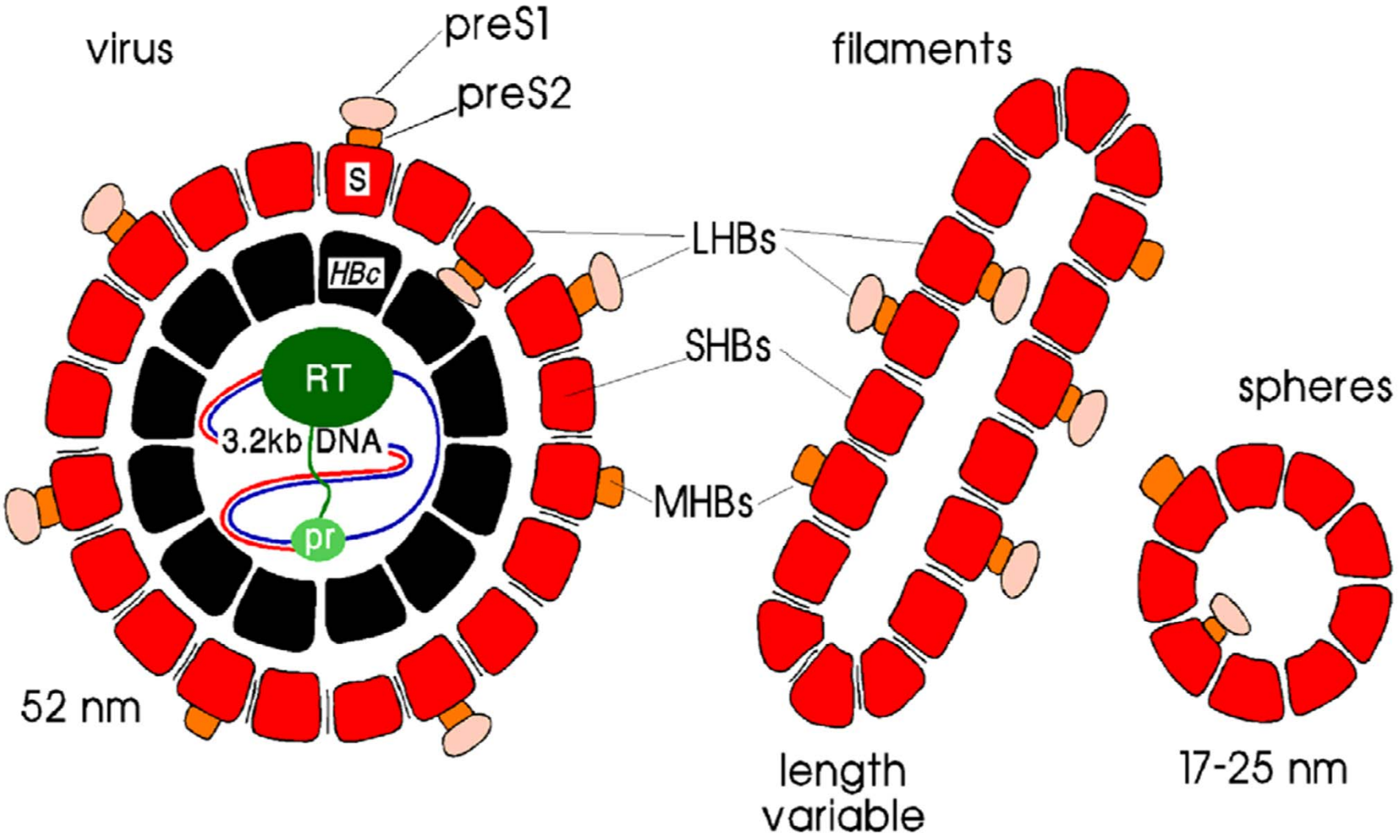
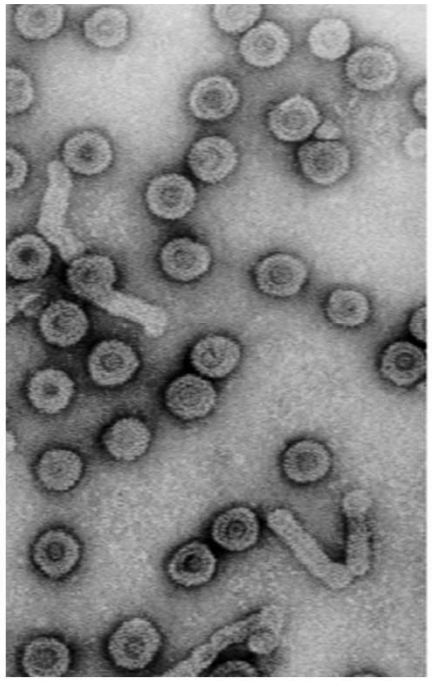
Professor of Virology

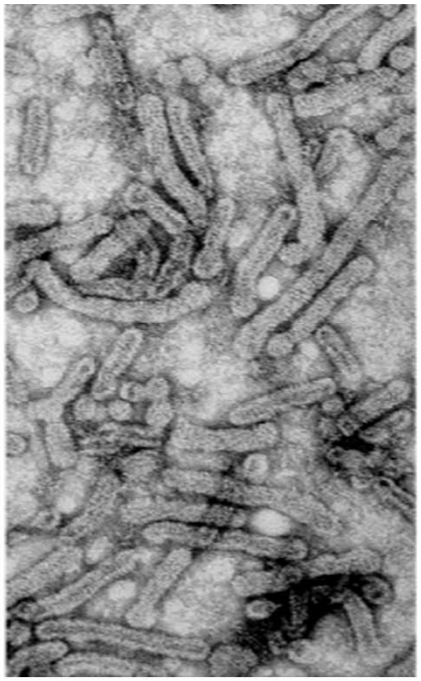
Structural Components of HBV and HBsAg Particles

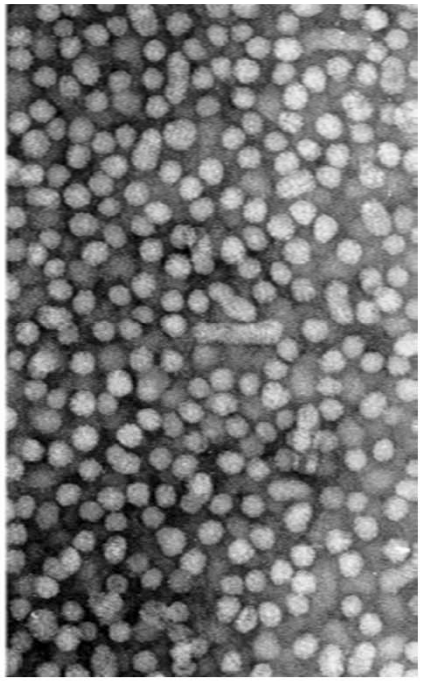


Virus and HBsAg Particles in Blood From Highly Viremic HBV Carriers

HBV particles | **HBsAg filaments** | **HBsAg 20nm particles**







? 10^{9} - 10^{11} /ml

10^{11} - 10^{12} /ml

10^{12} - 10^{14} /ml

From: Doerr & Gerlich Medizinische Virologie Thieme Verlag, in print

HBV Genotypes

- 8 HBV genotypes (A-H) have been defined.
- Genotype A,B,C and D are found worldwide.
- In Iran the only genotype D is reported.
- Current management and treatment guidlines do not recommend a role for genotype determination.

Serological Markers for HBV Diagnosis

- HBs Ag
- HBe Ag
- HBc Ag
- Anti HBc Ab (IgM, IgG)
- Anti HBs Ab
- Anti HBe Ab

HBs Ag

- HBs Ag circulate in excess ~10^{12} particles/mL equivalent of 500 µg protein in the serum.
- Clinical assays can detect as little as 1 ng of HBsAg per mL of serum providing a **highly sensitive** assay for HBV infection.
- Mutations in the S gene can lead to false-negative result.

HBs Ag (cont.)

- HBs Ag is the first marker of acute HBV infection.
- HBs Ag becomes detectable 6 weeks (1 to 10 weeks) after an acute exposure to HBV .
- In resolving infection, HBs Ag level begin to fall 4 to 6 months after exposure as HBs Ab increase.
- If HBs Ag remains positive for more than 6 months, HBV infection has become chronic.

HBs Ag :

- HBs Ag detection is mostly by using an enzyme immunoassay (EIA).
- 5% of adult patients with acute hepatitis B progress to chronic infection.
- 0.5% to 1% of patients with chronic HBV infection clear HBs Ag per year.

Quantitative HBsAg

- HBs Ag is composed of both the Dane particle as well as sub-viral particles.
- The amount of the cccDNA and relaxed circular DNA may affects HBs Ag level in the serum.
- The reduction in HBs Ag correlated well with that of cccDNA and total intrahepatic HBV DNA.

HBc Antigen

- HBc Ag is an intracellular antigen that is expressed in infected hepatocytes
- **Not** detectable in serum

Anti HBc *IgM*

- Anti HBc IgM is a reliable marker of acute infection.
- Anti HBc IgM may also be detected during flares of chronic hepatitis B.
- Anti HBc IgM appears shortly after HBsAg and persist for 6 to 24 months.

Anti HBc *IgG*

- IgG reactive to HBc Ag is a marker of past or ongoing infection .
- Anti HBc Ab found in both resolved infection and in chronically infected individuals .
- anti HBc Ig G eventually replaces IgM anti HBc.

Isolated Anti-HBc Ab Positive

- 5% of healthy blood donors have isolated anti HBc Ab in the serum.
- In Iran 10 to 30% of healthy donors have isolated HBc Ab in their serum.
- Among HIV infected as high as 42% are isolated anti HBc Ab positive

Isolated anti –HBc (cont.)

- *Isolated anti-HBc may occur:*

  - During the window period.
  - During chronic infection.
  - After resolved infection in the remote past
  - As a false-positive result.

Isolated anti HBc (cont.)

- 20% of people with Anti-HBc have chronic HBV infection with low level of circulating HBV DNA in their serum.
- These people represent the so called “Occult” HBV infection.
- Other 80% of cases represent a false-positive or resolved HBV infection in the remote past.

Anti HBs Antibody

The presence of Anti HBs Antibody could be due to :

1) Vaccination

2) Recovery from infection

-Positive for Anti HBc Antibody

-Higher antibody titer

In most patients persists for life

HBe Antigen

- HBeAg is secreted in the serum and can be detected by ELISA.
- HBeAg is detectable 6 to 12 weeks after infection.
- Persistence of HBeAg in serum beyond 3-4 months usually is accompanied by development of chronic HBV infection.

HBe Antigen (cont.)

- The presence of HBsAg & HBeAg is generally considered a signal of high titer (>10 5 copies/ml virus production and hence a high infectivity) .
- With seroconversion to anti-HBe, HBe Ag level declines.

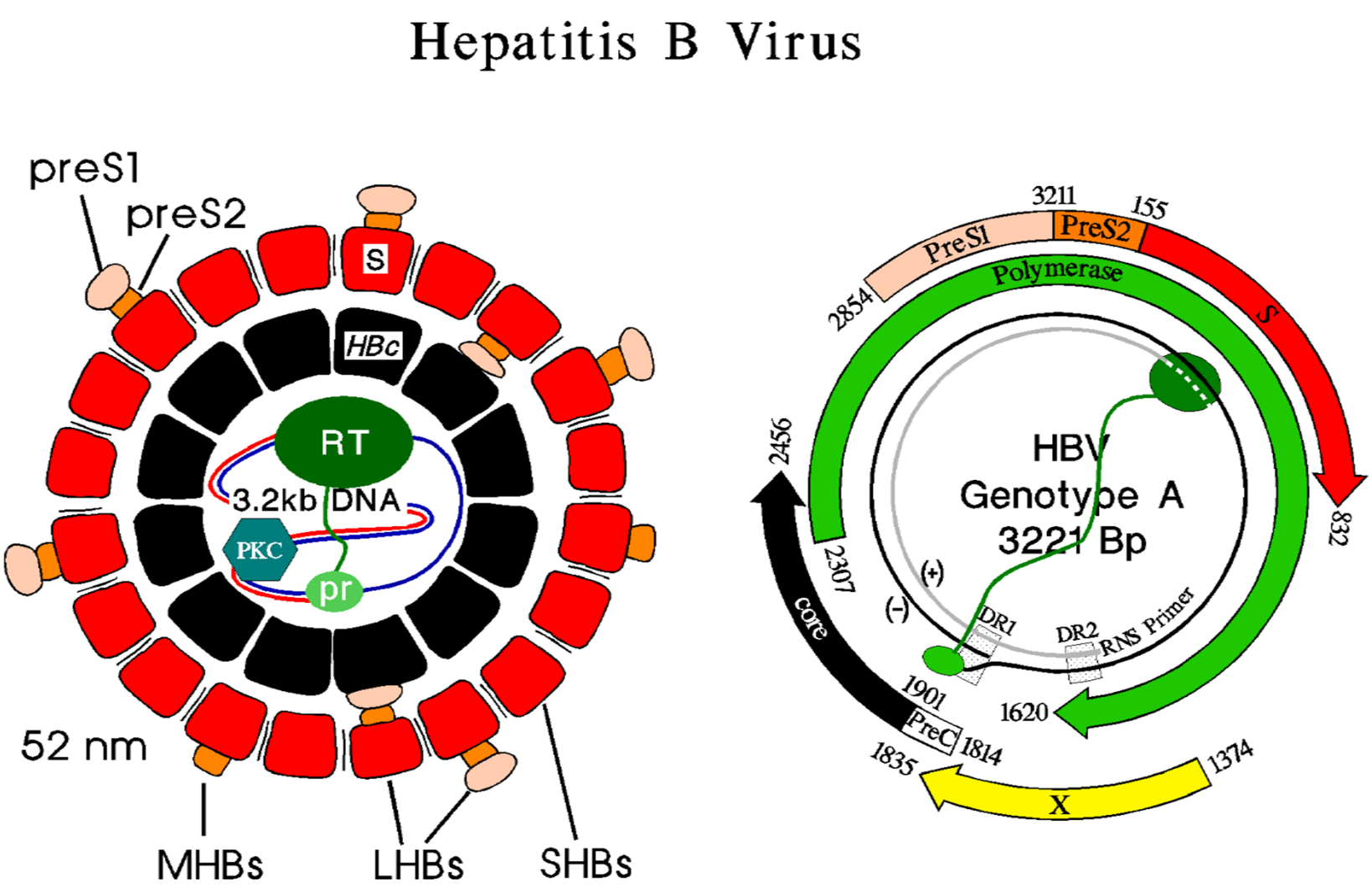
Loss of HBeAg

- With seroconversion : with the appearance of HBeAb
- Without seroconversion

- A decline in the amount of virus in the liver.

