*The quantity of target nucleic acid may be insufficient, or the patient specimen may contain substances that interfere with or cross-react in the hybridization and signal-generating reactions.

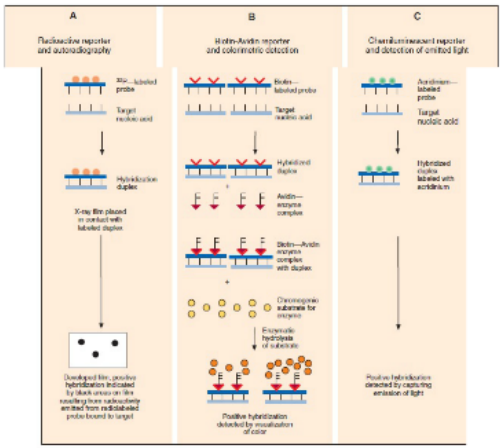
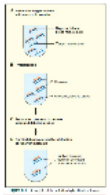
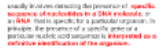


Figure 8–2 Reporter molecule labeling of nucleic acid probes and principles of hybridization detection. Use of probes labeled with a radioactive reporter, with hybridization detected by autoradiography (A); probes labeled with biotin-avidin reporter, with hybridization detected by a colorimetric assay (any probe labeled with chemiluminescent reporter (i.e., acridinium), with hybridization detected by a luminometer to detect emitted light (C).

Detection of Hybridization:
The method of detecting hybridization depends on the reporter molecule used for labeling probe nucleic acid and on the hybridization format (see Figure 8–2). Detection of hybridization using **radioactively labeled** probes is done by exposing the reaction mixture to radiograph film (i.e., autoradiography). Hybridization with **nonradioactively labeled probes is detected using colorimetry, fluorescence, or chemiluminescence**, and detection can be somewhat automated using spectrophotometers, fluorometers, or luminometers, respectively.
The more commonly used nonradioactive detection systems (e.g., **digoxigenin, chemiluminescence**)





ORGANISM IDENTIFICATION USING GENOTYPIC CRITERIA

usually involves detecting the presence of **specific sequence of nucleotides in a DNA molecule**, or an **RNA** that is specific for a particular organism. In principle, the presence of a specific gene or a particular nucleic acid sequence is **interpreted as a definitive identification of the organism.**

NUCLEIC ACID HYBRIDIZATION METHODS

Hybridization methods are based on the ability of two nucleic acid strands that have complementary base sequences (i.e., are homologous) to specifically bond with each other and form a double-stranded molecule, or duplex or hybrid.

Hybridization Steps and Components

The basic steps in a hybridization assay include:
1. Production and labeling of single-stranded probe nucleic acid
2. Preparation of single-stranded target nucleic acid
3. Mixture and hybridization of target and probe nucleic acid
4. Detection of hybridization

