1530 Lindbergh Dr • Beaumont, TX 77707-4131
Local 409-842-3714 • Toll free 800-231-5663 • www.helena.com
Email (Orders) orderentry@helena.com (General) helena@helena.com

Technical Bulletin - SPIFE Nexus Urine Template Application

Urine samples may be run on the SPIFE Nexus using a template. Advantages of the template application include the low sample requirement (only 3 µL per UPE sample, 18 µL per urine IFE sample) and the ability to run both urine and serum samples on the same gel. (Due to differences in the running parameters, urine specimens cannot be processed on the SPIFE Nexus in combination with serum samples unless urine specimens are applied using template application.)

Template applications may be used in one row, multiple rows, or all rows. To allow serum and urine samples to be run in combination on the same gel, the gel is prepared with urine samples manually applied to the bottom row or rows of the gel by template application while the SPIFE Nexus is processing serum samples. The prepared gel is then placed on board the instrument and the test continues with blade application of the serum samples.

See specific procedures for step by step guidance:

- SPIFE Nexus ImmunoFix-6 Procedure in Combination with Urine Template Application, Pro 281
- SPIFE Nexus ImmunoFix-9 Procedure in Combination with Template Urine Application, Pro 84
- SPIFE Nexus ImmunoFix-15 Procedure in Combination with Template Urine Application, Pro 280
- SPIFE Nexus Split Beta SPE Procedure in Combination with Template Urine Application, Pro 85

Concentration

A total protein range of 500-3,000 mg/dL is generally sufficient for optimum sensitivity. For the majority of samples run with the template procedure, concentrating to a target of 1000-1500 mg/dL is a reasonable target. Urine IFE is more sensitive than UPE, samples may need slightly higher concentration for UPE. There are several factors to consider with regard to concentration; sensitivity, relative distribution of the bands, accuracy of the total protein measurement, and sample characteristics.

1. **Sensitivity:**
   The sensitivity for SPIFE Nexus Urine IFE is 1-3 mg/dL for a single band. Note that sensitivity may vary, depending on sample characteristics. For example, a monoclonal migrates in gamma against a hypogamma background is more readily detectable than a monoclonal migrating in a polyclonal background or with alpha/beta proteins. In addition, every monoclonal is a unique protein with unique epitopes. The affinity of the antisera/antibody reaction may vary slightly for any given monoclonal.
2. **Sample characteristics:**
   a. Normal urine does not contain a significant quantity of protein, and clinically significant monoclonals may be present in low quantities. Thus urine samples should be concentrated if a higher sensitivity is desired. Concentration schemes are based on the initial total protein measurement to arrive at a target final concentration to best visualize bands. Too low of a concentration with too little protein, and a band can't be seen. Too much, and the bands may prozone, or be too dark and fuzzy to interpret.
   b. Unfortunately no single concentration scheme is optimal for all samples. A total protein measurement, by definition, includes the total of all fractions, but prior to electrophoresing the sample, the relative percentages of each fraction are unknown. A sample with a total protein of 1 mg/dL could be 99% albumin and 1% monoclonal, or 100% monoclonal. Thus the monoclonal component could range from 1 mg/dL and be at the limit of detection, or 100 mg/dL. The final gel will look very different for these two samples. A sample in which the majority of the protein consists of one band may have a prozoned/dark

appearance. A urine sample with a large albumin band may appear to have a faint monoclonal that is difficult to visualize.

c. Concentrating to a higher final total protein will increase sensitivity to small monoclonals, however a greater percentage of samples may need to be rerun for improved visualization. For the majority of urine samples run with the template procedure, concentrating to a target of 1000-1500 mg/dL is a good target. See table 1 for examples of starting and finishing volumes for different concentration factors.

d. In addition, the appearance of individual monoclonal proteins may vary. Each monoclonal is a unique protein containing unique epitopes. The strength of the antisera/epitope reaction may vary from sample to sample. In some situations, the structure of the monoclonal can partially obstruct the binding site of the antisera. Monoclonals of the same quantity won't always appear to be of the same intensity. (This is also why the SP lane, heavy chain, and light chain do not always appear of the same intensity)

3. **Accuracy of the total protein measurement:**

Total protein measurements should not be obtained from dipsticks. Dipsticks are known to be insensitive to Bence Jones proteins. This can cause an underestimate of total protein values in samples with significant monoclonals, and lead to overconcentration and prozoning in positive samples.

Tips on Technique

1. Urine samples can degrade quickly; alpha and beta proteins are the least stable. Degraded samples will look fuzzy, and while the monoclonal protein will be stable longer, there may be a loss of sensitivity.

2. Excess moisture left on the gel can cause fuzzy patterns. Before running, carefully blot the gel with a Blotter C and then place the templates on the gel in the correct position and orientation.