- Mutation in the precore gene or precore/core promoter.

Loss of HBeAg



Loss of HBeAg (cont.)

Precore and Core Region (PreC/C)

- Translation of protein from first AUG in PreCore region produce HBeAg
- Translation from second AUG produce HBcAg.

Loss of HBeAg (cont.)

In precore or precore/core promoter mutation there is ongoing disease activity because of immune selection of virus that has lost capacity to produce HBeAg.

HBV precore mutants are found in chronic HBV infection in:

- 10-15% USA & Europe
- 40-80% Southern Europe, Middle East ,Asia
- 60-90% Iran

Presence of HBe Ag (cont.)

In some Immunotolerant Patients (mostly in vertical transmission), HBeAg Positive Persists without Seroconversion and Precore Mutation.

Application of HBV Viral Load

- Diagnose cases of acute HBV.
- Diagnose replicative and non-replicative chronic HBV.
- Monitor patient response to antiviral therapy.

COBAS TaqMan (Roche)

- Lower Limit of Quantification: 6 IU/mL
- Upper Limit of Quantification: 100,000,000IU/mL
- Conversion Factor: 5.82

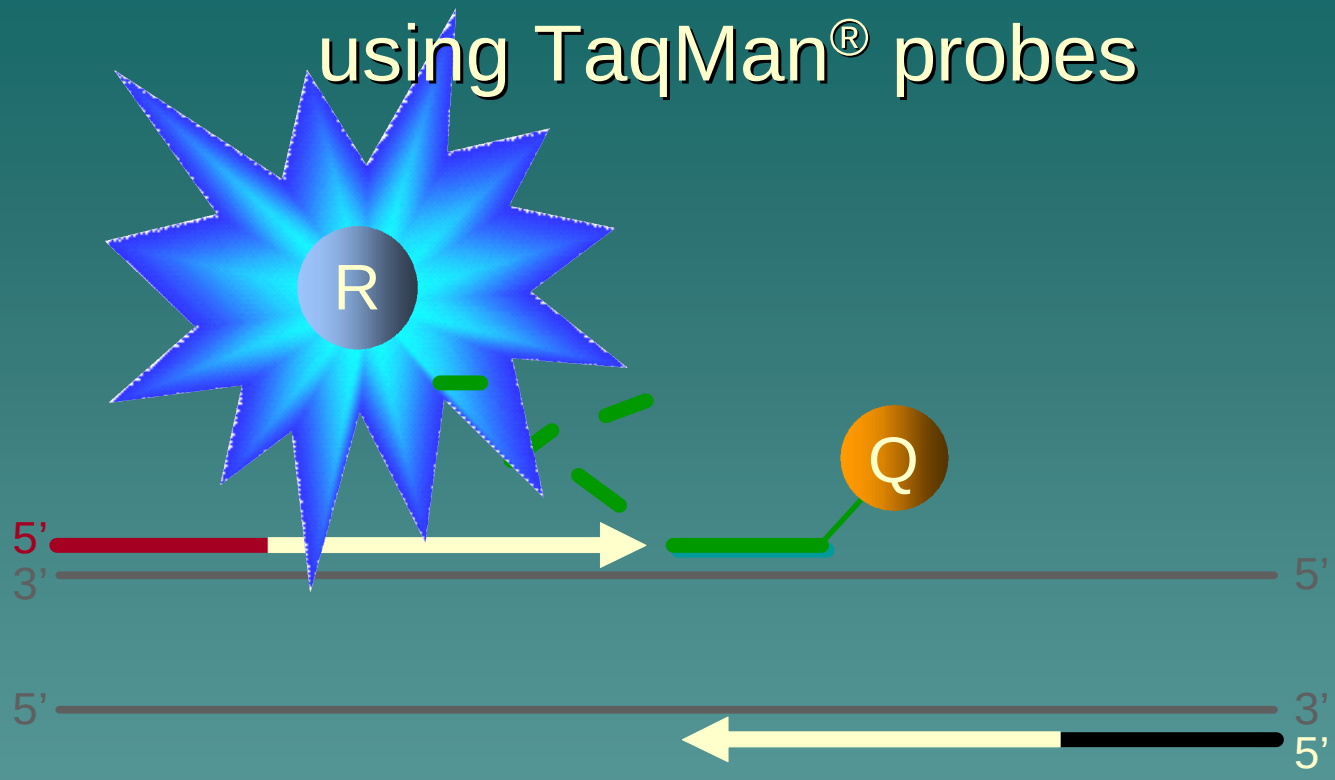
Real time PCR Methods

5' Nuclease Assay
using TaqMan® probes

- **PCR specificity (primer)**
- **Hybridization specificity (probe)**

5’ Nuclease Assay

using TaqMan® probes

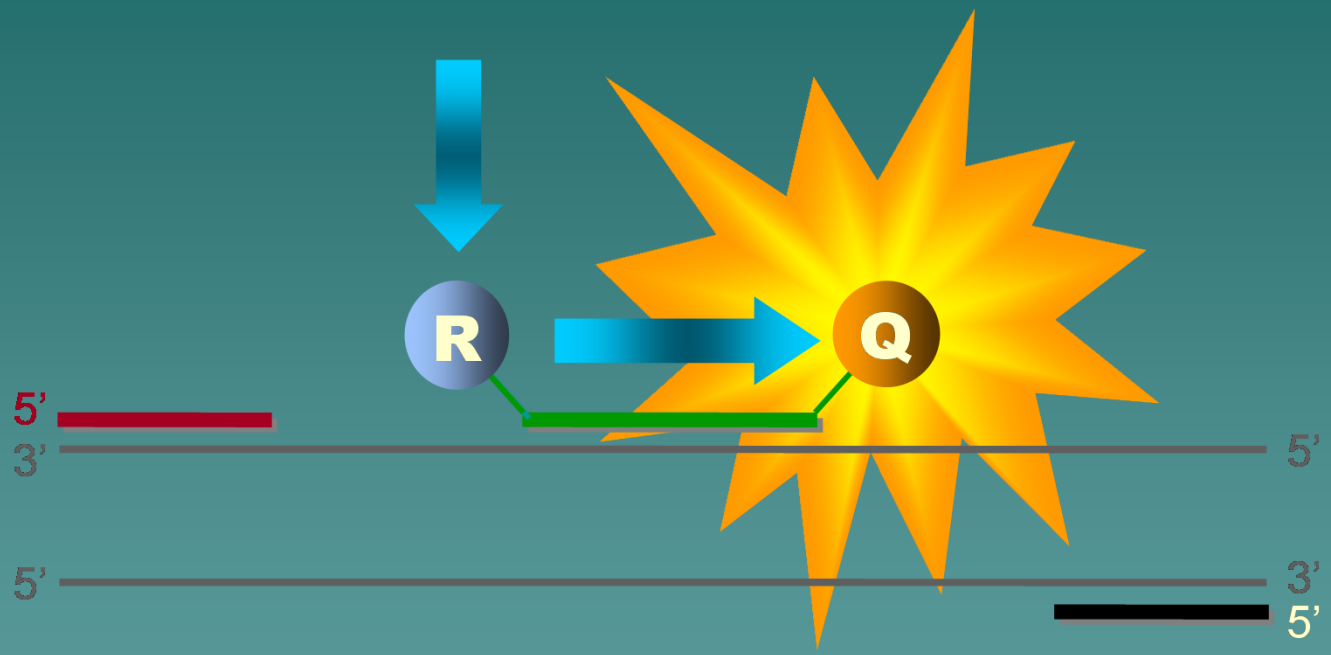


Cleavage of probe by 5‘ nuclease activity of Taq polymerase

➔ FRET disabled, generation of reporter signal

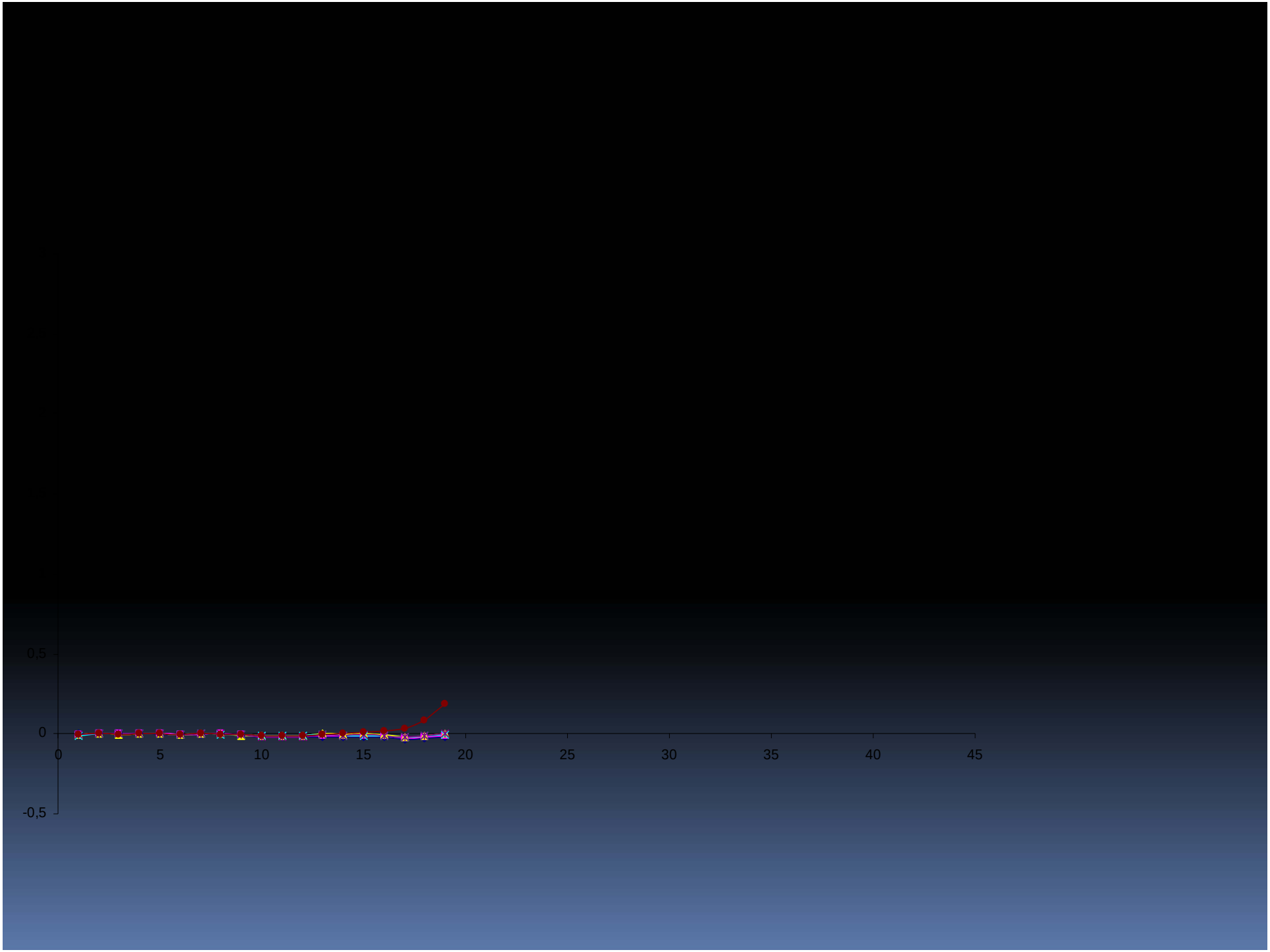
5’ Nuclease Assay

using TaqMan® probes



Fluorescence Resonance Energy Transfer (FRET) from high energy to low energy dye

➔ No reporter signal with intact probe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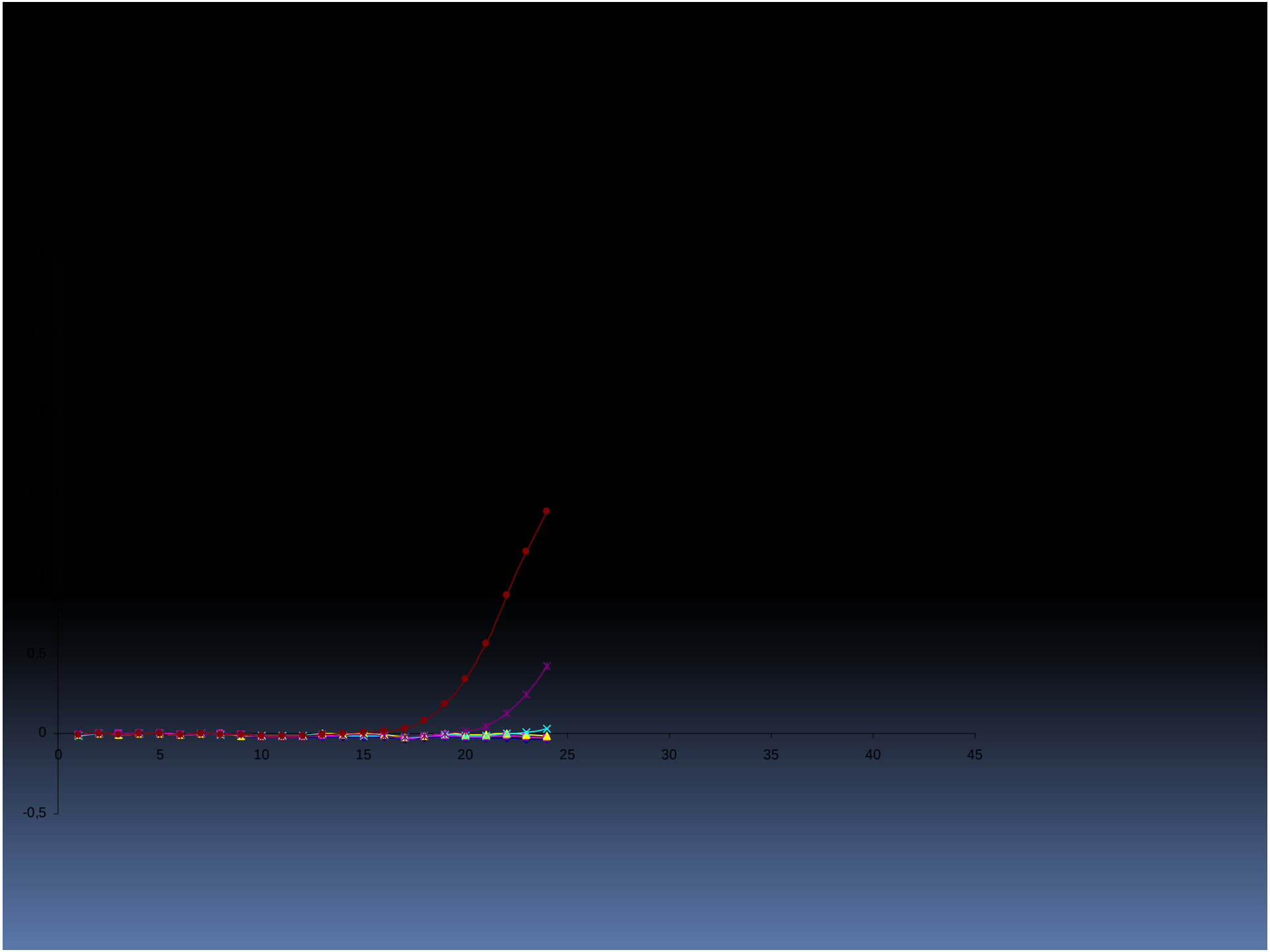
