Hydrogen Peroxide Monitoring: Ampoules vs. Test Strips – What's Right for You?

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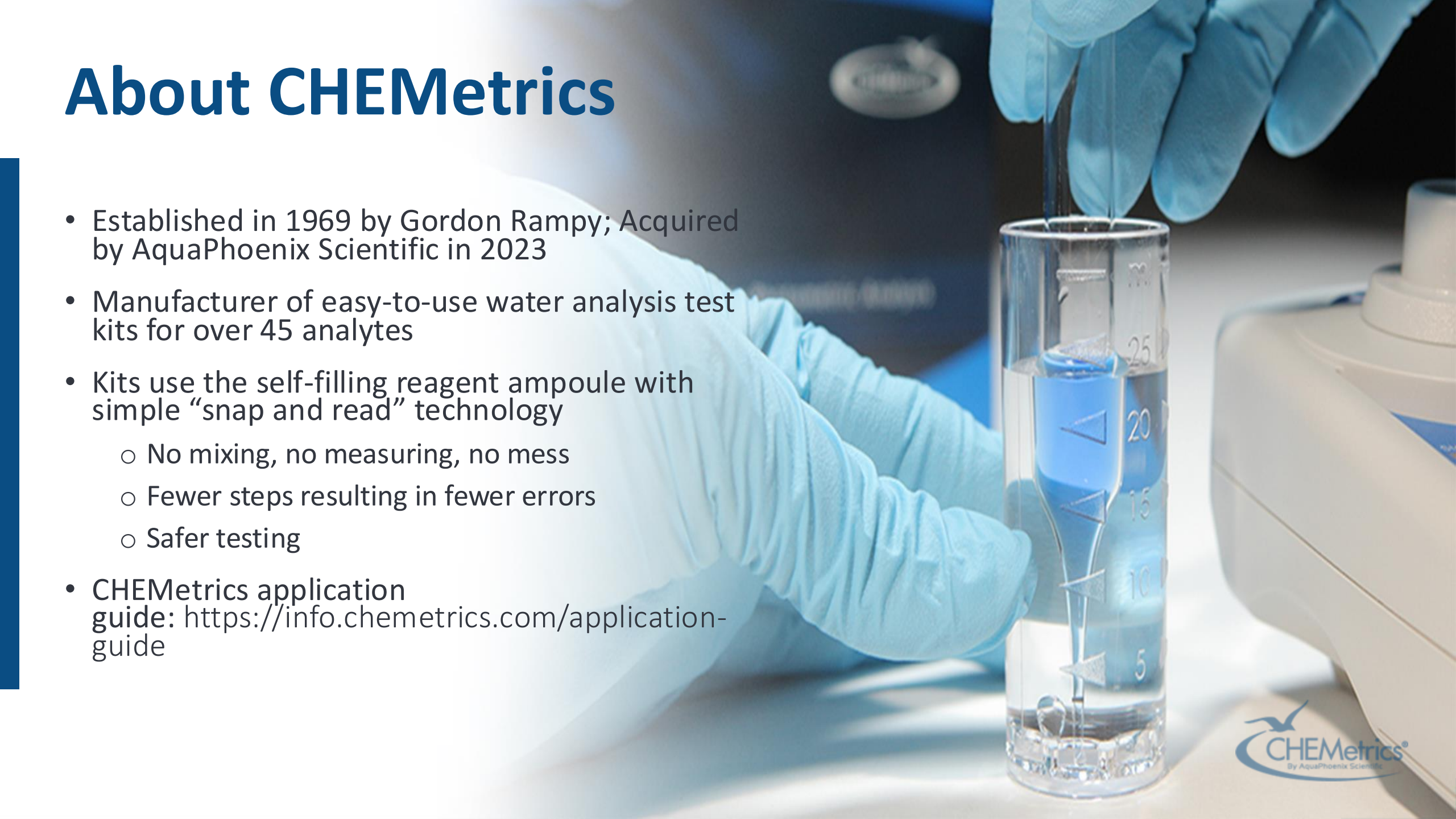
CHEMetrics®
By AquaPhoenix Scientific

About CHEMetrics

- Established in 1969 by Gordon Rampy; Acquired by AquaPhoenix Scientific in 2023
- Manufacturer of easy-to-use water analysis test kits for over 45 analytes
- Kits use the self-filling reagent ampoule with simple “snap and read” technology
  - No mixing, no measuring, no mess
  - Fewer steps resulting in fewer errors
  - Safer testing
- CHEMetrics application guide: https://info.chemetrics.com/application-guide



Hydrogen Peroxide Intro and Role in Sterilization

Mechanism of Action:

- **Oxidative Stress Induction:** H_2O_2 decomposes into water and oxygen, generating reactive oxygen species (ROS) such as hydroxyl radicals (•OH). These ROS aggressively attack microbial cell components—lipids, proteins, and nucleic acids—leading to irreversible damage and cell death.
- **Broad-Spectrum Antimicrobial:** Demonstrates efficacy against a wide range of microorganisms including Gram-positive and Gram-negative bacteria, viruses, yeasts, and molds.
- **Application in Sterilization:** Commonly used at 35% concentration for sterilizing food and pharmaceutical packaging. Post-application, hot air drying ensures residual H_2O_2 levels remain below the FDA limit of 0.5 ppm.

Advantages in Sterilization:

- **High Efficacy:** Capable of inactivating resistant spores such as *Bacillus subtilis*, often used in validation protocols.
- **Environmentally Sustainable:** Breaks down into non-toxic byproducts (H_2O and O_2), eliminating concerns about harmful residues or environmental contamination.
- **Worker Safety:** Safer than many chemical sterilants when used in controlled environments with proper ventilation and handling protocols.
- **Regulatory Compliance:** Widely accepted by regulatory bodies (e.g., FDA, EPA) for use in aseptic processing and sterilization.

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Aseptic Packaging

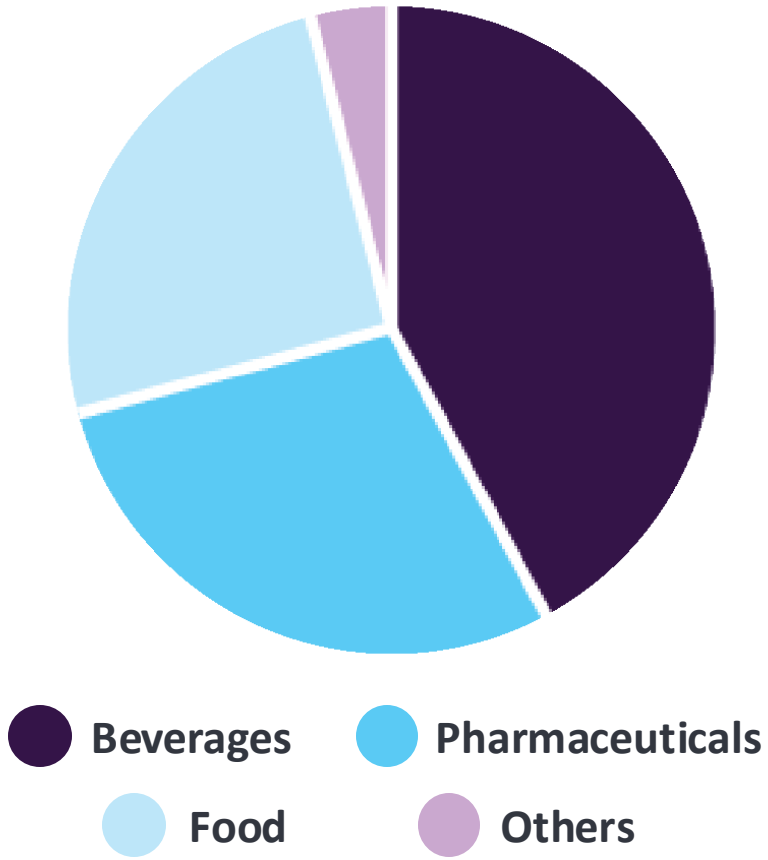
“Aseptic packaging is the process by which microorganisms are prevented from entering a package during and after packaging.

During aseptic processing, a sterilized package is filled with a commercially sterile food product and sealed within the confines of a hygienic environment.”

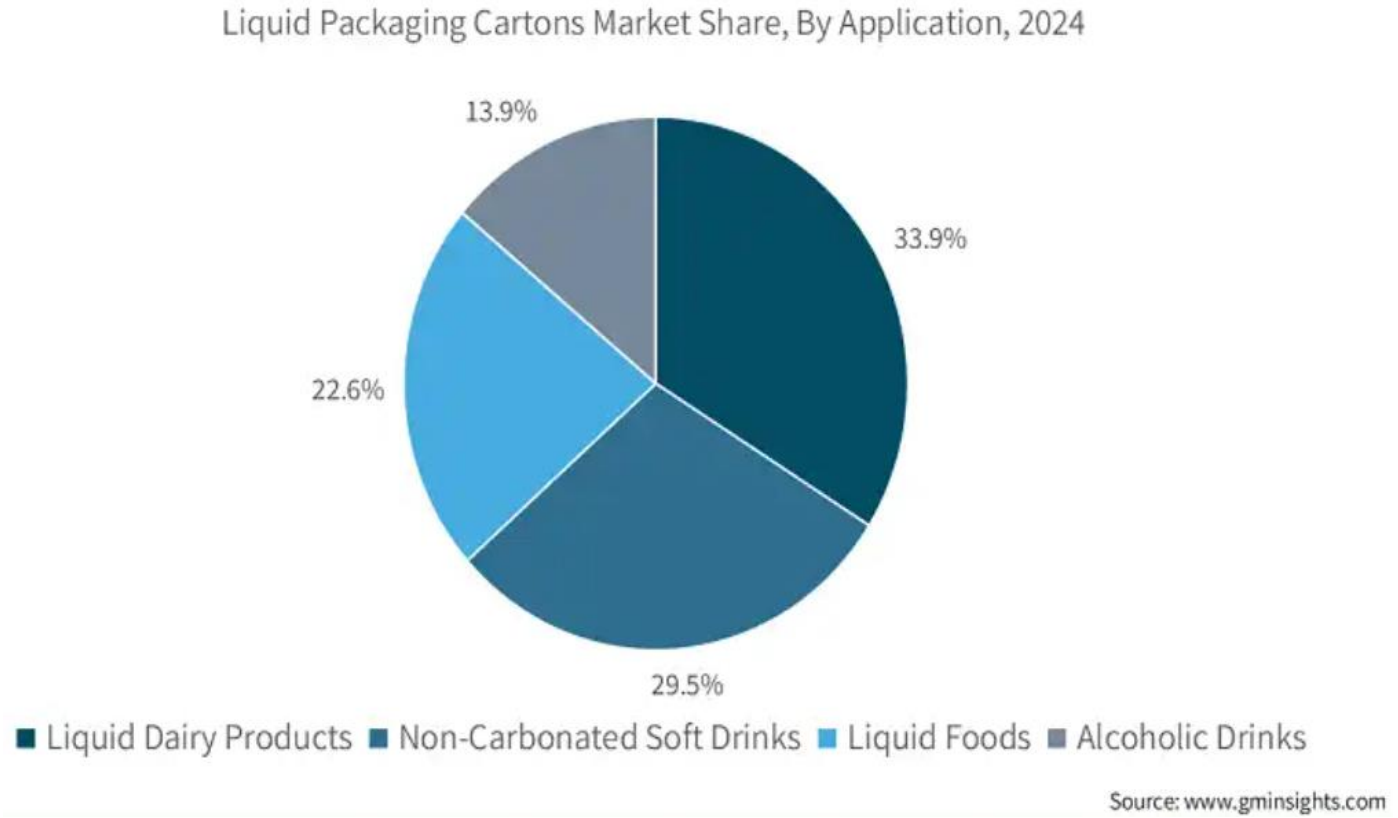
Current market evaluation for Aseptic Packaging

