Essential Insights: New FDA Controls Over Laboratory Developed Tests (LDTs)

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Summary

The FDA is tightening controls over 'Laboratory Developed Tests' (LDTs) used for In Vitro Diagnostics in Clinical settings.

In Europe, similar regulation change is also in progress through recent amendments regarding 'In-house' tests, to the Medical Device Regulations (MDR) and In Vitro Diagnostic Regulations (IVDR). In the US and Europe Quality Management in line with the Medical Device standard ISO13485, is required.

Purified Water is a component commonly used in many LDTs and is subjected to these new controls. Use of a high quality water purification system that is validated and calibrated by a reputable manufacturer can help LDT providers satisfy the new expectations that will come into force.

An introduction to LDTs

In Vitro Diagnostics (IVD) in the clinical setting are essentially tests carried out on samples taken from patients in order to determine the state of health. These can take place using highly controlled auto-analysers or as 'Laboratory Developed Tests' (LDTs). Commercially manufactured assays using auto-analysers make up the majority of clinical laboratory tests.

LDTs, however, are designed and developed for use in a single laboratory, sometimes referred to as "in-house" tests. LDTs are developed in facilities ranging from physicians' offices, hospitals, and academic medical centres to large testing companies. Though LDTs may contain the same or similar components as FDA-reviewed tests, they must be developed and used within the same facility.[1]

To address concerns over the safety and control of LDTs, given their increasing prevalence in healthcare decisions and increasing complexity, the FDA plans to enforce stricter regulation surrounding their delivery.

There are many FDA controls placed over the auto-analysers that are defined as a 'medical device' in the regulations, however, the FDA now plans to define LDTs as 'IVDs manufactured by laboratories' and also classify them as 'devices' under the Federal Food and Drug Cosmetic Act (FD&C Act). The result is that many regulations will also become enforceable to LDT providers.

Changes are planned to be implemented over a four-year period from May 2025.

Regulation change summary:

Stage 1 - from May 2025 compliance with:
Medical device reporting (MDR) requirements, correction and removal reporting requirements, and quality system (QS) requirements regarding complaint files

Stage 2 - May 2026 compliance with:
Requirements not covered during other stages of the phaseout policy, including registration and listing requirements, labelling requirements, and investigational use requirements

Stage 3 - May 2027 compliance with:
QS requirements (other than requirements regarding complaint files)

Stage 4 November 2027 compliance with:
Premarket review requirements for high-risk IVDs offered as LDTs (IVDs that may be classified into class III or that are subject to licensure under section 351 of the Public Health Service Act)

Stage 5 06/05/2028 compliance with:
Premarket review requirements for moderate and low-risk IVDs offered as LDTs

(2)

The Importance of ‘Laboratory Developed Tests’

LDTs are often complex and are developed and carried out by highly skilled specialists; the expertise of the analytical specialists and clinicians are often relied on to interpret results.

LDTs offer versatility and are able to satisfy unmet current or local clinical needs, where the development of automated methods is not practicable.

LDTs are often extremely useful where the FDA's regulatory process has struggled to keep pace with current knowledge[3] and for fostering innovation within IVD.

Examples of LDTs include those for:

- New viruses such as SARS-CoV-2,
- Evolving illicit recreational ‘Designer Drugs’,
- Local exposure to poisons,
- New genetic markers indicating cancer risk

Limitations

LDTs are in theory required to be developed and carried out only at individual certified laboratories. This means that straight transfer of methods between different laboratories should not be permitted. Many LDTs are now carried out on a much bigger scale than in the past, by laboratory corporations offering nationwide services. These LDTs are often carried out using instruments and components not legally marketed for clinical use. This is an imbalance that the FDA seeks to address.

The FDAs general ‘enforcement discretion approach’ has meant many regulations around IVD have not been enforced for LDTs allowing them to circumvent a variety of the requirements including those for Medical Devices, Quality Systems, pre-market review and post-market compliance. With an increasing complexity and prevalence of LDTs, the apparent lack of FDA control, in comparison with standard IVDs, has raised concerns regarding the validity, reliability and safety, and led to the changes.

Commonly Used Equipment

State of the art highly specialised and sensitive analytical equipment and detectors can be used in LDTs, for example, High Performance Liquid Chromatographies (HPLC) and Time of Flight Mass Spectrometry (TOF-MS)[4]. Operated by skilled and knowledgeable personnel, these methods and complex machines can offer particularly powerful tools to support clinicians. This includes high-resolution identification and quantitation of diversified biological markers such as hormones, amino acids, fatty acids, proteins and peptides, from trace to high levels in complex matrices.