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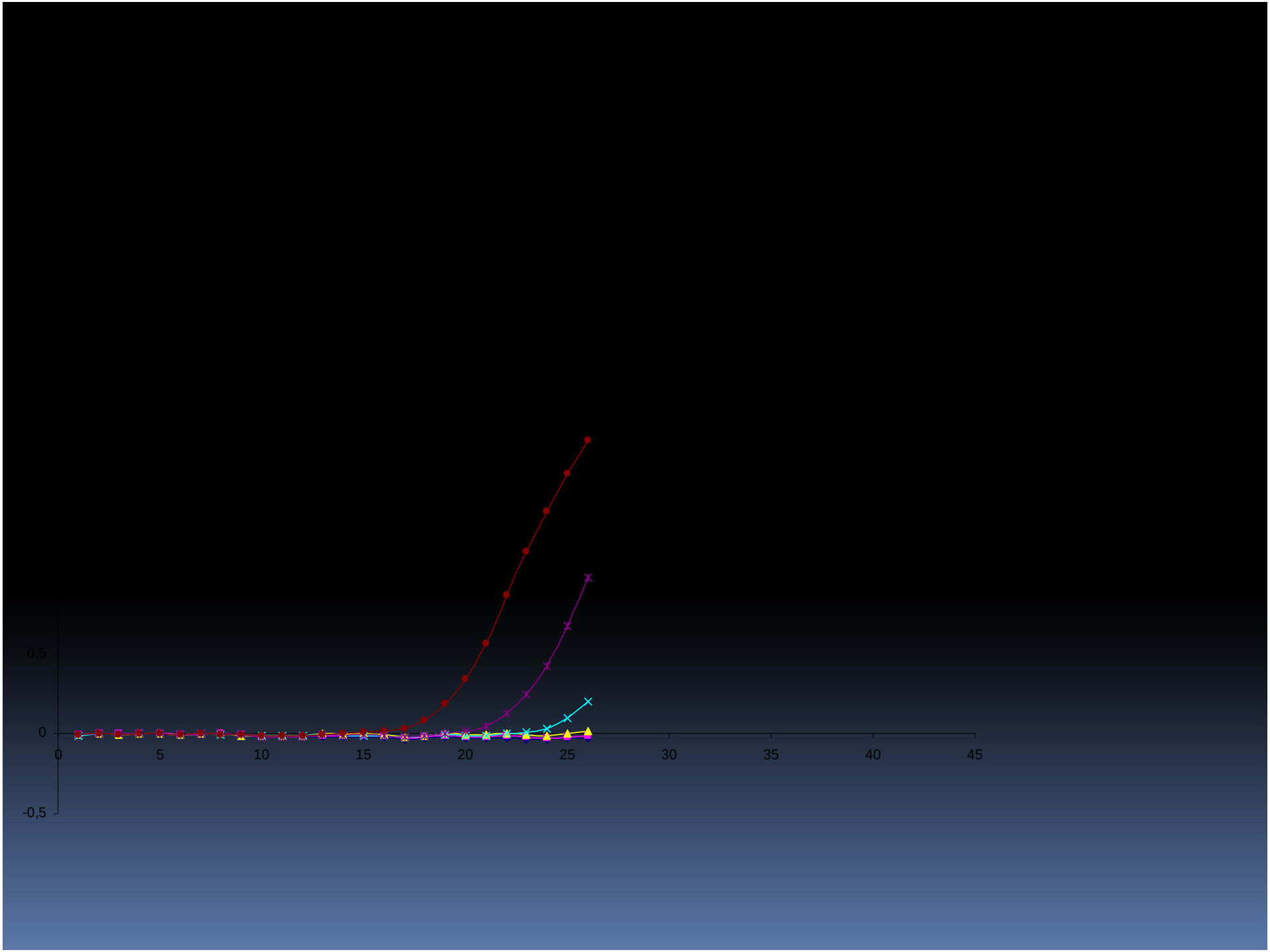
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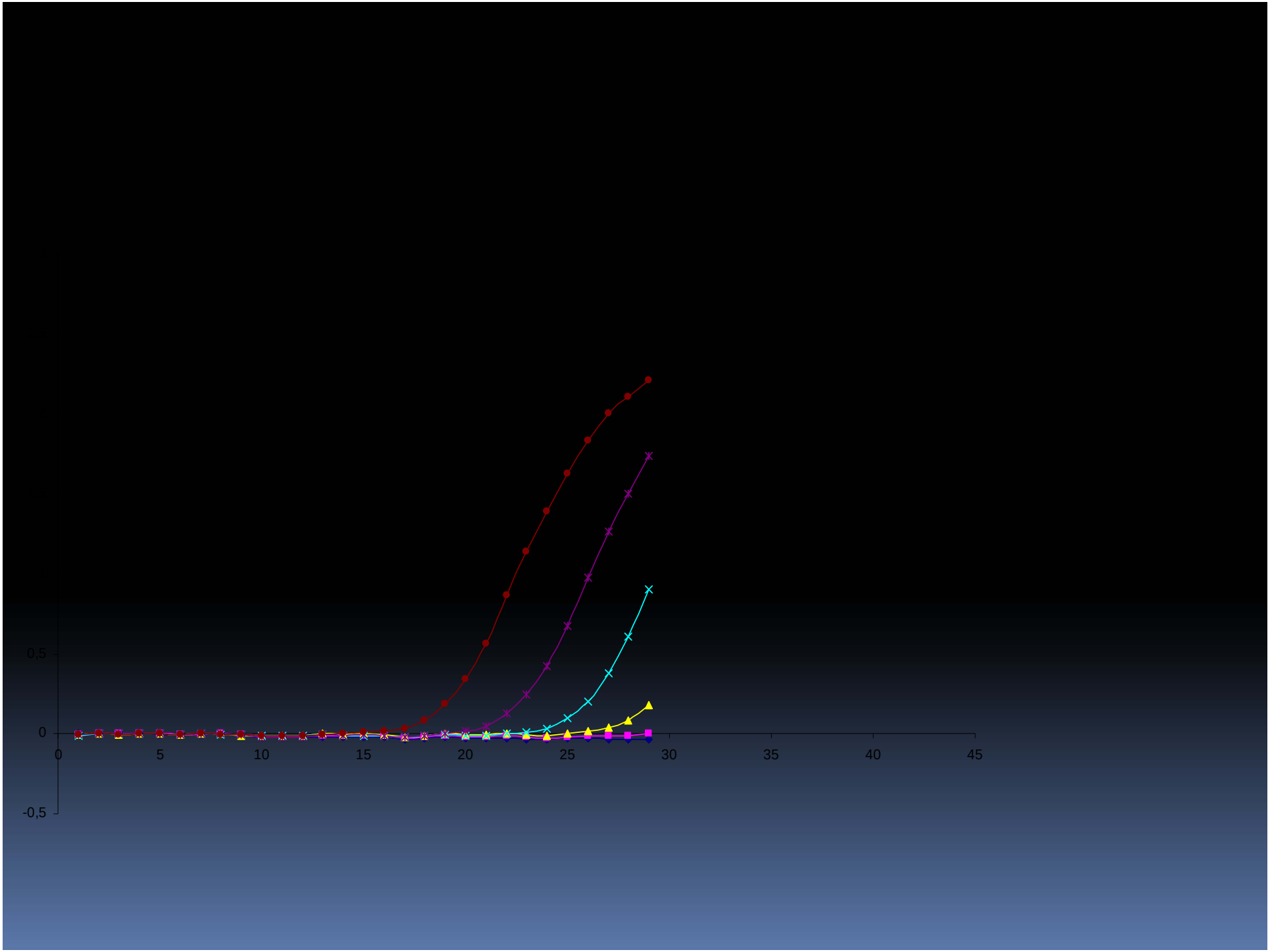
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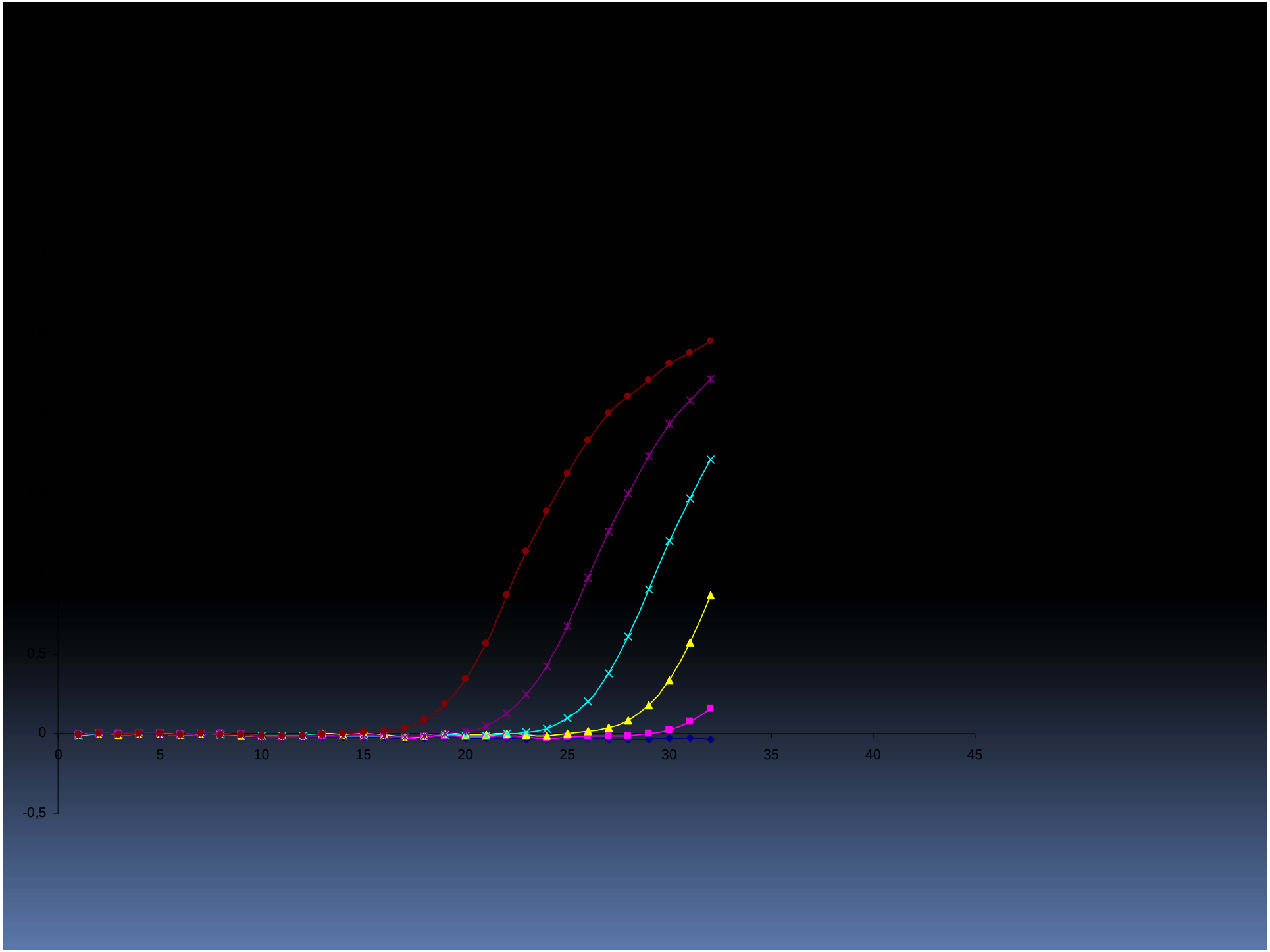
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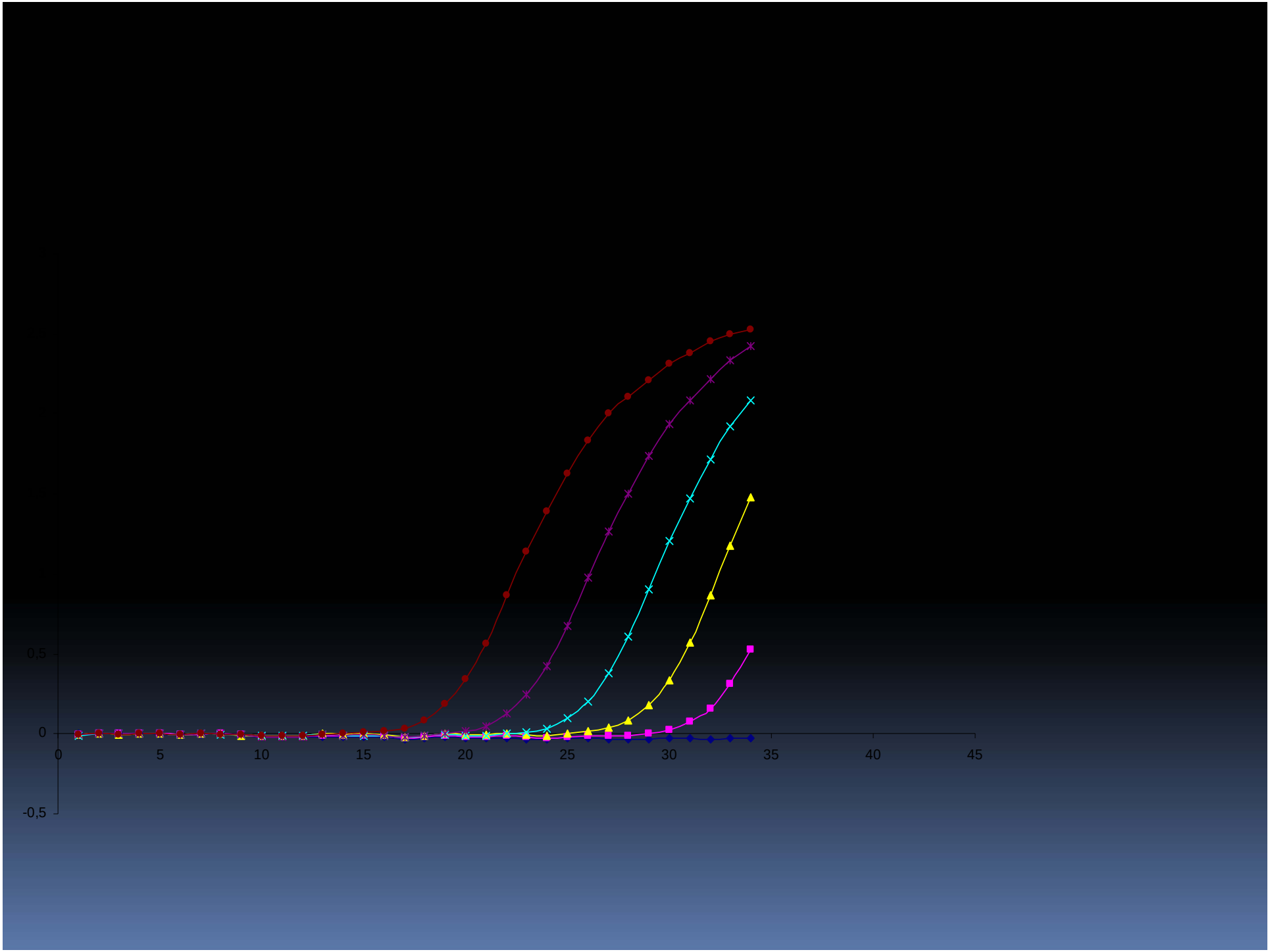
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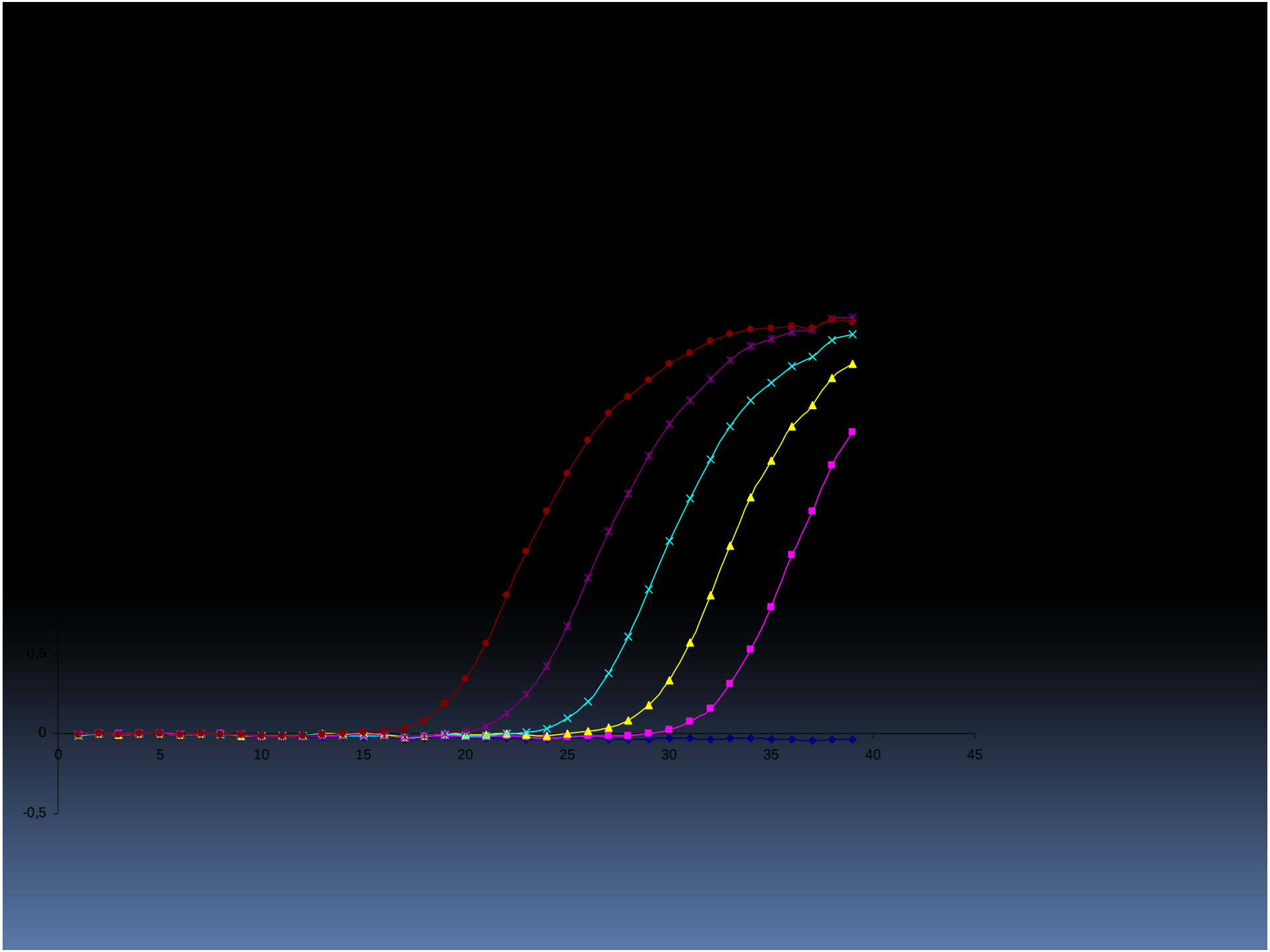
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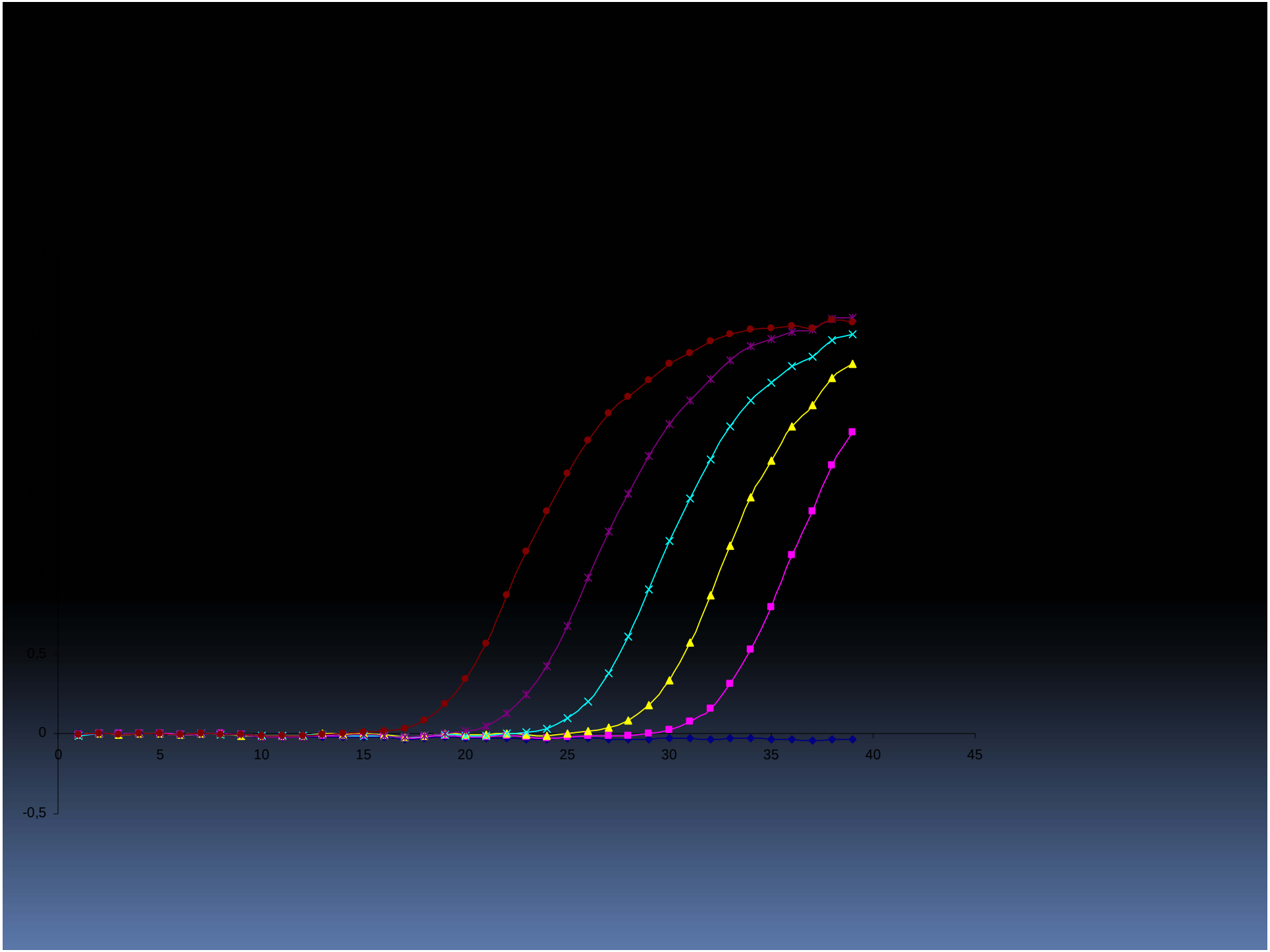
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- ✓ Given the varied technologies used to determined HBV DNA titer, considerable variation in results may occur when using different viral load tests.