Aseptic Packaging Market

Share, by Application, 2024 (%)



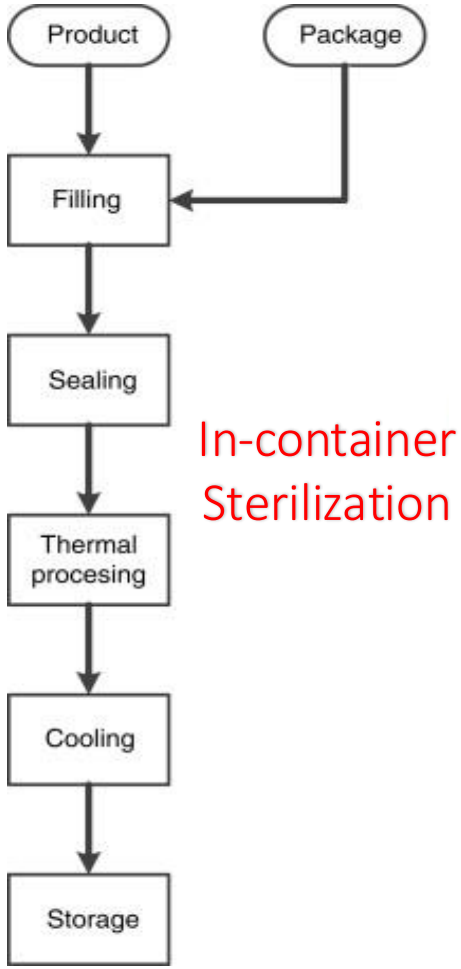
Aseptic packaging industry-wide market size is estimated at 72 billion USD according to Fortune Business Insights, comprising primarily Pharmaceuticals, Food and especially Beverages.



Overview of Aseptic Filling and Packaging Systems

Category	Examples of Systems
1. Metal and rigid containers sterilized by heat	Dole canning systems
Steam/metal containers	Drum fillers (e.g., Scholle, FranRica, Imdec)
Hot air composite can	Dole hot air system
2. Webfed paperboard sterilized by H2O2	Tetra Pak (Brik Pak), International Paper
3. Preformed paperboard containers	Combibloc, LiquiPak
4. Preformed rigid/plastic containers	Metal Box, Fresh Fill, Gasti, Croschecke
5. Thermoform-fill seal	Benco AsepacK, Bosch Servac, Conofast, Thermoforming USA
6. Flexible plastic containers	
• Bag-in-box type	Scholle, LiquiBox
• Pouches	Asepax, Prepac, Prodo Pak
• Blowmolded	Bottlepack, Serac

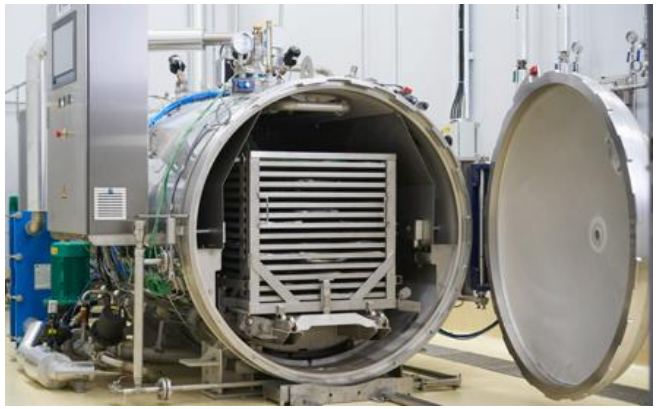
Conventional (Terminal) Retort Processing



In-container Sterilization

Retort Processing is a form of Terminal Processing

- Method of thermally sterilizing food in its packaging
- Three-Stage Sterilization Cycle
- Limited to some materials like metal, glass, and aluminum-foil laminates
- The necessary prolonged heat exposure degrades the product's nutritional value and sensory qualities



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Sterilization Techniques

High Temperature

Moist Heat / Steam
Saturated steam at under pressure. Gold Standard for the sterilization of many products.

Dry Heat
Dry, heated air, either by natural convection or forced air circulation.

Low Temperature

Radiation

Gamma
Ionizing radiation generated from radioisotopes of Cobalt-60

Electron Beam
Ionizing radiation generated by the bombardment with accelerated electrons

UV-C
Surface level radiation in the 200 – 280 nm range that damages DNA / RNA and disrupts cellular function

Chemical

Oxidizers

Hydrogen Peroxide Vapor
Exposure to vapors from concentrated hydrogen peroxide solution within a sealed chamber

Peracetic Acid Vapor
Highly biocidal oxidizer; Diluted solution circulated within a chamber

Chlorine Dioxide

Ozone

Alkylating Agents

Ethylene Oxide (EtO)
Exposure to EtO gas under controlled humidity and temperature within a sealed chamber

Glutaraldehyde

Low Temp Steam Formaldehyde

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Hazard Analysis and Critical Control Points (HACCP) For Low Acid Food Processing

1. Hazard Identification for Low Acid Beverages (e.g. Milk):

• The primary hazards include:

- **Biological**: *C. botulinum*, spoilage organisms.
- **Chemical**: Residues from cleaning agents or packaging materials.
- **Physical**: Foreign objects from packaging or equipment.

2. Critical Control Points (CCPs)

- **Sterilization of the product:** Ensuring the beverage is heated to a temperature that destroys pathogens, achieving commercial sterility
- **Sterilization of packaging materials:** Often done using hydrogen peroxide or other validated methods.
- **Aseptic filling and sealing:** Must be done in a sterile environment to prevent recontamination; Sterile air delivery system

3. Monitoring and Verification

- Continuous monitoring of temperature, pressure, and time during sterilization.
- Routine microbiological testing of the product and environment.
- Validation of cleaning and sanitizing procedures, including re-validation when changes occur.

4. Documentation and Traceability

What Are Low-Acid Beverages?

- Beverages with a **pH above 4.6**, which makes them susceptible to microbial growth, e.g *Clostridium botulinum*.
- **Not acidic enough** to inhibit microbial growth = requires stringent thermal processing and aseptic packaging to ensure safety.

Commercial Sterility of:

Thermally processed food is achieved by applying heat (or heat combined with water activity control) to eliminate microorganisms that can reproduce under normal nonrefrigerated conditions and those that pose public health risks.

Equipment and containers used in aseptic processing and packaging is achieved through **heat, chemical sterilants, or other treatments** to eliminate all viable microorganisms, including those of public health concern and those capable of reproducing in food under normal nonrefrigerated conditions.

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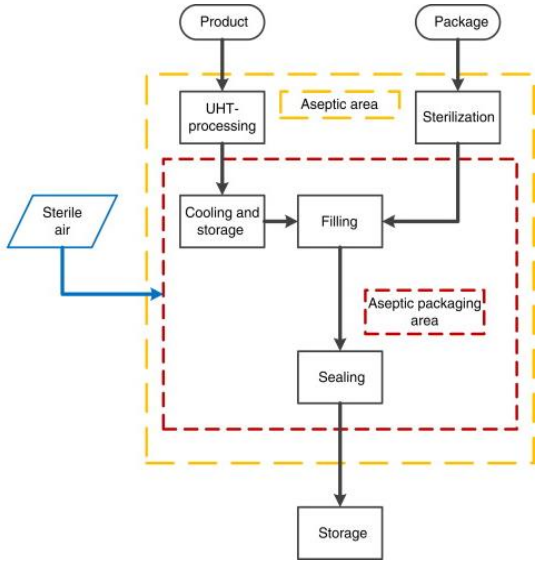
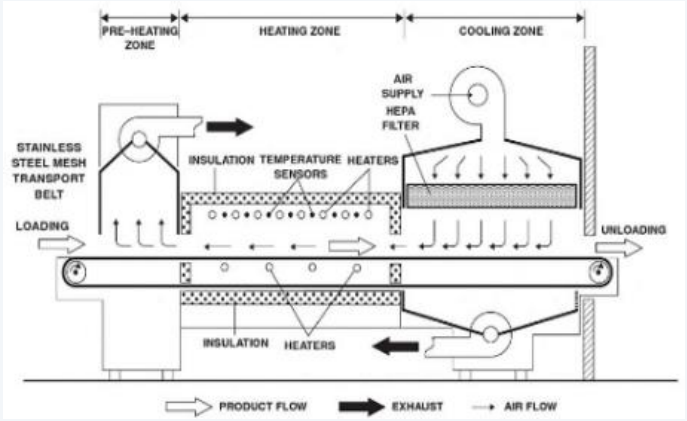
Aseptic Processing