Operation of such sensitive equipment requires care and skill; controlled environments such as clean-rooms and highly purified chemical reagents are often used to minimise potential contaminants that can compromise results and detection limits. During the development of LDTs, parameters critical to success such as the correct chemicals are established and subsequently required by the certified method.

In contrast, IVD assays carried out by auto-analysers use chemical reagents evaluated by the particular manufacturer, ensuring that they are fit for purpose and secure in supply and that documentation to demonstrate this is available and traceable. The methods and analytical equipment have been carefully developed in line with the Quality Management Systems (QMS) for medical devices such as ISO13485.

Compliance with the QMS for Medical Devices is a major challenge that providers of LDTs need to tackle. This is necessary to give the required confidence in the reliability of analytical equipment and their test results, and to satisfy the FDAs new stance. Section 7.4 of the standard covers compliant purchasing such as selection and definition of supplied components, consumables and chemicals needed for the IVD testings.

Purified Water is a reagent commonly utilised for the delivery of many LDTs and is discussed in the following section.

Purified Water for IVD Auto-Analysers

Purified water is without doubt, the reagent used in the largest quantities for IVD work; Due to its importance and the sheer quantities required, most auto-analysers used for IVD are accompanied by equipment generating high purity water and supplying it directly to the assay. This eliminates the potential for contamination events through handling and the environment.

The purity of water for clinical analysis has been determined by the Clinical & Laboratory Standards Institute (CLSI) in their Clinical Laboratory Reagent Water (CLRW) standard. This has been proven to be of suitable quality for reliable operation of IVD auto analysers and is detailed in the table below.

Parameter	CLSI CLRW Specification
Resistivity	> 10 MΩ.cm
TOC (Total Organic Carbon)	< 500ppbC
Particles	0.22µm filtration
Bacteria	< 10CFU/ml

Resistivity indicates low levels of dissolved ions (inorganic impurity), TOC low levels of organic matter, filtration removes particles in suspension and the bacteria specification indicates low microbiological activity. Water purification equipment incorporate sensors monitoring purity and are often 'Validated' by manufacturers, both on installation and periodically, to confirm correct operation and performance in situ. This monitoring and testing in comparison with the recognised CLSI standard gives confidence and traceability to the FDA that adequate water is being used to support successful clinical decisions.

Purified Water for LDTs

Highly purified water is also used in many ways as part of IVD via LDTs, however, the purity requirements can be less well defined and adhered to. While water matching CLRW has been shown to be adequate for auto-analysers, the high complexity and sensitivity of equipment used in many LDTs demand a much higher level of purity.

The purity of water required is often close to Type 1 Ultrapure which has a resistivity of > 18.2 MΩ.cm (at 25˚C) and TOC of < 10ppbC. In HPLC analysis, which is commonly used in clinical applications, examples of compromised baselines are clear with reagent water TOC of 100ppbC, and experience indicates degradation in performance can be expected with TOC raising above 5-10ppbC[5]. The importance of low TOC in chromatography applications is reflected in the ASTM D1193 specification for reagent water used for IC, which is <50ppbC and accompanied by a specification for water resistivity of > 18MΩ.cm @25˚C.

Purified water is used in large quantities in numerous LDTs, such as those involving liquid chromatography, and due to this, the performance effects of contamination can be magnified.

Packaged, application-specific waters are sometimes used to carry out laboratory analysis, however water purity cannot be guaranteed; Although certificates of purity can accompany commercially bottled water, they may not accurately represent the water's quality at the time of use. This is due to the potential for contamination during the time elapsed between analysis and utilisation, as well as the ease with which contamination can occur.

Contamination from organic impurities can occur via plastic bottles or elastomeric closures and glass bottles may contribute inorganic ions degrading resistivity. Once bottles have been opened further contamination can arise from various unexpected sources, including local air, laboratory fixtures, water storage containers and transfer equipment, operators, and personal care products. Levels of contamination can increase over time and with multiple exposures.