Clinically Important Mutations in HBV

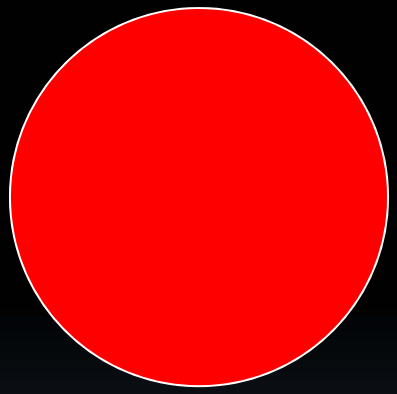
- PreCore/Core mutations
- Drug resistance Mutations

Drug Resistance & Viral fitness

- Generally monotherapy result in rapid suppression of viral replication but often not sustained because of emergence of drug resistance HBV variants.

- HBV generates up to 10^{12} viruses/day and because of the selective pressure of antivirals, mutants emerge.

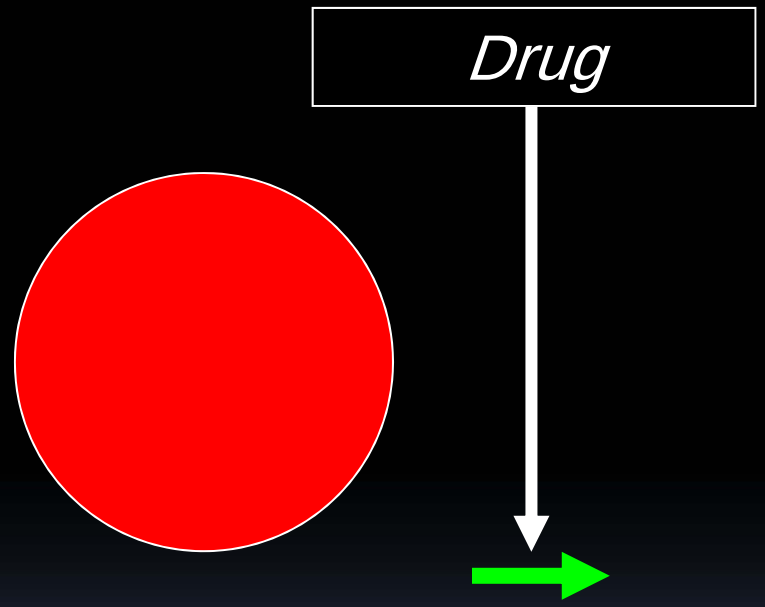
Mechanisms of Resistance



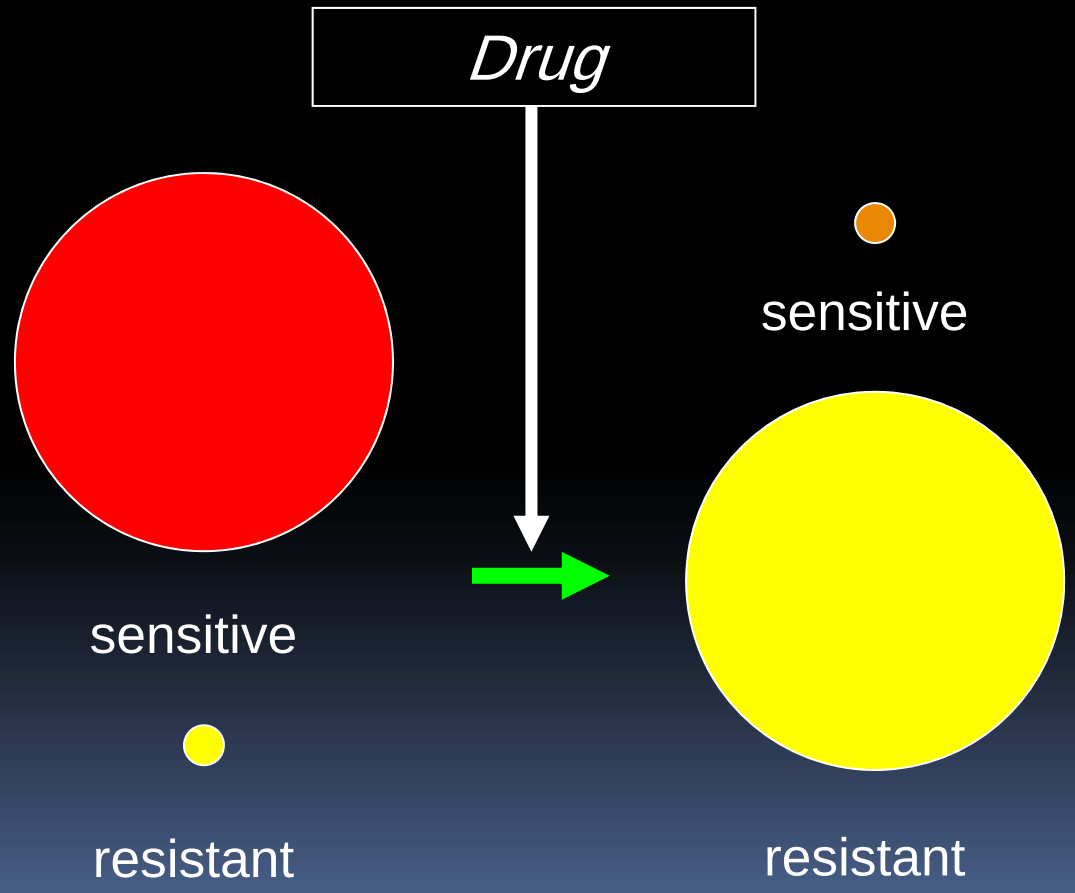
sensitive

resistant

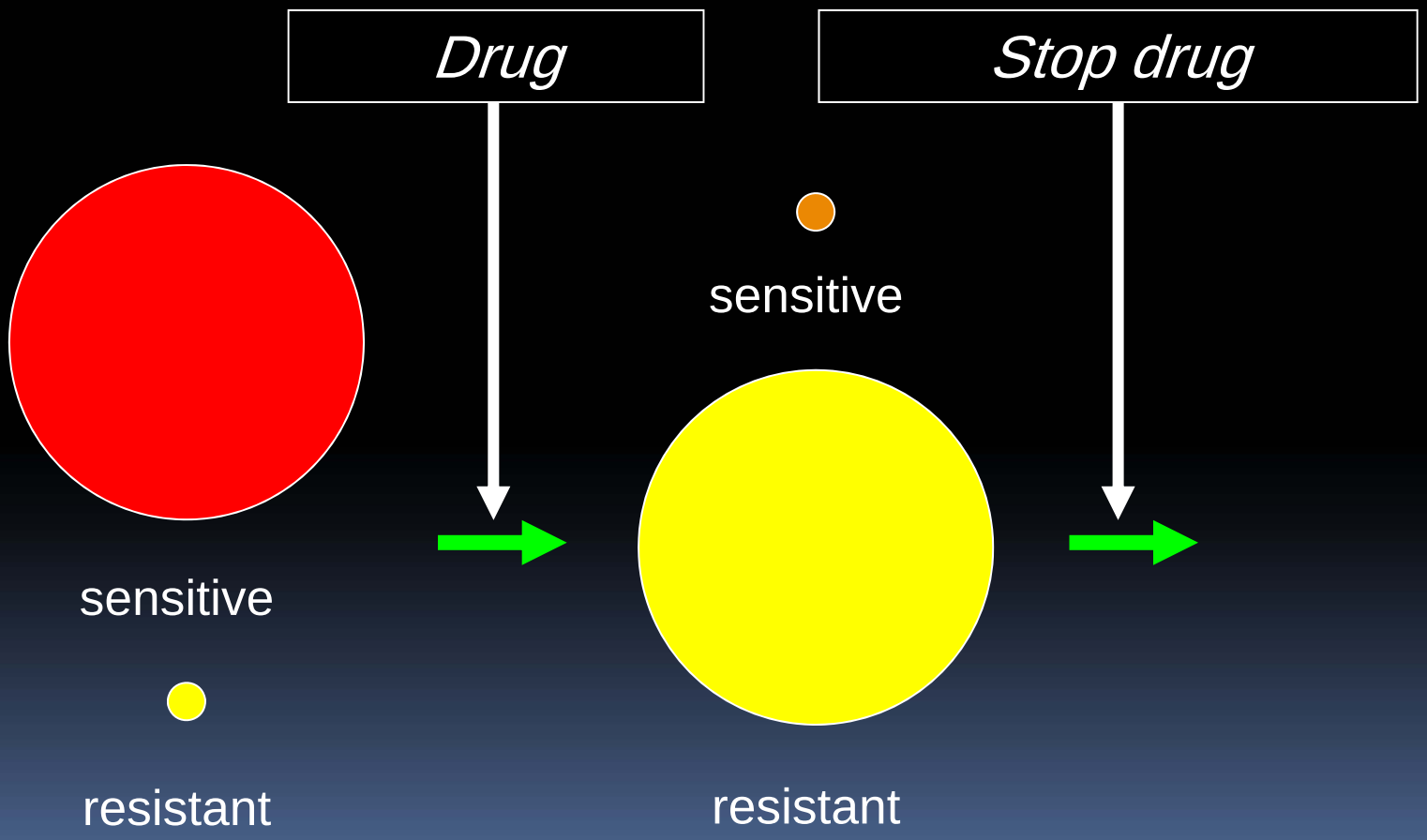
Mechanisms of Resistance



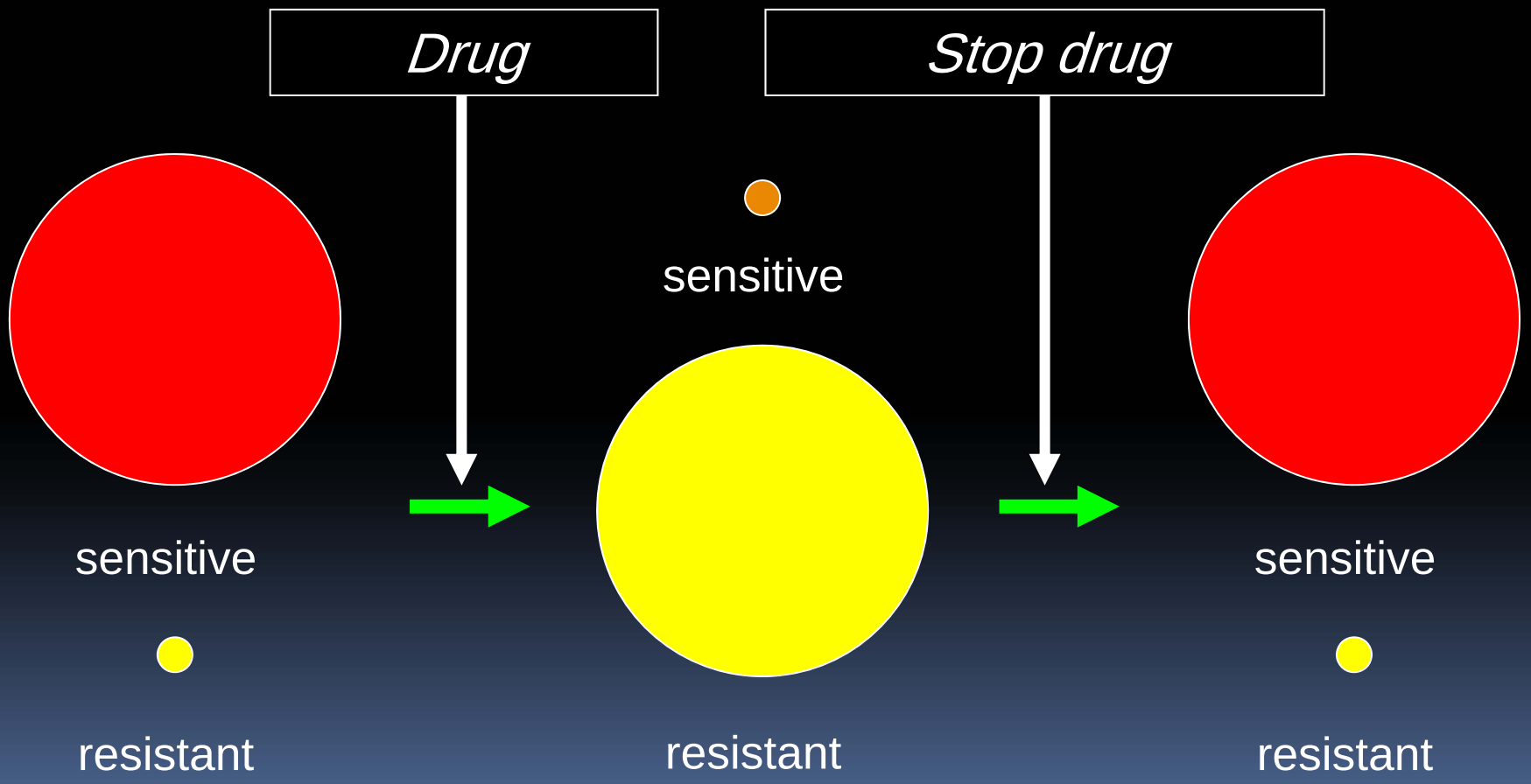
Mechanisms of Resistance



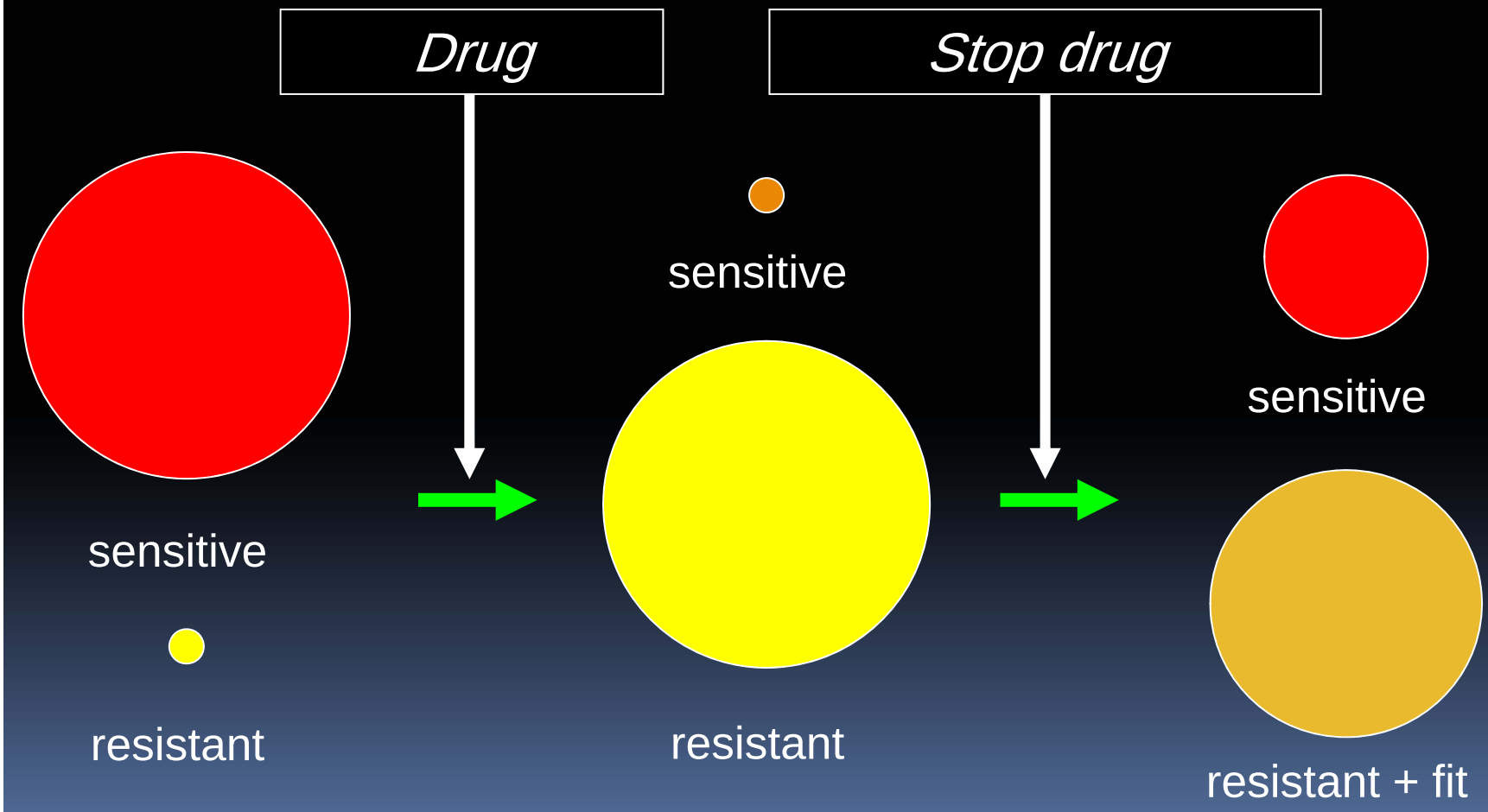
Mechanisms of Resistance



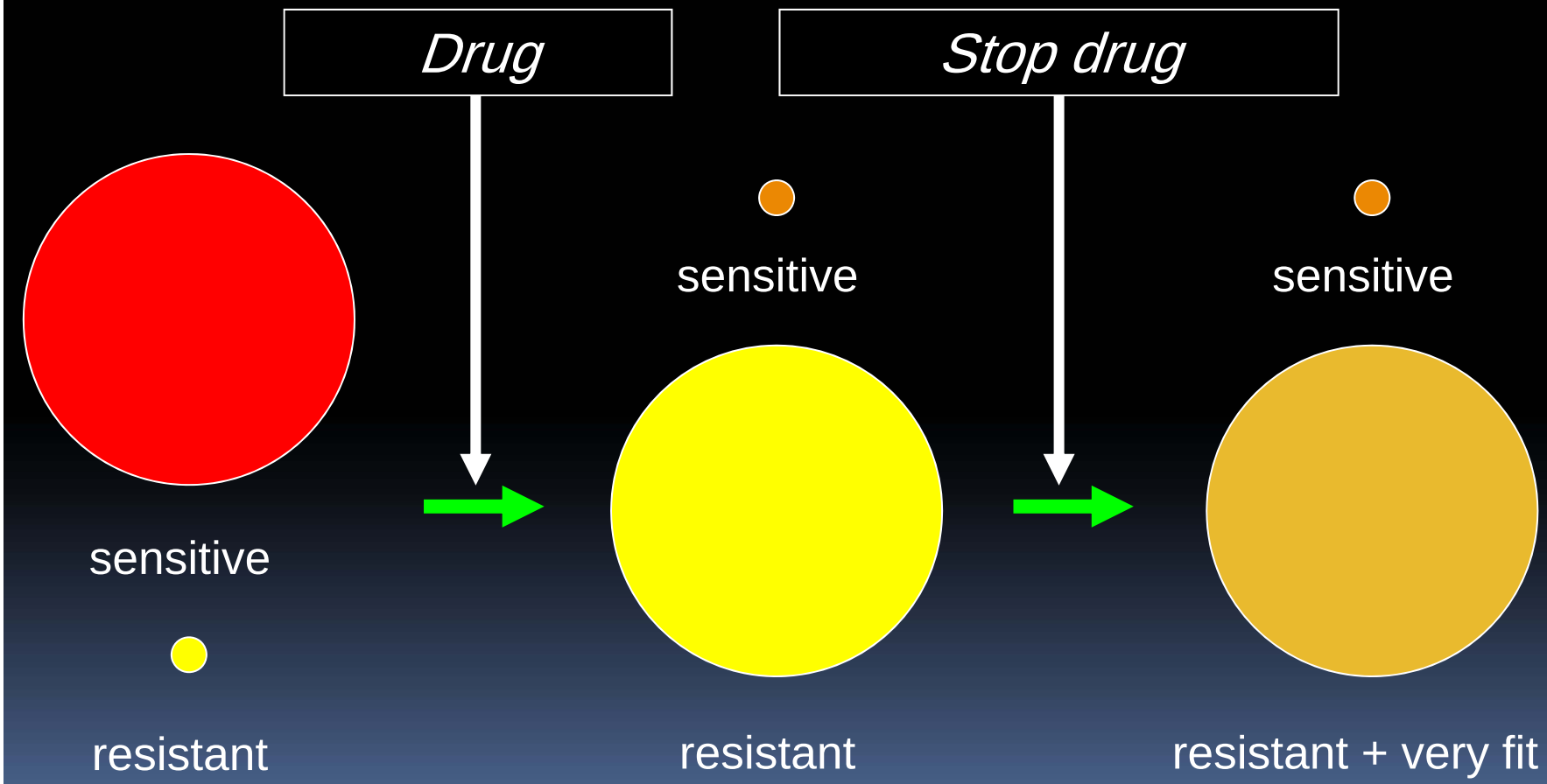
Mechanisms of Resistance