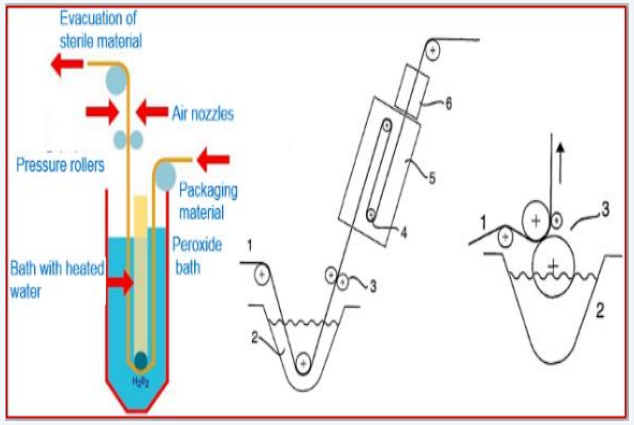


Flowchart Diagram of Aseptic Processing

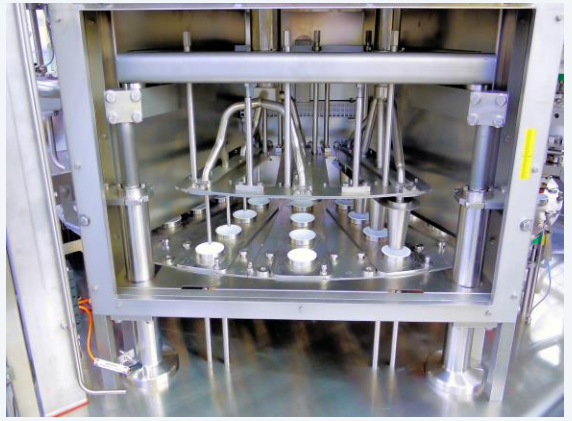
Packaging Sterilization



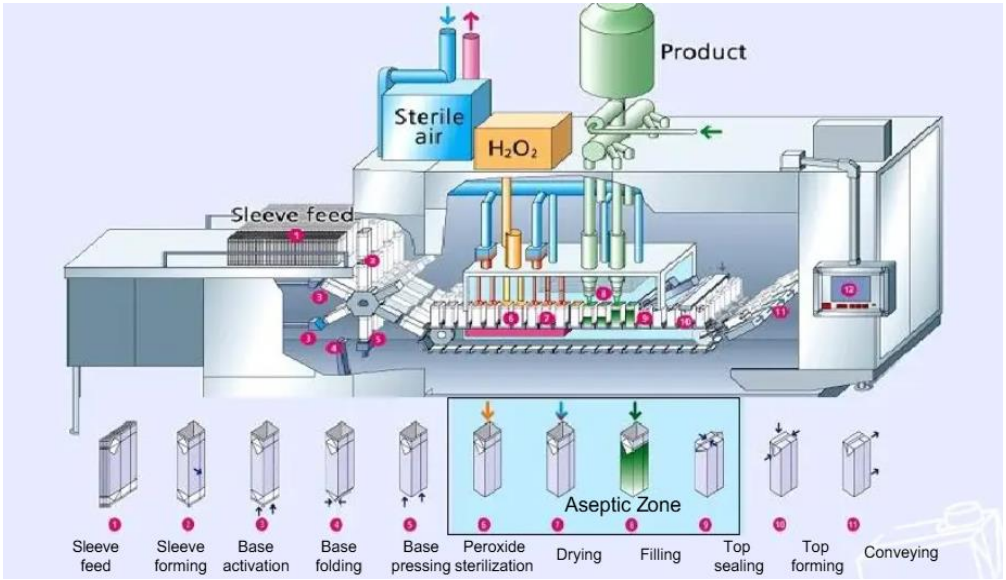
Dry Heat Sterilization

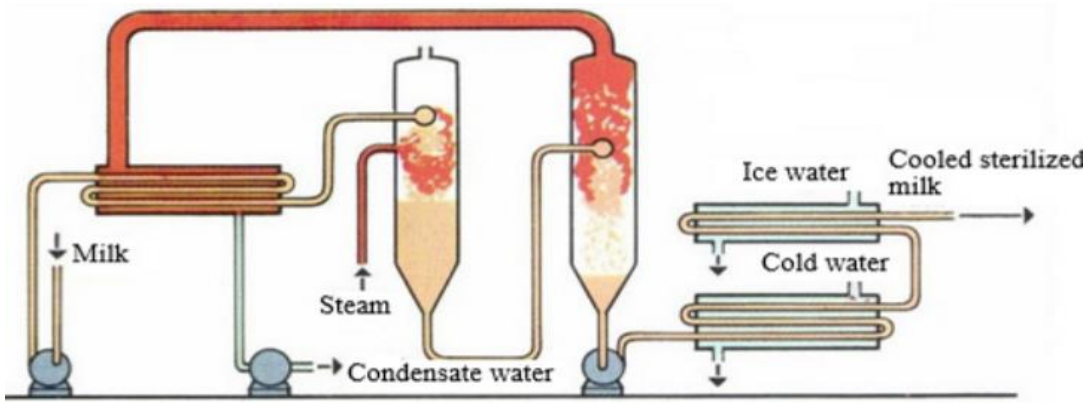


Hydrogen Peroxide Bath For Package Sterilization



H2O2 Mist applied to yogurt cups prior to filling. Image courtesy of Waldner North America.





***Product Sterilization**: Milk Pasteurized by Steam Infusion*

Are ESL products Aseptic?

Extended Shelf Life (ESL)

ESL products are products that have been treated in a manner to reduce the microbial count beyond normal pasteurization, packaged under extreme hygienic conditions, and which have a defined prolonged shelf life under ***refrigerated*** *conditions.*

Key difference between Aseptic and ESL products

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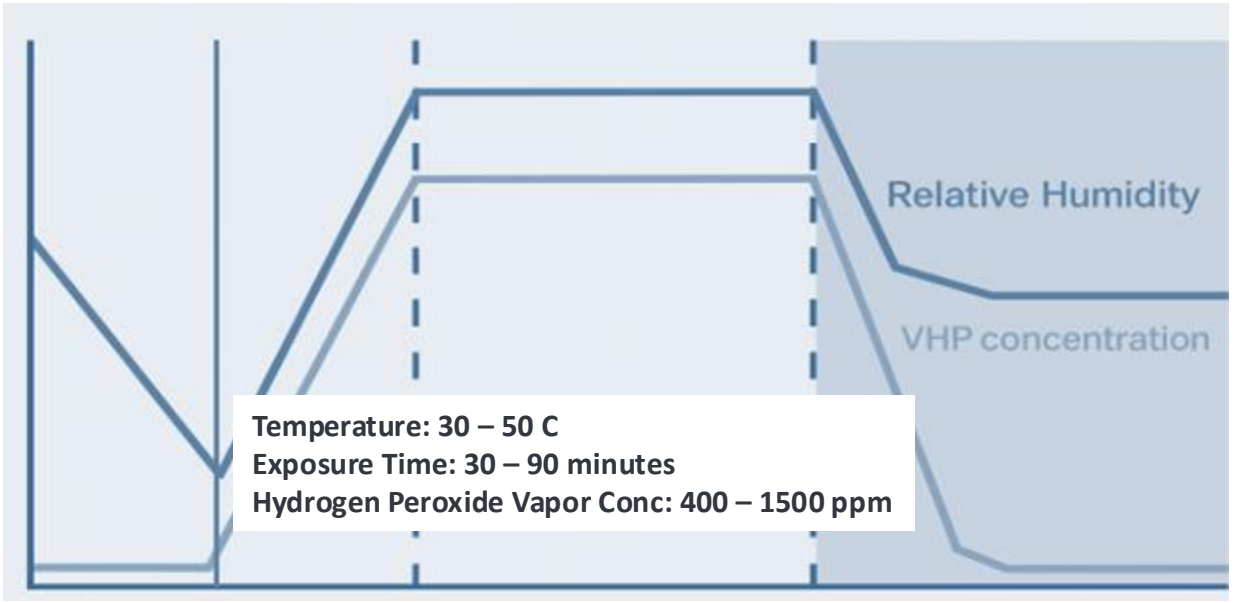
Medical / Pharmaceuticals: Sterilization Chambers & Isolators

Vaporized Hydrogen Peroxide Sterilization Process



1. **Conditioning / Dehumidification**: Sets the isolator's temperature and humidity to ensure consistent starting conditions.
2. **Gassing / H2O2 Injection**: Vaporizes aqueous hydrogen peroxide and introduces it into the isolator until the target VHP concentration is reached.
3. **Decontamination**: Maintains a stable VHP level by continuous injection to offset losses from uptake, breakdown, and microbial kill. The goal is a 6-log reduction in biological indicators, which can take less than an hour.
4. **Aeration**: Removes VHP from the isolator atmosphere until residual levelas drop to a safe target—typically **below 1 ppm**, or even lower for oxidation-sensitive products.

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Considerations for Sterilization Process by VH2O2

Material Compatibility

Material Susceptibility To Oxidation (e.g. Transition Metals)

Material Susceptibility To Uptake of VH2O2

- Peroxide Residual Levels (Polyurethane, Nylon, etc.) requires longer aeration times
- Reduction of VH2O2 Sterilization Efficacy by Cellulosic Materials

Material Reactivity of certain Transition Elements
Catalytic decomposition of VH2O2 reduces Sterilization Efficacy

Partial compatibility of certain plastics / metals

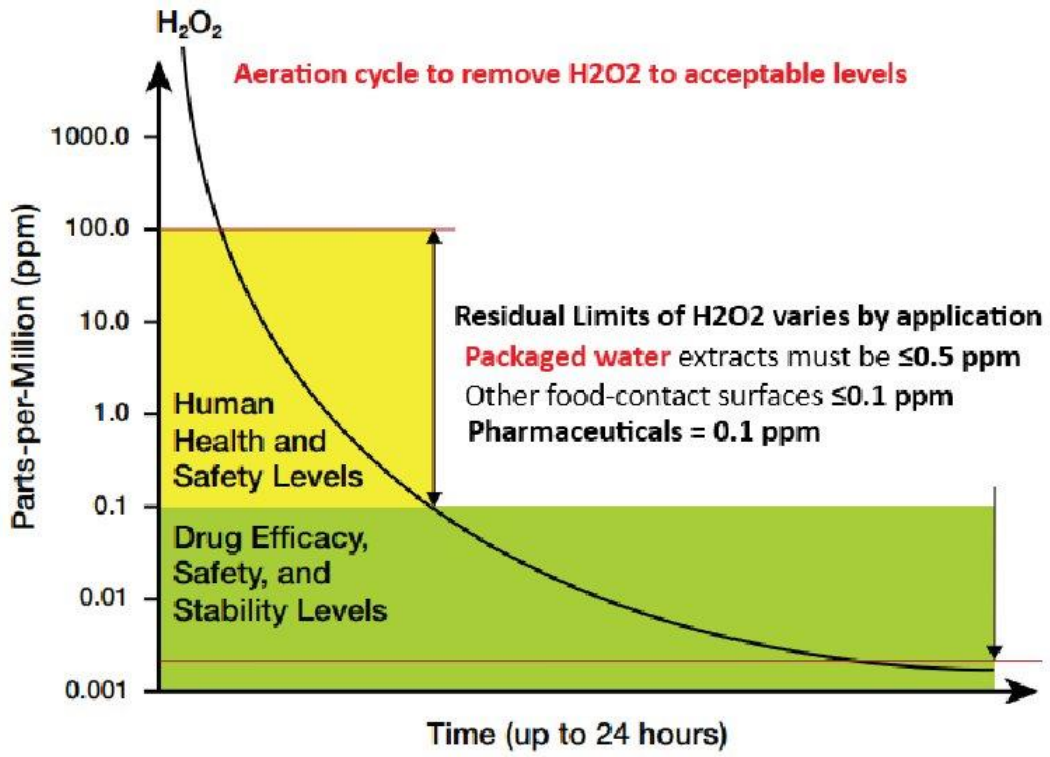
Exposure Time: Prolonged exposure VH2O2 can increase the likelihood of material degradation in some materials.