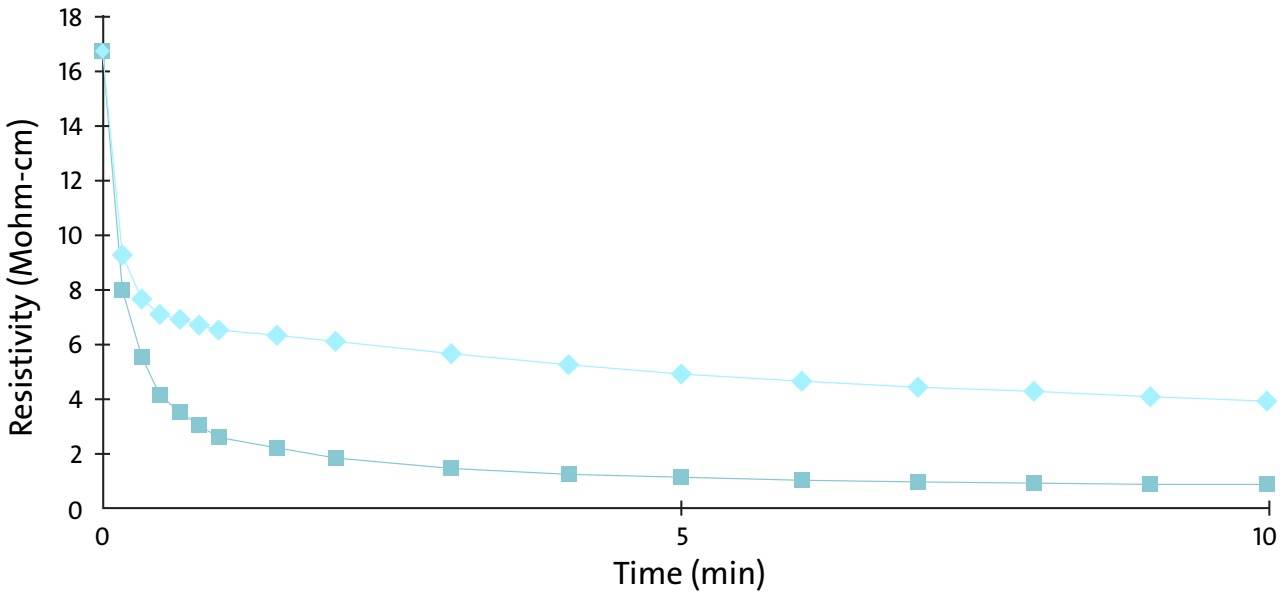
This limits confidence in the purity of packaged water, as any quality testing may be carried out a long time prior to use and even before packaging. Multiple uses of the same packaged water introduces further risks of deterioration in

purity and can lead to inconsistencies in analysis.

Ultrapure water is one of the purest substances used in laboratory analysis, and water as 'the Universal Solvent' can dissolve almost anything to some degree. Once generated, it is therefore extremely easy to contaminate and this can occur unexpectedly.

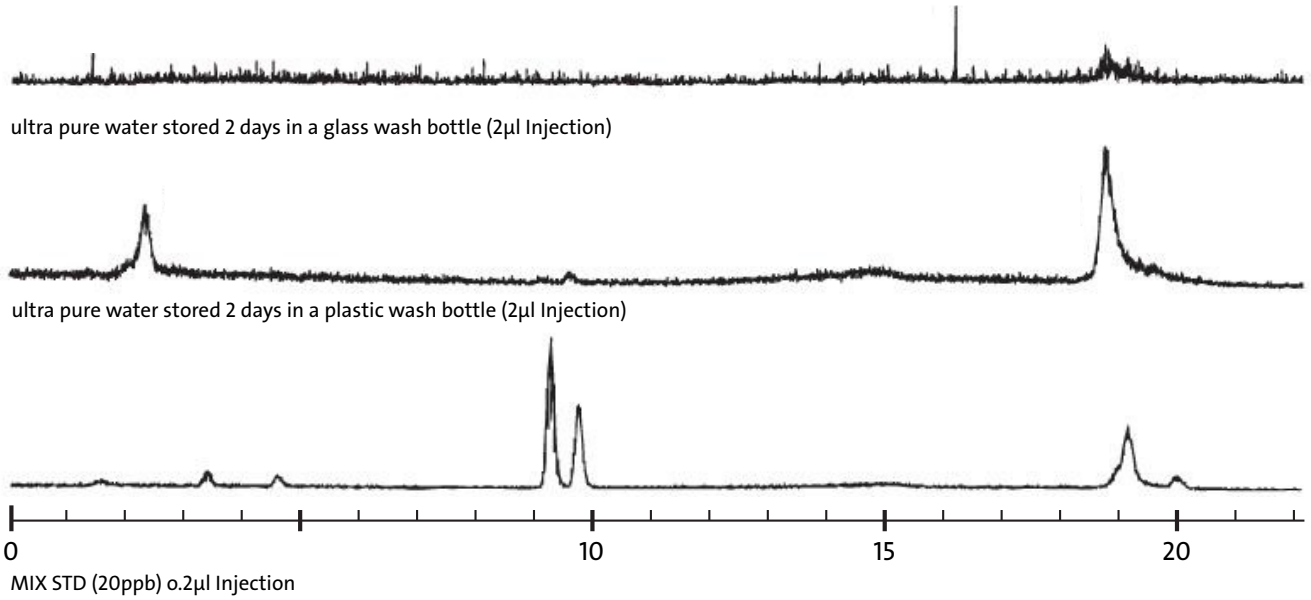
Contamination of ultrapure water can be almost instantaneous, as shown in the figures below through the decreasing water resistivity with exposure to air, the appearance of impurity peaks in HPLC traces due to contamination during storage, and MS detection of plasticizers in ultra pure water which has briefly contacted PVC material.

