can detect oxygen content to below 1% vol/vol oxygen. However, contamination of the injection needle or electrode chamber by reconstituted product can be problematic and regular servicing of the container-piercing assembly is required. Noninvasive oxygen measurement involves the use of the frequency-modulated signal absorption of near infrared laser light at 760 nm by oxygen in the headspace gas. This method is nondestructive, is independent of the formulation and indeed appearance of the freeze-dried cake (providing it has not broken up to such an extent to contaminate the sides of the container), and so is more versatile and easier to operate. A limitation is that a standard curve is required for each diameter of container used (as the signal will depend on the path length of the gas and so the diameter of the container). These can be supplied by the manufacturer traceable to NIST certified oxygen/nitrogen mixtures. The method is rapid and eliminates the risks associated with breaking open a container for the destructive method. A comparative study between the two methods was performed and similar results were obtained.

## Product Stability

Of prime importance as a test of the success of the freeze-drying of standards is the retention of good reactivity after reconstitution. In practice, some loss of activity may be acceptable on freeze-drying but the resultant product should maintain its activity once dried. Within NIBSC, this assessment is often by specialist functional assays, some of which may have confidence limits that are wider than others. However, we offer the option of laying down snap frozen sample stored in liquid nitrogen (frozen baselines) as a means of checking whether it is the freezing or drying process that is causing the deterioration. Activity of the standards after freeze-drying is generally well preserved as can be seen from the illustrations of four biologicals in Table 4.

Vials may be readily stoppered within the freeze-drier and so do not pose the same degree of problem in maintaining the internal environment during sealing. However, these rubber closures may prove less suitable in terms of long-term storage, particularly at subzero temperatures, compared with flame-sealed ampoules that are impervious to gas exchange.

**TABLE 4** Preservation of Biological Activity in Some Typical Biological Reference Materials After Freeze Drying

| | Activity (IU/mL) | | |
|---|---|---|---|
| | Activity pre-drying (defined as 100%) | Activity post-drying | Percent |
| 01/592 Heparin[a] | 1312 IU/mL | 1270 IU/mL | 96 |
| 01-037-MA factor VIII in plasma | 0.5 IU/mL | 0.46 IU/mL | 92 |
| 01/586 Thrombin[b] | 24 IU/mL | 22.3 IU/mL | 93 |
| 04/150 Tetanus toxoid[c] | 701 Lf/mL | 646 Lf/mL | 92 |

[a]Measuring factor Xa activity.
[b]Measured by chromogenic substrate assay.
[c]Flocculation activity units, measured by ELISA.
*Abbreviation*: LF, limes flocculationis.
*Source*: Data Courtesy of Drs Elaine Gray, Anthony Hubbard, Colin Longstaff, and Thea Sesardic, National Institute for Biological Standards and Control.