Temperature and Humidity: These environmental conditions affect the rate of hydrogen peroxide vaporization, distribution, and condensation on surfaces, which can impact both efficacy and material compatibility.

Concentration of Hydrogen Peroxide Vapor: Higher concentrations of VH2O2 generally lead to faster microbial inactivation but may also increase the risk of material degradation.

Significance of the Aeration Cycle:

Monitoring Residual Levels Post-sterilization



Comparing Methods of Monitoring

Monitoring Method	Result Type	Speed	Quantitative	Microbial Kill Insight	Additional Notes
Chemical Indicators (CIs)	Visual (Color Change)	Immediate	No*	No	Simple and cost-effective; only confirms H_2O_2 presence, not efficacy.
Biological Indicators (BIs)	Microbial Growth (Incubation)	Slow (Up to 7 days)	Yes	Yes (SAL 10^{-6})	Required for regulatory compliance; confirms sterility at specific locations.
Enzyme Indicators (EIs)	Bioluminescent Reading	Fast (Post-cycle)	Yes	Yes (SAL 10^{-6})	Instant results but manual handling required; not real-time.
Continuous Sensor Probes	Digital Sensor Readout	Real-time	Yes	No	Measures H_2O_2 concentration, temperature, and humidity; best used alongside BIs.

Various ways to measure hydrogen peroxide

Titrimetric
(Permanganate / Cerimetry)

Biological

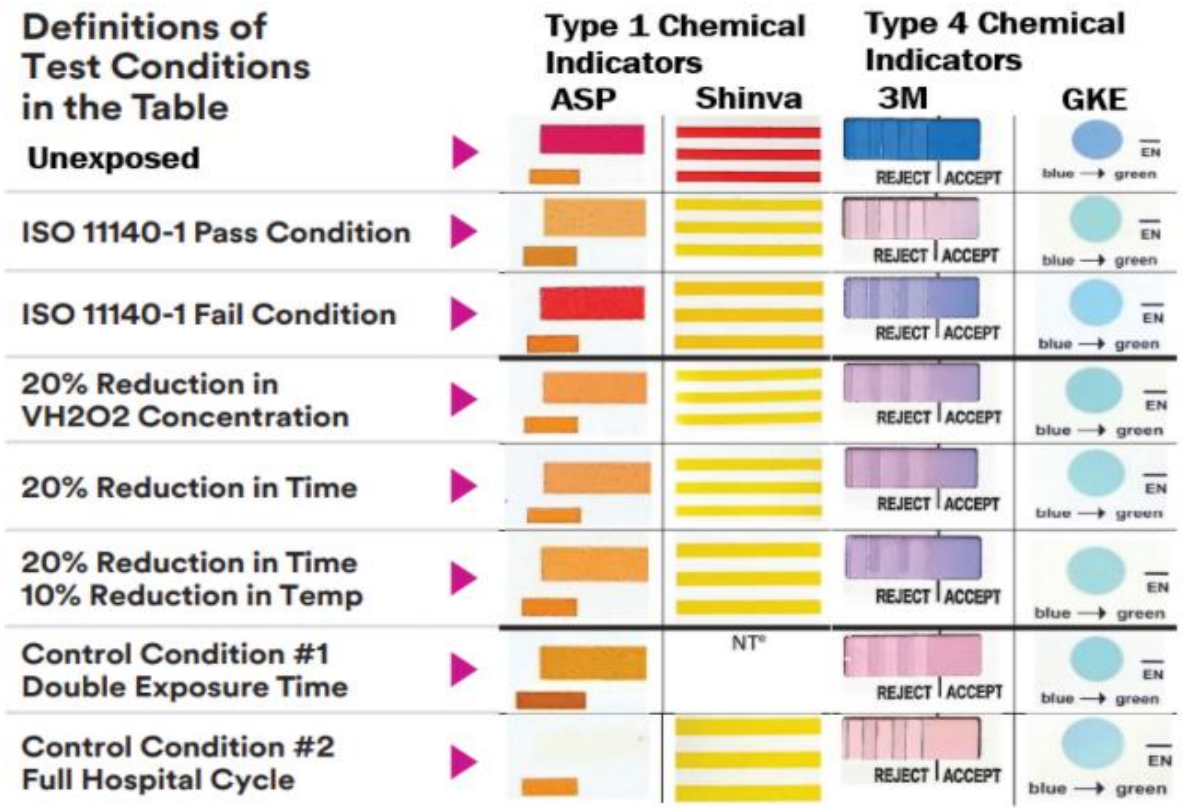
Enzyme Indicators

Fluorometric / Enzyme Assays

Spectrophotometric / Colorimetric

Gas / Vapor Detection

How VH_2O_2 Chemical Process Indicators Perform



Evaluation of a number of chemical indicators for monitoring vaporized hydrogen peroxide (VH2O2) sterilization processes.
Zentralsterilization | Volume 28 | 4/2020, Dr. Brian Kirk.

ISO 11140-1:2014 outlines general requirements for chemical indicators used in sterilization, including VH_2O_2 processes.

CIs are essential for verifying exposure and monitoring sterilization parameters, but they **do not confirm sterility** on their own.

- **Type 1 CIs**: Used externally and internally to show exposure and detect gross process failures. For VH_2O_2, they must be tested both with and without hydrogen peroxide under specific conditions.
- **Type 4 CIs**: Placed inside packs to assess whether critical process variables (time, temperature, H_2O_2 concentration) are met. They react to multiple variables and are more resistant than Type 1 CIs.

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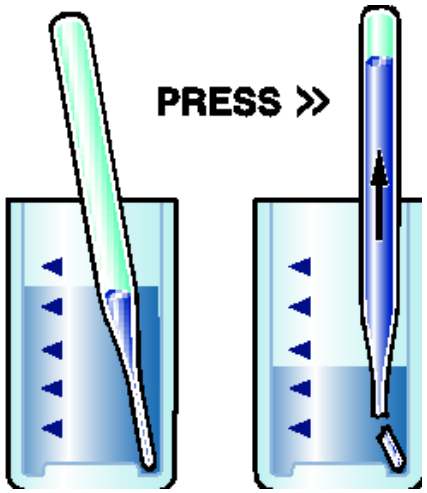
CHEMetrics Product Offerings For Hydrogen Peroxide Monitoring

colorimetric

Simple CHEMets® Test Procedure

1. Immerse the CHEMet into the sample and snap off the tip—the vacuum in the ampoule quickly pulls in the correct volume of sample.
2. Mix sample and reagent—in less than a minute, the hydrogen peroxide in the sample reacts with the reagent to form a color.
3. Match the color intensity to the standards in the comparator to obtain quick, accurate quantitative test results.



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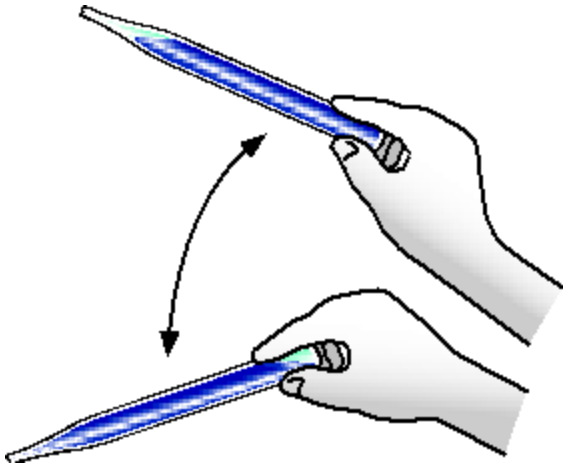

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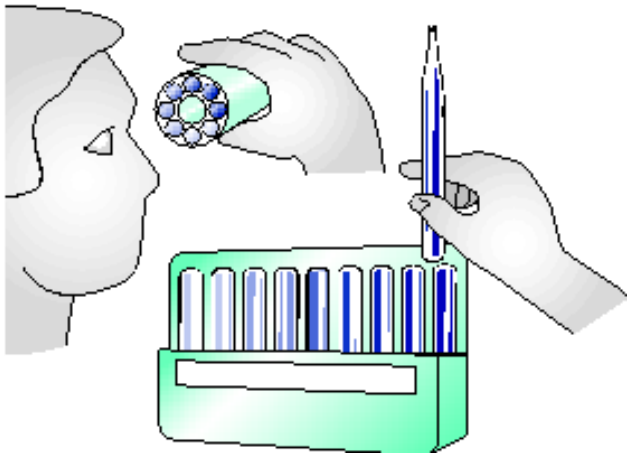

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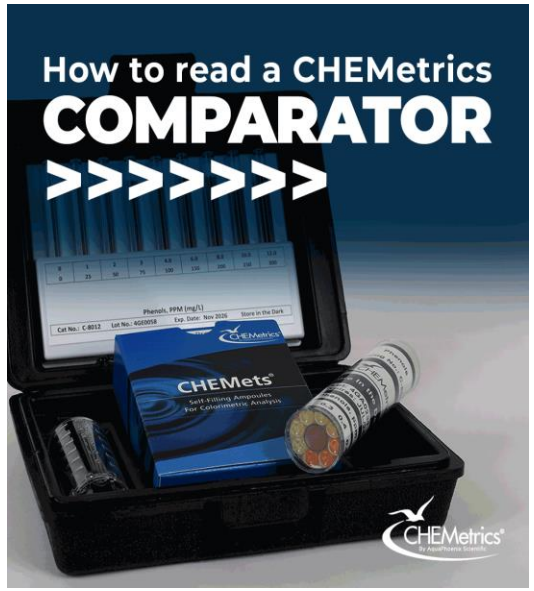

CHEMetrics® Instrumental Offerings for Hydrogen Peroxide

Hydrogen Peroxide Vacu-vials K-5543

V-3000 Multi-Analyte Photometer

Single Analyte Photometer (SAM)

- Vacu-vials® ampoules utilize the ferric thiocyanate chemistry.
- Measuring Range of 0 – 6.00 ppm Hydrogen Peroxide, allowing a robust range of applications
- Requires CHEMetrics **direct-readout photometers** or spectrophotometers capable of accepting a 13mm diameter round cell.
  - Single Analyte Meter (SAM)
  - V-3000

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Single Analyte Meters (SAMs)

Dedicated photometers use Vacu-vials® simple-to-use, accurate, economical

- SAMs provide accurate results equivalent to more expensive dedicated meters and probes.
- SAMs main target are for users who:
  - ✓ Prefer objective measurements over visual scrutinization of colors / have color blindness
  - ✓ Want Portability and Compactness
  - ✓ Need Sample Blanking (Sample color/turbidity)
  - ✓ Have tight budget / Not planning to measure more than 2 analytes from our instrumental test kits
- Available for chlorine, chlorine dioxide, COD low range, COD high range, detergents, hydrogen peroxide, dissolved oxygen, ozone (DPD), ozone (indigo), peracetic acid.