Resistivity of pure water in air



On exposure to air purified water resistivity is immediately compromised through dissolution of impurities

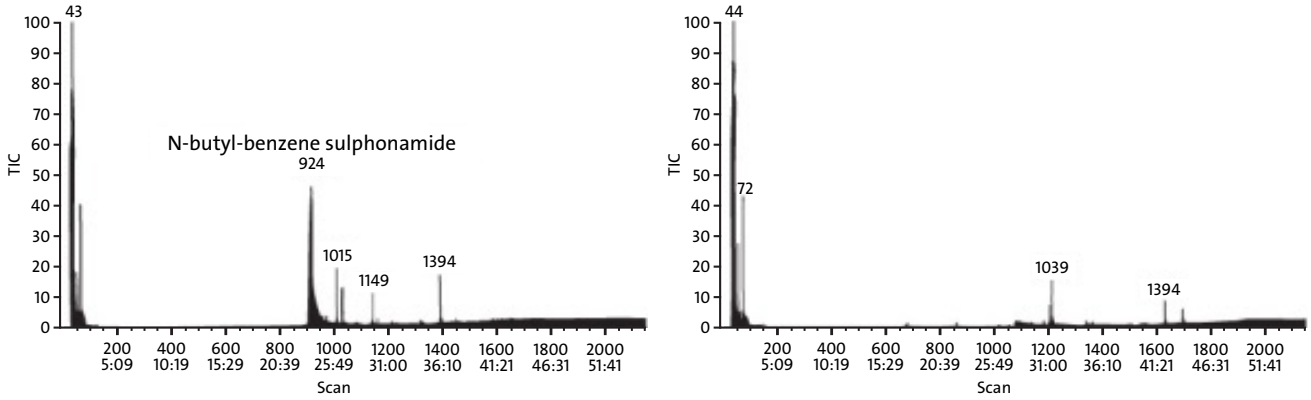
Contamination from a wash bottle



Chromatograms demonstrate compromised analytical results through storage of Ultrapure Water.[6]

Ultra pure water passed through 50cm of flexible PVC tubing

Ultra pure water



MS traces clearly show plasticizer contamination of ultra pure water via brief contact with PVC plastic.

While the above effects of water impurities on analytical equipment are obvious, this may not always be the case; a build up of material within the process, such as particulates for example, can cause gradual degradation of performance which can go unnoticed. A leading manufacturer of HPLC-MS equipment states that ultrapure water (i.e. particle-free, chemically clean, resistivity of >18MΩ.cm) will reduce potential for impurity accumulation[7].

Hence, where water purity is critical to analytical success, such as in many LDTs developed using highly sensitive equipment, the water should be used soon after generation by the purification system and protected from contamination.

Where water is generated using a local purifier system, water-purity can be monitored in 'real-time' through calibrated sensors within the water flow path. These can immediately highlight unexpected variations in the purity before it can impact test data.

Reputable manufacturers of laboratory water purification equipment also provide in-situ Validation packages to confirm that expected process performance is met; this may be on installation and periodically thereafter.

This Calibration and Validation, provides traceable confidence in purity of the important water reagent which therefore contributes to confidence in consistent LDT performance, and is particularly beneficial when demonstrating Quality Management to regulators such as the FDA.

Conclusion

- New restrictions bring challenges for LDT providers which may reduce the flexibility of the service provided.
- The first stages of FDA enforcement change to be implemented bring increased controls over Quality Management, including purchasing reagents and parts used for LDT delivery.
- Purified water is a reagent critical for many IVD tests including LDTs where it will come under increased scrutiny through new Quality Management System requirements.
- Purified water quality for LDTs can be ensured through using high quality water purification systems to generate water as required; as is the approach taken in most auto-analyser IVD work.
- Reputable suppliers of water purification equipment offer validation packages to confirm correct and expected performance of equipment in situ, and calibration of internal sensors that monitor purity in real-time; these can be used to demonstrate quality control of water used for IVD.

References

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