3. Be sure to use an Alignment Guide with the same number of paired template alignment pegs that correspond to the number of rows on the gel type used. An incorrect Alignment Guide may result in patterns positioned too high or too low on the gel.

4. Be sure to use a template with the correct number of slits. An incorrect template will result in patterns that do not line up with the antisera application and labeled antisera lanes for the gel type used.

5. Orient the template so that the small positioning hole is in the lower left side. After placing the template on the gel place a Blotter A Plus on the template and smooth the template down gently to seal it to the gel. Be careful to not push too hard or damage the gel. Excessive pressure can damage the gel, resulting in distorted patterns and/or arcing.

6. Pipette 3 µL of concentrated urine to the template slit in a drop. There's no need to spread the sample, and you do not want to touch the slit with the pipette tip.

7. After the samples have absorbed for 5 minutes, use the same Blotter A Plus. Blot off the sample that didn't absorb in 5-minutes. Remove the blotter and then carefully remove the template. When running multiple rows of template urines, it is suggested to set a 5 minute timer per row for equivalent absorption per row.

8. Cover the gel with the top half of the clamshell until the SPIFE Nexus has completed the serum sample processing to prevent drying. Dry gels may have shorter patterns, especially on the outer edges.

9. Gel size: It is recommended that if running a template method, a single template size is selected for optimum calibration of antisera placement. Options include SPIFE IFE 9 and 15 gels or SPIFE IFE 6 gels.

Necessary Components



1) SPIFE Nexus ImmunoFix-6 Procedure in Combination with Urine Template Application, Pro 281
    a. SPIFE Urine IFE Alignment Guide 3380
        i. The guide is yellow.
        ii. **There are three sets of pegs for 3 rows.**
    b. SPIFE Urine IFE-3/6 Accessories 3390
        i. Templates have 12 slits.
        ii. Orient the template so that the small positioning hole is in the lower left side.

2) SPIFE Nexus ImmunoFix-9 Procedure in Combination with Template Urine Application, Pro 84
    a. SPIFE Urine IFE Alignment Guide 3380
        i. The guide is yellow.
        ii. **There are three sets of pegs for 3 rows.**
    b. SPIFE Urine IFE-9/15 Accessories 3391
        i. Templates have 18 slits.
        ii. Orient the template so that the small positioning hole is in the lower left side.





3) SPIFE Nexus ImmunoFix-15 Procedure in Combination with Template Urine Application, Pro 280
    a. SPIFE Urine IFE-15 Alignment Guide 3407
        i. The guide is yellow
        ii. **There are five sets of pegs for five rows.**
    b. SPIFE Urine IFE-9/15 Accessories 3391
        i. Templates have 18 slits.
        ii. Note that as templates are placed onto the Guide, they will overlap.
        iii. Orient the template so that the small positioning hole is in the lower left side.

4) SPIFE Nexus Split Beta SPE Procedure in Combination with Template Urine Application, Pro 85
    a. SPIFE Urine IFE Alignment Guide 3380
        i. The guide is yellow.
        ii. **There are three sets of pegs for 3 rows** .
    b. SPIFE Urine Protein Accessories 3427
        i. Templates have 20 slits.
        ii. Orient the template so that the small positioning hole is in the lower left side.



Table 1: Urine Concentration: Starting and Finishing Volumes

Concentration Factor	Initial total protein (g/dL)	Initial total protein (mg/dL)	initial volume (mL)	Final volume (µL)	**Final total protein (g/dL)**
200x	0.005	5	10	50	**1.0**
	0.01	10	10	50	**2.0**
	0.02	20	10	50	**4.0**
100x	0.01	10	5	50	**1.0**
	0.02	20	5	50	**2.0**
	0.05	50	5	50	**5.0**
50x	0.01	10	5	100	**0.5**
	0.02	20	5	100	**1.0**
	0.05	50	5	100	**2.5**
	0.075	75	5	100	**3.8**
25x	0.01	10	5	200	**0.3**
	0.02	20	5	200	**0.5**
	0.05	50	5	200	**1.3**
	0.075	75	5	200	**1.9**
	0.1	100	5	200	**2.5**
10x	0.01	10	1	100	**0.1**
	0.02	20	1	100	**0.2**
	0.05	50	1	100	**0.5**
	0.075	75	1	100	**0.8**
	0.1	100	1	100	**1.0**
	0.25	250	1	100	**2.5**
	0.5	500	1	100	**5.0**
5x	0.01	10	1	200	**0.1**
	0.02	20	1	200	**0.1**
	0.05	50	1	200	**0.3**
	0.075	75	1	200	**0.4**
	0.1	100	1	200	**0.5**
	0.25	250	1	200	**1.3**
	0.5	500	1	200	**2.5**
	1	1000	1	200	**5.0**

For more information, contact Technical Services by calling 409-842-3714 or 800-231-5663 (USA) or by email at technicalservice@helena.com.

399-107
2/26