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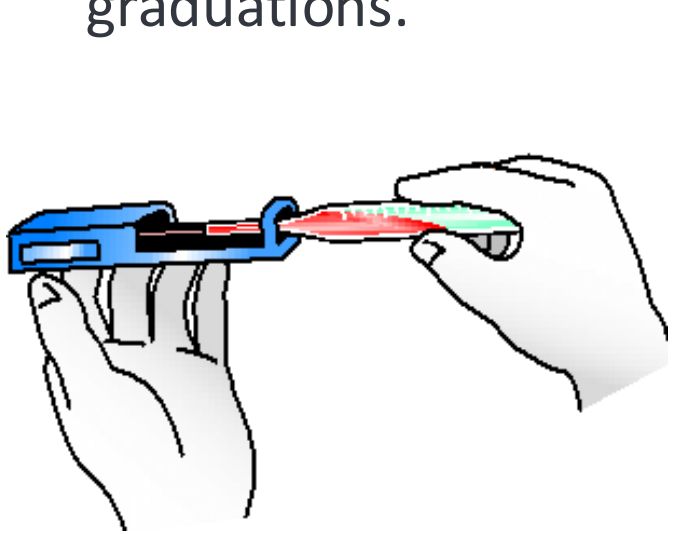
Titrets® Hand-held Titration Kits

Simplified visual titrimetric analysis

Titrimetric

1. Reverse titration quickly determines analyte concentrations.
2. Extremely simple—draw small volumes of sample using the ball-valve assembly on the ampoule tip until the color changes, indicating the equivalence point of the titration.
3. Quantitative results obtained by reading the scale off graduations.



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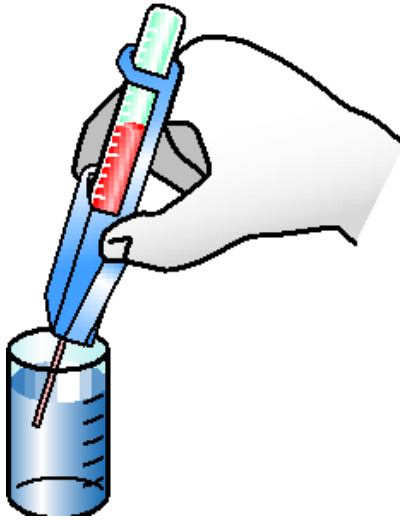


2

3

Catalog No.	K-5530
Range	0.1-1.0% (up to 20% with dilution)
MDL	0.10%
Method	Ceric Sulfate Titrant with Ferroin Indicator

Features of Titrets Technology

- ✓ Dependable, sealed single-use titrants
- ✓ Fast, easy procedure — no need to count drops
- ✓ No burettes or other equipment necessary
- ✓ Perform a titration ANYWHERE

CHEMetrics Hydrogen Peroxide Test Kit Methods

DPD

References: USEPA Methods for Chemical Analysis of Waters and Wastes, Method 330.5 (1983). APHA Standard Methods, 23rd ed., Method 4500-Cl G-2000. D.F. Boltz and J.A. Howell, eds., Colorimetric Determination of Nonmetals 2nd ed., Vol. 8, p. 303 (1978).

With the DPD Method, hydrogen peroxide reacts with DPD (N, N-diethyl-p-phenylenediamine) in the presence of potassium iodide to form a pink color. Various oxidizing agents such as halogens, ozone, and peracetic acid will produce high test results. Results are expressed as ppm (mg/L) H_2O_2.

Ferric Thiocyanate

Reference: APHA Standard Methods Online, Method 4500-H_2O_2 B-2020.

The ferric thiocyanate method consists of ammonium thiocyanate and ferrous iron in an acid solution. Hydrogen peroxide oxidizes ferrous iron to the ferric state, forming a red thiocyanate complex. Chlorine will not interfere with this method. Ferric iron, peracetic acid, and cupric copper will interfere. Results are expressed as ppm (mg/L) H2O2.

Ceric Sulfate Titrimetric

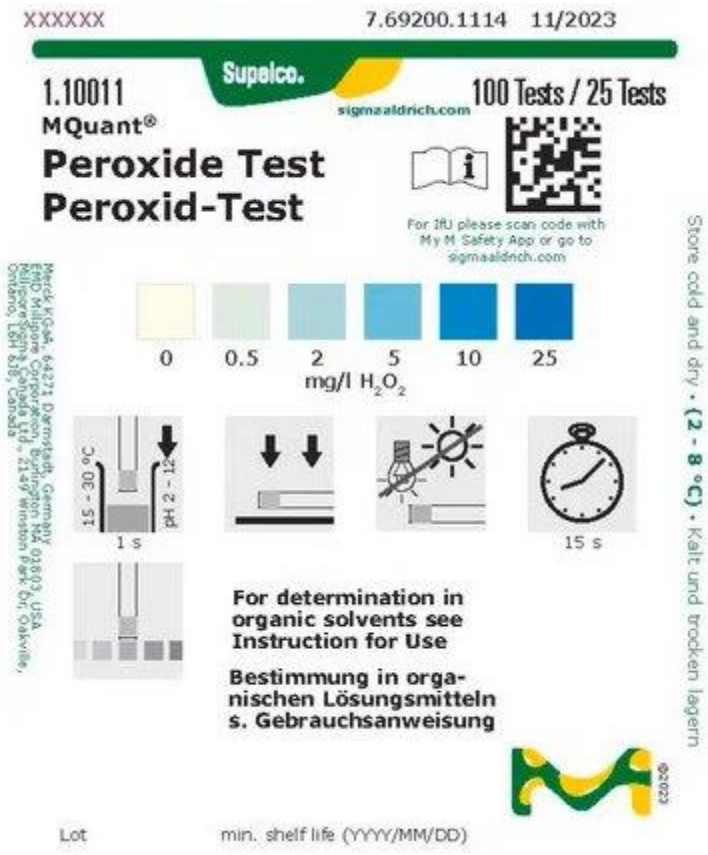
Reference: CHEMetrics, LLC

Using ceric sulfate as an oxidizer and ferroin as a redox indicator, the hydrogen peroxide will reduce the Cerium(IV) into Cerium (III) and eventually turning the ferroin back to its reduced state. A color change from green to orange signals the end of the titration. Results are expressed as percent (%) H_2O_2. The test range can be modified by performing a sample dilution. Details are provided in the kit instructions for ranges of 0.01 – 0.1% through 2- 20%.

The Visual Test Matchup: Ampoules vs Test Strips

- The ferric thiocyanate method consists of ammonium thiocyanate and ferrous iron in an acid solution. Hydrogen peroxide oxidizes ferrous iron to the ferric state, forming a red thiocyanate complex. Chlorine will not interfere with this method. Ferric iron, peracetic acid, and cupric copper will interfere.
- **Requires up to 25 mL sample***
- Increments in ppm:
  - 0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.8
  - 1, 2, 3, 4, 5, 6, 7, 8, 10
  - Higher range possible by dilution
- 30 – 60 seconds test
- $1.20 per test (refills), comparators cost $18 - 25

- Peroxidases catalyze the reaction between hydrogen peroxide and a substrate, often producing a colored product that can be measured to quantify the peroxide. Susceptible to reducing agents, phenolic compounds and other enzymatic factors
- **Just a drop**
- Increments in ppm:
  - 0, 0.5 , 2, 5, 10, 25
- 15 seconds per test
- $ 0.86 per test (if 100/pk)
- $ 2.40 per test (if 25/pk)



Hydrogen Peroxide Standard and Traceability

CHEMetrics® Hydrogen Peroxide Standard (A-5505) offers the analyst an easy method to confirm the accuracy and reliability of Hydrogen Peroxide measurements.

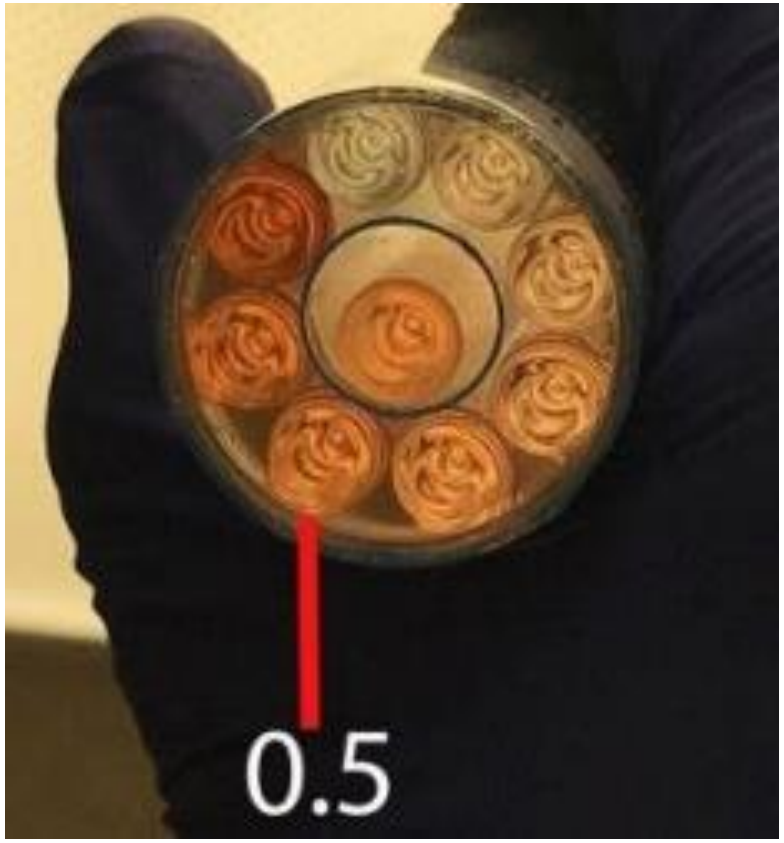
- Use to verify hydrogen peroxide kit reagent and instrument performance
- NIST Traceable
- Simple standard preparation using dispensing ampoules
- Stable, certified standard

To prepare the 0.5 ppm standard, simply dispense liquid from the double-tipped ampoule and dilute to 100 mL.