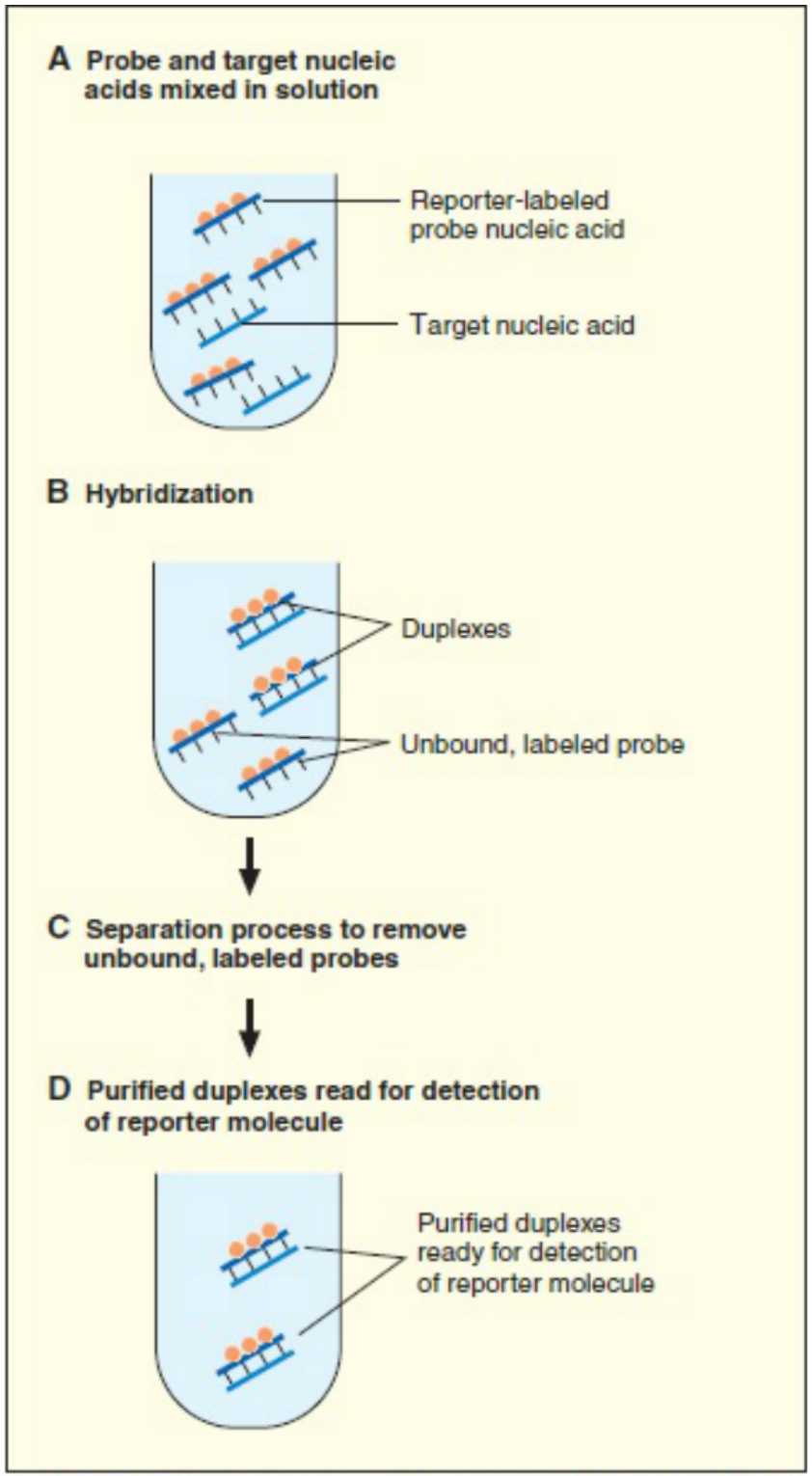


Figure 8-3 Principle of the solution hybridization format.

Detection of Hybridization:

The method of detecting hybridization depends on the reporter molecule used for labeling probe nucleic acid and on the hybridization format (see Figure 8-2). Detection of hybridization using **radioactively labeled** probes is done by exposing the reaction mixture to radiograph film (i.e., autoradiography) . Hybridization with **nonradioactively labeled probes is detected using colorimetry, fluorescence, or chemiluminescence**, and detection can be somewhat automated using spectrophotometers, fluorometers, or luminometers, respectively.
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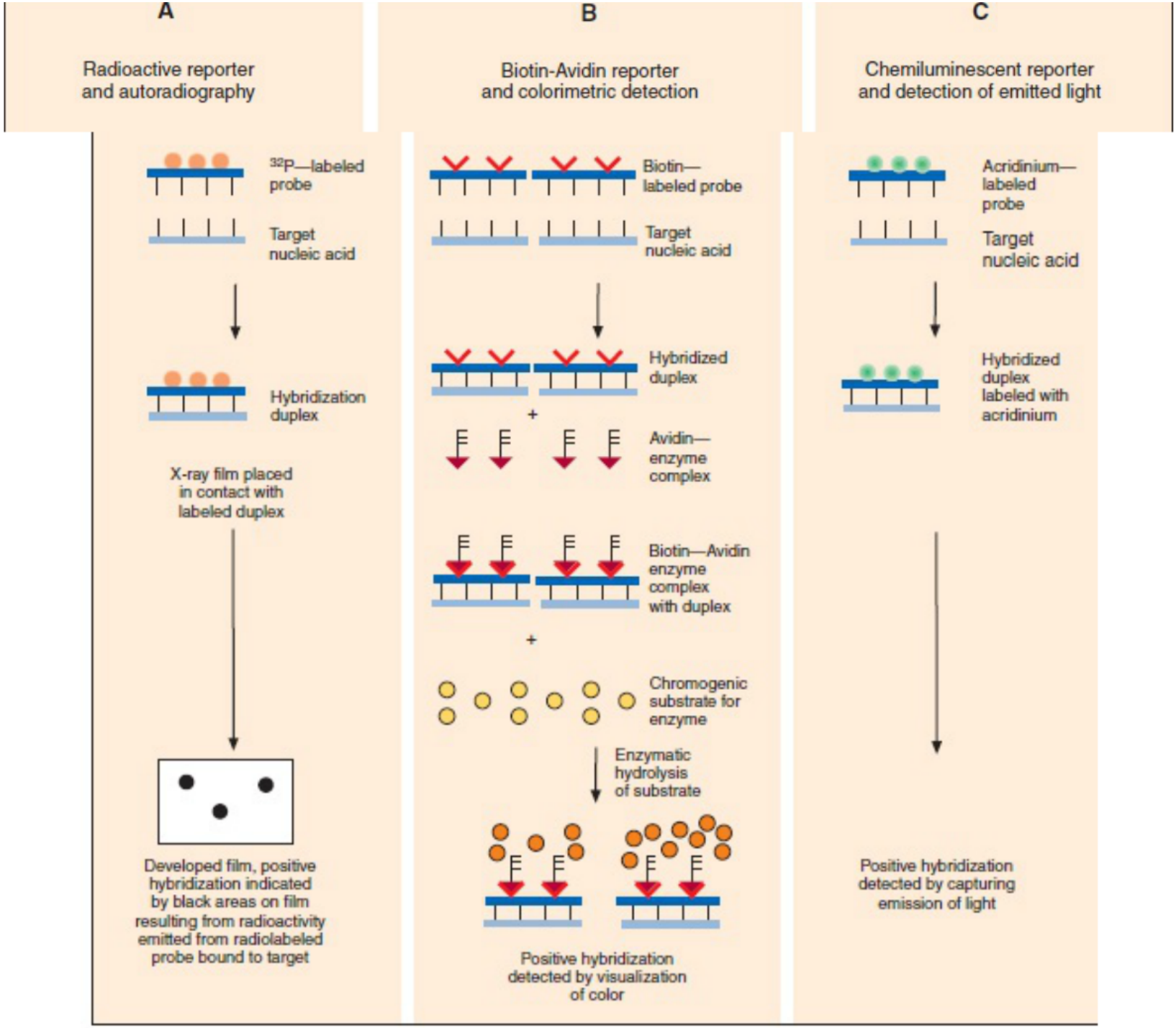

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