

3.2.S.2.4. IN-PROCESS TEST METHODS [BNT MAINZ AND RENTSCHLER]

Descriptions of the analytical procedures used for in-process testing as identified in the process flow diagram in [Section 3.2.S.2.2 Manufacturing Process \[BNT Mainz and Rentschler\]](#) are provided below.

3.2.S.2.4.1. In-Process Testing for Control (IPT-C)

Descriptions of the in-process test methods used for control are provided below.

3.2.S.2.4.1.1. Determination of RNA Concentration by Ultraviolet (UV) Spectroscopy

The UV spectroscopy method is used to quantitate the RNA concentration in the ultrafiltration/diafiltration (UFDF) pool (pre- or post-dilution) and is used to determine UFDF step yield. Details of the analytical procedure are described in [Section 3.2.S.4.2. UV Spectroscopy](#). A summary of the method validation is detailed in [Section 3.2.S.4.3 UV Spectroscopy](#).

3.2.S.2.4.2. In-Process Testing for Monitoring (IPT-M)

Descriptions of the in-process test methods for monitoring are provided below. These IPT-Ms with established action limits are used to routinely monitor the manufacturing process and ensure that the process remains in a state of control.

3.2.S.2.4.2.1. Determination of RNA Concentration by Ultraviolet (UV) Spectroscopy

The UV spectroscopy method is used to quantitate the RNA concentration in the proteinase K pool sample to determine IVT yield and at various steps during the UFDF recovery operation. Details of the analytical procedure and the corresponding method validation, are provided in [Section 3.2.S.4.2. UV Spectroscopy](#) and [Section 3.2.S.4.3 UV Spectroscopy](#), respectively. Prior to analysis, RNA is isolated from the proteinase K pool sample to remove matrix components that would cause inaccuracies during UV spectroscopy.

3.2.S.2.4.2.2. Bioburden

The bioburden procedure is performed to determine the microbial load of viable microorganisms in the proteinase K pool (pre- and post-hold) and in the UFDF pool (diluted pool and pre-filtration) samples. The analytical procedure is performed following the principles described in the local compendia, USP <61>, Ph. Eur. 2.6.12, and JP 4.05 using membrane filtration methodology.

3.2.S.2.4.2.3. Endotoxin

The purpose of bacterial endotoxin testing is to measure the level of bacterial endotoxins in the proteinase K pool (pre- and post-hold) and in the UFDF pool (diluted pool and pre-filtration) samples. The analytical kinetic turbidimetric limulus amoebocyte lysate (LAL) procedure is performed following the principles described in the local compendia, USP <85>, Ph. Eur. 2.6.14, and JP 4.01.