The 0.5 ppm standard will remain stable for 24 hours in a closed flask that is stored in the dark at room temperature.

Ampoule vs Test Strip Resolution

Picture 1: CHEMetrics® Color Comparator at 0.5 ppm

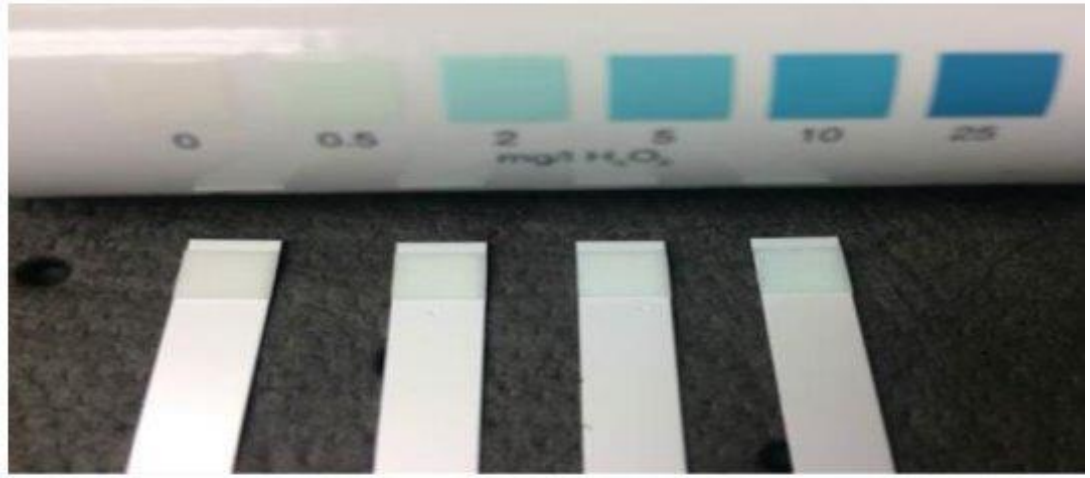


Accuracy claims for Visual Tests are +/- 1 ColorStandard (CS) increment for the end-user but were formulated with +/- 0.5 CS acceptance criteria.

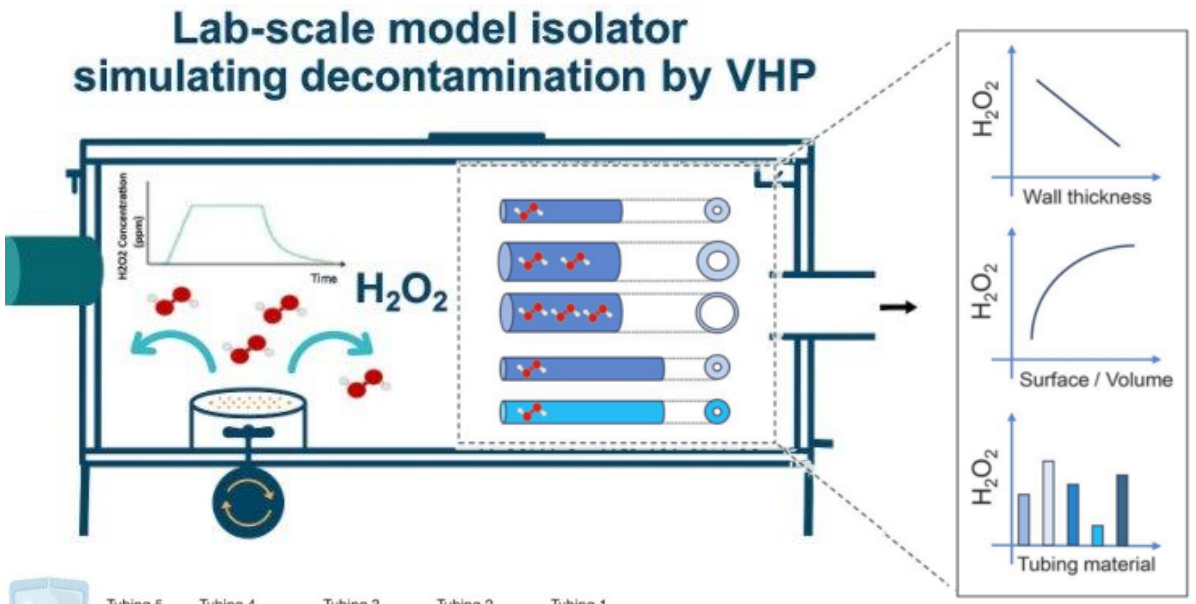
Table 1: Average Hydrogen Peroxide Test Results CHEMetrics® Hydrogen Peroxide Self-filling Ampoules vs. Test strips

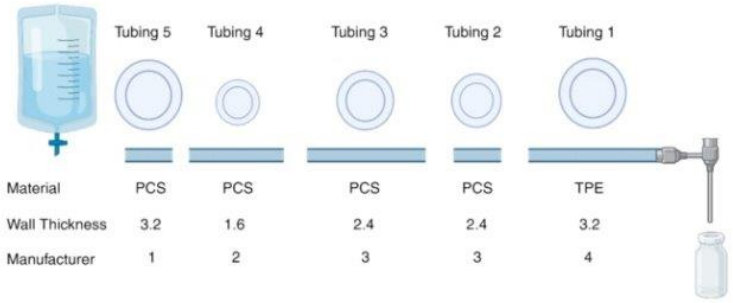
Peroxide Conc., ppm	CHEMetrics K-5510, ppm	CHEMetrics K-5543, ppm	MQuant™ Test Strips, ppm
0	0	0.00	0
0.15	0.15	0.13	> 0
0.25	0.25	0.24	0.25
0.50	0.50	0.48	0.5

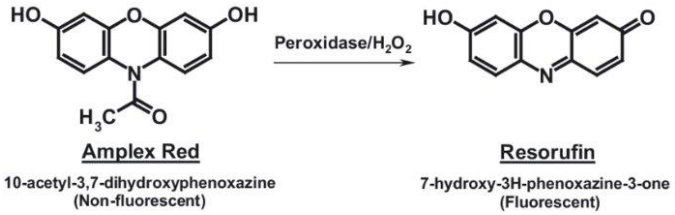
Picture 2: MQuant™ Test Strips Color Intensity

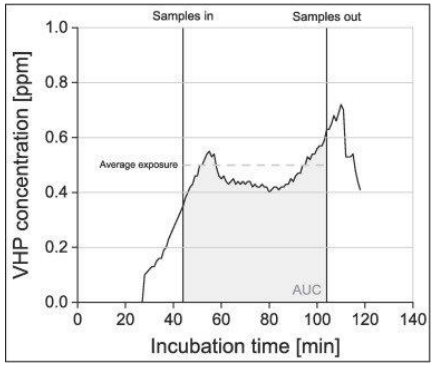


VHP Uptake by Different Tubing Materials / Dimensions









Research paper

Vaporized Hydrogen Peroxide Uptake by Tubing used for Aseptic Fill-Finish Manufacturing of Biopharmaceutical Drug Products

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- VHP uptake by tubing can lead to residual (<= 0.5 ppm) hydrogen peroxide (H_2O_2) contamination in oxidation-sensitive drug products such as monoclonal antibodies, causing protein degradation and decreased therapeutic efficacy.
- Platinum-cured silicone (PCS) tubing showed significant uptake of vaporized hydrogen peroxide (VHP)
- Thermoplastic elastomer (TPE) and thermoplastic vulcanizate (TPV) tubing showed no measurable VHP uptake under tested conditions.
- VHP uptake decreased linearly with increasing wall thickness, higher S/V ratios, smaller inner diameters (ID) and larger outer surface areas (OS).
- Pre-autoclaved tubing can reduce VHP uptake, while gamma irradiation may increase it
- **Amplex Red Fluorometric Enzyme Assay used for H2O2 determination => CHEMetrics CHEMet Technology might be applicable with some modification assuming enough sample (~ 5 mL) can be obtained**

Ampoules for vapor H_2O_2 analysis

For capturing gas / vapor samples, air sampling and **bubbler / impinger systems** are the possible ways to convert vapor samples to liquid. Gas flow rates must be accurately controlled to estimate how much total gas volume is being collected. (See related study on Peracetic Acid Vapor Analysis). Acidic pH might help.

OSHA has a procedure for Time-Weighted Average (TWA) Measurement of Hydrogen Peroxide Workplace Exposure Limit. Air Sampling method with **Titanium Oxysulfate Hydrate-coated cassette filter**, then extracted into solution.

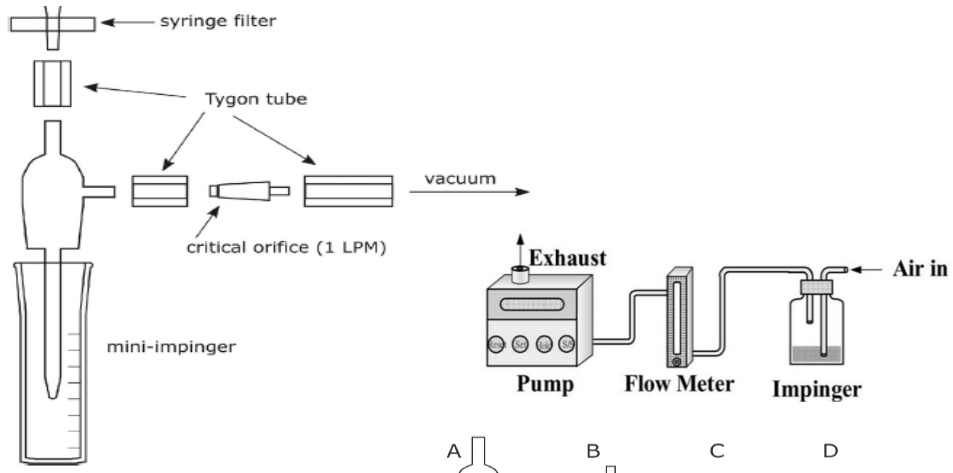
ALL CHEMetrics Tests require a **liquid** sample, typically between 15 – 25 mL depending on the test; it cannot directly measure analytes in vapor or gaseous phases.