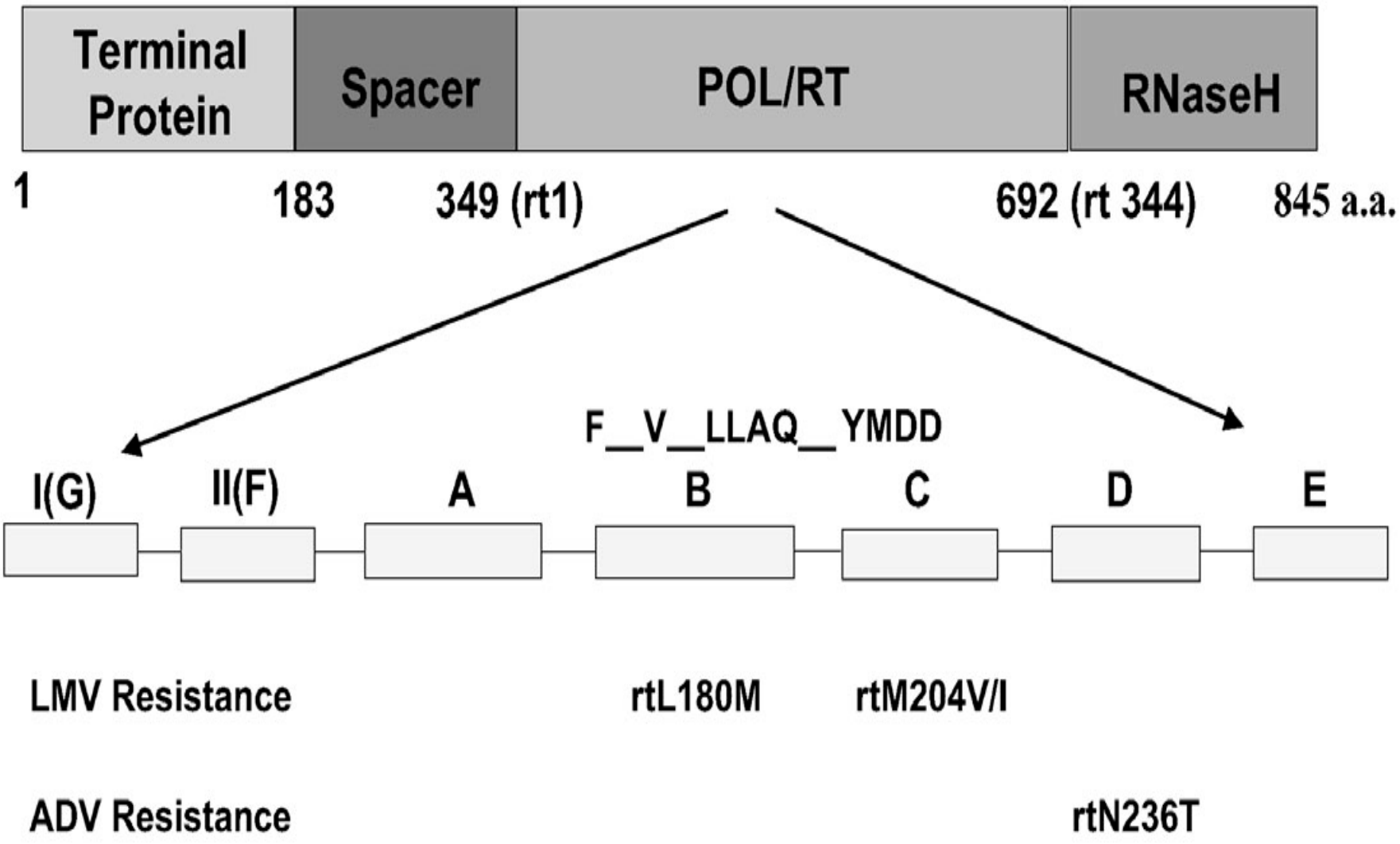


Mechanisms of Resistance



Mechanisms of Resistance

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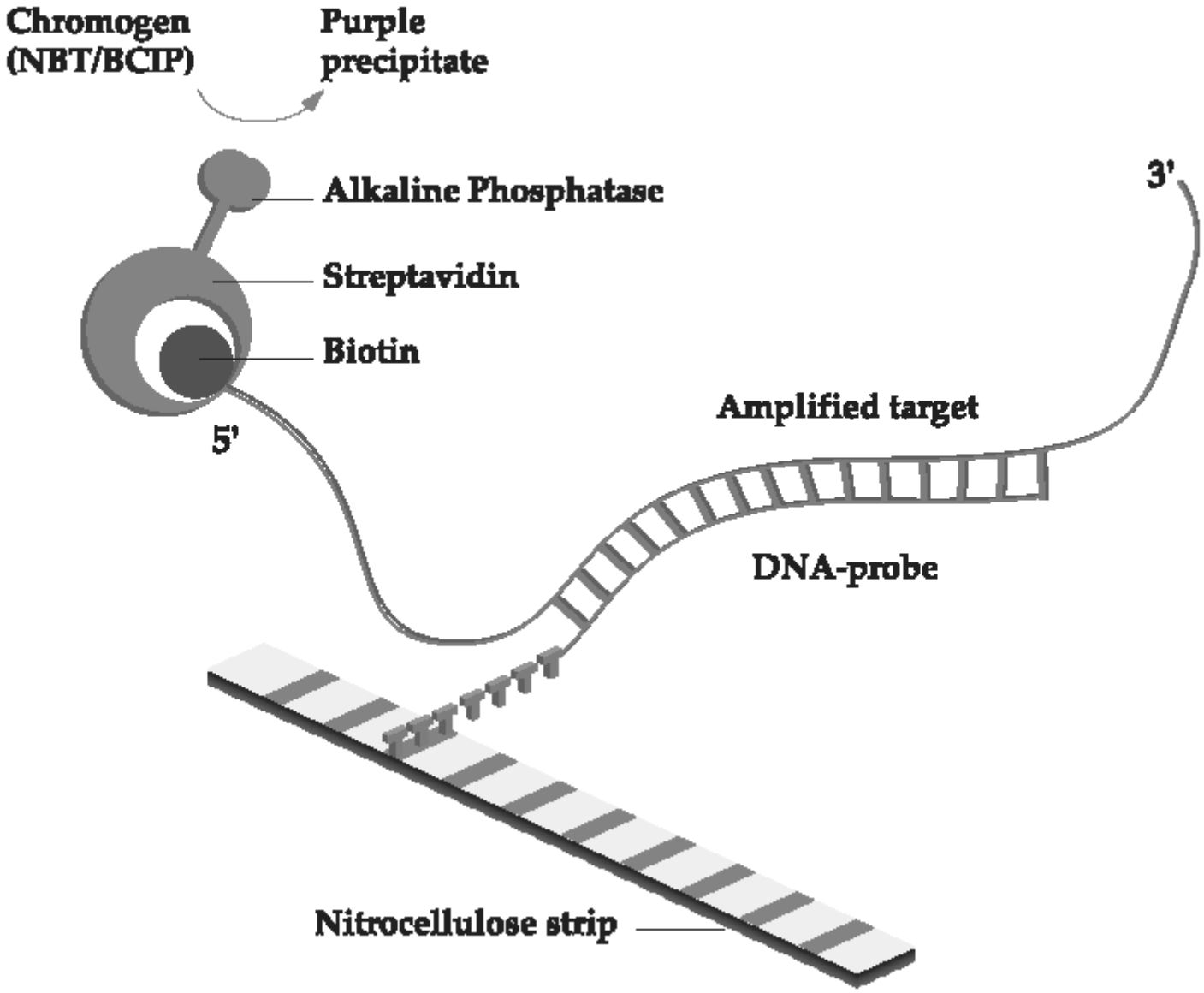
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Streptavidin
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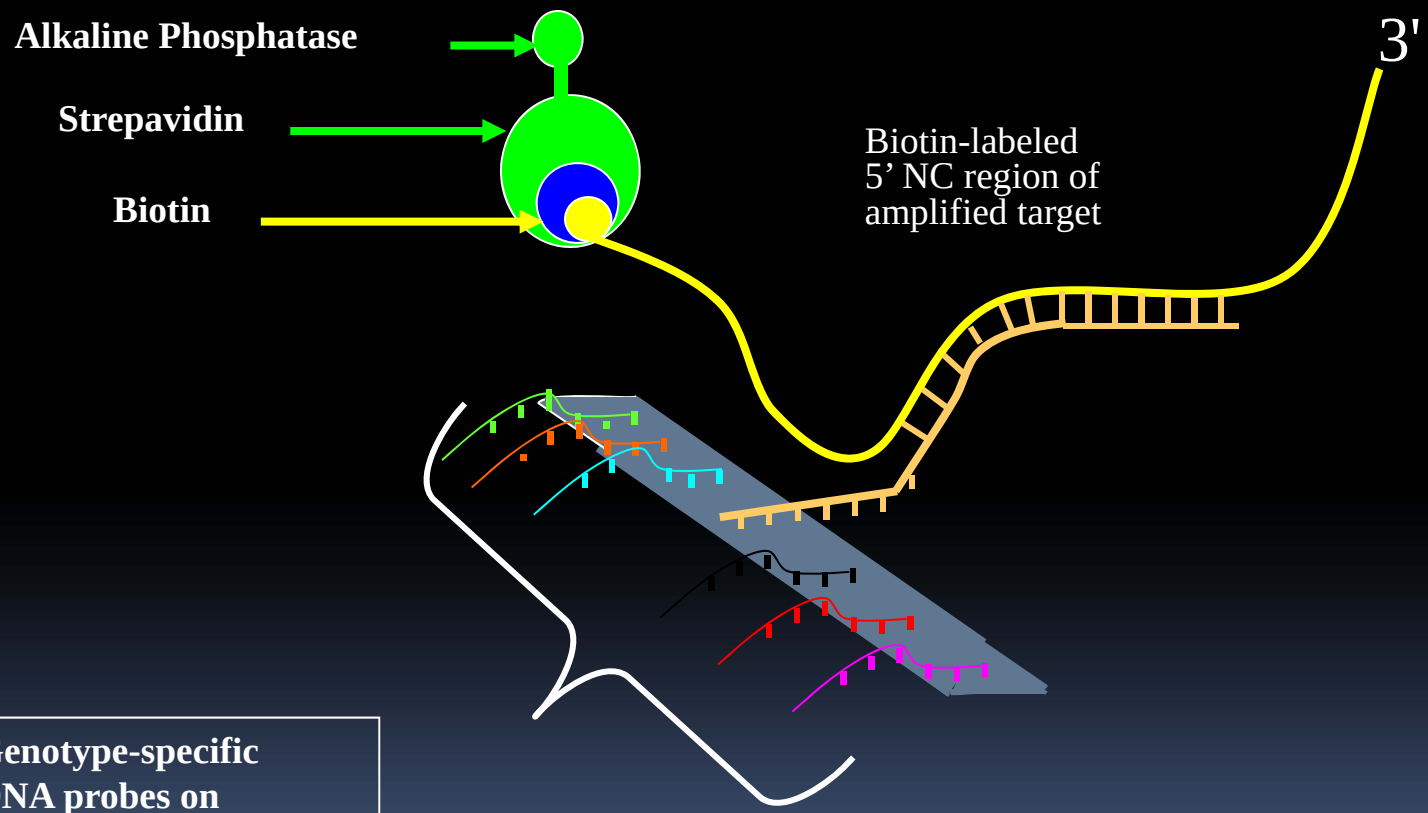
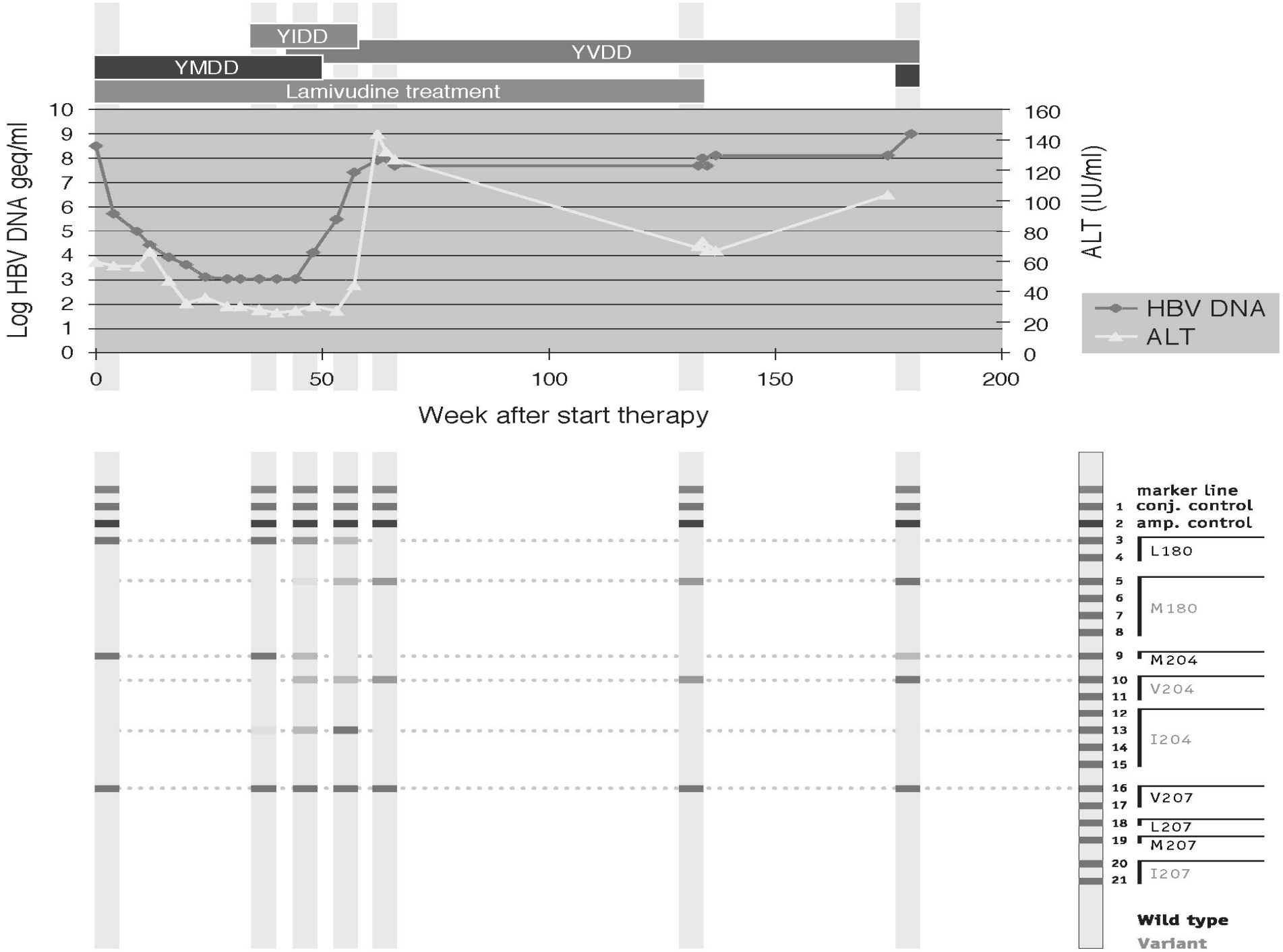
Amplified target
DNA-probe
Nitrocellulose strip