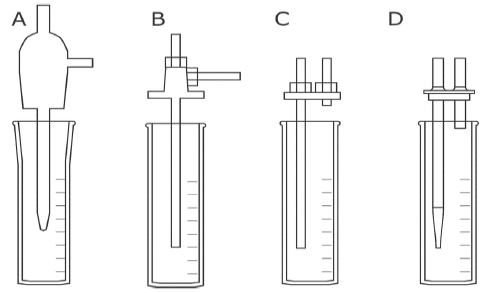
Deviating from prescribed sample sizes is generally not recommended / endorsed, but ***can be made to work for your situation***. Requires careful consideration and appropriate judgement call.

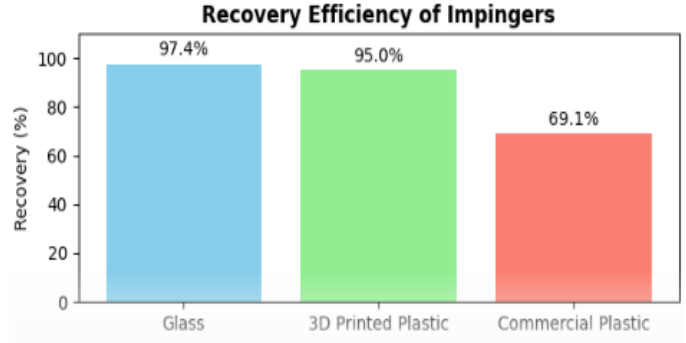
- Visual tests technically only require 2 mL of sample to fill the ampoule, but a **minimum** of 5 mL is recommended for proper aspiration into the ampoule. Technique is important.
- Instrumental tests use a larger volume at around 6 mL, requiring a **minimum** of 10 mL for proper aspiration. Technique is important.
- Titrimetric methods require up to 7 mL of sample as a reverse titration to reach an end point.

A field-portable colorimetric method for the measurement of peracetic acid vapors: a comparison of glass and plastic impingers

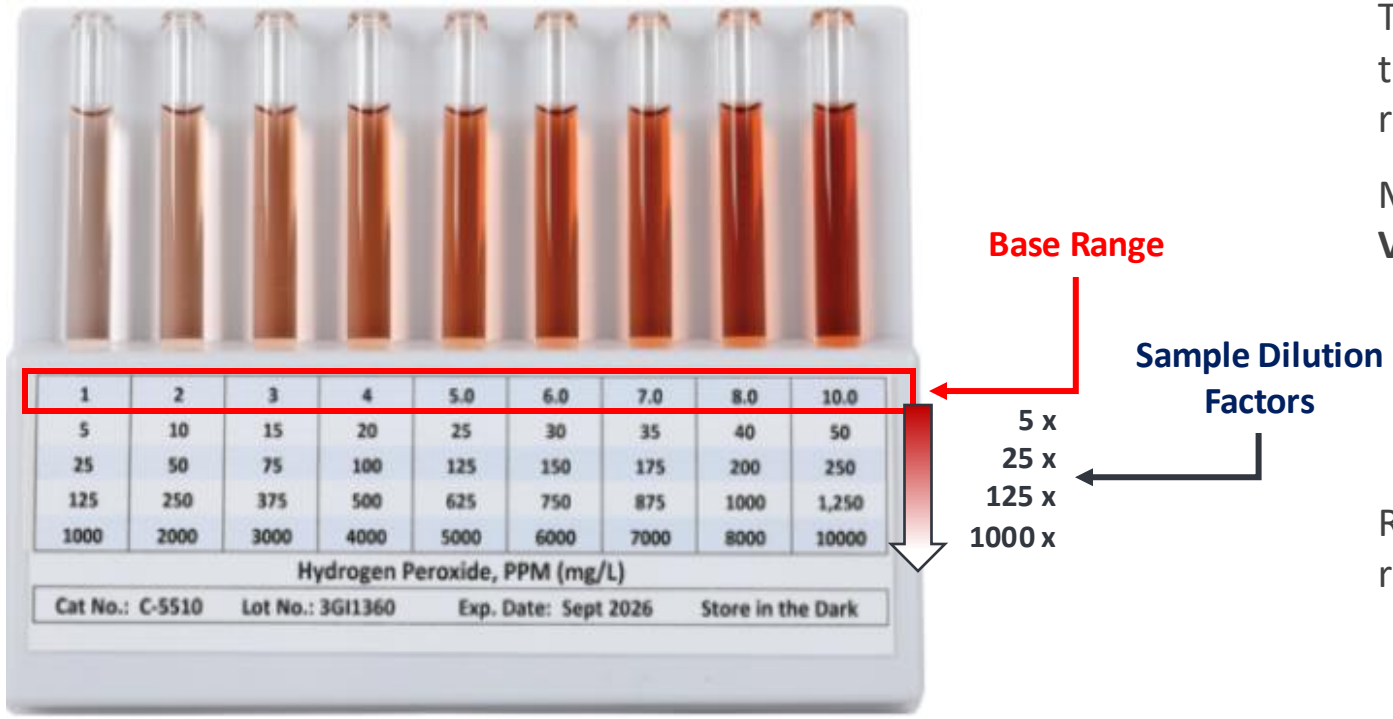
Angela L. Stastny, Amos Doepke & Robert P. Streicher



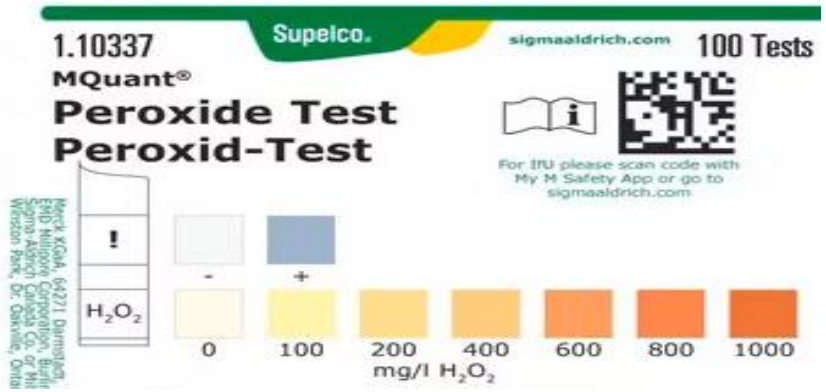




High-Range Hydrogen Peroxide Testing: What you should know



High Range (HR) Comparator for Hydrogen Peroxide (Ferric Thiocyanate)



The **CHEMetrics HR CHEMets** is designed to provide users the ability to measure much higher concentrations beyond residual levels, currently endorsed up to 10,000 ppm.

Meant as a direct replacement of the discontinued **VACUettes**.

- No more dilutor tips and fumbling around with technique.
- More flexibility and modularity

Reagent is formulated to cater to the **0 – 10 ppm** base range.

- Relies heavily on dilution to extrapolate the range.
- May involve a little trial and error to find the right dilution
- Technically works for any range if end-user is savvy

Side Effects (Maybe good or bad)

- Diluted interfering agents
- May affect results slightly due to other factors

High-Range CHEMets Testing, Understanding the kit offerings

www.chemetrics.com

Visual Kits

Range	MDL	Method	Kit Catalog No.	Refill Catalog No.
0-0.5 ppm	0.025 ppm	DPD	K-5502	R-7904
0-0.8 & 1-10 ppm	0.05 ppm	Ferric Thiocyanate	K-5510	R-5510
5-50 ppm	5 ppm	Ferric Thiocyanate	K-5520D	R-5510
25-250 ppm	25 ppm	Ferric Thiocyanate	K-5520A	R-5510
125-1250 ppm	125 ppm	Ferric Thiocyanate	K-5520B	R-5510
1000-10,000 ppm	1000 ppm	Ferric Thiocyanate	K-5520C	R-5510
0.1-1.0% (up to 20% with dilution)	0.10%	Ceric Sulfate Titrant with Ferroin Indicator	K-5530	

Parent kit of these HR CHEMets

Dual range kits like this includes BOTH
- **Low Range (Round) comparator**
- **High Range (Flat) comparator**

Kit Catalog Numbers with letters:
D, A, B or C are High Range CHEMet Kits

HR CHEMet Kits include ONLY the High Range (Flat) Comparator

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Testing for Non-Residual Levels of Hydrogen Peroxide

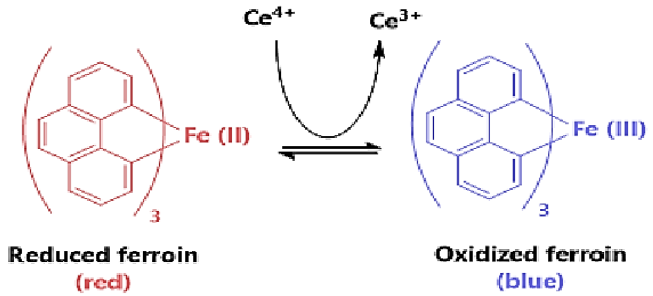
- ***Titration by Potassium Permanganate, $KMnO_4$***

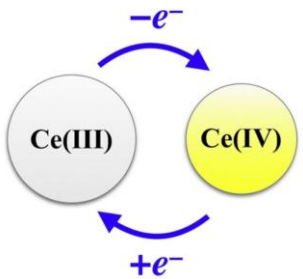
$$2KMnO_4 + 3H_2SO_4 + 5H_2O_2 \longrightarrow 2MnO_4 + K_2SO_4 + 5O_2 + 8H_2O$$

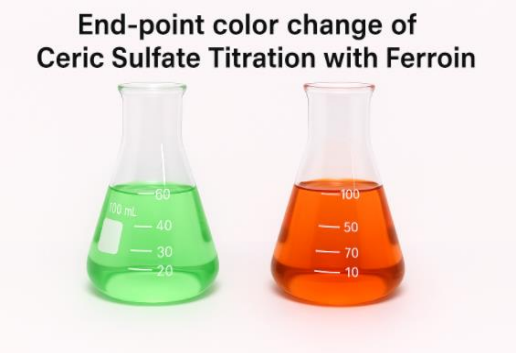
- ***Titration by Cerium Sulfate, $Ce(SO_4)_2$***

$$2Ce^{4+} + 3H_2O_2 \rightarrow 3Ce^{3+} + 2H_2O + 2O_2 + 2H^+$$

- $KMnO_4$v and $Ce(SO_4)_2$C act as oxidizing agents, H2O2 acts as reducing agent.
- Ce(IV) is bright yellow, upon reduction to Ce(III), turns colorless.
- In CHEMetrics version of the test, Ferroin is used as a sharper end-point indicator.
- Oxidizable species like nitrites and ferrous iron (Except copper) causes false positives.
- Chromates mask the end-point.
- Peracetic acid and Ethylene Glycol poses little to no interference with this method.