REVERSE DOT BLOT METHOD



Genotype-specific DNA probes on nitrocellulose strip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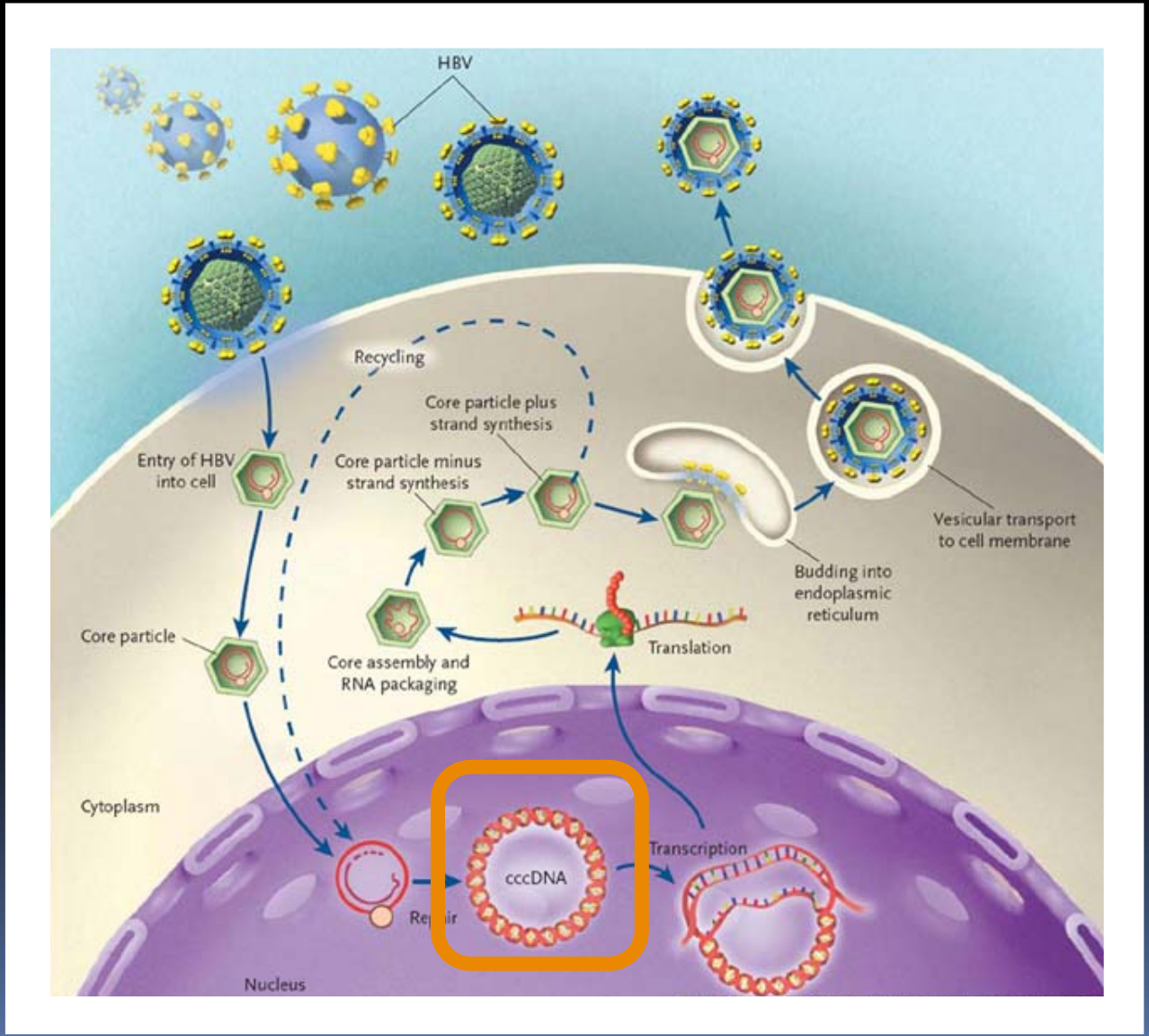
HBV resistance mutations can be archived in cccDNA



Thanks for your attention

Serologic markers

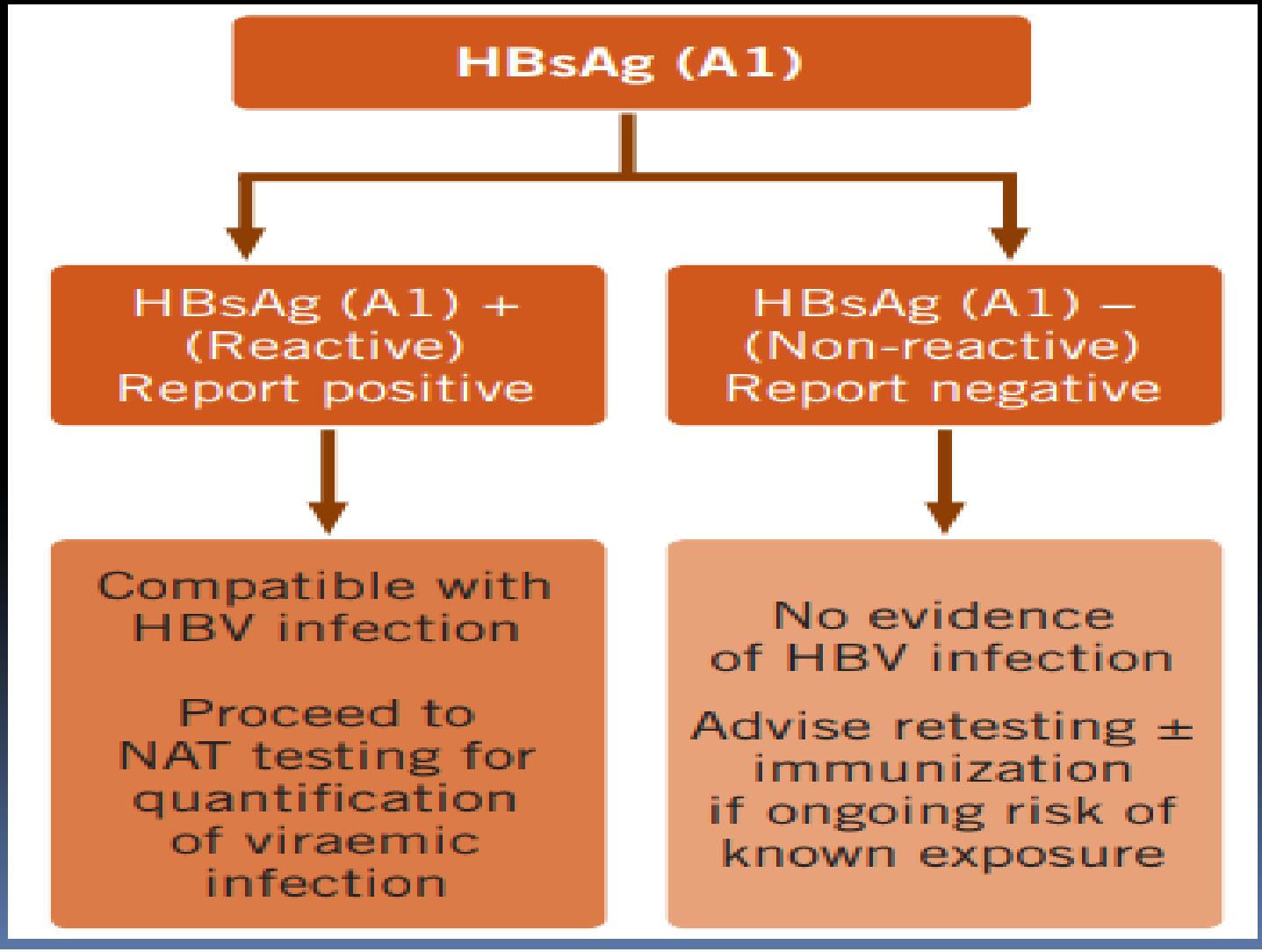
2. anti-HBs:

- In some patients, may not be detectable until after window period (the detection marker is IgM anti-HBc)
- in 5 -30% of anti-HBs-positive individuals the antibodies are unable to neutralize the circulating virions (carriers of the HBV)

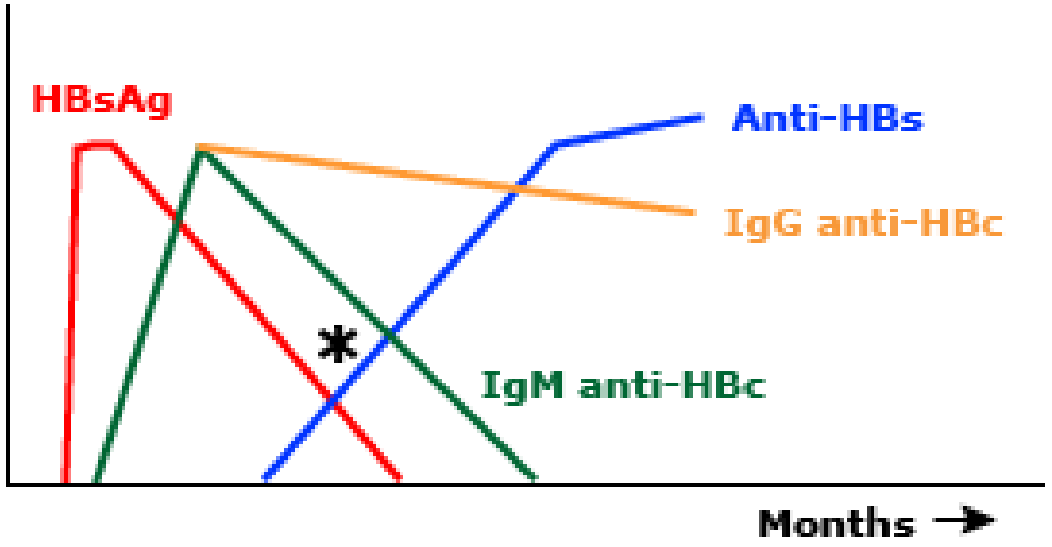
Quantitative HBsAg (cont.)

- HBs Ag clearance only means good immune control of the virus ,but HBV still can be detected inside the liver.
- Among patients who achieved HBsAg clearance after antiviral therapy, no virologic relapse has been reported.
- A negative HBsAg is actually a very low titer of HBsAg that is less than the detection limit of the commercial assay.

WHO-recommended for diagnosis of chronic HBV infection with HBsAg



Serologic marker



4. Anti-HBc:

- **IgM anti-HBc**

✓ During acute infection

✓ During the window period

✓ May remain detectable up to 2 years after the acute infection (during exacerbations of chronic hepatitis B)

It makes a diagnostic problem

1) Incorrectly suggesting acute hepatitis B, particularly in endemic areas with HBsAg+
2) Superinfection with hepatitis D virus (delta virus) or hepatitis C virus

Serologic markers

5. IgG anti-HBc:

- Persists along with anti-HBs in patients who recover from acute hepatitis B
- Persists along with HBsAg in those who progress to chronic HBV infection

Serologic markers

- **Isolated anti-HBc** in the absence of HBsAg and anti-HBs has been reported in 0.4 to 1.7 % of blood donors in low prevalence areas. It can occur in several settings:

Con …

- During the window period of acute hepatitis B
- When anti-HBs has fallen to undetectable levels after recovery from acute hepatitis B
- HBsAg mutations which lead to false-negative HBsAg results (This occurs when monoclonal instead of polyclonal hepatitis B surface antibodies in EIA).
- False-positive test result.

✓ To determine the etiology, repeat testing for anti-HBc, HBsAg, anti-HBs, and anti-Hbe.

Con…

- Transmission of HBV infection has also been reported from blood and organ donors with isolated anti-HBc. This may be due in part to the high percentage of patients with isolated anti-HBc (>70%) who have HBV DNA detected in the liver. Transmission can occur even when the serum HBV DNA is negative.

Serologic markers

6. HBeAg:
   - a marker of HBV replication and infectivity (higher rates of transmission)

Serologic markers

7. anti-HBe:

- associated with a decrease in serum HBV DNA and remission of liver disease
- some patients due to have low levels of wild-type HBV or HBV variants with pre-core mutations.

Serum HBV DNA assays

- Qualitative test
- Quantitative test

Con…

- Most patients who develop HBeAg seroconversion during nucleos/tide analogue therapy have undetectable serum HBV-DNA (<2 log(10) IU/mL). In fact, they remain HBeAg positive despite having undetectable serum HBV DNA for months or years.
- The explanation: the lack of direct effect of nucleos/tide analogues on ccc DNA and viral RNA transcription and viral protein expression

Con …

- The major clinical role of serum HBV DNA is to assess HBV replication and candidacy for antiviral therapy
- A cutoff of 20,000 IU/ml is proposed for treatment initiation in $HBeAg^{+}$ patients, and
- A lower threshold of 2000 IU/mL for $HBeAg^{-}$ patients

Diagnostic algorithms

Diagnostic tests to determine phase of acute or chronic hepatitis B virus infection

HBsAg	HBeAg	IgM anti-HBc	Total anti-HBc*	Anti-HBs	Anti-HBe	HBV DNA	ALT¶	Interpretation
Acute HBV infection								
+	+	+				+++	Elevated	Early phase
		+				+	Elevated	Window phase
			+	+	+	±	Normal	Recovery phase
Chronic HBV infection (HBsAg-positive for >6 months)								
+	+		+	-	-	+++ (Serum HBV typically >1 million international units/mL)	Normal or mildly elevated	Immune-tolerant phase
+	+		+	-	-	+++ (Serum HBV >20,000 international units/mL)	Persistently elevated	Immune-active, HBeAg-positive
+	-		+	-	+	++ (Serum HBV >2000 international units/mL)	Elevated	Immune-active, HBeAg-negative
+	-		+		+	- to ++ (Serum HBV ≤2000 international units/mL)	Normal or mildly elevated	Inactive chronic HBV
-	-		± (generally +)	±	±	+ in liver; - to + in serum	Normal	Occult HBV