Titration Video Demonstration & Outcomes

K-5530 Hydrogen Peroxide Ceric Sulfate Titrets

- Sample cup
- Titrettor (Titret Housing)
- Titret Ampoule
- Ferroin Tips

Three possible scenarios during titration:

Over Range: Reagent turns orange immediately after drawing in a bit of sample. Dilute sample, apply factor.

Under Range: Reagent stays green without reaching end-point.

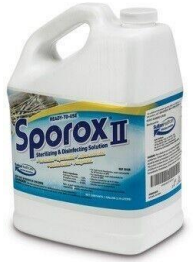
Correct Range: End point is reached after a reasonable amount of sample.

Efficacy Testing of Sporox: A H_2O_2 Sterilant

Sporox is a commercial sterilizing and disinfecting solution widely used in dental and medical settings, primarily for heat-sensitive instruments.

- Contains **7.5% nominal hydrogen peroxide**; glutaraldehyde-free = safer and less irritating
- For high-level disinfection and chemical sterilization, especially for heat sensitive devices
- Achieves high-level disinfection in 30 minutes and chemical sterilization in six hours at room temperature.
- No mixing, heating, or activation; poured directly into a soaking container and can be reused for up to 21 days, provided periodic testing confirms hydrogen peroxide activity remains effective.
- Test vials available to indicate when the solution needs to be refreshed and specialized trays for instrument soaking.

This is a Pass / Fail Test designed to test if the product H2O2 concent is at the very least at or above the **Minimum Effective Concentration (MEC) of 6.0%, where 7.0% is guaranteed to be effective**.



Comparison to typical iodide-starch based test strips:

- Both provide PASS/FAIL results.
- Test strips require **15 minutes**; Test vials need **2 minutes**.
- Test vials are not affected by humidity, unlike test strips.
- Stable under various conditions: temperature, freeze/thaw cycles, light exposure, and contamination.

H2O2 Concentration	Test Strips PASS rate	Test Vials PASS rate
MEC 6.05%	0 %	0 %
6.6 %	25 %	75 %
7.0 %	96 %	95 %

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Comparing Methods of Testing for H2O2 in the presence of PAA

Available Methods	PAA Interference
Cerium Sulfate Titration	PAA has minimal interference.
Ferric Thiocyanate Chemistry	PAA interference can be mitigated with a pre-treatment
DPD-based Chemistry	PAA is measured quantitatively along with H2O2 along with other common oxidizing agents.

- PAA is also a strong oxidizer that can easily oxidize Ferrous iron to Ferric, hence causes false positive.

$$CH_3COOOH + 2\,Fe^{2+} + 2\,H^+ \rightarrow CH_3COOH + 2\,Fe^{3+} + 2\,H_2O$$

$$Fe^{3+} + 3\,SCN^- \rightarrow [Fe(SCN)_3]$$

- Acetic acid produced from PAA with iodide has ***no consequence*** for Ferric Thiocyanate chemistry.

➔ PAA + KI = NO more PAA = NO potential contribution to H2O2 = NO False Positives

- its presence in samples causes high DPD response ➔ DPD chemistry is a poor choice here.

$$CH_3COOOH + 2\,I^- + 2\,H^+ \rightarrow CH_3COOH + I_2 + 2\,H_2O$$

$$I_2 + DPD \rightarrow DPD^{\cdot+} + 2\,I^-$$

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Potassium Iodide mitigates effect of PAA interference

Procedure for testing H2O2 in presence of PAA:

https://chemetrics.b-cdn.net/uploads/2024/02/peroxide_mod_procedure_PAA_copper.pdf

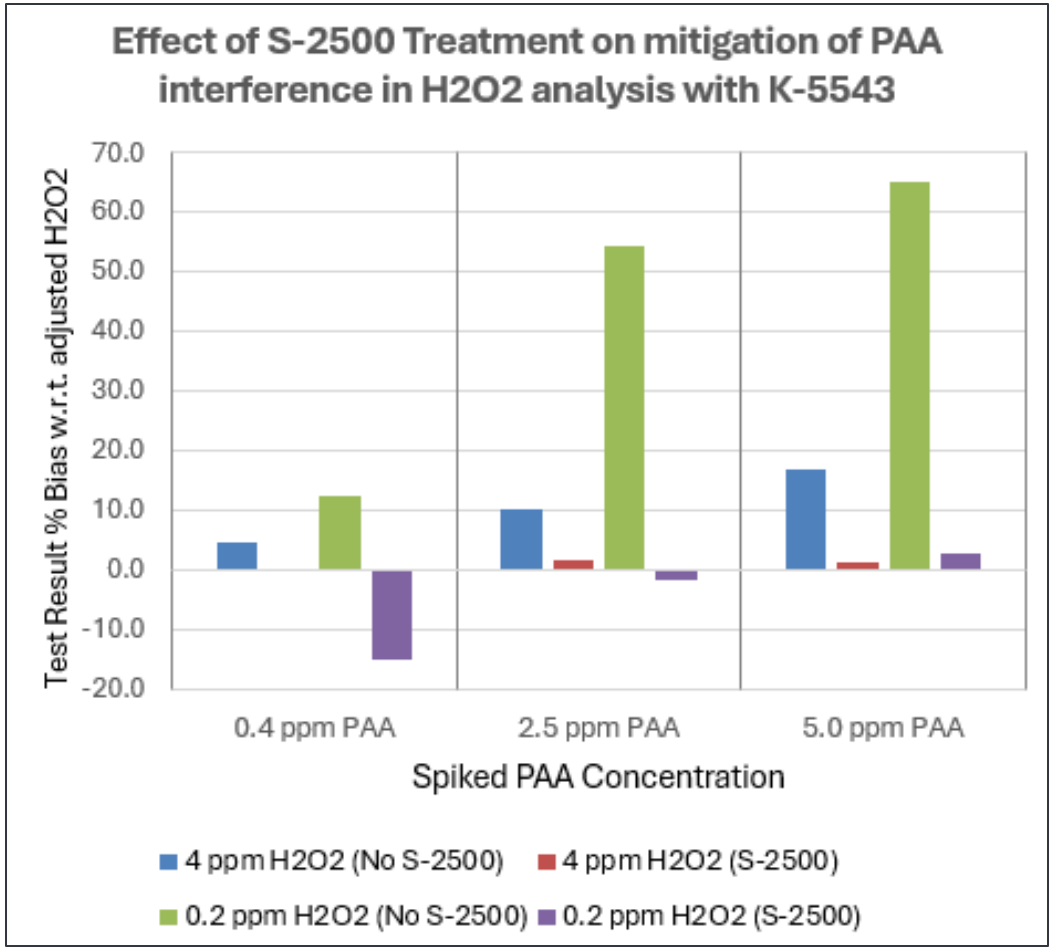
CHEMetrics By AquaPhoenix Scientific — SIMPLICITY IN WATER ANALYSIS | TECHNICAL BULLETIN

Hydrogen Peroxide Analysis in the Presence of Peracetic Acid and Copper Using CHEMetrics® Hydrogen Peroxide Test Kits, Cat. Nos. K-5543 and K-5510

Version 5 / Feb 2024

Question 1- Does the addition of S-2500 impact the Ferric Thiocyanate Chemistry?

		Without S-2500		Added S-2500	
Lot#	Target ppm H2O2	Measured ppm H2O2	AVERAGE Measured ppm H2O2	Measured ppm H2O2	AVERAGE Measured ppm H2O2
79062	0.0	-0.02	-0.02	-0.02	-0.01
78677		-0.02		0.00	
78925		-0.02		-0.01	
79062	0.2	0.18	0.20	0.18	0.19
78677		0.24		0.19	
78925		0.18		0.19	
79062	0.6	0.59	0.58	0.58	0.60
78677		0.58		0.64	
78925		0.58		0.58	
79062	3.0	2.97	2.99	2.92	2.94
78677		2.98		2.95	
78925		3.03		2.95	
79062	5.0	5.00	5.02	4.90	4.91
78677		5.07		4.88	
78925		5.00		4.94	



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Known Interference For CHEMetrics H2O2 Testing Methods

	Ferric Thiocyanate	DPD	Ceric Sulfate
H2O2	Too high = Color develops, then fades to colorless		Too low = Stays green, no end-point till the end. Too High = Turns Orange immediately
PAA	Positve interference; mitigated by S-2500	Positive Interference	No interference (tested with 5:1 ratio H2O2/PAA)
Iron	Positive interference	Off-color **yellow** hue impeding color match @ > 10 ppm at low H2O2 concentrations	Fe2+: Positive interference
Copper	Cu2+: Negative interference with aging reagent; Measurement after Acidication with S-6901 mitigates this.	Off-color **blue** hue impeding color match @ > 10 ppm at low H2O2 concentrations	No interference; May see blueish transition from green to orange near endpoint
Chlorine	Co-reacts with H2O2; If no H2O2, no color up to 40 ppm, positive interference above.	Positive Interference; Exceeding high range suppresses color development	
Bromine		Positive Interference; Exceeding high range suppresses color development	
Iodine		Positive Interference; Exceeding high range suppresses color development	
Monochloramine		Positive Interference > 1 minute	
Permanganate		Positive interference when no H2O2, low bias with H2O2--- might suggest co-reaction with H2O2.	
Ozone	Co-reacts with H2O2;	Possible positive Interference	
Nitrites		Positive Interference	Positive Interference
Chromate		May interfere; Barium chloride masks this interference	Masks End-point; Pre-endpoint green color smeared to yellow/gold
Persulfate	Positive Interference	Positive Interference if > 1 minute and D45 > 10 ppm	
Ethylene Glycol			No interference up to 50%
High pH	Negative interference; Precipitates iron, no result	Negative interference	Compromises test; Needs low pH to work.
High Alkalinity		No interference	

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Questions & Answers

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